THE ROLE OF ANTIOXIDANTS IN BOAR SEMEN PRESERVATION

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Abstract

The aim of the research was to test the biologically active substance obtained by the Institute of Microbiology of the Science Academy from Moldova. It was introduced in the basic medium as an additional component for boar semen dilution. The obtained experimental data showed that the introduction of 7% of the biologically active substance in the basic medium had positive results in the preservation of the semen material quality at 16-18° C. After 96 hours of conservation at 16-18° C the sperm motility of the spermatozoa was 54,3%.

Key words: boar, sperm, medium, speed of movement, preservation, hypothermic temperature

For the first time, it has been demonstrated that the capacity of the biologically active substance has demonstrated specific and non-specific characteristics to increase the preserved sperm resistance at 16-18° C. There was studied the average speed of the sperm with velocity average path (VAP), the average speed of the sperm with straight linear velocity (VSL) and curvilinear velocity (VCL) during the preservation at the hypothermic temperatures. The proposed medium for the dilution and preservation of the boar sperm at 16-18° C contains: glucose, sodium citrate, helaton, ammonium sulphate and the biologically active substance. The application of this medium allows to use more efficiently valuable reproduction characteristics of boars in the artificial insemination system.

INTRODUCTION

One of the important technological issues in the pig industry is to improve the reproduction of the herd when using artificial insemination. Pork production enterprises use fresh, or transported in a chilled state, semen of high-value boars instead of the traditional delivery of boars in order to eliminate inbreeding and to improve breeding.

One of the urgent problems of the animal husbandry science is to increase the efficiency of herd reproduction by maximizing the use of high-value producers, which is of great importance for ensuring further progress in animal husbandry. The use of artificial insemination is an important direction of improving the breeding and productive qualities of animals. One of the determining factors for the effectiveness of artificial insemination is the improvement of sperm storing methods, (Epshina, 2011).

Considering the peculiarities of boar spermatozoa, which react differently to thermodynamic processes and, consequently, to the formation of protective reactions and adaptive properties during storage outside the body, this is an urgent problem, (Narizhny, 2001).

In this regard, it seems promising to develop more efficient medium for the short-term storage of boar semen using biological active substances and other cry protectants.

The speed of sperm movement is a characteristic feature of all animal species. Its evaluation is done by different methods. It was established that sperm motility is normal if the spermatozoa pass the field of view of the counting chamber 0.06 ml in 0.9 seconds. The deviation from the normal forms of sperm movement often occurs; it depends on the influence of various factors of functional or morphological nature, (Zăhan, 2017). As the author notes, the assessment of sperm

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motility by computer programs is considered more modern and more effective than the traditional methods for assessing the sperm quality. A computer assessment of sperm motility was used to study boar semen, (Holt et.al. 1977), rams, (Martiner and Maswell, 1999), stallions, (Kovak et al, 2008).

The determination of the quality of diluents by the method of a test medium for motility and survival of spermatozoa outside the body is important. However, the most important biological properties of sperm, and namely the speed of sperm advance, remain relevant. That is why, in a detailed study of the quality of diluents, along with a bio control method, more functional methods should be applied to test the action of the tested diluents on the biological properties of spermatozoa, such as their speed of movement, which seems to correlate with fertilizing ability.

MATERIAL AND METHOD

In our research, we took as a basis, the production medium GCChS - glucose, citrate, chelate, sulphate medium for dilution of sperm from boars.

It was necessary to identify the optimal concentration of the biologically active substance preparation introduced as an additional component into the basic medium and prolonged exposure of the sperm at 16-18^0 C, as well as to determine the physical state of the germ cells under these storage conditions.

The study was conducted on the sperm of the boars of Landrace breed. After an eye evaluation, each ejaculate was divided into 11 parts and diluted with production medium - GCChS containing from 1 to 10 % of the biologically active substance preparation developed by the Institute of Microbiology and Biotechnology of the Academy of Sciences of the Republic of Moldova. The diluted sperm was kept at a temperature of 16-18^0 C. The sperm motility was determined every 24 hours.

These indicators were determined using the CEROS program.

VAP is a measurement of a common path taken by spermatozoa for a certain period of time. VSL is a straightforward path traveled by spermatozoa from one to the other for a certain observation period. VCL - the total path traveled by the sperm head for the period of observation.

RESULTS OF RESEARCH

The experiment tested the effect of the biologically active substance preparation on the biological properties of spermatozoa during their storage at 16-18^0 C.

The data on the study of the effect of the biologically active substance preparations an additional component in the composition of the basic medium are presented in Table 1.

Table 1 Spermatozoa motility depending on the preparation concentration, %

Concentration of the biologically active substance, %	1 day		2 days		3 days		4 days	
	Motility, %	Straight linear velocity, %	Motility, %	Straight linear velocity, %	Motility, %	Straight linear velocity, %	Motility, %	Straight linear velocity, %
1	84.5±5.5	42.8±7.9	75.0±2.8	29.8± 2.4	73.3±10.3	28.8±7.3	45.3±18.5	14.8±6.7
2	87.0±5.6	45.0±6.1	79.3±8.7	36.0±8.9	66.0±9.0	22.5±6.1	49.8±17.6	16.5±6.0
3	85.8±5.1	44.3±6.4	81.0±6.1	33.5±5.8	71.3±9.7	27.3±7.1	33.3±20.0	10.5±6.5
4	83.8±6.0	41.5±4.9	78.8±7.1	32.3±6.2	70.0±9.0	24.5±4.3	39.0±19.2	14.8±8.2
5	89.3±3.3	45.5±3.0	78.8±8.8	35.8±6.8	73.5±8.9	29.8±6.2	44.3±20.6	16.0±8.4
6	87.3±1.7	43.0±1.9	73.0±7.6	34.5±4.9	73.3±9.7	31.5±6.0	52.5±18.6	20.0±7.8
7	85.3±3.2	47.0±4.6	81.5±3.0	37.5±1.2	77.0±9.1	32.0±6.4	54.3±15.4	18.3±7.4
8	83.0±3.5	39.8±5.5	78.3±6.8	38.8±4.6	62.5±10.7	21.3±7.1	23.8±18.5	9.8±7.3
9	92.3±1.7	47.8±1.7	78.0±9.7	27.8±6.4	73.3±8.9	31.3±6.2	45.3±10.4	12.3±4.8
10	86.8±2.4	41.8±4.4	78.3±8.5	33.3±7.7	64.5±8.9	25.3±5.6	41.3±16.3	14.3±6.3

The research results show that biologically active substance preparation introduced into the basic medium (GCChS) is not toxic to spermatozoa within the studied concentration. Sperm motility after dilution in all the studied media corresponded on

average from 83.0% when 8% of the biologically active substance was introduced into the medium and 92.3% when 9% of the biologically active substance was added to the medium.

During the storage of sperm at 16-18^0 C sperm motility has changed. The best motility results were obtained when 7% of the biologically active substance preparation was administered to the basic medium. After 96 hours of semen storage, the motility was 54.3%. The same changes occurred in the number of sperm with straight linear velocity. If, immediately after dilution, the number of sperm cells with straight linear velocity was on average 40% in all the tested media, then during the storage process of the diluted sperm this indicator decreased. The best results were obtained when 7% of the biologically active substance preparation was introduced into the basic medium; after 96 hours storage, the amount of spermatozoa with straight linear velocity was 18.3%.

When measuring the speed of sperm movement, the CEROS program determines three indicators: average speed - VAP is the average speed passed by the spermatozoa over a certain period of time. VAP measurement data are presented in Table 2.

Table 2 Velocity average path of the boar semen (VAP) μм/s

concentration of the biologically active substance, %	24 h	48 h	72 h	96 h
1	98.1±1.5	85.1±7.6	72.1±7.6	47.7±16.7
2	108.7±9.5	89.8±9.2	74.4±10.0	54.3±18.5
3	95.6±7.1	83.2±12.7	79.3±8.8	29.3±17.2
4	90.0±3.2	81.7±8.2	77.8±11.7	45.5±17.2
5	104.6±9.1	76.1±9.0	77.2±9.7	50.2±17.6
6	98.7±1.6	82.3±4.1	82.6±7.2	49.0±16.9
7	96.8±5.4	80.0±6.0	85.4±12.6	65.2±11.9
8	94.8±5.8	85.4±6.1	67.9±10.6	34.1±19.7
9	99.0±6.6	76.3±9.1	85.3±8.2	55.2±12.0
10	97.1±2.6	79.4±10.8	70.4±7.4	47.9±16.1

The data presented in Table 2 show that the total path traveled by the spermatozoa for a certain period of time immediately after the dilution of the sperm in all the tested media has remained almost unchanged. However, in the research process the quality of sperm has changed. The best results were obtained when 7% of the biologically active substance preparation was introduced into the main diluent. The total path passed by the sperm after 96 hours of the sperm storage was 65.2 ± 11.9 μm / s.

When the speed of sperm movement is measured by the CEROS program, the straight-line path passed by sperm from one point to another is measured for a certain period of observation. This is the lowest digital value compared to the other measurements carried out by the CEROS program.

The data are presented in table 3.

Table 3 Straight linear velocity of boar spermatozoa (VSL) μм/s

concentration of the biologically active substance, %	24 h	48 h	72 h	96 h
1	51.4±3.7	44.6±6.5	36.6±3.8	33.5±15.3
2	59.1±6.1	48.0±5.7	37.8±5.7	27.9±9.5
3	50.4±6.0	44.5±5.1	39.9±5.5	15.7±9.3
4	47.6±2.9	43.0±4.2	37.6±4.0	25.0±9.1
5	56.2±4.0	41.7±4.1	40.9±4.9	25.6±9.3
6	53.7±0.5	44.4±1.7	41.3±3.8	26.4±9.1
7	52.5±1.9	44 5±2.3	44.7±6.2	33.8±5.5
8	52.4±2.9	48.3±2.7	36.0±6.3	18.4±10.6
9	54.5±2.6	41.8±3.3	43.2±2.8	29.3±3.7
10	51.6±2.8	42.8±5.5	36.8±3.4	25.4±8.7

Measuring the straight path traveled by the sperm from one point to another for a certain time we have established that the best results were obtained when 7% of the biologically active substance preparation was introduced into the basic medium. After 96 hours of sperm storage at 16-18^0 C, the speed of spermatozoa movement was 33.8±5.5 μm/s.

The data on the study of the total path traveled by the sperm head VCL for the observation period are presented in Table 4.

Table 4 Curvilinear velocity of boar spermatozoa (VCL) μм/s

concentration of the biologically active substance, %	24 h	48 h	72 h	96 h
1	186.1±8.1	150.1±10.1	115.4±32.1	82.7±28.5
2	191.2±10.5	159.3±15.3	131.7±17.1	96.7±32.4
3	178.8±10.9	147.2±23.1	139.8±14.4	52.3±30.2
4	167.2±10.9	144.2±15.3	143.8±18.8	84.9±30.3
5	191.3±12.6	138.1±14.2	139.3±15.5	87.1±30.7
6	179.7±7.1	148.0±12.3	155.9±14.9	85.8±28.9
7	185.9±13.8	144.6±12.6	155.6±21.0	115.2±22.2
8	176.8±8.6	152.8±10.5	123.2±18.3	57.0±32.9
9	187.4±10.9	134.9±18.7	157.6±15.3	97.7±20.3
10	179.2±6.6	145.8±20.5	131.1±12.3	83.6±28.1

The obtained data show that this is the largest digital indicator in comparison with other measurements. It was also found that the best results were obtained when 7% of the biologically active substance preparation was introduced into the primary diluent. The total path traveled by spermatozoa in this variant of the experiment was 115.2 ± 22.2 μm/s.

Despite the fact that the CEROS program conducts a lot of researches to determine the quality of sperm, recently in the specialty literature there have been disputes whether CEROS indicators can be used to predict the fertilizing ability of the sperm.

CONCLUSIONS

For the first time, the possibility of using biologically active substance preparation, a substance with specific and nonspecific actions to increase the biological ability of spermatozoa, introduced as an additional component in the composition of GCChS - medium, has been studied.

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