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CONTENT

BOTANY

PHYSIOLOGY OF ANIMALS

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MODERN MICROBIOLOGICAL APPROACHES TO THE REMEDIATION OF BREEDING BULLS SPERM

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Abstract

As a research result, it was established that the activities of germ cells in frozen-thawed semen with the introduction of gentamicin in the diluent in comparison with spermosan tended to a higher activity by 0.2-0.33 points.

Sperm with 5-7.6% gentamicin had a higher fertilizing ability among the experimental breeds. We can only talk about the tendency of higher efficiency of sperm of the Holstein red and black-spotted breed bulls.

The use of sperm with gentamicin for artificial insemination of cows and heifers will increase the efficiency of reproduction and will allow to obtain additional products for each cow by UAH 761.5.

It is recommended to use the drug gentamicin to rehabilitate the sperm of breeding bulls in order to reduce the duration of the service period and increase the efficiency of artificial insemination of cows and heifers. We recommend application of laboratory equipment HBO Bio-Test laboratory or IMV-2.

Keywords: breeding bulls, semen, sanitizing drugs, spermosan, gentamicin, activity, sperm survival, frozen-thawed sperm, the effectiveness of artificial insemination.

Topicality. Nowadays, the main task of animal husbandry is to increase production and reduce production costs. It can be achieved by further intensification of animal husbandry improving the animal's productive qualities and providing scientifically sound standards of feeding and keeping.

Large-scale selection is impossible without the biotechnological methods application in animal breeding, i.e. artificial insemination using deep-frozen sperm. The genetic improvement of animals is carried out due to the use of sperm of the most valuable breeding bulls tested for the offspring quality.

The most intensive use of bulls-breeders for artificial insemination has been achieved in dairy farming, where tens and hundreds of thousands of highly productive animals are obtained from individual bulls-breeders.

The role of heredity of high-quality breeders in the genetic improvement of dairy breeds of cattle reached 90-95%. They are have become a golden fund of animal husbandry.

The ability to reproduce is one of the physiological functions of mammals, it is under the constant influence of internal and external factors. I. Smirnov's research found that sperm outside the body are able to live in artificially made environment, i.e. diluents without losing hereditary properties.

However, sperm as a carrier of hereditary information is very sensitive to the influence of various exogenous factors affecting its functional properties. It is not always possible to create the necessary conditions for aseptic production of sperm of breeders, to comply with the necessary conditions for its evaluation and breeding.

Microbial contamination of bull semen is the main cause of reduced sperm fertility, impaired embryogenesis, abortion, metritis, birth of dead or weak, non-viable calves (A. Kruhliak, 2018; S. Shalovilo, 1983).

The topicality of the research is caused by the fact that the existing methods of obtaining and storing sperm do not provide sperm free of microorganisms.

Analysis of recent research and publications. At breeding enterprises various microorganisms can get into the sperm of the bull from its skin, air, unsatisfactorily prepared artificial vaginas and utensils. Sperm is an ideal environment for the microorganisms' development. They emit harmful metabolic products that adversely affect the vital functions of sperm. Thus, the problem of remediation of farm animals' semen is relevant, and methods of remediation of sperm need further development and improvement.

Antibiotics are organic substances synthesized by microorganisms in nature to protect against the intervention of other species of microorganisms, and have the ability to inhibit the development or kill these microbes.

As a rule, antibiotics are isolated from live bacteria or fungi. There are also a large number of synthetic antibiotics differing in modifications of the functional chemical groups of natural antibiotics. Such modified compounds are often more effective or more resistant to neutralization due to the resistance acquired by microorganisms [4, 32].

A characteristic feature of antibiotics is their ability to disrupt certain metabolic links of microorganisms or the action of some enzymes.

A characteristic feature of antibiotics is the selectivity of their action against microbes, i.e. each antibiotic affects only certain species. It is characterized by a specific antimicrobial spectrum of action.

Antibiotics gradually slow down the reproduction of microorganisms in the body and they gradually degenerate. The manifestation of the antimicrobial action of the antibiotic requires a constant continuous action over a period of time. They are distinguished by the mechanism of antimicrobial action as bactericidal and bacteriostatic [18].

Antibiotics exhibit primarily antimicrobial action (i.e. act on microbial cells). The point is the difference in the intensity of division and activity of microorganisms and cells of the body or sperm. Microbes multiply faster in the body; and antibiotics act on cells that multiply rapidly. So, antibiotics act primarily on microorganisms, and then (with a longer action) they act on cells [22].

Polymyxins are a group of narrow-spectrum antibiotics acting on gram-negative microorganisms. Antibiotics only help our defenses to fight infections that are unable to deal with them on their own. The most pronounced inhibition of hematopoiesis (leukopenia) in chloramphenicol [2].

Gentamicin is colorless or slightly yellow solution; 1 ml of solution contains 40.0 mg of gentamicin. Pharmacotherapeutic group is an antibacterial agent for systemic use.

Gentamicin is a broad-spectrum antibiotic of the aminoglycoside group. It has a bactericidal effect, irreversibly disrupting the protein synthesis of the pathogen. It is active against most grams of negative aerobic bacteria (Klebsiella spp., Shigella spp., Serratia spp., Escherichia coli, Proteus spp., Enterobacter spp., Pseudomonas aeruginosa, Acinetobacter spp.), tagram-positive aerobic cocci, (Staphylococcus spp., including strains resistant to penicillin and other antibiotics). It does not affect Neisseria meningitidis, Treponemapallidum, some strains of Streptococcus spp., and anaerobic microorganisms.

The need to rehabilitate sperm was first pointed out by I. Ivanov. He used alcohol, salversan, neganol and others for this purpose.

Nowadays various antibiotics are used for the rehabilitation of animal sperm (penicillin, streptomycin and others), and sulfonamide drug (streptocide white) doth in Ukraine and abroad.

According to the existing norms, freshly obtained sperm of breeding bulls must have a negative coli-titer and contain no more than 5,000 microbial bodies in 1 ml. The permissible coli-titer of the prepuce wash should not exceed 1 to 100.

Many species of microorganisms introduced with sperm into the genitals of females cause inflammation, the death of zygotes and embryos, as well as the death of newborns in the first days of life [12, 28].

The quality of sperm and the perfect diluents application helping to maintain the sperm viability after processing are among the main factors ensuring intensive reproduction of cattle [9, 25].

Thus, bactericidal and bacteriostatic substances are introduced into the composition to inhibit the development of the microflora diluting sperm with synthetic diluents.

The glucose-citrate-yolk (GCY) diluent is used to dilute the sperm of breeding bulls. It includes distilled or double-distilled water, glucose-medical anhydrous, pentahydrate sodium citrate, chicken egg yolk, spermosan-3 in the amount of 0.09-0.12 g per 100 ml amounting to 75-90 thousand units.

It should be noted that different amounts of antibiotics are used in the diluent for sperm of different animals' species. So, 75-90, 50-70 and 25-30 thousand units of bulls, sheep and boars' sperm are introduced per 100 ml.

Various bacteriostatic substances of crystalline penicillin (sodium or potassium salt), streptomycin (sulfuric or hydrochloric acid), and streptocide have been used for sperm rehabilitation for 15 years.

Spermosan is used for simplicity of synthetic diluents manufacturing. The drug is a non-toxic to animal sperm mixture of antibiotics, 250 thousand units of penicillin; 250 thousand units of streptomycin; and 0.5 g of streptocide.

Each first ejaculate of frozen semen must be examined for bacteriological total contamination, coli-titer, the presence of fungi, Pseudomonas aeruginosa and anaerobic microflora [9, 16].

However, the disadvantage of long-term use of spermosan as an envoronment for dilution of sperm is that developing strains of microorganisms resistant to this drug.

The most rapidly developing antibiotic-resistant strains are Staphylococci, Escherichia coli, Proteus, Pseudomonas aeruginosa, and Mycoplasma.

The rehabilitation of sperm is its particular importance to species. Sperm of breeding bulls are more sensitive to antimicrobial drugs than sperm of other animal species.

Rehabilitation by high concentrations complex combined drugs reduces their biological parameters and fertilizing ability. The effectiveness of artificial insemination depends on the quality of sperm, the good quality of the environment and sanitizing drugs as their part. Increasing the dose of sanitizing drugs increases the sanitizing effect, it also increases toxicity.

Prolonged application of bactericidal drugs in sperm dilution environments promotes the microorganisms' resistant strains development.

They have similar action on our body and microorganisms, they are less toxic. They differ in the effect substitution, action duration, efficiency, ability to accumulate in organs and tissues, and the activity against some microbes [25]

There are lots of research results on the sanitizing drug spermosan's effect on the sperm of breeding rams.

The introduction of spermosan into the ram's semen at the rate of 25, 50, 75, 100, 125, 150 thousand units per 1 ml of diluted semen differently affects its quality. The spermosan dose of 25, 50, 75 thousand units did not have a negative effect on the ram's semen after its dilution for 3-4 hours at a temperature of 2-3^{0}C. Sperm activity was in the control (without spermosan) [21].

The spermosan doses of 100, 125, 150 thousand units decreased sperm activity by 0.4 points and was 7 points for these options. A similar situation was observed after 3-4 hours of adaptation, i.e. the motility of sperm was 7.1 points in the control variant, it was 7.15 points in the variants with the addition of spermosan doses of 25, 50, 75thousand units.

The mobility after adaptation decreased to 6.3; 6.3; 6.5 points in variants with the addition of a 100, 125, 150 thousand units spermosan dose.

The most significant effect of spermosan high doses was observed in frozen-thawed ram's semen. Sperm motility averaged 2.3 points in the control variant, the introduction of spermosan doses of 25, 50, 75, 100, 125, 150 thousand units decreased it to 1.8, 1.75, 1.5, 1.0 points [24].

It should be noted that spermosan application into the ram's semen reduces the absolute sperm survival rate. Thus, this figure decreased more than 5 times (from 39.8 to 7.44 absolute units) after freezing-thawing, spermosan dose of 150 thousand units killed all sperm after freezing-thawing. The spermosan reduced the survival rate of native sperm from 39.8 to 8.5 absolute units.

The research results indicate the inexpediency of spermosan indication for sanitation of breeding rams' sperm in doses above 50-75 thousand units.

The spermosan doses of 100, 125, 150 thousand caused a decrease in sperm motility by 1.4 points, the spermosan negative effect on sperm motility was observed in frozen-thawed sperm, too.

Spermosan doses of 25, 50, 75 thousand units does not have a noticeable negative effect on the ram's semen after dilution and 3-4-hour adaptation at a temperature of 2-3^{0}C.

Research of microbial contamination of native sperm of breeding bulls indicate that from 37.3 to 45.4% of ejaculates did not meet the requirements of veterinary and sanitary rules in the reproduction of farm animals.

According to scientific literature, the level of semen contamination is influenced by the obtaining method. Using the Kharkiv technology of aseptic sperm production, 337 samples of native sperm were obtained from 60 breeding bulls, 126 samples (37.3%) were found to be inconsistent with veterinary and sanitary norms.

Using the generally accepted technology 245 samples were obtained from 28 bulls; they did not meet the requirements [13, 34].

The research results showed that the number of ejaculates meeting the requirements of veterinary and sanitary norms was by 5.5-8.1% higher than with the Kharkiv technology of sperm production.

The application of various sanitizing drugs in the diluents allows to improve the veterinary and sanitary quality of sperm. Thus, the results of the use of various antibiotics for sperm remediation show that the number of sperm samples not meeting the standards in the process of dilution before equilibration, after equilibration and freezing decreases compared to native sperm by 21.5, 34.1 and 40.8 %.

It is established that microbial contamination of sperm is significantly reduced due to the action of the sanitizing drug after its equilibration and freezing.

Decomsan was developed to rehabilitate sperm. It consists of benzylpenicillin sodium, streptomycin sulfate, and decamethoxine [30].

Freezing of sperm sanitized with spermosan revealed a decrease in microbial contamination compared to untreated sperm from 1.3 to 25 times, and when sanitizing sperm with decomsan microbial contamination compared with sanitized spermosan decreased from 1.9 to 10.6 times, and by 37.5% of samples did not detect the growth of microorganisms.

Thus, there is a 5 times probable reduction of microbial contamination of semen of bulls in comparison with unsanitized, it decreases by 2 times during remediation with spermosan.

Sanitary quality of native sperm of breeding bulls depends on the season. In autumn it is 1.41 times higher than in summer; it is 1.16 times higher in summer than in the winter-spring.

There are some data on the sanitizing drugs effect on sperm activity. Spermosan is the main antibacterial agent used to rehabilitate sperm. It contains a series of non-toxic to sperm antibiotics. According to scientific data [41], the spermosan solution has a certain osmotic pressure. 6.2% solution is isotonic for sperm.

According to O.Buhrov and P. Skliarov, under the action of gentamicin (treatment of sperm before in vitro fertilization) the survival of zygotes did not decrease compared with the control, it even had a tendency to improve. You can add the antibiotic gentamicin at a concentration of 25 mg and 100 units per 1 ml of environment to inhibit microbial contamination of zygotes.

When diluting sperm with synthetic diluents, bactericidal and bacteriostatic substances are introduced into their composition and have the ability to inhibit the development or kill these microbes. However, long-term use of bactericidal drugs in media for dilution of sperm promotes the development of resistant strains of microorganisms.

Research methods. Laboratory, microscopic, analytical and biometric methods were used for the research. The experiments were performed at the laboratory of UGC (Ukrainian Genetic Company). Sperm from breeding bulls is obtained in the arena. IMV laboratory equipment is used to evaluate sperm.

The obtained evaluation results allow to determine automatically the degree of sperm dilution. Evaluated semen was diluted with synthetic diluents and frozen. Sperm has been stored in quarantine for one month. It is necessary to determine the quality of sperm, its bacterial examination for pathogens, and pathogenic microflora. It was stored in liquid nitrogen in stationary Dewar vessels at a temperature of - 196^{0}C.

Cryopreserved semen is sold to agricultural enterprises of various forms of ownership. Breeding bulls of Holstein red and black-spotted breeds, Simmental breed, and Aberdeen-Angus breed are kept in special rooms.

Breeding bulls belong to the leading lines of their Cavalier breeds, i.e. selection index SI + 2100, Starbuck – SI + 1553, Elevation – SI + 1150.

Sperm from breeding bulls is obtained 2 times a week. The fetuses during the studies were clinically healthy, daily exercise in the circular corridor. Sperm

was obtained on a shortened, aseptically prepared artificial vagina with a disposable sperm receiver.

Sperm was evaluated organoleptically and microscopically according to SSU 3535-97 technical conditions on sperm of native bulls [8].

Sperm volume and germ cell concentration were determined in 1 ml of ejaculate. Each ejaculate was examined separately.

The volume of ejaculate was determined by weighing, i.e. 1 g of semen was equal to 1 ml.

Sperm was assessed for activity using the IVOS computer system. The program allows you to assess the concentration and activity of sperm, it also provides information about the amount of solution required for sperm dilution.

Sperm activity is assessed on a ten-point scale. The number of 9-point sperm is reduced to 90%; 8-point sperm is reduced to 80%, 7-point sperm is reduced to 70%. Oscillated moving germ cells were considered conditionally dead and were not taken into account in the assessment.

Sperm of breeding bulls with an activity of 8 or more points was allowed for application.

The effect of such sanitizing drugs spermosan and gentamicin was studied on ejaculates divided into 2 parts. The composition of glucose-citrate-yolk diluent included 30.0 g of glucose (medical, anhydrous); 14.0 g of sodium citrate (three substituted pentahydrate); 200.0 ml of chicken egg yolk; 1.000 ml of distilled water, and sanitizing drug.

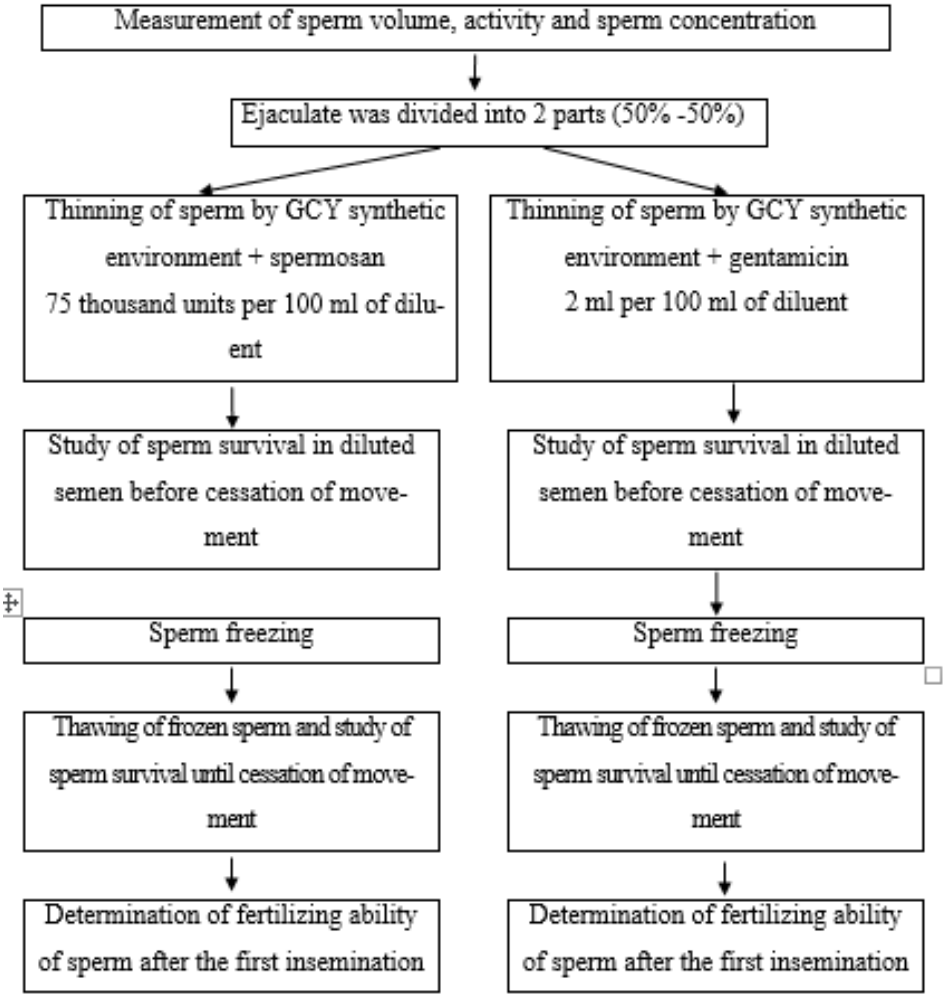

Fig. 1. Research scheme

Sperm survival was studied by determining the activity after each hour of incubation at $t = 37 \pm 0.5^0C$ in diluted and frozen-thawed semen sanitized with various antibiotics until complete loss of motility; fertilizing ability of sperm after the first insemination [8].

The fertility of sperm containing various antibiotics was researched taking into account their use in production (according to the books of artificial insemination). Cows and heifers were inseminated at farms of Zhytomyr and Vinnytsia regions. The obtained results were systematized, the data were statistically processed using M. Plokhynskyi biometric method on a personal computer using Microsoft Office Excel.

The duration of the service period was calculated by the formula.

$$SP = \frac{365 \times 100}{Bm} - 285, \quad (1)$$

where SP – average herd service period;

365 – days of the year;

100 – per 100 calves;

Bm – the actual number of calves per 100 cows;

285 – number of pregnancy days.

Meat and milk shortages were counted from 91 days after calving. 3 kg of milk and 0.3 kg of meat are lost every

day of the extended service period [25]. Economic evaluation was performed according to existing requirements [10].

The statistically significant result was considered to be the difference between the values at which the coefficient * P> 0.9, ** P> 0.95 [29].

Research results. Sanitizing drugs used for the manufacture of synthetic diluents have a certain effect on the survival of germ cells outside the body (Table 1).

Table 1

Sperm survival in freshly diluted semen of bulls of Holstein red-spotted breed

Nickname of the breeding bull and number	Incubation duration, hours									
	Sperm activity, points									
	0		3		5		7		8	
	I	II	I	II	I	II	I	II	I	II
Chancellor Red 768305280	8.5	8.5	5.0	6.0	4.5	5.0	2.0	2.0	SM	1.0
Klariti Red 53476816	9.0	9.0	4.0	3.0	1.0	1.0	SM	-	-	-
Rubinrot Red 579530275	8.5	9.0	5.5	6.0	4.5	5.0	1.5	2.0	-	1.0
Average	8.67± 0.17	8.83± 0.18	4.83± 1.17	5.0± 1.73	3.5± 1.17	3.67± 1.17	1.75± 0.57	2.0	-	0.67

Note: I – semen sanitized by spermosan; II – semen sanitized with gentomycin; SM – signs of movement; 0 – sperm activity immediately after dilution.

Analysis of survival (Table 1) of germ cells in the semen of breeding bulls of Holstein red-spotted breed shows that sperm in semen with the addition of gentamicin had a minimum activity of 0.67 points after 8 hours of incubation, and sperm signs of movement were absent (Fig. 1).

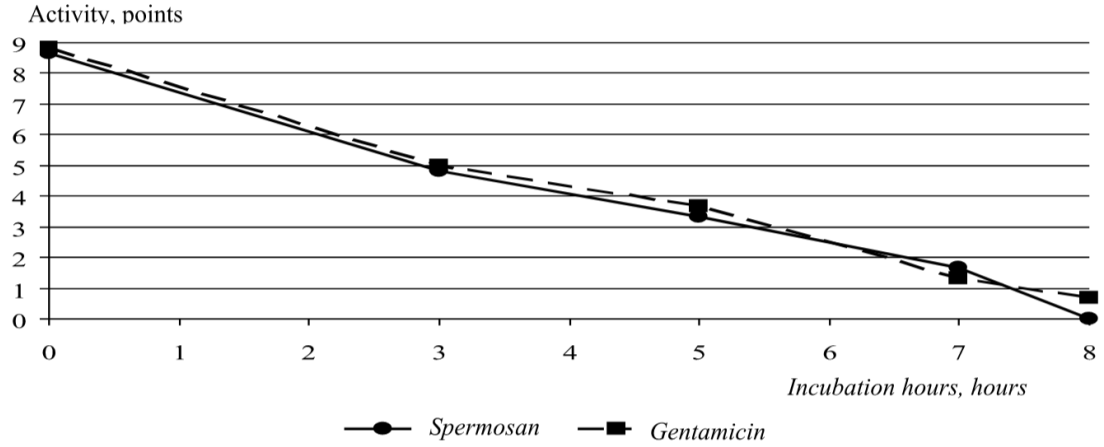

Fig. 2. Dynamics of sperm survival in the semen of bulls-breeders of Holstein red-spotted breed

Analysis of Figure 2 shows that a significant difference in the dynamics of sperm survival in semen sanitized by different antibiotics is not observed until the 7th hour of incubation. The activity of germ cells was slightly higher in the semen of the 2nd group. Incubation of semen with the addition of gentamicin to the diluent had an activity of 0.67 points in 8 hours, and sperm showed no signs of movement.

It is known that if the sperm after 8 hours of incubation has greater sperm activity, it has a better preservation of motility in the genitals and greater fertility.

The sperm of Aberdeen-Angus bulls with the addition of spermosan had a 22% higher activity compared to that with gentamicin. However, the difference in the activity of sperm in the composition of diluents which were injected with different antibiotics was not detected for 7-8 hours of incubation, the activity was, respectively, 1.33 and 0.67 points (Table 2).

The analysis shows that the semen in the diluent with gentamicin had a higher activity compared to spermosan from the 3rd to the 7th hour of incubation (Fig. 3).

Table 2

Sperm survival in freshly diluted sperm of Aberdeen-Angus bulls

Nickname of the breeding bull and number	Incubation duration, hours									
	Sperm activity, points									
	0		3		5		7		8	
	I	II	I	II	I	II	I	II	I	II
Avar 0580607151	8.0	8.5	4.0	4.5	2.5	3.5	2.0	2.0	1.0	1.0
Rastelli 2280847058	8.0	2.0	4.0	4.0	2.0	3.0	SM	SM	-	-
Goldstar 0665165485	8.5	8.5	4.5	5.0	3.0	3.5	2.0	2.0	1.0	1.0
Average	8.17±0.17	6.17±2.61	4.17±0.28	4.5±0.5	2.5±0.5	3.33±0.17	1.33	1.33	0.67	0.67

Figure 3 shows that the activity of sperm of the second and first experimental groups decreases after five hours of incubation. However, it reduces the activity of sperm, which was diluted using gentamicin.

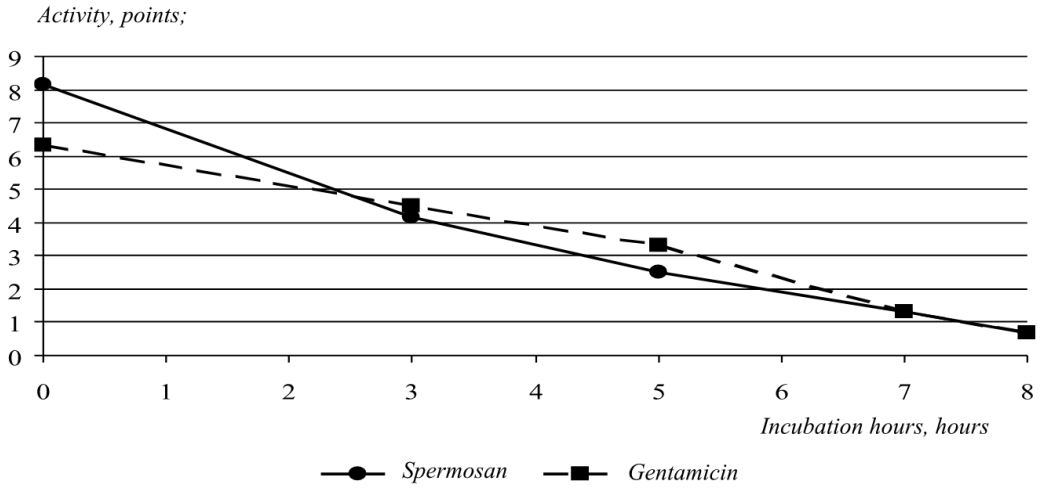

Fig. 3. Dynamics of sperm survival in the semen of Aberdeen-Angus breed bulls

It was found that sperm with various antibiotics had the same activity of 0.67 points in 8 hours (Table 3).

Table 3

Sperm survival in freshly diluted sperm of Simmental bulls

Nickname of the breeding bull and number	Incubation duration, hours									
	Sperm activity, points									
	0		3		5		7		8	
	I	II	I	II	I	II	I	II	I	II
Valet 0696	6.5	7.0	4.5	5.0	3.0	3.5	2.0	1.0	1.0	-
Inkubus 577790071	7.0	7.0	5.0	5.0	3.5	3.0	2.0	1.0	1.0	SM
Mikron 9078	7.0	7.5	5.0	5.5	3.0	3.0	2.0	1.5	-	SM
Average	6.83±0.17	7.17±0.18	4.83±0.17	5.17±0.19	3.17±0.17	3.17±0.17	2.0	1.17±0.18	0.67	SM

The study of sperm survival in diluted sperm of Simmental bulls showed that sperm with the introduction of gentamicin had a 6% higher sperm activity immediately after dilution. Table 5 shows that the activity of sperm in the diluted sperm of Simmental bulls is 22-28% less than in other experimental breeds. Studies of the dynamics of sperm survival in the semen of breeding Simmental bulls sanitized with gentamicin did not have a rectilinear translational motion, and every sixth spermatozoon moved in a rectilinear translational manner. However, during the first 5 hours of incubation, semen treated with gentamicin was more active (Fig. 4)

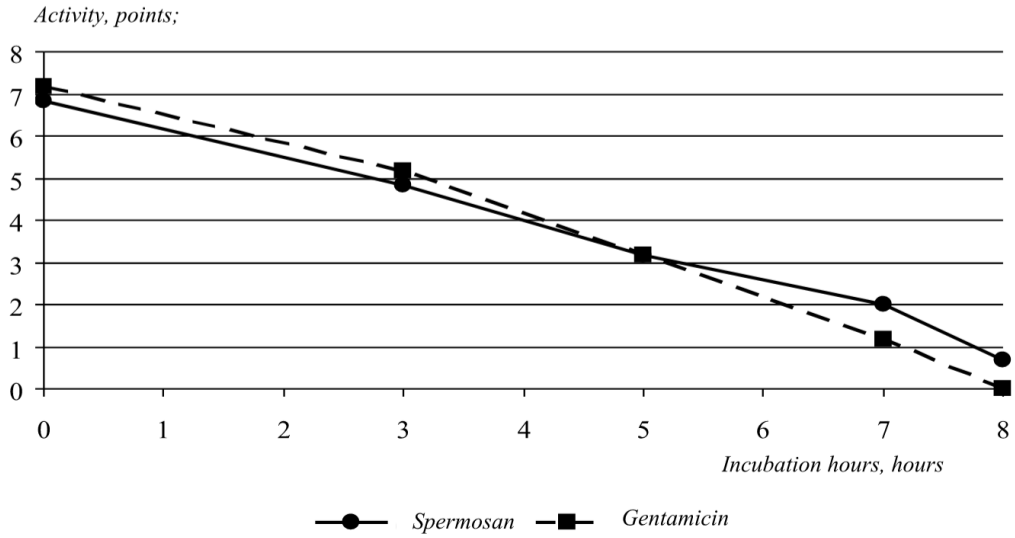

Fig. 4. Dynamics of sperm survival in the semen of Simmental breed bulls

According to Fig. 4, the activity of the seed decreases in two groups from the first hour of incubation. However, the number of active sperms in the diluted semen with the addition of spermosan decreases more rapidly. The activity of sperm in the diluent with gentamicin decreases sharply in the 5 hours, and their activity is 0 in 8 hours. It should be noted that the activity of sperm in the diluent with spermosan had an activity of 0.67 points in 8 hours.

The sperm activity in the sperm of Holstein black-spotted bulls decreases during the incubation period, regardless of the use of various antibiotics. However, the number of sperms in the semen decreases more rapidly with the use of spermosan in synthetic solvents (Table 4).

Table 4

Sperm survival in freshly diluted semen of Holstein black-spotted breed bulls

Nickname of the breeding bull and number	Incubation duration, hours									
	Sperm activity, points									
	0		3		5		7		8	
	I	II	I	II	I	II	I	II	I	II
Chantal 370975117	8.9	9	4	3	1	1	SM	-	-	-
Tirso 1601859425	8.6	8.6	5.1	6.1	4.4	5	2	2	SM	1
N. Bolta 114151975	8.5	9	5.5	6	4.6	5	1.5	2	-	1
Average	8.67±0.1	8.870.11	4.87±1.21	5.03±1.76	3.33±1.19	3.67±1.3	1.75	2	-	1

The activity of sperm (Table 6) in semen diluted with synthetic diluents decreases most intensively during the first 3 hours (Fig. 5).

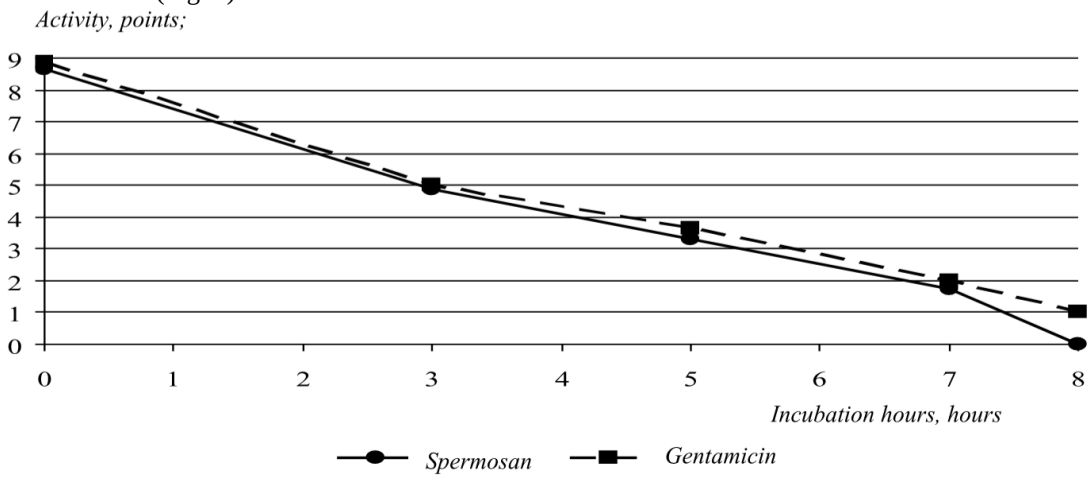

Fig 5. Dynamics of sperm survival in the semen of Holstein black-spotted breed bulls

According to scientific data (Fig. 5) there is no difference in the activity of sperm sanitized by different antibiotics within 7-8 hours of incubation.

After thawing of untreated sperm granules in 2.9% sodium citrate solution, sperm activity should be not less than 4 points, and the viability of sperm in a water bath at a temperature of 38 ± 0.50 C should be not less than 8 hours.

Sperm activity after thawing is the main indicator determining its suitability for artificial insemination of cows and heifers.

Studies of the activity of germ cells in frozen-thawed semen indicate the presence of a certain relationship between the breed of bull-breeder and the duration of their survival during incubation for 8 hours (Table 5).

Table 5

Sperm survival in frozen-thawed sperm of Holstein red-spotted breed bulls (M ± m)

Nickname of the breeding bull and number	Incubation duration, hours									
	Sperm activity, points									
	0		3		5		7		8	
	I	II	I	II	I	II	I	II	I	II
Chancellor Red 768305280	4.0	4.5	3.0	3.0	2.5	2.5	2.0	2.0	1.5	1.5
Klaritti Red 53476816	4.0	4.5	4.0	4.0	2.5	3.0	2.0	2.5	1.0	1.5
Rubinrot Red 579530275	5.0	5.0	4.0	4.0	3.0	3.0	2.0	2.5	1.5	1.5
Average	4.33± 0.67	4.67± 0.17	3.67± 0.67	3.67± 0.67	2.67± 0.17	2.83± 0.17	2.00± 0.0	2.33± 0.17	1.33± 0.17	1.50 ±0.0

Table 5 shows that after thawing, after 5 and 8 hours of incubation, the semen of breeding bulls of Holstein red-spotted breed sanitized with gentomycin had a higher activity. However, it should be noted that the difference is insignificant, and we can only talk about the trend of higher activity.

Analysis of the sperm activity dynamics in sperm sanitized with gentamicin shows that during the incubation period, the percentage of spermatozoa with rectilinear motion was higher than during sanitation with spermosan. However, it should be noted that after 3 hours of incubation, the difference in the activity of sperm in the sperm sanitized by different antibiotics was not detected (Fig. 6).

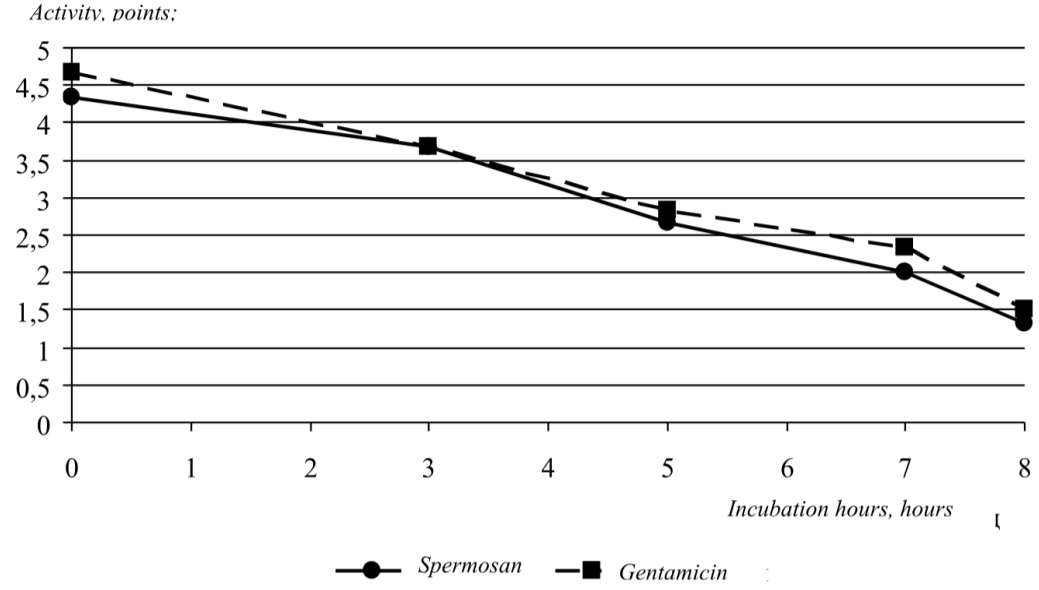

Fig. 6. Dynamics of sperm survival in frozen-thawed sperm of Holstein red-spotted breed bulls

Analysis of the dynamics (Fig. 5) of survival of germ cells during incubation shows that after thawing sperm sanitized with gentamicin it had a higher percentage of sperm with rectilinear motion by 6%.

Immediately after thawing, the activity of sperm in the semen of Aberdeen-Angus breeding bulls sanitized by spermatozoa was lower compared to other antibiotics by 8%. The difference is not probable, we can only talk about the tendency of less activity.

The analysis shows that the number of spermatozoa with rectilinear motion in the sperm sanitized with gentamicin was increased by 37-64% during the whole incubation period.

The number of germ cells with rectilinear translational motion decreases, but the intensity of the decrease in the sperm diluted with the addition of spermosan is greater (Table 6).

Table 6

Sperm survival in frozen-thawed sperm of Aberdeen-Angus bulls (M ± m)

Nick-name of the breeding bull and number	Incubation duration, hours									
	Sperm activity, points									
	0		3		5		7		8	
	I	II	I	II	I	II	I	II	I	II
Avar 0580607 151	4.0	4.0	2.5	3.5	2.0	3.0	1.0	2.0	0.5	0.5
Rastelli 2280847 058	4.0	4.5	3.0	4.0	2.0	3.0	2.0	2.5	0.5	1.0
Goldstar 0665165 485	4.0	4.5	3.5	4.0	2.0	3.5	1.5	2.5	0.5	1.0
Average	4.00± 0.0	4.33±0. 17	3.00± 0.5	3.83±0. 17	2.00± 0.0	3.17±0. 17	1.50± 0.5	2.33±0. 18	0.50± 0.0	0.83±0. 17

The analysis of the obtained results (Table 6) shows that the introduction of gentamicin into the diluent has a positive effect on male gametes, which is expressed in their higher activity during the entire incubation period (Fig. 7).

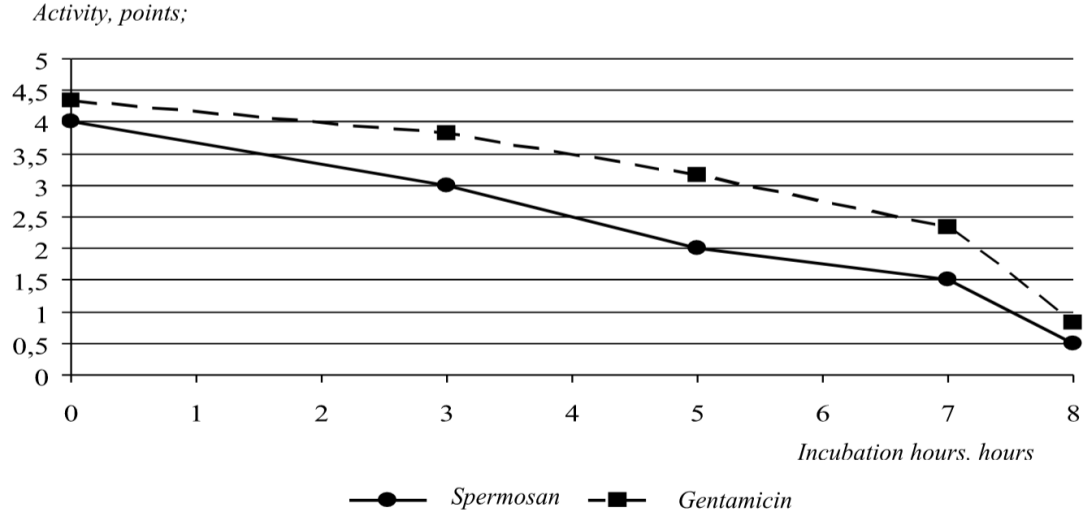

Fig. 7. Dynamics of sperm survival in frozen-thawed sperm of Aberdeen-Angus breed bulls

The research of the dynamics (Fig. 7) of the Aberdeen-Angus bulls sperm activity shows that in 8 hours of incubation spermatozoa with gentamicin has by 70% more spermatozoa with rectilinear motion in comparison with spermosan. However, the difference is not probable and we can only talk about their higher activity.

Analysis of the results shows that the introduction of the diluent antibiotic gentamicin has a positive effect on male gametes of Aberdeen-Angus bulls, which is expressed in higher sperm activity at 8 hours of incubation. After thawing, the activity of germ cells of sperm bulls of Simmental breed is significantly inferior in this indicator to other experimental breeds, regardless of the diluent drug (Table 7).

Table 7

Sperm survival in frozen-thawed sperm of Simmental bulls (M ± m)

Nickname of the breeding bull and number	Incubation duration, hours									
	Sperm activity, points									
	0		3		5		7		8	
	I	II	I	II	I	II	I	II	I	II
Valet 0696	2.5	3.0	2.5	3.5	1.0	1.5	0.5	0.5	-	SM
Inkubus 577790071	3.0	3.0	2.0	2.5	1.0	1.5	-	0.5	-	-
Mikron 9078	3.0	3.5	2.0	2.5	1.0	1.0	0.5	0.5	-	-
Average	2.83±0.17	3.17±0.18	2.17±0.18	2.83±0.67	1.00±0.0	1.33±0.18	0.33±0.17	0.50±0.0	-	-

The analysis shows (Table 7), regardless of the antibiotic diluent, active sperm were not detected after 8 hours of semen incubation.

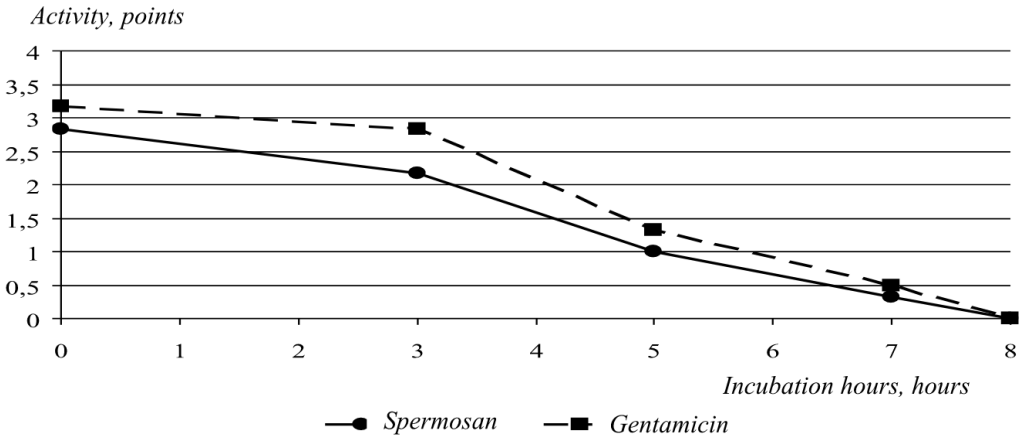

Fig. 8. Dynamics of sperm survival in frozen-thawed sperm of Simmental breed bulls

It should be noted that after 5 hours of incubation, the activity of sperm was at the minimum allowable value (Fig.8).

Analysis of sperm activity dynamics shows that during the whole incubation period the sperm of breeding bulls of the second experimental group, in which the diluent was introduced gentamicin, had a higher activity after thawing and after 5 hours of incubation by 12 and 35% compared to the first. The difference in activity is not probable, we can only talk about their higher activity. Sperm activity is affected by the breed of breeding bulls (Table 8).

Table 8

Sperm survival in frozen-thawed sperm of Holstein black-spotted breed bulls (M ± m)

Nickname of the breeding bull and number	Incubation duration, hours									
	Sperm activity, points									
	0		3		5		7		8	
	I	II	I	II	I	II	I	II	I	II
Chantal 370975117	4.2	4.6	3.1	3	2.6	2.5	2	2	1.5	1.5
Tirso 1601859425	4	4.5	4.1	4	2.5	3.2	2.1	2.6	1.1	1.5
N. Bolta 114151975	4.9	5.1	4.2	4.1	3.1	3.1	2	2.7	1.6	1.5
Average	4.37 ±0.45	4.73 ±0.21	3.80 ±0.74	3.70 ±0.64	2.73 ±0.21	2.93 ±0.29	2.03 ±0.01	2.43 ±0.29	1.40 ±0.14	1.50 ±0.0

The analysis shows that the activity of sperm in the gentamicin diluent tends to greater activity after thawing (Fig. 9).

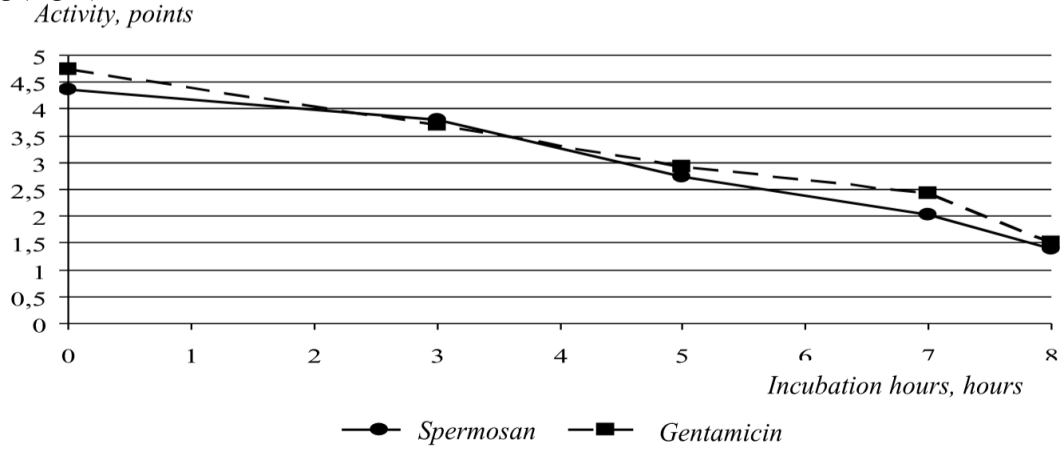

Fig. 9. Dynamics of sperm survival in frozen-thawed sperm of Holstein black-spotted breed bulls

Analysis of the dynamics (Fig. 10) of sperm activity shows that a probable difference in the activity of sperm sanitized by different antibiotics is not observed. Semen treated with various antibiotics has minimal activity after 8 hours of incubation.

Fertility of sperms, the diluents of which included spermosan and gentamicin were studied at farms of Zhytomyr and Vinnytsia regions.

The efficiency was determined by the results of artificial insemination of 1,780 heads of cows and heifers. According to the research results, sperm had a greater

fertilizing ability in the diluent with decomsan on average from 5 to 7.6% (Table 9).

Table 9

The fertility level of bull sperms using sanitizing drugs spermosan and gentamicin

Breed	Inseminated cows and heifers, heads		Pregnant cows and heifers, heads		Fertility level, %	
	I	II	I	II	I	II
Holstein black-spotted	269	251	185	186	69.0±2.0	74.1±2.7
Holstein red-spotted	203	216	140	160	69.3±7.0	74.3±4.2
Simmental	222	229	155	176	70.0±2.1*	76.8±3.2*
Aberdeen-Angus	190	200	133	154	70.0±3.2*	77.6±2.1*

Table 9 shows the Aberdeen-Angus and Simmental breeds sperm sanitized by Decomsan had the highest fertilizing ability among the experimental breeds of breeding bulls. The difference between sperm sanitized by spermosan and gentamicin is probable ($P \leq 0.5$) (Fig. 10).

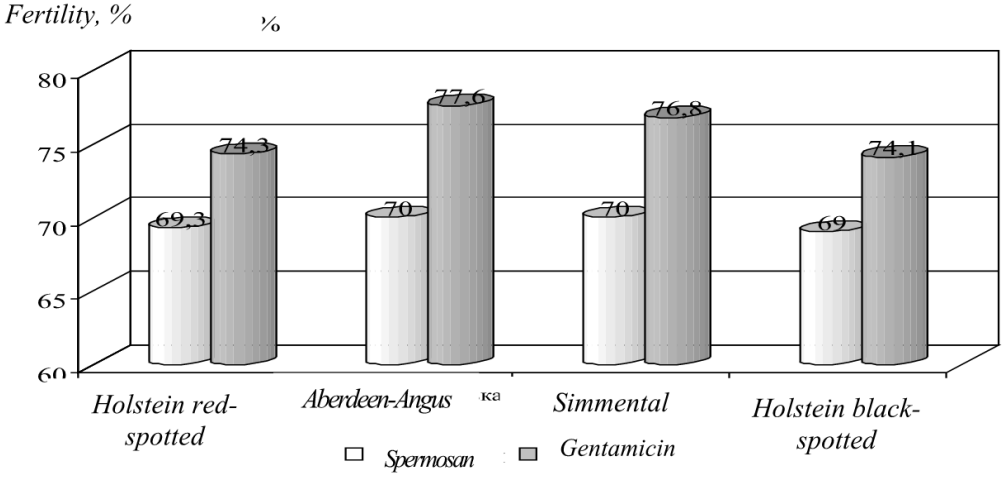

Fig. 10. Fertilizing ability of bulls semen sanitized by various antibiotics

Fig. 10 shows that the higher fertility of Holstein red and black-spotted breeds sperm with gentamicin diluent can illustrate the tendency of higher efficiency.

CONCLUSIONS

1. A probable difference in the activity of sperm sanitized by different drugs was not found during incubation. After 8 hours of incubation, the activity of sperm in the diluted sperm of breeding bulls of different breeds sanitized with spermosan and gentamicin had a minimum activity in the range of lim = 0.02-0.67 points. The difference between the experimental groups was unlikely.

2. The activities of germ cells in frozen-thawed semen with the introduction of the diluent gentamicin in comparison with spermosan tended to a higher activity by 0.2-0.33 points.

3. Sperm with gentamicin (5-7.6%) had a higher fertilizing ability among the experimental breeds. Probable difference was found in the fertilizing ability of sperm of the Aberdeen-Angus and Simmental breeds bulls ($P > 0.95$). We can only talk about the tendency of higher efficiency about the higher fertilizing ability of the Holstein red and black-spotted breed bulls.

4. The use of semen for artificial insemination of cows and heifers with gentamicin will increase the efficiency of reproduction and reduce the duration of the service period by 24 days and will allow an additional 73 kg of milk and 7.3 kg of beef per cow. The cost of additional products is 761.5 UAH.

Suggestions to production. It is recommended to use gentamicin at a dose of 2 ml per 100 ml of synthetic diluent to rehabilitate the sperm of breeding bulls in order to increase the efficiency of artificial insemination of cows and heifers.

It is recommended to use a set of laboratory equipment for collection, evaluation, packaging, storage of sperm of breeding bulls and its cryopreservation NVO Bio-Test laboratory, or IMV-2.

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