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Study of the Effect of a Complex of Preparations Containing *Aloe arborescens* Mill. Extract on the Quality and Quantity of Sperm

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ABSTRACT

In recent decades, there has been an increased interest in practical medicine in medicines obtained from raw materials of animal and plant origin – biostimulants and adaptogens. The use of such drugs in precise doses can increase the body's natural resistance indicators, increase the level of morphological and biochemical parameters of the blood, and improve metabolism. In this scientific study, the effect of a combination of iodine, amylodextrin, E-selenium, and preparation from the *Aloe arborescens* Mill. extract on the preparation of rams in the seasonal season was studied. The results of the experiment showed that the rams of the experimental group had a faster recovery and preparation of the body for the seasonal season after the winter period precisely due to the use of the non-hormonal stimulation means proposed by us. Our proposed means for stimulating rams in their preparation for the breeding season have a positive effect on the processes of metabolism and spermatogenesis in the animal body, and these drugs can be recommended for use. It has been found that the non-hormonal means used by us to prepare rams for the transition period do not hurt the body and allow us to improve spermatogenesis indicators and increase testosterone levels.

Keywords: Extract, *Aloe arborescens* Mill., Biostimulating supplement, Seminal fluid, Sexual activity.

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INTRODUCTION

In recent decades, there has been an increase in the interest in practical medicine in medicines obtained from raw materials of animal and plant origin – biostimulants and adaptogens [1-4].

Biostimulants have been used since the time of Hippocrates, but their thorough empirical verification and scientific justification of their use

began only in the twentieth century. In the preparations prepared according to the method of V. P. Filatov, substances produced by living cells in the process of their vital activity in extremely unfavorable conditions have a stimulating effect on the body. As a result, substances are formed in such tissues that stimulate specific biochemical processes in the same tissues, which contribute to the preservation of the vital activity (survivability) of tissues in unfavorable conditions [5-7].

The use of strictly dosed amounts of drugs containing such substances increases the indicators of natural resistance of the body (lysozyme and bactericidal activity of blood serum, phagocytic activity of leukocytes), increases the level of morphological and biochemical parameters of blood (the number of erythrocytes, leukocytes, hematocrit, ESR, hemoglobin, total protein), improves metabolism, increases reproductive function and stimulates growth [8-11].

The liver, spleen, testes, skin, placenta, peat, estuarine mud, plantain leaves, and other components, including *Aloe arborescens* Mill., are used for the manufacture of such preparations.

For example, when preparing animals for the seasonal season, many specialists intensively use such drugs to stimulate the reproductive qualities of animals. This improves the quality and quantity of semen produced in males and improves the quality of eggs in females. However, there is a fairly small number of studies on exactly how a drug or a complex of drugs affects the metabolic processes in the body, and whether the means used have a hidden negative effect on the body [12, 13].

The genus *A. arborescens* is represented by perennial tropical and subtropical plants with large thick juicy leaves. *A. arborescens* is a large perennial plant, reaching 10 m in height in its homeland in Africa, and leaves up to 65 cm long. The lower part of the stem turns woody and gradually gets rid of the leaves so that the rosette of leaves remains only at the top of the shoot [14, 15].

The leaves of *A. arborescens* contain oxymethylanthraquinone – Aloe-emodin (about 2%) and other anthra derivatives – aloin, nataloin, and hormonal-loin. In addition, anthraglycosides, dicarboxylic acids, fatty acids, aromatic acids, vitamins, enzymes, and phytoncides are isolated from the plant. The chemical composition of the leaf juice is shown in **Table 1** [16].

Table 1. Chemical composition of *A. arborescens* juice.

Set of compounds	Content	
	Mg/ml of juice	% of the dry matter weight of the juice
Dry substances	23.41	100
Ash content	6.29	26.88
Organic acids, including:	4.92	21.02
-free	0.95	4.06
-related	3.97	16.90
Amino acids free	0.36	1.54
Carbohydrates are common, including:	11.62	49.64
-free carbohydrates	10.52	44.94
-polysaccharides	1.10	4.70
Pyron compounds	1.05	4.49

The leaves of *A. arborescens* are xiphoid in shape, 15-45 cm long, 2-5.5 cm wide at the base, 0.7-1.5 cm thick; concave on the upper side, convex on the lower side, glabrous, thick, fleshy, covered with a waxy coating, with a serrated edge. The leaves have a faint peculiar smell and a very bitter taste. It is allowed to lose at least 92% of weight during drying, and at least 2% of the dry residue in the juice taken from fresh leaves before canning [17, 18].

A. arborescens is an old remedy. It was used to treat purulent wounds, and ulcers in ancient Egypt and Greece more than 3000 years ago.

In the last century in Russia, *A. arborescens* preparations were used only as a laxative. When applied externally, *A. arborescens* preparations have a wound-healing, anti-inflammatory, and radioprotective effect [19].

For peptic ulcers of the stomach and duodenum, *A. arborescens* extract is used as a means of nonspecific stimulation in the form of subcutaneous injections, and a liquid extract of *A. arborescens* is prescribed inside or Aloe-coated tablets [20].

The purpose of the research is to study the effect of the manufactured complex preparation based on *A. arborescens* extract, E-selenium, iodine, and amylodextrin on the quality of the obtained seed and testosterone production during the preparation of rams for the breeding season.

MATERIALS AND METHODS

Taking into account the research methodology of the Institute's research project number 20.80009.5107.20: "Managementul potenţialului genetic ai a producţiilor animalelor de rasă reproduce ai exploit în condiţiile pedoclimaterice ale Republicii Moldova", we, after consulting with specialists of Stavropol State Medical University, Rostov State Medical University, Saratov State University of Genetics, Biotechnology, and Engineering, Stavropol State Agrarian University and North Caucasus Federal University, conducted experiments to determine the effect of a complex of drugs containing *A. arborescens* plant extract on the quality of the seed and the production of testosterone in the preparation of rams for the transition period.

2 groups were formed to conduct the experiments: experimental and control. Each group consisted of 12 male individuals (rams), aged from 18 to 36 months, healthy, with average weight indicators.

Animals of the experimental group were given 5 ml of a drug containing iodine with amylodextrin daily for 50 days with feed, 1.5 ml/ head of E-selenium, and a tissue preparation of 0.5 ml/head mixed with 1 ml of 0.5% novocaine solution were intramuscularly injected.

The tissue preparation was manufactured in the Laboratory of Biotechnologies in Reproduction and Embryo Transfer of Scientific-Practical Institute of Biotechnologies in Animal Technology and Veterinary Medicine. The leaves of the *A. arborescens* plant were used for at least 2 years. The cut leaves of *A. arborescens* were kept for 10-12 days in the dark at a temperature of 4-8 ℃, the teeth and yellowed ends were removed, cut into small pieces, and ground until a pulp formed. A threefold amount of distilled water was added and boiled for 2-3 minutes (for protein coagulation), after which it was filtered. Sodium chloride was added to the filtrate (7 g per 1 liter), boiled for 2 minutes, and filtered, the pH level was measured (batches of the drug with a pH in the range of 5.0-5.6 were taken into operation), poured into 50 ml vials and sterilized in an autoclave at 120 ℃ for one hour.

Animals of the control group were not prescribed or given any drugs. Before the start of the research and at the end of the experiment, semen and blood samples were taken. Animals of both groups were kept in the same conditions and on the same diet. Qualitative and quantitative analysis of semen samples was carried out using the computer program «CEROS». The results obtained were formalized by test certificates.

RESULTS AND DISCUSSION

The statistically processed research results are summarized in **Tables 2 and 3**. When evaluating the results of the studies, it should be taken into account that the sexual function of rams in both groups increases as they approach the breeding season [21, 22].

Table 2 presents the results of studies of testosterone levels in the blood of rams in their preparation for a random period in a non-random season.

Table 2. Results of studies of testosterone levels in the blood of rams in their preparation for a random period in a non-random season.

Groups	Testosterone Level (ng/ml)			
	The beginning of the experience	The end of the experience	Difference	
			(ng/ml)	%
Experience	4.57 ± 0.39	7.5 ± 0.578	+ 2.93	+ 39.06%
Control	4.55 ± 0.43	6.81 ± 0.23	+ 2.26	+ 33.81%

Having studied the results of the studies, we see that at the beginning of the experiment, the testosterone level in rams was almost the same in both groups and was in the range of 4.57 ± 0.39 ng/ml and 4.55± 0.43 ng/ml. At the end of the experiment (taking into account the fact that the sexual function of rams in both groups increases as they approach the breeding season), it was found that the amount of testosterone in the experimental and control groups increased by 39.6% and 33.81%, respectively. In the rams of the experimental group, this indicator was 7.5 ± 0.578 ng/ml and was 0.69 ng/ml or 0.67% higher than in the animals of the control group, where the same indicator was 6.81 ± 0.23 ng/ml.

Tables 3 and 4 present the results of studies of rams' semen samples in their preparation for the random period in the non-random season.

Table 3. Characteristics of the volume and concentration of sperm of rams

Groups	Volume, ml				Sperm concentration billion/ml			
	The beginning of the experience	The end of the experience	Difference		The beginning of the experience	The end of the experience	Difference	
			ml	%			billion/ml	%
Experience	0.83 ± 0.09	1.05 ± 0.12	+ 0.22	+ 21%	1.05 ± 0.03	1.58 ± 0.13	+ 0.53	33.41
Control	0.63 ± 0.07	0.73 ± 0.07	+ 0.17	+ 16%	1.06 ± 0.03	1.22 ± 0.11	+ 0.15	12.57

The sexual activity and sperm production of rams in both groups increase as they approach the breeding season, therefore, when calculating the data in **Table 3**, this factor was necessarily taken into account for both groups of animals.

The rams of the experimental group ranged from 0.9 to 1.3 ml per cage, and in the rams of the control group, the same indicator ranged from 0.7 to 0.9 ml per cage.

Analysis of the data in **Table 3** allowed us to find out that the volume of ejaculate received in the experimental group increased from 0.829 ± 0.086 to 1.05 ± 0.122 ml and in the control group from 0.609 ± 0.067 to 0.725 ± 0.072 ml. Consequently, in comparison with the initial volume, the volume of ejaculate received in the experimental group increased by 21% or by 0.221 ml, and in the control group by 16% or 0.116 ml, which is less by 5% compared to the experimental group.

The concentration of spermatozoa in rams of the experimental group increased from 1.052 ± 0.028 billion/ml to 1.58 ± 0.126 billion/ml, and in the experimental group, this indicator increased from 1.064 ± 0.0351 billion/ml to 1.217 ± 0.105 billion/ml. In comparison with the initial data, it can be seen that the concentration of sperm in the ejaculate of the experimental group increased by 33.41% or 0.528 billion/ml, and the concentration of sperm in the ejaculate of the control group increased by 12.57% or 0.153 billion/ml, which is 20.84% or 0.375 billion/ml less than in the experimental group.

Table 4. Characteristics of the quality of sperm of rams

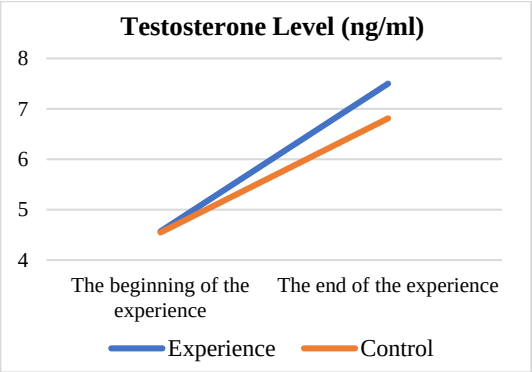
Groups	Mobility %							
	Live spermatozoa				Rectilinearly translational			
	The beginning of the experience	The end of the experience	Difference		The beginning of the experience	The end of the experience	Difference	
			-	%			-	%
Experience	78.58 ± 3.22	83.41 ± 8.69	4.83	5.79	39.41 ± 3.29	53.2 ± 11.71	13.79	25.92
Control	73.91 ± 5.44	77.75 ± 4.33	3.841	4.9	31.545 ± 3.4	33.75 ± 4.88	2.205	6.53

Data on sperm motility are presented in **Table 4**. It can be seen that at the end of the experiment, the percentage of motile live sperm in the experimental group changed from 78.58 ± 3.22 to 83.41 ± 8.69, and in the control group from 73.909 ± 5.44 to 77.75 ± 4.33 percent of motile live sperm, which is 5.66 less than in the experiment. In comparison with the indicators at the beginning of the experiment, these parameters in the experimental group increased by 5.79%, and in the control group by 3.841%, which is 5.66 units less than in the experiment.

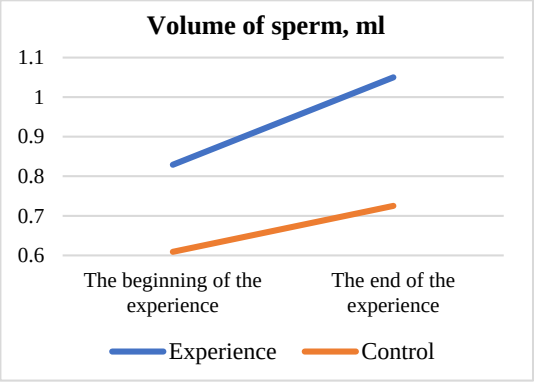
The level of motile sperm with rectilinear translational motion in the rams of the experimental group by the beginning of the experiment was 39.41 ± 3.29 and increased to 53.2 ± 11.71, and in the control group, this parameter increased from 31.545 ± 3.398 to 33.75±4.88, which is 19.45 less than in the experimental group. In comparison with the indicators at the beginning of the experiment, these parameters in the experimental group increased by 25.92%, and in the control group by 6.53%, which is 19.39% less than in the experimental group.

Analysis of data from all tables shows that testosterone levels and qualitative and quantitative indicators of spermatogenesis at the end of the experiment were higher in animals of the experimental group **(Figure 1)**. Considering that the animals were kept in the same conditions and on the same diet, we believe that these changes in the animals of the experimental group with better dynamics of growth of the reproductive system in preparation for the seasonal season were the result of the use of

stimulants. So, in addition to selenium, iodine, and vitamin E, the animals' body was also influenced by a bio-stimulator obtained from *A. arborescens*. This plant contains polysaccharides, vitamins, enzymes, amino acids, phytoncides, esters, phenols, resins, and more than 200 biologically active compounds that stimulate cellular metabolism, trophism, and tissue regeneration, increase the overall nonspecific resistance of the body and the resistance of mucous membranes to the action of damaging agents, accelerate regeneration processes. All this both directly and indirectly contributes to improving the quality and quantity of semen produced in males.



a)



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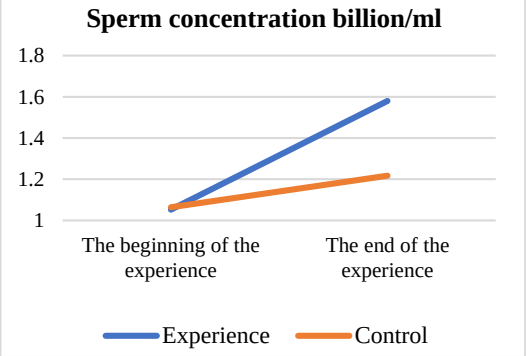

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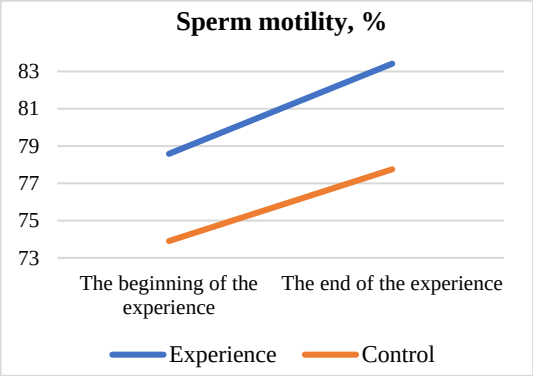

d)

Figure 1. Graphical representation of the results of the experiment.

We believe that the rams of the experimental group had a faster recovery and preparation of the body for the seasonal season after the winter period precisely due to the use of the non-hormonal stimulation means proposed by us. The means we offer to stimulate rams in their preparation for the breeding season have a positive effect on the processes of metabolism and spermatogenesis in the body of animals and these drugs can be recommended for use.

CONCLUSION

Studies conducted on two groups of young rams identical in composition (12 individuals in each group) showed a positive effect of the drugs used on the quantitative and qualitative characteristics of sperm. For 50 days, the following drugs were added to the experimental group: a complex of iodine and amylodextrin together with feed, 1.5 ml of E-selenium intramuscularly, *A. arborescens* extract with a solution of novocaine intramuscularly.

During the research, it was found that the non-hormonal means used by us to prepare rams for the transition period do not hurt the body and allow us to improve spermatogenesis and increase testosterone levels.

ACKNOWLEDGMENTS: The work was carried out under the Institute's research project number 20.80009.5107.20: "Managementul potențialului genetic ai a producțiilor animalelor de rasă reproduse ai exploit în condițiile pedoclimaterice ale Republicii Moldova".

CONFLICT OF INTEREST: None

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ETHICS STATEMENT: The protocol for experiments with laboratory animals complied with the requirements of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes.

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