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Investigation of the Impact of *Aloe arborescens* Mill. Extract-Based Preparations on Sperm Quality and Quantity

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ABSTRACT

In recent years, there has been growing interest in the use of biostimulants and adaptogens derived from animal and plant sources in practical medicine. These substances, when administered in precise doses, can strengthen the body's natural defenses, improve blood morphology and biochemical parameters, and support better metabolism. This study investigated the effect of a combination of iodine, amylodextrin, E-selenium, and *Aloe arborescens* Mill. extract on the preparation of rams for the seasonal period. The findings showed that the rams of the experimental group experienced faster recovery and better preparation for the breeding season after the winter period, which was primarily due to our proposed non-hormonal stimulation method. Our approach to stimulating rams during the transitional period had a positive effect on metabolism and spermatogenesis, making these preparations suitable for use. In addition, it was observed that our non-hormonal method did not harm the animals and contributed to improved spermatogenesis and higher testosterone levels.

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

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Introduction

In recent decades, there has been a growing interest in biostimulants and adaptogens derived from animal and plant sources in practical medicine [1-4]. While the use of biostimulants dates back to the time of Hippocrates, their systematic scientific validation began only in the 20th century. According to V. P. Filatov's method, substances produced by living cells under extreme conditions have a stimulating effect on the body. These

substances are formed in tissues under stress and activate specific biochemical processes that help preserve tissue function and survival in challenging environments [5-7].

When administered in precise doses, these substances enhance the body's natural resistance, boosting indicators such as lysozyme and bactericidal activity in blood serum, as well as phagocytic activity in leukocytes. They also improve various blood parameters, including the count of erythrocytes, hematocrit, leukocytes, ESR, hemoglobin, and total protein, while enhancing metabolism, reproductive function, and promoting growth [8-11]. Preparations containing components like liver, testes, spleen, skin, peat, placenta, plantain leaves, estuarine mud, and A*loe arborescens* Mill. are commonly used for these purposes.

For instance, many specialists use such drugs to enhance the reproductive capabilities of animals during the seasonal period. This approach improves both the quantity and quality of semen produced in males and enhances egg quality in females. However, there is a limited amount of research exploring the specific impact of these drugs or drug combinations on metabolic processes within the body, as well as whether these substances may have any hidden adverse effects [12, 13].

The *A. arborescens* consists of perennial plants native to tropical and subtropical regions, characterized by large, thick, succulent leaves. *A. arborescens*, which can grow up to 10 meters in height in its native environment in Africa, has leaves that can reach up to 65 cm in length. The lower portion of the stem becomes woody over time, and the leaves are shed, leaving a rosette of leaves at the top of the stem [14, 15].

The leaves of *A. arborescens* contain compounds such as oxymethylanthraquinone (Aloe-emodin, approximately 2%) and other anthra derivatives like nataloin, aloin, and hormonal-loin. Additionally, substances like anthraglycosides, fatty acids, dicarboxylic acids, aromatic acids, enzymes, vitamins, and phytoncides are extracted from the plant. A detailed chemical composition of the leaf juice is provided 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* have a xiphoid shape, ranging from 15-45 cm in length, 2-5.5 cm in width at the base, and 0.7-1.5 cm in thickness. The upper side of the leaves is concave, while the lower side is convex, smooth, thick, fleshy, and covered with a waxy layer. They also have serrated edges. The leaves emit a faint, distinctive odor and possess a very bitter taste. During drying, they are allowed to lose up to 92% of their weight, while at least 2% of the dry residue remains in the juice extracted from fresh leaves before canning [17, 18].

A. arborescens has been a traditional remedy for thousands of years, used by ancient Egyptians and Greeks more than three thousand years ago to treat ulcers and purulent wounds. In the 20th century, *A. arborescens* preparations in Russia were primarily used as a laxative. When applied externally, *A. arborescens* preparations have demonstrated anti-inflammatory, wound-healing, and radioprotective effects [19].

For treating peptic ulcers in the duodenum and stomach, *A. arborescens* extract is utilized as a nonspecific stimulant through subcutaneous injections. Additionally, a liquid extract of *A. arborescens* or Aloe-coated tablets is prescribed for internal use [20].

This research aims to investigate the impact of a combined preparation made from *A. arborescens* extract, E-selenium, iodine, and amylodextrin on semen quality and testosterone production during the preparation of rams for the breeding season.

Materials and Methods

Based on the research methodology outlined in the Institute's project number 20.80009.5107.20: "Management of the Genetic Potential and Production of Breeding Animals under Pedoclimatic Conditions of the Republic of Moldova," and after consulting with experts from 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, we carried out experiments to examine the impact of a drug complex containing *A. arborescens* extract on sperm quality and testosterone production during the preparation of rams for the transition period.

Two groups were established for the experiment: an experimental group and a control group, each consisting of 12 male rams, aged eighteen to 36 months, healthy, and with average weight. The experimental group animals received five ml of a drug containing iodine and amylodextrin daily for fifty days mixed with their feed, along with 1.5 ml of E-selenium per head. Additionally, they were intramuscularly injected with 0.5 ml of tissue preparation per head, mixed with one ml of 0.5% novocaine solution.

The tissue preparation was produced in the Laboratory of Biotechnologies in Reproduction and Embryo Transfer at the Scientific-Practical Institute of Biotechnologies in Animal Technology and Veterinary Medicine. The *A. arborescens* plant leaves used for the preparation were at least two years old. After being harvested, the leaves were stored in the dark for 10-12 days at a temperature of 4-8 °C. The yellowed tips and spines were removed, and the leaves were cut into small pieces and then ground into a pulp. Distilled water was added in a 3:1 ratio, and the mixture was boiled for two to three minutes to promote protein coagulation. The mixture was then filtered, and sodium chloride (7 grams per 1 liter) was added, followed by an additional 2-minute boil and filtration. The pH of the filtrate was measured, and only batches with a pH between 5.0 and 5.6 were used. The preparation was then transferred to 50-milliliter vials and sterilized in an autoclave at 120 °C for one hour.

No drugs were administered to the animals in the control group. Blood samples and Semen were collected before the research began and at the end of the experiment. Both groups of animals were kept under the same conditions and provided the same diet. A quantitative and qualitative analysis of the semen samples was performed using the "CEROS" computer program, and the results were documented through test certificates.

Results and Discussion

The research findings, after statistical analysis, are compiled in **Tables 2 and 3**. It is important to consider that the sexual activity of rams in both groups tends to increase as the breeding season approaches [21, 22].

Table 2 displays the testosterone levels observed in the blood of rams as they prepared for an arbitrary period during an off-season.

Table 2. Findings on the testosterone concentrations in the blood of rams during their preparation for an arbitrary period in an off-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%

Upon reviewing the study outcomes, it was observed that at the start of the experiment, the testosterone levels in rams were nearly identical across both groups, measuring at 4.57 ± 0.39 nanograms per milliliter and 4.55 ± 0.43 nanograms per milliliter. By the conclusion of the experiment, considering that sexual activity in rams tends to increase as they approach the breeding season, testosterone levels in both the control and experimental groups rose by 39.6% and 33.81%, respectively. In the experimental group, the testosterone level reached 7.5 ± 0.578

nanograms per milliliter, which was 0.69 nanograms per milliliter (0.67%) higher than the control group, where the testosterone level was 6.81 ± 0.23 nanograms per milliliter.

Tables 3 and 4 summarize the findings from the semen samples of rams during their preparation for the random period in an off-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 sperm production and sexual activity of rams in both groups increase as the breeding season approaches, so this factor was carefully considered when analyzing the data in **Table 3** for both groups.

In the experimental group, the volume of ejaculation ranged from 0.9-1.3 milliliters per cage, while in the control group, the range was from 0.7-0.9 milliliters per cage.

Upon reviewing the data in **Table 3**, we found that the ejaculate volume in the experimental group increased from 0.829 ± 0.086 ml to 1.05 ± 0.122 ml, while in the control group, it rose from 0.609 ± 0.067 ml to 0.725 ± 0.072 milliliters. This shows that, compared to the initial volume, the ejaculate volume in the experimental group increased by 21% (0.221 ml), while in the control group, it increased by 16% (0.116 milliliters), which is 5% lower than the increase in the experimental group.

The sperm concentration in the experimental group increased from 1.052 ± 0.028 billion/ml to 1.58 ± 0.126 billion per milliliter, whereas, in the control group, it rose from 1.064 ± 0.0351 billion per milliliter to 1.217 ± 0.105 billion per milliliter. Comparing the initial data, the concentration of sperm in the ejaculate of the experimental group rose by 33.41% (0.528 billion/ml), while the concentration in the control group increased by 12.57% (0.153 billion per milliliter), which is 20.84% (0.375 billion per milliliter) 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

Table 4 presents the data on sperm motility. After the experiment, the percentage of live motile sperm in the experimental group increased from 78.58 ± 3.22 to 83.41 ± 8.69, while in the control group, it rose from 73.90 ± 5.44 to 77.75 ± 4.33. The experimental group showed a 5.66 percentage point higher increase compared to the control. About the starting values, the experimental group's motility increased by 5.79%, while the control group experienced a 3.84% increase, 5.66 percentage points lower than the experimental group.

The proportion of motile sperm exhibiting rectilinear translational motion in the experimental group was initially 39.41 ± 3.29, rising to 53.2 ± 11.71 by the end of the study. Meanwhile, in the control group, this parameter increased from 31.55 ± 3.40 to 33.75 ± 4.88, 19.45 percentage points lower than the experimental group. When compared to the initial values, the experimental group saw a 25.92% increase, while the control group had a more modest rise of 6.53%, which is 19.39% lower than the experimental group.

An analysis of the data from all tables reveals that after the experiment, testosterone levels, along with both quantitative and qualitative indicators of spermatogenesis, were significantly higher in the experimental group **(Figure 1)**. Given that both groups of animals were kept under identical conditions and received the same diet, it is likely that the observed improvements in the experimental group, particularly in the development of the reproductive system in preparation for the breeding season, were due to the administration of stimulants. In addition to selenium, vitamin E, and iodine, the animals also benefited from a bio-stimulator derived from *A.*

arborescens. This plant is rich in polysaccharides, enzymes, vitamins, amino acids, phytoncides, phenols, esters, resins, and over two hundred biologically active compounds that support cellular metabolism, and tissue regeneration, and enhance overall nonspecific body resistance. It also strengthens the mucous membranes' defenses against harmful agents and accelerates the regeneration processes. These combined effects, both direct and indirect, contribute to the improved quantity and quality of semen produced by the males.

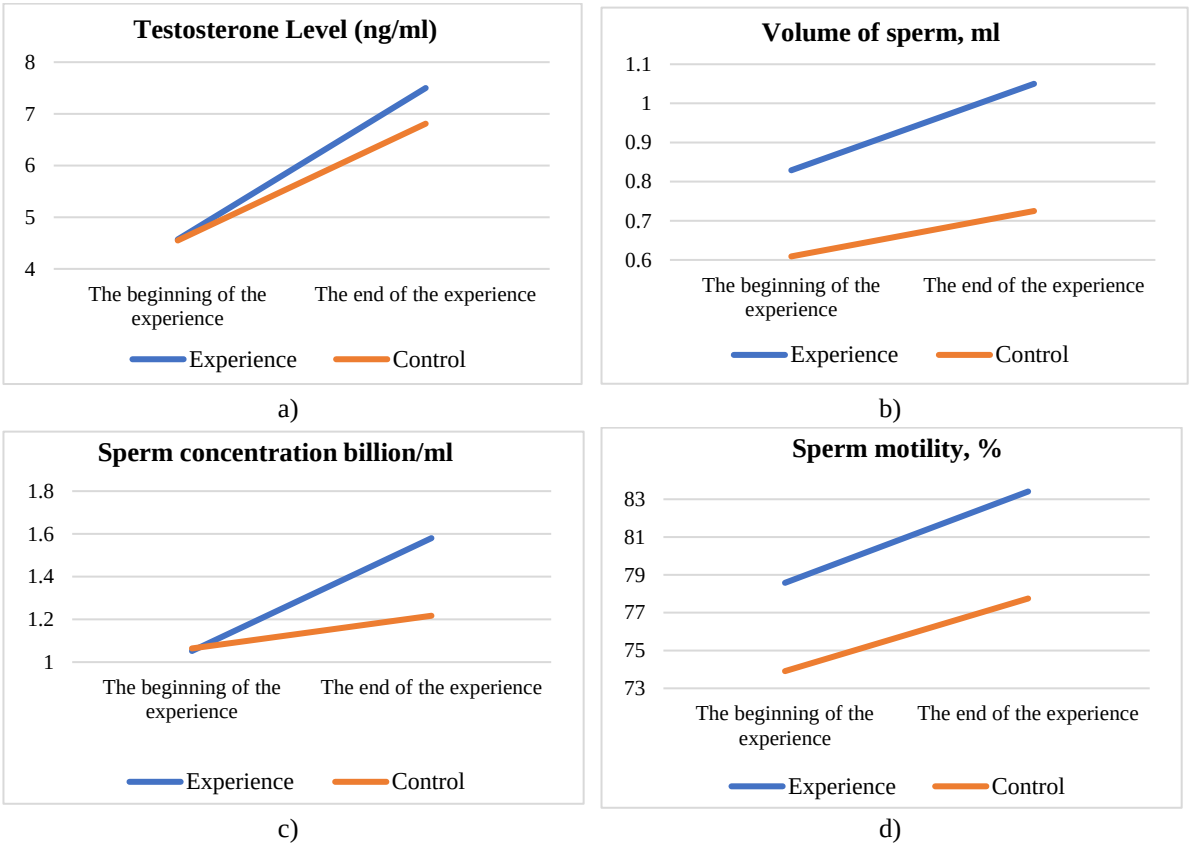

Figure 1. Graphical representation of the results of the experiment.

We propose that the rams in the experimental group exhibited a quicker recovery and preparation for the seasonal breeding period following the winter due to the non-hormonal stimulants introduced in our study. The substances we recommend for stimulating rams during their preparation for the breeding season positively influence metabolic processes and spermatogenesis in the animals. Based on these findings, we suggest that these treatments be considered for practical use.

Conclusion

Research conducted on 2 groups of young rams, each comprising 12 animals, demonstrated the beneficial impact of the administered drugs on sperm quantity and quality. Over 50 days, the experimental group received a combination of iodine and amylodextrin with their feed, 1.5 milliliters of E-selenium via intramuscular injection, and *A. arborescens* extract with a novocaine solution also administered intramuscularly. The findings revealed that the non-hormonal treatment used in preparing the rams for the transitional period had no detrimental effects on the animals and contributed to improved spermatogenesis and elevated testosterone levels.

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Conflict of Interest: None

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Ethics Statement: The experimental protocol adhered to the guidelines of the European Convention for the protection of vertebrate animals used in scientific and experimental procedures.

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