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## Utilization of Biologically Active Compounds from Plant Materials in Specific Physiological States of Cows

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### ABSTRACT

This study aimed to investigate the effect of drugs used to treat catarrhal postpartum endometritis in cows on the therapy duration, frequency of insemination, the time from calving to insemination, and certain hematological and biochemical blood parameters. Cows that calved and were diagnosed with postpartum pathologies between days 14-18 were divided into two groups: an experimental group and a control group. The experimental group received four subcutaneous injections of E-Selen at a dose of 1 ml per 50 kg of body weight once a week, along with a plant-based drug at a dose of 1 ml per 100 kg mixed with 0.5% novocaine. Additionally, 150 ml of a drug containing amyloextrin and iodine was administered intrauterine daily for 6-7 consecutive days. When using bio-stimulators containing biologically active substances (BAS) from plant materials in combination with E-Selenium and iodine-based amyloextrin for the treatment of catarrhal postpartum endometritis, the therapy duration was reduced by 38%, lasting only 6.4 days compared to the use of Metristar and Estrophan. The time from calving to the first insemination was shortened by 13% and reached 59 days. The time from calving to successful insemination was reduced by almost 10% and totaled 63 days. Overall, faster recovery processes were observed in the experimental group, indicating better glucose utilization in the presence of triglycerides and higher levels of creatinine and globulins. The results also confirm the safety and efficacy of the proposed therapeutic approach for the treatment of postpartum endometritis.

**Keywords:** Catarrhal postpartum endometritis, Biologically active substances, Cows, Therapy

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### Introduction

Biostimulants and adaptogens have been utilized since the times of Avicenna and Hippocrates, but the investigation into their composition and the scientific understanding of their mechanisms of action began only in the 20th century [1, 2]. Research revealed that certain preparations contain unique substances produced by living

cells in various animal and plant tissues under prolonged exposure to harsh conditions [3]. These biologically active substances (BAS) are formed in such tissues, where they stimulate specific biochemical processes that help sustain the tissues' vital functions under unfavorable conditions [4-6].

When used in precise dosages, drugs containing BAS enhance the body's natural resistance (such as the bactericidal activity of blood serum and the phagocytic activity of leukocytes), promote hematopoiesis, improve metabolism and reproductive functions, stimulate animal growth and weight gain, and boost feed conversion efficiency [7-10]. The production of these preparations involves various components, such as the liver, testes, spleen, skin, estuarine mud, placenta, peat, plantain leaves, and tree aloe, among others [11-13].

Currently, BAS-containing drugs are widely used by specialists in the comprehensive treatment of reproductive organ pathologies, including catarrhal postpartum endometritis in sows and cows [14-18]. These treatments have been shown to improve egg quality in females and accelerate myometrial tissue regeneration after metropathies and calving [15]. However, few experts thoroughly consider the precise effects these drugs or their combinations have on metabolic processes within the body, nor whether they may have any hidden negative impacts.

This study aims to examine the effects of the drugs we have developed and utilized for treating catarrhal postpartum endometritis in cows. Specifically, we will assess their impact on therapy duration, the frequency of insemination, the time from calving to insemination, and certain hematological and biochemical blood parameters in cows.

## Materials and Methods

In this study, experiments were conducted to assess the impact of therapeutic drugs developed in our laboratory on various health indicators of cows. These indicators included therapy duration, insemination frequency, the time from calving to insemination, and specific hematological and biochemical blood parameters.

After evaluating cows for postpartum pathologies between days 14 and 18 post-calving, two groups were created from those diagnosed with catarrhal postpartum endometritis: the experimental and the control groups.

The experimental group received four injections of the E-Selen drug (1 ml per 50 kg of body weight, subcutaneously) once a week, alongside an additional drug derived from plant tissues (1 ml per 100 kg body weight) mixed with 0.5% novocaine. Furthermore, for six to seven days, one hundred fifty ml of a drug containing amyloextrin and iodine was administered intrauterinely daily.

The tissue-based drug was manufactured in the Laboratorul Biotehnologii în Reproducție și Transfer de Embrioni NPIBZMV laboratory. The preparation process involved using leaves from *Aloe arborescens* Milli plants, aged at least two years. The aloe leaves were stored in the dark at 4-8 °C for 10-12 days. After removing the tips and any yellowed parts, the leaves were chopped, ground into a pulp, and combined with three times the amount of distilled water, then boiled for two to three minutes to induce protein coagulation. The mixture was filtered, sodium chloride (7 g per liter) was added, and it was boiled for an additional 2 minutes before being filtered again. The pH of the solution was measured, and only batches with a pH between 5.0 and 5.6 were used. The final solution was bottled in 50 ml vials and sterilized for 1 hour at 120 °C in an autoclave.

For the control group, between days 14 and 18 post-calving, a single rectal uterine massage was performed. Additionally, on days 14-18, 21, and 28 post-calving, the contents of one tube of Metristar (19 g) were injected into the uterine cavity, and two ml of Estrophan were administered on days 18 and 28 post-calving.

Animals were considered clinically healthy after the cessation of any uterine discharge, which typically occurred by day 3. However, even after discharge ceased, treatment with amyloextrin and iodine continued for an additional 3 days. The minimum duration of therapy was 6 days, even if the discharge stopped after just two days of treatment.

Blood samples were taken from both groups before the experiment began and at its conclusion. The animals from both groups were maintained in identical conditions and on the same diet. The results of the experiment were documented in official test certificates.

## Results and Discussion

The results of the study, after statistical analysis, are presented in **Tables 1 and 2**.

**Table 1** summarizes the findings of the investigation into the impact of the drugs developed and utilized by us for treating catarrhal postpartum endometritis in cows. It includes data on the therapy duration, insemination frequency, and the time between calving and insemination.

**Table 1.** Effectiveness of the proposed means of therapy for catarrhal postpartum endometritis of cows.

Groups	Intensive care period, days	Multiplicity of inseminations	Pregnancy after the first insemination	The period from calving to first insemination, days	The period from calving to 100% fertile insemination, days
Experimental	$6.4 \pm 0.273$	$1.2 \pm 0.22$	80%	$58.8 \pm 1.85$	$63 \pm 5.82$
Control	$10.4 \pm 0.44$	$1.2 \pm 0.22$	80%	$67.6 \pm 3.85$	$69.8 \pm 3.0082$
Difference (days)	4.0	-	-	8.8	6.8
Difference (%)	38.46	-	-	13.01	9.74

The data in **Table 1** indicate that the minimum duration of intensive therapy required to cleanse the uterine cavity and restore the endometrial structure was  $6.4 \pm 0.273$  days for the experimental group. This represents a 38.46% reduction compared to the control group, where the therapy took an average of  $10.4 \pm 0.44$  days, which is 4 days longer.

The frequency of insemination was consistent across both groups, with an average of  $1.2 \pm 0.22$  inseminations per animal. Additionally, the pregnancy rate following the first insemination was identical in both groups, with 80% of the cows becoming pregnant.

In the experimental group, the time from calving to the first insemination was on average  $58.8 \pm 1.85$  days, which was 8.8 days or 13.01% shorter than the  $67.6 \pm 3.85$  days observed in the control group. Some animals in both groups did not become pregnant after the first insemination, but they were re-inseminated after 20-21 days during their next estrus. As a result, the period from calving to successful insemination was  $63 \pm 5.82$  days in the experimental group, whereas in the control group, it was  $69.8 \pm 3$  days, a difference of 6.8 days or 9.74%.

**Table 2** presents the findings from the study on the effects of the drugs developed and used for treating catarrhal postpartum endometritis in cows, focusing on various hematological and biochemical blood parameters.

**Table 2.** Some hematological and biochemical parameters of cows' blood.

Parameters	Groups	Norms	1st blood draw, before the experience	2nd blood draw, after the experience
Hemoglobin (g/l)	Experimental	99-129	$102.6 \pm 2.16$	$107.4 \pm 3.81$
	Control		$104.5 \pm 3.94$	$106.1 \pm 4.56$
Erythrocytes ( $\times 10^{12}/l$ )	Experimental	5-10	$6.78 \pm 0.22$	$7.7 \pm 0.4$
	Control		$7.04 \pm 0.38$	$7.2 \pm 0.61$
Leukocytes ( $\times 10^9/l$ )	Experimental	4-12	$13.12 \pm 1.2$	$6.67 \pm 1.1$
	Control		$13.64 \pm 1.6$	$6.71 \pm 0.8$
Protein (g/l)	Experimental	62.-82	$39.3 \pm 2.3$	$42.7 \pm 15.2$
	Control		$39.7 \pm 2.1$	$40.4 \pm 4.1$
Albumin (g/l)	Experimental	28-39	$29.7 \pm 1.6$	$26.2 \pm 1.8$
	Control		$25.9 \pm 3.2$	$29.2 \pm 2.2$
Globulin (g/l)	Experimental	29-49	$9.4 \pm 2.34$	$16.7 \pm 4.1$
	Control		$13.8 \pm 2.8$	$11.4 \pm 3.1$
Triglycerides (mmol/l)	Experimental	0.3-0.6	$0.24 \pm 0.047$	$0.76 \pm 0.07$
	Control		$0.15 \pm 0.023$	$0.7 \pm 0.12$
Glucose (mmol/l)	Experimental	2.3-4.1	$4.63 \pm 0.63$	$3.1 \pm 0.42$
	Control		$4.81 \pm 0.59$	$3.7 \pm 0.44$
Urea (mmol/l)	Experimental	2.8-8.8	$11.6 \pm 1.14$	$9.2 \pm 1.6$
	Control		$12.2 \pm 2.11$	$9.3 \pm 1.6$

Creatinine (nmol/l)	Experimental	56-156	136.57 ± 32.2	166.11 ± 30
	Control		193.04 ± 33.06	182.56 ± 24.67
P (mmol/l)	Experimental	1.4-2.5	2.49 ± 0.35	2.5 ± 0.4
	Control		3.16 ± 0.41	2.41 ± 0.44
Calcium (mmol/l)	Experimental	2.1-2.8	8.67 ± 1.84	10.88 ± 1.16
	Control		10.5 ± 1.72	12.47 ± 2.09

When analyzing the blood sample results from cows diagnosed with catarrhal postpartum endometritis before and after treatment, it is observed that the levels of hemoglobin and erythrocytes were within normal physiological ranges. Initially, both groups had hemoglobin and erythrocyte levels close to the lower end of the normal range, with values between 102.6-104.5 grams per liter and 6.78-7.04 10<sup>12</sup>/l, respectively. After completing the therapy, both groups showed an increase in hemoglobin and erythrocyte levels, which ranged from 107.4-106.1 g/l and 7.4-7.210<sup>12</sup>/l. Notably, in the experimental group, the hemoglobin level rose by 4.7%, which is 2.85% (three times) higher than the 1.55% increase observed in the control group. Furthermore, the erythrocyte count in the experimental group increased by 13.2%, which is 6.63% (twice) more than the 6.6% increase in the control group. This suggests that the animals were diagnosed and treated early, preventing significant changes in these indicators. Leukocyte levels in both groups were elevated at the start of the study, with values of 13.12 ± 1.2 and 13.64 ± 1.6 \*10<sup>9</sup>/l, respectively. After treatment, the experimental group showed a decrease in leukocytes to 6.67 ± 1.1 10<sup>9</sup>/l, 3.3% lower than the control group, where leukocytes dropped to 6.71 ± 0.8 10<sup>9</sup>/l. Both groups experienced a significant reduction in leukocyte counts, with decreases of 49.2% in the experimental group and 50.8% in the control group, reaching physiological levels. These findings suggest that recovery processes in the experimental group were more successful and faster.

At the beginning of the study, protein levels in the experimental and control groups were 39.3 ± 2.3 grams per liter and 39.7 ± 2.1 grams per liter, globulin levels were 9.4 ± 2.4 and 13.8 ± 2.8 grams per liter, and triglycerides were 0.24 ± 0.047 and 0.15 ± 0.023 mmol/l, respectively. These values were all below the normal range. Additionally, urea levels were elevated in both groups, with the experimental group showing 11.6 ± 1.14 mmol/l and the control group 12.2 ± 2.11 mmol/l. This suggests that the animals were experiencing body depletion due to recent pregnancy and calving, initial signs of intoxication from postpartum endometritis, and an increased energy demand due to rising milk production and flow.

Following the therapy, both groups showed an increase in protein levels, although these remained slightly below the normal range. In the experimental group, the protein level rose to 42.7 g/l, a 5.4% increase compared to the control group, where it reached 40.39 g/l. It is important to note that this increase occurred alongside milking and a rise in milk production.

Although the globulin level remained below normal, there was a significant increase in the experimental group, where the globulin content rose to 16.7 ± 4.1 g/l (a 77.8% increase), which was 30.6% higher than in the control group. In contrast, globulin levels in the control group decreased to 11.4 ± 3.1 g/l, a 17.3% reduction.

At the end of therapy, the urea levels in both groups showed a slight decrease, with values of 9.2 ± 1.6 millimoles per liter and 9.3 ± 1.6 millimoles per liter, respectively. Although this level remained slightly above the upper normal limit, it is characteristic of dairy cows during the onset and peak of milk production.

An analysis of the globulin, protein, and urea data reveals that in the experimental group, these values were closer to the normal range. This suggests that the recovery processes in these cows progressed more quickly after calving and the treatment for endometritis. Furthermore, these indicators reflect better metabolism and immunity development, even as the cows experienced an increasing workload due to milk production. This is further confirmed by the increase in creatinine levels to 166.11 ± 30 nanomol/l in the experimental group after therapy. Conversely, in the control group, creatinine levels were above normal at the start of the experiment and only slightly decreased after therapy, indicating a slower recovery and metabolism.

Albumin levels in both groups showed only slight fluctuations. Notably, in the experimental group, the albumin level decreased by 11%, reaching 26.2 ± 1.8 g/l after therapy, while globulin levels rose by 77%. This suggests a rapid development of immunity. In contrast, in the control group, albumin levels increased to 29.2 ± 2.2 g/l, while globulin levels decreased by 17.3%, indicating a greater strain on the immune system and slower recovery processes after calving and the endometritis treatment.

At the beginning of the study, the triglyceride levels were below the normal range, while glucose levels were slightly elevated, with values of  $4.63 \pm 0.63$  mmol/l in the experimental group and  $4.81 \pm 0.59$  millimoles per liter in the control group. This indicates a disruption in glucose utilization, likely because of a lack of energy resources related to milking and the presence of metropathies. By the end of therapy, triglyceride levels had increased in both groups, while glucose levels decreased, suggesting that the processes of glucose utilization normalized as the endometrium began to recover. Specifically, triglyceride levels in the experimental group increased to  $0.76 \pm 0.07$  millimoles per liter (a 3-fold increase), while in the control group, they increased to  $0.7 \pm 0.12$  millimoles per liter (a 4.5-fold increase). Meanwhile, glucose levels in the experimental group decreased to  $3.1 \pm 0.42$  millimoles per liter (a 33.8% reduction), while in the control group, it decreased to  $3.7 \pm 0.44$  millimoles per liter (a 23.1% reduction). These findings suggest that the experimental group experienced a greater reduction in glucose and a smaller increase in triglycerides, indicating a higher metabolic rate and improved metabolic processes.

Both before and after the study, the calcium levels in the animals were four to five times higher than the norm. However, this excess calcium was of exogenous origin, as the animals were consuming water with a naturally high calcium content. The local water sources, derived from a well and the Dniester River, are rich in calcium due to the underlying calcareous deposits from the Sarmatian Sea that existed in the region millions of years ago. Consequently, the calcium levels observed were attributed to the high calcium content of the water rather than pathological processes.

Additionally, the fluctuations in triglyceride, urea, and creatinine levels throughout the study, corresponding to the treatment of postpartum endometritis, also suggested that the calcium observed was of exogenous origin. Elevated levels of these components are often seen in cases involving musculoskeletal disorders like osteoporosis, hepatopathy, or myositis. However, the changes observed in these indicators during the treatment period were not significant enough to suggest pathological alterations, further supporting the exogenous nature of the calcium.

Phosphorus concentrations were within acceptable limits throughout the study, fluctuating between 2.4 and 3.2 mmol/l in both groups.

Overall, the results indicate that recovery processes occurred more rapidly in the experimental group, as evidenced by better glucose utilization, higher triglycerides, and elevated levels of globulins and creatinine. These findings not only confirm the efficacy and safety of the therapeutic agents used for postpartum endometritis but also highlight the positive impact of BAS-containing preparations on the recovery process. Since both groups were kept under identical conditions and diets, it can be concluded that the faster recovery and shorter therapy duration observed in the experimental group were a direct result of the BAS-containing therapy.

## Conclusion

The biologically active substances evaluated in this study, when combined with E-selenium and amylopectin containing iodine, effectively shortened the intensive therapy period for postpartum catarrhal endometritis in cows to  $6.4 \pm 0.273$  days. Additionally, the time from calving to 100% successful insemination was reduced to  $63 \pm 5.82$  days. The analysis of biochemical and hematological blood parameters demonstrated that the drugs used in the treatment of metropathy were non-toxic and safe for the animals.

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**Conflict of Interest:** None

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**Ethics Statement:** The animal experimental protocol adhered to the guidelines set forth by the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes.



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