

Field Application of Ovarian Cytotoxic Serum for Reproductive Stimulation: A Practical Tool for Managing Ewe Infertility

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ABSTRACT

This study aimed to evaluate the effect of Ovarian Cytotoxic Serum (OCS) on the physiological, immunological, and reproductive parameters of ewes. A total of 200 Kazakh Merino ewes were randomized into two groups: experimental (n = 100) and control (n = 100). The experimental group received two subcutaneous injections of OCS (2.5 and 3–3.5mL) two weeks before the insemination campaign, while control animals received no treatment. Hematological, biochemical, and immunological parameters, including erythrocytes, hemoglobin, leukocytes, total protein, immunoglobulins (IgA, IgM, IgG), and phagocytic activity, were measured at five time points: before administration, 7 days post-injection, on the day of estrus, and 14 and 21 days after insemination. Administration of OCS significantly increased erythrocyte count by 30% and hemoglobin by 11.1% on the day of estrus compared to 6.7 and 1.1% in controls (P<0.05). Total protein in treated ewes rose by 26.6% versus 8.2% in controls. Immunoglobulin concentrations increased substantially, with IgA, IgM, and IgG reaching 0.81±0.17, 2.64±0.23, and 28.62±1.16mg/mL, respectively, on the day of estrus, and remained higher than controls throughout early gestation. Phagocytic activity and phagocytic number were also enhanced in the experimental group (maximum phagocytic activity: 50.5±2.8%; phagocytic number: 2.8±0.04), indicating improved cellular immunity. These results demonstrate that OCS positively modulates blood morphological and immunological parameters, accelerates estrus, improves fertilization, and supports pregnancy and fetal development. Implementation of OCS in breeding programs can enhance reproductive performance, offspring viability and overall herd productivity.

Keywords: Fertility enhancement, Ovarian cytotoxic serum effect, Reproductive biotechnology, Phagocytic activity, Sheep reproduction.

INTRODUCTION

The main problems in the agro-industrial sector are preserving livestock, increasing animal reproductive capacity, obtaining healthy offspring, significantly increasing productivity, and thoroughly satisfying needs for products. Inadequate feeding and improper animal husbandry practices lead to metabolic disorders, which compromise resistance and result in functional disorders (Sammad et al. 2022; Tufarelli et al. 2024). This leads to a discrepancy between the productivity and fertility of animals and their physiological capabilities (Mekuriaw 2023; Sadykov et al. 2023; Yagoubi et al. 2024).

In recent years, many countries have reported an increase in sheep populations, driven by rising demand for meat and wool products. For instance, Australia and New Zealand continue to expand production by applying intensive technologies and genetic improvement programs, which ensure both high productivity and sustainability of the industry. A similar trend is observed in Kazakhstan, where sheep farming has experienced both growth and substantial challenges. As of 2025, the total sheep population in Kazakhstan has reached approximately 23.1 million heads, placing the country among the leading sheep producers in Central Asia (Orkara et al. 2025). The livestock production sector is showing signs of recovery,

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with output indices increasing year on year — for example, in the first six months of 2025, livestock production increased by about 3.2% compared to the same period in 2024 (Bureau of National Statistics of the Republic of Kazakhstan 2025). Moreover, government strategies are being developed to adopt the so-called “Australian method” of intensive sheep farming (Prime Minister’s Office of Kazakhstan 2023). However, despite this positive dynamic, experts emphasize that Kazakhstan’s sheep industry is experiencing a deep crisis linked to insufficient infrastructure, high production costs, and limited access to modern reproductive technologies. Therefore, while quantitative growth is evident, qualitative development remains constrained, with reproductive inefficiencies, including low fertility, losses during gestation, poor lamb survival, and suboptimal reproductive rates, continuing to undermine the economic viability of sheep enterprises. These limitations highlight the urgent need for the implementation of innovative biotechnological tools to improve reproductive efficiency and ensure the long-term competitiveness of sheep farming.

Several physiologically active substances have been proposed to stimulate the function of farm animal organs and tissues, with a leading focus on immunological interventions and sera with immunomodulatory effects. Several authors have reported positive effects of immunological preparations on health and productive parameters that indirectly affect reproductive function, including autogenous vaccines, passive sera, and other immune stimulants (Li et al. 2023; Davis et al. 2024; Lei et al. 2024).

Large-scale scientific and practical experiments conducted on laboratory and farm animals have shown that organ-specific immune preparations can influence ovarian function and systemic physiology. Despite numerous earlier reports, many unresolved issues require further study. To date, organ-specific preparations have historically been obtained for many tissues, but only a few immunological products have seen sustained practical use; one historically reported preparation is ovarian cytotoxic serum (OCS) obtained by hyperimmunization with ovarian tissue.

The influence of OCS on reproductive function and on humoral and cellular components of natural resistance in ewes at different stages of the reproductive cycle remains practically unexplored in contemporary peer-reviewed literature, while recent studies emphasize mechanistic ovarian immunobiology and controlled immunological interventions that are directly relevant to understanding how immune preparations might affect reproduction (Li et al. 2023; Bazzano et al. 2024; Davis et al. 2024; Lei et al. 2024).

Summarizing the literature review, we can say that while immune sera (including OCS) have shown efficacy in improving reproductive outcomes in various etiologies, there remains a lack of comprehensive data on their mechanism of action, safety, and effectiveness under field conditions. Most work has focused on physiological phenomena rather than linking these effects to concrete reproductive outcomes in sheep under commercial or semi-commercial conditions (Martin 2022; Skarzynski et al. 2022; Han et al. 2023; Salama et al. 2024). The formation and regulation of animal sexual function directly depend on

protein metabolism processes; therefore, general protein metabolism disorders cause functional disorders of the reproductive system (Ali et al. 2021; Sheng et al. 2021; Kotsampasi et al. 2025).

Immunoglobulins play an essential role in regulating sexual function. They enhance immunobiological properties and affect physiological processes, such as growth and development (Castillo-Lopez et al. 2025; Wicki et al. 2025). Existing studies on the influence of cellular and humoral immune factors on ewes’ reproductive function are insufficient; therefore, this issue requires a deeper study (Machin et al. 2021; Bauer et al. 2022; Schiller et al. 2023; Pratelli et al. 2024).

This study aimed to evaluate the effects of Ovarian Cytotoxic Sera (OCS) on the reproductive function of ewes by studying the dynamics of biochemical and immunological blood parameters at different stages of the reproductive cycle, both under normal conditions and under OCS stimulation, to provide physiological substantiation for the use of OCS in sheep reproduction.

MATERIALS AND METHODS

Study site and animals

Scientific and production experiments were carried out to study the effect of Ovarian Cytotoxic Sera (OCS) on the indicators of natural resistance and reproductive function of ewes at the Azat farms in the Raiymbek district, Almaty region. The determination of indicators of nonspecific resistance was carried out in the laboratory of the Department of Pharmacology and Animal Pathology of the Kazakh National Agrarian Research University from 2020 to 2025.

During our scientific and production experiment, we used an OCS manufactured at the Department of Pharmacology and Animal Pathology of the Kazakh National Agrarian Research University. The experiments were conducted on Kazakh merino sheep. The farm was safe from infectious and invasive diseases during the experimental period.

Preparation of OCS

Ovarian tissues taken during the slaughter of ewes were used as an antigen to obtain OCS. The ovaries, cleaned of dense connective tissue and washed several times in sterile saline solution, were crushed in a homogenizer. The preparation of the antigen was carried out under sterile conditions. The resulting homogeneous mass was placed in a sterile container and preserved by freezing.

Healthy donkeys were selected as producers for OCS; the animals were subcutaneously injected with a 5% suspension of antigen in sterile saline solution in increasing doses, with intervals between injections of antigen of 5-7 days.

At the beginning of immunization, the reaction to the introduction of the antigen appeared after 5-7 hours and was characterized by some depression and refusal of food. At the end of immunization, this reaction occurred faster, within an hour of the introduction of the antigen, and lasted for several hours. By the end of the first day after the introduction of the antigen, the reaction completely disappeared, and the animals felt healthy. 7-8 days after the

last injection of the antigen, a small amount of blood was taken from the producers, serum was obtained, and its activity (titer) was determined. A serum is considered active if it has a titer of at least 1:100 according to the complement fixation test (CFT). In our case, the serum had a titer of 1:110 and was considered active. From 500 to 1,000mL of blood (per donkey) was taken from the jugular vein of immunized donkeys with strict observance of the rules of asepsis and antiseptics.

The obtained serum's toxicity was tested on white mice and rabbits using the generally accepted method. The serum was preserved with a 5% phenol solution, allowed to settle for 7 days, and then poured into ampoules. The ampoules were stored in the refrigerator at +4°C. Ampoules with OCS were examined before use and thoroughly shaken to distribute the suspension evenly. Ampoules with mold or film on the surface of the preparation and a putrid odor were discarded. The opened ampoules were used on the same day.

Experimental design

In the experiments, we used 200 heads of Kazakh merino breed ewes selected according to the principle of analogs with an average live weight of 50kg. The sample size of 200 ewes (100 per group) was determined based on the expected differences in reproductive performance between treated and control animals, taking into account data obtained from previous farms. An a priori power analysis assumed an average fertility rate of 70% in untreated ewes and an expected increase to 85% following OCS administration. To detect this absolute difference of 15% with 80% statistical power and a significance level of $\alpha=0.05$ (two-sided test), at least 92 animals were required in each group. To account for potential dropouts and to increase the reliability of the results, 100 ewes were allocated to each group. In addition, the chosen sample size was consistent with similar experimental designs reported in the literature (Hutchison et al. 2022). After screening, 200 ewes that met the selection criteria were randomized into two groups (experimental and control, 100 animals each). From each group, 20 animals were randomly selected for serial hematological and immunological examinations. The animals were kept under the same conditions and on the same diet. Baseline characteristics such as age, weight, number of lambing, and body condition score (BCS) did not differ significantly between the groups ($P>0.05$).

Inclusion and exclusion criteria

Animals were selected according to the following criteria: age 2–5 years (confirmed by tooth eruption), number of lambing 1–3, live weight 45–55kg, and a body condition score (BCS) between 2.5 and 3.5 on a 5-point scale. At the time of inclusion in the study, all ewes were non-pregnant, at least 60 days postpartum, and had exhibited regular estrous behavior in the previous cycle. The flock was free of infectious and invasive diseases during the study period, and all animals had undergone routine vaccination and deworming. Only animals without a history of reproductive system disorders in the previous cycle and those that had not recently received systemic antimicrobial, hormonal, or immunobiological therapy were included in the study.

Exclusion criteria were age younger than 2 years or older than 5 years, number of lambing 0 or ≥ 4 , BCS lower than 2.5 or higher than 3.5, pregnancy at the time of screening, lambing less than 60 days prior, clinical signs of disease (body temperature $>39.5^{\circ}\text{C}$, lameness, mastitis, respiratory or gastrointestinal diseases), and recent treatment with hormonal preparations, cytotoxic sera, or other stimulants of reproductive function.

Treatment protocol

The experimental group's animals were injected with OCS twice, subcutaneously in the middle third of the neck, with an interval of 3 days between injections, 2 weeks before the planned insemination campaign started. The serum was administered in the following doses: the first was 2.5mL, and the second was 3-3.5mL. No serum was administered to the control animals, and the animals of the experimental and control groups were monitored. We recorded the date when the sheep went into sexual heat, the date of insemination, the date of re-insemination, and the duration of the insemination campaign in both groups.

Blood sampling and laboratory analysis

20 ewes' from each group were selected for hematological and immunological blood tests. Blood was taken for analysis five times: before administration, on the 7th day after the introduction of OCS, on the day of sexual heat, and on the 14th and 21st days after insemination. Blood was taken from the jugular vein of clinically healthy animals in the morning before feeding. Before taking blood, the injection site was trimmed and disinfected with 96% alcohol. The needles and tubes used were sterile, dry, and clean. The blood was stabilized for hematological studies by adding heparin at five units per 1mL of blood. We determined the number of leukocytes, erythrocytes, hemoglobin, total protein, immunoglobulins A, M, G (IgA, IgM, and IgG) and phagocytic activity in the blood.

Biochemical and morphological blood parameters were determined using the StatFax® 2100 IFA Reader (Awareness Technology Inc., Palm City, FL, USA) and the ImmunoLight® 1000 automated ELISA analyzer (ImmunoChem Ltd., Moscow, Russia). Serum protein concentrations were measured with an Atago digital refractometer (Atago Co., Ltd., Tokyo, Japan). Protein fractionation was carried out using a vertical electrophoresis apparatus (Helena Laboratories, Beaumont, TX, USA). Total protein was determined by refractometric analysis, and protein fractions were determined by vertical electrophoresis on agar gel. Clinical and laboratory blood tests were performed in the Sana Medical Center's clinical diagnostic laboratory (Almaty).

Determination of phagocytic activity

The phagocytic activity of the blood serum and the quantitative content of IgG, IgA, and IgM in the blood serum allowed us to assess the state of natural resistance. The phagocytic activity of leukocytes was determined using an opsonophagocytic reaction. For this reaction, 0.5mL of blood was taken from the jugular vein, and 0.25mL of a 2% sodium citric acid solution or 2-3 drops of heparin were poured into a sterile tube and carefully mixed. Then, we added to the test tube 0.25mL of a 2×billion suspension of *E. coli*, *Staphylococcus albus*, or

Staphylococcus aureus culture inactivated by heating for 30min at T=70°C. Our experiment used *E. coli* strain No. 817 as a test culture (reference strain, obtained from the Culture Collection of the Kazakh Scientific Research Veterinary Institute, Almaty, Kazakhstan. The strain is maintained in the institute's microbial repository and supplied under controlled laboratory conditions. Prior to use, cultures were inactivated by heating for 30min at 70°C.).

The test tube with the prepared mixture was gently shaken and placed in a water bath or thermostat at 37-38°C for 30min. The tube was shaken every 10min, and then fine blood smears were prepared, fixed and stained with the Romanowsky method. The number of phagocytizing leukocytes was calculated by microscopy of the smear. The total number counted must be at least 100 to obtain reliable results. In addition, during microscopy, we counted the number of microbes absorbed by leukocytes. Based on the results of the smear study, several indications of phagocytosis were calculated.

The phagocytic activity (PA) is expressed in %. This is the ratio of leukocytes involved in phagocytosis to the total number counted. Example: When examining the smears, it was found that out of 100 leukocytes studied, 56 participated in phagocytosis, which is equal to 56% PA. The Phagocytic index is determined by the average number of phagocytized microbes per active leukocyte. This indicator characterizes the intensity of phagocytosis. For example, of the 100 leukocytes counted, 56 participated in phagocytosis and 205 microbes phagocytosed; therefore, the phagocytic index is 4.6 (205:56). The phagocytic number characterizes the aggressiveness and activity of leukocytes. It is calculated by dividing the number of phagocytized bacteria by the total number of counted leukocytes. Example: Microscopy revealed 205 phagocytized microbial bodies per 100 counted leukocytes. The phagocytic number will be 2.05 (205:100). The phagocytic capacity characterizes the total phagocytic activity of the blood. It is determined by the number of microbial bodies of phagocytized leukocytes in 1mm³ of blood. Example: By counting smears, the phagocytic number was 2.05, and 6,000 leukocytes were found in 1mm³ of blood. Therefore, the phagocytic capacity will equal 12,300 microbial bodies.

Determination of immunoglobulins

To determine IgM, 0.28g of veronal, 0.21g of medial, and 0.024g of zinc sulfate were dissolved in bidistilled water in a measuring flask per 1 liter. The pH, which should be 7.5, was checked before finally bringing the solution to the mark. 0.1mL of the test serum was added to 6mL of zinc solution and measured with a nephelometer. The amount of IgM (macroglobulin) was determined by optical density. A reagent was prepared to determine IgA. The reagent contained 189.0g of ammonium sulfate and 29.3g of NaCl. The reagent can be stored for a long time in a sealed container.

0.1mL of the test serum was added to 6mL of zinc solution and measured with a nephelometer. The amount of IgA was determined using a calibration graph based on the obtained optical density. The graph was constructed using reference immune blood sera from humans and animals with a known immunoglobulin concentration. For example,

one ampoule (1mL) of the reference serum contains immunoglobulins of the following classes: (a) IgG: 11.94mg/mL (b) IgM: 1.33mg/mL (c) IgA: 1.88mg/mL.

One standard serum ampoule was taken, and 1mL of distilled water was added and dissolved without foaming. Then, nine test tubes were placed, each previously filled with 1mL of saline solution. 1mL of the serum solution was transferred to the first tube and thoroughly mixed, and then 1mL of this solution was taken and transferred to the second tube. In the 9th tube, 2mL was obtained after mixing. Thus, the required dilution of a standard solution with a pre-determined amount of protein was achieved. The optical density of the solution was measured on a color density meter (CDM) at a wavelength of 400nm using 10mL cuvettes. The control was tubes with the same solutions but without serum.

The determination of IgG was carried out in 2 test tubes. A zinc-salicylic reagent of high ionic strength was prepared for the first tube, which contained 1.875g of zinc sulfate and 57.14g of salicylic sodium. The pH value of such a solution should be 7.3. In the first test tube reaction, serum was used where B-lipoproteins were removed since they were also precipitated by zinc-salicylic solution, which increased the parameters. To remove them, 2mL of 0.025 M calcium chloride was poured into a test tube, 0.2mL of test serum, and 0.04mL of 1% heparin solution were added. The mixture was stirred; the solution became cloudy from B-lipoproteins' precipitation. This mixture was placed in the refrigerator for 30 minutes to improve the flocculation reaction. The precipitate was then separated by centrifugation for 20 minutes at 4,000rpm. The resulting supernatant was used for the reaction in an amount of 1.1mL. Then, 1.1mL of the supernatant was added to 5mL of zinc-salicylic reagent while IgG precipitated intensively. Nephelometric changes also occurred, and the amount of immunoglobulins was determined by optical density.

Statistical Analysis

All data were processed using standard methods of variational statistics. Arithmetic means (Mean±SE) were calculated. The normality of data distribution was verified using the Shapiro–Wilk test, and homogeneity of variances was assessed with Levene's test. Since all variables met these assumptions, comparisons between groups and time points were conducted using Student's t-test for independent or paired samples, as appropriate. The level of statistical significance was set at P<0.05. In the case of multiple comparisons across time points, Bonferroni correction was applied to control for type I error.

RESULTS

Hematological parameters

We were tasked with studying the dynamics of morphological parameters of ewes' blood under the influence of OCS. The initial blood count in both experimental and control animals had no significant differences and was within the physiological norm.

The number of erythrocytes (Table 1) in the blood of ewes of the experimental group after the 2-fold administration of OCS significantly exceeded the indicators of the control group. Thus, 7 days after the introduction of OCS, the number of erythrocytes increased

Table 1: The effect of OCS on morphological parameters of ewes' blood in different periods of sexual activity

Indicators	Measurement unit	Animal group	Days of the study				
			Before administration		After administration		
			1	7	Sexual heat day 14	21	
Total protein	g/L	e	70.1±0.14 ^a	74.9±0.11 ^b	88.8±0.09 ^c	74.7±0.06 ^b	70.4±0.09 ^a
		c	70.3±0.14 ^a	71.6±0.13 ^a	76.1±0.09 ^a	72.9±0.08 ^a	71.6±0.08 ^a
Erythrocytes	10 ¹² /L	e	7.1±0.24 ^a	8.2±0.32 ^b	9.3±0.32 ^b	8.3±0.33 ^b	7.9±0.31 ^a
		c	7.2±0.22 ^a	7.4±0.24 ^a	7.7±0.21 ^b	7.6±0.22 ^a	7.6±0.32 ^a
Leukocytes	10 ⁹ /L	e	7.1±0.30 ^a	8.5±0.37 ^b	10.9±0.32 ^c	9.7±0.53 ^b	9.6±0.32 ^b
		c	6.9±0.20 ^a	7.2±0.32 ^a	7.5±0.30 ^a	7.4±0.32 ^a	7.4±0.31 ^a
Hemoglobin	g/L	e	75.6±2.16 ^a	82.05±1.9 ^b	83.96±2.03 ^b	79.16±2.13 ^{ab}	78.2±1.86 ^a
		c	73.51±1.16 ^a	74.1±1.94 ^a	74.26±2.12 ^a	74.21±1.89 ^a	74.2±1.94 ^a

Values are mean±SE. Different superscripts within a row indicate significant differences (P<0.05). E = experimental, C = control.

to 8.2±0.32×10¹²/L versus 7.1±0.24 of the initial value (P<0.05). By this time, the number of erythrocytes in the control animals increased to 7.44±0.24×10¹²/L versus 7.2±0.22 (P>0.05). The most significant increase in erythrocytes and leukocytes in experimental and control animals occurred on the day of sexual heat (Fig. 1b), while total protein and hemoglobin showed parallel upward trends (Fig. 1a). However, the manifestation rate was not the same. In the experimental animals, erythrocytes increased by 30%, while in the control animals, they increased by only 6.7% (P<0.01). Subsequently, the erythrocyte level declined and by 21 days after insemination had returned to values not significantly different from baseline (P>0.05).

Our studies established that hemoglobin dynamics in the blood of experimental and control groups of ewes coincide with erythrocyte dynamics. The lowest values occur 15 days before the start of sexual heat and are 75.6±2.16g/L in the experimental animals and 73.51±1.86g/L in the control animals (P>0.05). The hemoglobin level increases in the following days and reaches its maximum during the sexual heat. The amount of hemoglobin increases by 11.1% compared to the initial value in the experimental animals (84.0±2.34g/L) and by only 1.1% in the control animals (74.3±2.07g/L; P<0.05). By the 21st day after insemination, the hemoglobin levels in the control and experimental groups decreased, but they did not reach the baseline values.

The results of the content of leukocytes in sheep blood under the influence of OCS study are presented in Table 1. The data show that the level of leukocytes 15 days before the onset of sexual heat in both groups had a minimum value of 6.9±0.2 (P<0.05). Changes in leukocyte levels become more significant by the onset of sexual heat, and they are more important in the experimental animals. Thus, on the day of sexual heat, the number of leukocytes in the experimental group increased by 53.2% (10.9±0.32×10⁹/L) and in the control group by 8.1% (7.5±0.30×10⁹/L; P<0.01). In subsequent periods of sexual activity, the level of leukocytes decreased slightly: in the experimental group, from 10.88±0.32 on the day of sexual heat to 9.56±0.32 21 days after insemination, and in the control group, respectively, from 7.46±0.3 to 7.37±0.31 (P<0.05).

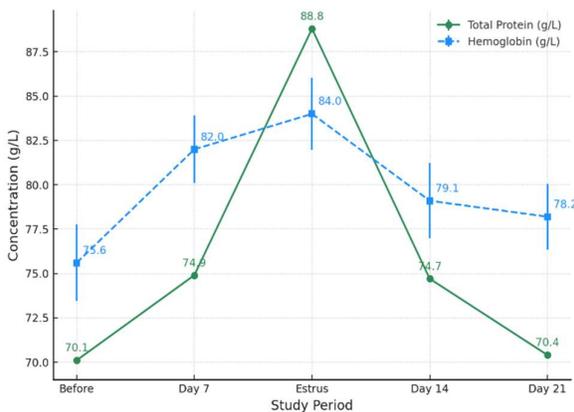


Fig. 1a: Total Protein (green) where is +SE with proteins and Hemoglobin (blue) with Mean±SE.

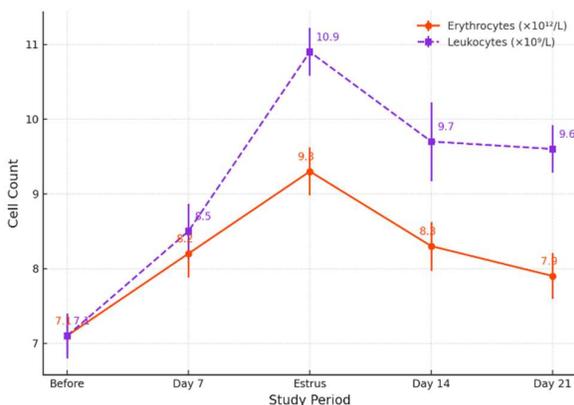


Fig. 1b: Erythrocytes (orange) and Leukocytes (violet) with Mean ± SE.

Biochemical parameters

Table 1 shows the numerical data we obtained from the study of the dynamics of total protein. It can be seen from the data that before the introduction of serum, there was no significant difference in total protein in the experimental and control groups. The total protein level in the experimental group was 70.1±0.14g, and in the control group, 70.3±0.14g (P>0.05). Differences in indicators occurred after the introduction of the OCS. The total protein level in the blood of the experimental and control groups gradually increased and reached its maximum value on the day of sexual heat. The protein level in the experimental group animals increased by 26.6% (88.8±0.09g/L), and in animals without the preparation, the total protein increased by only 8.2% (76.1±0.09g/L; P<0.01). The amount of total protein in the experimental group decreased from 88.8±0.09g on the day of sexual heat to 74.7±0.06g on 14-21 days after insemination, but it did not reach the initial values (P<0.05); in the control group,

on the 14th day after insemination, the level of total protein reached the initial values.

Immunological responses

For the quantitative determination of immunoglobulins in the blood serum, reference human and animal immune blood sera with a known concentration of immunoglobulins were used (Table 2). Using reference immune sera is necessary to build a calibration graph to determine the quantitative content of IgA, IgM, and IgG. The administration of a stimulating dose of OCS to sheep significantly affects the concentration of IgA, IgM, and IgG. Table 3 shows the effect of OCS on the content of immunoglobulins in ewes' blood serum depending on the functional state of the ovaries. The graphical dynamics of immunoglobulin levels in the experimental and control ewes during the reproductive cycle are presented in Fig. 2 (a–c).

Table 2: The content of immunoglobulins in 1mL of the reference serum

Name of immunoglobulins	Content	
	mg/mL	%
IgA	1.88	188
IgM	1.33	133
IgG	11.94	1194

The concentration of IgA, IgM, and IgG in the blood serum in the experimental and control groups fluctuated within narrow limits before the preparation was administered. After OCS was introduced, changes in the dynamics of immunoglobulins in the blood of the experimental and control groups occurred. Thus, 7 days after the introduction of OCS the level of class IgA increased by 27.4%, IgM by 5.8%, and IgG by 6.1%. They reached their greatest value on the day of sexual heat, respectively, IgA increased by 30.6% ($0.81 \pm 0.17 \text{ mg/mL}$); IgM by 12.2% ($2.64 \pm 0.23 \text{ mg/mL}$); IgG by 29.9% ($28.62 \pm 1.16 \text{ mg/mL}$), and in the control group, at the same time, there was an increase in IgA by 9%; IgM by 3%; and IgG by 4% ($P < 0.05$). On the 21st day after OCS was introduced, the concentration of immunoglobulins in the experimental groups decreased slightly and approached the initial values ($P < 0.05$). Nevertheless, the immunoglobulin levels in the experimental group remained high compared to the control group. We also studied and analyzed the dynamics of the amount of immunoglobulins. We found that the amount of immunoglobulins tended to increase under the influence of a stimulating dose of OCS. Thus, by the 7th day after stimulation, the increase in the number of immunoglobulins was 6.6%, and on the day of heat, it was 28.1%; in the control group, it was 3.1% and 4.5%, respectively. The total amount of immunoglobulins in the experimental group increased from 24.91 ± 1.23 to 31.91 ± 1.53 . In conclusion, by days 14 and 21, values in treated ewes declined slightly but remained significantly higher than in controls

Phagocytic activity

Table 3 indicates that in experimental groups, after treatment with the preparation, the proportion of immunoglobulins increases in relation to the total protein

and the total amount of immunoglobulins. Thus, the proportion of IgA was 0.88% before the introduction of OCS, and on the day of sexual heat, it increased to 1.13%. In the control animals, it ranged from 0.94% to 1.02%. Such an increase has also been noted for IgM (from 3.23% to 3.4%). IgG accounted for the main quantity of all immunoglobulins in the experimental and control groups. The percentage of IgG to total protein ranges from 31.5–39.31% in the experimental group; in the control group, it is 31.45–32.61%.

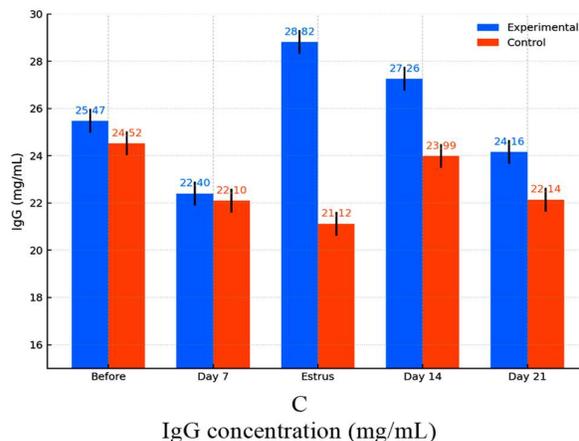
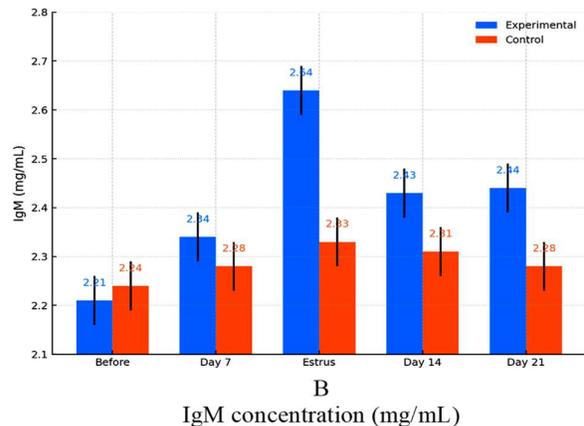
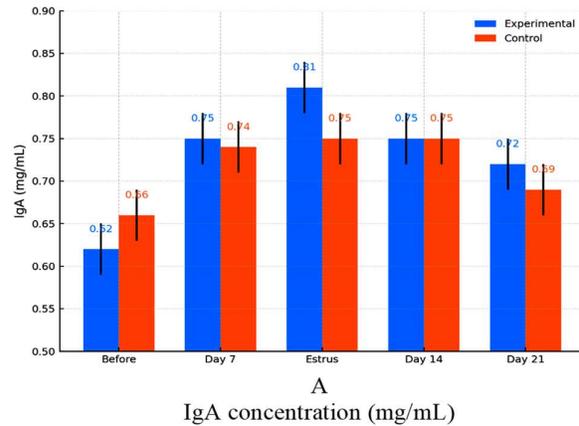


Fig. 2: Effect of Ovarian Cytotoxic Serum (OCS) on immunoglobulin concentrations in ewes during different periods of sexual activity (Mean ± SE).

Table 3: The effect of OCS on the number of immunoglobulins in the blood serum of ewes during various periods of sexual activity (mg/mL)

Immunoglobulins	Animal group	Time of blood collection				
		Before administration		After		
		1	7 days	Sexual heat day	14 days	21 days
A	E	0.62±0.17	0.79±0.18	0.81±0.17	0.79±0.18	0.72±0.18
	C	0.66±0.16	0.74±0.17	0.78±0.12	0.74±0.12	0.69±0.16
M	E	2.21±0.19	2.34±0.2	2.64±0.23	2.48±0.26	2.44±0.19
	C	2.24±0.17	2.26±0.18	2.33±0.19	2.31±0.22	2.28±0.18
G	E	22.08±0.96	23.44±1.01	28.62±1.16	27.96±1.09	24.16±1.03
	C	22.11±0.84	22.8±0.95	23.12±1.06	23.09±1.02	22.14±0.98
Total Ig	E	24.91±1.23	26.57±1.3	31.91±1.53	31.39±1.46	27.3±1.31
	C	25.01±1.08	25.81±1.21	26.16±1.06	26.16±1.34	25.11±1.25

E: experimental group; C: control group.

Table 4: The effect of OCS on the phagocytic activity of leukocytes in the blood of ewes during various periods of sexual activity

Days of the study	Animal group	PA, %	Phagocytic index	Phagocytic number	Phagocytic capacity
Before the introduction of the OCS	e	41.1±2.5	4.79±0.3	1.97±0.07	13,228.7±670.7
	c	41.0±2.1	4.9±0.26	2.00±0.04	11,394.2±547.5
7 days after administration	e	43.6±2.4	4.7±0.26	2.34±0.07	14,437.5±885.7
	c	42.2±1.9	4.7±0.21	2.00±0.03	12,041.0±841.1
Sexual heat day	e	50.5±2.8	4.5±0.25	2.80±0.04	15,870.7±663.4
	c	44.7±1.5	4.5±0.14	2.13±0.04	11,996.6±523.1
14 days after insemination	e	46.5±2.0	4.6±0.17	2.43±0.04	16,854.7±526.7
	c	45.4±1.14	4.5±0.17	2.14±0.04	12,018.6±428.8
21 days after insemination	e	45.8±2.3	4.6±0.15	2.06±0.06	14,641.3±765.3
	c	41.3±1.8	4.7±0.23	2.10±0.03	12,546.3±666.8

Values are mean±SE. E = experimental, C = control.

The phagocytic activity of blood serum was studied to assess the state of natural resistance under the influence of OCS. Table 4 presents the results of the study of the phagocytic activity of leukocytes. The levels of phagocytic activity of leukocytes in animals of both groups before administration of the preparation had no significant differences and were within 41.1±2.5-41.0±2.1% ($P>0.05$). The difference in the phagocytic activity of leukocytes appears after OCS is introduced. Thus, after the introduction of OCS, the PA of blood in the experimental ewes increases to 43.6±2.4% on the 7th day, to 50.5±2.8% on the day of sexual heat, and to 46.5±2.0% on the 14th day after insemination. This indicator was in the range of 42.2±1.9% and 45.4±1.14% in animals of the control group ($P>0.05$). The phagocytic index in ewes of the control and experimental groups for 15 days before the onset of sexual heat was 4.8±0.26 and 4.9±0.26. It did not undergo significant changes during the entire study period.

After the introduction of OCS, the phagocytic number (Table 4), which shows the intensity of phagocytosis, increased in the experimental group on day 7 from 1.97±0.07 to 2.34±0.07 and on the day of sexual heat from 1.97±0.07 to 2.8±0.04 ($P<0.01$). In animals of the control group, the phagocytic number increased at the same time, from 2.00±0.03 to 2.13±0.04. In the following days, phagocytic numbers decreased but remained higher in the experimental group. In the experimental animals, the PA of leukocytes increased by 9.14% on day 7 and 19.9% on the heat day. The maximum increase of 27.4% was noted on the 14th day after insemination. The increase in the control animals during the same period was 5.6%, 5.2%, and 5.4%.

DISCUSSION

The morphological composition of blood is closely related to the general vital activity. In industrial conditions,

blood can be used as the primary test for assessing the physiological state and is an objective indicator of an animal's adaptability to environmental changes (Očenáš et al. 2025; Turini et al. 2025).

Recent studies have examined immunological and hematological changes in ewes under experimental treatments, providing a basis for comparison with our findings (Seibel et al. 2021). Machín et al. (2021) reported increased IgG levels and leukocyte counts in sheep vaccinated against *Teladorsagia circumcincta*, complementing our observation that OCS elevates IgG, IgA, and leukocyte levels around estrus. Skarzynski et al. (2022) noted that few studies link physiological endpoints to reproductive success in commercial conditions; our results align with this, showing significant changes in erythrocytes, hemoglobin, proteins, and immune parameters, though the impact on fertility requires further documentation. Thus, the presented digital data allow us to conclude that OCS has a pronounced general stimulating effect on cellular immunity factors, increasing their activity and thereby creating more favorable conditions for the manifestation of the sexual cycle phenomena (Machín et al. 2021).

Our studies showed that the absolute values of most of the metabolites identified by us were at the lower limit of the physiological norm, which indicates a decrease in the level of metabolic processes. A decrease in metabolism is accompanied by a slowdown in the involution of the genitals and sluggish manifestations of estrus and sexual heat, which undoubtedly affects the fertilization process. The current circumstances require more active and effective measures to intensify metabolic processes and increase ewes' reproductive function. For this purpose, we used OCS, which has a general stimulating property and an organ-specific effect on the ovaries. The use of OCS showed that, in optimal doses, it positively affects the

course of metabolic processes and reproductive function, as evidenced by higher rates of the studied metabolites and high fertilization of ewes.

Recent studies confirm a close relationship between metabolic status and reproductive efficiency in ewes. Low metabolite levels are associated with reduced reproductive performance, weaker estrus expression and lower fertilization success (Zhai et al. 2023; Sha et al. 2024). Conversely, animals with higher body condition scores and elevated metabolites show improved estrus manifestation and lambing rates (Luridiana et al. 2025). These findings support our results showing that OCS stimulation enhances metabolic activity and create favorable conditions for estrus and successful fertilization.

Erythrocytes perform several functions in the body. Due to their hemoglobin content, they are oxygen carriers. Another essential function of erythrocytes is their ability to transport amino acids absorbed from the gastrointestinal tract to body tissues, thus participating in metabolism. Erythrocytes play a well-known role in immunity that absorb various toxins on their surface (Dobkin and Mangalmurti 2022). It transports them to the cells of the reticuloendothelial system, where they are neutralized. The respiratory pigment hemoglobin accounts for about 34% of the total and 90% of the dry mass of erythrocytes. The lack of hemoglobin in the blood leads to negative consequences (anemia, decreased productivity, and fertility). Therefore, the study of hemoglobin in the blood should be crucial.

Under the influence of OCS, the most significant increase was recorded on the day of sexual heat: erythrocytes increased by 30% in the experimental animals ($9.3 \pm 0.28 \times 10^{12}/L$) compared with only 6.7% in controls ($7.7 \pm 0.25 \times 10^{12}/L$, $P < 0.01$). After 14 to 21 days after insemination, the level of erythrocytes decreases but remains higher than in the control group ($P > 0.05$).

A recent study by Han et al. (2023) demonstrated increased ovulation rates and changes in hormonal milieu in small ruminants, tied with elevated IgG and other immune markers. Though their work was carried out under rather strict experimental conditions, the patterns, such as timing of immune response peaks just before estrus, followed by decline, mirror what we observe with OCS treatments. This suggests that OCS may act through similar immunomodulatory and endocrine pathways, and that future research should directly measure ovulation, conception, and prolificacy under practical flock management.

OCS also significantly affects hemoglobin content. The hemoglobin concentration in the experimental animals was higher throughout the study than in the control animals. The maximum increase in hemoglobin occurs on the day of sexual heat: 11.1% in the experimental group and 1.1% in the control group. Similar observations have been reported in other studies where metabolic or hormonal interventions in ewes led to increased hemoglobin levels, providing optimal conditions for ovulation and successful fertilization (Akhtar et al. 2024; García-Casillas et al. 2024; Luridiana et al. 2025).

These dynamics of erythrocytes and hemoglobin are probably explained by the fact that the fertilization process requires high energy costs, and the main source of energy is oxidation. The higher rates of erythrocytes and hemoglobin in stimulated animals are probably due to the

activation of hematopoiesis, increased circulating blood mass, and increased metabolism, which provides the best conditions for ovulation and fruitful fertilization. Supporting this, Al-Thuwaini (2021) reviewed the impact of hematological parameters and hemoglobin types on sheep's adaptation and reproductive performance, emphasizing the link between blood parameters and reproductive success. Additionally, El-Sayed et al. (2024) observed significant increases in TEC and Hb levels during late pregnancy in ewes, suggesting higher oxygen demand and increased metabolic rate. Darwish et al. (2025) found elevated expression of metabolic and oxidative stress-related genes in ewes affected by postpartum complications, indicating metabolic activation's role in reproductive health. Furthermore, they found elevated expression of metabolic and oxidative stress-related genes in ewes affected by postpartum complications, indicating metabolic activation's role in reproductive health.

Given the critical role of leukocytes in the formation of immunity in animals, we wanted to study the content of leukocytes in sheep blood under the influence of OCS. A change in the number of leukocytes indicates the stimulating effect of OCS. We found that the number of leukocytes in the stimulated animals was higher throughout the entire study period than in the control animals. This indicator reached its highest value by the time of the onset of sexual heat in the experimental group (53.2%) and in the control group (8.1%). A more significant increase in the number of leukocytes in the animals injected with OCS indicates an increase in protective functions. This is the most important biological reaction to increase resistance and protection from various influences and ensure the best conditions for ovulation and fertilization. Supporting this, Yang et al. (2022) observed baseline T-lymphocyte and cytokine indices in sheep, providing insights into immune responses. Battányi et al. (2023) also highlighted the role of leukocytes in protective host immune responses against gastrointestinal nematodes, and previously the study by Ahmed et al. (2020) revealed a sharp increase in total leukocyte counts and neutrophil counts in periparturient sheep, indicating significant immune responses.

Analyzing the results of our studies on the quantitative content of leukocytes, erythrocytes, and hemoglobin, it should be noted that the morphological parameters of the blood of the control group did not undergo significant changes throughout the experiment and remained within the baseline values. On the contrary, morphological parameters in the experimental group showed a significant increase. After fertilization and during embryo formation, the activity of metabolic processes slightly decreases. These days, the main function of erythrocytes and hemoglobin is to participate in embryo formation. Thus, on the 14th day after insemination, the number of erythrocytes and hemoglobin decreased: erythrocytes from 9.3 ± 0.32 to $8.3 \pm 0.33 \times 10^{12}/L$; hemoglobin from 83.96 ± 2.03 to $79.16 \pm 2.03 g/L$. Similar trends have been reported in previous studies, where reduced metabolic demand after fertilization was associated with lower erythrocyte and hemoglobin levels in ewes (Spanner et al. 2024).

The introduction of OCS significantly affects the dynamics of total serum protein. Under its influence, total protein increased from the first day and remained high until the end of the study, reaching its maximum on the day of

sexual heat, from 70.1 ± 0.14 to 88.8 ± 0.09 g/L. Changes in the total protein content of the control group animals had the same dynamics but were less pronounced in quantitative terms. This increase in total protein is probably due to increased protein synthesis, metabolic intensification, and mobilization of protective forces essential for ovulation and fertilization (Turini et al. 2025). The decrease in total protein concentration after fertilization on the 14th and 21st days after insemination is associated with increased plastic processes in the genitals and embryo development. This happens because proteins are actively involved in the formation of the embryo and its further development.

The results of the study conducted during various periods of sexual activity show that in stimulated and non-stimulated ewes, immunoglobulins have a one-sided orientation: their levels are low two weeks before sexual heat and high on heat days, but the degree of their manifestation in experimental ewes is higher. Thus, in ewes injected with OCS, the level of IgA increased by 27.4% on the 7th day after administration and by 30.6% on the day of heat; in controls at the same time, by 12.1 and 18.2%, respectively. Following fertilization on days 14 and 21, the concentration of IgA decreased, but in the experimental animals, this indicator remained higher than in the control animals. The same dynamics were observed in the IgM and IgG content, and with the onset of signs of sexual heat, experimental ewes' maximum IgM increased from 2.21 ± 0.19 to 2.64 ± 0.23 mg/mL, and IgG increased from 22.08 ± 0.96 to 28.62 ± 1.16 mg/mL. During this period, the amount of IgM increased from 2.24 ± 0.17 to 2.33 ± 0.19 , and IgG from 22.11 ± 0.84 to 23.12 ± 1.06 in control ewes. After fertilization, the level of immunoglobulins decreased. However, in the stimulated animals, the immunoglobulin level did not return to the baseline values.

The study of the ratio of immunoglobulins to each other and in relation to the total protein has shown that OCS causes certain shifts among individual classes of immunoglobulins. Therefore, it can be concluded that the use of OCS has a beneficial effect not only on the total protein and the number of immunoglobulins but also on the ratio of individual classes of immunoglobulins, on the level of which the reproductive ability of ewes depends in general. This aligns with findings by Wu et al. (2025), who identified significant diversity in sheep immunoglobulin gene expression, potentially influencing immune responses during reproduction.

An increase in the concentration of immunoglobulins, especially on the day of the onset of sexual heat, indicates that during this period, the level of metabolic processes and defenses is at its maximum, thereby creating a favorable condition for the full manifestation of sexual heat and fertilization. This is consistent with the observations of Lichtmannsperger et al. (2024), who noted that various physiological factors can alter protein and immunoglobulin levels during the periparturient period. The decrease in the number of immunoglobulins after fertilization is probably due to the increased use of proteins by the developing zygote and embryo formation. Supporting this, Wang et al. (2025) reported dynamic changes in protein expression during pregnancy, reflecting cellular and tissue-level functional adaptations.

The administration of a stimulating dose of OCS to

sheep has a significant effect on phagocytic capacity. Phagocytosis is a physiological function of cellular elements that is vital to stability. The level of blood PA is an indicator of the stimulating effect of OCS. The indicators of PA of leukocytes in the animals that were injected with OCS throughout the experiment were higher than the control ones and obeyed general patterns. They had maximum values on the days of the onset of sexual heat. During this period, PA increased by 22.8% and in the control group, by 9.1%.

The increased activity of humoral and cellular protective factors on the day of sexual heat and in the days after insemination is an integral part of a complex mechanism that provides the necessary conditions for egg fertilization and embryo development (Shehabeldin et al. 2025). The most significant increase in the level of humoral and cellular immunity in the experimental animals indicates that OCS stimulates the body. An increase in nonspecific resistance helps to accelerate the onset of sexual heat, make it more pronounced, and increase the fertilization of female farm animals.

Our data indicate that OCS in stimulating doses has a positive effect on the animals' reproductive ability and the course of pregnancy. Better conditions are created for fetal development in the bodies of stimulated animals, as evidenced by high viability and a greater number of twins (Nwachukwu et al. 2021; Sha et al. 2024; Shehabeldin et al. 2025). The beneficial effect of OCS on the body of ewes is also confirmed by the fact that their pregnancy proceeded without pathology, and they gave birth quickly and without any complications.

Conclusion

This study demonstrates that the functional state of the reproductive system strongly influences blood morphological and immunological parameters in ewes, with the lowest values observed during the anestrus season and the highest during estrus. The functional restructuring after fertilization and during early pregnancy leads to moderate decreases in these parameters, reflecting their active involvement in supporting embryo development. The effect of Ovarian Cytotoxic Serum (OCS) significantly enhances both cellular and humoral immunity throughout the reproductive cycle, providing improved conditions for ovulation, fertilization, and early embryogenesis. Treated ewes showed better reproductive outcomes, including higher lambing rates and increased twin births, with healthier, heavier, and more viable offspring compared to controls. These findings highlight the practical value of OCS as a biologically active intervention to optimize reproductive performance in sheep. Implementation of OCS in breeding programs can improve fertility, support embryo development, and increase the productivity and health of lambs. Future research may explore the molecular mechanisms underlying OCS effects and assess its applicability across different breeds and environmental conditions.

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Author's Contribution: YK, SN conceived the study and drafted the manuscript; AZh and KO designed the field survey and sampling strategy; AS and ZhK performed microbiological and molecular analyses; DK curated data and validated records; AI, AA provided technical support; ShT supervised the project and critically revised the manuscript. All authors read and approved the final version.

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