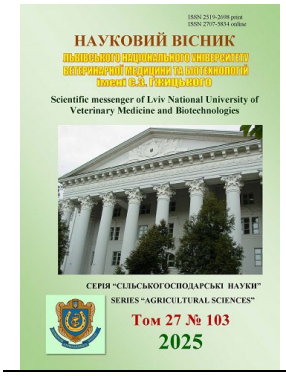

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Dynamics of immune system indicators in pregnant cows with physiological gestation and with endotoxicosis

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Particular attention in veterinary medicine is given to the study of the immune system status of pregnant cows, since this period is accompanied by significant physiological changes and increased sensitivity of the organism to stress factors and intoxications. One of such pathological conditions is endotoxicosis, which is associated with suppression of humoral, cellular, and nonspecific immunity, potentially leading to the development of secondary immunodeficiency. The aim of the study was to determine the dynamics of immune system indicators in pregnant cows with a physiological course of calving and in the presence of clinical signs of endotoxicosis. The work included analysis of humoral, cellular, and nonspecific immune components. It was established that the bactericidal activity of blood serum in the experimental group at the 8th and 9th months of pregnancy was lower by 11.3–18.7 % compared to the control, while lysozyme activity was reduced by 20.2–22.0 %. In addition, a significant decrease in neutrophil phagocytic activity and phagocytic index was observed, indicating suppression of nonspecific defense mechanisms. Cellular immunity studies showed that the number of T-lymphocytes in the blood of diseased cows was significantly lower by 23.2 % compared to the control group at the 9th month of pregnancy, and the number of B-lymphocytes decreased by 2.8–18.9 %. A decrease in serum immunoglobulin levels was also found: by 16.0 % at the 8th month and by 24.6 % at the 9th month of pregnancy. The most pronounced decline was observed in the concentration of immunoglobulins of class G, which decreased by 26.3 % compared to physiological pregnancy. The obtained results indicate that the development of endotoxicosis in pregnant cows causes systemic suppression of the main components of the immune system. This is manifested by a reduction in bactericidal and lysozyme activity of blood serum, numbers of T- and B-lymphocytes, neutrophil phagocytic activity, as well as decreased immunoglobulin levels. Such changes reflect the formation of secondary immunodeficiency, which has important practical significance for timely diagnosis, prevention, and therapy of endotoxicosis in cattle.

Keywords: *pregnancy, cows, endotoxicosis, immune system, humoral immunity, cellular immunity, non-specific resistance.*

Introduction

The clinic, course, and outcomes of many diseases are to some extent determined by the development of endogenous intoxication. By most clinical and immunological criteria, it can be characterized as a nonspecific manifestation of the imbalance between the production and elimination of products of normal and impaired (in the case of pathology) metabolism (Andersen, 2003; Levchenko et al., 2005; Gutyj et al., 2016; 2017; Bashchenko et al., 2021; 2023; Sidashova et al., 2024).

Pregnancy in cows is an extremely complex physiological process accompanied by significant changes in the functioning of the organism, particularly in the immune system (Eades, 1993; Grymak et al., 2020; Mylostyvyi et al., 2021; 2023; 2024). During this period, animals are

especially sensitive to the effects of stress factors, metabolic disorders, and intoxications, which may lead to the development of pathological conditions. One of the most dangerous factors is endotoxicosis, which arises as a result of the accumulation of toxic metabolites or impairment of the barrier functions of the digestive system (Culbertson & Osburn, 1980; Hrymak, 2015; Shcherba & Korda, 2019). It triggers activation of the immune response, alteration of quantitative and qualitative characteristics of cellular and humoral immunity, which may negatively affect the condition of the dam and the development of the fetus (Bomko et al., 2018; Broda et al., 2013; Denkovich et al., 2021; Slivinska et al., 2021; Klimkovetskaya et al., 2024).

The damaging effects of endogenous intoxication factors in pregnant cows are concentrated in three main directions: in the form of the arrest of metabolic processes due to the delayed elimination or excretion of metabolic end products; in the form of a switch of synthetic processes to the production of non-physiological compounds, up to the so-called "lethal synthesis," which leads to the appearance of highly toxic substances in the internal environment; and in the form of cellular membrane damage, which is the most harmful. In any case, modern concepts of the mechanism of endotoxin action on the body of pregnant cows are based on the leading role of the immune system (Zaviriukha et al., 2009; Kraievskyi, 2000; Lozynskyi et al., 2023; Chabanenko et al., 2024).

It is believed that all forms of harmful effects of endotoxins on the organs and systems of the whole organism are realized in the specific response to the primary damaging action of these substances. Such a response not only limits but also amplifies both the harmful effects of these substances and their penetration into the internal environment and can be defined as endotoxicosis (Boosman et al., 1991; Clark et al., 1991; Borshch et al., 2020; 2021).

Thus, the study of the dynamics of immune system indicators in cows at different stages of pregnancy in the presence of signs of endotoxicosis has important theoretical and practical significance. Understanding the nature of changes in the cellular and humoral branches of immunity will enable timely diagnosis of the initial manifestations of immune dysfunction, as well as the development of effective preventive and therapeutic measures aimed at preserving cow health and improving the reproductive efficiency of the herd.

Objective of the study

The aim of this work was to investigate the dynamics of immune system indicators in cows determined by the course of pregnancy with signs of endotoxicosis.

Materials and Methods

The study was conducted on Ukrainian Black-and-White Dairy breed cows. To achieve the objectives, two groups of animals were formed, each consisting of 10 pregnant cows: control and experimental. The cows of the experimental group exhibited clinical signs of endotoxicosis, such as congestive edema of the external genital organs, udder edema, mucosal anemia, depression, appetite disorders, and functional disturbances of the forestomachs and intestines. The control group consisted of healthy cows with a physiological course of pregnancy.

During the study, the rules mandatory for zootechnical experiments were observed, including the selection and maintenance of animal analogues in groups, as well as the technology of feed preparation, use, and recording of consumed feed. The animals' diet was balanced in nutrients and minerals to fully meet their nutritional requirements.

Blood samples for analysis were collected from the jugular vein at the 8th and 9th months of pregnancy. Neutrophil phagocytic activity (PA) and phagocytic index (PI) were determined using generally recognized methods in the modification of V. E. Chumachenko et al. The total number of T-lymphocytes (E-RFC) was determined by the method of spontaneous rosette formation with sheep erythrocytes according to M. Jondal et al. and the total number of B-lymphocytes was determined according to N. F. Mendes et al. (Vlizlo, 2012).

The research results were subjected to biometric analysis using mathematical statistics methods accepted in biology and medicine, using the Microsoft Excel program ("Statistica 5.0", "Bio-stat"). The degree of significance compared to the control group's data was determined – $P < 0.05$ – *, $P < 0.01$ – **, $P < 0.001$ – ***.

Results and Discussion

Immunity is a set of the body's defense mechanisms aimed at maintaining its genetic stability. It helps the organism resist various foreign factors, such as bacteria, viruses, toxins, and foreign bodies (Martyshuk & Hutyi, 2021; Kuljaba et al., 2022; Gutyj et al., 2023).

It is known that humoral immunity is ensured by specific macromolecules that function in the internal fluids of animals. Blood plasma contains special proteins capable of neutralizing microorganisms and the toxic products of their vital activity that enter the body's fluids.

The bactericidal activity of blood serum (BABS) is an integral factor of humoral natural resistance, reflecting the ability of blood to self-purify. This activity is determined by the presence of a complex of substances in the serum–complement, antibodies, lysozyme, and properdin–capable of neutralizing or destroying microbial cells.

As shown in Table 1, the bactericidal activity of blood serum in pregnant cows differed somewhat between the control and experimental groups. At the 8th month of pregnancy, BABS in the control group (cows with a physiological course of pregnancy) was 90.60 ± 2.80 %, whereas in pregnant cows with signs of endotoxicosis it was 80.32 ± 2.75 %. The lowest BABS values were observed in the experimental group at the 9th month of pregnancy, where it decreased by 18.7 % compared to the control group.

The lysozyme activity of blood serum (LABS) in cows with a physiological course of pregnancy at the 9th month was slightly lower than at the 8th month. In cows of the experimental group, a decrease in LABS was noted at both the 8th and 9th months of pregnancy, where compared to the control group, LABS decreased by 22.0 % and 20.2 %, respectively (Table 1).

Table 1
Indicators of humoral immunity in cows with a physiological course of pregnancy and in cows with the development of endotoxicosis, M ± m, n = 10

Months of pregnancy	Animal groups	LABS, %	BABS, %
8	C	28.82 ± 0.85	90.60 ± 2.80
	E	22.47 ± 0.75***	80.32 ± 2.75*
9	C	24.43 ± 0.80	85.23 ± 2.96
	E	19.50 ± 0.82***	69.27 ± 2.45***

Note: C – control group (physiological pregnancy); E – experimental group (endotoxicosis); LABS – lysozyme activity of blood serum; BABS – bactericidal activity of blood serum; *, *** – statistically significant differences compared to control (*P < 0.05, ***P < 0.001)

Thus, when studying the values of humoral immunity indicators, it was established that in cows affected by endotoxicosis, both the bactericidal and lysozyme activity of blood serum decreased.

Subsequently, we investigated nonspecific immunity–the immune defense system not associated with antigens and antibodies, which includes phagocytosis and overall nonspecific resistance. Along with the reduction in humoral immunity activity in cows showing signs of endotoxicosis, suppression of the nonspecific immune system was also found, manifested by decreased neutrophil phagocytic activity and a reduced phagocytic index.

It was established that at the 8th and 9th months of pregnancy, neutrophil phagocytic activity and the phagocytic index in the blood of the control group animals were within 63.90 ± 2.10–62.13 ± 2.25 % and 5.67 ± 0.15–7.82 ± 0.20 units, respectively. In pregnant cows with the development of endotoxicosis during the indicated study periods, neutrophil phagocytic activity was reduced by 7.2 % and 7.9 %, and the phagocytic index by 25.7 % and 25.3 %, compared with the control values (Table 2).

Thus, the obtained research results indicate that in cows with clinical signs of endotoxicosis, the nonspecific components of the immune system are suppressed.

The indicators of cellular immunity in cows with a physiological course of pregnancy and in cows with the development of endotoxicosis are presented in Table 3. It was established that the number of T-lymphocytes in the blood of cows in the control group at the 8th month of pregnancy was 47.52 ± 2.10 %. By the 9th month of pregnancy, the number of T-lymphocytes in the blood of this group of animals had slightly increased, rising by 13.2 % compared to the previous values.

Table 2
Indicators of nonspecific immunity in cows with a physiological course of pregnancy and in cows with the development of endotoxicosis (M ± m, n = 10)

Months of pregnancy	Animal groups	Phagocytic activity, %	Phagocytic index, units
8	C	63.90 ± 2.10	5.67 ± 0.15
	E	59.34 ± 2.12*	4.21 ± 0.19***
9	C	62.13 ± 2.25	7.82 ± 0.20
	E	57.12 ± 2.18*	5.84 ± 0.16***

Note: C – control group (physiological pregnancy); E – experimental group (endotoxicosis); *, *** – statistically significant differences compared to control (*P < 0.05, ***P < 0.001)

Table 3
Indicators of cellular immunity in cows with a physiological course of pregnancy and in cows with the development of endotoxicosis (M ± m, n = 10)

Months of pregnancy	Animal groups	T-lymphocytes, %	B-lymphocytes, %
8	C	47.52 ± 2.10	17.40 ± 0.85
	E	45.12 ± 2.35	16.92 ± 0.74
9	C	53.81 ± 2.44	22.01 ± 0.95
	E	41.32 ± 2.25**	17.85 ± 0.80**

Note: C – control group (physiological pregnancy); E – experimental group (endotoxicosis); **, – statistically significant differences compared to control (**P < 0.01)

In the blood of cows with clinical signs of endotoxicosis, a lower number of T-lymphocytes was observed throughout the entire study. The lowest number of T-lymphocytes in the blood of the experimental group of pregnant cows was recorded in the 9th month of pregnancy, where it decreased by 23.2 % compared to the control.

Similar changes were observed in the determination of B-lymphocyte counts. The study of cellular immunity indicators revealed that in pregnant cows affected by endotoxicosis, the number of B-lymphocytes was lower by 2.8 % and 18.9 % at the 8th and 9th months of pregnancy, respectively.

We explain the decrease in the number of T- and B-lymphocytes in cows with endotoxicosis by the action of toxins on the immune system of the affected animals. Thus, the reduction in lymphocyte counts indicates a

weakening of the resistance of pregnant cows during the development of endotoxicosis.

A critical moment in the immune response is the recognition of a chemical marker characteristic of a "foreign" agent, as opposed to a "self" one. This task is carried out by specific proteins that display remarkable diversity in molecular structure. The best-known recognition proteins are immunoglobulins. The most informative indicator of the immunological status of animals turned out to be the level of immunoglobulins in the blood serum of cows. The serum immunoglobulin levels of cows with a physiological course of pregnancy and those with the development of endotoxicosis are presented in Table 4.

The results of the study showed that the serum immunoglobulin level in cows with a physiological course of pregnancy was within 20.38 ± 1.28 mg/ml (8th month of pregnancy) and 19.46 ± 0.39 mg/ml (9th month of pregnancy). The decrease in immunoglobulin content in the blood serum of control group cows in the 9th month of pregnancy is explained by the need to provide calves with maternal antibodies, which accumulate in the mammary gland through colostrum.

The serum immunoglobulin level of diseased cows in the experimental group was slightly lower than that of the control group: it decreased by 16.0 % in the 8th month of pregnancy and by 24.6 % in the 9th month.

Table 4

Serum immunoglobulins of cows with a physiological course of pregnancy and cows with the development of endotoxicosis (M ± m, n = 10)

Months of pregnancy	Animal groups	Immunoglobulins, mg/ml	IgG, mg/ml	IgM, mg/ml	IgA, mg/ml
8	C	20.38 ± 1.28	17.85 ± 1.26	2.12 ± 0.05	0.41 ± 0.08
	E	17.12 ± 0.62*	14.80 ± 0.56*	2.01 ± 0.16	0.31 ± 0.03
9	C	19.46 ± 0.39	16.45 ± 0.54	2.49 ± 0.25	0.52 ± 0.12
	E	14.68 ± 0.36***	12.13 ± 0.41***	2.15 ± 0.23	0.40 ± 0.07

Note: C – control group (physiological pregnancy); E – experimental group (endotoxicosis); *, *** – statistically significant differences compared to control (*P < 0.05, ***P < 0.001).

Upon detailed analysis of immunoglobulins, it was found that in cows of the experimental group, the level of class G immunoglobulins significantly decreased in the 9th month of pregnancy, being 26.3 % lower compared to the control. Similar changes were observed in the study of class M and A immunoglobulins.

Conclusions

As a result of the conducted studies, we established that in pregnant cows with clinical manifestations of endotoxicosis, suppression of cellular, humoral, and nonspecific immunity occurs, leading to the development of secondary immunodeficiency. This is indicated by the decrease in bactericidal and lysozyme activity of blood serum, reduction in the number of T- and B-lymphocytes, decreased phagocytic activity and phagocytic index in the blood of diseased cows, as well as reduced levels of immunoglobulins.

Changes in immune system parameters serve as an important objective indicator of the condition of cows in the development of endotoxicosis of various etiologies.

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Conflict of interest

The authors declare that there is no conflict of interest.

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