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IMMUNOLOGICAL STATUS OF FETAL FLUIDS IN COWS OF DIFFERENT GENOTYPES OF THE BLACK-AND-WHITE BREED

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Abstract. *The immunobiological properties of allantoic and amniotic fluids in cattle at different stages of gestation (3–7 months), taking into account the animals' genotypes, were investigated. The aim of the study was to assess the levels of immunoglobulins G and M, lysozyme concentration and activity, as well as the bactericidal activity of the fetal fluids in order to elucidate the mechanisms of nonspecific immune protection of the fetus within the mother–fetus system. The experimental material consisted of four groups of pregnant Black-and-White cows, including hybrid genotypes with different proportions of Holstein blood. The concentrations of IgG, IgM, as well as lysozyme and bactericidal activity of the fetal fluids, were determined using standard generally accepted methods. The results demonstrated that IgG, IgM were absent or detected in trace amounts in the majority of fetal fluid samples, indicating limited activity of adaptive humoral immunity during the intrauterine period. The bactericidal activity of the fluids was low and inconsistent, whereas lysozyme remained a stable and dominant factor of nonspecific humoral defense. Its concentration in allantoic and amniotic fluids significantly exceeded the levels observed in the blood serum of both fetuses and dams, suggesting autonomous synthesis of this enzyme by fetal organs and immunocompetent cells. The highest lysozyme values were recorded at the fifth month of gestation, and a tendency toward increased enzyme levels was observed in animals with a higher proportion of Holstein blood, indicating a possible genetic determination of this parameter. The obtained data confirm the leading role of lysozyme in the formation of the primary immune barrier of the fetus and in maintaining physiological immune balance within the fetal environment. Within the framework of the One Health concept, these findings are of practical importance for reducing the risk of intrauterine infections, improving neonatal viability, decreasing the need for antimicrobial therapy, and ensuring the safety of livestock products. Therefore, the immunological properties of allantoic and amniotic fluids represent a key factor in normal fetal development and reproductive health in cattle.*

Keywords: cattle, intrauterine development, fetal fluids, IgG, IgM, lysozyme, bactericidal activity, nonspecific immunity, One Health concept

Immune reactions play a significant role in animal reproduction, since the fetus represents a kind of allotransplant for the maternal organism (Berry, 2011; Bigliardi, 2022; Maciag, 2022; Hemberg, 2023). A characteristic feature of these reactions is the predominance of a leading immune component at a certain stage of pregnancy, with clearly defined functions. Moreover, a permanent of the leading component occurs as pregnancy progresses, which ensures its normal course (Jobe, 2012; Horiuk, 2025). The dependence of prenatal development on the state of the maternal immune system is well known, as is the negative effect of immunodeficiency in the maternal organism on embryo survival (Fair, 2015).

It has also been suggested that the maternal immune response to embryonic antigens of the paternal haplotype is a necessary component of normal embryogenesis, determining growth, development, formation, and differentiation of embryonic tissues and organs. Thus, the immune system exerts an immunotrophic effect on the embryo (Aluvihare, 2004; Nancy, 2012). There is also evidence suggesting that alpha-1-fetoprotein, which appears during pregnancy, may influence fetal organogenesis (Mizejewski, 2004).

Rational livestock management is impossible without a deep understanding of the physiological processes occurring during pregnancy and fetal development. One of the key factors ensuring the normal course of intrauterine development is the condition of the fetal environment. Among the components of this environment, fetal (amniotic and allantoic) fluids play a particularly important role, performing not only mechanical and metabolic but also immunological functions.

Immunological parameters of fetal fluids reflect the interaction between the maternal organism and the fetus, as well as the state of fetal immune protection at different stages of gestation. Investigation of these parameters is important for the diagnosis of pregnancy pathologies, prediction of neonatal

viability, and optimization of housing and feeding conditions for pregnant animals in order to enhance reproductive performance.

Despite a considerable number of studies devoted to the general characteristics of amniotic and allantoic fluids, their immune status in different species of farm animals remains insufficiently studied. Of particular relevance is the investigation of changes in immunological parameters throughout the gestational period and under the influence of various external and internal factors.

In the context of the One Health concept, the results of such studies are of interdisciplinary importance, since intrauterine infections in farm animals represent a common link between animal health, farm biosecurity, and the safety of livestock products (Shaheen, 2022). The formation of effective fetal immune protection reduces the risk of weak offspring at birth, decreases the need for early antimicrobial therapy, and consequently lowers the risk of pathogen spread and the development of antimicrobial resistance. Therefore, the study of the immunological properties of amniotic and allantoic fluids can be considered an integral component of a preventive strategy consistent with the principles of One Health and of practical significance for veterinary medicine and the production of safe animal products.

The aim of our study was to investigate the immunological parameters of fetal fluids in pregnant Black-and-White cows of different genotypes at various stages of pregnancy, specifically the levels of immunoglobulins, lysozyme (lysozyme activity), and bactericidal activity, in order to gain a better understanding of immune interactions within the «mother–fetus» system.

Materials and methods. For the experiment, four groups of pregnant Black-and-White cows were formed according to the principle of analogs, with 15 animals in each group. In the first (control) group, fetuses of purebred Black-and-White cattle were obtained; in the second group, fetuses with a genotype of 5/8 Black-and-White (BW) × 3/8 Holstein (H); in the third group, 7/16 BW × 9/16 H; and in the fourth group, 5/16 BW × 11/16 H. Prior to slaughter, all animals were kept on a 24-hour fasting diet. Blood samples from pregnant cows were collected from the jugular vein. The fetuses together with the uteri were placed in a specially designed container that excluded the influence of external factors and were immediately transported by vehicle to the research laboratory. Before transportation, a ligature was applied to the cervix to prevent leakage of fetal fluids.

All manipulations with the uteri and fetuses were performed under laboratory box conditions. After opening the uterus, the cotyledons were carefully separated from the caruncles, removing the chorion that contained the fetus, fetal membranes, and fluids. A puncture was made in the allantochorion, and allantoic fluid was collected into a sterile syringe. Amniotic fluid was collected in a similar manner.

The concentrations of lysozyme and immunoglobulins G and M were determined in allantoic and amniotic fluids, and bactericidal and lysozyme activities were also assessed. The levels of immunoglobulins G and M were determined by the method of simple radial immunodiffusion using a commercial kit (Hussain, 2024), with agar plates containing antibodies against bovine immunoglobulins of the corresponding classes. Bactericidal activity of blood serum was determined according to Markov's method, and lysozyme activity was assessed using the diffusion method (de Souza, 2021).

All obtained experimental data were processed using methods of descriptive and inferential statistics with the help of Microsoft Excel and Statistica 10.0 software. Results are presented as mean values (M) and standard deviations (SD). To determine the significance of differences between group means, the Student's t-test was used for normally distributed data, while the Mann–Whitney U test was applied when the data distribution deviated from normality. Differences between groups were considered statistically significant at $p < 0.05$. In cases where $p < 0.01$, the differences were considered highly significant.

Results and Discussion. Immunobiological processes occurring in the bodies of animals during intrauterine development have a number of specific features. In particular, it has been suggested that the maternal immune response to embryonic antigens of paternal origin is an important component of normal embryonic development. This response may influence the growth, development, formation, and differentiation of embryonic tissues and organs, meaning that the immune system performs an immunotrophic function. In addition, there is evidence for the existence of so-called «enhancing antibodies» presumably maternal blocking immunoglobulins with high biological activity that may stimulate fetal development (Mor, 2010; Wang, 2024). In the context of the One Health concept, such mechanisms are of particular importance, as effective immune regulation within the mother–fetus system reduces the risk of intrauterine infections, the development of immunodeficiency states in newborns, and the subsequent need for antimicrobial therapy, which is a key factor in the prevention of antimicrobial resistance in livestock production (Jutel, 2023).

Section 3

As established in our study, the vast majority of allantoic fluid samples from cows throughout the investigated period of pregnancy did not contain immunoglobulins G and M, and when present, they were detected in negligible amounts (Table 1). The absence or low levels of immunoglobulins in fetal fluids indicate limited involvement of the adaptive humoral immune response and may be considered a physiological mechanism for preventing immune conflict between the maternal organism and the fetus. Such an immune strategy ensures gestational stability and the development of viable offspring.

Table 1

Immunological parameters of allantoic fluid in cows of different genotypes of black-and-white cattle (M±SD, n=35)

Indicator	Animal groups (n=5 in each group for each age period)				Average across groups II–IV in each gestation period (n = 15)
	I	II	III	IV	
3 months					
Bactericidal Activity, %	-	17.97±2.14 (3)	15.67±2.20 (3)	6.36 (1)	15.32±1.97 (7)
Lysozyme, µg/mL	20.20±0.86	22.50±0.83	21.10±1.47	22.60±1.34	22.07±0.62
Lysozyme Activity, units	410.06±17.43	456.75±16.83	428.33±29.90	458.78±27.19	447.95±12.65
IgG, mg/mL	-	-	-	-	-
IgM, mg/mL	-	-	-	-	-
5 months					
Bactericidal Activity, %	-	17.02±2.56(4)	4.69 (1)	-	14.55±3.31(5)
Lysozyme, µg/mL	23.10±1.61	23.70±1.45	24.30±1.56	26.40±0.67	24.80±0.69
Lysozyme Activity, units	468.93±32.58	481.11±29.51	493.29±31.61	535.92±13.62	503.44±14.01
IgG, mg/mL	-	0.01(1)	-	0.03(1)	0.02±0.01(2)
IgM, mg/mL	-	-	0.05(1)	0.05(1)	0.05(2)
7 months					
Bactericidal Activity, %	9.71±0.06(2)	11.74±1.36(2)	9.83±5.15(2)	8.58(1)	10.34±1.50(5)
Lysozyme, µg/mL	21.70±1.13	22.10±1.83	24.20±1.29	22.60±1.80	22.97±0.83
Lysozyme Activity, units	440.51±22.87	448.63±37.19	491.26±26.27	458.78±36.49	466.22±16.84
IgG, mg/mL	-	-	-	0.03(1)	0.03(1)
IgM, mg/mL	-	0.03(1)	-	0.05(1)	0.04±0.02(2)

Note. The number of positive samples is indicated in parentheses

IgG and IgM were detected in 3 and 4 out of 45 samples (6.67% and 8.89%, respectively) in animals of the experimental groups and were completely absent in cows of the control group. The bactericidal activity of allantoic fluid in cows of group I was absent at 3 and 5 months of gestation, while the average across groups II–IV was generally low, detected in 46.67% and 33.33% of samples, respectively. Notably, this parameter exhibited high variability in group II (Cv = 24.97%) and in the average across groups II–IV (Cv = 45.55%) at 5 months of gestation. At 7 months of gestation, the aforementioned parameter was relatively low, reaching its maximum in cows of experimental group II (11.74%) and minimum in group IV (8.58%). Overall, in cows of the control group at 3, 5, and 7 months of gestation, only 2 out of 15 allantoic fluid samples were positive for bactericidal activity (13.33%), whereas in cows of the experimental groups, 17 out of 45 samples were positive (37.78%).

The low and inconsistent bactericidal activity of the allantoic fluid indicates that its immune function is realized not through direct microbial elimination, but by maintaining immune homeostasis and controlling microbial load, which is characteristic of the physiological course of pregnancy (Shaheen, 2022).

Allantoic fluid in cows of all groups from 3 to 7 months of gestation contained exceptionally high amounts of lysozyme and, accordingly, exhibited high lysozyme activity. At 3, 5, and 7 months of gestation, the lysozyme content in group I cows was lower compared to group II by 11.39%, 2.60%,

and 1.84%; compared to group III by 4.45%, 5.20%, and 11.52%; compared to group IV by 11.88%, 14.29%, and 4.15%; and compared to the average of groups II–IV by 9.26%, 7.36%, and 5.85%, respectively. Thus, only a slight tendency toward increased lysozyme levels was observed in experimental groups whose fetuses had a certain proportion of Holstein blood in their genotype, and this difference was not statistically significant. The dynamics of lysozyme activity in allantoic fluid at 3 months of gestation followed a similar pattern. The predominance of lysozyme as the main immune factor in fetal fluids indicates the leading role of nonspecific natural resistance mechanisms in fetal protection. This type of immune defense is evolutionarily advantageous, as it provides antimicrobial action without triggering an excessive inflammatory response (Shaheen, 2022; Jutel, 2023).

The highest lysozyme concentrations in maternal allantoic fluid across all groups were observed at 5 months of gestation. At this time, the levels exceeded those in group I at 3 months by 1.14-fold (14.36%), group II by 1.05-fold (5.33%), group III by 1.15-fold (15.17%), group IV by 1.17-fold (16.81%), and the average across groups II–IV by 1.12-fold (12.37%). At 7 months of gestation, these values were 1.06 (6.45%), 1.07 (7.24%), 1.004 (0.41%), 1.17 (16.81%), and 1.08-fold (7.97%), respectively.

The lysozyme content in allantoic fluid at 3 months of gestation was higher than that in the blood serum of both the cows and their fetuses: in group I by 2.36- and 11.48-fold, group II by 2.63- and 10.71-fold, group III by 2.34- and 10.60-fold, group IV by 2.33- and 10.97-fold, and on average across groups II–IV by 2.43- and 10.77-fold. At 5 months of gestation, the corresponding values were 2.58- and 11.00-fold; 2.24- and 9.18-fold; 2.13- and 8.56-fold; 2.69- and 9.30-fold; 2.46- and 9.15-fold, and at 7 months of gestation, 1.81- and 12.12-fold; 1.74- and 9.82-fold; 1.94- and 12.39-fold; 1.98- and 9.89-fold; 1.88- and 10.61-fold, respectively. Differences in this parameter across all age groups were statistically significant ($P < 0.05$).

The significantly higher level of lysozyme in fetal fluids compared to its concentration in the serum of mothers and fetuses indicates local synthesis of this enzyme and its key role in establishing the primary immune barrier of the fetus. From 3 to 7 months of gestation, amniotic fluid in cows of the control group did not contain immunoglobulins G or M. In cows of groups II, III, and IV, and in the average across these groups, IgG and IgM were detected in negligible amounts, corresponding to 6.67% and 6.67%; 0% and 6.67%; 13.33% and 13.33%; 6.67% and 8.89% of samples, respectively (Table 2).

In amniotic fluid of group I cows at 3 months of gestation, bactericidal activity was absent, while in cows of the experimental groups it was detected in almost half of the samples (46.67%). However, its activity was low, and the variability of this parameter, especially in group V cows (average across groups II–IV), was relatively high ($C_v = 28.91\%$). Bactericidal activity in amniotic fluid at 5 months of gestation also remained low, with a high coefficient of variation, and was not consistently detected in all experimental animals. At 7 months of gestation, amniotic fluid exhibited low bactericidal activity in 40% of samples from cows in groups I, II, and III, in 20% of samples from group IV, and in 33.33% of samples on average across groups II–IV (Ukrainian Black-and-White dairy breed). Thus, throughout gestation, bactericidal activity in most amniotic fluid samples from all groups was absent.

Regarding lysozyme activity, amniotic fluid in cows of the experimental groups showed a slight, statistically non-significant advantage over the control group throughout the studied gestational period. At 3 months, this advantage was 9.13% in group II, 1.44% in group III, 10.10% in group IV, and 6.89% on average across groups II–IV. At 5 months, the corresponding values were 4.80%, 7.42%, 10.48%, and 7.55%, and at 7 months, 3.23%, 12.44%, 3.96%, and 6.54%, respectively. The lysozyme content in amniotic fluid at 3 months of gestation was significantly higher ($P < 0.001$) than in the blood serum of both the cows and their fetuses: in group I by 2.43- and 11.80-fold, group II by 2.65- and 10.81-fold, group III by 2.34- and 10.60-fold, group IV by 2.36- and 11.12-fold, and on average across groups II–IV by 2.45- and 10.84-fold. At 5 months, these values were 2.56- and 10.90-fold, 2.27- and 9.80-fold, 2.48- and 8.66-fold, 2.58- and 8.91-fold, and 2.44- and 9.09-fold, and at 7 months, 1.81- and 12.12-fold, 1.74- and 9.82-fold, 1.94- and 12.39-fold, 1.98- and 9.89-fold, and 1.88- and 10.61-fold, respectively.

Cows at 5 months of gestation had higher lysozyme levels in amniotic fluid compared to 3-month samples: group I by 1.10-fold (10.10%), group II by 1.06 (5.73%), group III by 1.17 (16.59%), group IV by 1.10 (10.48%), and the average across groups II–IV by 1.11-fold (10.80%). At 7 months, the corresponding values were 1.06 (5.53%), 1.07 (7.14%), 1.01 (0.82%), 1.12 (12.15%), and 1.07-fold (6.53%), respectively. Thus, from 3 to 5 months of gestation, amniotic fluid exhibited a gradual increase in lysozyme content and activity, followed by a slight decrease by the 7th month.

Immunological parameters of amniotic fluid in cows of different genotypes of black-and-white cattle (M±SD, n=35)

Indicator	Animal groups (n=5 in each group for each age period)				Average across groups II–IV in each gestation period (n = 15)
	I	II	III	IV	
3 months					
Bactericidal Activity, %	-	17.44±1.58 (3)	15.56±2.11 (3)	6.75 (1)	15.11±1.78 (7)
Lysozyme, µg/mL	20.80±0.65	22.70±0.89	21.10±1.47	22.90±1.23	22.23±0.62
Lysozyme Activity, units	422.24±13.23	460.81±18.16	428.33±29.90	464.87±24.97	451.34±12.65
IgG, mg/mL	-	-	-	-	-
IgM, mg/mL	-	-	-	-	-
5 months					
Bactericidal Activity, %	-	17.02±2.56(4)	4.69 (1)	-	14.55±3.31(5)
Lysozyme, µg/mL	23.10±1.61	23.70±1.45	24.30±1.56	26.40±0.67	24.80±0.69
Lysozyme Activity, units	468.93±32.58	481.11±29.51	493.29±31.61	535.92±13.62	503.44±14.01
IgG, mg/mL	-	0.01(1)	-	0.03(1)	0.02±0.01(2)
IgM, mg/mL	-	-	0.05(1)	0.05(1)	0.05(2)
7 months					
Bactericidal Activity, %	9.71±0.06(2)	11.74±1.36(2)	9.83±5.15(2)	8.58(1)	10.34±1.50(5)
Lysozyme, µg/mL	21.70±1.13	22.10±1.83	24.20±1.29	22.60±1.80	22.97±0.83
Lysozyme Activity, units	440.51±22.87	448.63±37.19	491.26±26.27	458.78±36.49	466.22±16.84
IgG, mg/mL	-	-	-	0.03(1)	0.03(1)
IgM, mg/mL	-	0.03(1)	-	0.05(1)	0.04±0.02(2)

Note: The number of positive samples is indicated in parentheses

It is known (Essawi, 2020; Tal, 2022; Pacheco, 2026) that fetal fluids contain humoral factors of natural resistance, including lysozyme and complement. Moreover, lysozyme concentrations in allantoic and amniotic fluids significantly exceed those in the serum of both fetuses and dams. The statistical correlation between enzyme activity in fetal fluids and fetal serum indicates its independent synthesis by the fetal organism and temporary embryonic organs, particularly since lysozyme is synthesized by macrophages and microphages, whose presence and function have been confirmed in fetal fluids.

Analysis of the obtained results indicates that between 3 and 7 months of gestation, bactericidal activity in fetal fluids was not consistently observed and, when present, was at a relatively low level. The vast majority of fetal fluid samples lacked immunoglobulins G and M. In practice, allantoic and amniotic fluids consistently contained only lysozyme. Lysozyme concentrations in these fluids were substantially higher than in the serum of both fetuses and their dams. Lysozyme content and activity in allantoic and amniotic fluids largely coincided. Regardless of gestational age, a tendency was observed for higher lysozyme levels in fetal fluids of cows whose fetuses had an increasing proportion of Holstein blood in their genotype. The results obtained indicate a similarity in the immune defense mechanisms of amniotic and allantoic fluids, emphasizing their coordinated role in ensuring the immunological safety of the intrauterine environment. From the perspective of the One Health concept, the identified features of the immune status of fetal fluids have practical significance, as they contribute to reducing the risk of intrauterine infections, improving the viability of newborns, and decreasing the need for antimicrobial therapy in livestock, which is directly related to the issues of antibiotic resistance and the safety of animal-derived food products.

Conclusion. Immune factors in allantoic and amniotic fluids throughout gestation in cows were inconsistent and generally at low levels. Only lysozyme and its high activity were consistently detected in these fluids, with peak values observed during the 5th month of gestation. The levels of these indicators in allantoic and amniotic fluids did not differ significantly, indicating their similar role in shaping the fetal immune environment.

In terms of lysozyme content, allantoic and amniotic fluids had a significant advantage over the serum of both the fetuses and their mothers during all studied periods of gestation — by 1.74–2.69 and 8.56–12.39 times, and by 1.74–2.65 and 8.66–12.72 times, respectively. This highlights the leading role of fetal fluids in providing nonspecific immune protection to the fetus and maintaining physiological immune balance within the mother–fetus system, which is crucial for normal intrauterine development and overall reproductive health of the animals.

Ethical consideration. Plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been checked by the authors.

Use of AI. During the preparation of this manuscript, artificial intelligence tools, specifically ChatGPT, were used. Their application was limited to auxiliary editorial tasks, including stylistic refinement of individual sentences and transitions between paragraphs, improvement of readability and logical flow, and clarification of wording. All changes were fully reviewed and verified by the authors, and the use of AI did not affect the scientific content of the study.

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ІМУНОЛОГІЧНИЙ СТАТУС НАВКОЛОПЛІДНИХ РІДИН У КОРІВ РІЗНИХ ГЕНОТИПІВ ЧОРНО-РЯБОЇ ПОРОДИ

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Резюме. Досліджено імунобіологічні властивості алантоїсної та амніотичної рідин великої рогатої худоби на різних стадіях тільності (3–7 місяців) з урахуванням генотипу тварин. Мета роботи полягала у вивченні вмісту імуноглобулінів класів G та M, рівня лізоциму та його активності, а також бактерицидної активності навколоплідних рідин для оцінки механізмів неспецифічного імунного захисту плода у системі «мати–плід». Вибірка складалася з чотирьох груп тільних корів чорно-рябої породи, включно з гібридними генотипами з різною часткою голштинської крові. Рівні імуноглобулінів класів G і M, лізоциму та бактерицидну активність навколоплідних рідин визначали за стандартними загальноприйнятими методиками. Результати показали, що імуноглобуліни G та M у більшості проб навколоплідних рідин були відсутні або виявлялися у мізерних кількостях, що свідчить про обмежену активність адаптивного гуморального імунітету у внутрішньоутробний період. Бактерицидна активність рідин була низькою та непостійною, тоді як лізоцим залишався стабільним та домінуючим фактором неспецифічного гуморального захисту. Його рівень в алантоїсній та амніотичній рідинах значно перевищував показники у сироватці крові плодів і матерів, що вказує на автономний синтез ферменту плодовими органами та імунокомпетентними клітинами. Найвищі значення лізоциму спостерігали на 5 місяці тільності, а вміст ферменту мав тенденцію до зростання у тварин з більшим вкладом голштинської крові, що свідчить про можливу генетичну детермінованість. Отримані дані підтверджують провідну роль лізоциму у формуванні первинного імунного бар'єру плода та забезпеченні фізіологічної імунної рівноваги навколоплідного середовища. У контексті концепції «Єдине здоров'я» це має практичне значення для зниження ризику внутрішньоутробних інфекцій, підвищення життєздатності новонароджених, зменшення потреби в антимікробній терапії та підтримання безпечності продукції тваринництва. Отже, імунологічні властивості алантоїсної та амніотичної рідин є ключовим чинником нормального розвитку плода та репродуктивного здоров'я великої рогатої худоби.

Ключові слова: велика рогата худоба, внутрішньоутробний розвиток, навколоплідні рідини, IgG, IgM, лізоцим, бактерицидна активність, неспецифічний імунітет, концепція «Єдине здоров'я»

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