

# Characteristics of collagenogenesis in the blood-thymus barrier of macrosomic fetuses

I. V. Sorokina<sup>id</sup>A,B,C,D, P. S. Talapova<sup>id</sup>C,D, G. I. Gubina-Vakulik<sup>id</sup>B,E, O. M. Pliten<sup>id</sup>E,F,  
O. V. Kaluzhyna-Biletska<sup>id</sup>D,E

Kharkiv National Medical University, Ukraine

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article;  
E – critical revision of the article; F – final approval of the article

## Keywords:

pregnancy, fetus, maternal-fetal exchange, fetal macrosomia, thymus, collagen, blood-thymus barrier.

## Pathologia.

2026;23(1):42-48

**Aim:** the primary objective of this study was to improve the pathomorphological diagnosis of macrosomia in stillborn fetuses. This was achieved by analyzing the characteristics of collagen synthesis in the structural components of the blood-thymus barrier in macrosomic stillborn fetuses and relating these findings to specific anthropometric parameters.

**Material and methods.** Our study examined 34 thymus specimens from macrosomic stillborn fetuses, which we divided into three groups (SF1, SF2, SF3) based on their Height and Weight Index (HWI). Group of medium-weight fetuses, randomly selected as the control group (SFc) (n = 9). Research methods: histological, immunohistochemical, morphometric. Statistical analysis was performed in accordance with the current methodological requirements.

**Results.** In endothelial basement membranes the synthesis of type IV collagen was significantly reduced in macrosomic fetuses of the SF1 group ( $p \leq 0.05$ ), with a tendency to increase in macrosomes of the SF3 group. In epithelial basement membranes, there was a significant increase in the production of type IV collagen in the thymus of SF3 group ( $p \leq 0.05$ ) and a tendency toward a decrease in the expression of this collagen in SF1 group of macrosomes. Compared to the control, in the reticular fibers and amorphous substance of the blood-thymus barrier the optical density of the luminescence of type III collagen was maximal in observations of the SF3 group and minimal in large fetuses of the SF1 group.

**Conclusions.** In the structure of the blood-thymus barrier of large fetuses, the expression of type IV collagen was detected in the composition of the epithelial and endothelial basement membranes. Vascular basement membranes contained a greater amount of type IV collagen compared to the epithelial basement membranes of blood-thymus barrier, depending on the anthropometric characteristics of the fetuses. In the reticular fibers and amorphous substance of the blood-thymus barrier of stillborns, irrespective of their anthropometric characteristics, type III interstitial collagen was detected.

## Ключові слова:

вагітність, плід, макросомія, тимус, колаген, гемато-тимусний бар'єр.

## Патологія. 2026.

Т. 23, № 1(66).  
С. 42-48

## Особливості колагеноутворення у гемато-тимусному бар'єрі при макросомії

I. В. Сорокіна, П. С. Талапова, Г. І. Губіна-Вакулик, О. М. Плітень, О. В. Калужина-Білецька

**Мета роботи** – покращити патоморфологічну діагностику макросомії у мертвонароджених; проаналізувати характеристики синтезу колагену в структурних компонентах гемато-тимусного бар'єра у великих мертвонароджених плодів, ці результати співвіднести зі специфічними антропометричними параметрами.

**Матеріали і методи.** Досліджено 34 зразки тимуса від макросомних мертвонароджених плодів, які поділили на три групи (SF1, SF2, SF3) залежно від індексу зросту та ваги (HWI). Групу плодів середньої ваги випадково обрано як контрольну (SFc) (n = 9). Методи дослідження: гістологічний, імуногістохімічний, морфометричний. Статистичні дані опрацьовано відповідно до чинних вимог.

**Результати.** В ендотеліальних базальних мембранах синтез колагену IV типу значно знижений у макросомних плодів групи SF1 ( $p \leq 0,05$ ) з тенденцією до збільшення у макросомних плодів групи SF3. В епітеліальних базальних мембранах виявлено значне збільшення продукції колагену IV типу в тимусах групи SF3 ( $p \leq 0,05$ ) та тенденцію до зниження експресії цього колагену в макросомних плодів групи SF1. Порівняно з контролем, у ретикулярних волокнах та аморфній речовині гемато-тимусного бар'єра оптична щільність люмінесценції колагену III типу максимальна у спостереженнях групи SF3 та мінімальна у великих плодів групи SF1.

**Висновки.** У структурі гемато-тимусного бар'єра великих плодів виявлено експресію колагену IV типу у складі епітеліальної та ендотеліальної базальних мембран. Судинні базальні мембрани містять більшу кількість колагену IV типу порівняно з епітеліальними базальними мембранами гемато-тимусного бар'єра. Це залежить від їхніх антропометричних характеристик. У ретикулярних волокнах та аморфній речовині гемато-тимусного бар'єра мертвонароджених, незалежно від антропометричних характеристик, виявлено інтерстиціальний колаген III типу.

Perinatal diagnosis plays an important role in ensuring the health of the mother and child. In recent years, technological progress has significantly enriched its arsenal of tools for diagnosing conditions and anomalies in the prenatal period. Nevertheless, as long as humanity exists, there are and will be many ways to improve perinatal diagnostics.

Pregnancy is a multifaceted biological phenomenon that requires the synchronized functioning of numerous physiological systems to guarantee the proper growth and development of the fetus. However, if the pregnancy is complicated by macrosomia, or a large fetus (LF), the risk of morbidity and mortality for both mother and child increases. However, the molecular mechanisms responsible for excessive fetal development are remain insufficiently understood.

LF is diagnosed in cases where the estimated weight of the fetus exceeds 4000 g or the 90th percentile according to gestational age or the weight of the child at birth is more than 4500 g [1]. According to studies published by M. Najafian et al., births with a fetus with a body weight of 4.0–4.5 kg are registered in 7.6 % of cases, children with a body weight of 4.5–5.0 kg – in 1.2 % of cases, and births with a giant fetus – with a body weight of more than 5.0 kg – is noted in 0.2 % of cases [2]. This problem is relevant, since the global prevalence of macrosomia is from 5 % to 20 % in developed countries and 0.5–15.0 % in less developed countries [3,4], while various factors can contribute to the birth of LF, such as maternal obesity, gestational diabetes, maternal age, multiple pregnancies in the family history [5].

Given the substantial incidence of macrosomia in newborns, researchers are increasingly focusing on its potential health implications for both the infant and the mother. Data from literary sources indicate an increased risk of pregnancy complications associated with LF, in particular preeclampsia, which is one of the most common causes of maternal and perinatal mortality. The prevalence of preeclampsia ranges from 2 % to 8 % and may be associated with the prevalence of dietary and constitutional obesity among mothers with macrosomia [5,6,7,8]. However, for substantiating these assertions, we advocate for the execution of comprehensive, large-scale observational studies to thoroughly investigate the incidence of macrosomia and comorbidities in newborns.

Undoubtedly, of great medical importance and concern are the well-known complications of LF births, such as birth trauma and fetal distress. Studies indicate that babies with macrosomia are at an increased risk of experiencing shoulder dystocia. This condition can result in the brachial plexus injury and potentially lead to both intra- and postnatal asphyxia, adversely affecting the nervous system [9,10].

In addition, childbirth complications can have far-reaching consequences for the child's immune system. Clinical observations suggest a link between fetal macrosomia and an elevated risk of immune system disorders, potentially resulting in impaired immune function in newborns. Studies also indicate that fetal weight can affect the development of the thymus, spleen, and lymph nodes, which can lead to a decrease in T-lymphocyte production and a decrease in the activity of natural killer

cells (NK-cells) [11,12]. In addition, a large fetus may have an altered cytokine profile, leading to changes in the inflammatory response and activation of innate immune cells in the fetal body [13]. However, the morphofunctional state of the central and peripheral organs of the immune system of the LF remains insufficiently studied. All this emphasizes the need for additional research to understand the relationship between fetal macrosomia and the development of the immune system.

In the early stages of human development, the thymus plays a pivotal role in the establishment of immunity. This is due to its function in facilitating the antigen-independent proliferation and differentiation of a T-lymphocyte subpopulation, essential for cellular immunity. These T-lymphocytes subsequently populate the T-dependent zones of secondary lymphoid organs via hematogenous pathways [14].

The blood-thymus barrier (BTB) is a histochemically separation between the circulatory system and the thymus, is formed in the early stages of intrauterine development and plays a crucial role in the formation and functioning of the thymus. After all, it is BTB that creates the conditions for the maturation and differentiation of a unique population of cortical thymocytes (cortical thymic epithelial cells, or cTECs), which contribute to the development of the T-cell population of lymphocytes outside of contact with foreign antigens [15]. However, several studies have demonstrated that antigens circulating in the blood can enter the thymus via the transcapsular route and via transendothelial migration [16]. Impaired BTB function is associated with several immunological disorders, including thymic aplasia, autoimmune diseases, and impaired T-cell development [17].

Collagens play a key role in ensuring the stability of BTB function, and the process of their cross-linking and maturation is responsible for the strength of the barrier. Currently, it is known that type I collagen is the main component of the extracellular matrix of BTB, type III collagen is involved in the formation of fibrillar networks, and type IV collagen is the main component of the epithelial and endothelial basement membranes [18,19].

Earlier, scientists of the Kharkiv School of Pathologists, under the leadership of A. F. Yakovtsova, discovered significant morphofunctional changes in the central and peripheral organs of the immune system of macrosomic fetuses depending on their mass-growth index [20]. In this paper, we returned to the scientific task set by Antonina Fedorivna to establish the specific features of collagen formation in the structural components of the BTB of large stillborn fetuses depending on their anthropometric indicators in order to improve the quality of pathomorphological perinatal diagnostics.

## Aim

The primary objective of this study was to improve the pathomorphological diagnosis of macrosomia in stillborn fetuses. This was achieved by analyzing the characteristics of collagen synthesis in the structural components of the blood-thymus barrier in macrosomic stillborn fetuses and relating these findings to specific anthropometric parameters.

**Table 1.** Anthropometric features of the research groups

Group	Number of samples	Height average value, m	Weight average value, kg	HWI average value, kg/m <sup>3</sup>
SF1	7	0.59 ± 0.02	4.2 ± 0.04	20.48 ± 0.13
SF2	11	0.58 ± 0.06	4.7 ± 0.08	24.10 ± 0.09
SF3	16	0.54 ± 0.07	4.5 ± 0.04	28.66 ± 0.17
SFc	9	0.52 ± 0.04	3.3 ± 0.03	23.57 ± 0.12

**Table 2.** Density of cortical lymphocytes per square millimetre (1 mm<sup>2</sup>) in the thymuses of stillborn fetuses with different anthropometric parameters

Observation group	Number of observations	Density of lymphocytes in the cortex (units/mm <sup>2</sup> )
SFc	9	11321.2 ± 175.2
SF1	7	15670.4 ± 246.4*
SF2	11	10987.3 ± 199.0
SF3	16	9447.0 ± 181.6*

\*:  $p \leq 0.05$  compared to the control group.

## Materials and methods

For a more comprehensive understanding of the topic and to facilitate the comparison of data across different time periods, we conducted a retrospective study. This study involved archival material from 2 sources: 1) the Department of Pathological Anatomy at Kharkiv National Medical University where we utilized paraffin-embedded thymus samples of stillborn full-term fetuses, collected between 2000 and 2004, with varying body weights (specific number and percentage ratio needed); 2) we examined thymus preparations from stillborns (specific number and percentage ratio needed), with autopsies conducted at the communal healthcare facility "Kharkiv City Perinatal Center" between 2012 and 2019.

When obtaining research material, the involved specialists followed the "Rules of Ethical Principles of Conducting Scientific Medical Research with Human Participation", in accordance with the Declaration of Helsinki (1964–2013), EU Directive No. 609 (from November 24, 1986), orders of the Ministry of Health of Ukraine No. 690 dated September 23, 2009, No. 944 dated December 14, 2009, No. 616 dated August 3, 2012 and ICH GCP (1996). According to the decision of the Expert Committee on Ethics and Bioethics of Scientific Research at Kharkiv National Medical University, this study was conducted in compliance with international ethical requirements and adheres to the ethical norms of science and standards for biomedical research (Minutes of the Bioethics Committee Meeting: No. 9, dated November 5, 2025).

In total, our study examined 43 thymus specimens from macrosomic stillborn fetuses (SF) and analyzed the corresponding anthropometric records of these fetuses. The study participants were categorized based on their Height and Weight Index (HWI) and divided into four groups: SF1, consisting of low-weight macrosomic fetuses ( $n = 7$ ); SF2, a group of medium-weight macrosomic fetuses, designated as the study group ( $n = 11$ ); SF3, comprising heavy-weight macrosomic fetuses ( $n = 16$ ); and SFc, a group of medium-weight fetuses, randomly selected as the control group ( $n = 9$ ) (Table 1).

The thymus specimens were fixed in a 10 % solution of formalin, processed through alcohols of varying

concentrations, and then embedded in paraffin. Serial sections, each 4-5 micrometers ( $\mu\text{m}$ ) thick, were prepared for histological examination. The analysis followed standard procedures, including staining the samples with hematoxylin and eosin, picrofuchsin according to van Gieson, and Mallory's trichrome method. Observations were conducted using an Olympus BX-41 microscope. For collagen typing, monoclonal antibodies specific to collagen types I, III, and IV were applied (Novocastra Laboratories Ltd.), and the samples were examined under an Axioskop 40 fluorescence microscope. The optical density (OD) of the immunofluorescence for the collagens was measured using an Axioskop 40 microscope and Biostat.exe software.

Further study investigated the relationship between the expression of type IV collagen in the endothelial and epithelial basement membranes, type III collagen in reticular fibers and amorphous substance, thymocyte density, and the anthropometric parameters of different groups of SF. For statistical analysis and visualization, we utilized RStudio, employing packages such as "psych", "stats", "tidyverse" (inclusive of "dplyr", "ggplot2", "tidyr"), and "corrplot". Our analytical approach included descriptive statistics, tests for the normality of distribution, tests for the equality of means and variances, linear regression modeling, and the Student's t-test ( $p \leq 0.05$ ) to determine the statistical relationships between dependent and independent variables, as well as to evaluate statistical significance. Additionally, scatter plots and Pearson's correlation coefficient were employed to assess the strength and direction of the relationships between variables.

## Results

In all the examined thymuses, histological examination revealed the presence of post-capillary venules in the cortical zone. These venules were encircled by thin, fibrous, pyroninophilic connective tissue structures, within whose loops macrophages, fibroblasts, and lymphocytes were situated (Fig. 1).

The walls of the capillaries, along with the surrounding pericapillary reticular fibers, were distinctly visualized using Mallory's trichrome stain, which manifested as a blue color of varying intensities. Additionally, basal epithelial membranes and the epitheliocytes situated on them were observed in a perivascular arrangement.

Through morphometric analysis, quantitative data were gathered and analyzed to determine the density of cortical lymphocytes per square millimeter (1 mm<sup>2</sup>) in the thymuses of stillborn fetuses, taking into account their varying anthropometric data (Table 2).

The immunohistochemical examination, utilizing monoclonal antibodies selective for type IV collagen, precisely demarcated the basement membranes, integrating the structural components of both epithelial and vascular (endothelial) elements (Fig. 2).

Concurrently, type III collagen was evidenced by a pattern of linear and discontinuous fluorescence, exhibiting heterogeneous intensities along the reticular fibers and within the interstitial matrix, as illustrated in Fig. 3.

We investigated the expression of type IV and type III collagens in the thymuses of stillborn fetuses across

different weight categories. The analysis focused on the endothelial and epithelial basement membranes, and amorphous substance and reticular fibers of the thymus (Table 3).

The findings revealed a notable trend: type IV collagen expression was highest in the heavy-weight SF3 group, both in the endothelial ( $0.042 \pm 0.005$ ,  $p > 0.05$ ) and epithelial basement membranes ( $0.039 \pm 0.003$ ,  $p \leq 0.05$ ), with the latter showing a statistically significant increase. This contrasted with the lowest expression observed in the low-weight SF1 group, underscoring the correlation between increased collagen type IV expression and fetal macrosomia.

This pattern was mirrored in the type III collagen expression within the amorphous substance and reticular fibers, with the highest expression again in the SF3 group ( $0.020 \pm 0.004$ ), but with no statistically significant difference when compared with the control group. However, the SF1 group displayed a statistically significant decrease of the protein synthesis. The minimum content of type IV collagen in the vascular basement membranes was revealed in the SF1 group, as evidenced by a significant decrease in OD of the immunofluorescence of this collagen in the endothelial basement membranes ( $p \leq 0.05$ ) compared to the control group. On the other hand, in the SF2 group this indicator practically did not differ from the control level. Additionally, there is a tendency towards a synchronous decrease or increase in the optical density of type IV collagen in both epithelial and vascular basement as well as type III collagen.

To visualize the correlation between variables, we constructed a heat map showing the strength and direction of relationships between variables.

In Table 4, there are the relationships and correlation coefficients between the various variables that we analyzed.

## Discussion

Our findings indicate that an increase in height is generally associated with an increase in the OD of collagen type IV fluorescence in the epithelial basement membranes of the thymus. This observation aligns with the study by Marvin Paulo Lins, which reported similar trends in collagen distribution with respect to height [21]. Furthermore, our results also suggest that taller individuals tend to have a higher density of lymphocytes in the thymic cortex, potentially indicating a more robust thymic function, especially in the terms of adaptive immune system, that is confirmed by R. Sender et al. [22].

The study also revealed that an increase in body mass is likely to lead to a decrease in the OD of collagen type IV in the epithelial basement membranes of the thymus. This phenomenon could be attributed to the morphological and functional changes in the thymic tissue due to increased body mass, altered thymic loading, and mechanical stress, which may impact collagen distribution, as discussed in the findings of other authors [23,24]. However, higher body mass is typically associated with greater height, suggesting a complex interplay between body mass, height, and collagen distribution within the thymus. This relationship underscores the intricate dynamics of thymic

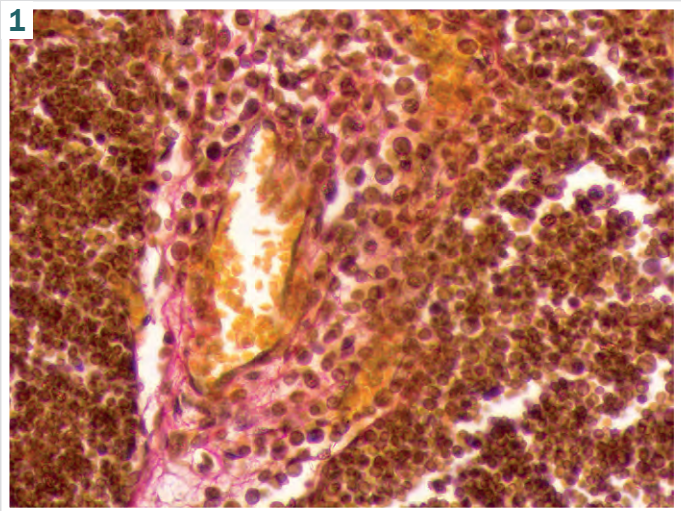


Fig. 1. Post-capillary venules and high thymocyte density in the cortical zone in the thymus of a large fetus (SF1). van Gieson method,  $\times 600$ .

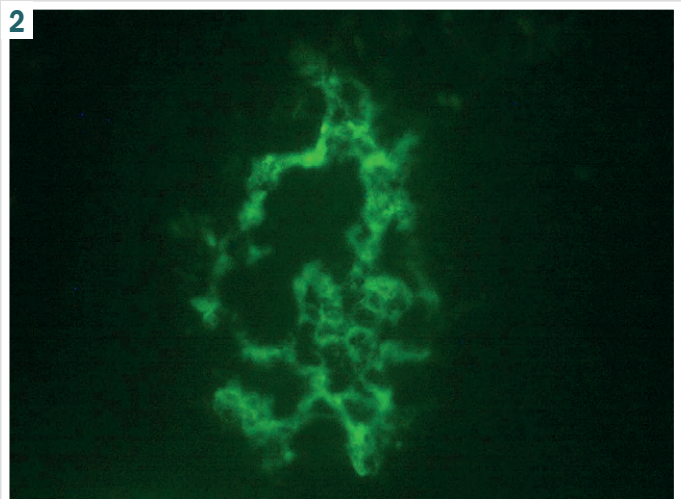


Fig. 2. Type IV collagen fluorescence in the epithelial endothelial basement membranes. Direct Koons method, MCA type IV collagen,  $\times 600$ .

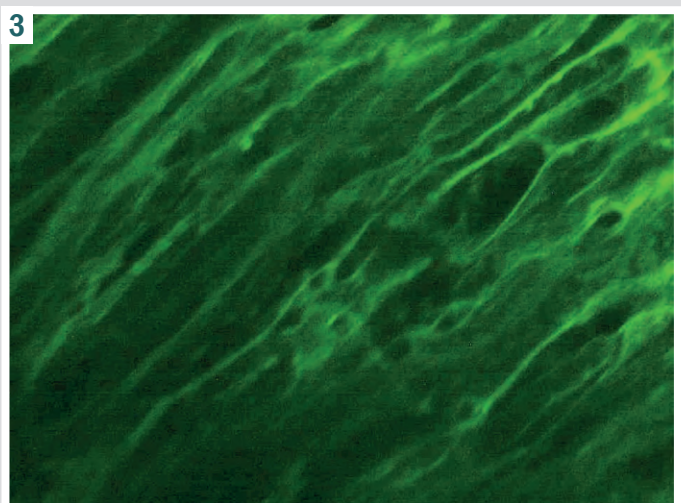


Fig. 3. Type III collagen fluorescence in the interstitial matrix. Direct Koons method, MCA type III collagen,  $\times 600$ .

**Table 3.** Average values of the OD (in conventional units of optical density) of the fluorescence of collagens in the structural elements of the BTB of stillborns with different anthropometric data

Group	Type IV collagen		Type III collagen
	Endothelial basement membranes	Epithelial basement membranes	Amorphous substance and reticular fibers
SFc	0.036 ± 0.004	0.022 ± 0.003	0.015 ± 0.005
SF1 (low-weight)	0.017 ± 0.007*	0.015 ± 0.008	0.009 ± 0.005*
SF2 (middle-weight)	0.034 ± 0.006	0.024 ± 0.005	0.014 ± 0.002
SF3 (heavy-weight)	0.042 ± 0.005	0.039 ± 0.003*	0.020 ± 0.004

\*:  $p \leq 0.05$  compared to the control group.

**Table 4.** Correlation analysis of anthropometric and collagen-related variables in the study

Variables	Correlation coefficient	Correlation type	Interpretation
Height and Col3	-0.27	weak negative	Taller individuals may have less type III collagen in their amorphous substance and reticular fibers
Col4epi_bm and Weight	-0.36	weak negative	Increase in mass may lead to a decrease in OD of Collagen type IV fluorescence in epithelial basement membranes
Col3 and CelDen	-0.87	very strong negative	Type III collagen's presence in amorphous substance and reticular fibers is inversely related to density of lymphocytes in the thymic cortex
Col4epi_bm and Height	0.25	weak positive	Increase in height may be accompanied by an increase in OD of collagen type IV fluorescence in epithelial basement membranes
Weight and CelDen	0.49	moderate positive	Larger individuals often have higher density of lymphocytes in the thymic cortex
Height and CelDen	0.51	moderate positive	Taller individuals tend to have higher density of lymphocytes in the thymic cortex
Height and Weight	0.83	strong positive	Higher body mass is typically associated with greater height
Col4end_bm and Col4epi_bm	0.99	very strong positive	Both variables reflect collagen protein mass and tend to increase or decrease together

Heatmap showing the strength and direction of relationships between variables: Height, Weight, CelDen – Density of lymphocytes in the thymic cortex (units/mm<sup>2</sup>); Col4end\_bm – OD of Collagen type IV fluorescence in Endothelial basement membranes, Col4epi\_bm – OD of Collagen type IV fluorescence in epithelial basement membranes, and Col3 – OD of Collagen type III fluorescence in the amorphous substance and reticular fibers.

A correlation matrix was calculated between the variables "Height", "Weight", "CelDen", "Col4end\_bm", "Col4epi\_bm" and "Col3". A heat map visualizing this correlation matrix was then constructed using the Corrplot package. In the heat map, color values are assigned to represent the strength and direction of the relationships between variables. Blue denotes a positive correlation, indicating that as one variable increases, so does the other. Conversely, red signifies a negative correlation, where an increase in one variable corresponds to a decrease in the other. White indicates no correlation.

structure and function in response to overall body growth and development.

Our data point to an inverse relationship between the presence of type III collagen in amorphous substance and reticular fibers, and the density of lymphocytes in the thymic cortex. This result is in line with the hypothesis proposed by Z. Sadjadi et al. [25] and Y. Li et al. [26], who suggested that the structural properties of collagen could influence lymphocyte distribution and density. This finding is particularly significant for understanding the structural-functional aspects of the thymic microenvironment.

Both variables reflecting the OD of collagen type III and IV fluorescence tend to increase or decrease together, suggesting a coordinated regulation of collagen expression in different thymic structures. This pattern of collagen dynamics is consistent with the synthesis pathways discussed in the seminal work of Mario Ruiz Pérez et al. [27].

In general, the characteristics of carrying a LF, which are significantly influenced by complex immune-endocrine interactions within the mother-placenta-fetus system, and factoring in prenatal stimulation, profoundly impact embryonic and fetal development. This effect encompasses not only the lymphoid and epithelial components of the fetal thymus, as previously demonstrated by the Kharkiv pathology school researchers [20], but also

extends to more intricate structures, such as the BTB. These observations underscore the substantial impact of maternal-fetal physiological processes on the intricate development of fetal thymic structures.

The specific patterns of collagen formation within the blood-thymus barrier structures in large stillborn fetuses could provide crucial insights into the morphofunctional characteristics of the thymus, particularly in relation to the anthropometric parameters of macrosomia. Our previous research has indicated that the thymuses of LF fetuses, which do not exhibit signs of obesity, display hyperplasia and disrupted maturation processes in cortical thymocytes [20]. A notable deficiency of type IV collagen in the vascular and epithelial basement membranes could lead to increased permeability of the BTB to maternal blood-borne antigens. It is well established that the primary function of the blood-thymus barrier is to inhibit antigen penetration into the thymic cortex. This barrier plays a pivotal role in facilitating the proper differentiation of cortical thymocytes. Hence, alterations in collagen composition and barrier integrity could have significant implications for fetal thymic development and functioning [28].

In response to antigenic stimulation, lymphocytopoiesis activation occurs on one hand, while on the other, the maturation of cortical thymocytes is disrupted, and apoptosis is also delayed. We associate the disruption in

thymocyte maturation with the hypofunction of the thymus' epithelial component, which is responsible for producing thymic hormones. This research reveals another potential factor in the morphogenesis of this complex immunobiological process.

Interesting results were observed concerning type III interstitial collagen. It is known that hypoxia is one of the stimuli for the formation of interstitial collagen [29,30]. For instance, the delivery of a large fetus is often complicated by chronic intrauterine hypoxia, which should manifest as increased collagen type III formation in various fetal organs, including the thymus. However, within the BTB, there was only a tendency towards an increase in the OD of collagen III type immunofluorescence, and this was observed solely in the SF3 group. In contrast, a significant decrease in this indicator was noted in the SF1 macrosomes compared to the control, a pattern that is consistent with the peculiarities observed in type IV collagen formation. This may be due to the unique functional status of type III interstitial collagen located within the blood-thymus barrier, as opposed to collagen in interstitial connective tissue. To date, there is a lack of literature addressing this specific issue.

All these findings suggest a direct correlation between macrosomia and variations in collagen expression within the blood-thymus barrier. This variation in collagen expression could be a physiological adaptation to the altered requirements or stressors associated with different fetal sizes. This understanding could have important implications for the study of fetal development and the management of pregnancies characterized by fetal macrosomia.

Future studies focusing on the endothelial lining of the blood-thymus barrier and the morphofunctional characteristics of macrophages localized in its perivascular spaces could provide a more comprehensive understanding of this critical structural component of the thymus.

## Conclusions

1. In the structure of the blood-thymus barrier of large fetuses, the expression of type IV collagen was detected in the composition of the epithelial and endothelial basement membranes. Vascular basement membranes contain a greater amount of type IV collagen compared to the epithelial basement membranes of blood-thymus barrier.

2. The degree of expression of type IV collagen differed depending on the anthropometric characteristics of the fetus. In endothelial basement membranes, compared with control group, the synthesis of type IV collagen was significantly reduced in macrosomes of SF1 group ( $p \leq 0.05$ ), with a tendency to increase in macrosomes of SF3 group. In epithelial basement membranes, there was a significant increase in the production of type IV collagen in the thymus of SF3 group ( $p \leq 0.05$ ) and a tendency towards a decrease in the expression of this collagen in SF1 group of macrosomes.

3. In the reticular fibers and amorphous substance of the blood-thymus barrier of stillborns, irrespective of their anthropometric characteristics, interstitial collagen type III was detected. Compared to the control, the optical density of the luminescence of type III collagen was

maximal in observations of the SF3 group and minimal in large fetuses of the SF1 group.

**Prospects for further research.** In-depth immunohistochemical analysis of the cellular constituents of the blood-thymus barrier. This detailed study will encompass endothelial cells, epithelial cells, and macrophages. We aim to explore the intricate interactions and functional dynamics of these cells, understanding their roles in the formation and regulation of the blood-thymus barrier. Such an investigation promises to shed light on the complex cellular mechanisms at play and their implications in broader physiological processes.

## Funding

The scientific study is a fragment of the scientific research work of Kharkiv National Medical University on the topic: "Pathological anatomy of the fetus and newborn in maternal-fetal infection", state registration No. 0120U102023 (2020–2024).

**Conflicts of interest:** authors have no conflict of interest to declare.  
**Конфлікт інтересів:** відсутній.

Надійшла до редакції / Received: 03.12.2025

Після доопрацювання / Revised: 28.02.2026

Схвалено до друку / Accepted: 09.03.2026

## Information about the authors:

Sorokina I. V., MD, PhD, DSc, Professor of the Department of Pathological Anatomy, Kharkiv National Medical University, Ukraine.

ORCID ID: [0000-0002-5945-2605](https://orcid.org/0000-0002-5945-2605)

Talapova P. S., MD, PhD, Assistant at the Department of Pathological Anatomy, Kharkiv National Medical University, Ukraine.

ORCID ID: [0000-0003-4147-1485](https://orcid.org/0000-0003-4147-1485)

Gubina-Vakulik G. I., MD, PhD, DSc, Professor of the Department of Pathological Anatomy, Kharkiv National Medical University, Ukraine.

ORCID ID: [0000-0003-3816-8530](https://orcid.org/0000-0003-3816-8530)

Pliten O. M., MD, PhD, Associate Professor of the Department of Pathological Anatomy, Kharkiv National Medical University, Ukraine.

ORCID ID: [0000-0001-6376-9376](https://orcid.org/0000-0001-6376-9376)

Kaluzhyna-Biletska O. V., MD, PhD, Associate Professor of the Department of Pathological Anatomy, Kharkiv National Medical University, Ukraine.

ORCID ID: [0000-0002-4181-9704](https://orcid.org/0000-0002-4181-9704)

## Відомості про авторів:

Сорокіна І. В., д-р мед. наук, професор каф. патологічної анатомії, Харківський національний медичний університет, Україна.

Талапова П. С., PhD, асистент каф. патологічної анатомії, Харківський національний медичний університет, Україна.

Губіна-Вакулік Г. І., д-р мед. наук, професор каф. патологічної анатомії, Харківський національний медичний університет, Україна.

Плітен О. М., канд. мед. наук, доцент каф. патологічної анатомії, Харківський національний медичний університет, Україна.

Калужина-Білецька О. В., канд. мед. наук, доцент каф. патологічної анатомії, Харківський національний медичний університет, Україна.



Iryna Sorokina (Ірина Сорокіна)  
[soririna@gmail.com](mailto:soririna@gmail.com)

## References

- Birene B, Ferreira A, Raimond E, Graesslin O, Ishaque U, Gabriel R. Impact of screening for large-for-gestational-age fetuses on maternal and neonatal outcomes: a prospective observational study. *J Perinat Med.* 2025;53(3):367-75. doi: [10.1515/jpm-2024-0522](https://doi.org/10.1515/jpm-2024-0522)

2. Najafian M, Cheraghi M. Occurrence of fetal macrosomia rate and its maternal and neonatal complications: a 5-year cohort study. *ISRN Obstet Gynecol*. 2012;2012:353791. doi: [10.5402/2012/353791](https://doi.org/10.5402/2012/353791)
3. Wolamo DD, Meskele M, Workie SB, Badacho AS. Determinants of fetal macrosomia among live births in southern Ethiopia: a matched case-control study. *BMC Pregnancy Childbirth*. 2022;22(1):465. doi: [10.1186/s12884-022-04734-8](https://doi.org/10.1186/s12884-022-04734-8)
4. Adjei-Gyamfi S, Musah B, Asirifi A, Hammond J, Aryee PA, Miho S, et al. Maternal risk factors for low birthweight and macrosomia: a cross-sectional study in Northern Region, Ghana. *J Health Popul Nutr*. 2023;42(1):87. doi: [10.1186/s41043-023-00431-0](https://doi.org/10.1186/s41043-023-00431-0)
5. Canday M. Identifying gestational diabetes mellitus and assessing risk factors in affected women: a comprehensive study. *Eur Rev Med Pharmacol Sci*. 2024;28:734-46. doi: [10.26355/eurev\\_202401\\_35073](https://doi.org/10.26355/eurev_202401_35073)
6. Mou AD, Barman Z, Hasan M, Miah R, Hafsa JM, Das Trisha A, et al. Prevalence of preeclampsia and associated risk factors among pregnant women in Bangladesh. *Sci Rep*. 2021;11(1):21339. doi: [10.1038/s41598-021-00839-w](https://doi.org/10.1038/s41598-021-00839-w)
7. Magee LA, Nicolaides KH, von Dadelszen P. Preeclampsia. *N Engl J Med*. 2022;386:1817-32. doi: [10.1056/NEJMra2109523](https://doi.org/10.1056/NEJMra2109523)
8. Vince K, Brkić M, Poljičanin T, Matijević R. Prevalence and impact of pre-pregnancy BMI on pregnancy outcome: a cross-sectional study in Croatia. *J Obstet Gynaecol*. 2021;41(1):55-9. doi: [10.1080/01443615.2019.1706157](https://doi.org/10.1080/01443615.2019.1706157)
9. Tsikouras P, Kotanidou S, Nikolettos K, Kritsotaki N, Bothou A, Andreou S, et al. Shoulder dystocia: a comprehensive literature review on diagnosis, prevention, complications, prognosis and management. *J Pers Med*. 2024;14(6):586. doi: [10.3390/jpm14060586](https://doi.org/10.3390/jpm14060586)
10. Chen P, Mu Y, Xie Y, Wang Y, Liu Z, Li M, et al. Trends of large for gestational age and macrosomia and their mediating effect on diabetes mellitus and obstetric hemorrhage. *Matern Child Nutr*. 2025;21(3):e70000. doi: [10.1111/mcn.70000](https://doi.org/10.1111/mcn.70000)
11. Feyaerts D, Urbschat C, Gaudillière B, Stelzer IA. Establishment of tissue-resident immune populations in the fetus. *Semin Immunopathol*. 2022;44(6):747-66. doi: [10.1007/s00281-022-00931-x](https://doi.org/10.1007/s00281-022-00931-x)
12. Chen S, Zhu H, Jounaidi Y. Comprehensive snapshots of natural killer cell functions, signaling, molecular mechanisms and clinical utilization. *Signal Transduct Target Ther*. 2024;9:302. doi: [10.1038/s41392-024-02005-w](https://doi.org/10.1038/s41392-024-02005-w)
13. Hart PM, Stephenson NL, Scime NV, Tough SC, Slater DM, Chapput KH. Second trimester cytokine profiles associated with gestational diabetes and hypertensive disorders of pregnancy. *PLoS One*. 2022;17(12):e0279072. doi: [10.1371/journal.pone.0279072](https://doi.org/10.1371/journal.pone.0279072)
14. Yayon N, Kedlian VR, Boehme L, Suo C, Wachter BT, Beuschel RT, et al. A spatial human thymus cell atlas mapped to a continuous tissue axis. *Nature*. 2024;635(8039):708-18. doi: [10.1038/s41586-024-07944-6](https://doi.org/10.1038/s41586-024-07944-6)
15. Kousa AI, Jahn L, Zhao K, Flores AE, Acenas DE II, Lederer E, et al. Age-related epithelial defects limit thymic function and regeneration. *Nat Immunol*. 2024;25:1593-606. doi: [10.1038/s41590-024-01915-9](https://doi.org/10.1038/s41590-024-01915-9)
16. James KD, Jenkinson WE, Anderson G. Non-epithelial stromal cells in thymus development and function. *Front Immunol*. 2021;12:634367. doi: [10.3389/fimmu.2021.634367](https://doi.org/10.3389/fimmu.2021.634367)
17. Bruss DM, Ely S. Anatomy, head and neck: blood thymus barrier. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [updated 2023 Jul 25]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK546601/>
18. Sun B. The mechanics of fibrillar collagen extracellular matrix. *Cell Rep Phys Sci*. 2021;2(8):100515. doi: [10.1016/j.xcrp.2021.100515](https://doi.org/10.1016/j.xcrp.2021.100515)
19. Sharma H, Moroni L. Recent advances in regenerative approaches for thymus rejuvenation. *Adv Sci*. 2021;8:2100543. doi: [10.1002/adv.202100543](https://doi.org/10.1002/adv.202100543)
20. Yakovtsova AF, Sorokina IV, Aleshchenko IE. Immunnaya sistema ploda cheloveka pri krupnoplodii i ZVRP [Immune system of the human fetus with macrosomia and intrauterine growth restriction (IUGR)]. *Kharkiv: Antiqua*; 2004. 153 p.
21. Lins MP. Thymic extracellular matrix in thymopoiesis: just a supporting? *BioTech (Basel)*. 2022;11(3):27. doi: [10.3390/biotech11030027](https://doi.org/10.3390/biotech11030027)
22. Sender R, Weiss Y, Navon Y, Milo I, Azulay N, Keren L, et al. The total mass, number, and distribution of immune cells in the human body. *Proc Natl Acad Sci U S A*. 2023;120(44):e2308511120. doi: [10.1073/pnas.2308511120](https://doi.org/10.1073/pnas.2308511120)
23. Han X, Caron JM, Brooks PC. Cryptic collagen elements as signaling hubs in tumor growth and metastasis regulation. *J Cell Physiol*. 2020;235(12):9005-20. doi: [10.1002/jcp.29752](https://doi.org/10.1002/jcp.29752)
24. Caron JM, Han X, Lary CW, Sathyanarayana P, Remick SC, Ernstoff MS, et al. Targeting the secreted RGDKGE collagen fragment reduces PD-L1 and inhibits tumor growth. *Oncol Rep*. 2023;49:44. doi: [10.3892/or.2023.8481](https://doi.org/10.3892/or.2023.8481)
25. Sadjadi Z, Zhao R, Hoth M, Qu B, Rieger H. Migration of cytotoxic T lymphocytes in 3D collagen matrices. *Biophys J*. 2020;119(11):2141-52. doi: [10.1016/j.bpj.2020.10.020](https://doi.org/10.1016/j.bpj.2020.10.020)
26. Li Y, Huamei C, Peng C, Meng G, Lu Y, Liu H, et al. Unraveling the spatial organization and development of human thymocytes through integration of spatial transcriptomics and single-cell multi-omics profiling. *Nat Commun*. 2024;15:7784. doi: [10.1038/s41467-024-51767-y](https://doi.org/10.1038/s41467-024-51767-y)
27. Ruiz Pérez M, Vandenabeele P, Tougaard P. The thymus road to a T cell: migration, selection, and atrophy. *Front Immunol*. 2024;15:1443910. doi: [10.3389/fimmu.2024.1443910](https://doi.org/10.3389/fimmu.2024.1443910)
28. Nelson BN, Friedman JE. Developmental programming of the fetal immune system by maternal western-style diet: mechanisms and implications. *Int J Mol Sci*. 2024;25(11):5951. doi: [10.3390/ijms25115951](https://doi.org/10.3390/ijms25115951)
29. Yang L, Gilbertsen A, Xia H, Benyumov A, Smith K, Herrera J, et al. Hypoxia enhances IPF mesenchymal progenitor cell fibrogenicity via the lactate/GPR81/HIF1 $\alpha$  pathway. *JCI Insight*. 2023;8(4):e163820. doi: [10.1172/jci.insight.163820](https://doi.org/10.1172/jci.insight.163820)
30. Graton ME, de Oliveira AA, Neupane A, Quon A, Kirschenman R, Spaans F, et al. Exposure to prenatal hypoxia impairs function and structure of carotid arteries in adult offspring. *Am J Physiol Heart Circ Physiol*. 2025;328(3):H518-H525. doi: [10.1152/ajpheart.00859.2024](https://doi.org/10.1152/ajpheart.00859.2024)