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# MICROSCOPICAL INDICES OF THYMUS IN NEWBORN FETUSES

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**ABSTRACT**

*The formation of the immunity system in ontogenesis, in particular the central organ of its thymus, is determined by the combination of the relationship between mother and fetus and the course of the period of early adaptation of offspring to conditions outside uterine life. As a rule, in early postnatal ontogenesis, the immune system is in a transient state and continues to form under the influence of various influences of external factors. The uncomplicated course of pregnancy largely determines the normal course of the immunological relationship between mother and fetus, and determines the development of a full-fledged immune system and its central and peripheral organs in the offspring in postnatal ontogenesis.*

Relevance. In the embryonic aspect, the thymus gland is one of the first endocrine organs and the first lymphoid organ. In the early stage of embryonic development, bone marrow lymphoblasts passing through the thymus become thymocytes that enter the blood and other lymphoid organs not only during the embryonic period, but throughout the life of the body. Thus, the thymus is an organ regulator of the immune system and at the same time a producer of lymphoid cells, which are the basis of the body's protective system against pathogens and foreign molecules [5,7,9,11,14,16,20]. The thymus is structurally a complex organ consisting of stroma and regenerative lymphoid tissue. The stroma is about 10% of the organ mass and consists of reticular and epithelial cells [1,4,14,16]. Thymic parenchyma is represented by thymocytes and lymphocytes migrating from the bone marrow. The impact of pathogenic climatoecological factors in pregnant women causes a malfunction of almost all the main systems of the body, mother and fetus, including the immune system [2,3,9,11]. It is known that the thymus gland, being the central organ of immunogenesis, can undergo transformations occurring under the influence of various pathogenic factors occurring in the mother's body during pregnancy. As a result of studying the

thymus by modern submicroscopic, radiographic, immunohistochemical and biochemical methods of research, new data on the morphofunctional organization of the organ have now been obtained. Histologically, the thymus represents, as if a three-layer organ consisting of a connective-woven stroma, an epithelial base and lymphoid contents, the latter in the developed thymus accounts for the bulk of the organ. The connective-tissue stroma is represented by interlobular partitions - septa and an organ capsule. The perivascular space is formed by intercellular matrix, fibroblasts, fibers, reticular cells and labrocytes and macrophages [10,13,19,20].

Research material and methods. The thymus of newborns born at term and killed mainly from asphyxia or traumatic brain injury at the time of birth has been studied.

Study methods: general morphological study of hematoxylin and eosin coloration; histometry: measurement of the thickness of the capsule, cortex and cerebral layer of the lobules; volume ratio of structural and cellular elements of thymus; statistical processing of the obtained digital data.

Autopsies for the review histological, histochemical, immunohistochemical, electron microscopic methods were taken no later than 30 minutes from the moment of death, followed by fixation in Carnois, Buen, Becker solutions and 10% neutral buffered formalin. Paraffin sections 4-5 μm thick were stained with Ehrlich hematoxylin with docraca eosin, Weigert iron hematoxylin with docraca according to Van Gison and Mallory. Glycosaminoglycans (GAGs) were differentiated with 0.1% toluidine blue on citrate phosphate buffer at different pH values (3.5-6.5) with bacterial and testicular hyaluronidase section control.

Results of the study. Depending on the significance of the fruit factor in the organ complex and its development, as well as in the formation of elements of the functional system "mother-placenta-fetus," the measurement parameters of the organs and morphological features of the thymus gland were compared with 15 somatometric parameters of the fetuses of 21-26 weeks of gestation with induced termination of pregnancy for medical reasons. Analysis of somatometric parameters of fetuses and newborns in induced and spontaneous termination of pregnancy at 21-26 weeks showed that harmonious and proportional somatic development is characteristic of uncomplicated course of pregnancy. With this type of somatic development, the weight and length of the body, the circumference of the head, chest, shoulder length, hip and weight and height in the coefficient increase evenly and directly proportional to the gestation period. Positive dynamics of the above somatometric parameters was revealed both in the whole group and in subgroups with a two-week interval: 21-22; 23-24 and 25-26 weeks. Thus, the increase in fetal weight in 23-24 weeks is 171 g, in 30 weeks - 193 g. Body length (parietal-heel size) every two weeks increases by 3-4 cm. The positive dynamics of parietal-heel size is directly associated with an increase in linear parameters of the lower limb, in particular, the hip, the length of which is 25-26 weeks is 1.5 times higher than the same parameter in 23-24 weeks. The increase in the linear parameters of the lower leg within the considered subgroups is not statistically significant. The length of

the upper limb varies in the range from 1.1 to 1.5 cm, while the shoulder grows most intensively (0.7-0.9 cm). The growth intensity of the upper limbs, as well as the lower limbs, stabilizes from 26-27 weeks of gestation. The circumference of the shoulder, forearm, thigh and lower leg, which characterizes the increase in muscle mass and subcutaneous fat, change in accordance with the increase in gestational term, reaching maximum numbers at weeks of gestation. It is unlawful to consider a complete analysis of anthropometric parameters without taking into account certain coefficients. The mass-growth coefficient during 21-26 weeks of gestation tends to increase progressively, especially during weeks of intrauterine development, which confirms the positive dynamics of weight and linear body parameters at this stage of ontogenesis. The thoracic-growth coefficient is 0,58±0,06 and remains stable within a given gestation period, which indicates proportional somatic development of fetuses in the mid-fetal period. Additionally, the proportionality of the development of fetuses was estimated using coefficients such as the ratio of the length of the upper and lower limbs to the length of the body. The coefficient (ratio of arm length to parietal-heel length), as well as the thoracic-growth one, changed statistically not significantly and amounted to 0.39 ±0,015. A slightly different dynamics was revealed from the side of the coefficient - the ratio of the length of the lower limb to the parietal-heel length. Given the stability of this parameter, fetuses 21-22 and 23-24 weeks of gestation showed an increase in this coefficient by weeks of development, which confirms the enhanced growth of the lower limb at this stage of ontogenesis. The harmony and proportionality of the somatic development of fetuses is confirmed by the presence of a strong straight line (r = 0.7) correlation between the mass of the fetus and the circles of the chest, head, shin length, as well as linear parameters of the arm and shoulder. Moderate positive relationship (r = 0.5) is established between fetal mass and leg and hip lengths, as well as circumference of both extremities in general and their components. The revealed dynamics of the somatometric parameters of the fetuses 21-26 weeks of gestation fits into the concept of the physiological type of intrauterine development, which is characterized by a relatively uniform increase in body weight and length, circumference of the head, chest, shoulder length, hip and mass-growth coefficient. The conjugation of somatic development with the formation of the central organ of the immune system in fetuses 21-26 weeks of gestation is confirmed by the presence of a moderate direct (r = 0.6) association between mass-growth parameters of the fetus and linear-weight parameters of the thymus. With a volume of significant correlation relationships equal to 21.4%, a direct positive relationship (r = 0.6) was found between the mass, length of the fetus, chest circumference and organometric parameters of the thymus, i.e. its mass, length and width. In the enlarged subgroups (21-24 and 25-26 weeks), as in general at the stage of 21-26 weeks of development, the organometric parameters of the thymus increase significantly in proportion to the gestation period ($p < 0.001$). The maximum increase in these values and a twofold increase in the volume of correlation relationships in

25-26 weeks of gestation indicates the intensity of development of both soma and the central organ of the immune system at this stage of fetogenesis. The growth rate of thymus mass in fruits 25-26 weeks of gestation is 3 times higher than the corresponding parameter in fruits 21-22 weeks of development and averages 2.7-3.2 g. A reliable increase in the linear size of the organ confirms its growth, both in length and in width. Cytokeratin expression by reticuloepithelial cells of the outer layer reflects the degree of their differentiation and indicates the ectodermal origin of the latter [2,3,7,8,14,20]. The second type of reticuloepithelial cell acquires a stellate shape through cytoplasmic processes. Epithelial cells of this type contain a rounded nucleus, 12 μm in diameter with 1-2 nuclei and finely condensed chromatin. A few tonofilaments, multivesicular bodies, vacuoles, short profiles of a rough endoplasmic network and a well-developed lamellar complex are visualized from ultrastructures in the cytoplasm. In the subcapsular zone, epithelial cells are found, in the cytoplasm of which, along with vacuoles and small electron-dense inclusions, there are single thymocytes. Cytoplasmic processes of reticuloepithelial cells are located between lymphocytes, contact them and are nanny cells [12,14,15,16,18]. In the early stages of the midfetal period, that is, in fetuses 21-22 weeks of gestation, the epithelial cells of a number of lobules migrate to the surrounding mesenchyma, forming various cell strands. The last 23-24 weeks of gestation in the thymus of the fruit, having high growth potency, perform a cambial function and contribute to the formation of growth zones on the periphery of the lobules. Among subcapsular zone lymphocytes, lymphoblasts are predominant, corresponding in antigenic composition to pre-T lymphocytes and expressing CD2 antigens on the membrane (expression coefficient - 2.79 units). The nucleus of lymphoblasts with a retinal chromatin structure, among which are 1-2 nucleoli. The cytoplasm covers a minimal area, contains free ribosomes, single cisterns of the granular endoplasmic reticulum and mitochondria. An additional indication of thymocyte proliferation activity is an increase to 0.42 AU in the mean histochemical coefficient (GCA) of deoxyribonuclein substances in subcapsular lymphocytes.The GCS value within all structural-functional zones increases parallel to the gestation period and averages 0.27 units.

In addition to lymphoid and epithelial cells, single macrophages with PAS-positive inclusions are found in the subcapsular zone, and with a higher frequency they are determined in the thymus of the fetuses of 26-27 weeks of gestation. The density of cell elements in the subcapsular zone is 17,76±02 cells and increases directly in proportion to the gestation period. In 26-27 weeks of development, the number of cells in a unit area increases to 22. The inner cortical zone of the lobules is dominated by medium-diameter lymphocytes expressing CD2, CD1 antigens, which indicates the ongoing antigen-independent differentiation of lymphocytes. However, the formation of receptors and antigen is also characteristic of this zone, which is confirmed by a significant increase in the expression of CD3 T lymphocytes. From the cell microenvironment of lymphocytes, dark-type epithelial cells prevail, characterized

by an abundance of ultrastructures, which indicates their functional activity [6,9,10,11,13,19]. T-lymphocytes of the medullary zone, unlike lymphocytes of the cortex, mostly have a mature phenotype, with antigens CD 2, CD3, which confirms the completion of the stage of their differentiation.

Conclusion. Thus, the intrauterine period from 21 to 26 weeks of gestation is characterized by intensive somatic development, which, according to the theory of intrauterine cell growth, is based on high proliferative activity of cells in combination with a progressive increase in their size. Comparative analysis of somatometric parameters in the enlarged groups (21-22 and 25-26 weeks) confirmed the intensity of somatic development of fetuses at the stage of 25-26 weeks. Taking into account the peculiarities of the dynamics of somatometric parameters and the factors specifying them, this stage of ontogenesis should be classified as critical. Exposure to pathogenic factors, including intrauterine infections, can cause compensation disruption at this stage of gestation, lead to preterm birth, and the birth of infants with intrauterine retardation syndrome. The thymus gland of the fruits of 21-26 weeks of gestation resembles a thymian leaf in shape, represented by two lobes connected at the base by an isthmus and covered with a thin connective tissue capsule. In 85 fetuses (74.7%), from 21-22 weeks of gestation, asymmetry of the lobes is detected; the left lobe in linear parameters exceeds the right. In addition to two lobes in 91 fruits (80%), additional lobes (up to 4) were found at the base of the thymus, rounded in shape from 0.4 to 0, 8 cm in diameter. Thus, the main criteria for the gestational maturity of the fetus thymus at the stage of 23-24 weeks are incomplete cortico-medullary differentiation in 18-20% of lobules, more than twice the prevalence of specific volume (58,29±2,11%) cortical matter above the brain (23,87±0,75%) with a cortico-medullary coefficient of 2.5 units and a specific volume of vessels in interstitial tissue - 0,46±0,07%, with a maximum density of cell elements (29,94±0,06 cells) in the internal cortical zone, with an average histochemical coefficient of deoxyribonucleic substances in the nuclei of lymphocytes - 0.27 conventional units and glycoproteins (0.36 conventional units).

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