TOSHKENT TIBBIYOT AKADEMIYASI URGANCH FILIALI
JANUBIY OROLBO‘YI TIBBIYOT JURNALI
1-TOM, 4-SON. 2025
14.00.00 - TIBBIYOT FANLARI ISSN: 3093-8740

UDK: 618.29+612.6481:61 2.438

MULTIPLEX AND CONFOCAL MORPHOMETRIC INDICATORS OF THE THYMUS IN EXTREMELY LOW BIRTH WEIGHT NEWBORNS

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ABSTRACT

The rehabilitation of extremely low birth weight (500-999 g) newborns represents one of the pressing challenges in modern neonatology. This study investigates the thymus tissues of stillborn and deceased newborns at 22-24 weeks of gestation using multiplex and confocal morphometric methods based on autopsy samples. Utilizing NanoZoomer scanning and QuPath-ImageJ software, the thickness of the cortex and medulla layers, as well as the volumetric indicators of blood vessels, septa, and Hassall's corpuscles, were analyzed. Results indicate that as thymus weight decreases, the cortical layer thickness reduces from 46.1 ± 0.2 µm to 20.74 ± 0.9 µm, showing a 55% developmental delay, a 57.4-fold reduction in blood vessel volume, and the predominance of stromal elements. These findings reflect degrees of thymic hypoplasia and aplasia, confirming morphological immaturity of the immune system. This research contributes to a deeper understanding of the factors influencing postnatal adaptation and survival in extremely low birth weight newborns.

Keywords: *extremely low birth weight newborns, thymus morphometry, cortex and medulla layers, Hassall's corpuscles, hypoplasia.*

Relevance of the topic. The problem of extremely low birth weight in newborns is one of the most serious issues in perinatology and child health. Infants with birth weights ranging from 500 to 1000 grams represent a vulnerable group with a high risk of complications and mortality [1]. According to data from the World Health Organization, preterm birth and low birth weight are associated with more than half of global child mortality [3].

One of the key aspects of health in low birth weight newborns is the development and functioning of the immune system. The thymus, as a component of the immune system, plays a crucial role in ensuring normal immune responses in children [4]. Studying the morphological characteristics of the thymus in extremely low birth weight newborns is essential for a deeper understanding of their impact on the infants' health and survival [2].

Materials and research methods. Microsamples prepared from thymus tissue obtained during autopsies of preterm stillborn and deceased newborns with extremely low birth weight were processed according to the guidelines for unification of general histological examination methods for the

obtained tissues and materials. Specifically, the obtained material was fixed in 10% neutral formalin buffered with phosphate (pH=7.4) for at least 24 hours, after which uterine fragments were washed in running water for 3-4 hours, then dehydrated in 70%, 80%, 90%, 96%, and 100% alcohols and chloroform, embedded in paraffin with wax, and blocks were prepared. From the paraffin blocks, sections with a thickness of 4-5 μm were prepared using rotary microtomes, and the paraffin in the sections was removed by melting with xylene in a thermostat at 57°C, stained with hematoxylin-eosin, and microscopic examinations were conducted. They were scanned using a NanoZoomer microscope. At least 10 sections were taken from each case, and measurements of the lost 5 μm intervals between consecutive sections were obtained with an average error of $P \leq 0.01$ and a reliability indicator not less than that value.

The main aspects in terms of the structural components of the thymus included: cells, fibrous structures, thickness of the cortex and medulla layers, structures in the cortico-medullary area, and the area occupied by each listed component, along with other indicators.

According to morphometric indicators for the thymus of newborns at 22-24 weeks gestation: In Group 1 (thymus weight 2.67 g), the cortical layer thickness was found to be 46.1 ± 0.2 μm, while in Group 2, this indicator was 39.18 ± 0.2 μm. This represents a 15% statistical difference between Groups 1 and 2 in relation to weight, indicating delayed maturation of the thymus gland. In Group 3, the cortical area of the thymus gland was 35.03 ± 0.9 μm, which is 24% behind Group 1 in development; statistically, this indicates delayed formation of the cortical area. This confirms that the development of the thymus gland in extremely low birth weight newborns is directly proportional to body weight and manifests as morphological immaturity at levels 3-4. In Group 4, the cortical area thickness of the thymus gland was 25.8 ± 0.9 μm, primarily indicating 44% developmental delay compared to Group 1, confirming morphological immaturity. In Group 5, this indicator was 20.74 ± 0.9 μm, indicating 55% developmental delay compared to Group 1, and morphologically signifying a state of thymic-lymphatic hypoplasia and aplasia in the thymus gland.

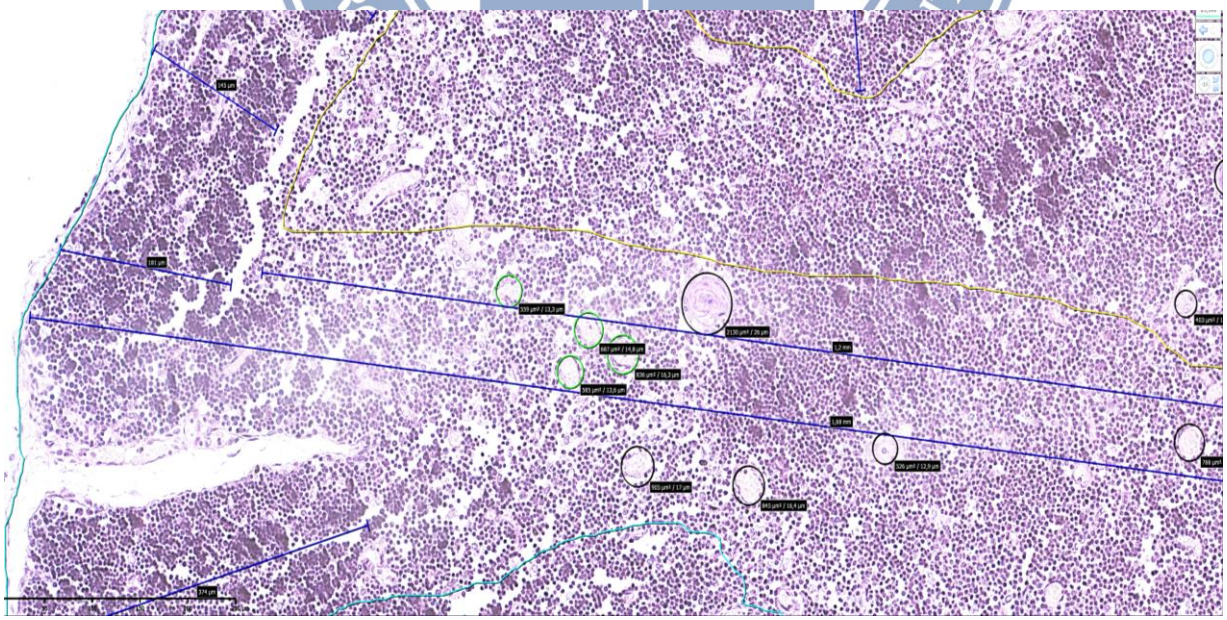

Figure 1. Group 1. Morphogram showing measurements of the cortex and medulla layers of the thymus gland. Indicators of various diameters of blood vessels and Hassall's corpuscles. Stained with H&E. Magnification 4x10. Scanned with NanoZoomer.

This confirms, through morphological examinations, that in Groups 2-5, the morphological features of the thymus glands show deep developmental delay in the number and volume of small lymphocytes, i.e., pre-thymocytes, as evidenced by the above morphometric indicators. Specifically,

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the initial abundance of small lymphocytes in the cortical area of the thymus gland and the paralysis of the differentiation process into large thymocytes in clusters of nurse cells or reticuloepithelial cells located in the cortico-medullary areas are confirmed.

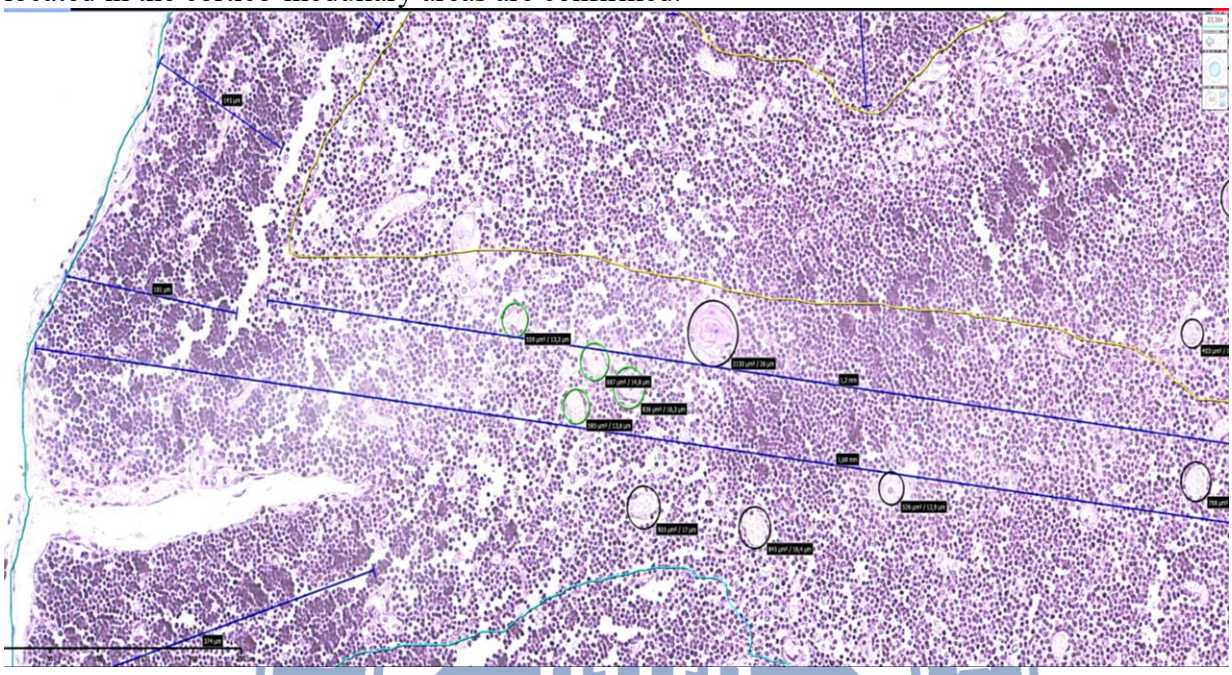

Figure 2. Group 2. Morphogram showing measurements of the cortex and medulla layers of the thymus gland with diameters oriented chordally. Indicators of various diameters of blood vessels and Hassall's corpuscles. Stained with H&E. Magnification 4x10. Scanned with NanoZoomer.

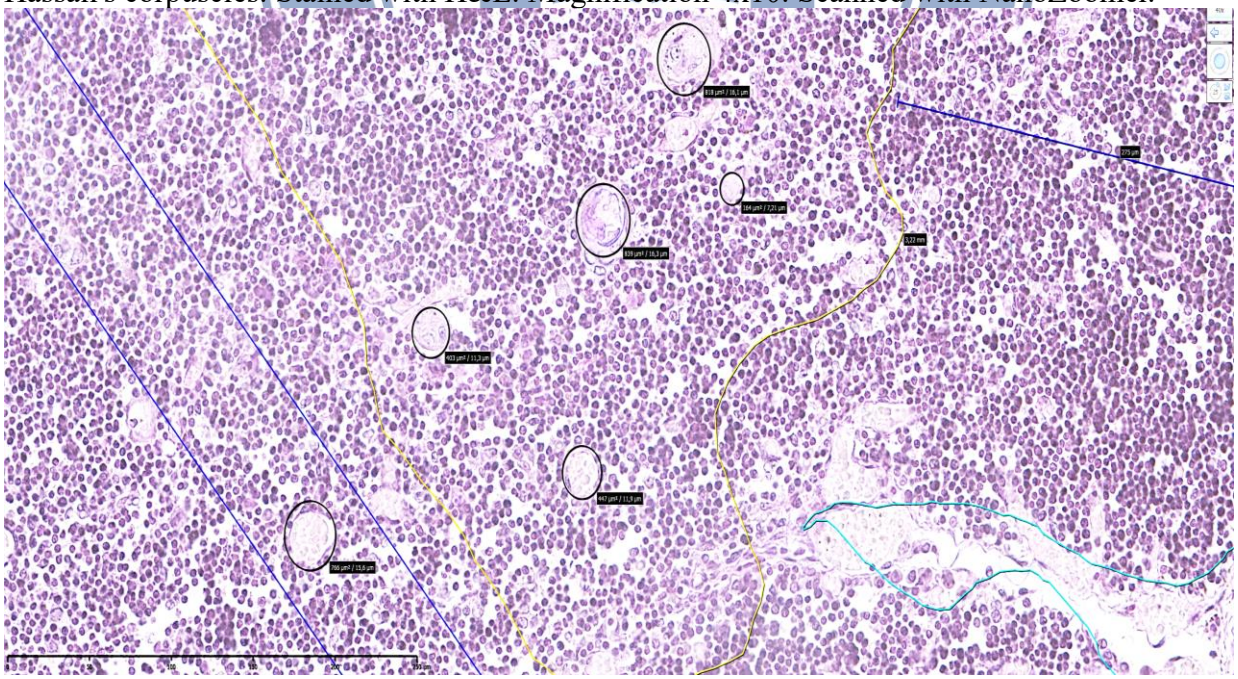

Figure 3. Group 3. Morphogram showing measurements of various areas of the medulla layer of the thymus gland. Indicators of various diameters of blood vessels and Hassall's corpuscles. Stained with H&E. Magnification 4x10. Scanned with NanoZoomer.

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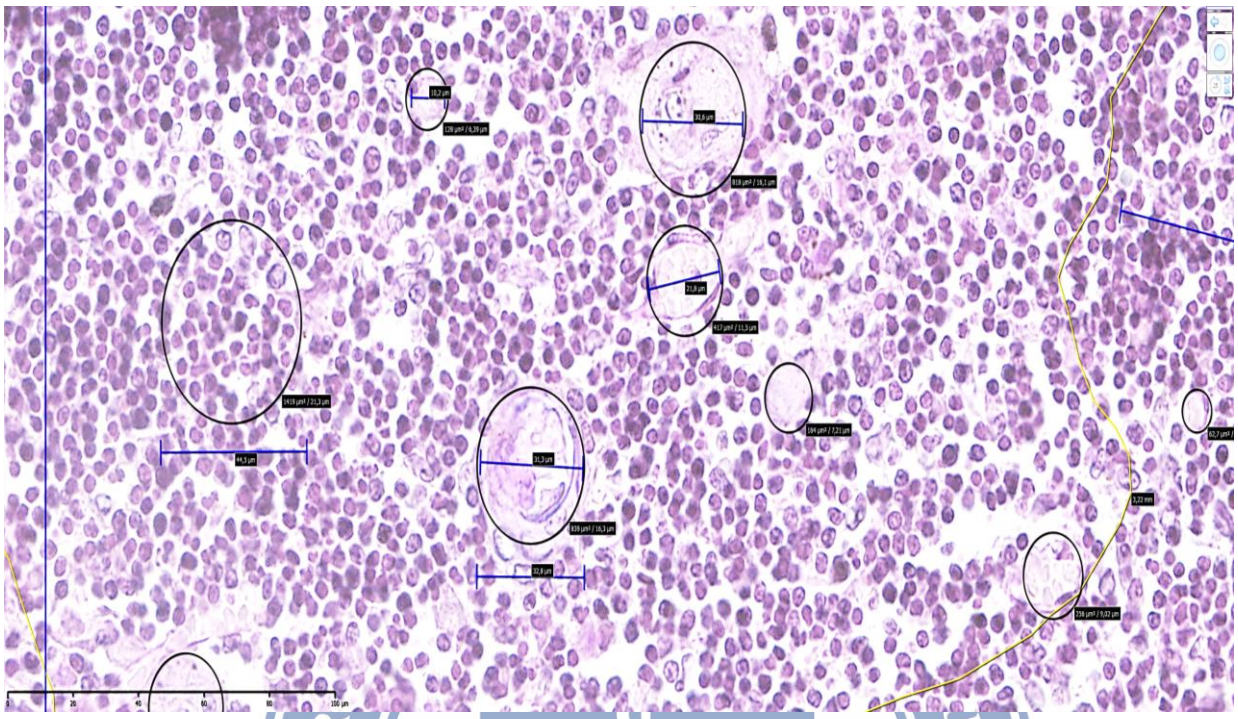

Figure 4. Group 4. Morphogram showing measurements of various areas of the medulla layer of the thymus gland. Indicators of various diameters of blood vessels and Hassall's corpuscles. Stained with H&E. Magnification 4x10. Scanned with NanoZoomer.

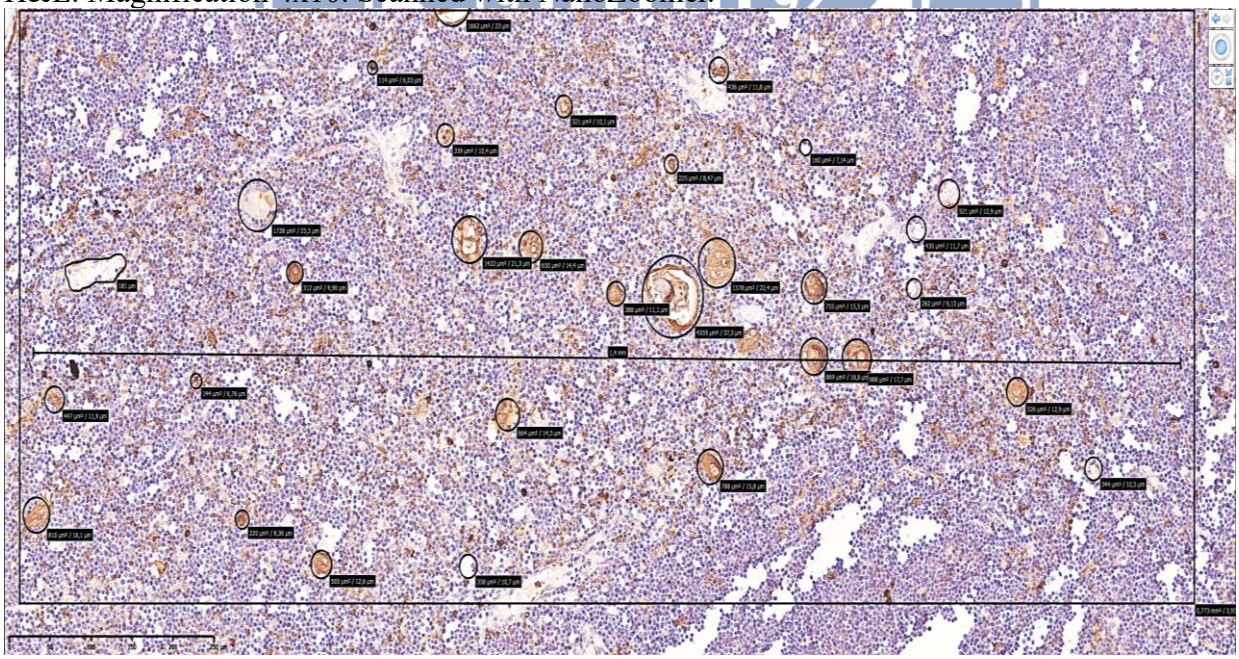

Figure 5. Group 5. Morphogram showing measurements of various areas of the medulla layer of the thymus gland, including diameters and areas of blood vessels in various forms. Indicators of various diameters of Hassall's corpuscles. Stained with H&E. Magnification 4x10. Scanned with NanoZoomer.

In the subsequent examination, the confocal morphometric indicators of the thymus were studied. The main aspects included volumetric indicators of blood vessels and stromal components within them. This procedure was performed by shaping a 2D plane into a volumetric surface as shown in Figure 9, uploaded and processed using QuPath-0.5.0 - ImageJ Pro software.

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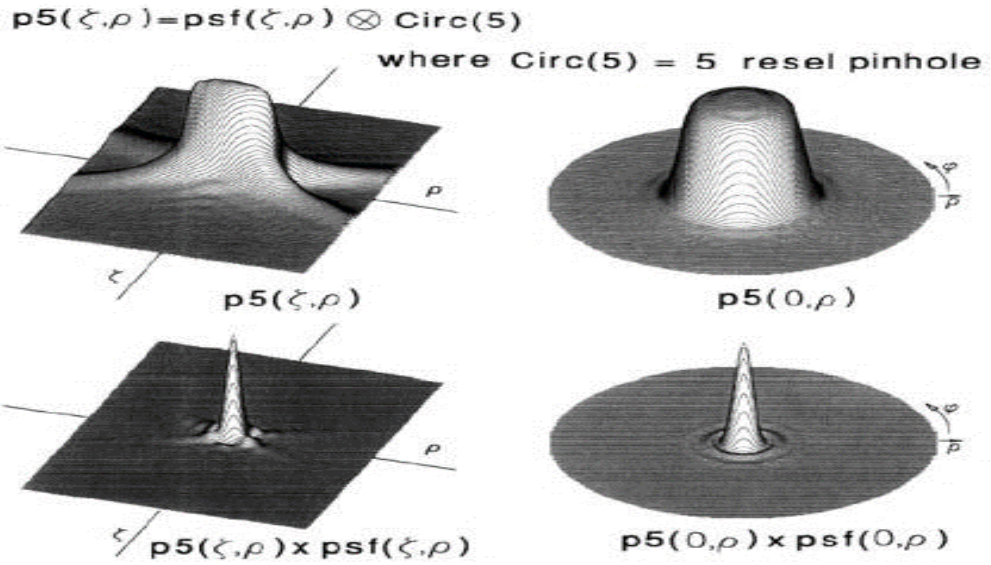

Figure 9. Confocal image is formed through the examples provided in this image for creating a 3D spatial shape in thymus tissue.

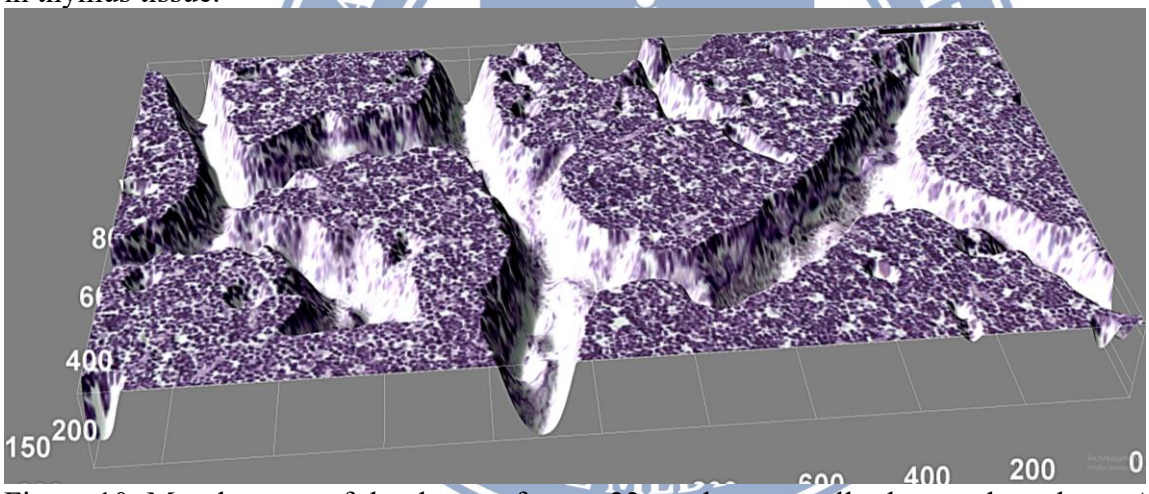

Figure 10. Morphogram of the thymus from a 22-week antenatally deceased newborn. A 3D image depicting numerous voids in the thymus parenchyma, showing a deeply furrowed, exposed stromal spatial shape. Scanned with NanoZoomer. Uploaded to QuPath-0.5.0 - ImageJ Pro software and spatial shape measured.

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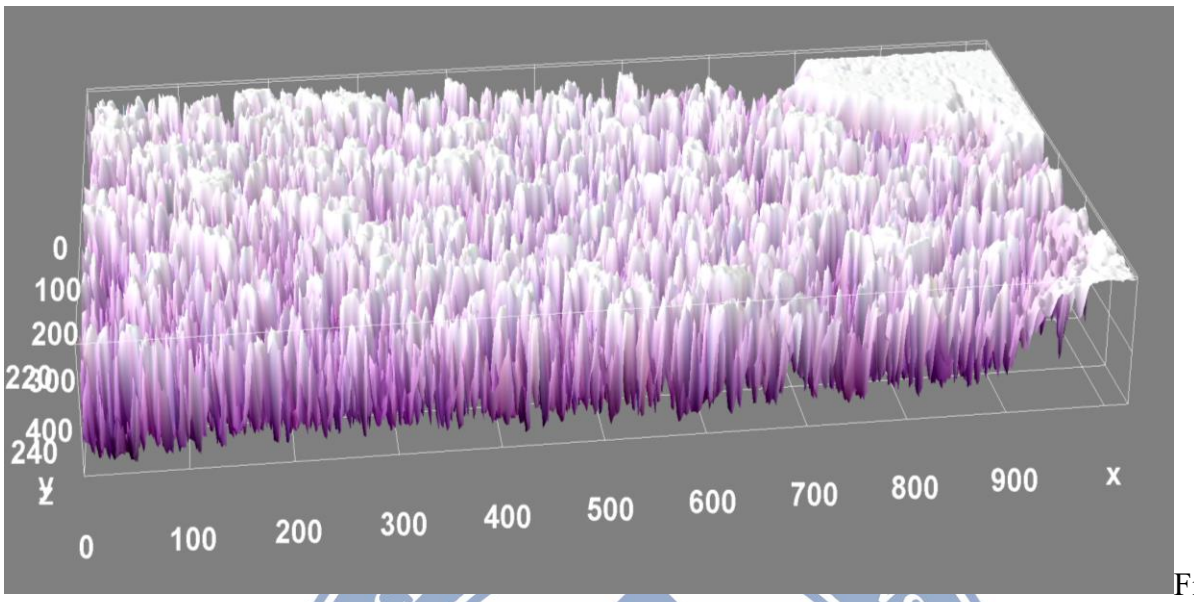
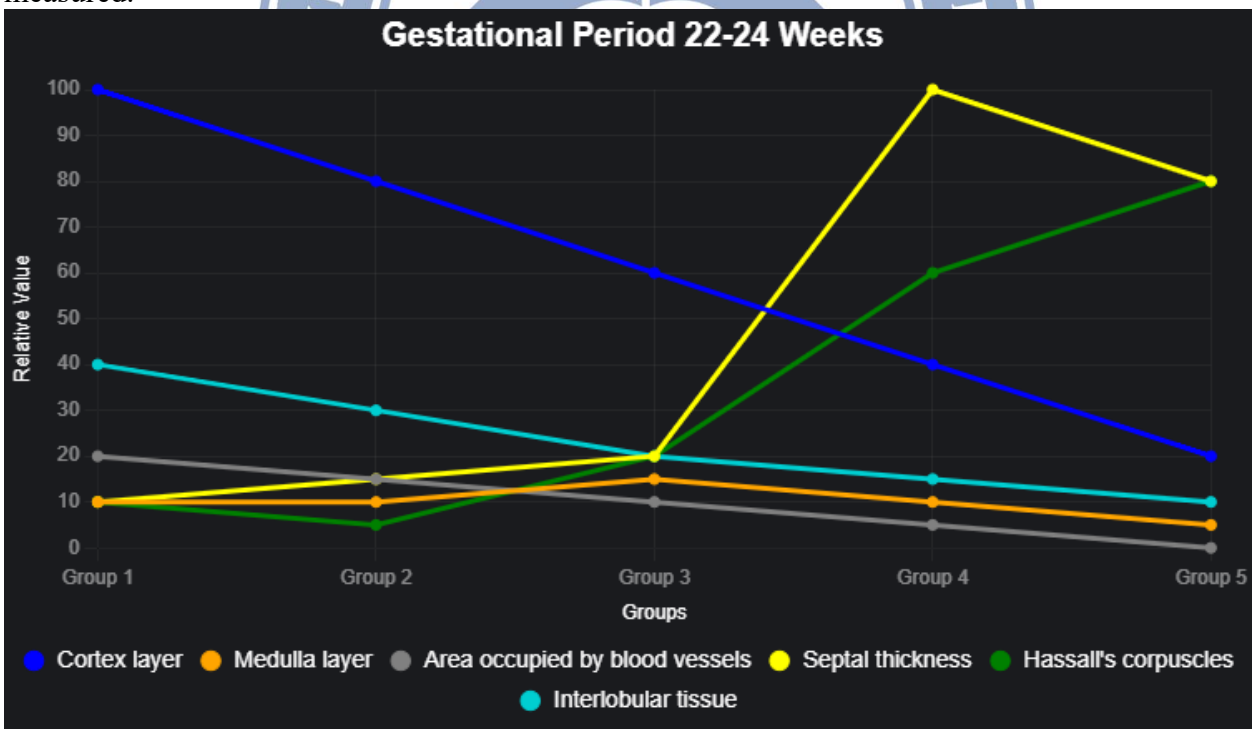


Figure 11. Morphogram of the thymus from a 23-week antenatally deceased newborn. A 3D image depicting numerous voids in the thymus parenchyma, showing a deeply furrowed, exposed stromal spatial shape. Scanned with NanoZoomer. Uploaded to QuPath-0.5.0 - ImageJ Pro software and spatial shape measured.



Confocal morphometric indicators of the structural components of the thymus gland during the gestational period were conducted in a volume of 320.76 mm³.

Conclusion

Based on the provided data, comparative differences in volumetric changes across groups and their distinctions from each other were identified. Accordingly, in our comparative analysis of the cortical layer thickness of the thymus from Group 1 to Group 5, the results showed an 11:1 ratio preserved, with a sharp decreasing pattern in the thickness changes of the cortex and medulla layers. In terms of the volumetric area occupied by blood vessels, in confocal morphometric aspects, the mutual comparative difference between Groups 1 and 5 was evident with high statistical indicators,

averaging a 57.4-fold reduction in blood vessel volume, manifesting as hypoplasia and aplasia in the angiogenesis process, indicating a sharp decline in the functional indicators of the thymus. This, in turn, confirms that the smaller the weight of the thymus gland presented in 5 different weights during the 22-24 week period, the more sharply its morphological maturity indicators lag behind. In septal thickness as well, comparative indicators from Group 1 to Group 5 showed an average thickening of 2.2-11.0 times, confirming that the main components in the thymus composition are stromal elements, with a sharp decrease in lymphocytes and compression of the thymus parenchyma. In terms of the volumetric area occupied by Hassall's corpuscles, the comparative differences between Group 1 and Group 5 showed an average increase of 1.92-10.9 times. This is morphologically explained by the fact that during the 22-24 week period, the main components of the thymus gland consist of morphofunctionally inactive stromal elements and various necrotic inclusions.

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