ISSN NO: 2770-2936
January, 2026

Histological Changes In The Ovaries At Early Stages Of Postnatal Development

Akramova Makhfuza Yuldashevna
[https://orcid.org/0009-0003-2372-7536]
Associate Professor at Tashkent State Medical University
223 Bogishamol St., Tashkent 100140, Uzbekistan.
E-mail: mahfuza-978@mail.ru,
Mobile: +998 94 653 31 41

Abstract. The article deals with the study the histotopographic changes in ovarian tissue of 6-month-old children. The ovaries of 6-month-old children who died of pneumonia were studied histologically and histochemically. The results show that the primordial eggs of a 6-month-old child are located in the middle zone of the cortex, have different sizes, some are atretized, others are vacuolized, and granulose and teka cells are activated randomly. they are localized and proliferate in interstitial tissue. Histochemically, it was found that collagen fibers stained with picrofuxin appeared around blood vessels between primordial eggs, acid glycosaminglycans stained with Altian blue are few due to the density of the tissue of the cortical layer and diffusely distributed due to the rarity of the cerebral layer. the tissue is swollen and myxmatous. At the age of 6 months, it is determined that the ovarian medulla is insufficiently developed, the blood vessels are small, the wall cells are hyperchromic due to lack of differentiation, and the cells and fibrous structures in the intermediate connective tissue are few and incomplete.

Key words: child, ovary, histology, histochemistry, histotopography, primordial eggs, atresia.

Гистологические Изменения Яичников На Ранних Этапах Постнатального Развития

Акрамова Махфуза Юлдашевна
[https://orcid.org/0009-0003-2372-7536]

Доцент Ташкентского государственного медицинского университета
100140, Узбекистан, г. Ташкент, улица Богишамол, 223.
Электронная почта: mahfuza-978@mail.ru,
Мобильный: +998 94 653 31 41

Резюме. Статья рассматривает исследование гистотопографических изменений ткани яичников детей 6-месячного возраста. Гистологическим и гистохимическим методами были изучены яичники детей 6-месячного возраста, умерших от пневмонии. Результаты показывают, что примордиальные яйцеклетки у 6-месячного ребенка расположены в средней зоне коры, имеют разный размер, одни атретизированы, другие вакуолизированы, а гранулезные и тека-клетки активируются хаотично. локализуются и пролиферируют в интерстициальной ткани.

Гистохимически установлено, что коллагеновые волокна, окрашенные пикрофуксином, появились вокруг кровеносных сосудов между примордиальными яйцеклетками, кислые гликозамингликаны, окрашенные альтианским синим, немногочисленны из-за плотности ткани коркового слоя и диффузно распределены из-за редкости мозгового слоя. ткань набухшая и миксматозная. В возрасте 6 мес определяют, что мозговое вещество яичника развито недостаточно, кровеносные сосуды мелкие, клетки стенки гиперхромны из-за отсутствия дифференцировки, а клетки и фиброзные структуры в промежуточной соединительной ткани немногочисленны и сформированы не полностью.

Ключевые слова: ребенок, яичник, гистология, гистохимия, гистотопография, примордиальные яйцеклетки, атрезия.

ISSN NO: 2770-2936
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Postnatal Rivojlanishning Dastlabki Bosqichlarida Tuxumdonlarning Gistologik O‘Zgarishlari

Akramova Mahfuza Yuldashevna
[https://orcid.org/0009-0003-2372-7536]

Toshkent davlat tibbiyot universiteti dotsenti
100140, O‘zbekiston, Toshkent, Bog‘ishamol ko‘chasi, 223.
E-mail: mahfuza-978@mail.ru,
Mobil: +998 94 653 31 41

Аннотация. Ушбу мақолада 6 ойлик чақалоқ тухумдони тўқимасининг гистотопографик ўзгаришлари ўрганилган. Пневмониядан ўлган 6 ойлик чақалоқлар тухумдони гистологик ва гистокимёвий жиҳатдан ўрганилди. Натижалар кўрсатишича, чақалоқлар 6 ойлик даврида тухумдонда примордиал тухум ҳужайралар пўстлоқ қаватининг ўрта соҳасида жойлашади, ҳар хил катталикда бўлади, айримлари атрезияланади, бошқалари вакуоллашган ҳолатда, оралиқ тўқимасида гранулёз ва тека ҳужайралар фаоллашади, бетартиб жойлашиб, пролиферацияланади.

Гистокимёвий жиҳатдан примордиал тухум ҳужайралар орасидаги қон томирлар атрофида пикрофуксин билан бўялган коллаген толалар пайдо бўлганлиги, пўстлоқ қават тўқимаси зич бўлганлигидан альциан кўки билан кўкга бўялган нордон гликозамингликанлари кам, мағиз қавати сийрак бўлганлигидан диффуз ҳолда тарқалганлиги, тўқиманинг шишга ва миксаматозга учраганлиги аниқланади. Чақалоқларнинг 6 ойлик даврида тухумдон мағиз қавати яхши ривожланмаганлиги, қон томирлари майдалиги, деворининг ҳужайралари кам дифференциялланганлиги сабабли гиперхромлилиги оралиқ бириктирувчи тўқимасида ҳужайралар ҳам, толали тузилмалар ҳам кам ва тўлиқ шаклланмаганлиги аниқланади.

Калит сўзлар: чақалоқ, тухумдон, гистология, гистохимия, гистотопография, примордиал тухум ҳужайралар, атрезия.

Level of Problem Investigation. At the 5th week of embryogenesis, the ovary begins to develop from the coelomic epithelium, mesenchyme, and gonocytes. The follicular epithelium and corpus luteum cells originate from the coelomic epithelium. The connective tissue of the ovarian stroma and the thecal tissue surrounding the follicles are formed from the mesenchyme. Gonocytes initially differentiate into oogonia and subsequently into primary and secondary oocytes [1,2,5,6,7,8].

At the 6th week of embryogenesis, primordial germ cells accumulate beneath the coelomic epithelium and migrate into the mesenchyme in the form of bundles. By the 7th week, the ovary undergoes an indifferent stage and becomes part of the female gonad. From the 12th week, the outer layer of ovarian tissue thickens due to the proliferation of gonocytes and the coelomic epithelium.

Between the 12th and 20th weeks, the cortical layer of the ovary is subdivided into germ cell cords consisting of proliferating gonocytes and pregranulosa cells. During this period, thecal cells appear in the interstitial tissue of the ovary; these cells are characterized by small, basophilically stained, oval-shaped nuclei [5,6,7,8,9,10].

With further ovarian development, a portion of the germ cells undergoes degeneration, and primordial follicles appear, predominantly located in areas adjacent to the medulla. By the 32nd week, fully developed follicles are formed, in which granulosa cells constitute 6–8 layers, surrounded by the formation of an inner thecal connective tissue layer. Data on histotopographic changes occurring in the ovary during various periods of postnatal ontogenesis in children, including the period up to the first 6 months of life, are scarcely represented in the scientific literature [5,6,7,8,9,10].

Aim of the Study. The aim of this study was to conduct a histological examination of the ovaries of newborns who died at the age of 6 months from various causes, and to clarify the histological changes

ISSN NO: 2770-2936
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occurring in the early period of postnatal ontogenesis in tissue structures performing reproductive and endocrine functions.

Materials and Methods. The study material consisted of ovaries obtained from 22 six-month-old children who died from pneumonia. The specimens were bisected through the middle of the ovary and fixed in 10% neutral formalin for 48 hours with the cut surface facing upward. Subsequently, the samples were washed in running water for 2–4 hours, dehydrated in graded alcohols and chloroform, embedded in paraffin, and sectioned.

Histological sections 5–7 μm thick were prepared from paraffin blocks and stained with hematoxylin and eosin. Fibrous structures of connective tissue in the ovarian tissue were examined using the Van Gieson staining method with picrofuchsin, while acidic glycosaminoglycans were stained with Alcian blue. The histological specimens were examined under a binocular light microscope, and representative areas were photographed.

Results and Discussion. Histological examination of the ovaries of 6-month-old children revealed diffusely distributed primordial oocytes of varying sizes in the intermediate part of the cortical layer. It was noted that the outer capsule of the ovary was somewhat thickened compared to the previous developmental period and consisted of eosinophilic connective tissue fibers. Beneath the outer capsule, swollen and inflamed granulosa and coelomic cells were observed.

It was established that structures composed of primordial oocytes and follicles within the cortical layer became thinner and relatively larger in size as they approached the medullary region. A distinctive difference in the structure of stromal tissue and the cells located between primary oocytes was observed.

By the 6th month, connective tissue cells and fibers between primordial follicles in the cortex were relatively increased in number (Fig. 1) and appeared denser, whereas in the inner cortical region and in areas adjacent to the medullary boundary, connective tissue cells in the interstitial space were more numerous and exhibited hyperchromatic staining. In certain cortical areas, primordial cells undergoing atresia were found among primordial follicles; their components stained dark blue with hematoxylin, indicating the development of calcinosis.

Further observations were made during microscopic examination of primordial oocytes and follicles in the ovarian cortex. In some primordial structures, an oocyte with a large nucleus and relatively hyperchromatic cytoplasm was identified, surrounded by proliferating and activated granulosa and theca cells (Fig. 2). These cells were irregularly arranged, with enlarged, hyperchromatic nuclei.

In the majority of germinal structures, homogeneous eosinophilic material lacking nuclei was detected, surrounded by granulosa cells with sparse and damaged layers. Between primordial oocytes, i.e., within the interstitium, pregranulosa and coelomic cells appeared in relatively large clustered accumulations in some areas, while in other regions they were sparsely distributed in small numbers.

Blood vessels of the interstitial tissue consisted mainly of small capillaries, most of which exhibited pale staining. Histochemical examination of the ovarian cortical layer in 6-month-old children demonstrated that among densely packed primordial oocytes there were numerous connective tissue cells and relatively few fibers. Picrofuchsin staining revealed small amounts of light violet and reddish fibers in certain areas (Fig. 3). It was established that these fibers were predominantly concentrated around blood vessels.

Histochemical analysis of immature ovarian tissue during this period showed abundant accumulation of acidic glycosaminoglycans in the interstitium, as evidenced by intense blue staining with Alcian blue. Since the cortical layer consists of relatively dense tissue with limited interstitial material, Alcian blue–positive acidic glycosaminoglycans in the cortex were mainly localized around primordial oocytes and blood vessels.

Due to the abundance of acidic glycosaminoglycans in the region between the cortical and medullary layers, as well as within the medullary tissue, these areas appeared edematous and myxomatous. The perivascular regions, in particular, demonstrated more intense Alcian blue staining.

ISSN NO: 2770-2936
January, 2026

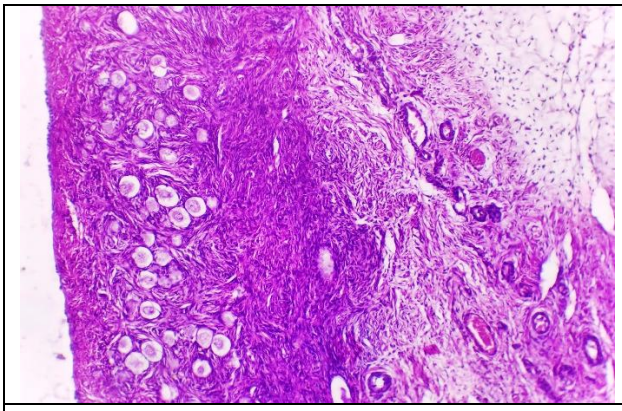

Figure 1. Cortical and medullary layers of the ovary of a 6-month-old child showing the density of interstitial connective tissue in the cortical layer. Staining: H&E. Magnification: 10×10.

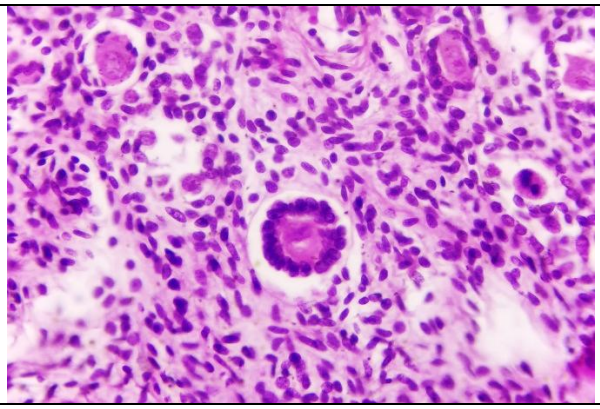

Figure 2. Hypertrophy and activation of granulosa and theca cells in the cortical layer and interstitial tissue of the ovary of a 6-month-old child. Staining: H&E. Magnification: 10×40.

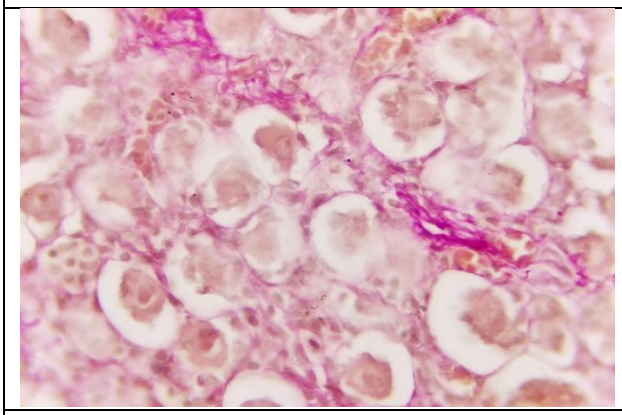

Figure 3. Cortical layer of the ovary of a 6-month-old child, showing the absence of connective tissue fibers. Staining: Van Gieson. Magnification: 10×10.

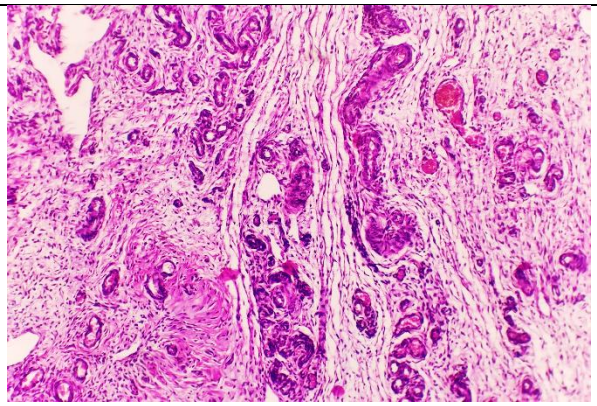

Figure 4. Medullary layer of the ovary at this period, rich in small blood vessels with poorly developed interstitial tissue. Staining: H&E. Magnification: 10×10.

Microscopic examination under the objective lens revealed the following findings: in the interstitial tissue between primordial oocytes, there was an abundance of acidic glycosaminoglycans, manifested by intense dark-blue staining and swelling of the interstitium. As a result, the cytoplasm of primary oocytes also exhibited marked swelling and vacuolization.

These data were obtained during the morphological examination of the ovarian medulla of a 6-month-old child. Based on its general appearance, the medullary layer was markedly lighter than the cortical layer and contained a large number of blood vessels of relatively small diameter. The blood vessels were arranged in groups between bundles of connective tissue within the interstitium and included both arteries and veins. It was established that the interstitial tissue of the medullary layer consisted of thin, loose connective tissue containing a small number of connective tissue cells.

It was observed that fibrous structures were relatively scarce, arranged parallel to one another, and mainly surrounded the blood vessels (Fig. 4). In addition, focal areas of connective tissue were identified within the ovarian medulla, in which the fibers were denser, homogenized, and more intensely stained with eosin.

To assess the amount of acidic substances in the ovarian medulla, it was determined that the medullary tissue was loose and thin, with a wide and swollen interstitium. The looseness and swelling of the connective tissue resulted in an increased content of acidic glycosaminoglycans, which impart hydrophilic properties to the tissue. The presented microphotograph demonstrates that the accumulation of acidic glycosaminoglycans

ISSN NO: 2770-2936
January, 2026

in the interstitium of the medullary connective tissue of the ovary and around blood vessels is confirmed by the edematous and myxomatous condition of the tissue.

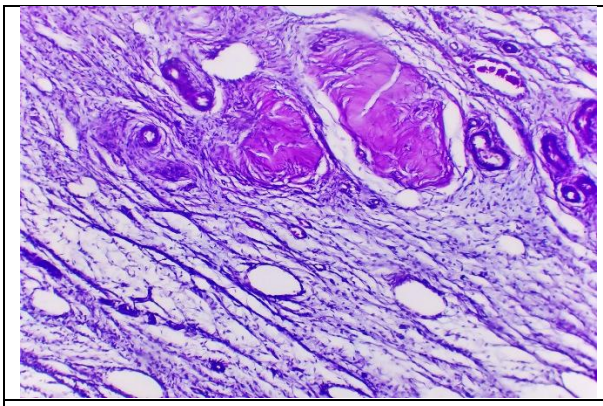

Figure 5. Medullary substance of the ovary appearing as a homogeneous matrix rich in collagen fibers. Staining: Alcian blue. Magnification: 10×10.

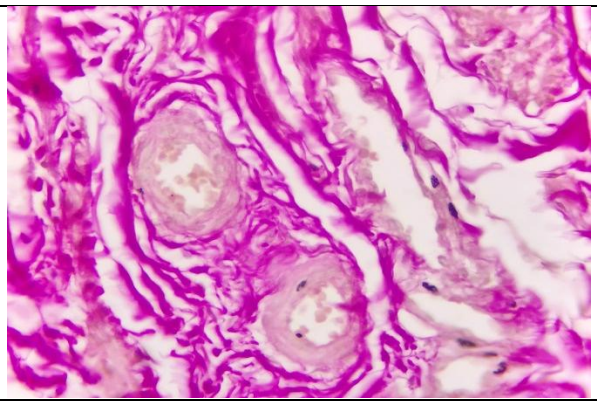

Figure 6. Picrofuchsin staining of the medullary tissue showing bundles of collagen fibers of varying thickness on the outer surface of the vessel wall, around vessels, and within the interstitial tissue. Staining: Van Gieson. Magnification: 10×40.

As mentioned above, specific structures were identified in the ovarian medulla, namely focal formations in the form of compact, oozing bodies resembling homogenized hyalinosis, which were stained dark red with picrofuchsin (Fig. 5). It was established that these structures were surrounded by blood vessels encased in connective tissue rich in fibers and acidic glycosaminoglycans.

Microscopic examination of numerous blood vessels in the ovarian medulla revealed that medullary arteries varied in diameter, and the tissue and cellular components of their walls appeared relatively dark, indicating a low degree of differentiation. It was noted that the endothelium protruded into the vascular lumen, smooth muscle cells were arranged circularly, and their nuclei were elongated and hyperchromatic. Proliferation and enlargement of pericytes surrounding the walls of certain arteries were also observed.

When the ovarian medulla was stained with picrofuchsin to identify fibrous connective tissue structures, it was found that the muscular layer of the arterial wall was not stained, whereas fibrous structures were present only in the outer layer of the vessel wall, which stained red (Fig. 6). In addition, bundles of collagen fibers of varying thickness were identified in the connective tissue between vessels; some of these fibers encircled the vessels, while others formed bundles oriented in different directions.

Conclusions.

The results demonstrate that in a 6-month-old child, primordial oocytes are located in the intermediate zone of the ovarian cortex and vary in size; some undergo atresia, while others are vacuolated. Granulosa and theca cells show irregular activation, localization, and proliferation within the interstitial tissue.

Histochemical analysis revealed that collagen fibers stained with picrofuchsin were present around blood vessels between primordial oocytes. Acidic glycosaminoglycans stained with Alcian blue were scant in the cortical layer due to its dense tissue structure and diffusely distributed in the medullary layer because of its loose organization; the tissue exhibited swelling and a myxomatous appearance. At the age of 6 months, the ovarian medulla was found to be insufficiently developed: blood vessels were small in caliber, the cells of the vessel walls were hyperchromatic due to a lack of differentiation, and cellular and fibrous structures in the interstitial connective tissue were few in number and incompletely formed. Dense homogenized foci of collagen fibers resembling hyalinosis were identified in the ovarian medulla.

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ISSN NO: 2770-2936
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