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Morpho-Immunohistochemical Characteristics Of The Ovaries In Early Childhood

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Annotation. This article aims to determine the specific morphological and immunohistochemical signs of the formation of a child's ovary in the embryonic and early postpartum period in girls. Morphologically and histochemically, the ovaries of 22 infants who died from asphyxia up to 3 months were studied. The results of the study showed that in the reproductive period, the development of ovarian dysfunction caused by pathologies in embryonic ontogenesis was detected. It was confirmed that the pathologies of the embryonic period affected the development of the ovary as a dangerous factor, leading to the development of hypoxia, dishormonal interaction, dystrophy and damage to ovarian tissue, the development of the main morphofunctional structures of the ovary, can be destroyed, necrosized in the fetal period, develop reparative inflammation or lead to hyperplasia.

Keywords: infant, early postnatal period, ovary, ontogenesis, morphology, histochemistry of ovaries, primordial egg, reproductive, endocrine.

Морфо-Иммуногистохимическая Характеристика Яичников У Детей Раннего Возраста

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Аннотация. В данной статье ставится цель определить специфические морфологические и иммунногистохимические признаки формирования яичника ребенка в эмбриональном и раннем послеродовом периоде у девочек. Морфологически и гистохимический изучены яичники 22-х младенцев, умерших в период от асфиксии до 3 месяца. Результаты исследования показали, что в репродуктивном периоде было обнаружено развитие нарушения функции яичников, обусловленного патологиями в

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эмбриональном онтогенезе. Было подтверждено, что патологии эмбрионального периода влияли на развитие яичника как опасный фактор, приводя к развитию гипоксии, дисгормонального взаимодействия, дистрофии и повреждению ткани яичника, развитию основных морфофункциональных структур яичника, могут разрушаться, некрозироваться в плодном периоде, развиваться репаративное воспаление или приводить к гиперплазии.
Ключевые слова: младенец, ранний постнатальный период, яичник, онтогенез, морфология, гистохимия яичников, примордиальная яйцеклетка, репродуктивная, эндокринная.

Эрта Ёшдаги Болалардатухумдонларнинг Морфо-Иммуногистокимёвий Тавсифи

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Аннотация. Ушбу мақолада қиз болаларда эмбрионал ва эрта постнатал (туғруқдан кейинги) даврда тухумдон шаклланишининг ўзига хос морфологик ва иммуногистокимёвий белгиларини аниқлаш мақсад қилиб қўйилган. Асфиксия ҳолатидан то 3 ойлик давргача вафот этган 22 нафар чақалоқнинг тухумдонлари морфологик ва гистокимёвий усулларда ўрганилди. Тадқиқот натижалари репродуктив даврда тухумдон функциясининг бузилиши эмбрионал онтогенездаги патологиялар билан боғлиқ эканлигини кўрсатди. Эмбрионал давр патологиялари тухумдон ривожланишига хавфли омил сифатида таъсир қилиб, гипоксия, дисгормонал ўзаро таъсир, дистрофия ва тухумдон тўқимасининг шикастланишига олиб келиши тасдиқланди. Натижада тухумдоннинг асосий морфо-функционал тузилмалари зарарланиши, емирилиши, ҳомилавий даврда некрозга учраши, репаратив яллиғланиш ривожланиши ёки гиперплазия юзага келиши мумкин.
Калит сўзлар: чақалоқ, эрта постнатал давр, тухумдон, онтогенез, морфология, тухумдон гистокимёси, примордиал тухум ҳужайраси, репродуктив, эндокрин.

Objective of the study: To identify the specific morphological and immunohistochemical features of ovarian development in girls during the early postnatal period.
Materials and Methods. The study material consisted of the ovaries of 22 children who died from asphyxia and pneumopathy before 3 months of age, resulting from umbilical cord aspiration or tight cord wrapping.

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Ovarian dysfunction may develop during the reproductive period as a consequence of pathologies in embryonic ontogenesis. Embryonic period pathologies can act as risk factors affecting ovarian development, leading to hypoxia, hormonal imbalances, dystrophy, and tissue damage. They may also delay the formation of the main morphofunctional structures of the ovary, cause tissue destruction, necrosis, trigger reparative inflammation, or result in hyperplasia during the fetal period.

Relevance of the problem. Ovarian pathologies occupy an important place in the structure of gynecological diseases [3,4,7–14]. The frequency of infertility among married couples is 10–15%, and if it is caused by endocrine disorders, it rises to 35–40%. Impaired folliculogenesis in the ovary plays a key role in the development of infertility. According to scientific literature, the level of oocyte development is directly related to the morphofunctional completion of folliculogenesis [1,2,4,8,9]. The ovary is one of the organs with the most complex structure in the human body. This complexity is due to the involvement of three embryonic tissues—endoderm, mesoderm, and ectoderm—in the formation and differentiation of the ovary. The complexity of ovarian structure also lies in the fact that, during its development, it passes through all stages of undifferentiated glands [3,5,6,8–14]. Follicles are an important structural unit of the ovary; they produce the corpus luteum, atretic bodies, and perform reproductive and endocrine functions of the organism in the post-embryonic period. Currently, as established by several researchers, there is a confirmed connection between ovarian pathologies—such as polycystic ovary syndrome, ovarian insufficiency, and other reproductive disorders—and the development of the gonads in girls during the perinatal period.

Results and Discussion. Morphological analysis showed that the outer surface of the child's ovary was covered with a single-layered epithelium, which was flattened in some areas but predominantly cuboidal in shape. Beneath the epithelium, a basal membrane was observed, the superficial layer of which consisted of thin fibers, while the deeper layer was composed of unformed connective tissue. From the unformed connective tissue layer of the basal membrane, fibrous bundles of varying sizes penetrated into the cortical layer of the ovary.

Scanning and topographic examination of histological sections prepared from the ovary revealed that the cortical layer occupies a relatively large area and contains a significant number of primordial follicles. In the ovaries studied, various histotopographic structures were identified. In some cases, the cortical layer occupies a large area; in others, it is relatively thin and covers a smaller area. In certain specimens, the boundary between the cortical and medullary layers was indistinct. In some cases, the cortical layer was wide in one area and narrow in another, and in other cases, primordial follicles were few in number and unevenly distributed within the cortical layer—sparse and chaotic in some regions and denser in others.

These observations confirm that the histotopographic structure of the ovaries in newborns is highly variable. The ovaries exhibit different histotopographic patterns, forming at different levels during the embryonic period and differentiating at distinct stages of development.

Histological Findings of Newborn Ovaries. Histological examination of newborn ovaries shows that the cortical layer is complete, while the marginal regions of the medullary substance are diffusely arranged, consisting of primordial primary oocytes of almost uniform size. The outer layer of the ovary is composed of connective tissue fibers with a relatively thin, eosinophilic structure. Beneath the outer layer, swollen and inflamed granulosa and coelomic cells are observed.

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Structures composed of primordial oocytes and follicles in the cortical layer become thinner and relatively larger in size as they approach the medullary substance. Differences are noted in the structure of the stromal tissue and the cells located between them. While connective tissue cells and fibers are relatively sparse and poorly distributed among the primordial follicles in the cortical layer, in the inner cortical regions and medullary interstitium, connective tissue cells are numerous and exhibit hyperchromatic staining. Among the primordial follicles in the cortical layer, atresia is observed, and their components are stained dark blue with hematoxylin, indicating calcification.

Microscopic examination of primary oocytes and follicles in the ovarian cortex yielded the following observations. In some primordial structures, oocytes with large nuclei and relatively hyperchromatic cytoplasm are surrounded by a single layer of granulosa cells. In most primordial structures, a homogeneous eosinophilic material without a nucleus is observed, surrounded by granulosa cells that are sparse and partially damaged.

In the interstitial spaces between primordial oocytes, pre-granulosa and coelomic cells appear in relatively large clusters in some areas, while in other regions they are sparsely distributed in small numbers. Blood vessels in the interstitial tissue consist of small capillaries, most of which appear pale.

Microscopic Findings in the Ovaries of 3-Day-Old Girls. Microscopic examination of the ovaries of 3-day-old girls revealed the following morphological changes compared with newborns. Primordial oocytes located in the cortical layer of the ovary were relatively rare due to proliferation in the interstitial tissue. Histotopographically, these oocytes varied in size and shape. In the center of the primordial structures, oocytes stained more intensely with eosin than others, with hyperchromatic nuclei stained by hematoxylin; such histological features indicate that these primordial oocytes are undergoing atresia. Granulosa cells surrounding these oocytes also showed signs of degeneration and pathomorphological changes.

Additionally, blood vessels in the interstitial tissue were filled with blood, and some contained diapedetic blood. Unlike the previous period, in some regions of the interstitial tissue, fibrous connective tissue proliferation was observed. Pre-granulosa and coelomic cells were arranged in clusters of varying size, in contrast to the arrangement seen in the ovaries of newborns.

Histotopographic changes developing in the ovaries of infants in the early postnatal period, 20–30 days after birth, were also variable. In some cases, a reduction in the number of primary primordial follicles was observed, along with the appearance of sclerotic and atretic follicles, isolated follicles, and nodular oocytes. In other infants, cystic atresia of follicles (Figures 1 and 2) was observed in the peripheral cortical regions. Mature and developed follicles were located in the deeper parts of the cortical layer, near the medullary layer, with their size determined by enlargement of both the nucleus and cytoplasm of the oocyte.

During the early postnatal period, primordial follicles in the cortex became variable in size, the epithelium lining their inner surface became hyperplastic, and some oogonia underwent apoptosis and degeneration. Follicles and corpora lutea were not observed in the ovarian tissue at this age. Proliferation and expansion of connective tissue were observed to varying degrees in the interstitium of both the cortical and medullary layers.

Depending on the level of connective tissue proliferation, ovaries can be classified as euplastic, hyperplastic, or hypoplastic. The presence of each type depends on the morphophysiological

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specifics of embryonic ontogenesis. The main morphofunctional structures in the ovarian tissue, including the number and quality of follicles, are determined by embryogenesis.
Thus, morphological examination of newborn ovaries reveals pathomorphological changes that reflect gestational pathologies. For example, children born to mothers with nephropathy had numerous cystic, obliterated, and atretic follicles, as well as hyperplasia of fibrous and thecal tissue in the stroma [3,4,5,10,12–14]. Ovaries of children born to mothers with cardiovascular diseases were 1.5 times smaller, and the vessel walls in their tissue were sclerosed.

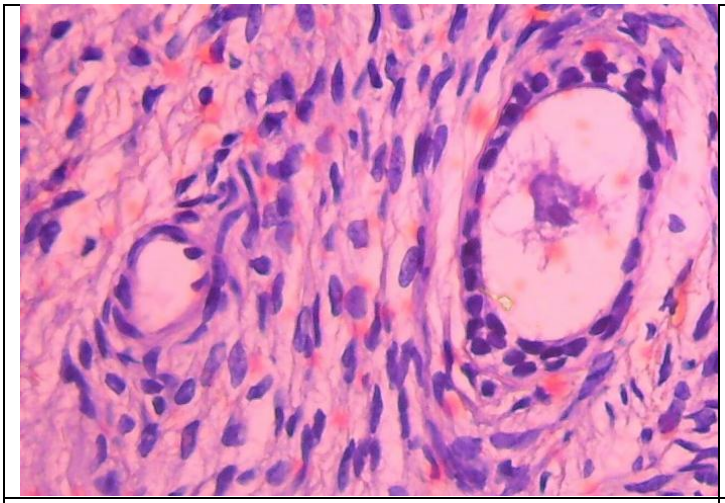

Figure 1. In an 18-day-old child, an ovarian follicle has transformed into a cyst. Staining: H&E (hematoxylin-eosin). Magnification: 10×40.

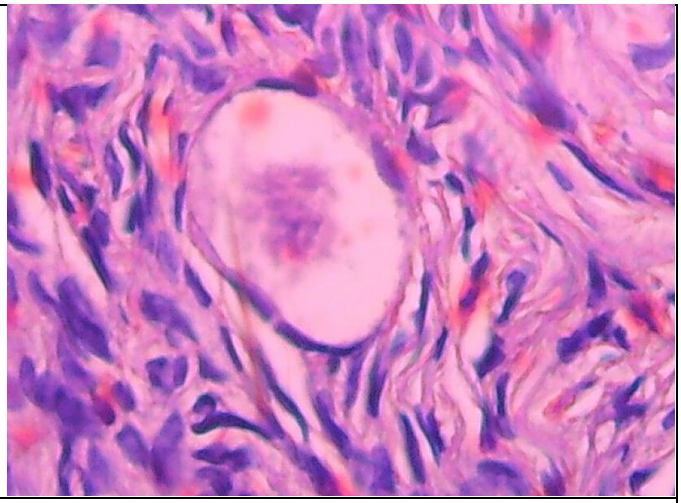

Figure 2. In a 24-day-old child, follicular atresia is observed in the ovary. Staining: H&E (hematoxylin-eosin). Magnification: 10×40.

Histological Analysis of the Cortical–Medullary Boundary in a 3-Month-Old Girl's Ovary. Histological examination of the cortical–medullary boundary in the ovary of a 3-month-old girl revealed the following findings. Unlike in newborns, primordial follicles in this region were relatively sparse and fewer in number. In some follicles, proliferation of granulosa cells led to the formation of cyst-like structures. Most primordial follicles in this area exhibited characteristic pathomorphological changes: in some cases, the central oocyte underwent destruction and disappeared, while surrounding granulosa cells experienced various degenerative changes and were arranged irregularly. In other follicles, granulosa cells proliferated, increased in number, and formed dense conglomerates; in several instances, follicle-like structures of varying sizes developed due to granulosa cell proliferation.
The interstitial tissue in this region also showed distinctive pathomorphological changes (Figure 3). The interstitial tissue was expanded due to edema and cellular infiltration; proliferation of pre-granulosa and coelomic cells resulted in abundant cellular infiltrates. Pre-granulosa and granulosa cells were concentrated around primordial follicles, forming clusters. Coelomic cells were relatively sparse in the interstitial tissue, and fibrous connective tissue proliferation was noted around blood vessels.

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By 3 months of age, the cortical layer of the ovary in infants shows a reduction in the number of primordial oocytes, most of which undergo degenerative and destructive changes. In the interstitial tissue, bundles of fibrous connective tissue appear.

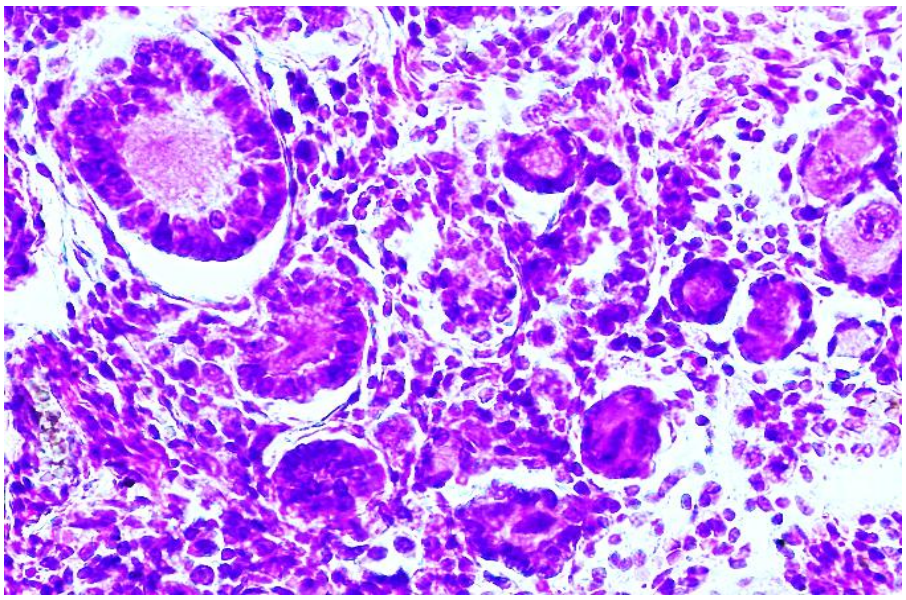

Figure 3. Cortical–medullary boundary of the ovary in a 3-month-old child. Enlargement of individual primordial follicles with the formation of cyst-like structures is observed. Staining: hematoxylin-eosin (H&E). Magnification: 10×40.

Proliferative Activity of Ki-67 in the Ovary at 3 Months of Age. At 3 months of the early postnatal period, the proliferative index of the immunohistochemical marker Ki-67 in the ovarian surface epithelium was 27.16 ± 0.13, confirming a high level of proliferative activity. A relatively high Ki-67 proliferative index (38.64 ± 1.41) was also observed in the connective tissue cells of the ovarian interstitial layer. Morphologically, this corresponded to strong Ki-67 expression, visible as intense dark-brown staining of the nuclei of most histiocytic cells surrounding the ovarian follicles (Figure 4).

Ki-67 expression in both the karyoplasm and nucleoli of epithelial cell nuclei indicates that the cells are in the middle phase of the cell cycle (G2 phase), confirming their functional activity. Thus, at 3 months of age, epithelial cells in the ovary develop relatively slowly, whereas interstitial connective tissue shows more intense growth and proliferation.

In the epithelium of developing ovarian follicles at 3 months, Ki-67 was positively expressed only in individual cells, indicating proliferative activity in both the basal and surface layers of the follicular epithelium, but only in a limited number of cells (Figure 5). At this stage, expression of this immunohistochemical marker in the walls of blood vessels and in the interstitial connective tissue cells was relatively weak.

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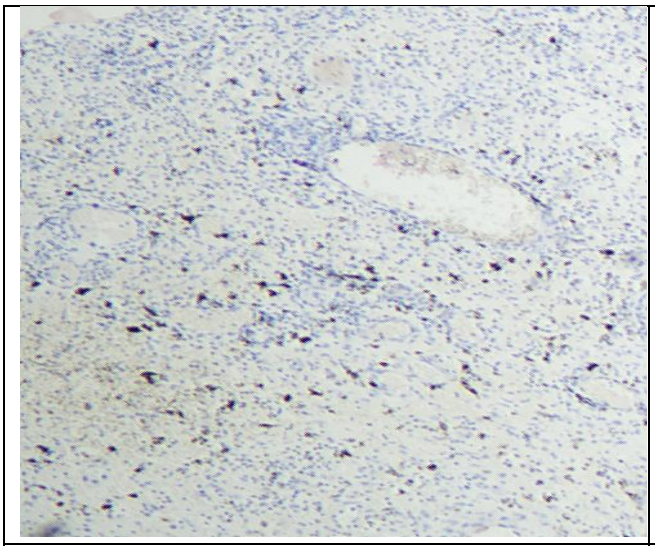

Figure 4. Early postnatal period, 3 months of age. Ki-67 expression in ovarian epithelial cells is weak, whereas it is more intense in stromal cells. Staining: immunohistochemistry. Magnification: 10×20.

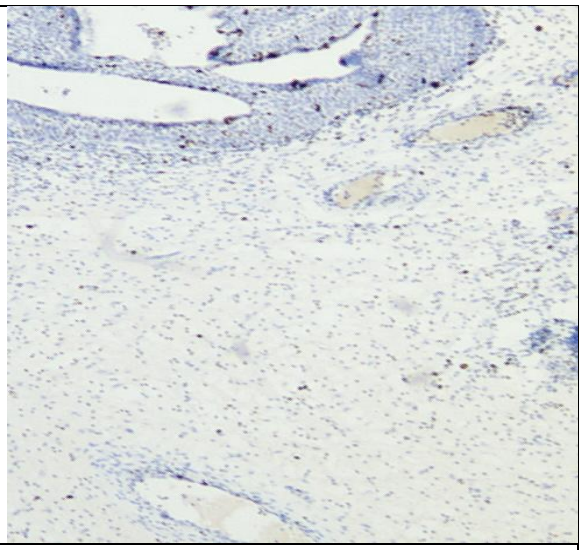

Figure 5. At 3 months of age, Ki-67 expression in the follicular epithelium and stromal cells of the ovary. Staining: immunohistochemistry. Magnification: 10×20.

Conclusions. During the reproductive period, ovarian dysfunction may develop as a result of pathologies in embryonic ontogenesis. Pathologies arising during the embryonic period can act as risk factors affecting ovarian development, leading to hypoxia, hormonal imbalances, tissue dystrophy, and damage to ovarian tissue. These conditions can delay the development of key morphofunctional structures of the ovary, cause destruction, necrosis, reparative inflammation, or hyperplasia during the fetal period. It is therefore important to prevent early and late toxicoses, taking into account critical developmental periods, and to maintain the integrity of the germ cell nuclei.

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