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HISTOLOGICAL AND HISTOCHEMICAL FEATURES OF OVARIES IN INFANTS DURING THE FIRST YEAR OF LIFE

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Abstract

This article is devoted to the identification of morphological changes occurring in the histotopography of the ovaries of 12-month-old children. As a material, the ovaries of girls whose abdomen died from asphyxia and pneumopathy were studied by methods of general histology, histochemistry and morphometry. It is noted that in the cortical layer of the ovary of newborns, rudimentary eggs are located in a dense state, follicular structures are formed as a result of the proliferation of granulose cells in the areas bordering the stratum corneum, and between them, i.e. in the interstitial, there are pregranular and celemic cells of different densities. From the earliest postpartum period in infants, destruction and atresia of part of the embryonic eggs that perform reproductive function in the ovary, granulosis and proliferation of cells of the uterine tissue in the intermediate tissue occur.

Keywords: Infant, early postpartum period, ovary, ontogenesis, morphology.

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Introduction

The ovary is a paired organ and performs two important functions: reproductive — it produces female gametes, and endocrine — it synthesizes sex hormones. According to scientific literature, there is a structural-metabolic relationship between the activity of the oocyte and the follicular histion [1,2,6,7,9,10,11,12]. Endocrine activity also depends on the morphofunctional state of the primary oocytes, follicular epithelium, theca tissue, and corpus luteum.

By the 5th week of embryogenesis, the ovary begins to develop from the coelomic epithelium, mesenchyme, and gonocytes. The follicular epithelium and corpus luteum cells develop from the coelomic epithelium. Connective tissue of the ovarian stroma and theca tissue around the follicles are formed from the mesenchyme. Gonocytes differentiate first into oogonia, and then into primary and secondary oocytes [4,5,6,7,8,9,10].

By the 6th week of embryogenesis, primordial germ cells gather between the cells of the coelomic epithelium and invade the mesenchyme in the form of clusters. By the 7th week, the ovary reaches the stage of an individual organ and begins forming the female gonad. From the 12th week, the outer layer of ovarian tissue thickens due to the proliferation of gonocytes and coelomic epithelium. Between the 12th and 20th weeks, the cortical layer of the ovary divides into sexual lobules, consisting of clusters of proliferating gonocytes and pregranulosa cells. During this period, small oval basophilic theca cells appear in the ovarian interstitial tissue.

Subsequently, during ovarian development, some germ cells degenerate, primordial follicles are formed, and in areas near the medullary layer, mature follicles appear. By the 32nd week, fully mature follicles are formed, in which granulosa cells form 6–8 layers, and an inner theca layer develops around them.

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Objective of the study

The scientific literature provides limited data on histotopographic changes occurring in the ovary of infants at different stages of postnatal ontogenesis [3,4,5,6,7,10,11,12]. Therefore, the aim of the present study was to perform a histological examination of the ovaries of newborns and children up to 12 months of age who died from various causes, and to investigate histological changes in the tissue structures responsible for reproductive and endocrine functions during the early postnatal period.

Materials and Methods

The study material consisted of ovaries from 98 newborns who died as a result of intrauterine asphyxia, pneumopathy, and pneumonia. The ovaries were cut in half along the midline, with the cut surface facing upwards, and fixed in 10% neutral formalin for 48 hours. They were then rinsed with running water for 2–4 hours, dehydrated in ascending concentrations of alcohol and chloroform, embedded in paraffin, and prepared into blocks.

Histological sections 5–7 µm thick were prepared from the paraffin blocks and stained with hematoxylin and eosin. Fibrous structures of the ovarian connective tissue were stained with picrosirius red according to the Van Gieson method, and acidic glycosaminoglycans were stained with Alcian blue. The histological specimens were examined and photographed using a binocular light microscope.

Results

Histotopographic examination of the ovaries of 12-month-old infants, compared to earlier periods, revealed significant maturation of tissue structures in both the cortical and medullary layers. During this period, the number of primordial oocytes in the cortical layer decreases markedly, with some showing atresia or vacuolization. The outer connective tissue layer, forming the white surface of the ovary, becomes slightly thicker, with an increase in the amount of fibrous structures.

Primordial oocytes are sparse, and the connective tissue between them—both cellular and fibrous—expands, forming bundles in various directions. Bundles of

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connective tissue in the interstitial region of the deeper cortical layer exhibit a relatively dense structure (see Fig. 1). Staining of cortical paraffin sections with picrosirius red using the Van Gieson method reveals collagen fibers stained red, oriented in multiple directions (see Fig. 2). The growth of fibers and formation of bundles in different orientations separates the primordial oocytes into distinct groups. Collagen fibers stained intensely red with picrosirius red in the outer white layer are highly dense and abundant. In the medullary layer, fibers are more sparse and fewer in number.

As noted earlier, during infancy, ovarian tissue is relatively acidic and is dominated by acidic glycosaminoglycans. This is confirmed by Alcian blue staining of the ovarian tissue: despite the density of the cortical layer, its interstitial matrix stains blue, indicating accumulation of acidic glycosaminoglycans (see Fig. 3). Although the primordial oocytes are small and vacuolized, an accumulation of acidic glycosaminoglycans stained blue is also observed around them.

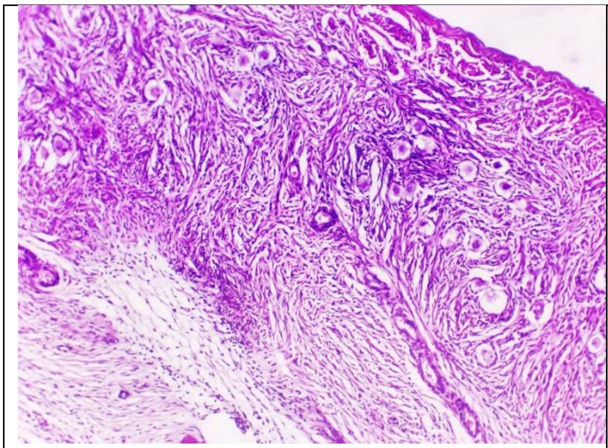

Figure 1. Ovary of a 12-month-old infant. In the cortical layer, the number of primordial oocytes has decreased, and the amount of interstitial connective tissue has increased. Staining: hematoxylin and eosin. Magnification: 10×10.

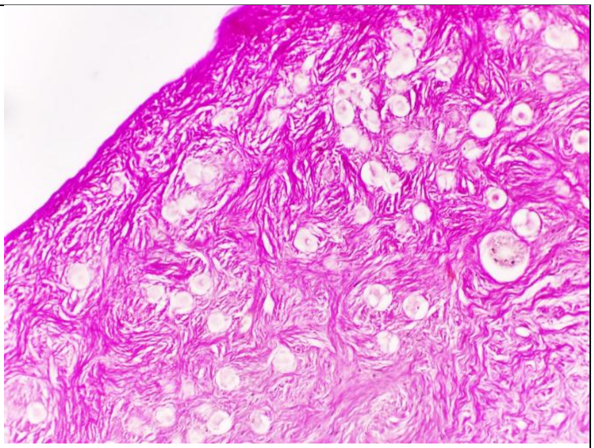

Figure 2. Chaotic arrangement of collagen fibers in the cortical layer of the ovary. Staining: Van Gieson. Magnification: 10×10.

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It was found that the boundary between the cortical and medullary layers stains less intensely due to a lower content of acidic glycosaminoglycans. Similar changes—namely, a low amount of acidic substances—were observed in the medullary tissue, with an uneven distribution: some areas contained more, while others contained less.

Examination of the cortical tissue of infant ovaries under high magnification revealed that primordial oocytes undergo changes of varying degrees: some are vacuolized and enlarged, some are atretic and fail to develop, and others transform into scar tissue (see Fig. 4). Another significant change is the development of connective tissue between the oocytes.

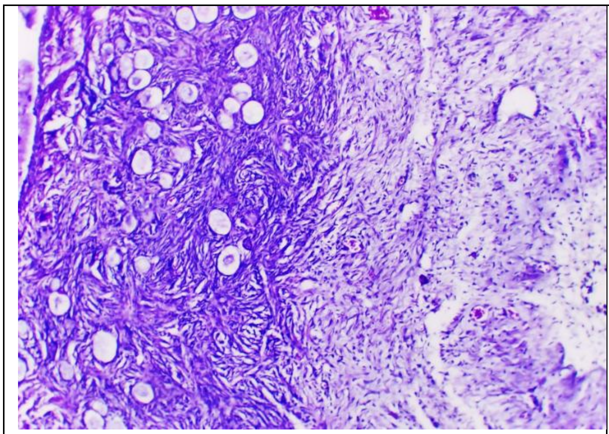

Figure 3. The cortical layer of the ovary is stained dark blue, and the medullary layer is stained light blue. Staining: Alcian blue. Magnification: 10×10.

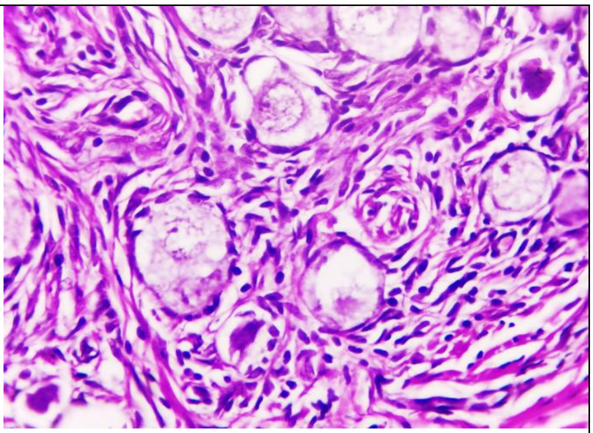

Figure 4. Vacuolization of primordial oocytes, their atresia and underdevelopment, and activation of stromal cells. Staining: hematoxylin and eosin. Magnification: 10×40.

Activation of connective tissue cells is confirmed by enlargement of their nuclei, hypertrophy, and hyperchromasia. Various degrees of collagen fiber development are observed between the cells: some form fibrous bundles, while others consist of newly formed homogeneous material. Upon closer examination of the cortical layer of ovaries from children of this age under high magnification, it was

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revealed that connective tissue and theca tissue develop intensively in the interstitial spaces of the cortical layer (see Fig. 5). Bundles of connective tissue cells and fibrous structures, hypertrophied and activated, are oriented in different directions, with monocytes and lymphocytes appearing within the tissue.

Within these extensively developed bundles of connective tissue, morphologically altered primordial oocytes are located, most of which are vacuolized and transformed into vesicles. The growth of connective tissue in the interstitial spaces of the ovarian cortical layer is confirmed by histochemical staining: picrosirius red staining shows an increased number of fibrous structures stained red (see Fig. 6).

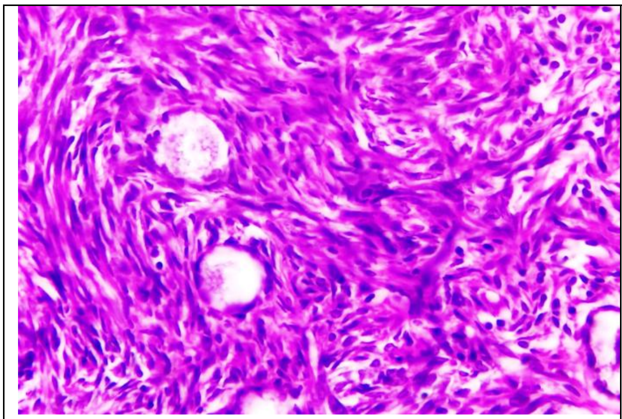

Figure 5. Activation and proliferation of interstitial connective tissue in the cortical layer of the ovary. Staining: hematoxylin and eosin. Magnification: 10×40.

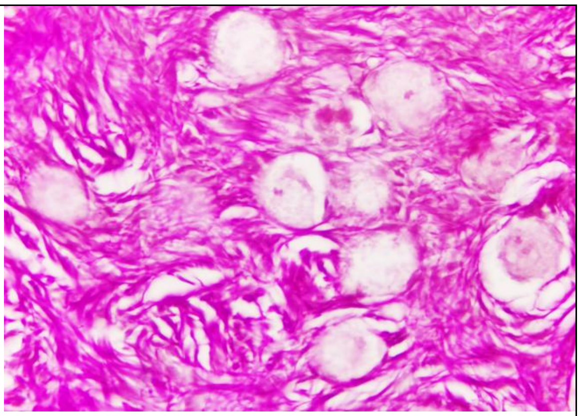

Figure 6. Increase in the number of collagen fibers in the cortical layer of the ovary of a 12-month-old child. Staining: Van Gieson. Magnification: 10×40.

As repeatedly noted, the ovarian tissue of infants under one year of age is rich in acidic glycosaminoglycans. It was established that these features persist in 12-month-old children: in the cortical layer, around the primordial oocytes and in the interstitial matrix, a high concentration of acidic glycosaminoglycans is maintained (see Fig. 7).

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Microscopic examination of the ovaries of 12-month-old infants revealed that some primordial oocytes in the cortical layer had transformed into primary follicles. At the same time, both the nucleus and cytoplasm of the central oocyte exhibited vacillation and destruction. The surrounding follicular cells formed several layers, most of which were also degeneratively altered and arranged irregularly (see Fig. 9).

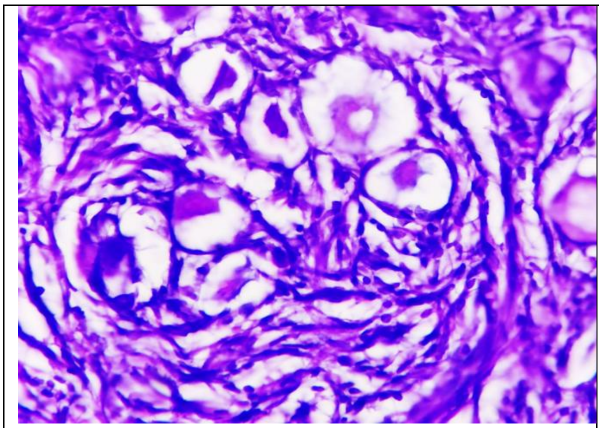

Figure 7. Presence of acidic glycosaminoglycans in the cortical layer of the ovary. Staining: Alcian blue. Magnification: 10×40.

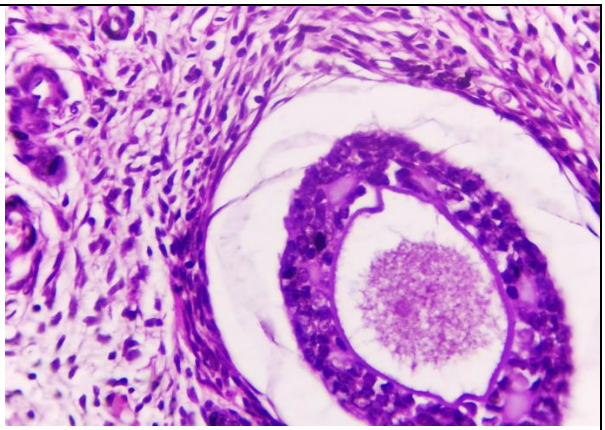

Figure 8. Transformation of a primordial oocyte into a follicle and cystic cavity. Staining: hematoxylin and eosin. Magnification: 10×40.

In the central and peripheral parts of the primary follicle, pronounced edema and vacuolization of cells were observed. Thus, during this period, some primordial oocytes develop into primary follicles, but at the same time undergo destruction and death.
Microscopic examination of the medullary layer of the ovaries of 12-month-old infants revealed a significant increase in its area, a looser tissue structure, and localized edema in some regions. Blood vessels were slightly enlarged compared to earlier periods, with mature walls and no hyperchromasia observed. Areas of pronounced edema and myxomatous changes appeared in the medullary tissue:

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the cytoplasm of cells was vacuolized and expanded, and the tissue itself resembled a reticular myxomatous structure (see Fig. 9).

Around these foci of edema, the tissue was denser, with concentrated fibrous structures, partially homogenized. To assess the prevalence of connective tissue fibers in the medullary layer, a histochemical method—picrosirius red staining using the Van Gieson technique—was applied. It was established that, compared to the cortical layer, there are fewer fibrous structures in the medullary layer, and therefore the tissue stains lighter (see Fig. 10).

The center of the edema and myxomatous focus transforms into a cavity, forming a cystic structure. The surrounding connective tissue is morphologically altered: fibrous elements are transformed into homogeneous protein, occasionally resembling hyaline, accompanied by a reduction in the number of vessels and cells, rendering the tissue pale (see Fig. 11).

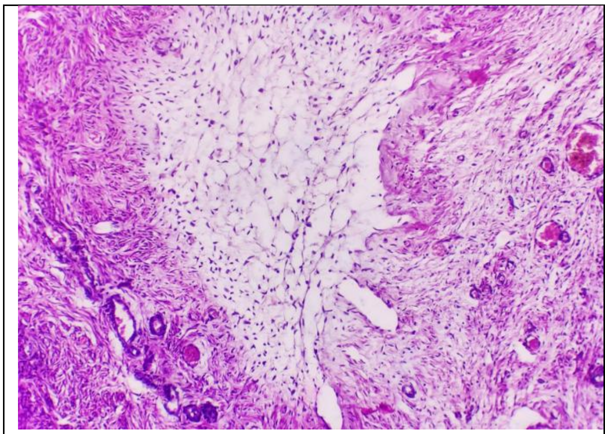

Figure 9. Medullary layer of the ovary of a 12-month-old child, showing the appearance of edema and myxomatous changes. Staining: hematoxylin and eosin. Magnification: 10×10.

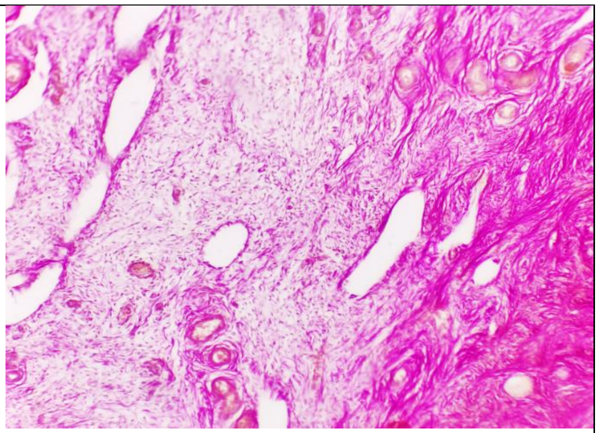

Figure 10. Deficiency of fibrous structures in the tissue of the ovarian medulla. Staining: Van Gieson. Magnification: 10×10.

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Histochemical staining with Alcian blue of the primary cystic cavities in the medullary layer revealed that the homogenized hyaline connective tissue immediately surrounding the cavity contains a high protein content and few acidic substances, and thus stains red (see Fig. 12). At the same time, the surrounding connective tissue is looser and thinner, with a predominance of acidic substances, and stains blue.

Thus, even at this stage, the ovarian tissue maintains an acidic environment with hydrophilic properties.

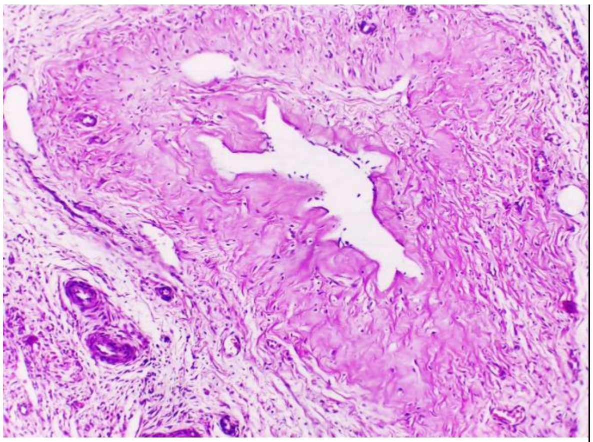

Figure 11. In the ovarian medulla, the connective tissue fibers have homogenized, forming a clear (white) zone. Staining: Hematoxylin–Eosin. Magnification: 10×40.

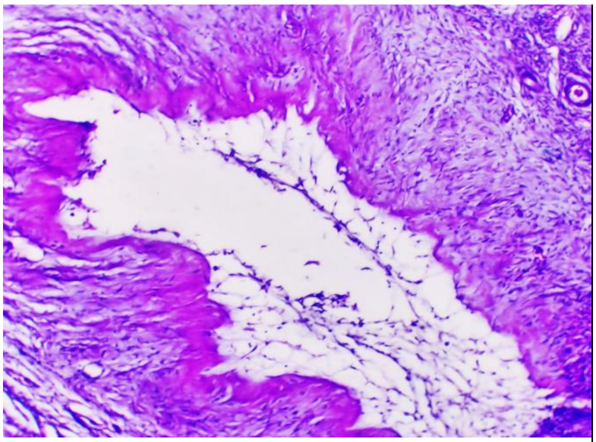

Figure 12. In the tissue immediately adjacent to the cystic cavity of the ovarian medulla, a high protein content is observed, while acidic substances predominate in the surrounding area. Staining: Alcian Blue. Magnification: 10×40.

Conclusion

During the first 12 months of life, a significant decrease in the number of primordial oocytes in the ovarian cortical layer is observed, along with changes in their shape and the appearance of atretic and vacuolated cells. During this period, proliferation of both cellular and fibrous connective tissue structures in the interstitial tissue of the ovarian cortex is confirmed. The ovarian medulla expands, showing edematous, myxomatous, cyst-like cavities and foci of hyaline-like tissue.

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