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ПОСЛЕДСТВИЯ ИНФЕКЦИИ HELICOBACTER PYLORI

Харченко А.В., Шерстюк О.А.

В работе показаны последствия влияния инфекции Helicobacter pylori на слизистую оболочку желудка. Применены как гистологические методики, так и молекулярно-биологическая методика (ISSR-PCR) с ипользованием ISSR –праймера S-2, со структурой (AGC)6G. Проанализированы изменения слизистой оболочки желудка при H.˚pylori-ассоциированных заболеваниях: хронической язвенной болезни двенадцатиперстной кишки, хронической язвенной болезни желудка, язвенно инфильтративном раке желудка. Гистологически в слизистой оболочке желудка обнаружены дисплазии трёх степеней тяжести (Д-1; Д-II; Д-III). Генотипически в слизистой оболочке обнаружены изменения соответствующие микросателлитным экспансиям, которые являются свидетельством предраковых изменений.

Ключевые слова: Helicobacter pylori, дисплазии, микросателлитные экспансии.

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CONSEQUENCES OF HELICOBACTER PYLORI INFECTION

Kharchenko A.V., Sherstyuk O.A.

The consequences of influence of infection of Helicobacter are in-process shown pylori on the mucous membrane of stomach. Both histological methodologies and molecular-biological methodology (ISSR-PCR) are applied with ISSR-praimer S-2, with the structure (AGC) 6G. The changes of mucous membrane of stomach are analysed at H.˚pylori- the associated diseases: chronic ulcerous illness of duodenum, chronic ulcerous illness of stomach, ulcerous infiltration cancer of stomach. Histological in the mucous membrane of stomach found out dysplasias of three degrees of weight (D-1; D-II; D-III). Genotype in a mucous membrane found out changes corresponding to microsatellite expansions that are the certificate of pre-cancer changes.

Key words: Helicobacter pylori, dysplasia, microsatellite expansions.

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THE PECULIARITIES OF THE PRENATAL MORPHOGENESIS OF THE EPIDIDYMIS

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Epididymis serves a critical function in the process of sperm cells maturation, since it provides with a unique liquid microbiomedium that allows maturation and survival of the spermatozoa. Therefore, the aim of our study is to clarify the patterns of the morphogenesis of the epididymis during the perinatal period of human ontogenesis. 27 series of the serial histological sections of the human embryos and prefetuses (4.0-80.0 mm of parietal-coccygeal length (PCL)) and 56 specimens of human fetuses (81.0-375.0 mm PCL) have been studied, using the methods of microscopy, macroscopy, graphic and plastic reconstruction and morphometry. It has been found that the anlage of the epididymal tubules occurs in the prefetuses 14,0-16,0 mm of PCL, and the formation of the epididymal segments (caput, corpus and cauda), as well as establishment of the correlation between the tubules of the testicle and epididymis is observed in prefetuses 18,0-65,0 mm of PCL. At the early fetal period the asymmetry of the shape and size of the right and left epididymises is observed; it is preserved during the entire fetal period and is accompanied by the process of their accelerated and slowed development. At the end of the fetal period of the ontogenesis the structure and shape of the epididymal tubular system is close to the definite state.

Keywords: epididymis, mesonephros, mesonephric duct, prenatal morphogenesis, human.

The study is a fragment of the research project "The features of morphogenesis and topography of the systems and organs in the human pre-and postnatal ontogenesis" (state registration No.0115U002769).

Knowing the nature and clarifying the time of specific transformations, which in general ensures the systemic genesis of the fetus, is extremely important in embryology. Numerous abnormalities that occur in the clinical practice can be mainly explained only on the basis of clarification of the origin and interaction of the organs and structures that eventually acquired their typical shape, and having studied the features of their topography and deeply realized relevant embryonic phenomena [1]. The epididymis serves a critical function in the process of sperm cells maturation, since it provides with a unique liquid microbiomedium that allows maturation and survival of the spermatozoa [2].

To perform its function the epididymis must undergo a complex path of the prenatal morphogenesis. The publications [4-6] report about intrauterine secretory activity of the human

epididymal epithelium. It is known that the nature of the epididymal tubules, the time of their origination, setting their embryotopography, the connection between the testicular and epididymal systems are not fully studied to date and require further clarification [3].

Congenital defects of the epididymis can lead to male infertility. However, the dysfunctions of epididymis may occur due to abnormal fetal development, though very little is known about the features of the epididymal morphogenesis and the nature and causes of its congenital defects [8]. Therefore, it is important to understand the patterns and features of the prenatal morphogenesis of epididymis and morphological prerequisites for the presupposed occurrence of options and congenital abnormalities of the development, namely, its cyst, isolated location of the epididymis relative to testicle, the development of agenesis of the epididymis or its segment (atresia of its caput, corpus and cauda), defects of the spermaduct development, absence of interconnection of the testicular and epididymal tubules [7, 8].

The purpose of the study was to clarify the patterns of morphogenesis of the epididymis during the prenatal period of human ontogenesis and to study the morphological prerequisites of the presupposed defects occurrence in its development.

Materials and methods. The study was performed on the 27 series within the serial histological sections of the human embryos and prefetuses 4.0-80.0 mm of parietal-coccygeal length (PCL) and 56 specimens of fetuses 81.0-375.0 mm PCL. The following methods have been used: microscopy, macromicroscopy, graphic and plastic reconstruction and morphometry.

The material was obtained from the obstetric-gynecological units of medical facilities of Chernivtsi city and region. The series of histological sections, taken from the M.G. Turkevych Museum of the Department of Anatomy at the HSEE of Ukraine "Bukovinian State Medical University", were also used for the study. Specimens of fetuses weighing more than 500.0 g were studied directly at the Chernivtsi Regional Children's Morbid Anatomy Bureau, within the framework of the cooperation agreement. During the performance of the medical scientific research no violations of moral and legal norms have been found by the Commission for Biomedical Ethics at the HSEE of Ukraine "Bukovinian State Medical University".

The periods of the intrauterine development (embryonic, prefetal and fetal) are organized according to G. A. Schmidt's classification (1968). The age of the objects of the study was defined according to the Tables suggested by B. M. Petten (1959) and B. P. Hvatov, Yu. N. Shapovalov (1969) on the basis of PCL measuring. After dissection of the organs of abdomen and pelvis in fetuses of different age groups, macro-and microscopic structure, shape and topography of epididymises, relation of the testicles and epididymises to the peritoneum, their relationship with the adjacent organs and structures was studied and their morphometry was made.

Results of the study and their discussion. It has been found that in the fetuses 4.0-4.5 mm long the mesonephros is located on the dorsal wall of the embryo in the form of longitudinal protuberances. The parenchyma of the mesonephros is represented by the mesenchymal cells and anlage of the mesonephric tubules, where the lumen is determined. In fetuses 5.5-6.0 mm of PCL the mesonephric tubules and glomeruli are well expressed, especially in the cranial part of the organ.

An intensive morphogenesis of the mesonephros is observed at the beginning of the week 7 of the intrauterine growth (prefetuses 14.0-14.5 mm of PCL). Starting from the prefetuses 16.0-17.0 mm of PCL the reduction of the mesonephros occurs, growing craniocaudally. The width of the cranial part of the mesonephros is 195-205 μm, whereas the width of the mesonephros of the medial and caudal parts is 320-395 μm. The diameter of the mesonephric bodies in the cranial part of the mesonephros is 38-52 μm, and 117-130 μm in its medial and caudal parts. The cranial part of the mesonephric Wolffian duct undergoes reduction, too, diameter of which is 26-30 μm. The diameter of the caudal part of the mesonephric duct is 75-82 μm. Clusters of epithelial cells and scarce primary germ cells occur in the mesonephric parenchyma between its reduced glomerules and tubules, in the part which corresponds to the anlage of the gonade, from which the epididymal tubules arise. The formation of the lumen in the chordas and its conversion into epididymal tubules begins at the end of the embryonic period, growing craniocaudally. Within 8-9 weeks of the intrauterine growth (prefetuses 21.0-41.0 mm of PCL) a rapid development of the epididymal tubular system occurs, which is characterized by the occurrence of the fissured lumens with the diameter of 5-6 μm in the clusters of cells (fig. 1). The anlages of the epididymal tubules are directed to the anlage of the gonads. Mesonephric ducts, similar to mesonephroses, are reduced in the cranial and, partially, in the medial portion. However, at the level of the gonads both mesonephric duct and mesonephros is detected. The diameter of the mesonephric duct varies from 48 to 62 μm.

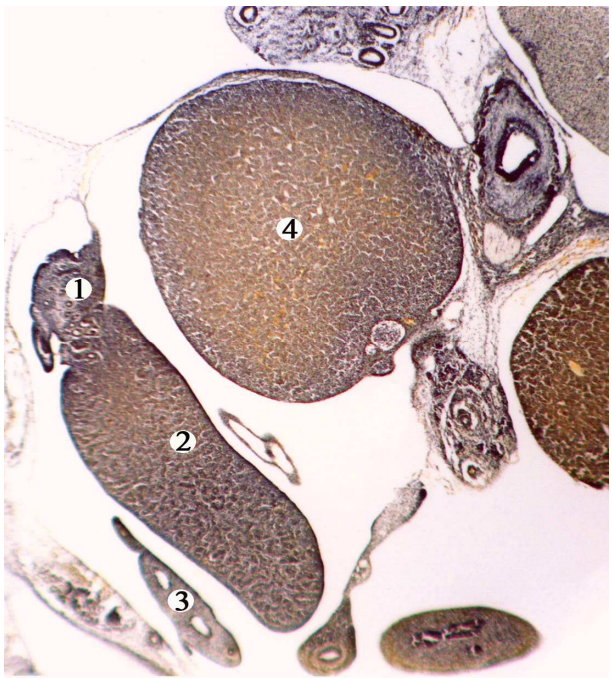

Fig. 1. Frontal section of the prefetus 23,5 mm of PCL. Van Gison stain. Slide mount. Lens 8, Ocular lens 7: 1 – anlage of the epididymis; 2 – gonad; 3 – mesonephric duct; 4 – adrenal gland.

In the prefetuses 35.0-38.0 mm of PCL the epididymal caput is represented by the tubules, the lumen of which is lined with the simple cuboidal epithelium. Stellate epithelial cells prevail. The lumen of the tubules of the epididymal caput is oblong with the diameter ranged from 8 to 10 μm. 2-4 layers of mesenchymal cells with fusiform nuclei are circularly located externally from the epithelium of the tubules. The mesenchymal cells of various shapes and orientation are located in the interspaces between the tubules of the epididymal caput. The coiled epididymal duct with small lumen, surrounded by the substantial layer of the mesenchyme, is found in the epididymal corpus. The lumen of the epididymal duct is lined with the simple cuboidal epithelium. The mesenchymal cells that are directly adjacent to the epididymal duct are located densely and have oblique and circumferential direction. It should be considered as an anlage of the muscle layer of the epididymal duct. Further formation of the tubular system of the epididymis, growing craniocaudally, is observed in the prefetuses 53.0-42.0 mm of PCL. The entire regions of the epididymis are formed macroscopically. The latter is mainly presented by the mesenchymal cells; the tubules are mainly developed in the epididymal caput and less in the corpus, and only scarce ones are detected in the cauda. The wall of the cavity of the epididymal tubules is more differentiated, as compared with the wall of the corpus and cauda. At this stage of the development the mesonephric duct is reduced in the cranial and medial regions. The diameter of the non-reduced portion of the mesonephric duct at the level of the gonad ranges from 58 to 68 μm. The lumen of the Wolffian duct is lined with simple cuboidal epithelium, where the cytoplasmatic ridges are found, directed into the lumen of the ducts. Externally, in the caudal region of the mesonephric duct the circumferential layer of the mesenchymal cells with oval nuclei is detected. Structural rearrangements in the wall of the caudal region of the mesonephric duct should be considered as the beginning of the formation of the spermaduct.

At the end of the prefetal period of ontogenesis (67.0-80.0 mm of PCL) the anlage of the testicular efferent tubules occurs. At the same time in the fold, connecting the testicle and epididymal caput the chordas of the epithelial cells appear, where the process of its canalisation is observed at the different stages of development.

At the beginning of the fetal period of ontogenesis the further development of all segments of the epididymis occurs. On the sagittal histological sections of the epididymises of fetuses 81.0-90,0 mm of PCL numerous lumens of the tubules of various shapes are detected, which are surrounded by the connective tissue with a small amount of blood vessels (fig. 2A). The diameter of the lumen of the tubules of the epididymal caput and corpus is 20-24 μm, and 16-18 μm in the cauda. Epididymal tubules within its caput and superior portion of the corpus are coiled; their wall is lined with simple cuboidal epithelium, among the cells of which scarce primary gametes are detected. 2-3 layers of mesenchymal cells, smooth myocytes and connective tissue fibers are circularly located externally from the epithelium. The wall of the cavity of the tubules of the inferior part of the epididymal corpus and cauda is lined with columnar epithelium, surrounded by 5-7 layers of the mesenchymal cells with fusiform or oblong nuclei (fig. 2B). The connective tissue fibers and scarce endocrynocytes (Leydig cells) are also visualized among the tubules of the epididymal corpus and cauda. Starting from the month 5 of the intrauterine growth a clear structuredness of the epididymal tubules is detected. On the sagittal histological sections of the epididymises of the fetuses 165.0-175.0 mm of PCL the tubules of the epididymal caput are coiled; their wall is lined with simple single-layer-, and in some places, double-layer cuboidal epithelium. 5-6 layers of the mesenchymal cells among which the smooth myocytes and connective tissue fibers are detected, are circularly located externally from the epithelium. Epididymal tubules within its corpus and cauda are less coiled. The diameter of the lumen of the tubules of epididymal caput is 28-34 μm, and 32-46 μm in the corpus and cauda.

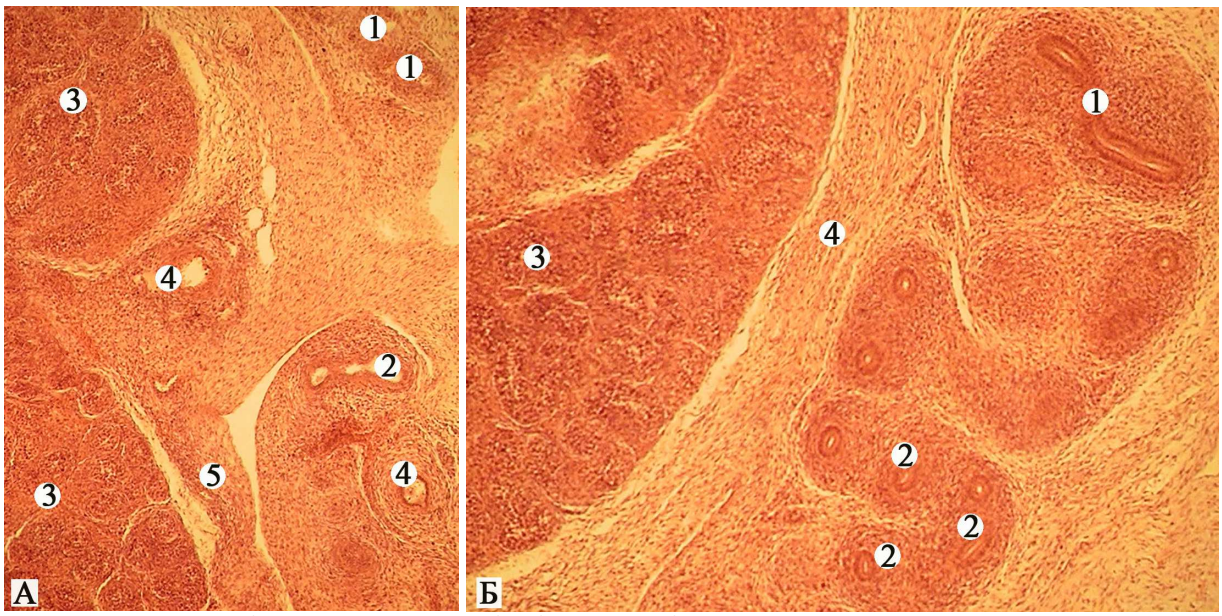

Fig. 2. Sagittal sections of the epididymis and testicle of the fetus 85.0 mm of PCL. Hematoxylin and eosin stain. Slide mounts. Lens 8, Ocular lens 10: A: 1 – tubules of the epididymal caput; 2 – lumen of the tubule of the epididymal corpus; 3 – testicle; 4 – spermaduct; 5 – protein coat of the testicle. B: 1 – tubule of the epididymal corpus; 2 – lumina of the epididymal cauda; 3 – testicle; 4 – protein coat of the testicle.

The wall of the cavity of the tubules of the superior portion of the corpus is lined with single cuboidal epithelium, outside of which 2-3 layers of the mesenchymal cells, smooth muscle cells and connective tissue fibers are located. The wall of the cavity of the tubules of the inferior part of the epididymal corpus and cauda in lined with simple prismatic epithelium surrounded by the mesenchymal cells, connective tissue fibers and scarce Leydig cells. The thickness of the protein coat in epididymal caput region reaches 36-44 μm, and its thickness in the corpus and cauda region is 32-38 μm. Microscopic study of the series of histological sections of the testicles and epididymises of the 6-month-old fetuses shows a protein coat of the organs, formed by the connective tissue fibers, among which mesenchymal cells are found. Minor 14-32 μm thick strata of connective tissue fibers are branched from the protein coat into the parenchyma of the epididymal caput and superior part of the corpus, dividing them into the lobules. The strata between the tubules of the epididymal caput are represented by the mesenchymal cells and connective tissue fibers, containing the blood and lymph vessels of the small diameter. Numerous tubules of predominantly orbicular and ovoid shape with minor lumen are found in all segments of the epididymis. The diameter of the lumen of the tubules of the epididymal caput is 38-46 μm, and 44-58 μm within its corpus and cauda. The thickness of the connective tissue strata between the tubules of the epididymal corpus and cauda is 56-68 μm. The wall of the epididymal duct is lined with simple multi-layer prismatic epithelium, outside of which there is a fine connective tissue proper plate, to which muscular coat adjoins. The latter is represented by a circular layer of the smooth muscle cells.

The study of histological specimens of the epididymises of the 7-month-old fetuses shows well-marked 105-110 μm thick protein coat, which is formed by the fibrous connective tissue. Numerous tubules with S-shaped lumen are detected in the epididymal caput region (fig. 3). The diameter of the lumen of the tubules of the epididymal caput and corpus is 46-50 μm, and 42-44 μm in the epididymal cauda region. The epididymal tubules of the fetuses of this age group become more coiled, as compared with the 6-month-old fetuses, within the inferior part of its corpus and cauda.

Starting from the early fetal period the marked asymmetry of the shape and size of the right and left epididymises is observed. During the fetal period of ontogenesis the epididymis is mostly retort-shaped and hook-shaped; in 9-10-month-old fetuses it is comma-shaped; rare shapes of the epididymis are helicoid, S-shaped, C-shaped and hooked ones. The width and the thickness of the right epididymis prevail over the similar size of the left one, and such pattern is preserved throughout the fetal period of ontogenesis. The intensive elongation of the right and left epididymises is observed on the 5, 7 and 10 months of the human fetal period. In the fetuses 311.0-345.0 mm of PCL the right and left testicular-epididymal complexes pass through the inguinal canal into the scrotum. This process is fairly well-described in the literature, so we will focus on some details that have not been elucidated yet. It has been found that as far as the testicle and its epididymis move through the inguinal canal into the scrotum their slight turn is observed. At the location of these organs in the abdominal cavity the testicle is located medially relative to the epididymis.

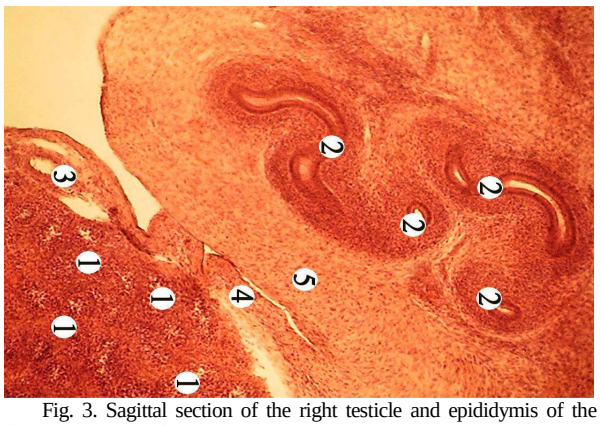

Fig. 3. Sagittal section of the right testicle and epididymis of the fetus 245,0 mm of PCL. Hematoxylin and eosin stain. Slide mounts. Lens 3.5, Ocular lens 10: 1 – tubules of the testicle lobules; 2 – tubules of the epididymal caput; 3 – spermaduct; 4 – protein coat of the testicle; 5 – protein coat of the epididymis.

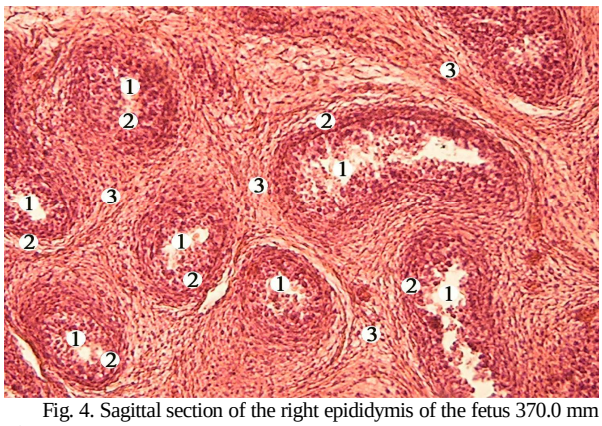

Fig. 4. Sagittal section of the right epididymis of the fetus 370.0 mm of PCL. Hematoxylin and eosin stain. Slide mount. Lens 8, Ocular lens 20: 1 – epididymal tubules; 2 – epithelium of the epididymal tubules; 3 – epididymal septa.

When passing through the inguinal canal the turn occurs: the testicle is gradually shifted anteriorly, and the epididymis – posteriorly, and in the discharge from the inguinal canal they occupy similar relative position. In the majority of the studied fetuses the right and left epididymises are located along the posterior end and lateral surface of the corresponding testicles. However, we have found the variants of the topography of the testicles and epididymises, indicating about the dynamics of the formation of the syntopy of the abovementioned organs during the fetal period and their strong correlation in the process of testicular descent. Microscopic study of the histological sections of epididymises of fetuses 350,0-370,0 mm of PCL has found that at the end of the fetal period of ontogenesis the epididymal tubules in the caput region are more coiled as compared with tubules of the epididymal corpus and cauda. The wall of the tubules of the epididymal caput is lined with simple double-layer cuboidal epithelium, among the cells of which scarce primary gametes are detected. 3-4 layers of the mesenchymal cells with elongated nuclei, as well as smooth myocytes and connective tissue fibers are circularly located externally from the epithelium (fig. 4). The wall of the tubules of the epididymal corpus and cauda is represented by the simple prismatic epithelium. 4-5 layers of the mesenchymal cells, soft muscle cells and connective tissue fibers that are located densely externally from the epithelial lining of the wall of the tubular cavity. The adjacent tubules sequentially conjugate in the direction from the caput to cauda, forming the epididymal duct. The thickness of the protein coat in the epididymal caput region is 210-235 μm, and 190-215 μm in the corpus and cauda region.

Based on the findings of the research, 3 stages of the epididymis development during the embryonic, prefetal and fetal periods of the human ontogenesis can be distinguished:

1 – the stage of the anlage of the epididymal tubules (prefetuses 14,0-16,0 mm of PCL);

2 – the stage of the formation of the epididymal segments, establishment of the relationship between the tubular systems of the testicle and epididymis (prefetuses 18,0-65,0 mm of PCL);

3 – passage of the testicle and epididymis through the inguinal canal into the scrotum (fetuses 311,0-345,0 mm of PCL).

Noteworthy, the disturbance of the processes of normal morphogenesis at these stages of the development can lead to the emergence of multiple abnormalities. The dystopy of the testicles and epididymises was observed in two cases (fetuses 280.0 and 315.0 mm of PCL). Pelvic ectopy of the left testicle and epididymis was detected in the in fetuses 190.0 and 260.0 mm of PCL. Perineal ectopy of the testicles and epididymises was conjoined with atresia of the cauda of the right epididymis, agenesia of spermaducts in fetus 135.0 mm of PCL. Three observations (fetuses 115.0, 180.0 and 190.0 mm of PCL) revealed almost isolated localization of the epididymises and testicles. Presumably, the prominence of the chorda of the testicular-epididymal complex in the human fetuses is one of the factors of the separate location of the epididymis and testicle. Cyst of the right epididymis was detected in fetuses 175.0 and 225.0 mm of PCL that, in our opinion, is the result of the processes of dysembriogenesis in the region of tubular system formation. In the fetus 215.0 mm of PCL doubling of the right epididymis was found.

Conclusions

1. The anlage of the epididymal tubules in the form of aggregation of epithelial cells and scarce germ cells occurs between the reduced mesonephric glomeruli and tubules in the prefetuses 14,0-16,0 mm of PCL.

2. The process of canalisation of the cellular aggregations starts in the first half of the prefetal period (prefetuses 22,0-29,0 mm of PCL).

3. In the prefetal period of the ontogenesis the epididymal segments (caput, corpus and cauda) are formed. The process of differentiation of the epididymal tubules occurs in craniocaudal region, and they are almost formed and coiled at the end of the prefetal period; however within the epididymal corpus and cauda they are poorly differentiated and are straight.

4. In the fetal period of the ontogenesis further differentiation of the epididymal segments and complication of the structure of its tubules occurs. The latter become coiled in all segments of the epididymis. At the end of the fetal period the structure and shape of the epididymal tubules are close to the definite state.

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Реферати

ОСОБЛИВОСТІ ПРЕНАТАЛЬНОГО МОРФОГЕНЕЗУ НАД'ЯЄЧКА

Хмара Т.В., Ризничук М.О., Сарафинюк Л.А., Кривчанська М.І., Бірюк І.Г.

Над'ячко має вирішальне значення для процесу розвитку сперматозоїдів, тому що воно забезпечує унікальне рідинне мікробіосередовище, що дозволяє дозрівати і виживати сперматозоїдам. Тому, ми хотіли з'ясувати закономірності морфогенезу над'ячка впродовж усіх періодів пренатального морфогенезу людини. Досліджено 27 серій послідовних гістологічних зрізів зародків і передплодів (4,0-80,0 мм тім'яно-куприкової довжини (ТКД)) та 56 препаратів плодів людини (81,0-375,0 мм ТКД) методами мікроскопії, макроскопії, графічного і пластичного реконструювання та морфометрії. Встановлено, що закладка канальців над'ячка відбувається у передплодів 14,0-16,0 мм ТКД, а формування частин над'ячка (головки, тіла та хвоста) та встановлення зв'язків між канальцями яєчка і над'ячка простежується у передплодів 18,0-65,0 мм ТКД. У ранньому плодовому періоді відзначається асиметрія форми, розмірів правого і лівого над'ячок, яка зберігається впродовж усього плодового періоду розвитку, та супроводжується процесом їх прискореного та уповільненого росту. Наприкінці плодового періоду онтогенезу канальцева система над'ячка за будовою і формою близька до дефінітивного стану.

Ключові слова: над'ячко, мезонефрос, мезонефральна протока, пренатальний морфогенез, людина.

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ОСОБЕННОСТИ ПРЕНАТАЛЬНОГО МОРФОГЕНЕЗА ПРИДАТКА ЯИЧКА

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Придаток яичка имеет решающее значение для процесса развития сперматозоидов, так как оно обеспечивает уникальную жидкостную микробиосреду, что позволяет созревать и выживать сперматозоидам. Поэтому, мы хотели выяснить закономерности морфогенеза придатка яичка на протяжении пренатального периода онтогенеза человека. Исследовано 27 серий последовательных гистологических срезов зародышей и предплодов (4,0-80,0 мм теменно-копчиковой длины (ТКД)) и 56 препаратов плодов человека (81,0-375,0 мм ТКД) методами микроскопии, макроскопии, графического и пластического реконструирования и морфометрии. Установлено, что закладка канальцев придатка яичка происходит у предплодов 14,0-16,0 мм ТКД, а формирование частей придатка яичка (головки, тела и хвоста) и установление связей между канальцами яичка и придатка яичка прослеживается у предплодов 18,0-65,0 мм ТКД. В раннем плодном периоде отмечается асимметрия формы, размеров правого и левого придатков яичек, которая сохраняется в течение всего плодного периода развития, и сопровождается процессом их ускоренного и замедленного развития. В конце плодного периода онтогенеза канальцевая система придатка яичка по строению и форме близкая к дефинитивному состоянию.

Ключевые слова: придаток яичка, мезонефрос, мезонефрический проток, пренатальный морфогенез, человек.

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