
ANATOMICAL FORMATION, SPATIAL ORIENTATION, AND DYNAMICS OF MORPHOLOGICAL CHANGES OF THE HUMAN ATRIOVENTRICULAR VALVE APPARATUS DURING PRENATAL DEVELOPMENT

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ABSTRACT

Background. The increasing incidence of Congenital Heart Defects (CHDs) drives interest in the study of cardiogenesis. Despite the successes of morphological research on the human heart, controversial issues remain. Understanding the precise chronology and dynamics of morphofunctional changes in the human atrioventricular heart valves provides a foundation for the development of prenatal diagnostics and pediatric cardiac surgery.

Aim. To investigate the initiation and formation of atrioventricular valve apparatuses in the human heart.

Materials and Methods. The hearts of 17 embryos, 24 prefetuses, and 40 fetuses were examined. Macroscopic, microscopic, histochemical methods, and 3D reconstruction were utilized. The study was qualitative and descriptive; no statistical analysis was performed. This study is a part of the planned research project of the Department of Histology, Cytology, and Embryology at BSMU "Patterns of Morphogenesis, Structural-Functional Organization and Anatomical Variability of Organs and Structures in Various Body Regions and Systems of Humans and Experimental Animals" (implementation period: 01.2026–12.2030), state registration number 0126U003125.

Research Ethics. The Biomedical Ethics Committee of BSMU (Protocol No.5 of February 19, 2026) found no violations of moral or legal standards during the course of this research work.

Results. It was revealed that the initiation of endocardial cushions, from which the atrioventricular valves are formed, begins at the end of the 4th week of intrauterine development. During the fetal period, the valve leaflets remain immature: they are covered with endothelium and contain interstitial cells. The matrix is represented by an amorphous substance and immature fibers. Chordae tendineae begin to form at the 15th week. The primary chordae tendineae are muscular. Subsequently, they differentiate into mature chordae, dominated by dense, regular connective tissue. Thus, chordae tendineae are classified into two types: muscular and fibro-muscular.

Conclusions. 1. Endocardial cushions are identified at the 4th week of embryogenesis and give rise to the atrioventricular valves. 2. In fetuses, the valve leaflets are covered with endothelium and contain interstitial cells and an extracellular matrix with an amorphous substance and immature fibers. 3. Primary chordae tendineae appear at the 15th week in the form of muscular bands. 4. They undergo remodeling and exhibit dense, regular connective tissue. The cords are classified into muscular and fibro-muscular types.

Keywords: *theoretical medicine, endocardial cushions, chordae tendineae, papillary muscles, intrauterine ontogenesis.*

Introduction

According to the Global Burden of Disease (GBD) studies, cardiovascular diseases remain the leading cause of disability and mortality worldwide, claiming over 17 million lives annually. Our country is among the global leaders [1].

According to the World Health Organization (WHO) forecasts, this statistic is projected to rise to 23 million by 2030. Low health literacy among the population and insufficient detection rates of cardiac defects are among the primary contributing factors. According to the data of the State Institution "National Institute of Cardiovascular Surgery named after M. Amosov of National Academy of Medical Sciences of Ukraine", every year, between 2,900 and 3,500 children are born with congenital heart defects in Ukraine. [2]. According to recent epidemiological data, the prevalence of congenital anomalies in children of the first year of life in Ukraine was dominated by Congenital Heart Defects (CHDs), accounting for 29.2% of all cases [3]. CHDs require surgical in

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tervention at an early age, but they are associated with a substantial mortality rate [4].

These figures demonstrate the magnitude of the problem, which demands particular attention from both clinical medicine [4] and morphologists. Researchers worldwide are studying cardiac development as a whole [5–9], with a specific focus on the pathogenesis of CHDs [10–12]. Among morphological studies, several works focus directly on the development of the cardiac valve apparatus [13–15], its structure [16–21], and age-related morphological changes [22; 23].

Despite the available data on the molecular and genetic features of cardiogenesis, the etiology of CHDs remains poorly understood. Given that abnormalities in the initiation and formation of endocardial cushions underlie the development of the cardiac valve apparatus, this area of morphological research remains highly relevant today. Improving existing morphological data will enhance diagnostic outcomes and clinical practices aimed at restoring morphological structure, normalizing functional hemodynamics, and saving human lives, starting in infancy.

Aim of the research was to study the development and structural morphogenesis of the human atrioventricular valve apparatus during intrauterine ontogenesis.

Material and Methods

The material consisted of 17 embryos (4.0–20.0 mm Crown-Rump Length (CRL, corresponding to CS 11–20), 24 early fetuses (21.0–79.0 mm CRL, from which 21.0–32.0 mm CRL, corresponding to CS 20–23), and 40 fetuses (81.0–375.0 mm CRL). Macroscopic, microscopic, histochemical, and 3D reconstruction techniques were used for the investigation. Biological material was fixed in a 10% neutral buffered formalin solution, followed by embedding in paraffin to prepare paraffin blocks. Serial histological sections with a thickness of 5–7 μ m were obtained using a microtome. To examine the general structure, the sections were stained with hematoxylin and eosin (H&E). For visualization of elastic and collagen fibers, as well as muscle tissue components, Van Gieson, Weigert-Van Gieson, and picro-Mallory staining techniques were employed. The 3D reconstruction method was performed utilizing an Olympus (C-4000) digital camera at $\times 100$ magnification, along with a software suite including Microsoft Office Picture Manager, Amira 5.0, and 3ds Max 8.0. This study was purely qualitative and descriptive. The quantitative statistical analysis was not performed.

Research Ethics

The Biomedical Ethics Committee of Bukovinian State Medical University (Protocol No.5 of February 19, 2026) found no violations of moral or legal standards during the course of this research work. The study was conducted in accordance with the ethical standards of the World Medical Association Declaration of Helsinki (1964–2024).

Results

The study revealed that Endocardial Cushions (EC) was initiated at the end of the 4th week of intrauterine development. Light microscopy demonstrated two large (dorsal and ventral) EC filled with cardiac jelly within the lumen of the Atrio-Ventricular (AV) canal in embryos of 6.0 mm CRL, corresponding to CS 13 (Fig. 1).



Fig. 1. Sagittal section of 6.0 mm CRL, corresponding to CS 13 human embryo. Van Gieson's stain. Microphotograph, $\times 56$.

1 – liver; 2 – atrial primordium; 3 – atrioventricular canal; 4 – dorsal endocardial cushion; 5 – ventral endocardial cushion; 6 – lung primordium; 7 – mesonephros; 8 – primordium of the vertebral column.

During the 6th–7th weeks of embryogenesis, the dorsal and ventral cushions of the AV canal increased in size. Subsequently, they fused, as a result of which the AV canal divided into two inlets: the right (tricuspid) and left (mitral) orifices.

In embryos of 11.0–12.0 mm CRL, corresponding to CS 17, two large (dorsal and ventral) EC

were already observed within the lumen of the AV canal, with two small lateral cushions. A higher cell density was visualized in the zone of future apposition of the EC compared to the rest of their surface. A gradual decrease in the volume of cardiac jelly, epithelial-mesenchymal transition, and migration of mesenchymal cells from the endocardium were noted.

Studies indicate that the fusion and transformation of the dorsal and ventral AV EC during the septation of the AV canal give rise to the anterior leaflet of the mitral valve (Fig. 2) and the septal leaflet of the tricuspid valve. The left lateral AV cushion formed the posterior leaflet of the mitral valve, while the right lateral cushion formed the anterior and posterior leaflets of the tricuspid valve.



Fig. 2. Sagittal section of the early fetus 15.0 mm CRL, corresponding to CS 17. Van Gieson's stain. Microphotograph, $\times 24$.

1 – left ventricle; 2 – mitral valve; 3 – aorta; 4 – lung; 5 – liver; 6 – adrenal gland; 7 – duodenum; 8 – metanephros; 9 – mesonephros; 10 – gonad.

By the beginning of the fetal period, the main stages of the valvular apparatus architecture were complete. Thereafter, relevant morphofunctional modifications took place.

Light microscopy of hematoxylin and eosin-stained sections of the leaflets of the AV valves demonstrated morphological similarity in human fetuses. The AV valve leaflets in human fetuses

were covered by endothelium on both sides. The endothelial cell nuclei were elongated and stained basophilically, while the cytoplasm was oxyphilic. Interstitial cells were detected within the leaflet thickness, uniformly distributed within the surrounding matrix (Fig. 3). These cells had a cell body with a basophilically stained nucleus and cytoplasmic processes. The matrix was dominated by a homogeneous ground substance, while the fibrous component remains immature.

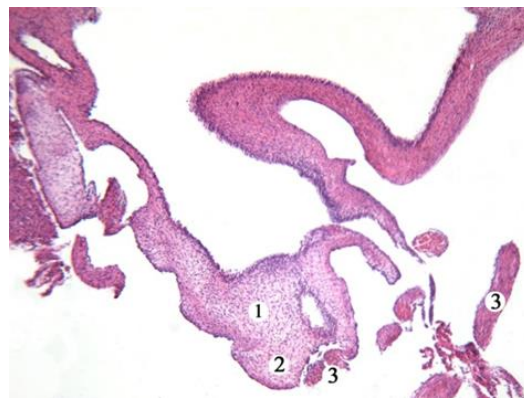


Fig. 3. Longitudinal section of the mitral valve leaflet of a 250 mm CRL fetus. Hematoxylin and eosin stain. Microphotograph, $\times 100$.

1 – interstitial cells; 2 – undifferentiated extracellular matrix; 3 – chordae tendineae.

Macroscopic examinations revealed that in the hearts of 81.0–90.0 mm CRL fetuses, Chordae Tendineae (CT) were absent, and the Papillary Muscles (PM) attach directly to the ventricular surface of the AV valve leaflets. CT in the form of thin, solitary strands began to be observed in the hearts of 130.0–135.0 mm CRL fetuses (Fig. 4).

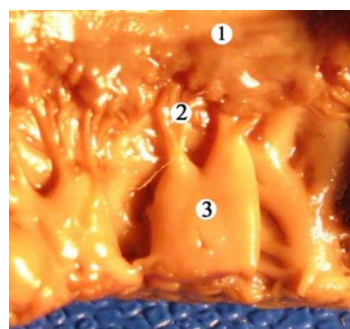


Fig. 4. Fetal tricuspid valve 135.0 mm CRL. Gross photograph, $\times 20$.

1 – valve leaflet; 2 – chordae tendineae; 3 – papillary muscles of the right ventricle.

Light microscopic examination of the hearts of 81.0–120.0 mm CRL fetuses showed that the PM attached to the ventricular surface of the AV valve leaflets, with their cardiac muscle tissue transitioning smoothly into the leaflet mesenchyme. The leaflet consisted of dense mesenchymal tissue with a small number of muscular elements located closer to its ventricular surface.

As a result of 3D modeling of the left AV valve in a 90.0 mm CRL fetus, it was revealed that the PM of the valvular apparatus transited directly into the AV valve leaflets.

In 125.0 mm CRL fetuses, light microscopic examination showed that primary CT appeared as thin strands between the PM and the AV valve leaflets. Primary CT of both the mitral and tricuspid valves were composed of a dense layer of cardiomyocytes with a small number of mesenchymal cells uniformly distributed within their thickness. A rather disorganized extracellular matrix was observed around the cells, in which collagen and elastic fibers of the connective tissue were not yet detectable. As gestational age increased, the cranial displacement of the AV valve leaflets increased the distance between the PM and the valves, accompanied by elongation of the primary CT. In 130.0–135.0 mm CRL fetuses, the CT had the appearance of muscular strands, which later differentiated into CT composed predominantly of dense, regular fibrous connective tissue.

Three-dimensional reconstruction of the valvular apparatus in a 135.0 mm CRL fetus demonstrated that the PM connected to the right AV valve leaflets via newly formed CT (*Fig. 5*).

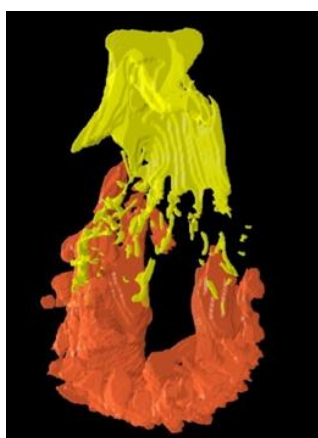


Fig. 5. Three-dimensional model of the fetal tricuspid valve 135.0 mm CRL.

Red color – papillary muscle of the right ventricle; yellow color – leaflets and chordae tendineae of the valve.

In 140.0–170.0 mm CRL fetuses, the CT were composed of both cardiac muscle and connective tissues, in which collagen fibers formed a continuous layer that connects the myocardium, the leaflet, and the PM.

The surface of the CT of both the mitral and tricuspid valves in 180.0–375.0 mm CRL fetuses was smooth and lined by a single layer of flattened endothelial cells. Beneath the endothelium of the CT in 180.0–375.0 mm CRL fetuses, a subendothelial layer was localized, within which elastic fibers were detectable as a thin band. They displayed a wavy course and run parallel to the collagen fibers, which exhibit an ordered arrangement at the base of the CT. When histological sections were stained using the Weigert–Van Gieson method, the elastic fibers stained brown.

The core of the CT was composed of dense, regular fibrous connective tissue, represented by a fibrous component, extracellular matrix, and cells of the fibroblastic lineage. The cells had smooth contours and an irregularly elongated shape with a few thin processes. The cell nuclei were mostly oval or elongated in shape and exhibit basophilic staining.

On histological sections of the cardiac CT in 320.0–375.0 mm CRL fetuses, a predominance of fibrous extracellular matrix structures over cellular elements was observed, along with a decrease in the amount of the amorphous component.

Upon microscopic investigation of the right ventricular CT in 180.0–375.0 mm CRL fetuses, it was established that fibrous strands penetrated throughout the entire thickness of the chordae. Picro-Mallory staining of histological specimens revealed that the strands stain bright red, which confirms they consist of muscle tissue. These structures were formed by individual rectangular cardiomyocytes linked in a chain-like fashion through intercellular contacts (*Fig. 6*).

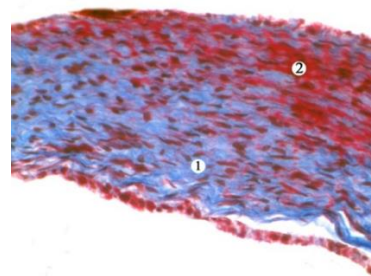


Fig. 6. Longitudinal section of the chorda tendinea of the fetal mitral valve 270.0 mm CRL. Picro-Mallory stain. Microphotograph, $\times 150$.

1 – bundles of collagen fibers; 2 – striated cardiac muscle fibers.

Examination of the right AV valve CT sections in 180.0–375.0 mm CRL fetuses established that thin-walled vessels of the microcirculatory bed lined by endothelial cells were present within the striated cardiac muscle tissue in the substance of the CT.

Discussion

The rising trend in the incidence of CHDs and impaired morphogenesis of the cardiac valvular apparatus poses a serious challenge for modern medicine [12]. Understanding the chronology of cardiogenesis during prenatal development provides the foundation for timely prenatal diagnosis and advanced pediatric cardiac surgery [4].

Our data demonstrated that in humans, the AV valvular apparatuses originate from the endocardial cushions, which develop and can be identified as early as the end of the 4th week, functioning as provisional valves. This is entirely consistent with current views on cardiac septation [5; 6]. During cardiac valve morphogenesis, cells undergo endothelial-to-mesenchymal transition. Specific molecular markers have been proven to regulate this process [7; 14].

Our findings regarding the morphological immaturity of the AV valve leaflets during the fetal period, characterized by the presence of endothelium, interstitial cells, and a predominant amorphous matrix component, align with previous reports [13]. The predominance of the amorphous component reflects the physiological requirements of fetal hemodynamics, whereas the definitive maturation of collagen continues throughout postnatal ontogenesis [22].

The chronology of the subvalvular apparatus development is of particular importance. The appearance of primary CT in the form of muscular strands at the 15th week of gestation confirms the embryonic link between the CT, the PM, and the trabecular layer of the ventricular myocardium [15]. In the course of development, the CT undergo a structural reorganization to form a central core of dense regular connective tissue. However, the persistence of the muscular component in certain CT allows for their classification in human fetuses into muscular and fibromuscular types, which aligns with findings from comparative anatomical studies of the adult human heart [17; 20]. Aberrations in the chronology of this fibrous transformation can lead to serious congenital anomalies affecting the subvalvular complex [10; 23].

Consequently, a detailed understanding of the spatial orientation and tissue remodeling dynamics

of the cardiac valves [10; 22] deepens fundamental knowledge of cardiogenesis [20] and serves as a morphological basis for improving prenatal ultrasound diagnostics.

Conclusions

Thus, based on the study results obtained using a combination of morphological methods, the following findings were established:

1. Endocardial cushions, identified at the end of the 4th week of intrauterine development, give rise to the atrioventricular valvular apparatus of the heart and function as provisional valves in the human embryo heart at this developmental stage.

2. During the fetal period, the atrioventricular valve leaflets are covered by endothelium and contain interstitial cells surrounded by the amorphous ground substance of the extracellular matrix, while the fibrous component remains immature.

3. Primary chordae tendineae appear as muscular strands at the 15th week of intrauterine development in fetuses with a crown-rump length of 125.0 mm. Arising from the apices of the papillary muscles, they insert into the leaflets of the cardiac atrioventricular valves.

4. In the course of development, primary chordae tendineae differentiate into mature structures characterized by a predominance of dense, regular fibrous connective tissue. This developmental process allows classification of atrioventricular valve chordae tendineae in human fetuses as muscular or fibromuscular.

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Declarations

Conflict of interest is absent.

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The authors of the manuscript state that in the process of conducting research, preparing, and editing this manuscript, they did not use any generative AI tools or services to perform any of the tasks

listed in the Generative AI Delegation Taxonomy (GAIDeT, 2025). All stages of work (from the development of the research concept to the final edi-

ting) were carried out without the involvement of generative artificial intelligence, exclusively by the authors.

Authors' Contributions

Contribution	A	B	C	D	E	F
Authors						
SemeniukT.O.	+	+	+	+	+	+
PentelejchukN.P.	+	+	+		+	+

Notes: A – concept; B – design; C – data collection;

D – statistical processing and interpretation of data; E – writing or critical editing of the article;

F – approval of the final version for publication and agreement to be responsible for all aspects of the work.

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