RESEARCH ARTICLE

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Morphological and histological characteristics of heart in adult domestic rabbit (Oryctolagus Cuniculus)

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ABSTRACT The heart is a vital muscular organ responsible for pumping blood throughout the circulatory system. This study investigates the morphological, morphometric, and histological development of the heart during prenatal and postnatal stages in rabbits. For the prenatal analysis, ten rabbit fetuses were collected from pregnant local-breed does, while ten healthy adult rabbits (Oryctolagus cuniculus) were examined postnatally. Histological observations revealed that cardiac differentiation begins between the 9th and 10th days of gestation, with the formation of a smooth-walled heart loop comprising the myocardial mantle, cardiac jelly, and endocardium. During embryogenesis, the heart undergoes distinct morphological transformations, including heart tube formation, looping, chamber development, and septation. Postnatally, the heart assumes a cone shape, pinkish coloration, and is positioned in the mediastinum, tilted toward the left, between the lungs and anterior to the diaphragm, spanning from the 3rd to 5th intercostal spaces. Histological examination of the heart wall showed three layers: endocardium, myocardium, and epicardium. The pericardium consists of parietal and visceral layers; the former comprises dense connective tissue fused with the fibrous pericardium, while the latter is closely bound to the myocardium and covered with a simple epithelial layer. The myocardium, the thickest layer, contains cardiac muscle fibers embedded in connective tissue with nerve bundles, lymphatics, and capillaries. The endocardium is a thin inner layer composed of endothelial, subendothelial, and subendocardial components. These findings provide a comprehensive understanding of heart development in rabbits and serve as a valuable reference for comparative and developmental cardiovascular studies.

KEYWORDS histology, morphological, adult, rabbit, heart, study

1. INTRODUCTION

Domesticated rabbits in Iraq have their origin from the European rabbit, Oryctolagus cuniculus, which is identified by having a second tiny pair of upper incisors or peg teeth. Rabbit, among laboratory animals, has been utilized as a model organism for research. The cardiovascular system is crucial to all living things. The heart beats rhythmically and systematically, circulating blood throughout the vascular system. The heart is the initial functional organ in an embryonic development, and the circulatory system is its initial functional unit. The heart is the muscular organ with the capability of frequently contracting and circulating blood throughout the circulatory system to the entire body [1]. The outermost is the epicardium, the middle and thickest is the myocardium, and the inner layer of the heart and continuous with the tunica intima of blood vessels is the endocardium [2]. The basic similarity of the heart structure and function in rats and human beings is specially interesting: the relative heart weight and the relative wall thickness of the right and left ventricles in rats are equivalent to comparable parameters in the human heart [3]. The ventricles provide the contractile function of the heart, and their histological structure contributes to knowledge about mechanisms of the heart. The wall of the ventricle consists of three layers: epicardium, myocardium, and endocardium [2]. The myocardium is the principal shell of the heart, and its contractility depends on its functional status [4]. The histological construction of ventricular myocardial fibers is responsible for cardiac function [5]. The histological construction of the heart is a multidimensional mechanism. The heart has a significant role in delivering oxygen- and nutrient-rich blood. The growth of the heart is extremely crucial at various ages in order to fulfill several needs and to prevent various congenital heart defects [6].

2. MATERIALS AND METHODS

2.1 Animal Ethics

The institutional animal ethics committee granted permission for the experimental procedures, and the euthanasia procedures were ethically approved by the AL-Mustaqbal University Institutional Animal Care Committee. In the present study, a total of ten rabbit fetuses collected from the uteri of local breed pregnant does were used, along with eight adult rabbits with an average weight of 4200 grams and aged 2–3 years.

2.2 Anatomical Inspection

We used ten fetuses of rabbits obtained from the uteri of local breed pregnant does. The does were bred to a buck for 1-2 hours, ear-marked, and maintained in separate cages. Five fetuses were fixed for each stage starting from 10, 12, 14, 16, 18, and 20 days of gestation (Figure 1). All the fetuses were measured in terms of their crown-rump lengths (CRL). CRL is taken from the vertex of the skull to the mid-point between apices of buttocks for fetal age only [7]. Adult rabbits, weighing around 4200±32.522 grams, were bought from local farms in Babylon province. They were housed in cages in the animal facility. All the animals that were chosen were euthanized prior to dissection with an intravenous overdose of sodium phenobarbital (Delmarva Laboratories, Euthasol, Midlothian) at a dose of 200 mg/kg [8]. The heart was dissected out of the thoracic cavity and photographed with a digital camera. Every anatomical measurement was taken, and samples were put into a suitable fixative for histology.

Heart tissue was harvested from atria and ventricles and fixed in 10% formalin for 24 hours. Following fixation, the samples were dehydrated with alcohol, cleared with xylene, and embedded in regular paraffin. Paraffin blocks were sectioned at 5 µm thickness. The sections were stained with Hematoxylin and Eosin for routine observation, Masson's trichrome to demarcate the pattern of connective tissue, and Periodic Acid-Schiff (PAS) stain to identify carbohydrate compounds in the cells of epithelium. The histological sections were viewed using an Olympus light microscope with a digital camera attached to a computer. All the sections were examined, and photographs were taken under a light microscope model 6300 (Japan) at magnifications of 4x, 10x, 20x, and 40x.

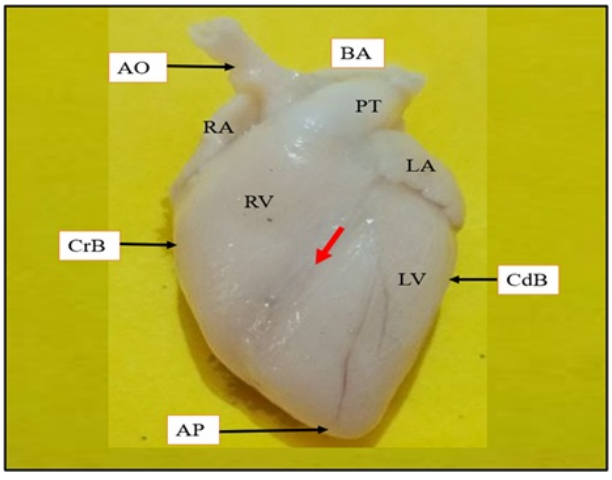

FIGURE 1. Morphological photograph of heart in adult rabbit (Oryctolagus Cuniculus) showing: (BA) base of heart, (AP) apex of heart, (RA) right atrium, (LA) left atrium, (RV) right ventricle, (LV) left ventricle, (CrB) cranial border, (CdB) caudal border, (red arrow) coronary groove, (AO) aorta, (PA) pulmonary trunk

3. RESULTS

3.1 Anatomical study

The development of rabbit heart pulsation starts during the 9th and 10th day of embryonic life, despite lacking valves and a conduction system. Morphologically, the heart of an adult rabbit is triangular in shape and located between the lungs, in the middle compartment of the thoracic cavity called the mediastinum. The heart extends between the third and fifth ribs. The heart's shape was trapezoidal when observed in frontal projection. The weight of the heart is about (4.28± 0.8) grams (Table 1). These results agree with [9], which reported that the average weight of the heart in sheep (Ovis aries) at the prenatal stage in the second half of gestation was about (10.694 ± 1.182) grams. The length of the heart from base to apex is about (42.464 ± 1.179) mm, and the width at the coronary groove is about (33.273 ± 1.197) mm. The length of the heart from base to apex is about (25.03± 0.4) mm, and the width of the heart is (15.2± 0.13 mm) (Table 2).

TABLE 1. Weight of animals and weight of heart (Mean ± SE)

Species	Anatomical parameters	Mean ± SE
Rabbit	Weight of animals	4200± 32.522 (gm)
Rabbit	Weight of heart	4.28± 0.08 (gm)

The heart has two surfaces (left and right), two borders (cranial and caudal), and two extremities—the base and the apex. The length of the cranial border is (22.5± 0.21 mm), and the caudal border is (24.5± 0.23 mm) (Table 2). The base is directed dorsally and formed by the right and left atria. The apex is directed ventrally, lying dorsally to the sternum. The cranial border is formed by the right ventricle, and the caudal border is formed by the left ventricle. The heart is composed of four chambers: two atria and two ventricles. The coronary groove (atrioventricular groove) separates the right and left ventricles. The paraconal interventricular groove descends on the left side of the heart and separates the two ventricles. The subsinuosal interventricular groove descends on the right side of the heart. The interatrial septum separates the right and left atria, and the ventricular wall is thicker than the atrial wall (Figure 1).

TABLE 2. Length of heart and width of heart and length of cranial and caudal border (Mean ± SE)

Species	Anatomical parameters	Mean ± SE
Rabbit	Length of heart	25.03± 0.4 (mm)
Rabbit	Width of heart	15.2± 0.13 (mm)
Rabbit	Length of cranial border	22.5± 0.21 (mm)
Rabbit	Length of caudal border	24.5± 0.23 (mm)

The right atrium forms the right-anterior part of the base of the heart and lies above the right ventricle. The right atrium receives blood from the body through two major veins: the superior vena cava, the inferior vena cava, and the coronary sinus (Figure 1). The wall of the right atrium has an oval-shaped depression known as the fossa ovalis. The internal

wall of the right atrium features prominent ridges of pectinate muscles, which are present in the right atrial appendage. The openings in the right atrium include the cranial vena cava, caudal vena cava, coronary sinus, and the right atrioventricular orifice (Figure 2).

The right ventricle constitutes the right-anterior part of the ventricular mass. It forms almost all of the cranial border of the heart but does not reach the apex. The right ventricle receives blood from the right atrium and pumps it into the lungs through the pulmonary trunk. The opening present in the right ventricle is the conus arteriosus, located at the beginning of the pulmonary trunk, which gives rise to the right and left pulmonary arteries.

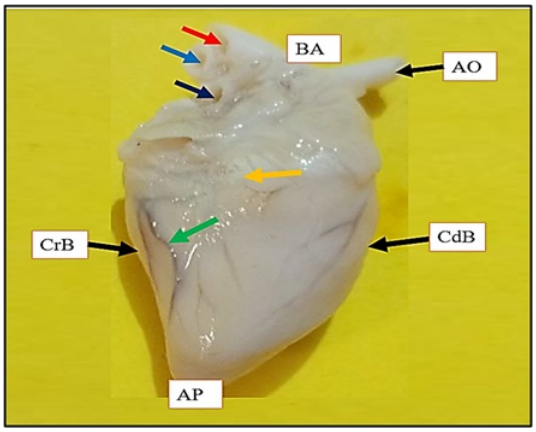

FIGURE 2. Morphological photograph of heart in adult rabbit (Oryctolagus Cuniculus) showing: (BA) base of heart, (AP) apex of heart, (CrB) cranial border, (CdB) caudal border, (AO) aorta of heart, (red arrow) cranial vena cava, (blue arrow) caudal vena cava, (black arrow) Pulmonary trunk, (green arrow) Coronary artery

The left atrium (atrium sinistrum) constitutes the posterior aspect of the base of the heart. It is situated behind the aorta and the pulmonary artery. The left atrium is above the left ventricle and takes oxygenated blood from the lungs via the pulmonary veins. The left atrium has an auricle, which is a blind-ended ear-like structure projecting laterally and cranially on the left side of the heart. It is situated caudal to the pulmonary trunk origin. Openings of the left atrium are the pulmonary veins and left atrioventricular orifice (Figure 3).

Left ventricle (ventriculus sinister) constitutes the posterior component of the ventricular mass. The left ventricle possesses a more muscular wall than the right ventricle due to the increased physiological load upon it. These findings concord with the fact that the left ventricular wall is denser than the right ventricular one and narrower lumen, attributed to intensive cardiac muscles and two big papillary muscles, as discussed by (Smerup et al., 2016). For the ovine species, the right ventricular wall possesses three papillary muscles that support the tricuspid valve, as supported by [10].

The openings available in the left ventricle are the aortic orifice, through which blood flows from the left ventricle to the aorta and subsequently to all the body organs. The aortic orifice is protected by the aortic valve, which is made of semilunar cusps connected to the aortic fibrous ring at the commencement of the aorta. Another opening is the left atrioventricular orifice, through which blood flows from the left atrium into the left ventricle and is protected by the bicuspid valve (Figure 3).

Both ventricles have shared structures: the papillary muscles—muscle projections used as sites of attachment for the chordae tendineae of the atrioventricular valves—and the chordae tendineae—elastic fibrous strands that fix the free margins of the atrioventricular valves to the papillary muscles and keep the valve leaflets from eversion into the atria during contraction of the ventricles.

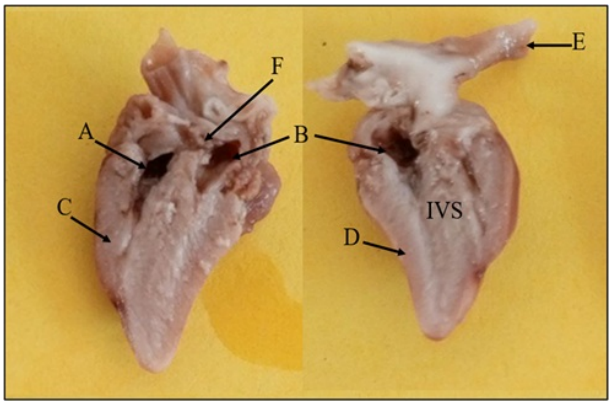

FIGURE 3. Anatomical photograph of the heart in adult rabbit (Oryctolagus Cuniculus) showing: internal structure (A) right atrium chamber, (B) left atrium chamber, (C) right ventricular wall, (D) left ventricle wall, (E) aorta. (F) interatrial septum, (IVS) interventricular septum

3.2 Histological Study

The atrial and ventricular walls of the present study consist of three layers: the endocardium (internal layer), myocardium (middle layer), and epicardium (outer layer). The inner layer, endocardium, consists of the endothelium layer, subendothelium layer, and subendocardium layer. These findings concur with earlier works that the endocardium is the innermost layer, which is lined by a thin layer of endothelium beneath the subendothelial connective tissue. The endothelium is a single layer of simple squamous epithelium. The subendothelium is the second endocardial layer, thicker than the endothelium, and supported by a thin zone of loose connective tissue. The subendocardium is the innermost layer of the endocardium, linking the endocardium to the myocardium, and bears two cell types: perinuclear clear zone cells and transitional cells. The perinuclear cells have scarce myofibrils in the cytoplasm, a single large central nucleus, and are smaller in size compared to cardiomyocytes. The transitional cells are rounded but smaller in size than perinuclear clear zone cells and have more myofibrils along with a large single central nucleus. The subendocardium layer of ventricles, which is a deep component of the ventricular wall, consists of Purkinje fibres. These fibres show few myocardial fibres and rich

carbohydrate content and strongly positive reactivity to PAS stain.

The myocardium is the middle and thickest wall of the atria and ventricles. Myocardium in the left ventricle is thicker than the right ventricle and constitutes the bulk of the ventricular wall. The myocardium of the left ventricle comprises larger and massive Purkinje fibres and cardiomyocytes because of increased pressure on the left ventricular wall. Myocardium consists of two forms of cardiomyocyte bundles: the working cardiac myocytes in the form of sheaths in intricate and varied directions. These bundles are enveloped with dense connective tissue termed epimysium and separated by perimysium. Endomysium surrounds each muscle fiber. Purkinje cardiomyocyte bundles are found in the subendocardium layer. In longitudinal section, cardiac myocytes are short, branch-like, striated, and anastomosed to form a net. The cardiomyocytes have one, oval, large, ovoid pale-staining nucleus that is centrally located and more euchromatic. There are binucleated cardiomyocytes.

The epicardium is the most external layer of the heart and is quite thin in comparison to the myocardium. The epicardium consists of mesothelium and the subepicardial layer. The subepicardial layer holds the myocardium together with the epicardium and consists of loose connective tissue primarily made up of collagen fibers. It is rich in blood vessels, lymphatic vessels, cardiac nerves, and numerous adipocytes. The heart is invested in a single layer of thin, flattened cells representing the typical simple squamous epithelial cells. The mesothelium rests upon a supporting subepicardial connective tissue.

4. DISCUSSION

The hearts in mammals are cone-shaped and have a pointed apex, except in dogs, which have oval hearts with a blunt apex. These results disagree with the morphology of the heart in *S. argus*, where it is positioned anteriorly in the peritoneal cavity and caudoventrally to the gills. It is likely that this has been investigated in some teleosts [11]. These results also disagree with [12], who noted that the pig heart's typical "Valentine heart" shape results from its position in the thoracic cavity, body orientation, and its two surfaces: the anterior (sternoventral) surface resting against the sternum, and the posterior (caudal) surface next to the diaphragm, with an apex and base and distinct upper and lower borders. The heart is composed of four chambers—two upper atria and two lower ventricles—which coincides with [13] in pigs and in bovines [14]. These results agree with the heart wall being normally composed of three layers: the outermost epicardium, the middle myocardium, and the innermost endocardium [11]. These results also agree with the presence of Purkinje fibers; in this study, they were observed within the myocardium, confirming their presence in both the endocardium and myocardium [15]. The endocardium consists of an inner, simple layer of endothelial cells (endocardial endothelium), a subendothelial layer (stratum subendothelial) of loose connective tissue, and a myoelastic layer (stratum myoelasticum) composed of connective tissue and smooth muscle cells [7].

5. CONCLUSION AND RECOMMENDATION

The purpose of our study was to examine the morphological and histological characteristics of the heart in adult domestic rabbits (*Oryctolagus cuniculus*). The results demonstrated that the epicardium is the thin outer layer of the ventricles and atria, composed of a subepicardial layer and mesothelial cells. The mesothelial cells are a single layer of simple squamous cells with flattened nuclei, supported by irregular, dense connective tissue. The subepicardial layer contains adipocytes in the atrium, as well as blood and lymphatic vessels that supply the cardiac muscle cells. Further histomorphological studies of the heart using light and electron microscopy in other domestic animals are recommended.

AUTHORS' CONTRIBUTION

Ahmed Jamil Abid, Ali Alhusainy, Dualfiqar Abbas Aswad, and Nadheema Bahaa Wetwet designed the research work and performed the anatomical and histological description of the heart. All authors reviewed and approved the final version of the manuscript.

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