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MICROSCOPIC STUDIES OF THE CARDIOVASCULAR SYSTEM IN SHEEP

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Abstract

This study was performed to determine the histostructural features of the heart wall and blood vessels in sheep. For this study, organs from normally developed, clinically healthy sheep of different ages were used. The samples were processed according to the usual histological techniques and stained by the methods Hematoxylin eosin, orcein and Mallory. Histological examination revealed that in the structure of the endocardium was observed the endothelium represented by a simple squamous epithelium, the endothelial cells have an elongated shape and arranged linearly. The subendothelial layer is represented by lax connective tissue and the subendocardial layer consists of well-represented lax connective tissue that continues with the interstitial connective tissue of the heart. The structure of the myocardium shows the contractile myocardium with a different thickness in the two ventricles and the excitoconductive tissue. The structure of the pericardium shows the visceral sheet represented by the serous pericardium and the parietal sheet represented by the fibrous pericardium. The circulatory system is made up of: macrovases made up of large caliber arterioles, elastic, muscular arteries, muscular veins and microvessels.

Key words*: endocardium, myocardium, epicardium, macrovessel, microvessel.*

INTRODUCTION

The function of the heart relies on the action of contractile cells, known as cardiomyocytes, specialized conducting cells that facilitate coordinating rhythmic contraction, extracellular matrix that provide mechanical support, as well as veins, arteries, and microvasculature to supply blood to the working muscle. Importantly, the heart vascular network, known as the coronary circulation, maintains perfusion of myocardial tissue with hemodynamics that are out-of-phase to the systemic circulation (Goodwill A.G., 2017).

Cardiac myocytes occupy approximately 75% of normal myocardial tissue volume, but they account for only 30-40% of cell numbers (Anderson, 2009). The majority of the remaining cells are non-myocytes, predominantly fibroblasts. Other cell types, such as endothelial or vascular smooth muscle cells, represent comparatively small populations (Lunkenheimer, 2006).

Histological examination, however, shows that the only muscular unit to be found within the myocardial walls is the cardiac myocyte itself. Our own investigations show that, rather than forming a continuous band, or being arranged as sheets, the myocytes are aggregated together as a three-dimensional mesh within a supporting matrix of fibrous tissue. Within the mesh of aggregated myocytes, it is then possible to recognize two populations, depending on the orientations of their long axes. The first population is aligned with the long axis of the aggregated myocytes tangential to the epicardial and endocardial borders, albeit with marked variation in the angulation relative to the ventricular equator. Correlation with measurements taken using force probes shows that these myocytes produce the major unloading of the blood during ventricular systole. The second population is aligned at angles of up to 40 degrees from the epicardium toward the endocardium (Anderson, 2009).

Concepts for ventricular function tend to assume that the majority of the myocardial cells are aligned with their long axes parallel to the epicardial ventricular surface. We aimed to validate the existence of aggregates of

myocardial cells orientated with their long axis intruding obliquely between the ventricular epicardial and endocardial surfaces and to quantitate their amount and angulation (Lunkenheimer, 2006).
Many animal studies have been performed to observe the formation sequences mature functional myocardocytes. In chicken embryos myocardocyte contractions may be observed only 36 hours after fertilization (Tokuyasu and Maher, 1987), and blood flow through the heart it begins 2 days after conception (Sissman, 1970). Myofibrils still uncompacted begin be visible in myocardocytes about 30 hours post-fertilization.
However, not much is known about the actual stage of sarcomere formation in human heart in vivo. At the age of 3-4 weeks, the human heart begins to contract, but does not he really knows to what extent sarcomeres are structurally developed (Mercola et al., 2011).
Although myocardial architecture has been investigated extensively, as yet no evidence exists for the anatomic segregation of discrete myocardial pathways (Smerup et al., 2009).
The visualization of the size, shape, and alignment of the myocytic arrays at any side of the ventricular wall is determined by the radius of the knives used, the range of helical angles subtended by the alignment of the myocytes throughout the thickness of the wall, and their angulation relative to the epicardial surface (Lunkenheimer, 2006).

MATERIALS AND METHODS

The research was conducted on permanent histological preparations of the heart and blood vessels of sheep clinically healthy. Histological specimens were prepared as follows: 10% formalin fixing, paraffin embedding and sectioning inclusion microtome. Large sections were stained on slides after staining following methods: hematoxylin eosin, orcein and Mallory (Bancroft, J.D. and A. Stevens, 1986).
By following successively and accordingly to the experimental plan, rigorous morphological studies were conducted using optical microscopy photomicrographs.
Histological preparations obtained were examined by light microscopy shooting device equipped with making photomicrographs.

RESULTS AND DISCUSSIONS

In the examined histological preparations it is observed as in sheep the endocardium of the left atrium is thicker and more opaque than that of the right atrium, and the collagen fibers are more numerous in the endocardium of the left atrium. As a histostructural aspect, the left ventricular endocardium appears thicker than the right ventricular endocardium.
The endothelium formed by a simple squamous epithelium arranged in a continuous layer on a thin basal membrane is observed. The subendothelial layer represented by a blade of loose connective tissue is observed. In its deep part, the collagen fibers appear more often, are arranged parallel to the surface of the endocardium and the elastic fibers form networks.
The level of the atria, the endocardium contains a continuous endothelium and a layer of loose connective tissue (Figure 1).

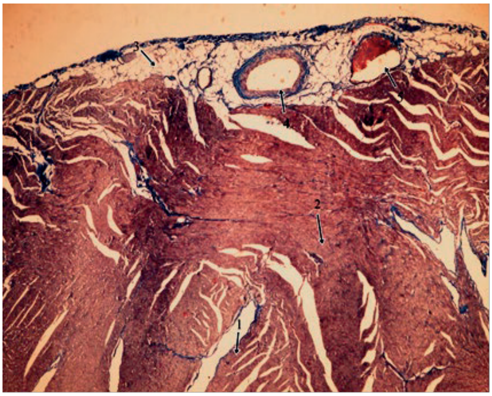

Figure 1. Heart, Mallory stain Overview, 4x objectiv: 1. Endocardium; 2. Myocardium; 3. Epicardium; 4. Artery; 5. Vein

The connective tissue is rich in elastic fibers, arterioles, veins, capillaries, nerve threads, adipocytes and elements of the excito-conductive system.
The right ventricular endocardium is thinner than the left ventricular endocardium.
In the structure of the myocardium, the myocardial fibers are organized in plexiform networks and are joined by means of intercalary discs or Eherth scalariform stria.
Their ends are often branched to form true anastomotic networks.

The areas of intercellular junctions appear in the form of transverse lines, with a winding trajectory, in a zig-zag pattern.
They are short and intersect cardiomyocytes at irregular intervals. The subendocardial space contains fenestrated elastic membranes and bundles of smooth non-muscular fibers, especially in the interventricular septum. At the level of the interventricular septum, blood vessels, nerves and elements of the excitoconductor system are observed.
In the wall of the right atrium, the sinoatrial node formed by a network of nodal cells is observed, in which the P cells are arranged centrally, and the T cells are arranged peripherally (Figure 2). Abundant loose connective tissue, blood vessels, nerve fibers, and nervous microglia are seen between the cells.
The cardiomyocytes have a cylindrical, elongated shape, the nucleus is located centrally (Figure 3).

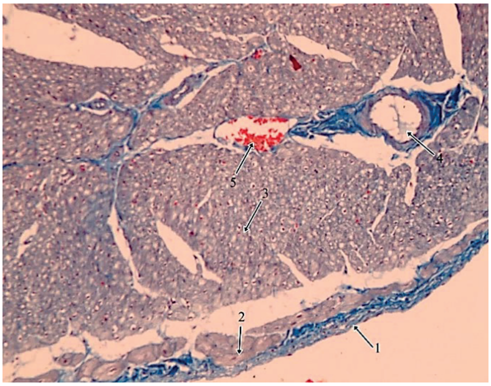

Figure 2. Heart, Mallory stain, 20x Objective: 1. Endocardium; 2. Nodal cells; 3. Myocardium; 4. Artery; 5. Vein

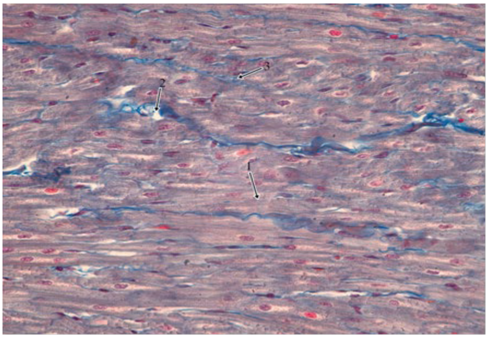

Figure 3. Heart, Mallory stain 40x Objective: 1. Cardiac muscle fibers; 2. Connective tissue; 3. Scalariform stria

Ventricular cardiomyocytes are larger than atrial cardiomyocytes. The working cardiomyocytes enters the structure of the atrial and ventricular myocardium. It is observed that they have morphological characteristics that clearly differentiate them from conducting cells.
The Purkinje anastomotic network is observed in the ventricular myocardium. These cells are smaller in size and are stacked with no orientation.

In the examined histological preparations it is observed that the contractile myocardium has a different thickness in the two ventricles (Figures 4, 5).
In the examined sections it is observed that the left ventricle has a much thicker wall than the right ventricle and the atria have a much thinner wall than the ventricular wall.

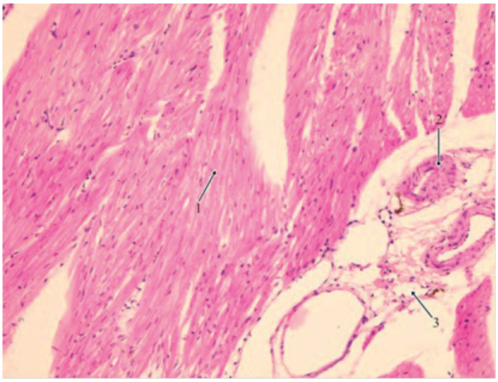

Figure 4. Heart, Hematoxylin and Eosin stain, 20x Objective: 1. Cardiac muscle fibers; 2. Blood vessels; 3. Connective tissue

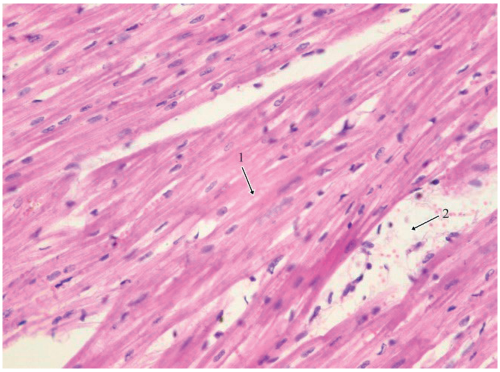

Figure 5. Heart, Hematoxylin and Eosin stain, 40x Objective: 1. Longitudinal section of muscle fibers; 2. Connective tissue

Mesothelium is represented by a simple, continuous squamous epithelium that covers the entire pericardium; The basal membrane of the mesothelium and the submesothelial connective tissue formed by a dense connective tissue, rich in elastic collagen fibers that form a continuous connective membrane, relatively thin in the superficial area, are observed.

In the deep part, the epicardial connective tissue has a looser structure and has numerous adipocytes (Figure 6).

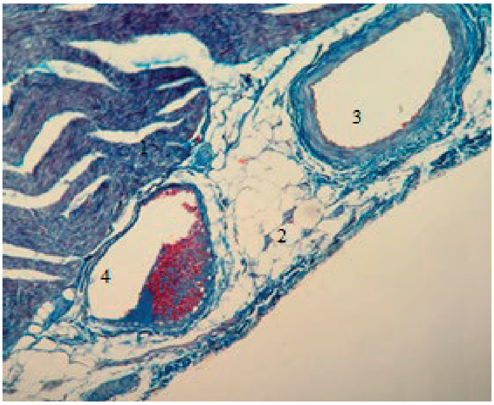

Figure 6. Heart, Mallory stain Overview, 20x Objective:
1. Myocardium; 2. Epicardium; 3. Artery; 4. Vein

It continues with the interstitial connective tissue of the myocardium.

Its structure shows the coronary arteries and their main branches, heart veins, lymphatic vessels and nerve fibers.

In the histological preparations examined from the arteries of elastic type it is observed that the endothelium is represented by a simple squamous epithelium, continuous, placed on a continuous basal membrane (Figure 7). Endothelial cells junction tightly with each other, but also with the basal membrane. They have numerous digitiform extensions.

The endothelium is a special type of epithelium with a semipermeable barrier function that separates the two compartments of the internal environment, blood plasma and interstitial fluid.

The endothelium has a high degree of specialization in mediating and actively monitoring the bidirectional exchange of small molecules, also having the role of restricting the transport of certain macromolecules.

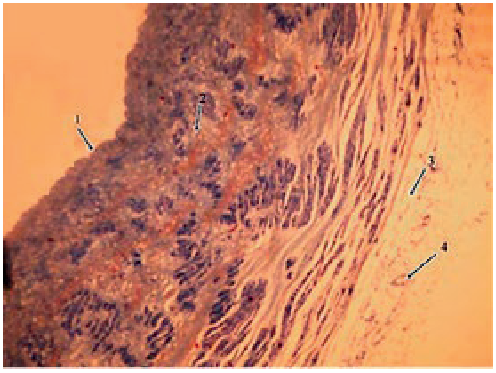

Figure 7. Aorta, Mallory stain Overview, 4x objective:
1. Intima; 2. Media; 3. Adventitia; 4. Vasa vasorum

The endarterium is located below the basal membrane and is made up of connective tissue rich in collagen, elastic and reticulin fibers. Among the fibers is connective tissue rich in glycoproteins and rare connective cells, especially fibroblasts.

The endarterium is separated from the tunica media by an internal elastic constraint. The internal elastic lamina is thin and difficult to distinguish from the first elastic blades of the tunica media.

Tunica media is the most developed structure. It consists of concentrically arranged fenestrated elastic slats, parallel, joined together by thin elastic fibers, few collagen fibers, smooth muscle fibers and sparse fibroblasts (Figure 8).

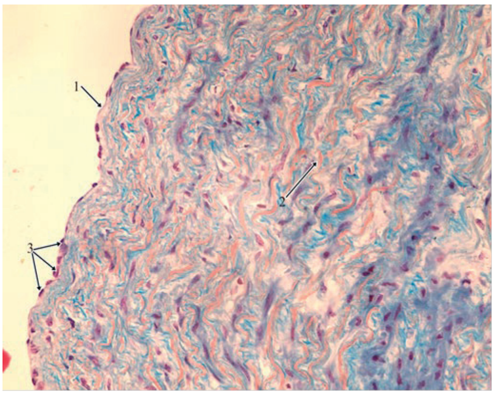

Figure 8. Aorta, Mallory stain, 40x Objective:
1. Intima; 2. Media; 3. Endothelial cell nuclei

The richness of lamellar elastic tissue and collagen fibers arranged on the lines of force creates a very elastic structure and at the same time very resistant to blood pressure.

Tunica externa or adventitia is well represented, consisting of areolar connective tissue in which there are blood vessels (vasa vasorum) and nerve extensions (Figure 9).

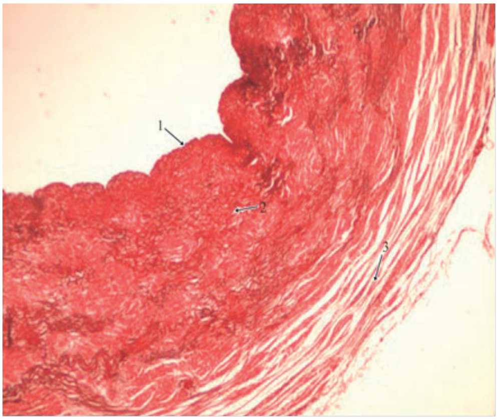

Figure 9. Aorta, Orcein stain Overview, 4x Objective: 1. Intima; 2. Media; 3. Adventitia

CONCLUSIONS

- The endothelium is a special type of epithelium with a semipermeable barrier function that separates the two compartments of the internal environment, blood plasma and interstitial fluid. The endothelium has a high degree of specialization in the mediation and active monitoring of bidirectional small molecule exchanges.
- Tunica intima is made up of a layer of endothelial cells supported by a basal membrane, a subendothelial layer made up of areolar connective tissue that may have a small number of smooth muscle fibers.
- Tunica media consists mainly of smooth muscle cells, arranged helically. Among them elastic and collagen fibers, proteoglycans and glycoproteins in are found varying amounts.
- Tunica externa or adventitia is composed mainly of collagen fibers and elastic fibers parallel to the long axis of the vessels. On the outside, the adventitia is lined by the connective tissue of the organ's blood vessels.
- The endocardium is similar to tunica intima of blood vessels and is bounded by a continuous endothelium consisting of a single layer of flattened cells. The left ventricular endocardium is thicker than the right ventricular endocardium.
- In the examined sections it is observed that the contractile myocardium has a different thickness in the two ventricles. The left ventricle has a much thicker wall than the right ventricle and the atria have a much thinner wall than the ventricular wall.
- The epicardium is a serous connective membrane covered by a single row of mesothelial cells.

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