



Peculiarities of organometry and morphoarchitectonics of the heart of the Domestic ram (*Ovis aries L.*, 1758)

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Abstract. Today, cardiovascular diseases are the dominant diseases of animals, which most often lead to their death both in Ukraine and worldwide. Therefore, morphological studies of the heart at the cellular, tissue, and organ levels are essential in clinical cardiology for in vivo ultrasound diagnosis of functional and organic heart lesions. The aim of the study was to perform a histological and cytomorphometric assessment of the morphological structures of the

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sheep heart using macroscopic, histological, and morphometric methods. Using morphological methods, the hearts of sexually mature clinically healthy animals (n=5) belonging to the class *Mammalia* – Mammals, species *Ovis aries L.*, 1758 – domestic sheep (ram) were studied. The absolute and relative heart weights of mature sheep were determined, which are, respectively, 208.4±9.82 g and 0.44±0.007%, and the weight without epicardial fat is 175.0±8.17 g. The heart of sheep, according to the development index (145.5±4.02%), belongs to the expanded-shortened anatomical type. The most developed components are the ventricles. At the same time, the ratio of the mass of the heart ventricles to its net mass is 1:0.78, respectively, the ratio of the mass of the atria is 1:0.22, and the ratio of the mass of the atrial myocardium to the mass of the ventricular myocardium is 1:0.29. The largest volume was found in the cardiomyocytes of the left ventricle – 3982.99±423.96 μm^3 , the smaller – in the right ventricle – 2463.02±318.04 μm^3 and the smallest – in the atrial cardiomyocytes – 1215.93±176.94 μm^3 . The volumes of cardiomyocyte nuclei in the left (53.42±5.18 μm^3), right (52.85±4.33 μm^3) ventricles and atria (50.16±4.57 μm^3) were almost identical. Nuclear-cytoplasmic ratio was the lowest in cardiomyocytes of the left ventricle – 0.0136±0.0062, slightly higher in cardiomyocytes of the right ventricle – 0.0219±0.0079 and the highest – 0.0430±0.0096 in atrial cardiomyocytes. The obtained results on the macro- and microscopic structure of the heart of bighorn sheep (*Ovis aries L.*, 1758) complement the information on the morphology of the sheep heart in the relevant sections of histology and comparative anatomy and are necessary for clinical cardiology

Keywords: morphology of organs; domestic animals; histostructure; development index; myocardium; cardiomyocytes

Introduction

The cardiovascular system is one of the most important systems of a living organism, which includes the heart, blood and lymphatic vessels that are systemically interconnected (Raiola et al., 2023). It is a system that performs a number of vital functions, including regulating blood supply to organs, ensuring blood pressure in the body and the outflow of lymph from organs and its transport to veins, and contributing to the functions of the immune defence, nervous and endocrine systems (Khomych et al., 2020). Thus, it plays an important role and participates in the regulation of homeostasis in the animal body and is one of the integrating systems of all living organisms. The heart is the central organ of the cardiovascular system in humans and animals. Due to the constant contraction of myocardial cardiomyocytes, the

heart drives blood flow in a closed system of blood vessels (Somberg, 2020). The study of the structural features of the internal structures of the heart is considered to be a relevant and essential link for the development of national morphology (Rykiel et al., 2020). This explains why veterinary cardiology, which studies diseases of the heart and blood vessels of animals, as well as cardiovascular surgery, are currently among the priority areas that are actively developing in veterinary medicine (Frąk et al., 2022; Pilz et al., 2022).

The sheep, as an experimental animal in this area of research, is often used as a model used in biomedical research and is perhaps one of the most influential models of human organ systems. At the same time, morphometric studies of organs and systems in clinically healthy

animals are a priority for timely and reliable diagnosis of diseases of various geneses, which are diagnostic criteria as indicators of normalcy for the diagnosis of diseases of contagious and non-contagious pathology (Hryhorieva & Cherniavskiy, 2018a; Shevchenko, 2018). The mathematical analysis of the structures of morphological objects has been recognized as a modern method that is distinguished by objectivity and reliability, which allows for a deeper understanding of the development of the pathological process and logical interpretation of the results of scientific research D. Baldrige *et al.* (2021) and I. Chala *et al.* (2021). This area is widely used in modern veterinary cardiology and provides objective information on the course of various physiological and pathological processes that occur in the organs and systems of the body due to damage to the cardiovascular system.

Currently, research of this nature is extremely relevant and is fundamental for the development, improvement, and application of new methods of prevention and treatment of cardiac diseases, which will be aimed at improving the quality of life of animals, including humans. The aim of the study was to investigate the morphological parameters of the heart of domestic sheep (*Ovis aries L.*, 1758) at the organ, tissue and cellular levels.

Literature Review

Over the past decades, many fundamental works have been published summarizing modern concepts and achievements of morphological and functional regularities in the structure and development of the heart in domestic animals of the mammalian and bird classes, structural components of its wall, etc. (Shevchenko, 2018; Hryhorieva & Cherniavskiy, 2018b), which is important for clinical cardiology to establish diagnoses in pets of certain species, including humans.

The study of the structure of the heart and its components in the comparative species and age aspects in different animals is devoted to the work of D. Solc (2007) and O.B. Slabyi *et al.* (2017). However, the literature often contains information on the structure of the heart mainly during the period of intrauterine development of animals or in laboratory, domestic animals and humans, less in newborn domestic maturational animals (Lelovas *et al.*, 2014; Slabyi, 2017). Thus, recent studies on the cardiovascular system of vertebrates, including domestic animals, have led to the discovery of new, previously unknown facts that require further in-depth study of the heart and its structures in comparative anatomical, species, breed, and age aspects (Stakhurska & Pryshliak, 2014).

Despite the fact that in domestic animals, morphological and haemodynamic parameters of the normal heart have a fairly wide range of fluctuations and constitute a certain number of studies of the cardiovascular system (Granger, 1998; Duncker & Bache, 2008; Hnatiuk & Slabyi, 2016), there are currently no clear objective morphological characteristics of structural and functional transformations obtained by morphological methods in the species aspect. There are numerous studies on the study of certain morphological aspects of the heart structure and structural features of its structure in domestic animals that have been studied by previous researchers (Anderson & Ho, 2003; Vansiatskaia & Kyrpaneva, 2014). At the same time, as the analysis of domestic and foreign scientific literature shows, the comparative and age-related morphology of the inner surface, its components at the macro- and microscopic level remain extremely poorly understood, so a significant number of issues remain unresolved and debatable in the problem of sheep heart morphology. Particularly poorly understood issues are the study of morphometric parameters of contractile muscle cells of the

heart myocardium (volume of cardiomyocytes, volume of their nuclei and nuclear-cytoplasmic ratio (NCR) of the ventricles and atria, which perform different functions: the atria receive blood returning to the heart from the body of animals; ventricles pump blood from the heart to the body of animals, while performing the greatest load.

Some authors (Solc, 2007; Sahni *et al.*, 2008) have shown a certain dynamism of the heart structure in the prenatal and early stages of postnatal ontogeny in domestic animals. For example, in mammals, as in other vertebrates, the heart has a dilated base and a narrowed apex. In large Mammals, the heart is located in the ventral part of the mediastinum and in most of them, the heart tends to have an elongated apex, except for sheep, in which the heart may have a blunted apex (DiVincenti *et al.*, 2014). In such animals, three types of hearts are distinguished: elongated-narrowed, cone-shaped, and dilated-shortened. As for the structure of the myocardium of domestic animals, there is a characteristic sharply expressed reticulation, which is due to the arrangement of fibres of the myocardium formed by cardiomyocytes, where thick layers of connective tissue are visible between the intermuscular fibres. The transverse striation of cardiomyocytes and insertion discs between myocardial contractile myocytes in cattle are poorly visible (Emam & Abugherin, 2020). Such characteristics of the heart structure, according to the literature analysis, are ambiguous, especially the dynamics of external parameters of the heart in sheep, its mass, internal architecture in the species age, etc. need to be clarified.

Therefore, the studies conducted by the authors on the structure of the heart and its morphometric assessment of morphological structures in sheep are important indicators of accurate morphological criteria at the macro- and microscopic levels, which is of exceptional

interest for biology, medical and veterinary practice. In particular, such data on the regularities of the structure and formation of the structural components of the heart in the normal state can be used in the diagnosis of diseases of various geneses in domestic animals.

Materials and Methods

In this study, sexually mature clinically healthy animals belonging to the class *Mammalia* – Mammals, species *Ovis aries L.*, 1758 – domestic sheep (ram) were investigated. The experimental animals were selected according to the principle of analogues, weight, breed, and age. Based on the objectives of the research, the following stages were performed: anatomical dissection of the hearts of the studied animals, description of the shape, structure and topography, determination of the absolute and relative weight of the heart, its components, microscopic examination, organ and cytometric parameters of the organ. The entire experimental part of the research was carried out in accordance with the requirements of the international principles of the European Convention for the Protection of Vertebrate Animals Used for Research and other Scientific Purposes (1986, March) and the Law of Ukraine No. 3447-IV “On Protection of Animals from Cruelty” (2006, February).

The sheep were dissected from the thorax and the hearts (n=5) were taken together with the pericardium for further macroscopic examination and organometric analysis, which included determination of the heart shape, location in the thoracic cavity, absolute and relative mass, height, width, and circumference. The absolute weight of the heart and its ventricles and atria was determined by weighing it on a laboratory balance, RADWAG PS 6000/C/2 (Poland). Relative mass of heart (RM) was calculated by the formula (1):

$$RH = \frac{AM}{MA} \cdot 100, \quad (1)$$

where: AM is the absolute mass of the heart; MA is the mass of the animal.

Determination of the linear parameters of the organ (height, width, and circumference) was carried out by direct measurement. The heart development index (HDI) was determined by the ratio of its total height to its width using the following formula (2):

$$HDI = \frac{HO}{WO} \cdot 100, \quad (2)$$

where: HO is the height of the organ; WO is the width of the organ.

For histological studies of the heart, general methods of fixation and preparation of histological sections were used (Horalskyi *et al.*, 2019). For this purpose, the corresponding pieces of longitudinal and transverse sections of the heart were fixed in a 12% chilled aqueous solution of neutral formalin for 24 hours or more, after which they were embedded in paraffin according to the schemes proposed by L. Horalskyi *et al.* (2019). Paraffin sections of the studied material (10-12 μm thick) were made on a sled microtome MS-2. To study the histological structure of the heart, such sections, after deparaffinisation, were stained with the classical staining method – haematoxylin and eosin (Diapath, Italy, 2020) and the Heidenhain method. The latter staining method allowed for better and clearer differentiation of cardiomyocytes, as well as detection of cardiomyocyte junctions (insertion discs).

Stained histological sections were used to perform histometric studies of the structural elements of the heart wall, including measurements of cardiomyocyte length and width, volume of cardiac cells and their nuclei, and, as a rule, the nuclear-cytoplasmic ratio. These studies were performed by light microscopy using Micros (Austria, 2012) and MBS-10 (Micromed, 1998) microscopes, according to the recommendations set out in L. Horalskyi *et al.* (2019).

The nuclear-cytoplasmic ratio (NCR) of cardiomyocytes was determined by formula (3):

$$NCR = \frac{V(n)}{V(c)-V(n)}, \quad (3)$$

where: NCR – nuclear-cytoplasmic ratio; $V(n)$ is the volume of the nucleus; $V(c)$ – cardiomyocyte volume.

The following formula (4) was used to determine the volume of cardiomyocytes:

$$Vc = P \times (B/2)^2 \times A, \quad (4)$$

where: Vc – cardiomyocyte volume; P – 3.14; B – width (diameter) of the cardiomyocyte; $B/2$ – cardiomyocyte radius; A is the length of the cardiomyocyte.

Determination of volume of nuclei of cardiomyocytes were determined by formula (5):

$$V(i) = P/6 \times A \times B^2, \quad (5)$$

where: $V(n)$ is the volume of nuclei of cardiomyocyte; P – 3.14; A is the length of the nuclei of cardiomyocyte; B is the width of the nuclei of cardiomyocyte.

The length and width of cardiomyocytes and their nuclei were measured using a microscope eyepiece ruler. Histological terms for structural parts of the organ are given according to the International Veterinary Histological Nomenclature and the International Veterinary Anatomical Nomenclature (Khomych *et al.*, 2020).

Histological sections were photographed using a 5.0 megapixel CAM V-200 video camera (InterMed, China, 2017) mounted in a microscope using a $\times 10$; $\times 40$ objective and a $\times 10$ eyepiece. The digital material was processed using variational and statistical methods on a personal computer using Microsoft Excel. The arithmetic mean (M) and the statistical error of the arithmetic mean (m) were determined, the probability of the difference between the arithmetic means of two variation series was

determined by the probability criterion (P) and Student's tables. The difference between two values was considered significant at $P < 0.05$, $P < 0.01$, $P < 0.001$.

Results and Discussion

The sheep heart is a hollow fibro-muscular organ that ensures continuous blood flow through a closed system of vessels. The heart has a conical shape, with a widened base and a narrowed apex (Figs. 1, 2). The organ is located in the mediastinum of the thoracic cavity between both lungs, in the area from the third to the sixth rib cranially of the diaphragm: cranially it reaches the third rib, caudally – the sixth rib. In relation to the median sagittal plane, the heart is displaced to the left by 5/7, adjacent to the left chest wall between the third and fourth ribs. The base of the heart is craniodorsal and is located at the height of the middle of the first or second rib. Accordingly, the apex of the heart is directed caudoventrally and is located opposite the fifth rib cartilage, or caudally from it, not reaching the sternum by two cm, and cranially from the diaphragm – from two to five cm.

The heart is contained in a thin but dense sac – the heart jacket (pericardium). The latter surrounds the organ from all sides, thus forming a closed serous sac, which is attached to the breastbone by two ligaments in the area of the sixth rib cartilage. Externally, the heart of mature sheep is divided into left and right ventricular outer grooves and a septum inside, which are not connected to each other. From the outside, each half of the heart is divided (left and right) into atria and ventricles by a transverse coronary furrow that runs across the heart closer to its base. The atria and ventricles of the same name (right and left) are connected to each other by the atrial-ventricular orifices.

The right and left atria in experimental animals are located at the very base of the heart, where they form bag-like protrusions – the

right and left heart ears, which are directed in the cranial direction and are located to the right and left, respectively, of the pulmonary arteries and aorta. The ventricles of the heart occupy the main part of the heart, externally they are separated from each other by the interventricular subaxillary and circumcondylar grooves, which are combined on the cranial surface of the heart, not reaching its apex, separating the right ventricle from the left. The apex of the heart in sheep refers to the left ventricle, which is located to the left in the caudal direction. The right ventricle of the heart, respectively, is located to the right in the cranial direction. The interventricular furrows have a similar location (subaxillary – in the caudal direction, near-cone – in the cranial direction) (Figs. 1, 2).

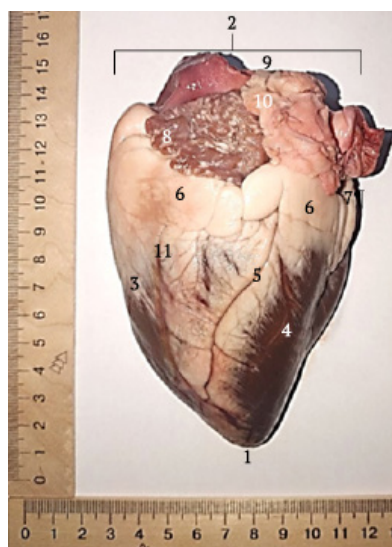


Figure 1. Macroscopic structure of a sheep's heart (left side)

Notes: Symbols: 1 – apex of the heart; 2 – the base of the heart; 3 – right ventricle; 4 – left ventricle; 5 – semi-conical interventricular groove; 6 – subepicardial fat; 7 – left atrium; 8 – left auricle; 9 – right auricle; 10 – pulmonary trunk; 11 – blood vessels. Photo from macropreparation

Source: author's development

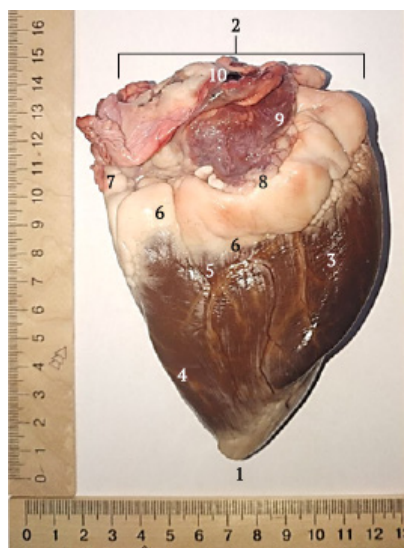


Figure 2. Macroscopic structure of a sheep's heart (right side)

Notes: 1 – apex of the heart; 2 – the base of the heart; 3 – right ventricle; 4 – left ventricle; 5 – axillary interventricular groove; 6 – subepicardial fat; 7 – left atrium; 8 – right atrium; 9 – right auricle; 10 – pulmonary trunk. Photo from macropreparation

Source: author's development

The criteria of organ development, which directly indicate its morphological and functional maturity, are absolute and relative weight, its linear parameters, etc. Indicators of morphometric parameters not only indicate

the development and morphological and functional maturity of the organ, but also have cognitive value and are the basis for determining the shape, establishing comparative anatomical types of certain organs (Linask, 2003; Belimenko *et al.*, 2021). According to the current studies, the absolute heart weight of mature sheep was 208.4 ± 9.82 g, and the relative weight was $0.44 \pm 0.007\%$. The net heart weight of the experimental animals (without epicardial fat) was 175.0 ± 8.17 g. Moreover, the height of the heart was 13.1 ± 0.4 cm, the width was 9.0 ± 0.3 cm, and the circumference was 22.2 ± 0.6 cm (Table 1).

Depending on the species, breed, and age of the animal, the following heart shapes can be distinguished in domestic mammals: narrowed-elongated (cattle), narrowed-shortened (rabbit), expanded-shortened (horse), and round-oval (dog). In dogs, it can be ellipsoidal (43%), cone-ellipsoidal (24%), ellipsoidal-spherical (26%) and spherical (7%), in cattle it can be elongated-narrowed, cone-shaped and expanded-shortened. Pigs have three main types of hearts: elongated-narrowed, conical; shortened, relatively narrowed; expanded-shortened, triangular (Rudyk, 2004; Demus, 2015). According to the analysis of the morphometry, in terms of linear parameters, the index of sheep heart development was $145.5 \pm 4.02\%$, so the heart of this species of animals is of the expanded-shortened type (Table 1).

Table 1. Linear parameters of the heart of sheep, $M \pm m$, $n=5$

Indicator	Numeric values
The height of the heart, cm	13.1 ± 0.4
The width of the heart, cm	9.0 ± 0.3
Circumference of the heart, see	22.2 ± 0.6
Heart development index, %	145.5 ± 4.02
The average value of the thickness of the wall of the ventricles, mm	12.42 ± 0.17
The thickness of the wall of the left ventricle, mm	16.2 ± 0.22
The thickness of the wall of the right ventricle, mm	8.04 ± 0.11
The average value of the thickness of the atrial wall, mm	6.62 ± 0.43

Table 1. Continued

Indicator	Numeric values
The thickness of the wall of the left atrium, mm	7.05±0.09
The thickness of the wall of the right atrium, mm	5.06±0.07

Source: author's development

The most developed anatomical structures of the heart are its left and right ventricles, then the left and right atrium, which correlates with linear indicators of the thickness of their walls and their absolute and relative mass, in relation to the net mass of the heart (without epicardial fat) (Table 1, 2).

Table 2. Morphometry of the heart, ventricles, and atria of sheep, M±m, n=5

Indicator	Absolute mass, g	Relative mass, %
Left atrium	27.9±3.31	15.94±1.49
Right atrium	11.2±2.02	6.4±0.82
Right and left atrium (together)	39.1±4.64	22.34±2.02
Left ventricle	90.3±5.21	51.6±3.06
Right ventricle	45.6±3.04	26.06±1.32
Left and right ventricles (together)	135.9±7.16	77.66±4.36
Heart weight (without apical fat)	175.0±8.17	100
The coefficient of the ratio of the mass of the ventricles to the mass of the heart	1:0.78	
The coefficient of the ratio of the mass of the atria to the mass of the heart	1:0.22	
The coefficient of the ratio of the mass of the myocardium of the atria to the mass of the myocardium of the ventricles	1:0.29	

Source: author's development

Thus, the wall thickness of the left ventricle was significantly greater than that of the right ventricle, respectively, by 2.01 times ($P<0.01$) and was equal to 16.2±0.22 mm, and the right ventricle was 8.04±0.11 mm. At the same time, the thickness of the atrial wall was 6.62±0.43 mm, respectively, of the left atrium – 7.05±0.09 mm, and of the right atrium – 5.06±0.07 mm (Table 1). With such linear parameters of the heart components, the average mass of the left atrium was 27.9±3.31 g (15.94±1.49%), the average mass of the right atrium relative to the left atrium was significantly ($P<0.01$) 2.5 times less and equalled 11.2±2.02 g (6.4±0.82%). The average weight of the atria of the sheep heart is 39.1±4.64 g (22.34±2.02%). The mass of the left ventricle is the largest and was 90.3±5.21 g (51.6±3.06%),

the weight of the right ventricle was intermediate and equalled 45.6±3.04 g (26.06±1.32%), the average weight of both ventricles was 135.9±7.16 g (77.66±4.36%). Consequently, the mass of the ventricles of the sheep heart was significantly ($P<0.001$) 3.5 times higher than the mass of the atria. Accordingly, the ratio of the mass of the ventricles of the heart of a mature sheep to its net mass (without epicardial fat) was 1:0.78, the ratio of the mass of the atria to its net mass was 1:0.22, and the ratio of the mass of the atrial myocardium to the mass of the ventricular myocardium was 1:0.29 (Table 2).

The wall of the sheep heart is formed by the respective membranes: endocardium – inner, myocardium – middle and epicardium – outer. The main structural component of the ventricular and atrial heart wall is the myocardium, a muscular

membrane consisting of cardiac myocytes (cardiomyocytes). Histological analysis of myocardial sections of the ventricular wall of the heart (left and right) stained with haematoxylin and eosin reveals five gradual layers: the outer and inner layers (their muscle fibres have an oblique longitudinal direction), then the outer and inner deeper layers and the deepest layer, whose fibres have a figure-eight direction (Fig. 3).

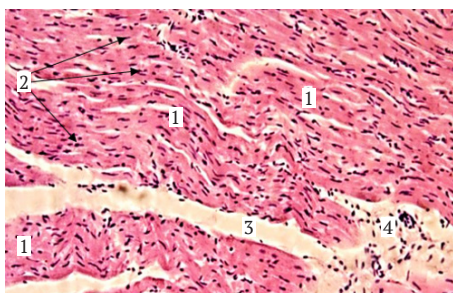


Figure 3. Microscopic structure of the myocardium of the left ventricle of a heart of a sheep

Notes: 1 – muscle fibres (longitudinal section); 2 – nuclei; 3 – intermuscular connective tissue; 4 – microcirculatory vessel. Haematoxylin and eosin. $\times 120$
Source: author's development

The myocardium of the atrial wall is formed by only two layers of muscle membrane – the outer (common to both atria) and the deep. The muscle fibres of the outer layer of the myocardium are located in a transverse direction from the right to the left ear. The muscle fibres of the deep myocardial layer of the right and left atria are located in the longitudinal direction. However, in the area of the myocardial venous orifices, circular bundles of muscle fibres are found. Due to the more intensive development of the ventricular myocardium relative to the atria, the ventricular walls are much thicker relative to the atrial wall, which is associated with their morphological and functional activity. The histoarchitecture of the ventricular

and atrial myocardial walls is formed by cardiac striated muscle tissue, which is represented by cardiomyocytes that form muscle fibres and intermuscular layers of loose fibrous connective tissue with the presence of blood and lymphatic vessels and nerves (Figs. 3, 4).

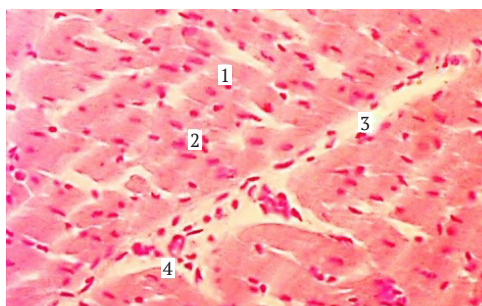


Figure 4. Microscopic structure of the myocardium of the left ventricle of a heart of a sheep

Notes: 1 – muscle fibres (transverse section); 2 – nuclei; 3 – intermuscular connective tissue; 4 – microcirculatory vessel. Haematoxylin and eosin. $\times 280$
Source: author's development

Cross-striated muscle fibres, built from cardiac myocytes (cardiomyocytes), which perceive colour in different ways (Fig. 5).

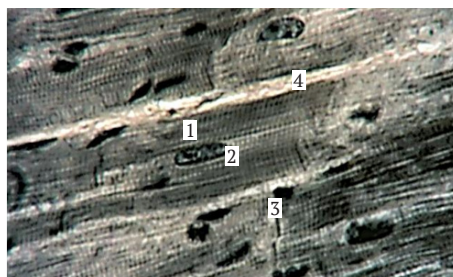


Figure 5. Microscopic structure of the myocardium of the left ventricle of a heart of a sheep

Notes: 1 – cardiomyocytes; 2 – nuclei of cardiomyocytes; 3 – insert disks; 4 – intermuscular connective tissue. Dyeing according to the Heidenhain method. $\times 600$
Source: author's development

Cardiac myocytes in the myocardium form a network of thin and thicker, porous striated muscle fibres, between which there is a gap space filled with intermuscular connective tissue. The parallel myocardial muscle fibres, formed by cardiomyocytes, are interconnected by anastomoses and form a mesh-like structure, forming a single contractile system of the heart. The central part of the cardiomyocyte sarcoplasm contains one, rarely two nuclei, which are oval or elongated and unevenly spaced. Their nuclear chromatin in the form of small or large grains is located around the perimeter of the caryoplasm (Fig. 5). Cardiomyocytes in the fibre structure, when staining histological specimens using the Heidenhain method, are arranged in a chain, interconnected by insertion discs (Fig. 6). When histological sections are stained with hematoxylin and eosin, cardiomyocytes in the cardiac muscle tissue form histological structures similar to the muscle fibres of somatic muscle tissue.

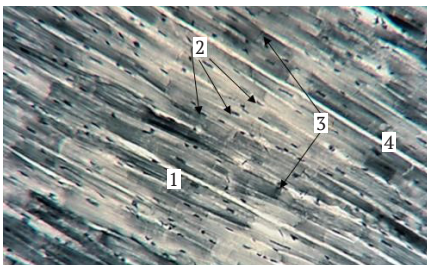


Figure 6. Microscopic structure of the myocardium of the left ventricle of a heart of a sheep

Notes: 1 – cardiomyocytes; 2 – nuclei of cardiomyocytes; 3 – insert discs; 4 – intermuscular connective tissue. Dyeing according to the Heidenhain method. ×280

Source: author's development

This connection of cardiomyocytes with each other by insertion discs, forming muscle fibres, provides a support function for the contractile elements of cardiac cells (myofilaments) and ensures a single contraction of the

myocardium and thus forms a functional syncytium. In light microscopy of histological specimens stained by the Heidenhain method, cardiomyocytes in the longitudinal section appear as dark stripes, rectangular (Fig. 7), and in the transverse section – rounded (Fig. 8), so their shape is cylindrical.

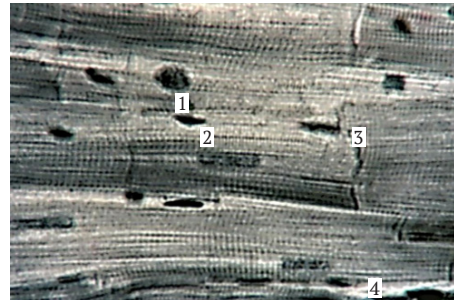


Figure 7. Microscopic structure of the myocardium of the left ventricle of a heart of a sheep

Notes: 1 – cardiomyocytes; 2 – nuclei of cardiomyocytes; 3 – insert discs; 4 – intermuscular connective tissue. Dyeing according to the Heidenhain method. ×600

Source: author's development

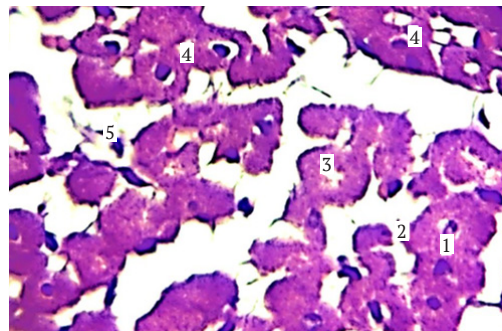


Figure 8. Microscopic structure of the myocardium of the right ventricle of a heart of a sheep

Notes: 1 – cardiomyocytes (transverse section); 2 – sarcolemma; 3 – sarcoplasm; 4 – nuclei of cardiomyocytes; 5 – intermuscular connective tissue. Hematoxylin and eosin. ×400

Source: author's development

In cardiomyocytes with such a histological structure, their sarcolemma, sarcoplasm, nuclei, and myofibrils are distinctly differentiated. They have clearly defined transverse (due to the presence of myofibrils) and longitudinal (due to the presence of actin and myosin proteins) striations (Fig. 9).

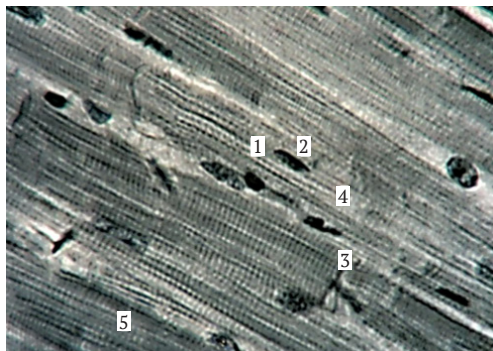


Figure 9. Microscopic structure of the myocardium of the left ventricle of a heart of a sheep

Notes: 1 – cardiomyocytes; 2 – nuclei of cardiomyocytes; 3 – insert disks; 4 – transverse striation; 5 – longitudinal striation. Dyeing according to the Heidenhain method. $\times 600$

Source: author's development

Myofibrils (special-purpose organelles) on the longitudinal section of cardiomyocytes, under a light microscope, look like thin filaments running longitudinally, parallel to each other, and having the length of the cardiomyocytes themselves (muscle fibres) (Fig. 9). In most cases, myofibrils are located along the entire perimeter of the sarcoplasm, which is visible in a cross-section of cardiomyocytes, where they appear as dots of several dozen in one cardiomyocyte (Fig. 10). Special-purpose organelles, often via anastomoses, pass from one fibre to another, thus ensuring the joint contractile function of the cardiac myocardium.

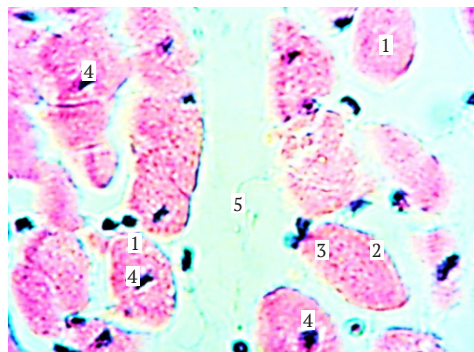


Figure 10. Microscopic structure of the myocardium of the left ventricle of a heart of a sheep

Notes: 1 – cardiomyocytes (transverse section); 2 – sarcolemma; 3 – sarcoplasm; 4 – nuclei of cardiomyocytes; 5 – intermuscular connective tissue. Haematoxylin and eosin. $\times 600$

Source: author's development

Myofibrils that are densely packed in the fibre structure and are located closer to its periphery are connected to other fibres by anastomoses. With a low density of myofibrils, the longitudinal striation of muscle fibres is clearly expressed, and the transverse striation is relatively weak. Thicker muscle fibres are much less susceptible to colour, so their transverse striation is weakly expressed, and myofibrils have a more elegant appearance. In muscle fibres of small thickness, myofibrils are more densely arranged.

In modern morphology, morphometric research methods are widely used, which make it possible to establish the relationship and interdependence of quantitative changes in individual structures of the animal body, quantitative and relative characteristics of certain morphological components at different stages of ontological and phylogenetic development of animals and different functional states of a particular animal body system, depending

on their species characteristics, etc. According to the analysis of literature sources (Demus, 2015; Slabyi et al., 2017) and the results of the author's research, the microscopic structure of the sheep heart, its histoarchitectonics of the atria and ventricles have a similar structure, but differ in histo- and cytometric parameters, which make it possible to establish even minor

changes in the microscopic structure at the tissue and cellular levels, depending on their functional load and haemodynamics. Thus, according to the results of histometry, cardiomyocytes forming layered muscle fibres, depending on their morphotopography (left, right ventricles, atria), are characterized by ambiguous cytometric parameters (Table 3).

Table 3. Histometric indicators of cardiomyocytes sheep, M±m, n=5

Indexes	Length of cardiomyocytes (µm)	Width of cardiomyocytes (µm)	Volume of cardiomyocytes (µm ³)	Volume of nuclei of cardiomyocytes (µm ³)	Nuclear-cytoplasmic ratio
Left ventricle	62.92±1.84	8.98±0.64	3982.99±423.96	53.42±5.18	0.0136±0.0062
Right ventricle	49.52±1.62*	7.96±0.56*	2463.02±318.04*	52.85±4.33	0.0219±0.0079**
Auricle	42.04±1.27**	6.07±0.38*	1215.93±176.94**	50.16±4.57	0.0430±0.0096***

Note: *P<0.05; **P<0.01; ***P<0.001 compared to the left ventricle

Source: author's development

At the same time, the quantitative indicators of contractile myocytes of the left ventricle of the sheep heart myocardium are greater than those in the right: the average length of cardiomyocytes of the left ventricle is significantly (P<0.05) is 1.27 times greater than that of the right ventricle and is equal to 62.92±1.84 µm, the width of cardiomyocytes, respectively (P<0.05) is 1.13 times greater and is 8.98±0.64 µm (Table 3). Similar morphometric characteristics were found in the calculation of cardiomyocyte volumes: the largest

volume of cardiomyocytes is characteristic of the left ventricle (3982.99±423.96 µm³), the volume of cardiomyocytes of the right ventricle, compared with the left, is significantly (P<0.05) less by 1.62 times and is equal to 2463.02±318.04 µm³. Similar changes in cytometric parameters were found in determining the volume of cardiomyocyte nuclei: a larger volume of cardiomyocyte nuclei was characteristic of the left ventricle (53.42±5.18 µm³), slightly smaller for the right ventricle (52.85±4.33 µm³) (Table 3; Fig. 11).

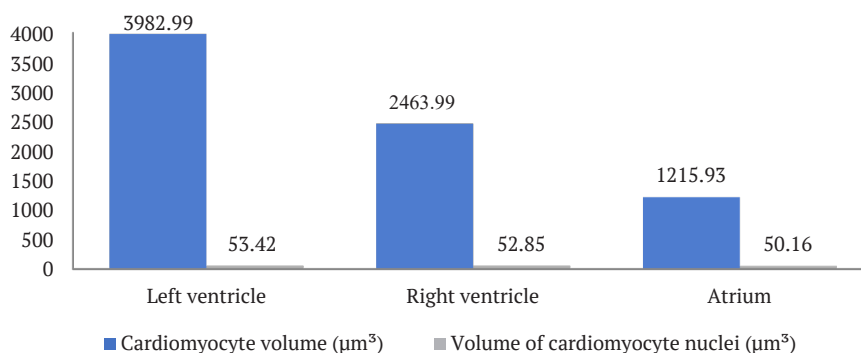


Figure 11. Histometric parameters of cardiomyocytes of heart myocardium of sheep

Source: author's development

The revealed ambiguous morphometric parameters of the volumes of cardiomyocytes and their nuclei in the right and left ventricles of the heart lead to different nuclear-cytoplasmic ratios: the lowest nuclear-cytoplasmic ratio is characteristic of cardiomyocytes of the

left ventricle (0.0136 ± 0.0062) and significantly ($P < 0.01$) 1.61 times higher for cardiomyocytes of the right ventricle (0.0219 ± 0.0079), which indicates the morphological and functional activity of cardiomyocytes of the left ventricle (Fig. 12).

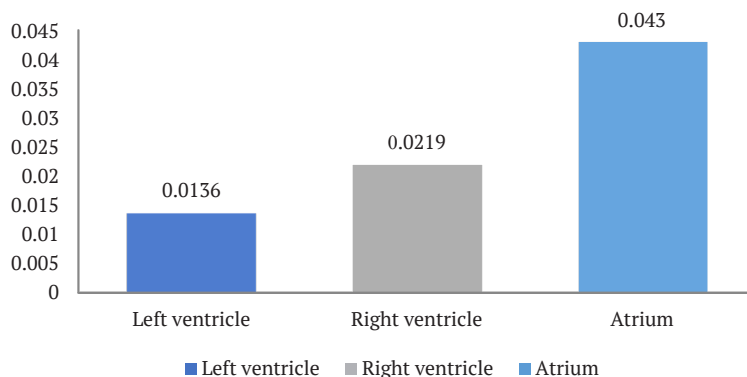


Figure 12. Nuclear-cytoplasmic ratio of cardiomyocytes of heart myocardium of sheep

Source: author's development

This should not be considered a coincidence, but an objective and realistic characteristic associated with differences in the activity of the heart ventricles, since the left ventricle functions mainly as a pump, and the right ventricle – as a volume ventricle (Haligür & Dursun, 2009). Therefore, an increase in cytometric parameters (length, width, volume) and a decrease in the nuclear-cytoplasmic ratio of cardiac cardiomyocytes in the left ventricle myocardium, compared to the right ventricle, are associated with the functional characteristics of the myocardial muscle tissue, which is capable of spontaneous rhythmic contractions, facilitating the movement of blood through the vessels. Accordingly, the cardiac contractile myocytes of the left ventricle of the heart myocardium provide a much higher load, thus promoting blood flow in the vessels of the large (somatic) circulation, and, accordingly, the cardiomyocytes of the right ventricle provide a

lower load, supporting blood flow in the vessels of the small (pulmonary) circulation.

The smallest cytometric parameters of the heart structure (length, width, volume) were characteristic of atrial cardiomyocytes, in which the nuclear-cytoplasmic ratio relative to that of the left and right ventricles was significantly ($P < 0.001$) 3.16 and 1.96 times ($P < 0.01$) higher and equalled 0.0430 ± 0.0096 (Table 3; Fig. 12). These cytometric parameters of cardiomyocytes indicated a significantly lower functional load of atrial contractile myocytes compared with ventricular cardiomyocytes. It is known that the most morphologically and functionally active and mature somatic cells in humans and animals are those characterized by a low nuclear-cytoplasmic ratio index and, conversely, cells with a high nuclear-cytoplasmic ratio are less functionally active (Anderson & Ho, 2002). Different cytometric and karyometric parameters of ventricular and atrial cardiomyocytes are

primarily associated with the functional activity of the heart: the atria receive blood returning to the heart from the body of animals, and the ventricles, respectively, pump blood from the heart to the body, performing a significant load.

At the same time, certain characteristics of cardiomyocytes and their nuclei have different meanings, depending on the morphological and functional activity of the myocardial tissue of the ventricles and atria. For example, morphometric parameters (thickness, length, average volume of sarcoplasm and cardiomyocyte nuclei) are larger in the left ventricle than in the right ventricle. Such ambiguous cytometric parameters of the left ventricular myocardium in experimental animals, relative to the right ventricle, are probably related to the activity of the ventricles themselves: the left ventricle functions, in most cases, as a pump, and the right ventricle functions as a volumetric compartment with morphological and functional features of myocardial tissue capable of spontaneous rhythmic contractions, which result in blood flow in the vessels. At the same time, the cardiomyocytes of the left ventricle provide a greater load, promoting blood flow through the vessels of the corporal (large) circulation, and the cardiomyocytes of the right ventricle, respectively, have a much lower load, ensuring blood flow through the vessels of the small circulation (Halgür *et al.*, 2009; Braile, 2013). Thus, the studies have shown that the morphological structure of the sheep heart myocardium has similar macro- and histoarchitectonics to other species of mammals, but differs in morphometric parameters.

Conclusions

A comprehensive morphological study of the heart of the domestic sheep was carried out, and new data on the morphological features and structural organization of the heart in the normal state were obtained. The morphological

analysis allowed systematizing, deepening and significantly supplementing the information about the microscopic structure and morphometric parameters of the heart structures of the normal bovine heart and can serve as a basis for further experimental studies of the heart in sheep and humans with various pathological conditions.

The absolute weight of the sheep heart was 208.4 ± 9.82 g, the relative weight was $0.44 \pm 0.007\%$, and the net weight of the heart (without epicardial fat) was 175.0 ± 8.17 g. According to the linear parameters of the heart (height – 13.1 ± 0.4 cm, width – 9.0 ± 0.3 cm, circumference – 22.2 ± 0.6 cm), the index of its development is $145.5 \pm 4.02\%$, so the heart is of the dilated-shortened type. The more developed components of the heart are the ventricles (left and right), then the atria (left and right):

- The mass of the left ventricle is the largest and was equal to 90.3 ± 5.21 g ($51.6 \pm 3.06\%$), the mass of the right ventricle was 45.6 ± 3.04 g ($26.06 \pm 1.32\%$), the average mass of both ventricles was 135.9 ± 7.16 g ($77.66 \pm 4.36\%$);
- The average weight of the left atrium was 27.9 ± 3.31 g ($15.94 \pm 1.49\%$), the average weight of the right atrium relative to the left atrium was significantly ($P < 0.01$) 2.5 times less and amounted to 11.2 ± 2.02 g ($6.4 \pm 0.82\%$), the average weight of both atria was 39.1 ± 4.64 g ($22.34 \pm 2.02\%$).
- The ratio of the mass of the ventricles of the sheep heart to its net mass was 1:0.78, the ratio of the mass of the atria was 1:0.22, the ratio of the mass of the atrial myocardium to the mass of the ventricular myocardium was 1:0.29.

The cardiomyocytes of the left ventricle had a larger volume ($3982.99 \pm 423.96 \mu\text{m}^3$), the right ventricle – $2463.02 \pm 318.04 \mu\text{m}^3$, and the smallest – atrial cardiomyocytes ($1215.93 \pm 176.94 \mu\text{m}^3$). The volumes of cardiomyocyte nuclei in the left ($53.42 \pm 5.18 \mu\text{m}^3$), right ($52.85 \pm 4.33 \mu\text{m}^3$) ventricles and atria

($50.16 \pm 4.57 \mu\text{m}^3$) of the heart were characterized by almost the same values. The nuclear-cytoplasmic ratio was the lowest in cardiomyocytes of the left ventricle – 0.0136 ± 0.0062 , significantly ($P < 0.01$) higher in cardiomyocytes of the right ventricle – 0.0219 ± 0.0079) and significantly ($P < 0.001$) the highest in atrial cardiomyocytes – 0.0430 ± 0.0096 , which is associated with the physiological characteristics of myocardial muscle tissue capable of spontaneous rhythmic

contractions. In the future, it is planned to conduct comprehensive morphological studies of the heart of domestic animals of the class Mammalia – Mammals in a comparative aspect.

Acknowledgements

None.

Conflict of Interest

None.

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Особливості органометрії та морфоархітектоніки серця барана свійського (*Ovis aries L.*, 1758)

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Анотація. На сьогодні домінуючими хворобами тварин є серцево-судинні захворювання, які найчастіше призводять до їх загибелі як в Україні, так і в усьому світі. Тому морфологічні дослідження серця на клітинному, тканинному та органному рівнях є необхідними у клінічній кардіології для прижиттєвої ультразвукової діагностики функціональних та органічних уражень серця. Мета роботи – за допомогою макроскопічних, гістологічних, морфометричних методів досліджень здійснити гісто- та цитоморфометричну оцінку морфологічних структур серця овець. З використанням морфологічних методів досліджено серце статевозрілих клінічно здорових тварин ($n=5$), які належать до класу *Mammalia* – Ссавці, виду *Ovis aries L.*, 1758 – баран (вівця) свійський. Обґрунтовано абсолютну та відносну масу серця статевозрілих овець, що становить, відповідно, $208,4 \pm 9,82$ г і $0,44 \pm 0,007$ %, масу без епікардіального жиру – $175,0 \pm 8,17$ г. Серце овець відповідно за індексом розвитку ($145,5 \pm 4,02$ %) відноситься до розширено-вкороченого анатомічного типу. Розвинутішими складовими є шлуночки. При цьому, коефіцієнт відношення маси шлуночків серця до його чистої маси дорівнює $1:0,78$, відповідно коефіцієнт відношення маси передсердь становить – $1:0,22$, а коефіцієнт відношення маси міокарду передсердь до маси міокарду шлуночків дорівнює $1:0,29$. Найбільший об'єм встановлено у кардіоміоцитів лівого шлуночка – $3982,99 \pm 423,96$ мкм³, менший – правого $2463,02 \pm 318,04$ мкм³ і найменший – у кардіоміоцитів передсердь – $1215,93 \pm 176,94$ мкм³. Об'єми ядер кардіоміоцитів у лівому ($53,42 \pm 5,18$ мкм³),

правому ($52,85 \pm 4,33$ мкм³) шлуночках серця та його передсердях ($50,16 \pm 4,57$ мкм³) майже однакові. Ядерно-цитоплазматичне відношення найменше у кардіоміоцитів лівого шлуночка – $0,0136 \pm 0,0062$, дещо більше у кардіоміоцитів правого – $0,0219 \pm 0,0079$ і найбільше – $0,0430 \pm 0,0096$ у кардіоміоцитах передсердь. Отримані результати щодо макро- та мікроскопічної будови серця барана свійського (*Ovis aries L.*, 1758) доповнюють відомості з морфології серця овець у відповідних розділах гістології та порівняльної анатомії і є необхідними для клінічної кардіології

Ключові слова: морфологія органів; свійські тварини; гістоструктура; індекс розвитку; міокард; кардіоміоцити