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# Morphology and morphometric features of the heart of the domestic horse (*Equus ferus caballus*)

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The article presents the results of studies of the macro- and microscopic structure, features of organo- and cytometry of the heart of the domestic horse - Equus ferus caballus Linnaeus, 1758. The obtained data are of great importance for fundamental and applied research in veterinary morphology, cardiology, and pathology. The results obtained significantly expand and supplement the information on the morphological structure of the horse's heart in relation to the species characteristics of domestic mammals in the relevant sections of histology, zoology, comparative anatomy, etc. According to the results of morphometry, the absolute mass of the heart of a physiologically mature horse was  $2988 \pm 97$  g, the relative mass was  $0.59 \pm 0.01\%$ , and the net mass of the heart was  $2807 \pm 93$  g. According to linear measurements of the heart (height  $-30.26 \pm 0.74$  cm, width  $-20.52 \pm 0.74$  cm, 0.55 cm, thickness  $-12.84 \pm 0.39$  cm, circumference  $-54.16 \pm 2.05$  cm), the development index (shape) of the horse's heart is  $147.5 \pm 8.1\%$ , therefore the horse's heart is defined as of the dilated-shortened type. The more developed components of the heart are its ventricles - left and right, then the left and right atrium. According to such absolute values, the ratio of the mass of the horse's ventricles to its net mass was 1:0.8, the ratio of the mass of the atria to its net heart mass was 1:0.2, and the ratio of the mass of the atrial myocardium to the mass of the ventricular myocardium was 1:0.24. According to the results of cytometry, the left ventricular cardiomyocytes had a larger volume - 12554 ± 878 μm³, the right ventricular cardiomyocytes had a slightly smaller volume  $-8402 \pm 681 \mu m^3$ , and the atrial cardiomyocytes had the smallest volume  $-5729 \pm 513 \mu m^3$ . The volumes of the cardiomyocyte nuclei were similar: in the left ventricle,  $133 \pm 9 \,\mu\text{m}^3$ ; in the right ventricle,  $132 \pm 8 \,\mu\text{m}^3$ ; and in the atrial cardiomyocytes,  $129 \pm 8 \mu m^3$ . And so, the smallest nuclear-cytoplasmic ratio was characteristic of cardiomyocytes of the left ventricle  $(0.0107 \pm 0.0007)$ , significantly higher is the value of cardiomyocytes of the right ventricle  $(0.0159 \pm 0.0010)$ , and significantly higher is the value of cardiomyocytes of the atrium  $(0.0230 \pm 0.0007)$ . We associate such ambiguous organo- and cytometric parameters of the ventricles and atria of the heart with the activity of their work - the functional features of the muscular tissue of the myocardium, capable of spontaneous rhythmic contractions, as a result of which blood moves through the vessels: the left ventricle (its cardiomyocytes) mainly performs the function of a pump, promoting the movement of blood through the vessels of the large circle of blood circulation, carrying out a correspondingly greater load; right ventricle (cardiomyocytes) - performs a predominantly volumetric function, promoting blood movement only through the vessels of the small circle of blood circulation, while performing a smaller load.

Keywords: macro- and microscopic structure; organometry; cytometry; myocardium; cardiomyocytes; nuclear-cytoplasmic ratio.

## Introduction

The cardiovascular system usually provides blood movement through a closed, dynamic tubular system in the human and animal body, resulting in metabolism (Savchuk et al., 2018; Christoffels & Jensen, 2020; Kots et al., 2021). It transports oxygen, which binds to hemoglobin in the lungs, hormones, and removes metabolic products from the body – carbon dioxide, aqueous solutions of nitrogenous wastes through the kidneys, etc. (Cesarovic et al., 2020; Raiola et al., 2023). This continuous exchange helps maintain homeostasis, regulates physiological processes, and ensures optimal functioning of all organs and systems of the body.

The cardiovascular system includes the heart (central organ) (Protsak et al., 2018) and anatomical structures: arteries, arterioles, capillaries, venules, and veins, through which the liquid connective tissue – blood circulates (Brown et al., 2020; Somberg, 2020).

Ensuring the full functioning of the cardiovascular system in humans and animals occurs in conjunction with other organs and tissues of the body: lungs (oxygen enrichment of blood through ventilation), liver (deactivation of toxic substances), hematopoietic organs (constantly replace formed blood elements that have died through necrosis or apoptosis), endocrine glands (secrete hormones into the blood). The lymphatic system, where the circulation of tissue fluid-lymph oc-

curs, is inextricably linked to the blood system (Borovkov et al., 2013; Vadzyuk & Huk, 2023). From a mechanical point of view, the heart can be considered as a mechanism consisting of two pumps mounted in a closed system of tubes through which fluid circulates. In this case, the pumps provide conditions for the directed flow of blood through the system, which is called the cardiac output (Boselli et al., 2015).

Today, improper and unbalanced nutrition of animals, their poor maintenance, and the use of animals for heavy work, in particular horses, leads to the occurrence of diseases of non-communicable and infectious etiology (Molesan et al., 2019; Townsend et al., 2022).

In recent years, cardiology of small and large productive animals has developed intensively, but there are still a number of unresolved issues. Among them are the etiological factors leading to the occurrence of some heart diseases, the lack of unambiguous reference indicators for animals of different species, breeds, and even animals of the same species and breed, but with different body weights (Zaragoza et al., 2011; Cesarovic et al., 2020).

Heart diseases are extremely common diseases among animals, which lead to a deterioration in their quality of life, and as a result, to a decrease in their productivity and working capacity (Khalesi et al., 2022). The causes of the development of acquired heart pathologies, according to the authors (Janus et al., 2016), can be various etiological factors, such as genetically inherited anomalies (Dukes-McEwan

et al., 2003), breed predispositions (Lobo et al., 2010; Gaar-Humphreys et al., 2022), excessive physical exertion, severe injuries, blood loss, poisoning, infectious and parasitic diseases (Maksymovich & Slivinska, 2013; Szatmári, 2020), non-communicable diseases (Chow & French, 2014).

Tsang et al. (2016) have noted that, according to statistical data, diseases of the cardiovascular system occupy a dominant place among diseases, which requires additional research. Cardiovascular pathology primarily includes primary heart diseases: some forms of myocarditis, cardiomyopathy, obesity, heart tumors, etc. (Tidholm & Jönsson, 2005; Siwinska et al., 2022).

When conducting a macroscopic study of heart pathologies, a significant increase in the mass of the organ is noted. Such a significant increase in the mass of the heart and cardiac index occurs mainly due to the ventricles, especially the left one. Very indicative are changes in the configuration of the heart, which tends towards spherical in shape. Thus, during macromorphometric analysis, a significant increase in its volume is noted, namely the width at the base of the heart and thickness, while the length from the base to the apex of the heart practically does not change (Lebedinets et al., 2013).

For early diagnosis of heart diseases in both humans and animals (especially in small animals), it is necessary to regularly undergo a cardiological examination, especially electrocardiography, ultrasound of the heart, X-ray examinations of the chest cavity organs, for a general assessment of the structure and morphophotography of the heart, its morphometric parameters, to identify or exclude independent or acquired pathologies due to heart failure (Legge et al., 2013; Mubanga et al., 2017). Therefore, the study of the features of the morphoarchitectonics of the heart and its morphometry in domestic animals is a topical problem in morphology. At the same time, the priority direction of today is organo- and histometric studies of the heart in clinically healthy animals, the results of which will serve as diagnostic criteria (as indicators of the norm) for timely and reliable diagnosis of diseases of the cardiovascular system (Ateş et al., 2017). This area of research has proven itself to be dominant in morphology and is widely used in modern veterinary cardiology, since the use of the results of heart studies (morphotopography, macro- and microscopic structure, organo- and histometry) in clinically healthy animals is a standard (test) for ultrasound and X-ray diagnostics, as indicators of the norm, or the course of physiological or pathological processes in cases of damage to the cardiovascular system (Levicar et al., 2022). To this end, we conducted studies on the features of the macro- and microscopic structure of the horse heart (Equus ferus caballus L., 1758 – domestic horse), which allowed us to significantly deepen and supplement data on the macro- and microscopic structure and morphometric parameters of the horse heart in normal conditions, the results of which are recommended for cardiological diagnostics in pathological conditions of the cardiovascular system.

### Materials and methods

Experimental studies were conducted in accordance with the requirements of the international principles of the "European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes" (Strasbourg, 1986), the "Rules for Conducting Work Using Experimental Animals", approved by the Order of the Ministry of Health No. 281 of November 1, 2000 "On Measures for Further Improvement of Organizational Forms of Work Using Experimental Animals" and the relevant Law of Ukraine "On the Protection of Animals from Cruelty" (No. 3447-IV of February 21, 2006, Kyiv).

The material for the study was the heart of physiologically mature, clinically healthy, sexually mature horses (Equus ferus caballus L., 1758 – domestic horse) (n = 5).

Morphological, organometric, cytometric and statistical research methods were used for the work (Horalskyi et al., 2019, 2023; Dukhnytskyi et al., 2024), in compliance with the general rules of good laboratory practice GLP (1981), morphological studies (Mishalov et al., 2007), the provisions of the "General Ethical Principles of Experiments on Animals", adopted by the 1st National Congress on Bio-

ethics (Kyiv, 2001) and in compliance with the relevant requirements and standards, in particular, they meet the requirements of DSTU ISO/IEC 17025:2005 (2006).

During anatomical dissection (for macroscopic and organometric studies), the heart was removed from the chest of the animals together with the pericardium. The heart was dissected immediately after measuring its parameters, then the ventricles were longitudinally dissected through the corresponding atrioventricular openings.

The absolute mass (AM) of the heart, its ventricles, and atria was determined by weighing on a laboratory balance of the brand "Radwag" PS 6000/C/2 (Poland). The relative mass of the heart (BM) was determined according to the formula (1): BM = absolute mass of the heart/body mass of the animal  $\times 100\%$ .

The linear parameters of the heart (height, width, thickness, circumference) were determined by direct measurement.

The index of development (shape) of the heart (IHD) was determined by the ratio of its total height to its width using the following formula (2): IHD = heart height/heart width  $\times$  100.

For microscopic and cytometric studies, generally accepted methods of fixation and preparation of histopreparations were used (Horalskyi et al., 2019). For this, pieces of the selected material were fixed in a 12% cooled solution of neutral formalin for 24 h or more (for staining with hematoxylin and eosin) and in Zenker-formol liquid at room temperature for 8–24 h (for staining according to the Heidenhain method). Then the material was embedded in paraffin according to the schemes proposed in the manual (Horalskyi et al., 2019). The preparation of paraffin sections, 10–12 µm thick, was carried out on a slide microtome MS-2. For microscopic examination of the heart at the tissue and cellular levels and for histo- and cytometry, histosections after deparaffinization were stained with hematoxylin and eosin and according to the Heidenhain method (Horalskyi et al., 2019).

Measurement of the length and width of cardiomyocytes and, accordingly, their nuclei was performed using an ocular ruler with a microscope "Micros" (Austria, 2012), according to the recommendations set out in the manual (Horalskyi et al., 2019).

Determination of the volume of cardiomyocytes was performed using the following formula (3):  $Vk = 3.14 \times radius$  of the cardiomyocyte.

Determination of the volume of cardiomyocyte nuclei was performed using the formula (4):  $V = 3.14/6 \times length$  of the cardiomyocyte nuclei  $\times$  width of the cardiomyocyte nuclei<sup>2</sup>.

The nuclear-cytoplasmic ratio (NCR) of cardiomyocytes was determined by the formula (5): NCR = (Vn)/(Vc-Vn), where NCR – nuclear-cytoplasmic ratio; Vn – nucleus volume; Vc – cardiomyocyte volume

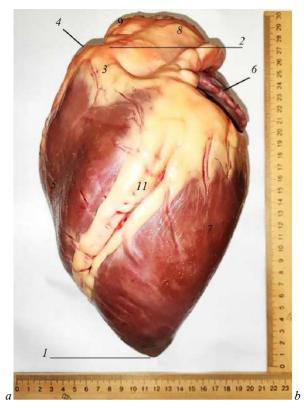
Morphological terms are given according to the International Veterinary Histological Nomenclature and the International Veterinary Anatomical Nomenclature.

Photographing of histological preparations was carried out with a CAM V–200 video camera (InterMed, PRC, 2017) mounted on a microscope. Digital processing of morphometric studies was performed statistically using the Statistica 7.0 software package (StatSoft, Tulsa, USA). Differences between values were determined using ANOVA, considered significant at P < 0.05 (taking into account the Bonferroni error) (Horalskyi et al., 2019).

### Results

From the outside, the horse's heart is located in a kind of cardiac bag (membrane) – the pericardium (pericardium), consisting of two layers, separated from each other by space. The inner layer is firmly connected to the cardiac bag; it is called the visceral leaf or epicardium. The outer layer is a rather inelastic connective tissue – the parietal layer. In the cavity between the membranes, there is a small amount of fluid, which performs the function of lubrication.

According to the morphometry of our studies, the absolute mass of the heart was  $2988 \pm 97$  g, which was  $0.59 \pm 0.01\%$  (relative mass) of the total mass of the animals. The net mass of the heart was  $2807 \pm 93$  g (Table 1).



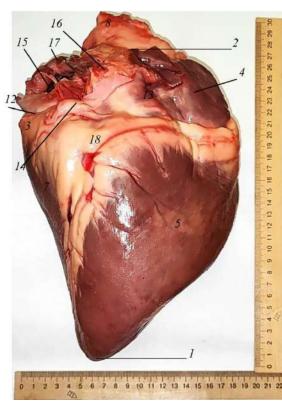


Fig. 1. Anatomical structure of the heart of a sexually mature horse: a – projection of the heart from the left side; b – projection of the heart from the right side: I – apex of the heart; 2 – base of the heart; 3 – subepicardial fat; 4 – right auricle; 5 – right ventricle; 6 – left auricle; 7 – left ventricle; 8 – aorta; 9 – brachiocephalic trunk; 10 – paraconal interventricular sulcus; 11 – blood vessels; 12 – left atrium; 13 – right atrium; 14 – fragment of the heart; 15 – caudal vena cava; 16 – cranial vena cava; 17 – pulmonary veins; 18 – axillary interventricular sulcus; macroscopic preparation

According to the linear measurements of the heart and its components, the height of the organ in the horse was  $30.26 \pm 0.74$  cm, width  $-20.52 \pm 0.55$  cm, thickness  $-12.84 \pm 0.39$  cm, and circumference  $-54.16 \pm 2.05$  cm. At the same time, the development index (shape) of the horse's heart was  $147.5 \pm 8.1\%$  (Table 1).

On the horse's heart, upon anatomical examination, its left and right lateral (side) surfaces, as well as the left and right ventricular edges were clearly expressed (Fig. 1). The horse's heart, as well as in other domestic mammals studied by us, is 4-chambered and consists of two atria (upper – dorsal small chambers) and two ventricles (lower – ventral large chambers, Fig. 1). On the cranial plane of the right and left atria, small protrusions are found – cardiac auricles, which are clearly outlined and are located to the left at the base of the aorta and pulmonary trunk (Fig. 1). On the outer surface of the heart between the atria and ventricles, a coronal groove is found.

The right ventricle of the horse's heart occupies a significant part of the cranial edge of the organ. On a transverse section, it has the shape of a moon. The left ventricle is located in the apical part of the heart and has a cone-shaped shape (Fig. 1).

The heart wall is formed by three membranes: epicardium (outer membrane), myocardium (middle membrane), and endocardium (inner membrane). The outer membrane of the heart is the thinnest; it is formed by delicate connective tissue. Nerves and large blood vessels are found in it. The middle membrane is the myocardium, highly developed and consisting of many layers of muscle tissue. It is the main muscle layer of the heart wall. In the atria, the muscular membrane is formed by two layers – the outer and the inner. The outer layer of the myocardium is common to both atria, where the muscle fibers are directed in the transverse direction from one atrium to the other. The deep layer of the myocardium in the right and left atria has longitudinal directions, and in the area of the venous openings, circular bundles of fibers are formed.

The myocardium of the ventricles of the heart is formed by five layers: superficial outer and inner (muscle fibers, which are located in the oblique-longitudinal direction); middle outer and inner (deeper layer) and the deepest layer, in which the direction of the fibers resembles a shape similar to the figure "eight". The inner lining (endocardium) of the heart is formed by a thin layer of endothelium, which is covered on the outside with a thin layer of loose connective tissue with smooth muscle fibers. It forms a soft coating of the inner surface of the heart chambers and valves.

The left atrium, like the right, is a thin-walled chamber in the dorsal part of the heart. The right and left atria are separated from each other by a rather thin interatrial septum. The left and right ventricles of the heart are thick-walled chambers that are separated from each other by an interventricular septum.

According to measurements of the wall thickness of the ventricles of the horses' heart, their total thickness was  $30.55 \pm 1.41$  mm. At the same time, the wall of the left ventricle (LV) (40.14  $\pm$  1.58 mm) of the horses' heart was significantly (P < 0.01) 1.92 times larger than the right one (20.92  $\pm$  0.91 mm). This indicator of an increase in the wall thickness of the left ventricle, compared to the right one, is associated with a significant development of the muscles (muscular sheath) of the heart, which in horses reaches up to 4 cm or even more as a result of the fact that the contractile cardiomyocytes of the left ventricle muscles during work perform an increased load, supplying blood (large blood circulation) to the whole body under pressure. Due to such a unique specific structure, the horse's heart in the body, respectively, performs the function (role) of a blood circulation pump, and therefore, in the circulatory system of the body, a constant movement of blood in a closed system of vessels is maintained, only in one direction. The decrease in the thickness of the wall of the right ventricle (RV) of the heart, compared to the left, is explained by the fact that the muscles of the right ventricle of the heart pump blood from the corresponding chamber into the small - pulmonary circulation, while performing a smaller functional load than the muscles of the left ventricle, pumping blood throughout the body.

According to the results of morphometry, the thickness of the wall of the left atrium of the horse's heart was  $11.02\pm0.32$  mm, respectively, the right –  $10.05\pm0.19$  mm, and therefore, the wall of the atrium has a less pronounced muscular membrane than the ventricles. At the same time, the average value of the thickness of the atrial wall of the horse heart was  $10.53\pm0.53$  mm, which is significantly (P < 0.001) 2.90 times less than this indicator in the ventricles (Table 1). This is explained by the fact that the main task of the atrial muscles is to pump blood in the ventral direction into the corresponding ventricles of the heart.

According to the results of morphometry of the absolute mass of the ventricles and atria of the heart, the average mass of the left atrium (LA) of the horse heart was  $338.7 \pm 14.5$  g, which was  $12.1 \pm 0.5\%$  of the pure (without epicardial fat) mass of the heart. The average absolute mass of the right atrium was  $212.9 \pm 10.8$  g ( $7.6 \pm 0.1\%$ ), which was significantly (P < 0.01) 1.60 times less than the left. The average mass of both atria of the horse's heart was  $551.6 \pm 42.3$  g ( $19.6 \pm 0.5\%$ ).

The absolute mass of the left ventricle was the largest and was  $1484.1 \pm 28.7$  g ( $52.7 \pm 4.1\%$ ), the absolute mass of the right ventricle was significantly (P < 0.01) 1.90 times smaller than the left ventricle and was  $771.6 \pm 19.3$  g ( $27.5 \pm 0.8\%$ ). In general, the average absolute mass of both ventricles is  $2255.8 \pm 88.7$  g, respectively, and the relative mass to the net mass of the heart as a whole was  $80.4 \pm 4.3\%$  (Table 2).

Accordingly, the ratio of the absolute mass of the ventricles of the heart of a physiologically mature horse to the net (without epicardial fat) mass of the heart is 1:0.8, the ratio of the absolute mass of the atria to its net mass of the heart as a whole is 1:0.20, and the ratio of the absolute mass of the atria to the absolute mass of the ventricles is 1:0.24. According to a detailed analysis of the organometry conducted by us, in terms of linear parameters and the development index  $(147.5 \pm 8.1\%)$ , the heart of the domestic horse is of the expanded-shortened type.

**Table 1** Linear parameters of the heart of a sexually mature horse (n = 5)

Indicators	$x \pm SD$	
Heart height, cm	$30.26 \pm 0.74$	
Heart width, cm	$20.52 \pm 0.55$	
Heart thickness, cm	$12.84 \pm 0.90$	
Heart circumference, cm	$54.16 \pm 2.05$	
Heart development (shape) index, %	$147.52 \pm 8.05$	
Mean ventricular wall thickness, mm	$30.55 \pm 1.41$	
Left ventricular wall thickness, mm	$40.14 \pm 1.58$	
Right ventricular wall thickness, mm	$20.92 \pm 0.91$	
Mean atrial wall thickness, mm	$10.53 \pm 0.53$	
Left atrial wall thickness, mm	$11.02 \pm 0.32$	
Right atrial wall thickness, mm	$10.05 \pm 0.19$	

**Table 2** Morphometry of the heart, ventricles, and atria of a sexually mature horse  $(x \pm SD, n = 5)$ 

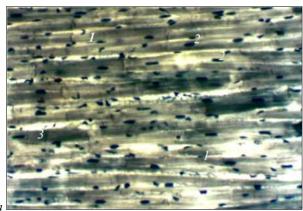
Indicators	Absolute mass, g	Relative mass, %	
Left atrium	$338.7 \pm 14.5$	$12.06 \pm 0.48$	
Right atrium	$212.9\pm10.8$	$7.58 \pm 0.12$	
Right and left atrium (together)	$551.6 \pm 42.3$	$19.64 \pm 0.51$	
Left ventricle	$1484.1 \pm 28.7$	$52.87 \pm 4.08$	
Right ventricle	$771.6 \pm 19.3$	$27.49 \pm 0.82$	
Left and right ventricles (together)	$2255.8 \pm 88.7$	$80.35 \pm 4.29$	
Heart weight (without apical fat)	$2807.3 \pm 92.8$		

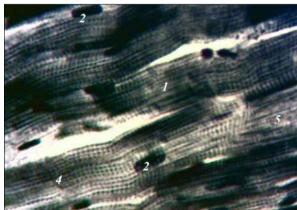
Microscopically, the structure of the myocardium is formed by cardiac muscle tissue, in the form of muscle fibers, between which layers of loose fibrous connective tissue, with the presence of blood and lymphatic vessels and nerves, are found.

Muscle fibers are built from cardiomyocytes (contractile myocytes), which are arranged in the form of a chain. Connecting cardiomyocytes with each other in a horizontal plane, they thus form structures similar to the muscle fibers of somatic striated muscle tissue (Fig. 2). Cardiomyocytes are connected to each other into muscle fibers by intercalated discs (Fig. 2), which perform a supporting function for the contractile elements of the cell (myofilaments) and ensure a

single contraction of the myocardium and, thereby, form a functional syncytium.

Contractile myocytes, according to their microscopic structure, are cylindrical in shape, in their sarcoplasm, especially when staining histopreparations according to the Heidenhain method, transverse and longitudinal striations are clearly differentiated (Fig. 2). When examining the preparations under a light microscope, cardiomyocytes on a longitudinal section have the form of dark transverse stripes. In them, the sarcolemma, myofibrils, and nuclei, which are located in the central part of the contractile cells, are clearly differentiated.





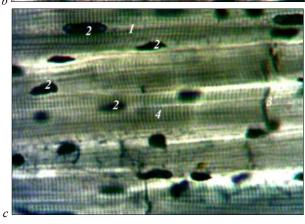


Fig. 2. Microscopic structure of the myocardium of a sexually mature horse: a – right ventricle; b, c – left ventricle: I – cardiomyocytes; 2 – cardiomyocyte nuclei; 3 – intercalated discs; 4 – transverse striation; 5 – longitudinal striation; staining according to the Heidenhain method

Thus, the volume of cardiomyocytes of the left ventricle of the myocardium of a domestic horse was significantly (P < 0.05) 1.49 times larger than the right ventricle (8401.7  $\pm$  681.0  $\mu m^3$ ) and was 12,554.4  $\pm$  877.5  $\mu m^3$ . The volume of cardiomyocytes of the atrium was the smallest and was equal to 5729.2  $\pm$  513.4  $\mu m^3$ , respectively (Fig. 2). When calculating the volume of the nuclei of the ventricles and atria, similar values were established: the volume of the nuclei of

cardiomyocytes of the left ventricle was  $132.9 \pm 9.1 \, \mu m^3$ , respectively, of the right ventricle  $-131.8 \pm 7.9 \, \mu m^3$  and of the atria  $-129.0 \pm 7.8 \, \mu m^3$  (Fig. 2; Table 3). With such ambiguous cytometric parameters of cardiomyocyte volumes and almost unambiguous characteristics of their nuclear volumes, a different nuclear-cytoplasmic ratio was formed for them: the smallest nuclear-cytoplasmic ratio was found in cardiomyocytes of the left ventricle  $(0.0107 \pm 0.0007)$ , significantly higher in cardiomyocytes of the right ventricle  $(0.0159 \pm 0.0010)$ , which indicates increased functional activity of cardiomyocytes of the left ventricle, since the left ventricle functions mainly as a pump, and the right as a volumetric one. Therefore, cardiomyocytes of the left ventricle of the heart perform a significantly greater load, contributing to the movement of blood through the vessels of the large circle of blood circulation, and, accordingly, cardiomyocytes of

the right ventricle – a smaller load, contributing to the movement of blood through the vessels of the small circle of blood circulation.

The highest nuclear-cytoplasmic ratio was found in atrial cardiomyocytes  $-0.0230\pm0.0007$  (Table 3), which is associated with a significantly lower functional load of atrial cardiomyocytes compared to ventricular cardiomyocytes. After all, according to Hnatyuk et al. (2016), more functionally active and mature cells are those characterized by a low nuclear-cytoplasmic ratio index, and, conversely, cells with a high nuclear-cytoplasmic ratio are less functionally active. Therefore, we associate different cyto- and cardiometric parameters of the volumes of cardiomyocytes of the ventricles and atria, and therefore different NCR of contractile myocytes, with the morphofunctional activity of the heart: the atria receive blood returning to the heart from the animal's body, and the ventricles pump blood from the heart to the body, performing the greatest load.

**Table 3** Histometric parameters of horse cardiomyocytes ( $x \pm SD$ , n = 30)

Indicators	Cardiomyocyte length, µm	Width of cardiomyocytes, µm	Cardiomyocyte volume, um <sup>3</sup>	Cardiomyocyte nuclear volume, µm <sup>3</sup>	Nuclear-cytoplasmic ratio
Left ventricle	$77.9 \pm 1.6^{b}$	$14.32 \pm 0.72^b$	$12554 \pm 878^{c}$	$132.9 \pm 9.1^a$	$0.0107 \pm 0.0007^a$
Right ventricle	$64.0 \pm 1.4^{a}$	$12.92 \pm 0.74^{ab}$	$8402 \pm 681^b$	$131.8 \pm 7.9^a$	$0.0159 \pm 0.0010^b$
Auricle	$60.9 \pm 1.4^{a}$	$10.94 \pm 0.73^a$	$5729 \pm 513^a$	$129.0 \pm 7.8^a$	$0.0230 \pm 0.0007^c$

Note: letters indicate significant differences between the subgroups within one column (P < 0.05) according to the Tukey test.

#### Discussion

The study of the morphology of the heart, the transformations of the shape of the organ as a whole, and the structural components of its wall in a comparative aspect is important for establishing diagnoses in domestic animals of certain species. The growth and development of the heart depend on the conditions of maintenance and feeding, as well as the physiological state of the animals (Trachsel et al., 2016). The horse's heart is a hollow, cone-shaped organ (Fig. 1). The heart is located in the thoracic cavity between the right and left lungs. A significant part of the heart is located to the left of the median (sagittal) plane, under the lungs, in the area of the 3rd-4th intercostal space. Cranially, the heart is limited by the third rib, and caudally by the costal cartilage of the fifth rib. The wide base of the heart is located at the level of the shoulder joint in the craniodorsal direction. The apex of the heart has a caudoventral direction and is extremely close to the sternum.

An indicator of the morphofunctional state of the animal organism is the absolute and relative mass of their organs and linear characteristics, the parameters of which are a reliable criterion of the development and morphofunctional state of maturity of the organism. Such indicators have cognitive value and are the basis for determining the shape, establishing comparative anatomical types of certain organs, etc. (Mits et al., 2016; Vernemmen et al., 2021).

According to Demus (2015) and Constantin & Tăbăran (2022) and the results of our research, which was described in our previous publications (Horalskyi et al., 2021), depending on age, breed, and species characteristics, the following heart shapes are distinguished in domestic animals of the class Mammalia: narrowed-elongated (*Bos taurus taurus* L., 1758 – domestic bull) – in cattle, the heart can be elongated-narrowed, conical, and expanded-shortened; narrowed-shortened (*Oryctolagus cuniculus* L., 1758 – European rabbit), expanded-shortened (*Equus ferus caballus* L., 1758 – domestic horse); round-oval (*Canis lupus familiaris* L., 1758 – domestic dog) – in dogs the heart can be elliptical (43%), conical-elliptical (24%), elliptical-spherical (26%) and spherical (7%); in pigs (*Sus scrofa*, forma *domestica* L., 1758 – domestic pig) three main heart shapes are distinguished – elongated-narrowed, conical; shortened, relatively narrowed; expanded-shortened, triangular.

Modern methods of research in morphology provide an objective characteristic and the ability to identify the relationships between individual structures of organs and systems of living organisms, their quantitative and relative characteristics at different stages of onto- and phylogenetic development of animals, their various functional states and during pathology. The use of morphometry in histology makes it

possible to determine changes in histoarchitectonics at the tissue and cellular levels (depending on their functional load), which cannot be detected by light microscopy (Gómez-Torres et al., 2021).

Therefore, analyzing the literature sources (Lelovas et al., 2014; Emam & Abugherin, 2020; Khvatov & Shchipakin, 2021) and according to the results of our own research, it was found that the microscopic structure of the heart of the domestic horse, its anatomical components, has similar histoarchitectonics, characteristic of domestic animals of the class Mammalia, but differs in cytometric parameters. Thus, according to the results of cytometry, cardiomyocytes, from which muscle fibers are built, depending on their functional load (right, left ventricle, atrium), have different quantitative cytometric values.

### Conclusion

The absolute mass of the heart of the domestic horse (*Equus ferus caballus* L., 1758) was  $2988 \pm 97$  g, relative  $-0.59 \pm 0.01\%$ . The net mass of the heart was  $2807 \pm 93$  g. According to linear measurements of the heart (height  $-30.26 \pm 0.74$  cm, width  $-20.52 \pm 0.55$  cm), the index of development of the heart of the domestic horse was  $147.5 \pm 8.1\%$ ; therefore, the heart is defined as of the expanded-shortened type.

The largest absolute mass in relation to the morphological structures of the heart is characteristic of the left and right ventricles, followed by the left and right atria. According to these indicators, the ratio of the mass of the ventricles of the horse's heart to its net heart mass was 1:0.8, the ratio of the mass of the atria was 1:0.2, and the ratio of the mass of the atrial myocardium to the mass of the ventricular myocardium was 1:0.24. The largest volume was characteristic of the cardiomyocytes of the left ventricle  $(12554 \pm 878 \, \mu m^3)$ , the smallest of the right ventricle ( $8402 \pm 681 \mu m^3$ ), and the smallest of the cardiomyocytes of the atrium (5729  $\pm$  513  $\mu$ m<sup>3</sup>). The volumes of the cardiomyocyte nuclei have similar values: in the left ventricle,  $133 \pm$ 9  $\mu$ m<sup>3</sup>; in the right ventricle,  $132 \pm 8 \mu$ m<sup>3</sup>; in the cardiomyocytes of the atrium,  $129 \pm 8 \mu m^3$ . The smallest nuclear-cytoplasmic ratio was characteristic of left ventricular cardiomyocytes  $-0.0107 \pm 0.0007$ , significantly (P < 0.01) higher for right ventricular cardiomyocytes –  $0.0159 \pm 0.0010,$  and significantly (P  $\! < \! 0.001)$  higher in atrial cardiomyocytes  $-0.0230 \pm 0.0007$ , which indicates the morphofunctional features of the muscular tissue of the myocardium, capable of spontaneous rhythmic contractions.

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