

## HISTOLOGICAL FEATURES OF ANGLO-NUBIAN GOATS' HEART VALVES

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### ABSTRACT

This study presents the histological features of the valvular apparatus of the heart of Anglo-Nubian goats. There were studying aortic semilunar valve, tricuspid valve, and morphometric data of its histological structures. As dated material, we used the hearts of Anglo-Nubian goats aged one year and older, obtained at slaughter from Gzhel Podvorie Farm in the Moscow, Russian Federation. A total of 10 animal corpses were examined, from which samples were taken by fine anatomical preparation in the form of one example of the cusp of the semilunar aortic valve and the tricuspid valve. For achieving this task, a complex of traditional histological methods and stains was used: Trichrome, according to Masson, toluidine blue, and Schiff-iodic acid, according to McManus. The Anglo-Nubian goat's semilunar heart valve's leaflets consist of aortic (fibrous), spongy (spongy), and ventricular layers. The aortic layer has formed by collagen and elastic fibers. The spongy layer has formed by loose small-cell connective tissue. The ventricular layer of the cusps of the semilunar aortic valve has also been formed by connective tissue and is much smaller than the aortic layer. The leaflets of the tricuspid heart valve of an Anglo-Nubian goat have formed by fibrous plates of dense high-celled fibrous connective tissue. In the spongy layer of which there are blood vessels of the mixed and muscleless type. The surfaces of the cusps of both valves had covered with endothelium. The obtained morphometric and histological data indicate the species and breed peculiarities of the heart of an Anglo-Nubian goat and the differences in the structure of the semilunar and atrioventricular valves at the micro-level.

**Disciplinary:** Histology, Animal Science, Veterinary, Bioscience.

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## 1. INTRODUCTION

The morphology of the heart's valvular apparatus in animals is currently a relevant and especially

important area of the cardiovascular system's anatomy. Pathologies such as endocardiosis of the heart valves, stenosis of the valves, and their insufficiency, are often found in veterinary practice for working with agricultural and small domestic animals. The study of the morphology of the heart valves, namely their anatomy and histology, in animals in terms of species and breed expands the field of knowledge of veterinary cardiologists and allows you to select an individual approach to a particular animal the treatment of heart pathologies (Sabyarzyanova et al., 2019; Sergeev and Kovalev 2020).

Anglo-Nubian goats in the Russian Federation and post-Soviet countries' agro-industrial complex breed in goat breeding. The combination of high productivity, quality of dairy products, and the taste of this breed of meat make Anglo-Nubian goats especially interesting for breeding (Nurushev et al., 2010; Sergeev et al., 2019).

The semilunar valve in farm animals' hearts has located in the aorta's orifices and the pulmonary trunk. Each valve consists of three cusps. In the aorta's semilunar valves, they are called septal, right and left, and in the pulmonary trunk's semilunar valves - intermediate, right, and left (Kuga 2012). Atrioventricular valves, in turn, are divided into bicuspid, which consists of parietal and septal cusps, and tricuspid, to which of the above is added an angular cusp. Both valves in agricultural and small domestic animals are located between the atria and ventricles of the heart's right and left chambers (Pomanskaya 2008; Prusakov et al., 2015).

The leaflets of all four heart valves in domestic animals develop from endocardial cushions formed during the endothelium conversion to the mesenchyme (Spicer et al., 2014).

In adults, the semilunar and atrioventricular valves' cusps contain connective tissue, including dense fibrous formed and loose fibrous unformed tissue. During a histological examination, fibroblasts and collagen fibers were found in the interstitial substance, and in the space between them, endotheliocytes are traced (Lobko and Rombalskaya 2010; Kozlovskaya 2014).

In the studies of domestic and foreign authors, it had found that the basis of the cusps of the heart valves is dense connective tissue, from the side of the atria or orifices of the aorta and the pulmonary trunk, elastic fibers prevail in it, and from the side of the ventricles - collagen fibers (Fletcher and Weber 2013; Kilany et al., 2019).

Xanthos et al., (2011) also indicate that the valve cusps are covered with endothelium and do not contain blood vessels.

After analyzing the available literature, we did not find a sufficient amount of information on the histology of heart valves in farm animals, particularly in small ruminants. The purpose of this work was to study features of heart valves histological structure of the Anglo-Nubian breed goat using for example tricuspid valve and semilunar aortic valve. And to establish the morphometric parameters of the histological structures of this area.

## 2. MATERIALS AND METHODS

The study's material was the carcasses of goats of the Anglo-Nubian breed older than one year, obtained during slaughter from Gzhel Podvorie farm, Moscow, Russian Federation.

For conducting the histological examination of the heart structures, the material was taken from

Anglo-Nubian goats' fresh hearts using fine anatomical preparation.

There were studying a total of 10 samples of the semilunar aortic valve and 10 samples of the tricuspid heart valve of the Anglo-Nubian goat.

The material was fixed in a 10% solution of neutral formalin for 24 hours (Gushchin and Muzhikyan 2014), after which it was embedding in paraffin according to the standard technique (Muzhikyan et al., 2014). Then sections with a thickness of 3-5  $\mu\text{m}$  were made, which were staining with hematoxylin and eosin.

Some of the sections were stained with Trichrome according to Masson, to luidine blue, Schiff-iodic acid, according to McManus. The histological preparations analysis was carried out using a CarlZeiss Axioskop 2 Plus light-optical microscope (Germany) at a magnification of 40, 100, 400, 1000. Microphotography was performed using a CarlZeissAxioCam ERc5s digital camera (Germany) and AxioVision 4.8 software. Morphometric measurements were performed manually using the AxioVision 4.8 software, ImageJ (Germany).

The variational-statistical processing of the research results was from using Excel and Statistika 6.0 (Statsoft, USA) with the calculation of the arithmetic mean and its standard error ( $M \pm m$ ).

In the statistical analysis of the data obtained, Student's t-test was used for independent samples, while the differences were considered significant at  $p < 0.05$ .

All anatomical and histological terms correspond to the "International Veterinary Anatomical Nomenclature" and "International histological nomenclature" (Semchenko et al., 1999; Zelenevsky 2013).

### 3. RESULT AND DISCUSSION

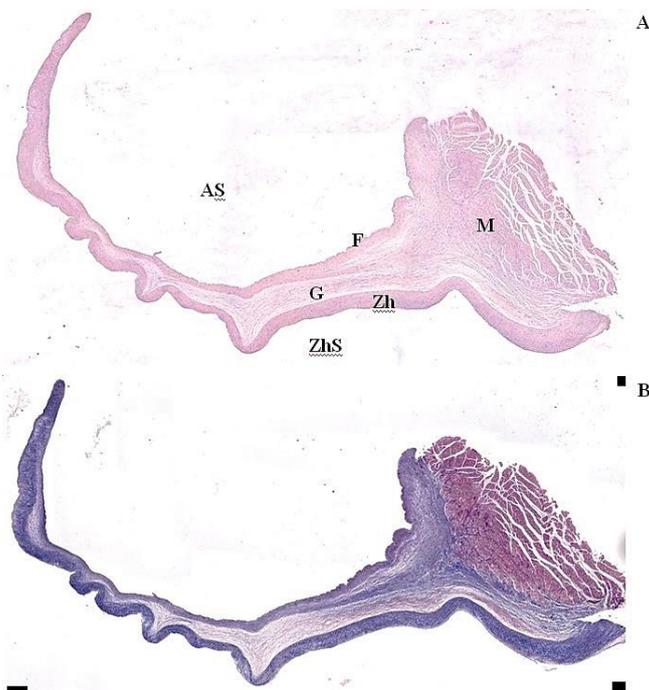
The Anglo-Nubian goat breed's semilunar heart valve's valves have a typical histological structure containing aortic (fibrous), spongy (spongy), and ventricular layers. Hill and Iaizzo (2015) point out that the valve cusps in humans consist of two layers surrounded by endothelial cells, which contradicts this study results.

At the base of each valve leaflet, the annulus fibrosus and myocardium structures, containing groups of cardiomyocytes of the cardiac conduction system, are revealed. The aortic (fibrous) layer is formed by densely intertwined, predominantly longitudinally oriented collagen and elastic fibers. The thickness of the fibrous layer varies within 150-200 microns and averages  $162.8 \pm 26.6$  microns. The spongy (spongy) layer is represented mainly by loose, few-celled connective tissue containing abundant extracellular matrix deposits in glycosaminoglycans. The spongy layer's thickness varies from 200-400  $\mu\text{m}$  at the base and up to 50-90  $\mu\text{m}$  closer to the distal part.

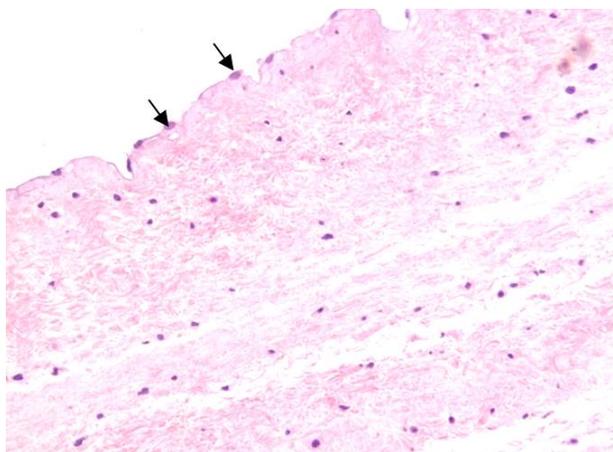
The ventricular layer of the cusps of the aortic semilunar valve of the Anglo-Nubian goat is also formed by connective tissue, and multiple undulating folds are formed. This layer of the studied valve's leaflets is relatively thinner than the aortic; D.E. obtained the same results in his studies (Borgarelli et al., 2011; Weerd and Christoffels 2016).

The valve's ventricular layer's thickness varies in the range of 90-140 microns and averages  $125.1 \pm 14.4$  microns in the studied animals.

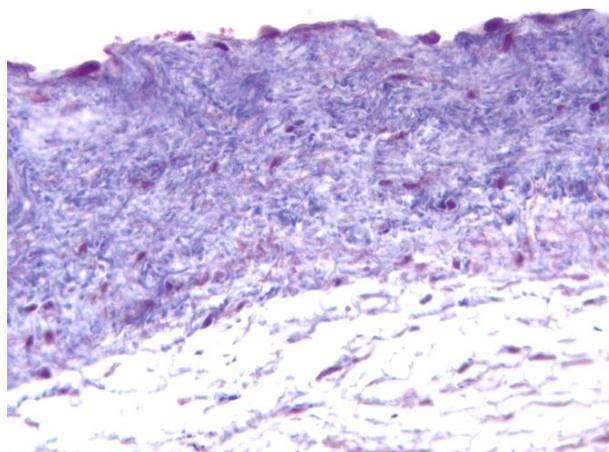
The surface of the valve leaflets is covered throughout by a single layer of endothelium. The endotheliocytes covering the aortic layer were characterized by larger oval and rounded, often closely spaced moderately hyperchromic nuclei. In contrast, the nuclei of endotheliocytes lining the aortic surface were predominantly flattened.



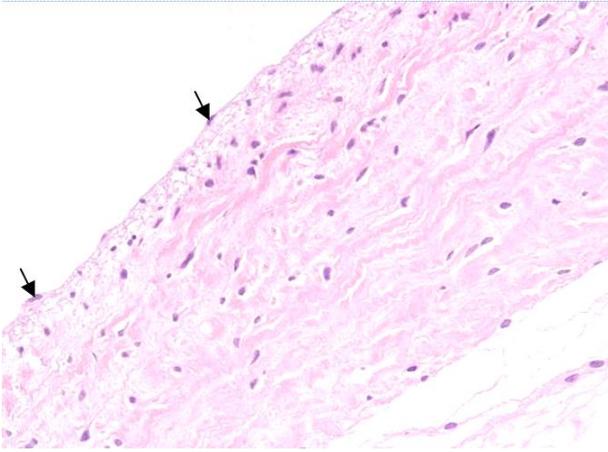
**Figure 1:** Semilunar valve, scanned histological sections stained with hematoxylin-eosin (A), and Masson's trichrome (B). Abbreviations: AS - aortic side; ZhS - ventricular side; F - fibrous (aortic) layer; G - spongy (spongy) layer; Zh - ventricular layer; M - myocardium.



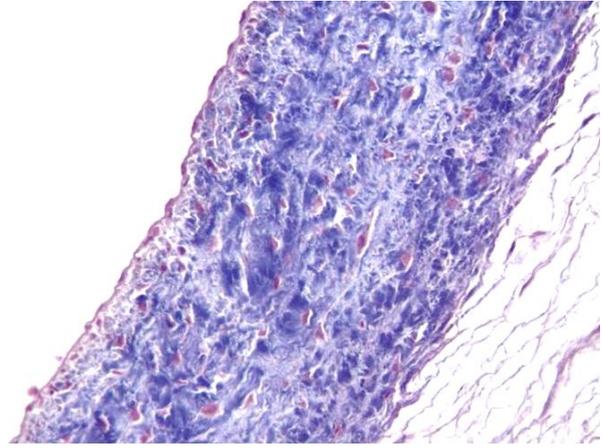
**Figure 2:** Semilunar valve, aortic (fibrous) layer. Endothelial cells are marked with arrows. Hematoxylin-eosin staining. x400



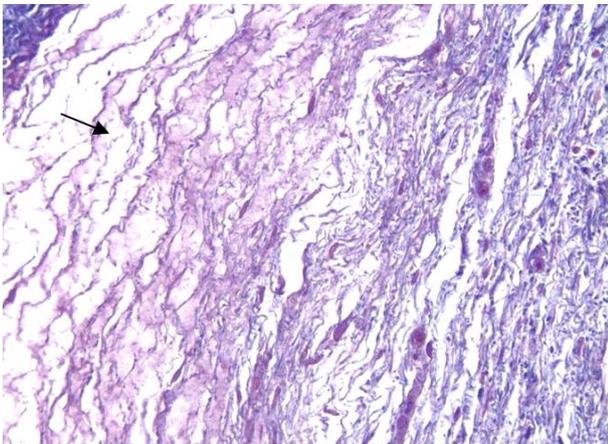
**Figure 3:** Semilunar valve, aortic (fibrous) layer. Collagen fibers are colored blue. Masson's trichrome staining. x400



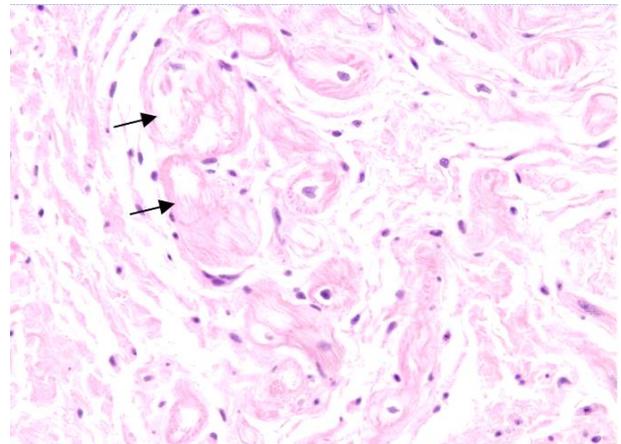
**Figure 4:** Semilunar valve, ventricular (ventricular) layer. Endothelial cells are marked with arrows. Hematoxylin-eosin staining. x400



**Figure 5:** Semilunar valve, ventricular (ventricular) layer. Collagen fibers are colored blue. Masson's trichrome staining. x400



**Figure 6:** Semilunar valve, cancellous (cancellous) layer. There is an accumulation of glycosaminoglycans in the extracellular matrix (arrow). Masson's trichrome staining. x400



**Figure 7:** Semilunar valve, the myocardium at the base of the valve, contains the conduction system's cells with a characteristic morphology (arrow). Hematoxylin-eosin staining. x400

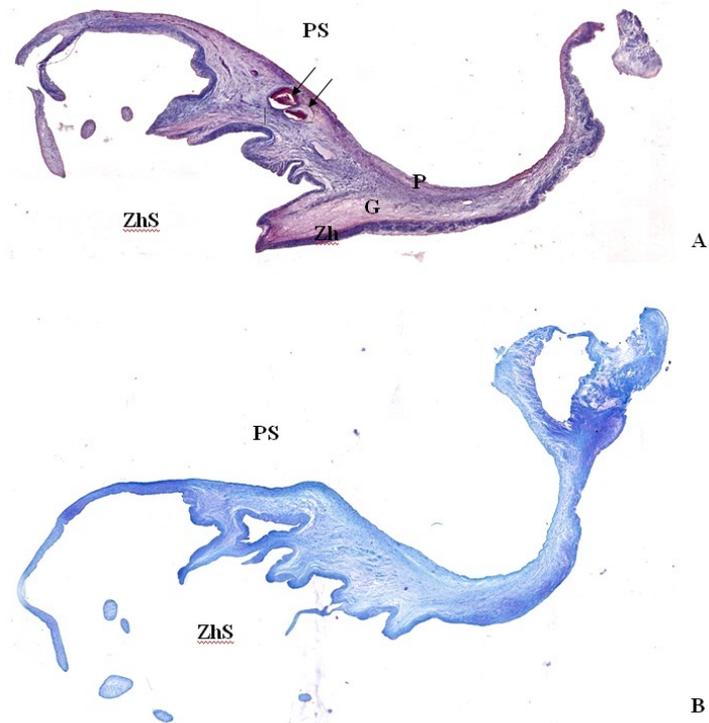
Thus, the large and small diameters of the endothelial nucleus of the ventricular part of the valve leaflets are on average  $7.2 \pm 1.0 \mu\text{m}$  and  $3.2 \pm 0.3 \mu\text{m}$ . In the aortic part,  $7.7 \pm 1.0 \mu\text{m}$  and  $5.6 \pm 0.6 \mu\text{m}$ , respectively. The thickness of the subendothelial layer on the ventricular and aortic surfaces is on average  $12.4 \pm 1.5 \mu\text{m}$  and  $13.7 \pm 1.9 \mu\text{m}$ , respectively.

The leaflets of the tricuspid heart valve of an Anglo-Nubian goat are formed by fibrous plates of dense high-celled fibrous connective tissue. The atrial side has a smooth surface, while the ventricular side is characterized by high outgrowths from which tendon filaments begin. The spongy layer of the valve leaflets is formed by loose, loose connective tissue rich in glycosaminoglycans in the intercellular substance, which had characteristic metachromatic staining when stained with toluidine blue. Perez et al. (2018) indicate that the aortic layer consists of spongy tissue containing collagen fibers, histiocytes, and fibrocytes, and the ventricular layer is made of fibrous tissue.

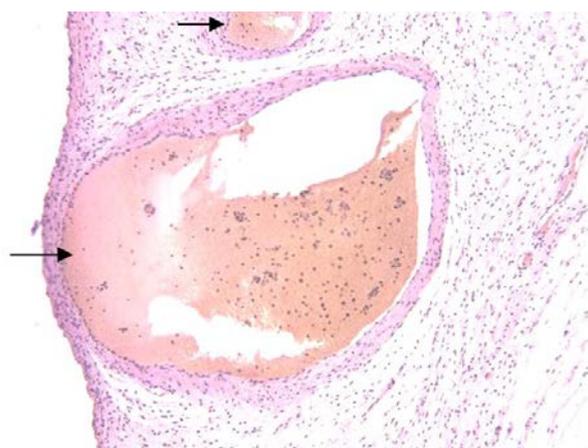
It is noteworthy that in the composition of the spongy layer of the tricuspid valve heart valves of the Anglo-Nubian goat, both small and enormous dilated blood vessels of the mixed and muscular type are revealed. When carrying out morphometric measurements, it was found that the thickness of the atrial layer is  $180.6 \pm 21.0 \mu\text{m}$ , that of the ventricular layer is  $134.8 \pm 19.6 \mu\text{m}$ , and that of the

spongy layer is  $294 \pm 55.4 \mu\text{m}$ .

The surface of the valve leaflets is covered throughout the entire length with one layer of endothelial cells, sometimes overlapping and forming "tiled" layers, which do not have pronounced differences on the atrial and ventricular surfaces. The nuclei of cells are oval, elongated, less often rounded. The large and small diameter of the endothelial cell nucleus averages  $9.5 \pm 0.8 \mu\text{m}$  and  $4.7 \pm 0.4 \mu\text{m}$ .



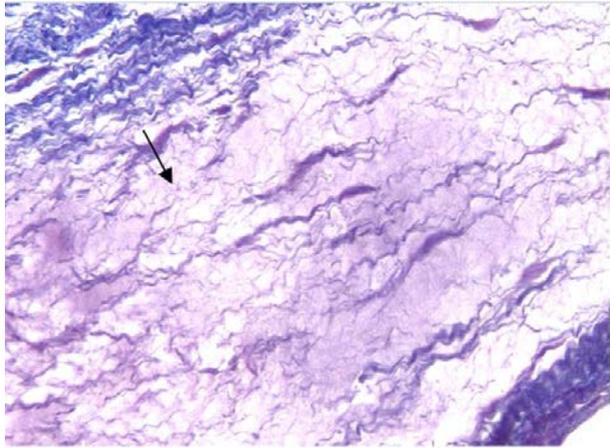
**Figure 8:** Tricuspid valve, scanned histological sections stained with Masson's trichrome (A), and toluidine blue (B). Legend: PS - atrial side; ZhS - ventricular side; P - atrial layer; G - spongy (spongy) layer; Zh - ventricular layer. Arrows indicate blood vessels.



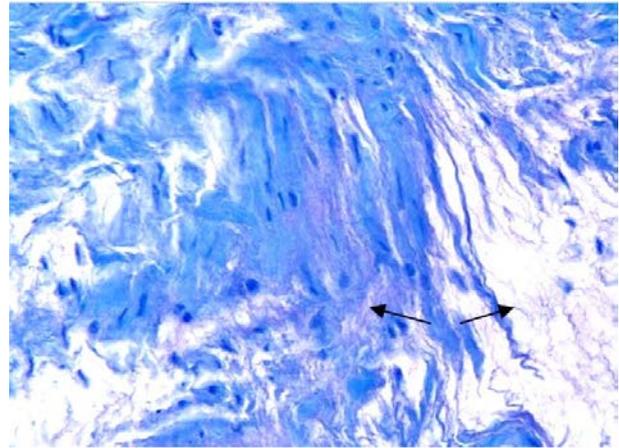
**Figure 9:** Tricuspid valve. In the spongy layer, closer to the atrial side of the valve, large blood vessels are visualized (arrow). Hematoxylin-eosin staining. Magnification x100



**Figure 10:** Tricuspid valve. In the spongy layer, closer to the atrial side of the valve, large blood vessels (muscleless type) are visualized. Hematoxylin-eosin staining. Magnification x100



**Figure 11:** Tricuspid valve. In the spongy layer, the accumulation of glycosaminoglycans in the extracellular matrix is noted (arrow). Hematoxylin-eosin staining. Magnification x100



**Figure 12:** Tricuspid valve. Accumulation of glycosaminoglycans in the spongy layer with characteristic metachromatic staining (arrow). Toluidine blue staining. Magnification x100

The thickness of the subendothelial layer in the atrial and ventricular surfaces is on average  $15.5 \pm 2.3 \mu\text{m}$  and  $16.9 \pm 1.8 \mu\text{m}$ , respectively. Research by Tarasevich and Ryadinskaya (2020), Ateş et al., Emam, and Abugherin (2019) do not contradict our results.

#### 4. CONCLUSION

From this result, we found that the Anglo-Nubian goat breed's heart valves have a typical histological structure for this species and consist of three layers: fibrous, spongy, and ventricular. There was determined the morphometric data of the heart valves' histological structures. It can be concluded that the ventricular layer of the semilunar aortic valve's cusps is significantly inferior in thickness to the aortic one.

We have established that the atrial side of the tricuspid valve cusps of the Anglo-Nubian goat has a smooth surface. The ventricular side is characterized by the presence of high outgrowths from which tendon filaments originate. The presence of blood vessels of mixed and nonmuscular types in the spongy layer of the tricuspid valve leaflets was found, which contradicts domestic and foreign authors' literature data and is an open issue for discussion research.

The data obtained are especially important for domestic and foreign morphologists. They can be used in scientific research for a comparative nature. The research results expand the database on the morphology of farm animals' hearts and can also be the basis for the certification of the Anglo-Nubian goat breed in the Russian Federation.

#### 5. AVAILABILITY OF DATA AND MATERIAL

Data can be made available by contacting the corresponding author.

#### 6. ACKNOWLEDGEMENT

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