GROSS ANATOMICAL AND HISTOMORPHOLOGICAL STUDIES ON HEART OF SHEEP

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Received: 14.09.2020; Accepted: 19.10.2020

ABSTRACT

The study revealed that heart of sheep was located in lower ventral part of mediastinal space between lungs from 3rd to 6th intercostal space. The heart was triangular in shape with blunt apex. The heart had two borders (anterior and posterior) and two surfaces (right and left). There were one transverse and three longitudinal grooves on external surface of the heart. The coronary transverse groove encircled the heart except conus arteriosus and indicated the separation of atria from ventricles and was occupied by the coronary vessels. The right atrium was having two parts; sinus venarum and auricle. Seven to eight pulmonary veins opened into the atrium behind and on the right side. The right ventricle was triangular and left ventricle was nearly circular in cross section. The histological sections revealed that all four chambers were composed of three layers i.e. endocardium, myocardium, and epicardium. The endocardium was subdivided into three layers i.e. endothelium, subendothelium and subendocardium. Myocardium was the middle and thickest layer of the heart. The cardiac myocytes were embedded in loose connective tissue that contained dense capillary network, lymph vessels and autonomic nerve fibres. Purkinje fibres were present in the subendocardium, between the myocardial bundles and as intramural fibres among the cardiac myocytes. These were large specialized cardiac muscle fibres and had greater diameter than the cardiac myocytes and are present as clusters. Each cell had only one or two centrally placed nuclei. The cytoplasm of Purkinje fibres was lightly stained because of large amount of glycogen. The cardiac cells of the atrium were smaller than the ventricle cardiac cells. Epicardium was composed of mesothelium (simple squamous epithelium) and subepicardial layer.

Keywords: Anatomy, Histomorphology, Heart, Sheep

How to cite: Sandhu, D., Gupta, A., Bansal, N., Uppal, V. and Mohindroo, J. (2021). Gross anatomical and histomorphological studies on heart of sheep. *Haryana Vet.* **60(1)**: 69-73.

The rural India economy depends on both agriculture and animal husbandry. Sheep are probably the first ruminant that humans have domesticated along with goat. Sheep is domesticated in the mountains of Iran, Turkistan and Baluchistan from their wild ancestors (Ovis oriantale vigani) (Arora and Garg, 1998). India is the seventh largest producer of the wool and accounts for nearly 2 to 3% of the total world wool production. The contribution of meat from sheep accounts for 7.68% of the total production (Indian Brand Equity Foundation, 2012). Improving sheep production is important for the upliftment of the agrarian economy. The heart is a hollow central organ which pumps blood continuously through the blood vessels by rhythmic contraction. Knowledge of basic anatomy and natural cardiac histology analysis is very important for disease diagnosis and corrective treatment of the procedure. Gross anatomical studies on the heart have been reported in sheep (Panditrao, 2014). The general description regarding heart of ox, horse, dog, goat and pig is available in many text books of veterinary anatomy. Histological studies on the ventricles of various animals and human were conducted (Motabagani, 2006). The present study describes the anatomical and histomorphological studies on heart of sheep.

MATERIALS AND METHODS

The gross morphological and histological studies were conducted on heart of sheep (n=12) collected from

slaughter house. The gross anatomical studies were conducted immediately after slaughtering. The heart was collected by opening the thoracic cavity and study was conducted on external and internal structures of all the four chambers of heart of sheep. After gross observations, the small pieces of heart tissue of 0.5 cm thickness were collected for histological observations. The tissue samples were fixed in 10% neutral buffered formalin (NBF) and processed for paraffin block preparation by acetone-benzene schedule (Luna, 1968). The blocks were prepared and sections of 5-7 μ m thickness were cut and obtained on clean glass slides with rotary microtome. The tissues sections were stained with haematoxylin and eosin stains to study the detail histomorphology.

RESULTS AND DISCUSSION

Gross Anatomical Studies

The study revealed that heart was located in lower ventral part of middle mediastinal space between lungs from 3rd to 6th intercostal space. The heart was triangular in shape with blunt apex (Fig.1). The base of the heart was directed dorsally whereas the apex lied above the last segment of the sternum. Similar findings have been reported in sheep (Panditrao, 2014), goat (Gumansing, 2015) and buffalo (Bhasin, 2017).

The heart had two borders (anterior and posterior) and two surfaces (right and left) (Fig. 1). The right surface was formed mainly by the right ventricle (Fig. 2). A small

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part of it was formed by left ventricle whereas the left surface was formed by more than one-third by right ventricle and rest by left ventricle. The anterior border was convex and curved ventrally and was present parallel to the sternum. The posterior border was vertical in all the hearts studied and laid with anterior border of 6th rib. Similar findings have been reported by Bhasin (2017) in buffalo, Panditrao (2014) in sheep and Gumansing (2015) in goat.

The divisions of heart into four chambers were indicated on surface by grooves. There were one transverse and three longitudinal grooves on external surface of the heart. These grooves were occupied by coronary vessels and fat. The coronary transverse groove encircled the heart except conus arteriosus and indicated the separation of atria from ventricles and was occupied by the coronary vessels. Similar findings have been reported by Malik *et al.* (1978) in caprine heart, Schummer *et al.* (1981) in ox, Panditrao (2014) in sheep and Bhasin *et al.* (2017) in buffalo.

All the three longitudinal grooves (left, right and intermediate) were seen on external surface of ventricles. The right longitudinal groove was on the right surface and was posterior in position. It began at coronary groove below the termination of posterior vena cava and moved downward towards apex and joined the left longitudinal groove almost in centre of anterior border (Fig. 2). The left longitudinal groove was more prominent than the right longitudinal groove. The short and shallow intermediate groove extended from coronary groove down the left side of posterior border up to middle of ventricle. This was in agreement with findings of Dyce *et al.* (1996) in dog and Pasquini *et al.* (2007) in domestic animals.

The right atrium was situated at right anterior part of the base of the heart and was having two parts *viz*. sinus venarum and auricle both (Fig. 2). The anterior and posterior vena cava and coronary sinus opened in the sinus venarum. The right auricle was blind pocket of aorta and was characterized as meshwork of pectinate muscles. Similar finding have been reported earlier by Getty (1975) in domestic animals.

The wall of right atrium was not uniformly thick because of presence of terminal crest and pectinate muscles on its internal surface. In between the pectinate muscles, small depressions known as vena cordis parva were found. In the posterior part of right auricle, anterior vena cava opened at upper and back part, whereas posterior vena cava opened at lower and back part just above the interventricular groove in straight line with anterior vena cava. Coronary sinus which delivered blood from heart itself opened ventral to posterior vena cava opening and vena hemiazygos opened into coronary sinus in all the heart studied. The opening into the right ventricle was right

atrio-ventricular opening. The present findings are in accordance with the Schummer *et al.* (1981) in ox, Panditrao (2014) in sheep and Bhasin *et al.* (2017) in buffalo.

Left atrium was situated at the back of base of heart, behind and to the left of the right atrium. It lied behind the pulmonary artery and aorta and above the left ventricle (Fig. 1). Similarly, Dyce *et al.* (1996) and Pasquini *et al.* (2007) reported that left atrium was seen on the left side of heart caudal to the pulmonary trunk where the veins opened.

The ventral border of left atrium had several notches (Fig. 1). Similarly, Bhasin (2017) noticed several notches which were more distinct in left auricle of buffalo. On contrary to this, indistinct notching in horse and smooth ventral border of left auricle was observed in dog (Schummer *et al.*, 1981).

Seven to eight pulmonary veins opened into the atrium behind and on the right side. The pointed blind end of the auricle was behind the origin of pulmonary artery. The left atrio-ventricular opening was triangular. This was in agreement with findings of Dyce *et al.* (1996) in dog and Pasquini *et al.* (2007) in domestic animals.

The right ventricle was triangular in shape with the base formed by right atrio-ventricle orifice. Its left part was projected higher and continued into root of pulmonary artery and was known as conus arteriosus (Fig. 1 & 2). Similarly, Bhasin (2017) reported in buffalo that right ventricle formed most of the right side of heart and wrapped around the cranial side of heart to continue as pulmonary trunk.

The anterior wall of right ventricle formed the anterior border of the heart while its posterior wall was formed by the interventricular septum. In the transverse section it was crecentric in shape and had tri-cuspid valve which was made of short cylindrical curtain of fibrous tissue which projected into ventricle formed margin of atrio-ventricle orifice (Fig. 3). The bases of valve touched one another and were named as infundibulum, marginal and septal depending on their position. Similar findings have been reported in male and female buffalo (Bhasin, 2017).

The chordae tendinae was the fine fibrous cord which attached the cusps to the papillary muscles projecting from the ventricle wall. Each cusp received chordae tendinae from two papillary muscles and viceversa in all the heart studied. These findings were in close proximity with the observations of Motabagani (2006) in human and mammalian species and Bhasin (2017) in buffalo heart.

Three types of trabeculae carneae were found in the ventricular wall. First one was attached to the wall of ventricle in the whole length and was known as ridges of relief. Second one was attached at both ends and was free in middle called moderator band while third one known as papillary muscle. Sathyamoorthy (2003) also found that right ventricle had three papillary muscles namely musculipapillaris magnus, musculipapillaris parvi and musculipapillaris sub arteriosus, and Motabagani (2006) reported that musculipapillaris was on the free wall while other two were on the inter-ventricular septum in human and some mammalian species.

The moderator band was a cord of myocardium crossing the lumen connecting the interventriculum septum to the opposite wall present midway between base and apex of heart. The right moderator band was single and unbranched. Sathyamoorthy (2003) also reported that moderator band extended from the anterior side of the base of musculipapillaris magnus in the base of musculipapillaris sub-arteriosus.

The left ventricle was conical in shape having base above and apex of left ventricle formed the apex of heart and was present approximately at sixth intercostal space (Fig. 1 & 2). The left ventricle formed left caudal part of ventricular mass and its wall was much thicker than right ventricle.

The chordae tendinae were larger in left than right ventricle. The trabeculae corneae resembled that of right ventricle while it had only two bundles of papillary muscles instead of three bundles as in right ventricle. On contrary to present findings, Ramsheyi *et al.* (1996) classified the papillary muscles of left ventricle into four types based on the way that they related to the leaflets via chordae. Moderator band was comparatively thinner than right ventricle. There were three moderator bands (small, medium and large sized) present in the left ventricle of sheep. Similar findings have been reported by Hareeswaraiah *et al.* (2019).

Interventricular septum was concave towards the left ventricle, so the transverse section of the cavity was nearly circular. The greater part of it had approximately same thickness as the left ventricular wall (Fig. 3) and was muscular in nature whereas the small portion of upper part was membranous and thin and lied between pulmonary and aortic orifices.

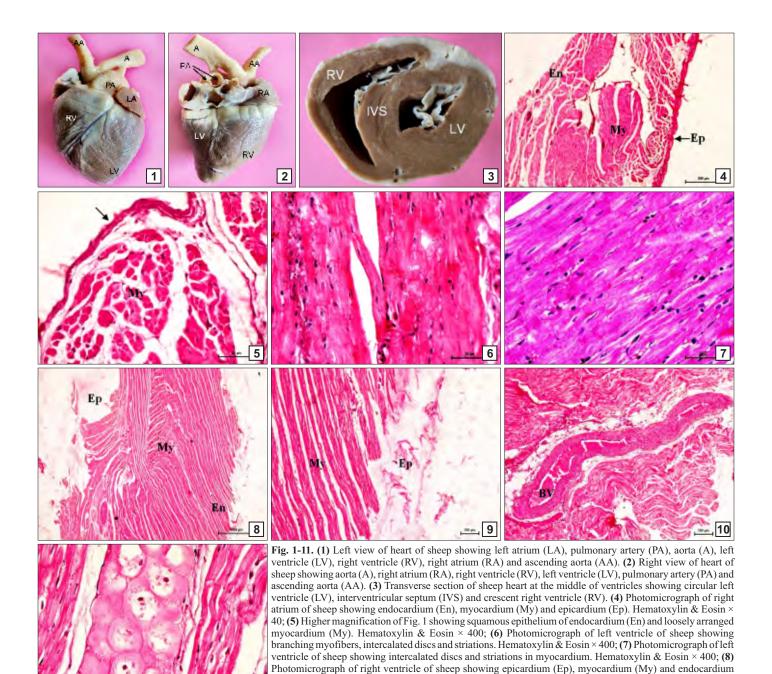
Histomorphology Studies

The histological sections from the heart of sheep revealed that all four chambers were composed of three different layers i.e. endocardium, myocardium, and epicardium.

Endocardium was the innermost layer lining the atria and ventricles and covered the cardiac valves and associated structures (Fig. 4 & 8). The endocardium was subdivided into three layers i.e. endothelium, subendothelium

and subendocardium layers (Fig. 4). Similar findings have been reported by Panditrao (2014) in sheep, Ghomini et al. (2014) in camel and Emam and Abugherin (2019) in Egyptian bovines. The endothelial layer was composed of simple squamous epithelium resting on the thin basement membrane in the atria and ventricles (Fig. 5). The subendothelial layer was the second layer of endocardium and was relatively thicker than endothelium and subendocardium. It contained dense irregular connective tissue with loosely arranged collagen and elastic fibres and few smooth muscle cells in the atrium, whereas the Purkinje fibres were seen in this layer in the ventricle (Fig. 5). Adipose tissue was also present along with blood vessels and conducting cells in the ventricles (Fig. 5). The adipose tissue was present as isolated bundles. Schummer et al. (1981) also noticed that the inner subendocardium of sheep and goat composed of small islets of adipose tissue. The subendocardium layer was the deepest layer of endocardium that connected the endocardium with myocardium and was composed of loosely arranged collagen, elastic fibres, adipose tissue, blood and lymph vessels and Purkinje fibres at certain locations. Purkinje fibres were varying in size but larger than myocytes. Its cytoplasm was pale as it contained few fibrils. It had single central large nucleus and sometimes binucleated. At the same time some of them appeared non nucleated. Similar findings have been reported by Ghomini et al. (2014) in camel.

Myocardium was the middle and thickest layer of the heart. The ventricular myocardium was characterized by housing massive, large numerous cardiac myocytes because of relatively high pressure and overload on ventricles (Fig. 4, 5 & 8). It was composed of bundles of cardiac muscle cells and Purkinje fibres. The cardiac myocytes were embedded in loose connective tissue that contained dense capillary network, lymph vessels and autonomic nerve fibres (Fig. 10). The bundles of cardiac muscles were comparatively larger in the ventricles as compared to the atrium. These cardiac muscles were arranged in sheath in a complex and spiral manner and were oriented in various directions viz. longitudinal, circular and oblique. These bundles of cardiac muscle cells were laterally separated from each other by a considerable amount of loose connective tissue that was rich with dense capillary network, lymph vessels and ganglionic plexuses. The intercellular connective tissue was less in ventricles where cardiac myocytes were numerous and much close to each other and appeared overcrowded. Most of these cells were elongated, branched and connected with each other (Fig. 7). In cross section, cardiac myocytes appeared irregular polygonal cells of different sizes with large round, centrally



placed, with single nucleus and sometimes binucleated. The cardiac muscle fibres showed striations and intercalated discs (Fig. 7). These muscle fibres showed different staining affinity i.e. some had darkly stained cytoplasm and others had lighter cytoplasm. The darkly stained muscle fibres were termed as hyper-eosinophilic as reported by Ghomini *et al.* (2014) in camel and Emam and Abugherin (2019) in Egyptian bovines.

Purkinje fibres were present in the subendocardium, between the myocardial bundles and as intramural fibres among the cardiac myocytes (Fig. 11). These fibres were large specialized cardiac muscle fibres and had greater diameter than the cardiac myocytes and cluster as group. Each cell had only one or two centrally placed nuclei (Fig. 11). Fine connective tissue was also observed between the cardiac myocytes along with blood vessels and nerve fibres (Fig. 11). The right ventricle contained more amount of interstitial connective tissue as compared to left ventricle (Fig. 8 & 9). The cardiac cells of the atrium were smaller than the ventricle cardiac cells. A thin layer of fibroelastic tissue supported the mesothelium; this layer was connected to the myocardium by a broad layer of adipose tissue. Nagpal (1977) also reported in goat that ventricular myocardium consisted of fibres which formed

(En). Hematoxylin & Eosin × 20; (9) Higher magnification of Fig. 8 showing epicardium (Ep) and myocardium (My). Hematoxylin & Eosin × 100; (10) Photomicrograph of left ventricle of sheep showing large blood vessel (BV) surrounded by adipose tissue in myocardium. Hematoxylin & Eosin×100; (11) Higher magnification

showing purkinje fiber (Pf). Hematoxylin & Eosin × 400

a more compact structure than that of atrium and its muscle bundles were comparatively larger and oriented in various directions *viz*. longitudinal, circular and oblique. Collagen and elastic tissue were relatively inconspicuous in the ventricles as compared to auricles. Whereas Sathyamoorthy and Geetha (2008) reported in pigs that muscle bundles were noticed as three layers *viz*. epimyocardium, endomyocardium and in between them, there was the thick middle layer.

Individual cardiac myocytes were not only connected in longitudinal direction but also made lateral contacts (Fig. 6). In rats, intercalated discs appeared as prominent undulating membrane demarcating the border between two adjacent heart muscle cells. Terasaki *et al.* (1993) noticed that the myocardial fibres of left ventricular papillary muscles were elongated and thinner at tips in human and animals.

The myocardium of heart was covered externally by epicardium. This layer was thin as compared to myocardium. It was composed of mesothelium (simple squamous epithelium) and subepicardial layer (Fig. 4 & 8). The later was made of loose connective tissue along with veins, nerves and adipose tissue. The atrium was having thick layer of epicardium as compared to the epicardium of ventricle (Fig. 5 & 9). Nagpal (1977) also found that atrial epicardium was comparatively thicker than that of the ventricles and right atrium was covered by a more compact structure than left atrium. Its outer part was covered by a single layer of mesothelial cells supported by the fibroelastic core. Fawcett (1994) noticed that epicardium was lined by a single layer of fattened epithelial cells resting on a thin layer of fibro-elastic connective tissue. Dellman and Eurell (1998) found that epicardium was covered externally by the mesothelial cells. Under the epithelium was a loose connective tissue layer rich in elastic fibers that formed protective sheath around blood vessels, nerves and ganglia. Sathyamoorthy and Geetha (2008) found that epicardium was lined by squamous cells.

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