



Special Issue:

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Histomorphological Development Study of the Heart of Local Awassi Sheep (*Ovis aris*) at Postnatal Stages

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Abstract | The present study aimed to morphological, histo-morphometric, histochemical and development-statements of the heart at postnatal in different stages of age of Awassi sheep. Through examination at the one-month age of the lamb indicated that morphologically heart was a triangular, hollow muscular organ and was located in the thoracic cavity extending from the 2nd to 5th ribs. Histologically, the heart wall was composed of three layers (pericardium, myocardium and endocardium), the pericardium was composed of the parietal and visceral layers, and the parietal layer is made up of dense connective tissue. The visceral layer was inner visceral epicardium and was attached to the heart, and epicardium was covered by single layer simple epithelial tissue. The endocardium was thin and inner layer, the composed of three layers (endothelial the simple squamous epithelial tissue, subepithelial and subepicardial). At the six-month age of animals, morphologically the heart was a muscular cone in shape with a sharp apex, the heart was located in the thoracic cavity and extended from the 3rd to 6th ribs, the two surfaces, two borders and composed of four chambers including two atria and two ventricles. The myocardium was the middle and thickest layer and was composed of myocardiocytes and Purkinje fibres, the myocardial fibres. Endocardium inner and thin layer lining cavities of the heart and contains Purkinje fibres in the deep part of the subepicardial layer. In conclusion the findings shown that the epicardium constitutes thin and outer layer of the atria and the ventricles were composed of mesothelium cells and a subepicardial layer. The mesothelium cells were composed of a single layer of simple squamous cells with flattened nuclei, and supported by irregular dense connective tissue and contain blood vessels and lymph vessels in subepithelial layer to the supply of heart muscle cells, subepithelial layer contain adipocytes in the atrium.

Keywords | Histology, Anatomy, Postnatal, Sheep, Heart

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INTRODUCTION

The sheep (*Ovis aries*) is the first animal, with a gestation period of 145–152 days, to be applied as a mammalian model of development, the heart is the first functional organ in a developing embryo, and the

circulatory system is its first functional unit (Gibert and Barresi, 2016). The heart is the muscle organ responsible for regularly contracting and pumping blood through the circulatory system to all body parts (Mescher, 2018). In mammals, the heart is composed of two atria and two ventricles, with the walls of the ventricles being thicker

than those of the atria (Archana and Kumar, 2010). The myocardium, the middle and thickest layer of the heart, the epicardium, the outermost layer of the heart, and the endocardium, the inner layer of the heart and continuous with the tunica intima of blood vessels, compose the heart's thick wall. The heart's cardiac muscle cells, which are capable of spontaneous rhythmic contraction, pump blood into the vascular system (Ghonimi et al., 2014).

The heart is a muscular and hollow organ situated in the thoracic cavity and is triangular. The heart in goats extends from the 3rd to 6th ribs and contacts the diaphragm at it is the posterior end, which is similar to the other ruminants, it is surrounded within the double-walled fibro-serous pericardial sac, which is oriented into the fibrous pericardium and serous pericardium is close to the aorta and enclosed the heart. In mammals, the circulatory system composed from blood vessels and the heart. In vertebrate embryos, heart development begins soon after gastrulation, when the three embryonic germ layers; mesoderm, endoderm, and ectoderm, are formed by the primitive streak (Sadler, 2022). The reversal process, which transforms the embryo's head end, carries the primitive heart, the single middle structure formed by a fusion of paired rudiments, ventral to the foregut (Ghazi and Al-Jebori, 2014).

The aim of this study is to investigate the developmental stages of Awassi sheep (*Ovis aris*) at postnatal life to guide the physiological parameters and animal health and production markers in the life of the animals.

MATERIALS AND METHODS

All animals (i.e., sheep) were bought from the Iraqi local market. All observations were conducted on the hearts of 20 healthy awassi sheep at the postnatal stage. All animals were divided into two groups based on age: One month = 10 and lamb at six months = 10. The approximate age of animals was determined according to the formula of milk teeth and eruption age of teeth (Sten, 2004).

$$2(0\backslash3 + 0\backslash1 + 3\backslash3) = 20$$

All samples of the heart were weighed using the sensitive balance (company name). A heart tissue was collected from atriums, ventricles and interventricular septum. These tissue samples were fixed using 10% neutral buffered formalin solution for 72 hours. After the fixation of tissue samples, the specimens were dehydrated in alcohol, specimens were cleared in xylene and embedded in routine paraffin. Paraffin blocks were cut with 5 µm thickness. The sections were stained with Haematoxylin and Eosin for general structures (Al -Aaraji & AL- Kafagy, 2016) periodic acid schiff (PAS) stain was conducted for reaction

for carbohydrate compounds in the epithelial cells, and Masson trichrome stain for identification of elastic and collagen fibres (Muhson and Dawood, 2023). The histopathological sections were examined using Olympus Light microscopic with the digital camera, which the connected to the computer. Gross pictures of the specimen were collected using digital camera.

RESULTS AND DISCUSSION

ASSESSMENT OF FIRST MONTH OLD LAMB

Morphologically, the heart was found to be triangular in shape, has two surfaces (right and left) and two borders (anterior and posterior). The anterior border was curved and carried long border, and the posterior border was a straight and short border and has two ends (base and apex) (Figure 1). The heart at this stage was situated between the two lung lobes on the left side of the mediastinum space, extended between the 2nd to 6th ribs. The hearts in mammals were long and have pointed the apex exception of dogs which have oval hearts with blunt apex. These results are in agreement with previous reports (Hill and Iaizzo, 2015).

The weight of the heart at this stage was about (64.75±0.32) grams, and the relative weight of heart was about (0.655%). The length of the heart from base to apex was about (73.41±2.23) mm, the width of the heart at coronary grooves was about (45.87±0.28) mm and the circumference of the heart from left to right was about (87.74±0.23) mm. The heart at this stage was also surrounded by the pericardium layer the fibroserous pericardium, and the pericardium contact with the diaphragm by the pherincopericardium ligament to fixation of the heart. These results correspond with previous report (Smith and Sherman, 2009) where heart in goats is a cone-shaped located thoracic cavity extended from the 3rd rib to the 6th, surrounded by the fibro-serous pericardium and closed with a diaphragm.

In the present study at this stage external morphology of heart was divided into four chambers: Two superior atria and two inferior ventricles. Two grooves, (atrioventricular groove, the longitudinal groove), and the coronary groove contained the coronary artery and was separated between the atrium and ventricles and longitudinal groove separated the right and left ventricles (Figure 1). The internal morphological structure of the chambers indicated that the walls of the left ventricle was thicker than the right ventricles. The cross-section analysis showed the left ventricle separated from the right ventricle by interventricular septum, and the internal wall of the ventricles showed present trabecular carnae. In right ventricle, the papillary muscles are attached chordae tendinae to the right atrioventricular tricuspid valve while in left ventricle, the papillary muscle was larger and attached to left bicuspid valve by chordae tendinae (Figure 2).

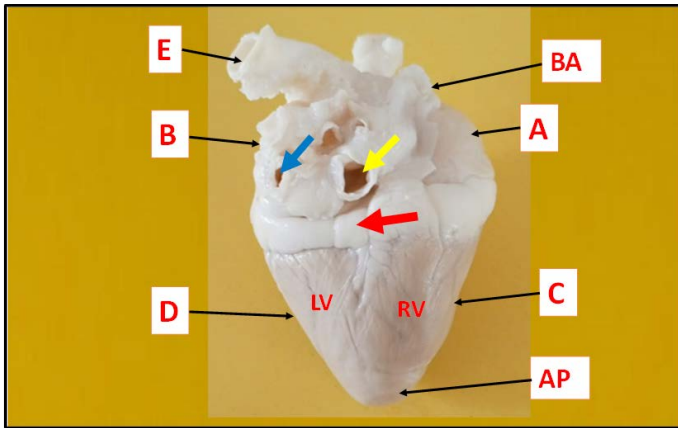


Figure 1: Anatomical photograph of heart in sheep at one month showing: (AP) apex of heart, (BA) base of heart, (A) right atrium, (B) left atrium, (RV) right ventricle, (LV) left ventricle, (C) cranial border, (D) caudal border, (E) aorta, (red arrow) coronary groove, (yellow arrow) cranial vena cava, (blue arrow) caudal vena cava.

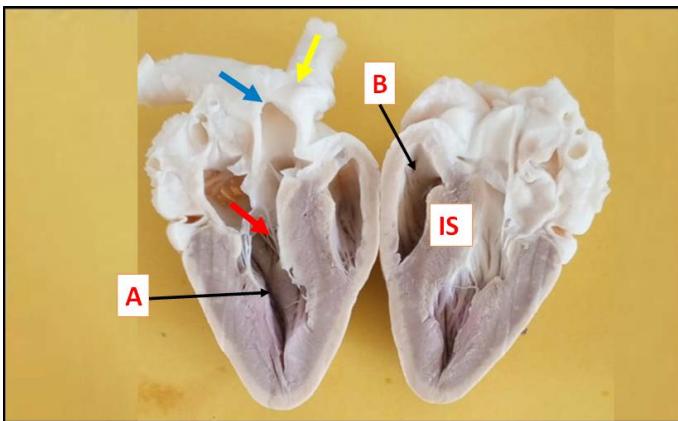


Figure 2: Anatomical photograph of heart in sheep at one month showing: (A) left ventricle, (B) right ventricle, (IS) interventricular septum, (red arrow) chordae tendineae, (yellow arrow) aorta, (blue arrow) brachiocephalic trunk.

Regarding two atria upper chambers of the heart, the wall of the left atrium was composed of two parts: Sinus venarum and auricle. The main orifice presents in the wall of the right atrium (the cranial and caudal vena cava), however for the right atrioventricular opening and coronary sinus were also identified. While the left atrium was composed of pulmonary veins and the left atrioventricular orifice, the right atrium was separated from the left atrium by interatrial septum.

These results disagree with the finding of Dai et al. (2020) where they have identified that the pig heart's classic Valentine heart shape was a result of its location in the thorax, body orientation, and two surfaces. The anterior (or sternovertral) surface resting against the sternum, and the posterior (or caudal) surface next to the diaphragm, and an apex and base with distinct upper and lower borders. The heart's shape was trapezoidal when observed in frontal projection.



Figure 3: histological section of the heart (left ventricle) at one month in sheep showing: Endothelium (En), myocardium (MYO), purkinje fibers (PF), (H & E stain 10X).

HISTOLOGICALLY STUDY

In the present study, the wall of ventricles at this stage was composed of three layers including the endocardium the inner layer, myocardium the middle layer and the epicardium layer. The endocardium of ventricles was composed of endothelial layer simple squamous epithelium with a flattened nucleus, sub-endothelium composed thin layer of loose connective, blood vessels and lymph vessels, the Purkinje fibres increased in number and more thickness and contain few myofibers the Purkinje fibres located in the deep part of the subendocardium layer (Figure 3). The thickness of the endocardium of the ventricles was more than the endocardium of the atria. The myocardium of the ventricles was in the middle and thickest layer and was composed of myocardiocytes and Purkinje fibres, as well as embedded in the connective tissue between myocardiocytes. The muscle fibres showed striated and less branched patterns. The myocardium at this stage was more apparent and joined myocardiocytes end to end, the cardiac muscle bundles in muscles surrounded by the epimysium, and a thin layer of connective tissue surrounding muscle bundles. Each muscle fibres were surrounded by delicate connective tissue (endomysium), and the thickness of myocardium of left ventricle was more than the right ventricle (Figure 4). The epicardium of the ventricles was a thin outer layer of the ventricles and atria, the epicardium was composed of a single layer of simple squamous epithelium and subepithelial tissue was composed of few collagen fibres, elastic fibres, blood vessels and lymph vessels in this layer.

They have observed that the endocardium is the most deeply embedded layer of connective tissue and covered in a thin layer of endothelium within the subendothelial layer. Fibrous connective tissue surrounds the endocardium, which was lined by endothelium. It also carried blood arteries, nerve fibres, and Purkinje fibres.

These results are similar to those observed by Atlases et

al. (2001). They have identified that the left ventricle's myocardium was thicker than the right ventricles, and the atrial myocardium was thicker than the right atrium's due to the blood circulation comparatively high pressure and resistance, and thickness of heart wall was determined by the amount of force it must develop during the contraction.

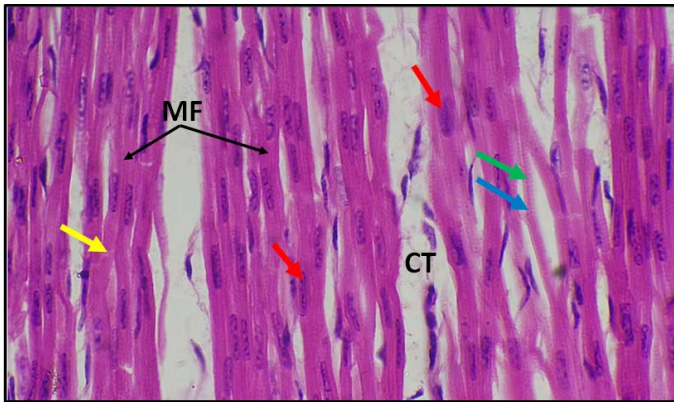


Figure 4: histological longitudinal section of the heart (right ventricle) in sheep at one month postnatal showing: muscle fibers (MB), nucleus (red arrow), A-band (green arrow), I-band (blue arrow), intercalated disks (yellow arrow), connective tissue (CT) (H & E stain 40X).

ASSESSMENT OF SIX-MONTH-OLD LAMB

Morphologically, weight of the heart was about (120.85 ± 0.32) grams, and length of the heart at the base to the apex was about (138.06 ± 0.15) mm, and the width of the heart at the coronary groove was about (85.03 ± 0.11) mm and the circumference of heart from right to left was about (170.99 ± 0.31) mm. The heart at this stage of sheep was hollow muscular organ and triangular in shape and located in the thoracic cavity and extended between the 3rd and 6th ribs, in the middle mediastinum space between left and right lungs, the heart surrounded by pericardium and contact with diaphragm by phrenicopericardium ligament. These results are similar to where they have observed that the heart of the sheep was situated between the right and left lungs in the lower mediastinal space. Its apex of the heart was above the last portion of the sternum, and its base of the heart was straight dorsally situated. In the present study at this stage, the external morphology of the heart consisted of four chambers (the upper two atria and lower two ventricles). Coronary groove was observed to be separating the atria from the ventricles and a longitudinal groove between the separated ventricles into right and left ventricles. We noticed that the heart composed of two surfaces (right and left), and two borders (cranial and caudal). The cranial border the convex and formed by right ventricle while caudal border the striated formed by the left ventricle. The heart chambers were divided into two grooves (longitudinal groove and coronary groove) (Figure 5).

The internal structure of the heart at this stage showed that the left ventricle become thickness than the atria due to the physiological load of the ventricle wall to the pumping of blood being greater than the atria. Three types of papillary muscles were present on the ventricular wall, the anterior and posterior and septal papillary muscles. The anterior and posterior papillary muscles were present on ventricular wall and septal papillary muscles present on interventricular septum wall. In the left ventricle, the papillary muscle was attached to the left atrioventricular bicuspid valve by chordae tendineae while the right ventricles were attached to the tricuspid valve by chordae tendinea. The wall of the left ventricle was thicker than the right ventricle, interventricular septum separated between right and left ventricle (Figure 6).

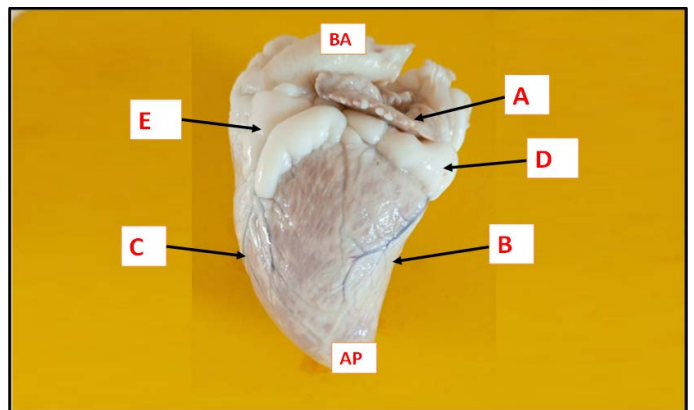


Figure 5: Anatomical photograph of heart in sheep at six months postnatal showing: (BA) base of heart, (AP) apex of heart, (A) right atrium, (B) cranial border, (C) caudal border, (D) coronary groove, (E) longitudinal groove.

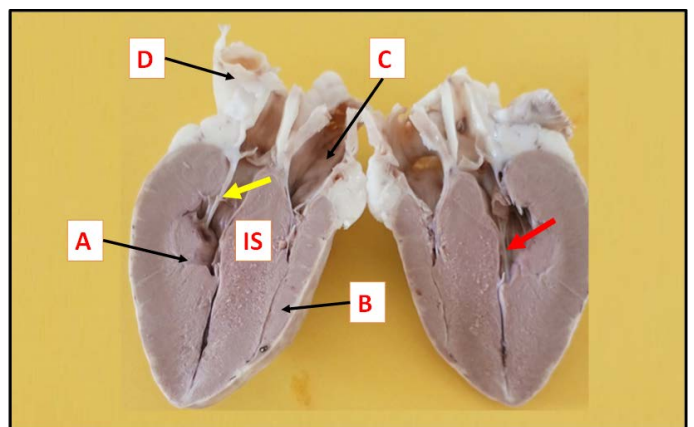


Figure 6: Anatomical photograph of heart in sheep at six months postnatal showing: (A) left ventricle, (B) right ventricle, (C) right atrium, (D) aorta, (IS) interventricular septum (IS), (red arrow) chordae tendineae, (yellow arrow) moderator band.

These results correspond with a previously published study (Al-Mahmodi et al., 2022). It was investigated that the right ventricle contains three papillary muscles (septal, angular, and parietal muscles) based on their location

and relationship to the heart's valves and ventricular wall. The left ventricle consists of two papillary muscles. These muscles are responsible for controlling blood flow into and out of the heart.

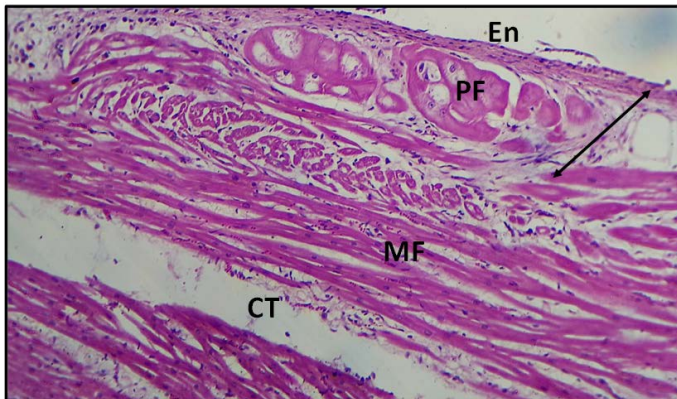


Figure 7: histological section of the heart (left ventricle) at six months in sheep showed: the endothelium layer (En), subendocardial (head double arrow), Purkinje fibres (PF), myocardium fibers (MF) (H & E stain 10X).

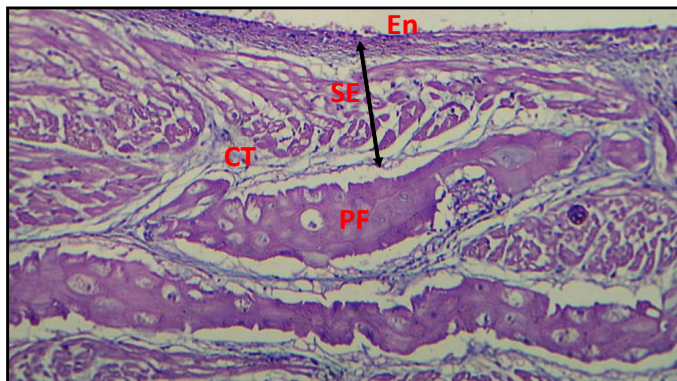


Figure 8: histological section of the heart (left ventricle) at six months in sheep showing the strongly PAS stain positive reactivity of the Purkinje fibers (PF) in the subendocardium (SE) (PAS stain 10X).

Histological analysis showed the wall of the ventricle was composed of three layers (endocardium, myocardium and epicardium). The myocardium at this stage becomes thickened and was filled with myofibrils due to an increased physiological load to pump blood, and the myocardium left ventricle was more myofibers and composed of myocardiocytes and Purkinje fibres (Figure 7). The longitudinal section of cardiac muscle cells showed the striated, long-branched, intercalated disk between myocardial cells joined end to end, and the nucleus of myocardiocytes was oval, large and centrally located in the muscle cells (Figure 7). Cross-section of the perimysium and epimysium covered of muscle bundles was evident (Figure 10). The atrial myocardium was the middle layer and was composed of muscle bundles that were randomly arranged longitudinally and were oblique. The muscle bundles were separated by a thin layer of loose connective

tissue and rich in blood capillary. The atrial myocardiocytes appear smaller and less striated than the myocardium of ventricles. The endocardium of ventricles and atria was composed of a single layer of the endothelial cell the simple squamous epithelium and sub-endothelium tissue was composed of loose connective tissue and was rich in collagen fibres. The sub-endothelium layer of ventricles was a deep part of ventricles and contain Purkinje fibres in this layer and these fibres appeared contain few of myocardial fibres and contain large amount of carbohydrate, and the more appear of positive reactive with (PAS stain) (Figure 8).

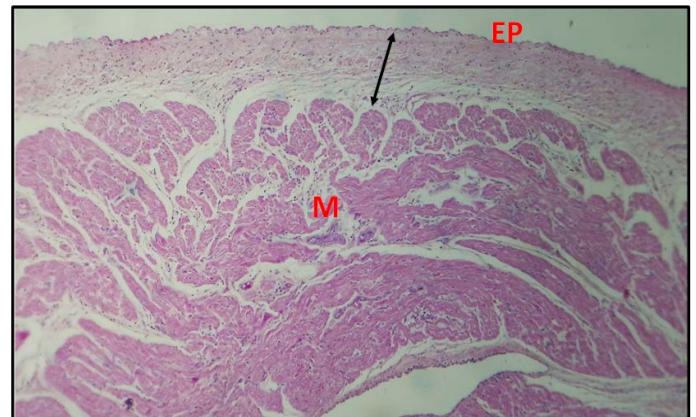


Figure 9: Histological cross-section of heart (right atrium) at (6) months postnatal showing: Epicardium (EP), subepicardial (double head arrow), myocardium (M) (H & E stain 4X).

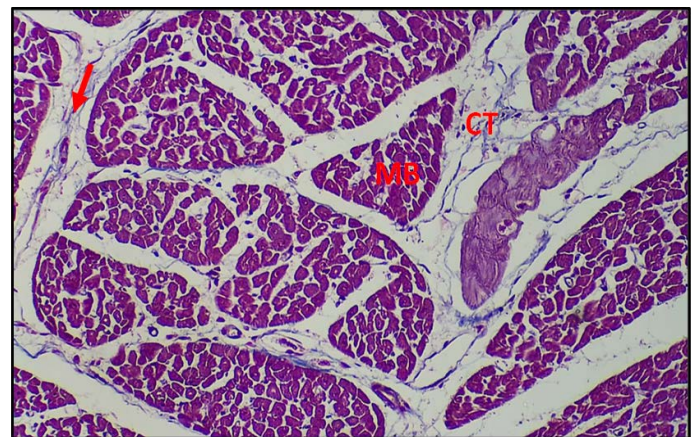


Figure 10: Histological cross-section of heart (left ventricle) at (6) months postnatal showing: Muscle bundles fibers (MB), connective tissue (CT), perimysium (red arrow) (Masson Trichrome stain 20X).

These results correlate with previous study (Veeresh et al., 2022) where they have identified that heart in sheep the myocardium's thick and middle layer was composed of a large number of myocardiocytes and Purkinje fibres. The loose connective tissue containing blood and lymph vessels between myocardiocytes, the ventricular myocardium contains muscle bundles more than atrium.

Previously, (Ghonimi et al., 2014) have showed that heart in Camel carried thickest myocardium in the middle of the left atrium and was made up of the bulk mass of the left atrium. The myocardium was composed of myocardiocytes through the wall of the atrium, and the cardiac muscle cells appeared striated, short and branched, and longitudinal and oblique in the orientation.

CONCLUSIONS AND RECOMMENDATIONS

The result of the current study showed that the epicardium constitutes thin and outer layer of the atria and the ventricles were composed of mesothelium cells and a subepicardial layer. The mesothelium cells were composed of a single layer of simple squamous cells with flattened nuclei and supported by irregular dense connective tissue and contain blood vessels and lymph vessels in subepithelial layer to the supply of heart muscle cells, subepithelial layer contain adipocytes in the atrium.

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NOVELTY STATEMENT

Presnt study can identifying breed-specific difference in anatomy and hostology of heart for awassi sheep.

AUTHOR'S CONTRIBUTION

Ahmed Jamil Abid: Research proposal, design, experimental process and writing pf report.

Ahlam Jaber Hamza: Did reseach defining, timelining, experimental process and drafting manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures of present study was approved by Veterinary Medicine College, University of Al-Qasim Green, in mean of ethical regulation and related rules such as animal welfare and standard ethical guidelines.

CONFLICT OF INTERESTS

The authors have declared no conflict of interest.

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