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Ahmed Jamil Abid
Department of Anatomy and Histology, Veterinary Medicine College, University of Al-Qasim Green, 51013 Babylon, Iraq

Ahlam Jaber Hamza
Department of Anatomy and Histology, Veterinary Medicine College, University of Al-Qasim Green, 51013 Babylon, Iraq

Corresponding Author:
Ahmed Jamil Abid
Department of Anatomy and Histology, Veterinary Medicine College, University of Al-Qasim Green, 51013 Babylon, Iraq

Histomorphological development study of the heart ventricles of local Awassi sheep during the first and second half of gestation

Ahmed Jamil Abid and Ahlam Jaber Hamza

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Abstract
Background and Objective: Study the morphological, histochemical, histomorphometric, and development statements of the heart in different prenatal stages of ages (First and second gestation). Also study of the morphological and histological structure associated with development of the heart in local awasi sheep.
Results: At the (70-75) days of gestation fetal heart was a narrow cone-shaped and hollow muscular organ pinkish in colour and located in the mediastinum more towards the left side of the midline in the crania-ventral part of the thoracic cavity in the frontal of the diaphragm at this stage, Histologically: present study the wall of atrium and ventricle composed from three layers (Outer epicardium, middle myocardium, inner endocardium), epicardium layer was formed by the simple squamous epithelium of mesothelium cells and a layer of loose connective tissue with vein and blood vessels and adipose tissue present beneath the surface of the epicardium. The myocardium middle and thick layer is composed of bundles of cardiac myocytes, these cells show eosinophilic cytoplasm with oval nuclei. The endocardium inner layer was made up of a single layer of simple squamous epithelium with central nuclei, subendocardium was composed of collage, elastic fibres blood and lymphatic vessels, and Purkinje fibres at **(**130-135) days of gestation, the heart triangular was in shape and was placed on the left side and extended from the 2nd to the 5th intercostal space and hollow muscular organ. Histologically: the wall of both atria and ventricles is composed of three layers (Outer epicardium, middle myocardium, inner endocardium), the epicardium is composed single layer mesothelium (Simple squamous epithelium), and the subepicardial (Loose connective tissue, blood, lymphatics vessels and adipose tissue), myocardium composed from more bundles of cardiac cells and Purkinje bundles embedded in loose connective tissue, endocardium was composed of three layers (Endothelium, subendothelium, subendocardium layers) the endothelial layer simple squamous epithelium, subendocardium the deepest layer was composed of collagen fibres and Purkinje fibres these layer connected between of endocardium and myocardium.

Keywords: Development, fetuses, heart ventricles, half, prenatal

Introduction
The heart is the first functioning organ and the circulatory system is the first functional unit in the developing embryo (Gibert and Barresi, 2016) [1]. The morphology and function of the sheep's heart are similar to those of goats, and it is situated in the lower thoracic cavity, extending more to the left side (Gupta, Pathak *et al*. 2018) [10]. After insemination, the heart's genesis begins in essential tissue called mesoderm. Among the three main germ layers, the mesoderm stratum is the first to differentiate during development and gives rise to all subsequent body tissues and organs (Zegyer, Al-Badri *et al*. 2022) [17]. The heart is a hollow organ that circulates blood to all organs in the body through coronary arteries (Archana and Kumar 2010) [3]. The heart of a mammal has two atria and two ventricles. The walls of the ventricles are thicker than those of the atria, and the physiological load that the ventricles have to carry to pump blood throughout the body is far more than the load that the atria bear to fill the ventricles (Emam and Abugherin 2019) [4]. Goats have a great, muscular heart with multiple chambers, two septums, valves, and other structures required to pump blood throughout the body (Gupta 2021) [8].

Materials and Methods
The study was conducted on 20 hearts of the prenatal sheep (Embryos and fetuses) ranging

from 70 days to 135 days. All the fetuses were weighed by using the sensitive balance and their crown-rump length (CRL) was measured by using verine calipers (Gall, Stier *et al*. 1994) [5]. (First half and second half of gestation) according to the gestational age. In the present study, the crown-rump length (CRL) of the first and second half of gestion specimens was calculated by using the formula (X=2.74Y + 30.15, where 'X' was the age of the fetus in days and 'Y' was the crown-rump length of the fetus in cm (Mohassen and Al-Jebori 2020) [14], (Hejazi, Issabeagloo *et al*. 2011) [12]. Tissue samples were fixed in a 10% neutral buffered formalin solution. After complete fixation of tissue, specimens were dehydrated in a graded series of alcohol, cleared in xylene then embedded in paraffin wax. The blocks were sectioned at 5-6 µm thickness of slice using a rotary microtome histological sections were stained with Haematoxylin and Eosin, Masson trichrome and PAS (Suvarna, Layton *et al*. 2018) [15]. The sections were studied using the Olympus light microscope with the digital camera, which was connected to the computer.

Results and Discussion

Group 1 foetus first half of gestation (70-75) days

The body weight of sheep embryo at (70-75) days of gestation is about (257.6±0.89) grams and the crown-rump length is about (17.32±0.42) cm.

Gross morphology: the heart of sheep fetuses was narrow and cone-shaped, and the position of the heart in the mediastinum, more towards the left side of the midline in the crania-ventral part of the thoracic cavity in front of the diaphragm. The heart was extended from the 2nd intercostal space to the 5th intercostal space (Figure, 1).

These results agree with the current (Gürbüz, Demiraslan *et al*. 2019) [11] observation in bovine fetuses at the first half of gestation, the heart was located from the third to the fifth intercostal space, revealing that the heart was located on the left side of the middle mediastinum in sheep fetuses.

The present study showed that heart weight at this stage was about (2.27±0.08) gm while dimensions of the heart recorded at the first half of the gestation were as follows: length of heart from base to apex about (20.01±0.4) mm and width of heart at coronary grove about (15.70±0.42) mm. the total weight of heart to body weight at this stage about the (1.59%).

The fetal heart in sheep is surrounded by fibroserous pericardium. The heart at first half of gestation consists of four chambers. External development formed the longitudinal and coronary grooves at this stage. The intermediate groove was not very evident. On the left side of the developing hearts the ventricles and atrium were in direct contact with the left thoracic wall at the deep notch formed between the cardiac and diaphragmatic lobes of the left lung, right lung apical lobe (Figure 1), whereas on the right side, it was surrounded by the different lobes of right lung except at the level of atrium and base of the right ventricle. The heart had two surfaces (Right and left) and two borders anterior and posterior border (Figure 2). The right ventricle contributes to the convex cranial border of the heart. The caudal border was almost straight ventrally and slightly convex dorsally and formed completely by the left ventricle. These results agree with the current (Malik, Tiwari *et al*. 1972) in the heart of the buffalo (*Bubalus bubalis*) the heart caudal border was thicker, shorter, and nearly straight or slightly concave.

Fig 1: Anatomical photograph of the heart at (70-75) days of gestation in sheep showing: the left side of the heart and its relation with other organs in the thoracic cavity, located between the 2nd rib to 5th ribs and its relation with other organs in thoracic cavity (A) base, (B) apex of heart (C) cranial lob right lung, (D) cranial lobe left lung, (E) caudal lobe left lung, (f) cranial lobe right lung, (G) caudal lobe right lung, (H) accessory lobe.

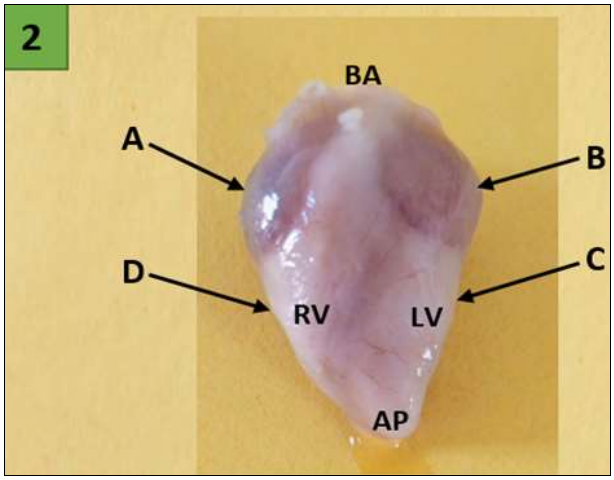

Fig 2: Anatomical photograph of the heart at first half of gestation (70-75) day showing: (A) right auricle (B) left auricle, left ventricle (LV), right ventricle (RV), (C) caudal border, (D) cranial border, the base of the heart (BA), the apex of the heart (AP)

Histological study at (70-75) days of gestation of the microscopic examination of the present study shows the heart ventricles wall consists of three layers (outer epicardium, middle myocardium, inner endocardium) the epicardium thin external layer of the ventricle was the epicardium composed of collagen fibres and covered by a single layer of mesothelial cells with simple squamous epithelium, the layer of loose connective tissue appears as a subepicardial layer (Figure, 3).

The results correspond with those (Al-Jebori 2014) [2] the heart in rabbit the composed of three layers; epicardium, myocardium, and endocardium are the three well-developed cardiac layers that make up the heart at this stage. The pericardium is a fibrous sac that encircles the heart. It is made up of fibrous and serous layers, which are then divided into parietal layers that border the fibrous layers and visceral layers, which are collectively referred to as the epicardium of the heart.

These results do not correspond with those (Gupta 2014) [6] in buffalo embryos at 50 days of gestation the initial ventricle and atrium wall are made up of two layers: the inner endocardium layer and the outer epimyocardium layer.

The presence of the current study shows the myocardium of

ventricles was the thickest layer composed of bundles of cardiac muscle fibres, the myocardial cells showed eosinophilic cytoplasm with spherical to oval nuclei, thin connective tissue between myocardial fibres with mesenchymal cells, few fibroblasts and capillaries, the myocardial fibres of the ventricle showed the branched appearance, the intercalated disks appeared connected the border between two adjacent cardiac myocytes (Figure, 5).

These results do not correspond with those (Gupta 2021) [8] in goat (*Capra hircus*) fetuses the ventricles of the myocardium were arranged in three distinct layers, with the muscle fibres arranged in more orderly organised fascicles and the layers demarcated by well-defined endomysium and perimysium. The myocardium layer was composed of the number of bundles of cardiac muscle fibres that were enveloped in the perimysium.

The results of the current study, the endocardium lines the cavities of the atrium and ventricles. It is made up of a single endothelial cell with simple squamous epithelium and flattened nuclei. The endocardium is also composed of bundles of Purkinje, elastic, and nerve fibres, as well as large amounts of collagen fibres that are formed in the subendothelium. The Purkinje fibres have spherical nuclei and are larger and less apparent than the myocardial fibres (Figure, 6).

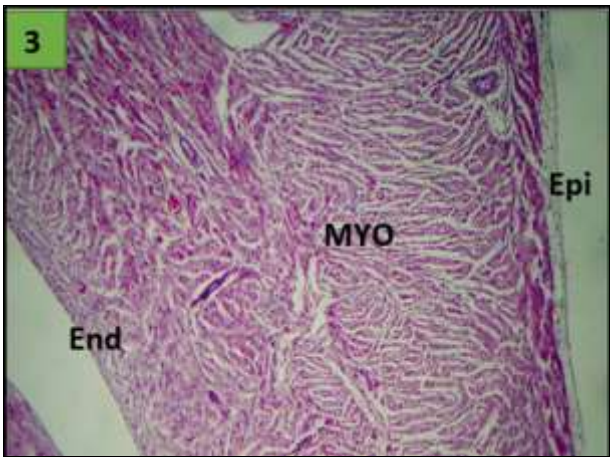

Fig 3: Histological section of the heart (right ventricle) at (70-75) days of gestation in sheep shows: epicardium (EPI), myocardium (MYO), endocardium (End) (H & E stain 4X)

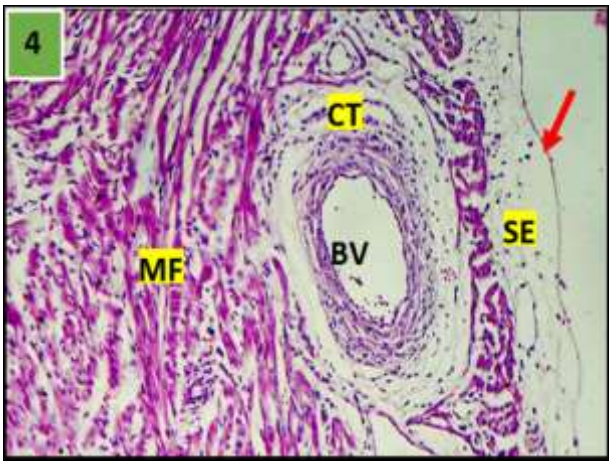

Fig 4: Microphotograph section of the heart (left ventricle) at (70-75) days of gestation in sheep showing: epicardium (red arrow), subepicardium (SE), blood vessel (BV), muscle fibres (MF), connective tissue (CT) (H & E stain 10X)

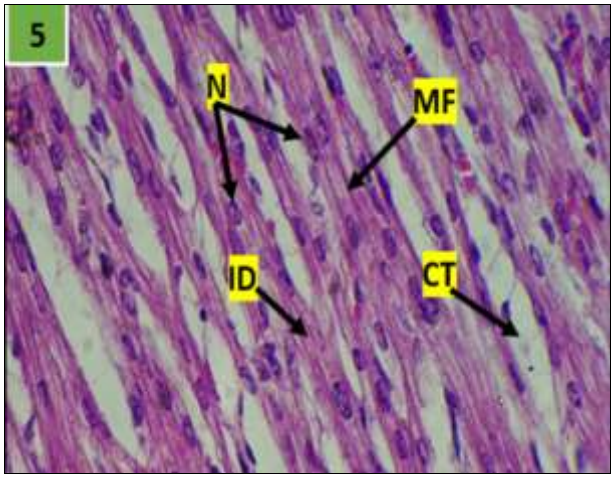

Fig 5: Microphotograph longitudinal section cardiac myocytes (striation) (left ventricle) at (70-75) days of gestation in sheep showing: cardiac muscle fibres (MF), nucleus (N), connective tissue (CT), intercalated disk (ID) (H & E stain 40X)

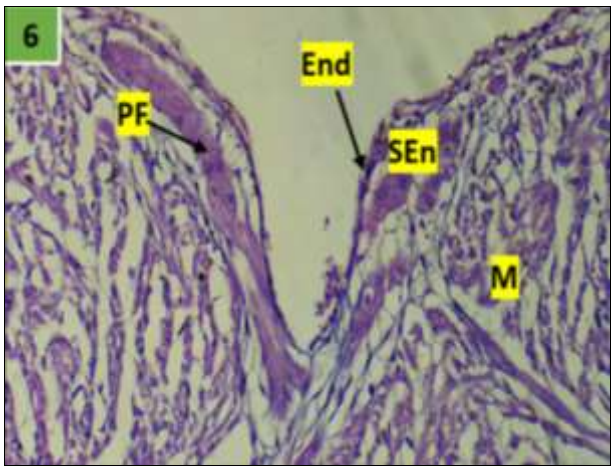

Fig 6: Microphotograph cross-section of the heart (left ventricle) at (70-75) days of gestation in sheep showing: Purkinje fibres (PF), endocardium (End), subendocardium (SE), myocardium (M) (PAS stain 20X)

Group 2 foetus second half of gestation (130-135) days

The body weight of sheep fetuses in the current study at (130-135) days of gestation is about (1080.12±0.65) grams and crown-rump length was about (37.75±0.32) cm. Gross morphology, the fetal heart at this stage was slightly shifted towards the caudal side of the thoracic cavity in front of the diaphragm. The heart of sheep fetuses was triangular with a pointed apex, and the colour of the surface of the heart became dark red, this colour was maintained until the terminal of the gestation period (figure, 7). The location of the heart at this stage was extended from the 3rd intercostal space to the 6th intercostal space.

These results agree with (Gupta, 2014) in buffalo fetuses the change in the position of the heart with the advanced age of the fetus due to rapid growth in organogenesis in the fetal period of gestation.

The present study showed that heart weight at this stage of second half gestion was about (11.05±0.21) gram while the dimensions of the heart recorded at the second half of gestation were as follows: length of heart from base to apex (39.39±0.56) mm, the width of heart at coronary groove (30.83±0.51) mm and circumference of the heart (74.65±3.120) mm. the total weight of heart to body weight

at this stage about (1.06%).

The present study showed at this stage heart is surrounded by the pericardium cavity and attached to the sternum by the sternopericardium ligament up to the xiphoid region, the posterior heart is attached to the diaphragm by the pherincopericardium ligament.

The results agree with (Veeresh, Lakshmi *et al*. 2022) [16] the heart in sheep fetuses the pericardium, which surrounds the heart, is fibrous and stronger than it was during the first half of gestation. The ligament that connects the heart to the sternum is called the sternopericardium ligament, and it becomes more fibrous and broader in the direction of the heart.

The present study showed at this stage heart is composed of four chambers two atria at the base and two ventricles at the apex, two surfaces (Right and left surface), and two borders (Anterior and posterior border) the anterior border was slightly convex at the base it did not reach up to the apex of heart while the posterior border was straight, on the external surface of the heart two sulci (Longitudinal and coronary sulcus) the longitudinal sulcus the separating between the right and left ventricles, the coronary sulcus also called atrioventricular groove the separating the atria from the ventricles, the intermediate groove was developed at this stage (Figure, 7, 8).

The present study showed at this stage the internal wall of the ventricles shows the presence of a muscular ridge that is different in size, the interventricular septum separates the right ventricle from the left ventricle, the wall of the left ventricle more thickness than the right ventricle, the wall right ventricle contains three papillary muscles (Anterior, posterior and septal) and left ventricle contain two papillary muscles (Anterior and posterior papillary muscle), the cordae tendinae show in the wall of ventricles the extended from apice of the valve to apice of papillary muscles (Figure, 9).

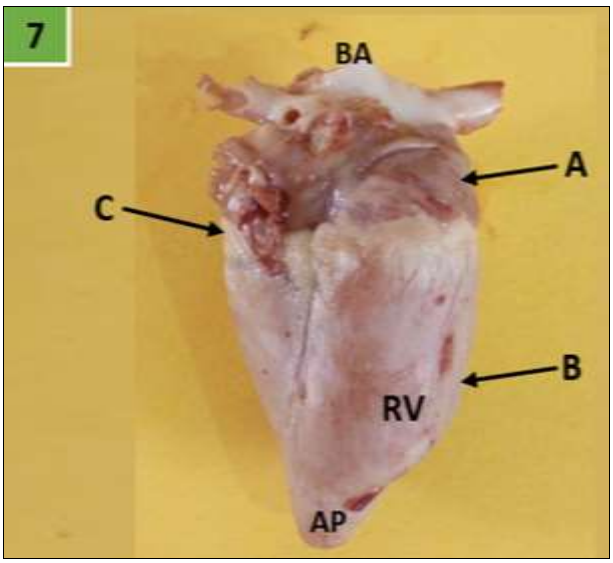

Fig 7: Anatomical photography of the right surface of the heart at (130-135) days of gestation showing: the right auricle (A), Cranial border (B), right ventricle (RV) coronary groove(C), the base of heart (BA), the apex of the heart (AP)

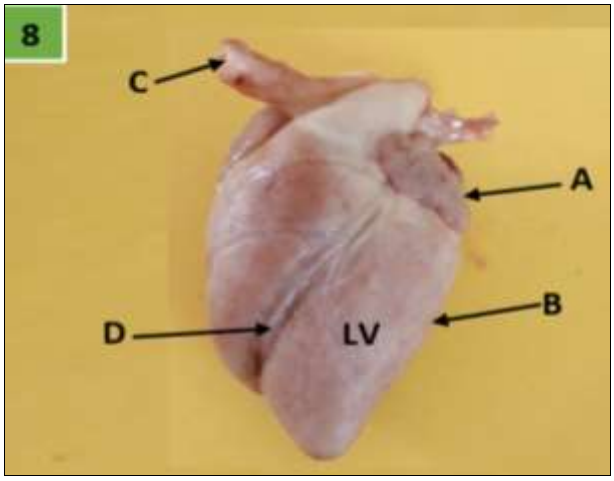

Fig 8: Anatomical photography of the right surface of the heart at (130-135) days of gestation showing: the left auricle (A), caudal border (B), left ventricle (LV), aorta (C), longitudinal groove (D)

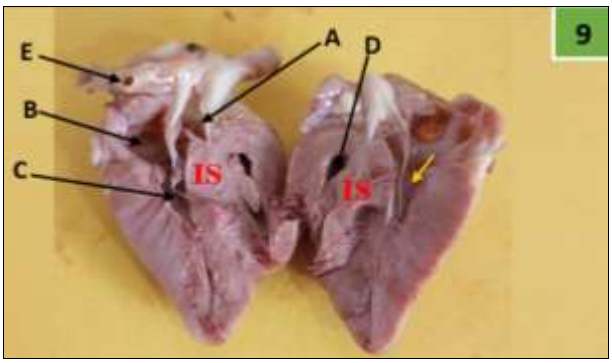

Fig 9: Anatomical photograph heart at the second half of gestation (130-135) days showing the internal structure of the heart: (A) right atrium, (B) left atrium, (C) left ventricle, (D) right ventricle, (E) Pulmonary trunk, (IS) interventricular septum, (Yellow arrow) chordae tendinae

Histological study at (130-135) days of gestation of the microscopic examination of the present study shows the heart ventricles wall consists of three layers (Outer epicardium, middle myocardium, inner endocardium) the epicardium is more vascular and increased in thickness more than the first half gestation, the epicardium of ventricular wall thin layer and external layer the composed of flattened epithelium cells the simple squamous epithelium and connective tissue in this layer (Figure, 10).

The presence of the current study shows the subepicardial layer of the ventricular wall is composed of large amounts of collagen fibres, elastic fibres, blood vessels, lymphatic, adipose tissue and nerve bundles in this layer (Figure, 11, 12). These results correspond with (Gupta, Bansal *et al*. 2014) [6] in buffalo fetuses the wall of the ventricular is comprised of three parts; the inner layer endocardium, the middle layer of the myocardium, and the outer layer epicardium. The epicardium layer was lined by simple squamous epithelium, subepicardial contained loose connective tissue, collagen and elastic fibres, blood vessels and a few smooth muscle cells, the epicardium layer of ventricles was continued with tunica adventitia of the aorta and pulmonary arteries. The presence study of the current

study showed the myocardium's middle and thickest layer of the ventricles composed of numerous cardiac muscle cells and Purkinje fibres. The bundles of cardiac muscle cells are embedded by a thin layer of loose connective tissue. The myocardial fibres showed branched, striation and were attached end to end junctions of cardiac cells by intercalated disks, the nucleus of the cardiac myocyte was spherical and single and large with uninucleated or sometimes binucleated located centrally the cardiac myocyte. The myocardial fibres are arranged in different orientations circular, oblique and longitudinal (Figure, 13).

The present study of the current study showed the bundles of cardiac muscle surrounded by a thin layer of connective tissue called perimysium, and each cardiac muscle fibre encircled by thin connective tissue called endomysium. The sarcoplasm of cardiac myocyte esinophlic and striation appeared dark band called anisotropic (A- band), and a light band called isotropic (I-band).

These results agree with (Gupta, Bansal *et al*. 2014) [6] in fetus buffalo the myocardium of the heart fetus in buffalo the myocytes formed of more trabeculated in ventricles, and the perimysium is thin layer of loose connective tissue surrounded by bundles of cardiac myocytes, and individual of the cardiac muscle fibres encircled by connective tissue called epimysium, endomysium, As the fetuses advancing age, the fibrous, cellular components of the endomysium and epimysium increased.

The present study of the current study the endocardium layer of ventricles attached to the myocardium layer, the endocardium is thin layer and consist into three layers (Endothelium, subendothelium and subendocardium), the endothelium was made up single of flattened cells with elongated and oval nuclei, the subendothelium second layer of endocardium and contain loose connective tissue and large amounts of collagen fibers, elastic fibers, the endothelium layer more thickness than endothelium, the subendocardium layer deep part of endocardium layer the connected of endocardium with myocardium, subendocardium contain purkinje fibers, collagen fibers and adipose tissue (Figure, 14).

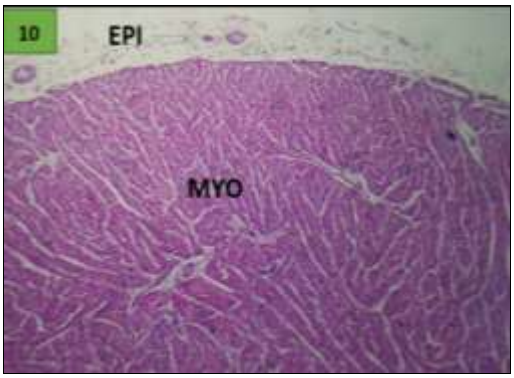

Fig 10: Microphotograph section of the heart (Right ventricle) at (130-135) days of gestation in sheep shows: (A) epicardium (EP), myocardium (MYO) (H & E 4X)

Fig 11: Microphotograph section of the heart (Right ventricle) at (130-135) days of gestation in sheep shows: sub epicardium (SE), connective tissue (CT), blood vessel (BV), nerve bundles (Red arrow) (H & E 10X)

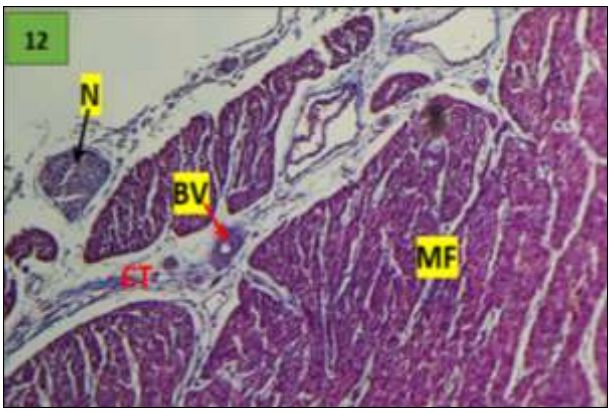

Fig 12: Microphotograph cross-section of the heart (Left ventricle) at (130-135) days of gestation in sheep shows: muscle fibres (MF), connective tissue (CT), nerve supply (Black arrow), Purkinje fibres, blood vessel (BV) (Masson Trichrome stain 20X)

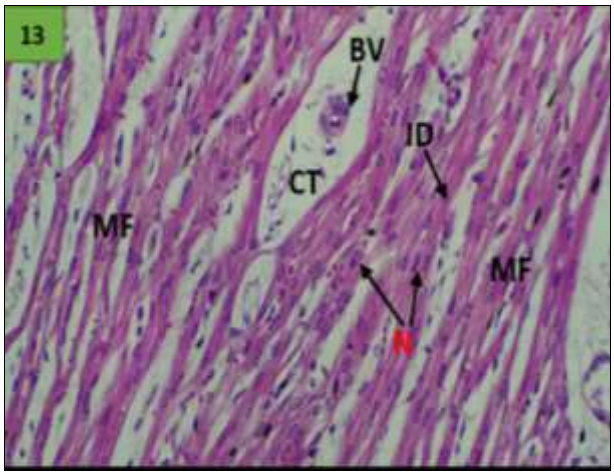

Fig 13: Microphotograph longitudinal section of the heart (Left ventricle) at (130-135) days of gestation in sheep shows: muscle fibres (MF), intercalated disk (ID), nucleus of cardiac cells (N), connective tissue (CT), blood vessel (BV) (H & E stain 40X)

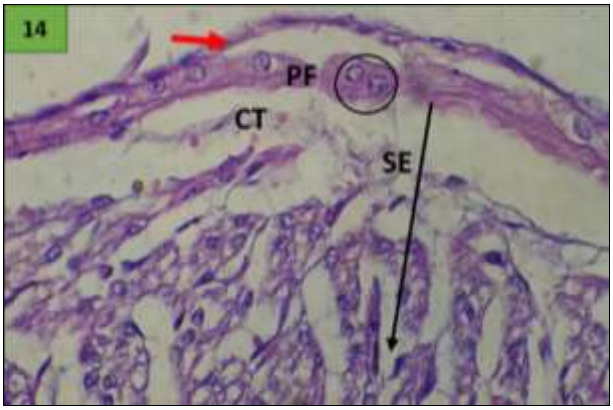

Fig 14: Microphotograph section of the heart (Left ventricle) at (130-135) days of gestation in sheep higher magnification shows Purkinje fibres (PF), endothelial layer (Red arrow), subendocardium layer (Black arrow), nucleus of Purkinje fibres (Black circle) (PAS stain, 40x)

Conclusion

In this study, the prenatal development of sheep hearts was comprehensively investigated, revealing significant insights into morphological and histological changes throughout gestation. Results showed that heart dimensions, weight, and structural features evolve notably from the first to the second half of gestation. Histological analysis highlighted the intricate development of cardiac layers and cellular composition, emphasizing the maturation process. Comparisons with previous studies on other mammalian species provided valuable context, elucidating both commonalities and unique characteristics in fetal heart development. Overall, this research contributes to a deeper understanding of sheep embryonic cardiac development, offering foundational knowledge for comparative studies and potential implications in developmental biology and veterinary medicine.

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