

## STRUCTURAL STUDY ON THE HEART VENTRICLES OF THE DOMESTIC DOGS (*Canis familiaris*) WITH SPECIAL REFERENCES TO CARDIOMYOCYTE ULTRASTRUCTURE

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**Abstract:** Our study aimed to investigate the light microscopic structure of the dog's ventricular wall in addition to the fine structure of the cardiomyocytes and the interstitial telocytes. For this purpose, tissue samples from the ventricles of 15 adult domestic dogs (*Canis familiaris*) were prepared and processed for histological, immunohistochemical, and ultrastructural analysis. The ventricular wall consisted of; epicardium, myocardium, and endocardium. The mesothelium and subepicardial connective tissue layer made up the epicardium. The myocardium was composed of bundles of cardiomyocytes separated by loose connective tissue. The endocardium was lined with endothelium located above the subendothelial connective tissue. Purkinje fibers were found only beneath the subendothelium in the subendocardial layer. The intercalated disc reacted positively to anti-E-cadherin protein. Under the transmission electron microscope (TEM) the ventricular cardiomyocyte appeared enveloped with a scalloped sarcolemma and had a striated sarcoplasm. The nucleus was oval, euchromatic, and centrally located. The mitochondria were multiple and polymorphic located either under the sarcolemma, between the myofibrils, or at the perinuclear pole. At the perinuclear pole, small electron-dense granules were observed. The intercalated disc was made up of desmosomes, adherens junctions, and gap junctions and bound the adjacent cardiomyocytes. Interstitial telocytes with cellular prolongations was evidenced. These interested microscopic structures of the dog's ventricular wall are expected to help researchers better understand the physiology of the heart ventricles, also provide a control picture that improves the differentiation of ventricular pathological conditions.

**Key words:** dog; heart; ventricles; E-cadherin; histology; electron microscopy

### Introduction

Dogs are considered as preferable animal species in cardiovascular research as their hearts share some anatomical and physiological properties with human (1). The cardiovascular system is vital to all organisms, the heart contracts rhythmically and continuously pumping the blood through the vascular system (2). The ventricles accommodate the contractile function of the heart and the histological

structure help in understanding the heart mechanism. The ventricular wall is composed of three layers endocardium, myocardium, and epicardium (3).

The Cadherins are transmembrane cell-cell adhesion molecules that play a major role in cell signaling and tissue morphogenesis (4). The classical cadherins are classified according to their distribution in the tissue into E-cadherin, N-cadherin, and P-cadherin, and all of them are localized in the ad

herens junctions (5). In the heart, the adherens junctions are components of the intercalated discs and allow the contractile force to be transported through cardiomyocytes (6).

The cardiac telocytes are interstitial cells distributed in all the heart layers. Depending on many previous studies, several functions are postulated for the myocardial telocytes as they are; predicted to play a role in intercellular signaling, acting as mechanoreceptors, and involved in cardiac renewal and repair (7).

Many studies have been conducted on the histological structure of the heart ventricles of human and different domestic animals (8, 9, 10, 11, 12). However, there are few studies that are interested in the histological structure of the dog's heart ventricles. So, this work was interested in studying the histological structure of the ventricular wall of the adult domestic dog, with special emphasis on the immunohistochemical expression of E-cadherin in the ventricular myocardium and the TEM structure of the ventricular cardiomyocytes and telocytes.

## Materials and methods

### *Animals and ethical considerations*

The current work utilized the heart of 15 clinically healthy adult domestic dogs (*Canis familiaris*) of both sexes used previously by (13) according to the ethics of AVMA guidelines for the euthanasia of animals to decrease pain and discomfort; the research protocol was reviewed and approved by the Institutional Animal Care and Use Committee, Zagazig University (ZU—IACUC; No. ZU-IACUC/2/F/7/2019). The dogs were collected from the surrounding local regions then transported to the Faculty of Veterinary Medicine, Zagazig University. The dogs were transported in well-ventilated steel cages and then housed individually in secure animal rooms at a moderate temperature and observed for 1–2 weeks before samples collection. Proper animal management, food, and water were delivered.

### *Specimens harvesting*

The animals were killed with an intravenously delivered euthanasia solution (sodium pentobarbital) at 1 mL per 10 lb of body weight. Then, the hearts were removed gently and immediately from the thoracic cavity and washed with the

physiological saline to remove the blood; then specimens from both right and left ventricular walls at the middle between the heart apex and base were gathered.

### *Tissue preparation*

#### Light microscopy

The tissue specimens were processed and stained according to Suvarna et al. (14). Briefly, the specimens were immediately fixed in 10% neutral buffered formalin for 72 hr. After that, they were processed and embedded in paraffin wax. Thin paraffin sections about 5–7  $\mu\text{m}$  were obtained by a rotary microtome. For studying the general structure, these sections were stained with Harris's Hematoxylin and Eosin (H&E), Masson's trichrome for identifying the collagen fibers, orcein stain for demonstrating elastic fibers, and the Periodic Acid Schiff (PAS) technique for detecting neutral glycosaminoglycans. All stained sections were examined and photographed with a digital Dsc-W 800 super steady cyper shot camera (Sony-Japan) connected to an Olympus BX 21 light microscope at the "Department of Histology and Cytology, Faculty of veterinary medicine, Zagazig University".

#### Histometric measurements

The thickness of the epicardium, the thickness of the endocardium, the diameter of cardiomyocytes and Purkinje fibers, the length of the sarcomere, and the diameter of mitochondria were all measured quantitatively using Image J software ([http:// Sb. Info. nih. gov/ij/](http://Sb.Info.nih.gov/ij/)) and expressed as mean  $\pm$  SE.

#### Immunohistochemical detection of E-cadherin

The paraffin sections were used for the detection of E-cadherin using rabbit polyclonal E-cadherin antibody (orb213706), Biorbyt Ltd. 5 Orwell Furlong, Cowley Road, Cambridge, CB4 0WY, United Kingdom., dilution 1:100) at 4°C. Then the sections were incubated with Goat anti-rabbit IgG H&L (HRP) ab205718, Abcam Inc. Kendall Square, Ste 341. Cambridge, USA., dilution 1:2000). The sections were incubated at horse reddish peroxidase polymer for 15 min at room temperature. DAB was used as chromogen and sections were incubated for 2–4 min at room temperature. Sections were counterstained with Mayer's hematoxylin, dehydrated, cleared, and mounted. The used technique was according to (15).

### Transmission electron microscopy

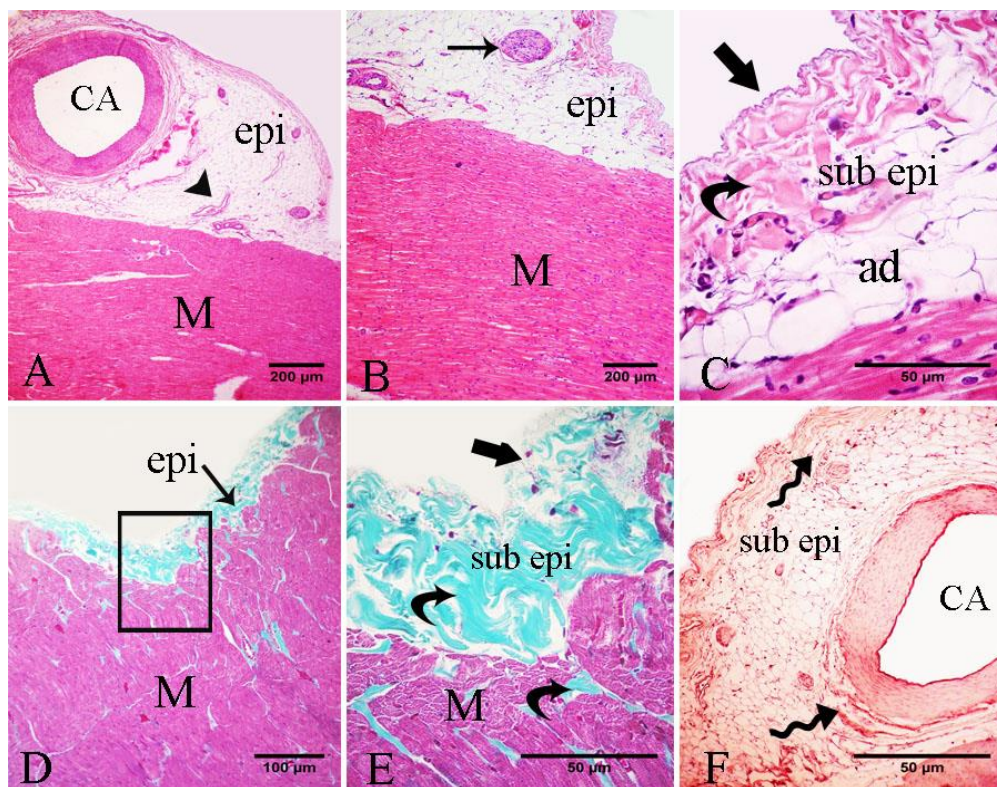
Tissue Pieces of about 1 mm<sup>3</sup> were obtained immediately after euthanasia from the myocardium of both ventricles of the dog and fixed in a buffered GA/FA fixative (1% glutaraldehyde and 10 % formaldehyde in 0.1 M Phosphate buffer) at pH. 7.4 and 4°C for 2 hours. Then the tissue pieces were processed for semi-thin sections and stained with toluidine blue according to Glauert et al (16). The ready ultrathin sections were examined and photographed at the Faculty of Agriculture, Mansoura University, Egypt by using a JEOL 2100 TEM at 80 KV.

### Results

#### *Light microscopy findings*

The wall of the ventricles was composed of three layers arranged from outward to inward as;

epicardium, myocardium, and endocardium. The epicardium was composed of two layers; a simple squamous epithelium called “mesothelium” and a layer of subepicardial connective tissue. The subepicardial layer was made up of loose connective tissue that was rich in nerve bundles, blood vessels, and a large amount of adipose tissue, especially at the sites of large coronary artery branches (Figure 1A, B, C). Moreover, the subepicardial connective tissue layer became denser and contained a large number of collagen fibers forming bundles at the sites without coronary artery branches as it was located directly above the myocardium without separating adipose tissue (Figure 1D, E). At the epicardium of the ventricular wall, only a few fine elastic fibers were detected (Figure 1 F). The thickness of the epicardium was about  $55 \pm 2.3$  in the right ventricle and  $150 \pm 23$  in the left one.

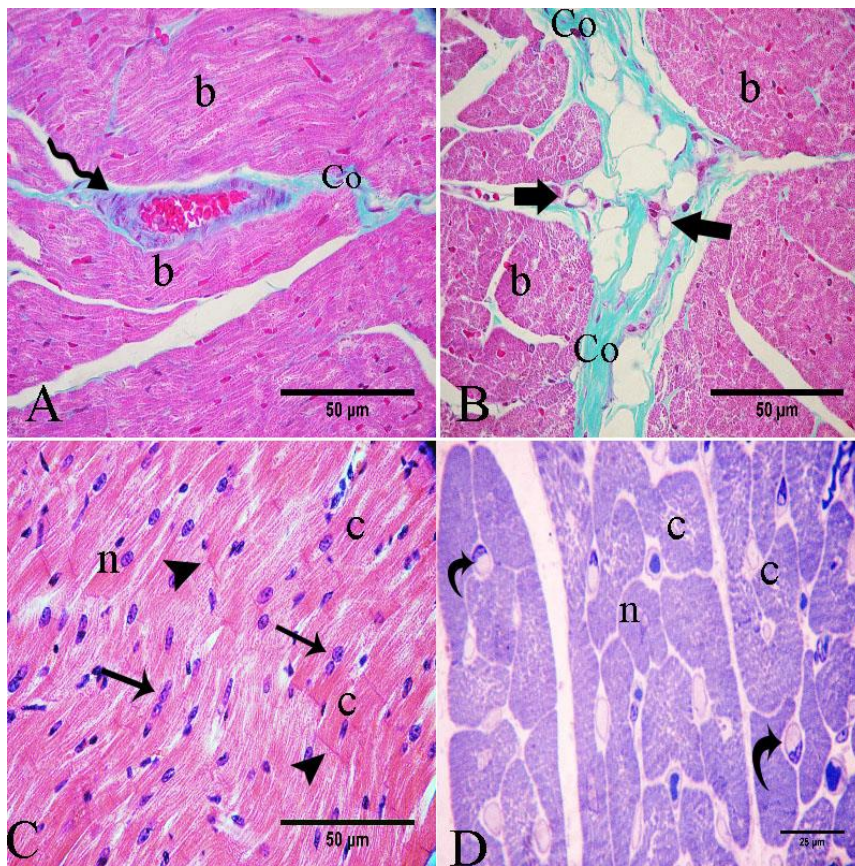


**Figure 1:** A photomicrograph showing the left ventricular wall of the dog. A. Showing the epicardium (epi) with a branch of coronary artery (CA) surrounded by adipose tissue and small blood vessels (arrowheads), and the myocardium (M). B. Showing nerve bundle (arrow) at the epicardium (epi). C. Showing mesothelium (thick arrow), and subepicardial layer (sub epi) that contained adipose tissue (ad) and collagen bundles (closed arrow). D. Showing the epicardium located directly above the myocardium (M). E. Higher magnification of the squared area in D showing the mesothelium (thick arrow) and the sub-epicardium with collagen fibers forming bundles (closed arrows) that extended to the myocardium (M). F. Showing fine elastic fibers (zigzag arrow) in the subepicardial connective tissue also in the tunica adventitia of the subepicardial coronary artery (CA). Stain. A, B&C. H&E. D&E. Masson's trichrome. F. Orcein

The myocardium of the ventricular wall is the middle thickest layer. The left ventricular myocardium was thicker than the right one. The myocardium was primarily composed of densely packed cardiomyocytes organized into bundles. The cardiac muscle bundles were separated by loose connective tissue including small blood vessels (Figure 2A), several blood capillaries, and collagen fibers (Figure 2B).

The ventricular cardiomyocytes were elongated and branched in the longitudinal section. The diameter of the cardiomyocyte of the left ventricle was thicker about  $12.4 \pm 1.4 \mu\text{m}$  than the diameter of the right ventricular cardiomyo-

cyte which was about  $7.5 \pm 0.6 \mu\text{m}$ . The ventricular cardiomyocyte sarcoplasm appeared eosinophilic with regular cross-striations. The nucleus was large, euchromatic, oval, and centrally located with a clear nucleolus. Additionally, some cardiomyocytes appeared to be bi-nucleated. The adjacent muscle fibers are connected through intercalated discs that appear as dark staining lines (Figure 2C). In the transverse section, the cardiomyocytes were polygonal with central rounded nuclei and they were separated from each other by fine connective tissue embracing several small capillaries (Figure 2D).



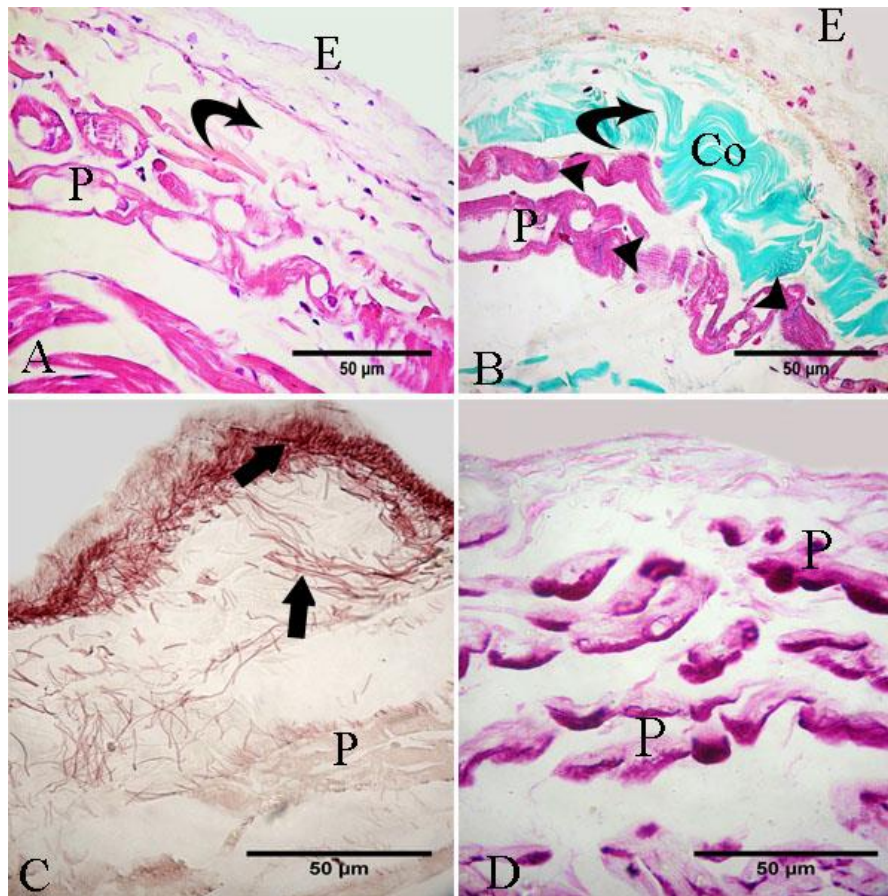
**Figure 2:** A Photomicrograph of the dog right ventricular myocardium (M). A. Showing small arteriole (zigzag arrow) among the cardiac muscle bundles (b) surrounded with collagen fibers (Co). B. Showing loose connective tissue between the cardiac muscle bundles (b) that consisted of collagen fibers (Co), and small blood capillaries (thick arrows). C. Showing a longitudinal section of the cardiomyocytes (C) with eosinophilic cytoplasm and oval central nucleus (n), bi-nucleated cardiomyocyte (arrows), and intercalated discs between cardiomyocytes (arrowheads). D. Showing semi-thin transverse section of cardiomyocytes (C) that appeared polygonal with central nuclei (n), it was crowded and embracing in between small blood capillaries (closed arrows) in the interstitial tissue. Stain. A&B. Masson's trichrome. C. H&E. D. Toluidine blue

The endocardium was equivalent to the tunica intima of the blood vessels, its thickness was about  $88.5 \pm 6.65$  in the right ventricle and  $115.85 \pm 16.3$  in the left one. It was composed of three

layers; the lining endothelium, the sub-endothelial layer, and the sub-endocardium (Figure 3A). The endothelium consisted of simple squamous epithelial cells and sometimes cuboidal ones. The

sub-endothelial connective tissue layer was composed of connective tissue rich in collagen (Figure 3B) and elastic fibers (Figure 3C). Purkinje fibers were located in the sub-endocardial layer and were arranged in parallel strands or bundles. It appeared distinct from the ordinary ventricular cardiomyocytes as it was thicker with a mean diameter of about  $16 \pm 0.74 \mu\text{m}$ . It had clear borders and ap-

peared paler than the ordinary cardiomyocytes as it had more glycogen and fewer myofibrils at the periphery. It was usually cylindrical but sometimes polygonal. It had a single oval large euchromatic nucleus that was either located centrally or peripherally displaced (Figure 3A, B). The Purkinje fibers also displayed a more strongly positive PAS reaction than the ordinary cardiomyocytes (Figure 3D).



**Figure 3:** A photomicrograph showing the left ventricular endocardium of the dog. A. Showing that the endocardium was composed of the endothelium (E), sub-endothelial layer (closed arrow), sub-endocardial layer with Purkinje fibers (P). B. Showing collagen bundles (Co) in the subendothelial connective tissue layer (closed arrow) surrounding Purkinje fibers (P). Intercalated discs (arrowheads). C. Showing elastic fibers (thick arrows) in the sub-endothelial layer. D. Showing Purkinje fibers (P) with a strong PAS reaction. Stain. A. H&E. B. Masson's trichrome. C. Orcein. D. PAS.

#### *Immunohistochemical findings*

In ventricular cardiomyocytes, E-cadherin immunohistochemistry revealed a strong positive reaction at the zigzag intercalated disc between adjacent cardiac muscle fibers. The sarcoplasm of the cardiomyocytes did not show any reaction (Figure 4 A, B).

#### *Ultrastructural findings*

The ventricular cardiomyocytes appeared closely packed to each other, elongated, and had a distinct cell polarity for most of their content as myofibrils and mitochondria. They were oval-branched fibers in the longitudinal section (Figure 5A) and polygonal in the transverse section (Figure 5B), surrounded by a scalloped shaped sarcolemma with deep invaginations where the

transverse tubules originate (Figure 5A). The cardiomyocytes were separated from each other by a highly vascularized loose connective tissue that was composed mainly of numerous blood capillaries (Figure 5B), collagen fibers, and nerve fascicles (Figure 6 A, B).

The cardiomyocytes were connected side by side through intercalated discs where there was a very narrow space between any two adjacent cardiac muscle fibers. It was located in the Z disc's zone, interrupting the I band. This disc was composed mainly of two portions; the transverse one that was made up of desmosomes and adherent junctions, and the longitudinal one that was composed mainly of the large gap junction (Figure 7A, B).

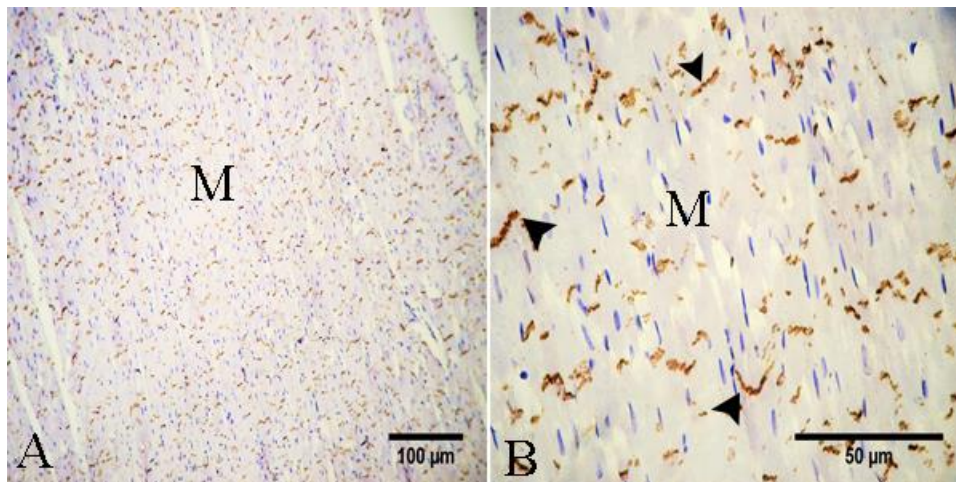
The sarcoplasm of the contractile cardiomyocytes consisted mainly of myofibrils that showed cross striations and had dark A and light I bands (Figure 8). The length of the sarcomere was about  $2.48 \pm 0.1 \mu\text{m}$ . The nucleus of the cardiac muscle fiber was single, oval, elongated, euchromatic, and centrally located. The perinuclear zone contained a little number of small electron-dense granules of different sizes and densities (Figure 5A, 9).

Multiple polymorphic mitochondria with tightly packed tubular cristae and a mean diameter of about  $1.66 \pm 0.17 \mu\text{m}$  were found at different sites in the cardiomyocyte as; aligned end- to- end

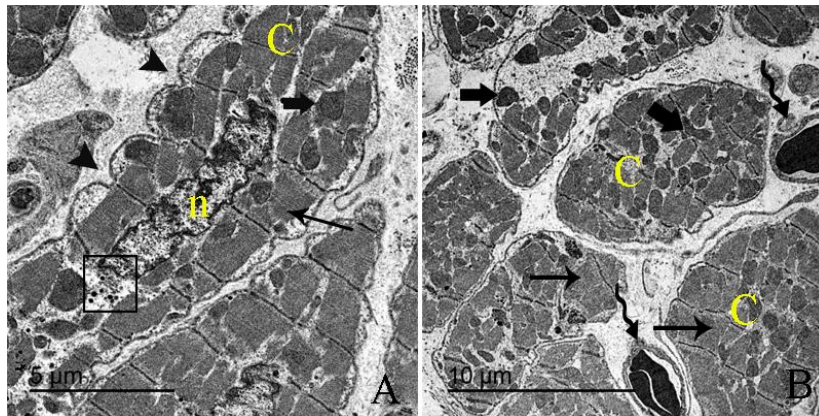
in the space between myofilaments (Figure 8), beneath the sarcolemma, and at the cardiomyocyte's perinuclear pole (Figure 9).

The sarcoplasmic reticulum was composed of very small cisternae that were observed within the sarcoplasm near the mitochondria, and nucleus; these cisternae with T- tubules forming diads (Figure 8,9) in the vicinity of the z line. The sarcoplasm of the ventricular cardiomyocytes was crowded by myofibrils and mitochondria. Therefore, other cellular organelles were very small and indistinct.

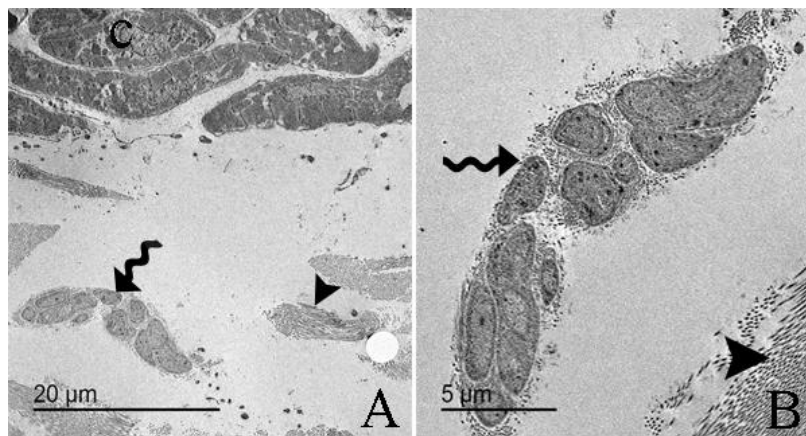
The cardiac telocytes of the ventricular myocardium were spindle-shaped interstitial cells observed surrounding the cardiomyocytes. They were characterized by having two parts; the cell body "cellular proper", and the cellular prolongation "telopode". The cell body had an oval nucleus that occupied most of the body and was surrounded by a narrow rim of cytoplasm with few cellular organelles, while the telopodes were long processes. It had two parts; very thin segments called podomers, and dilated parts called podoms. The podoms were characterized by the presence of cellular organelles such as mitochondria, rough, and smooth endoplasmic reticulum, intermediate filaments, vacuoles, lysosomes, and coated vesicles (Figure 10).



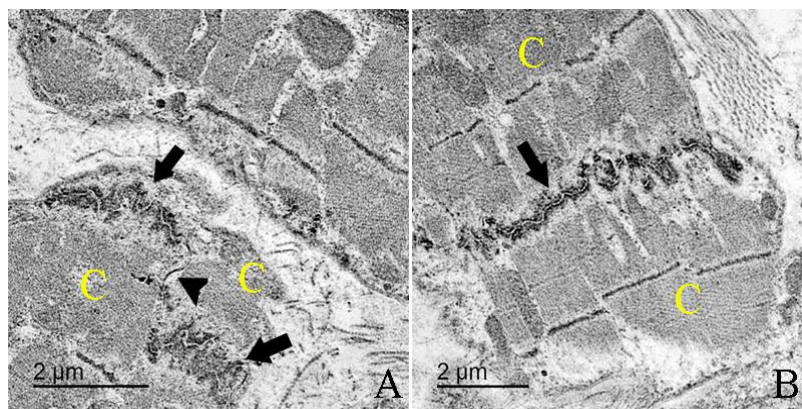
**Figure 4:** A photomicrograph shows the immunohistochemical localization of E Cadherin protein at the cardiomyocytes of the ventricular myocardium. A. Showing the positive brown color reaction in the myocardium (M). B. Higher magnification for A showing positive reaction in the myocardium (M) for E Cadherin was restricted to the intercalated disc (arrowheads) and appeared as a dark brown zigzag line between adjacent cardiomyocytes



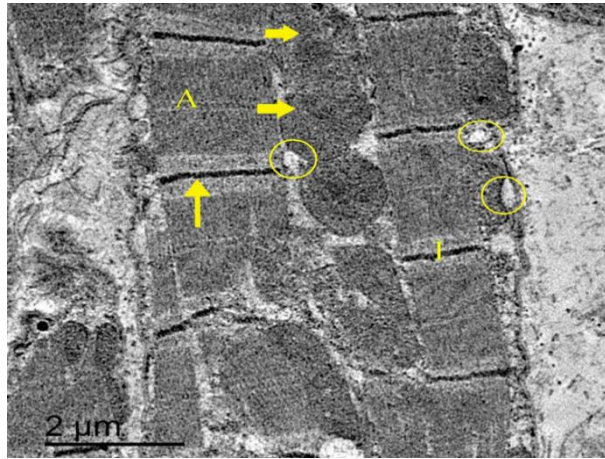
**Figure 5:** An electron micrograph of the right ventricular cardiomyocytes. **A.** Showing the scalloped sarcolemma (arrowheads) surrounding the cardiomyocyte (C), striated sarcoplasm with dark and light bands (arrow), a large number of mitochondria (thick arrow), and a central oval euchromatic nucleus (n) with prominent nucleoli that was surrounded with a small halo zone containing small secretory granules (squared area). **B.** Showing a transverse section of the ventricular cardiomyocytes (C) appeared closely packed and polygonal with striated sarcoplasm (arrow) and large numbers of mitochondria (thick arrows). Note the small blood capillaries (zigzag arrows) between the muscle fibers



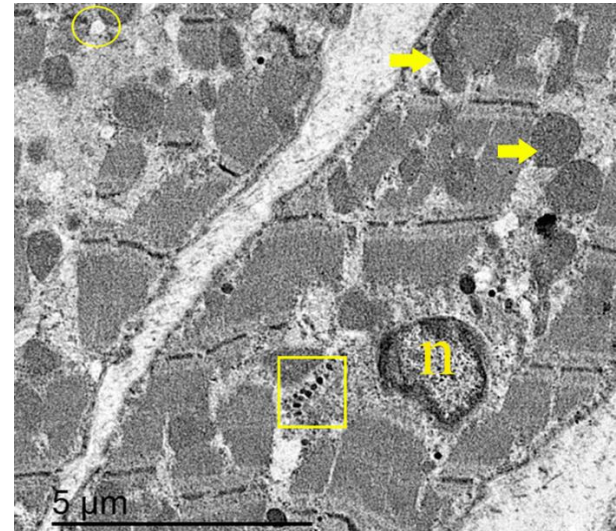
**Figure 6:** An electron micrograph of the cardiomyocytes (C) of the right ventricular myocardium. **A.** showing collagen fibers (arrowhead) and nerve fascicle (zigzag arrow) in the interstitial tissue. **B.** Higher magnification for A



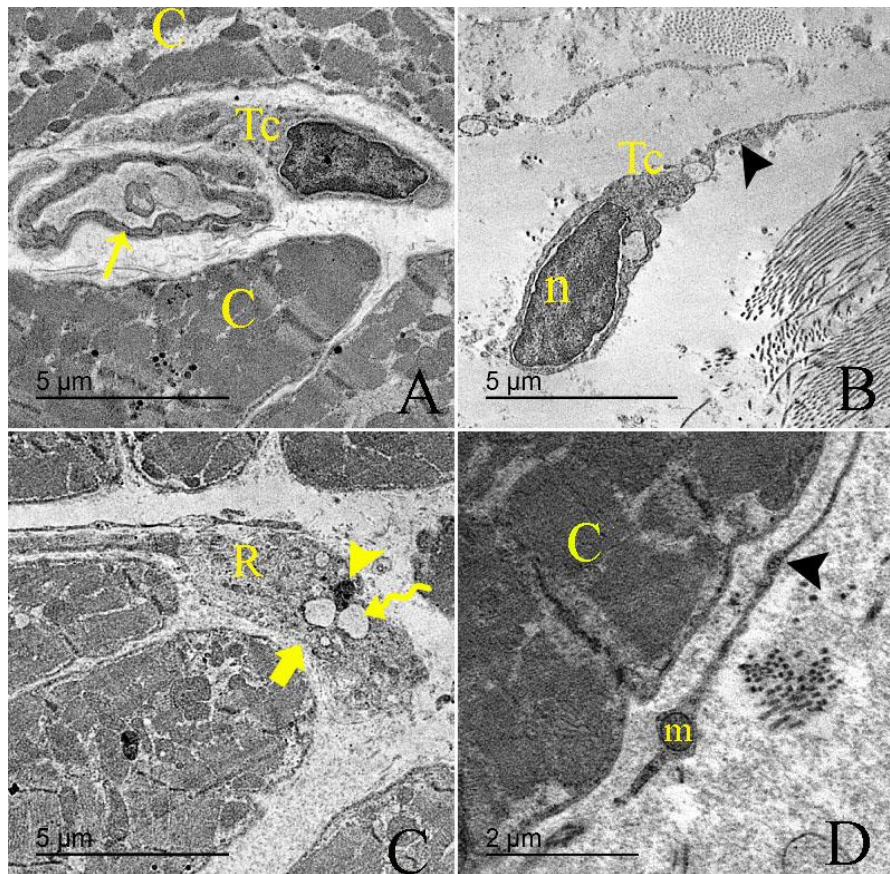
**Figure 7:** An electron micrograph of the left ventricular cardiomyocytes (C) of the left ventricular myocardium. **A.** Showing the intercalated disc between adjacent parts of two cardiomyocytes (C) that was composed of a transverse part (thick arrows) of adherent junctions and desmosomes and a longitudinal part of a gap junction (arrowhead). **B.** Showing the transverse part (thick arrow) of the intercalated disc between adjacent cardiomyocytes (C) at the zone of Z disc



**Figure 8:** An electron micrograph of the cardiomyocytes of the right ventricular myocardium shows a t-tubular system (circles) that originated from the sarcolemma and extended at the vicinity of Z lines (arrow) inside the sarcoplasm. The dark band of the sarcomere (A), the light band of the sarcomere (I), mitochondria between myofibrils were detected (yellow thick arrows)



**Figure 9:** An electron micrograph of the right ventricular cardiomyocyte shows very small electron-dense secretory granules (squared area) beside the nucleus (n), t-tubules (circle), mitochondria under the sarcolemma, and at the perinuclear zone (thick arrow)



**Figure 10:** An electron micrograph of the left ventricular myocardium. A. Showing the interstitial telocyte (Tc) between cardiomyocytes (c) near blood capillary (arrow). B. Showing a stellate-shaped telocyte (Tc) with oval nucleus (n) that occupied most of the cell body. Note the sharp conversion from the cell body to the telepole (black arrowhead). C. Showing a podom (thick arrow) that contained several cell organelles, rough and smooth endoplasmic reticulum (R), coated vesicles (zigzag arrow), and lysosomes (yellow arrowhead). D. Showing a podomere (arrowhead) and a podom with mitochondria (m) beside the cardiomyocyte (C)

## Discussion

Histologically, the ventricular wall of the dog heart consisted of the outer epicardium, the middle myocardium, and the inner endocardium as reported in previous studies (3, 9, 12,17,18). The epicardium was composed of the mesothelium and the subepicardial connective tissue layer. These findings were recorded partially in camel (3) and bovine (12).

Interestingly, this work revealed that the diameter of the right ventricular cardiomyocytes was about  $7.5 \pm 0.6 \mu\text{m}$  but the diameter of the left ventricular ones was about  $12.4 \pm 1.4 \mu\text{m}$ . We can attribute these findings to the adaptation of the cardiomyocytes to the pumping function and to the pressure. Hence, the right ventricle is responsible for pumping the deoxygenated blood into the lungs for oxygenation. However, the left ventricle must create great pressure to pump oxygenated blood into the aorta to supply the whole body. These results also go in line with previous study (19) which revealed that, the left ventricular myocardium was thicker than the right one. Owing to the greatest work required to pump the blood through the whole body by the left ventricle.

Referring to the physiological nature of the dogs, they have high respiratory and heart rates due to their high activity level. During the exercise, the dog's body temperature is increased, and to maintain it, the dog increases air flow through the mouth (panting), leading to increased evaporation and ventilation. Panting breaths are generally faster than normal breathing (20).

The dog ventricular cardiomyocytes were elongated, branched, and had cross-striated eosinophilic cytoplasm as well as a large euchromatic, oval centrally located nucleus. Similar findings were founded in (21). Additionally, some cardiomyocytes appeared to be bi-nucleated, as reported in albino rats (21) and camel (3). The cardiomyocytes in the transverse section were polygonal with a central nucleus and they were separated from each other by fine connective tissue embracing several blood capillaries, similar findings were reported in (2).

The current study indicated that the deep layer of the dog's ventricular endocardium contained bundles of Purkinje fibers. The same results were confirmed in camel (3), cat (9), and bovines (12). These Purkinje fibers were restricted to the dog's ventricular subendocardial layer as revealed in human (22) and dogs (23). However, the presence

of intramural Purkinje fibers at the depth of the myocardium among cardiac muscle bundles and individual cardiomyocytes was confirmed in camel (3), bovine (12), and sheep (22, 24).

Histometric results in this work revealed that the endocardium of the left ventricle was thicker than the right one. We can attribute this result to increasing the thickness of the Purkinje fibers bundles of the left ventricle more than that of the right one. This consideration can be directed toward the higher automaticity of the left ventricle as revealed in (25)

In this work, the dog's Purkinje fibers appeared strongly reacted to PAS. Similar results were reported (26). This finding may relate to the great glycogen content of the Purkinje fibers which can improve the mechanical resistance to anoxia as a result of enhancing the glycogenolysis and anaerobic ATP production.

The immunohistochemical results for E-cadherin at the cardiomyocytes of the ventricular myocardium showed intense reaction at the intercalated disc in both right and left ventricles. Our results were broadly in line with Mu et al. (27) who found N-cadherin expression at the intercalated discs of mature rat myocardial cells.

The ventricular cardiomyocytes were enveloped by scalloped-shaped sarcolemma as they formed deep invaginations. Similar results were observed by Ferrari (28) who revealed that these invaginations were the origin of the transverse tubules which are responsible for the propagation of the action potential.

The intercalated discs coordinate both electrochemical and mechanical communications among neighboring cardiac myocytes (29), our work revealed that it was composed of a transverse part that consisted of desmosomes and adherent junctions and a longitudinal part that consisted of the gap junction. These findings were confirmed previously in (30, 31).

According to Schaper et al. (32), the mitochondria make up about 22-23% of the heart's myocardial tissue in both human and canine hearts, and when the percent of mitochondria was compared to the body weights, the authors founded that the dog had more mitochondria per body weight which attributed high energy demands and reflected as dog's high-speed performance. Our study recorded multiple polymorphic

mitochondria with tightly packed tubular cristae that were located at various locations in the dog ventricular cardiomyocyte. These results were partially reported in albino rats (21).

This work observed the presence of a small number of very small-sized electron-dense granules in the perinuclear zone of the ventricular cardiomyocytes. These findings were in line with Larsen and Sætersdal (33) who revealed the presence of electron-dense granules at the rat ventricular cardiomyocytes which were similar to the immunoreactive ANP granules of the atrial cardiomyocytes. The last authors suggested that the production of these ANP positive granules is linked to the increased mechanical stretch of cardiomyocytes. The ANP is a peptide hormone that plays an important role in diuresis, natriuresis, and vasodilatation, so it causes a decrease in blood pressure (34, 35).

This work revealed that under TEM the myocardial telocytes of the dog's ventricles were composed of two parts; cellular bodies and the cellular prolongations "telopodes". The telopodes were long processes that had two parts; very thin segments called podomers and dilated parts called podoms. The telopodes have a major function in intercellular signaling owing to their location close to cardiomyocytes, blood capillaries, and nerve endings (36). The cell body of the dog's myocardial interstitial telocytes had an oval nucleus occupied most of it which was surrounded by a narrow rim of cytoplasm with few cellular organelles. The podoms were characterized by the presence of cellular organelles. These results were similar to those reported in a previous study (7)

## Conclusion

In conclusion, the ventricular wall of the dog's heart had the same layers of the ventricles of other domesticated animals and humans, but it was characterized by the presence of Purkinje fibers only in the endocardium. The epicardium, cardiomyocytes, and endocardium of the left ventricle were thicker than the right one owing to the greater pressure and higher automaticity of the left ventricle. As a result of the adherens junctions being part of the intercalated disc, the discs reacted positively to E-cadherin. The greatest part of the sarcoplasm of the ventricular cardiomyocytes was occupied by myofibrils and mitochondria. The ventricular cardiomyocytes express endocrine function as small electron-dense

granules noticed in the perinuclear region. The ventricular myocardium had interstitial telocytes with cellular prolongations surrounding the cardiomyocytes.

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