

Papillary Muscles in the Heart Ventricles of the Mature Dromedary Camel (*Camelus dromedaries*) with Special References to the Chordae Tendineae: Gross and Microanatomy

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Abstract

The present investigation was carried out on the papillary muscles of ten healthy mature dromedary camels. Anatomically, the papillary muscles are muscular projections from the walls of the right and left ventricles into their cavities. They are processing many nipples like processes. The apex of each muscle process is attached to cord-like tendons; chordae tendineae. The latter, are string-like in appearance and are sometimes referred to as "heart strings" that linking the papillary muscles to the tricuspid valve in the right ventricle and the mitral valve in the left ventricle. These cords divided, in turn, into sub branches before attaching to the apex, body and base of the cusp, , preventing eversion of the valve leaflets into the atria during the ventricular contraction. So histologically, there are great invasions of the chordae tendineae to inside the papillary muscles. These chordae tendineae are observed highly branched looked like the tree roots and the finger like projections in between the cardiac muscle fibers within the papillary muscles. Histologically, the papillary muscle is consisting of two major layers; the central myocardium and the peripheral endocardium which act as a capsule. The myocardium has two bundles; the contractile cardiomyocytes bundles and the purkinje cardiomyocytes bundles. The papillary muscles are completely covered externally by a single layer of simple squamous epithelium; endothelium that is reflected from the ventricular endothelium. The latter, is supported by a subendothelial loose connective tissue that is mainly composed of collagen and elastic fibers.

Keywords: Papillary muscle; chordae tendineae; Heart; purkinje cardiomyocytes; dromedary camel

Introduction

Getty, Nickel et al., Reece, Dyce, Smuts and Bezuidenhout, and Budras et al. [1-6] stated that the right atrioventricular orifice is guarded by right atrioventricular valve; tricuspid valve that has 3 large cusps. The peripheral edges of these cusps are attached to the atrioventricular opening while the central edges are connected with the ventricle by chordae tendineae. These chordae tendineae are attached with the ventricular wall by three papillary muscles. Two of these muscles are on the septum; interventricular septum and the third muscle is on the cranial wall.

In the left ventricle, the chordae tendineae are fewer but larger than it in the right ventricle. Moreover, the papillary muscles are two in numbers one on each side. Moderator band are also two; one extend from the interventricular septum to papillary muscles (the larger one) while, the other one present in various places especially in the apex [1,2].

Papillary muscle is the muscle that projected from the walls of the ventricles into their cavities. The apex of each muscle is attached to delicate cords called chordae tendinae, which are attached to the borders of atrioventricular valve cusps. Contractions of papillary muscles pull on chordae tendinae, which pull on atrioventricular valves and prevent them from opening towards the atria during the ventricular systole. Moreover, chordae tendineae are fibrous tendon-

like cords that attach the flaps of the atrioventricular valves of the heart wall [7].

The tendinous cord, trabecula carnea (papillary muscle, septomarginal band and false tendinous) and fibrous rings are essential structures that open and close the left and right atrioventricular orifices [8-10].

The pectinal muscles located on the wall of the atrium have changed their shape and become very eminent components on the wall of the ventricle and are called the trabecula carnea. This structure possesses 3 types: simply comprised ridges or prominent ridge, the so called moderator band or trabecula septomarginalis, for it lies between the anterior and septal walls of the ventricular cavity like a bridge [11], or false chordae tendineae; this kind comprises elements of the stimulus transmission even though it does not contain fibrous or musculo-fibrous structures [12], and the so called papillary muscle on the ventricular wall, which receives fibrous tendinous cords on its free edges [4,11].

The thicker the heart muscle is, the thicker and longer the musculus papillaris are. In the right ventricle, the musculus papillaris was observed to have 2-4 processing nipple like processes, which gave rise to chordae tendineae. All of the chordae tendineae divided, in turn, into subbranches before attaching to the apex, body and base of the cusps [13].

Ingels [14] claimed that as a requirement for the pumping function, the heart must have a determined arrangement of the ventricular muscle fibers. The papillary muscles are formed by the ventricular fibers originated from the cardiac muscle layers which project into the

ventricular cavity. Those structures are in direct contact with blood flow and actuate determining the adequate blood course, participating directly in the myocardium contractile function. The structure of left atrioventricular valve apparatus is composed by the mitral annulus, mitral valve leaflets, chordae tendineae and papillary muscles and has been studied elsewhere [15,16] concerning the diseases that attempt its integrity and function, including its morphogenesis [17,18].

Mechanical properties of mitral valve apparatus depend to large extend on the link between papillary muscle and valve that transmit contractions of the muscle to the valve leaflets. This link is represented by the tendinous chordae composed by a network of collagen and elastic fibers [16].

The septomarginal trabecula is present in all human hearts as well as in the hearts of other primates. It usually connects the interventricular septum with the anterior papillary muscle [19]. Moreover, Nerantzis et al. [20] clarified that the left ventricle papillary muscles are larger, attached to the left ventricle with a wider base, sustain a heavier workload and are exposed to an increased left ventricular intracavity pressure and higher oxygen content of arterial blood compared with the right ventricle papillary muscles arteries.

Gusukuma et al. [21] described that the junction between the chordae tendineae and the papillary muscles covered by the endocardium endothelium which was in continuity in the two anatomic structures. A single papillary muscle may originate multiple chordae tendineae which can divide into branches in swine and human hearts. Moreover, the fibers that constitute the chordae tendineae form bundles arranged in layers with a predominantly longitudinal disposition. In the chordae-papillary muscle junction region, the fibers compose a dense interwoven. In the human heart the collagen bundles showed a more organized arrangement, forming a meshwork with the fibers disposed in approximately orthogonal angles, whereas in the swine heart the fibers were randomly disposed.

Ghonimi et al. [22] claimed that in the cross section, the camel left atrial cardiac myocytes appeared as an irregular polygonal cells of various sizes with a large, round, pale-staining, euchromatic, centrally placed, single nucleus and also sometimes, binucleated. The cardiac muscle sarcoplasm is an eosinophilic, full of contractile myofibrils. Moreover, in the longitudinal sections, they appeared long, striated, branched and anastomosed, forming network. Furthermore, most of the cardiac muscle cells possess only a single, relatively large, oval, ovoid pale-staining, more euchromatic and centrally placed nucleus, however, some binucleated cells are occasionally observed. The cardiac muscle sarcoplasm is an eosinophilic, full of parallel contractile myofibrils that are consisted of myofilaments. They exhibit a very stronger cross-striated banding.

Purkinje fibers extend down the interventricular septum and continue up the lateral walls of the ventricles, but before they reach the lateral ventricular myocardium, they supply the papillary muscles of the heart. This conduction sequence ensures that the papillary muscles are the first parts of the ventricular musculature to depolarize and hence contract. Purkinje fibers conduct the wave of depolarization to the anteroapical region of the ventricular myocardium before it reaches the posterobasal region, so ventricular contraction begins at the apex of the heart and then spreads up the lateral ventricular walls [23].

Atrio-ventricular bundle is a group of specialized fibers which begin at the atrioventricular node and follow the membranous part of the interventricular septum beneath the endocardium. The trunk divides

into right and left crura. The right branch or bundle continues under the endocardium toward the apex to reach the right ventricular wall, the moderator bands and the papillary muscle. Its fibers form a subendocardial plexus of purkinje fibers in the wall of the right ventricle and the interventricular septum. The left branch also runs toward the apical region under the endocardium along the left surface of the septum. It fans out in the septal wall and breaks up into the conducting fibers of purkinje, which are distributed throughout the left ventricle, the moderator bands and the papillary muscle. The two bundles and their branches are surrounded by fibrous sheaths that separate or isolate them from the adjacent myocardium [1,2].

The objective of this investigation is the study of the papillary muscle architecture, their junction with chordae tendineae, the structure and distribution of the purkinje cardiomyocytes within the papillary muscle in the dromedary camel hearts.

Materials and Methods

Hearts of ten apparently healthy mature camels were collected from Zagazig slaughter house in Sharkia province, Egypt for anatomical and histological studies. For light microscopy; the papillary muscles were immediately fixed in 10% buffered neutral formalin and Bouin's fluid. The fixed specimens were processed using the usual histological techniques; dehydrated in ascending grades of ethanol series, cleared in benzene and embedded in paraffin. 5-7 μm thick sections were prepared and mounted on glass slides. These are dewaxed in xylene, hydrated in descending grades of ethanol series and stained with Harris's hematoxylin and eosin (H&E) for routine histological studies, Azan stain for demonstration of the muscle and collagen fibers, PAS (Periodic acid Schiff technique) for detection of neutral mucopolysaccharides [24], and Weigert's Resorcin Fuchsin stain for demonstration of the elastic fibers (Merck Millipore, Germany). The microphotography were taken using a digital Dsc-W 130 super steadycyper shot camera connected to an Olympus BX 21 light microscope.

Results

Anatomically, the papillary muscles are muscular projections from the walls of the ventricles into their cavities. They are processing many nipples like processes. The apex of each muscle process is attached to cord-like tendons; chordae tendineae. The latter, are tendons, string-like in appearance and are sometimes referred to as "heart strings" that linking the papillary muscles to the tricuspid valve in the right ventricle and the mitral valve in the left ventricle. These cords divided, in turn, into subbranches before attaching to the apex, body and base of the cusp, preventing eversion of the valve leaflets into the atria during the ventricular contraction (Plate 1: Figure 1 and 2). So histologically, there are a great invasion and invagination of the chordae tendineae to inside the papillary muscles. These chordae tendineae are observed highly branched looked like the tree roots and the finger like projections in between the cardiac muscle (Plate 2: Figure 3 and 4). Theses chordae tendineae are mainly consisting of collagen fibers (Figure 4a, 4b and 4c).

In the left ventricle, the chordae tendineae are fewer but larger, stronger and thicker than it in the right ventricle. Moreover, the papillary muscles in the left ventricles are larger and wider (Figures 1 and 2) because of the relatively high pressure and overload of blood circulation in the left ventricles than the right one. As the papillary muscles contract and relax, the chordae tendineae transmit the

resulting increase and decrease in tension to the respective valves, causing them to open and close. Moreover, they prevent the valves from opening towards the atria during the ventricular systole.

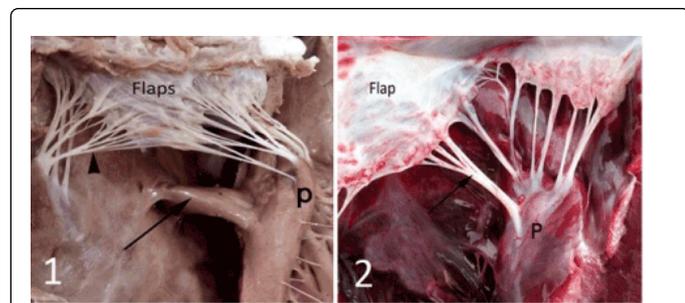


Plate 1: Figure 1: A photograph of the right ventricle of mature camel heart showing the papillary muscle (P), chordae tendineae (arrow head), valve flaps or cusps (Flaps) and the moderator band (arrow). **Figure 2:** A photograph of the left ventricle of mature camel heart showing the papillary muscle (P), chordae tendineae (arrow), and the valve flap (Flap).

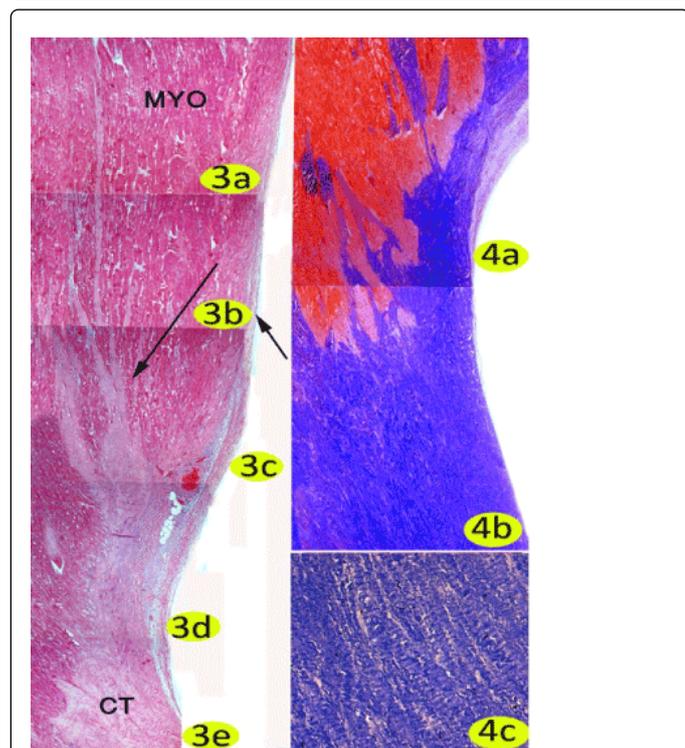


Plate 2: Figure 3a, 3b, 3c, 3d, 3e: A photomicrograph of the Papillary muscle and the chordae tendinae showing the endothelium (short arrow), the myocardium (MYO), branches of chordae tendinae (long arrow) and the chordae tendinae (CT) Stain: all H&E all Obj.x5 : Oc.x10. **(4a, 4b):** showing the papillary muscle (red) and the chordae tendinae (blue) Stain: Azan all Obj.x5 : Oc.x10. **(4c):** High magnification of (Fig. 96) showing the collagen fibers in chordae tendinae. Stain: Azan Obj.x20 : Oc.x10.

Histologically, the papillary muscles are consisted of two major layers; the central (core) myocardium and the peripheral endocardium

which act as the papillary muscles capsule (Figure 5). The central layer is considered the normal myocardial layer as in the moderator band and the ventricular wall where the cardiomyocytes; cardiac muscle fibers are extended from the interventricular septal wall to the papillary muscle and the parietal ventricular wall through the moderator bands (Figure 1), forming groups of longitudinal cardiac muscle bundles in one direction (Figure 5).

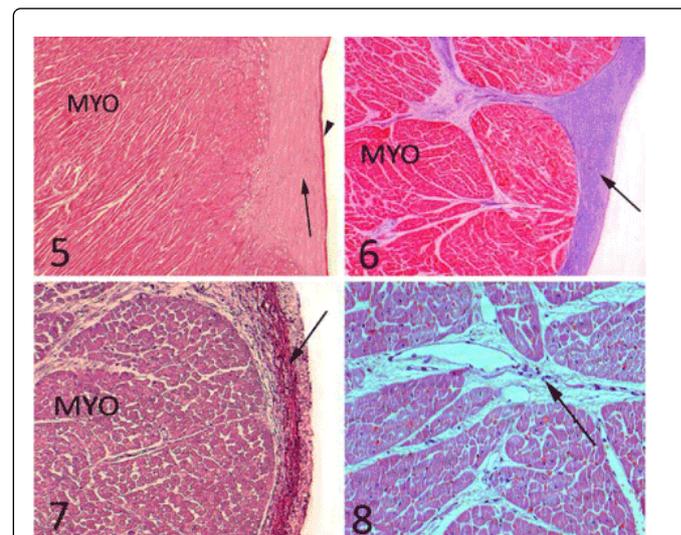


Plate 3: Figure 5: A photomicrograph of the papillary muscle showing the endothelium (arrow head), the subendothelium (arrow) and the myocardium (MYO) Stain: H&E Obj.x5: Oc.x10. **Figure 6:** showing the subendothelial collagen fibers (arrow) and the myocardium (MYO). Stain: Azan Obj.x5: Oc.x10. **Figure 7:** showing the subendothelial elastic fibers (arrow) and the myocardium (MYO). Stain: Weigert's Resorcin Fuchsin Obj.x10: Oc.x10. **Figure 8:** showing the highly vascularized inter bundle connective tissue (arrow). Stain: H&E Obj.x10: Oc.x10.

These longitudinal bundles of cardiac muscle cells are laterally separated from each other by a considerable amount of highly vascularized loose connective tissue (Figure 8), that is mainly composed of collagen (Figure 10), and elastic fibers (Figure 9). The intercellular connective tissue is few where the myocardiocytes in the papillary muscle are numerous, large in size and also much closed to each other and appeared overcrowded (Plate 3: Figures 5, 6 and 7). Moreover, most of these cells are elongated, branched and connected with each other, forming some sort of network-like structure (Figures 13, 14 and 15).

The cardiac muscle cells in longitudinal sections are appeared long, striated, branched and anastomosed, forming network and joined end to end and side to side at intercalated disks, forming the myocardial fibers. Furthermore, most of the cardiac muscle cells possess only a single, relatively large, oval, ovoid pale-staining, more euchromatic and centrally placed nucleus (Plate 5: Figures 13, 14 and 15), however, some binucleated cells are occasionally observed, occupying a central position in the muscle cell and some of them showed prominent nucleoli. The nuclear chromatin is dispersed and in most cases tended to be condensed peripherally (Figure 15). The cardiac muscle sarcoplasm is an eosinophilic, full of parallel contractile myofibrils that

are consisted of myofilaments. They exhibit a very stronger cross-striated banding pattern where, the sarcoplasm shows its characteristic striations of alternating dark and light bands (Figures 14 and 15). Furthermore, in the cross sections, the cardiac muscle fibers appear irregular polygonal cells of various sizes with a large, round, pale-staining, euchromatic, centrally placed, single nucleus and also sometimes, binucleated cells (Figure 16). Furthermore, the cardiac muscle showed moderately PAS positive reactivity (Figure 17), with presence of PAS positive granules in the perinuclear regions (Figure 19).

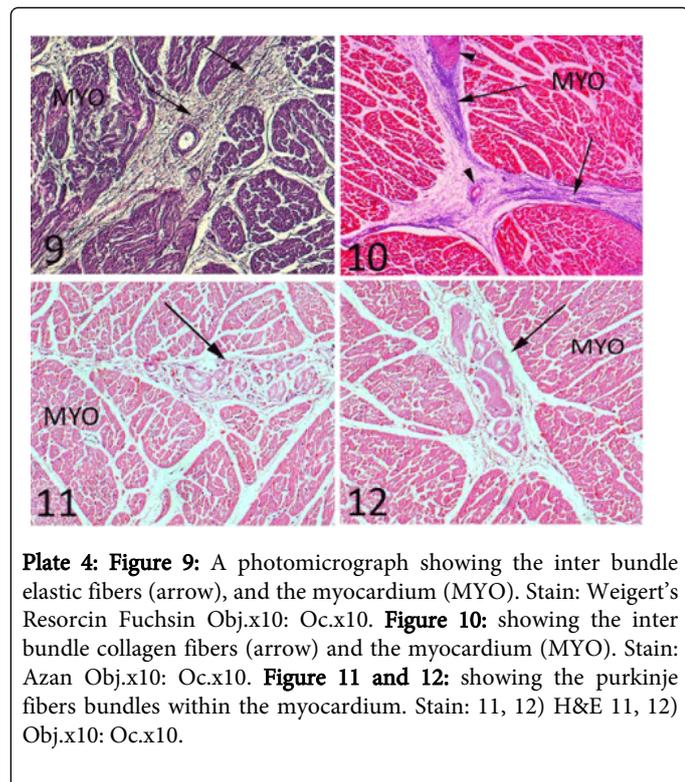


Plate 4: Figure 9: A photomicrograph showing the inter bundle elastic fibers (arrow), and the myocardium (MYO). Stain: Weigert's Resorcin Fuchsin Obj.x10: Oc.x10. **Figure 10:** showing the inter bundle collagen fibers (arrow) and the myocardium (MYO). Stain: Azan Obj.x10: Oc.x10. **Figure 11 and 12:** showing the Purkinje fibers bundles within the myocardium. Stain: 11, 12) H&E 11, 12) Obj.x10: Oc.x10.

Moreover, beside the longitudinal cardiac muscle bundles, the Purkinje fibers are present and organized into bundles in between and surrounding the cardiac muscle bundles, filling the core of the papillary muscle. These Purkinje cells were observed round, larger in size than the working cardiac myocytes, showing variety in number and size. Its cytoplasm is pale as it contains very fewer myofibrils than normal myocytes and rich in glycogen. It has single, central, large nucleus and sometimes binucleated with prominent nucleoli. The nuclear chromatin is dispersed and in most cases tended to be condensed peripherally. However, some non-nucleated cells are also observed (Plate 4: Figures 11 and 12). There was cell-to-cell communication between the Purkinje fibers within the bundle. Furthermore, these Purkinje cells were surrounded by a connective tissue sheath (Figures 11 and 12). Furthermore, the Purkinje fibers showed strongly PAS positive reactivity (Figure 17), with presence of numerous PAS positive granules in the cells cytoplasm (Figures 17 and 18). The Purkinje cardiomyocytes appeared darker with PAS stain in comparison with the contractile cardiomyocytes (Figure 17). Moreover, the inter-Purkinje cardiomyocytes connective tissue showed strongly PAS positive reaction (Figure 18).

The papillary muscles externally are completely covered by a single layer of simple squamous epithelium (endothelium) that is reflected

from the ventricular endothelium. The latter, is supported by a subendothelial loose connective tissue that is mainly composed of collagen (Figure 6), and elastic fibers (Figure 7).

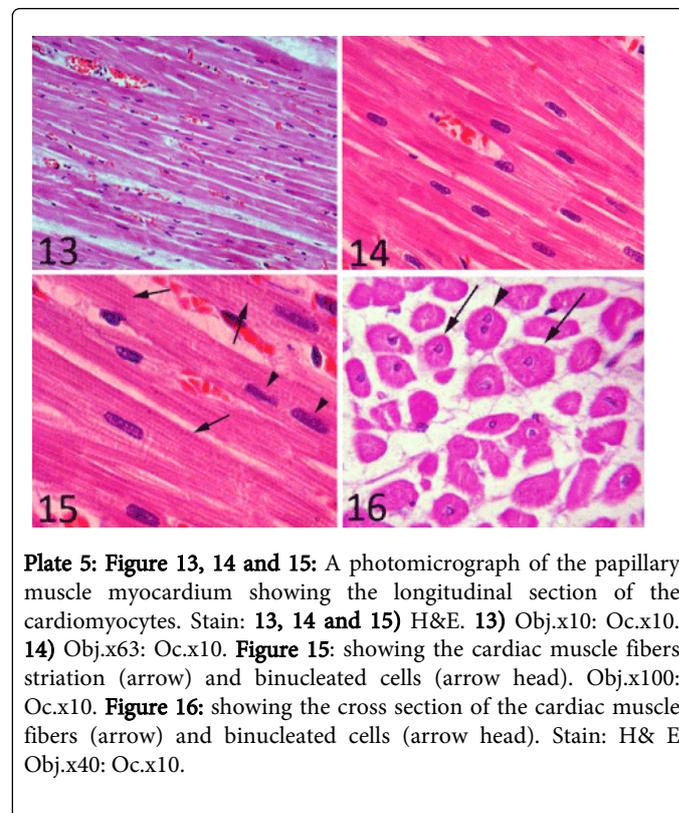


Plate 5: Figure 13, 14 and 15: A photomicrograph of the papillary muscle myocardium showing the longitudinal section of the cardiomyocytes. Stain: 13, 14 and 15) H&E. 13) Obj.x10: Oc.x10. 14) Obj.x63: Oc.x10. **Figure 15:** showing the cardiac muscle fibers striation (arrow) and binucleated cells (arrow head). Obj.x100: Oc.x10. **Figure 16:** showing the cross section of the cardiac muscle fibers (arrow) and binucleated cells (arrow head). Stain: H& E Obj.x40: Oc.x10.

The endothelial and the subendothelial layers are forming the endocardium that is completely surrounding and covering the papillary muscle from all directions (Figure 5). Moreover, the endothelial layer extends to cover the chordae tendineae (Plate 2).

Discussion

The papillary muscles are muscular projection from the walls of the ventricles into their cavities. They are processing many nipples like processes. The apex of each muscle process is attached to cord-like tendons; chordae tendineae. These cords divided, in turn, into subbranches before attaching to the apex, body and base of the cusp, preventing eversion of the valve leaflets into the atria during the ventricular contraction. This finding is in coincidence with [13] in donkey.

In the left ventricle, the chordae tendineae are fewer but larger and thicker than it in the right ventricle because of the relatively high pressure and overload of blood circulation. This finding is in parallelism with [1-4] in domestic animals and [5] in camel and [6] in horse.

The cardiac muscle fibers are extended from the interventricular septal wall to the papillary muscle and the parietal ventricular wall through the moderator bands, forming groups of longitudinal cardiac muscle bundles. This finding is in coincidence with [25-28] in pigs and [29] in ostrich.

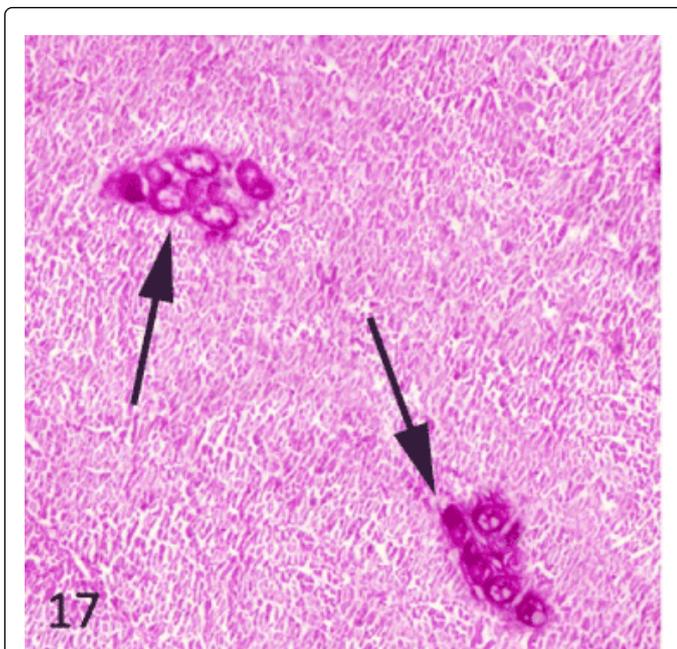


Figure 17: A photomicrograph of the papillary muscle myocardium showing the moderately PAS positive reaction of the cardiac muscles and strongly PAS positive reaction of the purkinje cardiomyocytes (arrow). Stain: PAS Obj.x5: Oc.x10.

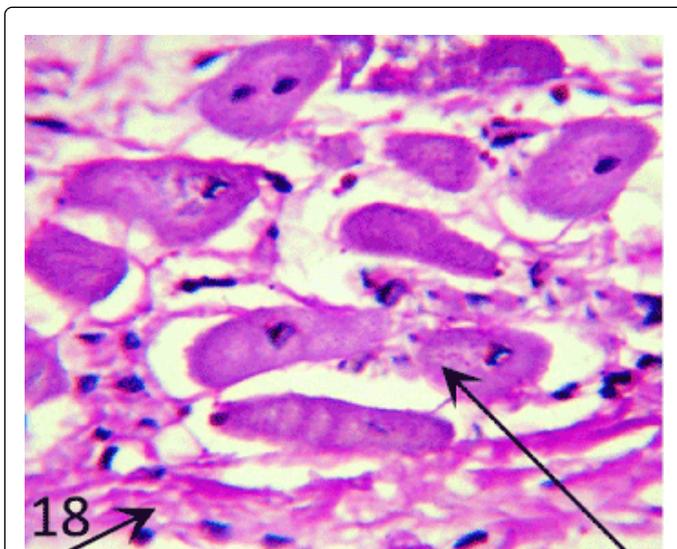


Figure 18: A photomicrograph showing the strongly PAS positive reaction of the purkinje cardiomyocytes with presence of numerous PAS positive granules within the cytoplasm (long arrow) and the strongly PAS positive reaction of the inter-purkinje cardiomyocytes connective tissue (short arrow). Stain: PAS Obj.x40: Oc.x10.

The central layer consists of contractile cardiac muscle fibers that arranged longitudinally from the interventricular septal myocardium to the moderator band and then to the myocardium of the ventricular wall and also run to inside the papillary muscles, forming groups of longitudinal cardiac muscle bundles. This result is indicating that the

cardiac muscle fibers in the interventricular septum, the moderator band, the ventricular wall and the papillary muscle are the same. This results is agree with [1,2] in domestic animals and [29] in ostrich who stated that in the right and left ventricles, the walls have a muscular moderator band which extend from the interventricular septum to the opposite ventricular wall especially at the papillary muscle. The function of this structure is to prevent over distention and dilatation of the right ventricle during contraction and also to allow the purkinje fibers to extend from the atrioventricular bundle branch to the papillary muscles and myocardium of right and left ventricular parietal wall.

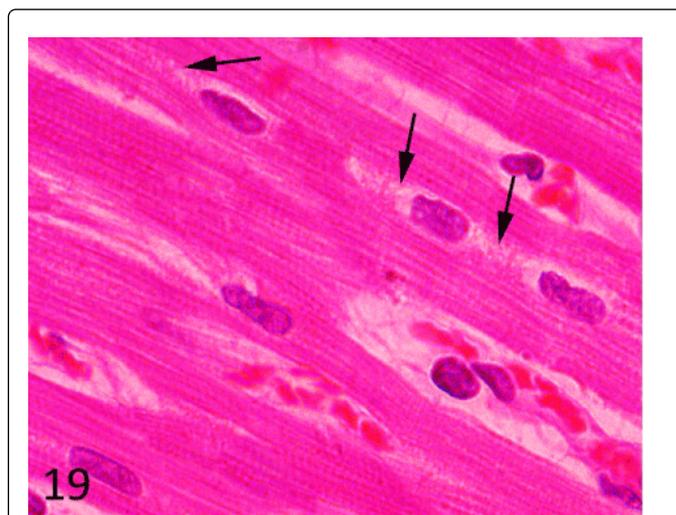


Figure 19: A photomicrograph showing the PAS positive reaction of the contractile cardiomyocytes with presence of numerous PAS positive granules in the perinuclear regions (arrow). Stain: PAS Obj.x100: Oc.x10.

There were two main cell types were recognized within the myocardial layer; the working myocardial cells and the conducting cells; Purkinje fibers. Moreover, the working cells were considered the predominant cell type. These results are very close and similar to the finding that described after [30] in camel.

The longitudinal bundles of cardiac muscle cells were laterally separated from each other by a considerable amount of loose connective tissue. Moreover, it was rich with a dense capillary network, lymph vessels, and autonomic nerve fibers. Most of these cells are elongated, branched and connected with each other, forming some sort of network-like structure. This finding is in close agreement with [22,30] in camel, [31] in bovines, [23] in human, [32-34] in bovines, [35,36] in human.

The cardiac muscle cells in longitudinal sections are appeared long, striated, branched and anastomosed, forming network. Furthermore, most of the cardiac muscle cells possess only a single, relatively large, oval, ovoid pale-staining, more euchromatic and centrally placed nucleus, however, some binucleated cells are occasionally observed. The cardiac muscle sarcoplasm is an eosinophilic, full of parallel contractile myofibrils that are consisted of myofilaments. They exhibit a very stronger cross-striated banding. Furthermore, in the cross sections, the cardiac muscle fibers appear irregular polygonal cells of various sizes with a large, round, pale-

staining, euchromatic, centrally placed, single nucleus and also sometimes, binucleated cells. These findings are goes hand in hand with the finding that described after [22,30] in camel.

Beside the longitudinal cardiac muscle bundles, the purkinje fibers are present and organized into bundles in between and surrounding the cardiac muscle bundles. These purkinje cells were round, larger in size than the working cardiac myocytes, showing variety in number and size. Its cytoplasm is pale as it contains very fewer myofibrils than normal myocardiocytes. It has single, central, large nucleus and sometimes binucleated with prominent nucleoli. However, some non-nucleated cells are also observed and showed strongly PAS positive reaction. These investigations are in parallelism with the findings that described after [30] in camel and [37] in human. Moreover, it goes hand in hand with [38] in horses and [29] in ostrich, who assumed that beside the longitudinal cardiac muscle bundles, bundles of purkinje fibers are present in between and surrounding the cardiac muscle bundles, filling the core of the moderator band. These purkinje cells were surrounded by a connective tissue sheath. As, the cardiac muscle fibers extends from the moderator band to the papillary muscle carrying the same cell architectures, so there are a great similarity in between the papillary muscle and the moderator bands.

The papillary muscles externally are completely covered by a single layer of simple squamous epithelium (endothelium) that is reflected from the ventricular endothelium. The latter, is supported by a subendothelial loose connective tissue that is mainly composed of collagen and elastic fibers. This investigation is in coincidence with the findings of [33,37].

The endothelial and the subendothelial layers are forming the endocardium that is completely surrounding and covering the papillary muscle from all directions. Moreover, the endothelial layer extends to cover the chordae tendinae. This result is in parallelism with the findings of [21] in human and swine who described that the junction between the chordae tendineae and the papillary muscles covered by the endocardium endothelium which was in continuity in the two anatomic structures.

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