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Moderator Bands (Trabecula septomarginalis) of Mature Buffalo (*Bos bubalis* L.) with Special Emphasis on the Structure and Distribution of the Purkinje Cardiomyocytes: Histological and Histochemical

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Abstract

Ten hearts of mature healthy Buffalo (*Bos bubalis* L.) were employed to investigate the histological and histochemical structures of the moderator bands. Hearts were gently dissected and the moderator bands were collected. The specimens were processed histologically and subjected to different stains. Grossly, the moderator band (Septomarginal trabeculae) was a single muscular band that was found only in the right ventricle of the buffalo heart. It extended from the interventricular septum to the free ventricular wall especially at the base of the papillary muscle, crossing and passing through the ventricular cavity. Histologically, the moderator band was invested in a dense capsule of connective tissue, endocardium that is mainly consisting of three layers; the endothelial layer of simple squamous epithelium, subendothelial layer of loose connective tissue and the subendocardial layer that connects the endocardium with myocardium. Internally, about 4/5 of the core, myocardium is of the cardiac muscle fibers that arranged in bundles of one direction while only about 1/5 is lodged by purkinje cardiomyocytes that were arranged in bundles distributing mainly in two areas within the moderator band; the subendocardial layer beneath the subendothelial connective tissue and myocardium in between the cardiac myocytes bundles. Centrally, a medium-sized artery of thick wall and a very distinct internal elastic lamina was observed running at the center of the band myocardium and is surrounded with the cardiac myocytes bundles.

Keywords: Buffalo (*Bos bubalis L.*); Trabecula septomarginalis; Moderator bands; Purkinje cardiomyocytes; Histological; Histochemical

Introduction

Single and branched fibromuscular strand, moderator band was described in the camel right ventricle extending from the interventricular septum to the ventricular free wall at the base of the papillary muscle, passing through the ventricular cavity and appeared fleshy and muscular in its consistency [1-3].

In other works, Lorenz and Hunigen [4], Lorenz [5], Lorenz and Guski [6], Crick et al. [7] and Rocha et al. [8] have shown that the muscle bundle in the septomarginal trabecula of pig hearts is generally a resistant crest that goes from the lower part of the septum to the lower part of the anterior papillary muscle.

Depreux et al. [9] described that the size and shape of the septomarginal trabecula depend on the size of the heart and the age of the animal. Moreover, Hsu and Du [10] assumed that the different morphological variations of the moderator bands have been described as congenital heart anomalies in the porcine right ventricle (including two parallel bands merging in the middle part or a band that does not cross the ventricular cavity, but connects the parietal papillary muscle with a deformed trabecula).

Bojsen-Moller and Tranum-Jensen [11] claimed that the right bundle branch of the conducting system of the heart passes down the interventricular septum and continues by way of the septomarginal trabecula to the base of the anterior papillary muscle. It was shown that when this trabecula contained muscle fibres, a Purkinje fibre group was located only at the periphery of the band section. Conductive cells were located in the central part in bands, which did not contain muscle fibres.

Conductive tissue in these moderator bands was represented by clumps of Purkinje cells, surrounded by myocardial fibres. The proportion of conductive cells and muscle fibres was approximately the same. Slight vascularization was observed in these moderator bands [12].

Gulyaeva and Roshchevskaya [12] described that the muscle fibres form the largest part of the moderator band section in the pig's right ventricle and conductive cells are few in number, so the main function of the band is not to conduct electric impulses but rather to prevent excessive ventricular dilatation during diastole through muscle fibers tension. Furthermore, Okamoto et al. [13] clarified that the moderator bands primarily provide quick transmission of electric impulses to the parietal wall and prevent excessive dilatation of the ventricles during diastole.

The aim of our investigation is to through more light on the histological and histochemical aspects of the buffalo moderator bands with special reference to the structure and distribution of the myocardiocytes; cardiac muscle fibers and purkinje cardiomyocytes.

Materials and Method

Hearts of ten apparently healthy mature Buffalo were collected

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from Zagazig slaughter house in Sharkia province, Egypt for histological studies. The collected bands 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, periodic acid schiff technique (PAS) for detection of neutral mucopolysaccharides, Alcian blue at pH (2.5) for detection of acidic mucopolysaccharides, Masson's trichrome (blue) stain for demonstration of collagen fibers and muscle cells cytoplasm, Weigert's Resorcin Fuchsin stain for demonstration of the elastic fibers, Toludine blue stain for detection of the mast cells and metachromatic substance [14] and Jone's methenamine silver stain for demonstration of the reticular fibers [15,16]. The microphotography were taken using a digital Dsc-W 130 super steady cyper shot camera connected to an Olympus BX 21 light microscope.

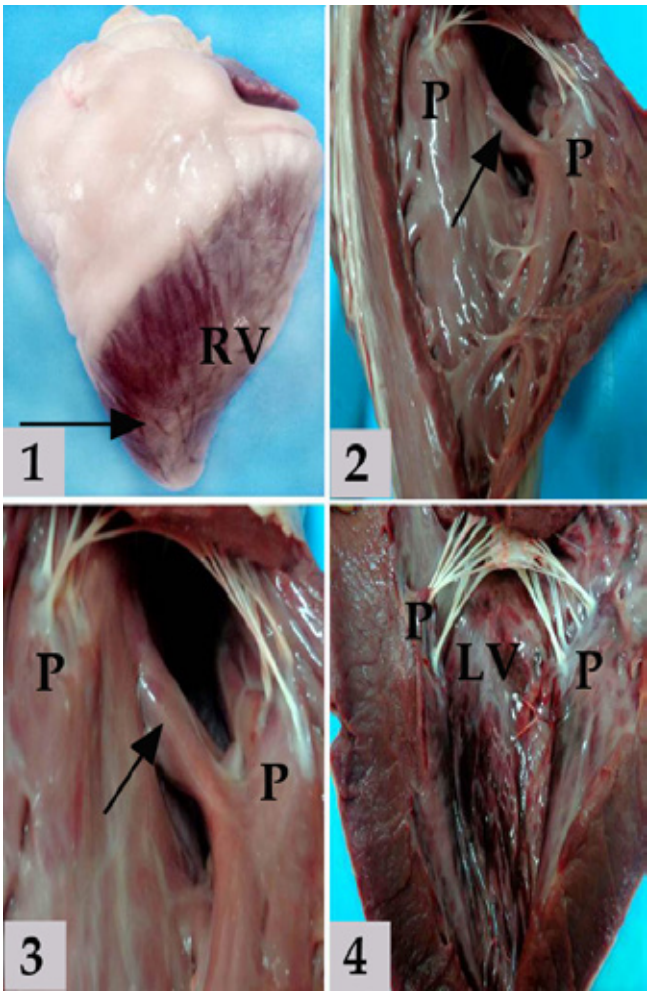

Figure 1: A photograph of the buffalo heart showing the right ventricle (RV) and left ventricle (arrow).
Figure 2: A photograph of the opened right ventricle of the buffalo heart showing the moderator band (arrow) and papillary muscles (P).
Figure 3: Higher magnification of Figure 2 showing the moderator band (arrow) and papillary muscles (P).
Figure 4: A photograph of the opened left ventricle of the buffalo heart (LV) showing the presence of papillary muscles (P) and devoid of any moderator band.

Results

Anatomically, the moderator band (Septomarginal trabeculae) is a single muscular band that is found only in the right ventricle of the buffalo heart. The right ventricle has one muscular moderator band which extends from the interventricular septum to the opposite ventricular wall especially at the papillary muscle, crossing and passing through the ventricular cavity and appearing as a fleshy and muscular in its consistency (Figure 1-3). Moreover, the left ventricle is observed devoid of any moderator band (Figure 4). The function of the moderator band is to provide quick transmission of electric impulses to the parietal wall and prevent excessive dilatation of the ventricles during diastole.

Histologically, the moderator band is observed consisting of two major compartments; the central (core) is myocardium and the peripheral is endocardium which acts as the moderator band capsule (Figure 5).

The endocardium is considered the outer most layer; a capsule that is completely surrounding and covering the band from all directions. It consists of three layers; the endothelial layer, subendothelial layer and subendocardial layer. From outside directing to the inside, the moderator band is completely covered externally by a single layer of simple squamous epithelium (endothelium) and sometimes with simple cuboidal epithelium. This endothelium is considered the outer most layer of the endocardium that is reflected from the ventricular endothelium (Figures 6 and 7).

The subendothelial layer is the second layer of the endocardium that supports the endothelium and consists of narrow compact zone of loose connective tissue (Figure 6 and 7) that is mainly composed of collagen fibers (Figure 9) and reticular fibers (Figure 10). It showed strongly PAS positive reaction (Figure 5).

The subendocardial layer is the deep layer of the endocardium that connects the endocardium with the myocardium (Figures 5, 6 and 9). It is composed mainly of loose connective tissue that housing blood vessels, nerve fascicles and some individual adipocytes (Figure 8). It showed PAS positive reaction (Figure 5). Moreover, it has bundles of modified cardiomyocytes, purkinje fibers with different size and shape that arranged in spherical manner (Figures 11 and 18) or longitudinal one (Figure 21 and 22).

The moderator band purkinje cardiomyocytes are observed large round and sometimes irregular polygonal in shape. Its cytoplasm is appeared vacuolated and pale as it contains very fewer myofibrils that arranged peripherally. It has single, central, large nucleus and sometimes binucleated with prominent nucleoli. The nuclear chromatin is dispersed and in most cases is tended to be condensed peripherally. Sometimes in some sections, these cells are observed non-nucleated (Figures 12, 13, 15 and 16).

The purkinje bundle is completely surrounded with highly vascularized loose connective tissue from different direction. It is covered externally with subendothelial loose connective tissue and is laterally separated from the surrounding myocardium by another one (Figures 11, 13 and 14) that is mainly consisting of collagen fibers (Figures 18-20) and rich with bundles of nerve fascicles that are distributed beside the purkinje bundle (Figures 14 and 17) and also in between the purkinje cardiomyocytes (Figure 15). Moreover, the purkinje cardiomyocytes are laterally separated from each other by a considerable amount of highly vascularized loose connective tissue (Figures 12 and 15) that is mainly consisted of collagen fibers (Figure 19).

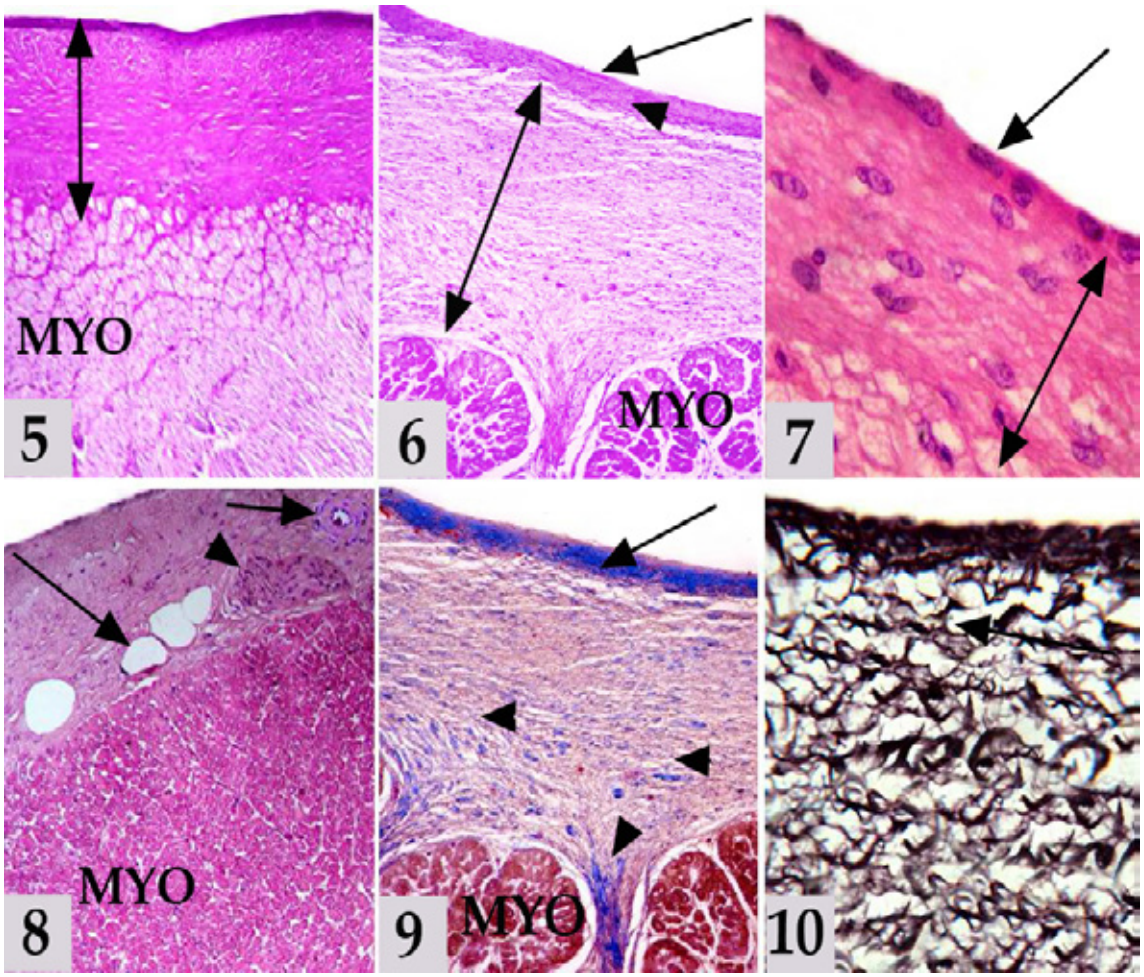

Figure 5: A photomicrograph of the moderator band showing the endocardium (double arrow heads) and myocardium (MYO). Stain: **PAS** Obj.x10: Oc.x10.
Figure 6: A photomicrograph of the moderator band endocardium showing the endothelium (arrow), subendothelium (arrow head), subendocardium (double arrow heads) and myocardium (MYO). Stain: **H& E** Obj.x10: Oc.x10.
Figure 7: A photomicrograph showing the endothelium of simple squamous epithelium (arrow), subendothelial loose connective tissue (double arrow head). Stain: **H& E** Obj.x40: Oc.x10.
Figure 8: A photomicrograph showing the subendocardial blood vessel (short arrow), nerve fascicles (arrow head), adipocytes (long arrow) and myocardium (MYO). Stain: **H& E** Obj.x10: Oc.x10.
Figure 9: A photomicrograph showing the subendothelial collagen fibers (arrow) and sub-endocardial collagen fibers (arrow head), and myocardium (MYO). Stain: **Blue Masson's trichrome** Obj.x10: Oc.x10.
Figure 10: A photomicrograph showing the distribution of the reticular fibers within the endocardium (arrow). Stain: **Jone's methenamine silver** Obj.x40: Oc.x10.

The central layer, core is considered the normal myocardial layer. It consists mainly of contractile cardiac muscle fibers that are arranged longitudinally in one direction from the myocardium of the interventricular septum to the moderator band and then to the myocardium of the ventricular wall, forming groups of longitudinal cardiac muscle bundles (Figures 23, 24, 30 and 31).

The longitudinal bundles of cardiac muscle cells are laterally separated from each other by a considerable amount of highly vascularized loose connective tissue that is rich in a dense capillary network, lymph vessels, and autonomic nerve fibers (Figures 23, 24, 26 and 27). And also, it is composed mainly of collagen fibers (Figure 26) elastic fibers (Figure 27) and reticular fibers that are mainly condensed around the wall of blood vessels (Figures 28 and 29). Wherever, groups of fibroblasts were present and surrounded with bundles of fibers with different directions (Figure 25).

The intercellular connective tissue is few where the myocardiocytes in the moderator band is numerous, large sized and also much closed to each other and appeared overcrowded (Figures 23, 24, 26, 30 and 31). Most of these cells are elongated, branched and connected with each other, forming some sort of network-like structure (Figures 30, 32 and 33).

The cardiac muscle cells in longitudinal sections of the moderator bands are appeared long, striated (Figure 32) branched and anastomosed, forming network and joined end to end and side to side at intercalated disks, forming the myocardial fibers (Figure 33). 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, 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

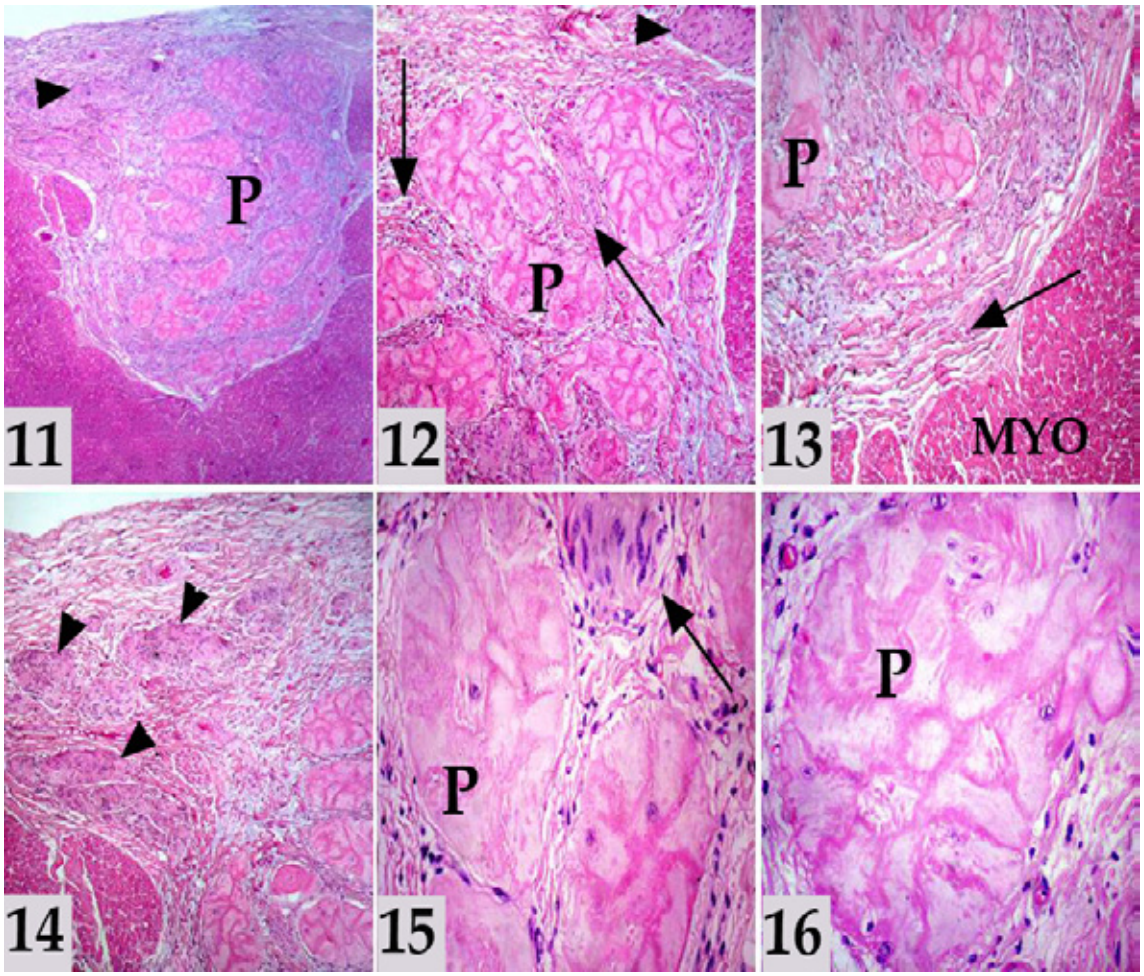

Figure 11: A photomicrograph showing the subendocardial loose connective tissue (arrow head) and clump of purkinje cardiomyocytes (P). Stain: **H& E** Obj.x4: Oc.x10.
Figure 12: Higher magnification of figure 11 showing the purkinje cardiomyocytes (P), inter purkinje cardiomyocytes loose connective tissue (arrow) and nerve fascicles (arrow head). Stain: **H& E** Obj.x10: Oc.x10.
Figure 13: A photomicrograph showing the purkinje cardiomyocytes (P), and loose connective tissue separating between subendocardial purkinje bundle and myocardium (arrow) and myocardium (MYO).Stain: **H& E** Obj.x10: Oc.x10.
Figure 14: A photomicrograph showing numerous nerve fascicles distributed in the subendocardial connective tissue beside the purkinje bundle (arrow head). Stain: **H& E** Obj.x10: Oc.x10.
Figure 15: A photomicrograph showing the purkinje cardiomyocytes (P) and nerve fascicle distributed in between the purkinje cells (arrow). Stain: **H& E** Obj.x40: Oc.x10.
Figure 16: A photomicrograph showing the purkinje cardiomyocytes (P). Stain: **H& E** Obj.x40: Oc.x10.

to be condensed peripherally (Figures 32 and 33). The cardiac muscle sarcoplasm is an eosinophilic, full of parallel contractile myofibrils that are consisting of myofilaments. They exhibit a cross-striated banding pattern. The sarcoplasm shows its characteristic striations due to alternating dark and light bands. The dark band is the A- band while, the light band is the I-band (Figure 32). Sometimes, a clear zone of perinuclear sarcoplasm, free of myofibrils, may be seen in some sections. Moreover, in semi thin sections stained with toluidine blue, fine granules were seen inside the cardiac myocytes. They were aggregated around the nucleus, being more localized along its two poles and sometimes localized in one pole. These granules are stained darkly, but did not show metachromasia (Figure 33). Furthermore, the myocardiocytes showed a relatively PAS positive reaction (Figure 35), but Alcian blue negative reaction.

Meanwhile, in cross sections, the cardiac muscle fibers appear irregular polygonal cells of various sizes with a large, round, pale-staining, euchromatic, centrally placed, single or binucleated nucleus and also with eosinophilic sarcoplasm (Figure 34).

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 moderator band (Figures 36-38). These purkinje cells are observed round, larger in size than the working cardiac myocytes, showing variety in number and size. Its cytoplasm is pale as the myofibrils are few, arranged peripherally and showed slightly striations of alternating dark bands with light bands. It has single, central, large nucleus and sometimes binucleated with prominent nucleoli. The nuclear chromatin is dispersed and in most cases tends to be condensed peripherally. However, some non-nucleated cells are also observed (Figures 39-41). Moreover, with the PAS reaction, these cells showed strongly PAS positive reaction (Figure 40). Moreover, the purkinje bundles are laterally separated from the surrounding cardiomyocytes bundles by a considerable amount of

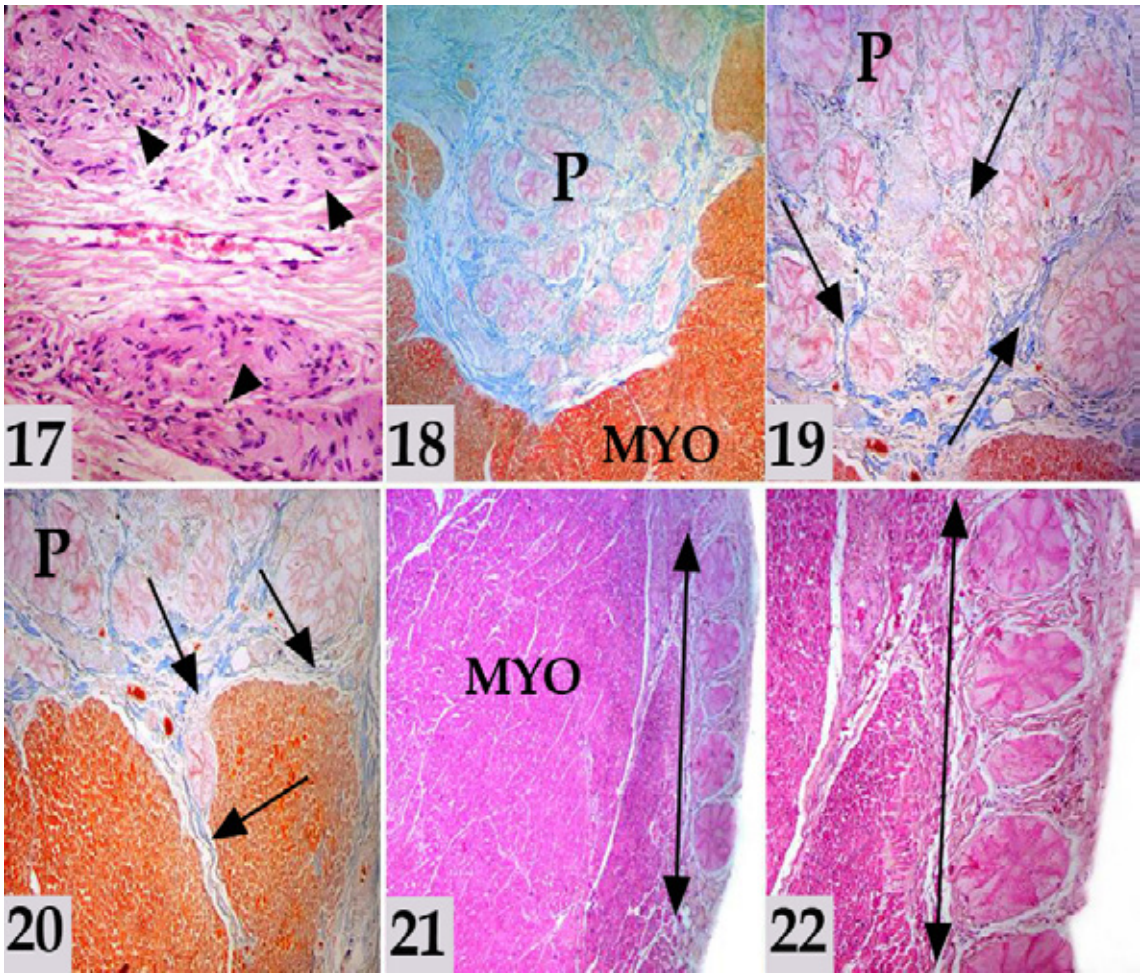

Figure 17: A photomicrograph showing the highly vascularized subendocardial loose connective tissue having numerous nerve fascicles (arrow head). Stain: **H& E** Obj.x40: Oc.x10.
Figure 18: A photomicrograph showing the subendocardial purkinje cardiomyocytes bundle (P) and myocardium (MYO). Stain: **Blue Masson's trichrome** Obj.x4: Oc.x10.
Figure 19: A photomicrograph showing the distribution of the collagen fibers in between the purkinje cardiomyocytes (arrow) and purkinje cardiomyocytes (P). Stain: **Blue Masson's trichrome** Obj.x10: Oc.x10.
Figure 20: A photomicrograph showing the collagen fibers surrounding the purkinje bundle and separate the later about the myocardium (arrow) and purkinje cardiomyocytes (P). Stain: **Blue Masson's trichrome** Obj.x10: Oc.x10.
Figure 21: A photomicrograph showing the subendocardial purkinje cardiomyocytes arranged in one row (double arrow head) and myocardium (MYO). Stain: **H& E** Obj.x4: Oc.x10.
Figure 22: Higher magnification of figure. 21 showing the same. Stain: **H& E** Obj.x10: Oc.x10.

highly vascularized loose connective tissue that is mainly composed of collagen fibers (Figures 37 and 38).

A medium-sized artery is observed running at the center of the band myocardium and is surrounded with the cardiac myocytes bundles. The wall of the artery is thick and characterized by a very distinct internal elastic lamina as well as well-marked intimal cushions and a thick tunica media of smooth muscle fibers and a wide tunica adventitia that is mainly composed of highly vascularized loosed connective tissue housing many blood vessels, lymph vessels, nerve fascicles and broad layer of adipocytes (Figures 42-44).

Discussion

The present investigation clarified that the moderator band (Septomarginal trabeculae) of the buffalo heart is a single muscular band that extends from the interventricular septum to the opposite right ventricular wall especially at the papillary muscle, crossing and passing through the ventricular cavity and appearing as a fleshy and muscular in its consistency. This finding is in coincidence with [2] in camel who described that the moderator bands are fibromuscular structures crossing the ventricular cavity with being attached to the base of the papillary muscles.

The wall of the right ventricle has one muscular band. This finding is goes hand in hand with [17,18] in domestic animals, [4-6,12] in pigs and [19] in ostrich. Meanwhile, the left ventricle is observed devoid of any muscular band. This investigation is in agreement with [3] in camel.

The moderator band is consisted of two major compartments; the central (core) is myocardium and the external (peripheral) is endocardium which acts as the moderator band capsule. These results are in close agreement with the findings in adult goat (*Capra hircus*) where the moderator bands are encased in a dense capsule of

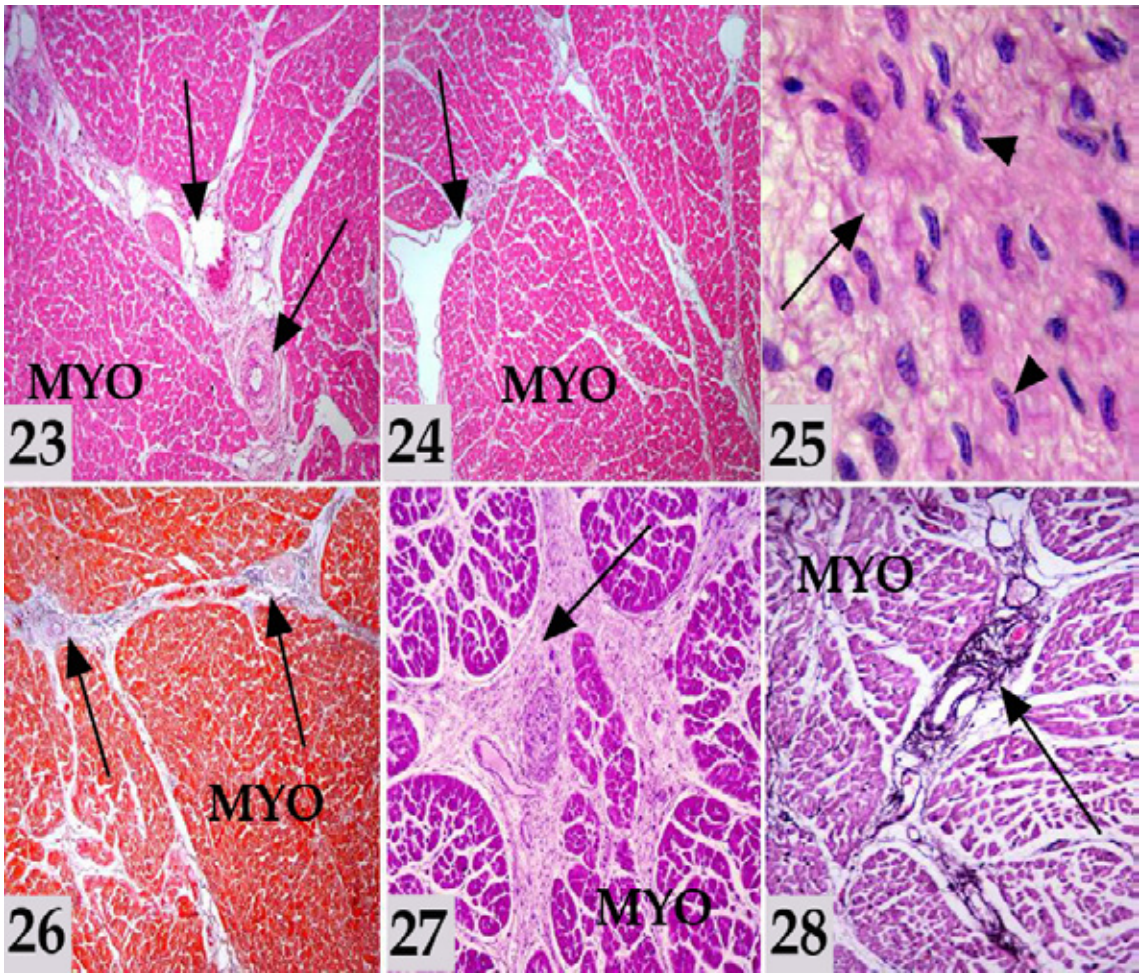

Figure 23: A photomicrograph of the band myocardium showing the highly vascularized loose connective tissue in between the cardiomyocytes bundle (arrow) and myocardium (MYO). Stain: **H& E** Obj.x10: Oc.x10.
Figure 24: A photomicrograph showing the inter cardiomyocytes bundles loose connective tissue. Stain: **H& E** Obj.x10: Oc.x10.
Figure 25: A photomicrograph of the inter cardiomyocytes bundles loose connective tissue showing the connective tissue fibers (arrow) and connective tissue cells (arrow head). Stain: **H& E** Obj.x40: Oc.x10.
Figure 26: A photomicrograph of the inter cardiomyocytes bundles loose connective tissue showing the collagen fibers (arrow) and myocardium (MYO). Stain: **Blue Masson's trichrome** Obj.x10: Oc.x10.
Figure 27: A photomicrograph of the inter cardiomyocytes bundles loose connective tissue showing the elastic fibers (arrow) and myocardium (MYO). Stain: **Weigert's Resorcin Fuchsin** Obj.x10: Oc.x10.
Figure 28: A photomicrograph of the inter cardiomyocytes bundles loose connective tissue showing the reticular fibers (arrow) and myocardium (MYO). Stain: **Jone's methenamine silver** Obj.x10: Oc.x10.

connective tissue rich in elastic and reticular fibers [12]. Internally, the major part of the core was of the cardiac muscle fibers while the other small part is clump of purkinje fibers. Moreover, these findings are in close agreement with [19] in ostrich who reported that the moderator bands are consist of longitudinal muscle fibers in central with purkinje cells in peripheral and are covered by endocardium. In some area, the myocardium and purkinje cells are mixed.

The endocardium consists of three layers; the endothelial, subendothelial and subendocardial layers. From outside directing to the inside, the moderator band is completely covered externally by a single layer of simple squamous epithelium (endothelium). This endothelium is considered the outer most layer of the endocardium that is reflected from the ventricular endothelium and is supported by a subendothelial loose connective tissue. This finding is in close agreement with [19,20] in ostrich and [21] in horses, who reported that the endocardium was thick and consisted of an outer endothelial layer situated over a fibro-elastic thick layer of subendothelium that composed of collagen, elastic and a few reticular fibers.

The subendocardial layer is the deep layer of the endocardium that connects the endocardium with the myocardium. It is composed of loose connective tissue that housing many blood vessels, lymph vessels, nerves that supply the band with clump of modified cardiac muscle cells; purkinje cardiomyocytes with different size and shape. These findings are in parallelism with [19] in ostrich and [21] in horses who clarified that the subendocardial connective tissue layer was loose and consisted of blood vessels and Purkinje fibers at some places. Moreover, this investigation is in agreement with the findings of [22] in human beings, [23] in domestic animals and [20] in adult goat (*Capra hircus*) who claimed that clump of purkinje fibers were apparently vacuolated and situated deep to the subendothelial layer.

The moderator band purkinje cardiomyocytes are observed round,

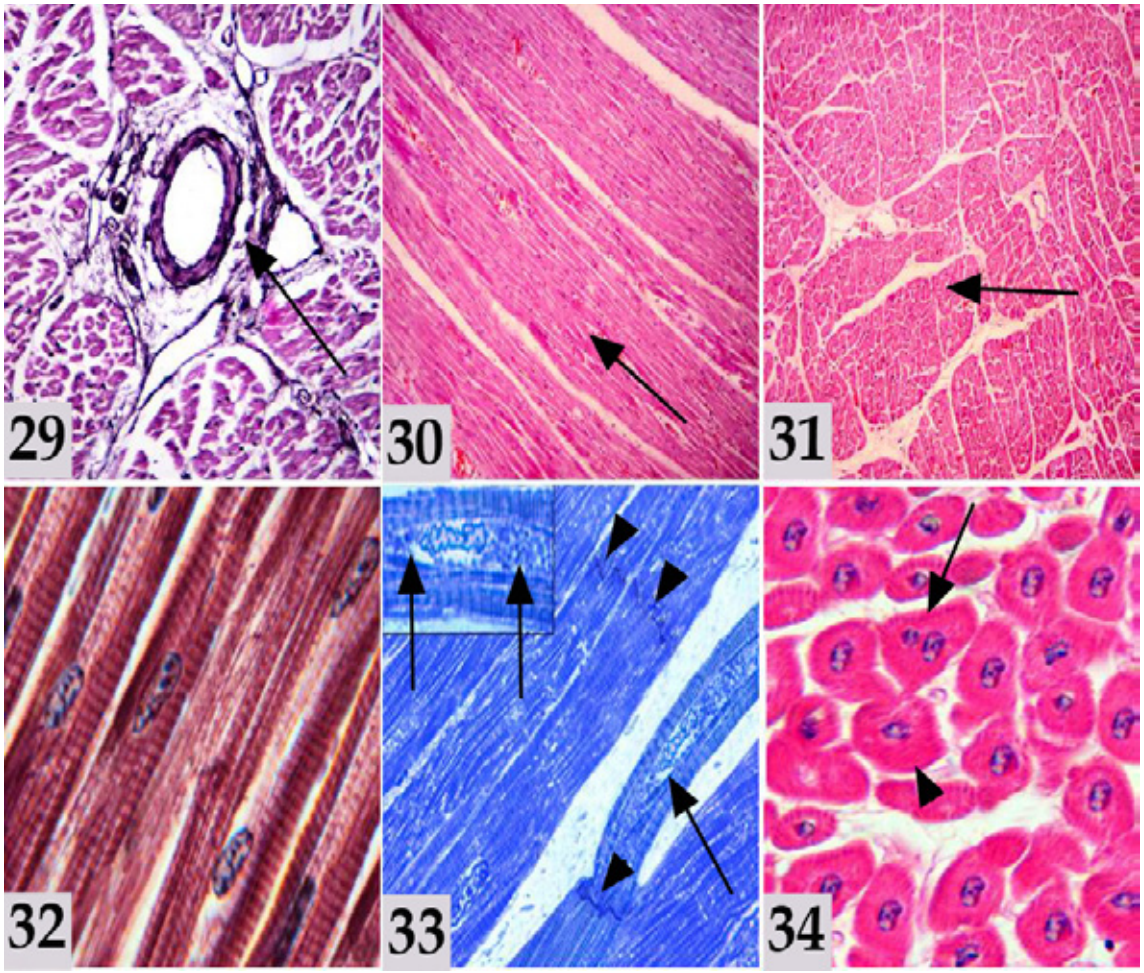

Figure 29: A photomicrograph of the inter cardiomyocytes bundles loose connective tissue showing the reticular fibers that condensed around the wall of the blood vessels (arrow). Stain: **Jone's methenamine silver** Obj.x10: Oc.x10.
Figure 30: A photomicrograph showing the one direction of the cardiomyocytes bundles of the moderator band, longitudinal section (arrow). Stain: **H& E** Obj.x20: Oc.x10.
Figure 31: A photomicrograph showing the one direction of the cardiomyocytes bundles of the moderator band, cross section (arrow). Stain: **H& E** Obj.x20: Oc.x10.
Figure 32: A photomicrograph showing the longitudinal section of the cardiomyocytes and the cross striation banding pattern of alternating dark band with light band. Stain: **Blue Masson's trichrome** Obj.x100: Oc.x10.
Figure 33: A photomicrograph showing fine secretory granules in the perinuclear region and euchromatic nucleus (arrow) and intercalated discs (arrow head). Stain: **Toluidine blue** Obj.x40: Oc.x10.
Figure 34: A photomicrograph showing the cross section of the cardiomyocytes (arrow head) and binucleated cardiomyocytes (arrow). Stain: **H& E** Obj.x40: Oc.x10.

larger in size than the perinuclear clear zone cells and also larger than the cardiac myocytes. Its cytoplasm is pale as it contains very fewer myofibrils 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. Sometimes in some sections, these cells are observed to be none nucleated. This finding is in close agreement with [19] in ostrich and [21] in horses who clarified that the Purkinje fibres were dispersed around the periphery with a vacuolated appearance in the center of the cell. The nuclei were round to oval and centrally placed, surrounded by a vacuolated surface. And also, this is in agreement with the findings of [22] who recorded that the vacuolated space was occupied by the glycogen.

The central layer of the moderator band is considered the normal myocardial layer where it 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 the statement of [17,18] in domestic animals and [19] 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 relaxation (diastole) 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 pariet alwall. Moreover, this investigation is goes hand in hand with [24,25] in human who demonstrated that in human, the moderator band extends between interventricular septum and ventricular free wall.

The cardiac myocytes in longitudinal sections appeared long,

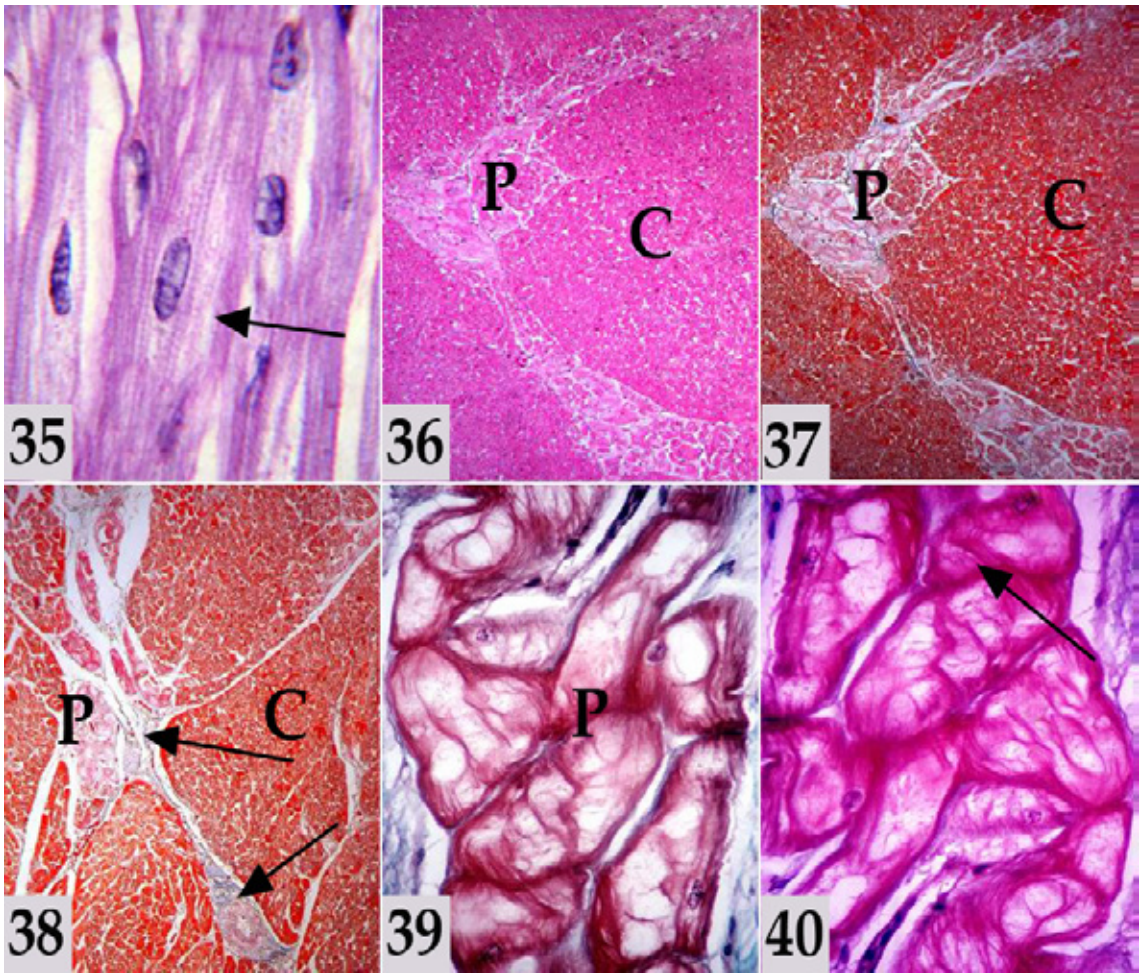

Figure 35: A photomicrograph showing the moderately PAS positive reaction of the cardiomyocytes with slightly cross striation banding pattern. Stain: **PAS** Obj.x100: Oc.x10.
Figure 36: A photomicrograph of the moderator band myocardium showing the purkinje cardiomyocytes bundles (P) and cardiac muscle bundles (C). Stain: **H& E** Obj. x10: Oc.x10.
Figure 37: A photomicrograph of the moderator band myocardium showing the purkinje cardiomyocytes bundles (P) and cardiac muscle bundles (C). Stain: **Blue Masson's trichrome** Obj.x10: Oc.x10.
Figure 38: A photomicrograph showing the purkinje cardiomyocytes bundles (P), cardiac muscle bundles (C) and inter bundles collagen fibers (arrow). Stain: **Blue Masson's trichrome** Obj.x10: Oc.x10.
Figure 39: A photomicrograph showing the myocardium purkinje cardiomyocytes bundles (P). Stain: **Blue Masson's trichrome** Obj.x40: Oc.x10.
Figure 40: A photomicrograph showing the strongly PAS positive reaction of the purkinje cardiomyocytes (arrow). Stain: **PAS** Obj.x40: Oc.x10.

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, 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. This result is very close and similar to the finding that described by [26] in albino rats and [27] in camel. Meanwhile, in the cross sections, the cardiac myocytes appear irregular polygonal cells of various sizes with a large, round, pale-staining, euchromatic, centrally placed, single nucleus and also sometimes, binucleated cells. This result is very close and similar to the finding that described after [2,3,27] in camel.

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**.** This result is very close and similar to the finding that described after [3,27] in camel.

In semi thin sections stained with toluidine blue, fine granules were seen inside the cardiac myocytes. They were aggregated around the nucleus, being more localized along its two poles and sometimes localized in one pole. These granules are stained darkly, but did not show metachromasia. Furthermore, the myocardiocytes showed a relatively PAS positive reaction, but Alcian blue negative reaction. These investigations are very close and similar to the finding that described after [3,27] in camel atrial cardiomyocytes that indicating the endocrine secretory nature of the cardiac myocytes.

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

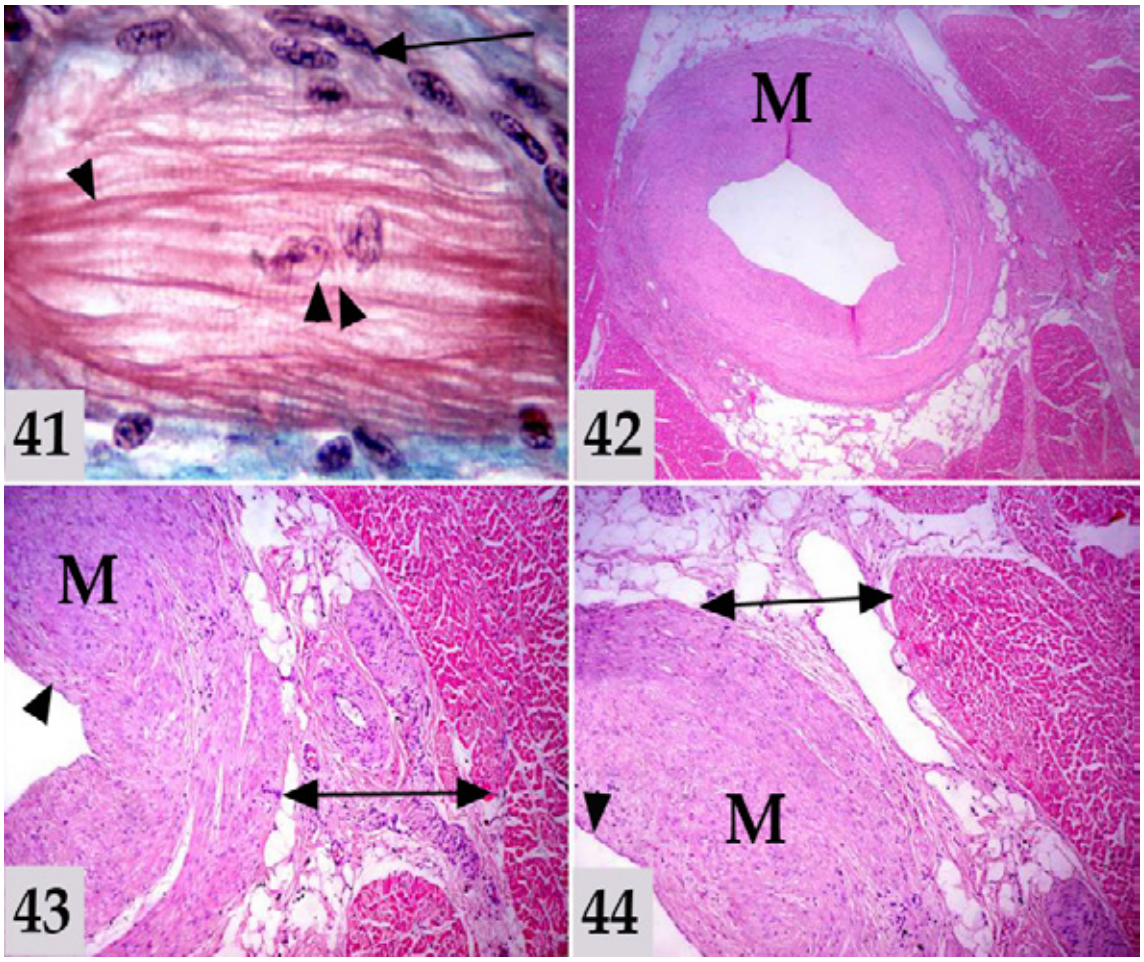

Figure 41: A photomicrograph showing the purkinje cardiomyocyte with central double nucleus (double arrow head) and peripheral few myofibrils with slightly striation (single arrow head) and surrounding connective tissue cells, fibroblasts (arrow). Stain: **Blue Masson's trichrome** Obj.x100: Oc.x10.
Figure 42: A photomicrograph showing a medium sized artery running at the center of the band myocardium (M). Stain: **H& E** Obj.x4: Oc.x10.
Figure 43 and 44: A photomicrograph of the medium sized artery showing the tunica intima (arrow head), tunica media (M) and tunica adventitia (double arrow head). Stain: **H& E** Obj.x10: Oc.x10.

moderator band. This result is in parallelism with [20] in adult goat (*Capra hircus*) and [19] in ostrich, who assumed that beside the longitudinal cardiac muscle bundles and 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.

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 myocardiocytes 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. Moreover, with the PAS reaction, these cells showed strongly PAS positive reaction where some PAS positive granules were identified, especially within the central pale cytoplasmic areas. This finding is in close agreement with [27] in camel and [21] in horses who reported that the ordinary myocardial cells were smaller than the Purkinje fibers. They showed striations and had centrally placed round or oval nuclei.

The function of the moderator band is to prevent over distention and dilatation of the right ventricle during diastole as the contractile muscle fibers represent the major part of the band. And also, provide rapid transmission of electric impulses to the parietal ventricular wall as it has purkinje cardiomyocytes that is considered one of the impulse conducting system of the heart. These results are in parallelism with the finding of [13] in human who clarified that the moderator bands primarily provide quick transmission of electric impulses to the parietal wall and prevent excessive dilatation of the ventricles during diastole. And also, goes hand in hand with [19] in ostrich and [12] in pigs who described that the muscle fibers form the largest part of the moderator band section in the pig's right ventricle and conductive cells are few in number, so the main function of the band is not to conduct electric impulses but rather to prevent excessive ventricular dilatation during diastole through muscle fibers tension.

A medium-sized artery is observed running at the center of the band myocardium and is surrounded with the cardiac myocytes bundles. The wall of the artery is thick and characterized by a very distinct internal elastic lamina as well as well-marked intimal cushions and a thick tunica media of smooth muscle fibers and a wide tunica adventitia that is mainly composed of highly vascularized loosed connective tissue housing many blood vessels, lymph vessels, nerve fascicles and broad

layer of adipocytes. These results are in completely agreement with the findings of [20] in adult goat (*Capra hircus*) who claimed that inside the moderator band a medium-sized artery with distinct internal elastic lamina and small nerve fascicles were observed.

Conclusion

The present investigation concluded that the moderator band is a single fibromuscular band and is found only in the right ventricle of the buffalo heart. It extends in one direction from the interventricular septum to the free ventricular wall, crossing the ventricular cavity. The moderator band is covered externally by the endocardium that is mainly consisting of the endothelial layer of simple squamous epithelium, subendothelial layer of loose connective tissue and the subendocardial layer that connects the endocardium with myocardium. Internally, about 4/5 of the myocardium is of the cardiac muscle fibers that arranged in bundles of one direction while only about 1/5 is lodged by purkinje cardiomyocytes bundles. Moreover, a medium-sized artery of thick wall and a very distinct internal elastic lamina is observed running at the center of the band myocardium and is surrounded with the cardiac myocytes bundles.

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