



Histological Study on Prenatal Purkinje Fibres (PF) Development in Dromedary Camel

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ABSTRACT

The development of the Purkinje fibre (PF) of the camel foetus was studied in dromedary camel foetuses during the three gestational periods. A total of 30 hearts were used, the hearts were collected from Tamboul and Al-Salam slaughterhouses, Sudan. The samples were prepared by routine histological procedures and stained by the general histological stain (H&E) and special stains. In the first trimester PF were embedded in cardiac muscle fibres close to the ventricular endocardium. In second trimester, some of PF were embedded in the connective tissue, some fibres contained two nuclei. The striation was clear in peripheral parts of some fibres. At 178 second trimester days of gestation most of fibres contained two nuclei and the cytoplasm around the nuclei was light. At the third trimester PF appeared as bundles of fibres parallel to myocardial muscles. They were also embedded in the endocardial connective tissue as groups or separate fibre. They were embedded in the myocardium in the first trimester and either between the endocardium and myocardium or within the myocardium in the second and third trimesters. It was concluded that most of Purkinje fibres were not found in their normal location till the last stages of pregnancy, which means that PF continue their developmental changes after birth.

Keywords: Purkinje fibre, Heart, development, dromedary camel

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INTRODUCTION

Development of purkinje fibres was studied by Canale, *et al.* (1987) sheep and Miquerol, *et al.* (2010), in mammals. In the camel foetus the development of purkinje fibres were lacking in the literature, hence this study was undertaken.

MATERIAL AND METHODS

Thirty hearts of camel fetuses obtained from Al-Salam and Tamboul slaughter houses, Sudan were used in this study.

Depending on the age, the foetuses were divided into three equal groups: first trimester (1-130 days), second trimester (131-260 days) and third trimester (261 days- birth). The age of foetus was determined by using the equation of crown vertebral-rump length (CVRL) as described by Elwishy *et al.* (1981).

The histological samples which were fixed in 10% buffered formalin included the entire hearts from the first and early second trimesters and one cm

thick specimens from the late second and third trimesters. Specimens were prepared by routine histological procedures (Bancroft and Stevens, 2008). General histological stains (H&E) and some special stains including Van Geison's for collagenous fibres, Verhoff's and Gomori's Aldehyde fuchsin for elastic fibres, Gordon and Sweet for reticular fibres were used.

RESULTS

Purkinje fibres appeared in 18 cm CVRL (115 days of gestation) embedded in cardiac muscle fibres close to the ventricular endocardium. They had a large size and lightly stained cytoplasm with oval eccentric nuclei; these fibres were surrounded by small cells which were eosinophilic and had oval nuclei (Figure 1).

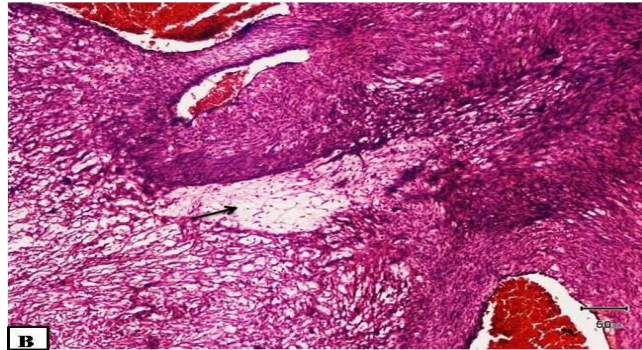


Figure 1: A photomicrograph showing Purkinje fibres (arrow) embedded in myocardium of 18 cm CVRL camel foetus. H&E (X10).

The formation of Purkinje fibres was very clear in the ventricular myocardium in 24.5 cm CVRL (132.5 days of gestation) (Figure 2). The fibres had different sizes and shapes and very dark cytoplasm laterally with

large empty vacuoles in the centre. They had central, spherical nuclei and some of the fibres contained two nuclei. Some Purkinje fibres were found embedded in the connective tissue (Figure 2).

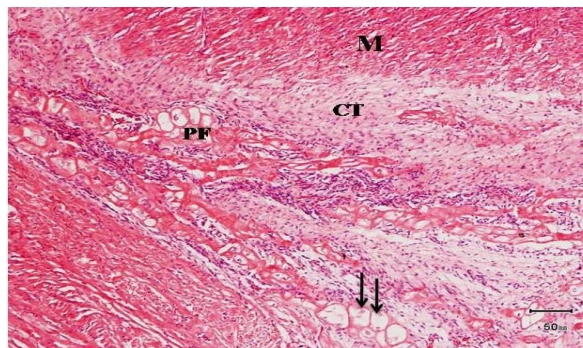


Figure 2: A photomicrograph showing morphogenesis of Purkinje fibres (PF) and cardiac muscles (M) of camel foetus at 24.5 cm CVRL. Purkinje fibres were embedded in a large amount of connective tissue (CT). Note that some of the fibres were binucleated (arrows) H&E (X10)

At the stage of 33 cm CVRL (156 days of gestation) the Purkinje fibres appeared as bundles embedded in the connective tissue with large amounts of collagenous fibres extending from

the epicardium to the myocardium. The fibres were irregular in shape with peripheral nuclei; their cytoplasm was dark peripherally (Figures 3, A and B).

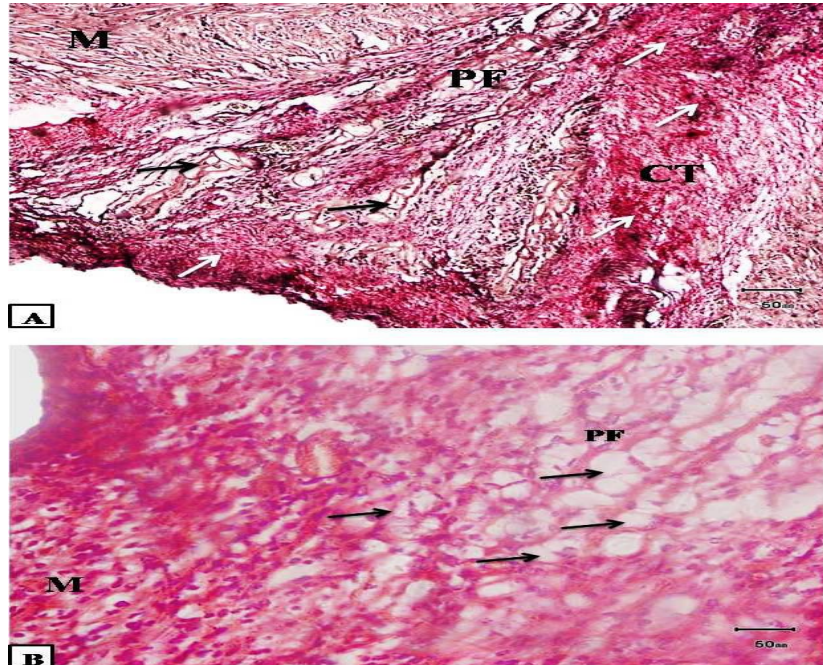


Figure 3: A: A photomicrograph showing morphogenesis of Purkinje fibres (PF), cardiac muscle (M) of camel foetus at 33 cm CVRL. They are embedded in connective tissue (CT) with large amount of collagen fibres (white arrows), irregular PF (black arrows) Van Gieson's. (X10). B: A high magnification of the same section in A showing Purkinje fibres (PF) irregular in shape. H&E (X40).

At the stage of 41 cm CVRL (178 days of gestation) Purkinje fibres appeared as lightly stained fibers embedded in the myocardium. The fibres had light

cytoplasm with spherical and central nuclei. Most of these fibres contained two nuclei and the cytoplasm around the nuclei was light (Figure 4).

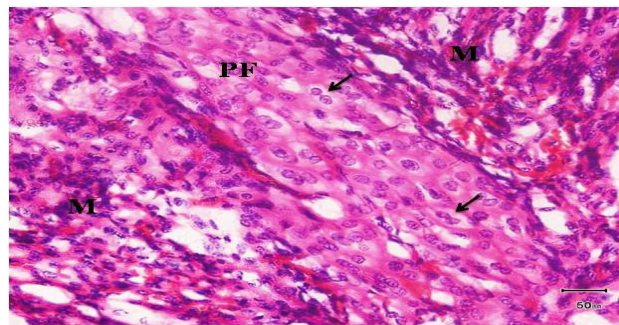


Figure 4: A photomicrograph showing histogenesis of Purkinje fibres (PF) from cardiac muscles (M) in camel foetus at 41 cm CVRL, PF appeared lighter than cardiac muscle with double nuclei (arrows). H&E (X40).

At the stage of 60 cm CVRL (230 days of gestation) Purkinje fibres were embedded in the myocardium as oval or spherical cells. Their cytoplasm was very light centrally and dark peripherally (Figure 5, A). In late stages of second trimester, 71 CVRL

(259.5 days of gestation) Purkinje fibres appeared as bundles of fibres embedded in the connective tissue of the myocardium (Figure 5, B). In some sections at the same stage (71 cm CVRL) Purkinje fibres were found in the subendocardium (Figure 6).

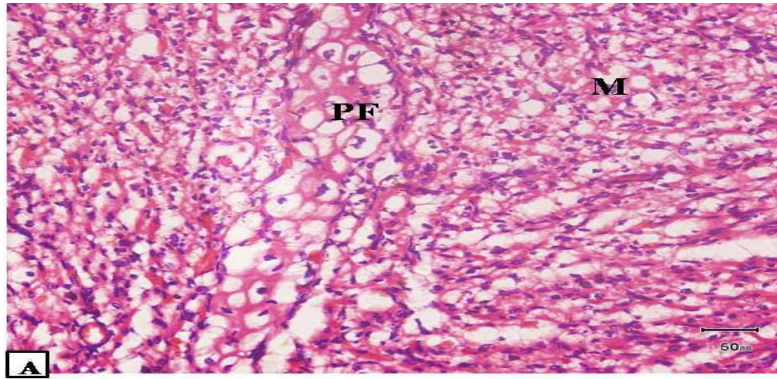


Figure 5.A: A photomicrograph showing Purkinje fibres (PF) embedded in the ventricular myocardium of camel foetus at 60 cm CVRL. H&E (X40).

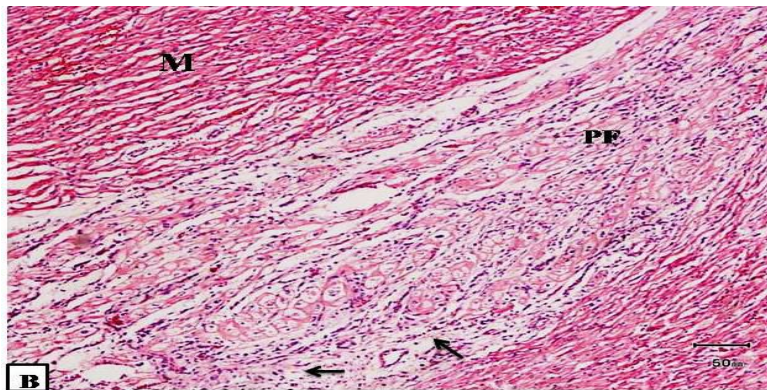


Figure 5. B: Showing (PF) of camel foetus at 71 cm CVRL as bundles embedded in the connective tissue (arrows) separating cardiac muscle from PF. H&E (X10).

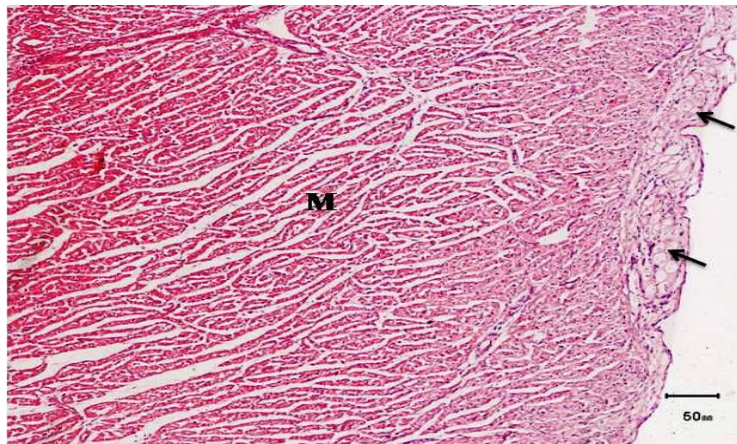


Figure 6: A photomicrograph showing Purkinje fibres (arrows) situated in the subendocardium, connected to the cardiac muscles (M) and covered by endothelium. Camel foetus at 71 cm CVRL. H&E. (X10).

At the early stages of the third trimester as shown at the stage of 74 cm CVRL (268 days of gestation) and 76 cm CVRL (273 days of gestation), Purkinje fibres appeared as oval fibres. They had a light cytoplasm and one or two oval, centrally located nuclei

(Figures 7, A and B). The fibers were found near the epicardium or in-between the myocardial muscles and were either attached to the myocardium or separated from it by connective tissue.

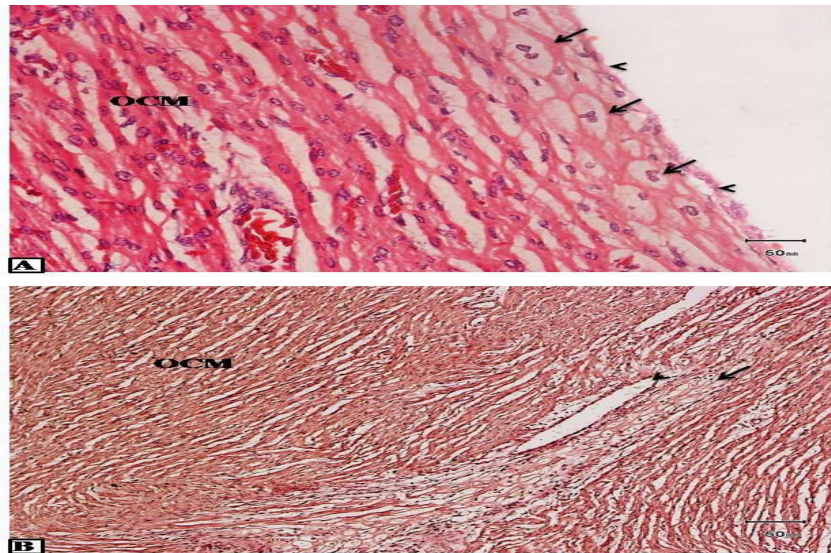


Figure 7.A: A photomicrograph showing Purkinje fibres (arrows) at 74 cm CVRL, adjacent to endothelium (arrowheads) and ordinary cardiac muscle (OCM). Some of the fibers are double nucleated (arrows). H&E (X40).

B: Showing Purkinje fibres (arrow) of the same age. It is accompanied by a blood vessel (arrowhead) and ordinary cardiac muscle (OCM). Van Gieson's (X10).

The fibres were embedded in the connective tissue (Figures 8, A, B and C) in the apical part of the left

ventricle at the stage of 79.5 cm CVRL (283 days of gestation). In this stage Purkinje fibres had dark cytoplasm.

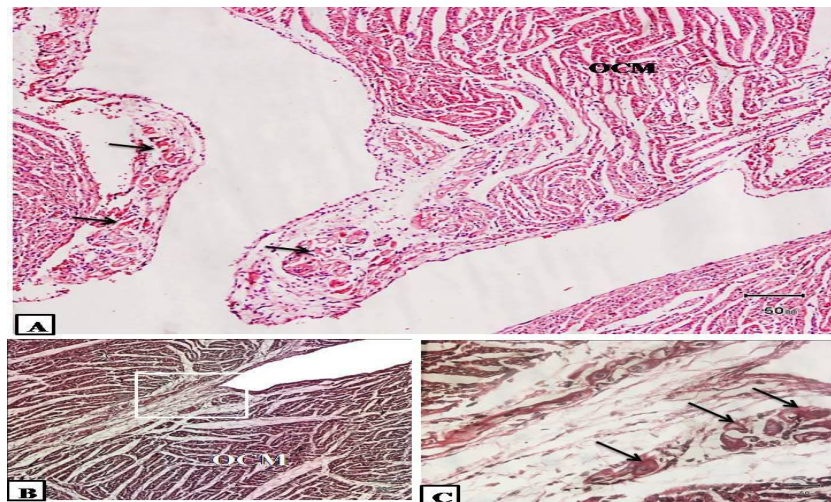


Figure 8.A, B and C: Photomicrographs showing apical part of left ventricle in heart of camel foetus at 79.5 cm CVRL. Purkinje fibres (arrows) embedded in endocardial connective tissue having dark cytoplasm. H&E (X10)

B: Showing the same section stained with Verhoff's (X10).

C: Is a magnification of the rectangle in B showing PF with dark cytoplasm (X40).

At the stage of 88 cm CVRL (306 days of gestation) Purkinje fibres had light cytoplasm compared to that of the cardiac muscle. However, it was darker than that of Purkinje fibres in other

stages (Figure 9, A and B). These Purkinje fibres were found as separate fibres, as small groups of fibres or as bundles embedded in connective tissue.

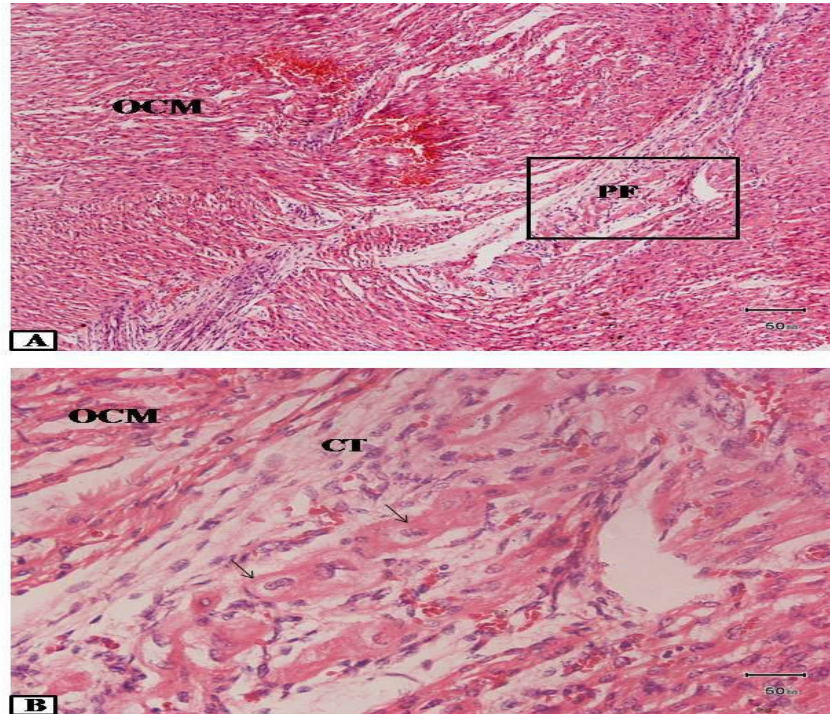


Figure 9.A and B: Photomicrographs showing PF (arrows) of camel foetus heart at 88 cm CVRL having a dark cytoplasm, separated from ordinary cardiac muscle (OCM) with connective tissue (CT). H&E.A (X10).

B: Is a magnification of the rectangle in A showing some of PF having double nuclei (arrows). (X40).

At the stage 89 cm CVRL (309 days of gestation), Purkinje fibres and myocardial muscles appeared as parallel bundles (Figure 10, A). In other sections, they were embedded in connective tissue beside blood vessels

branching in the myocardium and epicardium (Figure 10, B). The fibres which were also observed directly attached to the myocardium, contained one or two nuclei (Figure 10, C).

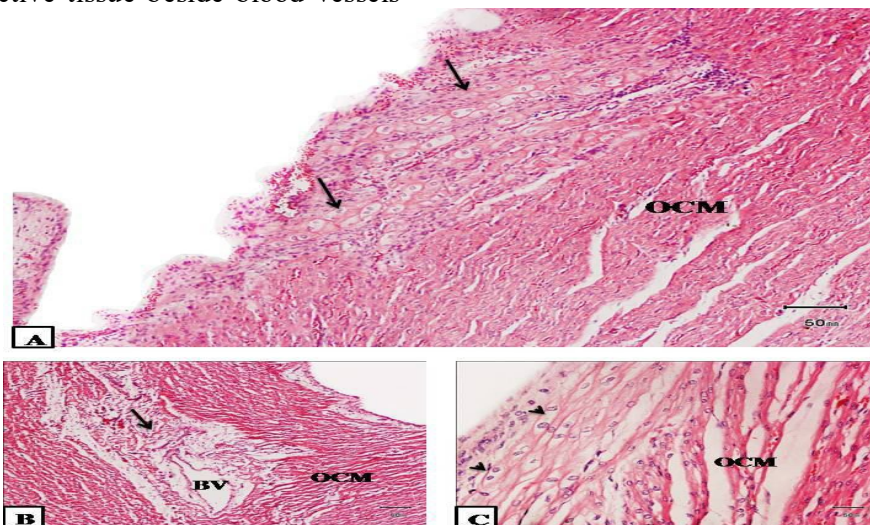


Figure 10. A: A photomicrograph showing PF (arrows) of camel foetus at 89 cm CVRL. They are appearing as bundles parallel to the ordinary cardiac muscles (OCM).

B: Showing the same age: PF beside blood vessels (BV). H&E (X10).

C: Showing PF (arrowheads) adjacent to the OCM having one or two nuclei. H&E (40).

At the stages of 91 cm CVRL (314 days of gestation) and 97 cm CVRL (330.5 days of gestation), Purkinje fibres appeared as bundles of fibres

parallel to myocardial muscles. They were also embedded in the endocardial connective tissue as groups or separate fibres (Figure 11, A and B).

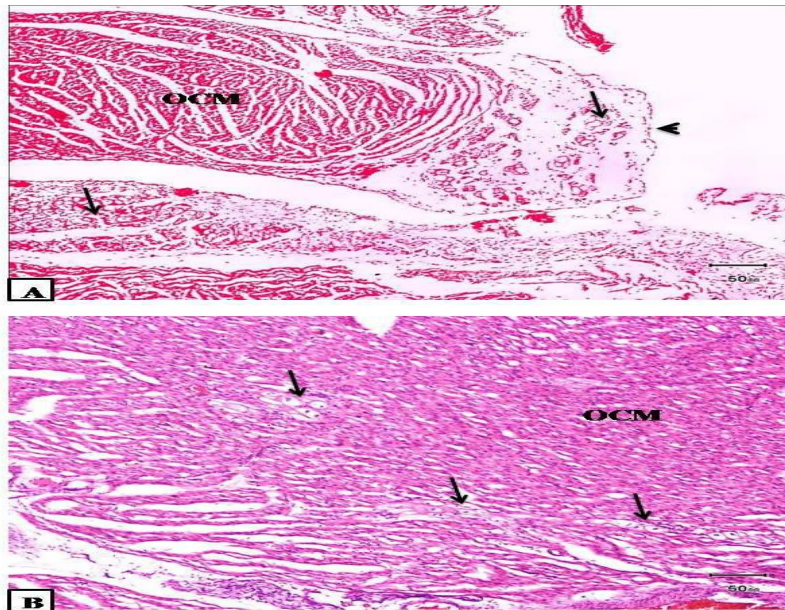


Figure 11.A: A photomicrograph showing Purkinje fibres (arrows) in camel foetus of 91 cm CVRL. They are embedded in the endocardial connective tissue. Endothelium, (arrowhead).

B: Showing PF (arrows) embedded in the ordinary cardiac muscle (OCM). H&E. (X10).

At the late stages of pregnancy, 131 cm CVRL (423.5 days of gestation) Purkinje fibres were also embedded as groups of fibres parallel to myocardial

muscles and in the myocardial connective tissue around a number of blood vessels (Figure 12, A and B).

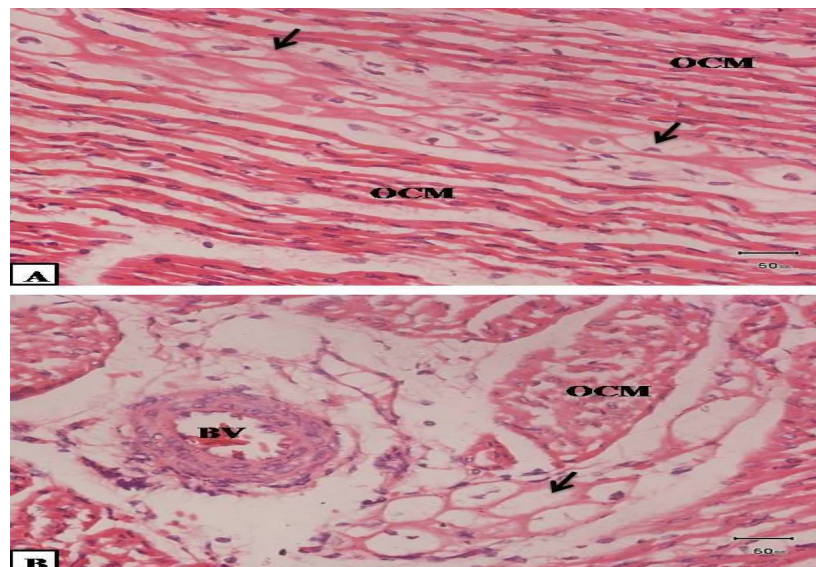


Figure 12.A: A photomicrograph showing Purkinje Fibres (arrows) in camel foetus of 131 cm CVRL. Purkinje fibers parallel to the ordinary cardiac muscle (OCM).

B: PF (arrow) near the blood vessel in the same heart in A. H&E (X40).

DISCUSSION

It is well known that Purkinje fibres are found in the subendocardium. They are larger than cardiac muscle cells, but have light glycogen content, fewer myofibrils and no T-tubules (Eliška, 2006). They are specialized conducting fibres which extend from the interventricular septum, to the papillary muscles and up the lateral walls of the ventricles. In this study, Purkinje fibres appeared to have many morphological shapes and locations during the three gestational periods, they were irregular, oval or spherical in shape, their nuclei single or double with dark or light cytoplasm or very light centrally and dark peripherally.

Differentiation of the ventricular Purkinje system extended distally from the region of bifurcation of the AVB from cells that were indistinguishable from the working myocardium and continuous with the AVB primordium. Differentiation of Purkinje-like AVB cells was complete by 46 days of gestation but Purkinje fibres were still differentiating within the ventricular wall at 60 days of gestation. Canale, *et al.*, (1987) stated that the main morphological changes included a large increase in cell size and organization into strands, development of characteristic glycogen-filled regions containing many intermediate filaments and early development of myofibrillar M lines compared to the working myocardium. Our study represented very important developmental changes during the three gestational ages especially in the Purkinje fibres locations. They were found near the epicardium or in-between the myocardial muscles and were either attached to the myocardium or separated from it by connective tissue, also in the myocardial connective tissue, around a number of blood vessels.

Parto *et al.* (2013) stated that Purkinje fibers or Purkinje cardiomyocytes were part of the cardiac conduction system and classified as specific heart muscle tissue responsible for the heart impulses. According to this author most of the Purkinje fibres in ostrich heart were composed of a clear structure with less sarcoplasm. The myofibrils tended to be confined to a thin ring around the periphery of the cells with one or more large nuclei centrally located within the fibre. This is in agreement with our study that the fibres contained one or two nuclei.

In this study the differentiation of PF from myocytes was very clear at the early stages of gestation in camel foetus. PF appeared as darkly stained striated fibres and double nuclei appeared in many fibres. PF were embedded in connective tissue or attached to the ordinary cardiac muscle. In the third trimester of gestation the PF had light cytoplasm and most of them were double nucleated.

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