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## Histological study on the heart ventricle of Egyptian bovines (*Bos aegyptiacus*)

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### Abstract

**Background:** The heart ventricles have thicker walls than atrium as they pump blood through blood vessels into all body organs.

**Aim:** This study aimed to describe the histological changes of the heart ventricles in Egyptian bovine (*Bos aegyptiacus*) with special reference to Purkinje fibers.

**Methods:** A total of 10 male Egyptian bovines of 1–10 years old were divided into three groups according to age; immature, mature, and adult animals.

**Results:** The histological sections from all examined animals' groups revealed three different layers of the wall of both right and left ventricles; endocardium, myocardium, and epicardium. The endocardium was lined with endothelium and filled with fibrous connective tissue. The endocardium of adult bovine was the thickest. Purkinje fibers appeared of pale cytoplasm with few myofibrils. They were present in the deep layer of the endocardium and in the myocardium. The size of Purkinje fibers and the amount of their myofibrils appeared to be increased with advanced age. Bundles of cardiac muscles were the main constituent of the myocardium. The myocardial bundles were separated by fine connective tissue in immature animals that showed an increased amount in the adult animals. The hypereosinophilic cardiac muscle cells were observed in the ventricles of both mature and adult animals suggesting hypercontraction during rigor mortis. An external layer of the ventricles was the epicardium which consisted of connective tissue and covered with mesothelium.

**Conclusion:** Overall, this study revealed histological changes in the wall of the ventricle and Purkinje fibers of Egyptian bovines (*B. aegyptiacus*) in relation to age. Additionally, the hypereosinophilia of the cardiac muscle cells was recorded in the ventricles of mature and adult bovines.

**Keywords:** Bovine, Heart, Histology, Purkinje fiber, Ventricle.

### Introduction

The heart is a hollow organ that pumps blood through blood vessels into all body organs (Pathak and Kumar, 2010). In mammals, it consists of two atria and two ventricles. The ventricles have thicker walls than atria. The required physiological load on the ventricles to pump blood throughout the body is much greater than the load exerted by the atria to fill the ventricles.

The histological architecture of ventricular myocardial fibers is critical for cardiac functions (Gouda *et al.*, 2015). The anatomical features of the ventricles in most animal species were reported (Crick *et al.*, 1998; Erdoğan *et al.*, 2014; Getty *et al.*, 1975; Haligür and Dursun, 2009; Panhwar *et al.*, 2007). Also, the histological studies on the ventricles of various animals and human were done (Ates *et al.*, 2017; Babiker, 2010; El-Khouly *et al.*, 2001; Motabagani, 2006; Nabipur, 2002). The reports about the histological changes of the ventricles in Egyptian bovine (*Bos aegyptiacus*) were little especially those discussing the effect of age on the ventricular structure and the Purkinje fibers. However, Ryu *et al.* (2009) referred

to Purkinje fibers in the sheep ventricles. Therefore, this study aimed to deal with the histological changes of the ventricles of Egyptian bovines (*B. aegyptiacus*) with the advancement of age. Also, a special reference to Purkinje fibers was studied.

### Materials and Methods

#### Animals

This study was conducted on the hearts of 10 male apparently healthy Egyptian bovines (*B. aegyptiacus*) collected from a local abattoir in Kalubya Governorate, Egypt. The animals were 1–10 years old. The animals were divided according to their ages into three groups: immature ( $n = 3$ ), mature ( $n = 3$ ), and adult animals ( $n = 4$ ).

#### Specimen collection

The heart of each animal was washed with physiological saline and then small specimens from both right and left ventricles were fixed in 10% neutral buffered formalin for 72 hours. Tissue specimens were dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin.

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### Histological examination

Paraffin blocks were cut into sections of 5  $\mu\text{m}$  thick. The sections were stained with hematoxylin and eosin for general structure and Masson's trichrome for the identification of collagen fibers as outlined by Bancroft and Gamble (2007).

### Results

The wall of the ventricles of all examined Egyptian bovines consisted of endocardium, myocardium, and epicardium (Fig. 1A and 1B). The endocardium is lined with endothelium (Fig. 1A) and filled with fibrous connective tissue. Also, it contains Purkinje fibers, blood vessels, and nerve fibers (Fig. 1C). The endocardium appeared the thickest in adult animals (Fig. 1D). Purkinje fibers are present in the deep part of the endocardium (Fig. 1A and 1D), between the myocardial bundles (Fig. 2A) as well as intramural fibers among the myocardial fibers (Fig. 2B). The cytoplasm of Purkinje fibers appears pale and contains few myofibrils that are thin fibers confined to the periphery of the cells.

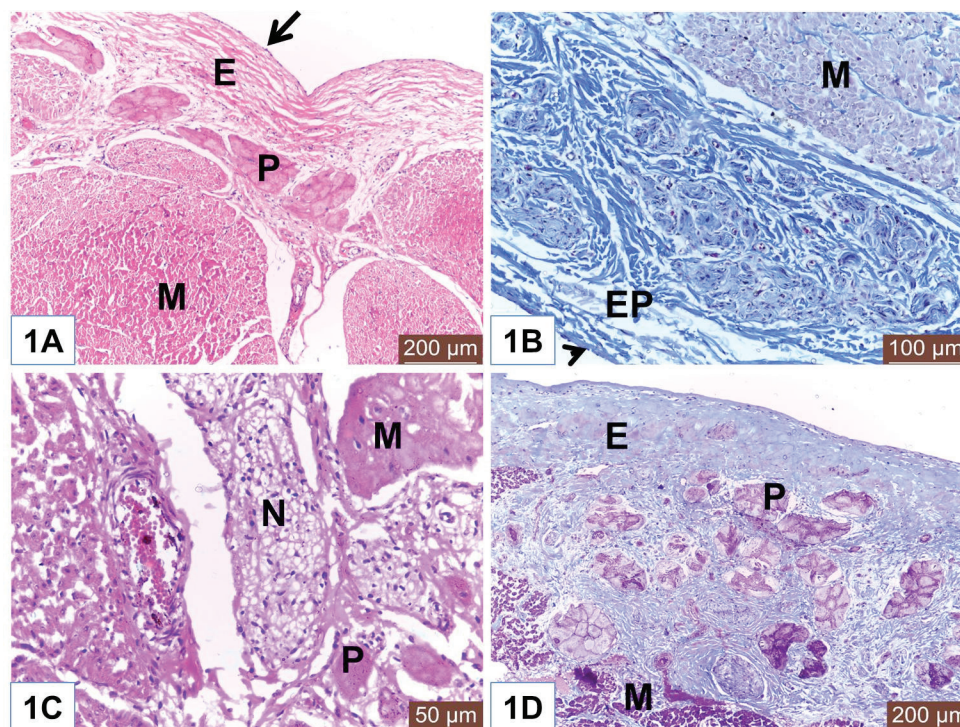
Additionally, the amount of myofibrils increased in the ventricles in adult animals (Fig. 2C).

Myocardium consisted of bundles of cardiac muscles (Fig. 2D). In both mature and adult animals, some cardiac muscles show dark cytoplasm (hypereosinophilia) and others had lighter cytoplasm (Fig. 3A and 3D). Fine connective tissue (CT) among myocardial bundles is observed in immature animal (Fig. 3B); however, the amount of CT is increased and become the widest in adult animal (Fig. 3C). Fine collagen fibers are surrounding myocardial cells (Fig. 3D).

Epicardium consists of CT and is covered externally with mesothelium. Nerve fibers can be seen within this layer (Fig. 2D).

### Discussion

The heart is a muscular pump that contracts rhythmically to propel blood around the body via the blood vessels. It is well known that the wall of the heart consists of three layers; endocardium, myocardium, and epicardium. The ventricles of Egyptian bovine, like



**Fig. 1.** (A) Photomicrograph of the ventricle from mature Egyptian bovine showed endocardium (E) and myocardium (M). Notice endothelial lining (Arrow) of the endocardium. Also, Purkinje fiber (P) in the endocardial layer. H&E. Scale bars = 200  $\mu\text{m}$ . (B) Photomicrograph of the ventricle from mature Egyptian bovine showed epicardium (EP) and myocardial bundles (M). Notice outer mesothelium (Arrowhead). Masson's trichrome method. Scale bars = 100  $\mu\text{m}$ . (C) Photomicrograph of the ventricle from immature Egyptian bovine showed myocardium (M), Purkinje fiber (P), and nerve fibers (N). H&E. Scale bars = 50  $\mu\text{m}$ . (D) Photomicrograph of the ventricle from adult Egyptian bovine showed wide endocardium (E). Notice Purkinje fibers (P) and cardiac muscles of the myocardium (M). Masson's trichrome method. Scale bars = 200  $\mu\text{m}$ .



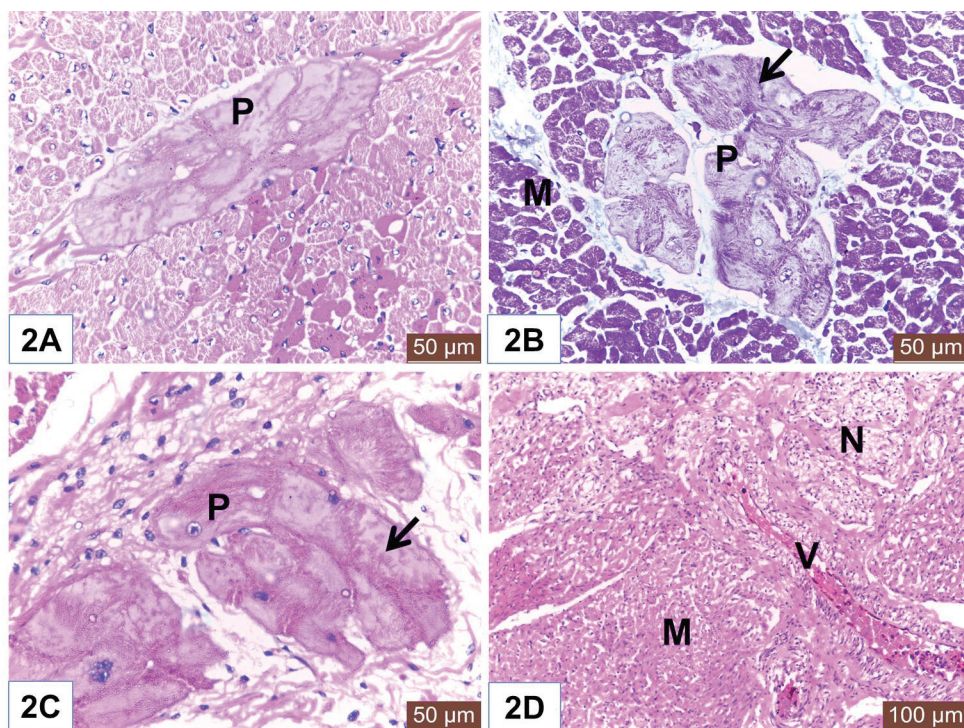
other animals, consisted of those three layers that were similar to reported by Ghazy *et al.* (2018).

The endocardium is the innermost that was covered with a thin layer of endothelium underlying the subendothelial connective tissue layer. This finding was similar to Gálfiová *et al.* (2017). Deep to the endocardium, clusters of Purkinje fibers were distributed, as a part of the conducting system of the heart. This supports the finding of Ansari *et al.* (1999). Like finding of Koprla and Nemeséri (1984), our histological results showed few amount of myofibrils in a pale cytoplasm of the Purkinje fibers. This histological feature varied depending on ages (Forsgren, 1982); therefore, our results revealed a higher amount of myofibrils in the Purkinje fibers of older animals.

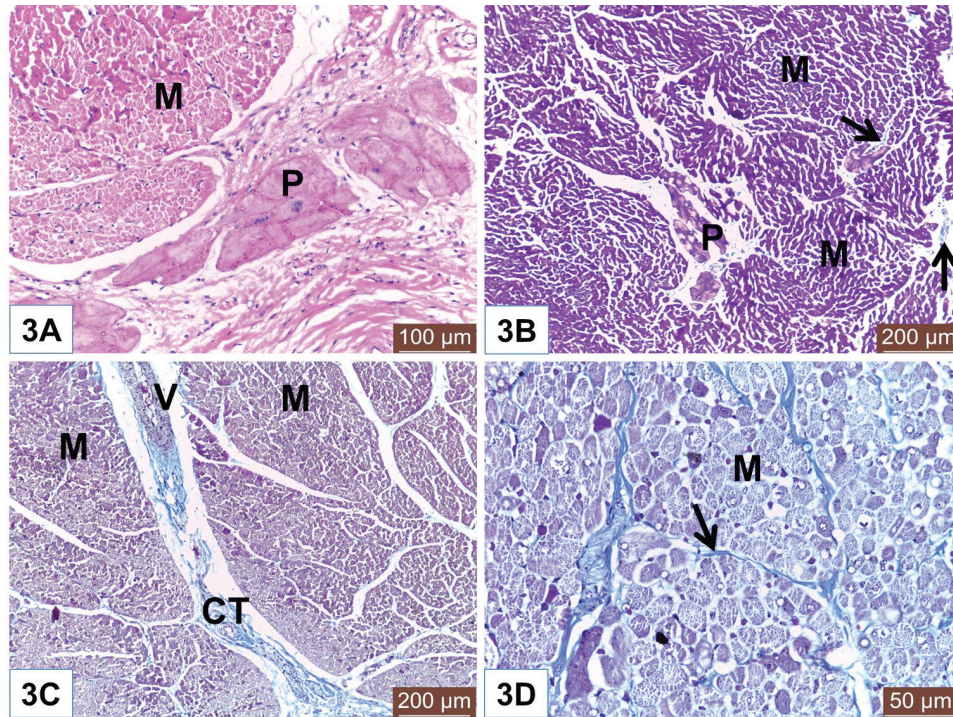
The present study observed the close localization of the nerve fibers to Purkinje fibers that point to the nerve-Purkinje fiber relationship as reported by Jensen *et al.* (1978) and Crick *et al.* (1999) as they referred to the importance of the nervous control for cardiac

performance in rhythm. Also, Kawano *et al.* (2003) demonstrated the presence of more autonomic nerves in the subendocardial area than in the subepicardial area of the human ventricle.

The intramural Purkinje fibers, in this study, were seen among the myocardium that confirms the ramification of Purkinje fibers within endocardium and myocardium as recorded by Ryu *et al.* (2009) in sheep and Ghonimi *et al.* (2014) in camel but, these fibers were not identified in the heart of human (Durrer *et al.*, 1970; Oosthoek *et al.*, 1993). The presence of intramural Purkinje fibers in the myocardium results in a more efficient excitation of the ventricles compared with the absence of intramural Purkinje fibers (Oosthoek *et al.*, 1993; Tranum-Jensen *et al.*, 1991) as they prevent intercytic pre-excitation of the ventricular myocardium. Our observation revealed a relative increase in the thickness of the endocardium with the advanced age that in accordance with Centurion *et al.* (2003).



**Fig. 2.** (A) Photomicrograph of the ventricle from mature Egyptian bovine showed intramural Purkinje fiber (P) in between myocardial bundles. Notice hypereosinophilia of some cardiac muscle cells. H&E. Scale bars = 50  $\mu$ m. (B) Photomicrograph of the ventricle from adult Egyptian bovine showed intramural Purkinje fibers (P). Notice myofibrils (Arrow) and cardiac muscles of the myocardium (M). Masson's trichrome method. Scale bars = 200  $\mu$ m. (C) Photomicrograph of the ventricle from mature Egyptian bovine showed Purkinje fiber (P) with pale cytoplasm and few myofibrils (Arrow). H&E. Scale bars = 50  $\mu$ m. (D) Photomicrograph of the ventricle from immature Egyptian bovine showed myocardium (M), blood vessels (V), and nerve fibers (N). H&E. Scale bars = 100  $\mu$ m.



**Fig. 3.** (A) Photomicrograph of the ventricle from mature Egyptian bovine showed different forms of muscles in the myocardium (M). Notice Purkinje fiber (P) in the endocardial layer. H&E. Scale bars = 100  $\mu$ m. (B) Photomicrograph of the ventricle from immature Egyptian bovine showed fine connective tissue (Arrows) in between myocardial fibers (M). Notice Purkinje fibers (P) among myocardial bundles. Masson's trichrome method. Scale bars = 200  $\mu$ m. (C) Photomicrograph of the ventricle from adult Egyptian bovine showed wide connective tissue (CT) in between myocardial bundles (M). Notice blood vessel (V). Masson's trichrome method. Scale bars = 200  $\mu$ m. (D) Photomicrograph of the ventricle from adult Egyptian bovine showed fine collagen fibers (Arrow) surrounding myocardial cells (M). Notice hypereosinophilia of the cardiac muscle cells. Masson's trichrome method. Scale bars = 50  $\mu$ m.

For the myocardial layer of Egyptian bovine, the most predominant constituent was the cardiac muscles which arranged in bundles that were inconsistent with that reported by Ghonimi *et al.* (2104) in camel. It was noteworthy to mention that some cardiac muscle cells of the ventricles of both mature and adult Egyptian bovine showed hypereosinophilic cytoplasm while the others had light ones. This feature was recorded by Dettmeyer (2011) and Turillazzi *et al.* (2014) and owed this to the hypercontraction of the myocardial cells during rigor mortis. Also, Faa *et al.* (2012) observed hypereosinophilia of the cardiomyocytes due to cardiac hypoxia in newborn piglets.

Another important notice was the increase in the amount of connective tissue among myocardial bundles in relation to the age. This was similar to the finding of Emam and Abo-Ahmed (2019) in the moderator bands of cattle.

The external layer of the ventricle was the epicardium which consisted of connective tissue and covered externally with mesothelium. The identification of

nerve fibers within the epicardium was similar to the finding of Crick *et al.* (1999).

In conclusion, the histological structure of the ventricle of Egyptian bovine (*B. aegyptiacus*) was similar to other animals and human. However, the intramural Purkinje fiber was characteristic of bovine and absent in human. Both the thickness of endocardium and the amount of connective tissue among the myocardial bundles were relatively increased with the advanced age. Additionally, the hypereosinophilia of the cardiac muscle cells was characteristic for the ventricles of mature and adult bovines.

#### **Conflict of interest**

The authors declare that there is no conflict of interest.

#### **Authors' contribution**

MAE collected and prepared the specimens for histological examination. Also, MAE captured the histological sections by Leica DM3000 microscope at the Histology Department, Faculty of Veterinary Medicine, Benha University, Egypt. Both MAE and BA interpreted the photomicrographs and shared the editing



of the manuscript. MAE responded to the comments of reviewers.

#### **Ethical Approval**

Slaughtering of the animals was carried out in accordance with the Guidelines Animal Ethics Committee at the Faculty of Veterinary Medicine, Benha University.

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