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The cardiac skeleton of the Egyptian Water buffalo (*Bubalus bubalis*)

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Abstract

In the present study, fifteen heart from clinically healthy buffaloes of different ages and both sexes are used to examine the skeleton of the heart. The techniques used are manual dissection, radiographs and Microscopical examination. The skeleton of the heart is composed of a group of fibrous structures encircles the margins of the inflow and outflow orifices of the ventricles in a form of rings. The right and left atrioventricular fibrous rings in addition to aortic and pulmonary rings are formed from collagen fibers. In addition to, the presence of a central fibrous body composed of one fibrocartilage encircled the origin of the aorta and two spongy bones; right and left ossacordis. The ossacordis are absent in the young calves and hyaline cartilage is present instead of the spongy bone. The results are discussed with relatives previous researches.

Keywords: Buffalo- Heart-Cardiac skeleton-Os-cordis

Introduction

The buffalo is a ruminant animal belongs to the class Mammilia, Subclass Ungulate, Order Artidactyla, Suborder Ruminantia, Family Bovidae, Subfamily Bovinae and tribe Bovini. This tribe includes Cattle, Asian (Water) buffalo and African buffalo. The Asian buffalo includes three species; Anoa, Tamaraw and Arni (The indian wild buffalo). The Arni has been domesticated and raised in the world under the name Water Buffalo (Bubalusbubalis). The water buffalo spread widely all over Asia, parts of Europe, Near east and Egypt and later Caucasian area and south America (**Mingala et al. 2017**). Buffalo population in the world around 168 million heads with Egypt have 3.7 million heads (2.2% of world production) in 2003 (**Borghese, 2005**). Buffalo population in 2006 reached 3.9 million heads and its productivity represents 31.05% and 46.97% of total Egypt meat and milk production respectively (**SADS, 2009**).

Skeleton of heart is part of the heart tissue composed of four fibrous rings formed of connective and partially cartilage or bone named fibrous trigones. Two fibrous rings are at the pulmonary trunk and aorta orifices and are festooned in conformity with cusps attached borders, and the two other fibrous rings of the atrioventricular orifices are present between atrial and ventricular musculatures (**Getty, 1975**and **Dyce et al., 2010)**. The fibrous trigones are present between aorta and the atrioventricular openings and together with the aortic fibrous ring forms the central fibrous body. The trigones composed of dense connective tissue that might contain fibrocartilage, hyaline cartilage and even bone in certain mammal's (**Loukas, 2016**).

The skeleton of the heart was important for normal heart function in domestic animals (**Schummer et al., 1981** and**Frandson and Spurgeon, 1992**), in human

(**Moore and Dalley, 2006**), and in the otter (**Egerbacher et al., 2000**) as it support the cusps of aorta, pulmonary trunk, as well as those of the atrioventricular valves and the presence of rings avoids over expansion of the valves. It also electrically isolates atrial and ventricular musculatureswhich is vital for cardiac contraction coordination (**Hill and Iaizzo, 2015**).One of the key factors for mitral valve repair success is the placing of sutures properly into the annulus fibrosus(**Istvan et al., 2008**).

The Cardiac skeleton was described in domestic animals (**Getty, 1975, Schummer et al.,1981** and **Frandson and Spurgeon, 1992**), in the donkey (**Wally, 2008**), in the sheep (**May, 1970**), in the goat (**Alloush, 2001**), in the dog (**Evans and De Lahunta, 2013**), in white rhinoceros (**Erdoğan et al., 2014**), in the cattle (**Islam et al., 2006**), in the camel (**Karkoura,1989**, and **Ghonimi et al., 2014**), in the pig (**Crick et al., 1998**), and in human (**Zhu, 2015, Li et al., 2016**, and **Loukas, 2016**).

Previous studies on buffalo cardiac anatomy were conducted including, cardiac veins (**Hemmoda and Amin, 1989** and **Karimi et al. 2010**), Coronary arteries (**Karimi et al. 2008**), Biometrical characteristics (**Panhwar et al., 2007** and **Gupta et al., 2012**), single oscordis (**Mia, 1973**) and mitral valve (**Hemmoda, 1988**).

Little is known about the skeleton of the heart in the Buffalo. So, the present study was carried out to describe the anatomical and histological structures of the cardiac skeleton aiming to give detailed information and increasing the anatomical knowledge for that animal which may be of help in clinical work.

Materials and Methods

Fifteen hearts of clinically healthy Buffaloes of different ages (1 month to 5 years) of both sexes obtained from Giza and El-fayum Governorate slaughter houses were used in the present investigation. Three hearts were freshly radio-graphed at exposure factors 50 kv and 13 mAs using Poskom digital X-ray unit model No. PXP-40HF (Goyang, Korea). Eight hearts were fixed in formalin solution (10% formalin, 4% phenol. 1% glycerin) and left for 3-4 days in a cold room (**Hildebrand, 1968**) before manual dissection. Dimensions of bones were measured using Vernier Caliber.

Four hearts were used forthe microscopical studies, after slaughtering the rings of the heart and ossacordis were immediately dissected out and sectioned into small pieces. Some of these specimens were fixed in neutral buffer formalin 10% and others fixed in Bouin's fluid. The blocks dehydrated in grades of ethanol, cleared by xylene and embedded in paraffin wax. Serial and step serial sections of 5-6 µm were obtained by rotatory-microtome and stained with Hematoxylin and Eosin (H&E), Masson's trichrome stain and Crossman’s trichrome stain for collagen fibers and smooth muscles (**Bancroft and Gamble, 2008**). Nomenclature was adopted according to **NominaAnatomicaVeterinaria (2012)**.

Results

The ventricular base of the heart (**Fig.1**) shows four orifices, right and left atrioventricular openings, aortic and pulmonary arterial openings. The aortic orifice occupies the center of the base, the right atrioventricular orifice is situated cranially and to the right while that of the pulmonary lies cranially and to the left. The left atrioventricular opening is located caudal to the aortic one.

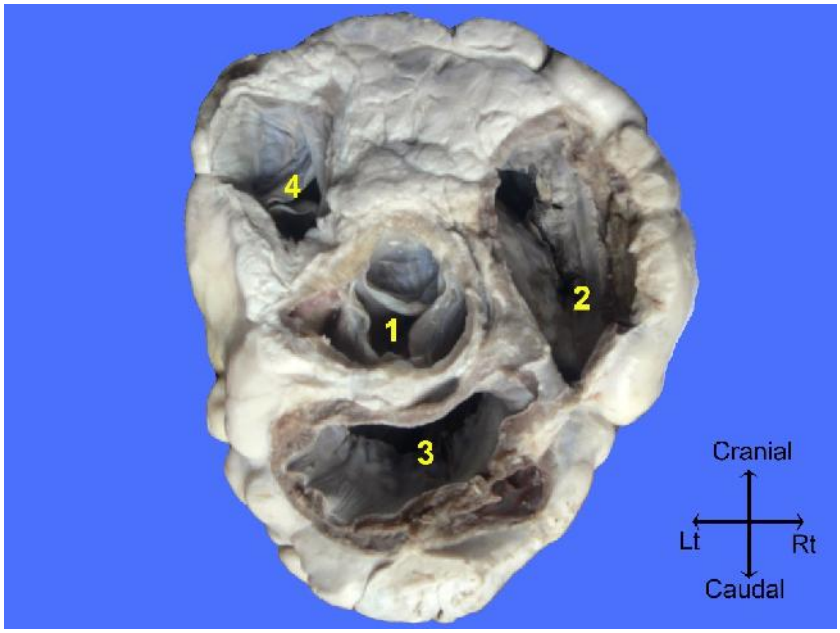

Fig. (1) A photograph showing the valvar system of the ventricular base of the Egyptian water buffalo heart after removing of the atria showing: 1. Ostium aortae. 2. Ostium atrioventricularedextrum. 3. Ostium atrioventricularesinistrum. 4. Ostium trunci pulmonalis.

A-Annulus fibrosusdextrum (Fig. 2):

The right atrioventricular orifice (Ostium atrioventricularedextrum) is located right and cranial to the aortic orifice (**Fig. 1**) and it has a tricuspid valve, each cusp has two borders attached (basal) and free. The basal border is attached while the free one is directed toward the ventricular cavity. Removal of the cusps shows its attachment to the right fibrous ring which encircles the right atrioventricular orifice (**Fig.2/A**). The fibrous ring extends between the atrial and ventricular musculature. The fibrous ring is formed of dense irregular connective tissue, mainly collagen fibers and white adipose tissue **(Fig. 2/B)**.

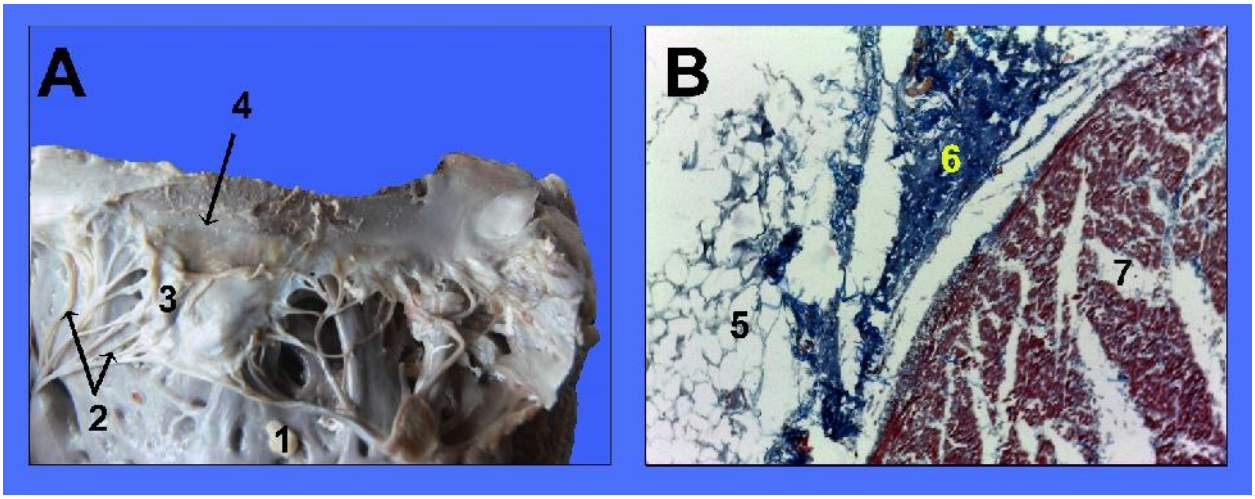

Fig. (2). Ostium atrioventricularedextrum of the Egyptian water buffalo heart. A. photograph showing the right atrioventricular opening showing reflected parietal cusp of the tricuspid valve. B. Photomicrograph of the Right atrioventricular ringof the water buffalo heart (Masson's Trichrome X100): 1.Ventriculusdexter. 2. Chordae tendineae. 3. Cuspisparietalis. 4. Fibrous ring. 5. Adipose tissue. 6. Collagen fibers. 7. Cardiac muscles.

B- Annulus fibrosuspulmonalis (Fig.3):

The pulmonary trunk orifice (Ostium trunci pulmonalis) lies cranially and to the left of the ventricular base(**Fig. 1**). The orifice is guarded by the pulmonary semilunar valve (Valva trunci pulmonalis)**(Fig 3/A)**which has three cusps.Each cusp had two borders; free and attached (basal). The free border is directed upward and the basal border is attached to the pulmonary fibrous ring (Annulus fibrosuspulmonalis).The pulmonary fibrous ring encircles the margin of the pulmonary orifice and in the form of three arches right, left and intermediate. Each of them gives attachment to the base of the corresponding semilunar cusp of the pulmonary valve**(Fig. 3/B)**. As line of attachment is curved and rising to the periphery of each cusp near their zones of apposition (commissures), It gives the festooned appearance to the pulmonary semilunar valve. The pulmonary fibrous ring is formed from dense irregular connective tissue, mainly collagen fibers (**Fig.3/C&D**).

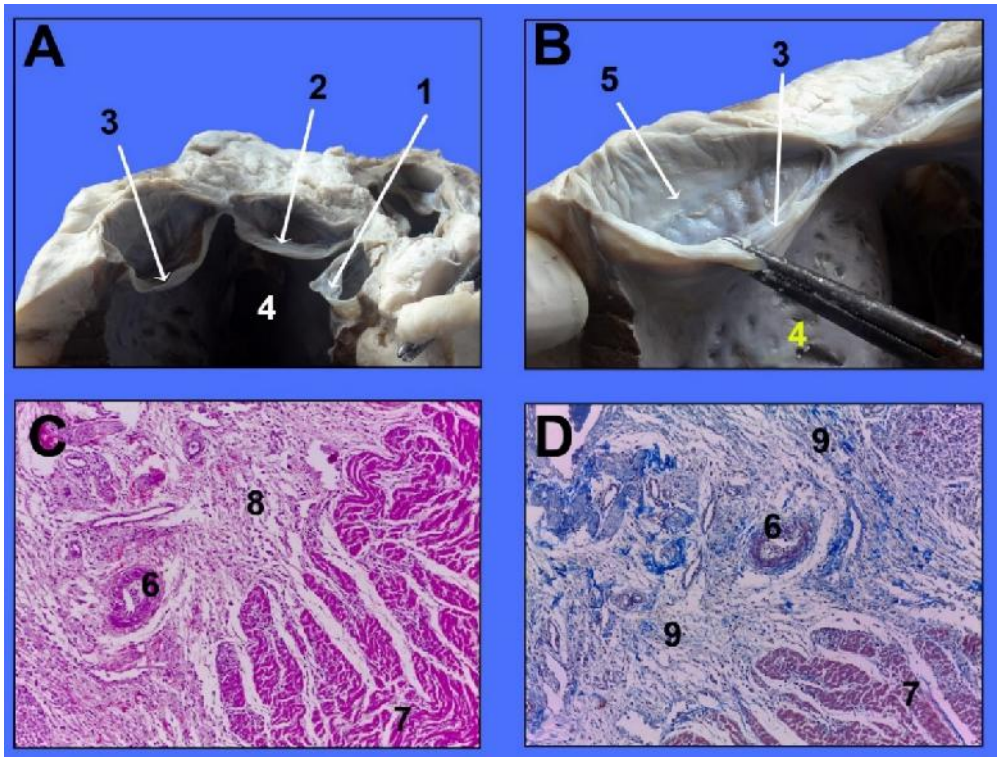

FIg. (3): Ostium trunci pulmonalis of Egyptian water buffalo Heart. A. Opened Valva trunci pulmonalisshowing the Valvulasemilunaris. B. Reflected Valvulasemilunarissinistra. C. Photomicrograph of the Pulmonary orifice (H&E X100). D. Photomicrograph of the Pulmonary orifice (Masson's Trichrome X100). Demonstrating: 1. Valvulasemilunaris intermedia.2. Valvulasemilunarisdextra. 3. Valvulasemilunarissinistra. 4. Ventriculusdexter. 5. Pulmonary fibrous ring. 6. Large blood vessels. 7. Cardiac muscle. 8. dense irregular connective tissue. 9. Collagen fibers.

C- Annulus fibrosussinistrum(Fig.4):

The left atrioventricular opening (Ostium atrioventricularesinistrum) is located caudal to the aortic orifice (**Fig. 1**) and it has a bicuspid valve, each cusp has two borders attached (basal) and free (**Fig. 4/A**). The basal border is attached while the free one is directed toward the ventricular cavity. Removal of the cusps shows its attachment to the left fibrous ring which encircles the left atrioventricular orifice (**Fig. 4/B**). The fibrous ring is formed of mainly collagen fibers and extends between the atrial and ventricular musculatureand appears thicker than the right fibrous ring (**Fig. 4/C**).

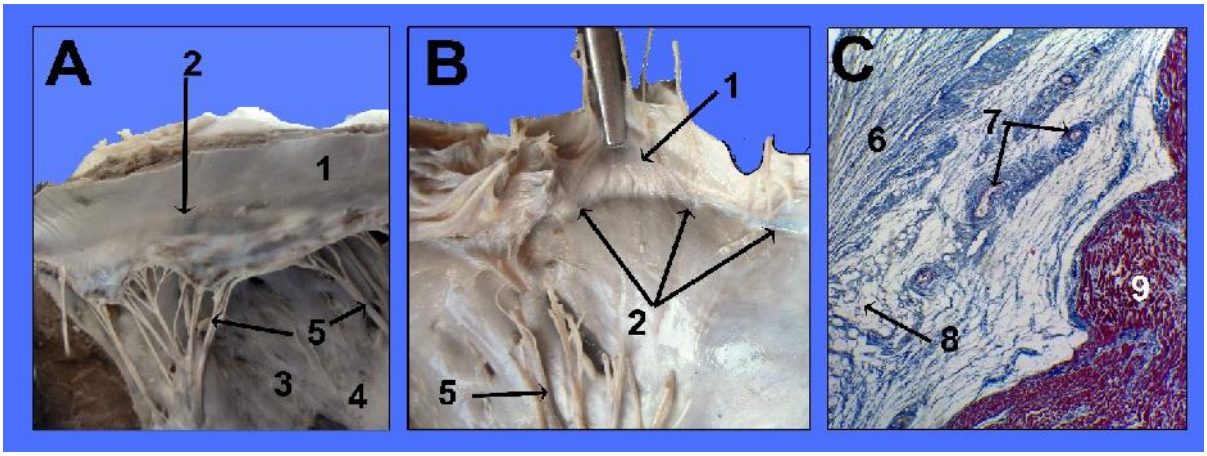

Fig. (4). Ostium atrioventricularesinistrum of the Egyptian water buffalo heart. A. photograph showing the left atrioventricular opening showing Valvaatrioventricularissinistra. B. A photograph showing the left atrioventricular opening showing reflected Cuspisparietalis.. C. Photomicrograph of left atrioventricular ringof the water buffalo heart (Masson's Trichrome X40) 1.Cuspisparietalis. 2. Fibrous ring. 3. Ventriculus sinister. 4. M. papillaris posterior. 5. Chordae tendineae. 6. Collagen fibers. 7. Large blood vessels. 8. Adipose tissue. 9. Cardiac muscle.

D- Annulus fibrosus aortae (Fig.5):

The aortic orifice (Ostium aortae) is located at the center of the ventricular base just cranial to the left atrioventricular orifice (**Fig. 1**). It has aorticsemilunar valve with three cusps, each cusp had two borders attached (basal) and free. The basal border is the ventrally attached while the free one is directed upward at the aortic orifice. The aortic orifice has an annular fibrous ring(Annulus fibrosus aortae) encircles the margin of the aortic orifice in the form of three arches; right, left and cranial. Each of them gave attachment to the base of the corresponding semilunar cusp of the aortic valve (**Fig. 5/B**). The line of attachment is curved, rising at the periphery of each cusp near their zones of apposition (commissures), giving the cusps a festooned appearance (**Fig. 5/A**).

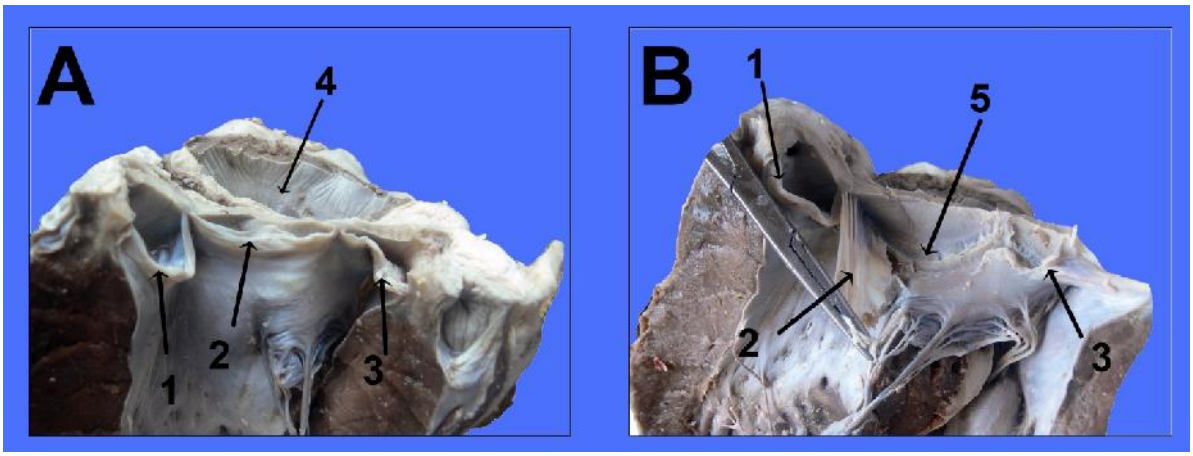

Fig. (5): Ostium aortae of Egyptian water buffalo heart. A. Photograph of a dissected specimens with intact aortic cusps. B. Photograph of a dissected specimen with reflected left and right cusps.: 1. Valvulasemilunarisseptalis. 2. Valvulasemilunarisdextra. 3. Valvulasemilunarissinistra. 4. Ostium atrioventricularesinistrum. 5. Aortic fibrous ring.

E-Central fibrous body (Fig.6, 7, 8 & 9):

The central fibrous ring appeared as thick cartilaginous structure around the aortic origin(**Fig. 7/A, B, C,&D,** and **Fig. 8/A& B**). It forms together with the two ossacordis (**Fig. 7/A & C,** and**Fig. 8/A**), the central fibrous body.It continues with the with the two atrioventricular fibrous rings. By microscopical examination, the aortic fibrous body at the level of right oscordis, it is formed of collagen fibers and fibrocartilage (**Fig. 7/ E &F**) and the oscordis is spongy bone.

In the young (30 days buffalo calf) the ossacordisis absent and by examining the central fibrous body thickened present structure at the same previous level histologically, it is formed of collagen fibers, fibrocartilage, and hyaline cartilage (**Fig.6/A, B, C&D**).

In the adult (3-5years), the central fibrous body is composed of full annular fibrocartilage ring (**Fig. 7/A, B, C& D,** and **Fig. 8/A& B**) and two ossacordis (**Fig. 7/A & C,** and **Fig. 8/A**). The annular cartilaginous ring has three dorsal projections; right (**Fig. 7/A & D, Fig 8/ A &B,**and **Fig. 9**), left (**Fig. 7/C& D, Fig 8/ A &B,**and **Fig. 9**) and caudal(**Fig. 7/B, Fig 8/ A &B,**and **Fig. 9**) that gave attachment and support to the semilunar cusps (**Fig.8/A&B**).

The Ossa cordis are represented by two right and left spongy bones (**Fig.7 /A & D, Fig 8/A, C & D, Fig. 9and Fig. 10**). They were developed within the central fibrous body and surrounded by the aortic fibrocartilage. By microscopical examination (**Fig. 8/ E &F**), they are formed of spongy bones with bone marrow content.

The right Oscordis (**Fig.7/A, Fig. 8/ B & C, Fig. 9 and Fig. 10**) is the largest appeared as elongated quadrilateral with pointed caudoventral angle and rounded cranial angles. It ranged from 5.3 - 6 cm in length, 0.83 -1.46 cm in width, 0.12-0.22 cm thickness at dorsal border and 0.42-0.52 thickness at ventral border. Its wide end is directed cranially toward the right atrioventricular opening, and its narrow end present caudally at the left atrioventricular opening (**Fig. 7/A & B**).

The left Oscordis (**Fig.7/ B & C, Fig. 8/ A, C & D, Fig. 9 and Fig. 10**) is small, narrow curved triangle. It is present just below the origin of left coronary artery with wide caudal end and narrow cranial pointed end. Its dimensions range from 2.3-2.63 cmin length, 0.12-0.15 cm thickness at its cranial pointed end and 0.24-0.35cm thickness at its caudal wider end.

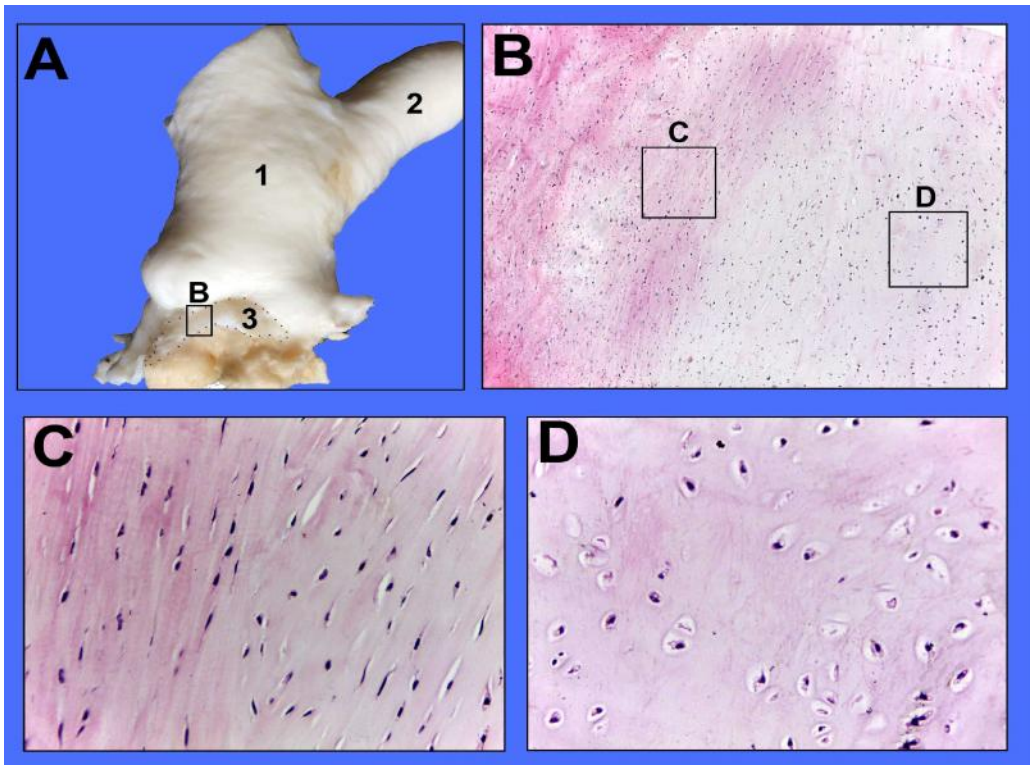

Fig. (6). Aorta of 30 days old Egyptian water buffalo calf. A. Aortic Origin from heart showing: 1. Aorta Ascendens. 2. Truncus brachiocephalicus. 3. Cartilaginous body. B. Transition between fibrocartilage and hyaline cartilage (H&E X100). C. Fibrocartilage (H&E X400). D. Hyaline cartilage (H&E X400).

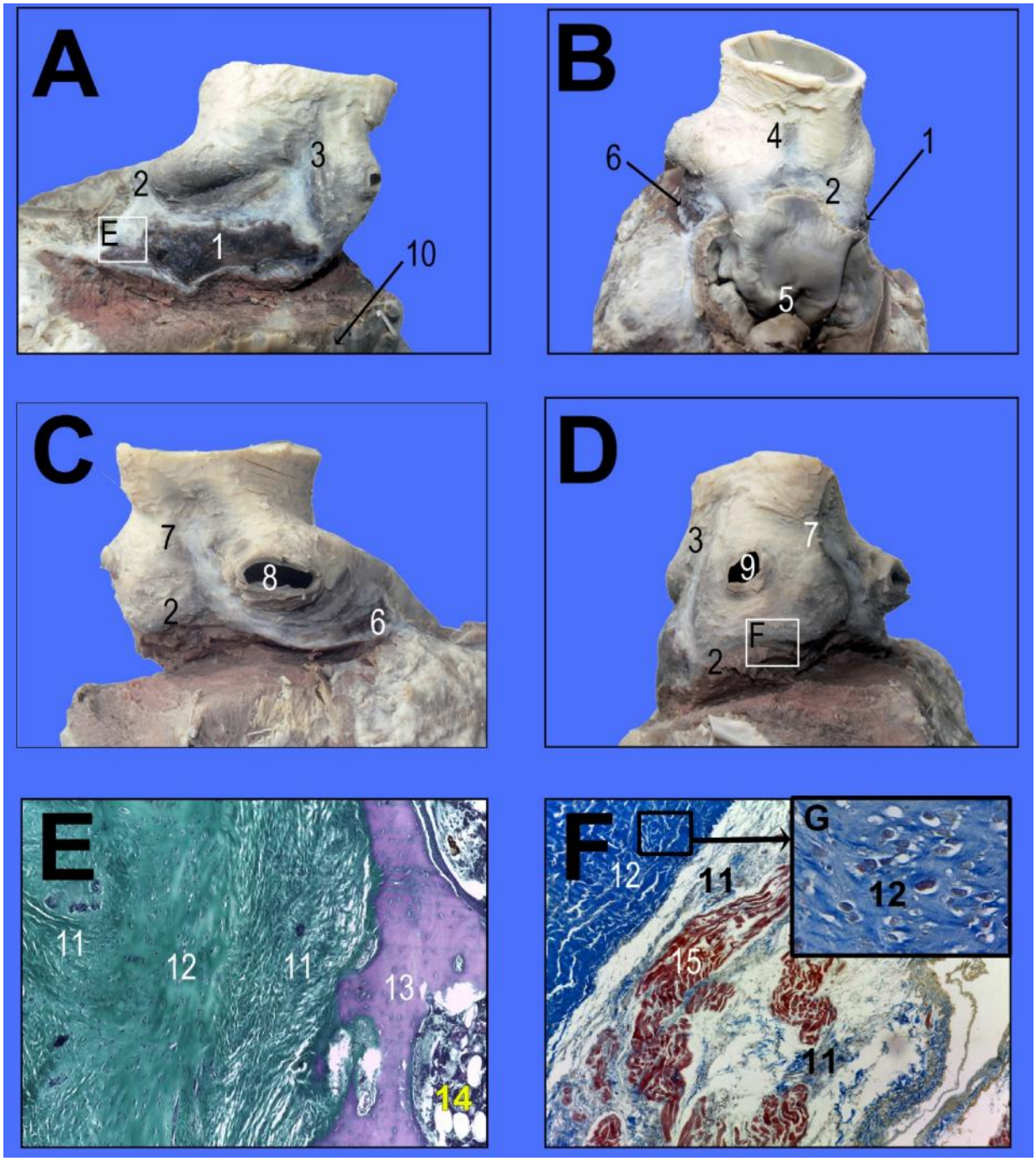

Fig. (7): Egyptian water buffalo Aortic fibrous ring. A. Left view Aorta. B. Caudal view Aorta. C. Right view Aorta. D. Cranial view Aorta. E. Photomicrograph of the Aortic fibrous ring (Crossman's Trichrome X100). F. Photomicrograph of the Aortic fibrous ring (Masson's Trichrome X100). G. Photomicrograph of the Aortic fibrous ring of the water buffalo heart (Masson's Trichrome X400) showing: 1. Right oscordis. 2. Aortic cartilaginous ring. 3.Right cartilaginous process. 4. Caudal cartilaginous process. 5. Ostium atrioventricularesinistrum. 6. Left oscordis. 7. Left cartilaginous process. 8. A. coronariasinistra. 9. A. coronariadextra. 10. Ostium atrioventricularedextrum. 11. Collagen fibers. 12. Fibrocartilage. 13. Spongy bone. 14. Bone marrow. 15. Cardiac muscle.

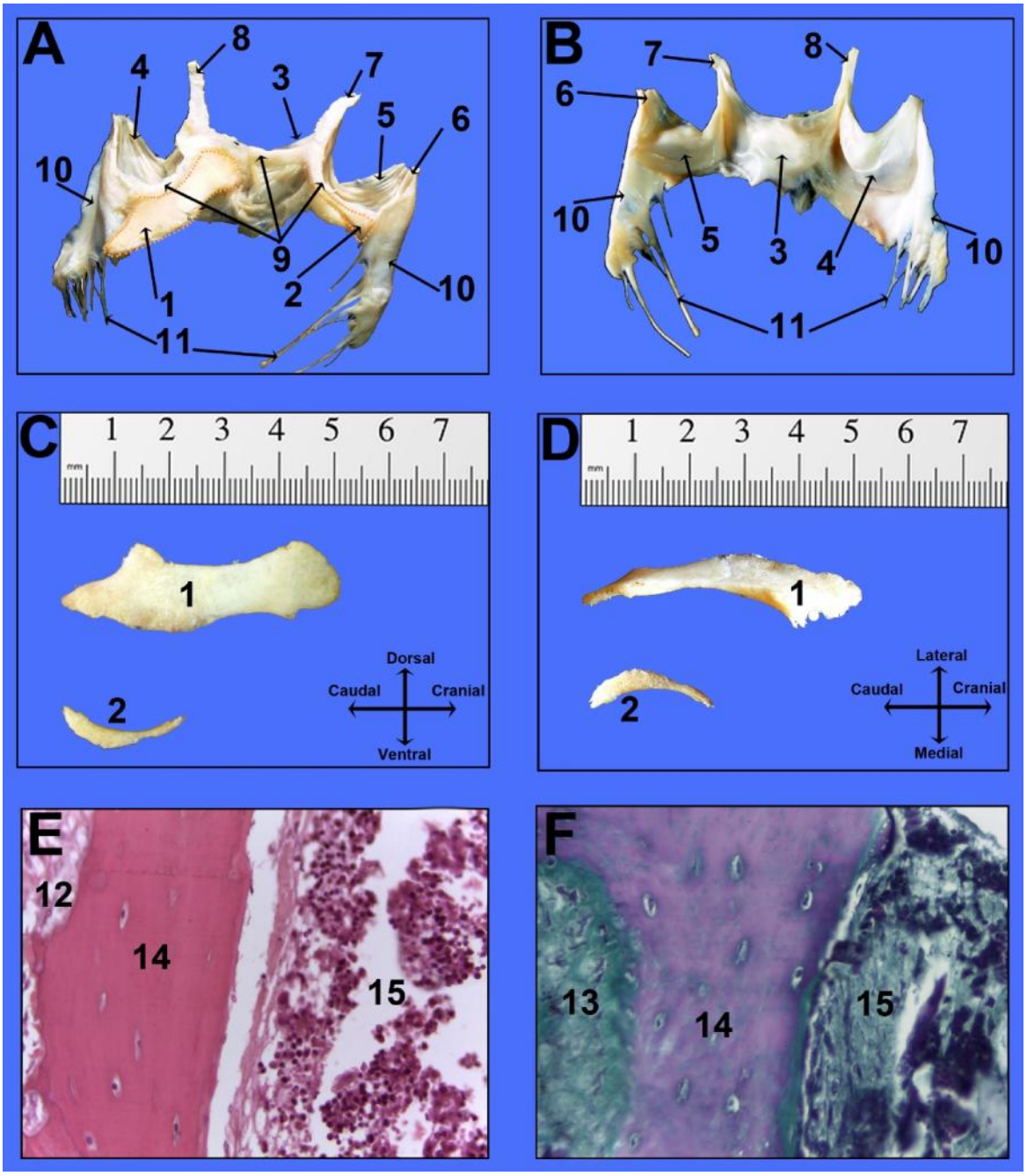

Fig. (8): Fibrous ring of Aorta in Heart of Egyptian water buffalo. A. External view of Aorta opened from mid caudal part after removal of aortic wall. B. Internal view of Aorta opened from mid caudal part after removal of aortic wall. C. Lateral view Oscordis. D. Dorsal View Oscordis. E. Photomicrograph of the Oscordis (H&E X400). F. Photomicrograph of the Oscordis (Crossman's Trichrome X400). 1. Right oscordis. 2. Left oscordis. 3. Valvulasemilunarisseptalis. 4. Valvulasemilunarisdextra. 5. Valvulasemilunarissinistra. 6. Caudal cartilaginous process. 7. Left cartilaginous process. 8. Right cartilaginous process. 9. Aortic cartilaginous ring. 10. Cuspisseptalis. 11. Chordae tendinae. 12. Dense irregular connective tissue 13. Collagen fibers. 14. Spongy bone. 15. Bone marrow.

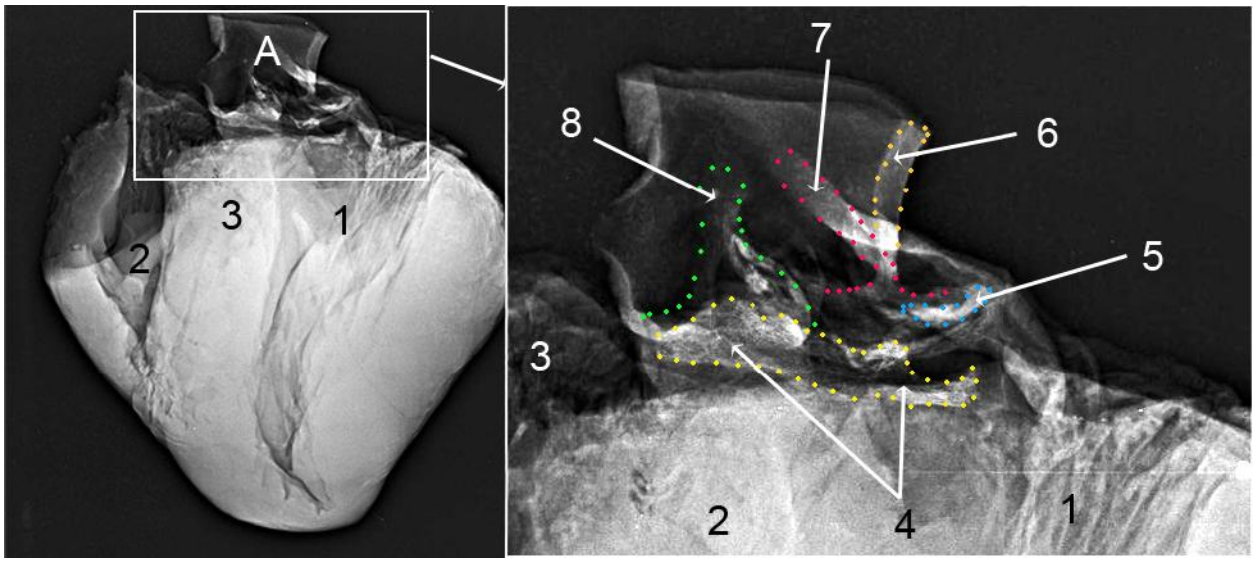

Fig. (9). A left lateral radiograph of Egyptian water buffalo heart at exposure factors 50 kv and 13 mAs demonstrating: A. Aorta. 1. Ventriculus sinister. 2. Ventriculusdexter. 3.Septum interventriculare. 4. Right oscordis. 5. Left oscordis. 6. Caudal cartilaginous process. 7. Left cartilaginous process. 8. Right cartilaginous process.

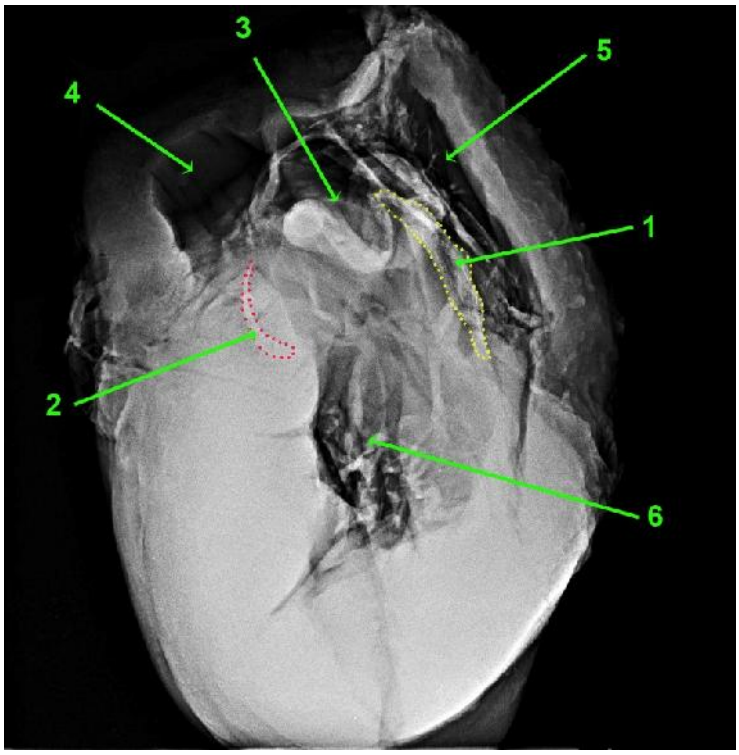

Fig. (10). A Ventrodorsal radiograph of Egyptian water buffalo heart at exposure factors 50 kv and 13 mAs demonstrating: 1. Right Oscordis. 2. Left Oscordis. 3. Aorta. 4. Pulmonary trunk. 5. Ventriculusdexter. 6. Ventriculus sinister.

Discussion

The present study revealed the fibrous skeleton of the heart of the water buffalo had a group of fibrous structures in the form of rings encircledthe margin of the inflow and outflow orifices at the ventricular base of the heart. Similar results was described in domestic animals (**Ghoshal, 1975, Schummer et al., 1981** and **Frandson and Spurgeon, 1992**), in the donkey (**Wally, 2008**), in the sheep (**May, 1970**), in the goat (**Alloush, 2001**), in white rhinoceros (**Erdoğan et al., 2014**), in the cattle (**Islam et al., 2006**), in the camel (**Karkoura, 1989**, and **Ghonimi et al., 2014**), in the pig (**Crick et al., 1998**), and in human (**Zhu, 2015, Li et al., 2016**, and **Loukas, 2016**).

These fibrous rings were formed mainly from collagen fibers in agreement with similar findings in human**(Loukas, 2016)** and in the donkey**(Wally, 2008)**. While in the camel **(Ghonimi, et al ,2014)** and in the otter **(Egerbacher et al., 2000)**the fibrous rings were composed of interwoven bundles of collagen fibers with a few elastic fibers,such elastic fibers were not observed in the present work. In human (**El-busaidy et al., 2012)** added that the ventricular annuli composition of collagen and elastic tissue was age dependent as by age collagen content increased and elastic content degenerated.

In the current investigation, the left fibrous ring encircled the left atrioventricular orifice appeared thicker than the right one, similar finding reported in the camel**(Karkoura,1989)**, in the goat**(Alloush, 2001)**, and in the donkey**(Wally, 2008)**.

The present study observed that the arterial fibrous rings in water buffalo were encircling the margins of the pulmonary and aortic orifices in the form of three arches,each of them gave attachment to the base of the corresponding semilunar cusp. The lines of attachment were curved, rising at the periphery of each cusp near their zones of apposition (commissures), giving the cusps a festooned appearance. This was simulating the findigs recorded in domestic animals(**Schummer et al., 1981**), in the camel(**Karkoura, 1989**), in the donkey(**Wally,2008**), in the dog(**Evans and De Lahunta, 2013)**, in the cattle(**Islam et al.,2006**) and in human **(Loukas, 2016)**.The later author added that proper valvular function depended primarily upon these semilunar attachments of the Cusps.

The present work revealed that the central fibrous body in the water buffalo was composed of full annular aortic cartilaginous ring and two ossacordis in accordance tofinding in the cattle(**Islam et al., 2006**).While the central fibrous body appeared as thick collagen fibers structure around aortic origin in the donkey (**Wally, 2008**) and considered as the structure connecting the mitral, tricuspid and aortic orifices in human **(Loukas, 2016)**.

The present study observed that, the aortic annular cartilaginous ring in the water buffalo heart had three dorsal projections; right, left and rostral that gave attachment and support to the aortic semilunar cusps and was composed of fibrocartilage. Similar finding reported in the dog (**Evans and De Lahunta, 2013**) but projections were hyaline cartilage in composition.
The two ossacordis were represent in the present study by alarge right and a small left spongy bone in all examined adult water buffalo heart specimens and were enclosed within the cartilage of the central fibrous body. The presence of two bones was in accordance to findings in the cattle (**Ghoshal, 1975**, **Schummer et al., 1981** and **Mohammadpour, 2004**). While other authors reported single bone in the water buffalo (**Mia, 1973**), in the sheep (**May, 1970**, and **Frink and Merrick,1974**), in the camel (**Ghonimi etal. 2014**), in the sheep and goat (**Tipirdamaz, 1987 and Mohammadpour, and Arabi, M., 2007).** Beside the single oscordis in the goat, a cartilage was also present (**Tipirdamaz, 1987).** Three heart bones were reported in the otter (**Egerbacher, 2000**). Two cartilages; right and left;were reported in the dog (**Schummer et al., 1981** and **Evans and De Lahunta, 2013**), in the horse (**Schummer et al., 1981**) in the donkey (**Wally, 2008**), butonly a single cartilage was present in white rhinoceros (**Erdoğan et al., 2014**),and in Equine (**Ghoshal, 1975**).

Regarding the length, width, thickness at dorsal border and thickness at ventral border of the right oscordis in the current investigated water buffalo hearts, results were5.3 - 6 cm, 0.83 -1.46 cm, 0.12-0.22 cm and 0.42-0.52 respectively. Length was reported in the water buffalo as 6 cm (**Mia, 1973**), ox as 4cm (**Ghoshal, 1975**), 5-6 cm (**Schummer et al., 1981**), Holstein cow as 4.085 cm and native Iranian cow as 3.092 cm (**Mohammadpour, 2004**), in the sheep were 1.25 cm

(**May, 1970**), 1-1.5 cm (**Frink and Merrick,1974)**, and 1.18 cm (**Mohammadpour, and Arabi, 2007),**and the later author added in the goat mean length was 1.699 cm**.** While width was reported in the water buffalo as 1 cm (**Mia, 1973**), 1.18 cm in Holstein cow, 0.77 cm in native Iranian cow (**Mohammadpour, 2004**), 0.5 cm in the sheep and 0.42 cm in the goat (**Mohammadpour, and Arabi, 2007),** whilewidth was 1-2 mm in the sheep(**Frink and Merrick,1974)**. Regarding thickness, it was reported in the water buffalo as 0.4 cm (**Mia, 1973**), 0.38 cm in Holstein cow, 0.253 cm in native Iranian cow (**Mohammadpour, 2004**), 0.23 cm in the sheep, 0.225 cm in the goat cm (**Mohammadpour, and Arabi, 2007).**

The left Oscordisdimensions of length, thickness at its cranial pointed end and thickness at its caudal wider end in the present work were 2.3-2.63 cm, 0.12-0.15 cm, and 0.24-0.35 cm respectively. Length and thickness were reported in Holstein cow as 1.99 cm and 0.325 cm respectively, and in native Iranian cow as 1.735 cm and 0.315 cm respectively (**Mohammadpour, 2004**). The left oscordis in the sheep was reported as 40% incidence of presence (**Frink and Merrick,1974)**.

Regarding our finding in the young buffalo calf of presence of Hyaline and fibrocartilage instead of bone was in agreement with findings in the cattle (**Islam et al., 2006**) and in the otter (**Egerbacher et al., 2000**) that oscordiswas entirely cartilaginous in young animals and the heart skeleton is age and species dependent.

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