

# Histological study of the atrioventricular node and bundle in the heart of ovine fetus

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## Summary

This study was conducted on the atrioventricular node (AVN) and atrioventricular bundle (AVB) of five four-month-old ovine fetuses. The histological structure of these components was studied by routine histological techniques and use of specific staining methods. The AVN was caudally located adjacent to the root of the aorta. It was almost spherical in shape and consisted of twisty cells. The node was mainly composed of "P" cells. There were not seen internodal pathways in the heart of four-month ovine fetus. The AVB was a direct continuation of the AVN and it passed through the fibrous ring toward the apex of the interventricular septum. At this place the right bundle branch (RBB) was ramified. The cells of the AVB were wider, shorter and lighter than normal myocardial cells. Some of the bundle cells have been changed to purkinje cells, whereas some others still did not represent typical characteristics of purkinje cells.

**Key words:** Atrioventricular bundle, Atrioventricular node, Heart, Histology, Ovine fetus

## Introduction

The cardiovascular diseases are one of the most causes of mortality in man throughout the world, especially in the developmental countries. In addition of higher mortality, the treatment expenses of these diseases are higher. The economical, social, industrial and psychological detriments of the affected people are extremely high. In order to understand cardiac function, it is necessary to do a histological study on the cardiac conduction system, especially the atrioventricular node (AVN) and atrioventricular bundle (AVB). For example, some of the cardiac arrhythmias are due to pathological lesions and anatomical defects in the AVN and AVB or their blood supply.

Histological study of the AVN and AVB in the fetus and comparison the fetal and adult AVN and AVB with regard to the developmental growth will provide us valuable information about these two structures.

The anatomy and histology of the AVN and AVB were studied in human being (Lev and Lerner, 1955; Titus *et al.*, 1963; James, 1970; Titus, 1973), dog and monkey (Nonidez, 1943; James, 1964), hoofed animals (Meyling and Terborg, 1957; Prasad and Sinha, 1980), rabbit (James, 1967), birds (Szabo *et al.*, 1986), lizard (Prakash, 1990), camel (Ghazi and Tadjalli, 1993, 2002), cat (Ghazi *et al.*, 1998; Tadjalli *et al.*, 1999), cattle (James, 1965), horse (Bishop and Cole, 1967), goat (Nabipour, 2002; Nabipour *et al.*, 2002) and recently in guinea pig (Nabipour, 2004). However, no comprehensive and precise information is available on the histology of the AVN and AVB of the ovine fetuses. The present study was, therefore, undertaken to deal with the histology of the AVN and AVB in the heart of four-month ovine fetus.

## Materials and Methods

Five ovine fetuses (four-month-old) were obtained randomly from a slaughter-

house in Mashhad. The age was determined by CRL method using the formula  $2.1 (CRL + 17)$  (Noakes *et al.*, 2001). After removal of the pericardium, the heart was flushed with warm (40°C) normal saline and for fixation perfused with 10% buffered formalin solution. The lower part of the interatrial septum (from the upper part level of the coronary sinus) along with the upper part of the interventricular septum was removed and submerged in the same fixative for 96 hrs. The selected samples were trimmed and processed histologically. The 6- $\mu$ m serial sections were made longitudinally starting from the right side of the samples. The sections preserved and then were selected by the interval of 3, stained with methods of Green Masson's Trichrome and Periodic Acid Schiff (Luna, 1968). The stained sections were studied under light microscope. The length and width of the AVN and AVB were measured by micrometry and their thickness was calculated by multiplying the number of sections by 6  $\mu$ m.

## Results

### The atrioventricular node (AVN)

The AVN of the four-month ovine fetuses was located at the lower part and

right side of interatrial septum and anterior to the coronary sinus near the root of aorta. Its shape was almost spherical. The nodal cells contained less myofibrils than normal myocardial fibers, so they were lighter than myocardial fibers. The average dimensions of the AVN in four-month ovine fetuses were  $0.13 \times 0.09 \times 0.28$  mm.

Within the AVN, there was a mass of interlacing bundle of fibers. There were two kinds of cells in the AVN; the "P" (pacemaker) cells and other cells with darker cytoplasm. The "P" cells were small and round or ovoid. Their cytoplasm were light and contained sparse myofibrils. They also had a large central nucleus (Fig. 1). The amount of carbohydrates in the AVN cells was more than myocardial fibers. There was a loose framework of collagen fibers and a few of vessels and nerve fibers between the AV nodal cells. Several arterioles were present at the posterior and lower parts of the AVN to supply it. These arterioles also supply the AVB and bundle branches. At the posterior margin of the AVN, many ganglia were observed. They were characteristically parasympathetic. However ganglia were not detected within the node and internodal pathways were not seen in the heart of four-month ovine fetus.

**Fig. 1: Photomicrograph showing the "P" cells in the AVN of four-month ovine fetus (Green Masson's Trichrome staining,  $\times 640$ )**

### **The atrioventricular bundle (AVB)**

Morphologically, the AVB was a continuation of the AVN. In the nodal-bundle junctional area, the irregularly dispersed fibers of the AVN assume a more orderly parallel arrangement and became the AVB. Generally, there was not detectable border between the node and the AVB (Fig. 2). The AVB passed obliquely through the fibrous ring to the apex of the inter-ventricular septum, where the RBB separated from the right lateral surface of the AVB as a compact structure. The RBB was passing through the muscular inter-ventricular septum. The LBB could not be observed.

The AVB was composed of cells that were aligned relatively parallel (Fig. 2). A few of these cells were purkinje-like cells. These purkinje-like cells were shorter, wider and lighter than the normal myocardial cells. Myofibrils were located at the periphery of the cell and a perinuclear clear zone was observed. Other cells of the AVB had darker cytoplasm. They did not represent any characteristics of the purkinje cells. In ovine fetus, multiple strands of cells in AVB were separated from one another by collagen fibers. The level of carbohydrates in the AVB cells was high. The percentage of purkinje cells was remarkably higher in the RBB than AVB. This branch was almost consisted of only purkinje cells (Fig. 3). The ganglia were not seen in the AVB and RBB, but blood vessels and nerve fibers were exit within and around the AVB and RBB which they were continuation of the blood vessels and nerve fibers of the AVN.

There was also fetal cartilage in the aortic fibrous ring of the four-month ovine fetus (Fig. 2). The os cordis was not seen in this age.

The AVB is difficult to define precisely. It may be considered to extend from the region of the nodal-bundle junctional area to the point at which the RBB is separated. With this definition, the AVB in four-month ovine fetuses had a dimension of 0.10 mm in length, 0.53 mm in width and 0.37 mm in thickness. The width and thickness of the RBB were 0.40 mm and 0.70 mm, respectively.

### **Discussion**

#### **The AVN**

The anatomical location of the AVN in the heart of four-month ovine fetuses is similar to that of rabbit (James, 1967) and guinea pig (Nabipour, 2004). Because the ostium of the coronary sinus is so large in the rabbit (James, 1967) and guinea pig (Nabipour, 2004), the AVN is displaced anteriorly and the entire region occupied by it and the AVB is foreshortened. Since mentioned animals normally have a left cranial vena cava, the ostium of the coronary sinus (embryologically derived from the terminal portion of the left cranial vena cava in most mammals) is unusually large. This effectively displaces the AVN and AVB anteriorly toward the root of the aorta. However, in sheep (Copenhaver and Truex, 1952), human being (Titus *et al.*, 1963), dog (James, 1964), horse (Bishop and Cole, 1967), cattle (James, 1965), camel (Ghazi and Tadjalli, 2002), cat (Tadjalli *et al.*, 1999) and goat (Nabipour, 2002) the AVN is located in the posterior portion of the interatrial septum, anterior to the coronary sinus. In ovine fetus, as that of the adult sheep (Copenhaver and Truex, 1952), the AVN is almost spherical in shape. It is oval or fan shaped in human being (Titus *et al.*, 1963). It is like a tiny spleen in dog (James, 1964). It has flattened oblong shape in horse (Bishop and Cole, 1967). It has an ovoid shape in cattle (James, 1965). It is an irregular elongated oval in shape in goat (Nabipour, 2002) and its shape resembled to an irregular ellipse in camel (Ghazi and Tadjalli, 2002). It has an irregular elongated oval shape in cat (Tadjalli *et al.*, 1999). It is almost spherical in shape in guinea pig (Nabipour, 2004). There is not morphologically definable AVN in avian hearts (Szabo *et al.*, 1986).

The dimensions of the AVN in four-month ovine fetuses are  $0.13 \times 0.09 \times 0.28$  mm. The dimensions of the node in human being (Titus *et al.*, 1963), horse (Schummer *et al.*, 1981), cattle (James, 1965), dog (James, 1964), rabbit (James, 1967), camel (Ghazi and Tadjalli, 2002), goat (Nabipour, 2002) and in guinea pig (Nabipour, 2004)

are shown in Table 1.

The AV node cells and their arrangement as a mass of interlacing bundles, interweaving with collagen fibers is similar to those of human being (Titus *et al.*,

1963), dog (James, 1964), horse (Bishop and Cole, 1967), cattle (James, 1965), camel (Ghazi and Tadjalli, 2002), goat (Nabipour, 2002), cat (Tadjalli *et al.*, 1999), rabbit (James, 1967) and guinea pig (Nabipour,

**Fig. 2: Photomicrograph showing the anatomical location of the AVB in the heart of ovine fetus; atrioventricular bundle (AVB); atrioventricular node (AVN); interventricular septum (IVS); fetal cartilage in the aortic fibrous ring (FC) (Green Masson's Trichrome staining, ×160). Note, only a part of the AVB is observed and the fibers of the AVB are more regular than the AVN fibers and are located in a parallel fashion**

**Fig. 3: Histological structure of the RBB in the heart of ovine fetus. Note the high number of the purkinje cells (P) (Green Masson's Trichrome staining ×640)**

**Table 1: The dimensions of the AVN in human being and some other species**

species	Length (mm)	Width (mm)	Thickness (mm)
Human being	7.5	3.7	1
Horse	6-10	5-7	0.6-2.5
Cattle	13	6-8	-
Dog	a little less than 2	a little more than 2	0.5-1
Rabbit	0.3-0.5	0.15-0.25	0.8-1
Camel	6.38±1.41	4.08±0.95	1.11±0.51
Goat	4.23	2.13	0.61
Guinea pig	0.34	0.27	0.20

2004) but there is small amount of elastic fibers scattered within the AVN in human being (Titus *et al.*, 1963), dog (James, 1964) and cat (Tadjalli *et al.*, 1999).

The AVN of four-month ovine fetus consisted of numerous of "P" cells. In this respect, it is similar to that of guinea pig (Nabipour, 2004), while the number of "P" cells in other animals is very less. The carbohydrates in the AVN cells of ovine fetus are high. However, there is not glycogen in the AVN cells of goat (Nabipour, 2002), camel (Ghazi and Tadjalli, 2002) and guinea pig (Nabipour, 2004). There is a small amount of nerve fibers within the AVN of four-month ovine fetus. In this respect, it is similar to those of human being (Titus *et al.*, 1963), dog (James, 1964), cat (Tadjalli *et al.*, 1999) and guinea pig (Nabipour, 2004). In contrast, in cattle (James, 1965), horse (Meyling and Treborg, 1957) and goat (Nabipour, 2002) abundant nerve fibers are present in the node. In the heart of four-month ovine fetus similar to those of human being (Titus *et al.*, 1963), dog (James, 1964), horse (Bishop and Cole, 1967), cattle (James, 1965), camel (Ghazi and Tadjalli, 2002), cat (Tadjalli *et al.*, 1999), rabbit (James, 1967) and goat (Nabipour, 2002) ganglia are present in the posterior part of the AVN and there were not seen ganglia in the node. There are not ganglia either at the periphery or within the node in the guinea pig. There are not seen internodal pathways in the heart of four-month ovine fetuses. While in human being (James, 1963), dog (Glomset and Glomset, 1940), rabbit (James, 1967) and guinea pig (Nabipour, 2004) the internodal pathways are exit and connected to the margins of the

AVN.

### The AVB

The AVB of ovine fetus is displaced anteriorly near the root of the aorta. This location is similar to those of rabbit (James, 1967) and guinea pig (Nabipour, 2004). The AVB is relatively small in guinea pig (Nabipour, 2004) and rabbit (James, 1967). This may be due to the foreshortening of the entire area produced by the presence of the large ostium of the coronary sinus, which drains not only the cardiac veins but normally present left cranial vena cava. The short length of the AVB in the ovine fetus is also similar to goat (Nabipour *et al.*, 2002), cattle and horse (Meyling and Terborg, 1957). In these animals due to the absence of the membranous part of the interventricular septum, the AVB extends to a shorter distance. However, in those animals which the membranous part is present, e.g. cat (Ghazi *et al.*, 1998), the AVB extends longer.

Histologically, there were two types of cells in the AVB of four-month ovine fetuses; purkinje cells and the cells that did not represent typical characteristics of the purkinje cells. In this respect, it is similar to that of guinea pig (Nabipour, 2004). The typical purkinje cells (Copenhaver and Truex, 1952), as seen in the AVB of ungulates (James and Sherf, 1971), have a distinct prnuclear light zone and have a much greater diameter than the cardiac cells. Ungulate purkinje cells are almost spherical or polyhedral and make contact with other cells at virtually their entire periphery, whereas the cells in the AVB of canine and human being are elongated and oblong in shape and make contact to some extent along their lateral margins but more often at their terminal end (James and Sherf, 1971). The partitioning of the AVB in the heart of ovine fetus is similar to that of other animals.

Unlike most of other animals, the AVB and its branches are weakly innervated in guinea pig (Nabipour, 2004).

In the heart of four-month ovine fetus, same as that of the adult sheep (Frink and Merrick, 1974), the first branch is the RBB. This pattern of branching is similar to those of dog (James, 1964), cat (Ghazi *et al.*,

1998), goat (Nabipour *et al.*, 2002) and guinea pig (Nabipour, 2004). Whereas, in the heart of human being (Titus *et al.*, 1963), horse (Bishop and Cole, 1967) and camel (Ghazi and Tadjalli, 1993) the first branch is LBB. In the heart of human being (Titus *et al.*, 1963), rabbit (James, 1967), cat (Ghazi *et al.*, 1998), goat (Nabipour *et al.*, 2002), dog (James, 1964) and guinea pig (Nabipour, 2004) the RBB is more compact and narrower than the LBB. The RBB in ovine fetuses is almost composed of only purkinje cells. This finding is similar to those of human being (James, 1970) and camel (Ghazi and Tadjalli, 1993). The RBB in guinea pig is composed of a mix of purkinje-like cells and some other cells similar to ordinary myocardial cells (Nabipour, 2004).

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