



Histomorphochemical studies on cardiac conduction system of prenatal buffalo

ANURADHA GUPTA¹, NEELAM BANSAL² and VARINDER UPPAL³

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141 004 India

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ABSTRACT

Histomorphological and histochemical studies were conducted on cardiac conduction system of buffalo heart during prenatal development. Foetuses (20) ranging from 13 cm CVRL (87days) to 104 cm CVRL (308 days) were used for study. The heart tissues were fixed in 10% buffered neutral formalin (BNF) and routinely processed. The paraffin sections of 5–7 µm thickness were stained with various stains to study the histomorphological and histochemical details. The study revealed that sinoatrial node was subepicardial and wedged at the junction of anterior vena cava with right atrium in 25 cm CVRL (130 days) foetus. Nodal and Purkinje type specialized cells were observed in sinoatrial node. The atrio-ventricular node was present in the base of atrial septum in front of coronary sinus under the endocardium. The branch of right bundle was first noticed in 13 cm CVRL (87 days) buffalo foetus. The sinoatrial node and right bundle branch showed intense reaction for acid and neutral mucopolysaccharides and basic proteins in all the age. A moderate to strong sudanophilic reaction was found in Purkinje fibers, nodal cells of sinoatrial node and branch of right bundle.

Key words: Buffalo, Cardiac conduction system, Foetus, Histochemistry, Histomorphology

Heart, the first organ to be formed and function during development (Sakabe *et al.* 2005), undergoes marked structural remodelling during foetal and postnatal development. The organogenesis of heart has always fascinated cardiologists (Allen *et al.* 1997). The conduction system of bovine heart is well developed as in ungulates and constituted by characteristic dense network of Purkinje fibres forming its ventricular conduction system (Sathyamoorthy 2003). The studies are available in adult buffalo (Borelli 1975, Prasad and Sinha 1979), goat (Shimada *et al.* 1984), camel (Ghazi and Tadjalli 1993) and pig (Crick *et al.* 1999) but detailed histomorphological and histochemical studies on cardiac conduction system had not been reported in buffalo during prenatal life.

MATERIALS AND METHODS

Heart samples of 20 buffalo foetuses of different gestational age were obtained from the Abattoir, Saharanpur. The foetal body length was measured as curved line in centimetre with the help of inelastic thread along the vertebral column between the most anterior part of frontal bone (crown) to the rump at ischiatic tuberosity and designated as curved crown rump length (CVRL) (Edward 1965). The approximate age of the foetuses was calculated by using the formula given by Soliman (1975).

$$Y = 28.66 + 4.496 X \text{ (CVRL < 20 cm)}$$

$$Y = 73.544 + 2.256 X \text{ (CVRL } \geq 20 \text{ cm)}$$

Present address: ¹anuradha71@in.com, ²bansal.neelam@rediffmail.com, ³v.uppal@yahoo.com, Department of Veterinary Anatomy.

Where Y is age in days and X is CVRL in cm.

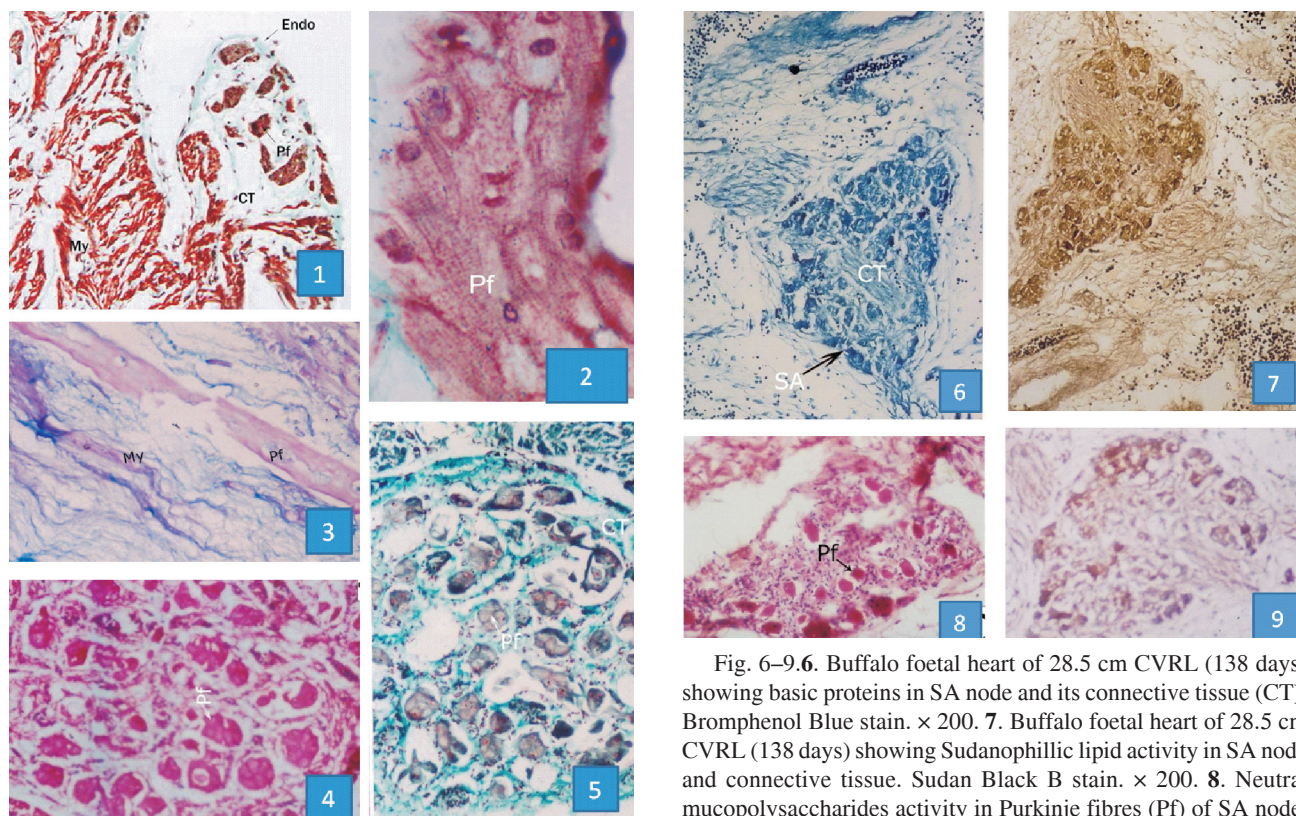
Based on CVRL the foetuses were divided into 3 groups :

- Group 1: Foetuses of CVRL between 0 – 20 cm (0–119 days)
- Group 2: Foetuses of CVRL between > 20 to 40 cm (120–164 days)
- Group 3: Foetuses of CVRL above 40 cm (165 days onwards)

The small tissue samples were collected at junction of anterior vena cava, interventricular septum and right moderator band and were fixed in 10% buffered neutral formalin (BNF) and Bouin's fixative. Once the fixation was achieved, the tissues were processed for paraffin block preparation by acetone-benzene schedule. The paraffin blocks were prepared and sections of 5–7 µm thickness were cut and obtained on clean glass slides with rotary microtome. The paraffin sections were stained with haematoxylin and eosin for routine morphology, Masson's Trichrome for connective tissue, Verhoeff's stain for elastic fibers and Gridley's stain for reticular fibers, Holme's for neuronal axons, Periodic acid Schiff for NMPS, Alcian blue for AMPS, Sudan black B for lipids and Bromphenol blue for basic proteins (Luna 1968).

RESULTS AND DISCUSSION

Cardiac conduction system is composed of sinoatrial node, atrio-ventricular node, His bundle and its bundle branches (left and right) as described by Moorman *et al.* (1998), Clements and Vigmond (2005) and Mikawa and Hurtado (2007).



Figs1–5.1. Buffalo foetal heart of 13 cm CVRL (87 days) showing the Purkinje fibers (Pf), connective tissue (CT) and endocardium (Endo) in the right bundle branch. Masson's trichrome stain. $\times 100$. 2. Buffalo foetal heart of 87.5 cm CVRL (271 days) showing mitotic figures in differentiating Purkinje fibers. Masson's trichrome stain. $\times 1,000$. 3. Ventricle of 40 cm CVRL (164 days) showing the acid mucopolysaccharides activity in Purkinje fibers (Pf). Periodic Acid Schiff / Alcian Blue. $\times 200$. 4. Neutral mucopolysaccharides activity in Purkinje fibers (Pf) and surrounding connective tissue. Periodic Acid Schiff stain. $\times 200$. 5. Collagen fibres in connective tissue (CT) surrounding Purkinje fibers (Pf). Masson's trichrome stain. $\times 200$.

Sinoatrial node (SA node): This node was subepicardial and wedged at the junction of anterior vena cava with right atrium and was seen in 25 cm CVRL (130 days) foetus. Its base was opposite the terminal crest. These findings were in agreement with findings of Borelli (1975), Kopria and Nemeseri (1984), Mathur and Shrivastava (1979), Prasad and Sinha (1979) and Sanchez – Quintana and Siew (2003). At the same age, SA node had a complex network of specialized myocyte cells with more fibrous and vascular connective tissue. Two types of specialized cells were observed in sinoatrial node i.e. nodal and Purkinje type cells and were interposed in connective tissue network. The connective tissue was composed of more collagen fibres with few elastic and reticular fibres (Figs. 6, 9). The nodal cells were thin, slender with oval or elongated centrally placed nuclei. Purkinje cells were much larger than nodal cells and were syncytially arranged. The nucleus was round or oval and centrally placed (Fig.2). Nerve bundles were also noticed at the periphery of the node. This was in

Fig. 6–9.6. Buffalo foetal heart of 28.5 cm CVRL (138 days) showing basic proteins in SA node and its connective tissue (CT). Bromphenol Blue stain. $\times 200$. 7. Buffalo foetal heart of 28.5 cm CVRL (138 days) showing Sudanophilic lipid activity in SA node and connective tissue. Sudan Black B stain. $\times 200$. 8. Neutral mucopolysaccharides activity in Purkinje fibres (Pf) of SA node. Periodic Acid Schiff stain. $\times 200$. 9. Buffalo foetal heart of 28.5 cm CVRL (138 days) showing neuronal elements in SA node. Holme's stain. $\times 200$.

agreement with the observations in canine (Kralios *et al.* 1999), pigs (Crick *et al.* 1999) and rats (Petrecca and Shrier 1998).

Atrio-ventricular node (AV node): The atrio-ventricular node was present in the base of atrial septum in front of coronary sinus under the endocardium. It was composed of specialized myocardial cells, connective tissue elements, blood vessels and nerves. This is in accordance with the observations in adult buffalo (Prasad and Sinha 1979) and pigs (Crick *et al.* 1999).

His bundle: The continuation of atrio-ventricular conduction occurred via the penetrating bundle of His in the central fibrous body. His bundle coursed down the inter-ventricular septum and divided into right and left bundle branches. The right bundle branch was first noticed at 13 cm CVRL (87 days) buffalo foetus (Fig.1). It passed through the septum musculature beneath the endocardium and gave rise to many branches. It also continued through moderator band connecting the medial and anterior papillary muscles. The left bundle branch coursed down the left side of inter-ventricular septum and divided into branches. The right and left bundle branches were also surrounded by the fibrous sheath that separated the specialized myocytes from ordinary myocardium except at the distal ramification. The right bundle branch in 13 cm CVRL (87 days) buffalo foetus was composed of Purkinje fibres surrounded by connective tissue sheath individually and in groups (Fig. 4). These fibres were syncytially arranged and resembled the

subendocardial and intra-myocardial Purkinje fibers (Fig. 5) of ventricle. The sarcolemma of Purkinje fibers was thicker than that of ordinary myocytes and their nuclei were round or oval and present in centre of cells. Mitotic figures were also noticed in nuclei, which suggested that these cells divide like other muscle cells (Fig. 2). The collagen elastic and reticular fibres were present in the connective tissue sheath. This was in agreement with the findings in goats (Shimada *et al.* 1984) and camel (Ghazi and Tadjalli 1993).

Histochemical studies: The specialized cells of sinoatrial node and right bundle branch showed intense PAS reaction (Fig.8). In groups 2 and 3 similar distribution of mucopolysaccharides was noticed with minor variations in intensity of reaction. The right bundle branch showed strong PAS positive reaction in group 3 (Fig. 8). These NMPS are used by the tissue for differentiation, development and maintenance. Prasad and Sinha (1979) reported more PAS positive reaction in Purkinje fibers and moderate in the connective tissue sheath of buffalo heart. However, Sathyamoorthy *et al.* (2008) found that PAS positive reaction was localized in myocardial cells, intense in Purkinje fibers and moderate in connective tissue.

The specialized cells of sinoatrial node and right bundle branch showed intense alcophilic reaction along with PAS positive material in group 3 (Fig. 3). The right bundle branch showed a strong alcophilic reaction in the different layers of myocardium. AMPS were mainly seen in ground substances and comprised chondroitin sulphate, hyaluronic acid and sialo mucins. These AMPS are responsible for strengthening connective tissue during development of heart in foetal life.

The intense reaction for protein was noticed in the specialized cells of conduction system, viz. sinoatrial node (Fig. 6) and right bundle branch. Similarly Prasad and Sinha (1979) noticed moderate to strong reactions in specialized cells of nodes for demonstration of protein and protein bound NH_2 .

A moderate to strong sudanophilic reaction was found in Purkinje fibers, conducting cells of sinoatrial node and right bundle branch (Fig. 7). However, the connective tissue component of the SA node showed weak reaction. The right bundle branch showed a strong sudanophilic reaction. Nagpal (1977) also reported in goat that numerous granular sudanophilic lipids were present throughout the muscle cells. Prasad and Sinha (1979) found high level of bound lipids in myocardial cells of SA node, AV node, Purkinje fibers and moderate level in the ordinary myocardial cells.

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