



Differentiation of vertebral column in buffalo during prenatal life

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The vertebral column is the defining feature of vertebrates and is clearly metameric (Pasquini *et al.* 2003). Prenatally, paraxial mesoderm gives rise to segmental somites, which dissociate to form sclerotomes and later on condense to form vertebrae (Morin-Kensicki *et al.* 2002). To date much attention has been focused on the development of vertebrae in human (O' Rahilly *et al.* 1980) and rat (Bergmann *et al.* 2006), but scanty literature is available on differentiation of vertebral column in buffalo. Hence the present study was designed to describe normal development of vertebral column in buffalo foetus.

The present study was conducted on 14 buffalo foetuses obtained from abattoir. The foetal age was calculated by using the formula given by Soliman (1975). Whole foetuses were fixed in 10% neutral buffered formalin (NBF) and processed as per acetone benzene schedule (Luna 1968). The paraffin sections of 5 to 7 µm thickness were stained with haematoxylin and eosin and Masson's trichrome stains to study the histomorphological details.

The differentiation of cervical, thoracic, lumbar, sacral and coccygeal vertebrae started at 2.0 cm CVRL in buffalo foetus. They vary in their morphology in various regions. Bergmann *et al.* (2006) stated that vertebrae differed in length-based on functional differences associated with different stresses applied to them by various muscles. In addition to minor differences in morphology between adjacent vertebrae, more substantial contrasts between vertebrae of different regions of the column have been reported by O' Rahilly *et al.* (1980). It was observed that the typical vertebra consisted of a cartilaginous body and two neural processes. These neural processes remained separated up to 2.5 cm CVRL and then began to unite with each other at 3.0 cm CVRL. At 4.2 cm CVRL each neural process was comprised of a pedicle, two articular processes, a transverse process and a lamina but the formation of spinous process could not be observed at this stage. The laminae of two sides

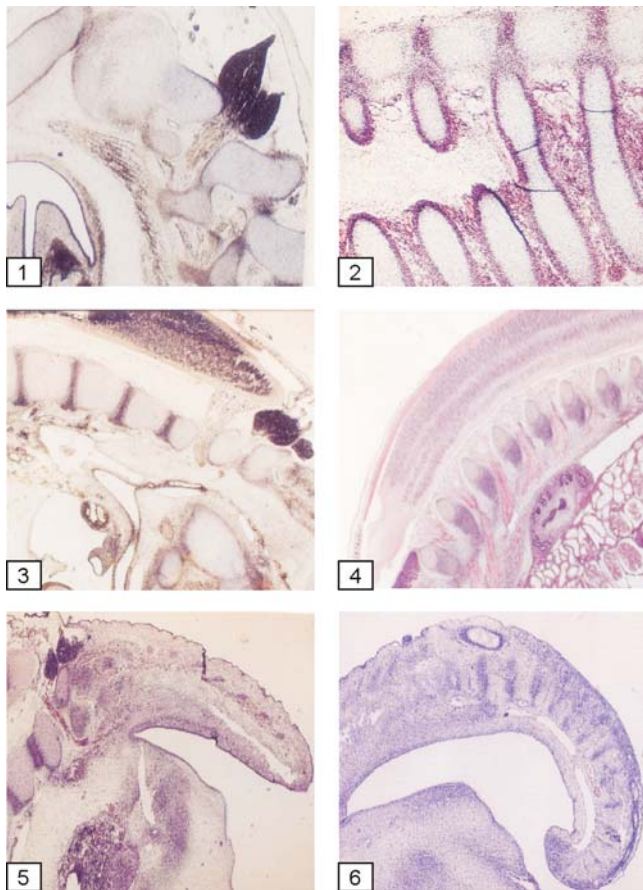
were joined by means of collagen fibres and complete fusion of two laminae of neural processes in buffalo foetii occurred at 6.0 cm CVRL, while in human embryos right and left laminae fused with each other at 5.0 cm CRL (O' Rahilly *et al.* 1980).

At initial stage of development (2.0 cm CVRL), the body of typical vertebra consisted of uniformly distributed mesenchymal tissue which condensed at 2.5 cm CVRL. At 3.2 cm CVRL the formation of chondroblast and perichondrium started. These chondroblasts migrated medially to surround the notochord at 4.0 cm CVRL. Gradually the notochord disappeared in the region of vertebral body in 4.5 cm CVRL foetus. But at the region of inter-vertebral discs notochord became expanded and formed nucleus pulposus (Castellana and Kosa 1999). Peripherally broader and centrally thin intervertebral discs were evident at 3.0 cm CVRL. These intervertebral discs were thicker in lumbar region than the cervical and thoracic region.

At 3.2 cm CVRL, typical cervical vertebra consisted of three independent osseous components i.e. a body and 2 neural arches, which are joined to each other by hyaline cartilage. The body of cervical vertebra was made up of hyaline cartilage and oval. Later on at 4.5 cm CVRL, these became narrow antero-posteriorly and wide on each side. The notochord passing through mid of the body was also visible at this stage. The cervical pedicles were long and broad with expanded free ends that articulate with the body at the neurocentral junctions. The cylindrical transverse processes arise between the articular processes and the pedicles. The first cervical ganglion was observed at the occipitoatlantal joint at 4.5 cm CVRL (Fig.1) similar to findings of Castellana and Kosa (1999) in human skeleton.

The cross section of the thoracic vertebra at 3.2 cm CVRL showed its relationship to lungs, spinal cord, spinal nerves and spinal ganglia. The facets for future ribs were also evident at this stage (Fig.2). The present study showed long vertebral lengths and large neural canal diameters around the posterior cervical and anterior thoracic region, which may coincide with the cervical enlargement and the positioning of the brachial plexus as reported by Johnson and O'Higgins (1994) in mouse.

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Figs 1–6. 1. Cervical ganglion at the occipitoatlantal joint at 4.5 cm CVRL. Haematoxylin & eosin. $\times 100$. 2. Thoracic vertebrae and facets for future ribs at 3.2 cm CVRL. Haematoxylin & eosin. $\times 100$. 3. Lumbar vertebrae at 8 cm CVRL. Haematoxylin & eosin. $\times 100$. 4. Formation of lumbosacral plexus at 3.2 cm CVRL. Haematoxylin & eosin. $\times 40$. 5. Mesenchymal proliferation of coccygeal vertebrae at 2.0 cm CVRL. Haematoxylin & eosin. $\times 40$. 6. Coccygeal vertebrae at 3.2 cm CRVL. Haematoxylin & eosin. $\times 40$.

The lumbar vertebrae showed well developed enlarged transverse processes at 8 cm CVRL in buffalo foetus. Two neural processes were also separated in this region as reported earlier in cervical and thoracic vertebrae. The body of lumbar vertebrae was flattened and larger in diameter as compared to other regions. It was also noticed that the size of body decreased from the L1 to L6 (Fig.3). These findings are in agreement with the findings of Johnson and O'Higgins (1994) in mouse. The lumbosacral plexus was clearly evident at 3.2 cm CVRL buffalo foetus (Fig.4).

The sacral vertebrae remained separated from each other and were slightly narrower than thoracic and lumbar vertebrae. The intervertebral foramina were evident and occupied by spinal ganglia. The intervertebral discs were clearly seen in sacral region of buffalo foetii at 4.5 cm CVRL.

At 2.0 cm CVRL, only mesenchymal proliferation of coccygeal vertebrae was observed, but the cartilaginous

formation was noticed at 3.2 cm CRVL (Figs. 5 and 6). The formation of 8–10 coccygeal vertebrae were observed with visible sacro- coccygeal and first inter-coccygeal space at 4.5 cm CVRL.

Thus it was concluded that the regional growth pattern of vertebral column showed at the initial stages of development (2 cm CVRL), the thoracic component was longest than other regions. It was observed that cervical region decreased and that of lumbar region increased with the advancement of foetal age which may be due to the functional demand associated with the flexibility of lumbar region as described by Pough *et al.* (2002).

SUMMARY

The present study was conducted on 14 buffalo foetuses from 0.9 to 8.0 cm CVRL (32 to 65 days) to observe the regional differentiation of vertebral column. The foetuses were fixed in 10% NBF, processed for paraffin sectioning and stained with haematoxylin and eosin and Masson's trichrome stains. It was revealed that the differentiation of cervical, thoracic, lumbar, sacral and coccygeal vertebrae started at 2.0 cm CVRL in buffalo foetus. The intervertebral discs were evident at 3.0 cm CVRL being broader at its periphery and thin centrally. The discs were thicker in the lumbar region than the cervical and thoracic regions. The length of the cervical region decreased and that of lumbar region increased with advancement of fetal age, whereas length of other regions remained unchanged.

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