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Histological study on the fibrous architectecture of the adrenal gland during prenatal development in buffalo

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ABSTRACT

The study was conducted on the adrenal glands of 43 buffalo foetuses ranging from 39 mm to 890 mm crown rump length (CRL). Very few and fine collagen fibres and reticular fibres were observed in the peripheral areas of the adrenal primordia and aggregates of neuroectodermal tissue by 109 mm CRL stage. The glands were fully encapsulated in the fibrous tissue, predominantly made up of collagen fibres by 210 mm CRL stage. The connective tissue trabeculae made up of collagen and reticular fibres arose from the inner aspect of the capsule by 250 mm CRL stage. The trabeculae pierced cortical parenchyma of the gland to reach the medulla along with the medium sized blood vessels and nerve fibres. The inner aspect of the capsule had higher proportion of reticular fibres that entered into cortical and medullary tissue and arranged the parenchyma into follicular form in 470 mm CRL and onward stages. The definite cortex and outer zone of medulla had a higher proportion of reticular fibres. The capsule became fibromuscular had high proportion of collagen fibres intermingled with the reticular fibres, smooth muscle cells and a good number of blood vessels and nerve fibres. The elastic fibres were not observed in any part of the gland up to 890 mm CRL stages, however, they were present in the wall of blood vessels and perivascular tissue.

Key words: Adrenal, Buffalo, Fibrous architecture

The prenatal development of the fibrous architecture had been reported in the adrenal gland of sheep (Boshier *et al.* 1980), goat (Hakeem *et al*. 1993), pig (Bielanska-Osuchowska 1989a) and rabbit (Coupland and Weakley 1968) and adult buffalo (Prasad 1969, Prasad and Yadava 1972) and foal (Webb and Steven 1981) but there is scanty information available on this aspect in buffalo foetuses. Hence, the present study was undertaken.

MATERIALS AND METHODS

Forty-three buffalo foetuses ranging from 39 mm to 890 mm in CRL were collected from non descript buffaloes sacrificed at abattoir, Saharanpur, Uttar Pardesh and Punjab Meats Limited, Dera Bassi, Punjab, India. Immediately after the collection of the foetuses their CRL was measured in millimeters (mm) with a calibrated inelastic thread as a curved line along the vertebral column between the most anterior part of frontal bone to the rump at ischiatic tuberosity and designated as crown rump length (Edward 1965). The approximate age of foetuses was determined on the basis of their CRL by using following formulae based on Soliman (1975)

$$Y = 28.66 + 4.496\ X \quad [CRL < 20\ cm]$$

$$Y = 73.544 + 2.256\ X \quad [CRL \geq 20\ cm]$$

where Y is the age in days and X is the CRL of foetuses in centimeters (cm). After measurement of CRL, the adrenal glands from the larger foetuses were removed, however, in the small foetuses the adrenal glands along with mesenephros and other viscera were resected and fixed in 10% neutral buffered formalin, Bouin's fluid and Orth's fluid. The fixed tissues were processed for paraffin blocks preparation by acetone benzene schedule (Luna 1968). The 5–6 µm thick paraffin sections were stained by Haematoxylin and eosin (Luna 1968), Masson's trichrome, Gridley's (Sheehan and Hrapchak 1973), Verhoeff's, Holmes and Bilenchowseky's method (Luna 1968).

RESULTS AND DISCUSSION

Collagen fibres

The fine collagen fibres were observed around adrenal primordial tissue in 39 mm CRL buffalo foetus (Fig. 1) as earlier described by Sethi and Roy (2007). At 109 mm CRL foetus very few and fine collagen fibres formed a complete

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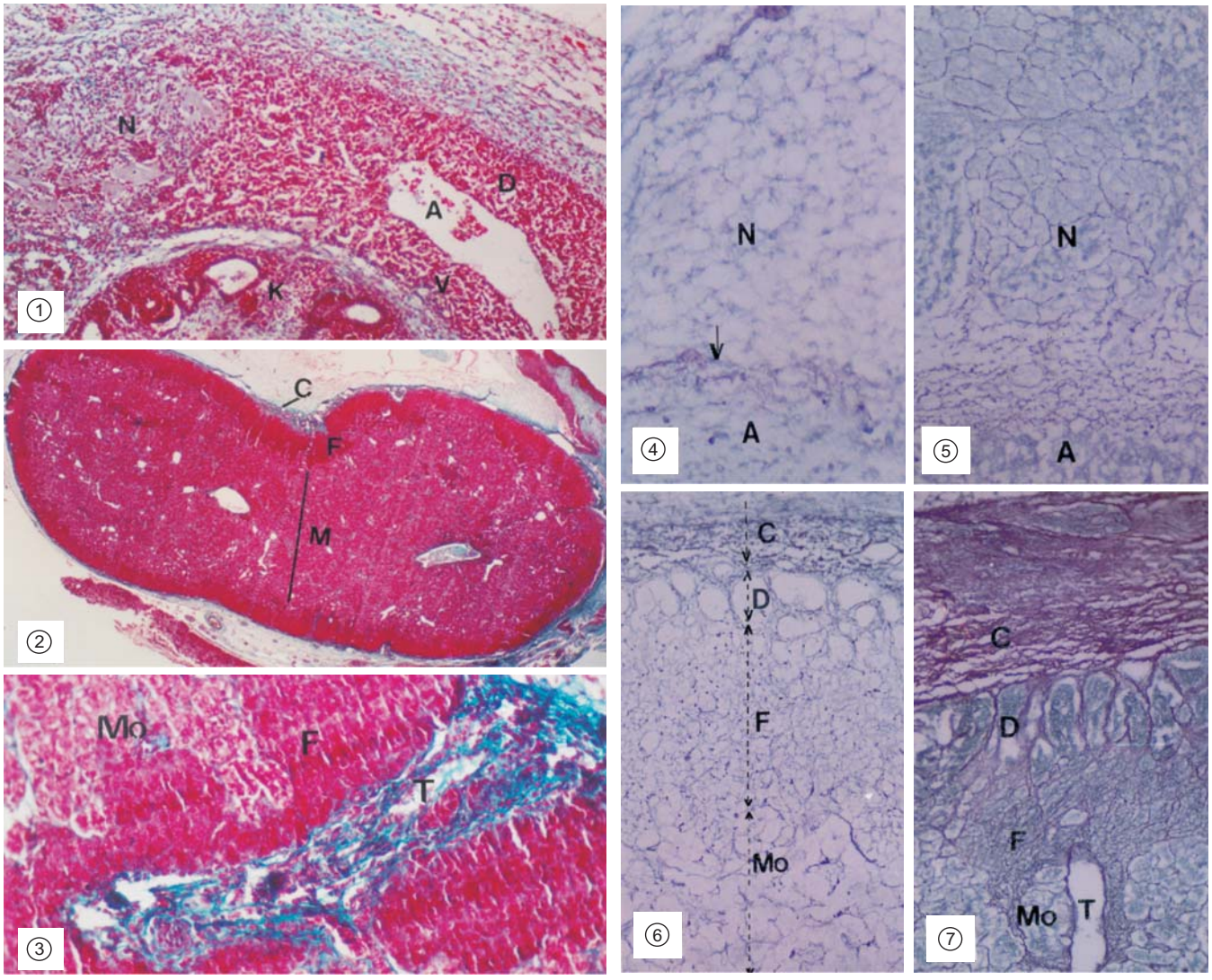
fine capsule in the peripheral areas of the adrenal primordia. Bielanska-Osuchowska (1989a) observed that in the pig foetuses between 22 mm to 35 mm CRL stage a fibrous capsule was seen on the periphery of the adrenal primordium. The fibro-collagen capsule appeared to be developed from the mesenchymal cells which surrounded the adrenal primordium. The spindle shaped cells and collagen bundles of the capsule run parallel to the surface of adrenal. However, a clearly differentiated capsule appeared around the adrenal gland of 40–80 mm CRL pig foetuses and consisted of 5–6 layers of fibroblasts and a layer of undifferentiated cells under the capsule. Bielanska-Osuchowska (1989a) further reported that between 85–150 mm CRL stages the capsule was thin and consisted of a dense layer of fibroblasts which blended into loose mesenchyme with arteries and capillaries and groups of chromaffinoblasts. In the present study, the collagen fibres were encircling the aggregates of neuroectodermal tissue.

By 210 mm CRL buffalo foetus the adrenal glands were found encapsulated in the fibrous connective tissue



Figs 1–7. Adrenal glands of buffalo foetus showing; **1**. differentiation of collagen fibrils in the dorsal (D) and ventral (V) segments of outer zone of adrenal primordium (A) at 39 mm CRL. Aggregates of neuroectodermal cells (N) and developing kidney (K) are also seen. Masson's trichrome stain × 100; **2**. a collagenous tissue capsule (C) and differentiated into foetal cortex (F) and medulla (M) at 325 mm CRL × 20; **3**. connective tissue trabeculae (T), foetal cortex (F) and outer zone of medulla (Mo) at 250 mm CRL × 200. Adrenal glands of buffalo foetus showing; **4**. fine reticular fibres (arrow) separating the neuroectodermal cells (N) and adrenal primordium (A) at 39 mm CRL. Gridley's stain × 200; **5**. well developed reticular fibres in the peripheral part of adrenal primordium (A) and neuroectodermal cells (N) at 90 mm CRL × 200; **6**. reticular fibres in capsule (C), definite cortex (D), foetal cortex (F) and outer zone of medulla (Mo) at 325 mm CRL × 100; **7**. a marked increase in the reticular fibres in the inner aspect of capsule (C), definite cortex (D), foetal cortex (F) and outer zone of medulla (Mo) at 785 mm CRL × 100.

predominantly made up of collagen fibres (Fig. 2). Fine collagen fibres were also noticed differentiating both in the cortical and medullary tissues. At location a group of medullary cells was found surrounded by collagen fibres and thus cell masses appeared in form of follicles. Coupland and Weakley (1968) also observed collagen fibres and fibroblasts in the superficial layer of the capsule of adrenal gland of rabbit foetuses as reported by Hakeem *et al.* (1993) in the capsule of the adrenal gland of goat foetuses.

In 250 mm CRL buffalo foetus the trabeculae arose from the inner aspect of the capsule piercing cortical parenchyma of the adrenal gland (Fig. 3). The medium sized blood vessels and nerve trunk found accompanying the trabeculae to the parenchyma. In some locations the trabeculae even reached up to the medulla. The connective tissue trabeculae were also reported in the adrenal gland of adult buffalo (Prasad and Yadava 1972) and foetuses of sheep (Boshier *et al.* 1980), goat (Hakeem *et al.* 1993) and pig (Bielanska- Osuchowska 1989a). Prasad and Yadava (1972) reported a fairly thick capsule in the adrenal gland of calves and adult buffaloes, which was richly supplied with blood vessels and nerve bundles. The outer part of the capsule was made up of compact bundles of collagen fibres and the trabeculae originated from the inner aspect of the capsule as reported in the adrenal gland of 136 to 147 days old sheep foetuses (Boshier *et al.* 1980). Hakeem *et al.* (1993) also observed that in the adrenal gland of goat foetuses the collagen and elastic fibres of the capsule formed the trabeculae and these trabeculae invaded the zona glomerulosa of the cortex at different points in the early foetal life. Contrary Dellmann (1993) described that in the adrenal gland of domestic animals the trabeculae originated from the capsule but rarely entered into medulla.

In the 300 mm CRL buffalo foetus the capsule was made up of high proportion of mature collagen fibres. The trabeculaes were also better developed. Some collagen fibres surrounded groups of cortical and migrating neuroectodermal cells in cortical zone. The latter cells passing through cortical zone were always encapsulated in a fibrous sheath which persisted even in the outer zone of medulla. In the 470 mm CRL and onward foetuses the capsule of the adrenal glands became fibromuscular. It was made up of high proportion of collagen fibres intermingled with the reticular fibres and smooth muscle cells. Prasad and Yadava (1972) also reported the presence of smooth muscle fibres intermingled with a few bundles of collagen fibres in the middle layer of the capsule of the adrenal gland of buffalo. In the present study, the capsule contained a good number of blood vessels and neuronal elements. These blood vessels and neuronal elements passed to the medullary tissue through cortical tissue along with trabeculae. The collagen fibres were found evenly distributed in the parenchyma of cortical and medullary zones. Webb and Steven (1981) also reported a thick and dense capsule in the adrenal gland of foal foetuses, which was due to the increased amount of collagen fibres. Bielanska-Osuchowska (1989b) observed increased collagen fibres in the capsule of adrenal gland of the pig foetuses with progression of age. In the present study, the connective tissue trabeculae became more prominent as gestation progressed and were seen passing through the cortex and terminated in the medullary tissue.

Reticular fibres

In the 39 mm CRL buffalo foetus the adrenal primordia showed the presence of fine reticular fibres in its peripheral part (Fig. 4) and in adjoining neuroectodermal cells. In the 57 mm CRL foetus differentiation of reticular fibres was almost similar as observed in the 39 mm CRL foetus. In the 90 mm CRL foetus, the reticular fibres were better differentiated. The fibres were also seen in the stromal tissue which enclosed the parenchymal cells in the form of follicles (Fig. 5). The parenchyma of the medullary primordia had better differentiated reticular fibres than the cortical primordia. Prasad and Yadava (1972) and Hakeem *et al.* (1993) also observed the presence of the reticular fibres in the adrenal gland of adult buffalo and goat foetuses, respectively. Further, Prasad and Yadava (1972) reported the blending of reticular and elastic fibres in the inner part of the capsule of adrenal gland of adult Indian buffalo. The reticular fibres continued in the capsule of the adrenal gland of goat even in the latter part of the foetal life (Hakeem *et al.* 1993).

In 325 mm CRL buffalo foetus the inner aspect of the capsule also had higher proportion of reticular fibres. These fibres entered into cortical and medullary tissue arranging the parenchyma into follicular form (Fig.6). The proportion of reticular fibres appeared to be more in the region of definite cortex and outer zone of medulla. This supports the earlier findings of Prasad (1969) in the adrenal gland of adult Indian buffalo.

In the 375 mm CRL buffalo foetus the reticular fibres became coarse and more organized in both capsule and trabeculae. There was a marked increase in the reticular fibres usually seen in the bundles at the inner aspect of capsule in 785 mm CRL buffalo foetus (Fig. 7). The fibres passed along with trabeculae from the inner aspect of capsule both into the cortical and medullary zones as reported earlier in the adult buffalo (Prasad 1969).

Elastic fibres

The Verhoeff's stained sections of foetal adrenal glands did not show the presence of elastic fibres in any part of the gland up to 890 mm CRL buffalo foetuses but they were observed in the wall and adjoining tissue of blood vessels. However, Prasad (1969) reported elastic fibres in the outer layer of adrenal capsule of adult Indian buffalo.

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