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Distribution of mucopolysaccharides in the adrenal gland of prenatal buffalo

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ABSTRACT

The study was conducted on the adrenal glands of 43 buffalo foetuses of 39 mm to 890 mm crown rump length (CRL) to investigate the distribution of mucopolysaccharides. The paraffin sections of adrenal glands were stained with PAS and AB-PAS methods to demonstrate the presence of neutral mucopolysaccharides (NMPS) and acid mucopolysaccharides (NMPS), respectively. The NMPS content was high in the inner aspect of the capsule, moderate in the wall of large blood vessels of the capsule, low in the definite and foetal cortex, and low to moderate in both the zones of the medulla. The AMPS content was low in the adrenal primordia and moderate in the aggregating neuroectodermal cells. The AMPS content was low in the capsule, negative to low in the definite cortex, low to moderate in the foetal cortex and both the zones of the medulla. The distribution of NMPS and AMPS varied in different regions of the adrenal gland of buffalo during prenatal development.

Key words: Adrenal gland, Buffalo foetus, Mucopolysaccharides

Mucopolysaccharides are the class of carbohydrates which are essential for life. The distribution of mucopolysaccharides in the adrenal gland had been reported in goat foetuses (Hakeem *et al.* 1993), pig foetuses (Bielanska-Osuchowska 1989a,b), sheep (Roy and Saigal 1987) and various domestic animals (Prasad and Sinha 1981). But scanty information is available on this aspect in the buffalo foetuses. Hence, the present study was undertaken to relate the secretory activity of the foetal adrenal gland with certain histochemical parameters at different foetal ages in buffalo.

MATERIALS AND METHODS

The study was conducted on 43 buffalo foetuses ranging from 39 mm to 890 mm in curved crown rump length (CVRL). They 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 CVRL was measured in millimeters (mm) with a calibrated inelastic thread. The approximate age of foetuses was determined on the basis of their CRL by using following formulae of Soliman (1975).

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

$Y = 73.544 + 2.256\ X$ [CVRL ≥ 20 cm]

where, Y is the age in days and X is the CVRL of foetuses in centimeters (cm). On the basis of the CVRL the buffalo foetuses were divided into 3 groups, viz. group 1 (39 mm to 200 mm CVRL; 46–118 days of gestation), group 2 (210 mm to 375 mm CVRL; 121–158 days of gestation) and group 3 (470 mm to 890 mm CVRL; 179–274 days of gestation). The adrenal glands from the larger foetuses were removed, however, in the small foetuses the adrenal glands along with mesonephros 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 cut by rotary microtome and stained with haematoxylin and eosin for routine histomorphology, periodic acid Schiff's (PAS) reaction for neutral mucosubstances and PAS-alcian blue (AB) at pH 1.0 and 2.5 for acid mucosubstances (Luna 1968).

RESULTS AND DISCUSSION

Neutral mucopolysaccharides

In group 1 (39 mm CVRL) buffalo foetus weak periodic acid Schiff (PAS) positive reaction was observed in the mesenchymal cells surrounding the adrenal primordia. However, the primitive capsule showed strong PAS positive reaction in 90 mm CVRL stage indicating the presence of high amount of NMPS (Fig. 1A). Hakeem *et al.* (1993) also observed the PAS positive reaction in the capsule of the adrenal gland of goat foetuses. Similarly Bielanska-Osuchowska (1989a) reported the presence of delicate PAS positive fibrils in the capsule of the adrenal gland of pig

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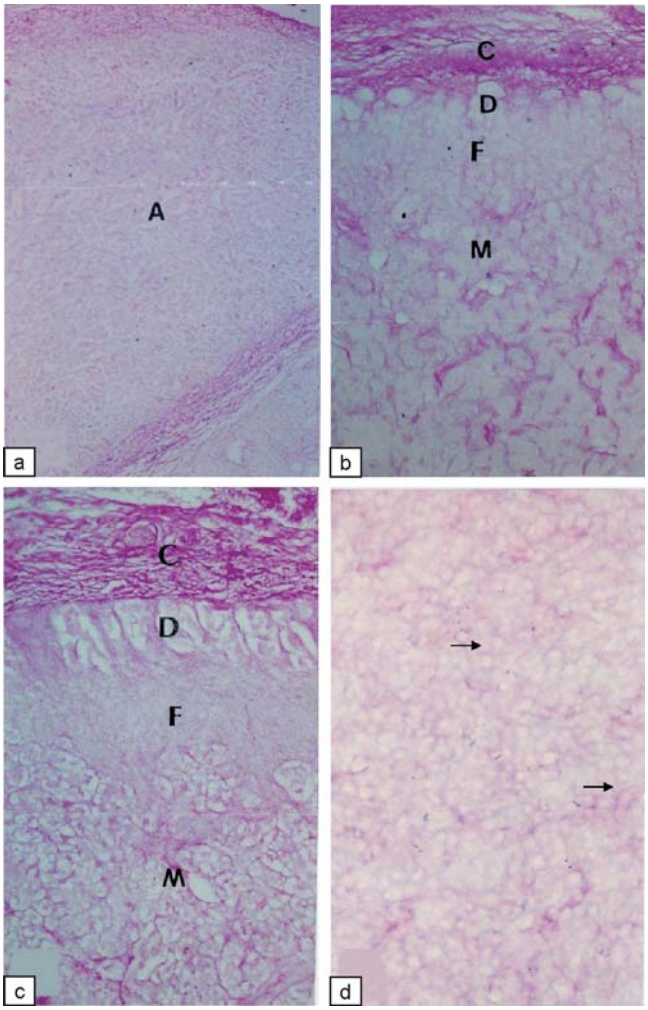

Fig. 1 (a-d). Adrenal gland of buffalo fetus showing PAS positive reaction **a.** in the mesenchymal tissue (m) surrounding the adrenal primordium (A) at 90 mm CRL stage. PAS×100; **b.** in the capsule (C), definite cortex (D), foetal cortex (F) and medulla (M) at 375 mm CRL stage×100; **c.** in the capsule (C), definite cortex (D), foetal cortex (F) and medulla (M) at 785 mm CRL stage×100; **d.** and a PAS positive rim (arrow) on the periphery of FC cells at 785 mm CRL stage×400.

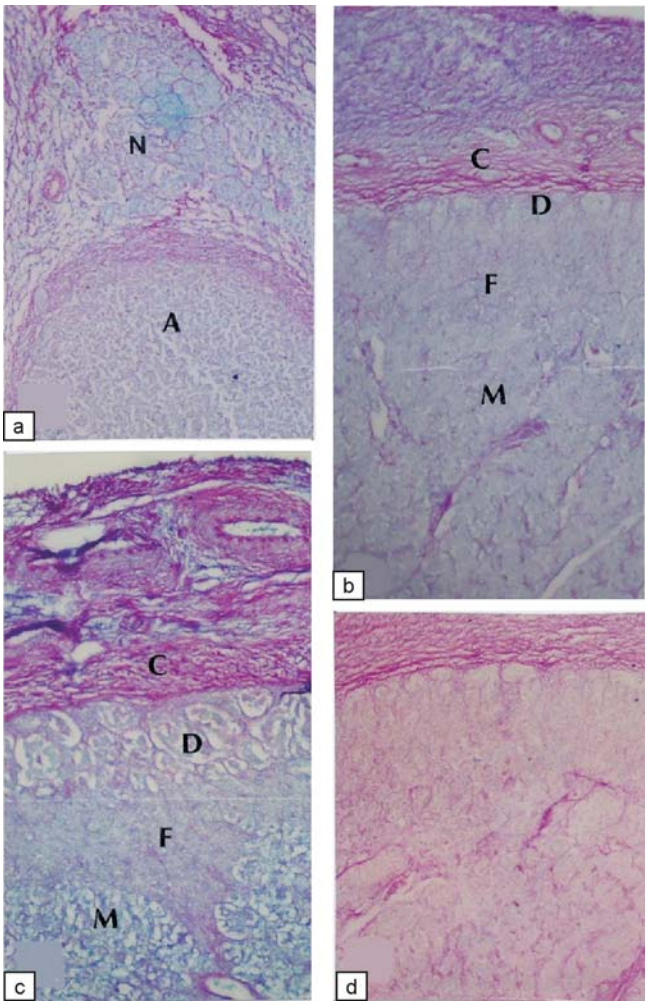

Fig. 2 (a-d). Adrenal gland of buffalo fetus showing the alcianophilic reaction **a.** in the neuroectodermal cells (N) and adrenal primordium (A) at 90 mm CRL stage. AB PAS×100; **b.** in the capsule (C), definite cortex (D), foetal cortex (F) and medulla (M) at 375 mm CRL stage×100; **c.** in the capsule (C), definite cortex (D), foetal cortex (F) and medulla (M) at 785 mm CRL stage×100; **d.** which is negative to very weak as compared to Fig. **2b.** PAS/AB at pH 1.0×100.

foetuses. A strong PAS positive reaction was also observed in the capsule of the adrenal gland of buffalo, ox, horse, sheep, goat, swine and dog (Prasad and Yadava 1974, Prasad and Sinha 1981). In the present investigation, the adrenal primordial cells were weakly PAS positive and no PAS positive reaction was observed in the aggregating neuroectodermal cells in the 90 mm CVRL buffalo foetus.

In group 2 (375 mm CVRL) buffalo foetus the capsule showed a strong PAS positive reaction especially in its inner zone and a moderate reaction was observed in the wall of large blood vessels of the capsule (Fig. 1b). The definite and foetal cortical (FC) cells were weakly PAS positive at this stage as reported earlier by Bielanska-Osuchowska (1989a,b) in pig foetuses. Prasad and Yadava (1974) also noticed a weak PAS positive reaction in the cortical cells of the fowl. The PAS positive reaction indicated the presence of glycogen as studied by Coupland and Weakley (1968) in the cortical cells of the adrenal gland of rabbit foetuses. In the present investigation, the PAS positive reaction was weak to moderate in both the outer and inner zones of the adrenal medulla. Roy and Saigal (1987) also reported a weak PAS positive reaction in the peripheral zone of the adrenal medulla of sheep foetus. However, the cells of the central zone were moderately PAS positive.

In group 3 (785 mm CVRL) buffalo foetus the inner portion of the capsule and its blood vessels were strongly PAS positive. Both definite and foetal cortical cells were weakly PAS positive (Fig. 1c). A distinct PAS positive rim

was observed on the periphery of the FC cells (Fig. 1d). Bielanska-Osuchowska (1989a,b) also observed a PAS positive rim around the FC cells of the adrenal gland of pig fetuses and electron microscopically microvilli were found on the surface of the FC cells. These microvilli formed a microlabyrinth in the intercellular spaces of the FC cells and these intercellular spaces were filled by the substances derived from plasma or steroid substances secreted by the cortical cells. The PAS positive reaction was due to presence of these substances in the intercellular spaces. The microlabyrinth facilitates metabolic exchange between the cortical cells and tissue fluid which is essential in respect to possible uptake of cholesterol from the blood and production of hormones or prohormones. Such PAS positive substance not corresponding to the basement membrane was also observed in the intercellular spaces in the foetal adrenal gland of hamster (Hillman *et al.* 1975). In the present investigation, the PAS positive rim was diffused or absent in the FC cells located in the medullary portion near the blood vessels suggesting that these were the degenerating FC cells. Bielanska-Osuchowska (1989a,b) also elucidated that the degenerating FC cells had a slightly diffuse PAS positive cytoplasm and the cells were located near the blood vessels. In the present investigation, the outer and inner zone of foetal adrenal medulla showed a weak to moderate PAS positive reaction. Sections stained for PAS with or without diastase digestion did not show any change in the intensity of the reaction for NMPS indicating that NMPS may not be glycogen as such rather it may be a conjugated carbohydrate like glycoprotein or glycolipid.

Acid mucopolysaccharides (AMPS)

In group 1 AMPS content was low in the adrenal primordia of buffalo foetuses. However, the aggregating neuroectodermal cells showed a moderate alcianophilic reaction (Fig. 2a). In group 2 (375 mm CVRL) buffalo foetus a weak alcianophilic reaction was noticed in the outer zone of the adrenal capsule (Fig. 2b). The definite cortical cells showed negative to weak alcianophilic reaction. However, a weak to moderate positive reaction was observed in the FC cells. Both outer and inner zones of adrenal medulla showed weak to moderate positive reaction for AMPS.

In group 3 (785 mm CVRL) foetus the outer zone of capsule and its blood vessels showed a negative to weak alcianophilic positive reaction (Fig. 2c). There was a weak to moderate alcianophilic reaction in the cells of the definite cortex while the reaction was weak in the FC cells. The outer and inner zones of medulla showed a moderate alcianophilic reaction. Almost similar observations were noticed with Alcian blue at pH 1.0 except that the intensity of the reaction wasvery low in all the structures (Fig. 2d) as compared to those observed with alcian blue at pH 2.5 suggesting the presence of both sulphated and non sulphated AMPS (Luna 1968).

The distribution pattern of NMPS and AMPS contents showed regional variations in the adrenal gland of buffalo during prenatal development.

REFERENCES

Bielanska-Osuchowska Z. 1989a. Prenatal development of the adrenal in pig (*Sus scrofa domestica*). 1. The differentiation and development of the adrenal cortex primordium in the first half of pregnancy. *Folia Morphol* (*Warsz*) **48** (1–4): 1–28.

Bielanska-Osuchowska Z. 1989b. Prenatal development of the adrenal in pig (*Sus scrofa domestica).* 2. Adrenal cortex development in the second part of pregnancy. *Folia Morphol* (*Warsz*) **48** (1–4): 29–58.

Coupland R E and Weakley R E. 1968. Developing chromaffin tissue in the rabbit: an electron microscopical study. *Journal of Anatomy* **102**(3): 425–55.

Hakeem N A, Sulochana S, Sharma G P and Murthy C T. 1993. Histological studies on the adrenal grand of the common Indian goat. *Indian Journal of Veterinary Anatomy* **5** (1–2): 66–77.

Hillman J E Seliger W G and Epling G P. 1975. Histochemistry and ultrastructure of adrenal cortical development in the golden hamster. *General and Comparative Endocrinology* **25**: 14–24.

Luna L G. 1968. *Manual of Histological Staining Methods of the Armed Forces Institute of Pathology*. 3rd edn. pp. 34–157 McGraw Hill Book Company, New York, USA.

Prasad G and Sinha R D. 1981. Histological observations on the adrenal medulla of domestic animals. *Indian Journal of Animal Sciences* **51**: 446–54.

Prasad G and Yadava R C P. 1974. Histological and histochemical studies on the adrenal glands of Indian buffalo. *Indian Journal of Animal Sciences* **44**: 243–48.

Roy K S and Saigal R P 1987. Histochemical study on the adrenal gland of sheep in early pregnancy. *Indian Journal of Animal Sciences* **57** (1): 29–30.

Soliman M K. 1975. Studies on the physiological chemistry of the allantoic and amniotic fluid of buffaloes at various periods of pregnancy. *Indian Veterinary Journal* **52**: 106–111.