

HISTOMORPHOCHEMICAL STUDY ON THE ADRENAL GLAND OF NEONATAL BUFFALO CALVES

Thesis

**Submitted to the Guru Angad Dev Veterinary and
Animal Sciences University
in partial fulfilment of the requirements
for the degree of**

**MASTER OF VETERINARY SCIENCE
IN
VETERINARY ANATOMY AND HISTOLOGY
(Minor Subject: Veterinary Physiology)**

By

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CERTIFICATE I

This is to certify that the thesis entitled **“Histomorphochemical study on the adrenal gland of neonatal buffalo calves”** submitted for the degree of M.V.Sc. in the subject of Veterinary Anatomy and Histology (Minor Subject: Veterinary Physiology) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Vishnudeo Kumar (L-2005-V-24-M)** under my supervision and no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE II

This is to certify that the thesis entitled
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ABSTRACT

The present study was undertaken on the adrenal gland of 29 buffaloes aging from day old to one year to investigate the histomorphological and histochemical changes during the postnatal development. The gross morphological observations revealed that the left adrenal gland was heavier, broader and thicker than the right adrenal gland. The adrenal gland was fully differentiated into cortex and medulla in the day old buffalo calf and was completely surrounded by a dense fibrous capsule. The adrenal cortex was further divided into zona glomerulosa of definite cortex and foetal cortex. The foetal cortex degenerated with progression of age; however, the remnants of foetal cortex were seen even in the one year old buffalo. The zona glomerulosa was the outer most zone under the sub capsular area and consisted of small columnar cells with spherical or oval nuclei and cells were arranged in clusters. By one week of age the zona fasciculata appeared between zona glomerulosa and foetal cortex. With progression of age the zona fasciculata became the widest zone among all the cortical zones and contained large cells with foamy cytoplasm. The zona reticularis was first time observed in the adrenal gland of one and half month old buffalo calf as the inner most zone of cortical parenchyma and had small cells with little cytoplasm and pyknotic and heterochromatic nuclei. The cells of zona reticularis were distinguished from the foetal cortical (FC) cells by the absence of PAS positive rim which was a characteristic feature of FC cells. An incomplete connective tissue condensation was observed at the corticomedullary junction in one and half month old buffalo calf which was well marked in Group – II and had large isolated cells with eosinophilic cytoplasm which were probably degenerating FC cells. In one week old buffalo calf the adrenaline and noradrenaline secreting cells constituted the outer and inner zone of the medulla, respectively and these two types of cells were differentiated by the Wood's and Giemsa methods. The medulla occupied the greater proportion of the gland and inner medullary cells were the main cellular constituent of the medulla. The neutral and acid mucopolysaccharide contents varied from low to high in the capsule, cortex and medulla. The cells of the zona fasciculata and zona reticularis showed rich contents of lipid. The Phospholipid content was more in the cortex as compared to the medulla. The concentration of basic protein was more in the cortical cells than the medullary cells.

Keywords: Adrenal, Buffalo, Histogenesis, Neonatal, Postnatal.

Signature of Major Advisor

Signature of Student

LIST OF ABBREVIATIONS USED IN THE TEXT

%	-	Per cent
µm	-	Micrometer
AMPS	-	Acid mucopolysaccharides
cm	-	Centimeter
FC	-	Foetal cortical
Fig.	-	Figure
g	-	Gram
NMPS	-	Neutral mucopolysaccharides
PAS	-	Periodic Acid Schiff
PAS/AB	-	Periodic Acid Schiff/ Alcian blue
SE	-	Standard error

CONTENTS

Chapter	Topic	Page No.
I	INTRODUCTION	1 – 2
II	REVIEW OF LITERATURE	3 – 31
III	MATERIAL AND METHODS	32 – 37
IV	RESULTS AND DISCUSSION	38 – 73
V	SUMMARY	74 – 82
	REFERENCES	83 – 90
	VITA	

LIST OF TABLES

Table No.	DESCRIPTION	Page No.
1	Particulars of buffalo used for the study	32
2	Histomorphological stains used in paraffin sections of adrenal gland of buffalo.	35
3	Histomorphological stains used in paraffin/cryostat sections of adrenal gland of buffalo.	36
4	Biometrical data showing Mean and Standard Error values of various parameters of right and left adrenal gland in different age Groups of buffalo.	64
5	Biometrical data showing coefficient of variation and correlation coefficient between age and various parameters in the adrenal gland of buffalo.	65
6	Micrometrical data showing Mean and Standard Error (in μm) values of various parameters of adrenal gland in different age Groups of buffalo.	66
7	Showing coefficient of variation and correlation coefficient of various micrometrical parameters in adrenal gland of various age Groups of buffalo.	67
8	Showing Mean \pm SE (in μm) of average density of cells per unit area in adrenal gland of different age Groups of buffalo.	68
9	Showing coefficient of variation and correlation coefficient of average density of cells per unit area in various zones of adrenal gland of different age Groups of buffalo.	69
10	Showing Mean \pm SE (in μm) of average cell size in various zones of adrenal gland of different age Groups of buffalo.	70
11	Showing coefficient of variation and correlation coefficient of average cell size in various zones of adrenal gland of different age Groups of buffalo.	71
12	Showing (percentage) proportion of different zones of cortex and medulla and corticomedullary ratio in adrenal gland of different age Groups of buffalo.	72
13	Showing histochemical distribution of mucopoly-saccharide in the adrenal gland of different age Groups of buffalo.	73

CHAPTER – I

INTRODUCTION

The adrenal gland has two embryologically distinct portions i.e. an outer cortex, which is of mesodermal origin, and an inner medulla, which develops from neuroectoderm (Dellmann and Eurell 1998). The adrenal gland in any individual is required for the beginning of the cyclic reproductive performance (Ali 1982). The adrenal cortex is further divided into three distinct zones: 1) The zona glomerulosa is the outermost zone which secretes mineralocorticoids; 2) The zona fasciculata is involved in the production of glucocorticoids and 3) The zona reticularis is the innermost zone responsible for secretion of androgens. The adrenal medulla consists of modified postganglionic sympathetic nerve cells and is subdivided into two distinct zones. The outer zone consist of large and intensely stained adrenaline cells and inner zone is made up of clusters of small polyhedral and lightly stained noradrenaline cells (Dellmann and Eurell 1998). The catecholamines secreted by the adrenal medulla play a key role in the adaptation of the new born to extrauterine life by providing both physiological and trophic signals (Slotkin and Seidler 1988). So any change in the functioning of adrenal gland brings about a series of changes in other endocrine glands (Das *et al* 1965). The postnatal development of adrenal gland has been studied in goat (Hakeem *et al* 1993a, b, kale *et al* 2003) and sheep (Boshier and Holloway

1989), but there is little information available on histological and histochemical aspect of adrenal gland with advancement of age in buffalo (Prasad and Yadava 1973). Therefore, keeping this in view the present research project is proposed on adrenal gland of buffalo with the following objectives:

- 1) To study gross morphology of adrenal gland during development in neonatal buffalo calves.
- 2) To establish the histomorphological changes of adrenal gland in neonatal buffalo calves.
- 3) To elucidate distribution of various histochemical moieties in the adrenal gland of buffalo during neonatal development.
- 4) To correlate the above finding to propose a development model of adrenal gland of buffalo with age.

CHAPTER – II

REVIEW OF LITERATURE

2.1 Gross Morphology

Nehls (1958) observed that the adrenal glands of horse were constant in form irrespective of size, sex, age or race of the animal. The weight differences between the right and left adrenal remained constant during the entire life. The ratio of cortex and medulla in the new born remained constant with the cortex being 70 to 80 per cent and the medulla 20 to 30 per cent of the gland's volume.

Groot and Fortier (1959) noticed that left adrenal gland of male albino rat was heavier than the right gland. Similar observations were recorded by Delost and Guerin (1962) in the adrenal gland of squirrel.

Howes *et al* (1960) described a comparative adrenal body weight ratio of herford and brahman (Zebu) cattle and found no significant difference between the two species. The adrenal weight in cattle was not influenced by sex. Left adrenal was larger than the right and the total adrenal weight was much smaller in relation to unit body weight than in any other mammalian species.

Last (1963) observed the right human adrenal gland as pyramidal in shape whereas the left adrenal gland was crescentic in shape and covered the medial border of the left kidney.

The gross biometrical study on the adrenal glands of ox, horse and dog was made by Raghawan (1964). The adrenal glands of ox were elongated, flattened and reddish-brown. The right adrenal was located in the anterior end of the respective kidney and occupied a part of the renal impression on the liver. The left gland was not embraced by the left kidney and was strongly curved forming a semi-circular shape. The gland was 5 to 6 cm long, 3.0 cm wide and 1.2 cm thick. Whereas in horse adrenal was reddish brown in colour and was in contact with the corresponding kidneys anteromedially. The average length and width of the gland was 8.5 to 10 cm and 2.5 to 4.0 cm respectively. The weight varied from 28 to 56 g. In dog the right adrenal was pointed at both ends lying between anterior part of the medial border of the right kidney and the posterior vena cava. The left adrenal was elongated and was along the posterior aorta.

Prasad (1969) studied the adrenal gland of buffalo and observed that the average weight of the right and left adrenal gland were 3.38 g and 3.64 g in young calves and 10.11 g and 10.23 g in adult, respectively. The average length, breadth and thickness of the right gland were 3.93 cm, 1.75 cm and 0.62 cm and that of the left were 4.33 cm, 2.16 cm and 0.67 cm, respectively in young calves. The average length, breadth and thickness of the right gland in adult buffalo were found to be 5.42 cm, 2.44 cm and 0.98 cm and in left glands 5.58 cm, 2.76 cm and 1.10 cm, respectively.

Sisson and Grossman (1977) reported that right adrenal of ox was pyramidal and left adrenal was irregularly triangular or heart shaped. Whereas in sheep and pig both the glands were bean shaped. But in dog the right adrenal was prismatic and pointed at either end and left was elongated and flattened dorsoventrally.

2.2 MORPHOGENESIS AND HISTOGENESIS OF ADRENAL GLAND

2.2.1 Organogenesis

Keene and Hewer (1927) observed that in human suprarenal gland the fetal cortex formed bulk of the gland at birth, however, it degenerated progressively during the last 10 weeks of fetal life and disappeared by the 12th month. The zona fasciculata and zona reticularis were differentiated by the 3rd week and 14th week, respectively. The medulla gradually increased in amount with progression of age.

Zwemer (1936) studied the cellular arrangement of the capsule and the zona glomerulosa of adrenal gland of rat and proposed the theory of capsular origin of cortical cells and their centripetal migration to the zona reticularis.

Cater and Lever (1954) observed that the zona intermedia was interposed between the zona glomerulosa and fasciculata in the adrenal gland of rat. The zona intermedia consisted of one or more cell layer and were clearly differentiated from the other zones.

Bloodworth and Powers (1968) reported that the adrenal gland of dog was made up of two morphologically and physiologically different endocrine glands i.e. the cortex and the medulla.

Mikhail (1973) observed that the zona reticularis appeared before the end of the first week of postnatal life in the adrenal gland of rat. The development of zona reticularis was never complete and the columns of fasciculata extended to the medulla in several part of the gland in 2nd and 3rd week. The gland consisted of an outer zona glomerulosa and a much wider inner fasciculata.

McNulty *et al* (1981) described that the inner four fifth of the fetal and new born adrenal cortex of *Macaca mulatto* was morphologically similar to the human adrenal cortex. The inner zone of the rhesus fetal adrenal was slowly remodeled into definitive cortex and the remodeling was accompanied by a loss of one-third of weight of the adrenal in the first 2 weeks postpartum. The weight of the gland remained constant up to 6 month and then showed an increase. The groups of small cells with little cytoplasm appeared in the outer part of fetal zone near parturition. These groups formed a dense band beneath the glomerulosa and showed a progressive increase during early postnatal period. The zona fasciculata appeared to its periphery. The remnants of the fetal zone were still visible adjacent to the medulla even after 6 months

of birth. According to McNulty *et al* (1981) this rim of fetal zone may be the precursor of the zona reticularis.

Boshier and Holloway (1989) observed that the basic pattern of the fetal ovine adrenal cortex was established by 100 days and after this stage the later development was primarily growth-related. There was no histological or histochemical evidence of the development of the zona reticularis by 2 days postpartum in the adrenal gland of sheep. The differentiation within the juxtacortical cells during postpartum period was largely a continuation of the changes present at 130 days. The central medullary cells increased in number but showed little changes ultrastructurally. The medullary cells during this period were more discretely organized, with several cells enclosed by a connective tissue sheath having the blood vessels and the innervating axons.

Mesiano and Jaffe (1997) described that in the adrenal gland of primate, the adult cortical zones developed from the persistent definitive zone. The postnatal remodeling of the primate adrenal cortex involved apoptosis of the fetal zone and the simultaneous expansion of pre existing zona glomerulosa and fasciculata.

Mitani *et al* (1999) observed that in the adrenal gland of rat the separation of the cortex and medulla and the development of functional zonation in the cortex started at around the time of birth. The cortical zonation was established after birth. DNA-synthesizing cells were localized near the undifferentiated zone

between zona glomerulosa and zona fasciculata, and then these cells migrated centripetally. The undifferentiated zone constituted the stem cell zone of the adrenal cortex and maintained the cell population of the cortical zones.

2.2.2 Histomorphology

2.2.2.1 Capsule

Meyers and Charipper (1956) described that the adrenal capsule of golden hamster contained both collagenous and reticular fibres. The reticular fibres were discrete and wavy, and were localized in the inner portion of the capsule, however, the collagenous fibres were found in the outermost layer of the capsule.

Maximow and Bloom (1957) observed that the capsule of human adrenal gland was made up of thick collagenous fibres, which extended through the cortex to varying depths of the parenchyma of gland as trabeculae.

Pauly (1957) described collagenous fibres in the capsule of adrenal gland of rat. There was a gradual transition from broad, wavy and red fibres to thin and pink fibres at the junction of capsule and outer most part of the cortex.

Stokoe (1959) described that the outer layer of capsule was rich in elastic fibres in the adrenal gland of sheep.

Sohal and Chaturvedi (1962) found that the outer part of the capsule was more compact as compared to the inner part in the adrenal gland of buffalo. The trabeculae originating from the inner part of the capsule penetrated the cortex.

Hewer and Foster (1966) observed that the adrenal gland of human was enclosed in a thick capsule of fibrous connective tissue with a few muscle fibres. The outermost part of the capsule was loosely arranged and merged with the surrounding areolar tissue. The trabeculae originating from the capsule penetrated the gland providing a framework and divided the glandular tissue into compartments.

Bloodworth and Powers (1968) reported that the capsule in adrenal gland of dog was composed of 5-10 layers of elongated connective tissue cells embedded in a collagenous framework. Most cells were surrounded by basement membrane, which varied in width.

Prasad and Yadava (1972, 1974) observed a fairly thick capsule richly supplied with blood vessels and nerves in the adrenal gland of adult buffalo. The compact bundles of collagenous fibres formed the outer part of the capsule, however in the inner part of the capsule there was blending of elastic and reticular fibres. Smooth muscle fibres were noticed at certain location in association with the connective tissue. Strong trabeculae made up of reticular and collagen fibres extended to the zona glomerulosa of the cortex.

Saleh *et al* (1974) described that the adrenal gland of dog was surrounded by a thick fibrocellular capsule. The connective tissue fibres extended from the Inner layer of the capsule as trabeculae.

The capsule was traversed at several points by numerous bundles of nerve fibres.

Copenhaver *et al* (1978) reported that the human adrenal gland had a tough connective tissue capsule merged with small blood vessels. Delicate trabeculae from the capsule penetrated into the cortex along with capillaries and arterioles. The reticular fibres were the main supporting tissue of the cortex.

Nagpal *et al* (1991) observed that the adrenal gland of camel was enclosed in a capsule of variable thickness which was made up of irregularly arranged connective tissue fibres with predominance of collagenous fibres over the reticular and elastic fibres. A large number of thick trabeculae descended deep from the capsule into the cortical parenchyma.

Hakeem *et al* (1993a) observed a thick fibrous capsule in the adrenal gland of goat. The measurement of the capsular thickness showed a gradual increase from birth to till maturity. The fibrous content of capsule was thick collagenous fibres in the periphery while the inner aspect consisted of loose elastic, reticular and smooth muscles fibres.

Dellmann and Eurell (1998) reported a thin capsule which was made up of dense irregular connective tissue and contained occasional smooth muscle fibres in the adrenal gland of various domestic animals. Thin trabeculae originated from the capsule and penetrated the cortex but rarely entered the medulla. The clusters

of cortical cells were also seen in the subcapsular region of the gland.

Trautmann and Fiebiger (2002) observed that the capsule was made up of elastic and smooth muscle fibres in the adrenal gland of various domestic animals. The pigment granules were found in large number in the adrenal gland of sheep and ox. Many thin and well vascularised processes extended from the capsule to the corticomedullary junction.

Kale *et al* (2003) observed that the adrenal gland in goat was covered by capsule of collagen and elastic fibres that increased in thickness with age. A number of trabeculae extended from capsule into the parenchyma. There were clusters of undifferentiated cells in the subcapsular area during early postnatal development.

2.2.2.2 Stroma

Stilling (1887) observed an extensive nerve plexus in the fibrous capsule, cortex, medulla and around the central vein of adrenal glands of horse, calf and cow.

Keene and Hewer (1927) observed that the stroma of the full time human adrenal gland was very richly supplied with nerve and collagen fibres.

Holmes (1961) found that the connective tissue at the corticomedullary boundary in adrenal gland of ferret was usually scanty and a distinct medullary connective tissue capsule had never been observed.

Mikhail and Mahran (1965) demonstrated that the nerve fibres gradually extended from capsule to zona reticularis in the adrenal gland of birth to five weeks old rat. The clusters of sympathetic and parasympathetic ganglion cells were commonly seen in the medulla.

Giordano (1967) described an intimate relationship between nervous trunks and the large vein of the central zone of the adrenal medulla in the adrenal glands of ox and sheep.

Bloodworth and Powers (1968) observed the presence of true capillaries and sinusoids throughout the adrenal gland of dog. Capillaries were more frequent at the junction of capsule and zona glomerulosa and the reticular-medullary border. The capillaries were also located in the perivascular space between the cell cords.

Prasad and Yadava (1972) noticed that stroma of the adrenal gland of buffalo was rich in blood vessels and nerves. The framework of corticomedullary portion of the gland was made up of reticular fibres and fine bundle of collagenous fibres surrounded each group of cells in the zona glomerulosa. The same fibres were arranged in radial columns in fasciculata, in irregular fashion in the zona reticularis and in alveolar fashion in medullary part. The corticomedullary junction was sharply distinguished by the presence of a thick connective layer of reticular fibres. Elastic fibres were seen scattered in the medullary portion and also in association with larger blood vessels.

Verhofstad and Lensen (1973) reported that no lymphatic vessel was found either in the fibrous capsule or the cortex and medulla in the adrenal gland of rat.

Saleh *et al* (1974) observed small and multipolar nerve cells scattered in the zona glomerulosa of the adrenal gland of dog. The nerve fibres possessed numerous dendrites that varied in length and extended in all direction and formed fine pericellular plexus around the glomerulosa cells. The zona fasciculata also contained numerous longitudinal nerve fibres and a rich plexus of nerve cells and fibres was seen in the zona reticularis.

Copenhaver *et al* (1978) noticed that the human adrenal gland was abundantly supplied with the sympathetic division of the autonomic nervous system. The majority of fibres passed to the medulla, however, a few ganglion cells were also present in the medulla.

Dellmann and Eurell (1998) reported that the stroma of adrenal gland of various domestic animals was penetrated by numerous cortical arteries that formed a dense arteriolar network which further branched into the sinusoidal capillaries of the cortex. Medullary arteries coursed through the cortex without branching and a large central venule drained the entire gland.

Trautmann and Fiebiger (2002) described that the sinusoids and capillaries were in close contact with the parenchymatous cells in the adrenal gland of ruminants. Lymphatics had been found in

association with the large blood vessels and smooth muscle fibres in the central vein of the adrenal medulla in the ruminant.

2.2.2.3 Parenchyma

2.2.2.3.1 Adrenal cortex

Keene and Hewer (1927) described that the human adrenal gland consisted chiefly of true and fetal cortex at full time of gestation. However, a very thin central zone of medullary cells was seen surrounding large blood sinuses. The small polygonal cells were present in the outer rim and replaced the fetal cortex to differentiate into true or definite cortex. The cell columns of the zona fasciculata were grouped by the third week of post natal life and the zona reticularis was defined by 3½ months.

Cater and Lever (1954) observed the cellular arrangement in the zona glomerulosa was very variable in different species. In horse, sheep and mouse single columns or double columns with arched ends were observed. While in the cat and ox the cells were arranged in small clusters, however, no definite arrangement was noticed in the adrenal gland of pig, cow and guinea pig.

Holmes (1961) described the differentiation of various cells layers of cortex in ferret adrenal. The zona glomerulosa was well developed and consisted of single or double column of cells forming loops immediately beneath the capsule. The zona fasciculata was made up of outer and inner parts without any distinct demarcation between the two. The zona reticularis was extremely variable both

in its prominence and in its cellular constitution. The cells of this zone were smaller and irregularly arranged with abundant connective tissue between them.

Bloodworth and Powers (1968) found that the adrenal gland of dog consisted of two morphological and physiologically different endocrine parts i.e. cortex and medulla. The cortex was divided into 4 zones i.e. glomerulosa, intermediate, fasciculata and reticularis. The zona glomerulosa was composed of glomeruloid groups of epithelial cells lying just under the capsule. The cells were arranged in arch like or circular groups or as large curving (corkscrew) cords. Each cord was surrounded by a basement membrane and a delicate connective tissue sheath containing capillaries. The group of glomerulosa cells was directly continuous with a cord of fasciculata cells. The cells of glomerulosa were usually of a tapering and/or columnar shape with the large end of the cell containing the nucleus located centrally in the cord and the small end of the cell was adjacent to the peripheral capillary in the sheath of the cord. The intermediate zone was observed as 4-5 flattened dark cells and was located between zona the glomerulosa and the zona fasciculata. The zona fasciculata was the widest zone of cortex and was made up of two types of larger polyhedral cells viz. clear (light cell) and compact cells (dark cells) which were arranged in radially orientated cords running at right angle to the capsule. The multicellular cords of the fasciculata with basement membrane-

collagen sheath seemed to fragment as they entered the reticularis. The reticular zone also had clear and compact cells and the clear cells were similar to those of fasciculate in all respects except that they had reduced lipid content. The compact cells were of uniform dense osmophilic type with sparse lipid droplets.

Prasad and Yadava (1974) observed that the adrenal cortex of Indian buffalo was divided into zona glomerulosa, zona intermedia, zona fasciculata and zona reticularis. The zona glomerulosa was located below the capsule and consisted of cells with lightly stained basophilic cytoplasm. Most of the cells were having scanty cytoplasm and centrally located spherical nuclei with single nucleolus. The cells were arranged in groups or cords and the two cords of cells were connected at the capsular end by an arch of cells. The zona intermedia was observed as an indistinct zone with many closely packed nuclei between the zona glomerulosa and the zona fasciculata. The zona fasciculata was the widest zone among the other cortical zones and was made up of large polygonal cells arranged in radiating columns. The centrally located nuclei were spherical in shape and cells had a homogenous cell cytoplasm. There was no distinct demarcation between the zona fasciculata and the zona reticularis. The zona reticularis was the smallest among the cortical zones and was consisted of eosinophilic cells with deeply stained nucleus arranged in small irregular groups.

The zona reticularis was comparatively better developed in adults than in young ones.

Jamdar and Ema (1982) observed incomplete cortical lobulation in the adrenal gland of donkey. The medullary tissue extended to the periphery of the gland in the form of podia.

Prasad and Sinha (1984) described that a distinct zona glomerulosa was discernible in the adrenal gland of buffalo and bullock. However, a clear arcuate zone was present in the horse and dog. The zona glomerulosa was comparatively less distinct in goat, sheep and pig. A few medullary cells trapped in the glomerulosa zone were also seen in the adrenal gland of sheep.

Boshier and Holloway (1989) identified a distinct zona glomerulosa and zona fasciculata in the adrenal gland of sheep by 100 days of gestation, however the zona reticularis was not differentiated even by 2 days postpartum.

Nagpal *et al* (1991) described that the cortical parenchyma presented three classical zones in the adrenal gland of camel. The glomerulosa cells were arranged in small clusters or in 1-2 cell thick cords separated by sinusoids. The cell cytoplasm was basophilic, granular and contained spherical to ovoid vesicular nucleus with prominent nucleolus. Binucleated cells were commonly seen. The zona fasciculata was the widest zone and was differentiated into an outer thinner and inner thicker sub zones. The outer part consisted of polyhedral cells with foamy and clear

cytoplasm and were oriented longitudinally whereas the inner part was formed by 2-3 cell layers. The cytoplasm of cells was granular and a little more acidophilic. The cells of zona reticularis resembled the cells of inner part of zona fasciculata.

Hakeem *et al* (1993a) observed that in the adrenal gland of goat in early fetal life the cortex consisted of cells without proper zonation. Whereas in later life cortex was demarcated from the rest of the zones. The cortex was subdivided into three zones - zona glomerulosa, zona fasciculata and zona reticularis. The zona glomerulosa was the outer most zone underlying the capsule, forming approximately the outer one sixth or one fourth of cortex. The zona fasciculata was the broadest zone and constituted 70 per cent of cortex in 6-9 months of postnatal life. The zona reticularis occupied approximately 15 per cent of the cortex.

Dellmann and Eurell (1998) described that the adrenal cortex was having three distinct zones in various domestic animals. The zona glomerulosa in ruminants consisted of irregular cluster and cords of cells with abundant smooth endoplasmic reticulum, tubular type mitochondria, little rough endoplasmic reticulum, a multilocular Golgi complex near to nucleus and a few lipid droplets. A zona intermedia occurred in ruminant. The zona fasciculata consisted of radially arranged cords of cuboidal or columnar cells similar to the zona glomerulosa and had more number of lipid droplets. The zona reticularis was made up of an irregular network

of anastomosing cell cords. The cells were polyhedral and contained fewer lipid droplets and more lipofuscin.

Kale *et al* (2003) observed that the adrenal cortex in goat differentiated into zona glomerulosa, zona fasciculata and zona reticularis. The thickness of zona glomerulosa was lesser than other two zones and glomerulosa cells were arranged in cluster of cuboidal cells with central and oval nuclei and peripheral cytoplasm in 0-8 months group animals. Whereas polygonal and oval cells with darkly stained nuclei in 9-18 months group and adult animals were observed in the zona glomerulosa. The zona fasciculata was widest zone arranged in single or double cell cord with the sinusoids in between. The zona reticularis was the innermost zone of the cortical parenchyma and cells of this zone were arranged in form of thick cords. The cells were polyhedral in shape and their nuclei were located in the center. The sinusoids in this zone were comparatively greater in size.

2.2.2.3.2 Adrenal medulla

Keene and Hewer (1927) observed that the medulla formed a central core in the adrenal gland of human during early postnatal life. The medullary cells were arranged in irregularly sized clumps and were of two types; i.e. small round cells with darkly staining nuclei and larger and some what irregular shaped cells of varying size showing a strong chromaffin reaction.

Wetzstein (1957) identified two types of chromaffin cells in the adrenal medulla of mouse, guinea pig and cat. The most numerous forms were the chief cells (adrenaline containing cells) while the other were named as the accessory cells (Noradrenaline storing cells). The accessory cells were having larger chromaffin granules, more osmophilic nucleus and more closely packed endoplasmic reticulum than the chief cells.

Sohal and Chaturvedi (1962) observed that the medullary cells of Indian buffalo were polygonal in shape with well-defined boundaries. The cells had scanty basophilic granular cytoplasm and were separated by sinusoids.

Duncan *et al* (1962) distinguished two types of medullary cells in the adrenal gland of hamster. The adrenaline storing cells had highly electron dense cytoplasm, osmophilic granules of 700-1000A° diameter and were mainly peripheral in distribution. The cytoplasm of the noradrenaline storing cells showed greater electron density and a constant large chromaffin granules (1000-3500A°).

Wood (1963) clearly differentiated the adrenaline and nor adrenaline secreting cells in the adrenal medullary tissue in 2-5 µm thick paraffin sections by differential fixation and staining. The two cell types were identified by differences in their staining ability and by visualization of characteristic cytological differences. The adrenaline secreting cells had a granular cytoplasm that was brown to purple in colour and were aggregated in islets with the nuclei

located towards the center of the islet. The noradrenaline secreting cells were stained yellowish and were having comparatively less homogenous cytoplasm. These smaller cells were more closely packed and were observed as solitary cells within patches of adrenaline secreting cells.

Coupland *et al* (1964) also differentiated the adrenaline and noradrenaline storing granules under the electron microscopy. In the glutaraldehyde fixed and osmicated sections of the adrenal medulla, the noradrenaline storing granules were intensely osmophilic and hence electron dense, while the adrenaline storing granules were moderately electron dense in rat, cat and mouse.

Elfvin (1965) identified two types of chromaffin cells in rat adrenal medulla. The most common cell type was characterized by having granules that were round to oval in shape and about 0.1-0.3 μm in diameter. A light space of about 50-300 \AA usually separated the opaque material from the surrounding membrane and this type of cell was referred as type-I. The granules of second cell type were mostly round and had a fusiform or more irregular shape. They contained material with a higher electron density and finer granularity than that of the granules of the type-1 cell. Part of the opaque material was always in close contact with the membrane. The cells of the intermediate granules type were frequently seen in the medulla of newborn and 2-day-old animals (Elfvin 1967). The cell having only the very dense type of granules were frequently

observed. At 4-8 days after birth, two cell types which closely resembled those found in the medulla of the adult animal were clearly distinguished on the basis of the structure of the granules.

Smollich (1966, 1967) clearly distinguished the adrenaline and noradrenaline secreting cells in different species of ruminants and observed that adrenaline secreting cells were always higher in ratio and increased proportionately with advancing age. The noradrenaline containing cells were limited to the centre of the medulla, whereas the adrenaline secreting cells formed the peripheral part of the medulla in the adrenal gland of cattle, sheep, swine and horses.

Zamora *et al* (1967) observed two distinct cellular layers in the adrenal medulla of the calf. The outer layer corresponded to the adrenaline and the inner one to the noradrenaline secreting cells.

Bloodworth and Powers (1968) reported that the bulk of the adrenal medulla in dog was composed of chromaffin cells, which were arranged in round groups of 10 to 15 cells or cords of 5-6 cells thick. Within the cords or groups of chromaffin cells, two distinct types of parenchymal cells were identified i.e. the light and dark cells which had been related to adrenaline and noradrenaline secreting cells, respectively.

Brown *et al* (1970) observed two distinct types of medullary cells in the adrenal gland of rat, however, an obvious cell difference was not found in the medullary cells of human. But the human

medullary cells showed a clear difference in density of chromaffin granules.

Prasad and Yadava (1973, 1974) reported that in the adrenal gland of adult Indian buffalo there was a distinct demarcation between adrenal cortex and medulla even in the unstained paraffin section. After fixation in the formal-dichromate solution the medullary portion was yellowish in colour, in contrast to the cortical portion which remained colourless. The adrenal medulla was distinctly arranged in two layers, viz. an outer portion corresponding to the adrenaline secreting cell zone and an inner or the central portion around a large central vein represented the noradrenaline secreting cell zone. The adrenaline containing cells were of brownish granular consistency and the noradrenaline cells were yellowish in colour after staining with Wood's method. The homogenous granular adrenaline secreting cells possessed a spherical nucleus located more towards the apical portion of cells with one or two eccentrically placed nucleoli. These cells were located in the outer portion of medulla. The noradrenaline secreting cells were having less homogenous and yellowish granular cytoplasm. The nucleus was located more towards the centre of the cell with one or generally two eccentrically placed nucleoli.

Copenhaver *et al* (1978) observed that the human adrenal medullary cells were ovoid in shape and arranged in groups or

anastomosing cords. The cell granules stained green with ferric chloride, yellow with iodine and brown with osmic acid.

Jamdar and Ema (1982) observed that in adrenal gland of donkey the medulla formed the common component to the cortex; however, island of cortical components was seen in the medulla. The juxta medullary zone contained most of the ganglion cells.

Dellmann and Eurell (1998) described that the adrenal medulla consisted of modified postganglionic sympathetic nerve cells and was divided into two zones. The outer zone was made up of large and intensely stained adrenaline secreting cells and inner zone was constituted by cluster of polyhedral and lightly stained nor adrenaline secreting cells.

Trautmann and Fiebiger (2002) described that the medullary cells were polygonal in shape in large ruminants, however columnar in swine and sheep. These chromaffin cells stained yellow or brown on chromic acid fixation.

2.3 Micrometry

Silver (1960) observed that the percentage of adrenaline secreting cells increased with age from 51 to 75 per cent during the period from 3 hours to 22 days in calves. The total adrenaline secreting cells per gland rose sharply from about the 10th day of postnatal life, while the noradrenaline secreting cells remained constant during the period of investigation.

Sohal and Chaturvedi (1962) found that the average thickness of the capsule was 149 μm in the adrenal gland of Indian buffalo. The average width of the zona glomerulosa was 166 μm and the average size of the glomerulosa cells was found to be 6 μm . The average density of cells was 25 cells per column. The average thickness of the zona fasciculata was 747 μm with average size of a single cell being 10 μm and the cells were polygonal in shape with a spherical nucleus. The average thickness of zona reticularis was 315 μm and the average cell size was 8 μm .

Dvorak (1972) observed a continuous upward trend in the adrenal weight of foetuses and piglets. A particularly rapid increase in absolute adrenal weight was found during the last day of prenatal development.

Prasad and Yadava (1974) described that the average thickness of the adrenal capsule was 150 μm and 253 μm in young and adult Indian buffaloes, respectively. The average width of the zone glomerulosa varied from 178 μm to 234 μm in young and adult animals. The average cell and nuclear size was 8.91 μm and 4.38 μm and 9.35 μm and 5.59 μm in young and adult animals, respectively. The average cell size of the zona intermedia was 7.10 μm . The zona fasciculata was the widest among the three cortical zones with average width of 337 μm and 474 μm in young and adult animals, respectively. The average size of cell and nucleus in the zona fasciculata was 10.96 μm and 6.62 μm and 11.77 μm and

7.10 μm in young and adult animals, respectively. The average width of the zona reticularis in young and adult animal was 66 μm and 143 μm , respectively. The average size of cell and nucleus in this zone was 10.80 μm and 6.32 μm and 11.47 μm and 6.39 μm in young and adult animals, respectively. The average width of medulla in young and adult animals was 875 μm and 1,235 μm respectively. The average size of medullary cell and nucleus in young and adult animal was 9.86 μm and 5.99 μm and 10.76 μm and 6.32 μm , respectively.

Kale *et al* (2003) observed that the thickness of adrenal capsule was $44.36 \pm 3.69 \mu\text{m}$ in 0-8 months old goat, which increased to $75.15 \pm 4.77 \mu\text{m}$ in adult animal. Similarly the thickness of zona glomerulosa increased from $191.63 \pm 7.06 \mu\text{m}$ in 0-8 months old goat to $243.75 \pm 4.77 \mu\text{m}$ in adult animals. The diameter of cells of this zone varied from $8.87 \pm 1.00 \mu\text{m}$ to $11.25 \pm 0.58 \mu\text{m}$ in day old to adult animal. The zona fasciculata also showed an increase in thickness from $548.30 \pm 13.34 \mu\text{m}$ to $898.18 \pm 15.76 \mu\text{m}$ with progression of age from 0-8 months to adult animals. The average diameter of cells of this zone varied from $9.44 \pm 1.09 \mu\text{m}$ to $11.99 \pm 0.82 \mu\text{m}$ in day old to adult animals. The thickness of zona reticularis was $240.17 \pm 8.52 \mu\text{m}$ and $255.85 \pm 6.36 \mu\text{m}$ and diameter of cells of this zone was $9.43 \pm 0.73 \mu\text{m}$ and $12.28 \pm 0.76 \mu\text{m}$ in 0-8 months old and adult animals, respectively.

2.4 Histochemistry

2.4.1 Carbohydrate

Hunt and Hunt (1959) observed that glycogen content was present in variable amounts in the capsule, zona fasciculata, zona reticularis and medulla in the adrenal gland of different species of rats in age group from 2 to 580 days. The collagen was uniformly present in the reticularis layer, however, did not occur in the zona glomerulosa.

Girod (1960) studied the glycogen content in the adrenal gland of monkey and rabbit by using Mac Manus technique and found the presence of glycogen in the zona glomerulosa of monkey, however no glycogen was found in rabbit adrenal.

Hopwood (1968) observed the PAS positive reaction in the cells of adrenal medulla of rat, rabbit, hamster, ox, pig and sheep. The PAS positive reaction was present only in the cryostat sections and potassium dichromate fixed material but not in formaldehyde fixed paraffin sections. The connective tissue elements and nerve fibres also showed PAS positive reaction in ox, sheep and pig.

Coupland and Weakley (1970) noticed that glycogen was not observed in the chromaffin cells of rabbit adrenal after birth.

Prasad and Yadava (1974) observed a strong PAS-positive reaction in the capsular connective tissue, trabeculae and cells of zona reticularis, a moderate reaction in the cells of zona fasciculate

and medulla and a negative reaction in the cells of zona glomerulosa in adrenal gland of Indian buffalo.

Hakeem *et al* (1993b) noticed a PAS positive reaction in the capsule, the trabeculae of the cortex and the fibres surrounding the cords of medulla in the adrenal gland of goat. The cells of zona glomerulosa was also positive for PAS, which was proved to be glycogen by PAS diastase reaction. The cells of zona fasciculata showed a lighter PAS positive reaction than the cells of the zona glomerulosa. The zona reticularis cells also showed the presence of glycogen. The fibres penetrating from zona reticularis into the medulla were also rich in glycogen.

2.4.2 Lipid

Keene and Hewer (1927) observed that in the full time human adrenal gland the fetal cortex contained no lipid, whereas the cells of the developing true cortex showed the presence of lipid contents which were increasing in amount with progression of age. After birth the adrenal cortex showed a well marked lipoid reaction in cells of the zona glomerulosa, fasciculata, and also in certain cells of the medullary region. At the corticomedullary junction there was a narrow band without having any lipid contents and this zone corresponded to the region of the degenerating remains of the fetal cortex.

Bennett (1940) observed little or no lipid contents in the cells of zona reticularis of the adrenal gland of rat.

Robinson and Yoffey (1950) showed a faint lipid reaction in the cells of zona reticularis by a special stain in the adrenal gland of rat.

Cater and Lever (1954) studied the comparison of lipid distribution in outer adrenal cortex of different animals. In rabbit and cat the zona intermedia was strongly sudanophilic and the glomerulosa contained few lipids, however, in rat, dog and horse in which the zona intermedia was sudanophobe the glomerulosa lipid contents were usually present in large quantity. In the cow, ox and sheep small quantities of lipids were equally distributed in the zona glomerulosa and intermedia. While in the mouse and guinea pig there was an upward lipid gradient from the zona glomerulosa through the zona intermedia to the outer zona fasciculata. The zona glomerulosa appeared completely devoid of lipid in contrast to the heavy content of the zona fasciculata in the adrenal gland of pig. At birth little amount of lipid was observed in the outer most zone of adrenal cortex but by 3rd day a lipid containing zona glomerulosa and a lipid free zona intermedia was clearly recognizable. The most of the lipid in the zona fasciculata was in its outer layers with a lipid free zone bordering on the medulla. The phospholipids were observed in large amount in the zona fasciculata however, small amount of phospholipids was found in the zona glomerulosa in the adrenal gland of pig. The zona intermedia were completely devoid of phospholipid content.

Mikhail (1973) demonstrated a large amount of lipid content in the zona glomerulosa with variable empty patches and a sudanophobic zone containing few or no lipid droplets under the zona glomerulosa. The outer fasciculata was heavily loaded with lipoids and its cells contained coarse to medium-sized lipid droplets. The fasciculata, reticularis and juxtramedullary zones appeared similar and were either depleted or contained tiny lipid droplets.

Badawy *et al* (1982) observed the presence of lipid droplets in the cells of the outer fasciculata in the adrenal gland of camel.

Nagpal *et al* (1991) also observed the presence of large quantities of lipid in the cortical cells of camel adrenal gland. The cells of outer part of zona fasciculata contained mostly the neutral lipid with scanty acidic components. The relative concentration of lipid decreased in the inner part of zona fasciculata and zona reticularis.

Hakeem *et al* (1993b) noticed that the capsule of the adrenal gland of goat was negative for lipid. The intensity of lipid was less in the zona glomerulosa. The glycolipid contents were more in zona fasciculata than the zona glomerulosa and reticularis.

Dellmann and Eurell (1998) observed that the cells of zona fasciculata possessed large number of lipid droplets as compared to the zona glomerulosa. The zona reticularis had fewer lipid droplets.

Singh *et al* (1999) demonstrated a strong reaction for sudanophilic lipid in the zona fasciculata in the adrenal gland of buffalo calves. The lipid contents were low in the zona glomerulosa and the zona reticularis. However, sudanophilic lipids were not observed in the cells of adrenal medulla.

2.4.3 Protein

Vyas and Jacob (1976) observed that the subcapsular and central zone of the interrenal cells showed high concentration of protein than the medullary cells in the adrenal gland of birds. The adrenal cells did not show any variations in the concentration of protein during sexually active and inactive period.

Sethi (2005) described that the concentration of basic protein in the adrenal gland of buffalo foetuses increased with the progression of age. The cortical cells contained high concentration of proteins than the medullary cells and concentration of protein was more in the adrenaline secreting cells than the noradrenaline secreting cells.

CHAPTER - III

MATERIAL AND METHODS

The present study was conducted on the adrenal glands from 29 buffaloes ranging from day old to one year of age. The adrenal glands were collected from non descript buffaloes sacrificed at abattoir, Saharanpur, Uttar Pradesh, college dairy farm, college clinic and Department of Vety. Anatomy. The age of the animals was calculated by the dental formulae (Saini *et al* 1982).

On the basis of age the animals were divided into three Groups viz. Group- I (Day old to 3 months), Group- II (more than 3 months to 6 months) and Group- III (more than 6 months to 1 year) as indicated in Table 1.

Table 1: Particulars of buffalo used for the study

Sr. No.	Age	Source
1	6 month	Slaughter house Saharanpur (UP)
2	6 month	-do-
3	1 year	-do-
4	7 month	-do-
5	8 month	-do-
6	8 month	-do-
7	5-6 month	-do-
8	6 month	-do-

9	5-6 month	-do-
10	4 month	-do-
11	9 month	-do-
12	1 year	-do-
13	9 month	-do-
14	8 month	-do-
15	1 month	College Diary Farm
16	9 month	Department of Anatomy
17	9 month	-do-
18	9 month	-do-
19	9 month	-do-
20	6 month	-do-
21	Day old	College clinic
22	1.5 month	Department of Anatomy
23	3 month	-do-
24	1 month	-do-
25	1.5 month	-do-
26	1 st week*	Slaughter house Saharanpur (UP)
27	2 nd week*	-do-
28	3 rd week*	-do-
29	4 th week*	-do-

* These sample were used for cryostat sectioning.

3.1 Fixation and Processing

Immediately after collection the adrenal tissues were fixed in different fixatives for the histomorphochemical study. Fixatives used during the course of the study were 10 percent neutral buffered formalin and orth fluid. Orth fluid was used for the demonstration of chromaffin cells. After complete fixation was achieved the tissue were processed for paraffin blocks preparation by acetone benzene schedule (Luna 1968). The paraffin blocks were prepared and sections of 5-6 μm thickness were obtained on glass slide with the help of rotatory microtome. These paraffin sections were used for various histomorphological and histochemical staining methods.

3.2 Cryotomy

Fresh unfixed samples of adrenal glands from four animals were collected and these were quickly frozen at -20°C in the deep freezer. These tissues were subjected to cryostat sectioning to obtain 10-12 μm thick sections. These cryostat sections were stained with Sudan Black-B, Baker's Acid Haematin and Schultz's method for demonstration of lipids, phospholipids and cholesterol, respectively (Chayen *et al* 1969).

3.3 Histomorphochemical Technique

The paraffin sections of 5-6 μm thickness were stained by different histological staining methods as given in Table 2 and Table 3.

Table 2: Histomorphological stains used in paraffin section of adrenal gland of buffalo calves

Purpose	Method	Source
Routine histomorphology	Hematoxylin and Eosin and Phaloxin	Luna (1968)
Collagen fibres	Masson's trichrome	Luna (1968)
Reticular fibres	Gridley's	Sheehan and Hrapchak (1973)
Elastic fibres	Verhoeff's	Sheehan and Hrapchak (1973)
Nerve cells and fibres	Holme's	Luna (1968)
Nerve cells and fibres	Bielschowsky's	Luna (1968)
Medullary cells	Wood's	Wood (1963)
Chromaffin cells	Giemsa	Pearse (1968)

Table 3: Histochemical techniques used in paraffin/cryostat sections of adrenal glands of buffalo calves

Purpose	Method	Source
Neutral mucopolysaccharides	Periodic acid schiff	Sheehan and Hrapchak (1973)
Acid-mucopolysaccharides	Alcian blue a) at pH 1.0 b) at pH 2.5	Luna (1968)
Lipid	Sudan Black B	Chayen <i>et al</i> (1969)
Phospholipid	Baker's acid Haematin	Chayen <i>et al</i> (1969)
Cholesterol	Schultz method	Chayen <i>et al</i> (1969)
Basic protein	Bromphenol blue	Chayen <i>et al</i> (1969)

3.4 Gross Anatomical Studies

The shape, weight, maximum length, maximum width and the average thickness of each adrenal gland were recorded to study the gross anatomy of the gland during various stages of postnatal development.

3.5 Micrometrical Observations

Micrometrical observations were recorded on the haematoxylin and eosin stained section of the adrenal gland of buffalo calves with the help of filar and ocular micrometer duly

calibrated with stage micrometer. The micrometrical observations recorded include thickness of capsule, foetal cortex, definite cortex (zona glomerulosa, zona fasciculata and zona reticularis), outer medullary and inner medullary zone and average diameter of cells in different zones of cortex and medulla. These observations were carried out on 4x magnification.

The average density of cells per unit area in different zones of the gland was recorded at ten different locations at 20x magnification. The average values of all parameters were subjected to statistical analysis.

chapter – IV

ResultS and discussion

The present study was conducted to study the histomorphological changes in the adrenal gland of neonatal buffalo calves during postnatal development. The adrenal glands from 29 buffalo calves were used for the present study. The buffalo calves were divided into 3 Groups on the basis of the age viz. Group – I (0 to 3 months), Group – II (more than 3 months to 6 months) and Group – III (more than 6 months to 1 year).

4.1 Gross Morphology

The gross morphological observations were made on the adrenal glands of neonatal buffalo calves to know the position, shape, size, weight and maximum length, width and thickness of the gland. The left adrenal gland was roughly 'C'-shaped and was situated at the cranial pole of the left kidney in one week old buffalo calf. The right adrenal gland was roughly 'V'-shaped and was located on the dorsomedial aspect of right kidney in close contact with liver in the renal impression. (Fig. 1) as reported earlier in various domestic animals (Sisson and Grossman 1977).

The biometrical observations conducted on the adrenal gland of buffalo revealed that the average weight of left and right adrenal gland was 1.48 ± 0.13 g and 1.47 ± 0.15 g in Group – I, 2.2 ± 0.24 g and 2.16 ± 0.14 g in Group – II and 3.44 ± 0.15 g and 3.37 ± 0.21 g in Group – III (Table 4). The left adrenal gland was heavier in all the

Groups as reported earlier by Howes *et al* (1960) in zebu cattle, Prasad (1969) in young buffalo calves and Groot and Fortier (1959) in rat. In the present study the coefficient of variation showed less consistency in Group – I (28.80%) as compared to Group – II (21.65%) and Group – III (9.15%) suggesting that the rate of increase in the weight of left adrenal gland was more in Group – I (Table 5). Similarly the rate of increase in the weight of right adrenal gland was more in Group – I (31.64%). The correlation coefficient between age and weight of the left and right adrenal glands showed a positive relationship that was significant at 5% and 1% levels of significance, respectively in Group – I (Table 5) suggesting that the weight of the gland increased with the progression of age in Group – I as reported earlier by Prasad (1969) in buffalo.

The average length of left and right adrenal gland was 2.93 ± 0.11 cm and 3.17 ± 0.19 cm in Group – I, 3.73 ± 0.26 cm and 3.80 ± 0.30 cm in Group – II and 4.40 ± 0.15 cm and 4.52 ± 0.58 cm in Group – III (Table 4, Graph 1). The right adrenal gland was larger than the left adrenal gland. However, Prasad (1969) reported that the left adrenal gland was larger in the adult buffalo. In the present study, the coefficient of variation showed more consistency in Group – III (7.18%) and Group – I (11.79%) as compared to Group – II (12.34%) suggesting that the rate of increase in the length of left adrenal gland was more in Group – II (Table 5). However, the rate of

increase in length of right adrenal gland was more in Group – III (25.65%). The correlation coefficient between age and length of left and right adrenal gland showed a positive relationship that was significant at 5% level in Group – III and 1% level of significance in Group – I, respectively (Table 5). However, Prasad (1969) reported that the average length of left adrenal gland was more in adult buffalo.

The average width of left and right adrenal gland was 1.83 ± 0.08 cm and 1.47 ± 0.06 cm in Group – I, 2.06 ± 0.20 cm and 1.73 ± 0.06 cm in Group – II and 3.05 ± 0.44 cm and 2.90 ± 0.45 cm in Group – III (Table 4, Graph 1). The left gland was broader than the right gland. The coefficient of variation showed more consistency in Group – I (13.91%) and Group – II (16.98%) as compared to Group – III (28.88%) suggesting that the rate of increase in the width of left adrenal gland was more in Group – III (Table 5). Similarly, the rate of increase in the width of right adrenal gland was more in Group – III (31.08%) as reported earlier by Prasad (1969) in adult buffalo.

The average thickness of left and right adrenal gland was 0.48 ± 0.01 cm and 0.45 ± 0.02 cm in Group – I, 0.54 ± 0.01 cm and 0.52 ± 0.02 cm in Group – II and 0.66 ± 0.02 cm and 0.64 ± 0.07 cm in Group – III (Table 4, Graph 1). The left adrenal gland was thicker than the right adrenal gland. The coefficient of variations showed more consistency in Group – II (5.36%) and

Group – III (9.94%) as compared to Group I (12.26%) suggesting that the rate of increase in thickness of left adrenal gland was more in Group – I (Table 5). However, the rate of increase in thickness of right adrenal gland was more in Group – III (23.83%). The statistical analysis did not show any positive correlation between age and thickness of left and right adrenal gland at 5% and 1% level of significance. Similarly, Prasad (1969) reported that the thickness of left adrenal gland was more in adult buffalo.

4.2 Histomorphological Observations

4.2.1 Capsule, trabeculae and stroma

4.2.1.1 Collagen fibres

The adrenal gland of day old buffalo calf was completely surrounded by a dense fibrous capsule (Fig. 2) as reported earlier in the adrenal gland of adult buffalo (Sohal and Chaturvedi 1962, Prasad and Yadava 1972, 1974), sheep (Stokoe 1959), goat (Hakeem *et al* 1993a, Kale *et al* 2003), camel (Nagpal *et al* 1991) and dog (Saleh *et al* 1974). The average thickness of the capsule of the gland was $230.66 \pm 33.45 \mu\text{m}$ (Table 6) and it consisted of high proportion of mature collagen fibres in Group – I (Fig 2). The capsule was made up of outer layer of loose connective tissue and inner layer of thick collagen fibres. The collagen bundles of the capsule run parallel to the surface of the gland (Fig 3). The outer part of capsule contained blood vessels and neuronal elements. The connective tissue trabeculae arose from the inner aspect of capsule

and descended deep into the cortical parenchyma. In some locations the trabeculae even reached up to the medulla. The trabeculae accompanied the medium size blood vessels and nerves. Similar type of connective tissue trabeculae were also reported in the adrenal gland of adult buffalo (Sohal and Chaturvedi 1962, Prasad and Yadava 1972), goat (Hakeem *et al* 1993a, Kale *et al* 2003) and camel (Nagpal *et al* 1991). In the present study, the collagen fibres were found in the zona glomerulosa and were present in high proportion in the inner zone of medulla (Fig. 3) Prasad and Yadava (1972, 1974) also observed few fine bundles of collagen fibres entering into the zona glomerulosa from the inner layer of capsule in the adrenal gland of young calves and adult buffalo. Similarly Hakeem *et al* (1993) reported that in the adrenal gland of Indian goat the fibrous content of capsule penetrated the zona glomerulosa. Similar findings were also reported in the adrenal gland of camel (Nagpal *et al* 1991). In the adrenal capsule of buffalo fine collagen fibres were found between the cortex and medulla which appeared to form a capsule around the medulla, however it was incomplete. The connective tissue capsule at corticomedullary junction varied in thickness from one part to another (Fig. 4) and was thin and irregular. The presence of a layer of connective tissue at the corticomedullary junction had also been reported in young calves and adult buffalo (Prasad and Yadava 1972). Similarly Holmes (1968) observed an incomplete

corticomedullary boundary in the adrenal gland of monkey. In the present study the average thickness of capsule increased to $240.13 \pm 9.04 \mu\text{m}$ in Group- II (Table 6) which was due to increase in the density of collagen fibres (Fig. 5). The proportion of collagen fibres were also more in the inner zone of medulla as reported earlier in young calves and adult buffalo (Prasad and Yadava 1972) and goat (Hakeem *et al* 1993, Kale *et al* 2003). In the present findings, the connective tissue capsule present at the corticomedullary junction was more defined in the five months old buffalo calves and often contained round to elongated cells with eosinophilic cytoplasm (Fig. 6). Similarly Holmes (1968) found two types of cells in the corticomedullary capsule viz: round cell with eosinophilic cytoplasm and cells with elongated nuclei resembling fibroblast. Howard-Miller (1927) suggested that degeneration of foetal cortex during the postnatal period was associated with development of corticomedullary fibrous zone and this zone disappeared with advancement of age.

The thickness of capsule further increased to $243.54 \pm 6.22 \mu\text{m}$ in Group-III. The proportions of collagen fibres were more in the zona glomerulosa and outer and inner zone of medulla (Fig. 7). The connective tissue capsule at corticomedullary junction also showed an increase in thickness and cellular density of eosinophilic cells (Fig. 8) however, distinct medullary connective tissue capsule was not observed. Similarly Holmes (1961, 1968) reported incomplete

connective tissue capsule in the adrenal gland of ferrets and monkey.

4.2.1.2 Reticular Fibres

In the day old buffalo calf the inner aspect of the adrenal capsule had a higher proportion of reticular fibres which entered into the parenchyma of the gland (Fig. 9). The proportion of reticular fibres was more in the zona glomerulosa and outer zone of medulla in Group- I (Fig. 10). Similarly Prasad and Yadava (1972), Hakeem *et al* (1993a) and Nagpal *et al* (1991) also observed the presence of the reticular fibres in the adrenal gland of adult buffalo, goat and camel, respectively. Further Prasad and Yadava (1972) reported the blending of collagenous, elastic and reticular fibres along with the smooth muscles fibres in the inner part of the capsule of adrenal gland of adult buffalo. The reticular fibres intermingled with loose collagenous, elastic and smooth muscles fibres in the capsule of adrenal gland of goat (Hakeem *et al* 1993a). In the present study the reticular fibres became more organized in the capsule and parenchyma in Group -II and Group-III. The proportion of reticular fibres appeared to be more in the zona glomerulosa and outer zone of medulla (Fig. 11), which supports the earlier findings of Prasad (1969) in adrenal gland of Indian buffalo.

4.2.1.3 Elastic fibres

The Verhoeff stain sections of buffalo adrenal gland in all the Groups did not show the presence of elastic fibres in any part of gland up to one year of age but the elastic fibres were observed in blood vessels. However, Prasad and Yadava (1972), Hakeem *et al* (1993a) and Nagpal *et al* (1991) reported the elastic fibres in the adrenal capsule of adult buffalo, goat and camel, respectively.

4.2.2 Adrenal parenchyma

4.2.2.1 Adrenal cortex

The adrenal gland of day old buffalo calf consisted of cortex and medulla. The cortex was made up of definite and foetal cortex. The definite cortex was observed in the subscapular area and consisted of zona glomerulosa only in day old buffalo calf (Fig. 2). The zona glomerulosa was made up of darkly stained cells having irregular nuclei and these cells were relatively smaller than the foetal cortical (FC) cells. A large number of sinusoidal capillary were observed in the zona glomerulosa (Fig. 3). Similarly foetal and definite cortical cells were observed in the adrenal gland of buffalo foetuses (Sethi 2005), pig foetuses (Bielanska-Osuchowska 1989a, b), human (Keene and Hewer 1927) and monkey (McNulty *et al* 1981). McNulty *et al* (1981) reported in monkey that the adult cortical zones developed from the persistent definite cortex and postnatal remodeling of the adrenal cortex included the atrophy of the foetal zone and differentiation of zona glomerulosa and fasciculata. In the present study the foetal cortex of adrenal gland

of day old buffalo calf consisted of small to elongated FC cells which were characterized by having eosinophilic cytoplasm and were arranged into irregular cords separated by a sinusoidal capillaries (Figs. 12,13). The FC cells were intermingled with medullary cells at the corticomedullary junction without any distinct line of demarcation between them. The cells in the outer part of the foetal cortex were larger with lightly vacuolated cytoplasm and were arranged in columns (Future zona fasciculata) (Fig. 12). No necrotic changes were observed in the foetal cortex however, wide spread hemorrhages were frequently seen in the foetal cortical region (Fig. 3). The demise of foetal zone was thought to occur by necrosis and hemorrhage in the adrenal gland of human (Brenner 1940) and monkey (Lanman 1957). However, Mesiano and Jaffe (1997) suggested that necrosis and hemorrhage were probably artifacts related to the cause of death and time elapsed before fixation. The postnatal remodeling of adrenal cortex involved apoptosis of the foetal zone and simultaneous expansion of preexisting definite cortex as reported by Spencer *et al* (1995) in human.

In the adrenal gland of one week old buffalo calf the zona glomerulosa was well differentiated and was relatively broader. The cells of this zone were larger (Fig. 14). The zona fasciculata was observed between the zona glomerulosa and foetal cortex at this stage. The cells of this zone were organized into radiating cords and

were larger in size. The FC cells were pushed towards the centre of the gland and were closely packed (Fig. 15). The FC cells were comparatively larger in size and were also observed around the central vein of medulla. Similarly the zona fasciculata was differentiated between the zona glomerulosa and foetal cortex during early postnatal period in the adrenal gland of monkey (McNulty *et al* 1981) and human (Keene and Hewer 1927).

In the adrenal gland of two week buffalo calf the zona glomerulosa and zona fasciculata were more differentiated. The cells of the zona glomerulosa were small columnar and had spherical or oval nuclei surrounded by a homogenous cytoplasm. The zona fasciculata consisted of cells having lightly vacuolated cytoplasm and arranged in radiating cords. No mitotic figures were seen in the zona fasciculata or at the junction of zona fasciculata and foetal cortex (Fig. 16). However, a smooth transition occurred between the zona fasciculata and foetal cortex. The foetal cortex showed further reduction in size and persisted as a narrow zone between zona fasciculata and outer zone of medulla. Similarly McNulty *et al* (1981) observed no mitotic figures in the zona fasciculata in the adrenal gland of six months old monkey and suggested that the definite cortex was progressively generated by transformation of cells from the preexisting dense band found closer to the medulla. As the dense band moved centrally zona fasciculata differentiated outside and shrinking foetal cortex was

seen between this band and medulla. In the present study the FC cells were closely packed and were having moderately vacuolated cytoplasm (Fig. 17). Similarly Keene and Hewer (1927) reported that the foetal cortex disappeared rapidly during the early postpartum period in the human adrenal gland.

In the adrenal gland of one month old buffalo calf the zona glomerulosa and zona fasciculata were more organized (Fig. 18). The FC cells were pushed towards the centre of gland and were seen as a thin rim between the zona fasciculata and medulla. The cells were having lightly stained cytoplasm and showed loss of nuclei (Fig. 19). Similarly Keen and Hewer (1927) reported that the FC cells first became swollen and then lost their nuclei during the postnatal remodeling of human adrenal gland. The foetal zone was transformed to adult cortex and then disappeared as reported by McNulty *et al* (1981) in the rhesus monkey.

By one and half month of age, the adrenal gland of buffalo showed first appearance of zona reticularis below the zona fasciculata. However, it was not well organized in the adrenal gland of three month old buffalo calf. The cells of the zona reticularis were smaller than the cells of the zona fasciculata and were having little cytoplasm with pyknotic and heterochromatic nuclei (Fig. 20). The zona reticularis was first time observed at six months of age in the adrenal gland of goat (Hakeem *et al* 1993a). Similarly Sucheston and Cannon (1968) reported the differentiation of zona

reticularis in the six months old human adrenal gland which appeared as a narrow incomplete rim of cells between the zona fasciculata and medulla. However, Keene and Hewer (1927) observed the zona reticularis by three and half months of age in the human adrenal gland. Boshier and Holloway (1989) reported that there was no histological and histochemical evidence of the development of zona reticularis by two days postpartum in the adrenal gland of sheep. In the present study a connective tissue stroma appeared to form an inner capsule around the medulla separating the cortex from the medulla (Fig. 4). This narrow condensation of connective tissue distinguished the cells of the medulla and the closely related zona reticularis as earlier reported in the adrenal gland of buffalo (Prasad and Yadava 1972), goat (Hakeem *et al* 1993a), camel (Nagpal *et al* 1991), human (Keene and Hewer 1927, Sucheston and Cannon 1968) and monkey (Holmes 1968). Prasad and Yadava (1972) reported that in the adrenal gland of adult buffalo a connective tissue capsule was present at the corticomedullary junction forming a distinct demarcation between the cortex and medulla. Keene and Hewer (1927) suggested that the FC cells were replaced to a certain extent by a temporary connective tissue which formed a well marked zone at the corticomedullary junction in the human adrenal gland as reported by Sucheston and Cannon (1968). Nagpal *et al* (1991) also observed the concentration of the connective tissue at the

corticomedullary junction however, the line of separation between cortex and medulla was irregular.

In the adrenal gland of four to five months old buffalo calves the bulk of the cortex was formed by the zona fasciculata and zona reticularis, however, there were no sharp distinction between these two zones (Fig. 21) as reported earlier in the adrenal gland of goat (Singh *et al* 1968), human (Bloom and Fawcett 1975) and domestic animals (Trautmann and Fiebiger 2002). In the present study the connective tissue capsule at the corticomedullary junction showed a gradual increase in thickness and was well marked at this stage. In this fibrous capsule large isolated cells with eosinophilic cytoplasm were observed (Fig. 6) which were probably degenerating cells of the foetal cortex as suggested by Howard-Miller (1927) in the human adrenal gland.

In the six months to one year old buffaloes the adrenal gland showed almost identical development as in the earlier stages. The zona glomerulosa formed the outer most zone of the cortex (Fig. 7). The zona fasciculata contained cells with foamy cytoplasm and the zona reticularis still lacked organization (Figs. 7, 22). There was sharp demarcation between cortex and medulla due to presence of more marked connective tissue capsule (Fig. 8). Remnant of foetal cortex was also observed at this stage. However, Prasad (1969) could not specify the development and structural differences that

occurred in the adrenal cortex during the postnatal development of adrenal gland in the buffalo calves.

The average thickness of foetal cortex, zona glomerulosa, zona fasciculata and zona reticularis was $353.45 \pm 46.88 \mu\text{m}$, $177.42 \pm 21.14 \mu\text{m}$, $411.53 \pm 45.55 \mu\text{m}$, and $141.23 \pm 23.40 \mu\text{m}$, respectively in Group - I (Table 6, Graph 2). The coefficient of variation showed more consistency in zona glomerulosa (26.65%) than zona fasciculata (16.65%) suggesting that the rate of increase in thickness of zona glomerulosa was more than the zona fasciculata (Table 7). The statistical analysis revealed a negative correlation between age and thickness of foetal cortex reflecting that foetal cortex was degenerating with the progression of age. Similarly, Prasad and Yadava (1974) and Kale *et al* (2003) reported increase in the thickness of zona glomerulosa in the early stages of postnatal development in young buffalo calf and goat, respectively. Keene and Hewer (1927) observed that the foetal cortex underwent degenerative changes with progression of age in the human adrenal gland. In the present study, the average density of cells per unit area was more in zona glomerulosa (41.78 ± 3.00) than zona fasciculata (30.84 ± 1.36) (Table 8). However, the statistical analysis showed a negative correlation between age and average density of cells per unit area of foetal cortex (Table 9) suggesting the degeneration of foetal cortex. The average cell size was $6.47 \pm 0.33 \mu\text{m}$, $6.37 \pm 0.12 \mu\text{m}$, $7.03 \pm 0.23 \mu\text{m}$ and $6.42 \pm 0.20 \mu\text{m}$ in the

foetal cortex, zona glomerulosa, zona fasciculata and zona reticularis, respectively (Table 10). The size of the cell in zona fasciculata was the largest as reported earlier in the adult buffalo (Sohel and Chaturvedi 1962, Prasad and Yadava 1974) and goat (kale *et al* 2003). The percentage contribution of zona fasciculata (14.44%) was more than the zona glomerulosa (6.23%) towards the adrenal cortex of buffalo in Group – I (Table 12, Graph 3).

The adrenal cortex showed an increase in thickness of all the cortical zones in Group- II and Group- III. The zona glomerulosa, zona fasciculata and zona reticularis found to be increasing significantly from Group – II ($202.75 \pm 11.78 \mu\text{m}$, $631.43 \pm 59.09 \mu\text{m}$ and $329.95 \pm 17.30 \mu\text{m}$) to Group – III ($252.73 \pm 11.51 \mu\text{m}$, $869.73 \pm 29.37 \mu\text{m}$ and $393.14 \pm 34.17 \mu\text{m}$) (Table 6, Graph 2). Kale *et al* (2003) also observed an increase in the area of all the zones of definite cortex during the postnatal development of adrenal gland in goat. In the present study the coefficient of variation showed more consistency in zona fasciculata suggesting that rate of increase in thickness of zona fasciculata was more in Group – II and Group – III (Table 7). The statistical analysis revealed a positive correlation between the age and thickness of zona fasciculata in Group – II and between zona reticularis and age in Group – III which were significant at 5% level of significance, suggesting that the rate of increase in thickness of zona fasciculata was significant in Group- II, however, zona reticularis showed significant increase

in thickness in Group- III. The zona glomerulosa was the narrowest zone among the three cortical zones in Group – III as earlier reported in the young buffalo calf (Prasad and Yadava 1974) and goat (Kale *et al* 2003). The average density of cells per unit area was maximum in the zona glomerulosa in all the Groups (Table 8). However, data suggested a more consistency in the zona reticularis in Group – III suggesting that zona reticularis was increasing in size (Table 9). The average cell size of the zona fasciculata was largest amongst all the cortical zones (Table 10) as reported earlier by Prasad and Yadava (1974) in young buffalo calf. The cortex constituted lesser proportion of the adrenal gland than the adrenal medulla in all the Group (Table 12) as reported earlier in the adrenal gland of buffalo foetuses (Sethi and Roy 2007), human (Keene and Hewer 1927) and foal (Yamauchi 1979).

4.2.2.2 Adrenal medulla

In the adrenal gland of day old buffalo calf the greater part of the gland was composed of medullary tissue (Fig. 2) which was formed from the neuroectodermal cells as reported earlier in the adrenal gland of buffalo foetuses (Sethi and Roy 2007), buffalo calves (Prasad and Yadava 1973), sheep (Boshier *et al* 1989), goat (Hakeem *et al* 1993a, Ashok *et al* 2007), foal (Comline and Silver 1971), dog (Carmichael and Blair 1973) and human (Keene and Hewer 1927). In the present study, the medulla occupied the central portion of the gland and was well differentiated from the

cortex. However, the outer and inner zones of the medulla were not well demarcated (Fig. 2). The medullary cells located at periphery were larger as compared to the cells found in the centre of the gland. Similarly Prasad and Yadava (1973) reported a distinct demarcation between adrenal cortex and medulla even in the unstained paraffin sections from the adrenal gland of buffalo, whereas Lever (1955) observed that the adrenal medulla was still a loose connection of cell island interdigitating with the cortex even at the birth in the adrenal gland of rat. In the human foetuses the separation of cortex and medulla occurred during late gestation and complete medulla was formed by full term (Keene and Hewer 1927). In the present study, adrenal gland of day old buffalo calf revealed numerous colloid filled follicles surrounded by fibrous tissue in the inner zone of adrenal medulla (Figs. 2, 23) as earlier reported in the day old goat (Ashok *et al* 2007) and various other domestic animals (Smollich 1966, 1967). Arthur *et al* (1982) correlated these colloid filled follicles with increased secretion of catecholamines from foetal adrenal medulla at birth, which were required for the adaptation of newborn to extrauterine life. In the adrenal gland of day old buffalo calf groups of nerve bundles were observed in the inner zone of adrenal medulla (Fig. 23) along with the nerve fibres which were also found transversing from the capsule to medulla. Similarly Prasad and Yadava (1973) observed the presence of nerves fibres in the adrenal medulla of Indian

buffalo as reported in horse, calf and cow (Stilling 1887) and rabbit (Evans 1947).

In the adrenal gland of one week to three months old buffalo calves two types of medullary cells were recognized arranged in the outer and inner zone (Fig. 24). The outer zone was made up of large adrenaline secreting cells which were arranged in a follicular form, however, the noradrenaline secreting cells were comparatively small and constituted the inner zone of medulla. The noradrenaline secreting cells were compactly arranged. The adrenaline secreting cells were mainly found in periphery of medulla, however, the noradrenaline secreting cells were observed in the central portion of the adrenal medulla around the central vein (Fig. 24) as reported earlier in bovine foetus (Sethi and Roy 2007), buffalo calf (Zamora *et al* 1967, Prasad and Yadava 1973), adult buffaloes (Prasad and Yadava 1973), goat (Hakeem *et al* 1993a) and different domestic animals (Smollich 1967, Dellmann and Eurell 1998). Smollich (1967) clearly differentiated the adrenaline and noradrenaline secreting cells in different species of ruminants and observed that adrenaline secreting cells were located at the periphery, however, noradrenaline secreting cells were limited to the centre of the medulla in the adrenal gland of cattle, sheep, swine and horses. In the present investigation, no colloid filled follicle was observed in the adrenal medulla of one week old buffalo calf and on-ward stages of development.

In Group II the adrenal medullary cells were more clearly differentiated in the outer and inner zone. The outer zone corresponded to adrenaline secreting cells and cells of this zone were arranged in follicular form (Figs. 25, 6). The adrenaline secreting cells had homogenous cytoplasm with a spherical to round nucleus located more towards the apical portion (Fig. 26) as reported earlier in the buffalo (Prasad and Yadava 1973) and various other domestic animals (Dellmann and Eurell 1998). In the present study the noradrenaline secreting cells were situated in the central portion of the adrenal medulla and were better differentiated in Group II. The noradrenaline secreting cells had a less homogenous cytoplasm with mostly centrally placed nucleus (Fig. 26). The degenerating FC cells with pyknotic nuclei were found on the periphery of blood vessels in the corticomedullary region of the gland (Fig. 27). Similarly Ashok *et al* (2007) observed the groups of cortical cells concentrating near the central vein in the adrenal gland of goat as reported earlier in the adult animals of several species by Prasad and Sinha (1981, 1984) and Pignatelli *et al* (1995) and in the foetal adrenal gland of buffalo (Sethi 2005) and pig (Bielanska-Osuchowska 1989a,b,c). McNulty *et al* (1981) reported that postnatal remodeling of the primate adrenal gland involved the degeneration of FC cells.

In Group-III the area of outer and inner zone of adrenal medulla was more at this stage. In the one year old buffalo more

number of nerve fibres were observed both in inner and outer zone of medulla (Fig. 28). The outer and inner zones of medulla were better defined at this stage (Fig. 29) as reported earlier in the adrenal gland of buffalo (Prasad and Yadava 1972), sheep (Prasad and Sinha 1981) and goat (Hakeem *et al* 1993a). Smollich (1966) reported that the adrenaline secreting cells increased proportionately with advancing age in different species of ruminant. Stevens (1977) suggested that increase in the thickness of adrenal medulla might be correlated to meet the increased demand of catecholamines for various metabolic activities.

The thickness of adrenal medulla was $1765.97 \pm 79.86 \mu\text{m}$ in Group – I and further the thickness of outer and inner medullary zone was $385.58 \pm 49.48 \mu\text{m}$ and $1380.39 \pm 70.42 \mu\text{m}$, respectively (Table 6, Graph 2). The data showed more consistency in thickness of inner medullary zone than the outer medullary zone (Table 7) suggesting that the rate of increase in the thickness of outer medullary zone was more than inner medullary zone. The percentage proportion of inner medullary zone (48.44%) was more than the outer medullary zone (13.53%) in the adrenal gland of buffalo in Group – I (Table 12, Graph 3) as reported earlier in the buffalo foetuses (Sethi and Roy 2007) and young buffalo calves (Prasad and Yadava 1974). The corticomedullary ratio was 1:1.62 indicating that the medulla contributed a larger proportion (61.97%) to the adrenal gland in this Group. The average density of

cells per unit area was more in the inner medullary zone (39.16 ± 2.30) than outer medullary zone (31.26 ± 2.22) (Table 8). The average cell size in the outer medullary zone was more than the inner medullary zone in Group – I (Table 10) and data showed more consistency in outer medullary zone suggesting that the rate of increase in cell size was more in the outer medullary zone (Table 11) as reported earlier in buffalo foetuses (Sethi and Roy 2006).

The thickness of adrenal medulla was $1833.40 \pm 342.18 \mu\text{m}$ and $1998.73 \pm 103.45 \mu\text{m}$ in Group II and Group III, respectively and further the thickness of inner medullary zone was more than the outer medullary zone (Table 6, Graph 2) in these Groups suggesting a progressive increase in the thickness of adrenal medulla with age in Group – II and Group- III. The coefficient of variation showed more consistency in thickness of outer medullary zone than the inner medullary zone (Table 7) suggesting that the rate of increase in thickness of inner medullary zone was more than outer medullary zone in Group II and Group III. Boshier *et al* (1989) reported seven fold increases in the proportion of inner medullary cells from 100 days of gestation to birth in sheep. In the present study the corticomedullary ratio was 1:1.57 in Group II (Table 12), however, showed a decrease in Group III (1:1.32) which may be due to rapid cortical growth at that time. Similar observations had been recorded in the adrenal medulla of neonatal sheep (Boshier *et al* 1989). The average cell density per unit area

was less in the outer medullary zone than the inner medullary zone in Group – II and Group – III (Table 8), however, the average cell size was more in the outer medullary zone (Table 10) as reported earlier in the adrenal medulla of buffalo foetuses (Sethi and Roy 2006).

4.2.2.2.1 Identification of adrenaline and noradrenaline secreting cells

The buffalo adrenal medulla was comprised of two cells secreting zones viz., outer adrenaline and inner noradrenaline in all the Groups. These two types of medullary cells were clearly differentiated by Wood's and Giemsa staining methods. Paraffin sections of orth fixed adrenal glands differentiated adrenaline and noradrenaline secreting cells when selectively stained with Wood's method (Figs. 30, 31, 32). The adrenaline secreting cells were stained brownish purple, however, the noradrenaline secreting cells were comparatively light yellowish in colour as reported earlier in bovine foetuses (Sethi and Roy 2007), calves and adult buffalo (Prasad and Yadava 1973) and different lab animals (Wood 1963). Wood (1963) developed a special staining method to clearly distinguished the adrenaline secreting cells from noradrenaline secreting cells by differential fixation and staining. The two types of cells were identified by morphological differences on the basis of granularity and size. The adrenaline secreting cells stained brown to purple and noradrenaline cells were yellow in colour. In the present study the Giemsa staining method also differentiated the

two types of medullary cells by selectively staining the noradrenaline secreting cells darker green than the adrenaline secreting cells (Fig. 33), but fixation in orth fluid was critical. Similar results were reported by Sethi and Roy (2007) in buffalo foetuses.

4.3 Histochemistry

4.3.1 Mucopolysaccharide

4.3.1.1 Neutral mucopolysaccharide (NMPS)

In Group – I the adrenal gland showed a strong Periodic Acid Schiff (PAS) positive reaction especially in the inner zone of the capsule and moderate reaction in the wall of large blood vessels of the capsule (Fig. 34). The strong PAS positive reaction in the inner aspect of capsule may be due to the reticular fibers seen in the histological stain. The PAS positive reaction in the adrenal capsule had been reported by Prasad and Yadava (1974) in young and adult buffalo and Hakeem *et al* (1993b) in goat. In the present study the zona glomerulosa was moderately PAS positive. Similarly Hakeem *et al* (1993b) proved by PAS diastase method that PAS positive reaction in the cells of the zona glomerulosa were due to glycogen. In the present findings the PAS positive reaction was weak in the zona fasciculata and zona reticularis in Group – I (Fig. 34, Table 13). The FC cells were strongly PAS positive and a distinct PAS positive rim was observed in the periphery of the FC cells (Fig. 35). Bielanska-Osuchowska (1989a, b) also reported a PAS positive rim

around the FC cells of the adrenal gland of pig foetus and confirmed that the microvilli found on the surface of the FC cells formed a microlabyrinth in the intracellular spaces of the FC cells. These intracellular spaces were filled by the substances derived from plasma or steroid substances secreted by the cortical cells. The PAS positive reaction was due to the presence of these substances in the intercellular spaces. Similar PAS positive substance was also observed in the adrenal cortical cells of man, pig and cat (Motta *et al* 1979). In the present study the PAS positive rim was diffused or absent in the FC cells located near the blood vessels of medulla suggesting that these were the degenerating FC cells (Fig. 36). Similarly Bielanska-Osuchowska (1989a, b) also found degenerating FC cells with slightly PAS positive cytoplasm in the adrenal medulla of pig fetuses. In the present investigations the outer and inner zone of buffalo adrenal medulla showed weak to moderate PAS positive reaction as reported earlier in the adrenal gland of young buffalo calf (Prasad and Yadava 1974). In Group – II and Group – III the inner portion of capsule and its blood vessels were strongly PAS positive and the cells of the zona glomerulosa were moderately PAS positive (Fig. 37), however, the zona fasciculata and zona reticularis showed a weak PAS positive reaction. The cells of zona reticularis lacked PAS positive rim around them, which was a characteristic feature of the FC cells. However, the cells of remnants foetal cortex showed moderate PAS

positive reaction (Fig. 38). Hakeem *et al* (1993b) reported the PAS positive reaction in all the zones of adrenal cortex of goat and the PAS positive reaction was more in the zone. However, Prasad and Yadava (1974) reported a moderate reaction in the cells of zona fasciculata and a negative reaction in the zona glomerulosa. In the present study the outer and inner zone of adrenal medulla showed a weak to moderate PAS positive reaction as reported earlier in young and adult buffalo by Prasad and Yadava (1974).

4.3.1.2 Acid mucopolysaccharide (AMPS)

In Group – I the alciphilic reaction was weak in the outer zone of the adrenal capsule of day old buffalo calf. The zona glomerulosa, zona fasciculata, zona reticularis and FC cells showed a moderate to strong alciphilic reaction in Group- I (Fig. 39). Both outer and inner zones of adrenal medulla showed weak to moderate positive reaction for AMPS.

In Group – II and Group – III the outer zone of capsule and its blood vessels showed a weak to negative alciphilic reaction. There was a weak alciphilic positive reaction in the cells of the zona glomerulosa and zona fasciculata, however, the reaction was strong in the cells of zona reticularis in the adrenal gland of one year old buffalo (Fig. 40, Table 13). The outer zone of medulla showed a weak alciphilic positive reaction whereas the reaction was moderate in the inner zone of medulla.

4.3.2 Lipid

4.3.2.1 Total lipid and phospholipid

The cryostat section of adrenal gland from Group-I were used to observe the distribution of lipid and phospholipid. The capsule of the gland showed a negative to low amount of fine lipid droplets (Fig. 41) as reported earlier in goat (Hakeem *et al* 1993b). The low amounts of sudanophilic lipid droplets were observed in the cells of zona glomerulosa whereas the cells of zona fasciculata had high amount of lipid droplets (Figs. 41, 42). Similarly Singh *et al* (1999) reported a strong Sudanophilic reaction in zona fasciculata and low proportion of lipid in the zona glomerulosa of three months old buffalo calves as reported earlier in camel (Nagpal *et al* 1991) and in various domestic animals (Bank 1993). Willims (1970) suggested that increased amount of lipid in the zona fasciculata may be related to secretion of glucocorticoids. In the present investigation, Sudanophilic lipid droplets were found to be absent in the outer and inner medullary zone in the adrenal gland of one month old buffalo calves (Fig. 41) as reported earlier by Singh *et al* (1999) in buffalo calves and Hakeem *et al* (1993b) in goat. However, Keene and Hewer (1927) reported low amount of lipid droplets in certain cells of human adrenal medulla. In the present study, the area separating the cortex and medulla did not show any lipid content which corresponded to the region of the degenerating remains of the foetal cortex (Fig. 42) as reported earlier in the human adrenal gland (Keene and Hewer 1927).

The phospholipid was demonstrated in the adrenal gland of buffalo by Baker's acid haematin method and the phospholipid content was more in the cortex as compared to the medulla (Fig. 43).

4.3.2.2 Cholesterol

In the present study a positive reaction to cholesterol was observed in the cortical cells of buffalo adrenal gland of Group – I. The moderate amount of cholesterol was observed in the cortex; however, the reaction was low to negligible in the cells of adrenal medulla (Fig. 44). Sethi (2005) reported low to moderate reaction in the definite cortex of buffalo foetuses. Greep (1966) also observed high amount of cholesterol in the cells of zona fasciculata in the adrenal gland of cow and related it to the steroid secretion activity of cortical cells. As the cholesterol is precursor of the glucocorticoids so the presence of lipid in zona fasciculata may be related to secretion of glucocorticoids (Willims 1970).

4.3.3 Basic protein

The Bromphenol blue positive reaction was observed throughout the adrenal gland of buffalo indicating the presence of basic protein in the adrenal gland. The concentration of protein was more in the cells of adrenal cortex and medulla in Group – III suggesting the better differentiation and physiological maturity of the corticomedullary cells in this age group. The high amount of protein content was observed in the subcapsular region of the

gland. The cells of the zona glomerulosa showed higher content of proteins than the cells of zona fasciculata, zona reticularis and foetal cortex in Group – I (Fig. 45). However, the concentration of protein was same in all the cortical zones in Group – II and Group – III (Figs. 46, 47). The cortical cells contained high concentration of protein than the medullary cells in all the Groups (Fig. 48) as reported earlier in the buffalo fetuses (Sethi 2005) and bird (Vyas and Jacob 1976).

Table 4: Biometrical data showing Mean and standard error values of various parameters of right and left adrenal gland in different age Groups of buffalo

Parameter	Group – I		Group – II		Group – III	
	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.
Weight (in g)	1.48 ± 0.13	1.47 ± 0.15	2.2 ± 0.24	2.16 ± 0.14	3.44 ± 0.15	3.37 ± 0.21
Length (in cm)	2.93 ± 0.11	3.17 ± 0.19	3.73 ± 0.26	3.80 ± 0.30	4.44 ± 0.15	4.52 ± 0.58
Width (in cm)	1.83 ± 0.08	1.47 ± 0.06	2.06 ± 0.20	1.73 ± 0.06	3.05 ± 0.44	2.90 ± 0.45
Thickness (in cm)	0.48 ± 0.01	0.45 ± 0.02	0.54 ± 0.01	0.52 ± 0.02	0.66 ± 0.02	0.64 ± 0.07

Table 5: Biometrical data showing coefficient of variation and correlation coefficient between age and various parameters in the adrenal gland of buffalo

Parameter	Coefficient of variation						Correlation coefficient					
	Group - I		Group - II		Group - III		Group - I		Group - II		Group - III	
	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.
Weight	28.80	31.64	21.65	11.11	9.15	12.82	0.77*	0.86**	0.86	0.50	0.10	0.89
Length	11.79	18.12	12.34	1.43	7.18	25.65	0.59	0.85**	0.00	-0.18	0.98*	0.94
Width	13.91	12.55	16.98	6.63	28.88	31.08	0.46	-0.27	0.56	0.00	0.93	0.91
Thickness	12.26	14.45	5.36	7.14	9.94	23.83	-0.18	-0.31	-0.53	0.86	0.03	-0.91

*Correlation is significant at 0.05 levels (2- tailed)

**Correlation is significant at 0.01 levels (2- tailed)

Table 6: Micrometrical data showing Mean and standard error (in μm) values of various parameters of adrenal gland in different age Groups of buffalo

Structure	Thickness (Mean \pm SE)		
	Group - I	Group - II	Group - III
Capsule	230.66 \pm 33.45	240.13 \pm 9.04	243.54 \pm 6.22
Fetal cortex	353.45 \pm 46.88	-	-
Zona glomerulosa	177.42 \pm 21.14	202.75 \pm 11.78	252.73 \pm 11.51
Zona fasciculata	411.53 \pm 45.55	631.43 \pm 59.09	869.73 \pm 29.37
Zona reticularis	141.23 \pm 23.40	329.95 \pm 17.30	393.14 \pm 34.17
Total cortex	1083.63 \pm 51.93	1164.13 \pm 83.80	1515.60 \pm 305.63
Outer medullary zone	385.58 \pm 49.48	449.46 \pm 70.26	600.79 \pm 56.22
Inner medullary zone	1380.39 \pm 70.42	1383.94 \pm 271.990	1397.94 \pm 137.70
Total medulla	1765.97 \pm 79.86	1833.40 \pm 342.18	1998.73 \pm 103.45

Table 7: Showing coefficient of variation and correlation coefficient of various micrometrical parameters in adrenal gland of various age Groups of buffalo

Structure	Coefficient of variation			Correlation coefficient between age and various parameters		
	Group - I	Group - II	Group - III	Group - I	Group - II	Group - III
Capsule	35.52	6.46	5.29	0.822*	0.942	0.52
Fetal cortex	29.55	-	-	-0.687	-	-
Zona glomerulosa	26.65	10.09	10.91	0.72	0.91	0.93
Zona fasciculata	16.65	16.21	51.39	0.59	0.99*	0.04
Zona reticularis	20.52	9.08	17.25	0.52	0.64	0.99*
Total cortex	11.73	12.46	34.92	0.01	0.96	0.19
Outer medullary zone	28.69	27.07	16.20	-0.17	0.55	0.99
Inner medullary zone	10.55	34.04	17.06	0.415	0.51	0.67
Total medulla	10.35	32.32	8.96	0.41	0.52	-0.36

*Correlation is significant at 0.05 levels (2- tailed)

Table 8: Showing Mean \pm SE (in μm) of average density of cells per unit area in adrenal gland of different age Groups of buffalo

Structure	Thickness (Mean \pm SE)		
	Group - I	Group - II	Group - III
Fetal cortex	25.92 \pm 3.80	-	-
Zona glomerulosa	41.78 \pm 3.00	46.90 \pm 6.85	51.26 \pm 4.13
Zona fasciculata	30.84 \pm 1.36	28.70 \pm 2.40	30.13 \pm 2.42
Zona reticularis	31.74 \pm 2.32	41.03 \pm 1.13	33.03 \pm 3.42
Outer medullary zone	31.26 \pm 2.22	30.40 \pm 1.30	29.50 \pm 2.81
Inner medullary zone	39.16 \pm 2.30	46.40 \pm 7.32	40.90 \pm 2.28

Table 9: Showing coefficient of variation and correlation coefficient of average density of cells per unit area in various zones of adrenal gland of different age Groups of buffalo

Structure	Coefficient of variation			Correlation coefficient between age and various parameters		
	Group - I	Group - II	Group - III	Group - I	Group - II	Group - III
Fetal cortex	32.78	-	-	-0.27	-	-
Zona glomerulosa	16.12	25.34	13.96	0.89	0.89	0.88
Zona fasciculata	9.91	14.49	13.94	-0.67	0.88	0.97
Zona reticularis	14.72	4.80	18.00	0.71	-0.91	0.99
Outer medullary zone	15.77	7.43	16.50	0.41	-0.81	0.99
Inner medullary zone	13.14	34.86	9.69	0.19	0.93	0.69

Table 10: Showing Mean \pm SE (in μm) of average cell size in various zones of adrenal gland of different age Groups of buffalo

	(Mean \pm SE)		
Structure	Group - I	Group - II	Group - III
Fetal cortex	6.47 \pm 0.33	-	-
Zona glomerulosa	6.37 \pm 0.12	5.72 \pm 0.31	5.66 \pm 0.47
Zona fasciculata	7.03 \pm 0.23	6.58 \pm 0.27	6.22 \pm 0.41
Zona reticularis	6.42 \pm 0.20	6.28 \pm 0.08	5.85 \pm 0.43
Outer medullary zone	6.88 \pm 0.20	6.10 \pm 0.52	6.00 \pm 0.46
Inner medullary zone	6.61 \pm 0.12	5.94 \pm 0.53	5.91 \pm 0.46

Table 11: Showing coefficient of variation and correlation coefficient of average cell size in various zones of adrenal gland of different age Groups of buffalo

Structure	Coefficient of variation			Correlation coefficient between age and various parameters		
	Group - I	Group - II	Group - III	Group - I	Group - II	Group - III
Fetal cortex	32.78	-	-	0.21	-	-
Zona glomerulosa	4.28	9.65	14.00	0.32	0.88	0.70
Zona fasciculata	7.38	7.18	11.46	0.76	0.64	0.94
Zona reticularis	-7.26	2.38	12.7	0.72	-0.15	0.90
Outer medullary zone	6.81	15.01	13.49	-0.38	0.86	0.74
Inner medullary zone	4.28	15.50	3.03	-0.07	0.63	0.90

Table 12: Showing (percentage) proportion of different zones of cortex and medulla and corticomedullary ratio in adrenal gland of different age Groups of buffalo

Structure	Group - I	Group - II	Group - III
Zona glomerulosa	6.23	6.76	7.19
Zona fasciculata	14.44	21.06	24.75
Zona reticularis	4.96	11.02	11.19
Foetal cortex	12.40	-	-
Total cortex	38.03	38.84	43.13
Outer medullary zone	13.53	14.99	17.09
Inner medullary zone	48.44	46.16	39.79
Total Medulla	61.97	61.17	56.88
Corticomedullary ratio	1: 1.62	1: 1.57	1: 1.32

Table 13: Showing histochemical distribution of mucopolysaccharide in the adrenal gland of different age Groups of buffalo

Structure	Group – I		Group – II		Group – III	
	NMPS	AMPS	NMPS	AMPS	NMPS	AMPS
Capsule	+++	+	+++	0/+	+++	0/+
Zona glomerulosa	++	++/+++	++	+	++	+
Zona fasciculata	+	++	+	+	+	+
Zona reticularis	+	++	+	+++	+	+++
Foetal cortex	++	++/+++	-	-	-	-
Outer medullary zone	+/++	+	+	+	+	+
Inner medullary zone	+/++	+	+	+	+	+

**- Not observed,
Moderate,**

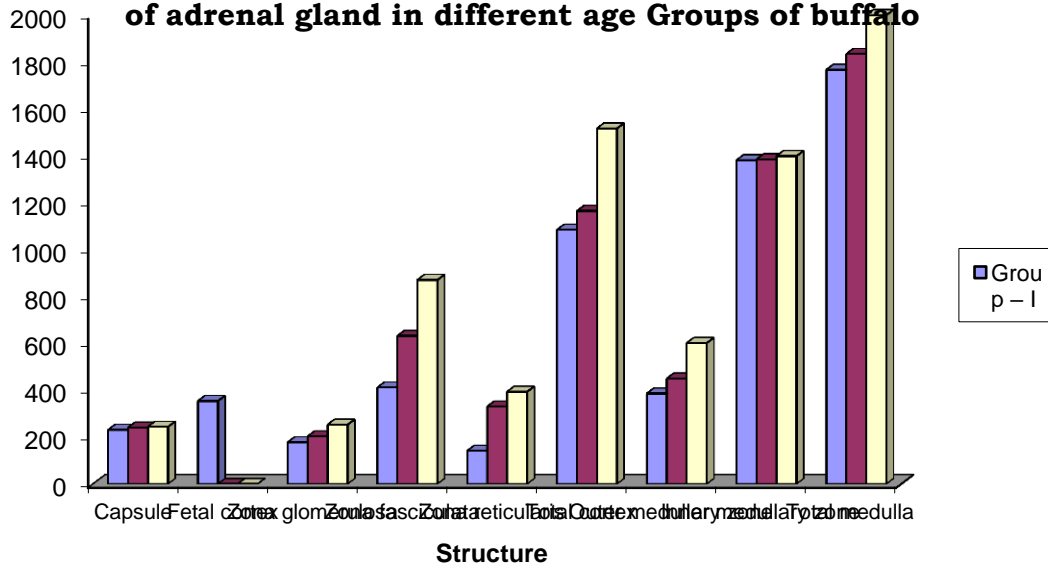
**0 Negative,
+++ Strong**

+ Weak,

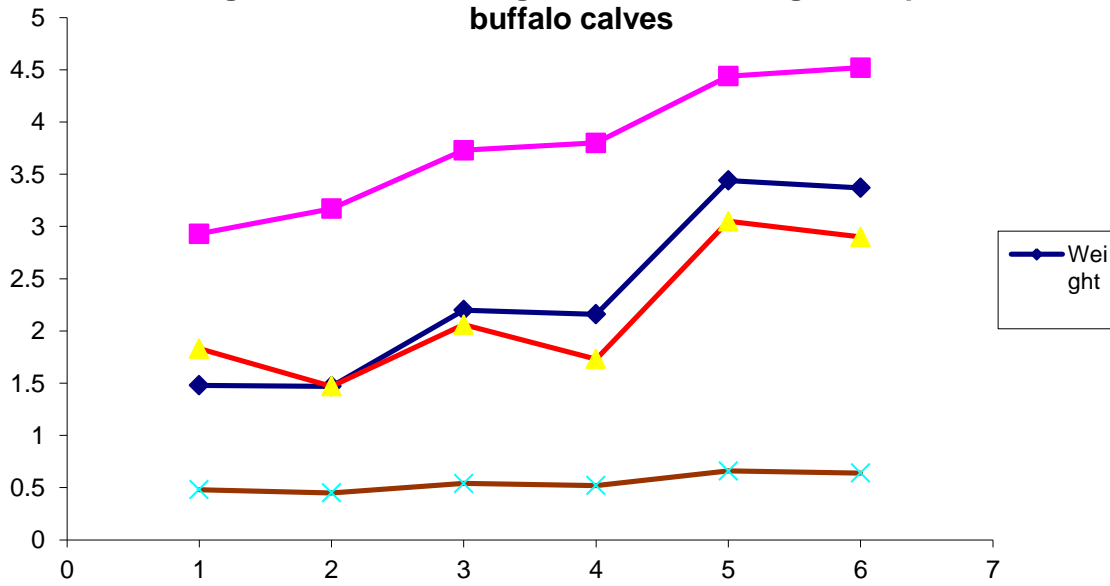
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- Fig. 1** Shape and size of right (RT) and left (LT) adrenal gland from the one weeks (1), two weeks (2), three weeks (3), and four weeks (4) old buffalo calves. X 3.5
- Fig. 2** Adrenal gland of day old buffalo calf showing the well developed dense fibrous capsule (C), zona glomerulosa (G) of definite cortex, foetal cortex (f) and colloid filled follicles (arrows) in the central part of medulla (M). Masson's trichrome stain x 40.
- Fig. 3** A part of cortex of Fig. 2 at higher magnifications showing capsule (C), zona glomerulosa (G) with large number of sinusoidal capillaries and hemorrhages (arrow) in the foetal cortex (f). Masson's trichrome stain x 100.
- Fig. 4** Adrenal gland from three months old buffalo calf showing collagen fibres in the outer part of medulla (Mo) and condensation of collagen fibres (arrow) at the corticomedullary junction. Masson's trichrome stain x 200.

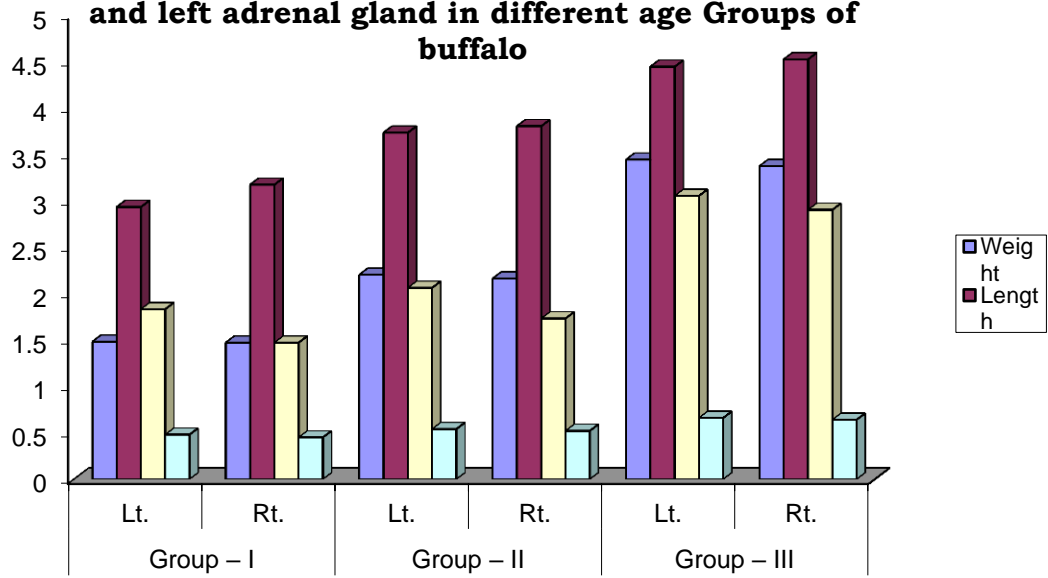
Graph 2: Micrometrical data showing Mean and standard error (in mm) values of various parameters of adrenal gland in different age Groups of buffalo



Biometrical observation (Mean ± SE) of various parameters of right and left adrenal gland in different age Groups of buffalo calves



Graph 1: Biometrical data showing Mean and standard error values of various parameters of right and left adrenal gland in different age Groups of buffalo



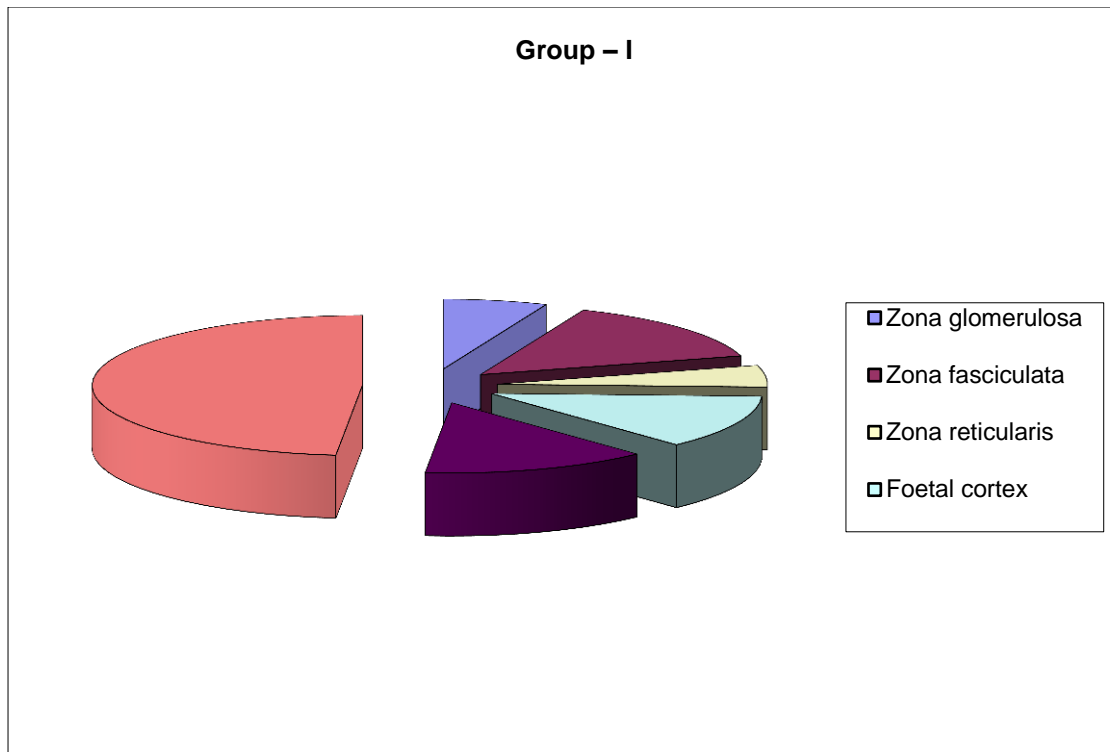


Fig. 5 Adrenal gland from five months old buffalo calf showing dominance of collagen fibres in the capsule (C), zona glomerulosa (G) and inner zone of medulla (Mi). Masson's trichrome stain x 40.

Fig. 6 Adrenal gland from five months old buffalo calf showing condensation of connective tissue at the corticomedullary junction in the form of incomplete capsule (arrows) containing round to elongated eosinophilic cells. Also seen are outer (Mo) and inner (Mi) zone of medulla. Masson's trichrome stain x 200.

Fig. 7 Adrenal gland from eight months old buffalo calf showing collagen fibers in capsule (C), zona glomerulosa (G), outer (Mo) and inner (Mi) zone of medulla. Also seen are zona fasciculata (F), zona reticularis (R) and central vein (Cv). Masson's trichrome stain x 40.

Fig. 8 Adrenal gland from one year old buffalo showing well marked connective tissue capsule at the corticomedullary junction with increased density of eosinophilic cells which are probably degenerating FC cells. Also seen are zona reticularis (R) and outer (Mo) zone of medulla. Masson's trichrome stain x 200.

- Fig. 9** Adrenal gland from day old buffalo showing reticular fibers in capsule (C), zona glomerulosa (G), foetal cortex (f) and medulla (M). Gridley's stain x 40.
- Fig. 10** Adrenal gland from three weeks old buffalo calf showing an increase in the reticular fibers in the inner part of capsule (C), zona glomerulosa (G) and outer (Mo) zone of medulla. Gridley's stain x 40.
- Fig. 11** Adrenal gland from one year buffalo showing reticular fibers in capsule (C), zona glomerulosa (G), outer (Mo) and inner (Mi) zone of medulla. Gridley's stain x 40.

Fig. 12 Adrenal gland from day old buffalo calf showing capsule (C), zona glomerulosa (G) in definite cortex and hemorrhage (arrow) in foetal cortex (f). Hematoxylin and Eosin stain x 100.

Fig. 13 A part of foetal cortex of Fig. 12 at higher magnifications showing FC cells (F) intermingled with medullary cells (M). Hematoxylin and Eosin stain x 200.

Fig. 14 Adrenal gland from one week old buffalo calf showing the appearance of zona fasciculata (F). Also seen are capsule (C), zona glomerulosa (G), foetal cortex (f) and outer (Mo) zone of medulla. Hematoxylin and Eosin stain x 100.

Fig. 15 A part of Fig. 14 at higher magnifications showing large and compactly arranged FC cells (f). Also seen outer (Mo) part of medulla. Hematoxylin and Eosin stain x 200.

- Fig. 16** Adrenal gland from two week old buffalo calf showing capsule (C), well marked zona glomerulosa (G), zona fasciculata (F) arranged in radiating cords and foetal cortex (f). Hematoxylin and Eosin stain x 100.
- Fig. 17** A part of Fig. 16 at higher magnifications showing FC cells (f) with moderately vacuolated cytoplasm. Hematoxylin and Eosin stain x 200.
- Fig. 18** Adrenal gland from one month old buffalo calf showing the blood vessels (v) in the outer part of capsule (C) and well organized zona glomerulosa (G) and zona fasciculata (F). Hematoxylin and Eosin stain x 100.
- Fig. 19** Adrenal gland from one month old buffalo calf showing the degenerating FC cells (f) with lightly stained cytoplasm and loss of nuclei. Hematoxylin and Eosin stain x 400.

- Fig. 20** Adrenal gland from three month old buffalo calf showing zona reticularis (R) below the zona fasciculata (F). Also seen are narrow condensation of connective tissue (arrows) at corticomedullary junction and outer zone of medulla. Hematoxylin and Eosin stain x 200.
- Fig. 21** Adrenal gland from five month old buffalo showing capsule (C), zona glomerulosa (G), zona fasciculata (F), zona reticularis (R), well marked connective tissue condensation at the corticomedullary junction and outer (Mo) zone of medulla. Masson's trichrome stain x 100.
- Fig. 22** Adrenal gland from nine month old buffalo showing zona fasciculata (F), pyknotic (arrows) and heterochromatic (arrow heads) cells in the zona reticularis (R) and outer (Mo) zone of medulla. Hematoxylin and Eosin stain x 200.

- Fig. 23** A part of medulla of Fig. 2 at higher magnifications showing colloid filled follicles (arrows) and groups of nerve bundles (arrow head). Masson's trichrome stain x 100.
- Fig. 24** Adrenal gland from one week old buffalo calf showing the outer zone of large adrenal secreting cells (A) arranged in follicular form and inner zone of small noradrenaline secreting cells (NA). Also seen is foetal cortex (f). Hematoxylin and Eosin stain x 100.
- Fig. 25** Adrenal gland from four month old buffalo calf showing adrenaline secreting cells (A) and noradrenaline secreting cells (NA). Also seen is foetal cortex (f). Masson's trichrome stain x 100.
- Fig. 26** A part of Fig. 25 at higher magnifications showing large adrenaline secreting cells (A) with homogenous cytoplasm and spherical to round nucleus and small noradrenaline secreting cells (NA) with a less homogenous cytoplasm and centrally placed nucleus. Also seen is foetal cortex (f). Masson's trichrome stain x 200.

- Fig. 27** Adrenal gland from four month old buffalo showing the foetal cortex (f) and degenerating FC cells with pyknotic nuclei (arrows) on the periphery of blood vessels (v) in the corticomedullary region. Hematoxylin and Eosin stain x 400.
- Fig. 28** Outer (Mo) and inner (Mi) zone of adrenal medulla from one year old buffalo showing nerve fibers. Bielschowsky's stain x 100.
- Fig. 29** Adrenal gland from nine month old buffalo showing well differentiated outer (Mo) and inner (Mi) zone of medulla. Also seen are zona reticularis (R) and connective tissue condensation at corticomedullary junction. Masson's trichrome stain x 100.

Fig. 30 Adrenal gland from one year old buffalo showing brownish purple stain adrenaline secreting cells (A) and slightly light yellowish noradrenaline secreting cells (NA). Also seen zona reticularis (R). Wood's stain x 100.

Fig. 31 A part of Fig. 30 at higher magnifications showing brownish purple stained adrenaline secreting cells. Wood's stain x 200.

Fig. 32 A part of Fig. 30 at higher magnifications showing comparatively light yellowish and compactly arranged noradrenaline secreting cells. Wood's stain x 200.

Fig. 33 Adrenal gland from six month old buffalo showing the adrenaline (A) and noradrenaline secreting cells (NA). Giemsa stain x 100.

- Fig. 34** Adrenal gland from one week old buffalo calf showing PAS positive reaction in the capsule (C), zona glomerulosa (G), zona fasciculata (F), foetal cortex (f), outer (Mo) and inner (Mi) zone of medulla. PAS x 40.
- Fig. 35** Adrenal gland from one week old buffalo calf showing a PAS positive rim (arrows) on the periphery of FC cells. PAS x 400.
- Fig. 36** Adrenal gland of three week old buffalo calf showing the degenerating FC cells (arrows) with diffuse or absence of PAS positive rim. PAS x 200.
- Fig. 37** Adrenal gland from one year old buffalo calf showing PAS positive reaction in the capsule (C), zona glomerulosa (G), zona fasciculata (F), zona reticularis (R), outer (Mo) and inner (Mi) zone of medulla. PAS x 40.

Fig. 38 Adrenal gland from nine month old buffalo calf showing a weak PAS positive reaction in the zona reticularis (R) and moderate reaction in the cells of remnant of foetal cortex (arrows). PAS x 400.

Fig. 39 Adrenal gland from day old buffalo calf showing the alcinophilic reaction in the outer part of capsule (C), zona glomerulosa (G) of definite cortex, foetal cortex (f) and medulla (M). AB PAS x 40.

Fig. 40 Adrenal gland from one year old buffalo showing alcinophilic reaction in the outer part of capsule (C), zona granulosa (G), zona fasciculata (F), zona reticularis (R), outer (Mo) and inner (Mi) zone of medulla AB PAS x 40.

Fig. 41 Cryostat section of adrenal gland from one month old buffalo calf showing lipid content in the capsule (C), zona glomerulosa (G), zona fasciculata (F) and foetal cortex (f) and no lipid contents are seen in the medulla (M). Sudan Black B stain x 40.

Fig. 42 A Part of Fig. 41 at higher magnifications showing the lipid contents in zona glomerulosa (G) and zona fasciculata (F). Sudan Blank B stain x 100.

Fig. 43 Cryostat section of adrenal gland of one month old buffalo calf showing phospholipid content in zona glomerulosa (G), zona fasciculata (F) and medulla (M). Baker's acid Haematin stain x 40.

Fig. 44 Cryostat section of adrenal gland from one month old buffalo calf showing moderate amount of cholesterol in cortex and low to negligible in the medulla (M). Schultz's stain x 80.

- Fig. 45** Adrenal gland from day old buffalo calf showing concentration of protein in the capsule (C), zona glomerulosa (G), foetal cortex (f) and medulla (M). Bromphenol blue stain x 100.
- Fig. 46** Adrenal gland from four months old buffalo calf showing the concentration of protein in capsule (C), zona granulosa (G), zona fasciculata (F) and foetal cortex (f). Bromphenol blue stain x 100.
- Fig. 47** Adrenal gland from nine month old buffalo showing concentration of proteins in capsule (C), zona glomerulosa (G), zona fasciculata (F) and zona reticularis (R). Bromphenol blue stain x 100.
- Fig. 48** Adrenal gland from four months old buffalo calf showing high concentration of protein in the cells of zona reticularis (R) than medullary cells (M). Bromphenol blue stain x 100.

CHAPTER – V

SUMMARY

The present study was undertaken to investigate the histomorphological and histochemical changes in the adrenal gland of neonatal buffalo calves during the postnatal development. A total of 29 animals aging from day old to one year were used in this study. Based on their age the animals were divided into three Groups viz., Group-I (day old to 3 months), Group-II (more than 3 months to 6 months) and Group-III (more than 6 months to 1 year).

The gross morphological observations revealed that the left adrenal was heavier (3.44 ± 0.15 g), broader (3.05 ± 0.44 cm), and thicker (0.66 ± 0.02 cm) than the right adrenal gland (3.37 ± 0.21 g, 2.90 ± 0.45 cm and 0.64 ± 0.07 cm) in Group – III, however, the right adrenal gland was longer (4.52 ± 0.58 cm) than the left adrenal gland (4.44 ± 0.15 cm) in the same Group.

The adrenal gland was fully differentiated into cortex and medulla in the day old buffalo calf and was completely surrounded by a dense fibrous capsule at this stage. The capsule consisted of outer layer of loose connective tissue and inner layer of thick collagen fibres. The connective tissue trabeculae made up of collagen and reticular fibres arose from the inner aspect of capsule and infiltrated the cortical parenchyma of the gland. The reticular fibres dominated in the inner aspect of the capsule and the proportion of reticular fibres was more in the zona glomerulosa and outer zone of medulla, however, elastic fibres were not observed in any part of the gland up to one year of age. The average thickness of the capsule increased with progression of age which was due to increase in the density of collagen fibres especially in the inner zone of capsule.

The adrenal cortex was made up of definite and foetal cortex in the day old buffalo calf. The zona glomerulosa was observed in the sub capsular area and consisted of darkly stained cells with irregular nuclei and large number of sinusoidal capillaries. The cellular mass in this zone was arranged in clusters of columnar cells and had spherical or oval nuclei surrounded by a homogenous cytoplasm. The zona glomerulosa was the smallest of the three cortical zones. The thickness of this zone was increased from 177.42 ± 21.14 μ m in Group-I to 252.73 ± 11.51 μ m in Group-III. The average density of cells per unit area was maximum in this zone. The foetal cortex was made up of small to elongated FC cells which were having eosinophilic cytoplasm and were arranged into irregular cords. These cells were intermingled with medullary cells at corticomedullary junction. The outer part

of the foetal cortex was characterized by the presence of comparatively larger cells with vacuolated cytoplasm. With progression of age the FC cells were pushed towards the centre of the gland, became closely packed and were moderately vacuolated. The remnant of foetal cortex was observed even in the Group-III.

The zona fasciculata was interposed between the zona glomerulosa and foetal cortex in the adrenal gland of one week old buffalo calf. The cells of this zone were arranged in single or double cords and were larger in size. No mitotic figures were seen in the zona fasciculata or at the junction of zona fasciculata and foetal cortex. The Zona fasciculata was more organized with the progression of age and contained cells with foamy cytoplasm. The zona fasciculata was the widest zone amongst all the cortical zones. The thickness of this zone increased from $411.53 \pm 45.55 \mu\text{m}$ in Group-I to $869.73 \pm 29.37 \mu\text{m}$ in Group-III. The average cell size of the cells in this zone was largest in all the Groups. Statistically the rate of increase in thickness of this zone was significant in Group-II.

The zona reticularis was first time observed below the zona fasciculata in the adrenal gland of one and half month old buffalo calves. The cells of this zone were smaller than the cells of zona fasciculata. These cells were having little cytoplasm with pyknotic and heterochromatic nuclei. However, this zone was not well organized even in the adrenal gland of one year old buffalo. The zona reticularis was the innermost zone of the cortical parenchyma of the adrenal gland and the thickness of this zone increased from $141.23 \pm 23.40 \mu\text{m}$ in Group-I to $393.14 \pm 34.17 \mu\text{m}$ in Group-III. Statistically the zona reticularis showed significant increase in thickness in Group-III. The diameter of cells of this zone varied from $6.42 \pm 0.20 \mu\text{m}$ to $5.85 \pm 0.43 \mu\text{m}$ in Group-I and Group-III, respectively.

The connective tissue condensation consisting of fine collagen fibres was observed at the corticomedullary junction in the adrenal gland in one and half month old buffalo calf. However, it was incomplete and varied in thickness from one part to another and distinguished between zona reticularis and outer zone of medulla. The connective tissue capsule at corticomedullary junction was well marked in Group-II and contained large isolated cells with eosinophilic cytoplasm which were probably degenerating FC cells. There was sharp demarcation between cortex and medulla due to presence of more marked connective tissue capsule in Group-III. The bulk of the adrenal cortex was formed by zona fasciculata and zona reticularis however, the cortical parenchyma constituted a lesser proportion of the gland than the medulla.

The adrenal medulla formed the greater part of the adrenal gland and occupied the central portion of the gland in day old buffalo calf. The medullary cells situated at the periphery of the medulla were larger as compared to the cells found in the centre of the gland, however, the two types of medullary cells were arranged in the outer and inner zone in the adrenal gland of one week buffalo calves. The large adrenaline secreting cells were arranged in follicular form and were seen in the outer zone of medulla. The noradrenaline secreting cells were observed in the central portion of adrenal medulla around the central vein and were small in size but compactly arranged. The adrenaline secreting cells were having homogenous cytoplasm with spherical to round nucleus located more towards the apical portion whereas the noradrenaline secreting cells had a less homogenous cytoplasm with a centrally placed nuclei. With progression of age the outer and inner zone of medulla were more clearly differentiated and more number of nerve fibres were observed in both the zones of medulla in Group-III.

The average thickness of adrenal medulla increased from $1765 \pm 79.86 \mu\text{m}$ in Group-I to $1998.73 \pm 103.45 \mu\text{m}$ in Group-III. The percentage proportion of inner medullar zone was more than the outer medullary zone in all the age Groups. Statistically the rate of increase in inner medullary zone was more than the outer medullary zone. The corticomedullary ratio decreased from 1:1.62 in Group-I to 1:1.32 in Group-III which may be due to

rapid cortical growth. The average cell density per unit area was less in the outer medullary zone than the inner medullary zone and the average cell size was more in the outer medullary zone in all the Groups.

The two types of adrenal medullary cells were clearly differentiated by Wood's, and Giemsa staining methods. The adrenaline secreting cells stained brownish purple whereas the noradrenaline secreting cells appeared light yellow by Wood's staining method however, fixation in orth fluid was must. The Giemsa staining method selectively stained the noradrenaline secreting cells darker green than the adrenaline secreting cells in the paraffin section of orth fixed adrenal gland.

The neutral mucopolysaccharide (NMPS) content was high in the inner aspect of capsule moderate in the wall of large blood vessels of the capsule and cells of zona glomerulosa, low in the cells of zona fasciculata and zona reticularis and low to moderate in the cells of both the zones of medulla.

The acid mucopolysaccharide (AMPS) content was low in the outer zone of capsule, the cells of zona glomerulosa and zona fasciculata, high in the cells of zona reticularis, low in the outer zone of medulla and moderate in the inner zone of medulla.

Sudan black-B positive lipid droplets content was negative to low in the capsule, low in the zona glomerulosa, high in the zona fasciculata and zona reticularis and negative in the outer

and inner zones of medulla and in the area separating cortex and medulla which corresponded to the region of degenerating foetal cortex. The Phospholipid content was more in the cortex as compared to the medulla, however, the cholesterol content was moderate in all the cortical zones and negative to low in the medullary cells.

The concentration of basic protein was more in the adrenal cortical cells than the medullary cells. The cells of the zona glomerulosa were having higher content of protein in Group-I, however, the concentration of protein was same in all the cortical zones in Group-II and Group-III.

CONCLUSIONS

1. The adrenal gland of day old buffalo calf was fully differentiated into cortex and medulla and was completely encapsulated by a fibrous capsule.
2. The adrenal cortex was made up of definite and fetal cortex in day old buffalo calf. The definite cortex was represented by zona glomerulosa only. The larger cells with vacuolated cytoplasm were the FC cells.
3. The zona glomerulosa was the smallest of the three cortical zones and consisted of columnar cells having homogeneous cytoplasm with spherical or oval nuclei and cells were arranged in clusters.
4. The zona fasciculata was observed in the adrenal gland of one week old buffalo calf and was situated between the zona glomerulosa and foetal cortex at this stage. The cells at this zone were comparatively larger in size.
5. The zona fasciculata was the widest zone amongst all the cortical zones and had cells with the largest diameter. With Progression of age the zona fasciculata contained cells with foamy cytoplasm.
6. The zona reticularis was first time observed in the adrenal gland of one and half month old buffalo calves. The cells of this zone were small in size with little cytoplasm and pyknotic and heterochromatic nuclei.
7. The zona reticularis was the innermost zone of cortical parenchyma and were not well organized even in the adrenal gland of one year old buffalo.
8. The foetal cortex degenerated with progression of age; however, the remnants of foetal cortex were seen in the adrenal gland of up to one year old buffalo.
9. An incomplete connective tissue condensation was observed at the corticomedullary junction in one and half month old buffalo calf which was

well marked in Group-II and had large isolated cells with eosinophilic cytoplasm which was probably degenerating FC cells.

10. The adrenaline and noradrenaline secreting cells were identified in the outer and inner zone of medulla. The adrenaline secreting cells were having homogenous cytoplasm with spherical to round nuclei, however, the noradrenaline secreting cells had a less homogenous cytoplasm with centrally placed nuclei.
11. The adrenaline and noradrenaline secreting cells were clearly distinguished by Wood's and Giemsa staining methods.
12. The medulla occupied the greater proportion of the gland and inner medullary cells were the main cellular constituent of the medulla.
13. The neutral and acid mucopolysaccharide contents varied from low to high in the capsule, cortex and medulla.
14. The cells of the zona fasciculata and zona reticularis showed rich contents of lipid. The Phospholipid content was more in the cortex as compared to the medulla.
15. The concentration of basic protein was more in the cortical cells than the medullary cells.

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