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K Rajalakshmi
Department of Veterinary Anatomy and Histology, Rajiv Gandhi Institute of Veterinary Education and Research, Kurumbapet, Puducherry, India

Geetha Ramesh
Department of Anatomy, Madras Veterinary College, Chennai, Tamil Nadu, India

Usha Kumari
Department of Anatomy, Madras Veterinary College, Chennai, Tamil Nadu, India

M Sivakumar
Department of Veterinary Anatomy and Histology, Rajiv Gandhi Institute of Veterinary Education and Research, Kurumbapet, Puducherry, India

S Uma
Department of Pathology, Rajiv Gandhi Institute of Veterinary Education and Research, Kurumbapet, Puducherry, India

Lakkawar AW
Department of Pathology, Rajiv Gandhi Institute of Veterinary Education and Research, Kurumbapet, Puducherry, India

Correspondence
M Sivakumar
Professor & Head, Department of Veterinary Anatomy and Histology, Rajiv Gandhi Institute of Veterinary Education and Research, Kurumbapet, Puducherry, India

Microanatomy of the parathyroid glands in sheep (*Ovies aries*)

K Rajalakshmi, Geetha Ramesh, Usha Kumari, M Sivakumar, S Uma and Lakkawar AW

Abstract
A study on the histomorphology and histochemistry of the parathyroid glands was conducted in the prenatal and postnatal age groups of sheep. For the prenatal study, the thyroid gland was collected from sheep embryos of early (42 days), mid (77.7 days) and late (128 days) gestation. Postnatal study included the collection of external parathyroid glands from young (<1 year), adult (1-5 years) and old (> 5 years) sheep, irrespective of the sexes. Tissue pieces were collected in different fixatives for routine paraffin embedding. Paraffin sections were used for routine and special staining techniques. Frozen sections were used for histochemical techniques. The internal parathyroid gland in the early, mid and late gestation embryos was observed within the section of the thyroid gland on the dorso-medial aspect. The general architecture of the parenchyma of the internal parathyroid in the prenatal age groups was similar to the postnatal age groups studied. In the postnatal age groups, the parathyroid gland was surrounded by a connective tissue capsule. From the capsule, thin indistinct trabeculae extended into the parenchyma, but did not form any compartments. The parenchyma of the internal and external parathyroids in all the age groups consisted of the light and dark chief cells. C cells were noticed in the internal parathyroids, but were absent in the external parathyroids. Three types of follicles namely, light, dark and mixed follicles were observed in the young and adult age groups. Older animals showed only light follicles. Some of the light follicles contained colloid in their lumen. The colloid in the light follicles of the internal and external parathyroids in all the age groups was PAS positive. The presence of lipids, acid and alkaline phospahatases were recorded in the parathyroid gland.

Keywords: Histochemistry, histomorphology, prenatal, postnatal, sheep, parathyroid glands

Introduction
The parathyroid gland was first reported by Raynard in 1835 and its structure was first described by Sandstrom in 1880 [1]. The number of pairs of parathyroid glands in domestic animals varies from one to two pairs. Sheep, cattle, dog and cat possesses two pairs [2], while the pig and horse have only one pair [3]. In sheep, the external parathyroids are not associated with the thyroid and are located at the branching of the common carotid artery [4]. The internal parathyroids are situated on the dorsomedial surface of the thyroid gland [2].

The parathyroid glands are endocrine glands producing parathyroid hormone which acts on the kidneys, intestine and bones to maintain the necessary concentration of calcium in the extracellular fluid of the body [5]. Parathormone is the principal hormone involved in the minute to minute fine regulation of blood calcium concentration in mammals [6, 7]. In severe parathormone deficiencies (eg, after parathyroidectomy), the decreased blood calcium level causes fibrillary twitching of various muscles, followed by clonic movements of the limbs and finally by rigid spasms and death [8].

Although an extensive work on the structure of the parathyroid glands has been carried out in various mammals, the structural details of these glands in the sheep are limited. Hence, the present work has been undertaken with an aim to achieve the following objectives. i) To study the histomorphology of the parathyroid glands of the sheep in the prenatal and postnatal age groups. ii) To study the histochemistry of the different structures of the parathyroid glands among the age groups studied. iii) To correlate the structures with the functional aspects. The attempt on the present study under light microscopy would give further scope in the ultra structural research of the parathyroid glands in sheep.

Materials and Methods

Materials

The thyroid and parathyroid glands were collected from sheep for the present study. The materials were collected from the Chennai Corporation slaughter house, Perambur.

For the prenatal study, the thyroid gland was collected from sheep embryos of early (42 days), mid (77.7 days) and late (128 days) gestation. Postnatal study included the collection of thyroid and external parathyroid glands from young (<1 year), adult (1-5 years) and old (> 5 years) sheep, irrespective of the sexes.

The tissue pieces after washing with normal saline were fixed in the following fixatives for routine paraffin embedding [9].

1. 10 percent buffered neutral formalin.
2. Formaldehyde solution (37-40 percent formalin).
3. Bouin's fluid.
4. Zenker's fluid.
5. Chilled formal calcium (4 ˚C).

The fixed tissues were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin (58 ˚C – 60 °C). 5-6 µm thin sections were cut and used for routine and special staining techniques.

For the localization of lipids and enzymes, 20-30 µm thick cryosections were used.

Methods

Histological staining methods

The following routine and special staining methods were used for histological observations.

1. Haemalum-Eosin phloxine method [10] for routine observations.
2. Masson's trichrome method [9].
3. Van-Gieson's stain for collagen fibres [10].
4. Weigert's method for elastic fibres [9].
5. Gomori's method for reticulum [9].
6. Mallory's phosphotungstic acid haematoxylin method [9].
7. Bielschowsky's method for Axis cylinders and dendrites [9].
8. Unna's method for mast cells [9].
9. Mallory-Heidenhain Azan stain [11].
10. Lead hematoxylin method [11].

Histochemical staining methods

1. Periodic acid-schiff reaction for neutral mucopolysaccharides [12].
2. Combined Alcian blue-PAS technique for acid and neutral mucopolysaccharides [12].
3. Oil Red '0' in propylene glycol method for fats [10].
4. Gomori's Alkaline phosphatase cobalt method [10].
5. Gomori's lead method for Acid phosphatase activity [12].

Results

Parathyroid Gland

Prenatal

In the embryos of early (42 days), mid (77.7 days) and late (128 days) gestation, the internal parathyroid tissue was observed within the section of the thyroid gland on the dorsomedial aspect and was separated by a thin connective tissue capsule from the thyroid gland (Fig. 1). The connective tissue was composed of a few collagen and reticular fibres. The general architecture of the parenchyma of the internal parathyroid in the prenatal age groups of the present study was similar to the postnatal age groups. The light and dark chief cells were the components of the parenchyma. The light cells were observed to form follicles, groups and cords (Fig. 2). As the age of the embryo advanced, the formation of follicles was also observed to be more. The interfollicular connective tissue was found to be highly vascular.

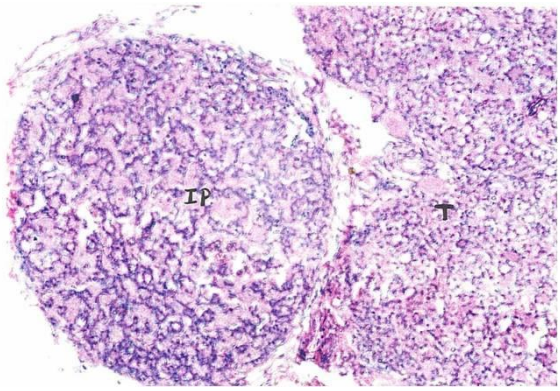

Fig 1: Photomicrograph of the thyroid gland of a mid gestation (77.7 days) sheep embryo showing the internal parathyroid on the periphery of the thyroid gland. IP-Internal parathyroid gland; T-Thyroid gland. H & E X 200.

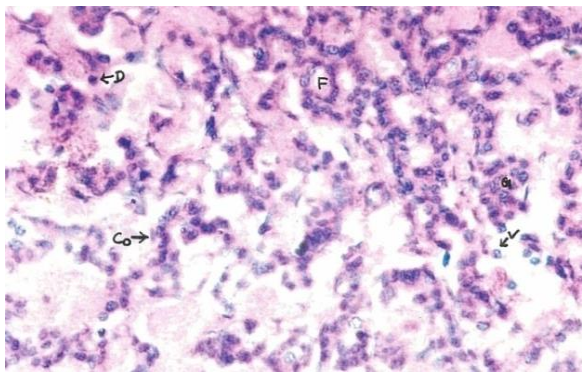

Fig 2: Photomicrograph of the internal parathyroid of a mid gestation (77.7 days) sheep embryo showing the organization of chief cells in the parenchyma (arrows). F-Follicle; G-Group; Co-Cord; L-Light Chief Cell; D-Dark Chief Cell. H&E x 630.

Postnatal

In general, the capsule of the external parathyroid gland was made up of dense irregular collagenous connective tissue, with lesser proportion of reticular and elastic fibres, in all the age groups studied. Three layers were noticed in the capsule. The external layer was a mesothelial layer lined by simple squamous epithelium. The middle layer was rich in fat cells, blood vessels and nerves with a few ultimobranchial follicles. The inner collagenous layer was closely adherent to the gland (Fig. 3). The presence of parenchymal cells in the capsule was very common in all the age groups studied. From the capsule, thin indistinct trabeculae extended into the parenchyma, but did not form any compartments. A proportional increase in the amount of connective tissue, in the capsule, trabecuale and the stroma was observed with advancing age. The internal parathyroid tissue was observed within the section of the thyroid gland on the dorsomedial aspect and was intermingled with the thyroid follicles in the < 1 year and the 3 year animals (Fig. 4). In the 1 year old animal, it was noticed on the dorsomedial aspect being separated by a connective tissue capsule from the thyroid gland. In the 2 year animal, it was found in the centre and towards the inferior pole of the thyroid section, intermingled with the thyroid follicles. In the 4 -5 year and > 5 year animals, the internal parathyroid gland was observed in the centre and towards the inferior pole of the thyroid gland. It was separated from the surrounding thyroid follicles by a thick connective tissue capsule (Fig. 5).

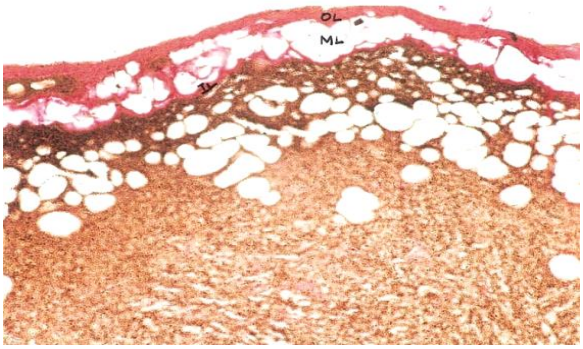

Fig 3: Photomicrograph of the external parathyroid gland of a >5 year-old sheep showing the three layers in the capsule. OL-Outer layer; ML-Middle layer; IL-Inner layer. Van Geison's x 100.

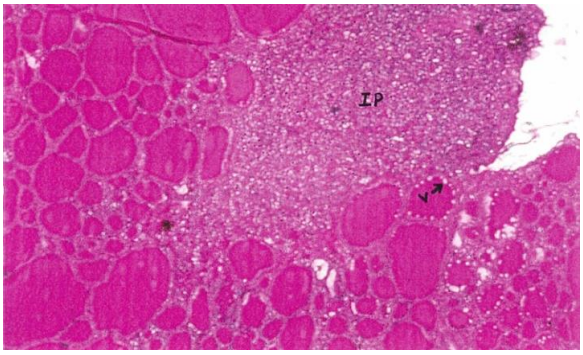

Fig 4: Photomicrograph of the thyroid gland of a <1 year-old sheep showing the internal parathyroid tissue intermingled with the thyroid follicles and the presence of peripheral vacuoles (arrow) in the colloid. IP-Internal parathyroid; V-Vacuoles.

Combined Alcian blue-PAS x 100.

The parenchyma of the internal and external parathyroid glands in all the age groups studied, consisted of a single cell type namely the principal or chief cells. Water-clear cells and oxyphil cells were not observed. The chief cells were of two types namely the light and dark chief cells. The light cells were of different shapes and sizes. Spherical, elongate, oval and polygonal light cells were recorded in the age groups studied (Fig. 6). A single layer of light cells which gathered around a sinusoidal capillary and solitary light cells with pale staining were observed in all the age groups studied. The nucleus of the light cell was large and lightly stained. The cytoplasm had lesser secretory granules. The dark cells were observed to be uniformly spherical in shape (Fig. 6).

Different sizes of the dark cells namely, small, medium and large cells were observed in all the age groups studied. The dark cells contained a nucleus with a condensed chromatin and were surrounded by a dark cytoplasm which had abundant secretory granules. In the periphery of the parathyroid gland, both light and dark cells were observed to be uniformly distributed in all the age groups (Fig. 6). In the internal parathyroids, the C cells were distributed in the periphery and frequently gathered as a large cluster in the central region and were polyhedral or elongate in shape. C cells were absent in the external parathyroids.

In all the age groups studied, the chief cells were arranged in the form of irregular anastamosing cords, clusters and also in a follicle-like manner (Fig. 7). The follicles were irregularly distributed in the gland parenchyma. Three types of follicles namely, light follicles lined exclusively by light cells, dark follicles lined solely by dark cells and mixed follicles, lined by both light and dark cells (Fig. 8) were noticed in the parathyroid of the young and adult age groups. In the animals aged over 5 years, only light follicles were observed in the parenchyma. Some of the light follicles had colloid in their lumen. Many follicles were noticed with degenerated cells in their lumen. The dark follicles found in the young and adult age groups were few and were confined to the centre of the parathyroid sections.

The interstitial connective tissue of the external and the internal parathyroid glands was highly vascular and increased as age advanced (Fig. 6 & 9). Sinusoids with distinct endothelial cells were also observed in the parenchyma. The mast cells were observed in the external parathyroid glands, in the vicinity of blood vessels, in the sections stained by Unna's method. They were few in young animals but were more frequent in the animals aged over 5 years. The presence of thymic tissue associated with the external parathyroid glands separated by a capsule, was also noticed in all the age groups of the present study.

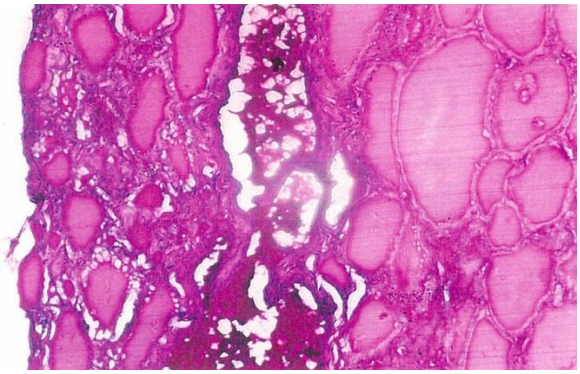

Fig 5: Photomicrograph of the thyroid gland of a >5 year-old sheep showing the PAS positive colloid in the internal parathyroid tissue of the thyroid gland (arrows). PAS x 200.

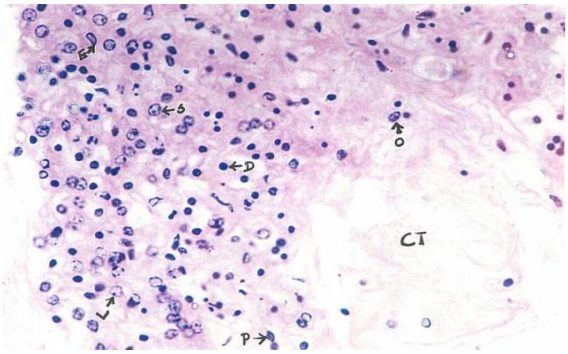

Fig 6: Photomicrograph of the internal parathyroid gland of a 3 year-old sheep showing the different shapes of chief cells (arrows). O-Oval light cell; E-Elongated light cell; S-Spherical light cell; P-Polygonal light cell; L-Light cell; D-Dark cell; CT-Connective tissue. H & E X 630.

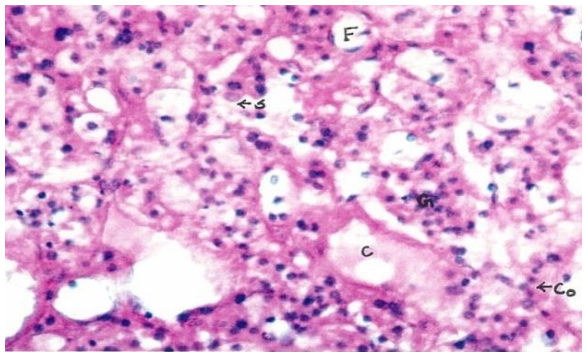

Fig 7: External parathyroid gland of a 4-5 year-old sheep showing the organization of chief cells in the paraenchyma (arrows). F-Follicle; G-Group; Co-Cord; C-Colloid; S-Solitary light cell. H & E x 630.

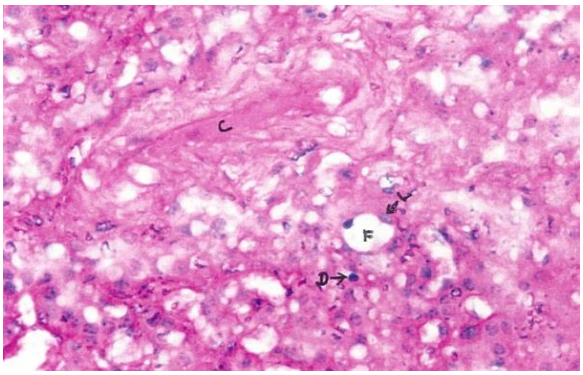

Fig 8: Internal parathyroid gland of a <1 year-old sheep showing the mixed follicle and PAS positive colloid. F-Follicle; L-Light cell; D-Dark cell; C-Colloid. Combined Alcian blue-PAS x 630.

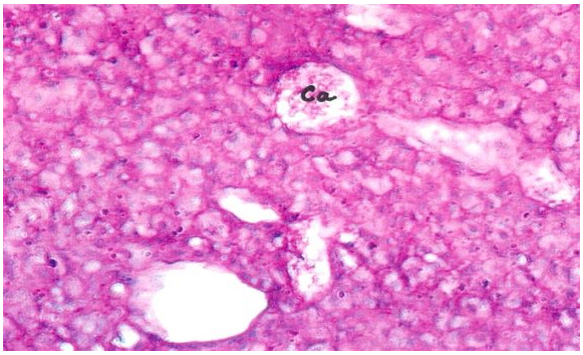

Fig 9: External parathyroid gland of a >5years-old sheep showing a sinusoidal capillary and the abundant interstitial connective tissue. Ca-Capillary. PAS X 630.

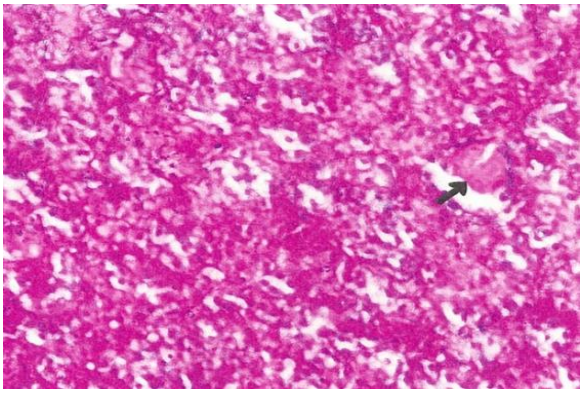

Fig 10: External parathyroid gland of a 4-5year-old sheep showing the organization of chief cells in the paraenchyma (arrows). F-Follicle; G-Group; Co-Cord; C-Colloid; S-Solitary light cell. H & E x 630.

The capsule of the external parathyroid glands was weakly PAS positive in all the age groups. The capsule was negative for acid mucopolysaccharides in the combined Alcian blue-PAS technique followed. The colloid in the light follicles of the external and the internal parathyroid glands in all the age groups were PAS positive (Fig. 5 & 10). The accumulation of lipids was observed in the capsule, the interstitial connective tissue and in some of the parenchymal cells in all the age groups, with an increased amount of lipid as age advanced. Acid and alkaline phosphatase activities were observed only in the capillaries.

Discussions

Prenatal

Mc Donald [2] observed the presence of internal parathyroid gland on the dorso-medial aspect of the thyroid gland in the post natal age groups of sheep. However, in the sheep embryos of early gestation (42 days) a similar location of the internal parathyroid was observed in the present study which confirms the earlier findings.

The general architecture of the parenchyma of the internal pararthyroid in the prenatal age groups of the present study was similar to the postnatal age groups. The light and dark chief cells were the components of the parenchyma. This concurs with the findings of Jordan *et al.* [13], who observed chief cells in the internal parathyroid of 30 day sheep embryos and reported that, at this stage the developing chief cells differed from the adult chief cells only in size, being considerably larger.

Postnatal

The capsule of the external parathyroid gland had three layers namely an external, middle and an inner layer. From the capsule, thin indistinct trabeculae extended into the parenchyma, but did not form any compartments. The presence of paranchymal cells in the capsule was very common in all age groups. The findings of the present study concur with the earlier observations in white leghorn birds [14]. However, Nagpal *et al.* [15] observed distinct lobes and lobules in the parenchyma of the cranial parathyroids in camel.The presence of a few ultimobranchial follicles in the capsule of the external parathyroids in the present study could be due to the close association of the pharyngeal derivatives.

The internal parathyroid tissue in the present study was found to be located on the dorso-medial aspect of the thyroid gland in some age groups corresponded with the earlier reports in sheep [2]. In some age groups, the same was found in the centre of the inferior pole of the thyroid gland as reported by Hecker [16] in sheep. This tissue was found to be either intermingled with the glandular tissue of the thyroid or was isolated by a connective tissue capsule as reported in goat [17].

The parenchyma of the internal and external parathyroids consisted of a single cell type namely the principal or chief cells. Water- clear cells and oxyphil cells were not observed in the present study, which corroborated with the earlier reports in sheep [18], cat [19] and other animals [20, 8], and in female Kuttanad ducks [21].

On the contrary to the findings of the present study, Roy *et al.* [17] reported the presence of oxyphilic and syncytial cells in the internal parathyroid of goat. In the parathyroids of dromedary camel, in addition to the chief and oxyphil cells, a third cell type namely the water-clear cell was distinguished by Al-Ramadan *et al.* [22]. In all the age groups studied, a single layer of light cells that gathered around a sinusoidal capillary was observed, as reported earlier by Nagpal *et al.* [15] in camel, who considered it to characterize the most advanced stage of specialization for synthesis of the hormone, since each cell had a minimum distance and obstruction from the blood stream.

In the periphery of both the internal and external parathyroid sections of all the age groups in the present study, both, light and dark cells were observed to be uniformly distributed. This concurs with the findings in the internal parathyroid of goat [17]. This is contrary to the findings of Dellmann [8], who reported that the periphery of the gland was occupied by light cells and the centre by dark cells.

In the internal parathyroids, the C cells were distributed in the periphery and the C cells were absent in the external parathyroids, as reported in sheep [23] and goat [24]. However, Tanimura *et al.* [25] had reported the presence of C cells even in the external parathyroids of horse. In the present study C

cells in the internal parathyroids, frequently gathered as a large cluster in the central region and was polyhedral or elongate in shape which concurs with the earlier findings in sheep [23].

The chief cells in all age groups of the present study were arranged in the form of irregular anastamosing cords, clusters and also in a follicle like manner as reported in camels [15], white leghorn birds [14] and cat [19].

Three types of follicles namely as light, dark and mixed ones were noticed in the parathyroid of the young and adult age groups. In the animals aged > 5years only light follicles were observed. Tsuchiya and Tamate [18] noticed light and dark follicles in the sheep parathyroids and stated that the follicles were irregularly distributed in the parenchyma, which concurs with the earlier findings. Some of the light follicles contained colloid in their lumen, which was also reported by in camel [15] and white leghorn birds [14].

The interstitial connective tissue in the internal and external parathyroids increased as age advanced as recorded earlier in goat [17] and camel [15]. Mast cells were observed in the parathyroid, in the vicinity of the blood vessels. They were few in young animals, but were more frequent in animals aged > 5 years. This is in agreement with the findings of Roy *et al.* [17] in the parathyroid of goat.

The presence of thymic tissue associated with the external parathyroid glands separated by a capsule, noticed in all the age groups of the present study is in accordance with the findings of earlier workers in sheep [26, 2].

Histochemistry

The colloid in the light cell follicles of the internal and external parathyroids in all the age groups studied was PAS positive. This concurs with the earlier findings in goat [17] and white leghorn birds [14]. Prasad *et al.* [27] recorded moderate PAS positive reaction in the parathyroid of duck.

The presence of lipids in the capsule, interstitial connective tissue and in some of the paranchymal cells observed in all the age groups in the present study is as reported earlier in birds [14]. Acid and alkaline phosphatase activities recorded in the parathyroids of the present study concurs with the similar findings in duck [27].

Conclusion

The present study concluded that the general architecture of the parenchyma of the internal parathyroid in the prenatal age groups was similar to the postnatal age. The light follicles were seen only in older animals. The parenchyma consisted of light and dark chief cells in all age groups. C cells were noticed in the internal parathyroids, while the same were absent in the external parathyroids. The colloid was found to be positive for PAS indicating the presence of polysaccharides. Lipids, acid and alkaline phosphatises were also recorded in the parathyroid glands in sheep.

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