Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(3): 1473-1476

Received: 18-03-2020
Accepted: 19-04-2020

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Prenatal development of thyroid gland in sheep (*Ovis aries*)

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Abstract
The primordium for thyroid gland in sheep fetuses was observed by 24 days of gestation at the floor of the pharynx. By 44 days of gestation, the gland showed presence of clumps of basophilic cells. It was surrounded by delicate connective tissue capsule whose thickness increased with gestational age. By 51 days of gestation, the clumps of cells were arranged to form curved cords. By 57 days of gestation, cells were organised into numerous follicles of different sizes filled with colloid. By 65 days of gestation, parafollicular cells were observed in between follicles. By 106 days of age vacuoles were noticed in periphery of the colloid in some follicles.

Keywords: Fetus, histogenesis, prenatal development, sheep, thyroid gland

Introduction
Thyroid is a reddish brown bilobed endocrine organ located in the neck region. Thyroid hormones are essential for normal development, neural differentiation and metabolic regulation in mammals [5]. They play an important role in endochondral ossification and are essential for skeletal development, linear growth, maintenance of bone mass and efficient fracture healing [6]. They are also essential for fetal and post-natal nervous system development and play a key role in the maintenance of adult brain function [13]. Thyroid disorders have a wide range of presentations and may affect many different organ systems [3].
Sheep are large mammals that have many similarities to humans in terms of physiology. They are easy to handle and suffer from many diseases which affect humans. They have short gestational period and give birth to young of similar weight to human babies making them excellent for studying development and genetics. Sheep is an excellent model for the study of major physiological systems such as cardiovascular, respiratory, renal reproductive and endocrinological systems [9].
The morphogenetic events that lead to the formation of thyroid gland are important to consider for understanding how developmental defects, which are the leading cause of congenital hypothyroidism may arise [16]. Present study will give scientific basis for understanding the normal development of thyroid gland in sheep which can form a basis helpful in various fields of science for comparison between the normal and abnormal development of the gland.

Materials and Methods
Present study was conducted at the Department of Veterinary Anatomy, Veterinary College and Research Institute, Namakkal. The pregnant sheep uteri with embryos and fetuses of sheep were collected immediately after slaughter from slaughter houses in Andhra Pradesh and Tamil Nadu. The Crown rump length (CRL) of embryos and fetuses were recorded. The approximate age in prenatal age groups was calculated by substituting obtained CRL in the formula given by Richardson [10].

$$X = 2.1(Y+17)$$

Where 'X' is the developmental age of fetus in days and 'Y' is crown rump length in centimeters.

In case of fetuses upto 3cm CRL standard values given by Bryden [4] were used for estimation of age. The crown rump length was measured from the most anterior part of the crown to the base of the tail [12].

The fetuses were dissected by making a mid ventral skin incision starting from the body of the mandible upto the inguinal region and thyroid was carefully dissected. The glands were carefully located, collected, rinsed in normal saline and were placed in the fixative.

Research material for present study was collected from three different age groups with 6 animals in each group. The groups are classified as follows

Prenatal age groups		
Group I	Group II	Group III
1-50 days	51-100 days	101-150 days of gestation

For studying histological structure, the tissue pieces were fixed in 10% neutral buffered formalin and Bouin's fluid. The fixed tissues were processed as per the methods described by Luna (1968). Paraffin sections of 3-5µm thickness were cut and subjected to the following techniques.

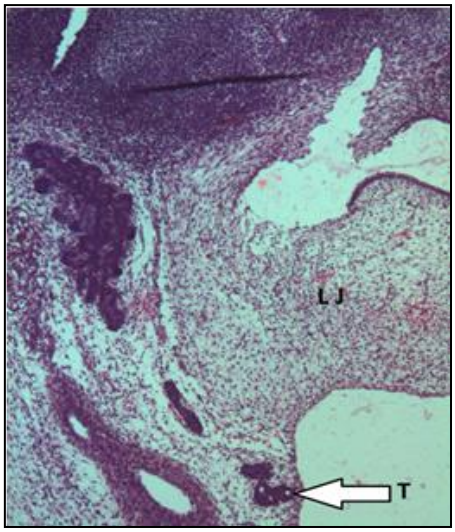
1. Standard Haematoxylin and Eosin (H&E) method for the routine histological study [2] (Bancroft and Gamble, 2003).
2. Gomori's reticulin method for reticular fibres [2] (Bancroft and Gamble, 2003).
3. McManus's method for glycogen (PAS) [14] (Singh and Sulochana, 1996).

Results and Discussion

Prenatal Groups

Group I

The primordium for thyroid gland was observed at 25 days of gestation at the floor of the pharynx. It consisted of group of basophilic cells. It was seen as a bilobed structure with cavities in it. (Fig 1) were in accordance with Taki [15] in humans Ramayya *et al.* [11] in buffalo fetuses and Al-jebori [1] in bovine fetuses.

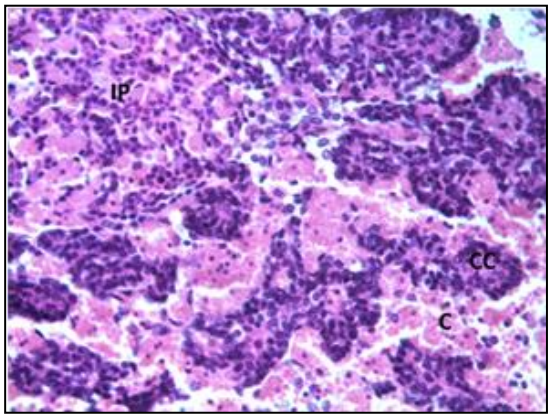


HE X10

Fig 1: Photomicrograph showing primordium of thyroid gland in 24 days sheep embryo HE X10. Lower Jaw (LJ) Thyroid (T)

At 44 days of gestation the thyroid gland was surrounded by a delicate connective tissue capsule surrounding the gland. Thick layer of red blood corpuscles were found surrounding the gland. Numerous highly basophilic rounded to oval basophilic cells grouped to form small and larger clumps. These clumps of cells were surrounded by numerous clumps of red blood corpuscles. Follicles were not observed.

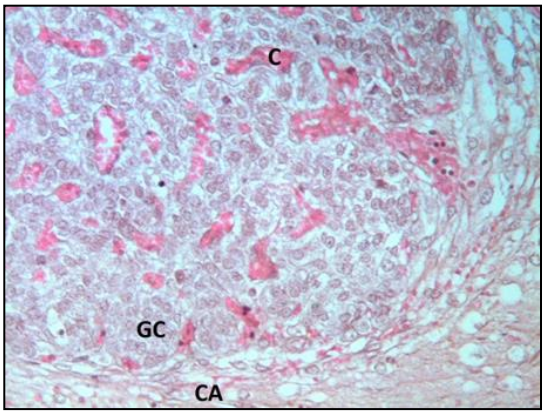
The internal parathyroid gland was also observed embedded within the parenchyma of the gland (Fig 2).



HE X 40

Fig 2: Photomicrograph showing organization of cells of thyroid into clumps in 44 days sheep fetus Clumps of cells (CC), capillaries (C), internal parathyroid (IP)

At 47 days of gestation, framework of the gland was laid by reticular fibres. Numerous sinusoidal capillaries were found. The capillaries were more concentrated at the periphery of the gland lobes than at the centre. Small round to oval cells with pale staining cytoplasm having granular appearance were found all over the parenchyma of the gland. These cells started to arrange into small groups ranging from two to twelve cells per group surrounded by reticular fibres. These groups of cells indicated the future follicles (Fig 3). These observations were in accordance with Al-jebori [1] in bovine fetuses.

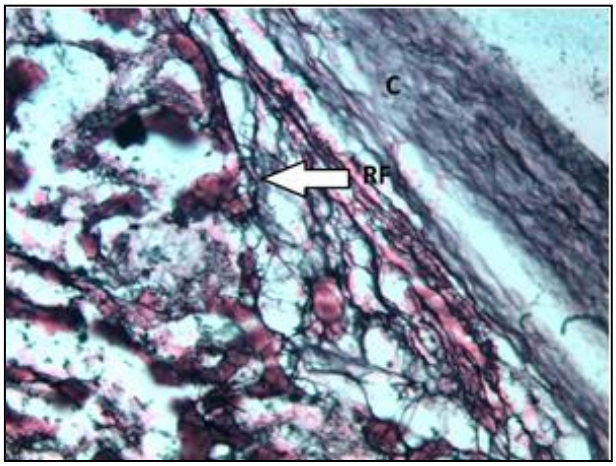


HE X40

Fig 3: Photomicrograph showing formation of cells into smaller groups in 47 days sheep fetus. Capillaries(C), Groups of cells (GC), Capsule(CA)

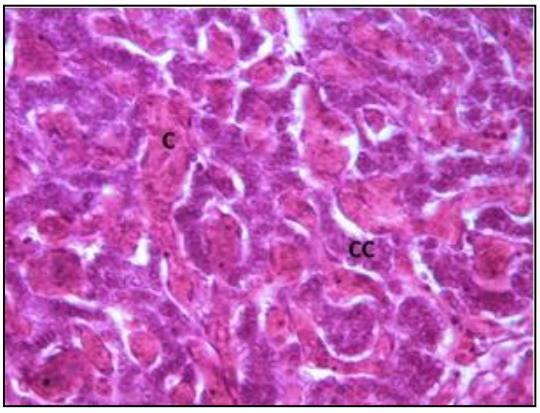
Group II

At 51 days of gestation the thyroid gland was surrounded by a capsule made up of numerous reticular and few collagen fibres (Fig 4). Just beneath the capsule large sinusoidal capillaries were seen. From here these capillaries made their entry into the parenchyma of the gland and were distributed throughout the gland. But however the capillary density was more at the periphery than at the centre. Numerous highly basophilic rounded to oval cells were seen arranged in the form curved cords. Between these cords of cells numerous capillaries were seen. Spaces were noticed surrounding the cords of cells (Fig 5).



Gomori's reticulin methodn X40

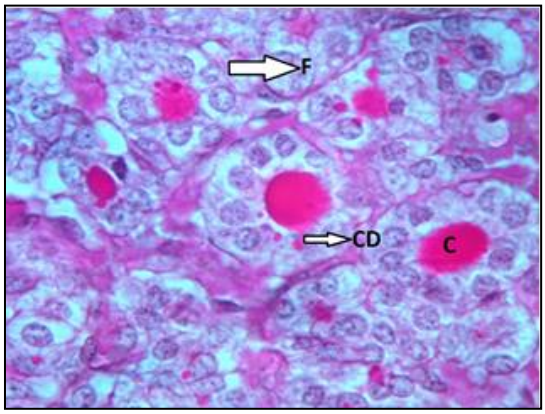
Fig 4: Photomicrograph showing reticular fibres in capsule and the stroma of gland in 51 days sheep fetus. Capsule(C), Reticular fibres (RF)



HE X 40

Fig 5: Photomicrograph showing curved cords of cells surrounded by capillaries in 51 days sheep fetus. Capillaries (C), Curved cords of cells (CC)

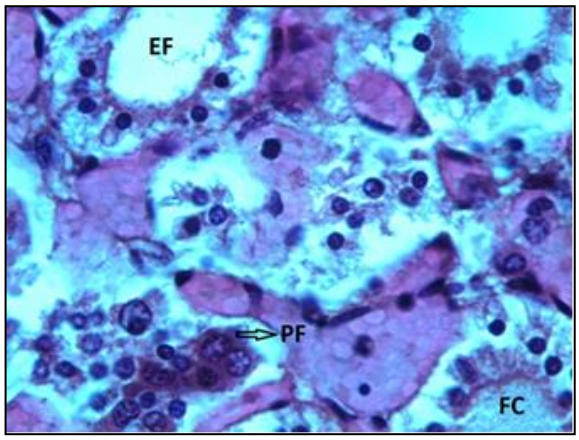
At 57 days of gestation numerous follicles of different sizes were formed which were lined by cuboidal follicular cells and vescicular nucleus. Many of them were filled with colloid. Larger follicles were seen at the periphery of the gland. Tiny colloid droplets secreted by the individual cells of the follicles were coalesced to form the colloid at the centre of each follicle (Fig 6).



Periodic Acid Schiffs X 40

Fig 6: Photomicrograph showing colloid droplets within follicular cells in 57 days sheep fetus. Follicle (F), Colloid droplets (CD), Colliod (C)

At 65 days of gestation the nuclei of the follicular cells were located close to the colloid and a clear empty area was noticed between the nucleus and the basement membrane. The follicular cells were high cuboidal with basophilic compact spherical nucleus located towards the base. All the follicles were surrounded by capillaries. Majority of the follicles were empty while a few of them contained colloid. At the periphery of the gland the lumen of these rings was empty with no colloid and nucleus of the cells is pale with granular appearance. Some follicles at the central region of the gland were filled with granular colloid. Parafollicular cells were observed in between follicles. They were larger with darker centrally placed large nucleus with granular basophilic cytoplasm (Fig 7). These findings were in accordance with Igbokwe and Ezeasor [7] in pigs.



HE X40

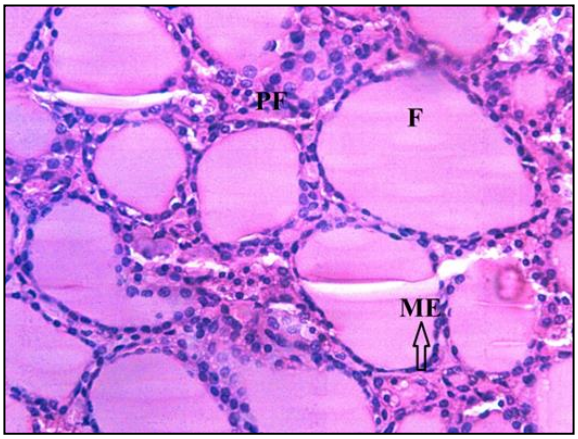
Fig 7: Photomicrograph showing empty thyroid follicles lined by high cuboidal follicular cells in 65 days sheep fetus Empty follicle (EF), Parafollicular cells (PF), Follicle with granular colloid (FC)

Group III

At 106 days of age thyroid gland was surrounded by thicker connective tissue capsule than that of 67 days of gestation (table 1). The follicles were irregularly round to oval in shape. The follicles contain homogenous basophilic thyroid colloid. In the periphery of the colloid vacuoles were noticed in some follicles. They were surrounded by cuboidal epithelium with rounded nucleus present at the centre. Follicles were surrounded by numerous capillaries. Sinusoids were more

concentrated towards the centre of the gland than at the periphery.

At 128 days of gestation the gland parenchyma was mainly made up of different sizes and shapes of follicles. Each follicle was made up of single layer of follicular cells which varies from cuboidal to low cuboidal with nucleus ranging from round to flat. The follicles were completely filled with basophilic colloid. The interfollicular area parafollicular cells, reticular fibres and few collagen fibres were noticed. Myoepithelial cells were noticed around each follicle (Fig 8). These findings were in accordance with Igbokwe and Ezeasor [8] in goat and were in contrary to the observations of Al-jebori [1] bovine foetuses.



HE X 40

Fig 8: Photomicrograph showing follicles with collid in 128 days sheep fetus. Parafollicular cells (PF) Follicle with colloid (F), Myoepithelial cells (ME)

Conclusion

The thyroid gland was surrounded by connective tissue capsule whose thickness increased with gestational age. The gland was not divided into lobes and lobules. The framework of the gland was mainly laid by reticular fibres and a few collagen fibres. By 57 days of gestation the PAS positive colloid material was seen within the follicular cells indicating the onset of the secretary activity of the gland. This colloid was secreted into the centre lumen during their further developmental stages. Follicular formation with central colloid was evident by 65 days of gestation. By this age of gestation parafollicular cells were also seen in the interfollicular region. The follicles were lined by high cuboidal epithelium at this stage of gestation which indicates their highest phase of secretary activity. Later on by 128 days of gestation, the follicular epithelium turned to low cuboidal with the follicles surrounded by myoepithelial cells indicating the contraction of follicles and release of the secretary product into the blood stream.

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