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Research Article

HISTOLOGICAL OBSERVATIONS ON PRENATAL DEVELOPMENT OF THYMUS IN SHEEP

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ABSTRACT

This study focused on the development of thymus in prenatal sheep. The primordium of thymus was observed at 37 days of gestation. The formation of thin connective tissue capsule was noticed at 50 days of gestation. At 56 and 60 days of gestation, thin connective tissue capsule that surrounded the lobe was clearly observed. It sent fine septa which divided the parenchyma into many lobules. At 84 days of gestation, the cortex was darker with densely packed small lymphocytes whereas, the medulla was occupied by loosely packed small, medium and large sized lymphocytes within the reticular cell network. At 93 days of gestation, the Hassall's corpuscles were observed in different sizes and shapes in the medulla. At 103 days of gestation, the connective tissue capsule was thick made up of collagen, elastic, reticular fibers. Numerous Hassall's corpuscles were observed in the medulla while very few corpuscles were noticed in the cortex and corticomedullary junction at 126 days of gestation. The mean thickness of the cortex and medulla increased with advancement of age.

Keywords: Histology, Prenatal, Thymus, Sheep

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Sheep plays a vital role among various small ruminants in India. It is rich source of not only meat but also milk, skin, wool and manure. The contribution of the sheep is noteworthy to the agrarian economy of India. The lymphatic system is part of the immune system and plays a significant role in defense mechanism against microorganism (Rahmoun *et al.*, 2019). Protection against the harmful effects of intruding foreign substances is the primary function of the lymphatic system, which entails the function of different organs and tissues. The lymphatic system performs a vital role in both healthy and diseased states. It facilitates in maintaining the equilibrium of normal tissue fluid balance, immune processes including cellular and antigen trafficking, and fatty acid and lipid-soluble vitamin absorption in the gut (Elizabeth and Fredric, 2011).

The thymus is a primary lymphoid organ found within the superior mediastinum, behind the upper part of sternum. The primary role of thymus is to produce 'T' lymphocytes, which are necessary for cell-mediated immunity thus recognized as a 'pacemaker' of lymphopoiesis. Thymus is also responsible for initiating the production of lymphocytes in other organs for instance spleen and lymph nodes (Ramayya, 2007). Understanding the normal morphological characteristics of the thymus and their variations is essential for assessing immune function which further helps to assess the typical involution of thymus. In the present study, histology of thymus has been studied at different gestational ages during prenatal period.

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MATERIALS AND METHODS

This study focused on the development of the thymus in 18 healthy foetuses (6 for each group) of either sex from a non-descriptive sheep breed. Foetuses collected in the South Indian region were categorized into three groups: early prenatal period (upto 50 days), mid prenatal period (51-100 days), and late prenatal period (101-till birth). The approximate age of the foetuses was estimated using following formula (Noakes *et al.*, 2009): X=2.1(Y+17), Where 'X' is the developmental age of foetus in days and 'Y' is crown rump length in centimeters. The CRL was estimated as described by Rao and Ramayya (2013). The entire embryo/thymus was fixed in 10% Neutral buffered formalin (NBF). The fixed tissue pieces from embryo/thymus were obtained and processed for paraffin sections. Paraffin sections of 5µm thickness were made using Leica Rotary Microtome (RM 2145) and stained with routine haematoxylin and eosin for studying the histoarchitecture, Van Gieson's and Masson's trichrome methods for collagen fibers.

RESULTS AND DISCUSSION

In this study, the primordium of thymus in sheep foetuses was observed ventral to the level of larynx as 6-7 individual cell clusters at 37 days of gestation (Fig. 1a). Similar primordium was noticed by Prasad *et al.* (2012) at 17 days of gestation in goat foetuses, Asha *et al.* (2011) at 22 days of gestation in goat foetuses, Ramayya (2007) at 40 days of gestation in buffalo calves and Gaber (2017) at 14th embryonic day in rabbit. There was no connective tissue component developed at this age. The clusters were

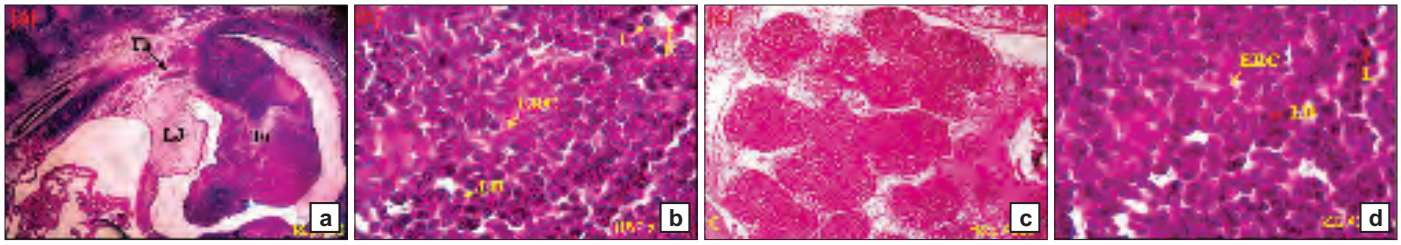

Fig. 1. (a) Photomicrograph of thymic primordium in sheep embryo at 37 days of gestation showing thymus (Th), lower jaw (LJ) and tongue (To). H&E×40. (b) Photomicrograph of thymus cell population in sheep embryo at 37 days of gestation showing lymphocytes (L), erythrocytes (E), lymphoblasts (LB) and epithelia reticular cell (ERC). H&E×1000. (c) Photomicrograph of thymic capsule in sheep foetus at 50 days of gestation showing capsule (C). H&E×100. (d) Photomicrograph of thymus cell population in sheep foetus at 50 days of gestation showing lymphocytes (L), lymphoblasts (LB) and epithelia reticular cell (ERC). H&E×1000.

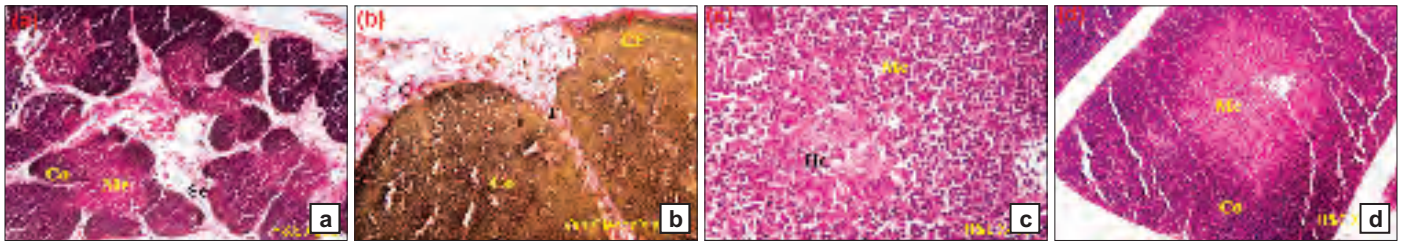

Fig. 2. (a) Photomicrograph of thymus in sheep foetus at 56 days of gestation showing cortex (Co), medulla (Me), Septa (Se) and capsule (C). H&E×100. (b) Photomicrograph of thymus in sheep foetus at 56 days of gestation showing collagen fibers. Van Gieson's×400. (c) Photomicrograph of thymus in sheep foetus at 56 days of gestation showing Hassall's corpuscle (Hc) formation. H&E×400. (d) Photomicrograph of thymus in sheep foetus at 84 days of gestation showing cortex and medulla. H&E×100.

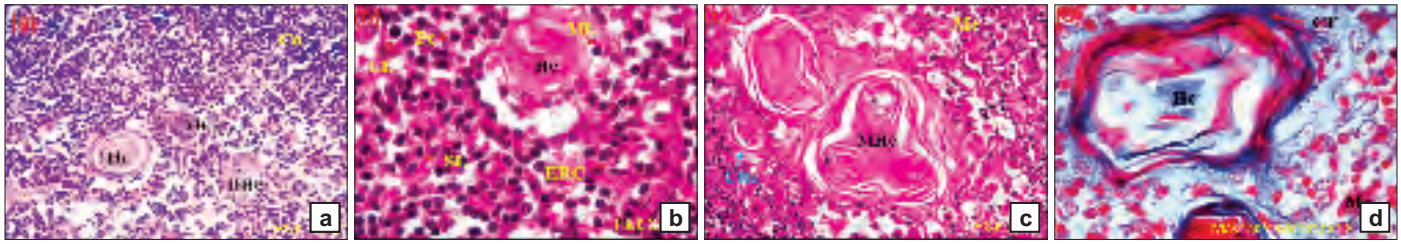

Fig. 3. (a) Photomicrograph of thymus in sheep foetus at 84 days of gestation showing developing Hassall's corpuscle (DHc) and medulla (Me). H&E×400. (b) Photomicrograph of thymus in sheep foetus at 93 days of gestation showing cell population (small lymphocytes (SL), medium lymphocytes, large lymphocytes (LL), plasma cells (PL) and reticular epithelial cells (ERC). H&E×1000. (c) Photomicrograph of thymus in sheep foetus at 93 days of gestation showing Hassall's corpuscle with keratinization (multilamellar Hassall's corpuscles (MHc). H&E×400. (d) Photomicrograph of thymus in sheep foetus at 93 days of gestation showing collagen fibers (CF). Masson's trichrome×1000.

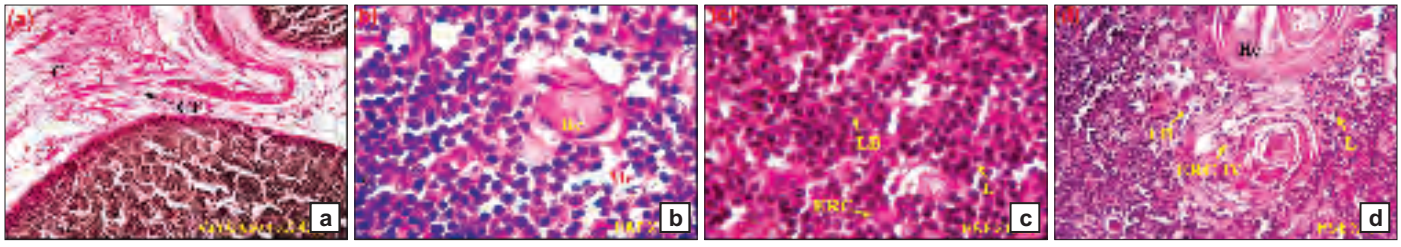

Fig. 4. (a) Photomicrograph of thymus in sheep foetus at 103 days of gestation showing collagen fibers. Van Gieson's×400. (b) Photomicrograph of thymus in sheep foetus at 103 days of gestation showing Hassall's corpuscle. H&E×1000. (c) Photomicrograph of thymus in sheep foetus at 126 days of gestation showing cellular population cortex. H&E×1000. (d) Photomicrograph of thymus in sheep foetus at 126 days of gestation showing cellular population. H&E×400.

composed only of reticulo epithelial cells and lymphocytes as described by Mainde *et al.* (2017) in goat foetuses (Fig. 1b).

The formation of thin connective tissue capsule was noticed after 45 days of gestation as described by Mainde (2007) in goat foetuses. The parenchyma was appeared as lobules. Cortex and medulla were not well demarcated until 50 days of gestation. No Hassall corpuscles were noticed at this age (Fig. 1c). These observations were in agreement with the findings of Gayathri *et al.* (2019) in human foetuses.

Lobules had more lymphoblasts, few lymphocytes and reticulo epithelial cells as described by Prasad *et al.* (2012) in goat embryo. The lymphoblasts were round cells with large round or oval nucleus which had scattered chromatin granules and nucleoli. The lymphocytes were

round cells with round condensed dense nucleus without nucleoli and had thin rim of cytoplasm. In both the cells, basophilic cytoplasm was observed (Fig. 1d). The reticulo epithelial cells were large pleomorphic lightly stained cells which had large spherical to ovoid vesicular nucleus and eosinophilic cytoplasm.

After 50 days of gestation, the thin connective tissue capsule surrounding the lobe was clearly observed fine septa coming from capsule divided the parenchyma into many lobules. The similar observations were noticed during 46-75 days in goat foetuses (Mainde, 2007), around 46-58 days of gestation in pig foetii (Igbokwe *et al.*, 2017) and 15th day of incubation in broilers (Dhande *et al.*, 2002). The differentiation of cortex and medulla was clearly observed at 56 days of gestation. In agreement with the present results, Ajita *et al.* (2006) observed the differentiation of cortex and medulla at 12-14 weeks in human foetuses, however, Ramayya (2007) did not observe it until 90 days of gestation in buffalo calves.

Each lobule had outer cortex which was divided by trabeculae extended up to corticomedullary junction into many compartments and common medulla (Fig. 2a). The capsule, interlobular septa and trabeculae consisted of collagen (Fig. 2b), very few elastic and reticular fibers. The reticular fibers were also noticed in the medulla. These findings are in consistence with the observations of Mainde (2007) in goat foetuses, Sarma *et al.* (2004) in bakarwali goat and Uppal *et al.* (2007) in buffalo calves. Similarly, Chandra (2004) stated that in goat foetuses the capsule was loose and the density of fibers increased with increasing age from 1-10 CRL.

The Hassall's corpuscles formation was initiated and noticed in the medullary region of some lobules as described by Mainde (2007) in goat foetuses and Ajita et al. (2006) in human foetuses (Fig. 2c). The cortex and medulla displayed reticular epithelial cells, lymphoblasts and lymphocytes. The cortex was darker and had densely packed small lymphocytes whereas the medulla was occupied by loosely packed large and medium sized lymphocytes within the reticular cell network at 84 days of gestation (Fig. 2d). The processes of adjacent reticulo epithelial cells formed the cytoreticulum which began to form Hassall corpuscles in the medullary region (Fig. 3a).

The cortex and medulla showed few microphages and more numbers of lymphoblasts with few lymphocytes in between (Fig. 3b). The Hassall's corpuscles were observed in different sizes and shapes in the medulla at 93 days of gestation. These results are in agreement with the findings of Mainde (2007) in goat foetuses. Two types of Hassall's corpuscles were observed in the medulla in which unilamellar Hassall's corpuscles were characterized by a single concentric layer of flattened epithelial reticular cells around the central degenerating acidophilic mass whereas in multilamellar Hassall's corpuscles two or more concentric layers of flattened epithelial reticular cells were present around central degenerating mass (Fig. 3c). These results are in correspondence with the findings of Chaurasia *et al.* (2018) in Surti goat and Ramayya *et al.* (2009) in buffalo. The size and number of Hassall's corpuscles was found to be increased and clearly displayed concentric arrangement with keratinized reticular epithelial cells and it showed collagen deposits at 93 days of gestation (Fig. 3d). The mean diameter of Hassall's corpuscles at mid gestational period (51-100 days) was 87.408±1.789 µm. The mean thickness of capsule, cortex and medulla during prenatal period were 19.602±0.897 µm, 213.700±2.874 µm and 274.915±2.356 µm, respectively.

At 103 days of gestation, the connective tissue capsule was thick made up of collagen, elastic, reticular fibers. The amount of collagen (Fig. 4a), elastic and reticular fibers were increased in all connective tissue septa and trabeculae with advancement of age. Few reticular fibers were noticed in the cortex and they were numerous in the medulla. The thymic cortex showed numerous lymphoblasts, lymphocytes and few reticulo epithelial cells. In the medulla, more lymphoblasts and reticulo epithelial cells were seen. These results are in consistence with the results of Mainde (2007) in goat foetuses. The keratinization in the Hassall corpuscle was noticed in the medulla (Fig. 4b). Macrophages and dendritic cells were also noticed in the cortex and medulla as described by Ramayya (2007) in buffalo calves.

The prenatal sheep thymus at 126 days of gestation, showed predominant cellular population of small lymphocytes within epithelial reticular cells in the cortex (Fig. 4c). The cellular population of the medulla was constituted by scattered small, medium and large sized lymphocytes with epithelial reticular cell network. The small lymphocytes had a small amount of basophilic cytoplasm with condensed nucleus. The medium lymphocytes had centrally positioned nucleus and thin border of moderate basophilic cytoplasm around it. The large lymphocytes consisted of round to oval nucleus with prominent chromatin with more basophilic cytoplasm. Numerous Hassall's corpuscles were seen in the medulla while very few were noticed in the cortex and corticomedullary junction. These findings were in consistent with the observations of Yogesh (2007) in goat.

Four types of reticulo epithelial cells were noticed at

this age. The type I reticulo epithelial cells had pale stained cytoplasm with oval to elongated nucleus. The type II cells showed irregular outline with large spherical nucleus. The type III cells were irregular in shape with irregular nucleus and had vacuoles in the cytoplasm. The type IV cells were noticed within the Hassall corpuscle and had darkly stained condensed nucleus (Fig. 4d). Roshdy and Derbalah (2017) reported similar observations in water buffalo. The mean diameter of Hassall's corpuscles late gestational period (100-150 days) was 51.212±1.024 µm. The mean thickness of capsule, cortex and medulla (prenatal period) were 19.136±0.265 µm, 254.750±3.025 µm and 365.236±5.632 µm, respectively.

CONCLUSION

The thymic primordium was noticed at the 37th day of gestation. The presence of the connective tissue capsule was seen at 50 days of gestation. The septa and connective tissue capsule were observed at 56 days of gestation. Cortex and medulla were not well demarcated until 50 days of gestation, but it becameclearly demarcated at 56 days of gestation. Hassall's corpuscles began to form at 56 days of gestation and by 93 days of gestation, they were observed in varying shapes and sizes within the medulla. With advancing age, the size of the cortex and medulla increased.

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