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Short Communication

GROSS AND HISTOLOGICAL STUDIES ON THE DEVELOPMENT OF THYMUS IN POSTNATAL AGE GROUPS OF SHEEP (*OVIS ARIES*)

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ABSTRACT: In the present study, the development of thymus in postnatal sheep was studied. At 2 months of age, the thymus appeared pale pink and changed to greyish yellow with age. The thymus of the sheep showed fat deposits at 4 months of age. Histologically, the sheep thymus showed an increase in connective tissue fibers *viz.,* collagen, reticular and elastic fibers in the capsule, interlobular septa, and trabeculae. The Hassall's corpuscles appeared round, elongated, and irregularly shaped. At 2 years of age, involution of the thymus was noticed with more deposition of fat. The depletion of lymphocytes was noticed in the cortex and medulla at 3 years of age.

Key words: Thymus, Sheep, Postnatal, Developmental stages, Morphology, Histology.

Thymus is a component of the lymphoid system that is distinct not only in appearance but also in its mode of development (Rahmoun 2020). The thymus is a bilobed organ located above the heart and beneath the sternum in the middle of the thoracic cavity that produces T lymphocytes. The thymus is also known as a lymphoepithelial organ that is required for the development of peripheral lymphoid tissue and its associated adaptive immunological activity. The study of various pathologies requires precise knowledge of the histogenesis and various components of the thymus organ (Tsuchiya 1989). Thymus performs a vital role in the development of immune response in the fetal period (Gayathri 2019). Additionally, the thymus initiates the generation of lymphocytes in other organs, such as the spleen and lymph nodes (Ramayya 2007). To the best of our knowledge, very few resources on the development of the sheep thymus are available. The current study is intended to assess the macroscopic and microscopic properties of the thymus in sheep at different postnatal age groups.

The study

The thymus organ was obtained from 18 sheep from a Corporation slaughterhouse, in Chennai, Tamilnadu, India. All animals were divided into categories, *viz.*, pre-pubertal (birth to 6 months, group-I), pubertal (6-8 months, group II), and post-pubertal (2-3 years, group III). The collected thymus gland was fixed in 10% neutral buffered formalin (NBF). The fixed tissues were processed as per Luna (1968). Paraffin sections of 5 µm thickness were sliced with Microtome (LRM2145). The biometrical measurements were obtained using vernier calipers and weighing balance. The histoarchitecture was studied using various staining methods, including Harris's Haematoxylin and Eosin (Bancroft and Stevens 1996), Van Gieson's (Bancroft and Stevens 1996), and Masson's Trichrome (Luna 1968).

Results and discussion

In the current research, the color of the sheep thymus was pale pink at 2 months of age which gradually changed to greyish yellow with age (Fig. 1). Similarly, Sivagnanam (2018) reported that the color of the thymus was pinkish at 4 months of age and changed to pale yellow with advancement of age in goat. Iman *et al.* (2017) noticed white to pale yellowish colored thymus in rabbits. At 4 months of age, fat deposits were noticed in the thymus of the sheep shown.

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Capsules with more amount of collagen, elastic and reticular fibers were noticed at 2 months of age (Fig. 2). In accordance with the current findings, Tamilselvan *et al.* (2017) reported reticular and collagen fibers with few elastic fibers in the thymic capsule and trabeculae of guinea fowl. The cortex was deeply pigmented and tightly packed with lymphocytes. The cellular population of the cortex and medulla in postnatal sheep thymus was similar to prenatal age groups. These results corresponded to the observations of Shailendra *et al.* (2018) in the Surti goat.

A well-developed Hassall's corpuscle made of central homogeneous acidophilic mass with degenerating reticular cells and cellular debris was noticed as described by Sivagnanam (2018) in goat (Fig. 3). Fat deposits were noticed in interlobular connective tissue septa at 4 months of age (Fig. 4). Following the present results, Medhi (2017) observed that the interlobular connective tissue and subcapsular areas were filled by adipose tissue, which appeared as numerous rounds to oval empty spaces in Assam hill goat.

The cortex had densely packed dark stained cells and the medulla showed loosely arranged lightly stained cells as stated by Tamilselvan *et al*. (2017) in guinea fowl. In the cortex, several lymphocytes, lymphoblasts, and reticulo-epithelial cells were observed. In the medulla, an almost equal distribution of lymphoblasts and lymphocytes was seen. Vacuolations appeared in reticulo-epithelial cells of the developing Hassall's corpuscle in the medulla. The Hassall's corpuscles of the medulla appeared round, elongated, and irregularly shaped which were surrounded by reticular epithelial cells with keratinized hyaline mass in the center. The results obtained were congruent with observations of Sivagnanam (2018) in goats. Three kinds of Hassall's corpuscles *viz*. type I with keratinized mass surrounded by reticular epithelial cells (Fig. 5), type II with completely keratinized concentric mass (Fig. 6), and type III with concentric hyalinization and calcified center

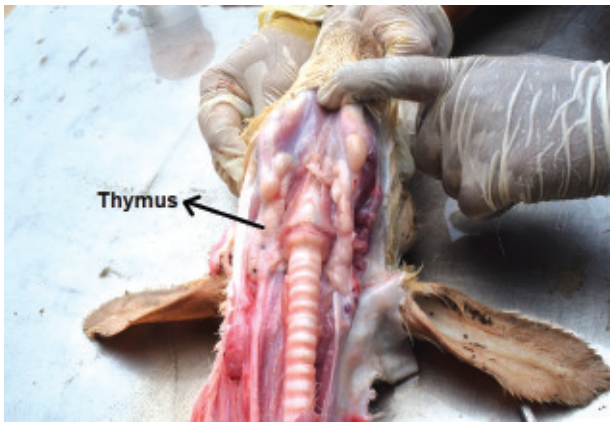

Fig. 1. Photograph showing thymus of sheep at 2 months of age.

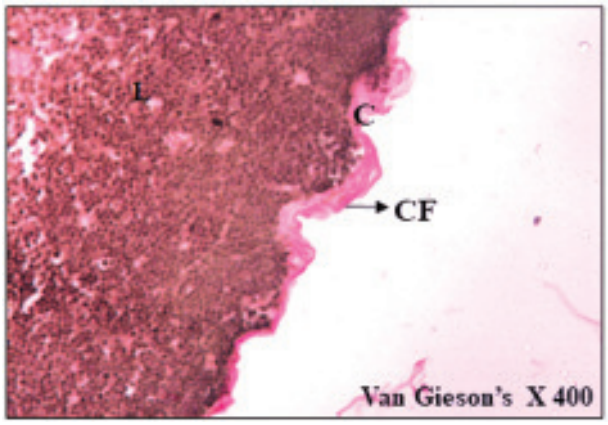

Fig. 2. Photomicrograph showing collagen fibers in thymus of sheep at 2 months of age (C=Capsule; CF= Collagen Fibers; L=Lymphocytes).

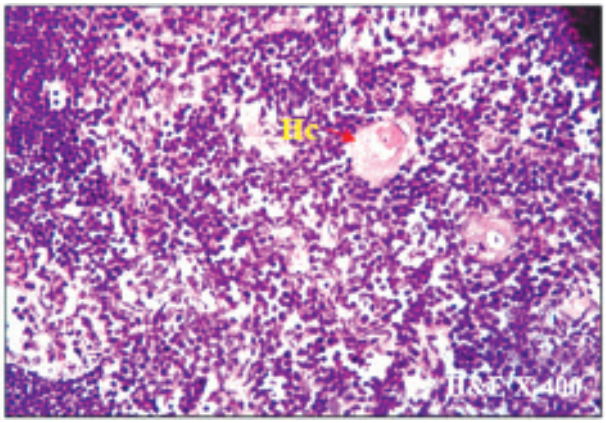

Fig. 3. Photomicrograph showing Hassall's Corpuscle in thymus of sheep at 2 months of age (Hc= Hassall's Corpuscle).

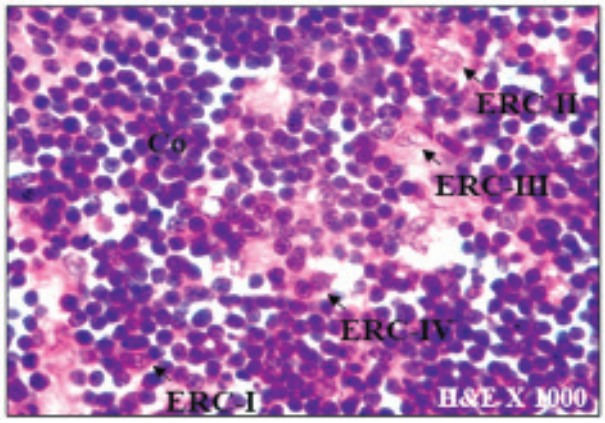

Fig. 4. Photomicrograph showing types of epithelio reticular cells in thymus of sheep at 4 months of age (Co = Cortex; ERC = Epithelio reticular cell).

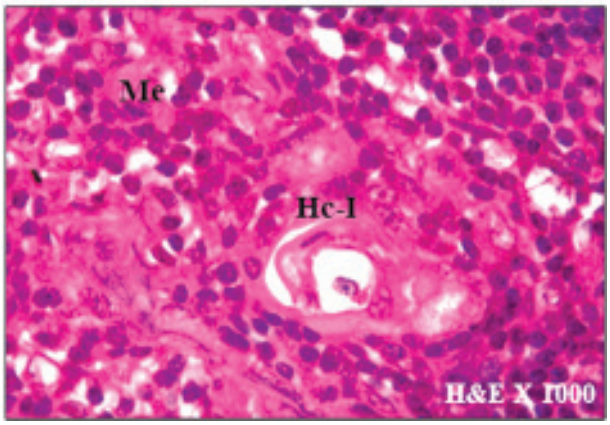

Fig. 5. Photomicrograph showing Hassall's Corpuscle with keratinized mass in thymus of sheep at 4 months of age (Hc-I = Hassall's Corpuscle type-I; Me = Medulla).

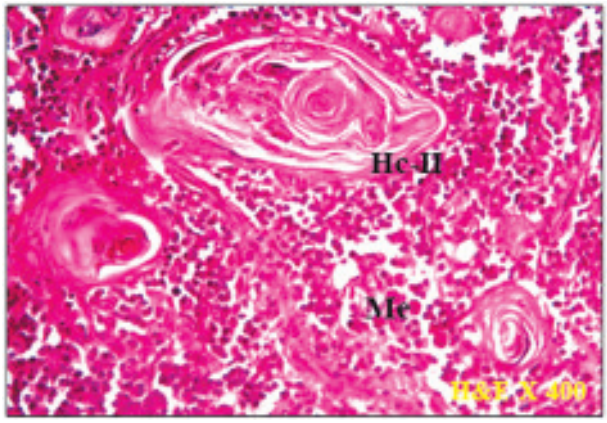

Fig. 6. Photomicrograph showing Hassall's corpuscle with calcification in thymus of sheep at 4 months of age (Hc-II = Hassall's Corpuscle type-II; Me = Medulla).

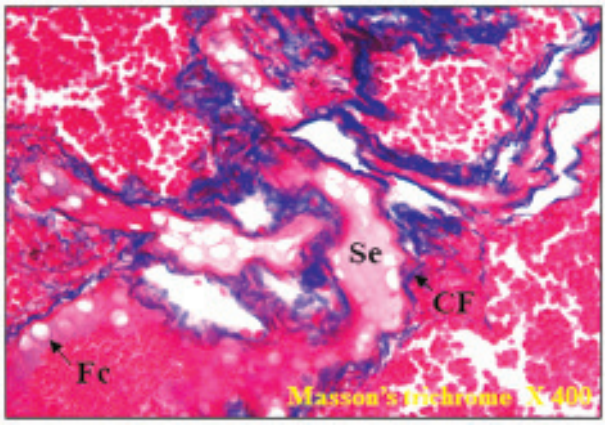

Fig. 7. Photomicrograph showing adipose tissue in thymus of sheep at 6 months of age (Fc = Fat Cells; CF = Collagen Fibers; Se = Septa).

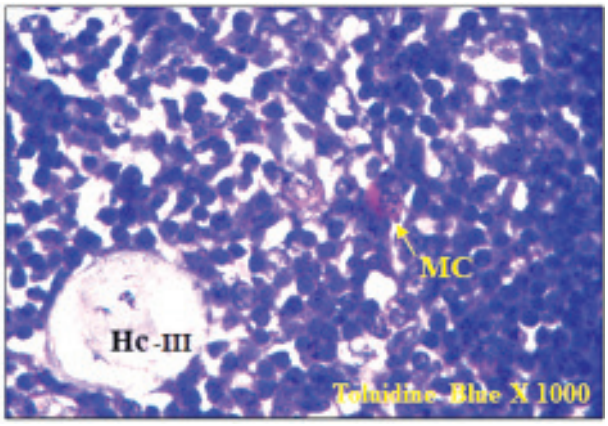

Fig. 8. Photomicrograph showing mast cell in thymus of sheep at 6 months of age (Hc = Hassall's Corpuscle; MC = Mast Cell).

were noticed at this age as reported by Jagapathi (2007) in buffalo calves (Fig. 8).

The color of the thymus was pale yellow and the size of cervical and thoracic parts was reduced at 6 months of age as described by Dyce *et al.* (2009) in mammals and Igbokwe and Ezenwaka (2017) in pigs. The quantity of collagen fibers in the capsule, interlobular septa, and trabeculae increased. Increased reticular fibers were noticed in the medulla. The appearance of adipose cells was noticed in the interlobular septa as observed by Medhi (2017) in Assam hill goat (Fig. 7). Hassall's corpuscles were found at the cortex and corticomedullary junction as noticed by Tamilselvan *et al.* (2017) in guinea fowl. Different characteristics of the thymus of quail, chicken, and duck were studied by Senapati *et al.* (2015) who found many differences in the structure of mammals of birds. The Hassall's corpuscle is noticed by them as some pale stained diffuse body consisting of vacuolated, squamous reticular cells.

In our study, some of Hassall's corpuscles showed a calcified mass in the center. The majority of parenchyma was constituted by lymphocytes. Few macrophages, dendritic cells, mast cells, and neutrophils were also seen as reported by Iman *et al.* (2017) in rabbits and Shailendra *et al.* (2018) in goats (Fig. 8).

From 2 years of age, the color of the thymus changed to greyish yellow (Fig. 9). Involution of the thymus was noticed with the deposition of fat. Ramayya (2007) also observed that the cervical part of the thymus disappeared by one year and the thoracic part was replaced by the adipose tissue at 2 years in buffalo.

The collagen and elastic fibers were greatly increased in both the capsule and septa. The increased collagen fibers surrounded the lymphocytic cell population and converted them into connective tissue-circumscribed compartments. Clusters of adipocytes were found to appear in the interlobular septa. The size of the cortex decreased. The size and cellular population of the medulla

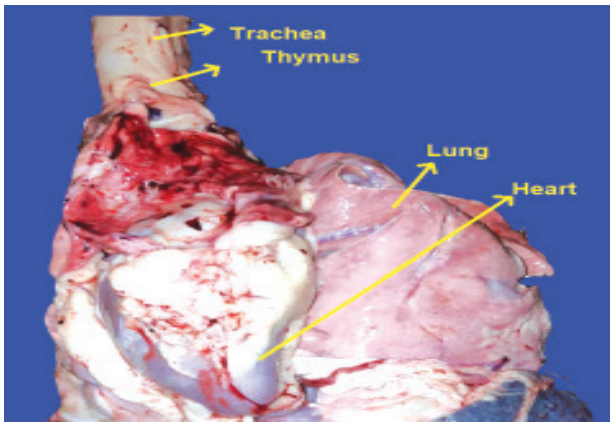

Fig. 9. Photograph showing thymus of sheep at 2 years of age.

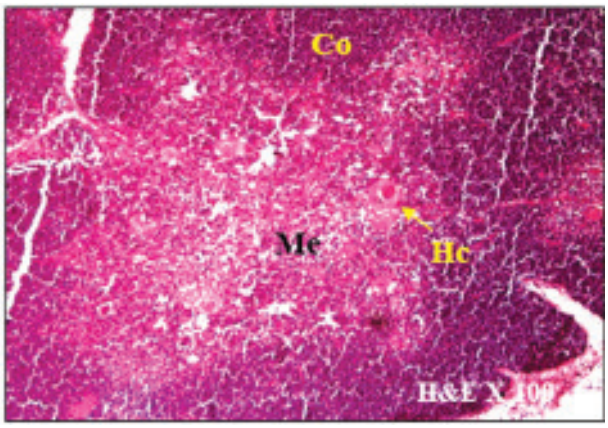

Fig. 10. Photomicrograph showing cortex and medulla in thymus of sheep at 2 years of age (Co = Cortex; Me = Medulla; Hc = Hassall's Corpuscle).

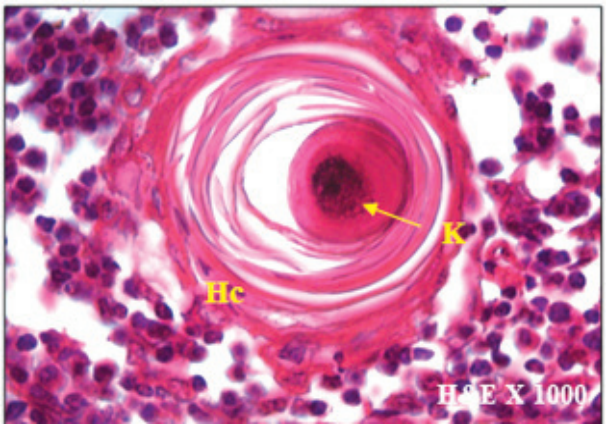

Fig. 11. Photomicrograph showing Hassall's corpuscle with keratohyaline granules in thymus of sheep at 2 years of age (Hc = Hassall's Corpuscle; K = Keratinized mass).

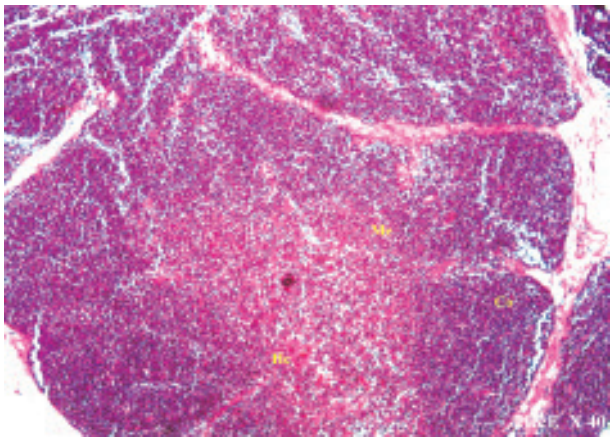

Fig. 12. Photomicrograph showing Hassall's corpuscle in thymus of sheep at 3 years of age (Hc = Hassall's Corpuscle; Me = Medulla; Co = Cortex).

also decreased as stated by Plecas *et al.* (2006) in rats (Fig. 10). Less number of collagen fibers was observed in the Hassall's corpuscles. Some of the degenerating reticular-epithelial cells of the medulla became flattened and keratinized (Fig. 11). These observations were in agreement with the findings of Sivagnanam (2018) in goats. The 3 years old adult sheep thymus showed few numbers of Hassall's corpuscles in the cortex and medulla (Fig. 12). The depletion of lymphocytes was observed in both the cortex and medulla. Clusters of fat cells were seen in the interlobular connective tissue septa.

Morphometry

The mean weight of the thymus in postnatal age groups reduced gradually from 14.787 ± 1.417 g to 8.259 ± 0.614 g as the age advanced in group I and group III. Following the present results, Ramayya (2007) recorded that the weight of the thymus in buffalo decreased from 166.074 + 13.54 g in 1st week-3 months to 35.51+16.14g in 9-12 months of age. Chaurasia (2019) also notice the variable trend of the thymus (group-I: 11.1 g, group II: 26.8 g, and group III: 3.6 g) in postnatal Surti goats. This gradual decrease in the weight of the thymus during the postnatal period showed a negative correlation between age and weight of the thymus.

Histometry

The average thickness of the capsule increased whereas the thickness of the cortex and medulla decreased. The maximum thickness of the capsule, cortex, and medulla were 36.573 ± 2.867 µm, 320.924 ± 4.021 µm, and 463.735 ± 5.264 in group III, group I and group II, respectively. The progressive increase in capsule thickness observed in the present study was consistent with the reports of Yogesh (2007) in goats.

The mean diameter of Hassall's corpuscles varied from 33.844 ± 2.265 µm to 84.721 ± 2.114 µm in all postnatal age groups whereas, Igbokwe and Ezenwaka (2017) recorded that the average diameter of Hassall's corpuscles varied from 21.6 ± 0.6 µm to 26.2 ± 0.8 µm in the postnatal period. The average diameter of the cortex, medulla, and Hassall's corpuscles showed significant variation between pre-pubertal and post-pubertal age groups. This might be due to age-related involutory changes in the postnatal sheep thymus.

Conclusion

In this study, gross and microscopic characteristics of the thymus in sheep at various age groups in postnatal life were observed. In the postnatal period, the weight of the thymus followed a variable trend with respect to age. Fat deposits were found in the thymus of sheep from 4 months of age. The Hassall's corpuscles appeared to be round, elongated, and irregular in shape. The mean thickness of the capsule increased whereas the mean thickness of the cortex and medulla got decreased. The average diameter of the cortex, medulla, and Hassall's corpuscles showed significant variation between pre-pubertal and post-pubertal age groups.

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