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**Research Article**

# HISTOCHEMICAL STUDIES OF THYMUS GLAND OF SHEEP IN PRE AND POSTNATAL AGE GROUPS

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## ABSTRACT

The present study investigates the distribution of key histochemical components in the thymus gland of sheep across various prenatal and postnatal age groups. In prenatal thymus, strong PAS positive reactions were observed in Hassall's corpuscles and blood vessels, while the capsule and trabeculae showed moderate PAS positive reaction, indicating presence of glycogen. The Hassall's corpuscle exhibited mild reaction for neutral mucopolysaccharides and strong reaction for acid mucopolysaccharides with combined Alcian blue-PAS technique in all prenatal age groups. The capsule, septa and parenchyma showed moderate to strong reaction for the presence of protein tyrosine in all prenatal age groups. The septa showed positive reaction for the presence of lipid deposits in all prenatal age groups. A strong activity in parenchyma of cortex, medulla and Hassall's corpuscle was noticed for the presence of enzyme alkaline and acid phosphatases in all prenatal age groups. In postnatal ages with combined Alcian blue-PAS staining a moderate neutral and acid mucopolysaccharide positive reaction was noticed in the Hassall's corpuscle of all postnatal age groups. The capsule and septa showed moderate PAS activity for the presence of glycogen. Capsule and septa showed weak reaction whereas Hassall's corpuscles showed moderate reaction for the presence of tyrosine in all postnatal age groups. Parenchyma of thymus showed mild reaction for lipids whereas strong lipid activity was noticed in the interlobular septa in all postnatal age groups. Both acid phosphatase and alkaline phosphatase showed mild reaction in the stroma however, a strong reaction was noticed in the parenchymal region in all postnatal age groups.

**Keywords:** Histochemistry, Postnatal, Prenatal, Sheep, Thymus

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Sheep serve as a vital livestock species in global agriculture, contributing substantially to the rural economy through the production of meat, milk and wool (Bhagyalakshmi *et al.*, 2024). Owing to their well-characterized gestational timeline, immunological relevance and physiological similarity to other ruminants, sheep are extensively utilized as a model organism in developmental biology and comparative immunopathology studies (Bhagyalakshmi *et al.*, 2025). The Greek word "warty excrescence" originates from the word thymus, which resembles the flowers of the thyme plant. It is located in the superior mediastinum and anterior part of the inferior mediastinum. The thymus, being a retrosternal and well-protected organ, can be utilised for foetal age determination, highlighting its importance in accurately estimating foetal age. The thymus develops as a bilobed structure from the endoderm of the third pharyngeal pouch during the 6th week of embryonic life (Tamilselvan *et al.*, 2017). This endoderm interacts with neural crest cells, which become encapsulated within a capsule. Therefore, the gross features and histogenesis of the thymus can be used to estimate the gestational age of foetuses in cases of grossly mutilated foetal deaths (Sandhya *et al.*, 2023).

Size of the thymus grows rapidly during the embryonic life and pre-pubertal life, reaches its maximum absolute size about the time of puberty and then degenerates (Prabavathy, 2021). The lymphatic system performs a vital role in both normal and pathological processes. It contributes in the maintenance of normal tissue fluid balance, the immune functions such as cellular and antigen trafficking, the absorption of fatty acids and lipid-soluble vitamins in the gut (Elizabeth and Fredric, 2011). The thymus is the one of the primary lymphoid organs, which appear during embryonic development. The main function of the thymus is the production of 'T' lymphocytes, differentiate and assist in the clonal expansion of lymphocytes, which are necessary for the cell-mediated immunity thus recognized as a 'pacemaker' of lymphopoiesis. Thymus performs a vital part in the development of immune response in the foetal period. The distribution of the epithelial cells gives rise to the thymic epithelial reticulum, which becomes occupied by blood vessels from the contiguous mesenchyma. Lymphoblasts derived from the bone marrow stem cells invade the interstices, filling the spaces between epithelial cells. Therefore, the thymus also referred to as a lympho-epithelial organ. Thymus is essential for the growth of the peripheral lymphoid tissue and their associated adaptive immune function. The thymus determines the number of lymphocytes in the lymphatic organs and regulates the immunologic competence of animals. It is also important in the production of the

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hormones (Prakash, 1988). The present study was undertaken to study the relative changes in histochemistry of thymus in different prenatal age groups of sheep foetuses.

## MATERIALS AND METHODS

The present study was conducted on thirty-six sheep (prenatal and postnatal) from 0 to 150 days of prenatal sheep and 1month to 2 years and above of postnatal age groups. These animals were divided into six groups and each group containing six animals; Group-I (0 to 50 days), Group-II (51 to 100 days), Group-III (101 to 150 days), Group-IV Birth to 6 months (Pre-pubertal) and Group-V 6-8 months (Pubertal) and Group-VI 2 years and above (Adult). The lymphoid organ thymus of prenatal and postnatal age groups of sheep collected separately in thermos containing ice cubes from slaughterhouses in Andhra Pradesh and Tamil Nadu.

The approximate age of the prenatal age groups was estimated by obtaining the crown rump length (CRL) and substituting in the formula given below (Noakes, 2009).

$$A = 2.1(B+17)$$

Where 'A' is the developmental age of foetus in days and 'B' is crown rump length in centimetres.

In case of foetuses up to 3 cm CRL standard values given by Bryden *et al.* (1972a) were used for estimation of age in days. The postnatal age of sheep was determined based on the eruption of teeth as per Dyce *et al.* (1996). The foetuses of non-descript breed and irrespective of sex were collected from the pregnant sheep uteri immediately after slaughter. The body weight and CRL of foetuses were recorded. The foetuses were dissected by making a mid-ventral skin incision starting from the body of the mandible up to the inguinal region. The lymphoid organ, thymus was located and collected. The collected thymuses were rinsed in normal saline. The gross morphology such as colour, shape, location and topography was recorded and fixed in the various fixatives such as 10% neutral buffered formalin, chilled 10% neutral buffered formalin and Bouin's fluid.

For histochemical study, fresh tissue samples of the thymus were fixed in chilled 10% neutral buffered formalin. After fixation, thymic tissue samples of 1-2 mm thickness size were cut at 9 µm thickness of frozen sections were prepared at -20º C and stained with oil red O for fats, Gomori's cobalt and Napthol AS-BI method for Alkaline phosphatase, Gomori's lead nitrate method for Acid phosphatase. These cryostat frozen sections were then processed separately with Gomori's Cobalt and Napthol AS-BI method for demonstration of alkaline phosphatase, Gomori's modified lead nitrate method for acid phosphatase (Carleton and Drury, 1980) and Oil Red O method for lipids. These cryostat sections for histochemical studies of the samples were carry out at the State Level Diagnostic Laboratory, Sri Venkateswara Veterinary University, Tirupati.

Paraffin wax-embedded sections were prepared and stained with periodic acid Schiff's reagent (PAS) for glycogen, Million's reaction for protein tyrosine and Combined Alcian blue- PAS technique for acid and neutral mucopolysaccharides. The slides were observed under a compound microscope at magnifications of 40, 100, 400 and 1000 times for interpretation. Hence this study aims in determining the histochemical components of thymus gland at different gestational ages in foetuses and adult sheep.

## RESULTS AND DISCUSSION

**Prenatal thymus:**

**(a) Carbohydrate analysis:**

In prenatal age groups the thymus of sheep, strong PAS positive reaction was observed in Hassall's corpuscles (Fig. 1a) and blood vessels whereas capsule and trabeculae showed moderate PAS positive reaction indicating the presence of glycogen. These results are in agreement with the reports by Yogesh (2007) in goat foetuses. The Hassall's corpuscle (Fig. 1b) showed mild reaction for neutral mucopolysaccharides and strong reaction for acid mucopolysaccharides with combined Alcian blue-PAS technique in all prenatal age groups (Table 1), which was in agreement with the reports of Yogesh (2007) in goat foetuses.

**b) Protein dynamics:**

The capsule, septa and parenchyma (Fig. 1c) showed moderate to strong reaction for the presence of tyrosine in all prenatal age groups (Table 1). The protein component was not noticed in the prenatal age group in any of the literature reviewed.

**c) Lipid storage:**

The septa (Fig. 1d) showed positive reaction for the presence of lipid deposits in all prenatal age groups (Table 1). Whereas Ramayya and Singh (2012) in buffalo foetuses and Geetha *et al.* (2016) in mice, rat and guinea pig reported that lipid content was less in prenatal age groups.

**d) Enzymatic activities:**

**i)** ***Alkaline phosphatase activity:***

A strong activity in parenchyma of cortex, medulla and Hassall's corpuscle was noticed for the presence of alkaline phosphatase (Fig. 2a) in all prenatal age groups whereas Ramayya

**Table 1. Histochemical observations of thymus in prenatal age groups of sheep**

| Thymus | Prenatal age groups | | | | | |
|---|---|---|---|---|---|---|
| | PAS | Combined Alcian blue -PAS | Tyrosine | Lipid | Alkaline Phosphatase | Acid Phosphatase |
| Capsule | - | - | ++ to +++ | - | - | - |
| Septa | - | - | ++ to +++ | + | - | - |
| Cortex | - | - | ++ to +++ | - | +++ | +++ |
| Medulla | - | - | ++ to +++ | - | +++ | +++ |
| Hassall's Corpuscle | +++ | + to +++ | ++ to +++ | - | +++ | +++ |
| Blood vessel | +++ | - | ++ to +++ | - | - | - |

+++ Strong; ++ Moderate; + Weak; - No reaction

**Table 2. Histochemical observations of thymus in postnatal age groups of sheep**

| Thymus | Prenatal age groups | | | | | |
|---|---|---|---|---|---|---|
| | PAS | Combined Alcian blue -PAS | Tyrosine | Lipid | Alkaline Phosphatase | Acid Phosphatase |
| Capsule | ++ | - | + | - | + | + |
| Septa | ++ | - | + | - | + | + |
| Cortex | - | ++ | - | + | +++ | +++ |
| Medulla | - | ++ | - | + | +++ | +++ |
| Hassall's Corpuscle | - | ++ | ++ | +++ | +++ | +++ |

+++ Strong; ++ Moderate; + Weak; - No reaction

(2007) noticed weak activity in capsule and stroma in all prenatal age groups of buffalo foetuses. In contrast to the present results, Yogesh (2007) and Geetha *et al.* (2016) stated that there was no alkaline phosphatase activity in all prenatal age groups of goat and mice, rat and guinea pig, respectively.

**ii)** ***Acid phosphatase activity:***

The cortex, medulla and Hassall's corpuscle showed strong activity for the presence of acid phosphatases (Fig. 2b) in all prenatal age groups whereas Yogesh (2007) stated that the acid phosphatase activity was moderate to intense in the capsule, septa, Hassall's corpuscles and blood vessels. Whereas, moderate activity was found in cortex and medulla in all prenatal age groups of sheep. In contrast to the present results, Ramayya (2007) reported no acid phosphatase activity during prenatal period in buffalo foetuses. Acid phosphatase activity noticed in the present study was indication of lysosomal activity in the developing phase of thymus because of continuous tissue variation with age advanced. The moderate to strong acid phosphatase activity in thymus could be described to the release of lysosomal hydrolases, which might be associated with the cellular modifications occurring during histogenesis of thymus with the advancement of age (Yogesh, 2007).

**Postnatal thymus:**

**a) Carbohydrate analysis:**

In postnatal age groups, a moderate neutral and acid mucopolysaccharide reaction was noticed in the Hassall's corpuscle and parenchyma of all postnatal age groups (Fig. 3a). In accordance with the present observations, Ramayya and Singh (2012) reported strong to moderate PAS positive activity in Hassall's corpuscles during pre-pubertal age groups of buffalo calves (Table 2).

**b) Protein dynamics:**

The capsule and septa showed weak reaction whereas Hassall's corpuscles (Fig. 3b) showed moderate reaction for the presence of tyrosine in all postnatal age groups (Table 2). The protein content was not noticed in the postnatal age groups in any of the literature reviewed.

**c) Lipid storage:**

The parenchyma of thymus showed mild reaction for lipids whereas strong lipid activity was noticed in the interlobular septa (Fig. 3c) in all postnatal age groups (Table 2). In contrast to the present results, Ramayya and Singh (2012) observed no activity for

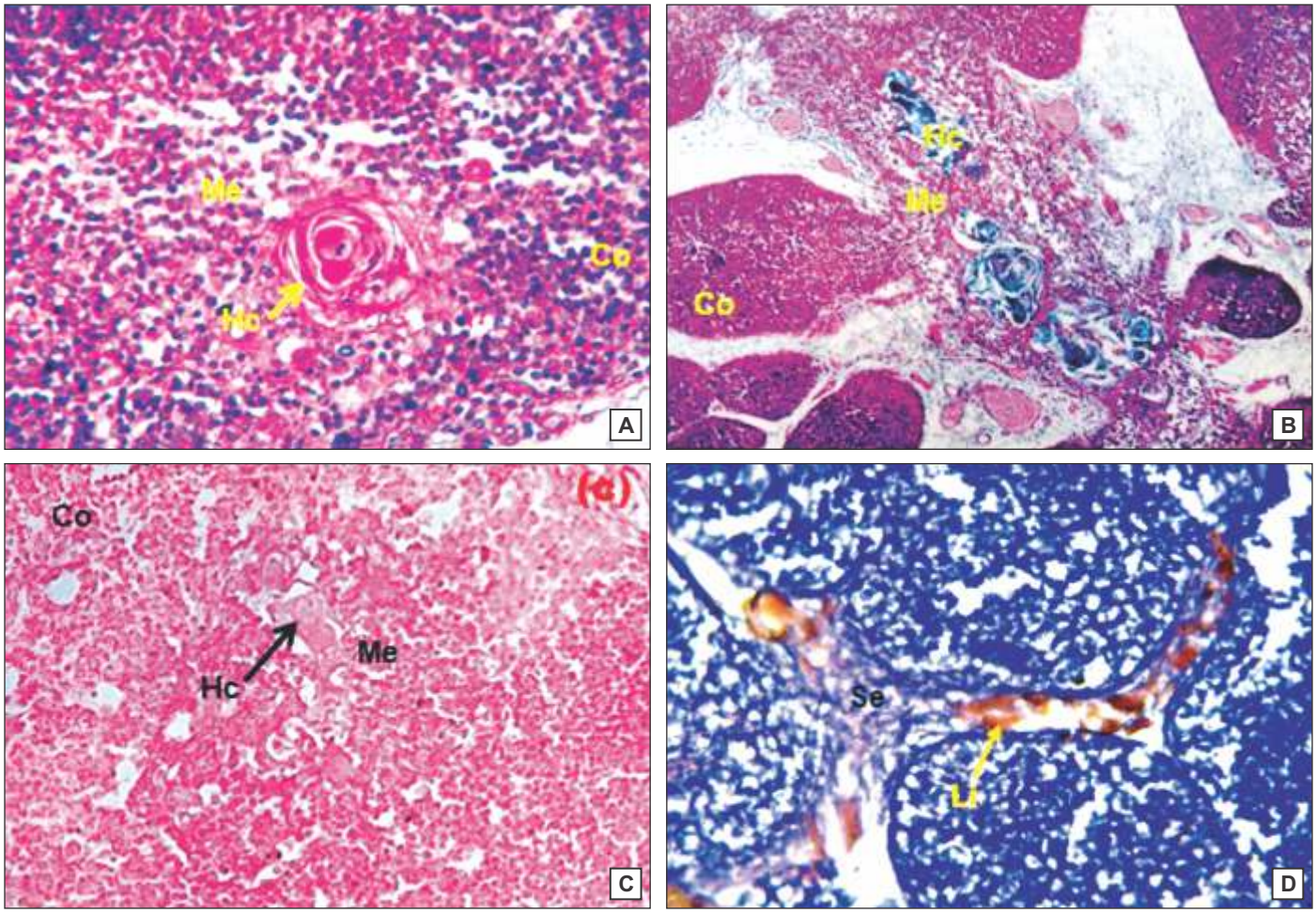


Fig. 1. **(a)** Photomicrograph showing PAS positive reaction in thymus of sheep foetus at 103 days of gestation (PAS×400). **(b)** Photomicrograph showing AB-PAS positive reaction in thymus of sheep foetus at 84 days of gestation (Combined Alcian blue-PAS×40). **(c)** Photomicrograph showing localization of tyrosine in thymus of sheep foetus at 126 days of gestation (Millon's reaction×400). **(d)** Photomicrograph showing lipid deposits in thymus of sheep foetus at 110 days of gestation (Oil Red O×400). [Hc- Hassall's corpuscle; Me- Medulla; Co- Cortex; Se- Septa; Li- Lipids].

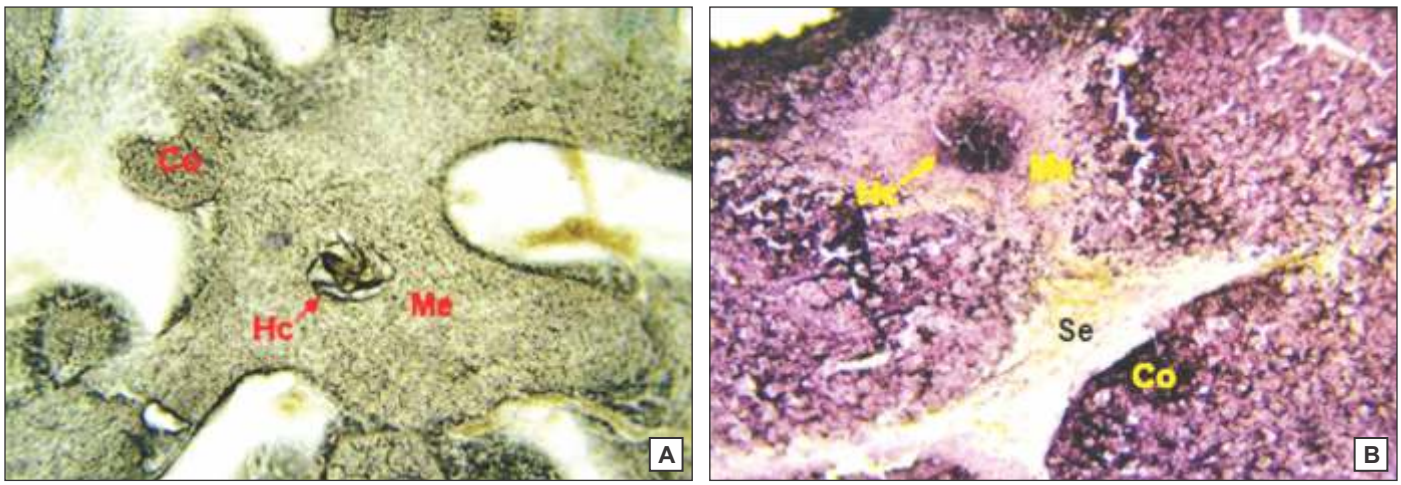


Fig. 2. **(a)** Photomicrograph showing alkaline phosphatase reaction in thymus of sheep foetus at 110 days of gestation (Gomori's cobalt ×100). **(b)** Photomicrograph showing acid phosphatase reaction in thymus of sheep foetus at 110 days of gestation (Gomori's lead×100). [Hc- Hassall's corpuscle; Me- Medulla; Co- Cortex; Se- Septa].

lipids in capsule of buffalo, but they have noticed lipids in reticular epithelial cells. According to Geetha *et al.* (2016), the accumulation of fats was more in the post-pubertal age groups when compared to prenatal and pre-pubertal age groups, which indicated the age-related involuntary changes

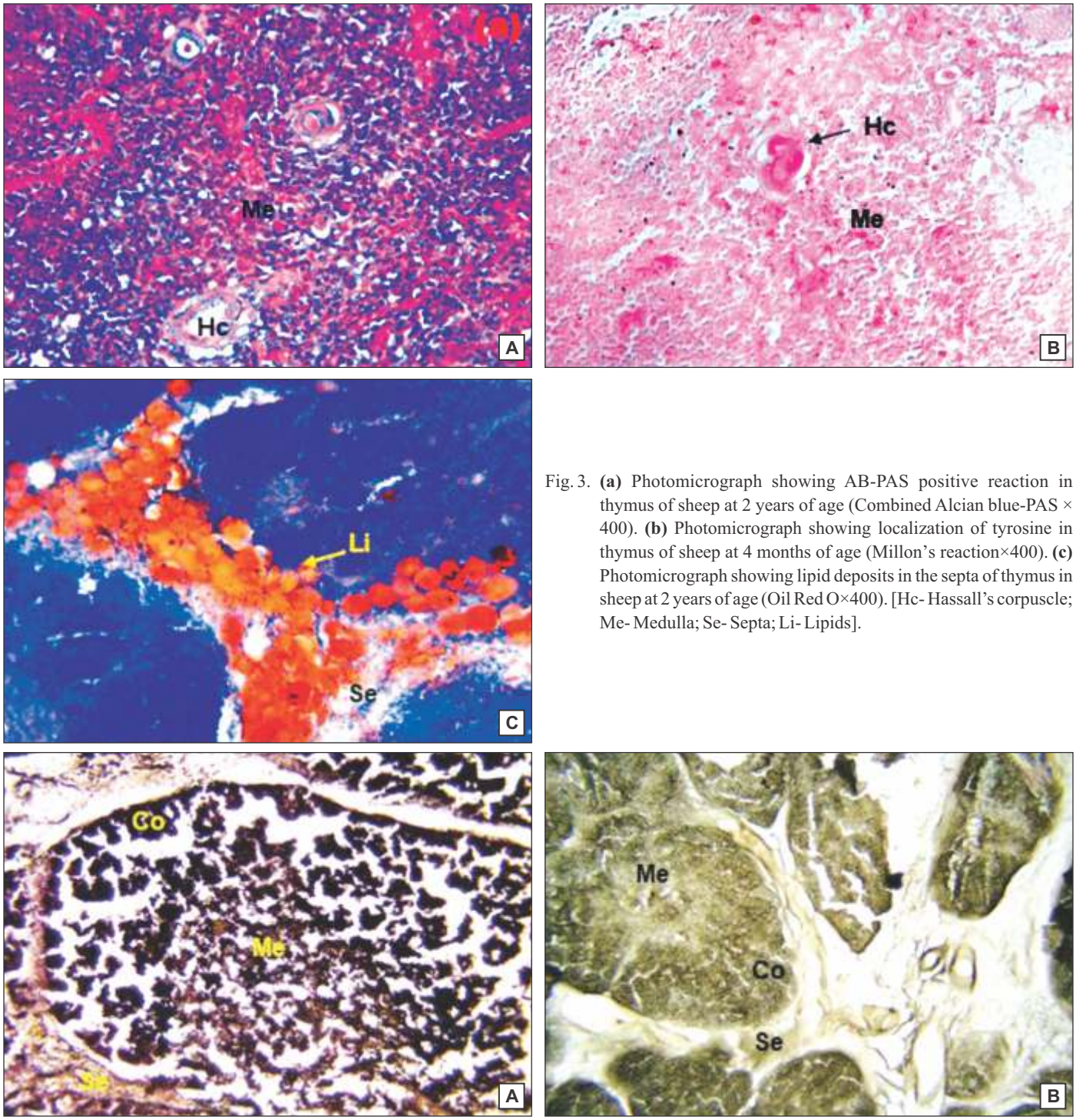


Fig. 3. **(a)** Photomicrograph showing AB-PAS positive reaction in thymus of sheep at 2 years of age (Combined Alcian blue-PAS × 400). **(b)** Photomicrograph showing localization of tyrosine in thymus of sheep at 4 months of age (Millon's reaction×400). **(c)** Photomicrograph showing lipid deposits in the septa of thymus in sheep at 2 years of age (Oil Red O×400). [Hc- Hassall's corpuscle; Me- Medulla; Se- Septa; Li- Lipids].

Fig. 4. **(a)** Photomicrograph showing acid phosphatase reaction in thymus of sheep at 7 months of age (Gomori's lead×100). **(b)** Photomicrograph showing alkaline phosphatase reaction in thymus of sheep at 7 months of age (Gomori's cobalt×100).

of thymus indicated by invasion of adipose tissue, loss of lymphocyte population and formation of small pits.

**d) Enzymatic activities:**

**i)** ***Alkaline phosphatase activity:***

The alkaline phosphatase showed mild reaction (Fig. 4a) in the stroma. However, a strong reaction was noticed in the parenchymal region in all postnatal age groups whereas Ramayya (2007) reported that the alkaline phosphatase activity was strong in cortex and medulla of buffalo calves during 1 week to 3 months and moderate in 6 to12 months of age.

**ii)** ***Acid phosphatase activity:***

The acid phosphatase showed mild reaction (Fig. 4b) in the stroma. However, a strong reaction

was noticed in the parenchymal region in all postnatal age groups (Table 2). In contrast to present results, Ramayya (2007) reported that there was no acid phosphatase activity in capsule, stroma and blood vessels during postnatal period.

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