RESEARCH ARTICLE

Histochemical Studies on Mesenteric Lymph Nodes in Pre- and Post-Natal Age-Groups of Sheep (*Ovis aries*)

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Abstract

This study investigated the histochemical properties of mesenteric lymph nodes from 36 sheep. Tissues were processed for cryosections to analyze various biochemical components including glycogen, mucopolysaccharides (acid and neutral), protein tyrosine, lipids and enzymes like alkaline and acid phosphatases. In prenatal specimens, lipid storage was evident with moderate periodic acid-Schiff (PAS) activity indicating glycogen presence, alongside moderate activity for acid and neutral mucopolysaccharides in the capsule across all groups, as evidenced by combined Alcian blue-PAS staining. Tyrosine showed moderate reactivity in the stroma and parenchyma, with lipid deposits primarily in the capsule. Enzymatic activity was generally weak in the capsule but moderate in the parenchyma. In postnatal specimens, moderate PAS activity was observed in the capsule, trabeculae and germinal centers, signifying glycogen presence. Alcian blue-PAS staining indicated strong activity for mucopolysaccharides in the capsule. Tyrosine and lipid reactivities ranged from weak to strong across different regions, with variable enzymatic activities noted in anatomical regions of the lymph nodes. These findings highlight significant histochemical differences in mesenteric lymph nodes between prenatal and postnatal age groups of sheep, with implications for understanding lymphatic biochemical dynamics across developmental stages.

Key words: Histochemistry, Mesenteric lymph node, Postnatal, Prenatal, Sheep.

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Introduction

Sheep are vital to global agriculture, offering meat, milk, wool, and manure, and are key to India's agrarian economy (Khalel, 2010). They also serve as models in biomedical research due to their physiological similarity to humans. The lymphatic system, crucial for immunity, includes lymph nodes that filter harmful substances and enable immune responses to infections (Rahmoun *et al.,* 2020). The lymphatic system is vital for immune defense, physiological regulation, and the absorption of fatty acids and vitamins from the gastrointestinal tract (Elizabeth and Fredric, 2011). Mesenteric lymph nodes, positioned along lymphatic vessels, act as filters, trapping pathogens and presenting them to immune cells, initiating immune responses and antibody production (Fails and Magee, 2018).

Mesenteric lymph nodes, specialized secondary lymphoid organs, are encapsulated, bean-shaped structures housing stromal and immune cells like lymphocytes, macrophages, and dendritic cells. Organized into the cortex, paracortex, and medulla, these regions support distinct immune functions. The cortex, rich in B lymphocytes and macrophages, facilitates antigen presentation and antibody production (Jubb *et al.,* 1985). Their structure and histogenesis are vital for diagnosing diseases, reflecting pathological changes, and supporting lymphocyte production, making them key indicators of systemic health (Patel *et al.,* 2016). This comprehensive understanding underscores the significance of lymph nodes in immune surveillance and response, emphasizing their role in both basic and clinical research. Therefore, this study was aimed to evaluate histochemistry of mesenteric lymph nodes in pre- and post-natal sheep.

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Materials and Methods

Collection of Sample

This study was conducted on 36 sheep, divided into prenatal and postnatal groups, spanning from 0 to 150 days for

prenatal specimens and from 1 month to over 2 years for postnatal specimens. The subjects were categorized into six distinct groups, each comprising six animals: Group I (0-50 days of gestation), Group II (51-100 days of gestation), Group III (101-150 days of gestation), Group IV (birth to 6 months, pre-pubertal), Group V (6-8 months, pubertal) and Group VI (2 years and above, adult). The mesenteric lymph nodes were collected from sheep at various slaughterhouses in Andhra Pradesh and Tamil Nadu, immediately stored in thermos containers with ice to preserve their integrity.

For histochemical analyses, lymph node samples of 1-2 mm in thickness were sectioned to 7-10 µm using a cryostat set at -20°C. These sections were subjected to several staining procedures: Gomori's alkaline phosphatase cobalt and Napthol AS-BI method for alkaline phosphatase, Gomori's modified lead nitrate method for acid phosphatase (Carleton and Drury, 1980) and the Oil Red O method for lipids. All histochemical procedures were performed at the State Level Diagnostic Laboratory, Sri Venkateswara Veterinary University, Tirupati (India).

Age estimation for the prenatal groups was performed using the crown-rump length (CRL), employing the formula A = 2.1(B+17), where 'A' is the developmental age of the foetus in days and 'B' is the CRL in centimeters (Noakes *et al.,* 2009). For foetuses up to 3 cm CRL, standard values were utilized from Bryden *et al.* (1972). Postnatal ages were determined based on tooth eruption patterns, following the guidelines set by Dyce *et al.* (1996).

The foetuses, non-descript in breed and irrespective of sex, were collected from the uteri of slaughtered pregnant sheep. Measurements including body weight and CRL were recorded immediately. The foetuses were dissected through a mid-ventral skin incision from the mandible to the inguinal region. The mesenteric lymph nodes were identified, collected and cleansed in normal saline. Observations on the gross morphology, including color, shape, location and topography were noted. The nodes were subsequently fixed in various fixatives, including 10% neutral buffered formalin, chilled 10% neutral buffered formalin and Bouin's fluid.

Histochemical Methods

Paraffin wax-embedded sections of mesenteric lymph node tissues were meticulously prepared and subsequently stained using several techniques to highlight specific cellular components. Periodic Acid SChiff's reagent (PAS) was applied to identify glycogen, while Million's reaction was used to detect protein tyrosine. Additionally, a combined Alcian Blue-PAS technique was employed to differentiate acid and neutral mucopolysaccharides. Prior to embedding, the tissues were fixed in chilled 10% neutral buffered formalin to preserve cellular integrity. For lipid analysis, frozen sections were prepared and stained with Oil Red O Method . Enzymatic activity was assessed using Gomori's cobalt and Napthol AS-BI method for alkaline phosphatase and Gomori's lead method for acid phosphatase, providing comprehensive insights into the biochemical properties of the lymph nodes.

Results and Discussion

Prenatal Mesenteric Lymph Nodes

Carbohydrate, Protein & Lipid Dynamics:

***Carbohydrate Dynamics*:** In the prenatal age groups studied, the mesenteric lymph nodes exhibited varied histochemical responses indicative of developmental biochemistry. Notably, the capsule of these nodes displayed moderate PAS activity suggesting the presence of glycogen, as observed in Fig.1a. This glycogenic activity mirrors observations in human foetuses by Gomori (1941) and in crossbred pigs by Geetha (1996), indicating a conserved metabolic feature across species. Further, the application of combined Alcian blue-PAS staining revealed moderate activity (Fig.1b) for acid and neutral mucopolysaccharides within the capsule across all prenatal groups, as detailed in Table 1. This staining highlights the glycosaminoglycan components essential for cellular functions such as cell adhesion and signaling.

***Protein Dynamics*:** Additionally, the stroma and parenchyma exhibited moderate tyrosine reactivity, as shown in Fig.1c and noted in Table 1, suggesting active protein synthesis. This contrasts with findings of Vikram (2014), who reported variable reactivity in buffalo foetuses, likely due to differences in gestational age and species-specific developmental patterns.

Lipid Storage: Lipid deposition was also analyzed, with the capsule showing moderate to strong lipid presence and the cortex demonstrating weaker activity, as depicted in Fig.1d and recorded in Table 1. These findings align with observations of Asha *et al.* (2012) in goat foetuses, underlining similar lipid storage mechanisms across different ruminant species

Enzymatic Activities:

Alkaline Phosphatase Activity: Alkaline phosphatase activity was found to be weak in the capsule but moderate in the cortex, as shown in Fig. 2a. This contrasts with reports from Geetha *et al.* (2016), who found no activity in prenatal groups of mice, rats and guinea pigs, suggesting a distinct difference in enzyme expression between ruminants and laboratory animals.

Acid Phosphatase Activity: In terms of acid phosphatase, the cortex exhibited strong activity, while the medulla showed moderate activity, as detailed in Fig. 2b and Table 1. This enzymatic pattern differs from that reported by Asha *et al.* (2012) in goat foetuses, where activity was weak in medullary cords and capillary epithelium, potentially reflecting the organ's functional status during different developmental stages. These variations emphasize the complexity of lymphatic system development and its regulation by both genetic and environmental factors.

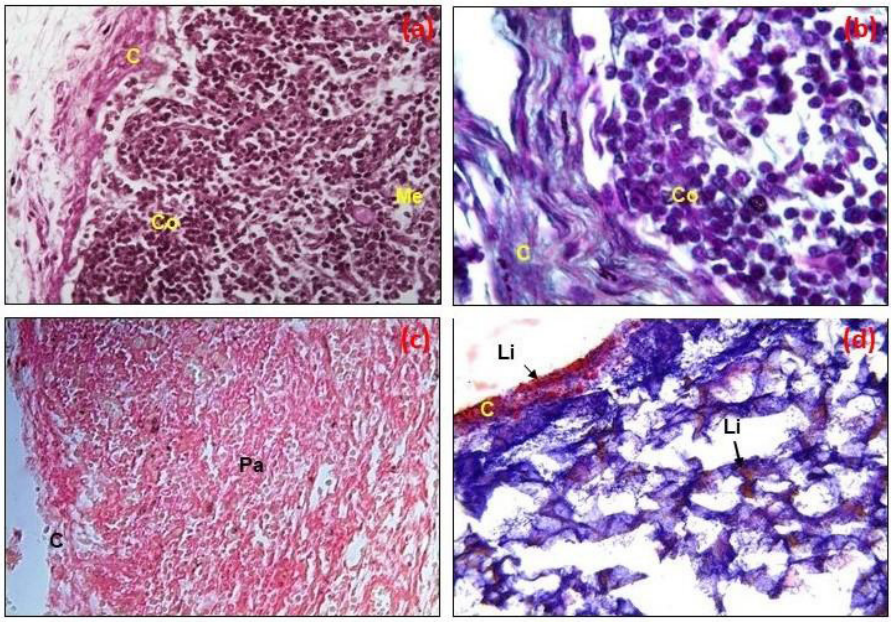

Fig. 1: Histochemical observations in mesenteric lymph node of sheep foetus at 103 days of gestation. (a) PAS positive reaction (PAS X 400), (b) AB-PAS positive reaction (Combined Alcian Blue-PAS X 1000), (c) Localization of tyrosine (Millon's Reaction X 400), (d) Lipid deposits (Oil Red O X 400).

Table 1: Histochemical observations of prenatal mesenteric lymph node (50-150 days) in sheep

Mesenteric lymph node	Prenatal age groups					
	PAS	Combined Alcian blue-PAS	Tyrosine	Lipid	Alkaline Phosphatase	Acid Phosphatase
Capsule	++	++	++	+++	+	+
Trabeculae	-	-	++	-	-	-
Lymphatic nodule	-	-	++	-	++	++
Cortex	-	-	++	+	++	++
Medulla	-	-	++	-	++	++

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

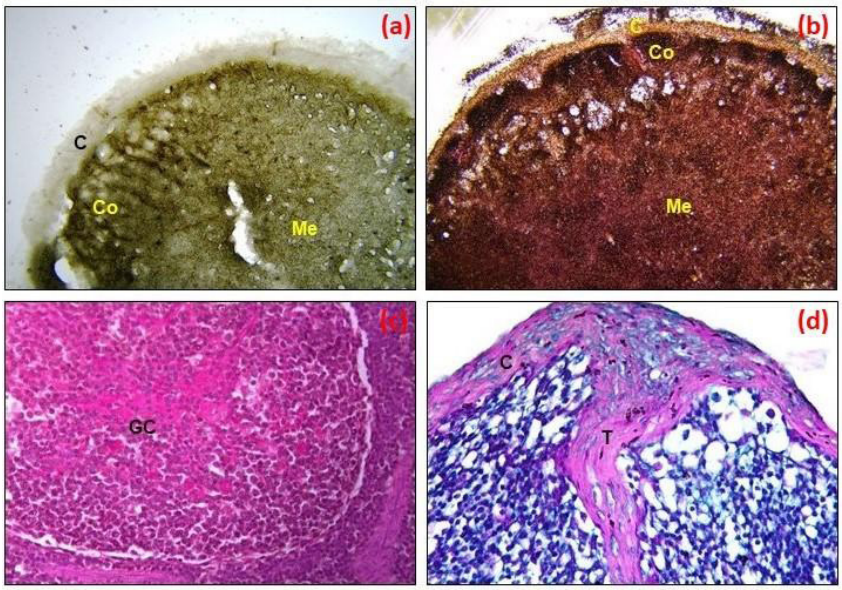

Fig. 2: Histochemical observations in mesenteric lymph node of sheep. (a) alkaline phosphatase reaction at 93 days of gestation (Gomori's cobalt X 100), (b) acid phosphatase reaction in at 126 days of gestation (Gomori's lead X 100), (c) PAS positive reaction in germinal center at 2 years of age (PAS X 400), (d) AB-PAS positive reaction at 6 months of age (Combined Alcian blue-PAS X 400)

Postnatal Mesenteric Lymph Nodes

Carbohydrate, Protein & Lipid Dynamics:

Carbohydrate Dynamics: In the postnatal age groups of sheep, the capsule, trabeculae and germinal centers of lymphatic nodules, as depicted in Fig. 2c, exhibited moderate PAS activity, indicative of glycogen presence. Additionally, intense Alcian blue staining was observed in the capsule, signifying substantial amounts of both neutral and acid mucopolysaccharides (Fig. 2d). This contrasts with findings of Geetha *et al.* (2016), who reported that in mice, rats and guinea pigs, both the capsule and macrophages across all age groups were consistently PAS-positive, highlighting interspecies variations in carbohydrate processing within lymphatic tissues.

Protein Dynamics: Observations revealed weak to moderate tyrosine activity (Fig. 3a) within the capsule, trabeculae and parenchyma of postnatal mesenteric lymph nodes. This suggests the presence of protein tyrosine, which is crucial for various cellular functions, including signaling and enzymatic reactions. Notably, there appears to be a lack of documented protein content in similar postnatal age groups within the existing literature, indicating a potential area for further research to explore protein profiles in ovine lymph nodes compared to other species (Table 2).

Lipid Storage: The lipid analysis showed strong activity in the capsule and moderate activity (Fig. 3b) within the germinal center of the lymphatic nodules in all examined postnatal age groups of sheep. This pattern of lipid deposition suggests active lipid metabolism and storage within specific regions of the lymph nodes. Interestingly, such lipid content has not been prominently noted in postnatal age groups in previously reviewed literature, pointing towards a unique aspect of lipid handling in sheep that might differ from other studied animals.

Enzymatic Activity:

Alkaline Phosphatase Activity: Alkaline phosphatase activity in the mesenteric lymph nodes of postnatal sheep exhibited a range from weak to moderate (Fig. 3c) in the capsule and trabeculae and consistently moderate within

Table 2: Histochemical observations of mesenteric lymph node in postnatal age groups (2 months to 2 years) of sheep

Mesenteric lymph node	Postnatal age groups					
	PAS	Combined Alcian blue-PAS	Tyrosine	Lipid	Alkaline Phosphatase	Acid Phosphatase
Capsule	++	+++	+ to ++	+++	+	++
Trabeculae	++	-	+ to ++	-	+	++
Lymphatic Nodule	++	-	+ to ++	++	-	-
Cortex	-	-	+ to ++	-	++	++
Medulla	-	-	+ to ++	-	-	-

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

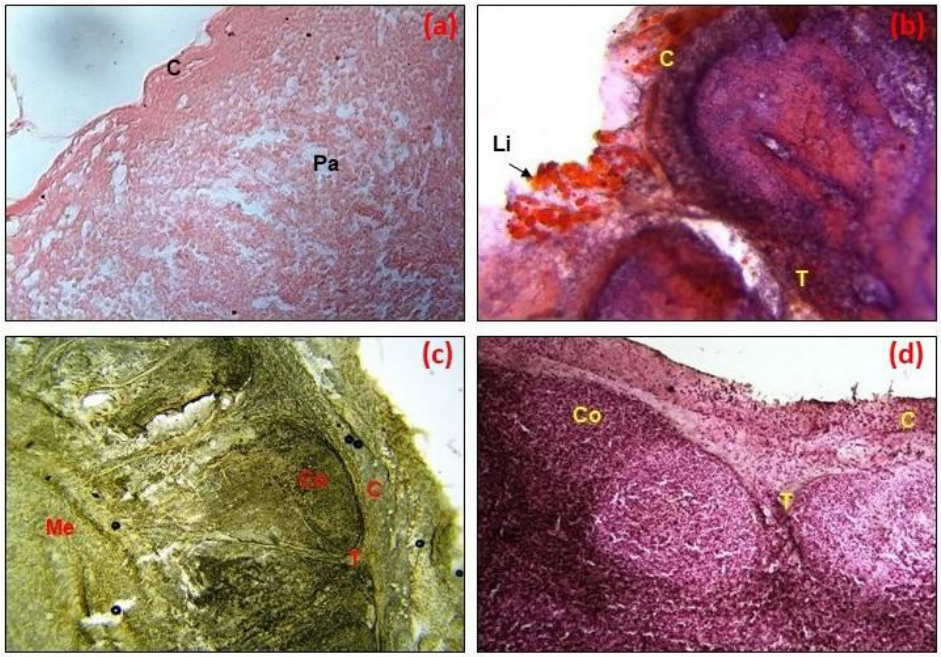

Fig. 3: Histochemical observations in mesenteric lymph node of sheep. (a) localization of tyrosine at 6 months of age (Millon's reaction X 400), (b) lipid deposits at 4 months of age (Oil Red O X 400), (c) alkaline phosphatase reaction at 2 years of age (Gomori's cobalt X 100), (d) acid phosphatase reaction at 7 months of age (Gomori's lead X 100).

the lymphatic nodules. These findings aligned with those reported by Sivagnanam (2018) in goats, suggesting a similar enzymatic profile in closely related species. However, this contrasts markedly with the observations made by Geetha *et al.* (2016), who noted an absence of alkaline phosphatase activity in all postnatal age groups across mice, rats and guinea pigs. This discrepancy underscores potential species-specific differences in enzymatic expression and function within the lymphatic system during the postnatal period.

Acid Phosphatase Activity: For acid phosphatase, the capsule, trabeculae and cortex of sheep lymph nodes showed moderate activity across all postnatal age groups (Fig. 3d). This level of activity contrasts with findings of Sivagnanam (2018), who observed predominantly weak reactions in the parenchyma of lymph nodes in goats. This variation may reflect differences in the metabolic and immunological demands placed on lymphatic tissues in different species or possibly different regulatory mechanisms controlling enzyme expression in these tissues.

Conclusions

In the prenatal age groups, the mesenteric lymph nodes exhibited varied histochemical responses indicative of developmental biochemistry. Notably, the capsule of these nodes displayed moderate periodic acid-Schiff (PAS) activity suggesting the presence of glycogen. The combined Alcian blue-PAS staining revealed moderate activity for both acid and neutral mucopolysaccharides within the capsule across all prenatal groups. The lipid analysis showed strong activity in the capsule and moderate activity within the germinal center of the lymphatic nodules in all postnatal age groups of sheep. Alkaline phosphatase activity in the mesenteric lymph nodes of postnatal sheep exhibited a range from weak to moderate in the capsule and trabeculae and consistently moderate within the lymphatic nodules. For acid phosphatase, the capsule, trabeculae and cortex of sheep lymph nodes showed moderate activity across all postnatal age groups.

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References

Asha, A., Maya, S., Harshan, K.R., & Chungath, J.J. (2012). Histochemical observations on the lymph nodes of goat foetuses. *Journal of Indian Veterinary Association, 90*(2), 17-19.

Bryden, M.M., Evans, H.E., & Binns, W. (1972). Embryology of the sheep. I. Extraembryonic membranes and the development of body form. *Journal of Morphology, 138*(2), 169-185.

Carleton, H.M., & Drury, R.A.B. (1980). *Histological Technique of Normal and Pathological Tissues and the Identification of Parasites*. 3rd edn., Oxford University Press, London.

Dyce, K.M., Sack, W.O., & Wensing, C.J.G. (1996). *Textbook of Veterinary Anatomy*. 2nd edn., W.B. Saunders Company, Philadelphia, Pennsylvania.

Elizabeth, A., & Fredric, L.D. (2011). *Comparative Veterinary Histology with Clinical Correlates*. 3rd edn., Manson Publishing Ltd., City, Country?

Fails, A.D., & Magee, C. (2018). *Anatomy and Physiology of Farm Animals*. 8th edn., John Wiley and Sons, Inc.

Geetha, R. (1996). Macroanatomical studies on the thymus, spleen and lymph nodes of certain laboratory animals. *Ph.D. Dissertation*. Tamil Nadu Veterinary and Animal Sciences University, Chennai.

Geetha, R., Kannan, T.A., & Sivakumar, M. (2016). Age related histochemical changes in thymus, spleen and mesenteric lymph nodes in mice, rat and guinea pig. *International Journal of Science and Research*, *5*(9), 1088-1091.

Gomori, G. (1941). Distribution of phosphatase in the normal organ and tissues. *Journal of Cellular and Comparative Physiology, 17,* 71-83.

Jubb, K.V.F., Kennedy, P.C., & Palmer, N. (1985). *Pathology of Domestic Animals*. 2nd edn., Academic Press, New York, p. 132, 273-282.

Khalel, M.K. (2010). Anatomical and histological study of the spleen in Iraqi Awasi sheep. *Basrah Journal of Veterinary Research*, *10*(2), 163-171.

Noakes, D.E., Parkinson, T.J., & England, G.C.W. (2009). *Veterinary Reproduction and Obstetrics*. 9th edn., Saunders, Elsevier.

Patel, S.K., Rajukumar, K., Kumar, R., Reddy, B.V.R., Singh, R., Munuswamy, P., & Rana, J. (2016). Histopathological changes in grossly normal caprine mesenteric lymph nodes. *Advances in Animal and Veterinary Sciences, 4*(1), 53-56.

Rahmoun, D.E., Barani, S., & Lieshchova, M.A. (2020). Anatomo-topographic and radiological study of the sheep lymph node in Algeria. *Journal of Veterinary Medical Research, 7*(4), 1195.

Sivagnanam (2018). Anatomy of the postnatal lymphoid organs in goats (*Capra hircus*). *Ph.D. Thesis*. Tamil Nadu Veterinary and Animal Sciences University, Chennai, India

Vikram, N. (2014). Prenatal development of superficial lymph nodes in buffalo (*Bubalus bubalis*). *M.V.Sc. Thesis*. Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India.