

## DEVELOPMENTAL DYNAMICS OF MESENTERIC LYMPH NODES IN SHEEP (*OVIS ARIES*): A GROSS MORPHOMETRIC ANALYSIS

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### ABSTRACT

Comprehensive gross anatomical and biometric analysis were conducted on the lymphoid organs, specifically targeting the mesenteric lymph nodes of 36 sheep across various developmental stages, encompassing both prenatal and postnatal periods. This study aimed to observe and quantify the morphological changes in these lymphoid structures over time. During the prenatal phase, observations began at 60 days of gestation, where the mesenteric lymph nodes in group-II were identified as tiny, spherical, cream-colored masses situated along the visceral border of the mesentery. By 84 days of gestation, these nodes evolved into an oval shape and assumed a creamish white coloration. Progressing to 93 days of gestation, a notable diversification in shape was observed, the nodes appeared in oval, circular and elongated forms, now exhibiting a grayish-red color. Postnatally, at two months of age, the sheep's mesenteric lymph nodes presented a variety of shapes including round, bean-shaped, oval and elongated, consistently maintaining a creamish color. This study provides a detailed chronological overview of the morphological evolution of mesenteric lymph nodes in sheep, contributing valuable insights into the developmental anatomy of lymphoid organs.

**Keywords:** Foetuses, Gross morphology, Postnatal, Prenatal, Sheep

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Sheep holds considerable significance in India, contributing extensively to various sectors of the agricultural economy (Sandhu *et al.*, 2021). Not only a vital source of meat, sheep also provide milk, skin, wool, and manure, thereby playing a pivotal role in enhancing agricultural productivity (Khalel, 2010). Lymph nodes, essential components of the immune system, are strategically dispersed along the lymphatic vessels. These nodes serve critical functions, filtering lymph and acting as primary defense sites against infections by housing lymphocytes, plasma cells, and macrophages (Fails and Magee, 2018). Upon activation, these immune cells within the lymph nodes proliferate and synthesize antibodies, forming a robust defense mechanism against diseases and infections. Consequently, understanding the histogenesis of lymph nodes is crucial for the accurate diagnosis and management of various diseases throughout both foetal and adult stages of development (Bhagyalakshmi *et al.*, 2023).

The lymph node represents a crucial nexus for the interaction between antigen-presenting cells, antigenic substances derived from lymph, and lymphocytes that migrate into the lymph nodes from the bloodstream. Given that the intestinal wall experiences a significant antigenic load, lymph nodes associated with the intestine, particularly the mesenteric lymph nodes, are of special interest in immunological studies (Khazaeel *et al.*, 2017). This has led to a focused examination of the mesenteric

lymph node's structure in the current research. The immune system in mammals, including sheep, undergoes continuous transformations throughout its lifespan, adapting and responding to various immunological challenges. Accordingly, this study aims to meticulously observe and compare the developmental changes in the topography of the mesenteric lymph node in sheep during both the prenatal and postnatal periods, providing insights into the dynamic structural and functional adaptations of this critical immune organ (Geetha *et al.*, 2016).

Lymph nodes are highly organized lymphoid organs characterized by an intricate architecture, where lymphocytes are embedded within a fine reticular stroma. Functionally classified as independent secondary lymphatic organs, lymph nodes are strategically situated along the lymphatic vessels, facilitating a vital role in mediating regional immune responses (Mack, 2006). This regional immunological activity not only provides a localized defense mechanism but also has significant implications in veterinary medicine, particularly in meat inspection processes. Lymph nodes serve as indicators of the health status of the specific bodily regions they drain, making them crucial in assessments of animal health (Patel *et al.*, 2015). The structural and functional attributes of lymph nodes have been the subject of extensive histological studies across various animal species, including humans. These studies aim to elucidate the complex interactions within lymph nodes that underpin their role in immune surveillance and response, offering

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valuable insights into both their physiological mechanisms and their implications in health and disease (Zidan and Pabst, 2015).

Lymph nodes, as dynamic components of the immune system, exhibit variations in color and size during infectious episodes, reflecting their critical role in immune response modulation. Although extensive research has been conducted on the morphometric characteristics of lymph nodes in mature and immature sheep, investigations into the morphometric development of lymph nodes in foetal sheep have been limited. Previous studies have indicated that morphometric parameters of lymph nodes in 4-month-old and 5-month-old goat fetuses show slight gender-based differences, with males exhibiting slightly larger dimensions. Similarly, the influence of gender on the morphometric development and evolution of cranial tracheobronchial lymph nodes in sheep fetuses has been explored, suggesting a potential neutrality (Fares *et al.*, 2023).

## MATERIALS AND METHODS

In this study, 36 specimens (prenatal and postnatal) were collected and divided into six different age groups, with six animals per group. Group-I (1-50 days), Group-II (51-100 days), Group-III (101-150 days), Group-IV (birth to 6 months), Group-V (6-8 months) and Group-VI (2 years and above). This structured sampling facilitated a comprehensive analysis of the morphological changes occurring in the mesenteric lymph nodes across various developmental stages, contributing valuable insights into both prenatal and postnatal lymphatic and immune development in sheep.

For prenatal specimens, the approximate developmental age was estimated using the crown-rump length (CRL). The age in days (A) was then calculated using the following formula, as proposed by Noakes *et al.* (2009).

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

Where 'A' is the developmental age of foetus in days and 'B' is crown rump length in centimetres. For fetuses with a CRL up to 3 cm, standard values from Bryden *et al.* (1972a) were utilized to estimate the age. For postnatal specimens, age determination was based on the eruption pattern of teeth, according to the methodology described by Dyce *et al.* (1996).

### Prenatal Groups

Foetuses of a non-descript breed, irrespective of sex, were harvested from the uteri of pregnant sheep immediately post-slaughter. Measurements of body weight and crown-rump length (CRL) were meticulously recorded. The total body weight of the prenatal subjects was determined using a digital monopan balance. Dissection of the foetuses was

performed through a mid-ventral incision extending from the mandible to the inguinal region. Mesenteric lymph nodes were located, excised, and subsequently rinsed in normal saline. Detailed observations of the gross morphology including color, shape, location and topography were documented. The extracted lymph nodes were then preserved in fixatives such as 10% neutral buffered formalin and Bouin's solution for further analysis.

### Postnatal Groups

Following slaughter, lymph nodes from postnatal sheep were collected. Initial observations recorded included the morphology specifically the color, shape, and position of the lymph nodes after rinsing them with normal saline.

### Gross Morphometry

Morphometric parameters including the length, width, and weight of the mesenteric lymph nodes were systematically measured across different age groups. A digital Vernier caliper was utilized to ascertain the length and width, while the weight of these lymphoid organs was quantified using an electronic balance.

## RESULTS AND DISCUSSION

### Group-I (1-50 days)

The mesenteric lymph nodes in Group-I were not discernible macroscopically but could be observed microscopically. This indicates a developmental stage where the lymph nodes are not yet sufficiently differentiated to be recognized through gross examination.

### Group-II (51-100 days)

In Group-II, the mesenteric lymph nodes were macroscopically identifiable by 60 days of gestation as small, rounded, creamish masses located at the visceral border of the mesentery (Fig. 1). These findings support with Mahabady *et al.* (2018), who documented similar observations in sheep fetuses at 59 days of gestation. By 84 days of gestation, the nodes had developed a creamish white color and an oval shape (Fig. 2), mirroring descriptions of human mesenteric lymph nodes by Johnston *et al.* (1958). By 93 days, the nodes exhibited varied shapes oval, round, bean, and elongated with a reddish-grey color (Fig. 3). This contrasts with observations by Asha (2009), who noted predominantly elongated mesenteric lymph nodes in goat fetuses.

### Group-III (101-150 days)

By 103 and 126 days of gestation, the mesenteric lymph nodes in Group-III were noted to be reddish-grey, situated along the visceral border of the mesentery adjacent to lymphatic vessels (Fig. 4). Both the weight and





Fig. 1. Photograph showing mesenteric lymph node in sheep foetus at 60 days of gestation. (MLN- Mesenteric lymph node; Ms- Mesentery; I- Intestine)



Fig. 2. Photograph showing the mesenteric lymph node of sheep foetus at 84 days of gestation. (MLN- Mesenteric lymph node; Ms- Mesentery; I- Intestine)



Fig. 3. Photograph showing various shapes of mesenteric lymph nodes (MLN) of sheep foetus at 93 days of gestation. (Lv- Lymphatic Vessel; I- Intestine; Arrow head- Bean shaped MLN; Arrow- Oval shaped MLN)



Fig. 4. Photograph showing mesenteric lymph nodes of sheep foetus at 126 days of gestation. (MLN- Mesenteric lymph Nodes; Lv- Lymphatic Vessel; I- Intestine)

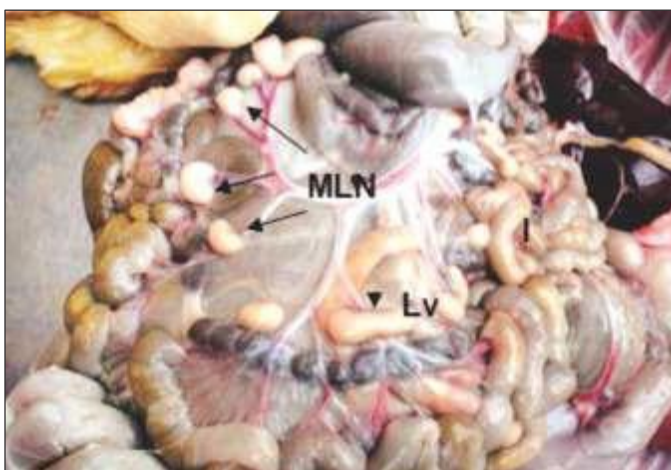


Fig. 5. Photograph showing various shapes of mesenteric lymph nodes (MLN) of sheep foetus at 2 months of age. (Lv- Lymphatic Vessel; I- Intestine; Arrow head- Elongated MLN; Arrow- Bean shaped MLN)

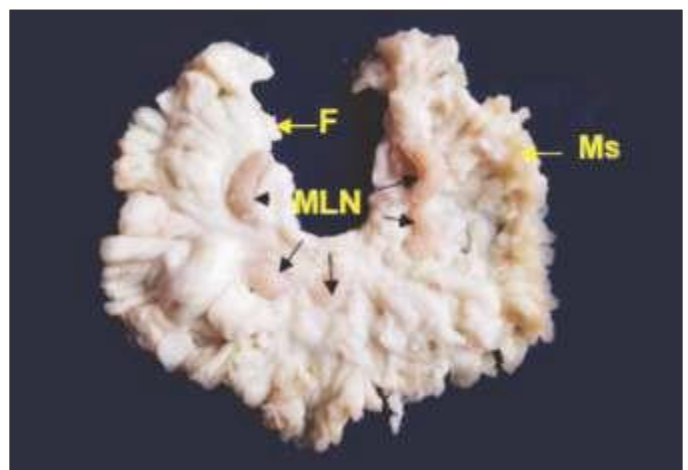


Fig. 6. Photograph showing mesenteric lymph nodes in sheep at 6 month of age. (MLN- Mesenteric lymph nodes; F- Fat; Ms- Mesentery; Arrow head- Elongated MLN; Arrow- Bean shaped MLN)

size of these nodes showed a consistent increase, suggesting progressive growth and development during the gestational period. These results illustrate a clear progression in the development of mesenteric lymph nodes during prenatal growth in sheep, with significant changes in size, color, and shape. The comparative analysis with existing literature underscores the consistency of certain developmental milestones while also highlighting species-specific variations in the gestational development of lymphatic organs.

### Morphometric Analysis

The morphometric analysis of the mesenteric lymph nodes demonstrated significant increases in mean length, width, and weight across the prenatal age groups. Specifically, measurements in Group-II showed an initial size of  $3.285 \pm 0.297$  mm in length,  $2.160 \pm 0.165$  mm in width, and a weight of  $0.027 \pm 0.0053$  g. As the gestational age advanced to Group-III, these dimensions expanded to  $10.368 \pm 0.465$  mm in length,  $3.683 \pm 0.156$  mm in width, and  $0.135 \pm 0.0042$  g in weight. This trend indicates a robust growth pattern as the developmental stage progresses. These findings are consistent with the observations reported by Mahabady *et al.* (2018), who documented that the dimensions of mesenteric lymph nodes in sheep foetuses varied from  $2.76 \pm 1.58$  mm in length,  $0.76 \pm 0.60$  mm in width, and  $0.02 \pm 0.02$  g in weight at early gestational ages (43-66 days), to  $30.39 \pm 21.01$  mm in length,  $4.29 \pm 1.35$  mm in width and  $0.21 \pm 0.18$  g in weight at later stages (109-138 days). These results underline a progressive increase in nodal size correlating with the foetal growth phase.

### Group-IV (Pre-Pubertal Stage)

During the pre-pubertal stage, both the size and number of mesenteric lymph nodes exhibited an increase. The observed shapes of these nodes included oval, round, bean, and elongated, predominantly creamish white in color, as corroborated by Sivagnanam (2018) in goats, Johnston *et al.* (1958) and Nigam and Knight (2020) in humans, and Rahmoun *et al.* (2020b) in camels. Notably, the bean-shaped lymph nodes were more prevalent than the elongated and round forms. There were no fat depositions observed surrounding the mesenteric lymph nodes during this stage (Fig. 5).

### Group-V (Pubertal Stage)

In the pubertal age group, the morphological characteristics of the mesenteric lymph nodes remained consistent with those observed in Group-IV in terms of shape and color. However, a significant change was noted in the surrounding mesentery, where fat deposition became evident, encasing the lymph nodes (Fig. 6). This development

marks a physiological adaptation as the animals mature through puberty.

### Group-VI (Post-Pubertal Stage)

Post-puberty, the mesenteric lymph nodes continued to maintain the shape and color characteristics similar to those seen in the earlier pubertal stage. Yet, a marked increase in the biometric parameters such as length, width, and weight of the lymph nodes was observed, aligning with findings by Sarma *et al.* (2008) in Kagan goats and Rahmoun *et al.* (2020a) in sheep. This growth reflects further developmental maturation and possibly an enhanced immunological role of the lymph nodes in the organism's defense mechanisms post-puberty.

### Morphometry

During the postnatal development of sheep, there was a notable progression in the morphometric parameters of the mesenteric lymph nodes across different age groups. Specifically, in Group-IV (pre-pubertal), the mean dimensions were recorded as  $13.475 \pm 0.378$  mm in length,  $8.060 \pm 0.189$  mm in width, and  $0.598 \pm 0.0029$  g in weight. As the sheep matured to Group-V (pubertal) and Group-VI (post-pubertal), these measurements increased substantially. By Group-VI, the mesenteric lymph nodes measured  $21.580 \pm 0.429$  mm in length,  $10.690 \pm 0.235$  mm in width, and weighed  $1.343 \pm 0.0085$  g. This trend of increasing size and weight with age is consistent with findings in other species, as reported by Sivagnanam (2018) in goats. In that study, the mesenteric lymph nodes showed an increase from an average length of  $4.2 \pm 0.24$  cm and width of  $0.4 \pm 0.02$  cm at ages 3-5 months, reaching dimensions of  $9.2 \pm 0.42$  cm in length and  $1.0 \pm 0.06$  cm in width by ages 2-3 years. The weight similarly escalated from  $2.0 \pm 0.12$  g to  $5.5 \pm 0.31$  g over the same age progression. These observations underscore a general biological principle that lymph nodes, as critical components of the immune system, undergo significant growth and development postnatally. The increase in size and mass of the lymph nodes not only reflects the physical growth of the animal but also likely correlates with a maturing immune response capability, essential for dealing with a wider array of antigens and pathogens as the animal age.

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## RETRACTION OF ARTICLE

This article earlier available at <https://www.luvas.edu.in/haryana-veterinarian/download/harvet2016-dec/1.pdf> entitled “Occurrence of some organochlorine pesticide residues in poultry feed and meat” has been retracted by the authors because of some error made during the data analysis process of the experimental observations due to counting the number of samples showing the concentration of pesticide below its corresponding Limit of Detection. All authors take full responsibility for this mistake and sincerely apologize for any inconvenience it may cause.

Editors