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Ontogenetic Morphological And Histochemical Evaluation Of The Abomasum In Local Lambs And Goat Kids (1–4 Months Of Age): A Comparative Study

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Abstract

Background:
The abomasum, known as the true stomach of ruminants, is central to enzymatic digestion and the establishment of mucosal immunity during early postnatal life. Although sheep and goats are critical for food security in developing countries, there is limited comparative evidence on the ontogenetic maturation of their abomasum, particularly in indigenous breeds.

Objective:
This study aimed to evaluate the morphological, histochemical, and morphometric development of the abomasum in local lambs and goat kids between one and four months of age, with emphasis on species-specific differences.

Methods:
A total of 48 abomasal samples (n = 8 per species per age group) were collected post-mortem from the Tikrit municipal abattoir in Iraq. Specimens were fixed in 10% buffered formalin, processed by routine paraffin embedding, and stained with hematoxylin–eosin and Masson's trichrome. Quantitative morphometric measurements of abomasal layers were obtained from five randomly selected fields per section. Statistical comparisons between species and age groups were performed using ANOVA followed by Tukey's test, with significance set at $p \leq 0.05$.

Results:
Both species exhibited progressive abomasal maturation with age. In lambs, glandular units compacted earlier, and collagen deposition in the lamina propria was evident by three months. Goat kids consistently demonstrated thicker tunica muscularis at all ages, indicating an emphasis on muscular reinforcement. Histochemical analysis confirmed a shift from delicate to dense collagen bundles with age, while lymphocyte and macrophage infiltration reflected early mucosal immune defense. Morphometric analysis showed significant species- and age-related differences across all layers.

Conclusion:
This study provides novel baseline data on abomasal ontogeny in local small ruminants. The divergent developmental patterns between lambs and goat kids highlight adaptive strategies linked to dietary behavior and digestive physiology. These findings have implications for veterinary anatomy, nutrition, and future molecular investigations.

Keywords: Abomasum; Collagen deposition; Goat kids; Histochemistry; Lambs; Morphometry; Ontogeny.

Introduction

Small ruminants, particularly sheep and goats, play an essential role in food security and the socio-economic stability of rural communities worldwide, especially in developing countries. They provide meat, milk, wool, and hides, and their adaptability to diverse environments makes them a cornerstone of sustainable livestock production (Saba et al.,2024; Singh et al.,, 2025). Iraq, like many countries in the Middle East, relies significantly on indigenous breeds of sheep and goats, where growth and health are closely linked to gastrointestinal development during early life.

The abomasum, often referred to as the "true stomach" of ruminants, is the primary site of enzymatic digestion and protein hydrolysis. It plays a central role in preparing microbial and dietary proteins for absorption in the small intestine through the secretion of gastric juices (Gaowa et al., 2021; Conrad, 2022; Gowtham et al., 2022). Structurally, the abomasum is composed of four tunics: mucosa, submucosa, tunica muscularis, and serosa. Its mucosal surface is characterized by gastric pits and tubular glands, lined by chief cells, parietal cells, and mucus-secreting neck cells (Betancourt et al., 2022; Ibrahim and Almundarij, 2023). Histochemical features, including collagen distribution and glycoprotein content, are critical for structural integrity, mucosal defense, and functional maturation (Nikoloudaki, 2021; Ibáñez-Cortés et al., 2023).

Several studies have described the histology of the abomasum in ruminants such as sheep, goats, and yaks, focusing on adult or diseased animals (Lu et al., 2024; Magdy et al., 2025). Others have examined the impact of parasitic infections such as Haemonchus contortus on abomasal histopathology (Flay et al., 2022; Lins et al., 2024).

However, comparative investigations on the ontogenetic development of the abomasum in local lambs and goat kids during the early postnatal period remain scarce, particularly studies integrating histological, histochemical, and morphometric parameters. This gap is significant because the first months of life represent a critical window in which structural and functional maturation of the abomasum directly influences growth performance, immune readiness, and nutrient utilization in small ruminants. The present study was designed to compare the histological, histochemical, and morphometric characteristics of the abomasum in local lambs and goat kids aged 1–4 months. The goal was to elucidate species-specific developmental patterns and provide baseline data that could support veterinary diagnostics, nutritional strategies, and future research on gastrointestinal physiology in small ruminants.

Materials and Methods

Animals and Sample Collection

The present study was conducted on abomasal samples collected from local lambs and goat kids aged one to four months. Animals were obtained from the modern slaughterhouse in Tikrit city, Salahaddin province, Iraq. Specimens were excised immediately after slaughter to ensure preservation of tissue integrity. Samples were consistently taken from the middle region of the abomasum to reduce variability between anatomical sites.

A total of n = 8 animals per species per age group (sheep and goats, 1–4 months; total N = 48) were included. Animals were randomly selected irrespective of sex but with balanced representation of males and females (1:1 ratio). The chosen number was based on power analysis for a two-way ANOVA ($\alpha = 0.05$, power 0.80), ensuring robust detection of inter-species and inter-age differences.

In histological and morphometric studies on ruminant gastrointestinal tissues, an acceptable sample size generally ranges between 5 and 10 specimens per age group or species. However, for robust statistical comparisons and to ensure adequate power in ANOVA and post hoc tests, a minimum of 6 samples per group is recommended, while 8–12 samples per group is often considered optimal in high-impact publications.

Tissue Fixation and Histological Preparation

Fresh specimens were immediately fixed in 10% neutral buffered formalin for 24 hours. The tissues were subsequently processed according to the standard paraffin-embedding protocol:

- **Dehydration:** serial immersion in graded ethanol solutions (50%, 70%, 80%, 90%, 100%).
- **Clearing:** immersion in xylol (two changes, 30 minutes each).
- **Embedding:** infiltration and embedding in paraffin wax with a melting point of 56–58 °C using metal molds.
- **Sectioning:** paraffin blocks were cut into 5–6 µm sections using a rotary microtome, and sections were mounted on glass slides coated with Mayer's albumin.
- **Routine Staining:** slides were stained with Hematoxylin and Eosin (H&E) for general histological examination.

Histochemical Staining

To evaluate connective tissue composition and specific mucosal elements, selected sections were subjected to histochemical staining:

Masson's Trichrome (MTC): for detection and differentiation of collagen fibers within the lamina propria, submucosa, and tunica muscularis.

In addition to H&E and Masson's Trichrome, selected sections were subjected to Periodic Acid–Schiff (PAS) and Alcian Blue (pH 2.5) staining to differentiate neutral and acidic mucopolysaccharides, thereby providing a more comprehensive assessment of mucosal glycoproteins.

Morphometric Analysis

Quantitative histomorphometric analysis was performed using a light microscope (Olympus BX51, Japan) equipped with a digital camera and an image analysis software package. Measurements were obtained from randomly selected, well-oriented fields (five fields per section) to calculate mean values for:

- Thickness of the mucosa (µm).
- Thickness of the submucosa (µm).
- Thickness of the muscularis mucosa (µm).
- Thickness of the tunica muscularis (µm).

Ethical approval

The study protocol was reviewed and approved by the Institutional Animal Ethics Committee, College of Veterinary Medicine, University of Tikrit (Approval No. VET/2024/112). Since the tissues were collected post-mortem from animals slaughtered at the local abattoir (Tikrit, Salahaddin, Iraq), no live animal experimentation was conducted.

Statistical Analysis

Data were expressed as mean ± standard error (SE). Statistical analysis was performed using SPSS software, version 25.0 (IBM Corp., Armonk, NY, USA). Differences between groups (species and age) were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Differences were considered statistically significant at $p \leq 0.05$. In addition to one-way ANOVA, a two-way ANOVA model was applied to test for interaction effects between age and species on abomasal layer thickness. Pearson's correlation coefficients were also calculated to examine the relationships between lamina propria thickness, collagen deposition, and epithelial height. Normality of data distribution was confirmed by Shapiro–Wilk test, and homogeneity of variances was assessed using Levene's test.

Results

Histological Observations

The abomasal wall of both lambs and goat kids at different ages (1–4 months) was composed of the four characteristic tunics: mucosa, submucosa, tunica muscularis, and tunica serosa (Figures 1–2).

At one month, the mucosa was lined with simple cuboidal epithelium forming gastric pits that extended into tubular gastric glands. These glands were relatively small, lined mainly by chief cells and fewer parietal cells, while the lamina propria showed wide connective tissue with lymphocytic infiltration and some macrophages (Figure 1A–B;).

By two months, the lamina propria displayed more organized groups of tubular glands, with parietal cells concentrated at the basal regions. The muscularis mucosa appeared as delicate strands of smooth muscle separating the mucosa from the submucosa, which contained loose connective tissue and blood vessels (Figure 1C–D;).

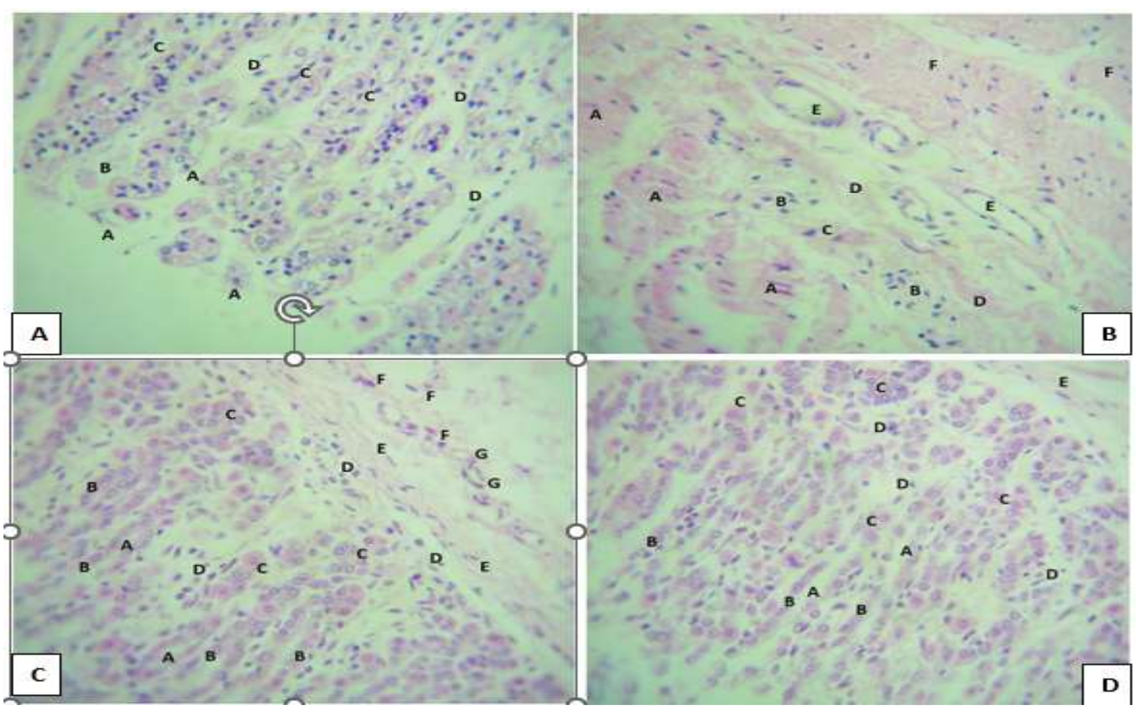

Figure 1: Histological sections of the abomasum in lambs at different ages (Hematoxylin & Eosin staining, ×40, Scale bar = 50 µm).
(A) One month: mucosa lined with simple cuboidal epithelium and scattered tubular gastric glands; lamina propria shows wide connective tissue with lymphocytic infiltration.
(B) Two months: tubular glands more organized, parietal cells localized at the basal regions; muscularis mucosa appears as delicate smooth muscle strands.
(C) Three months: taller and narrower gastric glands with increased parietal cells; lamina propria with dense lymphocytic infiltration.
(D) Four months: compact gastric glands with thickened muscularis mucosa and dense lamina propria.

At three months, the glands were taller and narrower with well-developed lumens, and the number of parietal cells increased. The lamina propria exhibited dense lymphocytic infiltration, particularly near the muscularis mucosa. The submucosa contained adipose tissue and vascular structures (Figure 2A–B;).

By four months, the abomasal wall demonstrated relative maturation. The mucosa contained compactly arranged glands lined with numerous chief and parietal cells. The lamina propria became denser, the muscularis mucosa markedly thickened, and the tunica serosa contained collagen bundles, nerve fibers, and blood vessels (Figure 2C–D;).

Species-specific differences were evident: goat kids exhibited a generally thicker tunica muscularis at all ages, while lambs showed earlier compaction of the lamina propria and more organized glandular units at three months.

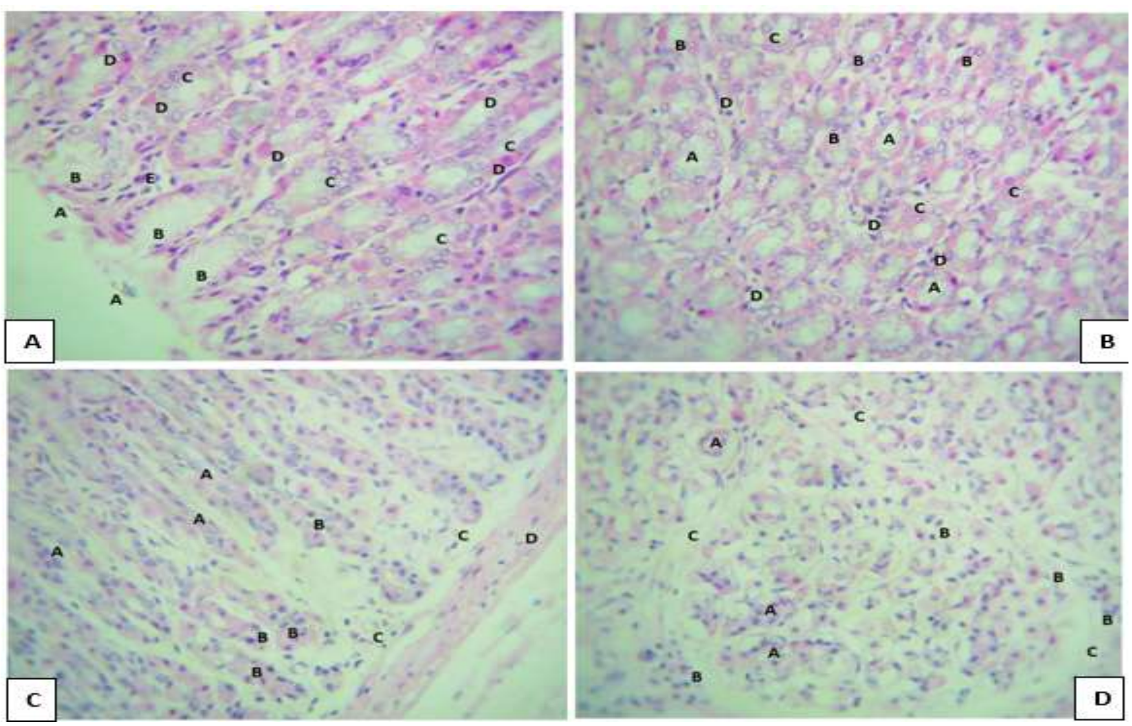

Figure 2: Histological sections of the abomasum in goat kids at different ages (Hematoxylin & Eosin staining, ×40, Scale bar = 50 µm).
(A) One month: mucosa lined by cuboidal epithelium with scattered gastric glands and wide

onnective tissue in lamina propria.
(B) Two months: tubular glands with parietal cells concentrated in the basal regions; submucosa with loose connective tissue and blood vessels.
(C) Three months: taller and narrower glands with well-developed lumina; lamina propria exhibits lymphocytic infiltration near the muscularis mucosa.
(D) Four months: compact gastric glands, thickened tunica muscularis, and dense connective tissue with vascular structures.

Histochemical Findings

Masson's Trichrome staining (MTC) confirmed collagen distribution in both species (Figure 3). At **1–2 months**, collagen fibers were delicate and sparse in the lamina propria and submucosa. At **3–4 months**, collagen bundles became denser and more organized, particularly in the tunica muscularis and around blood vessels ().

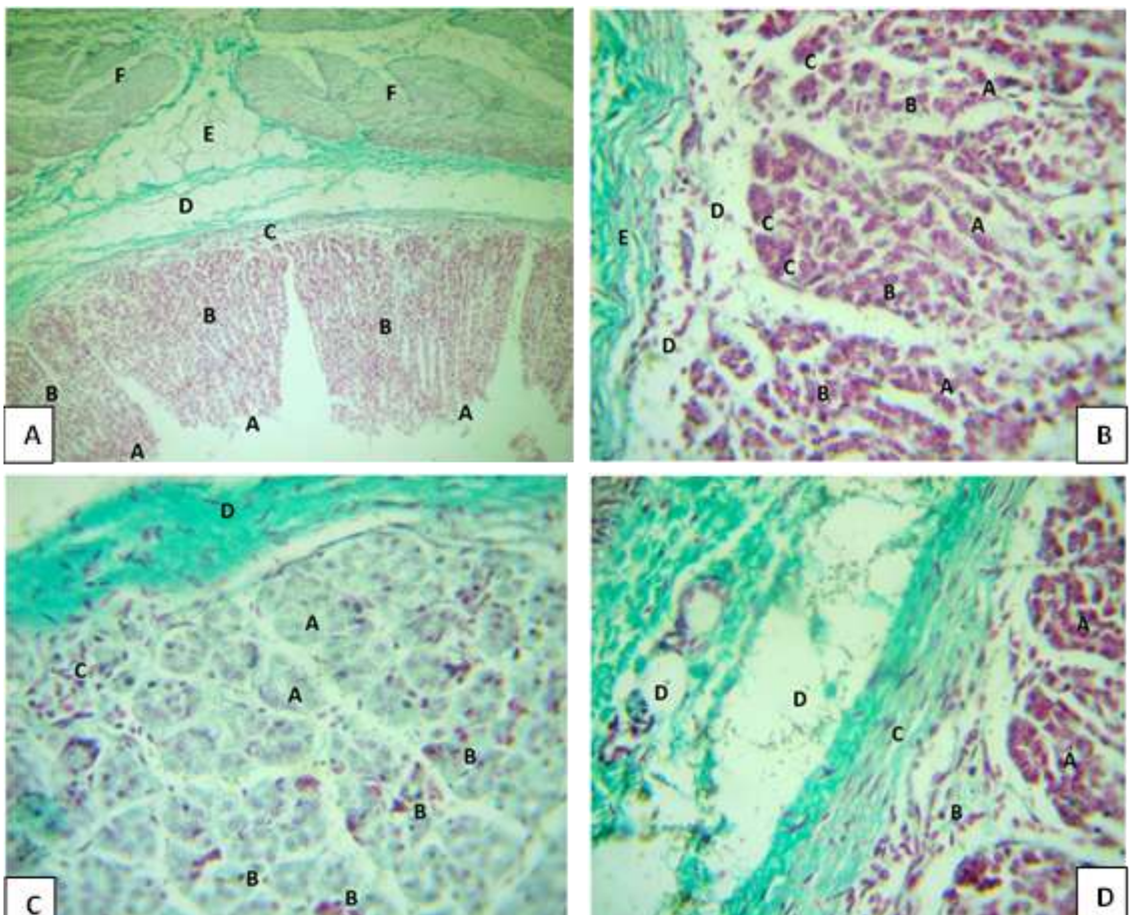

Figure 3: Histochemical sections of the abomasum in lambs and goat kids (Masson's Trichrome staining, ×40, Scale bar = 50 µm).
(A) Lamb, one month: delicate collagen fibers in lamina propria.
(B) Lamb, four months: dense collagen bundles in tunica muscularis.
(C) Goat kid, one month: sparse collagen fibers in submucosa.
(D) Goat kid, four months: well-developed collagen surrounding blood vessels and muscular layers.

Lambs demonstrated earlier collagen deposition at three months, correlating with lamina propria compaction (Figures 1–2). In contrast, goat kids exhibited delayed thickening, with prominent collagen bundles at four months. In both species, lymphocytes and macrophages were consistently present in the lamina propria and submucosa, indicating the progressive development of mucosal immune defenses.

Morphometric Analysis
Histomorphometric data (Table 1) revealed significant age- and species-related differences ($p \leq 0.05$) in most abomasal layers (Figure 4A–E).

- **Tunica muscularis:** increased with age in both species ($p = 0.022$); goat kids consistently had greater values (Figure 4A).
- **Submucosa:** highly significant increase ($p = 0.0004$), peaking at goat M3 (399.8 ± 38.8 µm) vs. sheep M3 (310.0 ± 35.9 µm) (Figure 4B).
- **Muscularis mucosa:** progressive increase in both species ($p = 0.0005$); maximum at goat M3 (45.75 ± 6.58 µm) (Figure 4C).
- **Lamina propria:** significant variation ($p = 0.003$); earlier thickening in lambs at M3 (450.0 ± 48.7 µm) vs. goats (422.5 ± 30.3 µm) (Figure 4D).
- **Gastric epithelium:** highest at M3 (sheep: 100.0 ± 14.0 µm; goat: 99.8 ± 12.8 µm, $p = 0.002$) (Figure 4E).

Morphometric Analysis
Quantitative histomorphometric measurements revealed statistically significant differences between ages and species in most abomasal layers (Table 1).

Table 1. Morphometric parameters (mean ± SE, µm) of the abomasum in lambs and goat kids (1–3 months).

Groups	Tunica muscularis	Submucosa	Muscularis mucosa	Lamina propria	Gastric epithelium
Sheep M1	323.8 ± 64.2^{c}	182.8 ± 18.7^{d}	27.40 ± 7.68^{bc}	332.5 ± 53.4^{cd}	63.75 ± 13.09^{bc}
Sheep M2	360.0 ± 51.7^{c}	253.8 ± 26.9^{c}	29.00 ± 4.89^{b}	355.8 ± 49.7^{c}	75.50 ± 12.59^{b}
Sheep M3	488.8 ± 82.9^{a}	310.0 ± 35.9^{b}	41.3 ± 9.72^{a}	450.0 ± 48.7^{a}	100.00 ± 14.00^{a}
Goat M1	410.0 ± 58.6^{b}	195.0 ± 28.8^{d}	24.00 ± 5.16^{c}	315.8 ± 31.3^{d}	56.0 ± 10.45^{c}
Goat M2	417.5 ± 43.1^{b}	269.3 ± 32.1^{bc}	29.25 ± 6.98^{b}	418.8 ± 37.4^{b}	90.8 ± 12.30^{a}
Goat M3	526.3 ± 35.7^{a}	399.8 ± 38.8^{a}	45.75 ± 6.58^{a}	422.5 ± 30.3^{ab}	99.8 ± 12.80^{a}

P-values: Tunica muscularis (0.022*), Submucosa (0.0004), Muscularis mucosa (0.0005**), Lamina propria (0.003**), Gastric epithelium (0.002**). Values with different superscript letters within the same column indicate statistically significant differences at $p \leq 0.05$**

Figure 4: Morphometric analysis of abomasal layers in lambs and goat kids at different ages (mean ± SE).

(A) Tunica muscularis thickness.

(B) Submucosa thickness.

(C) Muscularis mucosa thickness.

(D) Lamina propria thickness.

(E) Gastric epithelium thickness. Significant differences between groups are indicated by different superscript letters ($p \leq 0.05$, ANOVA with Tukey's post hoc test).

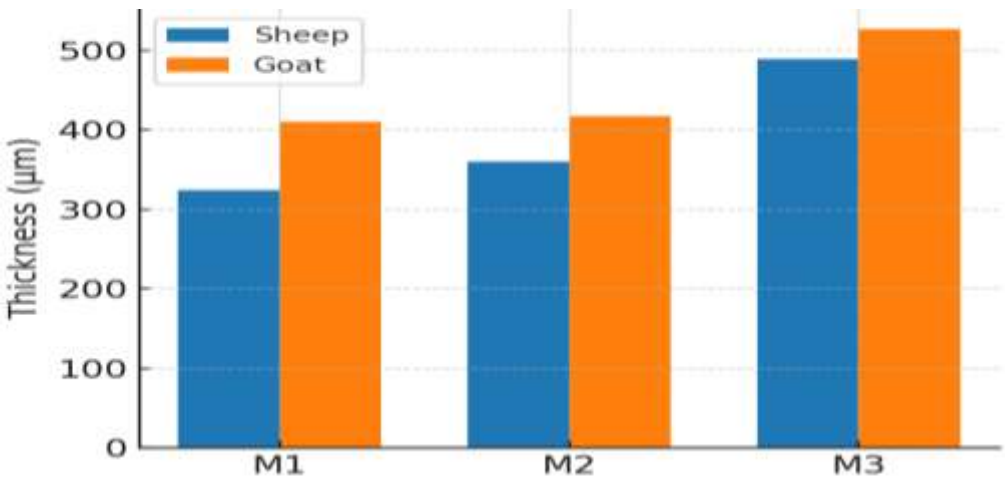

Figure 4A. Tunica muscularis thickness in lambs vs. goat kids (1–3 months).

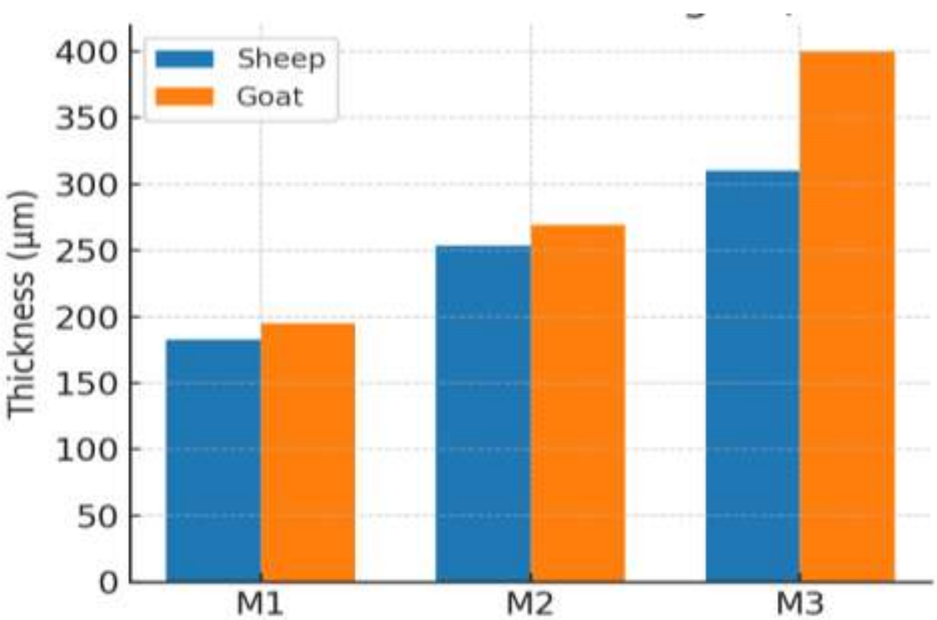

Figure 4B. Submucosa thickness in lambs vs. goat kids (1–3 months).

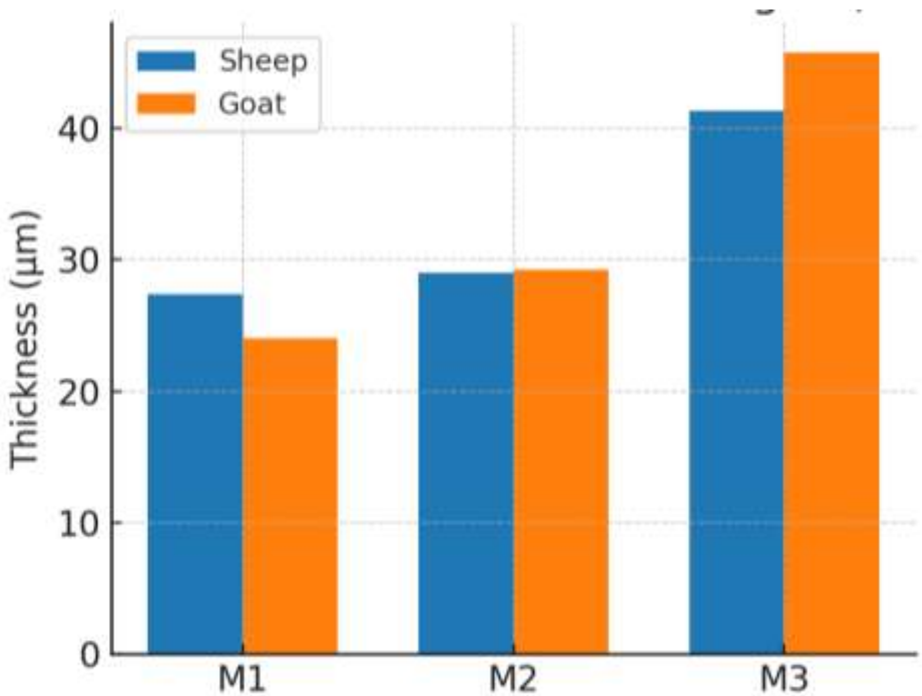

Figure 4C. Muscularis mucosa thickness in lambs vs. goat kids (1–3 months).

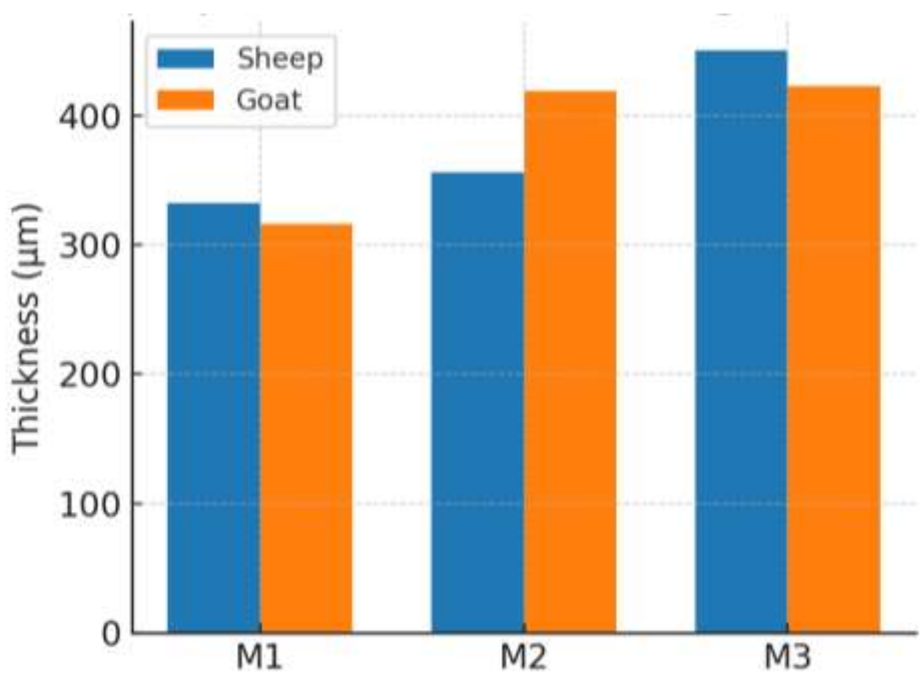

Figure 4D. Lamina propria thickness in lambs vs. goat kids (1–3 months).

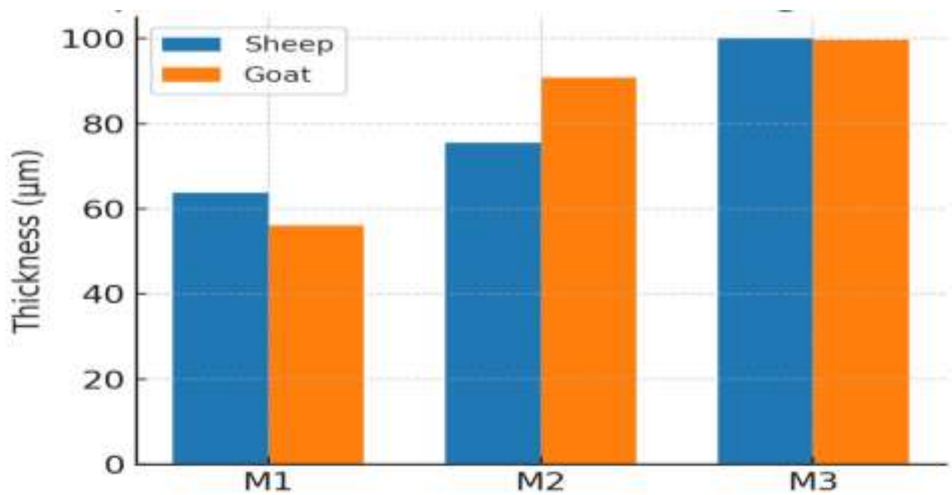

Figure 4E. Gastric epithelium thickness in lambs vs. goat kids (1–3 months).

Discussion

The present study provides a comparative analysis of abomasal development in local lambs and goat kids during the first four months of life, combining histological, histochemical, and morphometric approaches. The findings demonstrate a progressive maturation of abomasal structure in both species, with species-specific differences in glandular organization, collagen deposition, and tunica muscularis development. These observations are consistent with the general concept that early postnatal life represents a critical window for gastrointestinal development, directly influencing nutrient absorption, immunity, and growth performance (Jaswal et al., 2022; Geiger et al., 2023).

Histological development and species-specific differences

Our results showed that in both lambs and goat kids, the abomasal mucosa underwent substantial changes between one and four months of age. At early stages, the mucosa was lined by simple cuboidal epithelium with scattered glands, while later stages revealed taller, compact glands rich in parietal and chief cells. This aligns with observations in yak (Bos grunniens), where abomasal glands elongated and became denser with age, reflecting adaptation to dietary transition and high-altitude physiology (Jing et al., 2022; Ahmad et al., 2024). Interestingly, goat kids exhibited a thicker tunica muscularis than lambs at all ages, whereas lambs showed earlier compaction of the lamina propria and greater glandular organization by three months. Such interspecies differences may be explained by variations in feeding behavior and growth rates: goats typically adopt browsing diets with higher fiber and secondary plant compounds, requiring stronger muscular layers for gastric motility, whereas lambs, as grazers, rely more on rapid mucosal adaptation to fermentative substrates (Van Saun, 2022). At the molecular level, the observed thickening of muscularis mucosa and tunica muscularis likely involves upregulation of smooth muscle contractile proteins (e.g., α-actin, myosin heavy chain) under the control of growth factors such as TGF-β and IGF-1, which have been implicated in gastrointestinal muscle differentiation (Sanders and Perrino, 2022; Nolte and Markovits, 2024).

Histochemical features and extracellular matrix remodeling

Masson's Trichrome staining revealed progressive deposition of collagen fibers in the lamina propria, submucosa, and tunica muscularis with increasing age. Collagen density was greater in lambs at three months, whereas goats demonstrated more pronounced deposition at four months. This reflects differences in extracellular matrix (ECM) remodeling, which plays a pivotal role in structural maturation and mechanical stability of the abomasal wall. Collagen synthesis in the gastrointestinal tract is regulated by fibroblasts and smooth muscle cells under the influence of TGF-β1, connective tissue growth factor (CTGF), and matrix metalloproteinases (MMPs) (Pompili et al., 2021; Matsuoka and Yashiro, 2023).

Enhanced collagen deposition in lambs at earlier stages may correspond to accelerated activation of fibroblasts and earlier maturation of the mucosal barrier. Conversely, the delayed collagen remodeling in goats may reflect a prolonged phase of immune–structural interaction, as suggested by persistent leukocyte infiltration in the lamina propria.Similar findings were reported in goats experimentally infected with Haemonchus contortus, where collagen remodeling was pronounced around vascular structures in the abomasum (Liu et al., 2022). Our results extend this by showing that even in healthy animals, collagen distribution follows species-specific developmental trajectories.

Immune cell infiltration and mucosal defense

The presence of lymphocytes and macrophages in the lamina propria and submucosa at all ages indicates early establishment of mucosal immune defense. Previous studies have shown that gut-associated lymphoid tissue (GALT) in ruminants develops rapidly postnatally, providing protection against pathogens encountered during dietary transitions (Eurell & Frappier, 2013; König & Liebich, 2014; Torow et al., 2023). The increased infiltration at three months coincides with the transition to more solid diets, which is known to stimulate epithelial Toll-like receptor (TLR) signaling and cytokine production (IL-6, TNF-α), thereby recruiting lymphocytes and macrophages (Duan et al., 2022). These molecular signals may explain the histological observations of immune aggregates near the muscularis mucosa in both species.

Morphometric changes and functional significance

The morphometric data confirmed significant increases in the thickness of all abomasal layers with age, indicating structural reinforcement to support growing digestive demands. Goat kids showed greater tunica muscularis thickness, while lambs exhibited earlier lamina propria and epithelial maturation. This suggests species-specific developmental strategies: goats prioritize muscular strength for motility, while lambs emphasize glandular efficiency for protein digestion. Such differences are not merely anatomical but likely reflect divergent regulation of growth-related pathways. For instance, epidermal growth factor (EGF) and insulin-like growth factor 1 (IGF-1) are key drivers of epithelial proliferation and differentiation (Pham, 2022; Leśniak . and Filipek, 2023; Luján-Méndez et al., 2023). Earlier epithelial thickening in lambs may be attributed to higher responsiveness of abomasal mucosa to IGF-1 signaling, which enhances chief and parietal cell differentiation.

The earlier collagen deposition in lambs may be linked to accelerated activation of fibroblasts through TGF-β1 and matrix metalloproteinases (MMP-2, MMP-9), while the delayed muscular thickening in goats may reflect prolonged upregulation of smooth muscle contractile proteins such as α-actin and myosin heavy chain, mediated by IGF-1 signaling. These interpretations align with recent insights into ECM remodeling and gastrointestinal muscle differentiation in ruminants.

Although the primary focus was on H&E, Masson's Trichrome, PAS, and Alcian Blue staining, future studies should incorporate immunohistochemical markers such as α-smooth muscle actin (α-SMA), mucins, and Ki-67 to provide molecular confirmation of smooth muscle differentiation, mucosal barrier development, and cellular proliferation.

Integrative perspective

Taken together, these findings highlight that abomasal development in lambs and goat kids follows a common trajectory of progressive maturation but diverges in timing and magnitude of histological, histochemical, and morphometric changes. These differences likely reflect adaptive strategies shaped by species-specific feeding behaviors, growth demands, and molecular regulation of ECM remodeling and epithelial proliferation.

The study thus fills an important gap in veterinary anatomy and physiology by providing baseline ontogenetic data for local breeds, which can be applied in optimizing nutrition, diagnosing developmental abnormalities, and guiding comparative studies on ruminant gastrointestinal physiology.

Limitations. While the present study provides novel insights into ontogenetic abomasal development in local lambs and goat kids, certain limitations must be acknowledged. First, the sample size, although statistically acceptable, was restricted to 8 animals per group and may not capture the full variability of local populations. Second, the study relied primarily on H&E and Masson's Trichrome staining; the inclusion of PAS, Alcian Blue, or immunohistochemical markers (e.g., mucins, α-actin, IGF-1) would have provided deeper molecular insights. Third, the age range was limited to 1–4 months, which precludes conclusions on later developmental stages. Finally, all animals originated from a single abattoir in Iraq, which may restrict the generalizability of the findings to broader small ruminant populations. Future work should therefore integrate larger multi-breed cohorts, advanced staining techniques, and post-weaning stages to strengthen the translational value of the results. Another limitation is that the present work did not employ immunohistochemistry or molecular assays to validate the cellular mechanisms inferred from histological and histochemical findings. Integrating gene expression analysis (e.g., collagen isoforms, MMPs, growth factors) would provide stronger mechanistic support.

Conclusion

Abomasal development in local lambs and goat kids between 1–4 months showed progressive maturation of mucosal, submucosal, and muscular layers. Lambs exhibited earlier glandular organization and collagen deposition, while goats consistently developed a thicker tunica muscularis. These species-specific patterns suggest different adaptive strategies to dietary and physiological demands. The study provides essential baseline data for understanding gastrointestinal development in local small ruminants.

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conflicted interest: None**.**

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