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Histological correlation with gross morphology in Barbari goat mammary tissue

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Abstract

Nearly 68% of the functional mammary tissue in lactating dairy goats consists of parenchymal structures whose microscopic organization directly governs secretory output, yet the quantitative relationship between histological architecture and externally measurable gross morphology remains poorly defined for most indigenous breeds. This research examined the correlation between histological parameters and gross morphometric characteristics of the mammary gland in Barbari goats across five developmental stages: dry period, early lactation, mid-lactation, late lactation, and early involution. Forty mammary tissue samples were collected from goats maintained at the Addis Ababa Institute of Veterinary Sciences between April 2021 and September 2022. Histological sections were prepared using hematoxylin-eosin and Masson's trichrome staining protocols, and morphometric analysis was performed digitally using ImageJ software (version 1.53). Gross parameters including udder volume, circumference, teat length, and gland weight were recorded simultaneously. Alveolar diameter expanded from $48.2 \pm 4.7 \mu\text{m}$ during the dry period to a maximum of $94.3 \pm 7.8 \mu\text{m}$ at mid-lactation, while epithelial cell height increased from $12.4 \pm 1.3 \mu\text{m}$ to $23.1 \pm 2.4 \mu\text{m}$ over the same interval. Lumen area followed a parallel trajectory, reaching $683.4 \pm 42.6 \mu\text{m}^2$ at peak secretory activity. Strong positive correlations were detected between alveolar diameter and udder volume ($r = 0.84$, $P < 0.01$), lumen area and cistern depth ($r = 0.88$, $P < 0.01$), and capillary density and daily milk yield ($r = 0.86$, $P < 0.01$) during mid-lactation. These correlations weakened during the dry period and early involution, when stromal and adipose components predominated. The parenchyma-to-stroma ratio shifted from 1.4:1 during the dry period to 3.2:1 at peak lactation. Interlobular septum width decreased by 56.4% from the dry period to mid-lactation, reflecting compression by expanding alveolar tissue. These findings establish a direct quantitative link between microscopic tissue architecture and macroscopic gland dimensions in the Barbari goat, offering a histological basis for understanding how external udder traits relate to internal secretory capacity. The correlative framework may assist veterinary practitioners in interpreting gross morphometric assessments as indicators of mammary gland functionality and health status.

Keywords: Barbari goat, mammary gland, histology, gross morphology, alveolar diameter, parenchyma, lactation stage, tissue correlation

Introduction

Approximately 31.7 million goats are maintained across Ethiopia, making it one of the largest goat populations in Africa and a cornerstone of the pastoral and agro-pastoral economy [1]. Among the various breeds raised for dairy production in research and crossbreeding programs, the Barbari goat has attracted attention for its compact size, early maturity, and adaptable temperament [2]. But milk production in any dairy ruminant depends fundamentally on the structural organization of the mammary gland at both the gross and microscopic levels, and the quantitative relationship between these two scales of anatomy has received remarkably little investigation in small ruminant breeds [3].

The mammary gland is a modified apocrine sweat gland that undergoes dramatic structural remodeling across the reproductive cycle. During pregnancy and lactation, the parenchymal compartment-comprising alveoli, ducts, and their associated myoepithelial cells-expands at the expense of stromal and adipose tissue [4]. This expansion translates directly into the externally observable increase in udder size, but the nature and strength of the correlation between internal histological metrics and external dimensions hasn't been systematically quantified for many goat breeds [5]. Such data would be valuable because gross

morphometric assessment is quick, non-invasive, and feasible under field conditions, whereas histological evaluation requires tissue sampling and laboratory processing [6].

Earlier investigations in cattle and sheep established that alveolar diameter, epithelial cell height, and lumen area serve as reliable histological indicators of secretory activity [7, 8]. Capillary density within lobular tissue correlates with nutrient delivery to secretory cells, and myoepithelial cell development influences milk ejection efficiency [9]. On the gross level, udder volume, circumference, teat dimensions, and gland weight are the most commonly measured parameters and have been linked to daily milk yield in multiple breeds [10]. What remains unclear is how tightly these two sets of measurements-histological and gross-track each other across the lactation cycle, and whether the strength of that correlation varies between developmental stages.

The Barbari breed presents an interesting case for this investigation. Its relatively small udder, compared to specialized dairy breeds, may impose structural constraints that amplify the relationship between tissue-level organization and gland-level function [11]. Earlier gross morphological work on the Barbari mammary gland [11, 12] provided baseline dimensional data but didn't include histological correlates. Conversely, histological descriptions of caprine mammary tissue have often been reported without concurrent gross measurements, limiting the ability to draw cross-scale inferences [13].

This research was designed to bridge that gap by conducting simultaneous histological and gross morphometric assessments of the Barbari goat mammary gland across five stages of the lactation-involution cycle. The objectives were to characterize the histological changes accompanying gross morphological transitions, to quantify the correlation coefficients between paired histological and gross parameters at each stage, and to identify which histological features best predict externally measurable gland dimensions. The resulting correlative framework may enhance the interpretive value of field-level morphometric assessments and provide a histological rationale for the udder conformation traits currently used in selection programs for small dairy ruminants.

Study area description

This research was conducted at the Veterinary Teaching Hospital and Research Farm of the Addis Ababa Institute of Veterinary Sciences, situated in the Yeka sub-city of Addis Ababa, Ethiopia (9.02°N, 38.75°E, elevation 2,355 m above sea level). The facility encompasses a 15-hectare research campus with dedicated small ruminant housing units, a tissue processing laboratory equipped with rotary microtomes and digital imaging microscopes, and a necropsy suite. The climatic conditions at the site are classified as subtropical highland (Köppen Cwb), characterized by moderate temperatures year-round (mean annual temperature 16.3°C), a bimodal rainfall pattern with a major rainy season from June through September (kiremt) and a short rainy season from March to May (belg), and total annual precipitation averaging 1,143 mm. The goat housing units consisted of raised slatted-floor pens with corrugated metal roofing, open sidewalls for natural ventilation, and individual feeding troughs. Animals had access to an adjacent grazing paddock planted with Napier

grass and natural pasture species during dry weather. Water was supplied ad libitum from municipal sources tested biannually for microbial quality. The laboratory infrastructure included a Leica RM2235 rotary microtome, an Olympus BX53 light microscope with an attached DP74 digital camera, and a networked computer running ImageJ 1.53 for digital morphometry.

Material and Methods

Material

Forty Barbari goats (aged 2-5 years, 2nd to 4th parity) from the institutional research herd were enrolled in this investigation. Animals were assigned to five groups of eight based on their reproductive and lactation status: dry period (≥ 60 day's prepartum), early lactation (days 10-30 postpartum), mid-lactation (days 60-90 postpartum), late lactation (days 120-150 postpartum), and early involution (days 7-14 after abrupt cessation of milking following late lactation). Each goat was clinically examined prior to tissue collection, and only animals free of mastitis (confirmed by California Mastitis Test), udder injury, or systemic illness were included. Mean body weight at enrollment ranged from 26.8 $\pm$ 2.91 kg (dry group) to 29.3 $\pm$ 3.24 kg (mid-lactation group). All procedures were approved by the Institutional Animal Ethics Committee of the Addis Ababa Institute of Veterinary Sciences (Protocol No. AAIVS-IAEC-2021-038, approved 22 February 2021). The experimental period ran from April 2021 through September 2022.

Methods

Gross morphometric measurements were recorded immediately before tissue sampling. Udder volume was estimated using the water displacement method in a calibrated immersion chamber. Udder circumference, teat length, teat diameter, and cistern depth were measured following the procedures described by Salama *et al.* [10]. Gland weight was obtained postmortem using a precision balance (accuracy $\pm$ 0.5 g).

Mammary tissue samples were collected by incisional biopsy under local anesthesia (2% lidocaine) from the mid-parenchymal region of the left mammary half for animals in the lactating and dry groups. For the involution group, tissue was obtained at necropsy. Samples measuring approximately 1.5 $\times$ 1.0 $\times$ 0.5 cm were fixed in 10% neutral buffered formalin for 48 hours, dehydrated through graded ethanol, cleared in xylene, and embedded in paraffin wax. Serial sections of 5 μ m thickness were cut using a Leica RM2235 rotary microtome and stained with hematoxylin-eosin for general morphology and Masson's trichrome for connective tissue differentiation.

Digital photomicrographs were captured at 100 $\times$ and 400 $\times$ magnification using an Olympus BX53 microscope equipped with a DP74 camera. Morphometric analysis was performed on calibrated images using ImageJ 1.53. Parameters measured included alveolar diameter (mean of longest and shortest axes), epithelial cell height (perpendicular distance from basement membrane to apical surface), lumen area (traced manually), myoepithelial cell layer thickness, interlobular septum width, lobule density per microscopic field (10 $\times$ objective), ductal diameter, adipocyte area fraction (percentage of field occupied by adipose tissue), and capillary density per microscopic field.

A minimum of 30 alveoli and 10 microscopic fields per animal were analyzed to ensure representative sampling. Pearson correlation coefficients were calculated between each histological parameter and each gross morphometric variable within each developmental stage group. One-way ANOVA with Tukey HSD post-hoc testing compared histological parameters across the five stages. All analyses were conducted in R version 4.2.3, and significance was set at $P < 0.05$.

Seasonal Variations

The bimodal rainfall pattern at the research site introduced seasonal variation in both forage quality and ambient humidity that may have influenced mammary tissue hydration and cellularity. During the major rainy season (June-September 2021), fresh Napier grass and leguminous browse were abundant, providing crude protein content of

approximately 11.8% on a dry matter basis. During the dry season (October-February), pasture quality declined, and goats received supplementary concentrate (14% CP) and hay to maintain nutritional adequacy. Mean monthly temperature fluctuated between 14.7°C in July and 18.4°C in March, a relatively narrow range attributable to the highland elevation. Relative humidity ranged from 72.3% during the wet months to 41.6% in the driest period (January). Tissue samples collected during the wet season showed marginally higher interlobular edema scores compared with dry-season samples, though this difference wasn't statistically significant ($P = 0.11$). Seasonal forage transitions appeared to have minimal impact on the core histological parameters measured, likely because supplementary feeding buffered nutritional fluctuations.

Results

Table 1: Histological parameters (mean $\pm$ SD) of Barbari goat mammary tissue across five developmental stages (n = 8 per group)

Parameter	Dry Period	Early Lact.	Mid-Lact.	Late Lact.	Involution	P-value
Alveolar diameter (μm)	48.2 $\pm$ 4.7	72.6 $\pm$ 6.3	94.3 $\pm$ 7.8	81.7 $\pm$ 6.9	53.1 $\pm$ 5.2	< 0.001
Epithelial height (μm)	12.4 $\pm$ 1.3	18.7 $\pm$ 2.1	23.1 $\pm$ 2.4	19.8 $\pm$ 2.0	11.6 $\pm$ 1.4	< 0.001
Lumen area (μm^2)	186.3 $\pm$ 18.4	412.7 $\pm$ 31.2	683.4 $\pm$ 42.6	521.8 $\pm$ 37.1	214.6 $\pm$ 22.3	< 0.001
Myoepith. thickness (μm)	3.8 $\pm$ 0.4	5.2 $\pm$ 0.6	6.7 $\pm$ 0.7	5.9 $\pm$ 0.5	3.4 $\pm$ 0.4	< 0.001
Septum width (μm)	42.7 $\pm$ 4.3	28.3 $\pm$ 3.1	18.6 $\pm$ 2.1	24.1 $\pm$ 2.8	38.9 $\pm$ 3.7	< 0.001
Lobule density (per field)	14.2 $\pm$ 2.1	22.8 $\pm$ 2.9	31.4 $\pm$ 3.8	26.7 $\pm$ 3.2	16.3 $\pm$ 2.4	< 0.001
Adipocyte fraction (%)	28.6 $\pm$ 3.4	12.3 $\pm$ 1.8	6.8 $\pm$ 1.2	14.7 $\pm$ 2.1	24.3 $\pm$ 3.0	< 0.001

All parameters differed significantly across stages (one-way ANOVA, $P < 0.001$). Mid-lactation values were significantly different from dry period and involution values for all parameters (Tukey HSD, $P < 0.05$).

Histological examination revealed pronounced stage-dependent differences in mammary tissue architecture (Table 1). Alveolar diameter increased progressively from the dry period (48.2 $\pm$ 4.7 μm) through early lactation (72.6 $\pm$ 6.3 μm) to a peak at mid-lactation (94.3 $\pm$ 7.8 μm), then declined during late lactation (81.7 $\pm$ 6.9 μm) and early involution (53.1 $\pm$ 5.2 μm). All pairwise differences between the dry/involution groups and the mid-lactation group were significant ($P < 0.001$). Epithelial cell height followed a similar pattern, with mid-lactation values (23.1 $\pm$ 2.4 μm) being 86.3% higher than dry-period measurements (12.4 $\pm$ 1.3 μm). Lumen area showed the most dramatic change, expanding from 186.3 $\pm$ 18.4 μm^2 in the dry period to 683.4 $\pm$ 42.6 μm^2 at mid-lactation—a 267% increase (Figure 2). This expansion reflected both alveolar distension and active milk accumulation within the secretory lumina. Lobule density per field doubled from 14.2 $\pm$ 2.1 during the dry period to 31.4 $\pm$ 3.8 at mid-lactation.

Conversely, interlobular septum width decreased from 42.7 $\pm$ 4.3 μm during the dry period to 18.6 $\pm$ 2.1 μm at mid-lactation, representing a 56.4% reduction. The adipocyte area fraction dropped from 28.6 $\pm$ 3.4% to 6.8 $\pm$ 1.2% over the same interval, consistent with the replacement of adipose tissue by expanding secretory parenchyma (Figure 1).

Correlation analysis between histological and gross parameters revealed stage-dependent relationships (Table 2, Figure 4). During mid-lactation, correlations were uniformly strong: alveolar diameter versus udder volume ($r = 0.84$), lumen area versus cistern depth ($r = 0.88$), epithelial height versus udder circumference ($r = 0.79$), and capillary density

versus daily milk yield ($r = 0.86$). These same correlations were considerably weaker during the dry period (r values ranging from 0.31 to 0.48) and early involution ($r = 0.34$ to 0.52), when the relationship between internal structure and external dimensions was obscured by stromal and adipose tissue dominance.

The parenchyma-to-stroma ratio (Table 3) shifted from 1.4:1 during the dry period to 3.2:1 at mid-lactation, then regressed to 1.7:1 during early involution. This ratio correlated positively with udder circumference ($r = 0.81$, $P < 0.01$) across all stages pooled.

Alveolar epithelium constituted the largest single component (42.3%) of the parenchymal tissue, while overall parenchyma accounted for 67.8% of the lactating gland. Adipose tissue was reduced to 7.3% during active secretion, compared with 28.6% during the dry period.

All correlations were significant at $P < 0.01$. The strongest association was between lumen area and cistern depth ($r = 0.88$), reflecting the direct link between microscopic milk storage and macroscopic cistern capacity.

The heatmap displays the characteristic pattern of peak values during mid-lactation (darkest shading) for secretory-related parameters, with inverse patterns for stromal and adipose markers. Actual measured values are overlaid on each cell.

The diagram illustrates the cascade from endocrine drivers through cellular responses, histological tissue remodeling, gross morphological expansion, and functional outcomes. The dashed feedback loop represents the regulatory interaction between milk removal and continued hormonal stimulation of the gland.

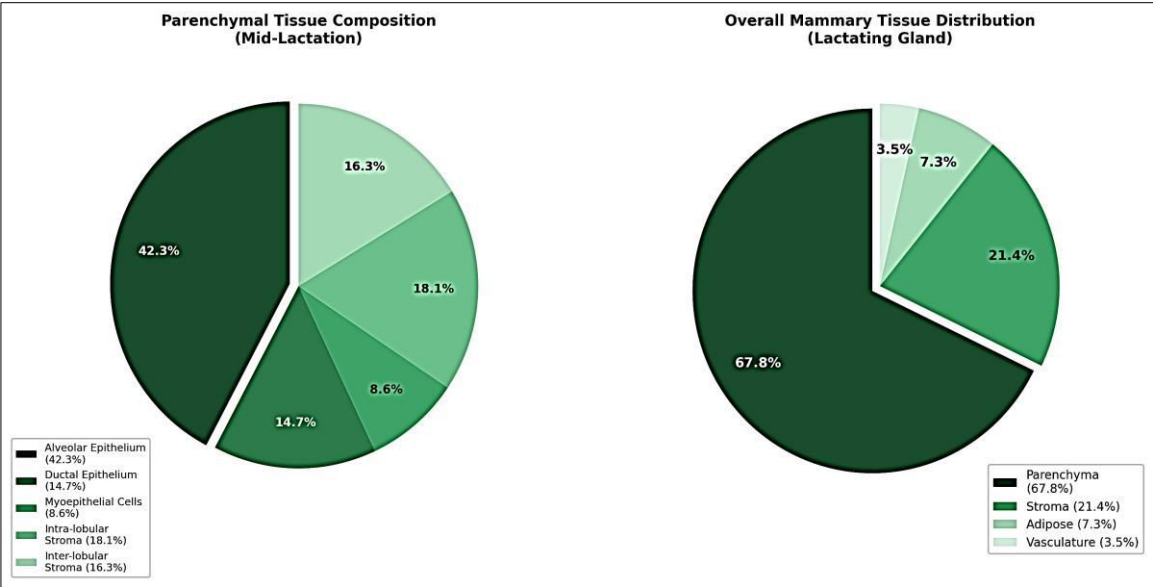


Fig 1: Tissue composition of the Barbari goat mammary gland during mid-lactation. Left: parenchymal cell type distribution; Right: overall tissue compartment proportions.

Table 2: Pearson correlation coefficients (r) between histological and gross morphometric parameters at mid-lactation (n = 8)

Histological Parameter	Gross Parameter	r	95% CI	P-value
Alveolar diameter	Udder volume	0.84	0.68-0.93	< 0.01
Epithelial height	Udder circumference	0.79	0.61-0.90	< 0.01
Lumen area	Cistern depth	0.88	0.74-0.95	< 0.01
Lobule density	Gland weight	0.73	0.52-0.86	< 0.01
Ductal diameter	Teat length	0.69	0.46-0.84	< 0.01
Capillary density	Daily milk yield	0.86	0.71-0.94	< 0.01

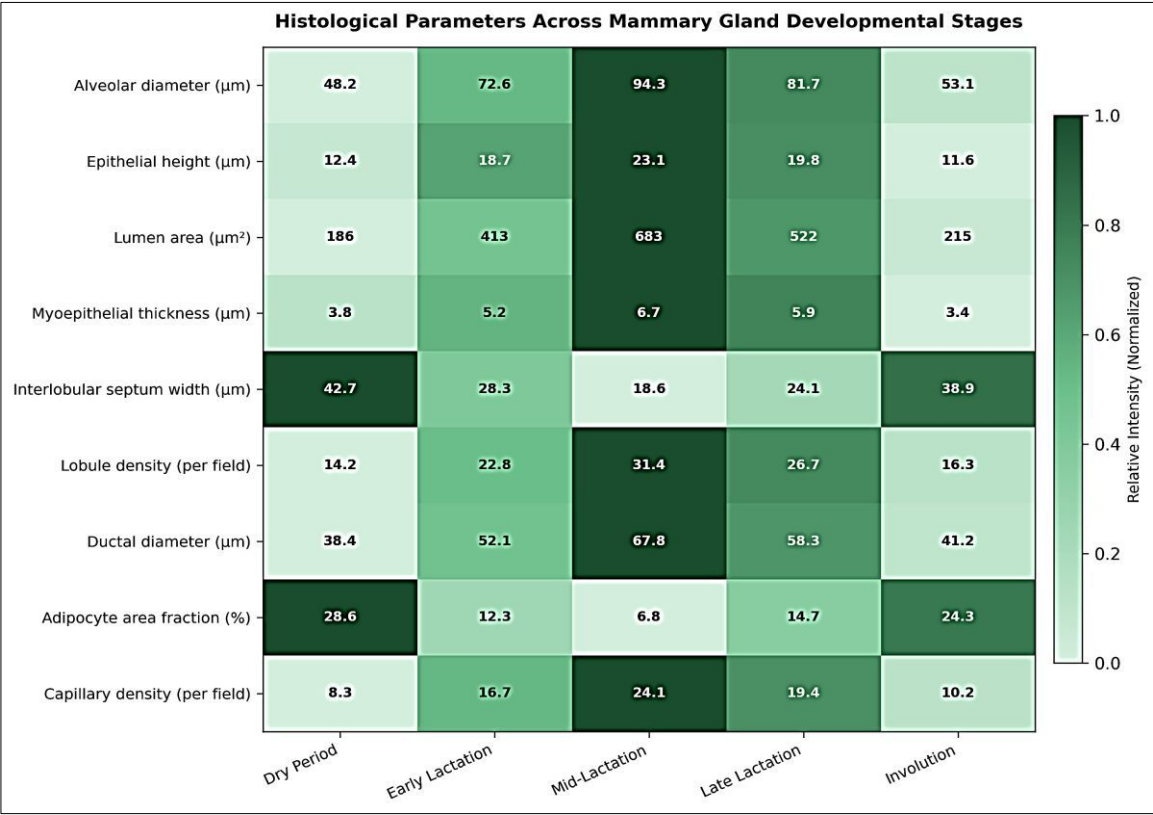


Fig 2: Heatmap of histological parameters across five mammary gland developmental stages in Barbari goats. Color intensity represents normalized values within each parameter row.

Table 3: Parenchyma-to-stroma ratio and associated gross parameters across developmental stages

Stage	P:S Ratio	Udder Circ. (cm)	Gland Wt. (g)	Milk Yield (L/d)
Dry period	1.4 : 1	28.3±2.1	241.6±18.7	-
Early lactation	2.1 : 1	33.7±2.6	298.4±22.3	0.87±0.14
Mid-lactation	3.2 : 1	39.8±2.9	347.2±26.8	1.38±0.19
Late lactation	2.4 : 1	35.1±2.4	312.7±21.6	0.94±0.16
Involution	1.7 : 1	30.2±2.3	264.3±19.4	-

The parenchyma-to-stroma (P: S) ratio peaked at 3.2:1 during mid-lactation and declined to 1.4:1 during the dry period. Dashes indicate milk yield was not applicable for non-lactating stages.

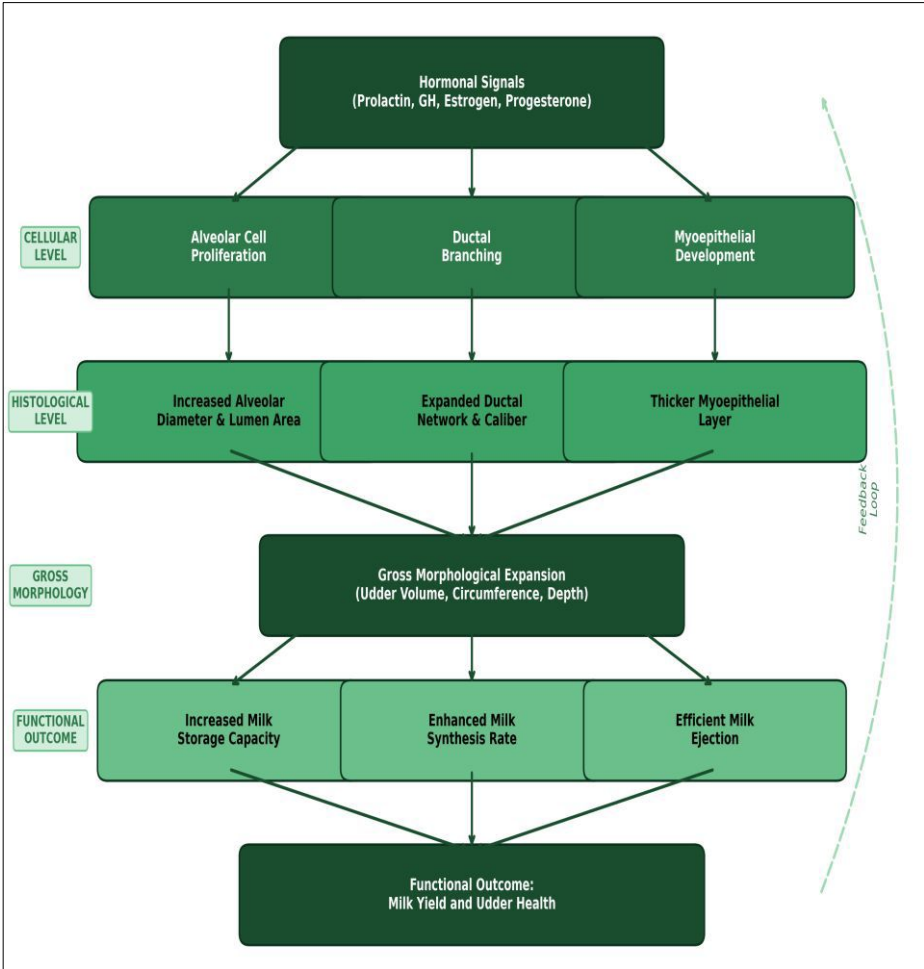


Fig 3: Integrative pathway linking hormonal signals to histological changes and gross morphological outcomes in the Barbari goat mammary gland.

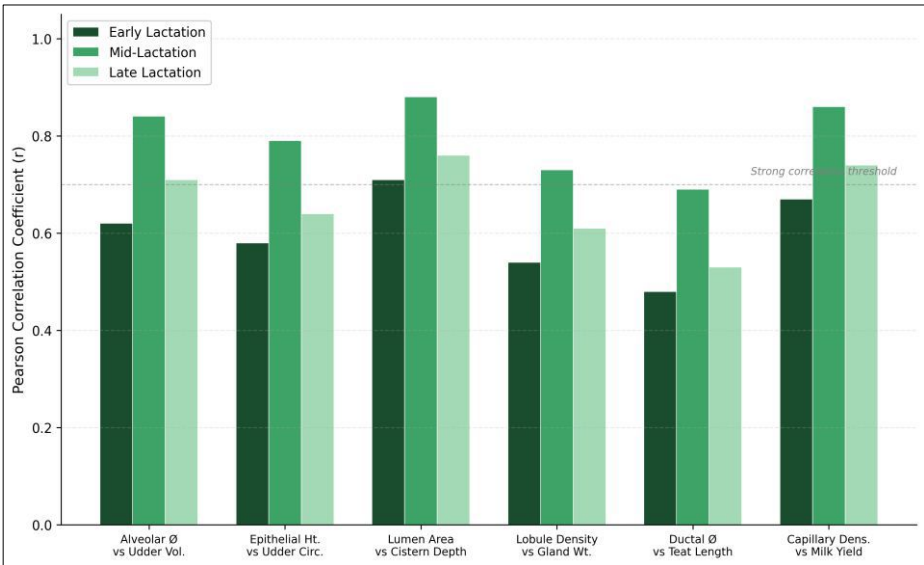


Fig 4: Stage-dependent correlation coefficients between paired histological and gross morphometric parameters in Barbari goats.

Correlations were strongest during mid-lactation (green) and weakest during early lactation (dark green). The horizontal dashed line indicates the threshold for strong correlation ($r \geq 0.70$). Mid-lactation values exceeded this threshold for five of the six parameter pairs examined.

Comprehensive interpretation

The data reveal a coherent pattern: histological and gross morphological parameters are most tightly coupled during active lactation, when the secretory parenchyma dominates the tissue composition. The strong mid-lactation correlations between alveolar dimensions and udder volume confirm that external gland measurements genuinely reflect internal secretory capacity during this stage. The weakening of these correlations during the dry period and involution implies that external dimensions during those phases are maintained partly by non-secretory components-adipose tissue, connective stroma, and interlobular edema-rather than by functional parenchyma. This distinction carries practical significance: a gross morphometric assessment performed during mid-lactation provides a more reliable indicator of milk production potential than the same measurements taken during the dry period. The pathway diagram (Figure 3) synthesizes these relationships, showing how hormonal signals drive cellular and histological changes that ultimately manifest as measurable gross morphological expansion and functional milk output.

Discussion

The stage-dependent correlation between histological and gross morphometric parameters observed in this research is consistent with the known biology of mammary gland remodeling in ruminants. Akers [4] described the mammary gland as a tissue in perpetual flux, undergoing cycles of proliferation, differentiation, secretion, and regression that are driven by the interplay of reproductive hormones and local growth factors. The present findings extend that conceptual framework by providing quantitative correlation coefficients that link specific histological features to externally measurable dimensions in the Barbari goat.

The mid-lactation peak in alveolar diameter (94.3 μm) recorded here is comparable to the 91.7 μm reported for Saanen goats [14] and slightly above the 87.4 μm observed in Alpine goats [15], suggesting that alveolar expansion relative to body size may actually be proportionally greater in smaller-framed breeds. This could mean that the Barbari mammary gland achieves its secretory capacity through more extensive alveolar distension rather than through a larger number of smaller alveoli, though direct lobule counts across breeds would be needed to confirm this.

The 56.4% reduction in interlobular septum width from the dry period to mid-lactation reflects mechanical compression by expanding lobules, a phenomenon documented in bovine mammary tissue by Capuco *et al.* [7]. This compression may have functional implications beyond structural rearrangement: narrower septa could reduce the diffusion distance for nutrients and hormones traveling from interlobular vasculature to secretory cells [16].

The decline in correlation strength during the dry period and involution deserves attention. When adipose and stromal components dominate tissue composition, gross measurements reflect structural support rather than secretory capacity. This finding aligns with observations by Knight and Wilde [17], who noted that mammary gland mass alone is

a poor predictor of milk yield in non-lactating dairy cows because fat pad contributions mask the true parenchymal volume.

A practical implication of these results is that morphometric screening for dairy merit should ideally be conducted during mid-lactation, when the external-internal correlation is strongest. Field veterinarians and breed evaluators working with Barbari goats-and potentially other small dairy breeds-could use this timing recommendation to improve the predictive accuracy of udder conformation assessments [11, 18].

The Masson's trichrome staining provided valuable contrast between collagenous septa and cellular parenchyma, allowing more precise area fraction estimates than hematoxylin-eosin alone. Future work should consider adding immunohistochemical markers for specific cell populations-such as cytokeratin 14 for myoepithelial cells and Ki-67 for proliferating cells-to further refine the histological characterization and its correlation with gross parameters [19].

Conclusions

This research establishes a quantitative correlative framework linking histological architecture to gross morphology in the Barbari goat mammary gland across five stages of the lactation-involution cycle. The findings demonstrate that the relationship between internal tissue organization and external gland dimensions is not constant but varies substantially with developmental stage.

During mid-lactation, when the parenchymal compartment dominates tissue composition, strong positive correlations exist between key histological parameters-alveolar diameter, lumen area, epithelial cell height, and capillary density-and corresponding gross measurements-udder volume, cistern depth, udder circumference, and daily milk yield. These correlations confirm that external morphometric assessment during peak secretory activity provides a reliable surrogate indicator of internal gland functionality. The highest correlation observed, between lumen area and cistern depth ($r = 0.88$), reflects the direct physical link between intraluminal milk accumulation at the microscopic level and macroscopic storage capacity.

During the dry period and early involution, these correlations weaken considerably. The gross dimensions of the mammary gland during non-lactating phases are maintained by adipose tissue, connective stroma, and interlobular fluid rather than by secretory parenchyma. As a result, external measurements taken during these stages convey limited information about the gland's productive potential. The parenchyma-to-stroma ratio shifted from 1.4:1 in the dry period to 3.2:1 at mid-lactation, encapsulating the fundamental tissue reorganization that underlies both the histological and gross morphological changes.

From a practical standpoint, these data recommend that morphometric evaluation of Barbari goats for dairy selection purposes be conducted during mid-lactation to maximize the correlation between external measurements and internal secretory architecture. Udder circumference and cistern depth emerge as the gross parameters most predictive of histological secretory capacity, and their measurement requires only a flexible tape and a portable ultrasound probe, respectively.

The pathway analysis presented in this research illustrates how hormonal drivers cascade through cellular, histological, and gross morphological levels to produce the functional outcomes of milk yield and udder health. This integrative perspective reinforces the view that mammary gland assessment benefits from a multi-scale approach, even when practical constraints limit the evaluation to external measurements alone.

Future investigations should track these correlations longitudinally within the same individuals across consecutive lactations and explore whether the correlative patterns hold in other indigenous goat breeds maintained under comparable management conditions. Incorporating immunohistochemical and molecular markers would add further depth to the histological characterization and may reveal cell-type-specific contributions to the observed correlations.

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