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Comparative Histological Quantification of Pulmonary Area and Connective Tissue of Lung in Cow and Sheep

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Abstract Histological quantification of lung includes measuring and analyzing the proportions of respiratory area, the respiratory bronchioles which lead to the alveolar sacs and alveoli where gas exchange occurs, and connective tissue quantification, the amount of collagen and elastic fibers, which provide support and elasticity for the lung. This can be done by techniques like image analysis software and histological staining methods, such as Hematoxylin and Eosin, Masson's trichrome and Van Gieson to differentiate connective tissue from air spaces. The present analysis aimed to quantify and compare the respiratory area and connective tissue between two adult, healthy of both sexes independent animal groups cow and sheep based on ten specimens per group (n = 10) were collected from local slaughterhouse in summer (2025). The analysis revealed no significant differences between respiratory area of cow and sheep (P > 0.05), that the average respiratory area in sheep (90.18 μm^2) was moderately higher than that in cow (85.21 μm^2), indicating a more efficient structure for gas exchange, which associated with higher metabolic demands and active lifestyle of sheep. In contrast, cow lung significantly demonstrated greater amount of connective tissue, suggesting increased structural support necessary for large body size but reduced respiratory efficiency.
Keywords: lung, quantification, pulmonary area, cows, sheep.

Introduction The lung is a specialized organ for efficient gas exchange, its consist of network of airways, alveoli and supporting connective tissue that work in unison to maintain respiratory function (1). Among mammals, variations in lung structure reflect adaptations to different environmental conditions, metabolic demands and body size (2). Ruminants such as cows (*Bos taurus*) and sheep (*Ovis aries*) are commonly studied livestock species and understanding their pulmonary histology was essential for veterinary sciences and comparative biology (3). Cow and sheep also similar in their ruminant digestive physiology but differ in metabolic rate and possibly lung architecture (4). Histologically, lung composed of pulmonary tissue, responsible for gas exchange occurs and includes the respiratory bronchioles, alveolar ducts alveolar sacs (5). The primary role of the respiratory zone is to facilitate the transfer of oxygen from inhaled air to the blood and to remove carbon dioxide from the blood into the exhaled air (6). Studies of connective tissue in the respiratory area examine its normal mechanical role, which involves collagen and elastic fibers providing stability and allowing for elastic passive recoil during breathing, playing a critical role maintaining airway dimensions and the structural integrity of the respiratory system (7). Pathological involvement in diseases, such as pulmonary fibrosis and pulmonary hypertension, often as a complication of connective tissue diseases like systemic sclerosis or lupus (8). The balance between these two components is crucial in lung elasticity and function, excess connective tissue (fibrosis) or reduction in respiratory area can severely impair respiratory efficiency (9).

Aim of study

The aim of this study to quantitatively compare the histological structure of the lung tissue in cow and sheep

Ethical approval

The project was approved (97/37/2025 in 9/9/2025) by the Committee for Research Ethics at the College of Veterinary Medicine, University of Basrah, Iraq.

Materials and methods

Quantitative histological evaluation was conducted to assess the proportion of respiratory area and connective tissue in lung sections collected from cow and sheep. Lung specimens were fixed in 10% neutral

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buffered formalin, embedded in paraffin, sectioned at 4–5 µm thickness, and stained using standard histological protocols such as Hematoxylin and Eosin, Masson's trichrome and Van Gieson, to differentiate connective tissue from air spaces (10). Image analysis was performed using Image J software (version 9). For each specimen, ten non-overlapping microscopic fields were randomly selected at consistent magnification and anatomical location to ensure uniformity and avoid sampling bias. Fields were captured using a light microscope equipped with a digital camera under identical exposure and resolution settings. In each field, the specific area occupied by either connective tissue or respiratory space was manually delineated and measured. The area percentage was calculated according to the following formula:

$$\text{Tissue Area Percentage} = \left(\frac{\text{Specific Tissue Area}}{\text{Total Field Area}}\right) \times 100$$

The results were expressed as mean ± standard deviation (Mean ± SD) for each group, based on measurements from n = 10 fields per sample. Statistical comparisons between the cow and sheep groups were performed using appropriate parametric tests (e.g., Independent Samples T-test), after confirming the normality of the data using the Shapiro-Wilk test. A p-value ≤ 0.05 was considered statistically significant. This method allowed for precise quantification of structural histological differences in the lungs of cow and sheep, particularly with regard to fibrotic remodeling and alveolar space integrity.

Results

Differences in respiratory area between two independent groups cow and sheep were analyzed using the Independent Samples T-test, after confirming the assumption of normality for both groups using the Shapiro-Wilk test. The test revealed p-values of 0.626 for sheep and 0.153 for cow, both exceeding the threshold of significance (α = 0.05), thus supporting the assumption that the data followed a normal distribution. The Independent Samples T-test indicated that the difference in mean respiratory area between cows and sheep was not statistically significant. The test statistic was t(18) = −1.49, with a corresponding p-value = 0.153. This result implies that there is no statistically significant difference in respiratory area between the two groups at the conventional significance level (p > 0.05). (Figure 1,2).

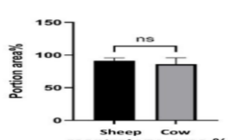


Figure1: Analysis of differences between respiratory area in sheep and cow n=10 mean ±SD

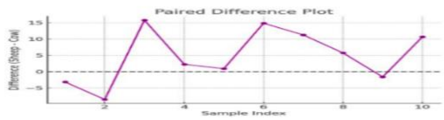


Figure (9) The Paired Difference Plot shows the difference between each pair of samples (sheep– cow). It shows that most specimens tend to favor cow.

Figure (2) cross section of lung show: respiratory bronchioles (RB), respiratory duct (RD) and alveoli (Al). (A) in cow, (B) in sheep. H&E stain, 10x.

Cohen's d (Effect Size): Although the difference was not statistically significant, the effect size was calculated to assess the practical magnitude of the difference. The computed Cohen's d was −0.67, which corresponds to a medium-to-large effect size according to Cohen's classification. The negative value indicates that the mean respiratory area in sheep was moderately higher than in cow, suggesting a practical tendency favoring sheep in terms of respiratory capacity (Figure 3,4).

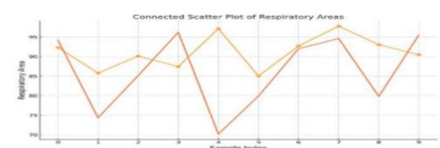


Figure (3) Scatter plot showing the distribution of the ten readings of the respiratory area of both sheep (orang) and cow (red)

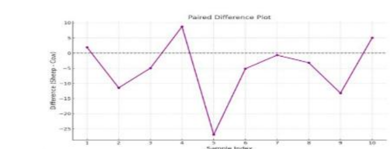
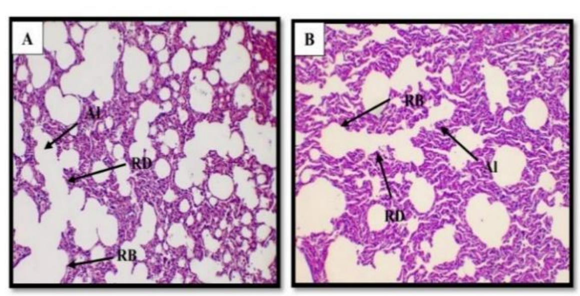


Figure (4) The Paired Difference Plot shows the difference between each pair of samples (sheep– cow). It shows that most specimens tend to favor sheep.

The bar chart illustrates the difference in the mean respiratory area between cow and sheep (n = 10 per group). The analysis revealed that the average respiratory area in sheep (90.18µm^2) was moderately higher than that in cow (85.21µm^2) (Table 1&2), with a mean difference of Δ = −4.98. This difference, while not statistically significant (p = 0.153), suggests a practical tendency favoring sheep in terms of respiratory area, as supported by the calculated effect size (Cohen's d = −0.67), which corresponds to a medium-to-large effect according to Cohen's classification. (Figure 5).



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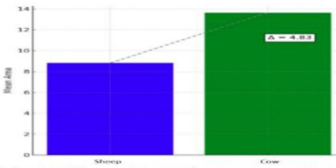

Figure 10: Value the difference between the average area of connective tissue between the two groups of sheep and cow n=10

Image NO.	Total area	Conn. area	Conn. area %	Respi. area	Respi. area %
1	9848832	752147	7.6	9096685	92.3
2	9843336	1402789	14.25115428	8446043	85.80468045
3	9848832	973150	9.880867092	8875682	90.11913291
4	9848832	1236872	12.55856532	8611960	87.44143468
5	9848832	283461	2.878117933	9565371	97.12188207
6	9848832	1472802	14.9540778	8376030	85.0459222
7	9848832	719573	7.306176001	9129259	92.693824
8	9848832	218428	2.217806132	9630404	97.78219387
9	9848832	686061	6.965912303	9162771	93.0340877
10	9848832	937171.6	9.51556083	8911660.4	90.48443917
total	9848282.4	868245.46	8.81282377	8980586.54	91.1827597

Table (2) show the differences of measurement unit between respiratory area and connective tissue in lung of cow (μm^2)

Image No.	Total area	Conn. area	Conn. area %	Respi. area	Respi. area %
1	9848832	569478	5.782188182	9279354	94.21781182
2	9848832	2524690	25.63441025	7324142	74.36558975
3	9848832	1462025	14.84465366	8386807	85.15534634
4	9848832	374493	3.802410276	9474339	96.19758972
5	9848832	2933073	29.78092225	6915759	70.21907775
6	9848832	1827018	18.5506058	7870108	79.90904911
7	9848832	785449	7.975047193	9063383	92.02495281
8	9848832	530529	5.386719968	9318303	94.61328003
9	9848832	1986338	20.16825955	7861701	79.82368874
10	9848832	440156	4.469118775	9408676	95.53088123
total	9848832	1343324.9	13.63943359	8490257.2	86.20572673

Differences in connective area between two independent groups, cow and sheep, were analyzed using the Independent Samples T-test, after verifying that the data of the two groups followed the normal distribution by the Shapiro-Wilk test, where the p-value for both sheep ($p = 0.626$) and cow ($p = 0.153$) was greater than the significance level of 0.05, indicating the applicability of the normal distribution hypothesis. The results of the T test showed that the difference in the average connective area between cows and sheep was not statistically significant, as the statistical value of the test was: $t(18)=-1.46$ $p=0.162$, and this indicates that there were no significant differences between the two groups at the significance level of 0.05. (figure 6,7).

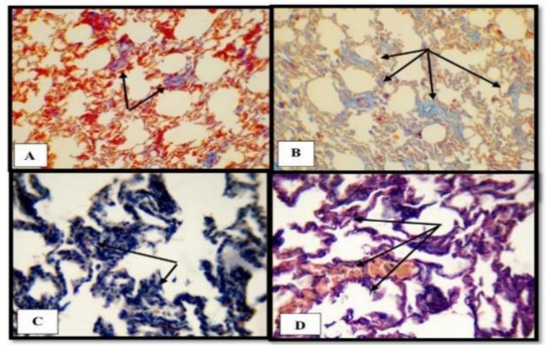

Figure (7) cross section of lung show: distribution of collagen fibers,(A) sheep, (B) cow. Masson trichrom,4x. Distribution of elastic fibers, (C) sheep, (D) cow, Van Gieson stain, 10x.

Cohen'sd analysis: Although there is no statistical significance in the T-test, the effect size (Cohen's d) was calculated to assess how significant the difference between the two groups was in practice. Cohen's $d = -0.65$ This value indicates an average effect size according to Cohen's classification, and indicates that the area of connective tissue in cows was moderately larger than that of sheep (a negative signal means that the average cow is higher than the average sheep) (Figure 8,9).

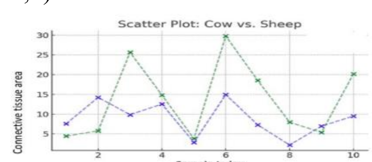

Figure (8) Scatter plot showing the distribution of the ten readings of the connective tissue area of both sheep (blue) and cow (green)

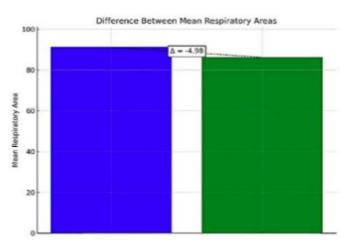

Figure (5) Value the difference between the average area of respiratory area between the two groups of sheep and cow n=10

When comparing the mean connective tissue area between the cow and sheep groups, the analysis showed that the mean in the cow group was significantly higher than in the sheep group. It reached: Average area of connective tissue in sheep: 8.81 units Average area of connective tissue in cow: 13.64 units. (Figure 10).

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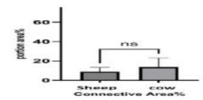

Figure (6) Analysis of differences between connective tissue area in sheep and cow n=10 mean ±SD

Discussion

The comparative histological quantification of pulmonary tissue in healthy, adult cow (*Bos taurus*) and sheep (*Ovis aries*) revealed distinct differences in the proportion of pulmonary area and connective tissue, this results was mentioned by (11,12,13) in domestic animals. Important parameters which studies of organs and systems for diagnosis diseases of infectious and noninfectious pathologies (14,15).Thus, respiratory system vitally important functions in organism, the main being gas exchange by inhaling oxygen from air and emitting carbon dioxide from organism into the air. Gas exchange is preformed in the lung between air and blood by diffusion of air and carbon dioxide through the alveoli into blood capillaries, were observed earlier by (16,17). Our results of histometric studies revealed the respiratory area of lung is more developed in sheep than cow parameters were much lower (18) in Tibetan sheep. Connective tissue of lung parenchyma was more notable in cow, indicating that sheep have significantly higher percentage of gas- exchanging area than cows. These differences based on body size, metabolic demands and physiological function, this results agreement with (19) in different species. This finding aligns with the higher metabolic rate of sheep which often live in more physically demanding environment, (hilly or mountainous), and thus require more efficient respiratory system (20). (21) showed that The expansive respiratory area in sheep allow for optimal oxygen diffusion to meet the energetic need. In contrast, cow lung showed relatively greater proportion of connective tissue . This finding can be refer to the cow larger body size, which require enforcement of the lung to maintain shape and function during respiratory cycle. The increased connective tissue may also serve biometrical role providing elasticity and flexibility against the weight of thoracic organs during prolonged standing and ruminating. These observations support previous histological and morphometric studies (6,9,22), which demonstrate that connective tissue provides mechanical support and prevent alveolar collapse, excessive connective tissue reduce effective surface area available for gas exchange potentially lowering respiratory efficiency.

Conclusion:

The comparative histological assessment between cow and sheep lungs demonstrates that while both species possess similar respiratory areas, sheep lungs exhibit a slightly higher respiratory area, suggesting more efficient structure for gas exchange. This may be related to their higher metabolic needs and more active lifestyle. Conversely, cow lungs contain a significantly greater amount of connective tissue, implying a structural adaptation to support their larger body mass, albeit at the cost reduced respiratory efficiency.

Conflict of interest

There is no conflict of interest in this study as stated by the authors.

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