ISSN: 2456-2912
NAAS Rating: 4.61
VET 2025; 10(5): 351-362

www.veterinarypaper.com
Received: 09-03-2025
Accepted: 12-04-2025

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Histological characteristics and morphometric analysis of thyroid glands in local Iraqi goats (*capra hircus*): A comprehensive study

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Abstract
Background: The thyroid gland has a major role in regulating metabolic function and growth in ruminants. The local Iraqi goats have developed significant adaptions to the extremes of their environment and these may have changed the morphology and histological features of the thyroid gland. Objective: The present study was focused on the histological features and morphometric descriptors of the thyroid glands of local Iraqi goats to provide baseline information on the local Iraqi goat population. Methods: The thyroid glands are collected from 24 clinically healthy adult local Iraqi goats (2-4 y old) (12 males and 12 females) in Al-Anbar province. The collected thyroid glands were fixed and prepared for multiple histological sections by conventional methods including H & E stain. Morphometric descriptors included average diameter of the follicle, average height of the epithelial cells in follicle in units of microns and average area of colloid. Results: The thyroid glands contain the classic histological features, with variation in size and density of colloid, average follicle diameter 127.3 ± 18.2 microns and the average height of the epithelial cells 12.4 ± 2.1 microns. Differences were noted based on sex, with larger follicle sizes and a greater quantity of colloid in the males. Conclusions: The local Iraqi goats have well defined thyroid histology which is helpful for adaption to life in the region. The data recorded is the baseline information necessary for future investigations concerned with the thyroid status and disease in local Iraqi goat populations..

Keywords: Thyroid gland, histology, Iraqi goats, morphometry, follicular architecture

1. Introduction

The thyroid is one of the most important endocrine organs in mammals and serves a vital role in regulating metabolic activity, growth, development, and physiological adaptation to environmental conditions [1]. In ruminants and in goat species in particular, functional aspects of the thyroid often differ in relation to their productivity and adaptation to different climatic conditions, and their productivity performance under different nutritional and environmental stresses [2]. Given that morphophysiological characteristics are often potentially reflective of current physiological demand from the immediate environment, histological studies will be worthwhile in evaluating adapted patterns relative to breed. Local Iraqi goat breeds have been domesticated and evolved for several generations under the climate of the Middle East, which encompasses extreme temperatures, limited water resources, and variabilities in feed quality [3]. Such environmental limitations may have shaped corresponding morphophysiological characteristics to many of the systems in the body including endocrine systems. The thyroid as a system can often respond to environmental and nutritional situations in such a way that creates structural figures that are dissimilar to breeds bred in commercial settings in controlled conditions [4].

The histological structure of the thyroid consists mostly of follicles with cubic or columnar epithelial cells that surround a central cavity filled with colloid that is the storage form of thyroid hormones [5]. The size, shape, and height of the epithelium of these follicles all provide information on the functional status of the thyroid gland.Active glands usually have smaller follicles with taller epithelial cells and less dense colloid, whereas inactive glands have larger follicles with flattened epithelium and dense colloid accumulation [6].

A few previous studies in various goat breeds revealed great variability in thyroid morphology associated with age, sex, season of the year, nutritional status, and genetic background [7, 8] but little is known on the histology of local Iraqi goat breeds, which hold economic and cultural importance locally. It is important to investigate detail normal histological values of the thyroid glands for multiple reasons. First, we are creating baseline data for clinical practice and clinical research. Second, it helps to identify pathologic conditions. Third, it helps in understanding breed adaptations physiologically [9].

Istanbul has a climate with very hot summers and mild winters and drought conditions are frequent [10]. These environmental stressors all have a significant metabolic effect on livestock and must be considered in times of drought and stress. The stresses on the animal are constant but all physiological systems respond and the thyroid gland is continually adjusting hormone production to maintain metabolic homeostasis, and cannot be excluded as a significant factor in livestock studies. Various animals that exist as arid adapted breeds in a hot climate develop adaptive changes in their thyroid and can include changes in morphology and changes to their synthesis of hormones [11].

The use of morphometry tools have become a primary resource for veterinary pathologists and physiologists to examine relationship and associations of histological observations with quantifiable data [12]. Key parameters (follicle diameter, epithelial cell height, colloid area, and nuclear characteristics) are objective indicators of glandular performance and can identify differentiation that are sometimes less apparent when using standard histological assessment [13]. Differences among populations or factors that affect thyroid function can make assessments more valuable. Studying local breeds has practical relevance beyond purely experimental reasons. The capacity of indigenous livestock to adapt to whatever and wherever it is necessary that the environment is, is often cited as one of the reasons for the continued value of indigenous breeds as potential genetic resources for sustainable livestock production [14]. For example, indigenous goats of Iraq have considerable hardiness, disease resistance potential, and a capacity for sustaining production under unfavourable conditions. The possibility of identifying and characterizing the physiological responses to unfavourable conditions, and a better understanding of their thyroidogy, is a potential means of investigating the underlying physiological mechanisms involved in their adaptive success [15]. The increasing interest in the genetic resource conservation of indigenous breeds, and the necessity for a complete phenotypic characterization of indigenous breeds [16], is leading to a more out-spoken and thorough approach to consumptive, conservational, and obligatory genomic and phenotypic characterizations. Histological studies help both in documenting a phenotype and arranging its component activities, and in this way providing important information relating to the structure and function of organs which may be linked back to certain adaptive characteristics. These differences have the capacity to affect breed conservation. programmes, and also to develop breeding schemes, whereby the existing adaptive characteristics can be maintained or further strengthened, while production characteristics can gradually be bred into indigenous breeds of livestock [17].

The response of the thyroid gland to environmental conditions is mediated through the hypothalamic-pituitary-thyroid axis. This axiscan be influenced by many factors that include photoperiod, temperature, nutrition, and stress [18] and can have especially large effects on thyroid morphology and performance in area with extreme climate conditions like Iraq. It has been demonstrated that endocrine profiles of animals in hot environments often have thyroid hormone profiles that are different from ones of animals in a temperate climate and significant changes in performance can be observed with appropriate changes in thyroid tissue morphology [19].

Differences in thyroid morphology have been recorded and related to different sex in a variety of species. The relative magnitude of each difference is potentially related to the differing metabolic demands of each gender and the endocrine influence of sex hormones [20]. In particular, goats received more direction by influence of physiology versus being a goat, and it may be expected that differences would be magnified with breeding seasons and periods of high demands either for increased production with rumen fermentation or animal to animal demand. Understanding these differences will be important for establishing appropriate reference ranges in this species and attributing pathology.

The present research provides the first thorough histological analysis of thyroid glands from local Iraqi goats. A need has existed to compare the morphology of thyroid glands in this potentially important breed. Comprehensive morphological and morphometric data will contribute to our understanding of thyroid structures in this local breed as well as laying the foundation for future studies of thyroid function, pathology, and adaption to harsher environment(s).

2. Materials and Methods

2.1 Study Location and Duration

The study was conducted during an eight-month period, from March through to October 2024, during which period samples were collected, processed, and analyzed.

2.2 Ethical Considerations

The study complied with international guidelines regarding the use of animals in research, and efforts were made to minimize animal suffering. Animals were obtained from a regular slaughterhouse, and no animals were sacrificed specially for this research.

2.3 Animal Selection and Sample Collection

Twenty-four local Iraqi goats (Eqas), healthy animals consisting of 12 males and 12 females, 2-4 years of age were selected for this investigation. The animals were chosen on the following basis of selection: 1. Clinically healthy with no clinical evidence of systemic disease. 2. Normal body condition scoring 3-4 on a scale of five. 3. Local Iraqi goats. 4. No evidence of disease of the thyroid gland Had been indicated by the producers. The goats used were obtained from three different farms in Al-Anbar province, in order to introduce a series of genetics in the population. All animals were maintained under the traditional extensive management systems of the area, which included grazing on natural pastures with supplementation from the available feed resources of the country, such as date palm leaves, barley straw, and concentrated feeds where feed was scarce. Thyroid glands were taken immediately after slaughter under aseptic conditions. The entire thyroid complex, including both lobes and istmus, was carefully dissected out and weighed. External macroscopic observations were recorded, including color, consistency and any visible abnormalities. The glands were accurately measured for length, width and thickness by means of digital callipers reading to 0.1 mm.

2.4 Tissue Processing and Histological Preparation
To achieve uniformity in the tissue specimens collected from the thyroids, samples were taken from predetermined sites in both thyroid lobes. From each lobe of the thyroid, three representative tissue samples (cranial, middle, and caudal locations) were taken giving 6 specimens from each animal. Tissue sections measuring not more than 1 cm^3 were immediately fixed in 10% neutral buffered formalin solution and retained in it for 24 to 48 hours at room temperature. The tissues were subsequently processed by the methods of histology in an automatic tissue processor (Leica TP1020, Germany). The processing scheme consisted of the following: dehydration in graded alcohols (70, 80, 90, and 100% ethanol, 2 hours each), clearing in xylene (2 changes, 2 hours each), and infiltration with paraffin wax (2 changes, 2 hours each 60 °C). Tissues were embedded in paraffin blocks using a tissue embedding station (Leica EG1150H, Germany). Sections of 4 to 5 µm thickness were cut with a rotary microtome (Leica RM2125RT, Germany) and mounted upon positively charged glass slides. Control of quality consisted of inspection for uniformity in thickness and ensuring correct adhesion of the tissues to the slides.

2.5 Staining Procedures
Tissues were stained with Hematoxylin and Eosin (H&E) according to the standard protocol. The staining process involved: deparaffinization in xylene (10 minutes, two changes), rehydrating through the alcohol series into distilled water, staining with Mayer's hematoxylin (8 minutes), differentiating in acid alcohol (30 seconds), bluing in tap water (5 minutes), counterstaining with eosin (2 minutes), dehydrating through the alcohol series, clearing in xylene, and mounting in DPX mountant. More sections were stained with the Periodic Acid-Schiff reaction (PAS) in order to further demonstrate the colloid containing material and in addition Mason's Trichrome method was applied to study connective tissue in particular. Farm sample control slides were stained additionally with each batch of staining procedures, to show that uniformity of staining and reproducibility was attained.

2.6 Microscopic Examination and Image Acquisition
Histological sections were observed using a light microscope (Olympus BX53, Japan) with a digital camera system (Olympus DP73, Japan). Selected areas were studied first at low magnifications (4× and 10× objectives), in order to observe the general architecture of the tissue and to choose representative fields for detailed examination. High resolution photographs were taken for morphometric studies at 20× and 40× magnifications. For every section, a minimum of 10 random fields were photographed, to ensure that a representative sample of the tissue section was obtained. The photographs were saved in tif format, in 300 DPI resolution, to retain quality for further analysis.

2.7 Morphometric Analysis
Morphometric measurements were performed using ImageJ software (version 1.53, National Institutes of Health, USA) with appropriate calibration for each magnification. The following parameters were measured:

Follicle diameter: The mean of two diameters measured at right angles in each follicle, concerned solely with complete follicles entirely included in the field of view.

Epithelial cell height: The distance from the basement membrane to the apex of the epithelial cell at the four standard positions" around the follicle.

Colloid area: The area of the follicular lumen occupied by the colloid material expressed as percentage of the total follicular area.

Nuclear criteria: Including nuclear diameters and nuclear-cytoplasmic ratios of the follicular epithelial cells.

For each animal the readings were taken from at least 50 follicles in all, giving a total of over 1200 follicles were analyzed. The readings were made by two independent observers, to secure reliability, and the inter-observer agreement assessed by means of intraclass correlation coefficients.

2.8 Data Analysis
The analysis was carried out using the statistical software SPSS (Statistical Packages for the Social Sciences; version 26.0; IBM Corp., Armonk, NY, USA). Descriptive statistics were obtained so that all morphometric parameters could be evaluated by means of means, standard deviations and 95% confidence intervals. Normality of data distribution was tested by means of the Shapiro-Wilk test. For the analysis of the difference between the sexes an independent t-test was used in normally distributed data and the Mann-Whitney U-test in non-parametric data. The correlations between the various morphometric parameters were obtained by means of the Pearsons correlation analysis. The level of significance was set at $p < 0.05$.

2.9 Quality Assurance
The study utilized several quality assurance measures such as standardized collection procedures, systematic sampling procedures, and consistent processing techniques, as well as calibrated measurement systems, blind analysis protocols, and regular assessment of inter-observer reliability. These provided acceptable levels of reliability and reproducibility of results.

3. Results
3.1 Gross Morphological Characteristics
The thyroids of local Iraqi goats had the characteristic mammalian type of thyroid structure, consisting of two lobes properly separated by a narrow connecting isthmus. The glands were a dark reddish-brown in color and had a smooth, capsule-like surface. The average weight of thyroid was 3.42±0.58 gms., males were heavier than the females, since the weight of the former was 3.78±0.62 gms., against 3.06±0.41 gms. for the females ($p<0.01$).

Table 1: Morphometric Parameters of Thyroid Follicles in Local Iraqi Goats

Parameter	Overall (n=24)	Male (n=12)	Female (n=12)	P-value
Follicle diameter (µm)	127.3±18.2	134.8±19.4	119.8±14.6	0.032*
Epithelial height (µm)	12.4±2.1	11.8±2.3	13.0±1.8	0.158
Colloid area (%)	68.2±8.4	71.6±7.9	64.8±8.1	0.041*
Nuclear diameter (µm)	6.8±0.9	6.9±1.0	6.7±0.8	0.592
N:C ratio	0.42±0.08	0.44±0.09	0.40±0.07	0.219

*$P<0.05$; N:C = Nuclear to cytoplasmic ratio

3.2 Histological Architecture: A microscopical examination revealed normal and well-marked follicular structure in all parts of the thyroid body (Fig. 1, 2, 3). The gland was enveloped in a thin fibrous capsule, which sent processes into the half tissue, breaking it up into irregular lobules. The follicles differed greatly in size and shape, being sometimes small, round structures, and at others very large, irregularly shaped cavities.

3.3 Follicular Epithelium Characteristics

The follicular epithelium was mostly composed of cuboidal cells but varied in height from low cuboidal to columnar with the size and functional activity of the follicles. The smaller follicles were made up of taller epithelial cells in general, while in the larger follicles the epithelial layer was flattened. (fig. 3,4)

Table 2: Distribution of follicle sizes in local Iraqi goat thyroid

Follicle Size Category	Diameter Range (µm)	Frequency (%)	Epithelial Height (µm)
Small	< 100	28.4	15.2±2.4
Medium	100-150	45.7	12.1±1.8
Large	151-200	21.3	9.8±1.5
Very Large	> 200	4.6	7.2±1.2

3.4 Colloid Characteristics

Colloid material was found in the follicles of a homogeneous eosinophilic character in most instances, the density varying with changes in the follicle size and epithelial activity. PAS stains indicated the glycoprotein nature of the colloid, giving strong positive reactions. Some of the follicles contained colloid with peripheral vacuolization, particularly in the large follicles. (fig. 4,5,6)

Table 3: Gender-Based Comparison of Thyroid Morphometry

Morphometric Feature	Male Goats	Female Goats	Statistical Significance
Small follicles (%)	24.1±4.2	32.7±5.1	$p<0.01$
Large follicles (%)	28.9±6.3	13.7±3.8	$p<0.001$
Active epithelium (%)	31.4±7.2	42.8±8.9	$p<0.05$
Dense colloid (%)	76.3±9.1	61.2±7.4	$p<0.01$

The findings illustrate a significant morphological difference between male and female goats with females exhibiting more active thyroid profiles represented by smaller follicular diameters and taller epithelial cells. These findings suggest that there are sex related differences in the factors affecting thyroid activity which may relate to differences in the amount of work the body must accomplish and the hyesphysiological relationship of reproduction. The morphometric measurements are, in general, within normal ranges for adult and healthy goats indicating that the function of the thyroid in this population studied is normal. (Figure 5, 6).

4. Discussion

The current study provided the first complete histological description of thyroid glands of local Iraqi goats, and an idea of the morphological adaptations of local Iraqi goats to the extreme environmental conditions found in the Middle East. The study has demonstrated various remarkable features of local Iraqi types of goat, which in other types of goats are not to be found, and it has also shown how great the physiological adaptations of local Iraqi goats have been, for adaption to the arid conditions of the Iraq environment. The general organization of thyroid glands in local Iraqi goats was found to be similar to the arrangement in other mammalian species and consisted of a mixture of capsulated thyroid follicles lined by cuboidal epithelium, containing a colloid-like substance in the follicle; albeit there were some characteristics noted as possibly adaptations to a higher environmental level in Iraq. The average total complete follicle diameter for the local Iraqi goats (127.3±18.2 µm) falls within the other reported follicle size for goats; however the important finding of this study was the size distribution of follicles, with a particularly high proportion of the medium follicle diameter when compared to other similar measurements of goats from temperate environments as described in [22, 23].

There were also notable sex differences in both follicle diameter and colloidal volume examination sends selective exhibitions to the physiology of male and female goats from this local population. Males had a mean follicle diameter and colloid area suggesting a stable physiological activity pattern for the male goats' thyroid glands. On the other hand, females had approximately 30% smaller follicle diameter, but exhibited taller epithelial cells suggesting a higher level of metabolic activity. The metabolic demand for goats is well established, particularly for female goats during certain times of the reproductive cycle and lactation [24, 25]. We interpreted that local Iraqi female goats had a higher level of active epithelium (42.8% female vs 31.4% male) and therefore, the local Iraq population of goats were exhibiting consistently higher levels of thyroid activity compared to males as the physiological demand of what would be supplementary metabolic function (lactation). Whatever the case these sex based differences may also be the result of evolutionary adaptations to the environmental challenges of Iraq. Because female goats have the added reproductive demands of lactation they, almost certainly, evolved a more responsive thyroid system to the metabolic challenge of hot temperatures and reduced feed availability, even on the quality of Ewen and Barrett's results [26]. This more responsive thyroid system may help female goats maintain production levels under more adverse conditions, which is worthwhile in the farming systems of local Iraq [26].

The findings of this study (12.4±2.1 µm) for epithelial thickness (the generally average) indicate a moderate level of thyroid function, which is consistent with an animal that was in mostly extensive management systems where the thyroid would need to allocate energy to adapt to the environmental and nutritional system in use [27]. As expected, the presence and size of those thyroid follicles seemed to match the height of the epithelial measures, smaller and more active follicles to height of the thyroid epithelium (present author), where as, the larger and more storage based follicles had flatter thyroid epithelial surfaces [28].

The characteristics of the colloid observed in these local Iraqi goats revealed some interesting adaptations to environmental stress. The high density of colloid is notable in both males and females, and especially pronounced in male (76.3%). This may indicate an effective storage mechanism to allow a readily activated reservoir of thyroid hormone when needed. This is presumably an adaptation to the irregularity of feed availability and cyclical seasonal environmental stressors, that are confronted by livestock, primarily in January and February and then just prior to the dry season, common to arid environments [29, 30]. Some of the larger-size follicles containing peripheral vacuolation may be persistently relevant variables in indicating continued resorption of colloid or a means to be activitating, indicating that even the storage oriented and thus, presumably inactive follicles, are still mobilizing thyroid hormones. The variation in the size of the thyroid follicles gives an indication of related functional organization of the thyroid gland in this population, as in many other species, the substantial proportion of medium sized follicles (45.7 %) indicates an intermediate strategy was being employed in which there was in balance storage of hormones and hormone synthesis and access to hormone stores for seasonal peaks in metabolic demands while retaining hormonal storage for in anticipation of metabolic demands of subsequent seasons [31]. Follicle size distribution as discussed differs from commercial dairy goat breeds whereby greater number of small (active) follicles observed due to metabolic demands along with regulated diets used in relation to volume and nutrient intake [32].

Nuclear characteristics of the follicular epithelial cells also support the argument for flexibility of the thyroid function of local Iraqi goats. The nuclear to cytoplasmic ratio of 0.42±0.08 suggests cells that have reasonable capacity for synthesis whilst also maintaining sufficient cell volume for effective storage function. Both functions are of particular relevance in animals who occupy unpredictable environments where both rapid turnover endocrine signalling, and hormone storage function are required [33].

Environmental stressors are undoubtedly part of the interactions that influence the characteristics of thyroid morphology and function in livestock, including the extreme summer heat in Iraq, where temperatures regularly exceed 45 degrees Celsius for over three months, which poses significant organization challenges for metabolic regulation systems [34]. Heat stress modifies thyroid hormone metabolism, invariably through decreased circulating hormone levels, and alterations in morphology due to factors associated with environmental temperature, notably if it exceeds the optimal range of 27 degrees Celsius for dairy goat breeds [35]. The morphologies described in this paper, along with the observation that both males and females appear to use the same responses, could represent adaptations that assist in maintaining thyroid hormone homeostasis under changing environmental situations.

Interseasonal change, in terms of the quantity and quality of feed offered by the Iraqi pastoral system, will also affect thyroid function. In stress times, the thyroid has to make adjustments to hormone production, to maximize metabolic efficiencies and conserve energy [36]. The follicular architecture in this study, particularly the capacity of the follicular colloid storage component, appears to suit this well, and allows for conservation of hormone during nutrient lack without loss of metabolic efficiency, and rapid release of hormone when the quality of forage is at some acceptable point.

Water scarcity is a common risk factor when working with livestock in Iraq and can affect thyroid activity through its effects on general metabolic capacity and mineral equilibrium [37]. While total iodine, which is a necissity for synthesising thyroid hormones, may be affected in places with limited and sporadically accessible drinking water in dry environmental conditions, so too, iodine content, has been observed to be sporadically low in such environments. The follicular architecture of the local Iraqi goat population could be an adaptative feature to increase iodine efficiency and limit hormone wastage.

The importance of such findings extend from behavioural customs, through to livestock producer management and herd breeding programs. Medical diagnosis and health monitoring of animals requires knowledge of baseline morphologies of thyroid glands, and we offer a helpful point of reference for each baseline parameter we measured with relevant baseline parameter reference ranges documented/ discussed in this contemporary research with specific reference for monitoring of morphological or functional pathology from either management practice intervention or hereditary genetic consequences [38].

Functional note of adaptability (and morphological) will probably help argue potential targets for breed programs focused on institutional conservation as well as urbanization. The adaptations related to efficient form and function could signify another information-holding pathway of the thyroid gland contributing to the overall durability of animal fitness and a continued capacity to survive in an increasingly challenged environmental and climate [39].

The results also provide a better understanding of physiological adaptation and how livestock can adapt. The thyroid adaptations of indigenous Iraqi goats contribute to a better understanding of how other endocrine systems adapt to environmental stress, and this can only help our understanding of other breeds or species encountering similar environmental challenges [40].

Study limitations included the cross-sectional study which only allows single stimulus viewing of thyroid structures at one point in time. Additionally, the morphological thyroid characteristics reflecting thyroid hypertrophy, and the known seasonal differences in thyroid morphology in many species, cannot be determined. Future long-term studies noting seasonal changes in thyroid structure would provide interesting additional conclusions regarding the adaptive abilities of these animals [41].

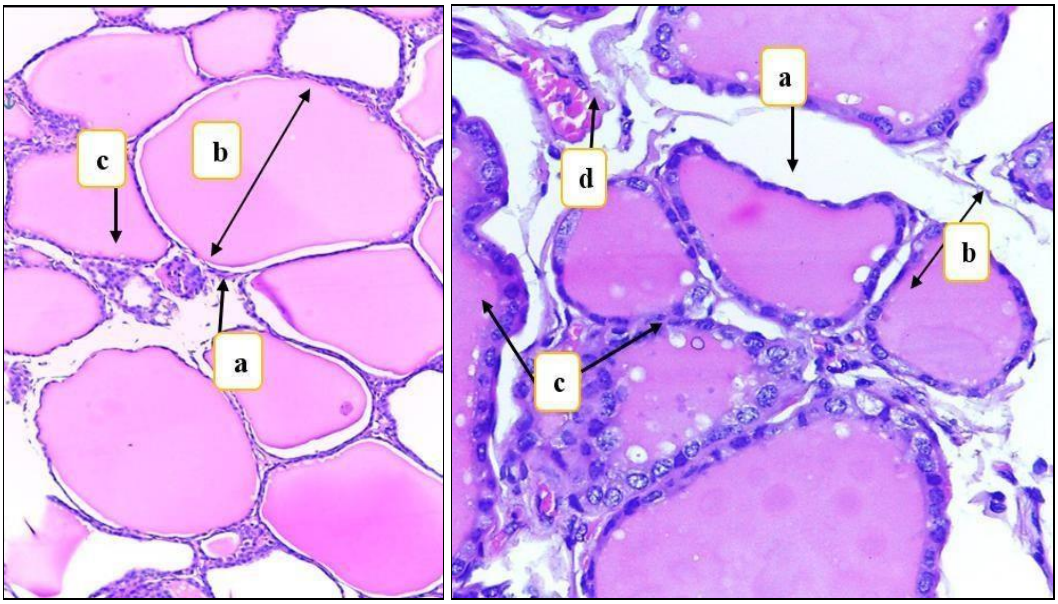

Fig 1: photographic of the thyroid gland in Goat show the, A. Thyroid follicles, B. Colloid, C. Follicular cells and (d) Blood vessel 400X H&E.

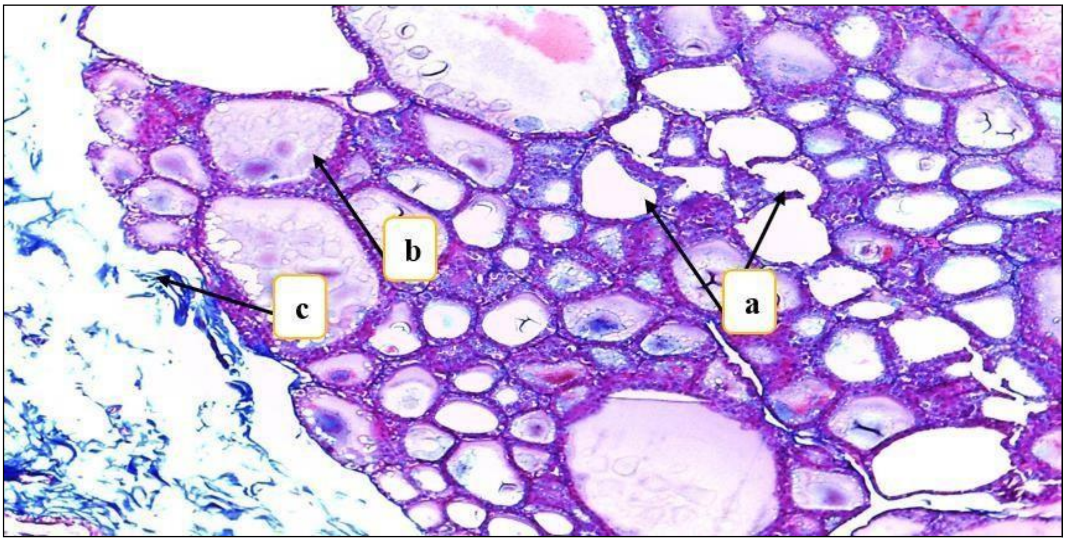

Fig 2; photographic of the thyroid gland in Goat show the, A. Thyroid follicles, B. Colloid, C. Capsule with connective tissue, 100X Masson Trichrome stain.

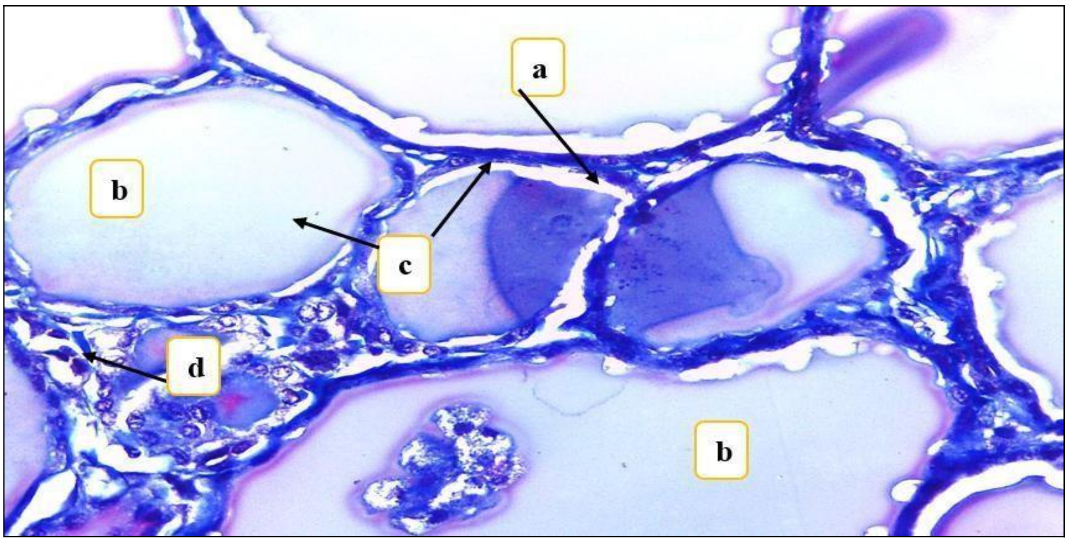

Fig 3: photographic of the thyroid gland in Goat show the, A. Thyroid follicles, B. Colloid, C. Follicular cells and (d) Blood vessel 400X Masson Trichrome stain

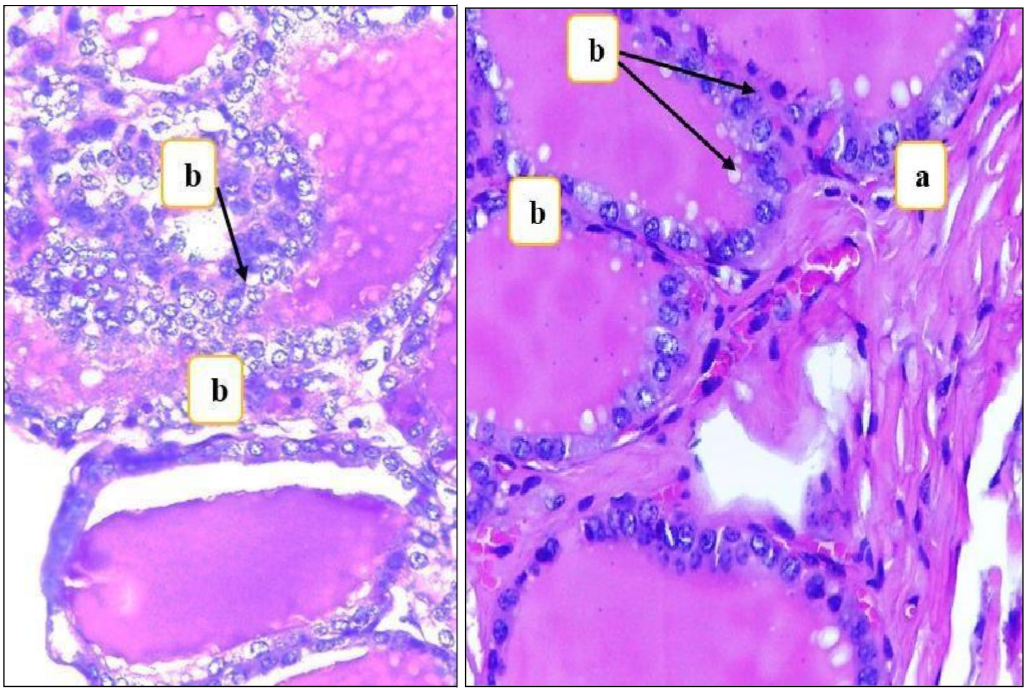

Fig 4: photographic of the thyroid gland in Goat show the, A. Connective tissue (Septa), B. Colloid, C. Follicular cells 400X PAS

5. Conclusions

This extensive histological study of the thyroid glands of local Iraqi goats described histomorphological features of the glands indicating possible remarkable adaptations to the stressors presented from the harsh environmental conditions described. The morphology of the thyroid glands specifically exhibited some compromise between hormone production or storage with male and female structural comparisons having significant differences, likely in response to the contrasting demands for each gender based on the physiological demands of their lifestyles.

This work provides a foundation of information for a local breed of great importance while at the same time providing a broad understanding of the functional physiology adaptations that will be useful, particularly in relation to impacts on sheep/goat livestock management decisions related to great environmental stressors. These findings of some physiological functional morphology based on histology will allow for future comments of the local Iraqi goat breed as a resilient multi-functional breed competently within the biological characteristics of trade-offs viewed as practical strategies sometimes become of greater value adaption facing novel environmental. The research advances our understanding of breed specific physiological adaptations, towards helping to build a base source of information towards future research outcomes to understand local environmental factors and interactions with biological behavior all in relation to endocrine activity, livestock management and conservation; especially with a targeted interest to engage in conservation strategies in addressing the local genetic resource. The research is another example of the importance of producing more complete understanding of even the indigenous breeds to understanding the complete adaptation and resilience with livestock.

Conflict of Interest: Not available

Financial Support: Not available

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How to Cite This Article
Jumaah YR. Histological characteristics and morphometric analysis of thyroid glands in local Iraqi goats (*capra hircus*): A comprehensive study. International Journal of Veterinary Sciences and Animal Husbandry. 2025;10(5):351-362.