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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 plays an important role in regulating metabolic function and growth in ruminants. The local Iraqi goats developed considerable adaptations to the harsh environments and these adaptations may have impacted thyroid gland morphology and histological characteristics.

Objective: This study focused on the histological characteristics and morphometric descriptors of the thyroid glands of local Iraqi goats to develop baseline data on the local Iraqi goat population.

Methods: Thyroid glands were collected from 24 clinically healthy adult local Iraqi goats (2-4 years old) (12 males and 12 females) in Al-Anbar province. After collection, thyroid glands were fixed and prepared for multiple histological sections via standard procedures including H & E staining. Morphemic descriptors included average diameter of the follicle, average height of epithelial cells in follicle, and average area of colloid.

Results: The thyroid glands had classic histological characteristics, with variation in size and colloid density, with an average follicle diameter measuring $127.3 \pm 18.2 \mu\text{m}$ and an average height of epithelial cells measuring $12.4 \pm 2.1 \mu\text{m}$. Differences based on sex were observed, with males having larger follicle sizes and more colloid material.

Conclusions: Local Iraqi goats have clearly defined thyroid histology that is beneficial for adaptation in the region. The collected data is important baseline information for future research on 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].

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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 ability of local livestock to adapt to whatever and wherever the environment imposes is often cited as a reason for the continued use of local breeds as potential genetic resources for sustainable livestock production [14]. For example, local Iraqi goats have substantial hardiness, potential for disease resistance, and can maintain production under adverse conditions. The potential to identify and characterize the physiological responses to adverse conditions and a better understanding of their thyroid morphology offers the potential to explore the underlying physiological mechanisms responsible for their adaptive success [15].

Increased interest in the conservation of local genetic resources and the need for a comprehensive phenotypic characterization of indigenous breeds [16] is shaping a robust approach to consuming, conserving, and documenting genomic and phenotypic characterizations. Histological studies serve to both document a phenotype and organize its component activities, providing valuable information concerning the structure and function of organs that may be linked back to particular adaptive characteristics. These variables have the potential to influence the design of breed conservation programs and also establish breeding schemes that maintain or further improve existing adaptive characteristics while selectively breeding on aspects of production to enhance the productivity of indigenous livestock [17].

The response of the thyroid gland to environmental conditions is mediated through the hypothalamic-pituitary-thyroid axis. This axis can 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

For the purposes of this study, twenty-four local Iraqi goats (Eqas) that were healthy were selected, balanced 12 males and 12 females, 2-4 years of age. The animals were selected based on the following criteria: [1] clinically healthy without clinical evidence indicating systemic disease [2] normal body condition score (3-4 on a 5-point scale) [3] local Iraqi goat, and [4] producers indicated no thyroid disease was present.

The goats were sourced from three different farms in Al-Anbar province to ensure genetic diversity within the sample population. All animals were maintained under traditional extensive management systems typical of the region, with grazing on natural pastures supplemented with locally available feed resources including date palm leaves, barley straw, and concentrate feeds during periods of feed scarcity.

Thyroid glands were collected immediately after slaughter under aseptic conditions. The entire thyroid complex, including both lobes and the isthmus, was carefully dissected and weighed. Gross morphological observations were recorded including color, consistency, and any visible abnormalities. Each gland was measured for length, width, and thickness using digital calipers accurate to 0.1 mm.

2.4 Tissue Processing and Histological Preparation

Tissue samples were collected from standardized locations in both thyroid lobes to ensure consistency across specimens. From each lobe, three representative sections (cranial, middle, and caudal regions) were taken, resulting in six samples per animal. Tissue blocks measuring approximately 1 cm³ were immediately fixed in 10% neutral buffered formalin solution for 24-48 hours at room temperature.

Following fixation, tissues were processed using standard histological techniques in an automated tissue processor (Leica TP1020, Germany). The processing schedule included: dehydration through graded alcohols (70%, 80%, 90%, and 100% ethanol, 2 hours each), clearing in xylene (two changes, 2 hours each), and infiltration with paraffin wax (two changes, 2 hours each at 60 °C).

Tissues were embedded in paraffin blocks using a tissue embedding station (Leica EG1150H, Germany). Serial sections of 4-5 µm thickness were cut using a rotary microtome (Leica RM2125RT, Germany) and mounted on positively charged glass slides. Quality control measures included monitoring section thickness consistency and ensuring proper tissue adhesion to slides.

2.5 Staining Procedures

Sections were stained with Hematoxylin and Eosin (H&E) using standard protocols. The staining procedure included: deparaffinization in xylene (10 minutes, two changes), rehydration through graded alcohols to distilled water, staining with Mayer's hematoxylin (8 minutes), differentiation in acid alcohol (30 seconds), bluing in tap water (5 minutes), counterstaining with eosin (2 minutes), dehydration through graded alcohols, clearing in xylene, and mounting with DPX mounting medium.

Additional sections were prepared using Periodic Acid-Schiff (PAS) staining to better visualize colloid material and Mason's trichrome staining to assess connective tissue distribution. Quality control slides were included in each staining batch to ensure consistency and reproducibility.

2.6 Microscopic Examination and Image Acquisition

Histological sections were examined using a light microscope (Olympus BX53, Japan) equipped with a digital camera system (Olympus DP73, Japan). Initial examination was performed at low magnification (4× and 10× objectives) to assess overall tissue architecture and identify representative areas for detailed analysis.

High-resolution images were captured at 20× and 40× magnifications for morphometric analysis. For each section, a minimum of 10 random fields were photographed, ensuring representative sampling across the entire tissue section. Images were saved in TIFF format at 300 DPI resolution to maintain image quality for subsequent 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: Measured as the average of two perpendicular diameters for each follicle, including only complete follicles entirely within the field of view.

Epithelial cell height: Measured from the basement membrane to the apical surface of follicular epithelial cells at four standardized positions around each follicle.

Colloid area: Calculated as the area of the follicular lumen filled with colloid material, expressed as a percentage of total follicle area.

Nuclear parameters: Including nuclear diameter and nuclear-to-cytoplasmic ratio of follicular epithelial cells.

For each animal, measurements were taken from a minimum of 50 follicles across all sections, resulting in over 1,200 follicles analyzed in total. Measurements were performed by two independent observers to ensure reliability, with inter-observer agreement assessed using intraclass correlation coefficients.

2.8 Data Analysis

Statistical analysis was performed using SPSS software (version 26.0, IBM Corp., USA). Descriptive statistics were calculated for all morphometric parameters, including means, standard deviations, and 95% confidence intervals. The normality of data distribution was assessed using the Shapiro-Wilk test.

Gender differences were analyzed using independent t-tests for normally distributed data and Mann-Whitney U tests for non-parametric data. Correlations between different morphometric parameters were assessed using Pearson correlation analysis. Statistical significance was set at $p < 0.05$.

2.9 Quality Assurance

Multiple quality assurance measures were implemented throughout the study: standardized collection protocols, systematic sampling procedures, consistent processing techniques, calibrated measurement systems, blind analysis protocols, and regular inter-observer reliability assessments. These measures ensured the reliability and reproducibility of results.

3. Results

3.1 Gross Morphological Characteristics

The thyroid glands of local Iraqi goats exhibited typical mammalian thyroid morphology, consisting of two distinct lobes connected by a narrow isthmus. The glands appeared dark reddish-brown in color with a smooth, capsulated surface. Mean thyroid weight was 3.42 ± 0.58 g, with males showing significantly higher weights (3.78 ± 0.62 g) compared to females (3.06 ± 0.41 g), ($p < 0.01$).

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: Microscopic examination revealed well-organized follicular architecture throughout the thyroid parenchyma (Figure 1, 2, 3). The gland was surrounded by a thin fibrous capsule with septa extending into the parenchyma, dividing it into irregular lobules. Follicles varied considerably in size and shape, ranging from small, round structures to large, irregularly shaped cavities.

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 $\pm$ 2.4
Medium	100-150	45.7	12.1 $\pm$ 1.8
Large	151-200	21.3	9.8 $\pm$ 1.5
Very Large	> 200	4.6	7.2 $\pm$ 1.2

3.4 Colloid Characteristics

Colloid material appeared homogeneous and eosinophilic in most follicles, with varying density depending on follicle size and epithelial activity. PAS staining confirmed the

3.3 Follicular Epithelium Characteristics

The follicular epithelium consisted predominantly of cuboidal cells, with variations from low cuboidal to columnar depending on follicle size and functional status. Smaller follicles typically showed taller epithelial cells, while larger follicles were lined by flattened epithelium. (fig. 3,4)

glycoprotein nature of the colloid, showing strong positive reactions. Some follicles contained colloid with peripheral vacuolation, particularly in larger 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 $\pm$ 4.2	32.7 $\pm$ 5.1	$p < 0.01$
Large follicles (%)	28.9 $\pm$ 6.3	13.7 $\pm$ 3.8	$p < 0.001$
Active epithelium (%)	31.4 $\pm$ 7.2	42.8 $\pm$ 8.9	$p < 0.05$
Dense colloid (%)	76.3 $\pm$ 9.1	61.2 $\pm$ 7.4	$p < 0.01$

The results demonstrate significant morphological variations between male and female goats, with females showing more active thyroid profiles characterized by smaller follicles and taller epithelial cells. These findings suggest gender-related differences in thyroid activity, possibly related to different metabolic demands and reproductive physiology. The overall morphometric values fall within expected ranges for healthy adult goats, indicating normal thyroid function in the studied population (Figure 5, 6).

4. Discussion

The current study offers the first full histological description of thyroid glands in local Iraqi goats, and provides insight into the morphological adaptations of local Iraqi goats relative to the extreme environmental conditions seen across the Middle East. The study has revealed multiple salient characteristics of the local Iraqi types of goat, which make them different from other goat types; moreover it highlights the significant physiological adaptations that local Iraqi goats have made to adapt to Iraq's arid 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 $\pm$ 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 $\pm$ 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 activating, 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 necessity 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 adaptive 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].

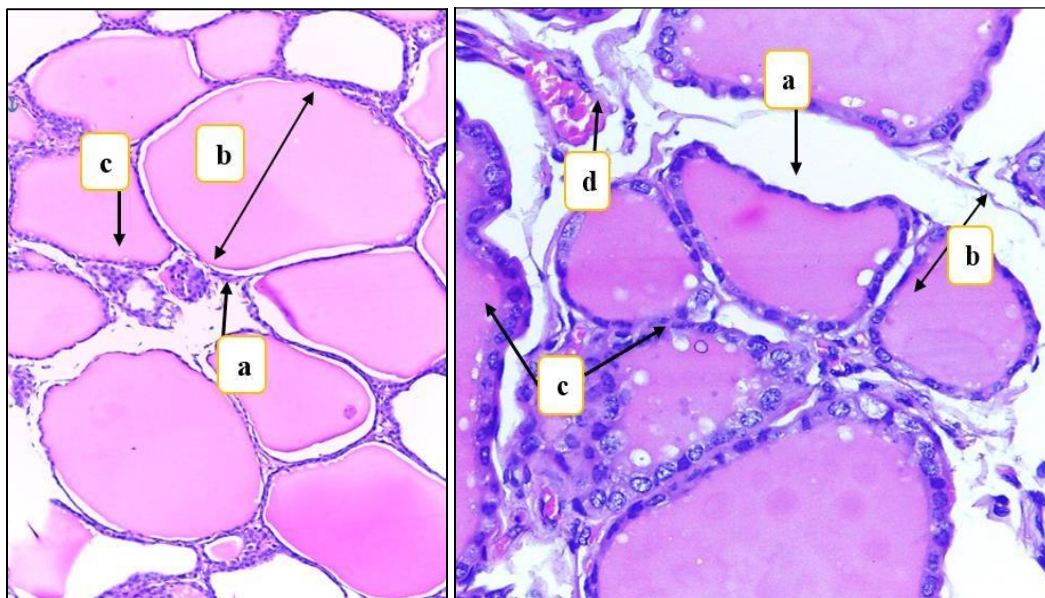


Fig 1: photographic of the thyroid gland in Goat show the, A. Thyroid follicles, B. Colloid, C. Follicular cells and (d) Blood vessel 400X H&E.

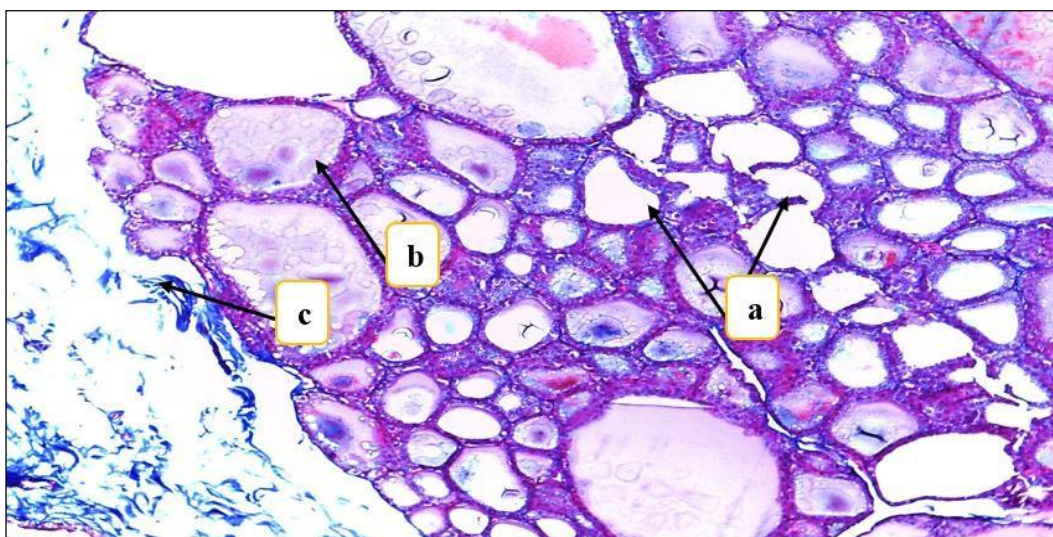


Fig 2; photographic of the thyroid gland in Goat show the, A. Thyroid follicles, B. Colloid, C. Capsule with connective tissue, 100X Masson Trichrome stain.

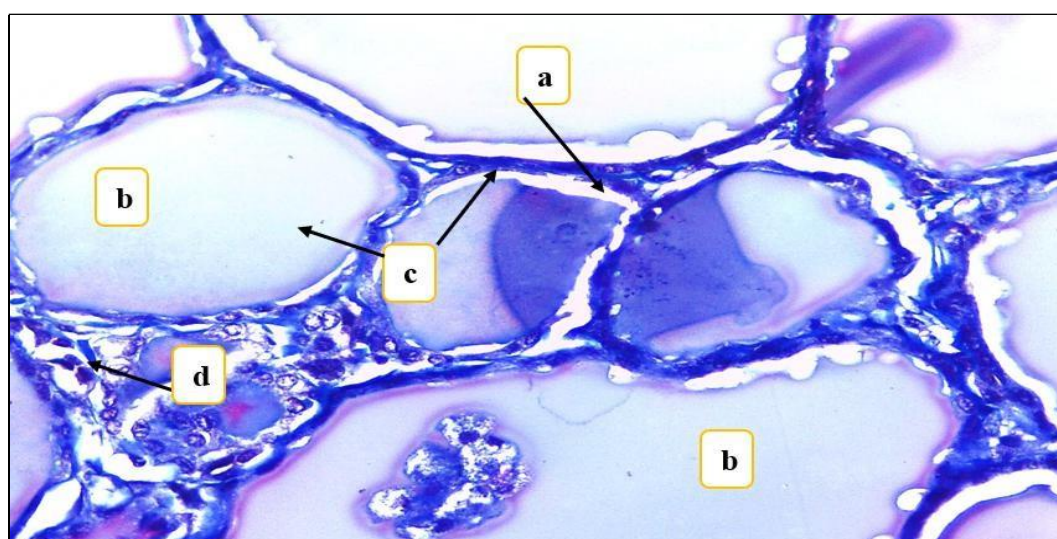


Fig 3: photographic of the thyroid gland in Goat show the, A. Thyroid follicles, B. Colloid, C. Follicular cells and (d) Blood vessel 400X Masson Trichrome stain

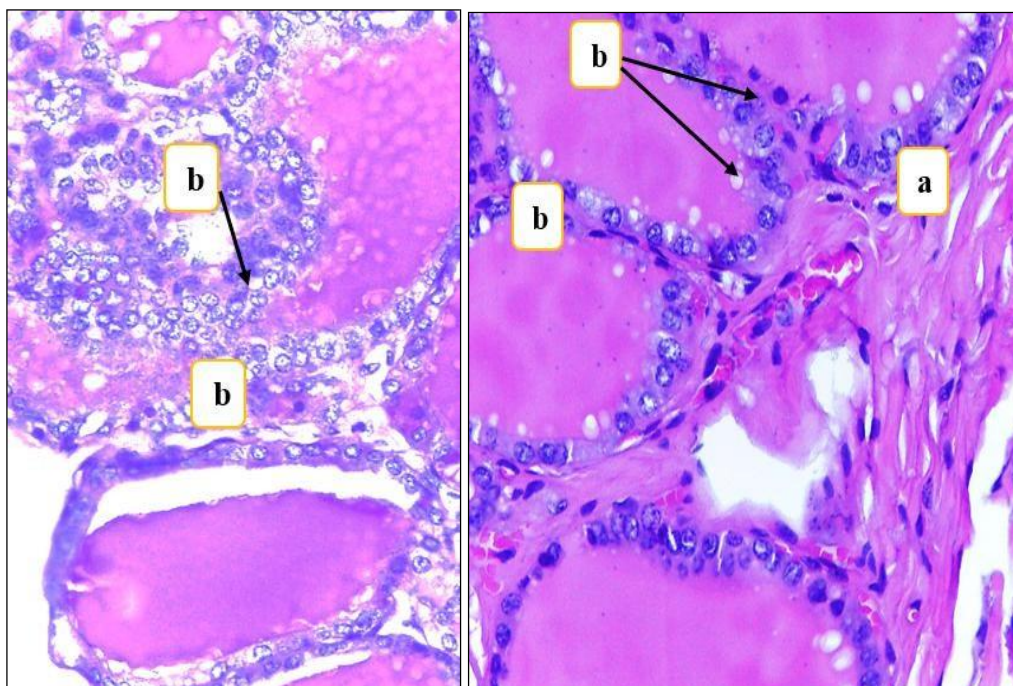


Fig 4: photographic of the thyroid gland in Goat show the, A. Connective tissue (Septa), B. Colloid, C. Follicular cells 400X PAS

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

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