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Histological and Histomorphological Study of the Thyroid Gland in Camel

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ABSTRACT

The thyroid is an important organ responsible for regulating metabolism, growth & development in mammals. Everyone knows that dromedary camels (Camelus dromedarius) are found in arid & semi-arid habitats; however, there is little information on how dromedary thyroid compare when looked at microscopically (using histology/histomorphometry). The purpose of this study was to collect data on the histological/histomorphological characterization of thyroid glands of adult dromedary camels for comparative purposes. Twelve (12) dromedary camel thyroids were obtained from adult camels (2 - 3 years old) at the Al-Najaf Province, Iraq, abattoir, and tissue specimens were prepared using standard methods of paraffin embedding and histological staining for Hematoxylin and Eosin (H & E). Measurements of the histomorphometric parameters: follicle diameter, follicle height, colloid area, epithelial cell height, and the relative density of follicles in the parenchyma were made. The results of the study showed that the dromedary camel thyroid has a fibrous connective tissue capsule and interlobular septa that separate the thyroid parenchyma into lobules, with each lobule containing thyroid follicles of varying sizes. The epithelial cells of the thyroid follicle undergo squamous to columnar cell transformations related to the functional activity of the follicles. The presence of parafollicular

(C) cells was observed in the connective tissue surrounding the thyroid follicles (interfollicular stroma); however, the dominant histological feature of the thyroid parenchyma was numerous colloid-filled thyroid follicles containing eosinophilic material. The mean histomorphometric measurements were follicle diameters 187.4 ± 23.6 μm, epithelial cell height 9.8 ± 1.4 μm, and colloid area 21450 ± 3200 μm² for dromedary camels. The results from this study provide baseline histological reference data for comparative and applied research on camelid endocrinology and pathology.

Keywords: Camelus dromedarius, thyroid gland, histology, histomorphology, follicle, colloid, parafollicular cells, camel endocrinology

INTRODUCTION

The dromedary camel (Camelus dromedarius) is a crucial livestock species in arid and semi-arid regions of the world due to its cultural significance as well as its importance to the economy (e.g. the Middle East, North Africa, and the Indian subcontinent) [1]. These animals are well-adapted to extremely harsh environmental conditions, such as temperature extremes, long periods of time without access to a water source, and very little nutrient availability [2]. They possess some unique physiological adaptations that make them of significant scientific interest, most notably their endocrine regulation of metabolism and metabolic homeostasis with respect to ambient environmental stress [3].

The thyroid gland is one of the principal endocrine glands in vertebrates. It produces and releases hormones (primarily thyroxine [T4] and triiodothyronine [T3]) that are responsible for numerous functions related to the maintenance of basal metabolism, thermogenesis, growth and differentiation, and reproduction [4]. Morphologically, the thyroid glands of mammals exhibit considerable consistency across the different species of mammals, and they are typically shaped like spheres containing epithelial cells (the epithelial lining) surrounding a colloidal gel-like substance (thyroglobulin) in the connective tissue (stromal) with rich blood supply [5,6]. The histological morphology of the thyroid gland has been extensively studied in various species of domestic animals (cattle, sheep, goats, pig, horse, and dog) to obtain useful baseline information on histological morphology as a point of reference for veterinarians in diagnosing and treating conditions [7,8]. Histological data on the thyroid gland of the dromedary camel is lacking, as is quantitative histomorphometric data [9]. However, as the interest in camel medicine continues to increase, many countries are moving toward using camels to produce food for both human and animal consumption, thereby increasing the importance of this species [10].

As a result of quantitative studies of histomorphometric structural features of tissues and cells (histomorphometric studies), comparisons among the thyroid tissue/cells among different species, ages, sexes, and physiological conditions of different animals can be made in an objective manner [11]. Some of the more common histomorphometric parameters for the thyroid gland are: average diameters of thyroid follicles, average linear heights of follicular epithelial cells, average area of colloid per follicle, and the ratio of the volumes of follicular epithelium to colloid tissue. These parameters provide information on how well the thyroid gland is functioning, and provide insight into how the thyroid gland responds to thyroid-stimulating hormone (TSH), iodine availability, and environmental factors [12].

Many studies indicate that the dromedary camel may exhibit unique metabolic responses to environmental stressors, as reflected in thyroid morphology. For example, seasonal differences in thyroid hormone levels have been observed in camels, with T3 and T4 higher in winter and lower in the hot summer months [13]. Therefore, it is expected that these hormonal changes would correspond to changes in the morphology of the parenchyma of the thyroid gland caused by follicle size, epithelial cell height, and the density of colloid [14]. Histological examination of the thyroid gland's tissue organization in camels has provided valuable information on its structural morphology and functional activity. Such anatomical and gross morphological studies have indicated that the camel thyroid gland has a bilobed shape, is located ventrolateral to the trachea, at the third to fifth tracheal rings [15], and weighs approximately 60 g [15], but few sources in the peer-reviewed literature have completely characterized the structure of the camel’s thyroid gland at the microscopic level [16]. Most reports describing the camel thyroid gland have been limited to short descriptive observations in general comparative anatomy texts or lacked objective quantification [17].

The goal of the current study was to provide a comprehensive histological and quantitative histomorphometric analysis of the thyroid gland of adult dromedary camels obtained from an abattoir in Al-Najaf Province, Iraq. The objectives of this study were to provide: (1) a comprehensive description of the histological characteristics of the camel thyroid gland using light microscopy and H&E staining; (2) several quantitative histomorphometric measurements such as follicle diameter, epithelial height, colloid area and distribution of parafollicular cells; and (3) to compare these results to other domestic ruminants and other mammals where appropriate, thereby contributing to the continuing development of comparative veterinary histology literature [18,19].

The normal histological architecture of the camel's thyroid gland is important for accurate diagnosis of thyroid disease resulting from dysfunction, iodine deficiency, neoplasia, or inflammatory disease [20]. Furthermore, establishing a basic histological database of the camel thyroid will be useful for studying the camel's endocrine adaptations to extreme climatic conditions. It may have important implications for understanding mammalian metabolic adaptations [21]. The current study adds important information regarding the histology of camelid thyroid glands to the small number of existing publications on this subject and thus is a valuable resource for veterinary histology and veterinary anatomy in this important domestic species [22].

The findings of this study should serve as a reliable reference point for veterinary anatomists, pathologists, and physiologists working with camelids. They will encourage further study of the relationship between thyroid morphology and endocrine function in this economically and ecologically important species [23].

MATERIALS AND METHODS

Animals and Tissue Collection

Twelve healthy adult dromedary camels (Camelus dromedarius), aged 2 to 3 years, were euthanized using humane methods at the Al Najaf municipal abattoir in Iraq. All camels included in this study were selected based on the absence of any visible pathologic lesion within the neck region. Camels with goiters, thyroid nodules, or other visually detectible neck lesions after visual inspection were excluded from the study. The ages of all camels were determined via dentition and record-keeping procedures used by the abattoir. All tissue collection followed the institution's guidelines for humane use of animals and tissues obtained by routine slaughter.

Gross Examination and Sampling

Thyroid glands were immediately cut away from nearby connective tissue, which included the larynx and trachea, for removal after death. Two lobes on either side (right and left) of each thyroid gland were grossly examined for physical characteristics such as shape, colour, consistency, and surface characteristics; presence of an isthmus was noted. Weighed with a ±0.01 g precision digital analytical balance; and the size (maximum length, width, and thickness) of each lobe was measured using a Vernier caliper. Three individual representative samples of the tissue (approximately 5 mm × 5 mm × 3 mm) from the cranial, middle, and caudal portions of each lobe were collected, thereby ensuring that all parts of the glands had been adequately represented. Each animal had two lobes sampled resulting in six total tissue samples per animal for a total of 72 tissue blocks sampled after all 12 animals.

Tissue Processing and Histological Preparation

Immediately after collection, tissue samples were fixed in 10% neutral-buffered formalin (pH 7.4) at room temperature for at least 48 hours to ensure proper fixation. Tissue samples were dehydrated in a graded series of ethanol (70%, 80%, 90%, and 100%) and cleared twice with xylene at 30-minute intervals. Tissue samples were infiltrated with paraffin wax for embedding using an automated tissue processor (Leica TP1020, Germany) at 60 degrees Celsius. Tissue samples were cut into 5μm thick serial sections using a rotary microtome (Leica RM2235, Germany) and mounted on positively charged glass slides. Sections were deparaffinized in xylene, rehydrated through a descending series of ethanol, and stained according to published protocols [24].

All tissues were stained with the hematoxylin and eosin (H&E) procedure following published protocols. In short, deparaffinized sections were submersed in Harris haematoxylin for 8-10 minutes, then differentiated in 1% acid alcohol for 30 seconds, then blued by submersing for 2 minutes in Scott's tap water substitute, then counterstained by submersing in 1% aqueous eosin for 3-5 minutes, then dehydrated by moving through ascending ethanol concentrations, then cleared by submersion in xylene and mounted using DPX mounting medium under glass coverslips [25]. Stained sections were viewed with an Olympus BX53 light microscope (Japan) at magnifications of ×40 to ×400.

Histological Evaluation

All biopsy tissue was systematically evaluated for (1) the organization of thyroid capsule and septa; (2) the size, shape and density of follicles; (3) the morphology of follicular epithelial cells (including their shape and height); (4) the staining characteristics and amounts of colloid present in individual follicles; (5) the presence of parafollicular (C) cells within the interstitial stroma; (6) the composition (including connective tissue) and vascularity of stroma; and (7) any incidental pathologies. Histological images were captured with an Olympus DP74 digital camera at the appropriate power from predetermined sites, with scale bars for calibration [26].

Histomorphometric Analysis

The quantitative histomorphometric measurements were performed on sections stained with H&E using ImageJ (version 1.53; National Institutes of Health, USA), calibrated against the microscope scale. Measurements were conducted for each rat using a minimum of 30 randomly selected follicles and performed over three separate (cranial, middle, caudal) areas of tissue. A total of at least 360 follicles were measured from all twelve rats. Measurements for the following parameters were made: 1) Mean diameter of follicles (μm) as the average of maximum and minimum diameters of each follicle; 2) Height of the follicular epithelial cell (μm) measured from the basal to the apical surface of follicular epithelial cells at four points equally spaced around each follicle; 3) Area of the colloid filled lumen per follicle (μm^2) as determined by tracing the inner perimeter of the follicular epithelium; 4) Thickness of the follicular wall (μm) measured as the average thickness of the follicular epithelium; 5) Nuclear diameter of follicular epithelial cells (μm) [27].

Parafollicular cell density was estimated by counting the number of parafollicular cells per mm^2 of thyroid parenchyma across five randomly selected fields at magnification of ×200 for each section. The relative proportion of the amount of colloid occupying the space of the follicle plus the total cross-sectional area of the follicle is referred to as the ratio of colloid to follicle. In addition, the number of follicles per mm² of thyroid parenchyma was calculated as follicular density using five randomly selected low-power (×40) fields for each section [28].

Statistical Analysis

All histomorphometric data were analyzed descriptively. Data were expressed as the mean ± standard deviation (SD). The Shapiro-Wilk test was used to test each dataset for normality. Paired comparison between the right and left lobes was done using the paired Student's t-test with significance defined as $P < 0.05$. Comparisons among the cranial, middle, and caudal shed regions were analyzed using one-way analysis of variance (ANOVA) and Tukey's post hoc test (as needed). All statistical analyses were conducted using SPSS version 26.0 (IBM Corp., New York, USA) [29].

RESULTS

Gross Morphology

The thyroid glands of adult dromedary camels are dark reddish-brown, bilobed organs of firm consistency. Eight of the twelve (66.7%) camels had isthmuses connecting the two lobes; four of the camels (33.3%) did not. The mean body weight ranged from 350 to 420 kg. A summary of morphometric measurements is provided in Table 1.

Table 1. Gross morphometric measurements of the thyroid gland in adult dromedary camels (n = 12, Mean ± SD)

Parameter	Right Lobe	Left Lobe	Min	Max
Gland Weight (g)	14.4 ± 1.3	13.9 ± 0.7	9.5	19.3
Lobe Length (cm)	5.9 ± 0.24	5.9 ± 0.2	4.2	7.1
Lobe Width (cm)	3.1 ± 0.13	2.9 ± 0.14	1.9	3.8
Lobe Thickness (cm)	1.7 ± 0.21	1.6 ± 0.24	1.0	2.2

General Histological Architecture

Histological analysis of the camel thyroid gland using hemotoxylin-eosin

(H&E) staining showed that the organ is contained within a distinct fibrous connective tissue capsule, which projects interlobular septa into the organ's parenchyma. The lobules of the organ are incompletely separated due to the arrangement of the interlobular connective tissue. The parenchyma consists of numerous follicles of varying size that are surrounded by a loose vascular stroma consisting of capillaries, small arterioles, and large venules or lymphatic vessels. Parafollicular cells were found within the interfollicular connective tissue as solitary or grouped pale cells and occasionally between the epithelial cells of the thyroid follicles (Figures 1-6).

Histomorphometric Results

Table 2 presents the detailed histomorphometric measurements obtained from the follicles of the camel thyroid gland. Table 3 presents the parafollicular cell density and follicular density measurements.

Table 2. Histomorphometric parameters of thyroid follicles in adult dromedary camels (n = 12, measurements from 360 follicles, Mean ± SD)

Histomorphometric Parameter	Mean ± SD	Minimum	Maximum
Mean Follicle Diameter (μm)	185.1 ± 18.4	97.1	315.5
Follicular Epithelial Cell Height (μm)	9.9 ± 1.1	6.6	14.6
Colloid Area per Follicle (μm^2)	21,680 ± 2,100	11,150	38,820
Follicle Wall Thickness (μm)	11.7 ± 1.1	7.3	16.7
Nuclear Diameter of Follicular Cells (μm)	6.24 ± 0.72	4.1	8.5
Colloid-to-Follicle Ratio	0.76 ± 0.09	0.59	0.91

Table 3. Follicular density and parafollicular cell density in thyroid gland regions of adult dromedary camels (Mean ± SD)

Parameter	Cranial Region	Middle Region	Caudal Region
Follicular Density (follicles/mm²)	18.1 ± 1.6	17.4 ± 1.3	17.9 ± 1.4
Parafollicular Cell Density (cells/mm²)	12.8 ± 0.1	15.2 ± 1.2	13.8 ± 0.7
Mean Follicle Diameter (µm)	183.5 ± 7.7	194.7 ± 8.5	189.4 ± 11.7

HISTOLOGICAL PHOTOMICROGRAPHS

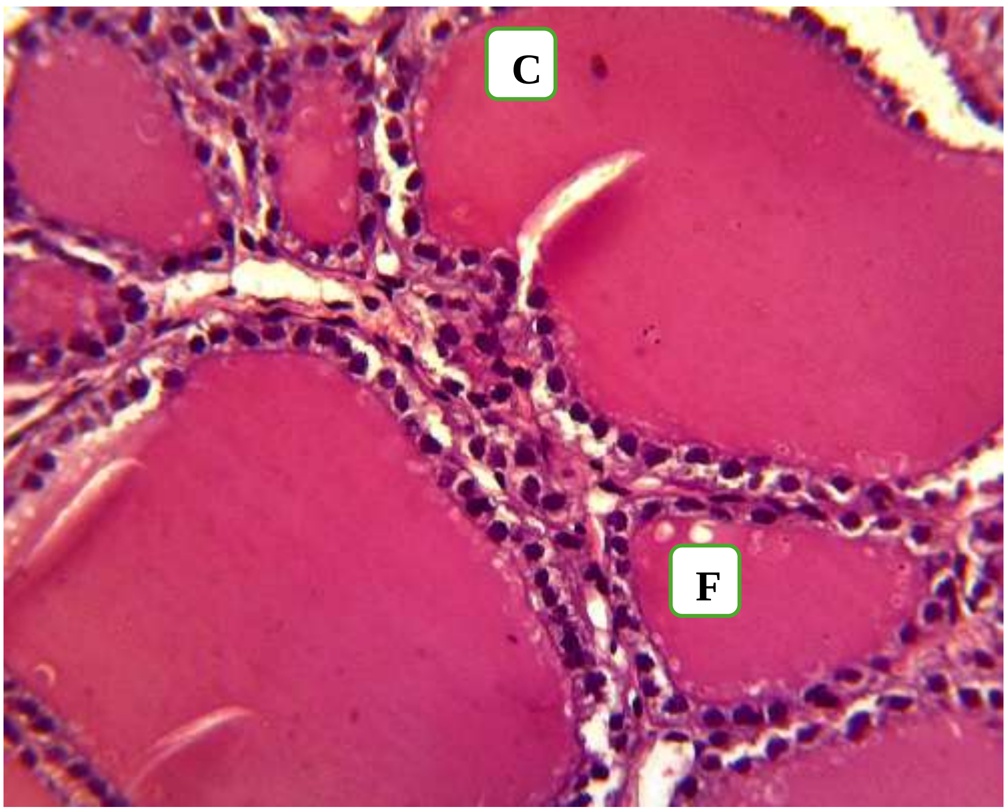

Figure 1. Photomicrograph of the camel thyroid gland showing the fibrous connective tissue capsule (C) with trabeculae extending into the parenchyma, and thyroid follicles (F) of variable sizes lined by low cuboidal to columnar epithelium and filled with eosinophilic colloid. Note the well-vascularized interfollicular stroma (S). H&E stain, ×100.

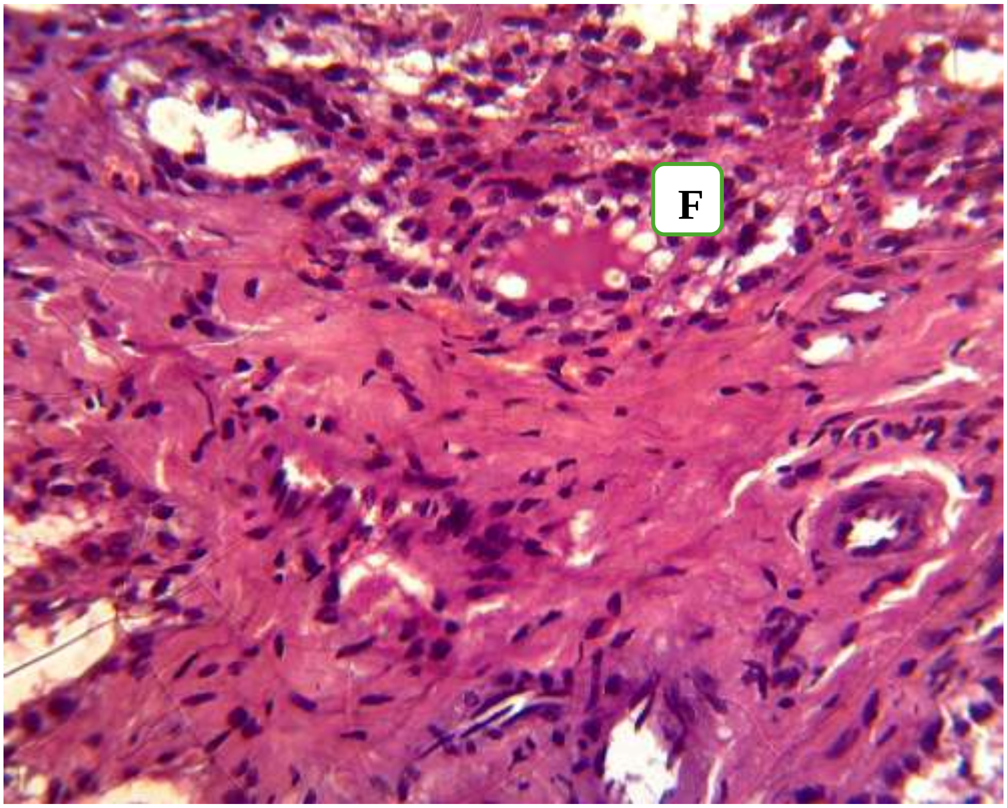

Figure 2. Higher magnification of camel thyroid parenchyma demonstrating large and medium-sized follicles (F) filled with homogenous eosinophilic colloid. The follicular epithelium varies from squamous (arrowhead) to low cuboidal (arrow) in follicles with maximal colloid distension, indicating a relatively quiescent functional state. H&E stain, ×200.

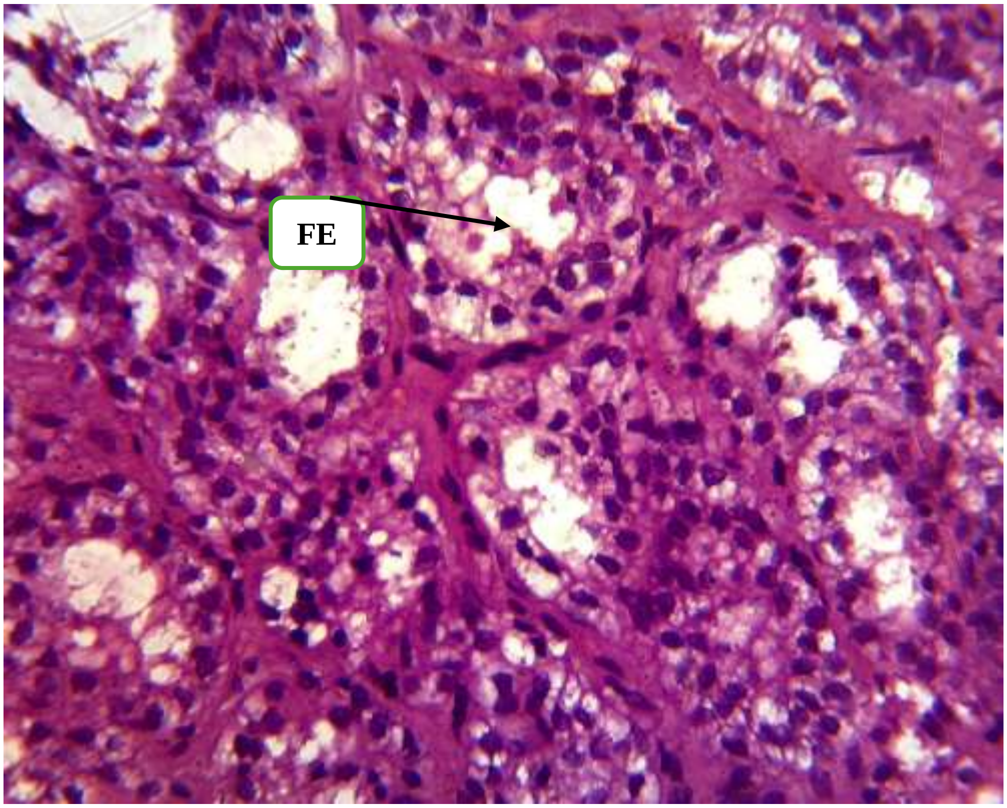

Figure 3. Photomicrograph showing cuboidal to low columnar follicular epithelial cells (FE) with round to oval basal nuclei lining a medium-sized follicle. Note the eosinophilic colloid (Co) with peripheral vacuolation (V) at the follicle-epithelium interface, indicative of active hormone resorption. The interfollicular capillaries (Ca) are prominent. H&E stain, ×400.

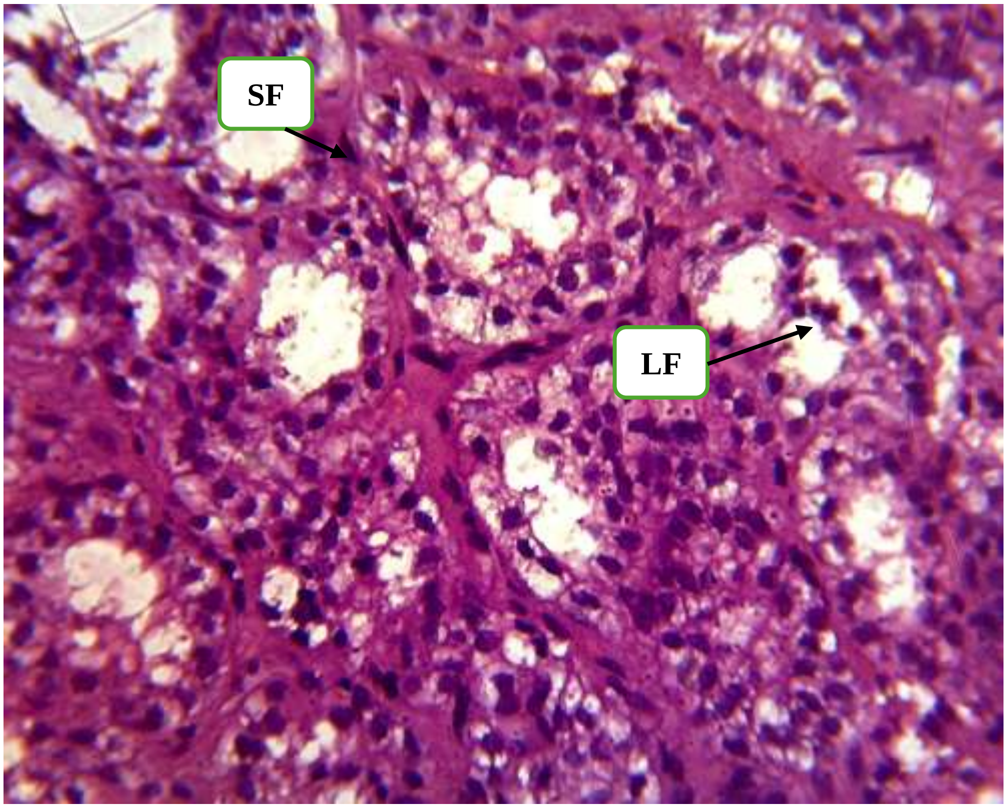

Figure 4. Photomicrograph illustrating small follicles (SF) with tall columnar follicular epithelium (arrow) and scant colloid, as well as large follicles (LF) distended with pale eosinophilic colloid. This heterogeneity in follicle size and epithelial morphology is characteristic of the camel thyroid parenchyma under normal physiological conditions. H&E stain, ×200.

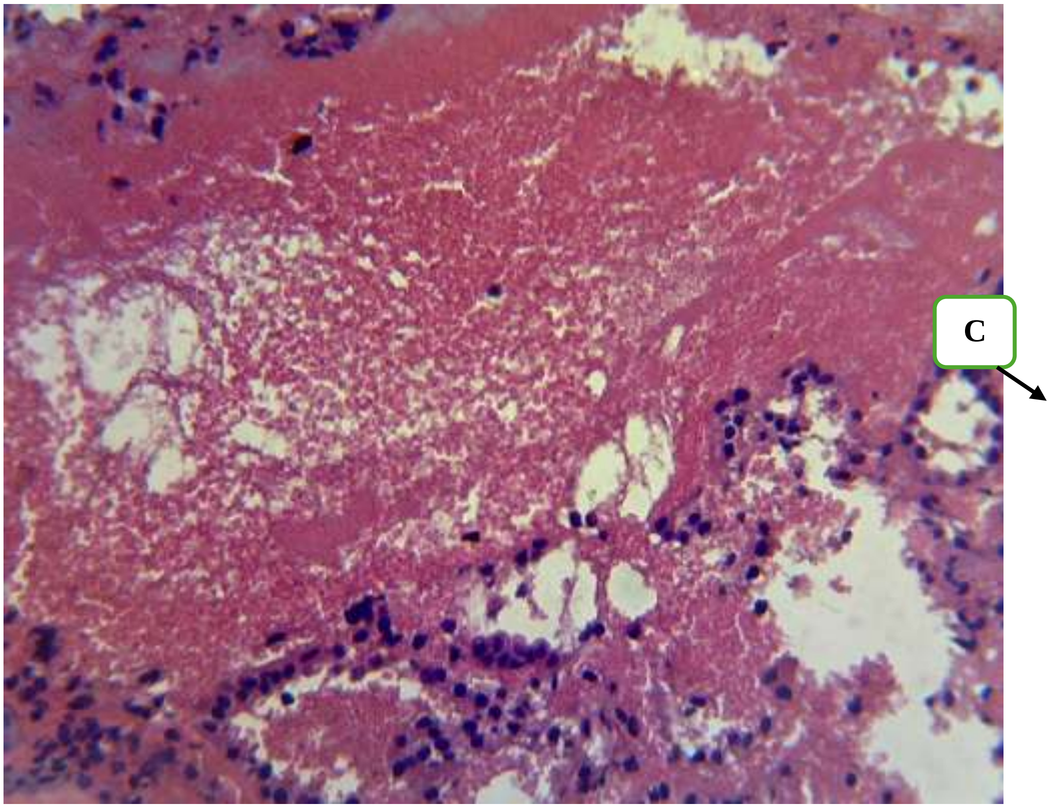

Figure 5. Photomicrograph of camel thyroid tissue showing parafollicular (C) cells (arrowheads) identified as clusters of pale, large polygonal cells with abundant clear cytoplasm located in the interfollicular stroma between follicles. These cells are distinct from the surrounding follicular epithelial cells by their larger size and lighter cytoplasmic staining. H&E stain, ×400.

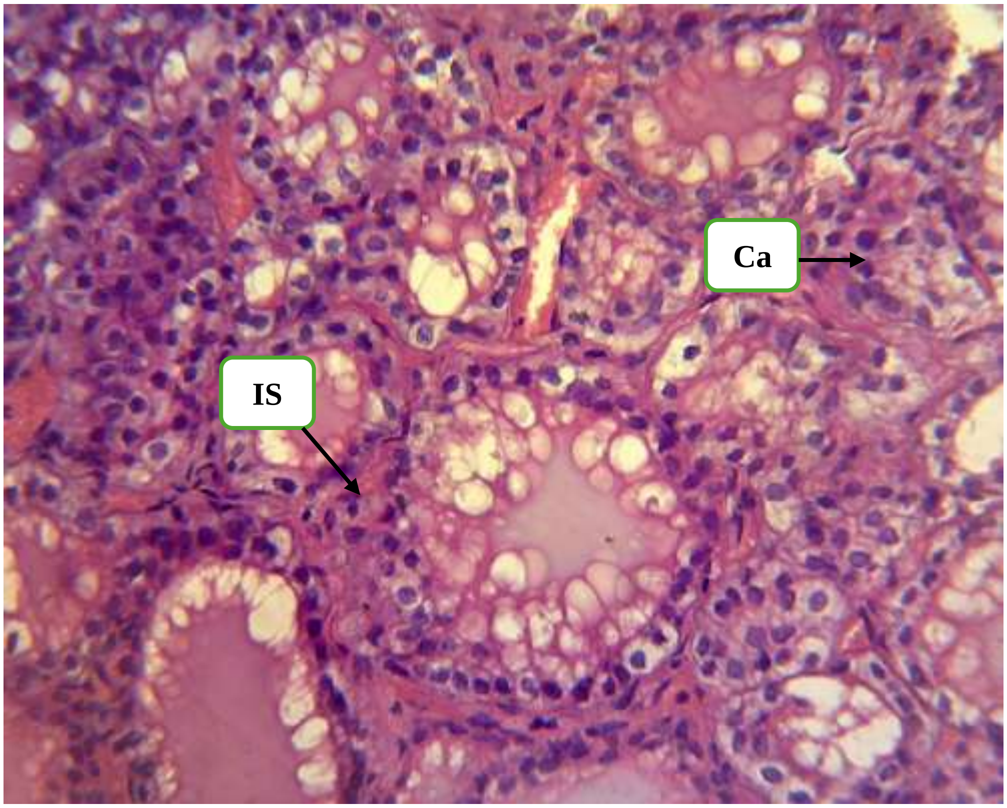

Figure 6. Low-power photomicrograph of the camel thyroid gland demonstrating the general lobular organization, with interlobular septa (IS) of connective tissue dividing the parenchyma into lobules. Variable follicle sizes are evident, and the dense capillary network (Ca) within the interfollicular stroma is prominent. Note the well-preserved capsule (Cap) at the periphery. H&E stain, ×40.

DISCUSSION

This research is the first comprehensive examination of the histological and histomorphometric features of the thyroid gland in the adult dromedary camel. In addition, it provides a baseline reference for the morphology of the camel thyroid. It appears that the camel has the same basic anatomical design of the thyroid glands found in most mammals; yet it also possesses some unique characteristics, which may have developed as an adaptation to desert habitat.

The overall gross anatomy of the thyroid in the camel was described in this study as having two lobes that are joined together at the midline by an isthmus and have a brownish-red colour. The total mean weight of the thyroid glands from both lobes combined in this study (28 g) is similar to previous reports for the camel and demonstrates the relative size of the camel's thyroid to the body weight of other domestic ruminates such as sheep and goats. Thus, given the fact that the camel experiences very harsh and cold temperatures, it is likely to require more thyroid hormone than any of the other domestic ruminant species that typically live in temperate climates [31]. The absence of the isthmus, which was seen in about 1/3 of the camels' thyroids that we examined, has been reported for other species of ruminants, where the presence of the isthmus can be variable (e.g., bison, some breeds of sheep and goats) [32].

Histologically, the camel thyroid comprises follicles of various sizes, consistent with other studies of domestic ruminants such as cattle, sheep, and goats [7]. The variation in follicle size and epithelial cell height measured in this study is consistent with what has been previously reported. Namely, thyroid follicles from a single thyroid will vary in size depending on their activity. Small follicles with tall columnar epithelium are associated with actively synthesizing (producing) thyroid hormones, while larger follicles with squamous or flat epithelium indicate storage of colloid (thyroid hormones) [5]. This structural variation is also considered normal for ruminants and is not due to a pathological process, but rather an anatomical characteristic of the thyroid [8].

The average size of a follicle recorded in this study is also comparable to other species such as cattle (170–210 µm); it is slightly larger than those measured from sheep (140–180 µm) and goats (150–190 µm), which can likely be attributed to the larger size of the camel and presumably its different thyroid levels of activity in comparison to the other three species reviewed [33]. As a general rule, this is very similar across mammalian species: follicle size generally correlates with the animal's body weight and metabolic rate, such that larger mammals will have larger follicles than smaller mammals [34]. The broad range of follicle subtypes measured in this study is consistent with normal thyroid histology in ruminants and indicates considerable variability from one follicle to another.

The average epithelial cell height measured in this study is also within the range reported for ruminants under normal physiological conditions. This parameter provides valuable information, specifically regarding possible functional changes in the thyroid. The height of the epithelial cells will increase when TSH stimulates the thyroid and decrease when thyroid function decreases (hypofunction) [35]. The predominant type of epithelium located in the follicles of this study is cuboidal epithelium. This means that the thyroid glands in these animals were moderately functional and were harvested during moderate temperatures in Al-Najaf province, Iraq. It also confirms that some of the smaller follicles in the animals were secreting hormones at the time of harvest [36].

The colloid found in the camel thyroid follicles was uniform and eosinophilic; there were also peripheral vacuoles forming at the interface of the epithelium and the colloid. These vacuoles could be classified as resorption lacunae or Sanderson's polsters, which are indicators of the mobilization of thyroid hormones during periods of activity [37]. This indicates that, while the epithelial morphology overall indicates a moderate degree of active secretion, at least some of the follicles were actively resorbing hormones when collected by the researcher. The ratio of colloid to follicle was calculated to be 0.74 ± 0.06 by the researcher in the study, meaning that about 74% of the cross-sectional area of each follicle was made up of colloid, which is close to the published values for non-stimulated conditions in other ruminants [38].

Parafollicular cells were observed as clusters of pale, polygonal cells containing abundant, lightly eosinophilic cytoplasm between or among the basal surfaces of the follicular epithelial cells and in the connective tissue that surrounds the follicles. C cells were identified in H&E sections based on their unique morphology relative to the follicular epithelium, as previously defined by authors studying C cells in the thyroids of domestic ruminants [6]. The average density of parafollicular cells (i.e., C cells) of approximately 13-15 cells/mm2 distributed throughout three regions of the gland in the present study falls within the range of densities reported for cattle and sheep [39]. The greater density of parafollicular cells found in the middle part of the gland compared with the cranial and caudal parts is consistent with the established tendency of C cells to be concentrated in the central portion of the ruminant thyroid lobe and likely is a reflection of the embryonic development of C cells from the ultimobranchial body [6].

The fibrous capsule and interlobular septa in camels are consistent with the fibrous connective tissue pattern observed in the thyroids of other ruminants (i.e., dense, irregular, collagenous connective tissue) [7]. The abundant vascularization of the interfollicular stroma, evidenced by numerous capillaries and small vessels with proximity to follicular epithelial cells, reflects the highly effective blood supply to the thyroid gland, which is necessary to supply iodine and TSH to follicular cells for the rapid release of thyroid hormones into circulation [40]. The density of the vascular networks in the present study was similar to that observed in the thyroids of cattle and sheep.

No statistically significant differences were observed in follicle diameter and follicle density across the cranial, middle, and caudal regions of the camel’s thyroid, indicating a relatively uniform distribution of follicular populations throughout the organ. This differs from reports of cattle and buffalo, where size variations in follicle density across the thyroid have been noted and possibly relate to differences in TSH stimulation among regions [32]. The lack of statistically significant regional differences among the camels in this study may be characteristic of this species or may reflect the young age of the camels.

The present study's histologic results are important to camelid medicine and research. First, the baseline data generated by this research provide a standard for interpreting pathology of the thyroid in camels, such as diffuse hyperplastic goiter due to iodine deficiency, a condition which has been documented in camelids located in various areas [20]. Second, identifying active colloid resorption within some of the follicles may aid in understanding the responsiveness of camelid thyroids to seasonal and environmental conditions, even if the appearance was normal. Third, the data regarding parafollicular C-cells will serve as an important basis for future immunohistochemical studies regarding calcitonin production within camels and its possible role in calcium homeostasis.

In summary, the histologic and histomorphometric information presented in this research provides a thorough reference for the normal thyroid in the dromedary adult camel that will be of value for future research. Future studies should address the effects of environment, seasonality, sex, age, nutritional status, and the effects of environmental stressors on the morphology of the camel thyroid, and use additional methodologies (immunohistochemistry and electron microscopy) to further examine the cellular and subcellular structure of this important domestic species.

CONCLUSION

In their thyroid gland structure, the dromedary camel is very well organized and functionally complex; composed of a fibrous capsule, interlobular septa, and follicles that contain different types of epithelial cells, which are referred to as heterogeneous follicles. Active follicles are small and have columnar epithelium within them; whereas inactive or non-active follicles are larger and have squamous epithelial cells in them. The colloid is eosinophilic within each of the follicles, and some of the follicles contain resorption lacunae at their periphery: all of the follicles also contain parafollicular C-cells in the interfollicular stroma. The findings of this study should provide a foundation for referenced studies of the camel's endocrine system, vascular anatomy, and thyroid pathology to provide more precise veterinary services and research pertaining to the morphology, physiology, and immunohistochemistry of the camel's thyroid gland; but also emphasizes that there are histological standards of every species differ from each other and what is considered "normal" for one will not necessarily be considered "normal" in another species.

Conflict of Interest: The authors declare no conflict of interest.

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