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## Gross and histomorphology of the thyroid gland in goats

**Poobitha S, Nair MG, Kumar R, Varshney KC, Uma Maheswari D, Uma S and Lakkawar AW**

**Abstract**

The morphometry of the thyroid glands from 72 apparently healthy cross-bred goat carcasses (age-groups: < 6 months, n=20; 6 months to 1 year, n=28, above > 1 year to 3 years, n=24) were studied. Progressive age-related increase was noticed in the length, width, and weight of the thyroid glands. Haematoxylin and eosin sections were subjected to image analysis and the histomorphometric parameters (median follicular diameter MFD, follicular area MFA, follicular epithelial height, MFH) were determined. The MFD of < 6 m, 6 m-1 y and > 1 y- 3 y was 84.24  $\mu\text{m}$ , 82.26  $\mu\text{m}$  and 82.29  $\mu\text{m}$  respectively. The MFA of < 6 m, 6 m-1 y and > 1 y- 3 y was 3824  $\mu\text{m}^2$ , 3542  $\mu\text{m}^2$  and 3492  $\mu\text{m}^2$  respectively. The MFH follicular epithelial height of < 6 m, 6 m-1 y and > 1 y- 3 y was 2.98  $\mu\text{m}$ , 2.8  $\mu\text{m}$  and 2.0  $\mu\text{m}$  respectively. The MFD of animals in 6 months and above was lower compared to < 6 months. The MFA and MFH showed a progressive decrease with increase in age the differences being significant ( $p < 0.05$ ) between the various age-groups.

**Keywords:** Goat, gross morphometry, histomorphometry, thyroid gland

**Introduction**

The thyroid is the largest endocrine organ consists of right and left lobes united by a narrow isthmus, which extends across the trachea anterior to second and third tracheal cartilages. Variations in hormone bioactivity allow the animals to adapt their metabolic balance to different environmental conditions, changes in nutrient requirements and availability, and to homeorhetic changes during different physiological stages. This is particularly important in the free-ranging and grazing animals, such as traditionally reared small ruminants, whose main physiological functions (feed intake, reproduction, hair growth) are markedly seasonal<sup>[1]</sup>. The morphology and location of the thyroid gland varies with species. The size of the gland is approximately 0.20% of bodyweight. It contains the highest concentration of iodine (0.2–5%) of dry weight and in the largest amount (70–80%) of total body iodine<sup>[2]</sup>. Morphometric evaluation of the thyroid gland is a sensitive indicator of the potential toxic effects of xenobiotics on the thyroid gland and also a valuable adjunct to histopathological evaluation and determination of thyroid weights to detect and quantitate subtle changes in follicular cells<sup>[3]</sup>. Goat (*Capra hircus*) is considered as an indicator species of iodine deficiency because of their browsing habits and less ingestion of soil compared to other grazing animals<sup>[2]</sup>. Gross morphology of the thyroid of goats have been reported<sup>[4-9]</sup>. Breed specific structural changes and variations within the goats have been reported from India<sup>[10-13]</sup>. Histologically, the thyroid gland has a capsule that consists of loose collagenous tissue. The perifollicular connective tissue consists of extensive quantities of reticular fibres. The structure or unit of thyroid gland is thyroid follicle. Follicles are hollow spheres whose size depends upon the activity of lining cells. The centre of each follicle is filled with a gel-like material called colloid, the storage form of follicular epithelial secretions. The lining epithelium varies from low cuboidal to high columnar. The thyroid epithelium consists of follicular lining cells and parafollicular cells (C cells). The size of follicles as well as their epithelial height is also an indicator of follicular activity<sup>[14]</sup>. Histomorphometry is a useful technique to evaluate the functional status of the thyroid gland by determining mean follicular diameter, mean follicular epithelium height, and proportions of glandular structural components and varies with the functional activity<sup>[15, 16]</sup>. Histometry of normal thyroid in man indicated that proportion of the gland occupied by the various histological components did not change appreciably with age, but the total thyroid volume increased quite rapidly with postnatal growth and declined

gradually in middle and old age [17]. Based on age-related morphological changes of thyroid gland in cattle and sheep, [18, 19, 20] it was recorded that the mean weight of thyroid gland increased and the mean follicular epithelial height decreased with age. Although hypothyroidism is reported to be the most common disorder affecting small ruminants, there are limited systematic studies carried out on the morphometry of the thyroid gland in goats [22]. The present study reports the gross morphometry, histology and histomorphometry of the thyroid gland in cross-bred goats and forms a part of the investigation on the occurrence of lesions of the thyroid glands in goats.

### Materials and Methods

The thyroid glands were collected from 72 apparently healthy cross-bred goat carcasses from the slaughter houses in Puducherry during the period from January 2014 to June 2014. In order to ascertain the influence of age and sex on thyroid gland, the goats were categorized into various age-groups (< 6 months, n=20, Male (M) 10 and Female (F) 10; 6 months to 1 year, n=28, M 14 and F 14; Above > 1 year to 3 years, n=24, M 12 and F 12). Both right and left thyroid lobes were carefully dissected out free of fat and a detailed gross examination with respect to the size, shape, colour and consistency was carried out. The length, width, circumference and weight of the right and left side of the lobes were determined. Lesions, if any, were recorded. After making longitudinal incisions, (Fig.1 & Fig. 2) the right and left lobes were fixed in 10% neutral buffered formalin (NBF) in separate containers. Representative tissues from each of the glands were processed by routine paraffin embedding and microtomy [22]. From paraffin embedded tissues, 4-5  $\mu$ m thick sections were prepared and stained by routine Haematoxylin and Eosin (H&E) staining procedure [22]. The H&E stained sections were examined to characterize and classify the microscopic changes. Histomorphometry was carried out on H & E stained sections of the thyroid glands. The follicular diameter, area and follicular epithelial height of the thyroid gland were determined as described [23]. All images were captured by using a trinocular microscope (Optika, Italy) fitted with Optika B5 camera and analyzed using Image J software (<https://imagej.nih.gov/ij/>, National Institutes of Health, USA). A single directional movement of the slide was used to avoid repeated measurement. For measuring the follicular diameter and follicular area, in each case, 3 fields were randomly selected from each lobe using the 20x objective. A representative histomorphometric measurement of follicular diameter is shown in Fig. 3. Total follicle area was identified for the 5 largest follicles (arbitrarily chosen to avoid introducing variability, as this parameter was highly dependent on the size of follicular cross section) that had no evidence of any artifactual distortion in each image by manually tracing the basement membrane. Once selected, the longest and shortest distance of follicle ( $L_f$  and  $W_f$ ) and longest and shortest distance of colloid ( $L_c$  and  $W_c$ ) were calculated (Fig. 4) for five follicles in each field and their mean follicular epithelial height were determined using formula  $((L_f - L_c) + (W_f - W_c))/4$  [23]. All data sets were tested for homogeneity of variance and normal distribution. The gross morphometric parameters (length, width, circumference, weight) were analyzed by Student's t test. The histomorphometric parameters were assessed by non-parametric test (Mann-Whitney U test). The level of significance used was  $p < 0.05$  [24].

### Results and Discussion

The thyroid gland of goats consisted of two fusiform shaped lobes connected by a fibrous isthmus and located on each dorso-lateral surface of the cranial trachea. The lobes were pale red to brown in colour. Variation in the shape of the thyroid in different breeds of goats have been reported to be either oval, pear or fusiform [8, 10, 14]. The length, width, circumference and weight of the left and right lobes of the thyroid glands were independently determined for both male and female goats categorized under various age-groups and are given in Table 1. Data of the left and right lobes as well as the combined data of male and female goats within an age-group and also between various age groups were compared and analyzed by Student's t' test. A progressive age-related increase was noticed in morphometric data of both the lobes of the thyroid gland. In general, the length of the left lobe was longer than the right. Progressive age-related increase was noticed in both left and right lobes. There was no significant difference in the length of the lobes between males and females. Combined data (male and female) of the length of the thyroid gland (mean, range) in the different age groups were: < 6 m (1.4, 0.98-2.2 cm); 6 m-1 y (2.2, 1.8-2.84 cm); >1 y -3 y (2.6, 1.8-2.9 cm). The thyroid glands of goats in the age group 6 months and above were significantly longer ( $p < 0.05$ ) than that of animals below 6 months. Further, the thyroid glands of the above 1 year group was also significantly longer  $p < 0.05$  than that of the < 6 m and 6 m to 1 y groups. Progressive age-related increase in width was noticed in both left and right lobes. There was no significant difference in the width of the lobes between males and females. Combined data (male and female) of the width of the thyroid gland (mean, range) in the different age groups were: < 6 m (0.8, 0.6-1.0 cm); 6 m - 1 y (0.9, 0.72-1.2 cm); >1 y -3 y (1.0, 0.8-1.2 cm). Although the width of the thyroid glands of goats showed an age-related increase, there were no significant differences between the three age groups. The data on the circumference indicated no significant differences either between the lobes or between males and females in the various age groups. Combined data (male and female) of the circumference of the thyroid gland (mean, range) in the different age groups were: < 6 m (2.0, 1.6-2.0 cm); 6 m - 1 y (2.1, 1.6-2.4); >1 y -3 y (2.0, 1.8-2.4). A progressive age-related increase in the weight was noticed in both left and right lobes. There were no significant differences in the weight between males and females. Combined data (male and female) of the weight of the thyroid gland (mean, range) in the different age groups were: < 6 m (0.398, 0.34-0.49g); 6 m - 1 y (0.52, 0.42-0.69 g); >1 y - 3 y (0.69, 0.48-1.12 g). The thyroid glands of animals of the age of 6 months and above were significantly heavier ( $p < 0.05$ ) than that of animals below 6 months. Further, the weight of the glands in above 1 year group was also significantly heavier ( $p < 0.05$ ) than that of the < 6 m and 6 m to 1 y groups. The observations made are accordance with earlier reports in various breeds of goats [10, 11, 12, 13]. Although the various histological components did not change appreciably with age in man, but the total thyroid volume increased quite rapidly with postnatal growth and declined gradually in middle and old age [17].

It was observed that the histology of both the lobes were not always uniform. The thyroid glands with normal histoarchitecture had characteristically, a thin capsule made of irregular connective tissue. The parenchyma comprised of thyroid follicles, parafollicular (C) cells, sparse interstitial loose connective tissue, and dense network of capillaries

(Fig.5). Follicles were round to oval in shape and lined by a single layer of follicular epithelial cells that were either flattened or low columnar, depending on the degree of activity of the gland (Fig. 6). Occasionally, follicular cells with abundant granular acidophilic cytoplasm (Hürthle cells) were noticed. In some of the cases, the thyroid gland appeared to be active. These cases were characterized by the presence of a row of small vacuoles located at the interface between follicular epithelium and the colloid (resorption vacuoles) and the phenomenon is referred to as scalloping (Fig. 7) and are in accordance with earlier reports in goats [5-8].

With the objective of ascertaining the influence of age and sex on thyroid gland, the histomorphometric parameters (median follicular diameter MFD, follicular epithelial height MFH and follicular area MFA) were determined for the sub-group of animals (< 6 m, 6 m to 1 y, > 1 y - 3 y). The combined histomorphometric data of the right and left lobes of both males and females are given in Table 2. The MFD of animals in 6 months and above was lower compared to the animals in below 6 months group. Combined data (male and female) of the MFD of the thyroid gland (median, range) in the different age groups were: < 6 m (84.24, 78.12-88.12  $\mu$ m); 6 m - 1 y (82.26, 77.89-85.45  $\mu$ m); > 1 y - 3 y (82.89, 79.85-86.12  $\mu$ m). Although the MFD of animals below 6 months was larger, there were no significant differences between the various groups. Using image analysis, assessment of the functional activity of the bovine thyroid indicated a reverse relation between follicular diameter and follicular height [15,16].

It has been recorded that epithelial height is a good indicator of thyroid activity [15]. Combined data (male and female) of the MFH of the thyroid gland in the different age groups (median, range) were: < 6 m (2.98, 1.6-2.82  $\mu$ m); 6 m - 1 y (2.8, 1.6-2.8  $\mu$ m); > 1 y - 3 y (2.0, 1.8-2.2  $\mu$ m). The MFH showed a progressive decrease with increase in age, the follicular epithelial height in animals of above 1 to 3 year group being significantly lower than the other two groups. It was recently reported in goats, [21] that the height of the epithelium of the active follicles (small) was  $7.72 \pm 0.44 \mu$ m and  $7.53 \pm 0.35 \mu$ m in the summer and the winter season respectively. However, the height of the epithelium of the inactive follicles (large) were  $5.83 \pm 0.44 \mu$ m and  $5.36 \pm 0.32 \mu$ m in the summer and the winter seasons respectively. The epithelial cell heights of the small, medium and large-sized follicles were  $7.52 \pm 0.05 \mu$ m,  $7.31 \pm 0.01 \mu$ m and  $7.12 \pm 0.06 \mu$ m respectively. The median follicular area (MFA) showed a progressive decrease with increase in age. Combined data (male and female) of the MFA of the thyroid gland in the different age groups (median, range) were: < 6 m (3824, 2957-3999  $\mu$ m<sup>2</sup>); 6 m - 1 y (3542, 2869-3896  $\mu$ m<sup>2</sup>); > 1 y - 3 y (3492, 3021-3912  $\mu$ m<sup>2</sup>). The differences in the MFA between the various age-groups were significant ( $p < 0.05$ ).

Establishing a baseline data on the gross and histomorphometry would be useful for interpreting radiographs, ultrasonographs and for toxico-pathological studies of the thyroid.

**Table 1:** Gross morphometry of the thyroid gland goats within various age-groups (n=72)

Parameters	Age group of goats														
	< 6 months (n=20)					6 months - 1 year (n=28)					> 1 year - 3 year (n=24)				
	Male (n=10)		Female (n=10)		Male & Female	Male (n=14)		Female (n=14)		Male & Female	Male (n=12)		Female (n=12)		Male & Female
	Left	Right	Left	Right		Left	Right	Left	Right		Left	Right	Left	Right	
L (cm)	1.5 (0.97-1.77)	1.2 (0.97-2.22)	1.35 (1.0-1.6)	1.15 (0.95-1.4)	1.4 <sup>***</sup> (0.98-2.2)	2.45 (1.95-2.85)	2.3 (1.97-2.85)	2.2 (1.8-2.85)	2.1 (1.8-2.4)	2.2 <sup>**b</sup> (1.8-2.84)	2.8 (1.7-3.0)	2.7 (2.0-3.0)	2.4 (2.05-2.97)	2.6 (2.4-2.9)	2.6 <sup>**c</sup> (1.8-2.9)
W(cm)	0.7 (0.6-0.82)	0.85 (0.6-0.92)	0.8 (0.57-0.92)	0.75 (0.57-1.0)	0.8 (0.6-1.0)	1.0 (0.97-1.12)	0.95 (0.87-1.12)	0.9 (0.7-1.0)	0.9 (0.67-0.92)	0.9 (0.72-1.2)	0.90 (0.8-1.07)	0.95 (0.82-1.0)	1.0 (1.0-1.2)	1.0 (0.9-1.17)	1.0 (0.8-1.2)
C (cm)	1.8 (1.8-2.1)	2.0 (1.8-2.2)	1.75 (1.4-1.92)	1.8 (1.47-2.0)	2.0 (1.6-2.0)	2.0 (1.8-2.25)	2.0 (1.8-2.2)	2.1 (1.8-2.6)	2.0 (1.6-2.4)	2.1 (1.6-2.4)	1.9 (1.8-2.0)	1.9 (1.8-2.15)	2.0 (2.0-2.3)	2.0 (1.8-2.2)	2.0 (1.8-2.4)
Wt (g)	0.416 (0.36-0.49)	0.432 (0.38-0.48)	0.378 (0.19-0.44)	0.336 (0.20-0.49)	0.398 <sup>**a</sup> (0.34-0.49)	0.492 (0.41-0.71)	0.56 (0.44-0.82)	0.54 (0.43-0.74)	0.50 (0.43-0.70)	0.52 <sup>**b</sup> (0.42-0.69)	0.639 (0.55-1.13)	0.838 (0.57-1.12)	0.512 (0.44-0.55)	0.488 (0.45-0.66)	0.69 <sup>**c</sup> (0.48-1.12)

n-number of animals, L-Length; W-Width; C-Circumference; Wt-Weight. Data analysed by Student's 't' test,

\*-Significant ( $p < 0.05$ ) and values with the same superscript indicates no significant differences between the groups,

**Table 2:** Histomorphometry of thyroid gland in goats within various age-groups (n=72)

Parameters	Histomorphometry														
	< 6 months (n=20)					6 months - 1 year (n=28)					> 1 year - 3 year (n=24)				
	Male		Female		Male & Female	Male		Female		Male & Female	Male		Female		Male & Female
	Left	Right	Left	Right		Left	Right	Left	Right		Left	Right	Left	Right	
MFD ( $\mu$ m)	82.19 (79.20-86.78)	85.25 (80.01-89.33)	86.56 (85.08-90.23)	86.29 (85.17-90.03)	84.24 (78.12-88.12)	80.47 (79.56-86.80)	80.12 (72.05-85.16)	84.97 (79.09-88.80)	82.75 (78.03-86.48)	82.26 (77.89-85.45)	85.24 (79.41-88.61)	85.37 (80.95-90.64)	84.75 (82.37-86.87)	84.84 (80.98-86.93)	82.89 (79.85-86.12)
MFH ( $\mu$ m)	3.25 (2.6-4.7)	2.95 (1.6-4.1)	2.65 (1.48-2.96)	2.32 (1.77-2.99)	2.98 <sup>**a</sup> (1.6-2.82)	2.0 (1.0-2.42)	3.0 (1.8-3.2)	2.1 (1.4-3.0)	2.5 (1.9-3.1)	2.8 <sup>**a</sup> (1.6-2.8)	2.5 (1.9-3.7)	1.85 (1.1-2.5)	2.3 (1.55-2.9)	2.3 (2.0-2.4)	2.0 <sup>**b</sup> (1.8-2.2)
MFA ( $\mu$ m <sup>2</sup> )	3957 (2965-4270)	3777 (3189-4568)	3844 (3636-4047)	3827 (3530-4005)	3824 <sup>**a</sup> (2957-3999)	3786 (3106-4020)	3332 (2996-3964)	3725 (2978-4002)	3626 (2987-4090)	3542 <sup>**b</sup> (2869-3896)	3675 (3131-3988)	3584 (3054-4091)	3526 (3364-3944)	3534 (3125-3689)	3492 <sup>**c</sup> (3021-3912)

Values are expressed in median (Q1, first quartile-Q3, third quartile), MFD- Median follicular diameter, MFH- Median follicular epithelial height, MFA- Median follicular area. \*-Significant ( $p < 0.05$ ) and values with the same superscript indicates no significant differences between the groups, n-number of animals.

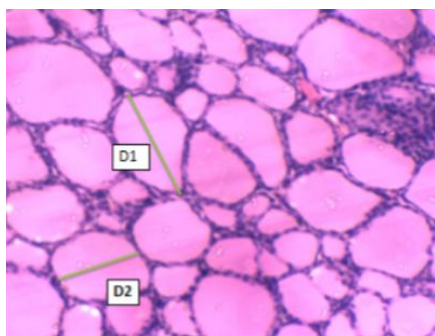




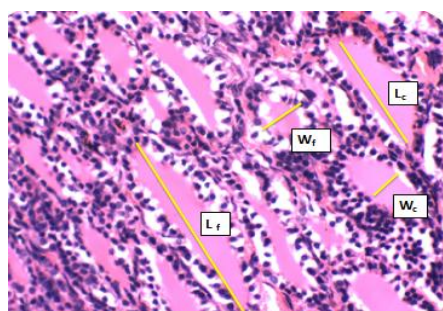
**Fig 1:** Normal sized lobes of the thyroid gland of goat L (Left lobe); R (Right lobe)



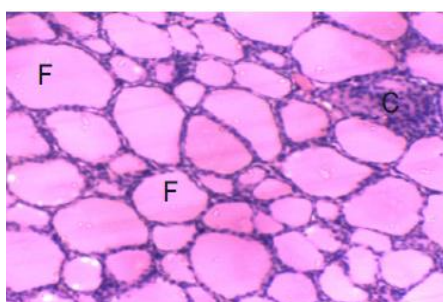
**Fig 2:** Longitudinal section of a lobe of thyroid



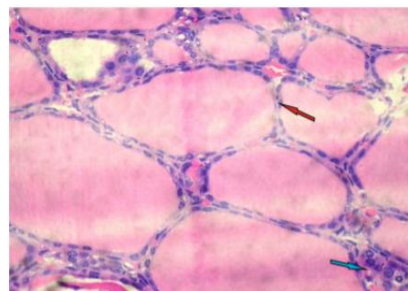
**Fig 3:** Image of normal thyroid gland showing measurements of follicle diameter (D1, D2, μm). (H&E x200)



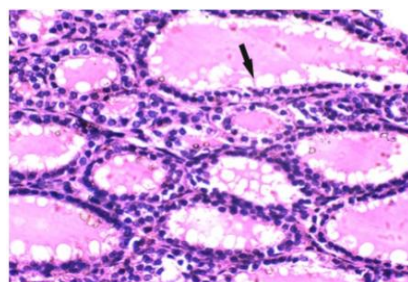
**Fig 4:** Image of normal thyroid gland showing measurements of follicular epithelial height: L<sub>f</sub>, L<sub>c</sub>- longest distance of follicle and colloid; W<sub>f</sub>, W<sub>c</sub>- shortest distance of follicle and colloid. H&E x200



**Fig 5:** Normal histoarchitecture of thyroid gland comprised of follicles (F) with sparse interstitial tissue. H&E x100.



**Fig 6:** Thyroid gland showing follicular epithelial cells (red arrow), C-cells (blue arrow). H&E x100



**Fig 7:** Thyroid gland showing resorption vacuoles (arrow) at the interface between follicular epithelial cells and colloid (Scalloping). H&E x200

### Conclusion

The thyroid glands of 72 carcasses were studied for morphometric and histomorphometric parameters. Gross morphometric studies showed an age-related increase in the length, width, circumference and weight of both left and right lobes of the thyroid glands. Histomorphometry was useful tool to quantitate follicular size and shape variations in the thyroid gland. Histometric measurements of the thyroid gland would be useful understand the structural adaptations to physiological conditions or due to disease process.

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