

## A histomorphometrical evaluation of the diameter of thyroid follicles at the proximal, middle, and distal extremities of the thyroid gland in Dareshuri horses (*Equus Caballus*)

R.A. Fatahian Dehkordi<sup>1</sup>, K. Forouhar Majd<sup>2</sup>, M. Hesamian<sup>2</sup>, H. Azadi<sup>3</sup>

<sup>1</sup> Department of Basic Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

<sup>2</sup> Graduated from the Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

<sup>3</sup> Graduated from the Faculty of Veterinary Medicine, Shahrekord branch, Islamic Azad University, Shahrekord, Iran

### \*Correspondence:

Author email:  
[Fatahian\\_1349@yahoo.com](mailto:Fatahian_1349@yahoo.com)

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**Abstract** The parenchyma of the thyroid gland is comprised of cells arranged in a spherical follicle, surrounded by a dense network of capillaries. These cells exhibit a range of morphologies, from squamous to cuboidal. The present study investigates the differences in follicle diameter between the proximal, middle, and distal regions of the thyroid gland in horses. While preparing tissue samples from five female Dareshuri horses and processing them in the histopathology laboratory of the veterinary faculty at Shahrekord University, paraffin blocks were prepared using a microtome with incisions of 5 µm in thickness. The data obtained from the histomorphometric evaluations of the proximal, middle, and distal regions of the thyroid gland revealed that the highest mean diameter of the small thyroid follicles was observed in the middle region, while the lowest mean diameter was observed in the distal end of the gland. The diameter of the large follicles reached their highest and lowest values, respectively, in the proximal and middle extremities of the thyroid gland. The results of this study indicate that there is a significant difference in diameter between the middle and distal extremities of the thyroid gland.

### Introduction

The thyroid gland, which is the largest endocrine organ, is found in the anterior part of the larynx and situated dorsolateral to the second and third tracheal rings in horses [1, 2, 3]. The structure is composed of two lobes that are joined ventrally by a narrow isthmus [3 - 5]. The thyroid parenchyma is composed of spherical follicles arranged in a network of dense capillaries. The cells within this structure exhibit a range of morphologies, from squamous to cuboidal [5 - 7]. The follicular lining cells are acidophilic and

contain a high concentration of secretory cytoplasmic vesicles. The thyroid gland is encased in a layer of connective tissue that extends as distinct septa between the follicles, providing a framework for lymphatic vessels, nerves, and the capillary network [3, 7, 8]. The capsule of each thyroid lobe is composed of loose collagenous connective tissue, which provides structural support to the organ through the formation of connective tissue walls. The connective tissue that surrounds the follicles, which contain reticular fibers, constitutes the structural units of the thyroid gland, which are

known as thyroid follicles [6, 9]. These are hollow, spherical bodies whose size is dependent upon the activity of the lining cells. The center of each follicle is filled with a gelatinous material, colloid, which serves as the storage form for secretory material [2, 3]. The height of the follicular epithelium exhibits a range of variation, from low cuboidal to tall columnar [4, 10]. The follicular epithelium is comprised of follicular cells, which constitute up to 90% of the cell population, as well as parafollicular cells [1]. The acidophilic follicular cells, which exhibit centrally placed nuclei, may contain pale-staining supranuclear Golgi complexes, which are sometimes considered to be secretory vesicles [7, 8, 11]. The parafollicular cells are situated between or in proximity to the follicular cells, extending to the follicular lumen. During the process of dissecting the follicles from the surrounding connective tissues, the remaining pale-staining cells are identified as the parafollicular cells. The colloid contains proteins, glycoproteins, and typically acidophilic enzymes that exhibit periodic acid-Schiff staining positivity [8, 12, 13]. An active follicle displays an acidophilic colloid with a vacuolated periphery, whereas inactive follicles, which may be basophilic or acidophilic, exhibit no peripheral vacuolation. The dimensions and height of the follicular epithelium serve as indicators of thyroid gland activity. Follicular activity is inversely correlated with follicular size [4, 8, 10]. The epithelium of small follicles is typically tall and the colloid volume is conspicuous, indicating greater activity. In contrast, large follicles have a low epithelium and a moderate colloid volume [1, 6, 9]. Given the dearth of research on histomorphometric analysis of thyroid follicle diameters and the subsequent assessment of the activity of disparate regions of the equine thyroid gland, this study seeks to elucidate the discrepancies in follicle diameters and activity among the proximal, middle, and distal regions of the thyroid gland in the Dareshuri horse (*Equus Caballus*). An understanding of the arrangement of thyroid follicles in horses provides insights into their physiological conditions and adaptation to environmental factors [1, 13, 14].

## Materials and Methods

This study was conducted on five female Dareshuri horses with the aim of examining the histomorphometric structure of various regions of the thyroid gland. The thyroid glands of the horses were transferred to the histopathology laboratory of the Faculty of Veterinary Medicine of Shahrekord University and placed in a 10% formalin buffered solution. To investigate the histomorphological structure of the thyroid glands, the right and left lobes were promptly separated immediately after slaughter. Subsequently, the glands were sliced into sections 5 millimeters in thickness and immersed in a 10% formalin-buffered solution for a period of 72 hours. Following fixation, the specimens were processed in an automatic tissue processor and underwent a tissue processing program in the histopathology laboratory of Shahrekord University. The tissue specimens were then dehydrated in solutions of increasing alcohol concentration, ranging from 70%, 80%, to 90%, and finally 100%.

Following the tissue clearing process with Xylol, the tissue samples were embedded in paraffin, and 5-micrometer-thick sections were prepared from the paraffin blocks. Upon staining with Hematoxylin-Eosin, the specimens were examined under a Nikon light microscope with an eyepiece micrometer and a  $\times 100$  magnification lens for the purpose of conducting histomorphometric studies of the follicle diameters in disparate regions of the thyroid gland. The data were analyzed using SPSS software to determine the diameters of small and large follicles in the various regions of the thyroid glands. One-way ANOVA and LSD tests were employed to ascertain the statistical significance of the observed differences in the data, with a significance level of  $P \leq 0.05$ .

## Results

From a histomorphometrical perspective, the thyroid gland is encased externally by a thin capsule of loose collagenous connective tissue, with trabeculae penetrating the gland and dividing it into irregular lobules, each comprising

numerous follicles of varying sizes and shapes. In the stroma between the follicles, there are efferent blood vessels responsible for the drainage of the follicular epithelium, which may be a single layer of short cuboidal to tall columnar cells surrounding each follicle. The interior space of the follicle is filled with colloid material, and the size of the colloid-secreting cells in each follicle is nearly uniform. Follicles can be classified into two groups based on their size: small follicles and large follicles. The diameters of large follicles in the middle and proximal regions exhibit maximum and minimum values, respectively.

**Table 1.** Comparison the mean diameter of small follicles in the proximal, middle, and distal parts of the thyroid gland of Dareshouri horses.

Extremities of small follicles in thyroid gland		Mean
Proximal extremity	Middle extremity	0.130
	Distal extremity	0.257
Middle extremity	Proximal extremity	0.130
	Distal extremity	0.024
Distal extremity of	Proximal extremity	0.257
	Middle extremity	0.024

The statistical analysis of the data indicates that there is no statistically significant difference in the diameters of large thyroid follicles between the different regions of the thyroid gland in horses. The highest mean

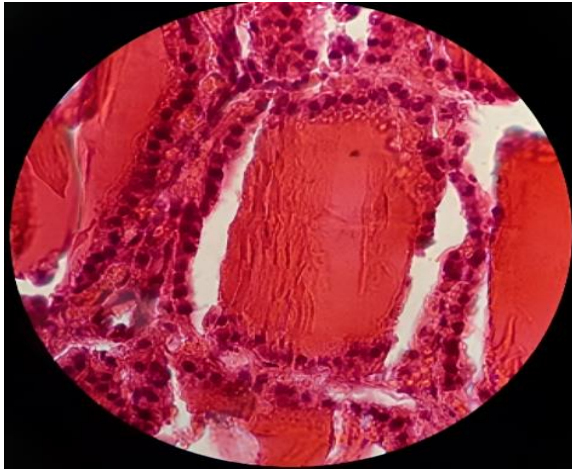
diameter of small thyroid follicles is observed in the middle region, while the lowest is in the distal region (Tables 1-3). The statistical analysis of the data yielded significant results with regard to the diameters of small follicles, indicating a notable disparity between the middle and distal regions (Figure 1-3). In large follicles, the height of the follicular epithelial cells is shorter in comparison with small follicles. In some cases, the cells appear as squamous cells due to the surrounding pressure of the larger follicles. In contrast, small follicles have taller cells in comparison with large follicles. The colloid in smaller follicles is typically completely acidophilic and dense, whereas the colloid in larger follicles is less acidophilic, thinner, and more uniform in appearance.

**Table 2.** Comparison the mean diameter of large follicles in the proximal, middle, and distal parts of the thyroid gland of Dareshouri horses.

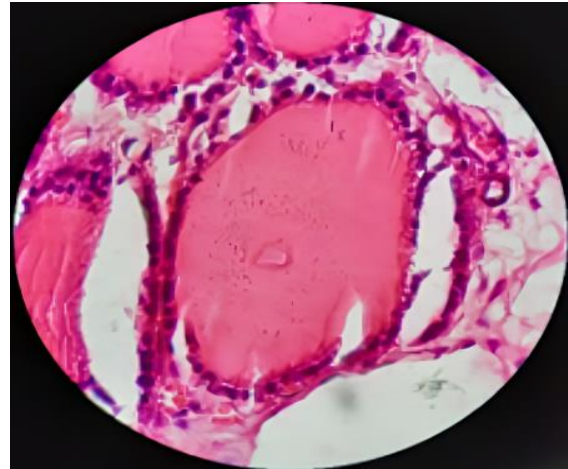
Extremities of large follicles in thyroid gland		Mean
Proximal extremity	Middle extremity	0.232
	Distal extremity	0.506
	Proximal extremity	0.232
Middle extremity	Distal extremity	0.575
	Proximal extremity	0.506
Distal extremity	Middle extremity	0.575

**Table 3.** Mean diameters of proximal, middle, and distal regions of thyroid follicles in thyroid gland of Dareshouri horses

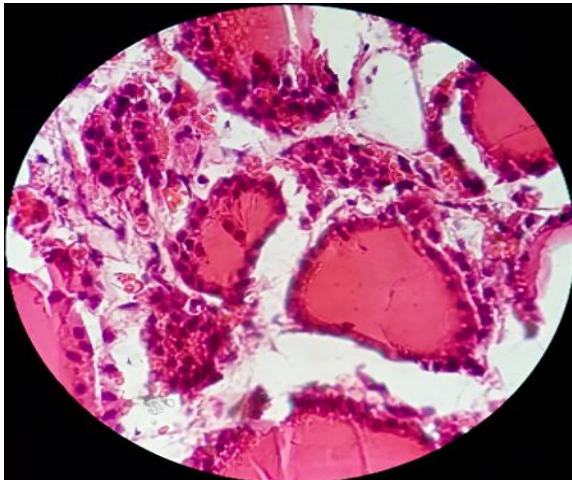
	Distal extremity in large follicles	Middle extremity in large follicles	Proximal extremity in large follicles	Distal extremity in small follicles	Middle extremity in small follicles	Proximal extremity in small follicles
Mean diameter of follicles	22.89	23.83	21.46	12.8	15.54	13.88
SD	2.11291	1.67246	1.94659	0.87611	1.12844	1.07219



**Fig 1.** Proximal extremity thyroid follicles of Dareshuri horses thyroid (H&E staining, x100 magnification).



**Fig 3.** Distal extremity of thyroid follicles of Dareshuri horses (H&E staining, x100 magnification).



**Fig 2.** Middle extremity of thyroid follicles of Dareshuri horses (H&E staining, x100 magnification).

## Discussion

From a histomorphometrical perspective, the thyroid gland of the horse exhibits notable similarities to that of other domestic mammals. Histological studies on the one-humped camel indicate that the thyroid gland is covered by a thick fibrous capsule and numerous follicles of varying sizes.

The lining epithelium of the follicles is composed of squamous to low cuboidal cells with a basally located round or spherical nucleus. In the male thyroid gland, the nucleus is slightly larger than in the female. The results indicate a slight increase in the epithelial height and follicular diameter of the thyroid gland in females compared to males [3, 15]. In a separate investigation involving dromedaries, the findings indicated that the mean follicular surface area is larger in females compared to males. The epithelial cells are observed to be at a higher level in males compared to females [16]. A review of the literature reveals a progressive decrease in follicular epithelial height with increasing age in goats [17].

The research findings indicate that the thyroid gland is composed of lobules, which are structural units of the thyroid gland. These lobules contain thyroid follicles, which are characterized by varying sizes and shapes due to the penetration of trabeculae from the glandular collagen capsule into the gland [10 - 12]. In horses, studies on smaller follicles, designated as active follicles, have revealed a discrepancy in the diameters of small follicles between the middle and distal regions of the gland. Conversely, no such variation was observed in the diameters of large follicles between the

different regions of the thyroid gland. Nevertheless, no distinction was discerned between the proximal and distal regions of the thyroid gland [4, 10]. The density of loose connective tissue, connective tissue cells, and the vascular bed is also higher in the center of the gland, which correlates with the activity of the central follicles [7, 11, 13].

As observed in other domestic mammals, the follicular epithelium of the thyroid follicles is composed of cuboidal cells that form the follicular wall, and the center of the follicles is filled with colloid. In follicles of a smaller size that demonstrate a high level of activity, the peripheral margin may exhibit vacuolization and scalloping. In follicles with reduced activity, a wall of short cuboidal cells is observed, which on occasion exhibit squamous characteristics [5, 7, 8].

## Conclusion

The results of this study indicate that there are variations in the diameters of small follicles between the middle and distal regions of the thyroid gland in horses. Conversely, no significant difference was observed in the diameters of large follicles across the different regions. An understanding of the arrangement of thyroid follicles in horses provides insights into their physiological conditions and adaptation to environmental factors.

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## Conflict of interest

The authors declare that they have no competing interests.

## Ethical approval

All ethical considerations including utilizing animals were considered cautiously.

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