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Histomorphochemical and Ultrastructural Studies on Thyroid Gland of Murrah Buffalo (*Bubalis bubalis*)

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ABSTRACT

Background: Buffalo is an important livestock of India that significantly contributes to the rural Indian economy through the milk and meat production. Murrah is a renowned and valuable dairy breed of buffalo found in Haryana and Punjab. The thyroid gland secretes thyroxin, triiodothyronine and calcitonin hormones which are crucial for body metabolism, growth, thermoregulation and production. So, the present study was aimed to explore histomorphochemical and ultrastructural details of thyroid gland of Murrah buffalo.

Methods: Total twelve samples of thyroid gland of adult Murrah buffalo were collected and fixed in 10% neutral buffered formalin and processed for acetone-benzene schedule for routine paraffin block preparation. The paraffin sections were stained with Hematoxylin and eosin and other special stains while fresh cryo-sections were used for demonstration of lipids. Three tissue samples were fixed in Karnovsky's fixative and processed for ultrastructural studies.

Result: Histologically, thyroid parenchyma was enveloped by a thick capsule. Follicles appeared in a wide range of sizes and shapes and the follicular epithelium was made up of both follicular and parafollicular cells. Vacuoles were mainly observed in the active follicles. Ultimobranchial follicles were found in various places. Colloid exhibited strong PAS reactivity, indicating the presence of neutral mucopolysaccharides. Capsule, Blood vessels and follicular cells all displayed moderate to strong reactions for the presence of basic proteins. Ultrastructurally, the follicular cells exhibited a large number of secretory vesicles, whereas parafollicular cells contained a small number of secretory granules.

Key words: Buffalo, Colloid, Follicles, Histological, Thyroid gland, Ultrastructure.

INTRODUCTION

Buffalo (*Bubalis bubalis*) is an essential livestock of India, particularly in the Haryana and Punjab states. Buffalo provides a substantial contribution to the production of meat, milk and hide. Since the buffalo alone accounted for 49% of all milk produced in India (MoFAH and D, 2019) and 12% of global milk production (Kumar and Pradhan, 2014), it is a crucial component of the Indian agricultural economy. Due to its physical and anatomical traits, the buffalo is ideally suited to muddy terrain and humid, hot weather. Murrah is essentially a dairy breed and often regarded as "Black Gold" (Kumar *et al.,* 2019). Unfortunately, the potential of buffalo has seldom been appreciated, recognized and explored (Bhat, 2010).

The thyroid gland of the buffalo is located on the lateral aspect of the thyroid cartilage and the first two tracheal rings, comprising of irregularly triangular left and right lateral lobes joined by a thin strand of isthmus (Poonia *et al.*, 2023). The thyroid gland contains follicular cells, which secrete thyroxin (T4) and triiodothyronine (T3) hormones and parafollicular cells that produce calcitonin hormone. The physiologically active hormones triiodothyronine and thyroxine are necessary to maintain appropriate levels of metabolic activity. Calcitonin antagonizes parathromone and directly inhibits osteoclasts reducing bone resorption and lowering blood calcium levels. The negative feedback of serum calcium concentration on C cells directly controls calcitonin release (Khaleel and Salih, 2017). Growth, metabolism, thermoregulation and the body's response to environmental changes are all influenced by these hormones (Kierszenbaum and Tres, 2019). Serious clinical diseases with visible symptoms can result from either excessive or insufficient thyroid hormone production. The literature reports histomorphochemical studies on the thyroid gland of buffalo (Hussain and Al-Taay, 2009) and ultrastructural studies of the thyroid gland of goats (Ali *et al.,* 2020). The present comprehensive study was designed to examine histoarchitecture, histochemical and ultrastructure characteristics of thyroid gland of buffalo to

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establish baseline data for the Murrah breed. The obtained information could be utilized to study the physiological significance of the thyroid gland in growth, development, reproduction and metabolism, as well as in the diagnosis of various diseases.

MATERIALS AND METHODS

Animals, sample collection and animal ethics statement

The thyroid gland was collected from twelve (n=12) adult buffalo (*Bubalis bubalis*) (3.5 to 8 years age) of either sex of Murrah and mixed Murrah breed soon after their slaughter from government-approved slaughterhouse, M/s Fairexports, Satakipur, District Nuh, Haryana, India. According to the University's IAEC, research on slaughterhouse specimens does not require permission and experimental design was duly reviewed and approved by Dean, Post-graduate studies, GADVASU. The experiment was conducted at the Department of Veterinary Anatomy, College of Veterinary Sciences, GADVASU, Ludhiana, Punjab during the years 2019-2021.

Histomorphochemical, micrometrical and transmission electron microscopic studies

The small tissue pieces of 0.5-2 cm thickness were collected from thyroid lobes and isthmus and fixed in 10% neutral buffered formalin and after appropriate fixation, the tissues were processed by the acetone-benzene schedule (Luna, 1968) to make paraffin blocks and then sectioning was done to get 5-6 µm-thick tissue sections. The paraffin sections were stained with Hematoxylin and eosin and other special stains (Luna, 1968) to demonstrate routine histomorphology and other special components. Fresh cryo-sections of 10-12 µ were subjected to Sudan black B method for demonstration of lipids (Chayen *et al.*, 1969). The micrometrical observations were recorded with the help of Image J Software and SPSS software and computed statistically (Snedecor and Cochran, 1994). For ultrastructure, three tissue samples (n=3) were fixed in Karnovsky's fixative and required protocol (Bozzola and Russell, 1999) was carried out at AIIMS, New Delhi.

RESULTS AND DISCUSSION

Histomorphological studies

The present investigation comprehensively studied the histological, histochemical and ultrastructural features of the thyroid gland of Murrah buffalo (*Bubalis bubalis*). The thyroid gland was enclosed in a tri-layered connective tissue capsule (Fig 1) comparable to cattle (Igbokwe and Ezeasor, 2015a) and goat (Joshi, 2016) but contrarily, Baishya *et al.* (1998) and Hussain and Al-Taay (2009) reported bi-layered capsule in yak and buffalo, respectively.

The outer layer contained collagen fibers parallel to the parenchyma (Fig 2) with few reticular fibers, fibroblasts and sparse elastic fibers similar to the cattle (Igbokwe and Ezeasor, 2015a). The middle layer was thickest and most loosely packed, comprised adipose tissue, blood vessels, nerves and collagen fibers. The inner layer adhered closely to glandular parenchyma similar to goat (Shehan, 2017). The mean capsular thickness of the thyroid gland in buffalo was 344.37±9.46 µ.

The glandular parenchyma was separated into incomplete lobules by the connective tissue trabeculae emerging from the capsule (Fig 3) and large-sized adipocytes were observed within prominent trabeculae

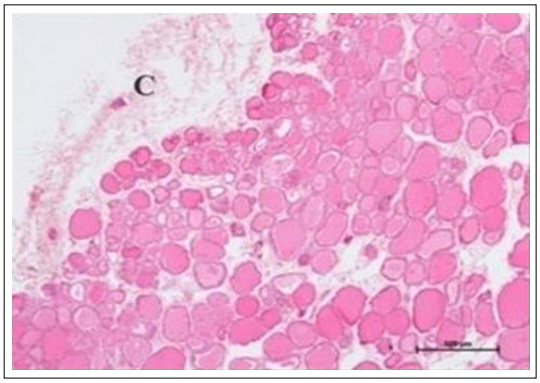

Fig 1: Photomicrograph of thyroid gland of buffalo showing Capsule (C) and parenchyma (Hematoxylin and Eosin × 40).

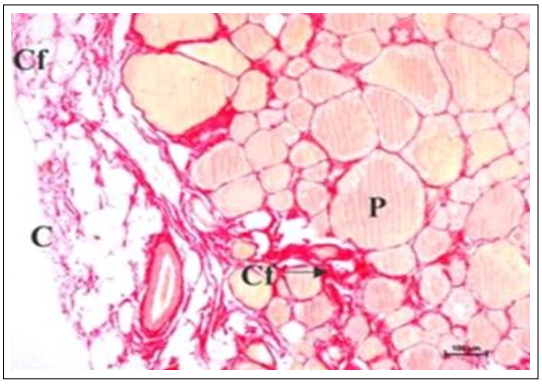

Fig 2: Photomicrograph showing presence of collagen fibers (Cf) in capsule (C) and parenchyma (P) (Picrosirius × 100).

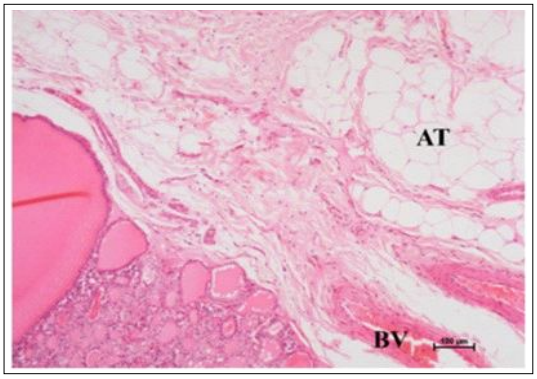

Fig 3: Photomicrograph showing presence of blood vessels (BV) and adipose tissue (AT) in trabeculae (Hematoxylin and Eosin × 100).

consistent with previous findings in goat (Joshi, 2016). The parenchyma was composed of clumps of follicles within the lobules, surrounded by a small amount of interfollicular stroma similar to goat (Shehan, 2017). Interfollicular spaces comprised of connective tissue (Fig 3), blood vessels, nerves, fibroblasts, fibrocytes, mast cells and parafollicular cells. Follicles varied greatly in shape and size (Fig 1 and 4) due to various irregularly arranged follicles cut at different planes in a particular section and according to glandular activity. The shape of follicles was predominantly round to oval, but triangular, quadrilateral, polygonal, pyriform, bilobed and tubular-shaped follicles were also observed (Fig 4 and 5). The follicles were classified as small (0 µ to 50 µ), medium (50 µ to 100 µ) and large (> 100 µ) follicles based on their diameter which aligned with the results in sheep (Khaleel and Salih, 2017). Small, medium and large thyroid follicles had mean diameters of 36.18±1.04 µ, 72.75±1.38 and 185.98±6.65 µ, respectively. However, according to Baishya *et al.* (1998), the average diameter of thyroid follicles in mithun and yak was 89±7.06 µ and 92.80±6.60 µ, respectively.

The follicular lumen was filled with a viscous, homogeneous fluid called colloid, which was eosinophilic and stained differently across follicles, suggesting varying stages of metabolic activity (Igbokwe and Ezeasor, 2015b). The majority of the small and medium-sized follicles were found closer to the margin of the gland (Hussain and Al-Taay, 2009) and were primarily round to oval in shape. Medium-sized follicles were the most numerous, followed by large and small follicles. The majority of small and medium-sized follicles contained light-colored, less viscous colloids with a variable number of peripherally located vacuoles (Fig 4). It could be considered in active metabolic stages, as they were lined mainly by low-columnar or high-cuboidal epithelium with a central spheroid nucleus and eosinophilic cytoplasm consistent to the previous findings in camel (Abdel-Magied *et al.*, 2000) and sheep (Nabi *et al.,* 2018).

The large follicles were mostly asymmetrical and found in the deeper portion of the gland, in consonance to the observations in sheep (Nabi *et al.,* 2018) while Peksa *et al.* (2011) reported the uniform follicular distribution in cattle. Presence of squamous epithelium and dark-colored, semi-solid colloid in majority of the large follicles consistent to the previous reports in sheep (Ali *et al.*, 2020). A few bilobed, half-filled, empty and ruptured follicles were also observed. A few ruptured follicles considered as degenerating follicles containing cellular debris of nucleated and some non-nucleated cells were encountered as reported previously in thyroid gland of human (Kelly *et al.*, 1984) and sheep (Roy and Saigal, 1986 and Rajalakshmi *et al.*, 2019).

Follicular and parafollicular (light or C) cells were the two cell types that made up the follicular epithelium (Fig 5). A few parafollicular cells were encountered in the follicular epithelium and none of them extended upto the follicular lumen (Fig 5). The follicular lining was predominantly simple cuboidal, low columnar, or squamous type (Fig 4 and 5) of epithelium, depending upon the functional state of the follicles consistent to observations in goats (Joshi, 2016) whereas, Hussain and Al-Taay (2009) did not observe columnar-type cells in buffalo. The mean epithelial height of squamous and cuboidal cells recorded in the follicular epithelium was 6.72±0.1 µ and 9.85±0.14 µ, respectively. However, Peksa *et al.* (2011) recorded the highest follicular height as 9.42±1.50 µ in bulls whereas, Igbokwe and Ezeasor (2015a) reported that the mean follicular cell height was 5.53±0.08 µ in adult cattle. A few follicles possessed two types of epithelial cells, *i.e.,* low columnar and cuboidal or cuboidal and squamous (Fig 5), which could be considered in the transition stage of metabolic activity in conformity with the observations in sheep (Khaleel and Salih, 2017). A few follicular cells were also found in the interfollicular area. The parafollicular cells resided mainly in the interfollicular areas, mostly solitary and occasional in clusters (Fig 6), as reported earlier in buffalo (Hussain and Al-Taay, 2009) while no C-cells were observed in camel (Kausar and Shahid, 2006). The number of parafollicular cells was higher in the deeper part of the gland than the

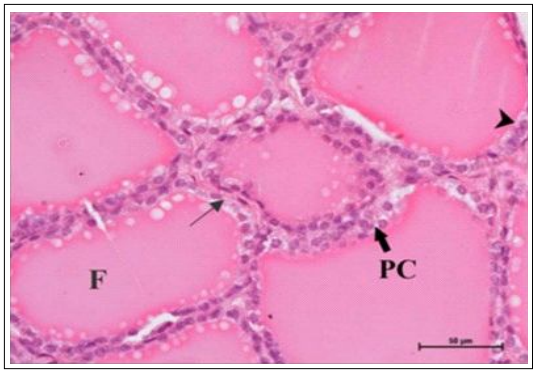

Fig 4: Photomicrograph showing parafollicular cells (PC) and cuboidal epithelium (arrowhead) of follicles (F) (Hematoxylin and Eosin × 400).

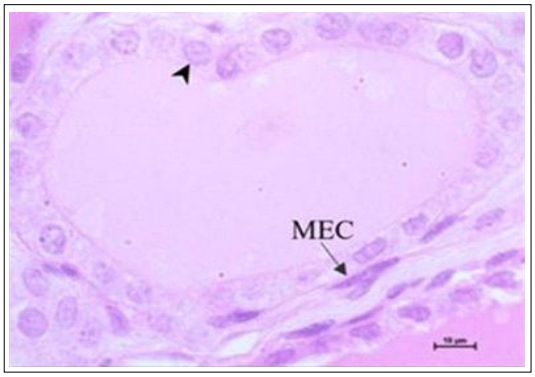

Fig 5: Photomicrograph showing presence of cuboidal epithelium (Arrowhead) and myoepithelial cell (MEC) (Hematoxylin and Eosin × 1000).

peripheral part of gland (Okada *et al.*, 1995 in sheep). Parafollicular cells were generally round or oval, with indistinct boundaries, lightly basophilic nuclei and lightly eosinophilic cytoplasm having larger nucleus than those of follicular cells (Hussain and Al-Taay, 2009). Myoepithelial cells and fibroblasts were also present near the follicular basement membrane.

The isthmus of the buffalo thyroid gland was also glandular and covered by a connective tissue capsule like cattle (Igbokwe *et al.*, 2015); whereas there was fibrillar appearance of colloid in isthmus of camel (Abdel-Magied *et al.*, 2000). Parafollicular cells were observed in the interfollicular area, albeit in smaller proportion as compared to lateral lobes (Khaleel and Salih 2017 in sheep); however, Okada *et al.* (1995) reported absence of C-cells in isthmus of sheep.

In current study, ultimobranchial bodies were observed in the thyroid gland of 3 out of 12 buffaloes. These ultimobranchial bodies varied greatly in structure, shape (round to pearl) and size and were mainly found in the deeper part of the glandular parenchyma (Fig 7), surrounded by numerous blood vessels. The capsule of ultimobranchial bodies was made up of stratified squamous epithelium and their lumen contained smooth muscle fibers intermingled with other cell types in consonance to the findings in sheep (Roy and Saigal, 1986).

A small number of ultimobranchial follicles (Fig 8) having light-colored, foamy colloid containing variable amounts of cellular debris and a stratified epithelium were also observed as documented previously by Roy *et al.* (1978) in goat. Sayed *et al.* (2005) observed that ultimobranchial body remnant of adult buffalo appeared in the form of irregular and elongated follicles of variable size and shape with predominance of calcitonin cells while Rajalakshmi *et al.* (2019) suggested that ultimobranchial follicles may act as a source of follicular cells in sheep.

Histochemical studies

The follicular colloid exhibited a strong PAS-positive reaction (Fig 9) indicating the presence of neutral mucopolysaccharides and glycoproteins as observed in sheep (Rajalakshmi *et al.*, 2019) and Chabro chicken (Vishen *et al.*, 2021). The strong PAS reaction in colloid could be suggestive of its synthetic and transformation activities. The connective tissue content, follicular cells, ultimobranchial bodies and blood vessels showed a weak to moderate PAS reaction, whereas parafollicular cells did not show any reactivity consistent with observations in sheep (Rajalakshmi *et al.*, 2019).

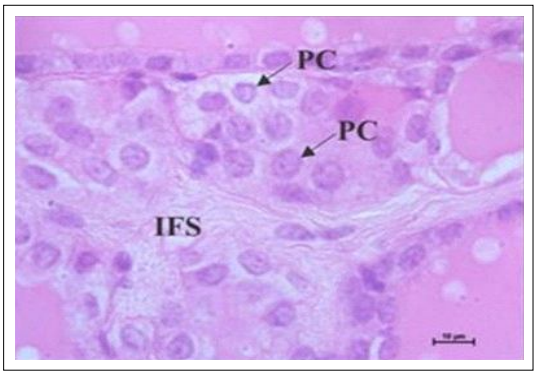

Fig 6: Photomicrograph showing presence of parafollicular cells (PC) in interfollicular space (IFS) (Hematoxylin and eosin × 1000).

Fig 8: Photomicrograph showing presence of ultimobranchial follicle (UBF) with stratified epithelium (Hematoxylin and Eosin × 1000).

Fig 7: Photomicrograph showing presence of ultimobranchial body (UBB) in glandular parenchyma (Hematoxylin and Eosin × 100).

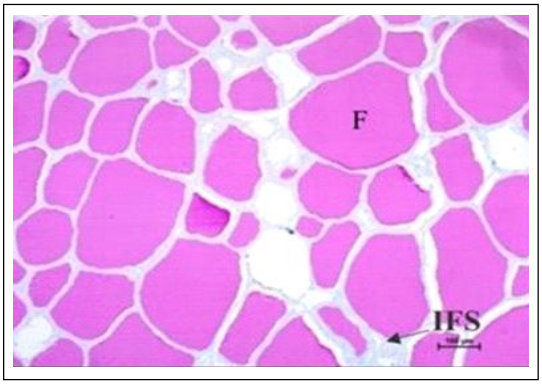

Fig 9: Photomicrograph showing PAS reaction in colloid of follicles (F) and Alcian Blue reaction in interfollicular space (IFS) (PAS-AB × 100).

The connective tissue content displayed moderate to strong; follicular cells showed moderate (Fig 9), whereas parafollicular cells and the colloid exhibited negative aclianophilic reaction for the presence of acid mucopolysaccharide reactions whereas, Sarma *et al.* (2013) documented very weak alcianophilic reaction in connective tissue components in goat.

Follicular cells exhibited a moderate reaction (Fig 10), while parafollicular cells and colloid expressed a negligible to weak reaction for lipids whereas in earlier report on sheep thyroid there was accumulation of lipids droplets in the follicular cells, C cells and ultimobranchial follicles (Rajalakshmi *et al.*, 2019).

Follicular cells demonstrated moderate, while parafollicular cells exhibited weak reaction for basic proteins; whereas, Sarma *et al.* (2013) reported that the parafollicular cells exhibited weak to strong reaction in goats; however, Vishen *et al.* (2021) reported intense reaction for basic proteins in thyroid follicular cells of Chabro chicken. The blood vessels exhibited strong reaction, whereas connective tissue and ultimobranchial bodies showed a mild reaction for presence of basic proteins.

Connective tissue, follicular cells, parafollicular cells and colloid showed no reaction for iron and a negligible to weak reaction for calcium, contrary to the observations of Joshi (2016) in goat thyroid. Blood vessels of the capsule and parenchyma of the gland showed a weak to moderate presence of iron and calcium.

Ultrastructural studies

Transmission electron microscopy confirmed light microscopy findings. Follicular cells (Fig 11) exhibited euchromatin and heterochromatin, with dilated rER cisternae, as observed in camels (Atoji *et al.*, 1999). Nuclei were lined by heterochromatin, with the nucleolus as the largest clump (Singh *et al.*, 2023). Follicular cells appeared low columnar, cuboidal (Fig 11) or squamous with polymorphic, often indented nuclei indicative of cellular activity. Igbokwe *et al.* (2015) revealed that younger goats had cuboidal cells while older ones had flattened cells indicating metabolic status. Follicular cells contained secretory vesicles of varying size and electron density, representing colloid droplets (Igbokwe *et al.*, 2015); electron light vesicles were less active, while electron dense vesicles were more active (Fig 11).

Follicular cells were polarized, with organelles distributed differently between the basal and apical regions. The apical surfaces were surrounded by colloid. rER strands were dispersed in the cytoplasm, some attached to the nuclear membrane and occasional phagocytic vesicles were present (Fig 11). Parafollicular cells (Fig 12) were oval to elongated, with numerous dense granules of variable electron density and size (Singh *et al.*, 2023) in cattle and camel, respectively. The isthmus parenchyma was loosely packed and richer in connective tissue than lateral lobes.

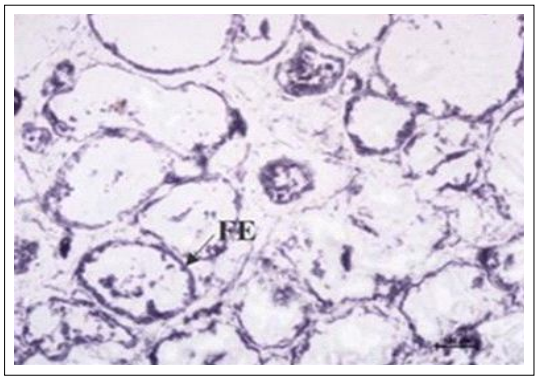

Fig 10: Photomicrograph showing lipids in follicular epithelium (FE) (Sudan Black B × 100).

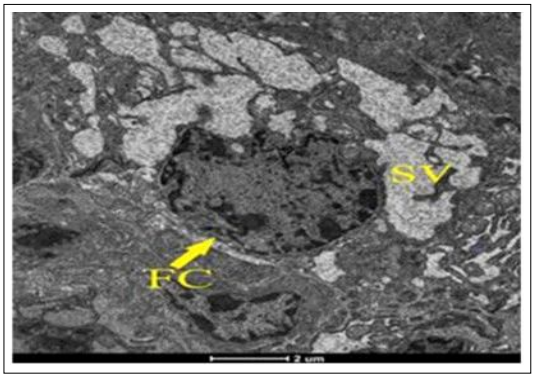

Fig 11: Transmission electron micrograph showing cuboidal folliclular cells (FC) and secretory vesicles (SV) (Uranyl acetate and lead citrate, Bar length 2 µ).

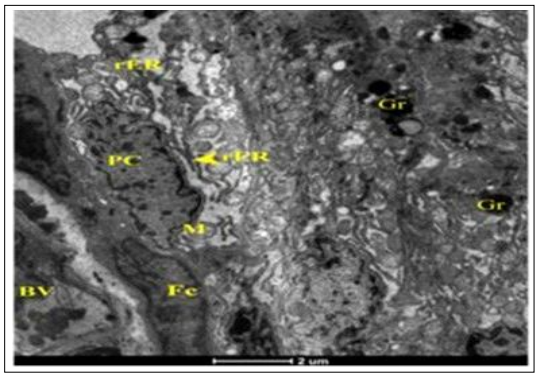

Fig 12: Transmission electron micrograph showing parafollicular cell (PC), mitochondria (M), rER, granules (Gr), Fibrocytes (Fc) and blood vessel (BV) (Uranyl acetate and lead citrate, Bar length 2 µ).

CONCLUSION

The thyroid gland was enveloped by a tri-layered capsule. The thyroid follicles varied widely in size and form. The follicular lining was made up of simple cuboidal, low columnar, or squamous type of epithelium. A few follicles featured two kinds of epithelial cells. The active follicles contained vacuoles at the periphery of the colloidal mass. Ultimobranchial bodies were observed in the thyroid gland

of 25% of the samples and varied greatly in structure, shape and size. Ultimobranchial follicles had a stratified epithelium with foamy colloid. Colloid exhibited strong reactivity for neutral mucopolysaccharides. Ultrastructural studies elucidated that the follicular cells exhibited a large number of secretory vesicles.

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Data availability

The data that support the findings of this study are available in this article and more data can be obtained from the corresponding author upon reasonable request.

Conflict of interest

The authors declare no conflicts of interest.

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