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Histoarchitectural, Micrometerical and Histochemical Analysis of Thyroid Gland in Beetal Goat (*Capra Hircus*)

Amit Poonia[1*], Anuradha Gupta[2], Varinder Uppal [3] and Devendra Pathak[4]
Department of Veterinary Anatomy, College of Veterinary Sciences
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab, India.

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ABSTRACT

The present study was undertaken to study histological, micrometrical and histochemical aspects of the thyroid gland of goat. The thyroid gland was covered by three layered connective tissue capsule which gave rise to trabeculae dividing the glandular parenchyma into incomplete lobules. The glandular parenchyma of thyroid gland was made up of follicles and small amount of interfollicular stroma. Follicles were variable in shape and size. The follicular epithelium was comprised of two kind of cells, *i.e*., follicular and parafollicular cells depending upon the size and functional state. The lumen of thyroid follicles was filled with homogenous, semi-solid colloid. The parafollicular cells had round or oval having lightly stained cytoplasm and nucleus larger than follicular cell and were found mainly in interfollicular spaces. The isthmus was also glandular in nature and covered by connective tissue capsule. Ultimobranchial follicles of variable structure, size and shape were observed at different locations in gland. Internal parathyroid gland was present in the center of the gland without any definite demarcation from the thyroid follicles and comprised of different cell types. Connective tissue of gland expressed moderate to strong reaction for acid mucopolysaccharides. Colloid demonstrated strong reaction for neutral mucopolysaccharides. Basic proteins exhibited moderate to strong reaction in capsule, follicular cells and blood vessels. Follicular cells and internal parathyroid exhibited moderate reaction for basic proteins in goat. Blood vessels of capsule and parenchyma showed weak to moderate reaction for iron and calcium.

Key words: Colloid, Follicle, Goat, Internal parathyroid, Isthmus, Parafollicular cells, Thyroid gland, Ultimobranchial follicles

INTRODUCTION

Goats are a significant component of livestock in rural India and Punjab contributing substantially in milk, meat, wool and hide production which is necessary for earning livelihood and daily household requirements. India has the world's largest goat population which grows at faster rate as compared to other livestock. Goat rearing is particularly well suited for the regions with adverse climatic conditions, little vegetation and limited water resources. Goat rearing is more cost-effective for deprived rural population as it provides extra income and food security (Acharya and Kumar 2013). The total goat Population of India and Punjab was 148.88 million and 347.95 thousand, respectively (MFAHD, 2019) contributing 3 % of the total milk production and 13.53% of total meat production in India. Beetal is one of the largest goat breeds of India raised mainly for milk production but also significantly for meat production. Beetal goats are the main source of income for the impoverished landless families of Punjab mainly in northern districts like Gurdaspur and Amritsar *etc*. due its production, prolificacy and sustainability under low input conditions (Tantia *et al*., 2001). Thyroid gland is one of the largest ductless glands located on lateral aspect of trachea and comprising of two lateral lobes and a connecting 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 productive performance like meat, milk and fiber production *etc*., in farm animals is thought to be highly dependent on normal hormonal activities of these animals. Thyroid hormones have very crucial role in physiological processes of animals by enabling survival and mating in variable environmental

1* Asst.Prof, LUVAS,Hissar 2 Prof & Head, COVAS,GADVASU, 3. Prof, COVAS,GADVASU, 4. Principal Scientist, GADVSU.
*Corresponding author email: amitpoonia263@gmail.com

conditions. Variations in thyroid hormone concentrations enable the animals to adapt their metabolic balance according to variable environmental conditions, nutrient requirements and availability. This is especially significant for traits that exhibit seasonal dependence in small ruminants (Pehlivan and Dellal, 2017). Hence, the current research investigation was designed to examine histomorphology and histochemistry of thyroid gland of goat as the results of this study could be utilized to better understand the physiological significance of thyroid gland in growth, reproduction and metabolism as well as in detection of various disorders.

MATERIALS AND METHODS

The histological, micrometrical and histochemical studies were conducted on thyroid gland of 12 adult goat (n=12) of either sex of beetal or mixed beetal breed collected from slaughter house and local meat shops of Ludhiana, Punjab. For histological and histochemical studies, the tissue samples of 0.5 cm thickness were collected from lobes and isthmus of thyroid gland of goat and fixed in 10% neutral buffered formalin (NBF) immediately after collection. After fixation, the tissues were processed for paraffin block preparation by acetone-benzene schedule (Luna 1968). Tissue sections of 5-6 µm thickness were cut with rotary microtome and obtained on clean glass slides. These paraffin sections were stained with Hematoxylin and Eosin stain (Luna, 1968) for studying routine histomorphology and by various special stains to study other histological and histochemical details (Luna, 1968 and Sheehan and Hrapchak, 1973). The various histological and histochemical observations were noted and desired photomicrographs were taken under digital microscope Nikon 80 i. Micrometrical observations were recorded on haematoxylin and eosin stained sections with help of Image J Software. All micrometrical parameters were statistically evaluated using Microsoft Excel version 2007 and SPSS software having inbuilt function for statistical analysis. Mean and standard error for morphometric measurements were computed and statistically analyzed for their significance (Snedecor and Cochran 1994). Presence of lipids was demonstrated on fresh cryostat sections (10 µm thick) by using Sudan black B stain (Chayen *et al.*, 1969).

RESULTS AND DISCUSSION

The present work was undertaken to study histological and histochemical studies on the thyroid gland of goat.

Histological studies

The thyroid gland was enveloped by tri-layered capsule made up of dense irregular connective tissue capsule in goat (Figs. 1 and 2) (Joshi, 2016 and Menaka, 2022). The outer layer of capsule was lined by a mesothelial layer and was composed of collagen fibers (Fig. 2) oriented parallel to parenchyma, few reticular fibers and scanty elastic fibers. The middle layer was thickest and most loosely packed. The innermost layer of gland was closely adherent to the glandular parenchyma consistent to the earlier published findings in Black Bengal goats (Adhikary *et al.*, 2003) and goats Bikaner region of Rajasthan (Joshi, 2016). Ultimobranchial follicles were found in middle layer of capsule (Roy *et al.*, 1978a in goat and Roy and Saigal, 1986 in sheep). The capsule gave rise to connective tissue trabeculae which invade the glandular parenchyma and further gave rise to loose interstitial connective tissue fibres wrapping around the thyroid follicles present in the form of aggregations within incompletely divided lobules (Fig. 3). Trabeculae were comprised of connective tissue fibers, blood vessels, nerves and adipocytes (Fig. 3) as reported earlier in iraqian goat (Shehan, 2017) and local sheep of Kashmir (Nabi *et al.*, 2018).

The glandular parenchyma of was made up of follicles and small amount of interfollicular stroma. These follicles were greatly variable in shape and size (Figs. 3 and 4). The variation in size and shape occurred due to various irregularly arranged follicles cut at different planes in the section and due to variable follicular activity states. The shape of follicles was predominantly round and oval but triangular, quadrilateral, polyhedral, pyriform, bilobed and tubular shapes were also observed (Fig. 4) (Rajalakshmi *et al.*, 2019 in sheep and Nandeshwar *et al.*, 2023 in goat).

On the basis of the diameter, follicles were categorized into small (0 µ to 50 µ), medium (50 µ to 100 µ) and large (> 100 µ) categories (Khaleel and Salih, 2017). Myoepithelial cells and fibroblasts were also encountered near the basement membrane of follicles (Joshi, 2016). The small and medium size follicles were mainly localized near the periphery and were predominantly round to oval but a few polyhedral follicles were also noticed. The medium sized follicles were most numerous followed by large and small follicles in partial agreement to Rajalakshmi *et al.* (2019) who reported that the small and medium follicles dominated in all age groups of sheep. Most of the small and medium sized follicles contained less viscous, light coloured colloid. These follicles could be considered in active metabolic stages as they were mostly lined by low columnar or high cuboidal epithelium with central spheroid nucleus and eosinophilic cytoplasm (Joshi, 2016 and Nabi *et al.*, 2018). The large sized follicles were mostly irregular in shape and were located in deeper part of the gland but a few large sized follicles were also present in the peripheral region of gland or near the trabeculae (Choudhary and Doley, 2017). However, Peksa *et al*. (2011) revealed the uniform distribution of follicles throughout the parenchyma of thyroid gland of cattle.

Large follicles were lined by squamous epithelium and contained semi-solid, darkly stained colloid, so these larger follicles can be considered in inactive stage (Joshi, 2016 in goat and Rajalakshmi *et al.*, 2019 in sheep), contrarily, Menaka *et al.* (2024) reported that the follicular cells of large follicles were high cuboidal in the thyroid gland of 64-days goat foetuses. Bilobed follicles were also encountered (Fig. 4) which might be in the transition state of division of large follicle into two small follicles. Some small and medium sized follicles contained peripherally placed vacuoles (Fig. 5) depending upon their active stage. Some empty, partially-filled and a few ruptured follicles containing cellular debris were also observed. The ruptured follicles containing cellular debris might be considered as the degenerating follicles (Roy and Yadava, 1975 in buffalo). The interfollicular spaces were comprised of connective tissue fibers (Figs. 2 and 6), arteries, veins, nerves (Fig. 7), fibroblasts, fibrocytes, mast cells and parafollicular cells. The mast cells were more numerous around the blood vessels or capillaries.

The follicular epithelium comprised of follicular and parafollicular cells (C cells) (Figs. 8 and 9). The follicles were generally lined by single layered cuboidal, low columnar or squamous (Figs. 8 and 9) epithelium depending upon the functional state of the follicles as reported earlier by Khaleel and Salih (2017) in sheep and Choudhary and Doley (2017) in goat. However, a few follicles possessed two types of epithelial cells i.e. either low columnar and cuboidal or cuboidal and squamous (Fig. 8) (Roy *et al.*, 1978b in goat and Khaleel and Salih, 2017 in sheep). These follicles appeared to be in transition stage of metabolic activity. A few follicular cells were also encountered in the interfollicular space (Roy *et al.*, 1978b).

The lumen of follicles was filled withcolloid i.e. homogenous fluid of viscous consistency. The colloid was eosinophilic in nature and different follicles stained differently suggesting the different metabolic stages. The active follicles had vacuoles at the periphery of colloidal mass (Joshi, 2016). The colloid of small and medium sized follicles was light coloured, less viscous possessing peripherally placed vacuoles (Fig. 5) (Khaleel and Salih, 2017 in sheep). The colloid of larger follicles was darkly stained having solid consistency (Adhikary *et al.*, 2003). The parafollicular cells were observed in the follicular epithelium (Fig. 9) and interfollicular spaces (Fig. 10) (Roy *et al.*, 1983). These parafollicular cells were generally present solitary but a few clusters of parafollicular cells were also noticed. Parafollicular cells of follicular epithelium (Fig. 9) did not reach upto the lumen of follicles consistent with findings of Khaleel and Salih (2017) in goat and Rajalakshmi *et al.* (2019) in sheep. The parafollicular cells were more numerous in the deeper part as compared to the peripheral part of gland as reported earlier by Okada *et al.* (1995) in thyroid gland of sheep. The shapes of parafollicular cells varied from round or oval in outline, lacking a distinct boundary (Roy *et al.,* 1983). These cells possessed lightly stained eosinophilic cytoplasm and very lightly basophilic nuclei nucleus which was comparatively larger than the nucleus of follicular cell (Khaleel and Salih, 2017 and Menaka, 2022).

The isthmus of thyroid gland of goat was also glandular in nature and wrapped by connective tissue capsule as revealed by Joshi (2016) and Nabi *et al.* (2018) in goat and sheep, respectively. The connective tissue components of the trabeculae and interfollicular space of isthmus were very loosely packed as compared to lateral lobes. The follicular distribution pattern and follicular epithelium in isthmus was almost similar to that of lateral lobes consistent to the findings in goat (Roy *et al.*, 1978b). The parafollicular cells were less numerous in isthmus as compared to lateral lobes (Khaleel and Salih, 2017). However, Okada *et al.* (1995) and Rajalakshmi *et al.* (2019) reported the absence of parafollicular cells in isthmus of thyroid gland of sheep.

Ultimobranchial follicles of variable structure, size and shape were observed at different locations in the thyroid gland of goat. Some duct like ultimobranchial follicles (Fig. 11) with scanty colloid were also observed. Few ultimobranchial follicles having stratified epithelium which distinguished these follicles from thyroid follicles were also observed (Roy *et al.*, 1978a). The colloid of these follicles was light coloured, less in quantity and contained variable amount of cellular debris. Similarly, Roy *et al.* (1978a) observed in pocketing of small follicles near tubular follicles in thyroid gland of goat. Ultimobranchial follicles may be acting as source of follicular cells as reported earlier in goat (Roy *et al.*, 1978a) and sheep (Rajalakshmi *et al.*, 2019).

The internal parathyroid gland was present in the center of the gland without any definite demarcation from the thyroid follicles (Fig. 12) consistent to the observations of Roy *et al.* (1984) in goat. It was comprised of chief cells (dark and light), oxyphil cells, syncytial cells intermingled with patches of collagen fibers (Fig. 13). These cells were mostly arranged in form of cords and clusters. The light chief and dark chief cells dominated in frequency and were uniform. The light chief cells had irregular cell boundaries, agranular foamy cytoplasm and dense irregular nucleus whereas the dark chief cells had distinct cell boundaries, granular cytoplasm and dense spherical nucleus. The oxyphilic cells were less in numerous and possessed dark red cytoplasm. Syncytial cells were found in group without distinct cell boundaries (Roy *et al.*, 1984).

Micrometrical studies

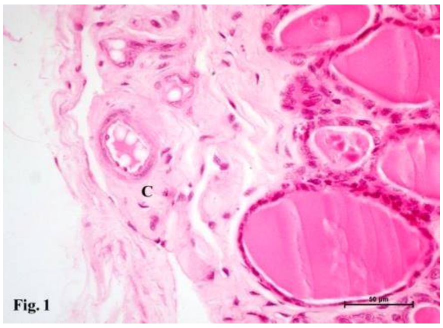
The mean capsular thickness in thyroid gland in goat was 287.47 ± 4.99 µ, which was in the range of observations of Adhikary *et al.* (2003) who reported the average thickness of capsule as 263.21 ± 46.67 µ in pubertal and 321.46 ± 34.18 µ in castrated Black Bengal goats. However, Igbokwe and Ezeasor (2017) observed that mean capsular thickness as 110.19 ± 1.04 µ in 1 to 2 years age group and 133.54 ± 1.93 µ in 2 to 3 years age group goats. The mean diameter of small thyroid follicle in goat was 33.59 ± 1.06 µ. However, Joshi (2016) observed that diameter of small sized follicles varied from 17.22 µ to 32.66 µ in goat whereas, Igbokwe and Ezeasor (2017) measured the mean small follicular diameter as 51.39 ± 0.32 µ in the 2 to 3 years age group of goat.The mean follicular diameter of medium thyroid follicles was 72.63 ± 1.17 µ whereas, Igbokwe and Ezeasor (2017) measured the mean medium follicular diameter as 96.28 ± 0.14 µ in goats of 2 to 3 years age group. The mean diameter of large follicles of thyroid gland in goat was 145.24 ± 6.93 µ respectively. These observations were in consonance with findings of Adhikary *et al.* (2003) who observed that minimum and maximum diameters of the largest oval follicle were 155.34 ± 26.59 µ and 215.75 ± 35.58 µ in pubertal group of goats. The mean epithelial height of squamous and cuboidal cells present in follicular epithelium of thyroid gland was 7.33 ± 0.31 µ and 9.13 ± 0.15 µ respectively which were in the range of earlier observations of Adhikary *et al.* (2003), who measured the average minimum and maximum height of follicular epithelium as 9.74 ± 0.76 µ and 11.86 ± 0.76 µ in pubertal group.

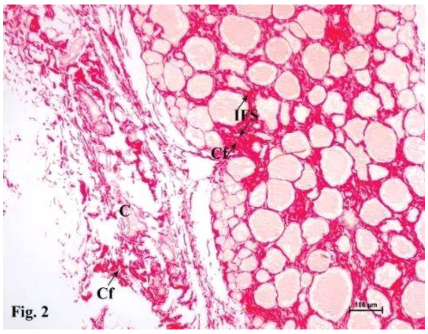
Histochemical studies

The capsule and trabeculae of thyroid gland exhibited weak PAS reaction for connective tissue, whereas, components of interfollicular space showed moderate PAS reaction (Fig. 14) (Rajalakshmi *et al.*, 2019 in sheep). The follicular cells demonstrated weak evenly distributed, non-granular reaction for PAS stain (Sarma *et al.*, 2013 in goat). Parafollicular cells did not express any PAS reactivity in consonance to the earlier published observation Joshi (2016) in goat. The colloid present in follicles was strongly PAS positive (Fig. 14) (Ali *et al.*, 2020). The colloid of smaller follicles showed comparatively weak rection than larger follicles for presence of neutral mucopolysaccharides as reported by Roy and Saigal (1987) in sheep. The blood vessels exhibited weak to moderate PAS positive reaction while internal parathyroid demonstrated negligible PAS reactivity (Fig. 14). The follicular colloid contained glycoproteins and its strongly PAS positive nature (Shehan, 2017 and Rajalakshmi *et al.,* 2018) is indicative of its bio-synthetic and metamorphising activities.Capsule and trabeculae demonstrated moderate to strong reaction while interfollicular space showed weak to moderate reaction for presence of acid mucopolysaccharides (Figs. 15 and 16) contrary to published findings of Sarma *et al.* (2013) who observed very weak reaction in the connective tissue of the capsule, septae and the interfollicular stroma in goat. Follicular cells expressed moderate alcianophilic reaction as reported earlier by Sarma *et al.*, (2013) but Rajalakshmi *et al.* (2019) revealed negative alcianophilic reaction in follicular cells. The parafollicular cells and the colloid (Fig. 15) showed negative Alcian blue reaction (Ali *et al*., 2015 in sheep). Blood vessels exhibited weak to moderate reactivity while internal parathyroid exhibited strong reactivity for acid mucopolysaccharides (Fig. 16).

Capsule, trabeculae, interfollicular space and colloid exhibited negligible to weak reaction for lipids (Ali *et al.*, 2015 in sheep). Follicular cells demonstrated moderate reaction while parafollicular cells exhibited negligible to weak reaction for presence of lipid (Roy and Saigal, 1987). Capsule exhibited strong reaction for basic proteins, however, Sarma *et al.* (2013) reported weak reaction for basic proteins goat. Colloid (Fig. 17) and follicular cells showed moderate while parafollicular cells showed weak reaction for basic proteins (Roy and Yadava, 1975). Whereas, Sarma *et al.* (2013) observed weak to strong reaction in the parafollicular cells in different age groups of goats. Blood vessels of capsule and parenchyma of gland showed intense reaction for basic proteins. Moderate reaction for basic proteins found in ultimobranchial bodies and internal parathyroid (Fig. 17) (Roy *et al.*, 1984 in goat).

Capsule showed negligible to weak amount of iron while trabeculae, interfollicular space, follicular cells, parafollicular cells, colloid, ultimobranchial follicles and internal parathyroid showed no reaction for iron. Contrarily, Joshi (2016) observed ample amount of iron particles in capsule and in lesser amount in follicular epithelial cells, colloid and parafollicular cells of thyroid gland of goat. Blood vessels of gland exhibited weak to moderate presence of iron. Capsule and trabeculae demonstrated weak reaction for calcium in goat. Follicular cells, parafollicular cells and colloid showed no reaction for calcium while Joshi (2016) observed the calcium deposits in the follicular cells, parafollicular cells, colloid and internal parathyroid. Blood vessels (Fig. 18) and ultimobranchial follicles expressed weak to moderate reaction for calcium. Conclusively, the thyroid parenchyma was made up of thyroid follicles of variable shape and size and lined by simple cuboidal, low columnar or squamous type of epithelium depending upon their metabolic states. Parafollicular cells were mainly observed in interfollicular spaces while ultimobranchial follicles were noticed in capsule and near the trabeculae.The internal parathyroid gland was comprised of dark and light chief cells, oxyphil cells and syncytial cells. Colloid exhibited strong reaction for presence of neutral mucopolys accharides. The baseline observations obtained by this study could be utilized for future anatomical, physiological and pathological studies.





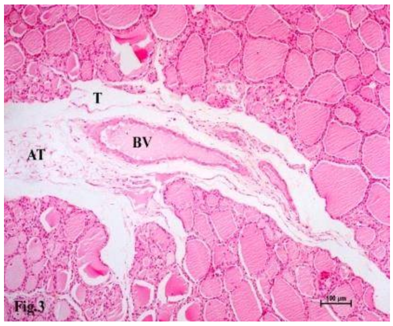
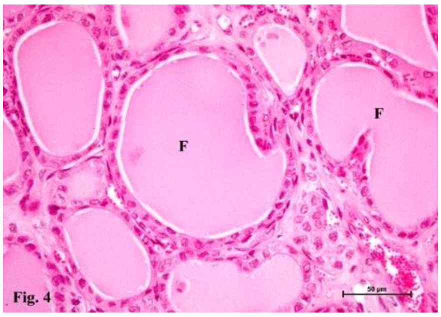
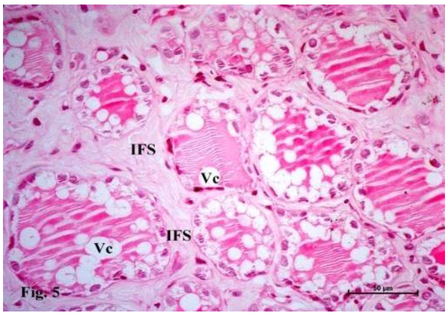


Fig. 1 Photomicrograph of thyroid gland of goat showing loose irregular connective tissue in capsule (C). Hematoxylin & Eosin X 400 **Fig. 2** Photomicrograph of thyroid gland of goat showing collagen fibers (Cf) in capsule (C) and interfollicular spaces. Picrosirus X 400 **Fig. 3** Photomicrograph of thyroid gland of goat showing blood vessels (BV) and adipose tissue (AT) in the trabeculae (T). Hematoxylin &Eosin X 100





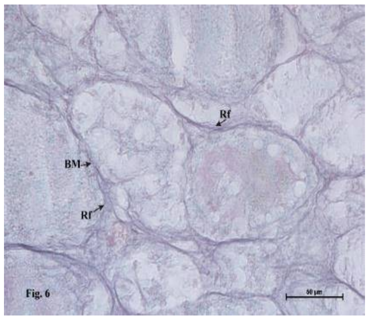
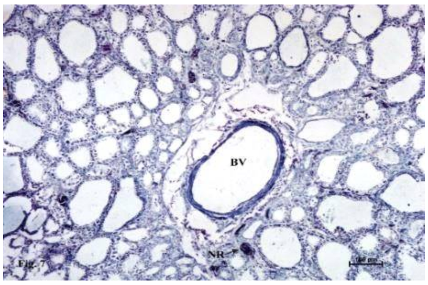
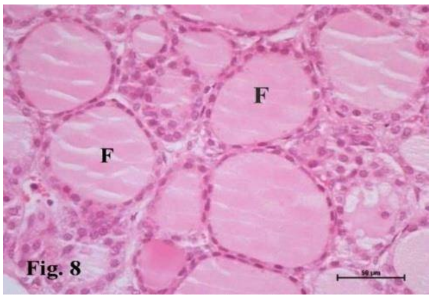


Fig. 4 Photomicrograph of thyroid gland of goat showing dividing follicles (F). Hematoxylin & Eosin X 400,**Fig. 5** Photomicrograph of thyroid gland of goat showing peripherally placed vacuoles (Vc) in the colloid. Hematoxylin & Eosin X 400,**Fig. 6** Photomicrograph of thyroid gland of goat showing reticular fibres (Rf) in the basement membrane (BM). Gridley's X 400





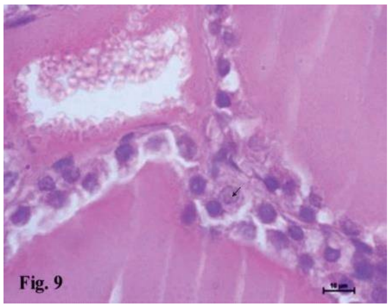
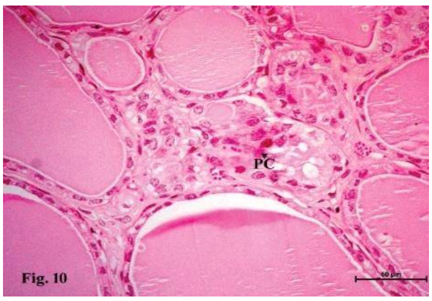
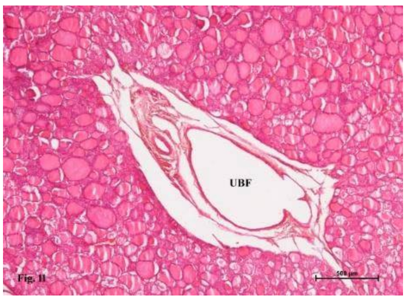


Fig. 7 Photomicrograph of thyroid gland of goat showing nerve (NR) and blood vessel (BV). Holme's X 400, **Fig. 8** Photomicrograph of thyroid gland of goat showing cuboidal and squamous cell epithelium in a single follicle (F). Hematoxylin & Eosin X 400, **Fig. 9** Photomicrograph of thyroid gland of goat showing cuboidal epithelium and parafollicular cell (↑). Hematoxylin & Eosin X 1000





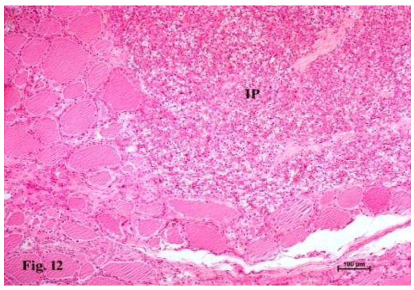
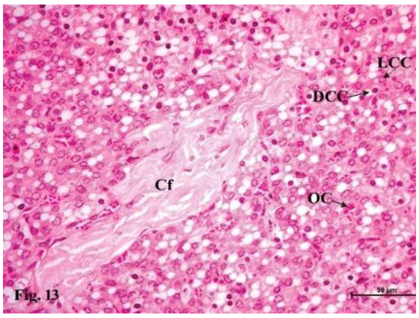
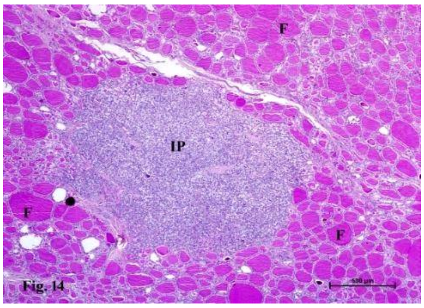


Fig. 10 Photomicrograph of thyroid gland of goat showing parafollicular cells (PC) in the interfollicular space (IFS). Hematoxylin & Eosin X 400, **Fig. 11** Photomicrograph of thyroid gland of goat showing ultimobranchial follicle (UBF). Hematoxylin & Eosin X 100,**Fig. 12** Photomicrograph of thyroid gland of goat showing internal parathyroid gland (IP). Hematoxylin & Eosin X 40





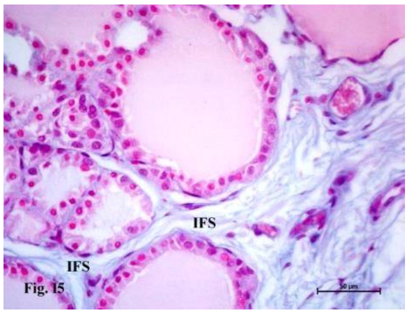
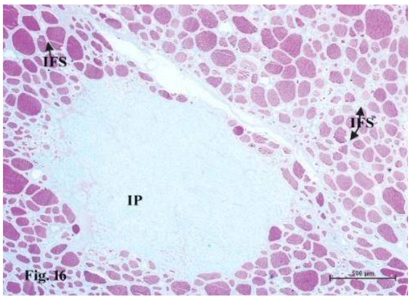
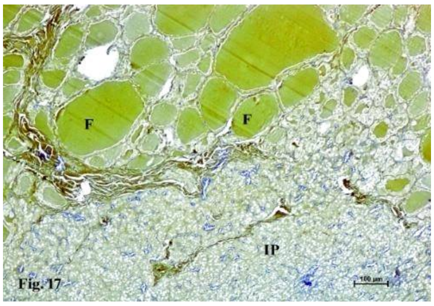


Fig. 13 Photomicrograph of internal parathyroid of goat showing patches of collagen fibres (Cf), dark chief cells (DCC), light chief cells (LCC) and oxyphil cells (OC). Hematoxylin & Eosin X 400, **Fig. 14** Photomicrograph of thyroid gland of goat showing negligible PAS reaction in internal parathyroid gland (IP) and strong PAS reaction in colloid of follicles. PAS X 40 **Fig. 15** Photomicrograph of thyroid gland of goat showing weak to moderate alcianophilic reaction in interfollicular space (IFS). Alcian blue X 400





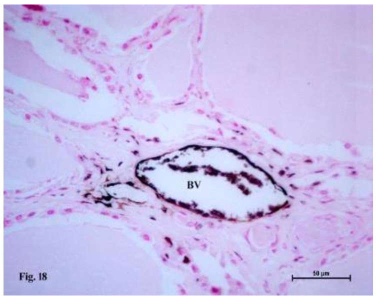


Fig. 16 Photomicrograph of thyroid gland of goat showing strong PAS reaction in colloid and weak to moderate alcianophilic reaction in interfollicular spaces (IFS) and moderate to strong alcianophilic reaction in internal parathyroid (IP). PAS-AB X 40, **Fig. 17** Photomicrograph showing moderate reaction in internal parathyroid (IP) and weak to moderate reaction for basic proteins in colloid present in follicles (F). Bromophenol blue X 100, **Fig. 18** Photomicrograph of thyroid gland of goat showing positive reaction for calcium in blood vessels (BV). Von Kosaa X 100

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