SEX RELATED AND SEASONAL VARIATIONS IN HISTOMORPHOLOGICAL, MICROMETRICAL AND HISTOCHEMICAL ARCHITECTURE OF THE STROMAL ELEMENTS AND FOLLICULAR EPITHELIUM OF THE THYROID GLAND IN LOCAL SHEEP OF KASHMIR

Neelofar Nabi[1], M.A. Baba[2], Ajaz Qadir[3] and Kamal Sarma[4]
Division of Veterinary Anatomy, Faculty of Veterinary Sciences and Animal Husbandry
Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir, Shuhama Campus,
Srinagar-190006, Jammu and Kashmir, India

Received on: 11.05.2017
Accepted on: 25.08.2017

ABSTRACT

The thyroid gland from 24 animals of both sexes viz. adult male and female sheep during summer and autumn seasons were used for the study. Sections of 5 µm thick were obtained and stained for various histological, micrometrical and histochemical studies. The thyroid gland was surrounded by thick capsule consisting of well developed collagen, reticular and very few elastic fibres and associated fibroblasts. The principal parenchymal units of thyroid gland were formed by closely packed thyroid follicles of varying shapes and sizes. Follicles were categorized as small (54.72±1.34 µm), medium (117.28±1.34 µm) and large (198.72±2.09 µm) sized based on the diameter of the follicles. There was a significant difference with respect to average thickness of capsule i.e. 226±47.72 µm and 175±8.92 µm; 188.83±21.45 µm and 262.33±34.23 µm in male and female sheep of summer and autumn season, respectively). The epithelial cell height in male and female sheep of both the seasons varied significantly (13.42±0.91µm and 16.26±0.31 µm; 15.93±0.52 µm and 13.09±0.61 µm in male and female sheep of summer and autumn season, respectively).Whereas, the values of follicular diameter varied non-significantly in both the sexes and both the seasons.

***Key words*:** Histology, micrometry, histochemistry, thyroid gland, sheep, season

Introduction

In ovine species, a notable interest has been excited by the involvement of thyroid hormones in seasonal reproduction and during pregnancy (Karsch *et al*., 1995). Local sheep is adaptive to the harsh winter of Kashmir and its rearing is economical compared to those of other domestic animals. Since the thyroid gland is very sensitive and its function in domestic animals is altered by many environmental factors, the detailed knowledge about its histomorphological structure in local sheep of Kashmir becomes imperative to study the effects of sex and season on the morphology of the gland. Paucity of literature on this aspect prompted this present study.

Materials and Methods

The present study was conducted in the laboratory of Division of Veterinary Anatomy F.V.Sc and A.H. SKUAST-K, Shuhama, Srinagar. The material for the study was formed by 24 samples of thyroid gland collected from adult male and female sheep. The collection of samples was done from district Bandipora immediately after the slaughter of the animals. After collection, the samples were immediately carried to the laboratory for gross observations and then preserved in 10% neutral buffer formalin, Bouin's fluid, Carnoy's fluid. For histological and histochemical studies, 5 µm thick sections were obtained by using a Rotary Microtome and stained for various histological and histochemical studies (Table 1).

For micrometrical studies, paraffin sections stained with haematoxylin and eosin were used. The various measurements, as detailed under, were recorded by using ocular micrometer calibrated with a stage micrometer.

a.Thickness of capsule
b.Diameter of follicles
c.Cell height of lining epithelium

Results and Discussion

Capsule and stroma

The thyroid gland was surrounded by a thick capsule which consisted of well developed collagen, reticular and very few elastic fibres and associated fibroblasts. Similar findings were reported by Ali (2014) in female donkey and Igbokwe and Ezeasor (2015) in White Fulani cattle. It was frequently thickened in the areas where arteries, veins and nerves were present on the surface of gland .The capsule (Fig. 1) presented three layers. The outermost layer was mesothelial layer lined by simple squamous epithelium; the middle layer was rich in fat cells, blood vessels and nerves and also consisted of loose collagen and elastic fibres. The inner most layer was closely adherent to the parenchyma. From the inner surface of capsule thick connective tissue septa or trabeculae were found to divide the gland into different lobes and lobules. The septa that divided the gland into different lobes and lobules were also rich in blood vessels. The collagen fibres predominated in the capsule followed by reticular and elastic fibres. Observations of the present study were in accordance with the findings of Adhikary *et al*. (2003) in Black Bengal goat, Frandson *et al.* (2003) in farm animals, Kausar and Shahid (2006) in one-humped camel. A differentiating feature was observed by Hussin (2003) in one- humped camel where the thickness of the trabeculae of the thyroid gland increased in winter season. Well developed venous sinuses were seen in the capsule.

[1]Part of M.V.Sc. Thesis and current address Ph.D. Scholar; [2]Professor & Head, [4]Associate Professor and corresponding author, Email kamalsarma73@yahoo.com.

Fig. 1: Thyroid gland, female sheep, autumn showing capsule (C), artery (A) and vein (V). (H&E x 100 X).

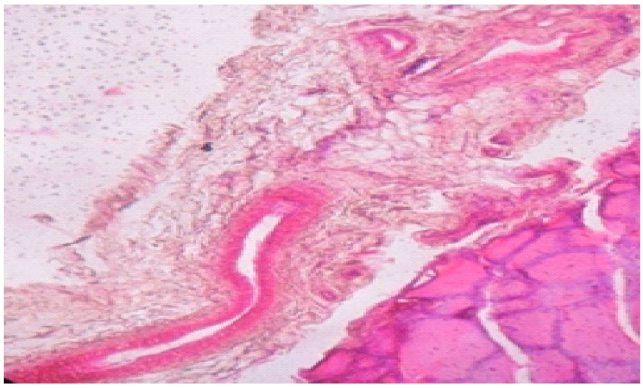


Fig. 2: Thyroid gland showing collagenfibres between the follicles. (Masson's Trichrome stain, 1000X).

Fig.3: Thyroid gland showing large (L), medium (M) and small (S) follicles with colloid (C) in female sheep. (H&E x 400 X).

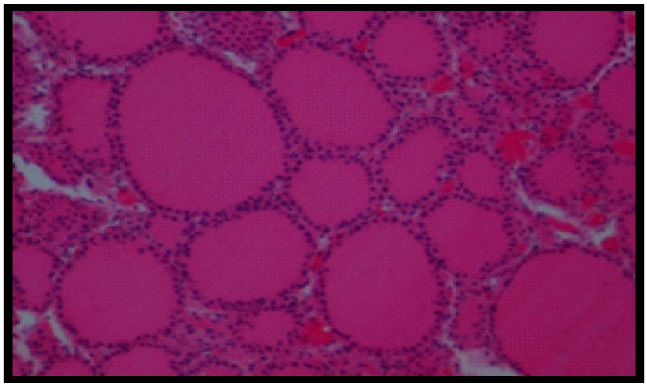


Fig.4: Thyroid gland showing parafollicular cells (arrow head) and myoepithelioal cells (arrow) in male sheep (summer). (H&E x 1000X).

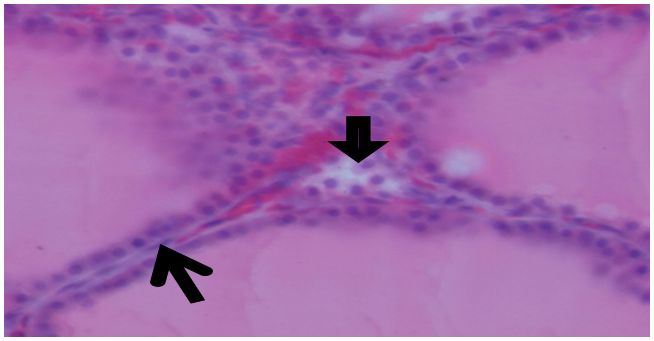


Fig. 5: Thyroid gland showing active follicle with tall columnar epithelium in male sheep (autumn). (H&E x 1000 X).

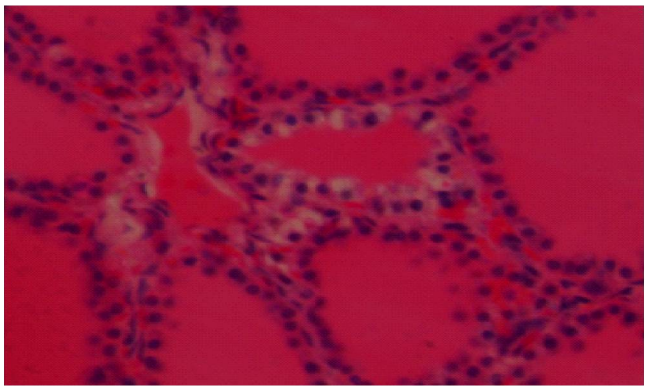


Fig.6: Thyroid gland showing hexagonal shaped follicles (arrow) in male sheep. (H&E 400X).

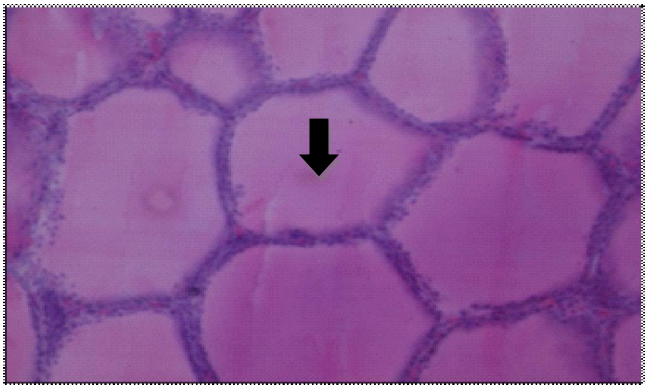


Fig. 7: Thyroid gland showing inactive follicles with simple squamous epithelium in male sheep. (H&E 400X)

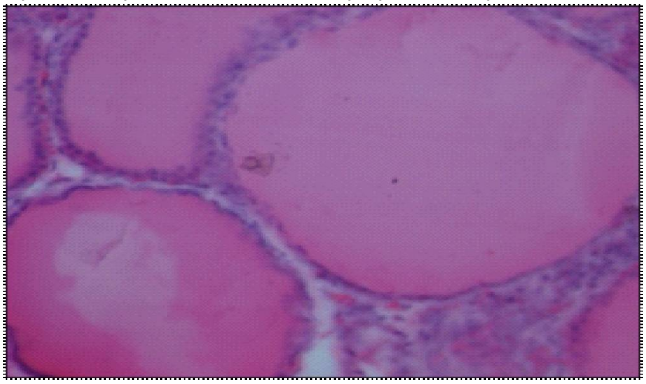
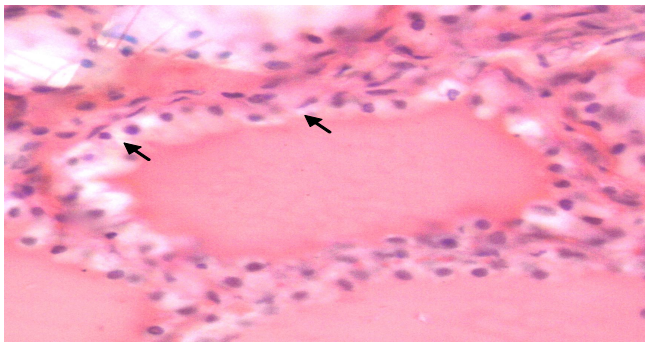


Fig. 8: Thyroid gland showing active follicle with vacuolation of colloid (black arrow) and myoepithelial cells (blue arrow). (H&E, 1000X).



The interstitial connective tissue was thick and consisted of collagen (Fig. 2) and reticular fibres extending from capsule. In between the follicles, the connective tissue together with arterioles, venules, lymphatic vessels and nerves were present. This has been even reported by Igbokwe and Ezeasor (2015) in White Fulani cattle in which the capsule penetrated the parenchyma through the trabeculae carrying enormous connective tissue elements, vascular structures and lymphatics. In the areas where the follicular walls were closely apposed, only the thinnest layer of connective tissue, a few flattened fibroblasts, myoepithelial cells and sinusoidal capillaries could be seen as reported earlier by Hussin and Al-Taay (2009) in Iraqi buffalo. The capsule was weakly positive for PAS-AB reaction. These observations were in accordance with Sarma *et al.* (2013) in male Assam goat.

Follicles and epithelial lining cells

The principal parenchymal units of thyroid gland were formed by closely packed thyroid follicles (Fig. 3) and follicular epithelium, which usually consisted of two types of cells; follicular lining cells, which lined the follicles and parafollicular cells (Fig. 4). The follicular lining cells were acidophilic in nature with basally positioned nucleus. The follicles were of varying shapes and size. The follicles presented round, oval and irregular shapes. Same observations were reported by Evans (1993) in dogs and Kausar and Shahid (2006) in camel. A distinct basement membrane was evident and the follicles were enmeshed in a highly vascular stroma. Blood vessels were present around the follicles in a basket like manner. Large venous sinuses could be seen in the trabeculaeas reported earlier by Eroschenko and Victor (2005) in the thyroid gland of horse and Ali (2014) in female donkey.

The follicles were categorized as small, medium and large sized based on the diameter of the follicles (Table 2, 3). The follicles with an average diameter of 198.72±2.09 µm were arbitrarily considered as large follicles whereas the follicles having average diameter of 117.28±1.34 µm and 54.72±1.34 µm were grouped as medium and small follicles, respectively. Same observations were reported by Evans (1993) in dogs and Kausar and Shahid (2006) in camel. In addition to this Kratochvíl (1998) and Krabacova (2002) reported that in cattle the size and shape of these follicles varied in accordance to the activity of thyroid gland. Small and medium follicles were restricted more to the periphery while as large sized follicles were present towards the centre of parenchyma. Small follicles were usually active with high epithelium and appropriate colloid characteristics as compared to large follicles. In many inactive follicles, the follicular epithelium was low cuboidal and even squamous. Frandson *et al*. (2003) also reported that the thyroid gland of domestic animals consisted of follicles lined by a simple epithelium ranging from cuboidal to columnar while as Igbokwe and Ezeasor (2015) observed in White Fulani cattle that highly flattened cells were common in the older pubertal animals.

In female sheep, the follicles were of varying shapes and the epithelium of the gland was more active in summer season as compared to autumn (Table 2). The epithelium of the follicles was high cuboidal in summer (Table 4). In most of the follicles the colloid exhibited vacuoles indicating high activity of the gland. These findings are similar to that of Todini (2007) in which he reported that in ewes, during late lactation, the increased activity of thyroid gland may be related to the decrease of milk production.

In autumn season, large, medium and small sized follicles with low cuboidal epithelial cells having rounded, prominent nucleus touching the basement membrane were observed in female sheep. In most of these follicles, the colloid in the lumen was homogenous indicating the low activity of the female thyroid gland during autumn season. Only a few active follicles with active cells, prominent nucleus touching the apex of the cell were seen (Fig. 5). The colloid in these follicles was observed with irregular outline. The seasonal pattern of thyroid gland activity often shows maximal values during winter (cold months) and minimal during summer (hot months) in sheep according to Salem *et al*, 1991; Menegatos *et al*, 2006. However, contrasting results have been reported by Yokus *et al*. (2006).

Whereas, in male sheep, the follicles were mostly hexagonal in shape than rounded in summer season (Fig. 6). The activity of the gland was less, lined by simple squamous and low cuboidal epithelium (Fig. 7). A very few follicles were active showing vacuolation in the colloid giving a web/astral appearance and were lined by tall cuboidal epithelium (Fig. 8). According to Esther lowe (1932) during summer months in cat, the glands of both sexes became much more passive in condition. The acinar epithelium was in most cases low cuboidal, that of the males being flatter than that of the females. However, in autumn season, the follicles were more organized in their conformity having round, oval and prismatic shapes in male sheep. The follicles were lined by tall cuboidal epithelium with prominent nucleus and the colloid in these follicles was heterogenous and eosinophillic in nature exhibiting high activity during this season. Similar results were found by Esther lowe (1932) that during the autumn months the glands of both sexes of cat were producing a certain amount of new secretion, but the amount of colloid was much greater in males than in females.

References

Ali SA (2014) Anatomical and histological study of thyroid gland in female local donkey *(Equs africanusasinus*) at Basrah city. *AL-Qadisiya J. Vet. Med. Sci.* **13**(1): 85-87

Adhikary GN, Quasem MA and Das SK (2003) Histological observation of thyroid gland at prepubertal, pubertal and castrated Black Bengal goat. *Pak. J.Bio. Sci.* **6**(11): 998-1004.

Adhikary GN, Quasem MA and Das SK (2003) A prospective study on histochemical observation of thyroid gland at prepubertal Black Bengal goat. *Mimensingh Med. J.* **12**(2): 108-111.

Bancroft JD and Gamble M (2003) Theory and Practice of Histological Techniques, 5th ed., Churchill Livingstone, Newyork.

Culling CFA, Allison RT and Barr WT (1985) Cellular pathology techniques. 4th ed. Butterworth and Co. Ltd., London.

Table 1: Showing different stains used for the histological and histochemical studies

1	Haematoxylin and Eosin for general histology	(Culling *et al*., 1985)
2	Masson's trichrome method for collagen and muscle fibres	(Bancroft and Gamble, 2003)
3	Hart's method for elastic fibres.	(Luna,1968)
4	Periodic Acid Schiff-Alcian Blue method for mucosubstances.	(Culling *et al*., 1985)
5	Mercuric Bromophenol Blue method for bound proteins.	(Culling *et al*., 1985)

Table 3: Average follicular diameter in male and female sheep during summer season (Mean ± SE)

S. No.	Summer season						Autumn season					
	Average follicular diameter (µm)						Average follicular diameter (µm)					
	Male			Female			Male			Female		
	Large	Medium	Small	Large	Medium	Small	Large	Medium	Small	Large	Medium	Small
1	224.16 ±0.01	141.83± 0.04	53.67 ±0.05	212.33 ±0.05	126.33± 0.03	51.12± 0.01	220.16 ±0.07	140.83± 0.04	53.67± 0.05	214.33 ±0.05	138.33± 0.03	53.12± 0.04
2	225.17 ±0.04	136.83± 0.04	55.51± 0.01	219.83 ±0.02	136.26± 0.06	52.50± 0.04	222.17 ±0.04	134.83± 0.04	55.51± 0.01	219.83 ±0.02	136.26± 0.06	52.50± 0.03
3	226.56 ±0.01	135.50± 0.04	56.00± 0.03	210.67 ±0.01	126.17± 0.01	53.33± 0.03	226.56 ±0.04	135.50± 0.05	54.00± 0.03	221.67 ±0.04	126.17± 0.01	51.33± 0.05
4	226.56 ±0.01	138.17± 0.04	56.21± 0.01	210.50 ±0.01	133.50± 0.02	52.67± 0.05	226.56 ±0.05	136.17± 0.06	56.21± 0.04	210.50 ±0.03	137.50± 0.02	52.67± 0.04
5	225.17 ±0.02	137.17± 0.04	53.50± 0.03	211.17 ±0.04	126.33± 0.03	53.33± 0.01	223.17 ±0.06	137.17± 0.04	52.50± 0.03	226.17 ±0.04	126.33± 0.03	52.33± 0.03
6	226.56 ±0.01	141.28± 0.04	55.33± 0.01	215.17 ±0.03	128.17± 0.07	53.51± 0.02	226.56 ±0.04	141.28± 0.03	55.33± 0.02	214.17 ±0.03	128.17± 0.07	53.51± 0.04

Table 4: Average cell height of active follicles in male and female sheep during summer season (Mean ± SE)

S. No.	Summer season		Autumn season	
	Female	Male	Female	Male
1	16.53±0.14	12.54±0.16	14.53±0.14	15.53±0.15
2	15.68±0.11	12.47±0.15	13.68±0.11	15.68±0.13
3	16.50±0.10	13.27±0.14	13.50±0.14	16.50±0.12
4	16.27±0.11	13.23±0.14	12.27±0.13	16.27±0.11
5	16.22±0.15	14.55±0.11	13.22±0.15	15.22±0.14
6	16.38±0.11	14.48±0.12	14.38±0.12	16.38±0.10

Evans (1993) Miller's anatomy of the dog. 3rd ed. WB Saunders Company, pp. 567-571.

Esther L (1932) Seasonal and sexual variation in the thyroid glands of cats. pp: 577-591.

Eroschenko P and Victor G (2005) Thyroid gland, Atlas of histology with functional correlation section **2**: 395.

Frandson (2003) Anatomy and physiology of farm animals. 6th ed. Lippincott Williams and Wilkins. pp. 195-197.

Hussin AM (2003) Seasonal histological changes in thyroid gland of one-humped camel (*Camelus dromedaries*) in middle of Iraq. Ph.D. Thesis submitted to University of Baghdad.

Hussin AM and Muntadhar MA (2009) Histological study of the thyroid and parathyroid glands in Iraqi buffalo (*bubalus bubalis*) with referring to the seasonal changes. *Basrah J. Vet. Res.* **8**(1): 26.

Igbokwe CO and Ezeasor DN (2015) Histologic and ultrastructural observations on the thyroid gland of the White Fulani (Zebu) cattle in Northern Nigeria. *Afr. J. Biotech.* **14**: 156-166.

Karsch FJ, Dahi GE, Hachigian TM and Thrun LA (1995) Involvement of thyroid hormones in seasonal reproduction. *J. Reprod. Fertil.* **49**: 409-422.

Kausar R and Shahid RU (2006) Gross and microscopic anatomy of thyroid gland of one-humped camel (*Camelus dromedarius). Pak. Vet. J*. **26**: 88-90.

Krabacova I (2002) Morphological and functionalchanges of the thyroid gland at differentiodine saturation of livestock.Dissertation, ZF JU v CeskychBudejovicich, Ceske Budejovice (in Czech).

Kratochvil P (1998) Morphological and functionalchanges of the thyroid gland in differentbreeding conditions. Dissertation, ZF JU v Ceskych Budejovicich, Ceske Budejovice (in Czech).

Luna LG (1968) Manual of histological staining methods of Armed Forces Institute of Pathology. 3rd ed. McGraw Hill Book Co., New York. pp. 153-173.

Manegatos J, Goula C and Kalogiannis D (2006) The productivity, ovarian and thyroid activity of eves in an accelerated lambing system in Greece. *Small Ruminant Res.* **65**:209-216.

Salem MH, Elsherbiny AA, Khalil MH and Yousef MK (1991) Diurnal and seasonal rhythm in plasma-cortisol, triiodothyronine and thyronine as affected by the wool coat in Barki sheep. *Indian J. Anim. Sci.* **61**: 946-951.

Sarma K, Kalita SN and Devi J (2013) Age related histochemical studies on the thyroid gland in male Assam goats (*Capra hircus*) from birth to ten months of age. *Indian J. Anim. Reprod.* **47**(3): 254-256.

Trautmann A and Fiebiger J (2002) Fundamentals of the histology of domestic animals. Translated and Revised by Habel, R.E. and Biberstein, E.L. Compstock Publishing Associates, Ithaca, New York.

Todini L (2007) Thyroid hormones in small ruminants: Effects of endogenous, environmental and nutritional factors. *Anim.* **1**(7): 997-1008.

Yokus B, Cakir DU, Kanay Z, Gulten T and Uysal E (2006) Effects of seasonal and physiological variations on the serum chemistry, vitamins and thyroid hormone concentrations in sheep. *J. Vet. Med. Ser. A***53**: 271-276.