J. Sylhet Agril. Univ. 1(2):147-153, 2014 **Research Article** **ISSN: 2308-1597**

HISTOLOGICAL OBSERVATIONS OF THYROID GLANDS AT PREPUBERTAL, PUBERTAL AND CASTRATED ADULT INDIGENOUS BULLS (*Bos indicus*)

G N Adhikary*[1], S K Chowdhury[2], S Sarkar[1], B Paul[1], K M Islam[3] and M N Islam[1]

[1]*Department of Anatomy and Histology, Sylhet Agricultural University, Sylhet-3100, Bangladesh*
[2]*Department of Pathology, Sylhet Agricultural University, Sylhet-3100, Bangladesh*
[3]*Department of Parasitology, Sylhet Agricultural University, Sylhet-3100, Bangladesh*

Abstract

The experiment was conducted to elucidate the histomorphology of thyroid gland of prepubertal, pubertal and castrated adult indigenous bulls (*Bos indicus*) using Hematoxyline and Eosin (H&E) during July 2013 to June 2014. Twelve bulls of three age groups namely prepubertal (below 1 year of age, n=4), pubertal (1.5 to 2.5 years, n=4) and castrated adult (above 4 years, n=4) were selected in this study. Thyroid glands of slaughtered bulls were collected for histological studies. The thyroid gland was found to cover by a thick connective tissue capsule consisted of dense collagen fibers having two layers, outer and inner layer. The follicles consisted of follicular epithelium and intrafollicular colloid substances. Shapes of the follicles were seen round, oval, pentagonal or hexagonal and irregular in appearance. In prepubertal group, most of the follicles were smaller. In pubertal group, most of the follicles were larger in sizes including some smaller follicles. Significant differences were found in the follicular diameters of the prepubertal from the pubertal bulls. Castrated bulls showed larger inactive follicles with few smaller follicles. Colloid was more homogenous, predominantly eosinophilic by H&E staining in most active follicles. Follicular epithelium become simple squamous to low cuboidal in prepubertal bulls, low cuboidal to columnar in pubertal bulls and squamous epithelium in castrated adult bulls. Para follicular cells were frequently found beneath the basal lamina in prepubertal bulls, but in pubertal and castrated bulls a fewer or no parafollicular cells were found. Finally, thyroid glands were found to undergo age related changes and histological features indicated the functional status of the thyroid gland.

Keywords: Histology, pubertal stage, thyroid gland, indigenous bull

Introduction

Thyroid gland is the most important unique gland of the body which accumulates iodine in large quantities and incorporates it into hormones. The metabolism of iodine is so closely related to thyroid function. Thyroid hormones have many functions in the body. In general, it increases the basal metabolic rate by increasing glycolysis, gluconeogenesis and glucose absorption from the intestine, stimulate new protein synthesis and heart rate, increases neural transmission and thyroxin stimulates oxygen utilization as well as heat production by all cells of the body. Tri-iodothyronine is the main stimulator of cellular metabolic rate and its action is very powerful and immediate, whereas tetra-iodothyronine (Thyroxin) is powerful but less rapid in action. Overproduction of both hormones causes thyrotoxicosis, hypo-secretion in adults produces myxoedema and in infants cretinism. Their synthesis and release are mainly controlled by adenohypophyseal Thyroid stimulating hormone (TSH). However, in Grave's disease antibodies to human thyroid stimulating immunoglobulin bind to TSH receptors sites in the follicular cells, interfering with this control and causing excessive hormone production. Thyroid diseases due to extrinsic and intrinsic causes, hyperthyroidism and hypothyroidism, thyroid neoplasm also diagnoses the developmental abnormalities of the thyroid intend to medicinal, surgical and pathological producers for the treatment. So, anatomical observation has got priority in clinical or histo-pathological studies. The histology of thyroid gland in different animals were studied by Agrawal and Bhattacharya (1981) in Barbari goat, Baishya *et al.* (1986) in Asam goat; Das *et al.* (1965) in bull and bullock; Mathur (1971) and Roy and Yaadava (1975) in Indian buffalo; Roy and Saigol (1987) in sheep; Sanap *et al.*(1998) in cattle. The objective of this study was to detect the variation of histoarchitectural features of thyroid gland of indigenous bulls of Bangladesh.

***Corresponding author: G N Adhikary,** Department of Anatomy and Histology, Sylhet Agricultural University, Sylhet-3100, Bangladesh, e-mail: adhikarygn.dah@sau.ac.bd.*

Materials and Methods

The experiment was conducted in the laboratory of the Department of Anatomy and Histology, Sylhet Agricultural University, Bangladesh from July 2013 to June 2014. Twelve bulls of three age groups were selected from the local market before Muslim sacrifice on year round: pre-pubertal group (below 1 year of age, n=4), pubertal group (1.5 to 2.5 years, n=4) and castrated adult group (above 4 years, n=4) which were castrated earlier in the pubertal stage, respectively. The age of bulls was determined based on tooth eruption patterns (Getty, 1975). The animals were reared by the local farmer neighboring the Sylhet Agricultural University. The bulls were kept individually in the stall and fed with *adlibitum* balanced ration for 4 weeks. Ante mortem examination of bulls was done before slaughter. The glands were then cut into few pieces and few pieces were collected for microscopic study. A part of each cut tissue samples were fixed in more than 20 times the volume of each in Bouin's fixatives for 24 hours, dehydrated in series of submerging progressively more concentrated ethyl alcohol (70%, 80%, 90%, 95%, 100%). The tissues were kept for 3 hours in each grade of ethyl alcohol. Tissues were then transferred in a hydrophobic clearing agent, xylene in two changes to remove the ethyl alcohol. In both cases, the tissues were kept for 40 minutes. Infiltrated the tissues through 2 changes of liquid paraffin in the micro oven taking two hours in each step to remove the xylene from tissues. Then they were placed in liquid paraffin for embedding, which was achieved by cooling them in room temperature.

The hardened tissue blocks containing the tissue samples were then sectioned at 6µm thickness using microtome (Microm GmbH, type HM 325, Germany) and the sections were floated in a water bath at 45ºC for flattening out on water. Then floated sections were picked up carefully on clean slides, which had been smeared with egg albumin and dried on the slide warmer. The serial sections were chosen position on at every 21 section to ensure the different position of tissue.

The sectioned tissues were deparaffinized by transferring them through xylene for two changes; 15 minutes in each case. Then they were transferred through descending grades of alcohol (100%, 95%, 90%, 80%, 70%). Afterward, they were passed through the normal water and distilled water. Tissues were stained with Hematoxyline and Eosin (Gridly, 1960). The tissue sections were then dehydrated by transferring and immerging in progressively more concentrated ethyl alcohol (70%, 80%, 90%, 95%, and 100%) and then were passed through xylene. Tissue sections were clean blotted and mounted in Canada balsam with a cover slip. Four slides were prepared for each bull in which each slide contained four tissue sections from each sample.

The microscopic evaluation of thyroid glands includes diameters of the follicles and epithelial height of the follicles through oculo-micrometer observation. The data generated from this experiment were processed for further analysis. Mean value, standard error (SE) and correlations were estimated using the software Statistical Analysis System (SAS, 1998).

Results and Discussion

Capsule

In the present study, the thyroid gland was found to cover by a thick connective tissue capsule consists of dense, compact collagen fibers as described in Black Bengal goat (Adhikary *et al.* 2003). The distinct two layers were observed as an outer and an inner layer which was differ completely with the reports of Adhikary *et al*. (2003) in goats. They stated that the thyroid capsule consisted of three layers outer, middle and inner and the middle layer consisted of adipose tissue which arranged in 2-10 layers in some animals. The present observation was in accordance with the findings in human (Ham and Carmack, 1979) and in Asam Barbari goats (Baishya *et al.* 1986). In the present study, adipose tissue was found in few areas in the capsule as a layer in between connective tissue strands with blood vessels especially in the outer layer (Fig. 1) observed in the prepubertal and pubertal bulls. Inner layer of the capsule remained as compact connective tissue without any adipose tissue. The thickness of the capsule was variable due to the adipose tissue in the outer layer present in all three groups of animals. On the other hand, compact connective tissue capsule was found at the same time. The connective tissue capsule was rich in blood vessels. The connective tissue septa arisen from the capsule and entered into the gland for dividing the gland into lobe and lobules. The present findings regarding to prepubertal, pubertal and castrated thyroid glands of indigenous bulls were in agreement with Roy and Yadava (1975) in buffalo and Adhikary *et al.* (2003) in goats. In addition, the

septa gradually became thinner as they reached the centre of the glands of follicles, separated each follicle from others by fine, irregular connective tissue composed of reticular fibers as stated by Junquera and Carneivo (2005) in man. In the present study, capsule represented smooth muscle fibers along with collagen and reticular fibers which were similar to the reports of Das *et al.* (1965) in bull and bullocks.

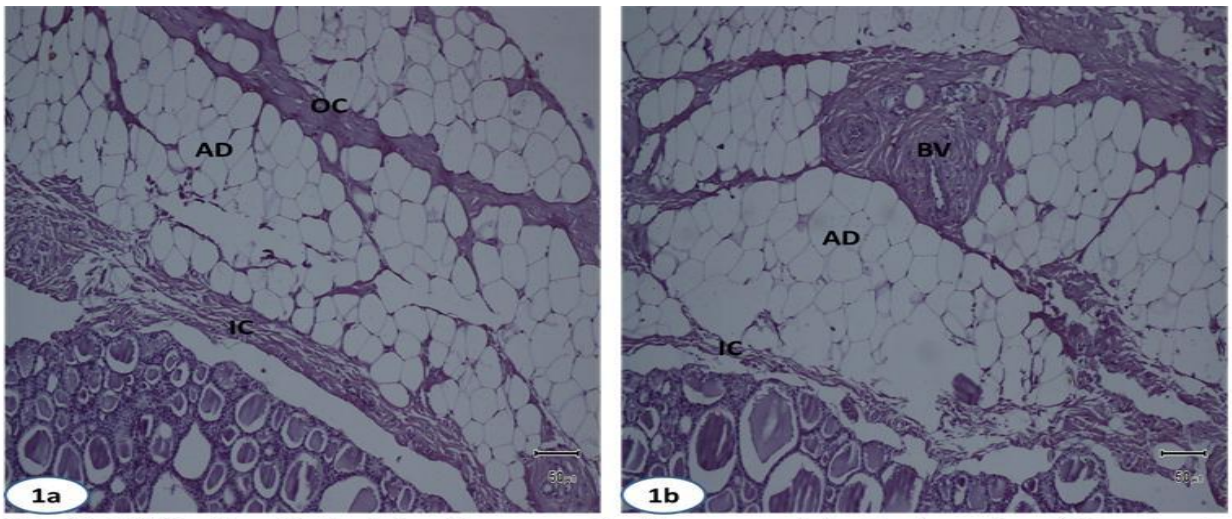

Fig. 1(a-b). The thyroid gland showing connective tissue capsule having inner layer(IC) and outer layer (OC), blood vessels(BV) and in between adipose tissue (AD) of the pubertal bull. H&E 10X

Follicles

The thyroid gland composed of numerous follicles with the interfollicular stroma. The number of follicles and the connective tissue stroma varied with age and physiological status of the animals. The follicles consisted of follicular epithelium and intrafollicular colloid substances. Follicles of various sizes and shapes were observed in the present study. They were smaller and larger arranged heterogeneously in the thyroid lobules without any homogenous pattern as described in Black Bengal goats (Adhikary *et al.* 2003). They noted that the small size follicles were found nearer to the capsule and larger follicles were found towards the centre of the gland with little exception. In the present study, the smaller follicles were nearer to the capsule, but larger follicles were observed closely to the capsule also (Fig. 2). Shapes of the follicles were seen round, oval and irregular with pentagonal or hexagonal in appearance in the present study. Oval shape small and large follicles were observed more frequently throughout the gland (Fig. 2). Colloid materials completely filled up the intrafollicular lumen with few empty follicles or less colloid materials observed in prepubertal, pubertal and castrated indigenous bulls.

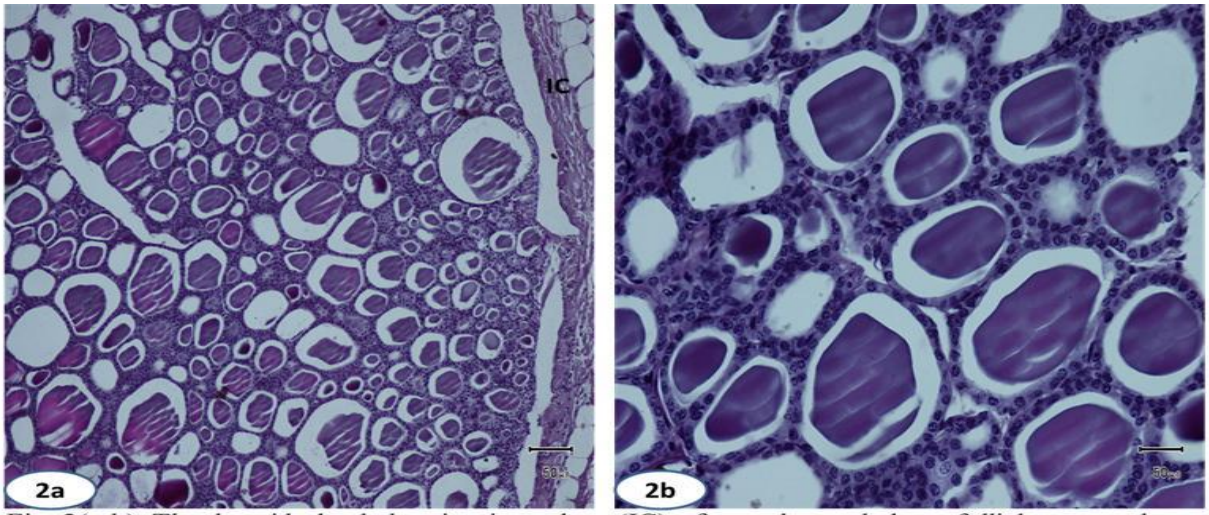

Fig. 2(a-b). The thyroid gland showing inner layer(IC) of capsule, oval shape follicles more than the round, smaller size follicles nearer to the capsule with few larger follicles of the pubertal bull. H&E, 2a 10X, 2b 40X

Each lobule of the thyroid gland contained 40-55 follicles in the present study, whereas, Harach, 1987 and Carlos and Muro (2000) reported 20-40 follicles in man (and 20-30 millions of follicles in the thyroid gland. In the prepubertal group, most of the follicles were small with a few larger follicles indicating that the follicles were in developing stage. Whereas, most of the follicles were larger in sizes together with few smaller follicles indicating the most of the follicles were active in function. Histological variation was found between prepubertal and pubertal indigenous bulls. The castrated group of bulls showed larger inactive follicles with few smaller follicles.

The follicular diameters were presented Table 1. The follicular diameters of the round follicles were minimum 158.12±3.13 µm and maximum 728.75±15.09 µm in prepubertal bulls, minimum 210.62±7.48 µm and maximum 890.62±10.13 µm in pubertal bulls, minimum 206.87±4.53 µm and maximum 972.50±6.76 µm in castrated adult bulls. Highest follicular developments occurred in castrated adult due to adult age of the bulls. The follicular diameters of the smallest oval follicles were minimum 187.50±4.92 µm and maximum 280.00±4.64 µm in prepubertal bull, minimum 218.75±5.16 µm and maximum 3.88.57±8.42 µm in pubertal bulls, minimum 267.50±5.16 µm and maximum 495.00±8.68 µm in castrated adult bulls indicated the parameters were highest follicular developments in castrated adult bulls. The follicular diameters of the largest oval follicles were minimum 661.25±7.20 µm and maximum 882.50±9.61 µm in prepubertal bulls, minimum 753.75±11.67 µm and maximum 1067.50±18.05µm in pubertal bulls, minimum 885.00±8.68µm and maximum 1266.25±31.71 µm in castrated adult bulls indicated the parameters were highest follicular developments in castrated adult bulls. The adult castrated group of bulls showed larger follicular diameters than in the pubertal and prepubertal stages. In human, the follicles that vary in diameters about 200-800 µm, depends on the size of the follicles, depends on the size and number of the follicular cells and the amount of the colloid (Antongiulio *et al.* 2004). The histometry of round follicles, smallest oval follicles and largest oval follicles at the level of minimum and maximum diameters were found to differ ($p<0.01$).

Table 1. Histometry of thyroid follicles of prepubertal, pubertal and castrated indigenous bulls Mean±SE (µm)

Parameters		Prepubertal	Pubertal	Castrated
Round follicle	Minimum diameters	158.12^{e}±3.13	210.62^{dd}±7.48	206.87^{d}±4.53
	Maximum diameters	728.75^{c}±15.09	890.62^{b}±10.13	972.50^{a}±6.76
Smallest oval follicle	Minimum diameters	187.50^{d}±4.92	218.75^{cdd}±5.16	267.50^{cc}±5.16
	Maximum diameters	280.00^{cc}±4.64	$3.88.57^{b}$±8.42	495.00^{a}±8.68
Largest oval follicle	Minimum diameters	661.25^{e}±7.20	753.75^{d}±11.67	885.00^{cc}±8.68
	Maximum diameters	882.50^{c}±9.61	1067.50^{b}±18.05	1266.25^{a}±31.71
Epithelial height		11.50^{b}±0.73	18.12^{a}±0.85	9.00^{c}±0.37

Means with different superscripts within each row for each parameter differ significantly

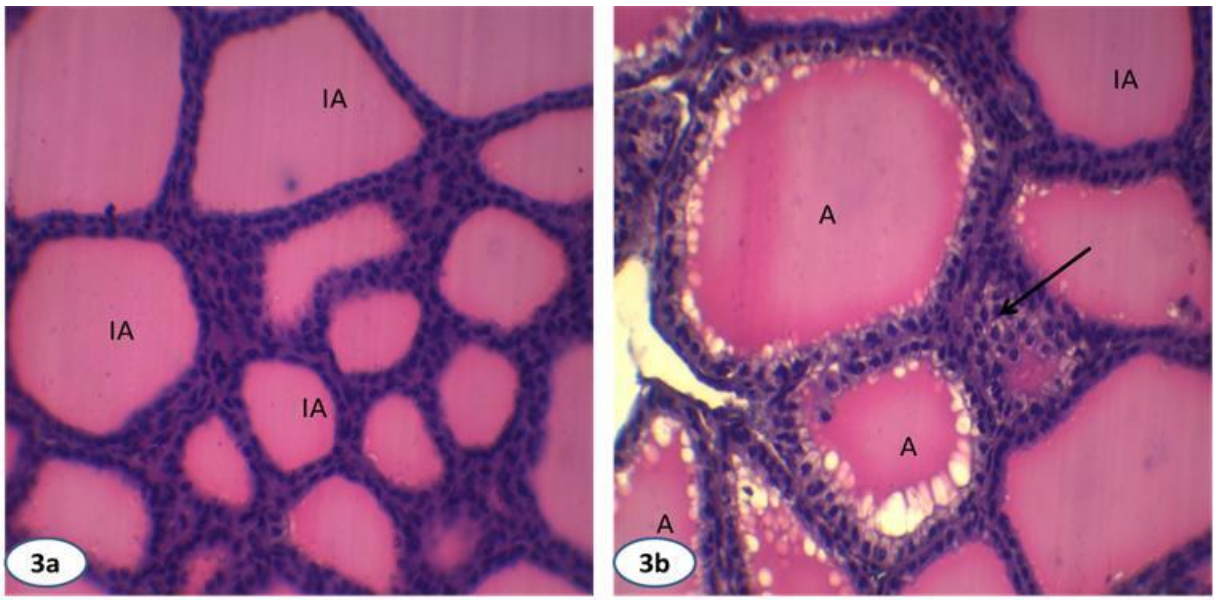

Fig. 3(a-b). The thyroid gland showing the inactive thyroid follicles (IA), active follicles(A) and Parafollicular cells (arrowhead) with the low cuboidal lining epithelium of prepubertal bull. H&E 40X

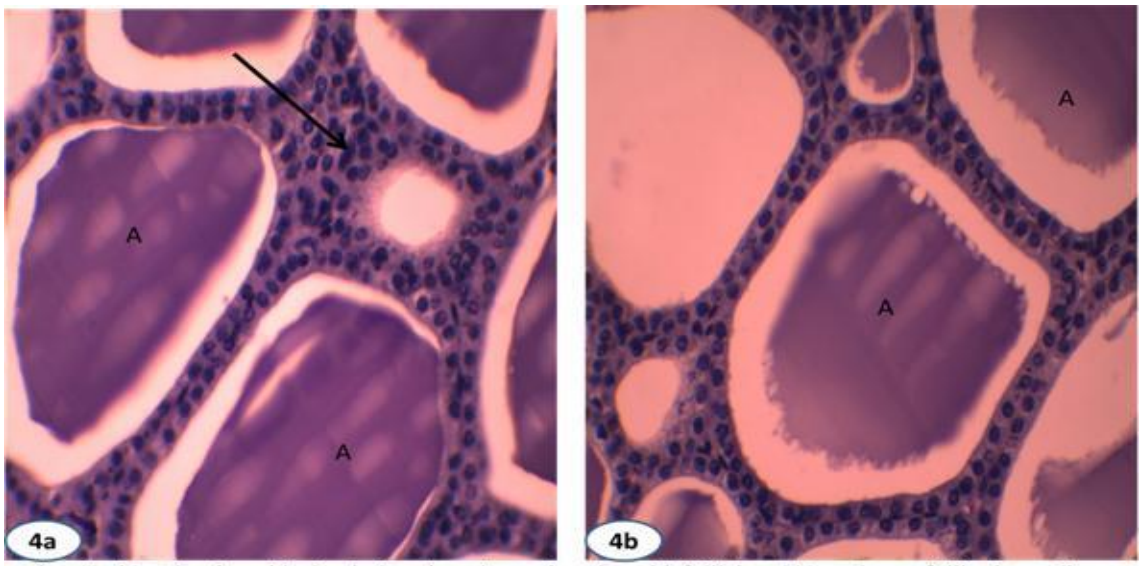

Fig. 4(a-b). The thyroid gland showing the active thyroid follicles (A) and parafollicular cells (arrowhead) with the cuboidal or columnar lining epithelium of pubertal bull. H&E 40X

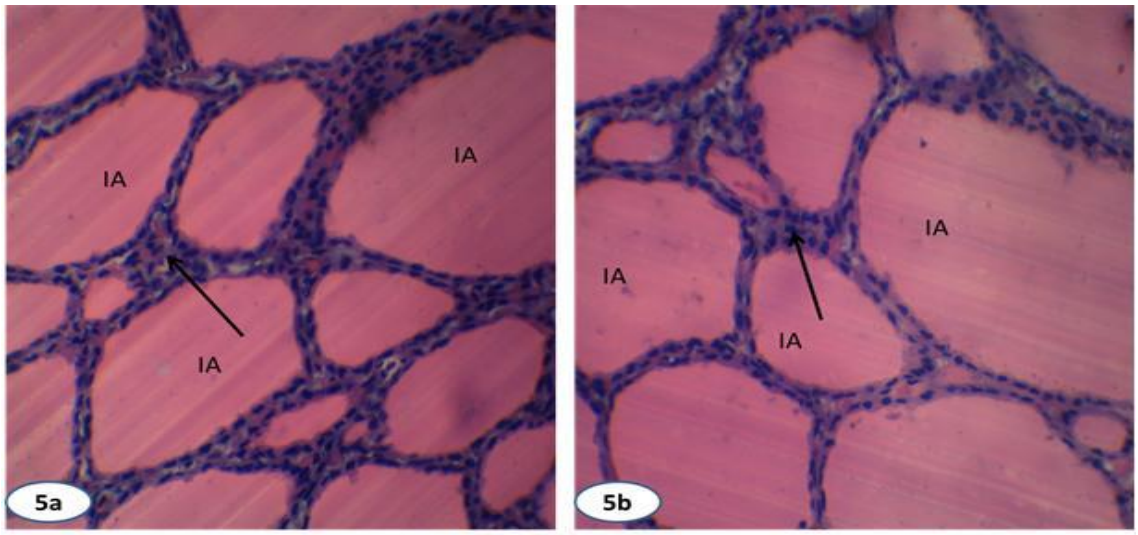

Fig. 5(a-b). The thyroid gland showing the inactive thyroid follicles (IA) and parafollicular cells (arrowhead) with the flattened or squamous lining epithelium of castrated adult bull. H&E 40X

In the present study, colloid was the amber colored homogenous, solid and lightly eosinophilic materials. Sometimes dead or degenerating cells were found in the lumen of the follicles due to sloughing of the follicular epithelium. This observation was in accordance with the reports of Adhikary *et al*. (2003) in goats. In prepubertal group, vacuoles were found inactive and some large follicles appeared which detached the colloids from the follicular epithelium indicated that the follicles were activated from inactive condition and at the same time follicular epithelium transformed into cuboidal or columnar from squamous type (Fig. 3). In pubertal group, spaces were found between follicular epithelium and colloid materials. Colloid were more homogenous, predominantly eosinophilic by Hematoxylin and Eosin stain in most active follicles, but few inactive follicles showed colloid materials of various degree of acidophilia. These observations were in agreement of the reports of Das *et al.* (1965) in bulls and bullocks and Roy and Yadava, (1975) in buffalo. In the present study, pubertal bulls showed mostly active follicles with few colloidless or inactive follicles indicating that at pubertal stage the thyroid gland were more functional than the other groups. In castrated adult bulls, colloid materials appeared lightly to be eosinophilic in the follicles. Most of the follicles were larger and inactive but appeared like prepubertal group with simple squamous epithelium indicated

lesser follicular activity of the thyroid. This observation indicated that the functional activity decreased with the older age of the animals. Few larger colloid free follicles were observed in castrated group of bulls which indicated decreased activity of the thyroid. This observation was in accordance with the report of Sanap *et al.* (1998) in prepubertal, pubertal and castrated cattle.

Follicular epithelium

Follicular epithelium of the thyroid gland varied with the age of animals. Epithelium became simple squamous to low cuboidal in prepubertal bulls, simple low cuboidal to columnar in pubertal bulls and simple squamous epithelium in castrated bulls in the present study (Figs. 3, 4 and 5). This might be due to different physiological activities and status of the thyroid glands. Epithelial heights of the prepubertal, pubertal and castrated bulls were 11.50±0.73 µm, 18.12±0.85 µm and 9.00±0.37 µm, respectively. This observation indicated the activity of the thyroid gland which was highest in pubertal group of bulls than in the prepubertal and castrated adults. Castrated adult showed lower epithelial height and the cells became completely simple squamous indicating very poor or non functional thyroid. In human, the thyroid glands in elderly person were characterized by mild atrophy, increased fibrosis and decreased size of the follicles. Functionally there was less pheripheral conversion of thyroxin (T4) to triiodothyronin (T3), decreased uptake of iodine and overall lower level of thyroxin noted in aged condition (Sirota, 1990).

Para follicular cells or interfollicular cells

The cells were observed in three groups of bulls, located beneath the basal lamina of the follicles instead of the follicular epithelium or in the interfollicular spaces. The cells were arranged in group or singly. Frequently observed in prepubertal group of bulls, but in pubertal and castrated group showed less or no parafollicular cells indicated that the functional activity of parafollicular cells decreased with the age and status of the animals (Figs. 3, 4 and 5). The observation was in agreement with the findings of Dellmann and Eurell (1998) in dogs and Sanap *et al.* (1998) in cattle. Light cells were oval or polyhydral and slightly larger than the follicular epithelial cells in the pubertal group of bulls. Thickness of interfollicular spaces were more in the prepubertal group than in pubertal and castrated adults indicated that the follicles were developing from prepubertal stage and increased in sizes with the advancement of age. The observation was similar to the findings of Adhikary *et al.* (2003) in goats and Roy and Yadava (1975) in buffaloes. Another study in man Sirota (1990) stated that the parafollicular cells showed negative correlation between the process of aging and the number of cells which represented 25% in childhood and in adolescence, while in the adult and elderly age they represented 8-10% of thyroid gland cells.

Histological features of the thyroid gland of prepubertal, pubertal and castrated adult bulls showed that prepubertal bulls showed lower histometric parameters of the thyroid follicles than the pubertal and castrated adult stage. Follicular epithelium become simple squamous to low cuboidal in prepubertal bulls, simple low cuboidal to columnar in pubertal bulls and simple squamous epithelium in the castrated bulls. So, the study may conclude that the activity of the thyroid gland increases from prepubertal to pubertal stage and decrease at castrated adult bulls. Finally, thyroid gland undergoes changes with age; in course of aging and physiological status, histological features and function also changes.

Acknowledgement

The authors are cordially grateful to the Department of Anatomy and Histology, Sylhet Agricultural University, Sylhet, Bangladesh for providing research facilities.

References

Adhikary G N, Quasem M A and Das S K. 2003. Histological observation of thyroid gland at prepubertal, pubertal and castrated black Bengal goat. Pak. J. Bio. Sci. 6(11):998-1004.

Agrawal K P, Bhattacharyya N K. 1981. An evaluation of thyroid status at different physiological stages in barbari goats. Indian J. Anim. Sci, 51:328-332.

Antongiulio F, Jeremy C, Nicolas B and Monique T. 2004. Age dependant variation of follicular size and expression of iodine transporters in human thyroid tissue. J. Nucl. Med. 45(2):232-237.

Baishya G, Ahmed S, Bhattacharyya M. 1986. Histomorphological and histochemical study of thyroid gland of prepubertal Assam goats. Indian J Anim. Sci. 56:667-669.

Carlos A, Muro M. 2000. Tumors of thyroid gland: Histologic and cytologic features. Am. J. Can. Cont. 7(3):276-278.

Das L N, Misra D B and Biswal G. 1965. Comparative histology of adrenal and thyroid gland of the bull and bullock. Indian Vet. J. 42:824-830.

Dellmann H D and Eurell J A 1998. A Text Book of Veterinary Histology. 5thedn., Williams and Wilkins, A Waverly Company, Philadelphia, USA. Pp 226-235. Getty R. 1975. The Sisson and Grossman's the Anatomy of the Domestic Animals. Vol 1, 5thedn.,W. B. Sounders Company. Philadelphia. London. Toronto.

Gridley M F. 1960. Mannual of Histologic and Special Staining Tecnique. 2ndedn., McGraw-Hill Book Company. USA.

Ham A W and Carmack D H. 1979. Histology, 8th edition. J B Lippincott company. Philadelphia and Toronto. pp.801-2.

Harach J R. 1987. Mixed follicles of Human thyroid gland. J. Acta Anat. 129:27.

Junquera L C and Carneivo J. 2005. Basic Histology, Text and Atlas, 11th edition. McGraw Hill Company, pp.411-415.

Mathur M I. 1971. Microscopic study of the thyroid gland of the Asiatic water buffalo (Bos bubalis). Am. J. Vet. Res. 32:363-66.

Roy K S and Yadava R C P. 1975. Histological and certain histochemical studies on the thyroid gland of the Indian buffalo (Bubalus bubalis). Indian J. Anim. Sci. 45:201-208.

Roy K S and Saigal R P. 1987. Histochemical study on thyroid gland of sheep during early pregnancy Indian J. Anim. Sci. 57:26.

Sanap S M, Mugale R R, Bhosale N S and Mamde C S. 1998. Histology of thyroid glands in prepubertal, pubertal and castrated cattle. Indian Vet. J. 75:813-816.

SAS, SAS Users' Guide. 1998. SAS Institute Inc., Cary, North Carolina, USA.

Sirota D K. 1990. Thyroid function and dysfunction in the elderly: a brief review. Mt. Sinai J. Med. 74:126-131.