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Histologic and ultrastructural observations on the thyroid gland of the White Fulani (*Zebu*) cattle in Northern Nigeria

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The histology and ultrastructure of thyroid gland of White Fulani (*Zebu*) cattle were examined during the postnatal developmental period to show if variations in morphology exist with age and in relation to tropical climate. Eight prepubertal and ten pubertal cattle of abattoir origin were used to carry out this study. Histologically, the thyroid consisted of well-developed capsule, interlobular connective tissue, follicular and parafollicular cells from prepubertal to pubertal age of 5 to 7 years. The follicular cells were cuboidal in prepubertal period with some variations, became predominantly columnar in pubertal age of 2 to 4 years and highly flattened cells were common in the older pubertal animals of 5 to 7 years. The colloid was observed in the lumen at all age and was PAS-positive with abundant peripheral vacuoles in the pubertal age of 2 to 4 years. Significant variations ($p<0.05$) in structure of the thyroids were observed at all age and the follicular size and number of follicles increased as the cattle matured. Follicular height was reduced significantly in the older pubertal cattle of 5 to 7 years. Ultrastructurally, the follicular cells showed highly dilated cisternae of rough endoplasmic reticulum which decreased with age. Flattened follicular cells seen in the older pubertal cattle contained few cytoplasmic organelles and microvilli. Apically placed pseudopods and blebs were commonly observed in the pubertal age of 2 to 4 years. Lysosomal bodies increased with age of development. The parafollicular cells were encountered frequently in the prepubertal age than in the older cattle. They consisted of numerous dense secretory granules which increased in number with age. The present results suggest an optimum thyroid function during the pubertal. Our findings would be useful in interpreting changes in thyroid morphology during experimental and pathological conditions.

Key words: Cattle, postnatal thyroid, histology, ultrastructure.

INTRODUCTION

The thyroid is the largest and the first recognizable endocrine gland during development in vertebrates. Marked variations in location, gross and histological features of the thyroid gland have been observed in different vertebrates (Dyce et al., 2002). Thyroid responses to environmental and nutritional influences

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differ across the phylum. In mammalian development, the thyroid hormones exercise significant effects on cell proliferation, differentiation and migration. The effects of thyroid hormones on growth and metabolism in all stages of mammalian development are well documented (Legrand, 1986). Calcitonin secreted by parafollicular cells lowers blood calcium levels by suppressing the resorptive action of osteoclasts and promotes calcium deposition in bones by increasing the rate of osteoid calcification (Mescher, 2010). The thyroid follicular and parafollicular cells undergo significant alteration during prenatal and postnatal development. The alterations pertain to the number of cells, their ultrastructure, as well as their hormone storage in the cells (Kameda et al., 1984; Fujita, 1975). The parafollicular cells produce mainly calcitonin that regulates calcium metabolism and it also produces few other regulatory peptides of the thyroid such as somatostatin, chromogranin A and neuron specific enolase (NSE) that are probably involved in intrathyroidal regulation of follicular cells (Sawicki, 1995). It is generally accepted that variations in the organelle content of the thyroid follicular cell reflect variations its hormone synthesis, secretion and absorption (Harrison and Young, 1970; Gorbman et al., 1983). Environmental factors such as temperature and photoperiods may influence follicular cell activity through nervous endocrine agents (Norris, 2007).

Available literature on the histological, ultrastructural studies on the development of the thyroid gland in several exotic wild and domestic animal species including cattle are abundant (Fujita, 1975; Fujita, 1988; Hernandez et al., 1972; Schafie and Mashaly, 1974; Roy et al., 1978; Pardehi, 1981; Baishya et al., 1985; Sawicki and Zabel, 1997; Sawicki and Zabel, 1999; Hajovska, 2002; Jelinek et al., 2003; Peksa et al., 2011). Although, these studies exist on thyroid morphology, there is scant published literature on morphology of thyroid of our indigenous domestic animals of the humid tropical environment. However, many of these were on exotic breeds of cattle in the Mediterranean and temperate climate. The white Fulani *(zebu)* cattle unlike some of the exotic breeds are reared in the semi-arid and arid regions of the country by pastoralists that move from place to place in search of water and pasture. It is reasonable to hypothesize that there should be variation in structure and function of the thyroid in tropical breeds due to variation in genome, environmental condition and husbandry methods. The present study was therefore undertaken to investigate such possible variations and to relate the findings to function in a humid tropical climate. This would facilitate the accurate diagnosis of congenital, and acquired pathological abnormalities and iodine-related disorders of the thyroid that may occur during rearing.

MATERIALS AND METHODS

Thyroid glands were obtained from eight prepubertal (less than 1 year) and ten pubertal (3 to 6 years) White Fulani (Zebu) cattle slaughtered at the Nsukka market abattoir after being certified free of disease. The age was estimated by dentition (Torrel, 1998; Pace et al., 2003). Following death, thin slices taken from different parts of each thyroid lobe and isthmus were fixed in 10% neutral buffered formalin and processed routinely for light microscopy. Some of the sections were stained with haematoxylin and eosin and others with periodic acid Schiff (PAS). An ocular micrometer gauge calibrated with a stage micrometer was used to obtain values of histological parameters of the thyroid gland from selected sections under a light microscope with × 40 objective lens. Data were analyzed statistically using analysis of variance. Duncan's multiple range tests was used to separate variant means, and significance was accepted at $p < 0.05$. For electron microscopy, small pieces of the organ were diced into 1 mm^3 cubes, fixed in 2.5% glutaraldehyde in 0.12 M Millonig's phosphate buffer at pH 7.4. They were post-fixed in 1% osmium tetroxide after rinsing in phosphate buffer for electron microscopy. The fixed pieces of the thyroid gland were dehydrated in graded ethanol, cleared in propylene oxide and embedded in epoxy resin. Ultra-thin sections (60 to 80 nm) were collected on copper grids, stained with uranyl acetate, and counterstained with Reynold's lead citrate and they were examined under Philips CM10 transmission electron microscope accelerating at 80 KV (FEI, Eindhoven, The Netherlands).

RESULTS

Histology

Thyroid gland of prepubertal cattle of 10 to 11 months showed a developed thyroid capsule with collagenous fibers, moderate fibroblasts and profiles of vascular and nervous tissues. The inner part of the capsule penetrated the parenchyma through the trabeculae septa carrying enormous connective tissue elements, vascular structures and lymphatics. The trabeculae divided the organ into very distinct lobule (Figure 1a and b). These follicular cells were columnar in most follicles, but occasionally cuboidal follicular cells were seen in some colloid-distended large follicles. Generally, follicular lumina predominated over cellular and vascular element and medium- sized follicles were predominant, followed by large follicles and few small follicles. Small follicles were mainly in the periphery. All follicles contained a homogenously stained colloid with colloid droplets apparent in the periphery of the colloid (Figure 1b). The colloid was PAS- positive (Figure 1c). Parafollicular cells were present as in the previous age group. The thyroid tissue of about 2 to 4 years showed similar histological structure in the maturation of the capsule, trabeculae and their various connective tissue components. Larger vascular tissue and nerves bundle were encountered at this age than the prepubertal. The predominance of medium-sized follicles, followed by large follicles in the centre of the lobules was maintained (Figure 2a). Colloid droplets seen in the prepubertal also persisted and the colloid was intensely PAS- positive (Figure 2b and c). The cells were cuboidal to columnar with little variation in height. Parafollicular cells were observed as in the previous age. In cattle of 5 to 7 years focal areas of cell hyperplasia were present. Large irregular follicles were greatly

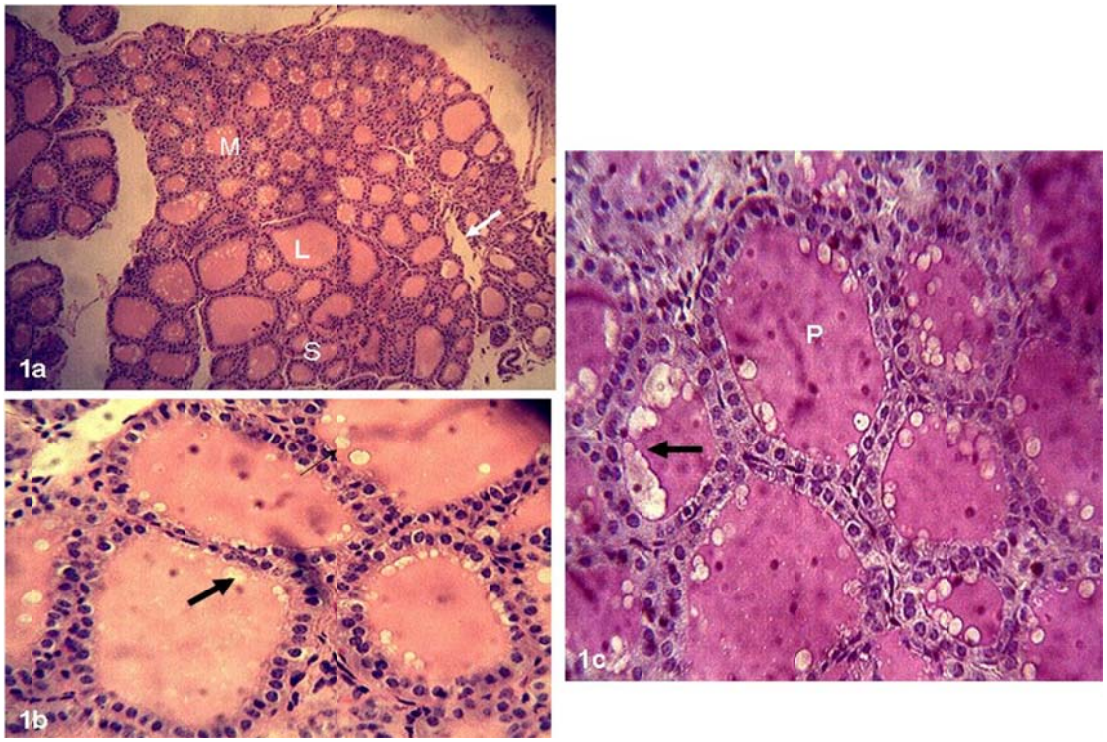

Figure 1. a and b, Histological section of prepubertal thyroid showing large follicles(L), medium follicles and small follicles (S) and trabeculae (arrow) and colloid droplets at higher magnification. **C.** A PAS-positive colloid lumen (P) with colloid droplets (arrow).

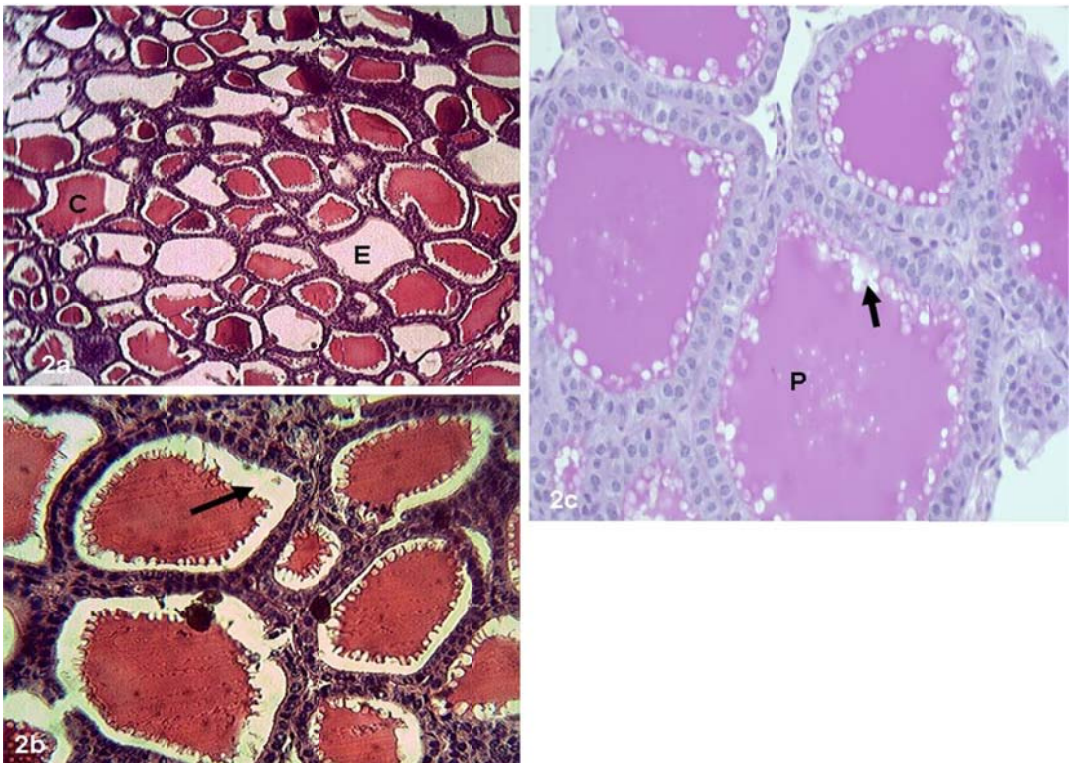

Figure 2. a. Histological sections of thyroid of 2 to 4 years cattle showing profiles of irregular large and medium- sized follicles, some with colloid (C) and others empty (E). **b.** Follicles at this age containing copious peripheral vacuole (arrow). **C.** The colloid was PAS-positive (P) colloid vacuoles (arrows).

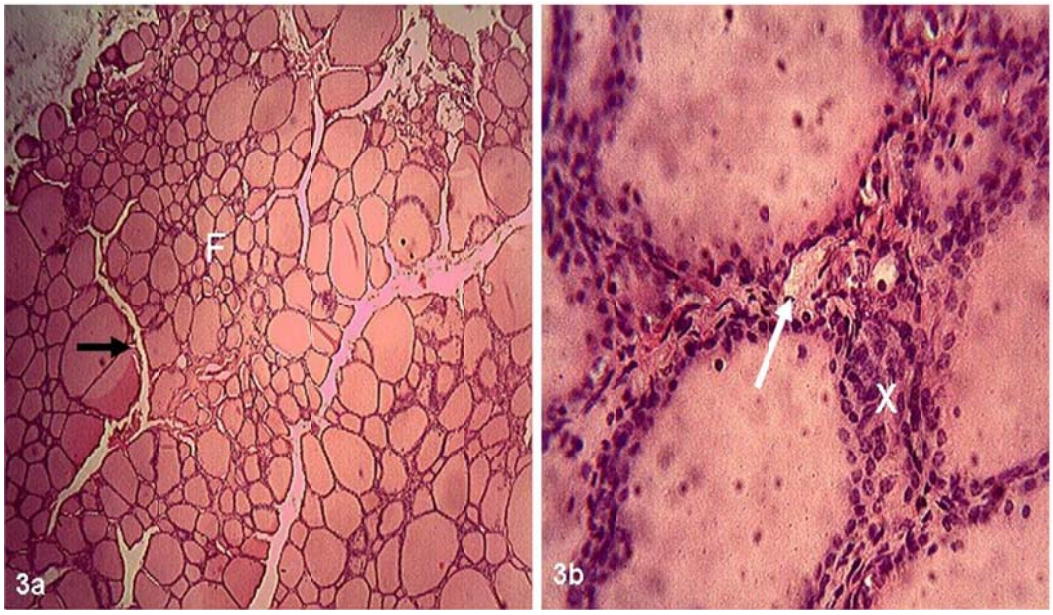

Figure 3a. Thyroid histological section of 5-7 years cattle with highly distended profiles of irregular large follicles (F), and increased lobulation of the parenchyma (arrow). **b.** Higher magnification of the large follicles showing areas of cellular hyperplasia (X) and accumulation of fibrous tissue (arrow).

Table 1. Histomorphometric of thyroid follicle of postnatal white Fulani *(zebu)* cattle with increasing development age.

Dev. Age d/year	SFD (µm)	MFD (µm)	LFD (µm)	CT (µm)	CH (µm)
Prepubertal	$53.36^{d} \pm 0.30$	$106.91^{d} \pm 0.65$	$237.97^{d} \pm 0.75$	$90.35^{d} \pm 0.33$	$7.75^{b} \pm 0.05$
Pubertal 2 to 4 years	$61.43^{e} \pm 0.48$	$109.55^{e} \pm 0.55$	$264.89^{e} \pm 1.04$	$93.44^{e} \pm 0.38$	$7.45^{c} \pm 0.04$
Pubertal 5 to 7 years	$73.37^{f} \pm 0.84$	$125.01^{f} \pm 1.13$	$284.00^{f} \pm 0.98$	$107.08^{f} \pm 1.21$	$5.53^{d} \pm 0.08$

^{a,b,c,d,e,F}Means in the same column with different superscript are significantly different from each other. **SFD**, small follicle diameter; MFD, Medium follicle diameter; LFD, large follicular diameter; **CT**, thickness of thyroid capsule; **CH,** follicular cell height.

distended with colloid that was less intensely PAS-positive and follicular cells presented flattened nuclei (Figure 3a and b). Evidence of fibrous tissue formation was observed in the interfollicular connective tissue. Parafollicular cells were present singly. Significant variations ($p<0.05$) in the histomorphometry of the thyroid gland were observed amongst the age groups during the postnatal developmental period. All histological parameters (Table 1) increased as the thyroid matured in age.

Ultrastructure

In prepubertal thyroids of about 10 to 11 months old cattle, the follicular cells appeared well differentiated and varied in size and shape, particularly in their vertical and lateral dimensions (Figure 4a). Some where columnar with indented nucleus and contained more cytoplasmic organelles and others were cuboidal with irregularly-shaped nucleus with few cytoplasmic organelles. In addition, very few flattened follicular cells were evident in some follicles.

The basement membrane was well differentiated and the interfollicular space contained connective fibroblasts, fibrocytes and collagen fibrils. Perifollicular vascular endothelium was well developed. The cuboidal follicular cells contained nucleus with heterochromatin (condensed chromatin) was concentrated marginally on the nuclear membrane as irregular layer and was also present as scattered clumps in the nucleus. The euchromatin was well-dispersed in the nucleus (Figure 4b). Profiles of well-developed rough endoplasmic reticulum were apparent in the cytoplasm and displayed well-marked dilated cisternae containing flocculent materials in some cells. Golgi complex was present in the supranuclear or atimes paranuclear position and was relatively extensive with lots of small-medium sized vesicles and secretory granules in the cytoplasm. The apical part of the cytoplasm showed numerous small dense secretory vesicles which were presumed to be primary lysosomes,

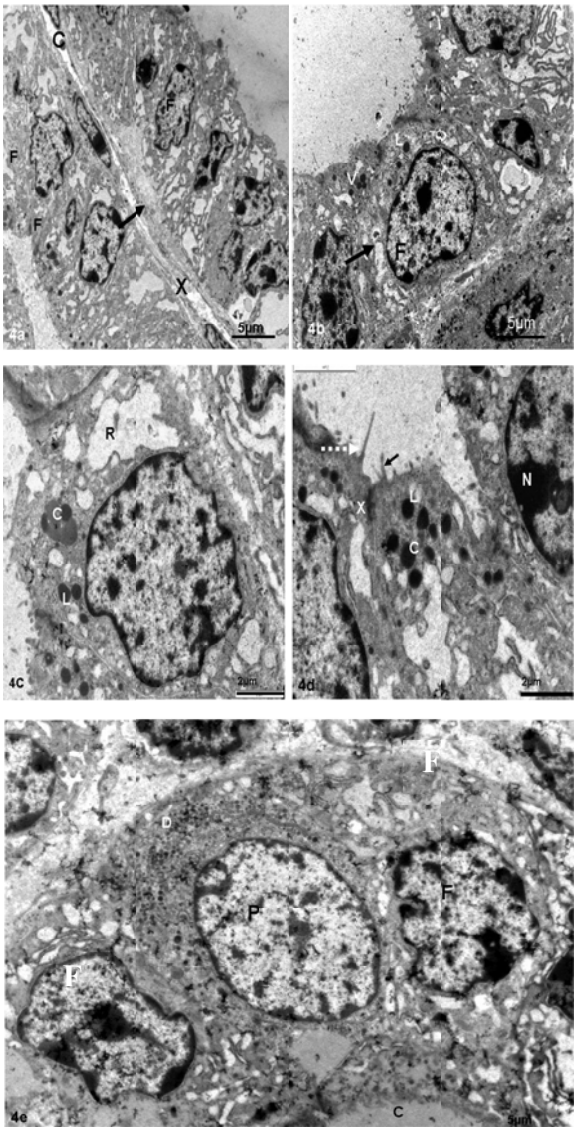

Figure 4. a. Electron micrograph section of thyroid follicular epithelium showing varying size and shape of thyroid follicular cells (F): Note the well delineated basement membrane (arrow) and interfollicular connective tissue (X), perifollicular capillary (C). **b.** EM section of thyroid follicular cell (F) of prepubertal thyroid showing highly developed dilated RER with some flocculent materials (arrow). Some small vesicles (V) and dense lysosome-like secretory granules are present (L). **c.** EM photographs of thyroid follicular cell of prepubertal showing highly dilated RER(R), dense secretory granules; probably lysosomes (L) that engaged in phagocytosis of large colloid droplets (C) to release thyroid hormones through the basal part of the epithelium (B).The apical cytoplasm studded with microvilli faced the colloid of lumen (X). **d.** EM photo graph of apical cytoplasm of follicular cell of thyroid of prepubertal cattle, showing large secretory vesicles that may be colloid droplets (C) and dense secretory granules probably lysosomes (L), microvilli(arrow), cilium (broken arrow), nuclei of follicular cells (N): Note the well delineated junctional complex (X). **e.** EM photograph of parafollicular cell (P)of prepubertal cattle thyroid interposed between two follicular cells (F).The parafollicular cells showed numerous dense granules(D) in their cytoplasm, in addition into some mitochondria, but lacked dilated cisternae of RER found in follicular cells, colloid (C).

also large less dense vesicles with same resemblance to the luminal colloid were identified as colloid droplets. Few lysosomal bodies were observed to have engulfed colloid droplets in the apical cytoplasm. Moderate number of mitochondria of varied shape was present in the apical position and few were scattered in the cytoplasm (Figure 4c).

Also, in the apical cytoplasm, profiles of short microvilli were present amongst single long cilium (Figure 4d) close to a well-developed junctional complex. But in some columnar follicular cells the microvilli appeared to be more than those in the cuboidal cells. The elements of the complex were identified between two columnar cells and included the tight junction, intermediate junction and desmosome and these followed each other in the order given in an apical-basal direction. Few parafollicular cells with large oval nuclei were identified by their basal location between follicular cells. They also did not make contact with the follicular lumen (Figure 4e). However, these relatively large, lightly stained cells contained strikingly numerous electron dense secretory granules in the cytoplasm. These cells also showed abundant mitochondria in the cytoplasm, but distended cisternae of rough endoplasmic is scant. The surface of closest contact between the follicular cell and the parafollicular cell lacked complex interdigitations or specialized areas of plasma membranes attachement.

In the thyroid of pubertal cattle of about 2 to 4 years, the matured follicular structure displayed in the pre-pubertal was maintained during this adult age (Figure 5a). The shape and size of these cells also varied from follicle to follicle, such that cuboidal and columnar epithelial cells were even present within a follicle, as previously noted. But columnar cells were more frequently encountered. In the follicular epithelial cells at this age, the arrangement showed well formed follicular cells with plasma membrane, basement membrane, nucleus and various profiles cytoplasmic organelles as seen in the pubertal age. A definite interfolllicular connective tissue containing perifollicular capillary endothelial cells, collagen fibrils and fibroblast amongst other connective tissue elements were copiously present. These components appeared to more abundant unlike in the prepubertal age. Junctional complexes were typical. Large, small dense secretary vesicles and colloid droplets were also prominent. In distinction from the prepubertal age, the follicular cells of pubertal age of 2 to 4 years, in addition to the numerous microvilli on the apical membrane, also showed moderate number of ciliated follicular epithelial cells in all of the sections examined at this age (Figure 5b and 5c).

Usually, one cilium per cell was present, although occasionally two cilia together have been observed in sections amongst numerous microvilli in thyroid sections of this age. Pseudopods (apocrine protrusions) with dome-shaped or balloon-like shape where observed on the apical surface, it contained fine granular matrix often

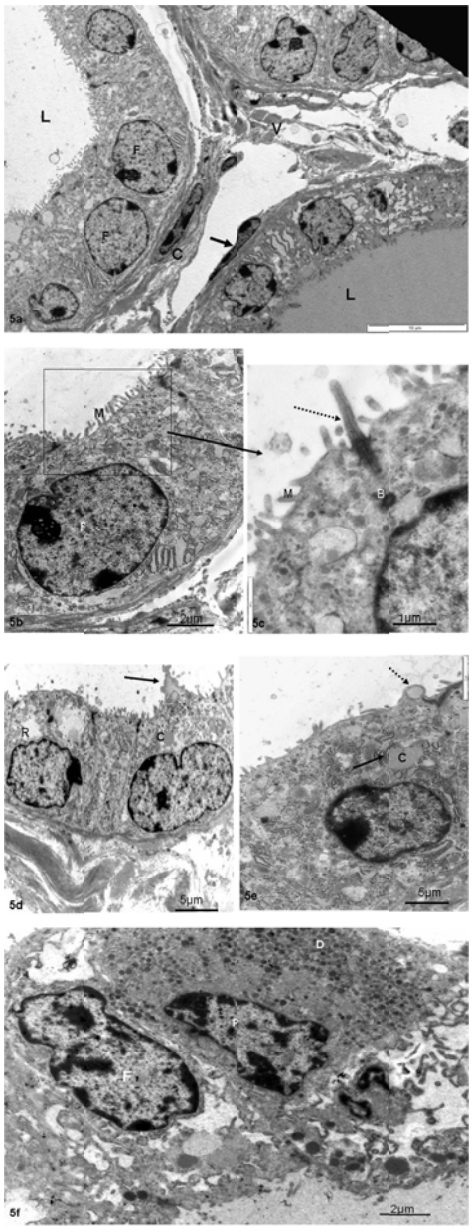

Figure 5. a. EM photographs of 2 to 4 years thyroid showing well differentiated epithelium of follicular cells (F) and highly vascularised interfollicular connective tissue (V) with some endothelial cells (arrow).Connective tissue fibroblasts and collagen fibrils (C) and luminal colloid (L) are shown, **b:** EM photograph of thyroid of 2 to 4 years thyroid showing thyroid follicular cell (F) with apparent increase in microvilli (M), with single cilium (box) occurring amongst them on the apical cytoplasm, **c:** Magnification of the apical cytoplasm showing a single cilium (broken arrow) and its basal bodies (b) and microtubule core. **d.** EM photograph of cuboidal follicular cell of thyroid of 2 to 4 years cattle showing apical pseudopods (arrow) and numerous colloid droplets (C). Note the highly abundant collagen fibrils (R) in the inter follicular connective tissue. **e.** EM photograph of thyroid follicular cell showing dome-shaped apocrine activity (broken arrow) in the apical cytoplasm, note the some colloid vacuoles(C) and Golgi complex (arrow). **f.** EM photograph of parafollicular cells (P) of thyroid of 2 to 4 years cattle showing increased accumulation of dense granules (D). These cells were not frequently encountered at this age as in the prepubertal, follicular cell (F).

with colloid droplet (Figure 5d and e). These pseudopods suggested evidence of phagocytosis of colloid droplets by the follicular cell. The intracellular organelles of the follicular cell appeared to be quantitatively similar to those of the previous age. The nucleus was also located basally, mitochondria occurred in all parts of the cell and some were closely associated with rough endoplasmic reticulum (RER). The RER was largely located between the nucleus unlike in the prepubertal groups. The cisternae of RER maintained highly distended profiles observed in the previous age groups. Several apical vesicles were present between the apical membrane and the nucleus, and large vesicles were recognized as colloid droplets. Dense lysosome-like bodies were rare in the apical position. The apical vesicles could be confused with dilated bits of RER except for the absence of ribosomes on the surface of the apical vesicles. Some parafollicular cells were however encountered in the sections examined and they appeared to display more dense secretory granules in the cytoplasm unlike in the previous age (Figure 5f).

In comparison to the prepubertal age, the adult thyroid differed in the sense that microvilli observed in the prepubertal age increased in number in the adult age and prominent single cilium was always present amongst them. Also, the lateral surface exhibited interdigitations in the prepubertal age, but these were only seldom observed on the basal half of the adult follicular cell but the junctional complex was similar in both groups, but more desmosomes were present amongst follicular cells. Parafollicular cell were encountered frequently in the prepubertal age unlike in this present pubertal thyroids of 2 to 4 years cattle. In the pubertal cattle thyroid gland of 5 to 7 years the follicular arrangement of cells were maintained, several of these follicular cells were squamous cells that intermingled with some cuboidal and even few columnar cells in some follicles (Figure 6a). Collagen fibrils with some connective tissue cells were remarkably increased in the interfollicular space. Generally, the follicular cells decreased in the profiles of organelles present. Some of the apical modifications like pseudopds were rare in sections, even though microvilli were present amongst a single cilium as previously observed. Highly dilated cisterna of RER common in the earlier age groups was highly reduced and Golgi complex was not commonly present. The preponderant apical vesicles, including colloid droplets and dense secretory granules were not a consistent finding in several of the flattened follicular cells (Figure 6b). Lysosome-like bodies appeared to have increased in the apical cytoplasm of the cuboidal cells in some follicles.

DISCUSSION

The present study used the prepubertal and pubertal White Fulani cattle to provide information on the comparative development, morphology and histochemistry

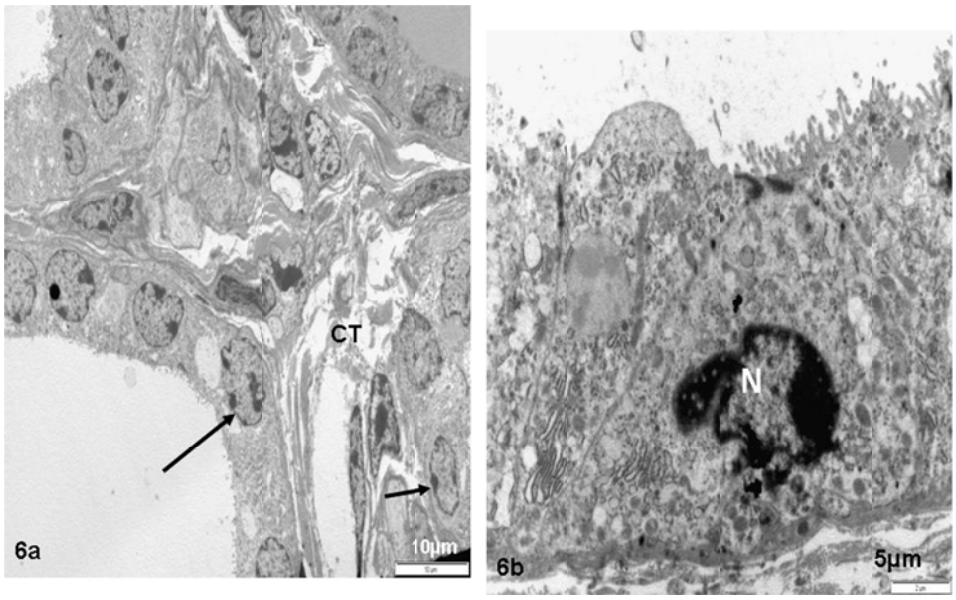

Figure 6. a. EM photograph of thyroid follicles of pubertal cattle of 5 to 7 years cattle showing follicular cells that were mostly squamous cells (arrows) and abundant interfollicular connective tissue (CT). **b.** EM photograph of follicular cell of thyroid of 5-7 years showing flattened cells devoid of dilated and the nucleus (N) appeared pyknotic.

of the thyroid gland. It is well-established that the role of the thyroid gland in vertebrates is crucial to the general metabolism of the organism and the expression of this role is dependent on the age, nutrition and environmental, especially climatic factors. It is therefore reasonable to expect certain structural and functional variations which modulate the function of the thyroid. The histologically components of the thyroids remained essentially the same in the postnatal age group has been described in several mammalian thyroids (Tomonari, 1958; Fujita, 1975; Fujita, 1988). Changes in the histometry of the thyroid components were also noticed during development and these were related to activity of the thyroid during postnatal development. Ultrastructural features of the thyroid gland were essentially the same in all age groups as have been described in some mammals (Klinck et al., 1970; Fujita, 1975; Atoji et al., 1999; Abdel-Magied et al., 2000). However, extreme ultrastructural variability of the shape and size of follicular cells in postnatal thyroids of all animals were noticed, even amongst cells of same follicle. Also, variable during development, were colloid droplets (resorption vacuoles), lysosomes and microvilli. This suggests that thyroid function is highly sensitive to several factors that include age, climate, feeding and environmental factors. The finding is supported by the study of fine structural fluctuations in rat thyroid during 24 h by Uchiyama et al. (1986) and is similar to the variability observed in the thyroid structure and hormonal profile in some domestic animals (Barnes et al., 1958; Fisher et al., 1977; Alwan, 2009). As development progressed from pre-pubertal to pubertal age in this study, there was increased structural and functional differentiation of the follicular cells with appearance of abundant cytoplasmic organelles, sub-apical vesicles, lysosomes and colloid droplets. However, the presence of pseudopods and dome-shaped apocrine secretion in the apical cytoplasm was remarkable in the 2 to 4 years pubertal cattle. An increase in the dense secretory granules of the parafollicular cells was encountered in the older pubertal cattle of 5 to 7 years.

The thyroid follicles varied histologically in size (follicular diameter, capsular thickness and cell height) and shape during development. This present finding is in agreement with histological findings of the thyroids by Blumenthal (1955) in European cattle, Mathur (1971) in Asiatic water buffalo, Roy et al. (1975) in goat and Sawicki et al. (1992) in European bison. It was observed variability in size and shape of follicles in young bison which increased significantly in older animals and large follicles were present in relatively great number in the very old bison.

However, Ranjan et al. (2011) observed no change in the follicular lining epithelium during histogenesis of foetal thyroid of buffalo. In our opinion, these variations in follicular size amongst developmental stages could be as result of the influence of the several internal and environmental factors influencing thyroid development and function. Concerning the existence of low and high cuboidal or columnar cells in same follicle, the present result showed that low cuboidal cell height had few organelles, indicating low activity and high cuboidal cells have more cellular organelles and were assumed to be

active; but, this opinion is contrary to that of Atoji et al. (1999), who observed no differences in the organelles content of low and high cuboidal cells in adult camel but showed the existence of low cuboidal and high cuboidal/columnar cell height in different follicles in this specie and concluded that there is no relationship between follicular cell height and function. However, the present result is supported by (Roy et al., 1978), with observation of changes in epithelial height of thyroids of different age groups in goat kids and buffalo calves (Marthur, 1971). In addition Young (1966), Klinck et al. (1970), Fujita (1975) and Uchiyama et al. (1986) noted in dogs, pigs, human and rats that tall follicular cells are highly active, whereas flat (squamous) follicular cells were hypoactive. The dissimilar observation in camel in relation to organelle content and functionality unlike in the present study may be due to the harsh environment exposed to camels unlike the cattle used in this study. The thyroid glands in the camel may have to remain very active with several organelles abundant in squamous cuboidal and columnar follicular cells to carry out its functions in the harsh, dry arid environment. It must be pointed that our study showed that follicular cells of varied height sometimes existed amongst follicles of within the same thyroid lobe in all the species. This common observations showed that the functional activity of each follicular cells were not always the same in each thyroid lobe during development, but when follicles with columnar cells or cuboidal predominate in the thyroid lobe, it is said to be active. However, low (squamous) or flat cells in several follicles seen in older pubertal cattle suggest low thyroid activity. The hyperplastic follicular epithelium seen in older pubertal cattle of 5 to 7 years is part of the degenerative changes seen in old thyroids and can give rise to some form of neoplasia of thyroid, as seen in ageing human thyroids (Kondo et al., 2006).

The follicular colloid showed a PAS-positive reaction in all of postnatal cattle with varying intensity. This histological finding indicated evidence of functional activity in the synthesis of thyroglobulin into follicular lumen to form colloid in the postnatal period. The presence of colloid was even reported in prenatal and postnatal cattle by Schafie and Mashaly (1974) and adult buffaloes (Roy and Yadava, 1975; Ranjan et al., 2011) with varying intensity as well. PAS-positive colloid was detected in human foetal thyroid at 13th to 14th week of gestation (Gaikwad et al., 2012). It therefore shows that the appearance of colloid and development of follicles in cattle occurs in the prenatal period. This opinion is supported by reports of Mitskavitch (1957) and Badawy et al. (1978). There were significant variations ($p<0.05$) in the mean thyroid weight, length, width and thickness of thyroids of all studied age groups. All measured gross parameters were increasing with advancing postnatal age. The present finding followed the pattern of growth of most organs in animal which normally increase in size and weight as the animal matured. The mass and volume of the thyroid depend on animal breed, body weight, nutrition, physiological status, and season of the year, geographical and climatic region. The significant variability in weight of thyroids during development may be caused by the above factors in addition to the nomadic pastoral management of the cattle used. The relationship between weights of the thyroid to body weight was not considered in this study, mainly because the factors that influence thyroid/body weight relationship such as sex and body weight were not available because of the abattoir-origin of samples. However, the absolute weight showed a significant increase in the absolute weight of thyroid gland with progress of development. The present observations on variations are in agreement with those of Das (1971) in the dog, Kausar and Shahid (2006) in camels, and Ekholm and Niemineva (1950), Bocian-Sobkowska et al. (1997) and Shepard et al. (1964) in developing human thyroid gland. However, in our study the absolute weight of the two lobes of thyroid cattle up to 5 years ranged from 18.90 to 22.05 g, this differ from the weight of 20 to 35 g (Peksa et al., 2011) reported in adult exotic cattle breeds of Eastern Europe and weights of about 12 to 34 g reported in calves and young cattle (Suuroja et al., 2003).

The ultrastructural features in the present study of colloid droplets remarkably showed the presence of these colloid vacuoles (droplets) as intracellular droplets on the apical cytoplasm of follicular cells in all postnatal cattle. They were observed amongst other components of the apical cytoplasm of follicular cells including primary lysosomes, profiles of RER and Golgi complexes. These two types of colloid droplets strongly show evidence of endocytosis of thyroglobulin. Colloid droplets seen in this study were membrane-bound, round or oval structures and their electron density were similar to those of the colloid. They are formed by engulfing of colloid by pseudopods on apical membrane of follicular cells (Capen and Martin, 2003). Fused colloid droplets were frequently encountered in this study suggesting that they gain size by the fusion of smaller droplet. Colloid droplets seen in the follicular cells at various age of development indicate thyroglobulin resorption. Their presence in large number suggest active endocytosis (Fujita, 1975), as observed frequently in most thyroid sections of prepubertal and pubertal cattle of 2 to 4 years used in this study. The reabsorption of colloid droplets from follicular lumen by endocytosis is considered the first step in the production of thyroid hormones (Ericson, 1981). Lysosomal digestion of colloid droplets facilitates the release of T3 and T_4 hormones from colloid droplets into the follicular cell cytoplasm (Norris, 2007), which will then be actively secreted into the adjacent capillary bed at the base of the cell. The presence of colloid droplets have been also described in several mammals (Fujita, 1975), including sexually immature camel (*Camelus dromedarius*) (Abdel-Magied et al., 2000) and several cold-blooded vertebrates (Lynn and Wachowski, 1951).

Some pseudopods with finger-like processes and apical apocrine protrusions (blebs) with a dome-like or balloon-like shape were remarkably encountered in the apical cytoplasm of the thyroid follicular cells of pubertal cattle of 2 to 4 years used in this study and suggested apocrine secretion. This is one of the few reports of this phenomenon in the thyroid gland of domestic animals. It is also possible that the frequent apical blebbing and pseudopods observed in the thyroid follicular cell of pubertal cattle may be due to the harsh nomadic system of rearing and mode of transportation of the cattle for slaughter, which enhanced the stimulation of the thyroid gland giving rise to numerous pseudopods and apical blebs observed in this study. During intense stimulation of thyroid in some severe conditions pseudopods are produced at the luminal surfaces of follicular cells facing the colloid and this phenomenon initiate endocytosis of stored thyroglobulin, resulting in large colloid vacuoles (droplets) (Zeligs and Wollman, 1977). Pseudopods equally encountered in the adult thyroid gland of cattle used in this study, may be similar to that encountered in one –humped camel (Atoji et al., 1999), which like cattle did not seem to enclose colloid. This finding in cattle may be related to low uptake of iodine into the thyroid gland in the normal camel (Abdel-Wahab and Osman, 1971; Atoji et al., 1999).

The occurrence of a single cilium per cell amongst some microvilli was observed on the apical membrane of the follicular cells in all postnatal pubertal age of development. It appeared to increase in number with age. This element has been reported in the follicular cell of many kinds of animal, including camel (Fujita, 1975; Atoji et al., 1999) and snake embryos (Rupik, 2011). Several studies of the ultrastructure of the thyroid glands of many species including human, dog, bat, salamander and *Xenopus levis* have noted the presence of an occasional cilium extending from apical border into the thyroid follicular lumen (Klinck et al., 1970; Setoguti, 1973). Some studies have suggested that the cilia mix the colloid mechanically (Sobrinho-Simoes and Johannessen, 1981) or by dissolving the newly synthesised thyroglobulin in the colloid by softening the colloid for uptake into the thyroid follicular cells during the secretory process. However, the study of Martin et al. (1988) on primary cilia in the follicular epithelium of the human thyroids showed that the structure and immotility of these cilia makes it unlikely to be related to motility function as in other cells. Cilia have also been suggested as vital cellular component for chemosensation (detection of a specific ligand, growth factor, hormone or morphogen) (Rupik, 2013). It physiological roles in chemical sensation in many endocrinal glands has also been suggested (Emmer et al., 2010). Therefore, it is also possible that the cilia in the thyroid follicular cells of cattle were sensory organelles which have been suggested for the pancreas (Zhang et al., 2005) and pituitary gland (Adams et al., 2008). Some membrane limited dense bodies of various sizes, were observed in thyroid follicular cells in the prepubertal and increased remarkably in the older pubertal animals in this study. They were highly abundant in the apical cytoplasm of the follicular cells of very old pubertal animals. This is because the activity and number of lysosomes increase during involution of thyroid gland which has been observed in ageing adult thyroid glands of several mammals (Pantic, 1974). An increase in size and number of lysosomes with developmental age was observed in this study, such that in very old pubertal cattle, the flattened (squamous) follicular cells that surrounded highly eosinophilic colloid were seen with large number of lysosome-like bodies, including multivesicular bodies. This indicate thyroid involution at this advanced age of cattle corresponding to- the so called 'cold' follicles of mouse thyroid (Nĕve and Rondeux, 1991). A large number of lysosomes in the cytoplasm of follicular cells possibly represent a common feature in the aging thyroid.

The secretory granules evident in the parafollicular (C-cells) increased in number and size with developmental age. The developing pubertal C- cells seemed to contain much more profiles of rough endoplasmic reticulum (RER) and few secretory granules in the cytoplasm suggesting synthesis phase of hormones. While the more mature C-cells of older animals have less RER but numerous granules of varying sizes suggesting storage phase of regulatory peptide hormones. The present observation of less number of dense secretory granules in the prepubertal cattle is consistent with the view that C-cells secrete actively during the neonatal and juvenile periods. This indicate higher turnover of calcitonin in prepubertal (young animals) than older pubertal animals. Moreover, the present observation of large number of lysosomes in the older pubertal animals than pubertal animals suggest that there is a slower rate of secretion in older animals. Lysosomes are usually required to remove secretory granules as they accumulate in the cytoplasm of older cells (Fujita, 1975). In the present study some pubertal ultrastructural sections did not show any C-cell. This observation is similar to that of Abdel-Magied et al. (2000) in sexually immature dromedary camel and suggested that the cells may be sparse or concentrated in one location within the gland and thus easily missed in sections. This reason is also the reason of our failure to encounter C- cells in some sections in this study.

In conclusion, the present research work remarkably showed variability in morphological shape and organelle content of the follicular and parafollicular cells even within the same follicle of the thyroid in all animals. This also varied amongst thyroids of the same age in each animal. This variability is attributed not only to age but to feeding, rearing management and several environmental factors (temperature and season). The presence of apical pseudopods and blebs were preponderant in the pubertal thyroid and suggested intense stimulation of the thyroid. This may be because of the nomadic management of

the White Fulani cattle in Nigeria.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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