Histological and Histochemical Study of Thyroid Gland in Bull (Bos Taurus) in Al Muthanna province

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Abstract
The current study aimed to finding of the histological and histochemical characteristics of the thyroid glands in adult males of bull . Fifteen adult male bull to was used in this study. Histological study was used to describe the general structure of thyroid, in addition to microscopic measurements included the thickness of capsule, diameter of different thyroid follicles. According to histological findings, the thyroid glands in each animal had a capsule covering them that was made up of two layers of collagen fibers. Thyroid gland follicles in three different shapes and sizes made up every lobe of the thyroid gland: large, medium, and small follicles .

The histological result revealed the Para follicular cells in the bull, also referred to as C-cells. C-cells are large, spherical, and oval in shape, and their cytoplasm was stained lighter than that of follicular cells. Their nuclei were also densely stained. They began as a single cell or in groups of two or three. In the tissue section, it was observed that certain C-cells were supported by a basement membrane, serving as the bases of both follicular and Para follicular cells. In contrast, other cells were arranged haphazardly in the intermolecular tissue, separating the Ccells from the follicular wall. The Para follicular cells, which were observed in tissue sections either singly or in small groups, have an oval or rounded shape, a spherical nucleus, and light stain cytoplasm .

Histochemical results showed that the capsule, colloid material, basement membrane and Para follicular cells of thyroid gland showed intense reactions. To PAS stain All parts of thyroid l glands was exhibited positive reaction to Alcian blue_ PAS and Masson Trachoma stain .

Keywords: *Histochemical, positive and Alcian blue_ PAS*

Introduction:

Domesticated bovine farm animals known as cattle are raised for their meat, milk, hides, or draft value. The term is most frequently used to refer to domesticated cattle from Europe or the West, as well as those from India and Africa. Nonetheless, a few other bovids—including the Tibetan yak, the Asian water buffalo, the gayal and banteng of Southeast Asia, and the plains bison of North America—have also undergone domestication or semi-domestication and are occasionally regarded as cattle. (*Rajathi 2019*) (Peksa et al,2011) .

The Artiodactyla order includes cows as members. The order is made up of animals with even toes, and cows are known for having unusual cloven hooves, which are made from the toenails on the middle two fingers of each foot. The names of all these animal groups are derived from the Latin word bos, which means cow. The family Bovidae (hollow-horned ruminants) includes antelope, sheep (Alwan, 2009), and goats (Solaiman *et al,2010*); the tribe Bovini (which includes cattle, bison, and yak); and the genus Bos. (*Yousef et al,2016*).

With a large mouth and teeth designed to break down tough vegetation, cows are ideally suited for grazing, or feeding on grass. Adults have 32 teeth, but instead of canines and upper incisors, they have a gummy pad that helps them tear up grass. Because of the moon-shaped ridges on the molars, which run parallel to the tongue, chewing effectively requires circular motions. *(Hussein and Dawood 2023*).

Material and Methods:

Collected the thyroid gland was fifteen healthy thyroid gland for bull . All samples have been collected from a local abattoir in Al-Samawa city fifteen thyroid gland during (October _ November _ December (The animals ages were from 2 to 3 years old. The thyroid have been gathered directly after slaughterin the morning. The thyroids are examined graphically in situ to determine their position. The thyroid have been washed with normal saline solution then put the thyroid in labeled container filled with 10% formalin solution for 48hours to complelet the mechanism of fixation.

To prepare tissue for histological sectioning, tissue samples are taken from different parts of the thyroid gland. The thyroid gland of fifteen bulls is subjected to histological examination. Thyroid sections with a diameter of 0.5 to 0.8 cm were preserved for 48 h in 10% formalin. To prepare a 10% formalin solution, ten milliliters of 37.9% formalin are mixed with ninety milliliters of distilled water (Vacca, 1985).

The histological sections were stained with Hematoxylin-Eosin stain (H&E) for general morphological features, Masson trichrome stain for identifying connective tissue, PAS for identifying carbohydrates and PAS – AB (PH 2.5) Stain for show the type of secretion. The histological sections were observed with an Olympus microscope (Leica Galen III) and were photographed with a digital camera mounted to a microscope (Leica with Dinocapture 2).

Results and discussions:

connective tissue capsule of thyroid gland in bull

The histological result of current study showed thin capsule covering the thyroid gland in Bull which made of irregular dense connective tissue that was containing strands of elastic and collagen fibers, so noted spindle-shaped of fibroblasts, tissue section appeared adipose tissue interaction through the tissue structures of capsule which effected on the cellular limit between collagen and elastic fibers in connective tissue capsule composition(Fig1). Theses findings of the connective tissue capsule were may be because of the mode of action of the thyroid gland during the study period, the our results were confirmed with (wang *,et al.,*2013) in donkey and (Kausar b, 2006) in monkeys, which they noted the tissue composition of the capsule were

variable in structures according to the seasonal variation, so composed of dense collagen fibers with a small amount of elastic and reticular fibers made up the outer layer. Adipose tissue made up the middle layer, and collagen and elastic fibers were found inside. Regarding the dimensions and form of the histological composition of the follicles.

The glandular parenchyma was divided into discrete lobules have different in size which included small, medium, and large follicles. Thin strands of trabecular that were extended from the shell which have important role in convert the medium blood vessels into small blood vessels (Fig. 2). The means thickness of capsule in the right and left lobes were 801.57± 64.84 µm and 807.2±56.01µm respectively (Table 1)**.** The mean thickness of capsule in the left lobe was greater than the right lobe significantly at $p < 0.05$ (Table 1)**.**

The thyroid gland in bulls is encased in a thin capsule that is composed of an inner layer of dense, irregular connective tissue that contains spindle-shaped fibroblasts, elastic fibers, and a thread of collagen; the outer layer is composed of thin adipose tissue that displays cellular boundaries clearly mixed with collagenous fibers and a few elastic fibers. The thyroid gland capsule of the bul showed positive responses to the hematoxylin and eosin stain, Masson's Trichrome and Periodic Acid Schiff both show high collagen content in the capsule., carbohydrates, and elastic fibers (Olukole *,et al.,*2016).

Thyroid Follicles in bull

The tissue section of thyroid gland in bull noted the left and right lobes have different in sizes of follicle which included(small, medium and large) (Fig.3), the thyroid follicles were varying in sizes which connected to form both left and right lobes of the thyroid gland. A thin framework of interfollicular connective tissue rich in fine collagen fibers and sinusoidal spaces which divided the follicle from the other ones. A basement membrane encircled every follicle., Follicular cells as epithelial layer that lined the inner surface of the follicles, the type of epithelial cell, varying in shape from flattened to columnar in shape, made up the follicular epithelium of the thyroid gland, all follicles were filled with colloid material. The result showed the three distinct follicle sizes present in both lobes of thyroid gland (Fig.4,5).

The (Table 1) included the biometrical result of the follicles in right lobe of thyroid gland which have means diameter of the three different follicle sizes: large, medium, and small were 1742 ± 23.26 µm, 1104.2 ± 22.01µm and 531 ± 23.29 µm, respectively, while in the left lobe were 1734 ± 41.03 µm, 1112 ± 27.96 µm and 539 ± 19.39 µm, respectively. The results of the statistical analysis showed that the mean diameter of large follicle in right lobe have higher significant increase in diameter when we compared with those in the left lobe at $p < 0.05$.

The mean diameter of medium follicle in left lobe have significant increase compared with those in right lobe significantly ($p < 0.05$) (Table 1).

The mean diameter of small follicle in left lobe didn't have significant differences when we compared with right lobe non-significantly at $p < 0.05$ (Table 1).

The current findings of the Bulls' thyroid gland appeared each follicle surrounded by a network of blood capillaries, the follicle wall was prominent when stained with P.A.S. stain, this reaction was referred to the wall of the follicle was rich with collagen fibers. These results were may be

to controlling on the hormonal secretion of the thyroid gland, which agreement with (Pankowski *,et al.,*2021) and (Skripkin ,et al.,2019) .

The current results of tissue section of thyroid gland that noted the shape of the follicular cells that lining the inner lumen of the follicles have simple elongated cuboidal epithelia, the epithelial cells have prominent spherical nuclei, acidophilic cytoplasmthat rests on a basal lamina. This result was similar to (Peksa *,et al.,*2011) the thyroid follicles are lined by simple cuboidal epithelium, which showed round nuclei with sparsely distributed chromatin.The cytoplasm showed some vacuolization and intense acidophilia. The thyroid follicles are filled with colloid showing an acidophilic characteristic.

The tissue results noted the small follicles were located at the gland's periphery under the capsule whereas the large follicles were predominant were dispersing at the gland's parenchyma . Additionally, the medium and small follicles have variety in shapes from polygonal, spherical, oval, tubular, and irregular. While the large follicles have uniform as spherical in shape. These results were may be to the gland activity and the amount of the colloid materials, the current findings were agreement with (Hussin and Altaay, 2009) who decided The follicles are irregular, polygonal, oval, spherical, and tubular in shape. Large follicles were present toward the center, whereas the majority of small sized follicles were dispersed around the periphery.

The histological finding of the thyroid gland showed the isthmus of the bull thyroid gland was made up of follicles separated by vascular structures which filled the interstitial connective tissues. The isthmus was encased in a lose connective tissue capsules. and have follicles with different in size as large, medium, and small follicles that filled with colloid material. The inner basement membrane and colloid substance of the follicles have positive reaction to P.A.S. staining. Our findings are consistent with those of (Benvenga ,et al., 2018) research,

The findings showed that the size and form of follicles in the gland that produces thyroid hormone weren't homogenous, as reported by (Yaglova et al. 2017) in bull , (Yousef *,et al.,*2016)in camels (Enemali *,et al.,*2016) within a mature male African giant rats the gambianus crustacean, (Igbokwe,2010) in grasscutter Thryonomys swinderianu, (Seraphim ,2014) in Hipposideros lankadiva (kelaart), and Sekulic *,et al.,* 2007) in pigs, so the (Mense and Boorman 2018) which noted Thyroid gland follicles in rats are divided by septa of connective tissue. The larger follicles can have a diameter of up to 270 µm and are spherical in shape, usually located in the periphery. The tiniest follicles can reach a diameter of up to 120 µm and are typically found in the center. Based on the functional state of the follicle, the follicular epithelium is cuboidal when it is at rest and columnar when it is active.

Also (Abdul-Hamid and Salah 2013) and (Maaruf *,et al.,*2017) observed that that the size of the follicles is not at all homogeneous, as larger follicles are usually

found in the periphery and smaller ones are found in the central, Thyroid follicles can have a wide range of shapes, but they are often irregularly shaped from oval to spheroid. The thyroid gland in adult rats was lined by more plump cuboidal epithelial cells and had smaller, less colloid follicles (Nilsson and Fagman 2017).

Thyroid follicles showed varying responses to PAS stain; depending on the density of the colloid material, some follicles showed a Some showed a weak reaction, while others showed

a strong reaction via a magenta color. The Alcian Blue _PAS pH 2.5 stain and Masson's Trichrome showed varying responses from each thyroid follicle(Alturkistani, *et al.* 2015) .

Para follicular cells or C-cells in bull

The histological result appeared the parafollicular cells, also known as Ccells, this cell rarely distrusted through the tissue sections of thyroid gland, C-cell have large, round, oval in shape, the cells appeared a lighter-stained cytoplasm than the cytoplasm of follicular cells, the C-cell have densely stained nuclei. They founded as a singly or in clusters of two or three cells(Fig.6). The tissue section showed some of C-cells supported by a basement membrane as the bases of both follicular and parafollicular cells, while other cells were randomly situated in the interfollicular tissue, C-cells were separated and unable to contact with the follicular wall. Our findings were similar to (Igbokwe, 2010) who noted the thyroid glands in wild African grasscutter, has large, oval parafollicular cells (C- cells) that exhibit a lighter-stained cytoplasm than follicular cells. Few of these cells were in the follicular epithelium, and the majority were in the interfollicular stroma.

These cells were situated as a little numbers between the follicular cells, and some of them came into contact with the colloid. The result noted the parafollicular cells found in the thyroid lobes also appear in the isthmus. These findings were agreement with (Prasanth et al., 2012). Which claimed the Cats' thyroid glands contain parafollicular cells, also known as C-cells, with lightly stained cytoplasm.

The tissue section of the thyroid gland showed the majority of parafollicular cells were found in the upper poles of the thyroid in bulls, which located in the interfollicular spaces or beneath the basal lamina of the follicles which instead of the follicular epithelium. This result was confirmed with (Pankowski *,et al.,* 2021) who noted the cells were arranged in groups or individually.

Colloid in bull

The current study showed the amount of eosinophilic colloid material in the intrafollicular spaces was varied in thickness and amount amongst all the follicles. Both homogenous and nonuniformly stained colloids were present, many or few peripheral visible empty vacuoles emerging through storage colloid , some follicles showed empty from colloid. in the one-humped camel (Rejeb *,et al.,* 2011). Colloid material, most likely an apocrine secretion from the lining epithelial cells, was present in the lumen of the follicles. (Fig. 7)

The colloid have varies in density through the follicles, some follicles containing eosinophilic colloid material that was highly stained (Fig. 8), the clearly the stained of colloid materials had a magenta color and have strongly positive reaction with P.A.S. stain (Fig. 9). Certain follicles had colloid material that was only slightly stained, indicating that the colloid in these follicles tested positive for PAS. Peripheral colloid vacuoles, which are found between follicular colloid and epithelial cells in certain follicles, are indicative of the metabolic activity of these cells. These results were in excellent agreement with (Peksa *,et al.,*2011) in camels and (Kausar and Shahid 2006) Researchers indicated that the majority of thyroid parenchyma consists of follicles.

Table 1. Histological measurement of right and left lobes of Thyroid gland in bull

Type of animal	Histological measurement(µm)	Right Lobe	Left Lobe	T- test
		Mean ± SE	Mean ± SE	
Bull	Thickness of capsule	801.57± 64.84	807.2±56.01	1.000 NS
	Diameter of large Follicle	1742 ± 23.26	1734 ± 41.03	1.306 NS
	Diameter of Medium Follicle	1104.2 ± 22.01	1112 ± 27.96	1.823 NS
	Diameter of small Follicle	531 ± 23.29	539 ± 19.39	2.005 NS
* (P<0.05) significant, NS: Non-Significant.				

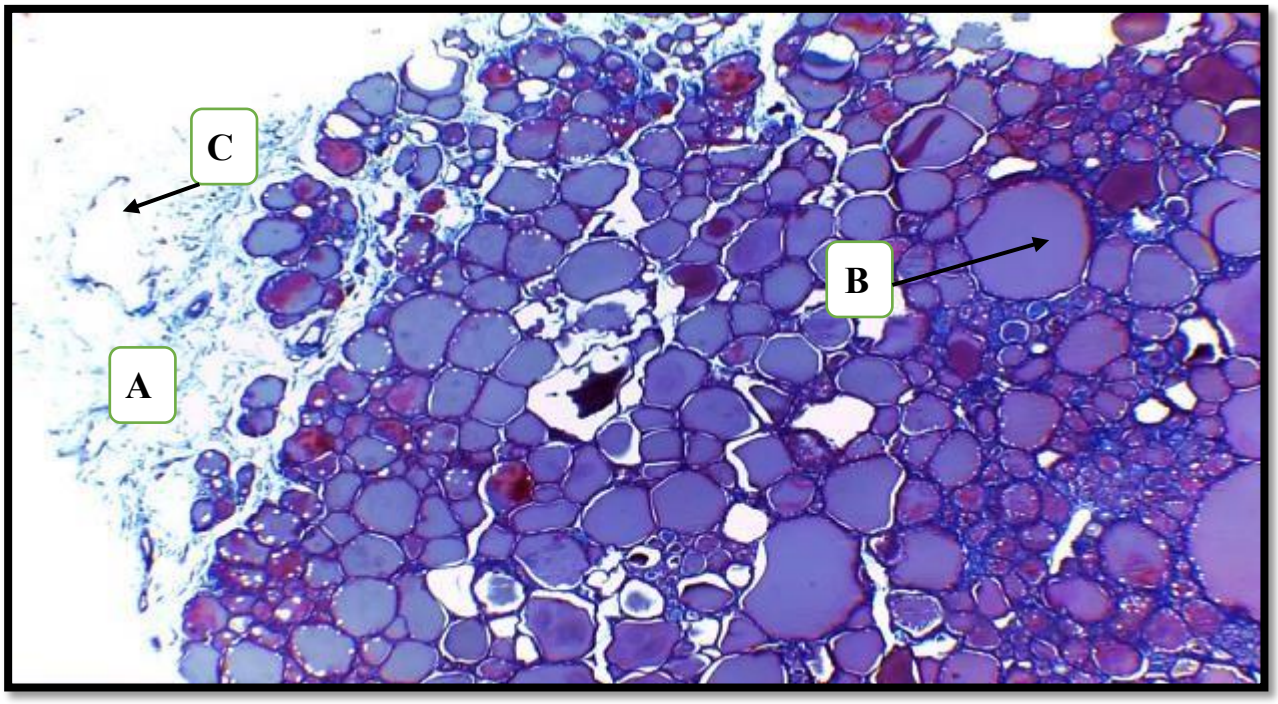

Fig.1. Microscopic section of the thyroid gland in bull show the : A. Capsule , B. Colloid, C. Blood vessels. **X40 Masson stain**

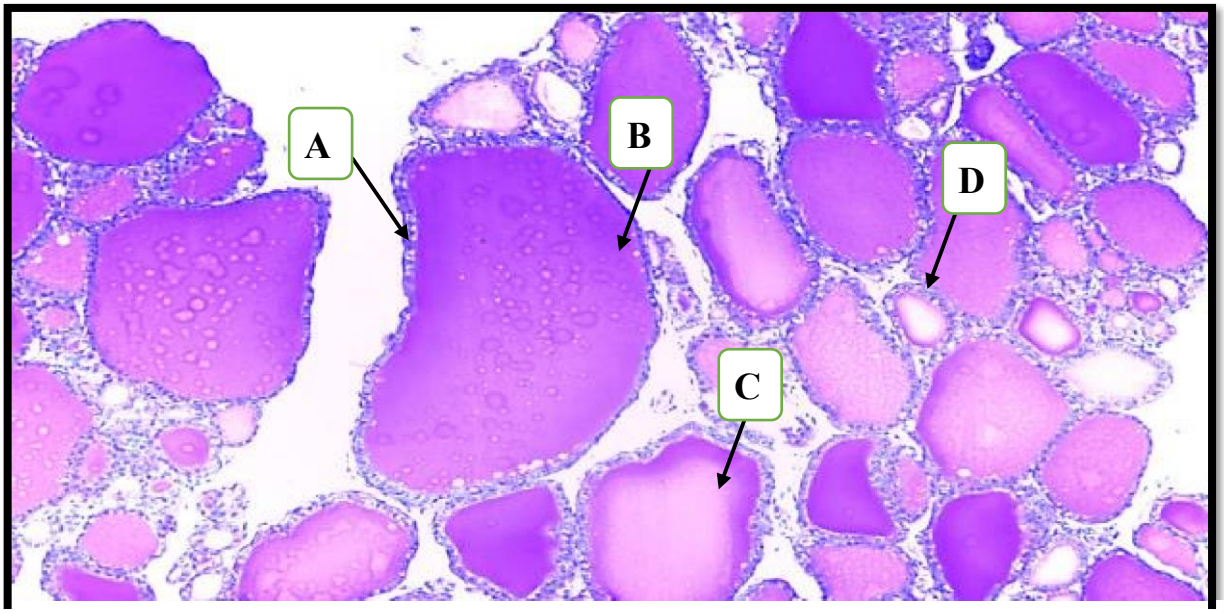

Fig.2. Microscopic section of the thyroid gland in bull show the : A. Basement membrane, B. Large follicles, C. Medium follicles, D. Small follicles **X100 PAS**

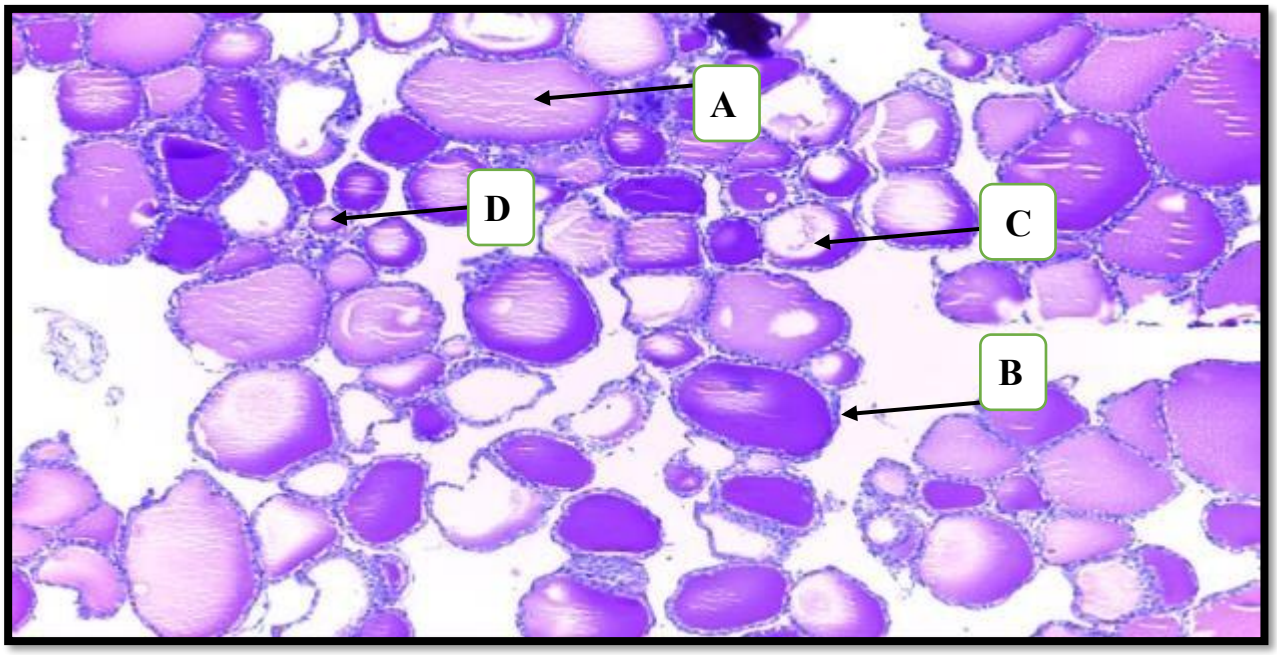

Fig.3. Microscopic section of the thyroid gland in buffalo show the : A. Colloid with mucin, B. Large follicles, C. Medium follicles, D. Small follicles **X40 PAS**

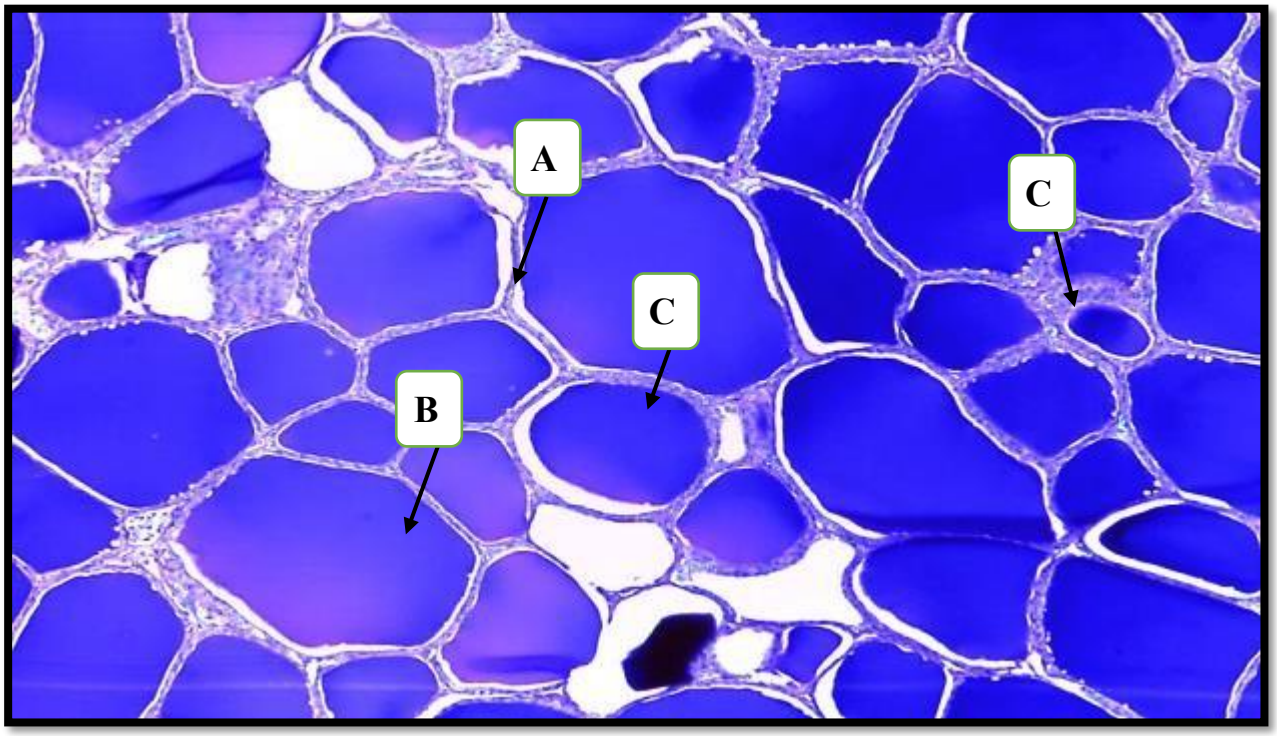

Fig.4. Microscopic section of the thyroid gland in bull show the : **A**. Basement membrane, **B**. Large follicles with mucin, **C**. Medium follicles, D. Small follicles **X100 PAS-AB**

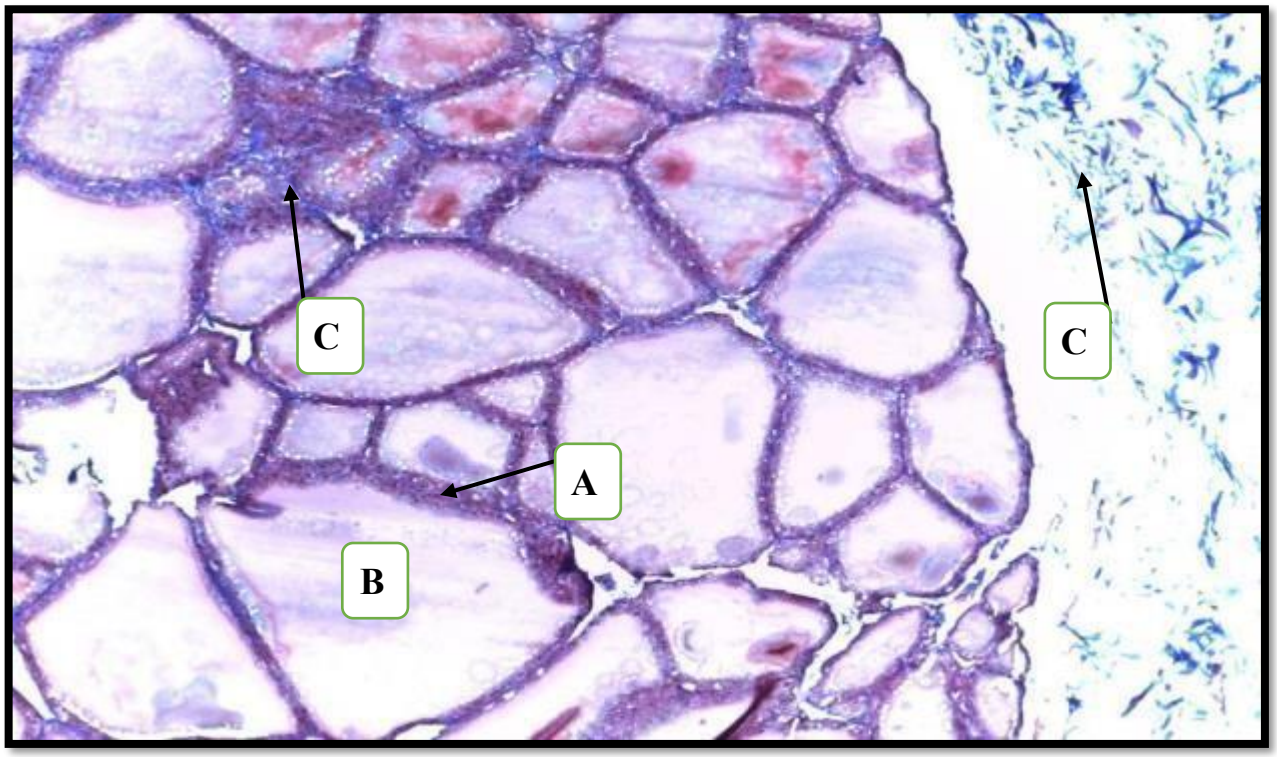

Fig. 5. Microscopic section of the thyroid gland in buffalo show the : A. Thyroid follicles, B. Colloid, C. Collagen fiber. **X10 Masson stain**

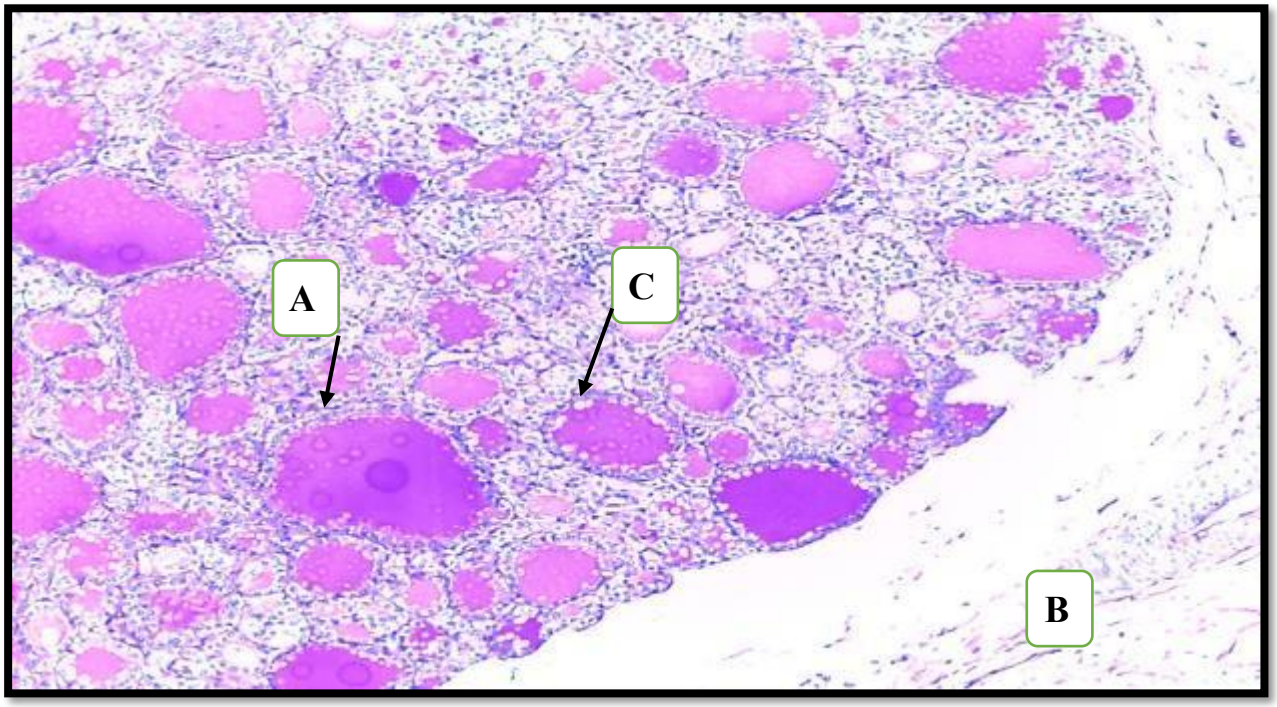

Fig.6. Microscopic section of the thyroid gland in bull show the : **A**. Basement membrane, **B**.

Capsule with Collagen fiber, **C**. Follicular cells. **X100 PAS**

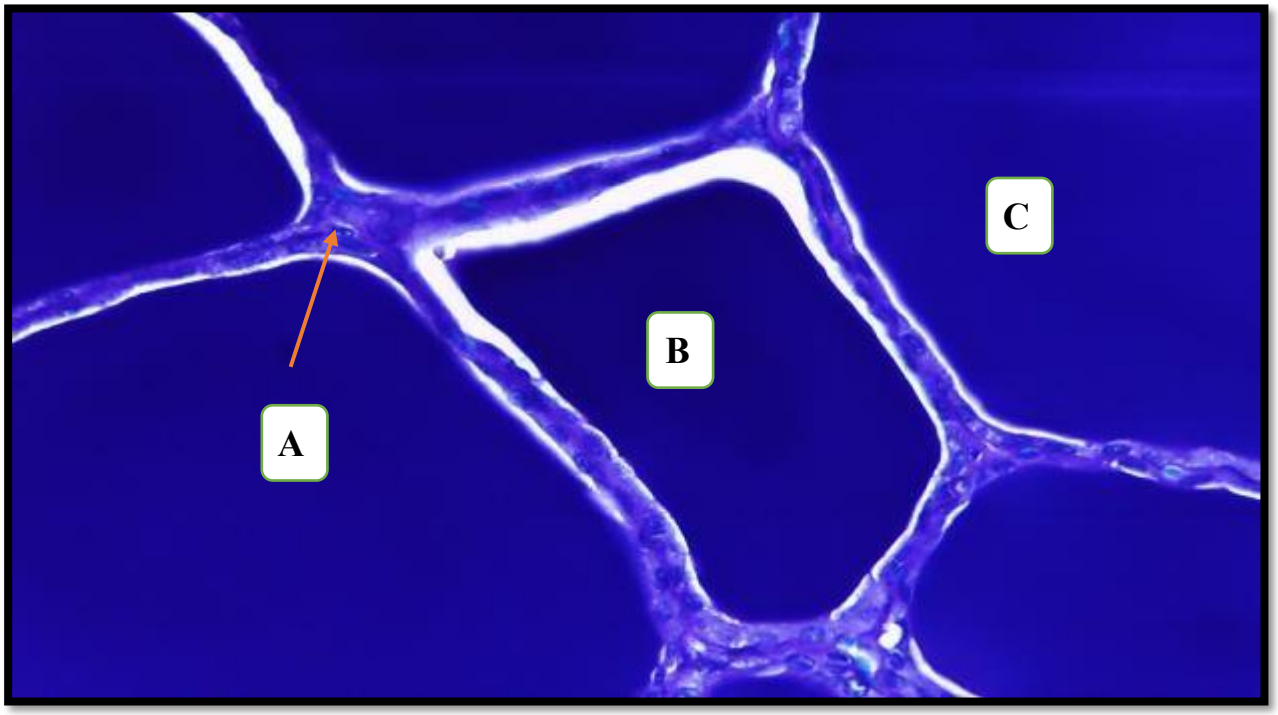

Fig.7. Microscopic section of the thyroid gland in bull show the : **A**. Thyroid follicles, **B**. Colloid with mucin , **C**. Mucin, **X400 PAS - AB**

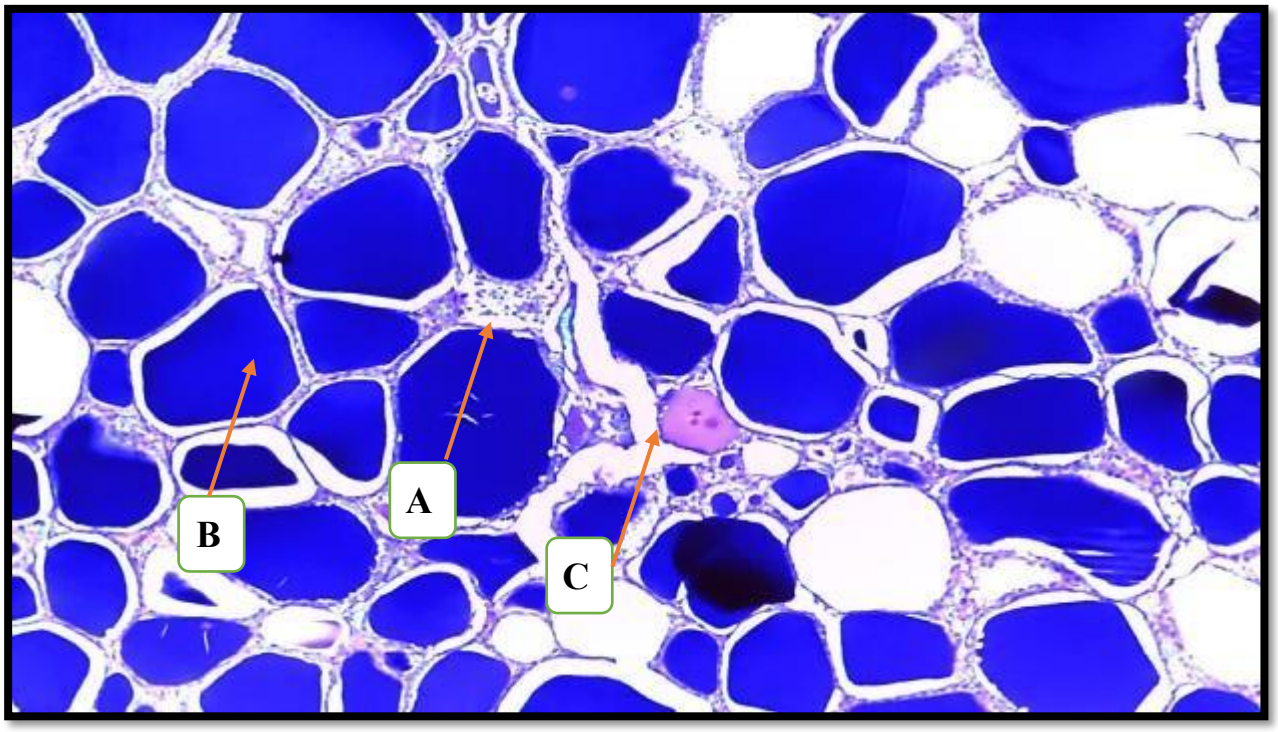

Fig.8. Microscopic section of the thyroid gland in bull show the : **A**. Thyroid follicles, **B**.

Colloid with mucin , **C**. Blood vessel, **X40 PAS - AB**

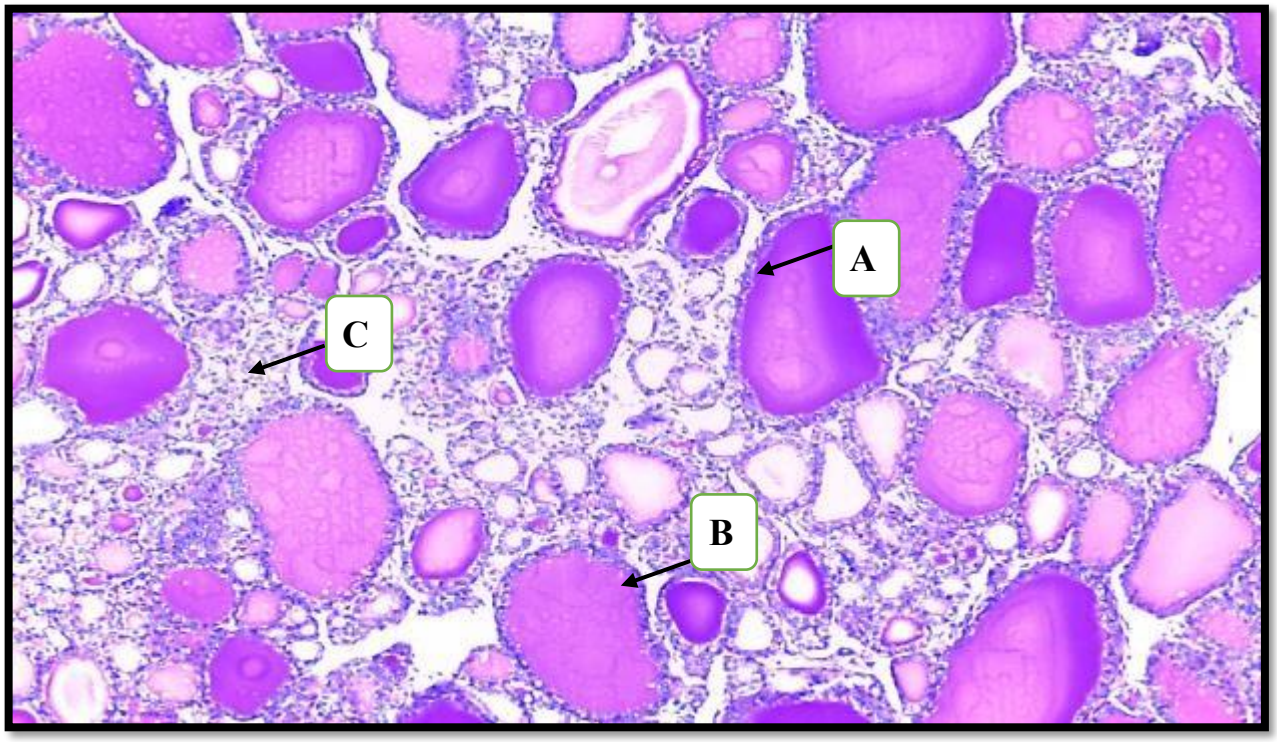

Fig.9. Microscopic section of the thyroid gland in bull show the : **A**. Thyroid follicles, **B**. Colloid, **C**. Interfollicular connective tissue, **X100 PAS.**

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