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Histological, Histochemical and Ultrastructural Study of Thyroid Gland in Mature Male Indigenous Rabbit (*Oryctolagus Cuniculus*)

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Abstract

This current study was designed to investigate the histological, histochemical structure and ultrastructural study of thyroid gland in mature male rabbits *(Oryctolagus Cuniculus*). Ten healthy mature male rabbits (six months in age) were used divided into two groups, each of which contains 5 male rabbits. The first group was used to histological and histochemical study of thyroid gland, while the second group was used to ultrastructural study, The histological study included the general histological structure description. Ultrastructural study included the knowing the microscopic structures that distinguish the different secretory cells found in the thyroid gland in mature male rabbits. The histochemical results shown thyroid follicles colloids of animal were showed positive reaction for PAS and alcian blue (PH 2.5) stains. Ultrastructural results showed the cytoplasm of follicular cells presented mitochondria in diverse shapes. The rough endoplasmic reticulum appeared as extensive elongated and irregular elliptical cisterns. The findings from this study contribute to a better understanding for deep details of thyroid gland in domestic animal.

Key words: Thyroid, Gland, mature, rabbit,ultrastructural .

I. Introduction

The European and Asian continent serves as the natural habitat for the native *Oryctolagus Cuniculus* rabbit population. Several scientific institutions agree that the rabbit served as an ancestor for its descendants (Parveen, et al., 2014). According to (DiVincenti and Rehrig ,2017) rabbits possess specific biological abilities because of their cognitive design to manage stress through diverse mechanisms. The utilization of these animals within lab experiments produces superior results by discovering analogies between human and animal physiological pathways.Longitudinal research involving rabbits enables scientists to develop disease-treatment solutions applicable for human and animal populations (Yamada et al., 2016). The thyroid gland, one of the main endocrine organs which responsible for controlling the metabolism of the body by increasing or decreasing the secretion of thyroxin which in turn regarded as important factor in regulating body temperature (Junqueira et al., 1992, Ross et al., 1995). The thyroid tissue contains two cell varieties consisting of follicular cells and parafollicular cells which also maintain their alternative name as C cells. The cells of this space located between thyroid follicles can be found in the parafollicular spot according to (Inyushkin et al., 2009, Enas and Hadaf ,2020).

II. Materials and Methods

Ten healthy Rabbits *(Oryctolagus Cuniculus*) of mature male rabbits (six months in age) , were used in the current study which were divided into two groups, each of which contains 5 males rabbits .The first group were used to histological and histochemical study of thyroid gland, while the second group were used to ultrastructural study of thyroid gland. were be bought from local market of (Baghdad city – Iraq). The sample animals were kept alive in plastic cages, given feed and water for one day before the research. After euthanasia using inhalation of chloroform in a closed

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container (Adeyemu and Oke, 1990, Ahmed et al., 2020, Abdulla et al., 2022, Younis and Mutlag ,2022 , Hadaf and Luay, 2023, Hanadi and Hadaf ,2023).

Histological and Histochemical study of thyroid gland :

Five mature of male rabbits were used for histological and histochemical studies. After extirpation of thyroid gland, they were washed by normal saline solution. The thyroid gland (right and left lobs) were fixed with (10%) formalin solution for 48 hours (Bancroft and Steven, 2012, Luna, 1968). Then proceed in routine histological technique. After staining of tissues sections with Hematoxylin and Eosin, Masson,sTrichrome,Verhoff,s ,HCL-Toluidin-blue and Combine AB-PAS PH 2.5.

Ultrastructural study:

Five mature rabbits which were used for ultrastructural study by using transmition electronic microscopic to study the thyroid gland . Very small pieces of thyroid gland samples were cut into 2-3 mm pieces to be sent for examination then fixed in Karnofsky's solution for 30 minutes, then trimmed into smaller pieces of 1 mm and kept in the solution for an additional 3-5 hours. The samples underwent three washes in cold (0.1 M) sodium cacodylate solution for 30 minutes, then fixed in 1.33% osmium tetroxide solution stored in cold sodium cacodylate for 2 hours. The samples were stained by placing them in a Petri dish and adding several drops of uranyl acetate for 10 minutes, then rinsing them with pure water and adding drops of lead citrate for 15 minutes, then storing them in a mesh box and sending them for examination under an electron microscope(Shi et al., 2013).

III. Result and Discussion

Histologically, The structure of thyroid gland in mature male rabbits (*Oryctolagus Cuniculus*) was comparable to the common shape of histological structure of thyroid in mammals and in domestic animals (Samuelson, 2007). The thyroid gland was enclosed by thin capsule composed of connective tissue of thin layer of collagen fibers , with some adipose tissue and a few elastic fibers(Fig.1,2 ,6 ,7). which showed thin strands of trabeculae contains connective tissue with small blood vessels were extended from the capsule and penetrate the glandular parenchyma and divided it into different size of lobules which made of variable size and shapes follicles(Fig.4,5)These results agreed with AL-Aamery and Dauod, (2016) but disagreed with Adhikary et al., (2003) who stated that the capsule of thyroid in goats was consisted of three layers. There were three main sizes of follicles; small, medium and large-size were identified, and different shapes of follicles were observed include; oval, Rounded, irregular, Polygonal shapes(Fig.4,5). All thyroid follicles contain variable amounts of eosinophilic colloid substance within follicular lumen. Some follicles recognized without colloid material (Fig.2,4), These results agree with Takashi et al., (1984) In white crossbred pigs and Batah and Shakir,(2019) in guinea pig and rat, some follicles contain densely stained eosinophilic colloid material which reacted positive with PAS stain and appeared magenta color(Fig.9) These results coincide with El-Desouki et al., (2014) in rabbits. The thyroid colloid showed positive reaction for alcian blue (PH 2.5) stain and appeared dark blue color (Fig.9), since its contents were mainly of glycolpro-teins. This disagrees with Batah and Shakir,(2019) in guinea pigs who found that colloid substance showed negative reaction for alcian blue (pH 2.5) stain and explain this finding due to its devoid of acidic mucopolysaccharide in content of colloid. This variation may be due to the differences in the activity of the thyroid gland .The isthmus of the thyroid gland of the rabbit was glandular and contains of follicles of different sizes and shapes. (Fig.3) These results agree with Ali et al., (2015) in sheep, Khaleel and Salih ,(2017) in gazelle, but disagreed with Dyce et al., (2002) The thyroid lobes in horses feature a tiny isthmus connection whereas large parenchymal tissue composes the isthmus in cattle with small ruminants showing inconsistent thyroidisthmic formations that comprise solely fibrous tissue. Few parafollicular cells were observed in contrast with the follicular cells, it is showed as oval - rounded large cells, display more lightly stained cytoplasm than of the follicular cells with densely stained nuclei (Fig.8) these results agree with Igbokwe, (2010) in African grasscutter,Abbas and Shakir,(2019) In Guinea pigs. Ultrastructural results, In the present study examined small and large follicle profiles through the investigation of mature male rabbits.The examination of these follicles by low-power magnification revealed colloid matter inside them combined

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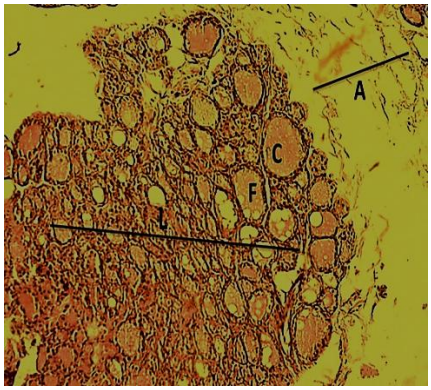
with follicular cells that displayed varying size and shape distribution around the edges.Under high magnification it became possible to observe the basement membrane together with connective fibrolasts and fibrocytes and collagen fibrils in the interfollicular area (Fig.10,12).The nuclear heterochromatin in the cuboidal follicular cells spread as uneven patterns between the membrane and throughout the nuclear regions (Fig.12) Our data confirms the findings which Igbokwe et al., (2015) reported when studying the West African Dwarf goat. The nucleus contained evenly distributed euchromatin (Fig.10). The follicular cells appeared cuboidal-shaped with spherical nuclear features (Fig.11 ,12). White Fulani cattle showed similar nucleus findings according to Igbokwe and Ezeasor, (2013). The microvilli on the cuboidal cells were numerous thinner and finger-like (Fig.12). This might be due to more in endocytotic activity. Like observation by French and Hodges,(1977) in normal domestic fowl.

Different shapes of mitochondria presented within follicular cell cytoplasm. The majority of organelles were positioned at the upper most part of the cytoplasm adjacent to the colloid (Fig. 12) these results agree with Abdel-Magied *et al.*, (2000) and Mubarak and Sayed ,(2005) in camel .The highly dilated rough endoplasmic reticulum as elongated,irregular, elliptical cisterns in the cytoplasm of thyroid follicular epithelium (Fig.10, 11) observation by Igbokwe et al., (2015) in West African Dwarf goats. The Golgi apparatus were well noticeable in thyroid sections of animal and consisted of flattened sacs, vesicles and colloid droplets were observed (Fig. 12), that might be an indication of more active thyroid in mature male rabbit, Agreeing to Abdel-Magied et al., (2000) in camel, the presence of Golgi complexes, RER and secretory vesicles indicated the activity of follicular cells in the synthesis and secretion of thyroglobulin towards the follicular lumen. Electron dense granules were seen in the apical cytoplasm of thyroid follicular cells supposed to be primary lysosomes (Fig.10,11) Similar observation by Igbokwe et al., (2015) in West African Dwarf goats, The research of Norris,(2007) demonstrated that follicular cell cytoplasm accessed T3 and T4 hormones more easily when lysosomes decomposed colloid droplets before the hormones entered the cap illaries below the cell.

(Fig.1). Histological section of thyroid gland in male mature rabbit shows: capsule (black arrow), (T) Trabeculae, (L) gland lobules. (H&E stain,X40).



(Fig.2). Histological section of thyroid gland in male mature rabbit shows: (A) adipose tissue, (L) gland lobules, (F) Follicles, (C) Colloid material .

(H&E stain, X100).

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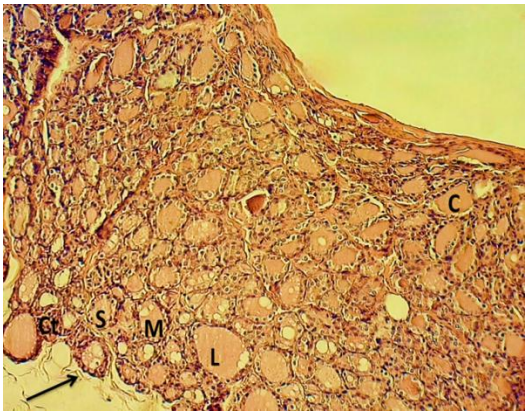
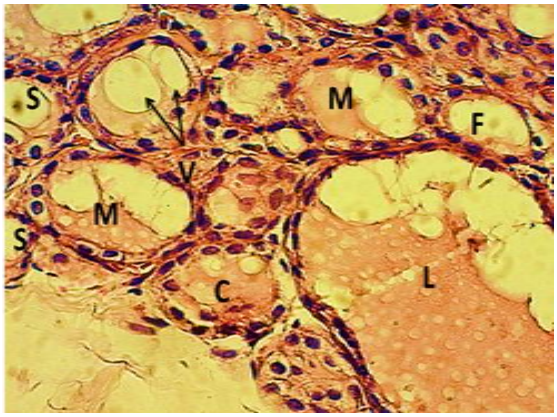
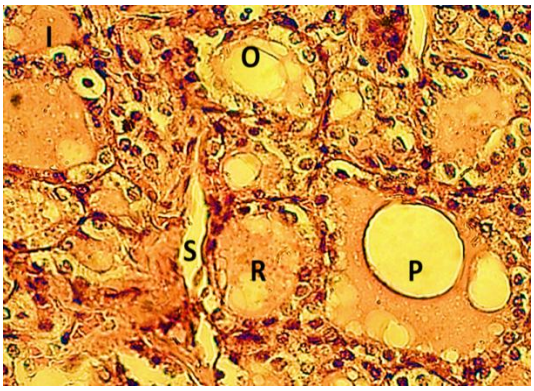


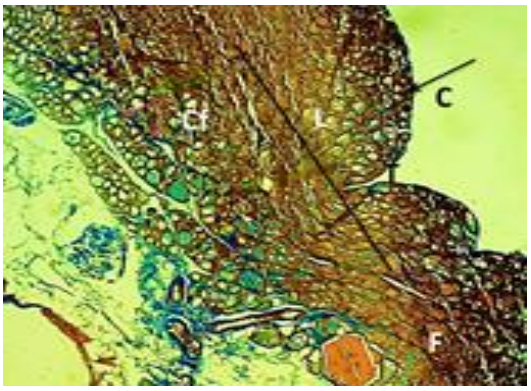
(Fig.3).Histological section of isthmus thyroid gland in male mature rabbit showFollicles: Capsule (blackarrow), (S) Small size follicle, (M) Medium size follicle,(L) Large size follicle,(C) Colloid material, (Ct)connective tissue.(H&E X100).



(Fig.4). Histological section of thyroid gland in male mature rabbit show Follicles: (S) Small size follicle,(M)Medium size follicle,(L) Large size follicle, (F) Follicle, (C) Colloid material,(V) Vacuole(black arrows).(H&E stain X400)



(Fig.5). Histological section of thyroid gland in male mature rabbit shows: (R)Rounded follicle,(O) Oval follicle,(P) Polygonal follicle, (I) Irregular follicle, (S) Sinusoid. (H &E stain X400).



(Fig.6). Histological section of thyroid gland in male mature rabbit shows:(C) Capsule(black arrows), (Cf) collagen fibers(red arrows), (L) Lobules, (F) Follicle, (T)trabeculae.

(Masson's Trichrome stain X40).

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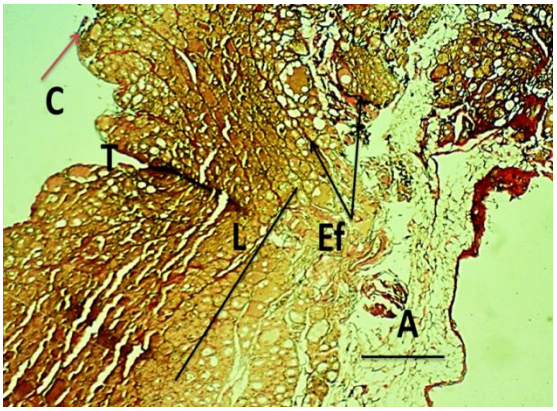
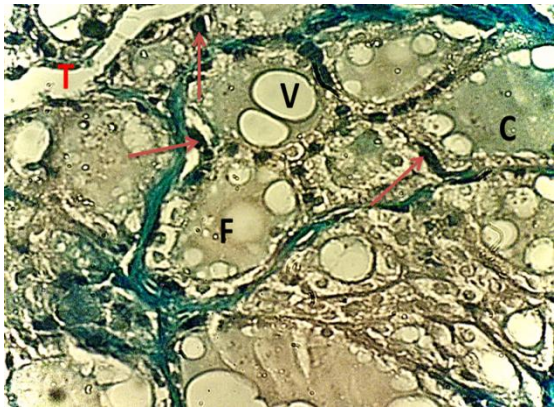
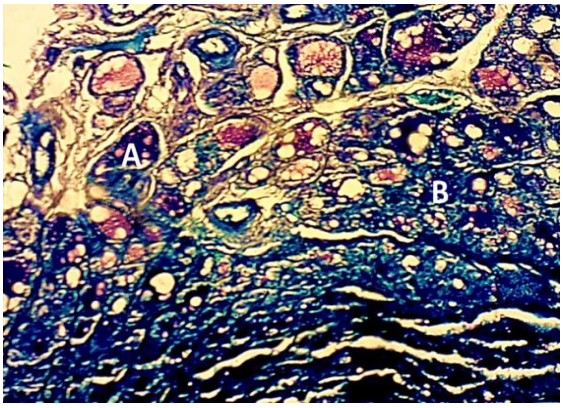


(Fig.7). Histological section of thyroid gland in male mature rabbit shows: (A) adipose tissue, (Ef) elastic fibers (blackarrow),(L)Lobules,(T)trabeculae, (C)Capsule (redarrows).

(Verhoffs stain X40).

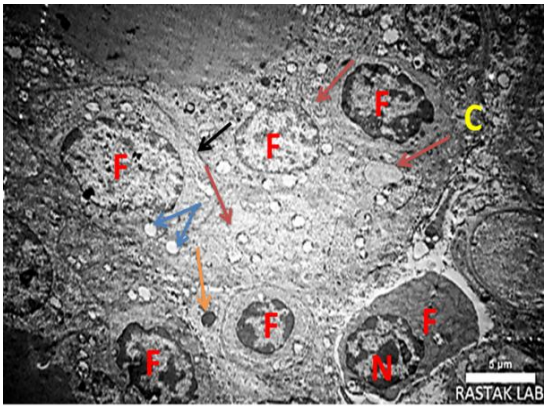


(Fig.8). Histological section of thyroid gland in male mature rabbit shows: C-cell (Parafollicular cells) (red arrow), (F) Follicle, (T) trabeculae, (C) Colloid material, (V) Vacuole. (Toluidine blue stain X400).



(Fig.9). Histological section of thyroid gland in male mature rabbit shows: (A) colloid material showed positive reaction for PAS stain, (B) colloid material gives positive reaction for AB stain .

(CombineAB-PAS,PH2.5 stain X100).



(Fig.10):Transmissionelectronmicrogrh section of thyroid follicular epithelium in mature rabbit shows: varying shape and size of thyroid follicular cells (F), basement membrane (blackarrow), highlydilatedRER(redarrow),interfollicularconnectivetissue(C),somevesicles(bluearrow),lysosme (orange arrow) and nuclei of follicularcell with euchromatic chromatin (N).

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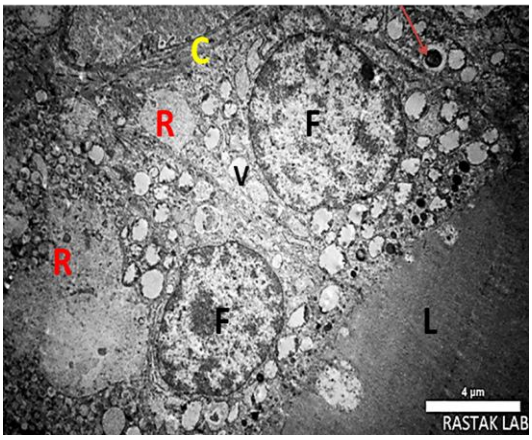
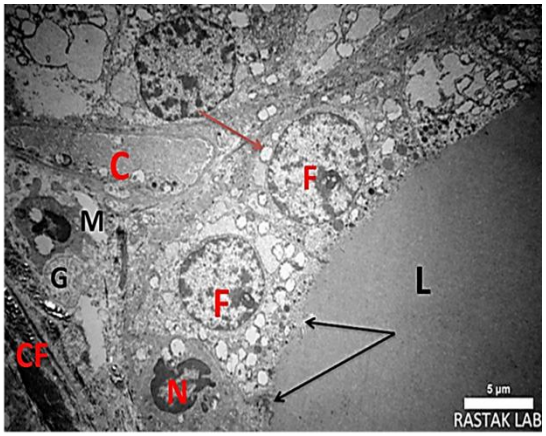


(Fig.11): Transmission electron micrograph section of thyroid follicular epithelium in mature rabbitshows: differentiated epithelum of follicular cells (F), highly vesicles (V), interfollicular connective tissue (C), highly dilated RER (R), lysosome (red arrow) and luminal colloid(L).



(Fig.12): Transmission electron micrograph section of thyroid follicular epithelium in mature rabbit shows:differentiated epithelum of follicular cells (F), Golgi apparatus (G), mitochondria (M), highly vesicles(red arrow), colloid droplet(C),luminalcolloid(L), microvilli in follicular lumen (arrows), Collagen fibers(CF) and nuclei of follicular cell (N) with peripheral chromatin .

IV. Conclusions

Histologically, thyroid gland in mature male rabbits was very active.

Histochemically, the colloid material showed positive reaction for PAS and AB stains of animal due to it's a glycoprotein substance.

Ultrastructural study, showed that the thyroid gland of mature male rabbit does not differ from that of mammalian species put more active .

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