The effect of maternal hypothyroidism on the postnatal development of the pituitary–thyroid axis in albino rats: a histological, morphometric, and immunohistochemical study

Mohamed R. Shehata, Dorreia A. Mohamed, Manal M. Samy El-Meligy, Ashraf E. Bastwrous

Department of Human Anatomy and Embryology, Faculty of Medicine, Assiut University, Assiut, Egypt

Correspondence to Ashraf E. Bastwrous, MSc, MD, Department of Human Anatomy and Embryology, Faculty of Medicine, Assiut University, Assiut, Egypt
Tel: +20 127 186 4467; Fax: 088-2332278; Postal Code: 71526;
e-mail: aedward77@yahoo.com

Received 08 September 2016
Accepted 03 October 2016

Journal of Current Medical Research and Practice
January-April 2017, 2:79–97

Background

The adequate functioning of the maternal thyroid gland plays an important role in ensuring that the offspring develop normally. Therefore, maternal hypothyroidism and hyperthyroidism are associated with offspring abnormalities.

Aim of the work

This study was carried out to examine the effect of maternal hypothyroidism on the postnatal development of the pituitary–thyroid axis in the albino rat.

Materials and methods

Thirty pregnant female albino rats were divided into two groups. Group I was the control group and group II was the hypothyroid group whose rats were given carbimazole in a dose of 5 mg/rat/day through the intragastric intubation from the gestational day 10 until the postnatal day 20. The offspring of both groups were killed at the ages of newborn, 10, 30, and 60 days. The pituitary and thyroid glands were extracted from the pups of control and treated animals and processed for light and electron microscopy and morphometric analysis. In addition, an immunohistochemical study was carried out on the pituitary specimens for the detection of thyrotrophs.

Results

The present study revealed that the maternal hypothyroidism caused morphological changes in the pituitary–thyroid axis of the offspring. The changes started to appear in the newborn age and persisted throughout the postnatal life. The light microscopic examination revealed shrunken thyroid follicles. The follicular epithelial height increased and was composed of tall columnar cells with a vacuolated cytoplasm. The colloid decreased or was completely absent. Regarding the pituitary gland, there were many large pale vacuolated cells with dark nuclei and sometimes the vacuolation affected most of the cells. The electron microscopic examination of the thyroid follicular cells and thyrotrophs showed ultrastructural signs of an increased activity, which included dilated endoplasmic reticula, well-developed Golgi, enlarged mitochondria, and a decreased number of the secretory granules. Large cytoplasmic vacuoles were also observed. The immunohistochemical study of the pituitary gland revealed an increased number of thyroid-stimulating hormone immunopositive cells. The morphometric analysis of the pituitary and thyroid sections showed a significant decrease in the thyroid follicular diameter and a significant increase in the thyroid follicular epithelial height and in the number of the thyrotrophs per reference area.

Conclusion

From this study, it could be concluded that the experimentally induced maternal hypothyroidism disturbed the pituitary–thyroid axis of the offspring.

Keywords:
antithyroid drugs, development, hypothyroidism, pituitary, thyroid

J Curr Med Res Pract 2:79–97

2357-0121

Introduction

Thyroid hormone (TH) is essential for the development and homeostasis of almost all the tissues and organs [1] and plays a crucial role in the physiological functioning of the different body organs, especially the brain [2].

Ahmed *et al.* [3] stated that the adequate functioning of the maternal thyroid gland played an important role in the normal offspring development. Thus, maternal hypothyroidism and hyperthyroidism were associated with offspring abnormalities.

Disorders of thyroid gland development and/or function are relatively common, affecting approximately one newborn infant in 2000–4000. The most prevalent form of thyroid disorders is congenital hypothyroidism, which is frequently caused by genetic defects of the transcription factors involved in the development of the thyroid or pituitary gland [4].

Thyroid diseases are more common in females than in males. These diseases are either due to thyroid gland overactivity resulting in hyperthyroidism or underactivity resulting in hypothyroidism [5,6]. Hypothyroidism is clinically linked with a decreased metabolic rate, which results in adverse effects on many organs and system activities [7].

Thyroid dysfunction is common during gestation. The prevalence of hypothyroidism during pregnancy is estimated to be 0.3–0.5% for overt hypothyroidism and 2–3% for subclinical hypothyroidism. Thyroid antibodies are found in 8–14% of the women in the childbearing age, and chronic autoimmune thyroiditis is the main cause of hypothyroidism during pregnancy, apart from iodine deficiency [8].

Materials and methods

Animals

Ethical approval for this study was provided by medical ethics comittee, faculty of medicine, Assiut university on 27/8/2011. A total number of 30 pregnant female rats were used in this study. They were obtained from the Animal House, Faculty of Medicine, Assiut University.

Experimental design

The rats were divided into two groups.
(1) Group I was the control group that consisted of 15 rats. Rats in this group were given distilled water daily from gestational day 10 until the postnatal day 20
(2) Group II was the experimental group that consisted of 15 rats. They were given carbimazole in a dose of 5 mg/day/pregnant rat [9]. The drug was administered through an intragastric intubation daily from the gestational day 10 until the postnatal day 20.

Six offsprings were obtained from each of the control and the treated groups and killed at the following ages: newborn, 10, 30, and 60 days. The pituitary and thyroid glands were extracted from the control and the treated animals and subjected to the following:

Light microscopic study

The specimens were fixed in Bouin's solution and 10% formalin for histological and immunohistochemical stains. After fixation the specimens were dehydrated and embedded in paraffin and the blocks were cut at 8 μm. The sections were processed for the following:
(1) Hematoxylin and eosin staining for the general histological structure of thyroid and pituitary glands
(2) Immunohistochemical staining for the pituitary gland to detect the thyroid-stimulating hormone (TSH) secreting cells using an anti-TSH antibody.

Electron microscopic study

Immediately after sacrificing the animals, small samples were taken from the thyroid and pituitary glands and fixed in 5% cold glutaraldehyde for 24 h. Then the specimens were washed in three to four changes of cacodylate buffer (pH 7.2), 20 min for each change, and then fixed in cold osmium tetraoxide for 2 h. After that, the specimens were washed in four changes of cacodylate buffer for 20 min each. Dehydration was done by using ascending grades of ethyl alcohol (30, 50, and 70%) each for 2 h, and 90%, and 100% two changes 30 min each. Embedding was done in Epon (TAAB-812, Embedding Resin Kit, England) 812 using gelatin capsules for the polymerization. The embedded samples were kept in an incubator at 35°C for 1 day, at 45°C for another day, and for 3 days at 60°C [10].

Semithin sections (0.5–1 μm) were prepared by using the LKB ultramicrotome (8800, Bromma, Sweeden). The sections were stained with toluidine blue, examined under the light microscope, and photographed.

Ultrathin sections (50–80 nm) from selected areas of the trimmed blocks were made and collected on a copper grid. The ultrathin sections were contrasted with uranyl acetate for 10 min and lead citrate for 5 min. Transmission Electron Microscpy (J. E. M. 100 CXII, Tokyo, Japan) and photographed at 80 kV at Assiut University Electron Microscopy Unit.

Morphometric and statistical analysis

Three morphometric parameters were measured in the present study.
(1) The diameter of the thyroid follicles
(2) The thyroid follicular epithelial height
(3) The number of positive-stained thyrotrophs per reference area.

The diameter of the thyroid follicles and the height of the thyroid follicular cells were measured by means of a millimeter eye-piece, under magnification of ×340. In each thyroid gland, 10 follicles were analyzed.

Regarding the number of the thyrotrophs per reference area, it was counted by a using a computer-assisted image analysis system at ×400 magnification using the Digimizer PC Image Analysis Software (version 4.0, 2011; MedCalc Software, Mariakerke, Belgium).

Data were tabulated and statistically analyzed using SPSS software, version 9 (SPSS Inc., Chicago, Illinois, USA). Comparison of significance between the different groups was carried out using an independent *t*-test. The significance of the data was determined by the *P*-value. A *P*-value greater than 0.05 was considered nonsignificant and *P*-value less than 0.05 as significant.

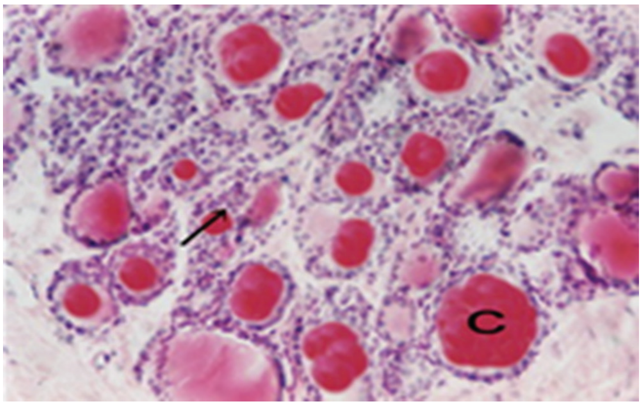
Results

The histological results

The age of newborn

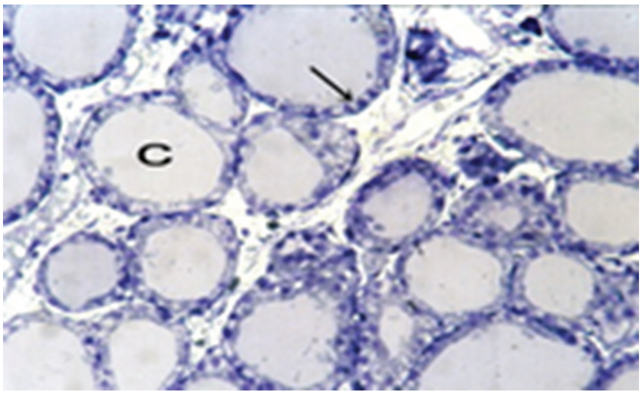
(1) The control group:
  (a) The light microscopic examination:
    The thyroid sections reveal multiple small thyroid follicles. The follicles are lined with a low cuboidal epithelium and filled with colloid (Figs. 1 and 2)
    The pituitary sections reveal anastomosing cords or groups of cells. The cells are of two types, chromophobes and chromophils. The chromophobes are generally smaller and more numerous than the chromophils. They have rounded vesicular, relatively large nuclei, and a pale cytoplasm. Two types of chromophils are observed, acidophils and basophils (Fig. 3)
    The semithin sections reveal the thyrotrophs that are distinguished by being branched cells located in close relation to the blood capillaries. The cells have very fine secretory granules uniformly distributed in their cytoplasm (Fig. 4)
    On using the immunohistochemical technique for the demonstration of TSH secreting cells, the cells appear large and their cytoplasm shows a positive reaction in the form of brown granules (Fig. 5)
  (b) The electron microscopic examination:
    The thyroid follicular cell possesses a round euchromatic nucleus. Rough endoplasmic reticula (rER) are abundant in the follicular cell cytoplasm. The rER is extensive and located toward the basal lamina of the follicular epithelium. Many electron-dense secretory granules are located in the apical part or center of the cell. Microvilli from the apical region of the cell project into the follicular lumen that contains an electron-dense colloidal substance (Fig. 6)
    The thyrotrophs are elongated with oval nuclei. Few amounts of mitochondria, ER, and small secretory granules are mostly located peripherally (Fig. 7)

Figure 1



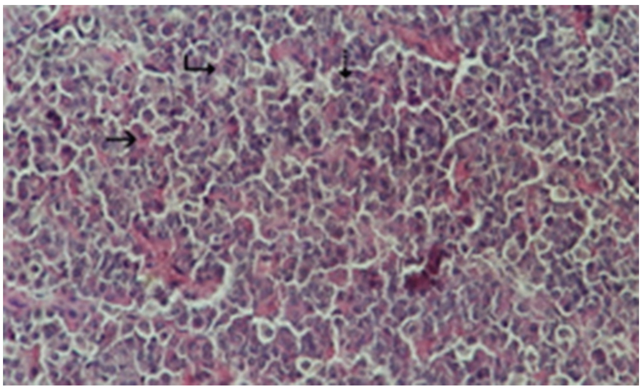
A photomicrograph of a control newborn rat's thyroid gland showing multiple thyroid follicles that are lined by a low cuboidal epithelium (→) and filled with colloid (c) (hematoxylin and eosin, ×400).

Figure 2



A photomicrograph of a semithin section in a control newborn rat's thyroid gland showing multiple thyroid follicles of variable sizes lined by a low cuboidal epithelium (→) and filled with colloid (c) (toluidine blue, ×400).

Figure 3



A photomicrograph of a control newborn rat's pituitary gland showing groups of cells that can be differentiated into chromophobes (↳) with rounded, relatively large vesicular nucleus and pale cytoplasm, acidophils(→) and basophils (↓) (hematoxylin and eosin, ×400).

(2) The treated group:
  (a) The light microscopic examination:

The treated thyroid gland reveals multiple thyroid follicles that are more or less reduced in size. The follicular epithelium shows an increase in height and has a vacuolated cytoplasm. The lumina of some follicles are obliterated and others have a decreased amount of colloid (Figs. 8 and 9)

The treated pituitary gland reveals large cells with eccentric nuclei and a severely vacuolated cytoplasm. The vacuolation affects most of the pituitary gland cells (Figs. 10 and 11)

On using the immunohistochemical technique for the detection of TSH secreting cells, an increase in the number of the immunopositive cells is observed (Fig. 12)

(b) The electron microscopic examination:

The thyroid follicular cell reveals a large indented nucleus, swollen ER, several large vacuoles distributed throughout the cytoplasm, and a small number of microvilli (Fig. 13)

The thyrotrophs reveal large oval cells possessing an abundant cytoplasm that contains dilated ER, several mitochondria, prominent Golgi complexes, and few randomly distributed secretory granules (Fig. 14).

The age of 10 days

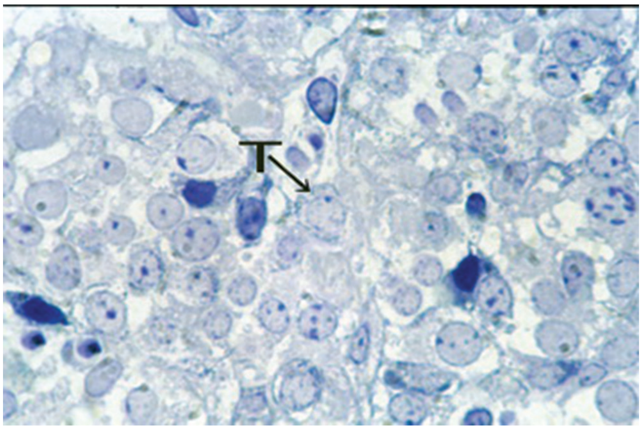
(1) The control group:

(a) The light microscopic examination:

The thyroid and pituitary sections reveal the same findings as those of the previous age (Figs. 15–18)

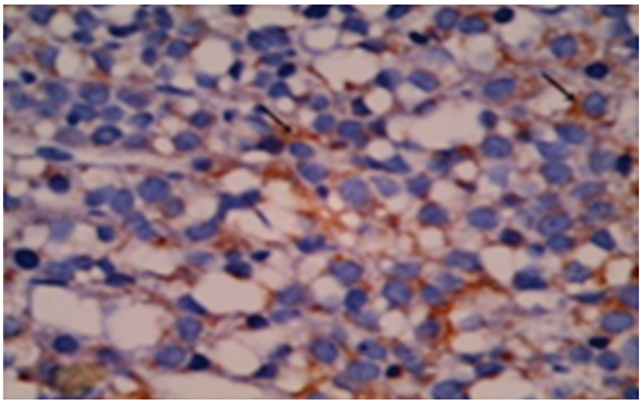
By immunohistochemistry, the thyrotrophs show a positive reaction in the form of brown granules (Fig. 19)

Figure 4



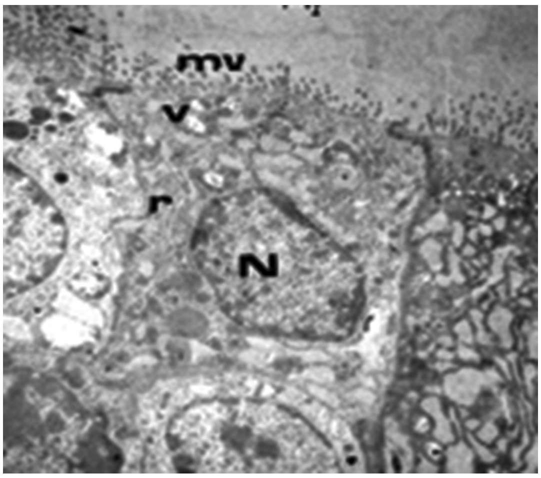
A photomicrograph of a semithin section in a control newborn rat's pituitary gland showing the thyrotrophs (t) that are characterized by their angular shape, rounded eccentric nucleus with a prominent nucleolus, and their finely granulated cytoplasm (toluidine blue, ×1000).

Figure 5



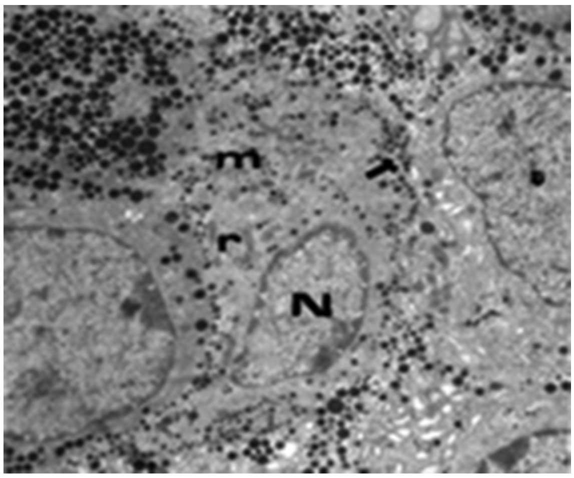
A photomicrograph of a section in a control newborn rat's pituitary gland immunostained with thyroid-stimulating hormone antibody showing a positive reaction in the thyroid-stimulating hormone producing cells in the form of brown granules (→). Immunostained with thyroid-stimulating hormone antibody (×1000).

Figure 6



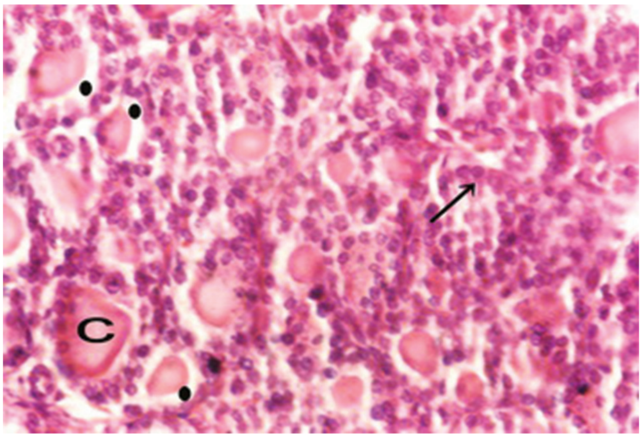
An electron micrograph of a control newborn rat's thyroid follicular cell showing an elongated basal nucleus (N), rough endoplasmic reticulum (r), apical secretory vesicles (v), and microvilli (mv) projecting from the apical surface of the cell (×5800).

Figure 7



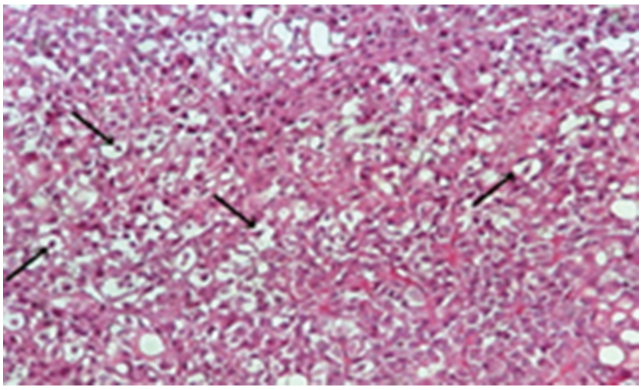
An electron micrograph of a control newborn rat's thyrotroph showing that the cell is elongated with an oval nucleus (N), few amounts of mitochondria (m), endoplasmic reticulum (r), and small secretory granules (arrow) (×5800).

Figure 8



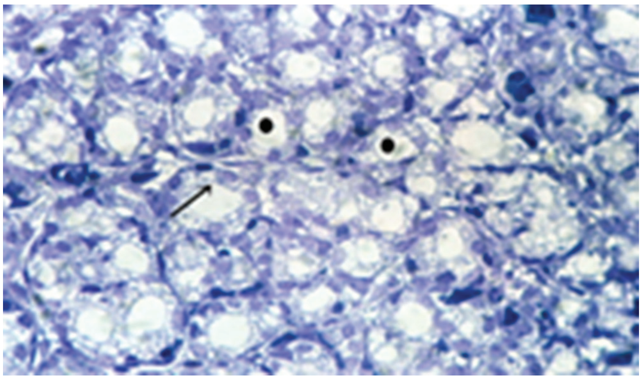
A photomicrograph of a treated newborn rat's thyroid gland showing an increase in the height of the follicular epithelium (→) in comparison with the control group. Some follicles show a decrease in the amount of the contained colloid (c) with an appearance of pericolloidal space (•) (hematoxylin and eosin, ×400).

Figure 10



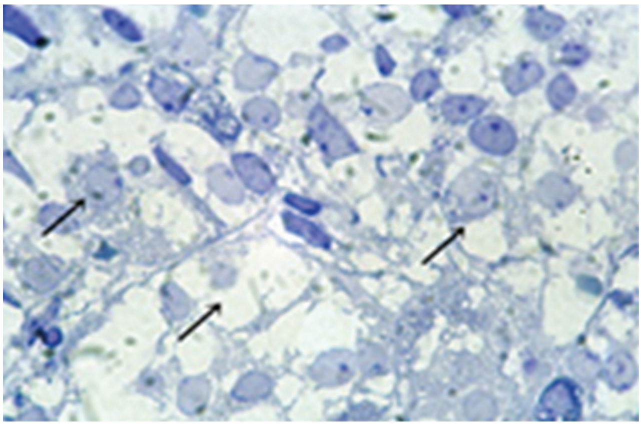
A photomicrograph of a treated newborn rat's pituitary gland showing a severe vacuolative degeneration that affects most of the cells (→). Most of the cells have piknotic nuclei (hematoxylin and eosin, ×400).

Figure 9



A photomicrograph of a semithin section in a treated newborn rat's thyroid gland showing numerous thyroid follicles that are reduced in size. The follicular epithelium is increased in height and has a severely vacuolated cytoplasm (→). The follicles are mostly devoid of colloid (•) (toluidine blue, ×400).

Figure 11



A photomicrograph of a semithin section in a treated newborn rat's pituitary gland showing the appearance of many cells (→) with rounded nuclei and a severely vacuolated cytoplasm (toluidine blue, ×1000).

(b) The electron microscopic examination:
Ultrastrucuturally, the thyroid follicular cells and thyrotrophs have the same features as those of the previous age (Figs. 20 and 21)

(2) The treated group:

(a) The light microscopic examination:
The examination of the thyroid and pituitary sections show the same changes as those in the previous age (Figs. 22–25)
The immuohistochemical examination reveals an apparent increase in the number of TSH-immunopositive cells (Fig. 26)

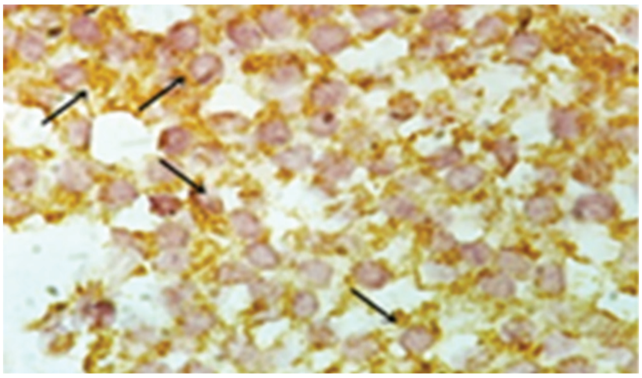
(b) The electron microscopic examination:
Ultrastrucuturally, the thyroid follicular cells and thyrotrophs reveal similar observations as those of the previous age (Figs. 27 and 28).

The age of 1 month

(1) The control group:

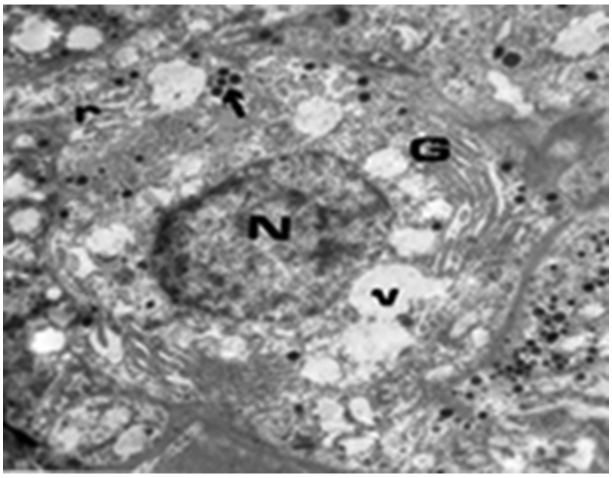
(a) The light microscopic examination:
The thyroid sections reveal thyroid follicles of different sizes; their cavities contain an acidophilic colloid. The thyroid follicles are lined with cubical follicular cells with rounded vesicular nuclei (Figs. 29 and 30)
The pars distalis of the control rat's pituitary gland is formed of cords of epithelial cells. The cells are of two types: chromophobes and chromophils. The chromophobes have unstained cytoplasm and rounded nuclei. The chromophils are larger and have a homogenously stained cytoplasm and vesicular nuclei with prominent nucleoli. The chromophils are further subdivided into acidophils, which have rounded vesicular nuclei and an acidophilic cytoplasm, and basophils, which have rounded eccentric nuclei and a relatively basophilic cytoplasm (Fig. 31)
The semithin sections stained with toluidine

Figure 12



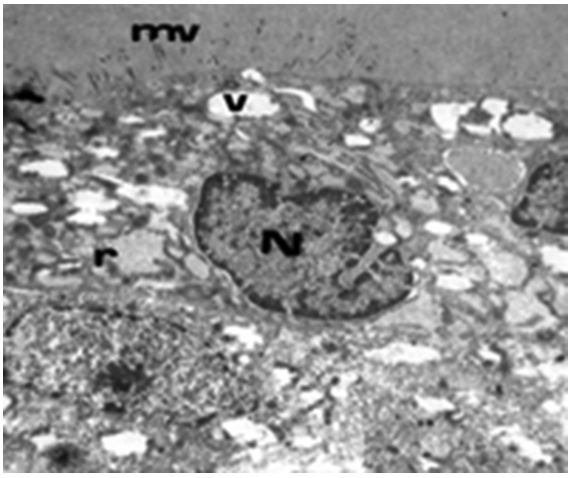
A photomicrograph of a section in a treated newborn rat's pituitary gland showing an increase in the number of the immunopositive cells (→). Immunostained with thyroid-stimulating hormone antibody (×1000).

Figure 14



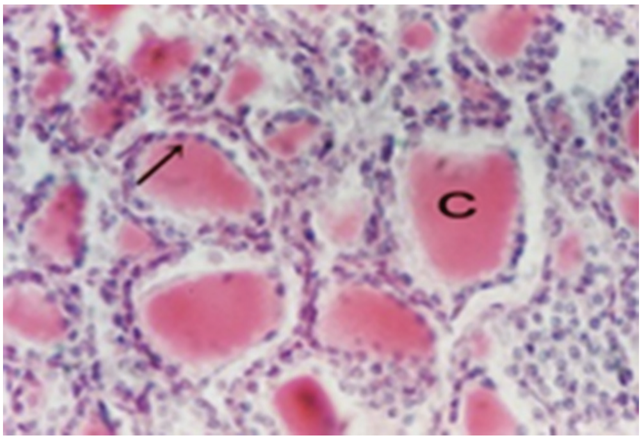
An electron micrograph of a treated newborn rat's thyrotroph showing a large rounded nucleus (N), well-developed Golgi (G), dilated endoplasmic reticulum (r), few secretory granules (→), and many large vacuoles (v) (×5800).

Figure 13



An electron micrograph of a treated newborn rat's thyroid follicular cell showing a large indented nucleus (N), swollen endoplasmic reticulum (r), several large vacuoles (v), and a little number of microvilli (mv) (×5800).

Figure 15



A photomicrograph of a control 10-day-old rat's thyroid gland showing multiple thyroid follicles that are lined by a low cuboidal epithelium (→) and mostly filled with colloid (c) (hematoxylin and eosin, ×400).

blue are used to differentiate the chromophils depending on their size, shape, density, and distribution of their secretory granules. The thyrotrophs are characterized by thick processes that project toward the blood vessels. They are oval with acute angles or polyhedral. They have eccentric vesicular nuclei with prominent nucleoli. The cytoplasm contains moderately stained fine granules (Fig. 32)

By using the immunohistochemical technique, the thyrotrophs show a positive reaction in the form of brown granules (Fig. 33)

(b) The electron microscopic examination:

The thyroid follicular cells are cuboidal and their apices are characterized by numerous irregularly arranged microvilli. The ER of the follicular cell are distributed throughout the cytoplasm. Numerous elongated mitochondria and lysosomes are observed. The cells contain apical vesicles and some colloid droplets (Fig. 34)

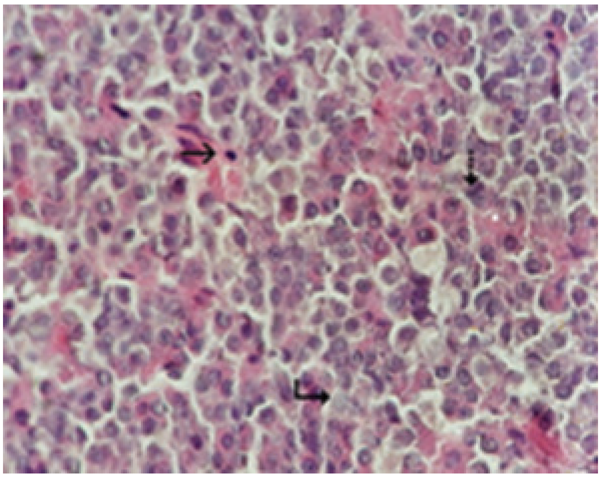
The thyrotrophs are distinguished from the other anterior pituitary cells by their small size, angular shape, and cytoplasmic organelles that include moderately developed ER and short mitochondria (Fig. 35)

(2) The treated group:

(a) The light microscopic examination:

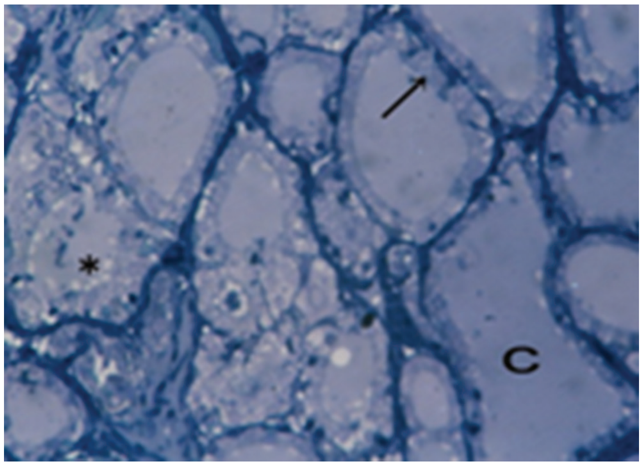
The thyroid sections reveal an apparent decrease in the follicle size. Groups of follicles are lined with vacuolated proliferating follicular cells. In some follicles, multiple layers of follicular cells are obviously seen (Fig. 36). Some thyroid follicles show an increase in the follicular epithelial height that consisted of cells with pale nuclei and

Figure 16



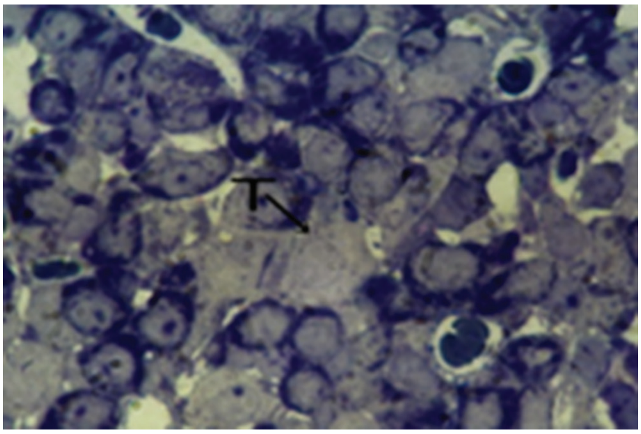
A photomicrograph of a control 10-day-old rat's pituitary gland showing clusters of cells including the acidophils(→), basophils (↑), and the lightly stained chromophobes(↳) (hematoxylin and eosin, ×400).

Figure 17



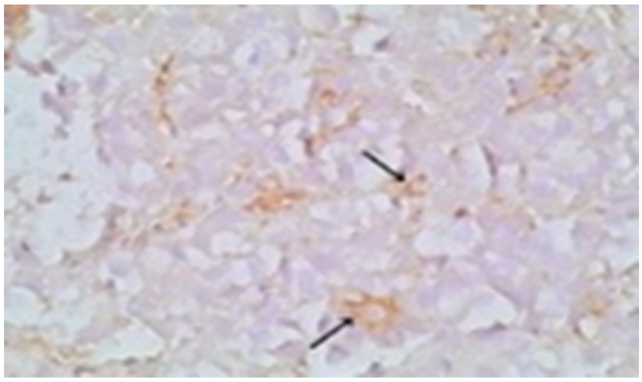
A photomicrograph of a semithin section in a control 10-day-old rat's thyroid gland showing numerous thyroid follicles lined by a low cuboidal epithelium (→) and filled with colloid (c). Notice the colloid peripheral vacuolations (*) adjacent to the follicular cells (toluidine blue, ×400).

Figure 18



A photomicrograph of a semithin section in a control 10-day-old rat's pituitary gland showing different cell types of the anterior pituitary from which the thyrotrophs (T) are distinguished by their angular shape, rounded nucleus with a prominent nucleolus, and pale finely granulated cytoplasm (toluidine blue, ×1000).

Figure 19



A photomicrograph of an immunostained control 10-day-old rat's pituitary gland showing a positive reaction in the thyrotrophs in the form of brown granules(→). Immunostained with thyroid-stimulating hormone antibody (×1000).

a vacuolated cytoplasm that nearly obliterate their lumina. Some follicles are completely devoid of colloid (Fig. 37)

The pituitary gland reveals that the cells appear larger in size compared with the cells of the control group, and their cytoplasm is somewhat vacuolated (Figs. 38 and 39)

The immuohistochemical examination reveals an apparent increase in the number of TSH-immunopositive cells (Fig. 40)

(b) The electron microscopic examination:

The thyroid follicular cell reveals that the ER consists of more dilated cisternae. The mitochondria are dilated and the nuclei are more oval. Moreover, there are many vacuoles throughout the cytoplasm (Fig. 41)

The thyrotrophs reveal a large rounded nucleus. The cytoplasm of these cells contains very few secretory granules and the rER are comparatively well developed with dilated cisternae. Enlarged mitochondria are also seen (Fig. 42).

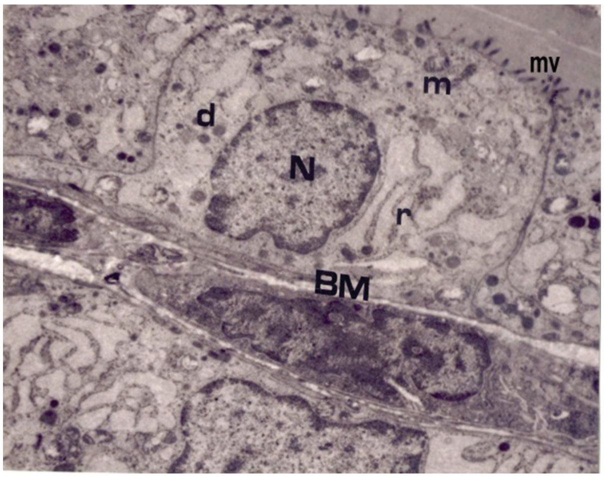
The age of 2 months

(1) The control group:

(a) The light microscopic examination:

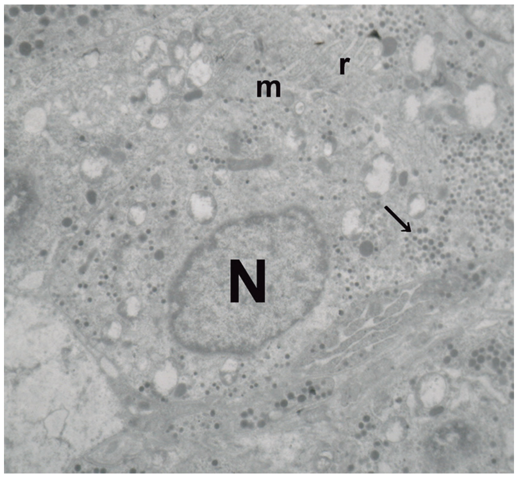
The thyroid gland consists of numerous follicles and an interfollicular stroma. The follicles are lined with a single layer of a cuboidal epithelium and filled with uniformly distributed colloid. In some follicles, the colloid has peripheral vacuoles (Figs. 43 and 44)

Figure 20



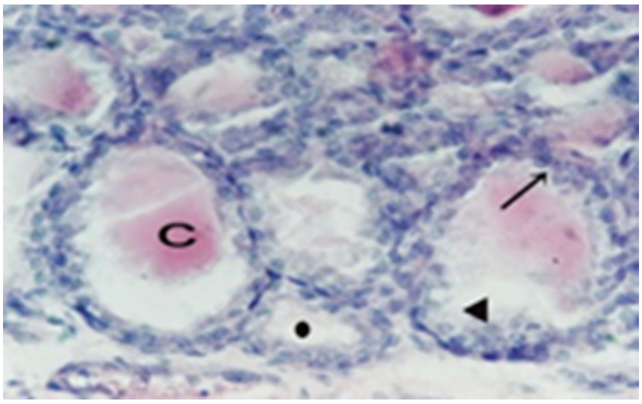
An electron micrograph of a control 10-day-old rat's thyroid follicular cell showing a nucleus (N) near the basal membrane (BM), dilated rough endoplasmic reticulum (r), mitochondria (m), few secretory granules, and dense bodies (d). There are few microvilli (mv) projecting from the luminal surface of the cell into the follicular lumen (×5800).

Figure 21



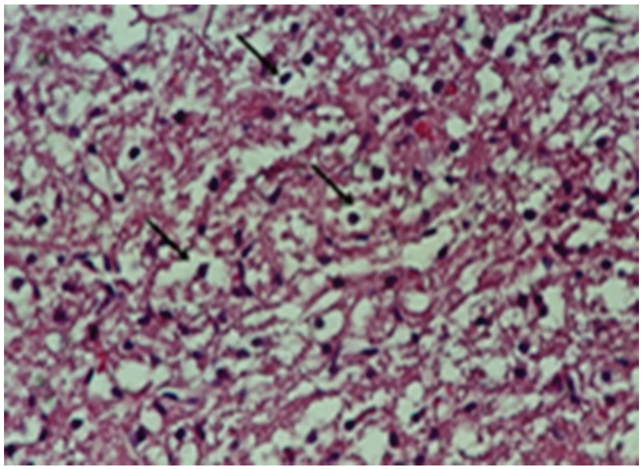
An electron micrograph of a control 10-day-old rat's thyrotroph showing that the cell is slightly elongated with an oval nucleus (N). Few mitochondria (m) and endoplasmic reticulum (r) are present. Notice the small secretory granules that tend to be peripherally situated (→) (×5800).

Figure 22



A photomicrograph of a treated 10-day-old rat's thyroid gland showing an increased height of the follicular epithelium (→) and follicular epithelial hyperplasia in some areas (arrow head). There is a decrease in the amount of colloid (c) and some follicles are completely devoid of colloid (•) (hematoxylin and eosin, ×400).

Figure 23



A photomicrograph of a treated 10-day-old rat's pituitary gland showing a severe vacuolative degeneration affecting most of the cells (→) (hematoxylin and eosin, ×400).

The pituitary gland is formed of anastomosing cords or groups of cells separated by blood sinusoids. The chromophobes have rounded vesicular, relatively large nuclei, and a pale cytoplasm. Two types of chromophils are observed: acidophils and basophils. The thyrotrophs are identified as branched cells present in close relation with the adjacent blood capillaries and have very fine secretory granules uniformly distributed in their cytoplasm (Figs. 45 and 46)

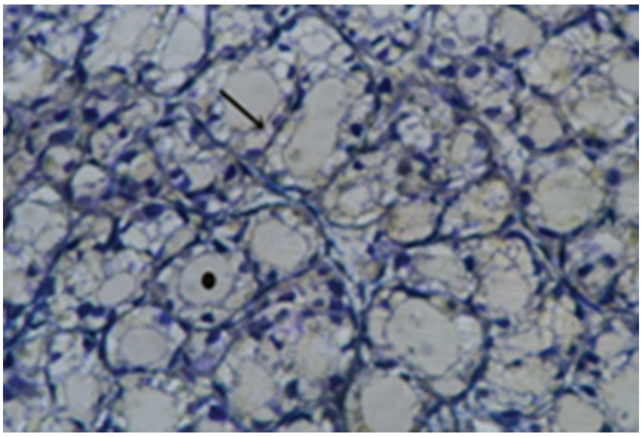
By using the immunohistochemical technique, TSH-immunopositive cells are mostly present in the central part of the pars distalis as small groups or single cells in close proximity to the blood capillaries. TSH immunopositivity is observed as a diffuse granular brown cytoplasmic stain. The cells vary in size and appearance (polygonal, elongated, or ovoid) (Fig. 47)

(b) The electron microscopic examination:

The thyroid follicular cell has numerous microvilli that project from the apical border of the cell into the follicular lumen. At the other pole of the cell, a basal lamina separates the basal surface of the cell from the extracellular spaces. A large nucleus is usually found in the basal half of the cell. Well-developed rER are observed. Many secretory granules that have a moderate density can be detected, especially in the apical cytoplasm. Some dense bodies (colloid droplets and lysosomes) can be also seen in the apical part of the cytoplasm (Fig. 48)

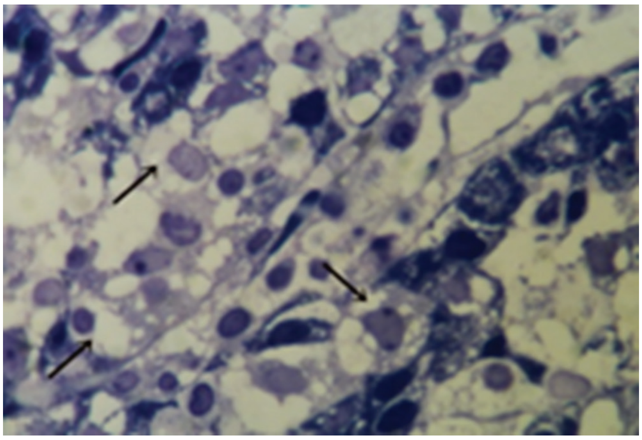
The thyrotrophs are distinguished from the other anterior pituitary cells by their small size,

Figure 24



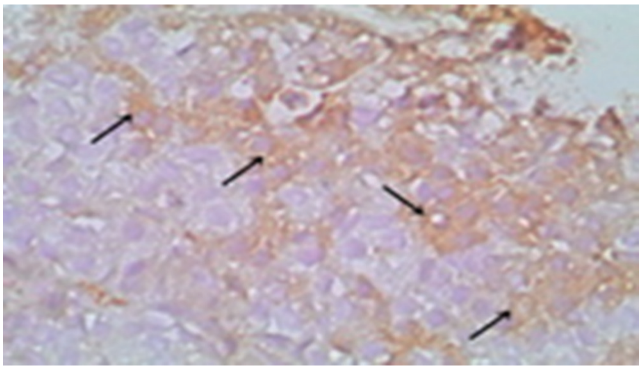
A photomicrograph of a semithin section in a treated 10-day-old rat's thyroid gland showing multiple thyroid follicles, which appear to be reduced in size and almost empty of colloid (•). The follicular epithelium (→) shows an increase in height and several vacuolations (toluidine blue, ×400).

Figure 25



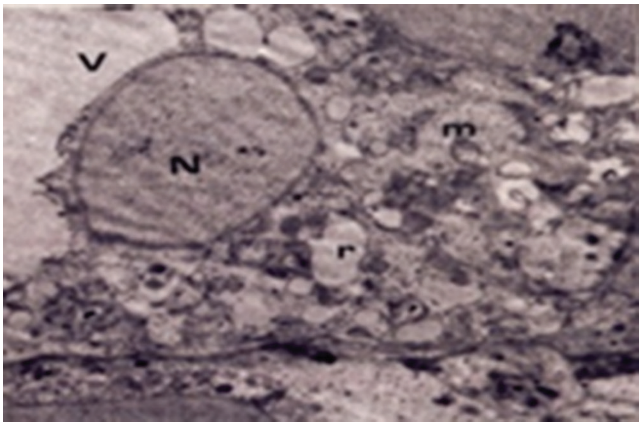
A photomicrograph of a semithin section in a treated 10-day-old rat's pituitary gland showing a severe vacuolation (→) affecting most of the cells (toluidine blue, ×400).

Figure 26



A photomicrograph of an immunostained treated 10-day-old rat's pituitary gland showing an increase in the number of the immunopositive cells (→). Immunostained with thyroid-stimulating hormone antibody (×1000).

Figure 27



An electron micrograph of a treated 10-day-old rat's thyroid follicular cell showing a large rounded nucleus (N), dilated damaged mitochondria (m), swollen rough endoplasmic reticulum (r), and many vacuoles (v) (×5800).

angular shape, and small cytoplasmic granules. They contain euchromatic oval nuclei, rER, mitochondria, long slender processes, and small secretory granules (Fig. 49)

(2) The treated group:

(a) The light microscopic examination:

The thyroid gland is composed of small-sized aggregations of follicles. Most of the follicles are markedly small with a tall columnar epithelial lining. The follicles are mostly rounded and filled with a scanty colloid material or completely devoid of colloid. Some follicles exhibit a papillary in-growth projecting into their lumina. The stroma between the follicles appears to be more dense (Figs. 50 and 51)

Regarding the pituitary gland, very large pale cells are observed throughout the adenohypophysis, often with a vacuolar degeneration of the cytoplasm and a large round nucleus (Figs. 52 and 53). By immunohistochemistry, TSH-immunopositive cells are strongly increased in number as compared with the control group. Some of these cells have a vacuolated cytoplasm (Fig. 54)

(b) The electron microscopic examination:

The thyroid follicular cell is increased in height with a large nucleus shifted to the luminal side of the cell and separated from the basement membrane by many vacuoles. The rest of the cytoplasm is studded with many large vacuoles (Fig. 55)

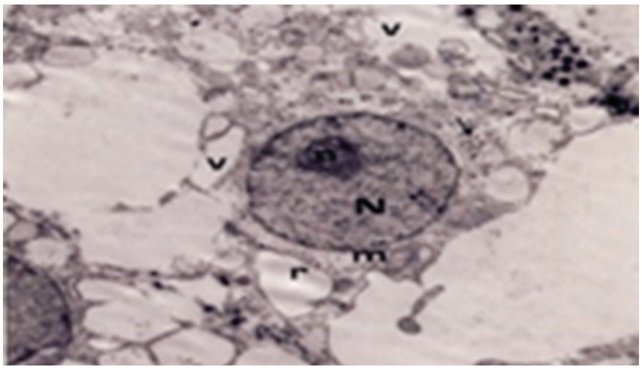
Regarding thyrotrophs, they present obvious ultrastructural changes including a large rounded nucleus, well-developed Golgi, many dilated ER, enlarged mitochondria, and a few amount of secretory granules (Fig. 56).

The morphometric results

The age of newborn

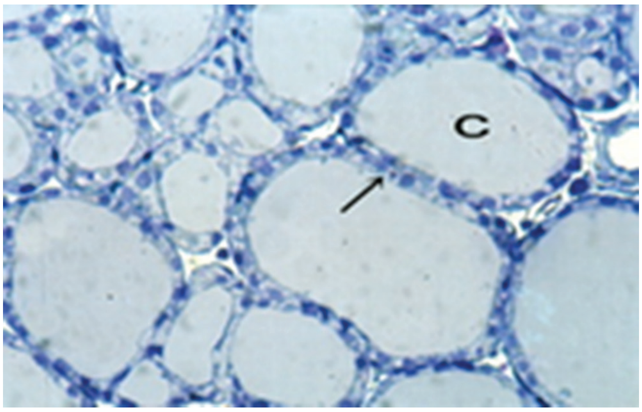
The statistical analyses of the pituitary and thyroid

Figure 28



An electron micrograph of a treated 10-day-old rat's thyrotroph showing a rounded nucleus (N) with a prominent nucleolus (n), dilated endoplasmic reticulum (r), enlarged mitochondria (m), few secretory granules (→), and many large vacuoles (v) (×5800).

Figure 30



A photomicrograph of a semithin section in a control 1-month-old rat's thyroid gland showing oval, circular, or irregular thyroid follicles. Most of the follicles are lined with low cuboidal follicular cells (→) with a scanty cytoplasm and rounded basal nuclei. The follicles are filled with colloid (c) (toluidine blue, ×400).

sections at the age of newborn reveal that there is a significant decrease in the mean diameter of the thyroid follicles, and a significant increase in the thyroid follicular epithelial height and in the mean number of the positive immunoreactive thyrotrophs in the treated group in comparison with the control group (Table 1 and Chart 1).

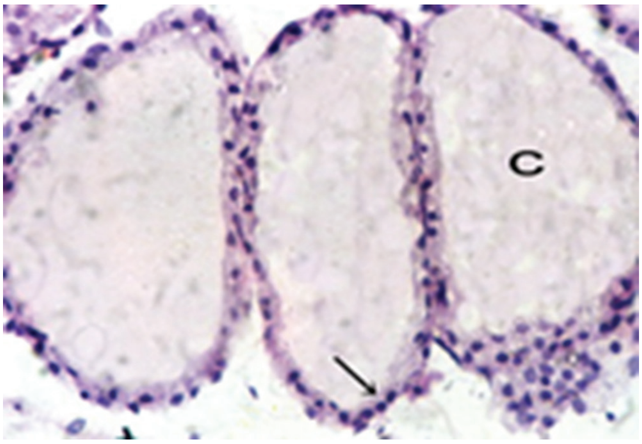
The age of 10 days

The statistical analysis reveals a significant decrease in the mean diameter of the thyroid follicles, and a significant increase in the thyroid follicular epithelial height and in the mean number of the positive immunoreactive thyrotrophs in the treated group in comparison with the control group (Table 2 and Chart 2).

The age of 1 month

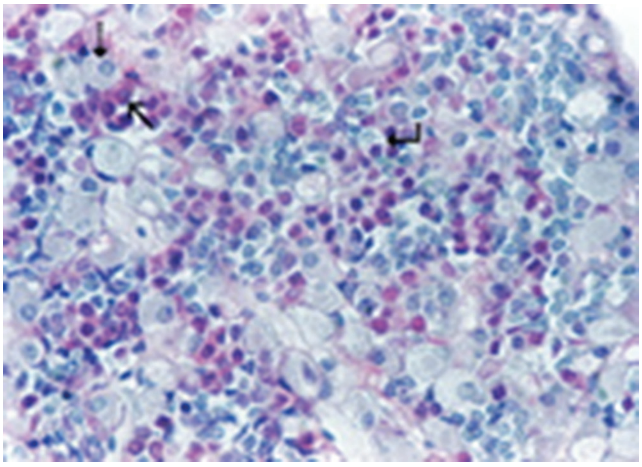
The statistical analysis reveals a significant decrease in the mean diameter of the thyroid follicles,

Figure 29



A photomicrograph of a control 1-month-old rat's thyroid gland showing thyroid follicles of different sizes lined by low cuboidal follicular cells (→) with rounded nuclei. Their lumina are filled with acidophilic colloid (c) (hematoxylin and eosin, ×400).

Figure 31



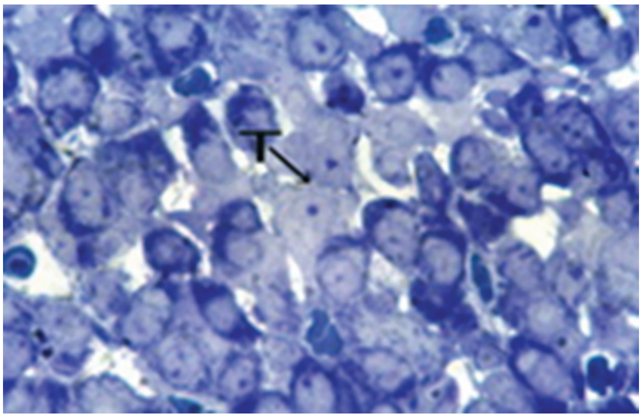
A photomicrograph of a control 1-month-old rat's pituitary gland showing lightly stained chromophobes (↵), large basophils (↓) with a basophilic granular cytoplasm, and eccentric nuclei and acidophils (↖) with an acidophilic cytoplasm as well as eccentric nuclei (hematoxylin and eosin, ×400).

and a significant increase in the thyroid follicular epithelial height and in the mean number of the positive immunoreactive thyrotrophs in the treated group in comparison with the control group (Table 3 and Chart 3).

The age of 2 months

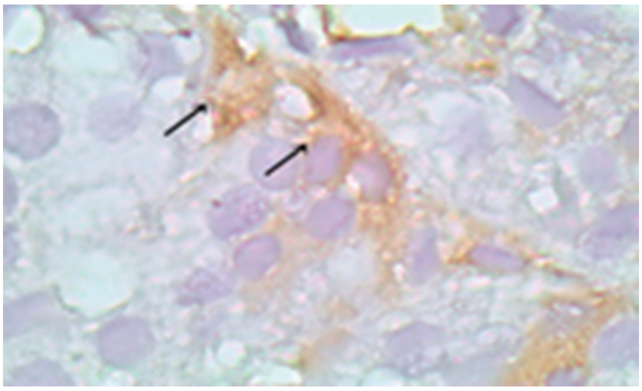
The statistical analysis reveals a significant decrease in the mean diameter of the thyroid follicles, and a significant increase in the thyroid follicular epithelial height and in the mean number of the positive immunoreactive thyrotrophs in the treated group in comparison with the control group (Table 4 and Chart 4).

Figure 32



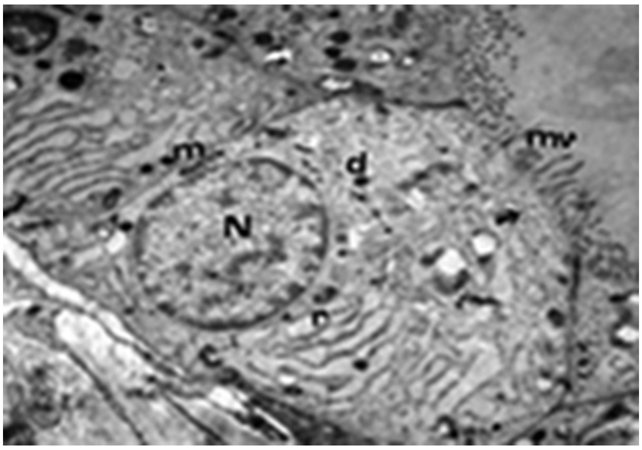
A photomicrograph of a semithin section in a control 1-month-old rat's pituitary gland showing branched thyrotrophs (T) having vesicular nuclei with prominent nucleoli and the smallest secretory granules in their cytoplasm (toluidine blue, ×1000).

Figure 33



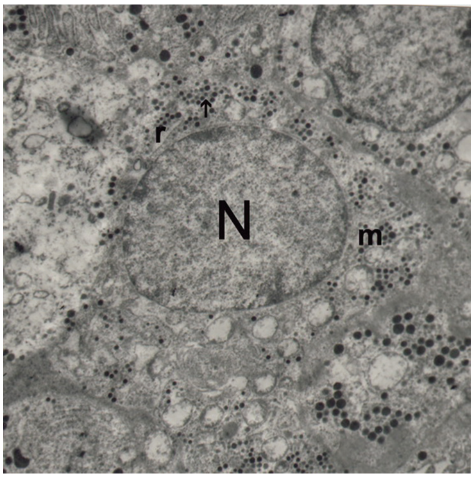
A photomicrograph of an immunostained control 1-month-aged rat's pituitary gland showing a positive reaction in the thyroid-stimulating hormone producing cells appearing as dark brown granules (→). Immunostained with thyroid-stimulating hormone antibody (×1000).

Figure 34



An electron micrograph of a control 1-month-old rat's thyroid follicular cell showing a basal nucleus (N), many dilated rough endoplasmic reticulum (r), and mitochondria (m). In the apical region of the cytoplasm there are secretory granules (→) and dense bodies (d). Notice the presence of microvilli (mv) projecting from the luminal surface of the cell (×5800).

Figure 35



An electron micrograph of a control 1-month-old rat's thyrotroph showing that the cell is polygonal with a large rounded nucleus (N), many mitochondria (m), endoplasmic reticulum (r), and small secretory granules (→) (×5800).

Discussion

Carbimazole was the drug chosen for the induction of hypothyroidism in this study. Carbimazole crosses the placental barrier [11], and is also excreted in the milk [12]. Thus, hypothyroidism could be induced before and after birth in the offspring during the period of lactation. The drug was given to the animals through a stomach tube. This method was preferable to mixing the drug with the diet [13] or with the drinking water [14] as the dose could be accurately adjusted.

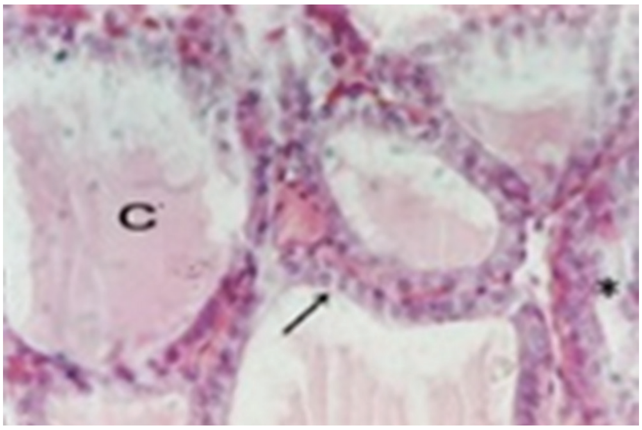
The drug was given to the pregnant mothers on the 10th day of gestation. This day was chosen because during it the eve of thyroid primordia formation occurs by means of the evagination from the foregut [15,16].

This study reveals that giving carbimazole to the pregnant and lactating mothers results in morphological changes in the pituitary–thyroid axis of their offspring. These changes start in the newborn and persist throughout the postnatal development.

Regarding the thyroid gland, this study reveals that the thyroid gland of the treated animals has small shrunken thyroid follicles, an increase in the height of the follicular epithelium with a vacuolated cytoplasm, and a decrease in the amount of colloid up to a complete absence in some follicles.

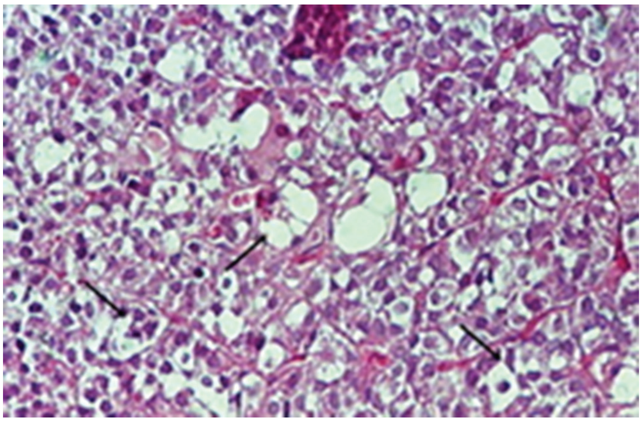
Similar findings were observed in previous studies that used measures other than carbimazole for induction of hypothyroidism. These measures included methimazole [17,18], propylthiouracil [19–21],

Figure 36



A photomicrograph of a treated 1-month-old rat's thyroid gland showing thyroid follicles of different sizes lined by proliferating vacuolated follicular cells (→). In some follicles, multiple layers of follicular cells are seen with an increase in the epithelial height (*). Notice the decrease in the amount of colloid (c) and the presence of dilated blood capillaries (ca) (hematoxylin and eosin, ×400).

Figure 38



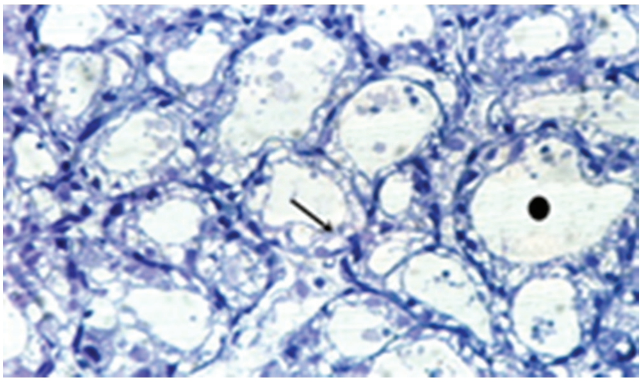
A photomicrograph of a treated 1-month-old rat's pituitary gland showing the presence of many large cells (→) with a vacuolar degeneration of the cytoplasm and rounded nuclei (hematoxylin and eosin, ×400).

2-mercaptobenzimidazole, which is a member of the thioureylene compound family known for their potent antithyroid activity [22], iodine-deficient diet [23], and sodium chlorate [24].

In addition, the results of the present study are in accordance with those of the previous researches that studied the effect of hypothyroidism on the thyroid gland in other animals such as dogs [25], Buforegularis tadpoles [26], mice [27], and sheep [28,29].

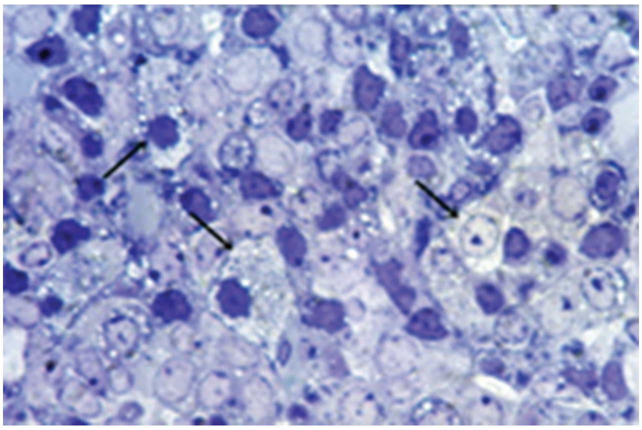
By the electron microscopic examination, this study reveals that hypothyroidism results in obvious changes in the thyroid follicular cell. They include a large sometimes indented nucleus, dilated mitochondria, swollen rER, some colloid droplets, and many large vacuoles. These ultrastructural findings are in

Figure 37



A photomicrograph of a semithin section in a treated 1-month-old rat's thyroid gland showing multiple thyroid follicles that appear to be smaller compared with the control group. They are lined by columnar follicular cells with a highly vacuolated cytoplasm (→). Most of the follicles are devoid of colloid (•) (toluidine blue, ×400).

Figure 39



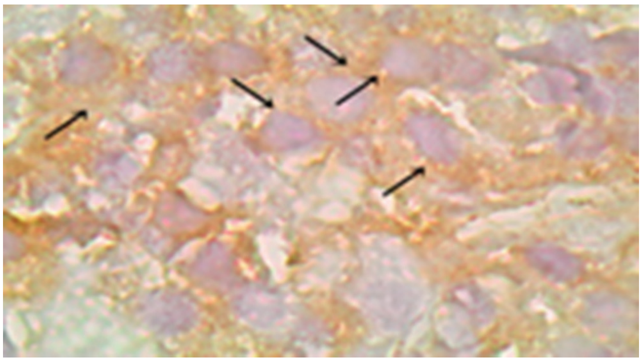
A photomicrograph of a semithin section in a treated 1-month-old rat's pituitary gland showing the appearance of large cells (→) with eccentric nuclei and a vacuolated cytoplasm (toluidine blue, ×1000).

agreement with those of Gosselin *et al.* [25], and Young and Baker [30], who reported that the hypothyroidism produced ultrastructural changes in the thyroid follicular cell in the dogs and the rats, respectively.

In agreement with our findings, Sakai *et al.* [31] studied the ultrastructural characterization of the thyroid follicular epithelial cells in the rdw rat, which is a model of rat dwarfism. They found that a large vacuole occupied almost all of the cytoplasm on the basal side of the cell. The nucleus was shifted to the luminal side. The Golgi apparatus had flattened or dilated cisternae. The ER was markedly swollen and the secretory granules could not be detected in the cytoplasm.

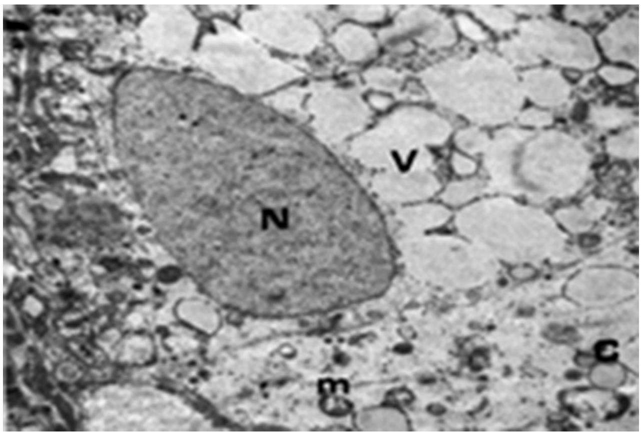
As per the morphometric analysis of the thyroid gland, this study reveals that hypothyroidism results in a significant decrease in the follicular diameter and a significant increase in the thyroid follicular

Figure 40



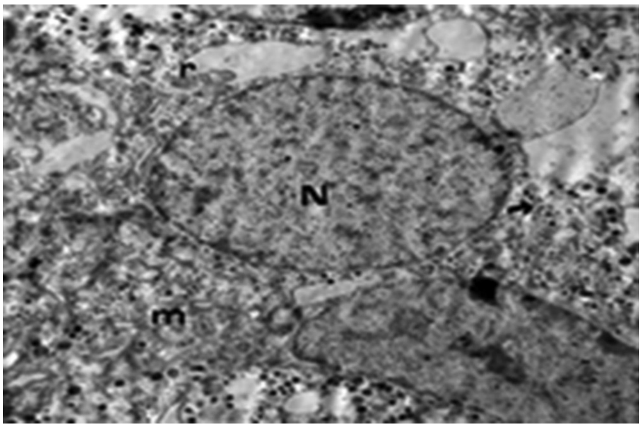
A photomicrograph of an immunostained treated 1-month-old rat's pituitary gland showing an increase in the number of the immunopositive cells (→). Notice that some of the positively stained cells contain vacuoles in their cytoplasm. Immunostained with thyroid-stimulating hormone antibody (×1000).

Figure 41



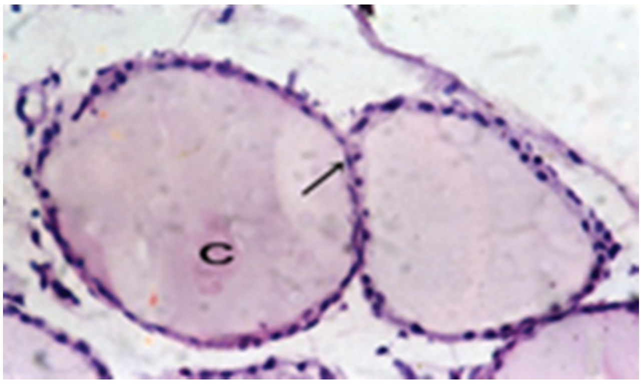
An electron micrograph of a treated 1-month-old rat's thyroid follicular cell showing a large oval nucleus (N), many dilated mitochondria (m), large colloid droplets (c), and many large vacuoles (v) occupying most of the cytoplasm (×5800).

Figure 42



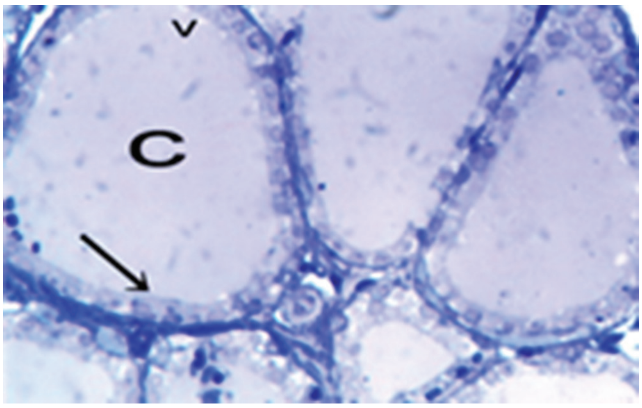
An electron micrograph of a treated 1-month-old rat's thyrotroph showing a large rounded nucleus (N), enlarged mitochondria (m), swollen endoplasmic reticulum (r), and a few amounts of secretory granules (→) (×5800).

Figure 43



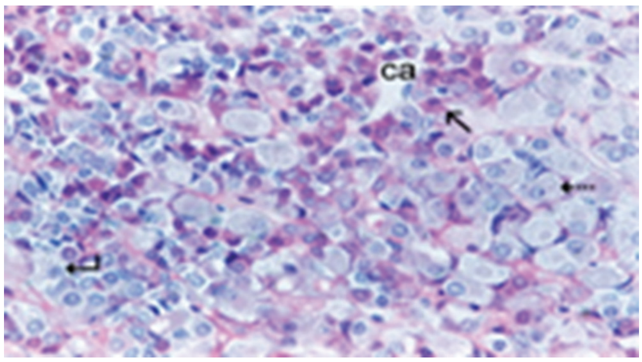
A photomicrograph of a control 2-month-old rat's thyroid gland showing large thyroid follicles. Each follicle is lined with a single layer of flat to low cuboidal epithelial cells (→) with a regular orientation and prominent nuclei. Colloid materials (c) are seen in the follicular lumen (hematoxylin and eosin, ×400).

Figure 44



A photomicrograph of a semithin section in a control 2-month-old rat's thyroid gland showing large thyroid follicles lined with low cuboidal epithelial cells (→) and filled with colloid material (c). Notice the presence of absorptive vesicles (v) in the colloid near the luminal aspect of the follicular cells (toluidine blue,×400).

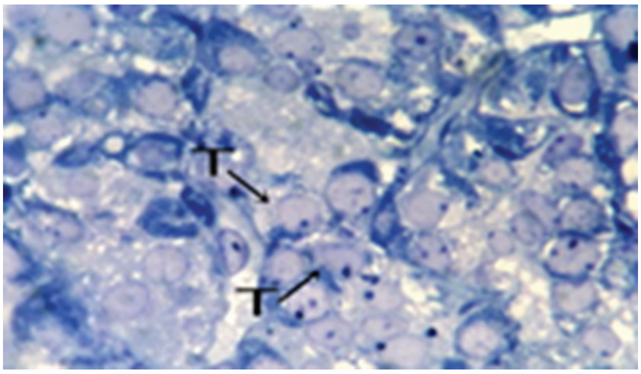
Figure 45



A photomicrograph of a control 2-month-old rat's pituitary gland showing that the cells are arranged in clusters with intervening capillaries (ca). The cells can be differentiated into basophils(⇠), acidophils (↖), and chromophobes(↵) (hematoxylin and eosin, ×400).

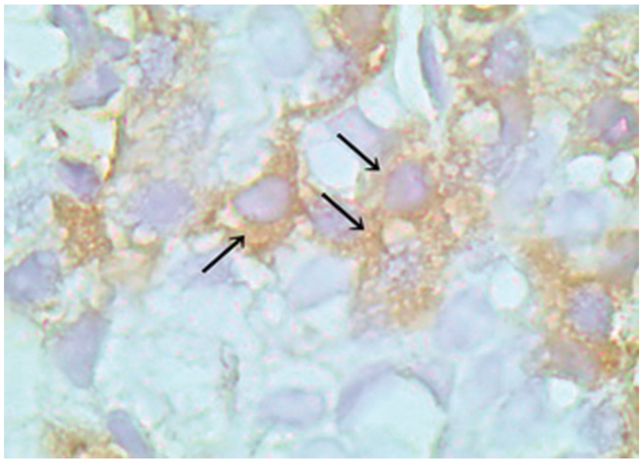
epithelial height. These results are in agreement with those of Elkalawy *et al.* [19], and Ferreira *et al.* [27], who found that the mean follicular cell height in propylthiouracil-treated mice and rats was significantly increased.

Figure 46



A photomicrograph of a semithin section in a control 2-month-old rat's pituitary gland showing different pituitary cell types from which the thyrotrophs (T) are distinguished by their angular shape, an eccentric nucleus with a prominent nucleolus, and a pale finely granulated cytoplasm (toluidine blue, ×1000).

Figure 47

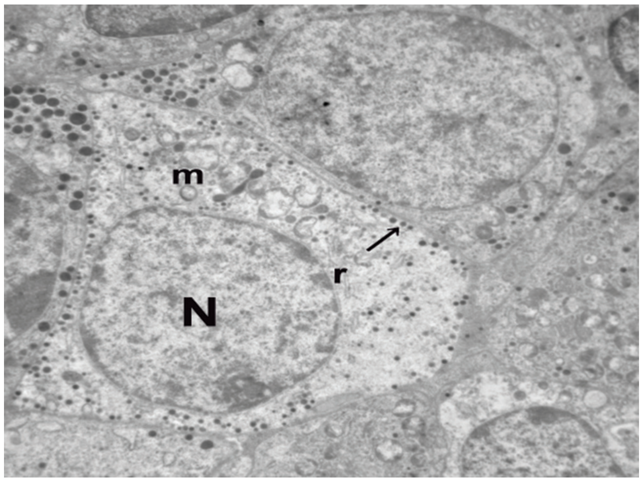


A photomicrograph of an immunostained control 2-month-old rat's pituitary gland showing a positive reaction in the thyroid-stimulating hormone producing cells appearing as dark brown granules (→). Immunostained with thyroid-stimulating hormone antibody (×1000).

Figure 48

An electron micrograph of a control 2-month-old rat's thyroid follicular cell showing a nucleus (N) near the basal membrane (BM), well-developed rough endoplasmic reticulum (r), and some mitochondria (m). Dense bodies (d) are seen near the luminal aspect of the cell. Notice the presence of microvilli (mv) projecting from the apical region of the cell (×5800).

Figure 49



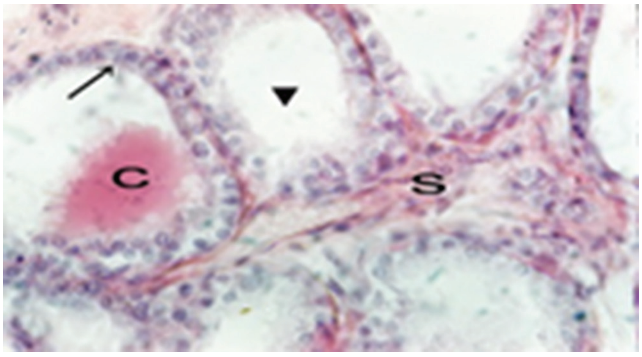
An electron micrograph of a control 2-month-old rat's thyrotroph showing a large rounded nucleus (N), mitochondria (m), endoplasmic reticulum (r), and small secretory granules (→) present mainly at the periphery of the cytoplasm (×5800).

The thyroid gland activity is regulated by the hypothalamic–pituitary– thyroid axis, including the negative feedback loop. TSH is a major growth factor for thyroid. The thyroid gland under TSH undergoes enlargement, hyperplasia, neovascularization, and morphological alterations of the thyrocytes related to their involvement in the production, processing, and release of the THs [17,32].

In this study, there was a significant increase in the height of the follicular epithelium in the hypothyroid rats, and some follicles appeared to be lined by multiple layers of follicular cells. This could be attributed to a low level of T4 that leads to increased TSH levels, which are responsible for the proliferative activity of the follicular cells. Serakides *et al.* [33], Ferreira *et al.* [27], and Mostaghni *et al.* [28] confirmed the previous suggestion and added that the intrafollicular adenomatosis that consisted of an increase in the number of the epithelial cells in the follicles, forming in some instances papillary projections into the lumen. The projections occasionally divided the follicle in the middle or even completely obliterated the lumen, giving an appearance of an adenomatous solidification.

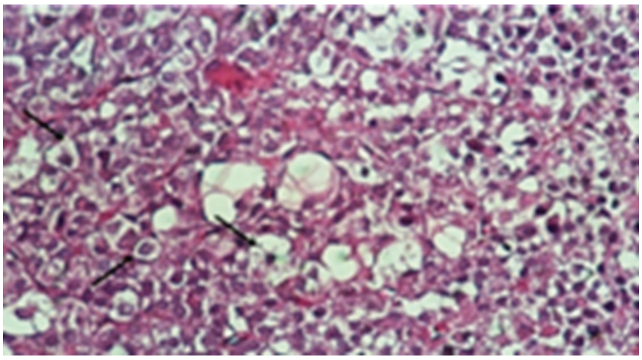
In this study, some thyroid follicles exhibited peripheral colloidal vacuolations that are more obvious when compared with the control group. It could be suggested that the follicular cells increase their activity in taking up and releasing the THs into the circulation to compensate for the increased demand. Gartner and Hiatt [34] confirmed this and reported that during the

Figure 50



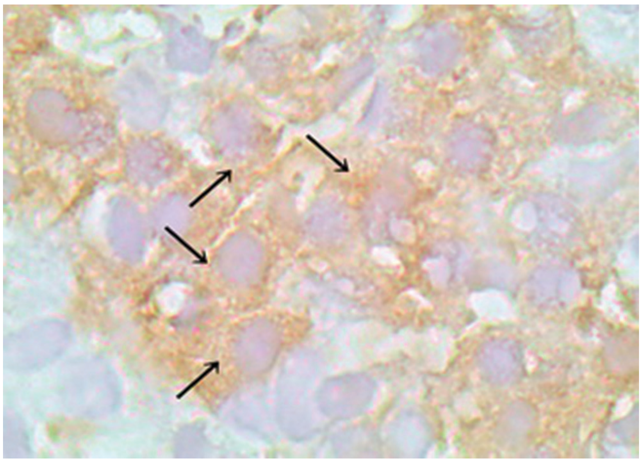
A photomicrograph of a treated 2-month-old rat's thyroid gland showing that the thyroid follicles are smaller than that of the control group. The follicular epithelium (→) is increased in height and some of its nuclei are shifted to the luminal side. The follicles are filled with a scanty colloidal material (c) or completely devoid of colloid (arrow head). The stroma (s) between the follicles appears to be more dense (hematoxylin and eosin, ×400).

Figure 52



A photomicrograph of a treated 2-month-old rat's pituitary gland showing the appearance of many large vacuolated cells (→) (hematoxylin and eosin, ×400).

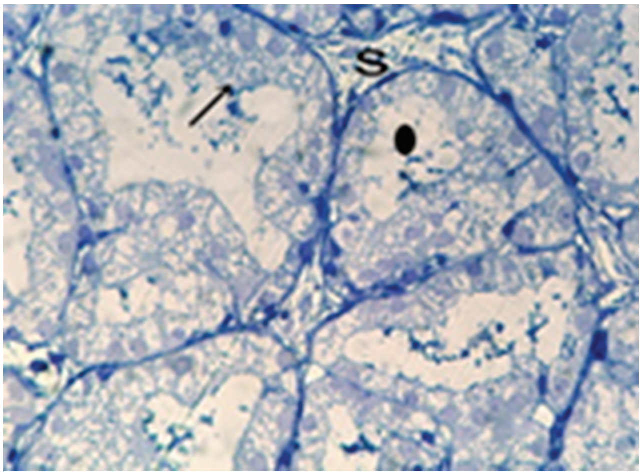
Figure 54



A photomicrograph of an immunostained treated 2-month-old rat's pituitary gland showing an increase in the number of immunopositive cells (→) in comparison with the control group. Notice the presence of cytoplasmic vacuolation in some of these cells. Immunostained with thyroid-stimulating hormone antibody (×1000).

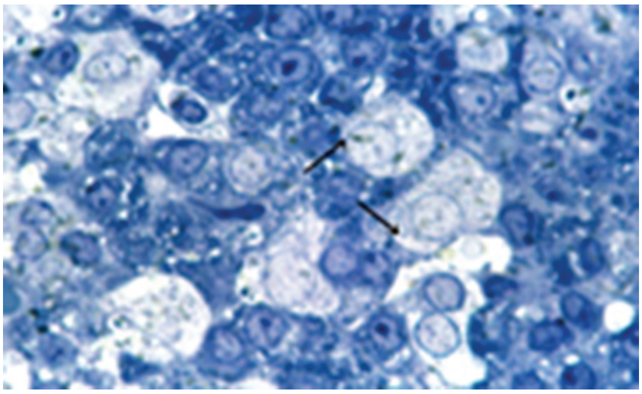
great demand for the TH, the follicular cells extended pseudopods into the follicular lumen to envelop and absorb the colloid.

Figure 51



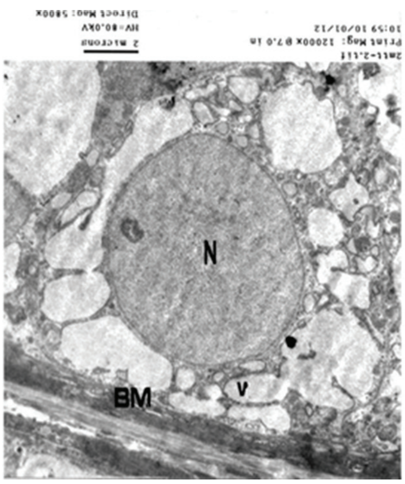
A photomicrograph of a semithin section in a treated 2-month-old rat's thyroid gland showing shrunken thyroid follicles lined with tall columnar epithelial cells (→) that have many vacuoles in the supranuclear region. The follicles are nearly devoid of colloid (•). The interfollicular stroma (s) appears to be more dense (toluidine blue, ×400).

Figure 53



A photomicrograph of a semithin section in a treated 2-month-old rat's pituitary gland showing the appearance of giant cells (arrows) with large vesicular nuclei and a pale vacuolated cytoplasm (toluidine blue, ×1000).

Figure 55

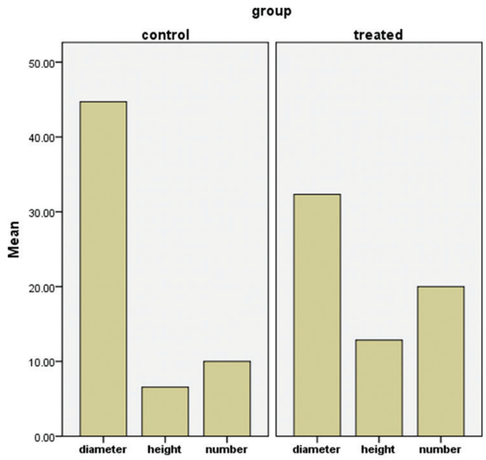


An electron micrograph of a treated 2-month-old rat's thyroid follicular cell showing a large rounded nucleus (N) that is separated from the basement membrane (BM) by many vacuoles (v). The rest of the cytoplasm is studded with many large vacuoles (×5800).

Figure 56

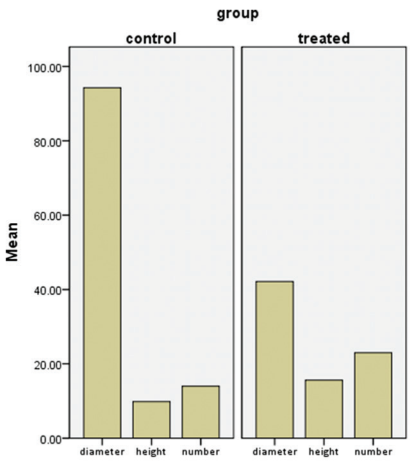
An electron micrograph of a treated 2-month-old rat's thyrotroph showing a large rounded nucleus (N), well-developed Golgi (→), many dilated endoplasmic reticulum (r), enlarged mitochondria (m), and few amounts of secretory granules (→) (×5800).

Chart 1



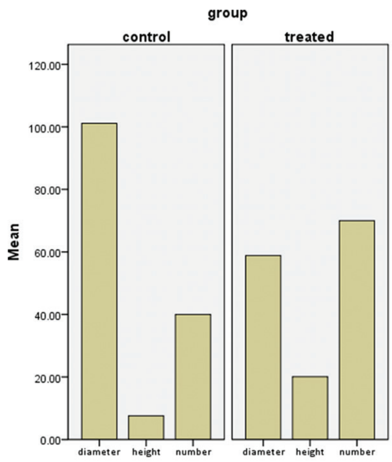
The mean diameter of the thyroid follicles, the mean thyroid follicular epithelial height, and the mean number of the positive immunostained thyrotrophs per reference area of the control and treated groups at the age of newborn.

Chart 2



The mean diameter of the thyroid follicles, the mean thyroid follicular epithelial height, and the mean number of the positive immunostained thyrotrophs per reference area of the control and treated groups at the age of 10 days.

Chart 3



The mean diameter of the thyroid follicles, the mean thyroid follicular epithelial height, and the mean number of the positive immunostained thyrotrophs per reference area of the control and treated groups at the age of 1 month.

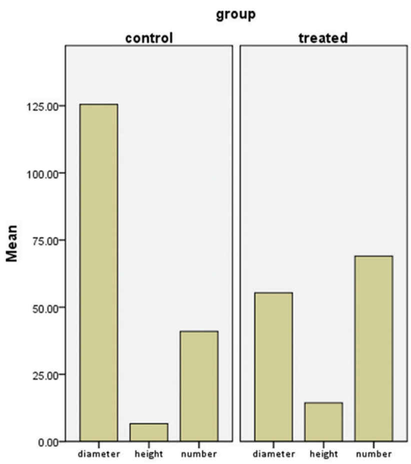
In the present study, the morphological changes observed in the semithin sections of the thyroid gland of the hypothyroid rats include tall columnar follicular cells. Some of the cells are filled with cytoplasmic vacuolations and have pale nuclei. Some follicles exhibit multiple follicular cells.

The increase in the cell size (hypertrophy) observed in this study might be due to a fluid accumulation and glandular overstimulation. The previous studies specified that hypertrophy occurred as a result of an increase in the cell size and functional capacity when the trophic signals or functional demand increased. The adaptive changes to satisfy these needs led to an increased cellular size (hypertrophy) and, in some cases, increased cellular number (hyperplasia) [35,36].

Another factor, suggested by Underwood [36], that could be responsible for cellular hyperplasia is that the increased functional demand or chronic injury stimulates the resting (G0) cells to enter the cell cycle (G1) to start multiplication.

Kum *et al.* [37] and Rubin and Strayer [35] explained that the hydropic swelling resulted from the impairment of the cellular volume regulation, a process that controlled ionic concentrations in the cytoplasm. They added that the injurious agents might interfere with the membrane-regulated process by increasing the permeability of the plasma membrane to sodium, as a result of which the capacity of the pump to extrude sodium was exceeded, damaging the pump directly, or interfering with the synthesis

Chart 4



The mean diameter of the thyroid follicles, the mean thyroid follicular epithelial height, and the mean number of the positive immunostained thyrotrophs per reference area of the control and treated groups at the age of 2 months.

of ATP, thereby depriving the pump of its fuel. The authors concluded that the accumulation of sodium in the cell led to an increase in the water content to maintain the osmotic conditions and consequently caused the cell swelling.

Available evidence indicated that there was a very little transplacental migration of the thyroid and pituitary hormones [38]. Although the thyroid would develop in the absence of the fetal pituitary, there was a fetal pituitary–thyroid feedback system [39]. The administration of the antithyroid drugs by the mother produced goiter in the fetus. This did not occur in the fetuses that had been hypophysectomized by decapitation *in utero* [40], indicating that the fetal, not maternal TSH, was responsible.

Regarding the pituitary gland, this research reveals that giving carbimazole to the pregnant rats produces morphological changes in the pituitary glands of their offspring. On the light microscopic examination, this study reveals the appearance of large pale cells with rounded nuclei and a vacuolated cytoplasm. These findings are in agreement with those of Diaz *et al.* [41], who studied the morphological changes in the adenohypophysis of the dogs with induced primary hypothyroidism and found many very large pale cells throughout the adenohypophysis, often with a vacuolar degeneration of the cytoplasm, a large round nucleus with a prominent nucleolus, and sometimes double nuclei.

As per the electron microscopic examination, this study revealed ultrastuctural changes in the thyrotrophs of the offspring.

Table 1 Comparison of the mean diameter of the thyroid follicles, the mean thyroid follicular epithelial height, and the mean number of the positive immunostained thyrotrophs per reference area between the control and treated groups at the age of newborn

	Control	Treated	*P*
The diameter of the thyroid follicle (μm)	44.7125±3.13227	32.3350±1.91959	0.001
The thyroid follicular epithelial height (μm)	6.5475±1.04541	12.8450±0.84870	0.000
The number of the thyrotrophs per reference area	10±0.95743	20±0.95743	0.000

Table 2 Comparison of the mean diameter of the thyroid follicles, the mean thyroid follicular epithelial height, and the mean number of the positive immunostained thyrotrophs per reference area between the control and treated groups at the age of 10 days

	Control	Treated	*P*
The diameter of the thyroid follicle (μm)	94.2875±15.77591	42.1475±4.16015	0.001
The thyroid follicular epithelial height (μm)	9.8250±2.23972	15.6125±2.63102	0.015
The number of the thyrotrophs per reference area	14±0.95743	23±1.50000	0.000

Table 3 Comparison of the mean diameter of the thyroid follicles, the mean thyroid follicular epithelial height, and the mean number of the positive immunostained thyrotrophs per reference area between the control and treated groups at the age of 1 month

	Control	Treated	*P*
The diameter of the thyroid follicle (μm)	101.13±6.87297	58.8567±3.73513	0.001
The thyroid follicular epithelial height (μm)	7.5933±1.12491	20.0900±2.24546	0.001
The number of the thyrotrophs per reference area	40±3.59398	70±2.21736	0.000

Table 4 Comparison of the mean diameter of the thyroid follicles, the mean thyroid follicular epithelial height, and the mean number of the positive immunostained thyrotrophs per reference area between the control and treated groups at the age of 2 months

	Control	Treated	*P*
The diameter of the thyroid follicle (μm)	125.49±10.99156	55.3533±4.25667	0.000
The thyroid follicular epithelial height (μm)	6.6133±0.65741	14.3667±1.37031	0.000
The number of the thyrotrophs per reference area	41±2.64575	69±1.70783	0.000

These ultrastructural changes are in agreement with those observed by Alkhani *et al.* [42], who studied the cytology of pituitary thyrotroph hyperplasia in the primary hypothyroidism and found that the thyrotrophs were enlarged with an ovoid eccentric nucleus, possessing a finely dispersed chromatin and a dense nucleolus, a vacuolation of the cytoplasm, cystically dilated ER cisternae, a prominent Golgi apparatus, randomly distributed few small secretory granules, and few large lysosomes.

Moreover, Franceschi *et al.* [43] reported that due to the loss of thyroxine feedback inhibition and the subsequent overproduction of thyrotropin-releasing hormone, the longstanding hypothyroidism resulted in a hyperplasia of thyrotrophs and a subsequent enlargement of the pituitary gland.

Regarding the immunohistochemical study of the pituitary gland, this study reveals that giving carbimazole to pregnant rats results in an increase in the number of TSH-immunopositive cells in the pituitaries of their offspring. This finding was ensured by the morphometric analysis where there was a significant increase in the number of thyrotrophs in the treated group.

These results are in agreement with those of Diaz *et al.* [41], who found that TSH-immunopositive cells were strongly increased in number in the hypothyroid dogs, and they added that many of the large vacuolated cells stained positive for TSH.

In addition, the results of this research are in line with the observations of Radian *et al.* [44], who found an increase in the number and size of TSH-immunoreactive cells in the methimazole-treated rats.

The mechanisms regulating the pituitary cytogenesis and cell proliferation are complex and poorly understood. Different hypotheses have been introduced to explain the nature and origin of the cells contributing to pituitary hyperplasia in such varied settings as primary hypothyroidism [45,46], pregnancy and lactation [47], and estrogen treatment [48,49].

Stratmann *et al.* [50] suggested that the presence of mitoses in the hyperplastic pituitary cells led to the assumption that hyperplasia of a pituitary cell type could only be attributed to the proliferation of such cells.

In contrast, others suggested that the cells of one line might transform to those of another, thus acquiring their morphologic features and secretory capacity – a process termed ‘transdifferentiation’ [51]. Such an interconversion was viewed not as a direct process but as occurring through bihormonal transitional cells exhibiting functional components common to both cells [52,53].

Although the division of the pre-existing thyrotrophs and the differentiation of the stem cells most likely contribute to the development of new thyrotrophs, the previous studies indicated that the transdifferentiation also played a role in thyrotroph hyperplasia as seen in primary hypothyroidism [54].

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1 Bernal J. Thyroid hormone receptors in brain development and function. Nature Clin Pract Endocrinol Metab 2007; 3:249–259.

2 El-Bakry AM, El-Gareib AW, Ahmed RG. Comparative study of the effects of experimentally induced hypothyroidism and hyperthyroidism in some brain regions in albino rats. Int J Dev Neurosci 2010; 28:371–389.

3 Ahmed OM, Abd El-Tawab SM, Ahmed RG. Effects of experimentally induced maternal hypothyroidism and hyperthyroidism on the development of rat offspring: I. The development of the thyroid hormones–neurotransmitters and adenosine-ergic system interactions. Int J Dev Neurosci 2010; 28:437–454.

4 Kratzsch J, Pulzer F. Thyroid gland development and defects. Best Pract Res Clin Endocrinol Metab 2008; 22:57–75.

5 Fabrizio M. Classification of thyroid diseases: suggestions for a revision. J Clin Endocrinol Metab 2003; 88:1428–1432.

6 James C, Richard D, Pramila R. *Clin Pathol.* New York, NY: Oxford University Press Inc.; 2007.

7 Golden SH, Robinson KA, Saldanha I, Anton B, Ladenson PW. Clinical review: prevalence and incidence of endocrine and metabolic disorders in the United States: a comprehensive review. J Clin Endocrinol Metab 2009; 94:1853–1878.

8 Krassas GE, Popper K, Glinoer D. Thyroid function and human reproductive health. Endocr Rev 2010; 31:702–755.

9 Severson B, Blomosbdt B, Einbron J. Effect of graded doses of carbimazole and propylthiouracil on the synthesis of thyroid hormone. Acta Endocrinol (Copenh) 1966; 57:149–152.

10 Gupta PD. Ultrastructural study on semithin section. Sci Tools 1983; 30:97–104.

11 Sachs C, Tebacher M, Mark M, Cribier B, Lipsker D. Aplasia cutis congenital and the antithyroid drugs during pregnancy. Case series and literature review. Ann Dermatol Venerol 2016; 143:423–435.

12 Legrand J. Comparative effects of thyroid deficiency and undernutrition on maturation of the nervous system and particularly on myelination in young rats. In: Hamburgh M, Barrington EJW, editors. *Hormones in development.* Appleton, NY: Appleton-Century-Crofts; 1971: 381–390.

13 Hamburgh M, Mendoza L, Burkat J, Weils F. Thyroid independent process in the developing nervous system. In: Hamburgh M, Barrington EJW, editors. *Hormones in development*, Appleton, NY: Appleton-Century-Crofts; 1971: 143–167.

14 Peterson RR, Young WC. The problem of placental permeability to propylthiouracil, thyroxine and thyrotrophic hormone in the guinea big. Endocrinology 1950; 50:218–225.

15 Edwards JA. The external development of the rabbit and rat embryos. In: Woollam DHM. *Advances in teratology.* London, UK: Logos Press; 1968: 239–263.

16 Phillips J, Schmidt B. A comparative study of the developing pituitary and thyroid glands of the fetal rat. J Exp Zool 1959; 141:499–518.

17 Cakic-Milosevic M, Korać A, Davidović V. Methimazole-induced hypothyroidism in rats: effects on body weight and histological characteristics of thyroid gland. Jugoslov Med Biohem 2004; 23:143–147.

18 Shibutani M, Woo GH, Fujimoto H, Saegusa Y, Takahashi M, Inoue K, *et al.* Assessment of developmental effects of hypothyroidism in rats from in utero and lactation exposure to anti-thyroid agents. Reprod Toxicol 2009; 28:297–307.

19 Elkalawy SA, Abo-Elnour RK, El Deeb DF, Yousry MM. Histological and immunohistochemical study of the effect of experimentally induced hypothyroidism on the thyroid gland and bone of male albino rats. Egypt J Histol 2013; 36:92–102.

20 Matheus G, Moraes NP. Histological study of the thyroids of rats treated with propyl thyouracil, parotidectomyzed and parotidectomyzed treated with propyl. Rev Odontol 1983; 12:47–52.

21 Ahmed KA, Al-Robai AA, Khoja SM, Ali SS. Can *Nigella Sativa* oil (NSO) reverse hypothyroid status induced by PTU in Rat? Biochemical and histological studies. Life Sci J 2013; 10:802–811.

22 Norford DC, Meuten DJ, Cullen JM, Collins JJ. Pituitary and thyroid gland lesions induced by 2-mercaptobenzimidazole (2-MBI) inhalation in male Fischer rats. Toxicol Pathol 1993; 21:456–464.

23 Iwata T, Yoshida T, Teranishi M, Murata Y, Hayashi Y, Kanou Y, *et al.* Influence of dietary iodine deficiency on the thyroid gland in Slc26a4-null mutant mice. Thyroid Res 2011; 4:4–10.

24 Sourour DA. Curcumin induces apoptosis in thyroid cells in rats: possible role of caspase 3. Int J Adv Res 2014; 2:790–801.

25 Gosselin SJ, Capen C, Marti SL. Histologic and ultrastructural evaluation of thyroid lesions associated with hypothyroidism in dogs. Vet Pathol 1981; 18:299–309.

26 Michael MI, Naur El Din AM. Effect of chemical thyroidectomy on the stages of the Egyptian Toad *Bufo Regularis* Ress III. Histogenesis of the thyroid gland. J Egypt German Soc Zool 1991; 6c: 101–122.

27 Ferreira E, Silva AE, Serakides R, Gomes AES, Cassali GD. Model of induction of thyroid dysfunctions in adult female mice. Arq Bras Med Vet Zootec 2007; 59:1245–1249.

28 Mostaghni K, Badiei K, Khodakaram-Tafti A, Maafi B. Pathological and biochemical studies of experimental hypothyroidism in sheep. Veterinarski Arhiv 2008; 78:209–216.

29 Potter BJ, Mano MT, Belling GB, McIntosh GH, Hua C, Cragg BG, *et al.* Retarded fetal brain development resulting from severe dietary iodine deficiency in sheep. Neuropathol Appl Neurobiol 1982; 8:303–313.

30 Young BA, Baker TG. The ultrastructure of rat thyroid glands under experimental conditions in organ culture. J Anat 1982; 135:407–412.

31 Sakai Y, Yamashina S, Furudate SI. Missing secretory granules, dilated endoplasmic reticulum and nuclear dislocation in the thyroid gland of rdw rats with hereditary dwarfism. Anat Rec 2000; 259:60–66.

32 Townsend CM, Beauchamp RD, Evers BM, Mattox KL. Thyroid. In: Hanks JB, Leslie J, editors. *Salmone Sabiston textbook of surgery*. 18th ed. Philadelphia, PA: Saunders/Elsevier; 2007: 917–954.

33 Serakides R, Nunes VA, Santos RL. Histomorphometry and quantification of nucleolar organizer region in bovine thyroid containing methylthiouracil residues. Vet Pathol 1999; 36:574–582.

34 Gartner LP, Hiatt JL. *Endocrine system. Color text book of histology*. 3rd ed. Philadelphia; London; New York: Saunders; 2007: 303–326.

35 Rubin R, Strayer DS. The endocrine system. In: Merino M, Quezado M, Rubin E, Rubin R, editors. *Rubin's pathology: clinicopathologic foundations of medicine*. 5th ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2007: 935–973.

36 Underwood JCE. Endocrine system. In: Cross S, Stephenson TJ, editors. *General and systemic pathology*. 4th ed. Edinberg; London; NewYork: Elsevier; 2007: 433–466.

37 Kum V, Abbas AK, Fausto N, Mitchell R. The endocrine system. In: Maitra A, editor. *Robbins basic pathology*. 8th ed. Philadelphia, PA: Saunders/Elsevier; 2006. p. 557–567.

38 Knobil E, Josimovich JB. Placental transfer of thyrotrophic hormone, thyroxine, triidothyronine, and insulin in the rat. Ann N Y Acad Sci 1959; 75:895–904.

39 Geloso JP. Thyroid hormone production and physiology in mammalian fetuses. *Proceedings of the Second International Congress of Endocrinology*. Amsterdam, The Netherlands: International Congress Series 83, Excerpta Medica Foundation; 1964: 764–768

40 Jost A. Action of propylthiouracil on the thyroid of foetal rats. Compt Rend Soc Biol 1957; 151:1295.

41 Diaz-Espiñeira MM, Mol JA, van den Ingh TS, van der Vlugt-Meijer RH, Rijnberk A, Kooistra HS. Functional and morphological changes in the adenohypophysisof dogs with induced primary hypothyroidism: loss of TSH hypersecretion, hypersomatotropism, hypoprolactinemia, and pituitary enlargement with transdifferentiation. Domest Anim Endocrinol 2008; 35:98–111.

42 Alkhani AM, Cusimano M, Kovacs K, Bilbao JM, Horvath E, Singer W. Cytology of pituitary thyrotroph hyperplasia in protracted primary hypothyroidism. Pituitary 1999; 1:291–295.

43 Franceschi R, Rozzanigo U, Failo R, Bellizzi M, di Palma A. Pituitary hyperplasia secondary to acquired hypothyroidism: case report. Ital J Pediatr 2011; 37:15.

44 Radian S, Coculescu M, Morris JF. Somatotroph to thyrotroph cell transdifferentiation during experimental hypothyroidism – a light and electron-microscopy study. J Cell Mol Med 2003; 7:297–306.

45 Khalil A, Kovacs K, Sima AAF, Burrow GN, Horvath E. Pituitary thyrotroph hyperplasia mimicking prolactin secreting adenoma. J Endocrinol Invest 1984; 7:399–404.

46 Scheithauer BW, Kovacs KT, Randall RV, Ryan N. Pituitary gland in hypothyroidism. Histologic and immunocytologic study. Arch Pathol Lab Med 1985; 109:499–504.

47 Scheithauer BW, Sano T, Kovacs KT, Young WF Jr, Ryan N, Randall RV. The pituitary gland in pregnancy: a clinicopathologic and immunohistochemical study of 69 cases. Mayo Clin Proc 1990; 65:461–474.

48 Asscheman H, Gooren LJ, Assies J, Smits JP, de Slegte R. Prolactin levels and pituitary enlargement in hormone treated male-to-female transsexuals. Clin Endocrinol 1988; 28:583–588.

49 Scheithauer BW, Kovacs KT, Randall RV, Ryan N. Effects of estrogen on the human pituitary: a clinicopathologic study. Mayo Clin Proc 1989; 64:1077–1084.

50 Stratmann IE, Ezrin C, Sellers EA, Simon GT. The origin of thyroidectomy cells as revealed by high resolution radioautography. Endocrinologu 1972; 90:728–734.

51 Horvath E, Lloyd RV, Kovacs K. Propylthiouracyl-induced hypothyroidism results in reversible transdifferentiation of somatotrophs into thyroidectomy cells. A morphologic study of the rat pituitary including immunoelectron microscopy. Lab Invest 1990; 63:511–520.

52 Frawley LS, Boockfor FR. Mammosomatotropes: presence and functions in normal and neoplastic pituitary tissue. Endocr Rev 1991; 12:337–355.

53 Porter TE, Hill JB, Wiles CD, Frawley LS. Is the mammosomatotrope a transitional cell for the functional interconversion of growth hormone- and prolactin-secreting cells? Suggestive evidence from virgin, gestating, and lactating rats. Endocrinology 1990; 127:2789–2794.

54 Vidal S, Horvath E, Kovacs K Cohen SM, Lloyd, RV, Scheithauer BW. Transdifferentiation of somatotrophs to thyrotrophs in the pituitary of patients with protracted primary hypothyroidism. Virchows Arch 2000; 436:43–51.