Bulletin of Faculty of Science, Zagazig University (BFSZU)
e-ISSN: 1110-1555
Volume-2025, Issue-4, pp-105-114
https://bfszu.journals.ekb.eg/journal
Research Paper DOI: **10.21608/bfszu.2025.337085.1447**

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Histopathological change in effect of Metformin Treatment on Ovarian Function in Hypothyroid Rats

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ABSTRACT **: Hypothyroidism is a common endocrine disorder in reproductive-aged women that is associated with insulin resistance and ovarian function disturbances.The aim of the present investigation was effect of metformin treatment was useful as an insulin sensitizer on TSH level, thyroid morphology in hypothyroidism and ovarian changes in PCOS. Forty adult healthy female albino rats of local strains weighing average 150-170 gm each were divided into four equal groups. Group I received distilled water orally (90 days), while Group II received distilled water orally daily in the 1st 45 days of the study, then received orally metformin (50 mg/100 g body weight) daily from the 46th day of the study till the end of the study (90th day), Group III and Group IV received propylthiouracil (PTU) dissolved in distilled water (15 mg/kg body weight) orally for 45 days, Then Group III received daily 1 ml distilled water orally from the 46th day of the study till the end of the study (90th day) while Group IV received metformin orally (50 mg/100 g body weight) daily from the 46th day of the study till the end of the study (90th day).Examination were thyroid and ovary histology .These results confirmed the association between thyroid hypofunction and disturbed metabolic and ovarian function, they declared that metformin treatment ameliorated ovarian and thyroid changes in rat model of hypothyroidism ,more confirmed that metformin has anti-inflammatory and ant-oxidant activity , it improved ovarian function changes ,it has no effect on ovary ,observed in hypothyroidism-induced rat model.These results confirmed that metformin doesn't affect ovarian structure in euthyroid rats.**

KEYWORDS: Metformin, propylthiouracil, hypothyroidism, ovary.

Date of Submission: 12-04-2025 Date of acceptance: 16-04-2025

I. INTRODUCTION

The thyroid gland is an endocrine gland that is concerned with production, storage, and release of thyroid hormones triiodothyronine (T3) and thyroxin (T4) which are essential for cell growth and development [40]. Thyroid diseases are highly prevalent worldwide [1,28]. Hypothyroidism is a disorder in which the thyroid gland does not produce enough thyroid hormones [20]. Thyroid disorders affect nearly 14% of adult women and are among the most common endocrinopathies in reproductive-aged women [33]. thyroid hormones had a strong effect on sexual and reproductive functions in both humans and animals[31]. Also recorded a decrease in FSH levels in hypothyroid rats that was associated with a decrease in number of ovarian follicles [24]. In addition noticed increased serum levels of LH in hypothyroid rats [39]. Unfortunately, many thyroid disorders are associated with adverse reproductive and metabolic health effects [36]. For instance, subclinical hypothyroidism (SCH) is associated with polycystic ovary syndrome (PCOS), a reproductive disorder characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology [11]. In women with PCOS, SCH is associated with comorbidities like dyslipidemia and insulin resistance [9]. Metformin, sold under the brand name Glucophage, among others, is the main first-line medication for the treatment of type 2 diabetes, [5,8,26], particularly in people who are overweight [5]. In addition, founded that long-term metformin treatment of overweight-obese women with PCOS and normal baseline glycemic homeostasis resulted in reduction and stabilization of BMI, improvements of androgen profile and low conversion rate to diabetes.Metformin is considered one of the safest anti-hyperglycemic agents [17]. The main side effect is gastrointestinal (GI) intolerance, including diarrhea, nausea, dyspepsia, and

abdominal pain. Although GI symptoms may be observed in up to 28% of patients, they are rarely the cause of discontinuation of therapy (in less than 2% of patients) [4]. Several studies have reported a decrease in TSH levels following the administration of metformin in patients with T2DM or polycystic ovary syndrome (PCOS) [14]. In addition, various reports have linked metformin use with decreased cancer incidence and mortality [6, 30]. . Metformin is currently one of the most widely used oral hypoglycemic agents in the world, and is also a core drug in the prevention and control of diabetes worldwide [10]. As the use of metformin becomes more widespread, numerous studies showed that metformin might also played essential roles in protecting the cardiovascular system, slowing down the aging process, and inhibiting tumor progression [22, 29, 43]. Moreover, many clinical trials have reported its positive effects on liver, lung, gastric, esophageal, and thyroid cancer [12, 16, 23, 25,34]. reported decreased estrogen levels in hypothyroid rats which was considered responsible for the increase in the number of atretic follicles and the reduced growing follicles as estrogen plays a vital role in viability of follicular cells [24].

II.MATERIALS AND METHODS

The research was conducted at Zagazig University's Faculty of Medicine's Scientific & Medical Research Centre, approved by the International Animals and Use Committee and the ZU-IACUC Committee. The approval number is (ZU-IACUC/1/F/44/2023).
Biochemical Agents

- Propylthiouracil (PTU, Thyrocil 50 mg tablets, Amoun, Egypt) which was given intraperitoneally to groups III and IV at doses (15 mg/kg bwt) orally for 45 days
- Metformin Hydrochloride (1g tablets, Chemical Industries Development, Giza). which was given intraperitoneally to groups I and IV at doses (50 mg/100 g bwt) daily from the 46th day of the study till the end of the study (90th).

Material Preparation
A suspension containing metformin dissolved in distilled water , and was administered orally at a dose of (50 mg/100 g body weight) (Verma et al., 1994).other suspension containing propylthiouracil (PTU) dissolved in distilled water (15 mg/kg body weight) (Cooper, 2005) , and was administered orally by gavage for 90 days to induce hypothyroidism .
Investigational Animals
The Veterinary Laboratory Animal Farm at Zagazig University provided Forty adult healthy female albino rats for the experiment. Rats were kept in metallic cages with ten rats per cage and were kept in the laboratory condition model with air exposure and temperature. The estimated animals were allowed unrestricted access to a standard meal (a rodent food that is viable) and unlimited water. Just prior to the ideal conditions already beginning the experimental period, all of the animals were housed under observation and acclimatization for a period of two weeks.
Experimental Design
Rats weighing 150-170g at baseline were split into four equal groups at random. Oral distilled water
was administered to Group I, which is the controlling group. Rats in Group II were administered distilled water orally for the 1st 45 days of the study, then received orally metformin (50 mg/100 g body weight) daily from the 46th day of the study till the end of the study (90th day), Group III and Group IV received propylthiouracil (PTU) dissolved in distilled water (15 mg/kg body weight) orally for 45 days, Then Group III received daily 1 ml distilled water orally from the 46th day of the study till the end of the study (90th day) while Group IV received metformin orally (50 mg/100 g body weight) daily from the 46th day of the study till the end of the study (90th day). All of the rats' thyroid and ovary were cleaned using standard cold saline, dried with filter paper, and then preserved in 10% formalin-Saline for the histology investigations.
Inspection
each rat's entire thyroid and ovary were taken, cleaned in regular saline, dried with filter paper, and preserved in 10% formalin-Saline at room temperature for the histology analyses. The international federation of clinical .

II. RESULTS AND DISCUSSION

results
Histopathological analysis results
H&E
Thyroid gland

thyroid section of the control and control-metformin treated groups revealed normal structure of Thyroid gland as it appeared formed of different size follicles filled with acidophilic colloid, lined with low cuboidal epithelium lining with rounded nucleus , with inter follicular cells and few blood vessels in between(Figure.1a&b). Thyroid gland of hypothyroid group appeared formed mostly of small size empty follicles ,lined with vacuolated epithelium lining , with congested blood vessel in the periphery and multiple congested blood vessels in between the follicles (figure.2a). while, thyroid section of the hypothyroid-metformin treated group appeared having different size follicles some were filled with acidophilic colloid while others were empty ,lined with low cuboidal epithelium lining with rounded nucleus ,some epithelial lining were vacuolated with few congested blood vessels in between the follicles (figure.2b) **[38].**

Ovary

The ovarian sections of control and control-metformin groups appeared formed of cortex and medulla .cortex had multiple stages of ovarian follicles, primordial follicle , primary follicle , corpus luteum . And Graafian follicle that had secondary oocyte surrounded by zona pellucida, corona radiata and attached by cumulus oophorus to the wall of follicle .the antrum of Graafian follicle was surrounded with granulosa cells , theca interna cells and theca externa cells (Fgure3&4) .

the ovarian cortex of hypothyroid group revealed multiple follicular stages with secondary follicles , primary follicles ,multiple corpus luteum , multiple cystic follicles and congested blood vessels in medulla . The cystic follicles were surrounded with vacuolated cells and corpus luteum cells showed marked vacuolation, while the medulla was filled with congested blood vessels . The secondary follicle was lined with multiple layered follicular cells with vacuolated cytoplasm and was containing primary oocyte was surrounded with zona pellucida.note small primary follicle appeared with containing primary oocyte(Figure. 5).

The ovarian sections of hypothyroid-metformin groups appeared formed of cortex and medulla .cortex had multiple stages of ovarian follicles, primordial follicle , primary follicle , corpus luteum . And Graafian follicle that had secondary oocyte surrounded by zona pellucida, corona radiata and attached by vacuolated cumulus oophorus to the wall of follicle .the antrum of Graafian follicle was surrounded with vacuolated granulosa cells , theca interna cells and theca externa cells.the medulla showed some congested blood vessels (Fgure.6) .

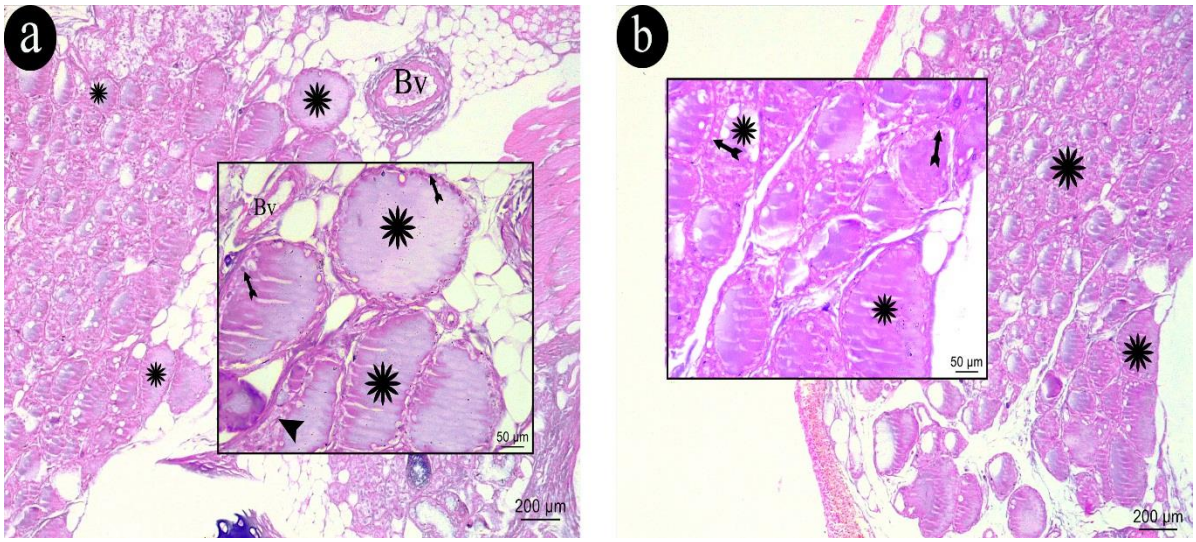


Figure 1 : Representative photomicrographs of H&E-stained sections of thyroid gland. **[a]** thyroid section of the control group, **[b]** thyroid section of the control-metformin treated group. Thyroid gland is formed of different size follicles filled with acidophilic colloid(asterisk),lined with low cuboidal epithelium lining with rounded nucleus (tailed **arrow**), with inter follicular cells (**arrow**) and few blood vessels (Bv) in between **(H&E x 100 & inset magnification x400).**

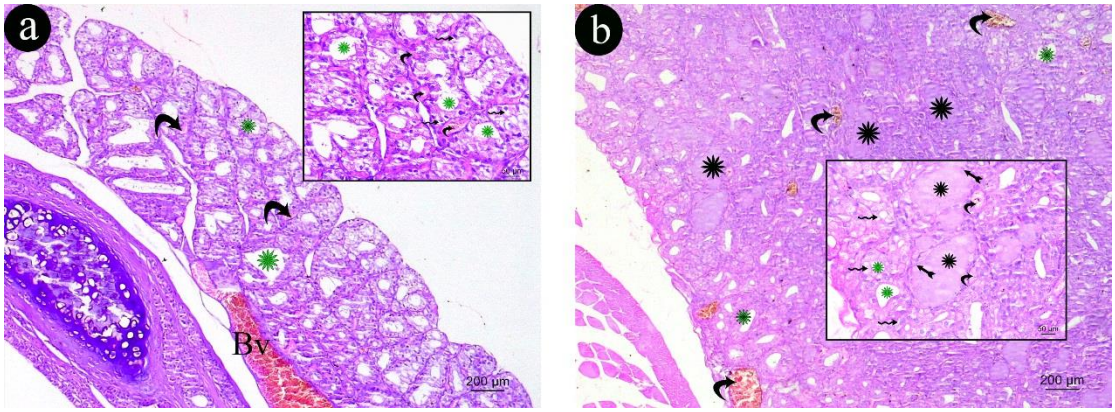
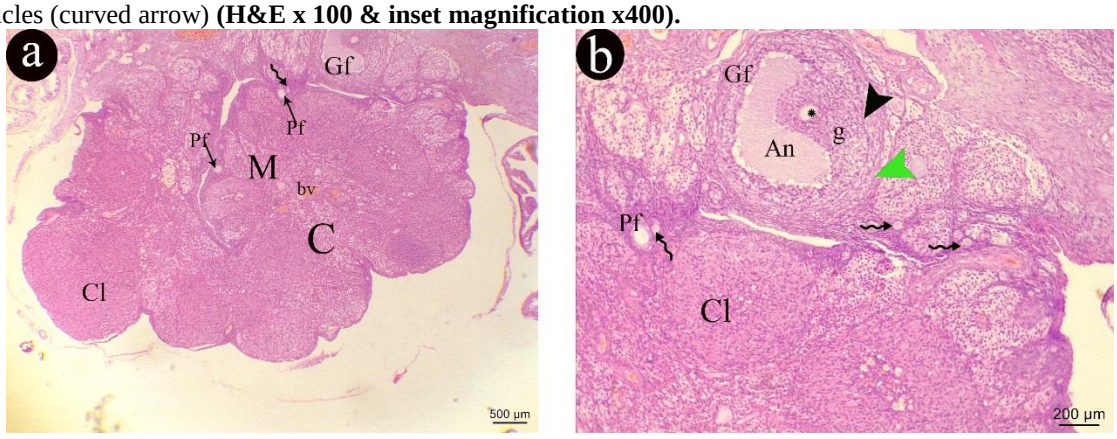


Figure 2 : Representative photomicrographs of H&E-stained sections of thyroid gland. **[a]** thyroid section of the hypothyroid group, Thyroid gland is formed of mostly small size empty follicles (green asterisk),lined with vacuolated epithelium lining (zigzag **arrow**), with congested blood vessel (Bv)in the periphery and multiple congested blood vessels in between the follicles(curved arrow) **[b]** thyroid section of the hypothyroid-metformin treated group. Thyroid gland is formed of different size follicles some are filled with acidophilic colloid(asterisk) while others are empty (green asterisk),lined with low cuboidal epithelium lining with rounded nucleus (tailed **arrow**),some epithelial lining are vacuolated (zigzag arrow) with few congested blood vessels in between the follicles (curved arrow) **(H&E x 100 & inset magnification x400).**



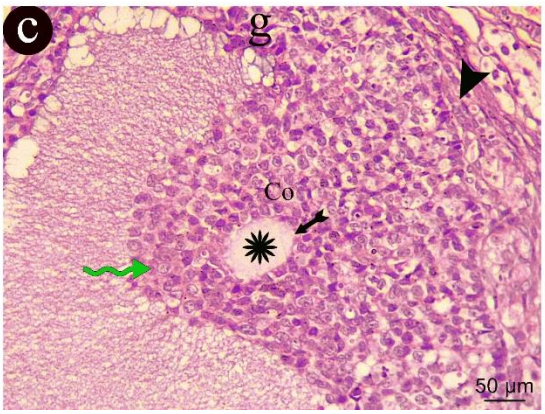


Figure. 3: A representative photomicrographs of H&E section in an ovary of control rat. **a)** The ovarian section appears formed of cortex (c) and medulla (M), with corpus luteum (Cl) , Graafian follicle (Gf) in cortex and small blood vessels(bv)within medulla (H&E, x40). **b)** The cortex appears with multiple stages of ovarian follicles, primordial follicle (zigzag arrow), primary follicle (Pf) and corpus luteum (Cl). The Graafian follicle (Gf)is containing secondary oocyte(asterisk) and appeared with antrum (An) is surrounded with granulosa cells (g), theca interna cells (black arrow head) and theca externa cells (green arrow head) (H&E, x100). **c)** The Graafian follicle(Gf) appears with antrum (An) surrounded with granulosa cells (g), theca interna cells (black arrow head). secondary oocyte (Asterisk), surrounded by zona pellucida (tailed arrow), corona radiata (green zigzag arrow) and attached by cumulus oophorus (Co) (H&E, x400).

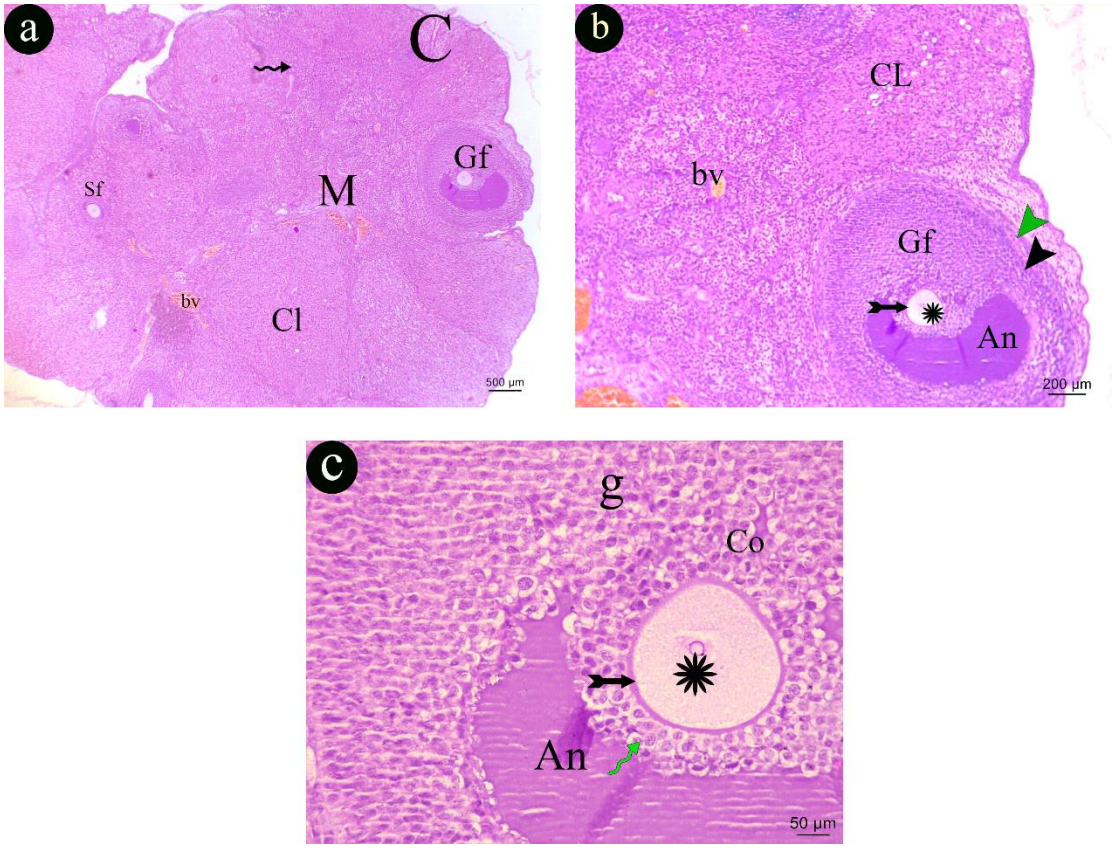


Figure. 4: A representative photomicrographs of H&E section in an ovary of control-metformin treated rat. **a)** The ovarian section appears formed of cortex (c) and medulla (M), the cortex appears with multiple stages of ovarian follicles, primordial follicles (zigzag arrow), secondary follicle (Sf) , corpus luteum (Cl) and Graafian follicle (Gf) with small blood vessels(bv)within medulla (H&E, x40). **b)** The cortex appears with corpus luteum (Cl) and Graafian follicle(Gf)is containing secondary oocyte(asterisk) and appeared with antrum (An) surrounded with granulosa cells (g), theca interna cells (black arrow head) and theca externa cells (green arrow head) (H&E, x100). **c)** The Graafian follicle (Gf) appears with antrum (An) surrounded with granulosa cells (g), theca interna cells (black arrowhead). secondary oocyte (Asterisk), surrounded by zona pellucida (tailed arrow), corona radiata (green zigzag arrow) and attached by cumulus oophorus (Co) (H&E, x400).

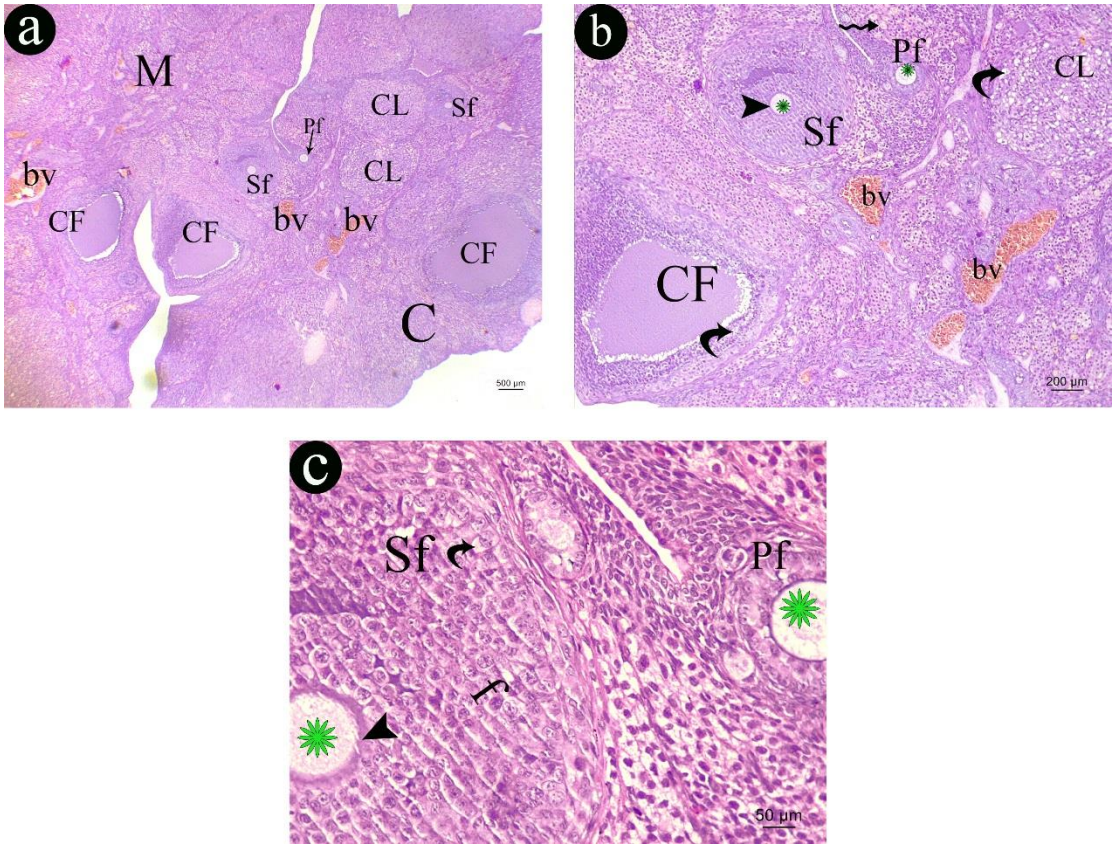


Figure5: A representative photomicrograph of H&E ovarian sections in hypothyroid rat. a) the ovarian tissue is formed of cortex (C) and medulla (M), with secondary follicles (Sf) , primary follicles (Pf) ,multiple corpus luteum (CL) , multiple cystic follicles (CF)in cortex and congested blood vessela (bv) in medulla (H&E, x40). **b)** The cortex is formed from multiple cystic follicles (CF) surrounded with vacuolated cells and corpus luteum with marked vacuolation of its cells, while the medulla is filled with congested blood vessels (C) (H&E, x100□). **c)** The secondary follicle (Sf) is lined with multiple layered follicular cells (f) with vacuolated cytoplasm (curved arrow) and is containing primary oocyte(green asterisk) surrounded with zona pellucida(arrow head).note small primary follicle (Pf) with containing primary oocyte(green asterisk) (H&E, x400).

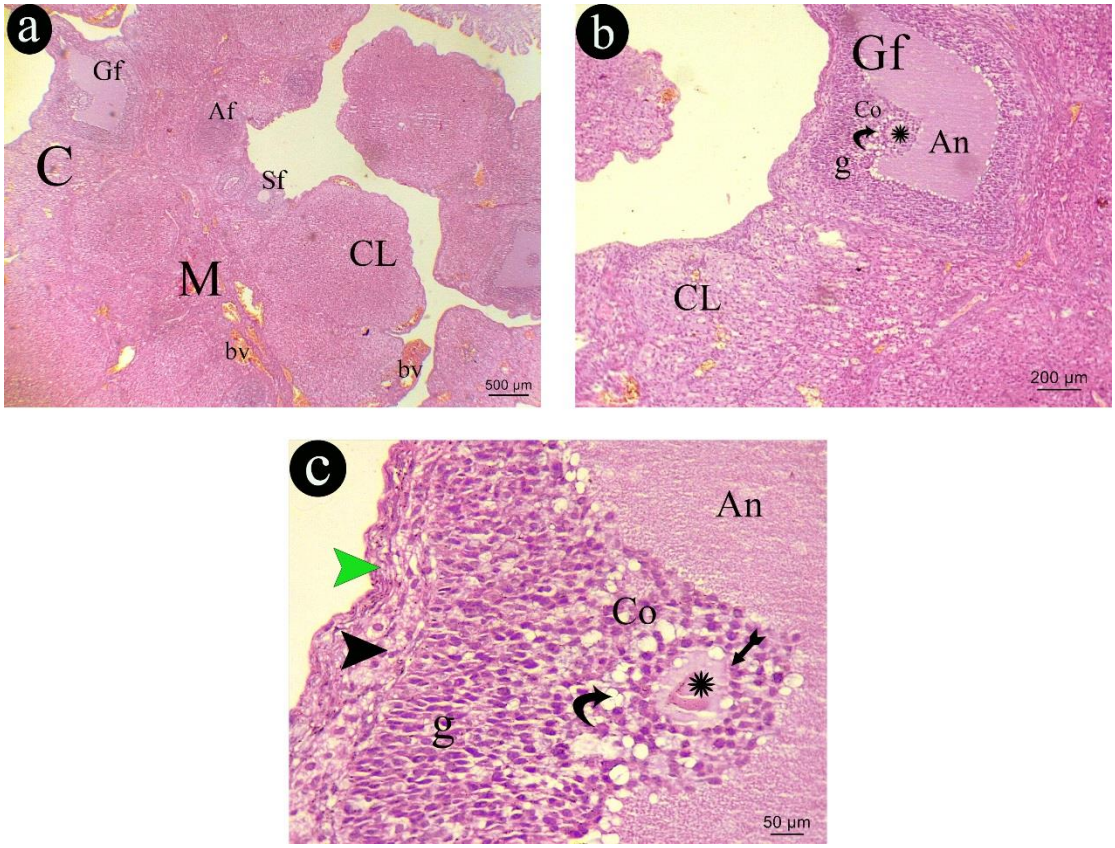


Figure .6: A representative photomicrographs of H&E sections in an ovary of hypothyroid- metformin treated rat. **a)** The ovarian section appears formed of cortex (c) and medulla (M), the cortex appears with multiple stages of ovarian follicles, secondary follicle (Sf) , corpus luteum (Cl) and Graafian follicle (Gf) and atretic follicle(Af) with congested blood vessels(bv)within medulla (H&E, x40). **b)** The cortex appears with corpus luteum (Cl) and Graafian follicle (Gf)is containing secondary oocyte(asterisk) and appeared with antrum (An) surrounded with vacuolated (curved arrow) granulosa cells (g) (H&E, x100). **c)** The Graafian follicle (Gf) appears with antrum (An) surrounded with granulosa cells (g), theca interna cells (black arrowhead). secondary oocyte (Asterisk), surrounded by zona pellucida (tailed arrow), and attached by vacuolated (curved arrow) cumulus oophorus (Co) (H&E, x400).

Discussion

In this study, thyroid section of the control and control metformin treated groups revealed normal structure of thyroid gland as it appeared formed of different size follicles filled with acidophilic colloid, lined with low cuboidal epithelium lining with rounded nucleus, with interfollicular cells and few blood vessels in between. These results confirmed that metformin doesn't affect ovarian structure in euthyroid rats.

Histopathological results showed that thyroid gland of hypothyroid group appeared formed mostly of small size empty follicles, lined with vacuolated epithelium lining, with congested blood vessel in the periphery and multiple congested blood vessels in between the follicles. These results emphasized the success of induction of hypothyroidism. different cells in the ovary, including oocytes, granulosa cells, and epithelium express receptors of thyroid hormone These results were supported by **[19]** .

Also, thyroid hormone is also found in follicular fluid. It plays an important role in the process of follicle development and its dysregulation may impair follicular development were supported by **[19]**.

Thyroid section of the hypothyroid metformin treated group appeared having different size follicles some were filled with acidophilic colloid while others were empty, lined with low cuboidal epithelium with rounded nucleus, some epithelial linings were vacuolated with few congested blood vessels in between the follicles. These results declared the ameliorative effect of metformin treatment on thyroid structure in hypothyroidism.

The ovarian sections of control and control metformin treated groups appeared formed of cortex and medulla. The cortex had multiple stages of ovarian follicles, primordial follicle, primary follicle, corpus luteum and Graafian follicle that had secondary oocyte surrounded by zona pellucida, corona radiata and attached by cumulus oophorus to the wall of follicle. The antrum of Graafian follicle was surrounded with granulosa cells, theca interna cells and theca externa cells. normal thyroid hormone levels are essential for regulation of growth, metabolism, development and differentiation of the ovary These results were in agreement **[3]** .

The ovarian cortex of hypothyroid group revealed multiple follicular stages with secondary follicles, primary follicles, multiple corpus luteum, multiple cystic follicles and congested blood vessels in medulla. The cystic follicles were surrounded with vacuolated cells and corpus luteum cells showed marked vacuolation. The secondary follicle was lined with multiple layered follicular cells with vacuolated cytoplasm and contained primary oocyte that was surrounded with zona pellucida.

These results confirmed that hypothyroidism deteriorates ovarian structure ,which hypothyroidism in females impaired fertility by disturbing endometrial and ovarian structure were supported by **[27]**. Also, the presence of large corpora lutea in ovaries of hypothyroid rats to reduction of the circulating thyroid hormones which are essential for follicular granulosa cells differentiation to granulosa lutein cells **[21]** .

In addition, hypothyroidism disturbed the function of pituitary-ovarian axis which affected follicular maturation causing cystic formations **[2]**.

In line rats of hypothyroid group showed marked congestion of medullary blood vessels which was attributed to ovarian hyperactivity in response to hormonal changes that occurs in hypothyroidism **[24]**. hypothyroidism is associated with retarded ovarian function and female fecundity through affecting the reproductive axis **[41]** .

Also, thyroid hypofunction causes a defect in the follicular growth and development, and anovulation **[18]**. In addition, thyroid abnormality causes ovulatory dysfunction, which is accompanied with endocrine reproductive disorders **[32]**. Moreover, **[35]** stated that thyroid hormones act through binding to their receptor in ovary with subsequent stimulation of follicles. Therefore, hypothyroidism can directly affect the function of ovary.

Furthermore, **[13]** reported that oocytes express cell surface receptors for thyroid hormones that affect the actions of follicle-stimulating hormone and luteinizing hormone through steroid biosynthesis.

The ovarian sections of hypothyroid metformin groups appeared formed of cortex and medulla. The cortex had multiple stages of ovarian follicles, primordial follicle, primary follicle, corpus luteum and Graafian follicle that had secondary oocyte surrounded by zona pellucida, corona radiata and attached by vacuolated cumulus oophorus to the wall of follicle.

The antrum of Graafian follicle was surrounded with vacuolated granulosa cells, theca interna cells and theca externa cells. The medulla showed some congested blood vessels. These findings declared that metformin treatment ameliorated ovarian changes with hypothyroidism which is supported by hypothyroidism-related ovulatory dysfunctions are resolved following the treatment of hypothyroidism **[32].**

Limitations of the study:

A small number of rats were used. Also, this study was conducted on rats and the results may be different from human. In addition, immunohistochemistry for thyroid hormone receptors in the ovary was not evaluated.

Conclusion:

Hypothyroidism deteriorates ovarian structure and function which are ameliorated with metformin treatment through improving body metabolism, hormonal profile, and its anti-inflammatory and antioxidant actions.

Recommendation

It is recommended to use large samples to validate the results. In addition, it is recommended to evaluate the effect of metformin treatment of hypothyroid women on their ovarian function. Moreover, immunohistochemistry for thyroid hormone receptors in the ovary should be evaluated in the future studies.

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