The Role Of Selnium On Some Hormones And Histological Structures In Ovaries Of Hypothyroid Female Albino Rats

Eman Ghanim Alhealy* , Mayson Alkazzaz , Professor Wahda Mohammad Taib Al-Nuaimy*****
***Department of Anatomy, Collage of Medicine , Ninevah University , **Department of Anatomy College of Medicine, University of Mosul , **Department of Pathology, College of Medicine, University of Mosul, Mosul, Iraq.**
Correspondence: eman.sheet@uoninevah.edu.iq

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ABSTRACT

Background: The appropriate function of the ovary depends on the proper function of other endocrine glands especially the thyroid gland, which is the largest endocrine glands in the body. Hypothyroidism is a common thyroid disorder which commonly affect females and influence the function of reproductive system.
Aim of this study : This work is to focus on the structural changes in ovary of adult female rats induced by hypothyroidism and the role of selenium.
Methods: Thirty adult female rats were used. They were classify into 3 groups (10 animals in each group): Group I (control) taken 0.5 ml distilled water orally for 3 months . Group II (hypothyroid) they were given antithyroid drug, carbimazole in a dose of 6 mg/ kg orally by gastric tube for 3 months. Group III (hypothyroid + selenium) this group were treated by carbimazole as same as group II then added selenium in the last 2month in a dose of 10 µg/ kg orally. At the end of experiment , blood samples were taken for assessment of serum TSH ,T4 , FSH, LH, and estradiol, then animals were sacrificed and the ovaries were fixed in 10% formalin solution and prepared to be examined under the light microscope.
Results: Serum concentration of TSH was significantly increase while the level of T4, LH, FSH, and estradiol concentrations were significantly decreased in both treated groups. The ovaries of hypothyroid groups showed degenerative changes of most growing follicles. Many atretic follicles and corpus luteum were observed with increase of collagen fibers and edema in the stroma associated with congestion of blood vessels. Treating of hypothyroid rats with selenium for two months produced an improvement in hormonal level and histological structure of the ovarian tissue.
Conclusion: Hypothyroidism affect the structure of ovarian tissues but adding selenium has ameliorative role on thyroid function and histological changes of ovary which may be attributed to its antioxidant activity.

Keywords: hypothyroidism, selenium, ovary.

دور السيلينيوم في بعض الهرمونات والتركيبات النسيجية فى مبايض إناث الفئران المصابة بقصور الغدة الدرقية

ايمان غانم شيت* ، ميسون محي الدين حسن القزاز** ، الاستاذ وحدة محمد طيب النعيمي***
فرع علم التشريح ، كلية الطب ، جامعة نينوى ، **فرع علم التشريح ، كلية الطب ، جامعة الموصل ،
***فرع علم الامراض ، كلية الطب ، جامعة الموصل ، الموصل ، العراق

الخلاصة

الخلفية : تعتمد وظيفة المبيض على وظيفة الغدد الصماء الأخرى وخاصة الغدة الدرقية ، وهي أكبر الغدد صماء في الجسم. قصور الغدة الدرقية هو اضطراب شائع في الغدة الدرقية يصيب عادة الإناث ويؤثر على وظيفة الجهاز التناسلي
الهدف من الدراسة : يركز هذا العمل على التغيرات الهيكلية في مبيض إناث الفئران البالغة الناتجة عن قصور الغدة الدرقية ودور السيلينيوم في تحسين هذه التغيرات.
الطريقة : تم استخدام ثلاثين أنثى بالغة من الفئران . تم تصنيفهم إلى ٣ مجموعات (١٠ حيوانات في كل مجموعة): المجموعة الأولى (مجموعة التحكم) أخذت ٠.٥ مل من الماء المقطر عن طريق الفم لمدة ٣ أشهر. المجموعة الثانية (قصور الغدة الدرقية)

تم إعطاؤهم عقار مضاد للغدة الدرقية كاربيمازول بجرعة ٦ مجم / كجم من وزن الجسم عن طريق أنبوب معدي لمدة ٣ اشهر. المجموعة الثالثة (قصور الغدة الدرقية + السيلينيوم) عولجت هذه المجموعة بكاربيمازول مثل المجموعة الثانية ثم أضيف السيلينيوم في آخر شهرين بجرعة ١٠ ميكروغرام / كغم عن طريق الفم. في نهاية التجربة تم أخذ عينات دم لتقييم مستوى TSH و T4 و FSH و LH و estradiol ، ثم تم قتل الحيوانات وتثبيت المبايض في محلول الفورمالين بنسبة ١٠٪ وتحضيرها وفحصها تحت المجهر الضوئي .

النتائج : زاد تركيز هرمون TSH في الدم بينما انخفض مستوى T4 و LH و FSH و estradiol بشكل ملحوظ في مجموعة قصور الغدة الدرقية. أظهرت معظم الجريبات النامية في مجموعة الغدة الدرقية تغيرات تنكسية .لوحظ وجود العديد من الجريبات المندثرة والجريبات الجسدية الصفراء مع زيادة ألياف الكولاجين والوذمة في السدى المصاحب لاحتقان الأوعية الدموية. أدى علاج الفئران المصابة بقصور الغدة الدرقية بالسيلينيوم لمدة شهرين إلى تحسن في المستوى الهرموني والبنية النسيجية لنسيج المبيض.

الخلاصة: يؤثر قصور الغدة الدرقية على بنية أنسجة المبيض ولكن إضافة السيلينيوم له دور محسن في وظيفة الغدة الدرقية والتغيرات النسيجية للمبيض والتي يمكن أن تعزى إلى نشاطها المضاد للأكسدة

الكلمات المفتاحية : قصور الغدة الدرقية ، السيلينيوم ، المبييض .

INTRODUCTION

Thyroid gland is the largest endocrine gland located in front of the trachea in the neck[1]. It secretes Thyroxine (T4) and Triiodothyronine (T3) which are important regulator for body development and rates of cells metabolism of all tissues in the body including the ovaries and endomerium[2]. Normal thyroid function is important for proper reproduction. Researchers in the field of infertility have noticed the close relationship between thyroid diseases and ovulatory problems in females, which may be considered an important cause of infertility in women[2].

Hypothyroidism is a common thyroid disorder, characterize by inadequate secretion of thyroid hormones affects 3-5% of population and it's more common in female[3].Abroad spectrum of reproductive disorder were observed in women with hypothyroidism ranging from menstrual irregularity to infertility[4].Inadequate thyroid hormones in female rats and mice affect the growth of ovarian follicles and ovulation[5].

Hypothyroidism reduces the capacity of the antioxidant defense system and accelerates the production of free radicles which is not only lead to lipid peroxidation and oxidative DNA damage but also interferes with physiological adaptation and intracellular signal transduction leading to altered cellular function[6].

Selenium (Se) is an essential trace element has a crucial role in many biological and physiological process[7]. In animals & human require selenium for appropriate function of a number of selenium dependent enzyme known as selenoprotein that plays an important role in reproduction, thyroid hormone metabolism, DNA synthesis and protection from oxidative damage by catalyzing the reduction of hydrogen peroxide H2O2.[8] It may protect against free radical damage and cancer[9].

For this reason, the present study aimed to evaluate the effect of hypothyroidism on reproductive efficiency of adult female rats and ameliorative effect of Se on reproductive function.

MATERIAL AND METHODS

Thirty adult female Wistar albino rats weighing about (185- 225) grams and aged about 12 weeks were purchased from animal house of veterinary college, university of Mosul. They were housed in polypropylene cages (55 x 37x15 cm) with stainless steel top grill, for period from the 1st of January till the 1st of April 2020. The animals were kept in separate well-ventilated cages, under standard conditions, with free access to the standard diet and water . All the animals had been examined carefully for general health status. Estrus synchronization had been done before the beginning of the experiment. The animals were divided into three groups(ten rats in each group) as following :Group I (control group) receive 0.5 ml distilled water daily for three months. Group II (hypothyroid group) the animals in this group received carbimazole (antithyroid drug) at a dose of 6 mg / kg b.w dissolvent in water and given as 0.5ml orally by gastric tube once daily for three months to induce hypothyroidism. Hypothyroidism was confirmed by the measurement of the serum TSH and total thyroxine (TT4) concentration. GroupIII (hypothyroid +Se) rats were given carbimazole as same as group II but we added sodium selenite (10 µg/kg BW) orally by gastric tube once daily for the last two months. Towards the end of the experiment blood samples were collected from the retro-orbital venous plexuses in a plain tube and centrifuged, serum was taken to

estimate levels of TSH , T4, ,estradiol (E2), luteinizing hormone (LH), and follicle stimulating hormone (FSH) levels by using minividus instrument . Then all animals were sacrificed by cervical decapitation, the ovaries were dissected out, fixed in 10% formalin and processed through a series of alcohol and embedded in the paraffin wax to get sections of 5µm thickness which stained with haematoxylin-eosin (H&E) to be examined under the light microscope.

Statistical Analysis

All data were reported as mean± SD (standard deviation). Statistical analysis of data was performed by Graph pad prism using one-way ANOVA. Followed by Tukey's multiple comparison test with the level of significance set at P ≤ 0.05.

RESULT

Observations of Activity and Physical Signs:

1-Control group (GI) :The animals of this group were healthy , active, quick to respond , alert throughout the experiment.

2- Hypothyroid group (GII): Rats of this group became lethargic with reduction in their activity and their food intake but no death occurred among the member of this group during the time of experiment.

3-Hypothyoid group receiving selenium(GIII): The member of this group looked more well with mild decrease in physical activity . also no death occurred among this group.

Hormonal level

Serum concentration of TSH level showed the higher significant increase , while T4 is the least significant decrease at (p≤0.05) in GII (hypothyroid) followed by GIII (hypothyroid +Se) in comparison to GI and there was a significant difference between both treated groups(GII and GIII)(table 1).

Compared to control group , serum concentration of estradiol (E2), LH and FSH showed least significant decrease in GII followed by GIII. There was a significant differences between both treated groups (table 2).

Morphological changes:

Group I (control): In this group the ovary showed normal histolgical features. The ovarian's parenchyma made up of cortex and medulla, in the cortex there was many follicles with different stages of development, the primordial follicles were found close to the surface, it appeared with oocyte surrounded by flat follicular cells. Primary follicle with normal oocyte surrounded by a single cuboidal layer of granulosa cells were found, other forms of growing follicles as secondary follicles, tertiary follicles with few number of corpora lutea and degenerated follicles were observed in the cortex (Figure 1).The ovarian's surface covered with a single layer of cuboidal cells beneath it there was a layer of connective tissues called tunica albuginea. (Figure 2). The pre-ovulatory follicle was formed of primary oocyte surrounded by distinctive zona pellucida and corona radiata and several layers of granulosa cells which were filled with follicular antrum , the entire follicle was enclosed with well-developed stromal cells which was differentiated into theca interna and externa (Figure 3).

Group II (hypothyroid group): The most histopathological evidence in the ovaries of hypothyroid female rats were increment in the number of atretic follicles and corpus luteum associated with reduction in the number of the normal ovarian follicles (Figure 4). The surface epithelium of ovary
appeared with different height of cells , the thickness of tunica albuginea was more than those of control group (Figure 5). Absent of typical appearance of many follicles were observed some follicles showed degenerative changes in which the oocyte had no nucleus and nucleolus associated with disruption and thinning of the zona pellucida and disorganization of granulosa cell layer and hypertrophy of lutein cells (Figure 6). In the stroma many congested blood vessels with increased interstitial cells and fibrosis were observed (Figure 7).

Group III (hypothyroid +Se): in this group the ovaries showed an improvement in histological features with appearance of primordial follicles (Figure 8) and primary follicle(Figure 9) and decreased number of atretic follicles but still there was dilated blood vessels (Figure 10)

DISCUSSION

Thyroid gland plays an important role in regulation of many body functions. Some researchers studied the role of thyroid hormones for a proper function of reproductive system[10]. Other author note that thyroid disorder affect fertility, pregnancy and postnatal development in human and rat[11]

In the present study, Induction of hypothyroidism by carbimazole resulted in a significant elevation (p≤ 0.05) of the serum TSH and significant suppression of T4 with least significant concentration was in GII followed by GIII compared to control groups .. These results were compatible with finding of other researcher who

said that carbimazole treatment dramatically decreased thyroid hormones level with elevation of TSH level [12]. This alteration of thyroid hormones may refer to action of carbimazole which acts principally on thyroid gland and reduces the synthesis of T4 by preventing iodination of tyrosine residues and inhibits the coupling of iodotyrosines, as a result the TSH secretion from anterior pituitary increased by positive feedback mechanism.[13]

Moreover, the serum concentration of female sex hormone , Estradiol (E2) and gonadotropin (FSH, LH) level were significantly reduced at ($p \leq 0.05$) in GII (hypothyroid) and GIII (hypothyroid + Se) compared to control group with least significant decrease at GII. The same results were observed by another researcher.[14] In contrast, other studies showed that FSH and LH did not differ in hypothyroid rats from the control one.[15] This reduction may be result from decreased production of estrogen- binding –globulin from the liver in hypothyroidism that lead to elevation of serum free estradiol which inhibit the secretion of gonadotropin from pituitary by negative feed-back mechanism[16]. In addition, the reduced number of healthy follicles in hypothyroidism induced farther reduction of estrogen level in this animals.[14]

In this study, the ovaries of hypothyroid rats showed overcrowded of surface epithelium associated with increased the amount of connective tissues in tunica albuginea and in ovarian stroma; this finding is concomitant with another worker[17] who attributed this increment in connective tissues amount to the effect of hypothyroidism on lysosomal enzyme which responsible for collagen degradation. On the other hand, the present study showed that induction of hypothyroidism in female rats resulted in degenerative changes and atresia of many ovarian follicles, the same finding were reported by another author[18]. while other researcher observed the development of follicular cyst associated with hypergonadism in hypothyroid animals.[19] Another study founded that ovaries of hypothyroid rats showed least number of antral follicles with more secondary follicles.[20] However, the effect of hypothyroidism on ovary differ according to the species, age, duration and method of induction of hypothyroidism in animals .[19]

Furthermore, in this study had been observed that granulosa cells in corpora lutea were hypertrophied; this condition may be attributed to the low thyroxin level which responsible for maintenance of differentiation of granulosa lutein cells from follicular granulosa cells.[21]

Co-administration of hypothyroid rats with Se showed some improvement in the thyroid function which demonstrated by increasing the level of thyroid hormones in blood. Hawazen and Lamfon[22] observed that the administration of Se to chlorpyrifos (CPF) - exposed rats could protect them from CPF induced thyroid dysfunction. Some authors stated that selenium is essential for thyroid hormones synthesis through its role in selenoenzyme's actions.[8]

Moreover adding of selenium in this study showed amelioration in the ovarian function of hypothyroid rats; such improvement includes elevation of female sex hormone(E2), LH and FSH towards the control level in addition to that enhances the histological modification produced in ovaries via hypothyroidism which demonstrated by improvement of folliculogenesis and decreased the number of atretic follicles, these finding were in agreement with other worker who stated that Se supplementation was able to protect ovaries and uterus from arsenic effect in rats[23]. Other study suggested that the antioxidant properties of Se and its role on DNA repair pathway may be responsible for chemoprotection effect of Se[24]. Some authors reported that selenium could prevent hepatotoxicity induced by carbimazole in rats through its action on lipid peroxidation and elevation of antioxidant level.[25]

CONCLUSION

The current study showed that hypothyroidism led to structural changes in the ovaries of rats by development of atretic follicles with decreased of growing follicles and hypertrophy of interstitial cells and reduction in the level gonadal hormones. These changes were improved when selenium was given.

Table 1: serum concentration of thyroid hormones in different groups of female rat Data were expressed as Mean±SD.

Hormone	Group of animals	Data
TSH (µlu/ml)	GI (Control) GII(Hypothyroid) GIII(Hypothyroid +Se)	3.09±0.23A 6.77±0.09B 4.62±0.21C
T4 (nmol/L)	GI (Control) GII(Hypothyroid) GIII(Hypothyroid +Se)	75.73±3.22 a 52.04±0.82 b 67.71±1.62c

-Different letters mean there is a significant difference at p≤ 0.05
-Similar letters mean no significant difference at p> 0.05

Table 2: serum concentration of gonadotropin (FSH ,LH) and estradiol E2 in different groups of female rats .

Hormone	animal's Group	Data
FSH(mlu/ml)	GI (Control) GII (Hypothyroid) GIII (Hypothyroid +Se)	1.58±0.24 A 0.97±0.23 B 1.08±0.11 C
LH(mlu/ml)	GI (Control) GII (Hypothyroid) GIII (Hypothyroid +Se)	1.42±0.18 a 0.52±0.14 b 0.84±0.48 c
E2(Pg/ml)	GI (Control) GII(Hypothyroid) GIII(Hypothyroid +Se)	51.21±4.04 D 27.49±6.02 E 42.40±5.22 F

-Different letters mean there is a significant difference at p≤ 0.05
-Similar letters mean no significant difference at p> 0.05

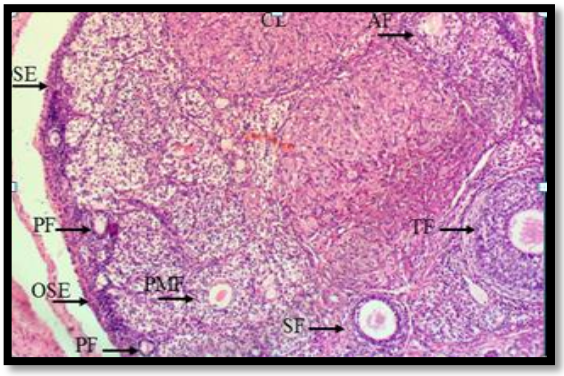

Figure 1: Microphotograph of rat' ovary Control group, showing different growing stages of follicles included primordial follicles (PF), primary multilayer follicles (PMF) , secondary follicle (SF), tertiary follicle (TF), this series of follicles start from ovarian surface epithelium (OSE), with distinct corpus luteum (CL) and atretic follicle (AF). H&E. 100X.

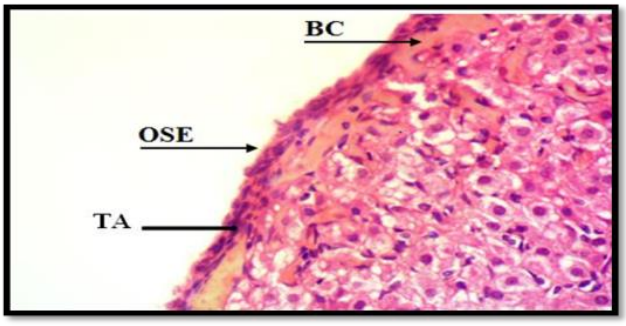

Figure 2:. Microphotograph of rat' ovary control group, showing The ovarian's surface covered with a single layer of cuboidal cells beneath it there was a layer of connective tissues called tunica albuginea . H&E, 400x

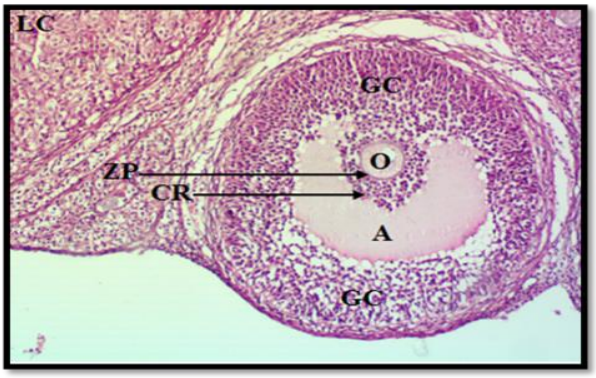

Figure 3: Microphotograph of rat' ovary control group, showing graafian (pre-ovulary) follicle showing oocyst (O), zona pellucida (ZP), corona radiata (CR), antrum (A), surrounded by granulosa cells (GC) in addition to lutein cells (LC). H&E, 400x

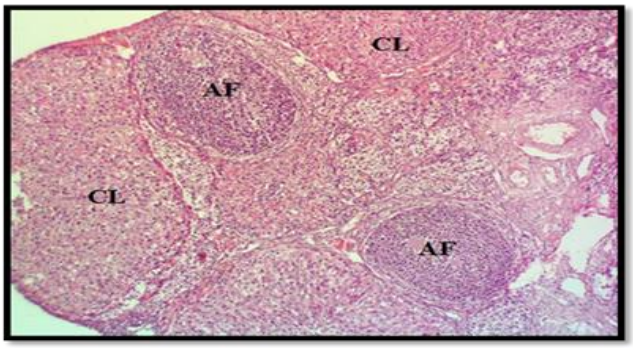

Figure 4: Microphotograph of rat' ovary hypothyroid group showing corpus luteum (CL), with many atretic follicles (AF). H&E, 100x

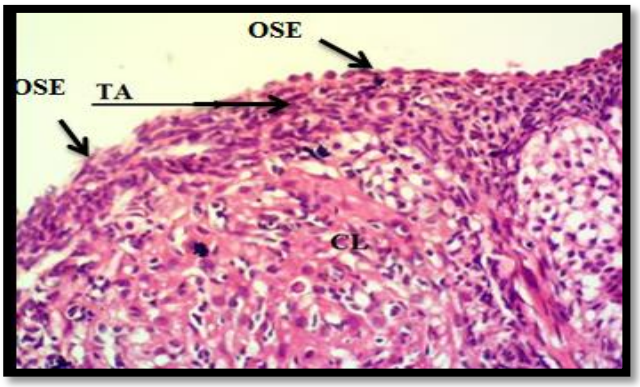

Figure 5: Microphotograph of rat' ovary Hypothyroid group, showing increase in thickness of tunica albuginea(TA). The surface epithelium of ovary appeared with different height of cells H&E, 400x.

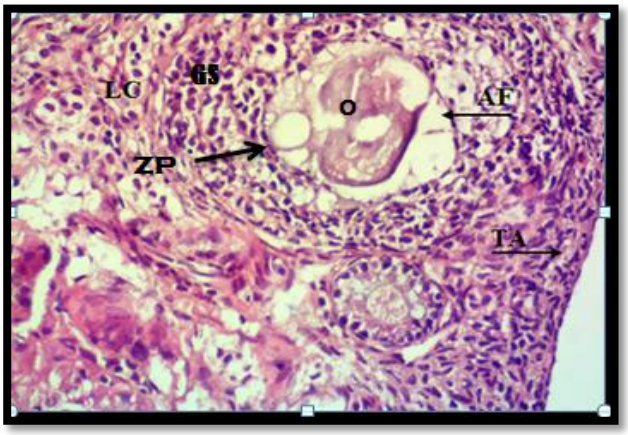

Figure 6 : Microphotograph of rat' ovary Hypothyroid group, showing atretic follicles (AF) in which the oocyte (o) had no nucleus and disruption of the zona pellucida (ZP) and granulosa cell layer (GS) , hypertrophy of lutein cells (LC). H&E, 400x

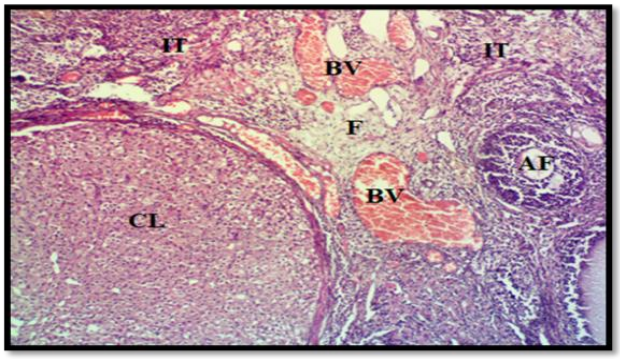

Figure 7: Microphotograph of rat' ovary Hypothyroid group showing corpus luteum (CL) congested blood vessels (BV), increase of interstitial tissue (CI), fibrosis (F), atretic follicles (AT). H&E.400x.

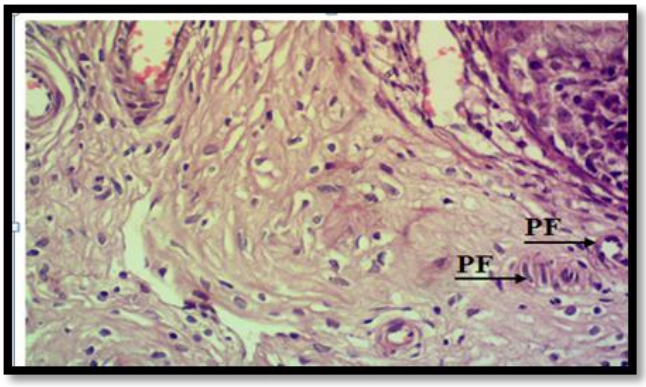

Figure 8: Microphotograph of rat' ovary Hypothyroid with Selenium group showing few primordial follicles (PF). H&E. 400x

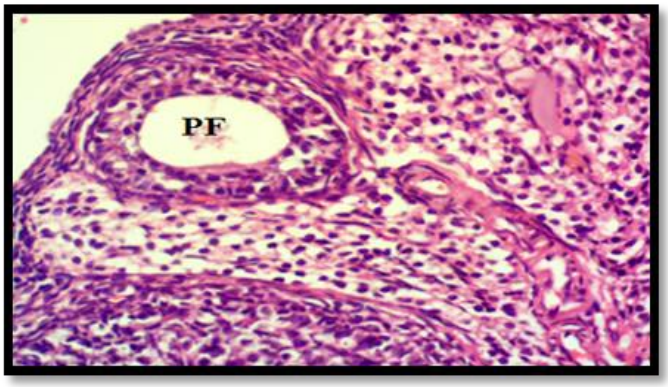

Figure 9: Microphotograph of rat's ovary Hypothyroid with Selenium group showing primary multilayer follicles (PF). H&E.400x

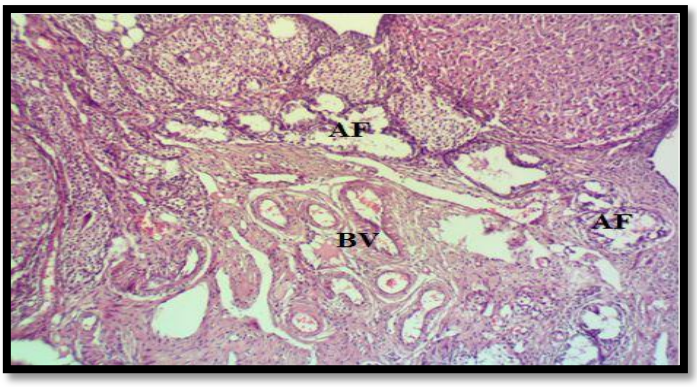

Figure 10:Microphotograph of rats ovary Hypothyroid with Selenium group, showing many blood vessels (BV) and atretic follicles (AF). H&E, 100x

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