Morphological and Biochemical Changes in the Rat Ovaries Following Electromagnetic Field Exposure

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Research

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Abstract

Purpose

This study aims to investigate the effect of electromagnetic field (EMF) exposure which is environmental toxic agent on rat ovary.

Methods

A total of 30 female Wistar albino rats are randomly divided into three groups (n=10) as Control, Sham, and EMF groups. The rats are not exposed any processes in Control group. The rats are kept in special cage for the same duration as in the EMF group without any EMF exposure in Sham group while the rats are exposed to 900 MHz EMF with a special mechanism in EMF group. At the end of day 28, the ovarian tissues are collected. Stereological and biochemical examinations are performed.

Results

Concerning the GSI values, there are statistical differences between the Control and EMF (p=0.001), and Sham and EMF (p=0.001) groups, respectively. There are also statistical differences between Control and EMF groups in terms of volumetric parameters including medullary, and follicular antral volumes. Regarding the biochemical parameters, there are statistically differences between the Control and EMF (p=0.000), and Sham and EMF (p=0.000) groups, respectively.

Conclusion

It may be concluded that exposure to a 900-MHz EMF leads to changes in ovarian follicle volumes which may affect the ovarian reservoir.

1. Introduction

Exposure to electromagnetic field (EMF) causes negative effects on human health such as fatigue, stress, pain and ache in muscles. The World Health Organization (WHO) has called these effects “idiopathic environmental intolerance attributed to electromagnetic fields” (IEI-EMF) [1]. In addition to these effects, EMF may be reason of some illness such as Alzheimer’s disease (AD), Amyotrophic Lateral Sclerosis (ALS), childhood asthma and cancer [2]. Widely used cell phones, Wi-Fi, and various kinds of connected devices generate high frequency electromagnetic fields (HF-EMF) and exposure to EMF becomes unavoidable [3].

EMF has negative consequences on male productivity. It can cause reduced sperm motility, elevated abnormal sperm percentage, loss of spermatocytes and spermatids, slightly elevated testes caspase-3 activity [4]. Also, it can cause morphological changes in sperm such as significant reduction in sperm head area and acrosome percentage of the head area, significant decrease in sperm binding to the hemizona. Sperm fertilization potential is affected negatively because of these changes [5]. The ovaries

may be further affected by environmental stress factors such as radiation, which may result in germ cell apoptosis. EMF exposure caused by infertility, separation of thecal layer of primary follicles, irregular thickness of the zona layer, and reduction number of ovarian follicles in rats [6,7]. When the effects of EMF on ovarian tissue are examined, the zona granulosa and thecal layers become thin, granulosa cells shrink, mitotic activity and leukocyte infiltration reduce [8]. Also, ovarian follicles are affected negatively such as isolation with dilated vessels showing infiltration in ovarian stroma is seen [7]. In another research, especially in prenatal period, exposure to 2450 MHz EMF cause postnatal growth restriction and delay in puberty in female rat are found [9]. In this experimental research, we aimed to investigate the effects of EMF which is one of the very important environmental toxic agents on ovarian tissues using unbiased stereological methods and biochemical approaches.

2. Methods

This experimental study was performed following Animal Experiments Local Ethics Committee of the Ondokuz Mayis University approval with the number of 2018-07. A total of 30 female rats of Wistar Albino (12 weeks old) were randomly divided into three groups (10 animals each) as Control, Sham, and EMF. During the experiment, subjects were kept under a constant dark light cycle of 12 hours; drinking water and standard rat feed ad libitum were given. The weight of all animals was measured, and data were recorded before starting the experiment. Groups and transactions are as follows:

Control group: This group will not be processed.

Sham group: This group was subjected to stress conditions in a special cage for the same duration as the electromagnetic field group. However, this group was never exposed to electromagnetic field.

EMF group: This group was exposed to 900 MHz electromagnetic field for 28 days (60 min / day) [10] with a special mechanism [11].

On the 28th day of the experiment, vaginal smear method was used to check the estrus cycle of the animals in all groups and the data were recorded. At the end of day 28, the weight of all animals was measured, and the data were compared with the initial results. After the experimental procedures, the subjects were perfused transcardially under ketamine (0.1) and xylazine (0.5) anesthesia and ovarian tissues were removed. Stereological and biochemical analyzes were performed on the obtained tissues samples.

Applied methods:

2.1. Gonadal Somatic Index

Gonadal somatic index (GSI) is an indicator for the cyclic status of the animals. It basically manifests maturation of Graafian follicles and decreases in the acyclic animals compared with cyclic counterparts. GSI calculated with the following formula [12];

GSI= (Gonadal weight/body weight) x100

2.2. Stereological Method

Ovarian tissues from rats in all groups were fixed in 10% formalin for 24-48 hours before embedded in paraffin for light microscopic examination. After deparaffinization and dehydration procedures, 20 µm thick sections (tissues were subjected to sectioning based on our previous studies for stereological methods) and stained with hematoxylin-eosin for histological examination and stereological analysis.

The sections prepared for examination were examined under Olympus BH 40 camera attachment light microscope and stereological and histopathological evaluations were made by taking photographs of all groups. Ovarian volume, cortex / medulla ratio, follicle volumes were calculated by using stereological method which is called Cavalieri method.

2.2.1. Cavalieri method

Cavalieri method was used to calculate ovarian volume, cortex/medulla ratio and follicle volume. This method is a branch of stereology which is one of the unbiased approach [13] and mainly is based on the calculation of the volume of an equally spaced and parallel sliced structure in sections taken by systematic random sampling. By calculating the surface area of the related structures on the surfaces facing the same direction, volumetric data are obtained by multiplying the total surface area and average cross-sectional thickness. For the area calculation, a point counting grid which consists of systematic (+) signs representing points separated at equal intervals from each other is used. The area occupied by a point is known in the point counting grid and is called the area associated with the point and shown as [a (p)]. In this context, the points falling on the area of interest were counted and the total number (Σp) was obtained. The obtained numbers were multiplied by a (p) to achieve surface area (A) measurements for ovarian volume, cortex/medulla ratio and follicle volume [14].

The following formula was used during the calculations;

$A = \Sigma p \, . \, [a(p)]$

The volumes of the respective parameters were calculated using histological images taken on x10 magnification.

2.3. Biochemical procedures

Regarding the biochemical parameters, ovarian tissues were harvested (200 mg) from each animal and homogenized inappropriate buffer solution (4800 ml). Catalase (CAT), superoxide dismutase (SOD), lipid peroxidation (LPO) and glutathione (GSH) levels were assessed in homogenates. CAT test was performed using H_2O_2 at 240nm whereas SOD measurement was performed using slightly modified version of Sun et al. LPO level was examined using thiobarbituric acid while GSH activities was measured according to slightly modified version of Sedlak and Lindsay.

2.4. Statistical Analysis

The data analyzed using SPSS 21.0 for Mac (IBM Corporation) software and the comparisons between the groups are performed by One Way ANOVA (Tukey-Post-Hoc) test. $p<0.05$ value is considered as statistically significant.

3. Results

3.1. Gonadal Somatic Index

Concerning the GSI values, there are statistical differences between the Control and EMF ($p=0.001$), and Sham and EMF ($p=0.001$) groups, respectively whereas no statistical difference is found between the Control and Sham groups ($p>0.05$) (Table 1).

3.2 Stereological Results

Regarding the stereological evaluation; statistical differences were observed between the Control and EMF ($p=0.001$), Sham and EMF ($p=0.001$) groups respectively in terms of ovarian volumes. There were statistical differences between the Control and EMF ($p=0.001$), Sham and EMF ($p=0.001$) groups respectively in terms of medullary volumes. Also, there were statistical differences between the Control and EMF ($p=0.001$), Sham and EMF ($p=0.001$) groups respectively in terms of primary follicle volumes. Similarly, statistical differences were observed between the Control and EMF ($p=0.005$), Sham and EMF ($p=0.005$) groups respectively in terms of secondary follicle volumes. In addition, there were statistical differences between the Control and EMF ($p=0.001$), Sham and EMF ($p=0.001$) groups respectively in terms of both secondary follicle antrum and Graafian follicle antrum volumes. No statistical differences were found between the Control and Sham groups ($p>0.05$) in points of above parameters, stereologically. Finally, there were also no statistical differences between the Control and EMF ($p>0.05$), Sham and EMF ($p>0.05$), Control and Sham ($p>0.05$) groups, respectively in terms of Graafian follicle volumes (Table 2).

3.3. Biochemical Analyses

Biochemical analyses show that CAT levels significantly decreased between the Control and EMF ($p=0.001$), and Sham and EMF ($p=0.000$) groups, respectively. Regarding the SOD levels, there are statistically significant differences between the Control and EMF ($p=0.000$), and Sham and EMF ($p=0.000$) groups, respectively. Similarly, there are significant statistical differences are found between the Control and EMF ($p=0.000$), and Sham and EMF ($p=0.000$) groups, respectively in points of LPO levels. There are no statistical differences between the Control and Sham groups in terms of the above parameters ($p>0.05$). Finally, no statistical differences are found between the all groups in point of GSH levels ($p=0.05$) (Table 3).

The obtained results are given in Figure 1 visually (Figure 1).

4. Discussion

Previous studies regarding the effect of magnetic fields on serum gonadotropin levels revealed conflicting results [15,16], this conflicting results may originate from cyclic changes of these hormones, for this reason in our study, we aimed to assess gonadal stromal index to figure out this effect, our data showed that exposure to EMF resulted in significantly lower GSI. This result was consistent with the previous study by Roshangaret al. [17] which showed exposure to EMF during the developmental period which interfered with both oocyte differentiation, folliculogenesis and reduced fertility, this result was claimed to be secondary to decreasing ovarian reservoir. Technological devices such as electrical appliances, communication devices, radio and television transmitters and sub-stations and mobile phone base stations emit electromagnetic waves and generate electromagnetic field. It causes negative health consequences and damage various tissues in living organism [18].Various studies have been done about the damage to tissues in various systems such as nervous system and endocrine system [19,20,21]. The effect of EMF exposure on the reproductive system was also investigated. Since there is no standard methodology for the evaluation of male and female reproductive systems, some contradictory results have been obtained in these studies. Several studies have reported that EMF reduces fertility potential [21], sperm concentration, motility, and seminiferous tubule diameter [22,23] and increases abnormal sperm morphology [8]. However, there are also experimental studies reporting that EMF does not affect sperm count in testes or epididymis and does not alter sperm motility or morphology. It has been reported in previous animal experiments that EMF increases oocyte DNA damage, apoptosis and oxidative stress in the endometrium and ovary and reduces the number of follicles in the female reproductive system [24,25]. Other studies have emphasized that EMF does not have a negative effect on the male and female reproductive system [6,26-28]. In our research, we found that there are significant statistical changes in volumes of medulla, primary follicle, secondary follicle, secondary antrum, graafian antrum.

In their work with RE-DNA, which can play an important role in cell functionality that can be replicated and critically affects gene and genome regulation, Del Re et al. (2019) found that EMF can affect the transcription of types of RE-DNA (L1, HERV-H, SATA) [29]. von Niederhäusern et al. (2019) found that EMF might cause to an impairment of mitochondrial function [30]. In their work with cancer cell, Storch et al. (2016) found that EMF causes to increase in reactive oxygen species (ROS) [31]. It is known that ROS and oxidative stress can cause DNA damage, general and specific gene expressions and cell apoptosis [18]. Because of these changes, volume changes may have been in the research.

Few studies have examined histopathologically the effects of EMF on the female reproductive system. In their studies on the effect of EMF to ovarian follicle reservoir in prenatal exposure, Türedi et al. (2016) found that EMF can cause increased apoptosis in the rat ovarium, impaired the follicular development process and as a result of these conditions, a decrease in follicular reservoirs in the ovarium can be seen [23]. In another study, Okatan et al. (2018) found that EMF cause a decreases in mitotic activity in follicle cells, follicle cell dimensions, thickness in the zona granulosa layer, thickness in the thecal layer and secondary follicle numbers whereas an increase in leukocyte infiltration, hyperchromasia in granulosa cells, and biochemical parameters [8]. Our findings suggest that, there is an increase in the volume of

secondary follicles. In the literature review on role of mitochondria in the oxidative stress induced by EMF on reproductive systems, Santini et al. (2018) found that EMF can cause morphological, histological and biochemical changes. These include delayed puberty, accelerated loss of primordial follicles in the adult, no differences in ovarian follicle count, changes in the volume and the primordial follicle content of newborn ovaries, reduction of primordial ovarian follicles. Our results are consistent with these results. There are also other studies in the literature that are manifesting increased apoptosis in ovaries, reduced primordial and tertiary follicles, increased atretic follicle, vasocongestion, stromal fibrosis, lost oocytes in primary follicles, extended degeneration, vacuolization, and loss of connection with cumulus cells in granulosa cells, and increased Total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) [26]. Increased apoptosis, TAS, TOS and OSI may be the cause of these volume changes.

Bakacak et al. (2015) reported a study and their results have shown that there is a significant decrease in the number of ovarian follicles in rats exposed to EMF(6). Alekperov et al. (2019) studied the effects of extremely low-frequency EMF on the ovaries in rats. They found exposure to EMF in the selected mode caused no significant defects in the structure and function of rat ovaries [32]. Okatan et al. (2018) demonstrated a study using histological and biochemical methods to evaluate ovarian changes following EMF exposure in middle and late adolescence [8]. In this context, their results showed that exposure to 900-MHz EMF in middle and late adolescence may cause changes in the morphology and biochemical content of the rat ovarium. Roshangar et al. (2014) evaluated the effect of EMF on oocyte differentiation and follicular development [18]. It is concluded that exposure to EMF during the developmental period could affect both oocyte differentiation and folliculogenesis and may result in reduced fertility, by decreasing ovarian reservoir.

Based on our study results, it may be concluded that EMF is one of the most important environmental toxic facts and exposure to 900-MHz EMF may negatively affect the ovarian tissues.

5. Conclusions

The vast majority of the electromagnetic field studies in the literature indicated that it has harmful effects of on human health. Especially, electromagnetic field emitted from cell phones, are thought to have many harmful effects including. However, despite molecular and epidemiologic studies on both experimental animals and humans, the effects of electromagnetic field on ovarian tissues which related to female reproductive system is still a topic for discussion. Based on our study results, it may be concluded that EMF is one of the most important environmental toxic facts and exposure to 900-MHz EMF may negatively affect the ovarian tissues.

6. Declarations

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Conflict of Interest: None Declared

Availability of Data and Material: All data generated or analysed during this study are included in this article (and its additional files).

Ethical Considerations & Disclosure(s): This experimental study is performed following the Animal Experiments Local Ethics Committee of the Ondokuz Mayis University approval with the number of 2018-07.

Running Head Title: Effect of EMF Exposure on Rat Ovaries

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Tables

Table 1: GSI values of the groups (Mean ± SEM).(Same capital letters manifest there is no statistical difference between two groups in the same line, while different capital letters do).

	GROUPS		
	Control	Sham	EMF
GSI	0.07 ± 0.005^{A}	0.12 ± 0.009^{A}	0.04 ± 0.005^{B}

Table 2. Ovarian follicle volume at various stages in the control and EMF groups The data represent mean ± SEM.

Ovarian follicle types	Control Group	EMF Group
Ovary	170662236±20370057.9	302742667±95286846.1
Medulla	32839051.4±1815796.99	53510729.6[a]±3707704.44
Primary	626794.800±44917.4636	931688.200[b]±44051.2557
Secondary	1722880.00±72962.3697	5231627.20[c]±909539.911
Secondary_Antrum	563069.400±16542.1636	1656707.20[d]±211647.756
Grafian	6516187.80±638388.704	6345220.60±476901.371
Grafian_Antrum	8437689.00±1261301.91	2353182.60[e]±218511.910

a:There is an advanced significant relation between Control and EMF groups in medulla volume (p=.001)

b: There is an advanced significant relation between Control and EMF groups in primary volume (p=.001)

c: There is a significant relation between Control and EMF groups in secondary volume (p=.005)

d: There is an advanced significant relation between Control and EMF groups in Secondary Antrum volume (p=.001)

e: There is an advanced significant relation between Control and EMF groups in Graafian Antrum volume (p=.001)

Table 3: Biochemical evaluations of the samples (Mean ± SEM). Relevant parameters indicate that CAT and SOD levels decreased whereas LPO level increased with EMF exposure.

	GROUPS		
	Control	**Sham**	**EMF**
CAT (µmol/min/mg)	278.16±21.24 [A]	302.24±18.46 [A]	108.20±12.02 [B]
SOD (mmol/min/mg)	9.94±1.03 [C]	11.83±2.02 [C]	5.01±0.86 [D]
LPO (nmol MDA/mg)	28.26±2.03 [E]	28.70±1 [E]	62.14±0.98 [F]
GSH (nmol/mg)	3.90±0.20 [G]	3.90±0.07 [G]	4.01±0.34 [G]

Furthermore, there is no statistical difference between the groups in point of GSH levels. (Same capital letters manifest there is no statistical difference between two groups in the same line, while different

capital letters do).

Figures

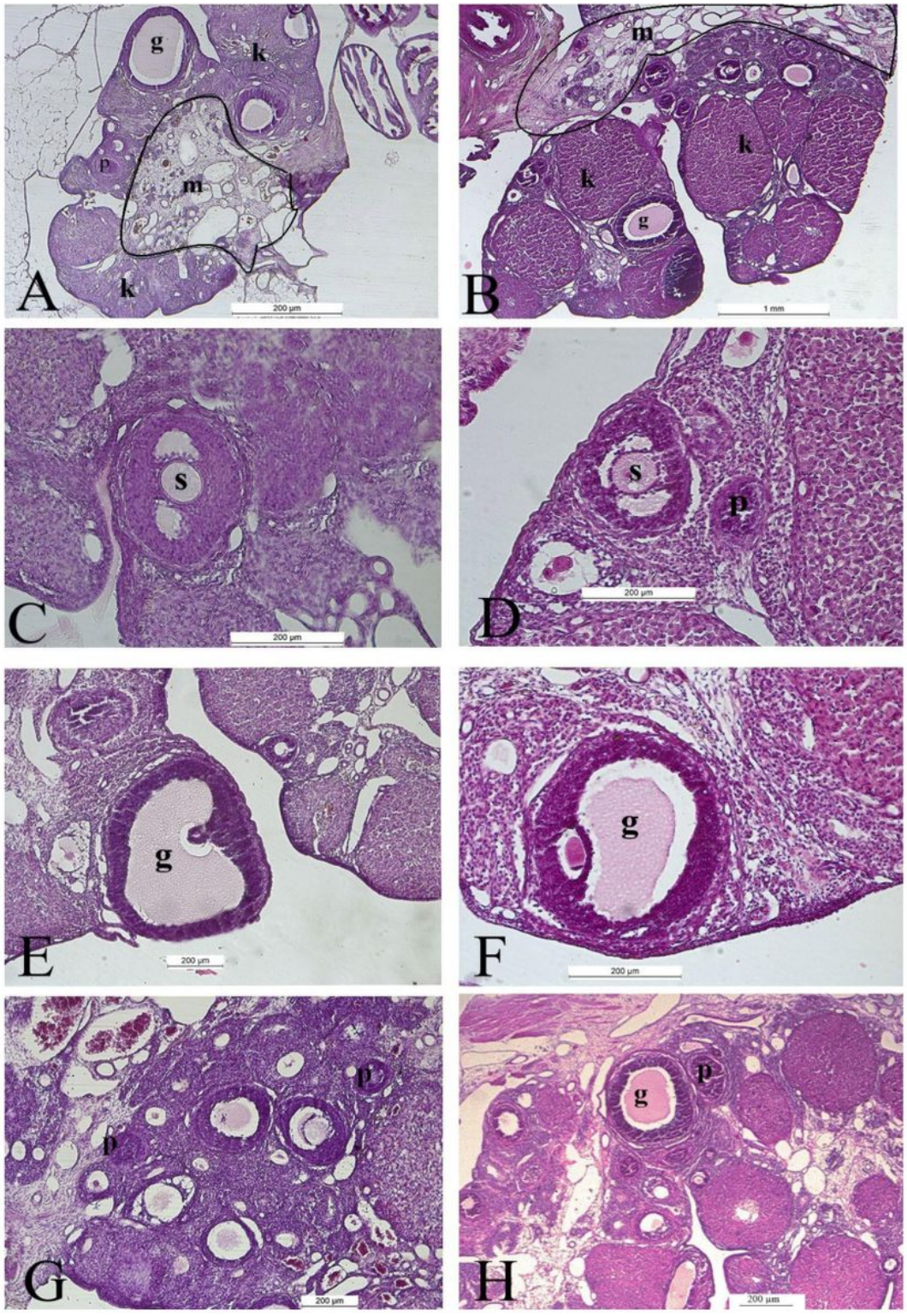
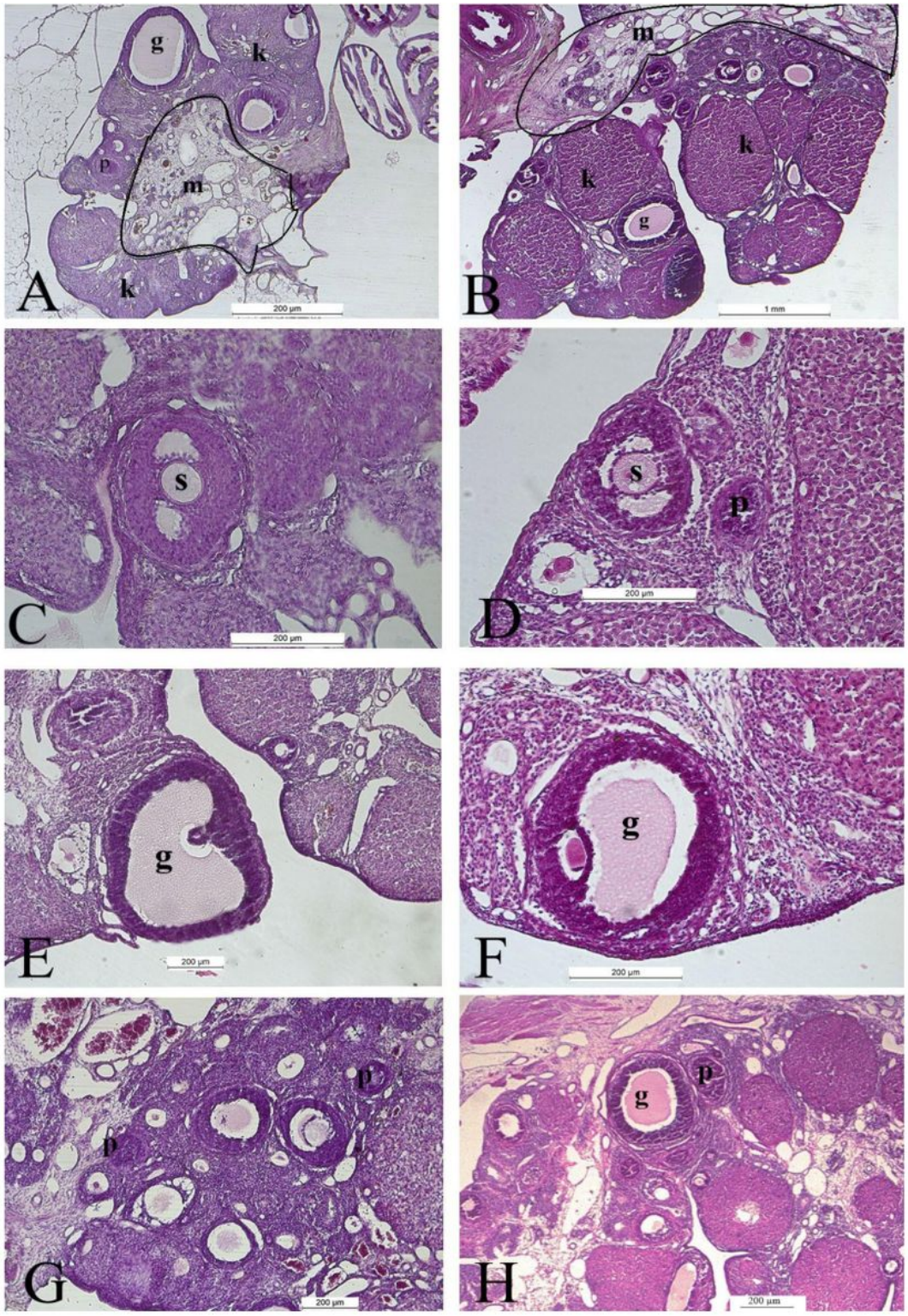


Figure 1

Panoramic view of the ovarian sections of Control (A) and EMF groups (B). Secondary follicles of the Control (C) and EMF (D) groups are seen. Similarly, Graafian follicles of the Control (E) and EMF (F) groups are also seen. Finally, Primary follicles of the Control (G) and EMF (H) groups are seen, respectively. (k: Cortex, m: Medulla, g: Graafian Follicle, s: Secondary Follicle, p: Primary Follicle. Hematoxylin-eosin staining)



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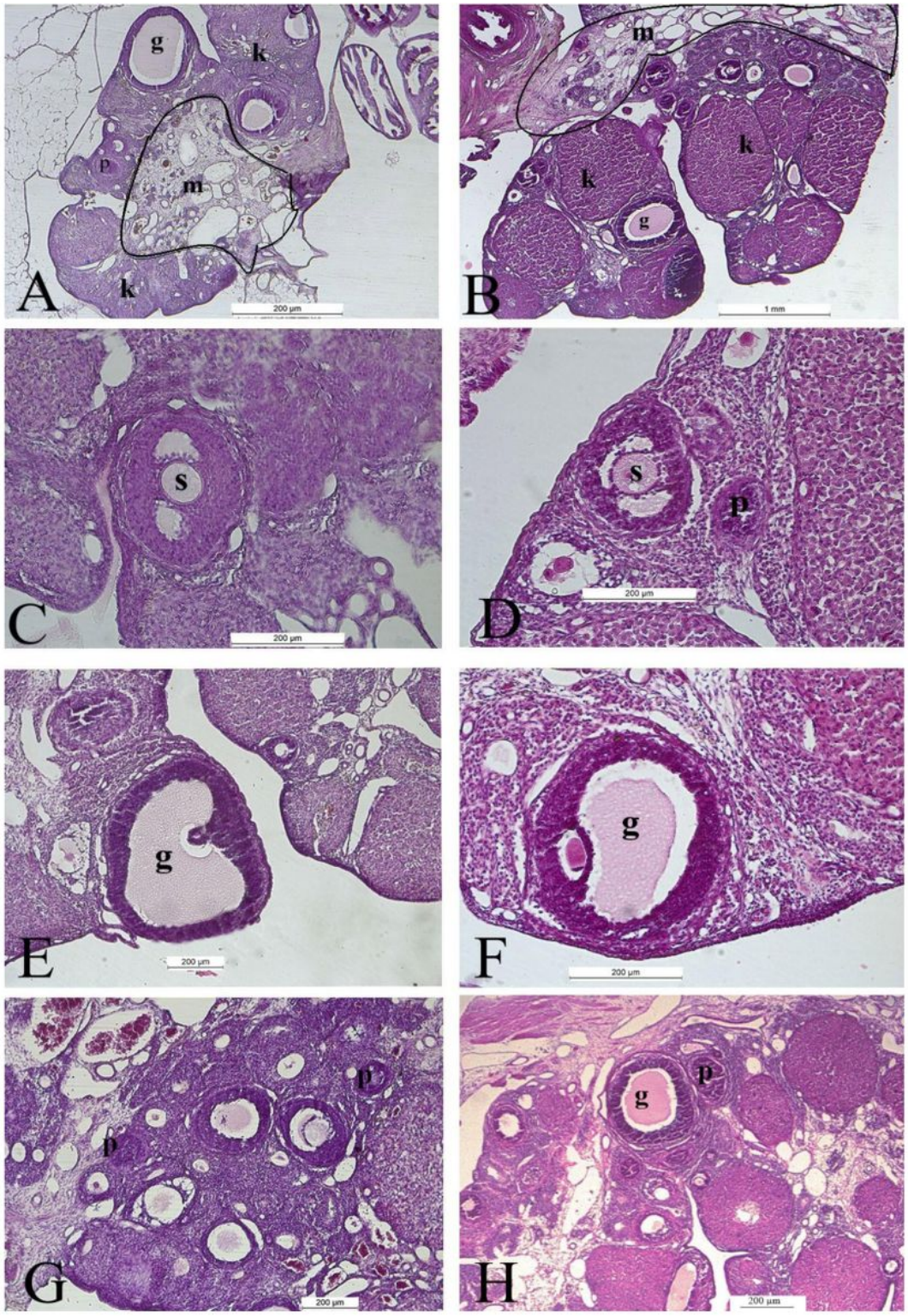

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