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ORIGINAL ARTICLE



Continuous 900-megahertz electromagnetic field applied in middle and late-adolescence causes qualitative and quantitative changes in the ovarian morphology, tissue and blood biochemistry of the rat

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ABSTRACT

Purpose: The purpose of this study was to use histological and biochemical methods in order to evaluate changes taking place in the ovarian of rats exposed to the effect of a 900-megahertz (MHz) electromagnetic field (EMF) in middle and late adolescence.

Materials and methods: Twenty-four 34-d-old female Sprague-Dawley rats were assigned equally to control, sham and EMF groups. EMF group rats were exposed to the effect of a 900-MHz EMF for 1 h a day, at the same time every day between postnatal days 35 and 59, while inside an EMF cage. Sham group rats were kept inside the EMF cage for the same time between postnatal days 35 and 59 without being exposed to any EMF effect. At the end of the study, rats' ovaries were removed and blood specimens were taken. Right ovary tissues were subjected to routine histological procedures and stained with hematoxylin and eosin, periodic acid shift and Masson's trichrome. Follicles were counted in ovarian sections stained with hematoxylin and eosin. The TUNEL method was used to evaluate apoptosis. Left ovarian tissue and blood specimens were investigated biochemically.

Results: Histopathological examination of EMF group ovarian tissue revealed thinning in the zona granulosa and theca layers, shrinking in granulosa cells, reduced mitotic activity and leukocyte infiltration in the follicles and stroma. Secondary follicle numbers in the EMF group were significantly lower than in the other groups. In terms of biochemistry, EMF and sham group superoxide dismutase, catalase and anti-Müllerian hormone levels and EMF group 3-nitrotyrosine values increased significantly compared to the control group. EMF and sham group serum catalase and 8-hydroxy-deoxyguanosine values increased significantly compared to the control group, and EMF group total oxidant status and oxidative stress index values were significantly higher compared to the sham and control groups.

Conclusions: A total of 900-MHz EMF applied in middle and late adolescence may cause changes in the morphology and biochemistry of the rat ovary.

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Introduction

The fact that numerous devices and systems that simplify daily life as technology progresses also establish an electromagnetic field (EMF) effect, that their numbers are increasing every day and that they are widely used for other than their originally intended purposes constitute serious threats in terms of human health (Davis et al. 2013). For example laptop computers, tablets and cell phones are used for many different purposes, such as entertainment and playing games, in addition to their intended purposes of accessing and transmitting information. This is more prevalent before adulthood, in children and adolescence. Exposure to the effect of EMF in childhood and adolescence can lead to various health problems, due to both the greater use of cell phones in these periods and to the fact that the tissues, organs and systems of children and adolescents have not yet reached

adult levels. Organs and systems that have not yet fully acquired their morphological characteristics and completed their development can alter their normal development under the effect of even a small environmental agent (Gultekin and Moeller 2013). The fact that there are approximately 7 billion cell phone subscribers in the world today (ITU 2015) and that a significant part of these are individuals in stages of life prior to adulthood (Lenhart et al. 2010) illustrates the importance of investigating the effect of EMF emitted by cell phones (Davis et al. 2013).

Scientists have suggested that long-term cell phone use can cause risk of glioma (Hardell et al. 2007) headache, clotting in the blood, tinnitus and hearing problems (RNCNIRP 2011; Davis et al. 2013; Morgan et al. 2015), and can affect reproductive functions in women (Hancı et al. 2013) and men (Türedi et al. 2016). However, although several studies have investigated the severity of these effects and the impact of

that severity on human health, the subject is still a matter of debate. Since many research reports contain insufficient information concerning the deleterious effects of cell phone use on children and adults, the need to appropriate precautions to be taken without delay and for the necessary warnings to be issued has also arisen (RNCNIRP 2011). It is also of considerable importance for the effects of cell phones on living tissue, and particularly systems that perform vital functions, such as the nervous and reproductive systems, and on the organs and tissues, such as the ovarium, liver, heart, brain, comprising those systems to be investigated using scientific methods.

The purpose of this study was therefore to investigate potential changes in the ovarian of rats exposed to the effect of a continuous 900-megahertz (MHz) EMF in middle and late adolescence using histological and biochemical methods. To the best of our knowledge, this is the first time that our study findings have appeared in the scientific literature. Cell phones worldwide commonly operate at a frequency of 900–1800 MHz. A frequency of 900 MHz is most common in Europe. Therefore, rats in middle and late adolescence were exposed to the effect of a 900-MHz EMF in this study.

Materials and methods

Study plan, ethical procedures, animals and groups

The experimental and surgical procedures described in this study were conducted in line with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. The study protocol was considered and approved by the Karadeniz Technical University (KTU) Animal Care and Ethics Committee (Trabzon, Turkey) (Study protocol license no. 2015/9; Confirmation Date. 03.03.2015). Twenty-four female Sprague-Dawley rats, 28.3–49.3 g in weight and 34 d in age, were supplied by the KTU Experimental Animals Surgical Research and Application Center (Trabzon, Turkey). These were kept in standard plastic cages under standard laboratory conditions at $22 \pm 2^\circ\text{C}$, $50\% \pm 5$ humidity and a 12 h light/12 h dark cycle. Commercial rat chow (Bayramoğlu Feed and Flour Industry Trading Corp., Erzurum, Turkey) and tap water were provided *ad libitum* for all animals apart from at the time of EMF and sham EMF applications. All the experimental and surgical procedures described were carried out in the KTU, Experiment Animals Surgical Research and Application Center (Trabzon, Turkey).

Rats were randomly assigned into one of three groups (eight in each group) – control, sham and EMF. No procedure was performed on the control group rats. Sham group rats were placed inside an EMF cage inside the EMF-application system for 1 h a day from postnatal day 35–59 (the 25 d from days 35 to 59 inclusive represent middle and late adolescence in rats) (Tirelli et al. 2003) every day at 09:00–10:00 am. However, they were not exposed to the effect of EMF during this time. EMF group rats were also placed inside an EMF cage inside the EMF-application system for 1 h a day from postnatal day 35–59 (the 25 d from days 35 to 59 inclusive represent middle and late adolescence in rats) (Tirelli et al. 2003) every day at 10:00–11:00 am, but were exposed

to the effect of continuous 900-MHz EMF for 1 h. Temperature and humidity in the EMF cage were measured every day at the beginning, during and at the end of sham and EMF applications, with rats inside the cage, using a digital thermometer (YCOM KMN-303, YuYao Shuanghe Electron Instrument Co. Ltd. Zhejiang, China). During exposure to 900-MHz EMF, rats were allowed to move freely within the application cage in order to model genuine cell phone users. This was in order to eliminate potential stress due to immobilization. Rats were also exposed to 1 h continuous EMF in order to model long-term cell phone users.

The EMF-application room, EMF-application system and the establishment of sham and EMF effects

Sham application in the sham group and EMF application in the EMF group were carried out in a separate room defined as the EMF-application room. Sham and EMF group rats were transferred to the EMF-application room during sham and EMF applications, respectively, and were later returned to the room where they were housed together with the control group rats. Sham application was performed first. Following the sham exposure of the sham group rats, EMF application was performed on the EMF group rats. Sham application was performed simultaneously on all the sham group rats and EMF application was performed simultaneously on all EMF group rats.

The EMF-application system in the EMF-application room was designed for sham and EMF applications. It was identical to those used in our previous studies concerning EMF and is described in detail elsewhere (Hancı et al. 2013; Odacı and Özyılmaz 2015; Türedi et al. 2016). To summarize, The system consisted of an oscillator (1218-BV, Lockable Oscillator, 900–2000 MHz, General Radio Company, Concord, MA, Serial No. 1483), a continuous fixed power source (1267-B Regulated Power Supply, General Radio Company, Concord MA, Serial No. 903) and an EMF cage. The oscillator, with an output power of approximately 300 mW and a frequency set at 900-MHz, capable of producing a high-speed frequency, was used to establish a 900-MHz EMF inside the cage. The continuous fixed power source attached to the electricity grid was used to run the oscillator and meet the need for continuous energy. The oscillator outlet was attached to a half-wave dipole antenna, 15 cm long and 1 mm in diameter, made from a copper rod. The antenna was attached to the center of a wooden rod inserted 11 cm from the top and between the right and left sides of the EMF cage ($40.5 \times 31.5 \times 40.5$ cm in size).

In order to determine the intensity of the electric field during application of EMF in the EMF group, EMF measurements were taken at the beginning and end of application from nine separate points on the floor and top of the EMF cage with EMF group rats inside it. These measurements were performed using a broadband electric field intensity measuring device operating at a range of 100 kHz–2.5 GHz (C.A 43 Isotropic Electrical Field Intensity Meter, Chauvin Arnoux Group, Paris, France). Electric field intensity, power

and specific absorption rate (SAR) values were calculated on the basis of these measurements.

Surgical and histological staining procedures

At the end of the experiment (postnatal day 60) rats were placed on a dissection table in the dorso-ventral position. An abdominal skin incision along the midline was made using a scalpel under anesthesia (Ketalar® 50 mg/kg, Eczacıbaşı Co., Istanbul, Turkey). The peritoneum was passed and the right and left ovaries were extracted. Surrounding tissues were carefully removed, and both ovaries were weighed on a sensitive scale. Blood specimens were collected from the abdominal aorta for serum biochemical analyses, and the rats were then sacrificed by desanguination. The left ovarian were quickly washed in iced 0.09% NaCl isotonic solution and stored at -80°C in Eppendorf tubes for biochemical analyses. The right ovarian were fixed in 10% formaldehyde for histological analyses. The blood specimens collected were centrifuged at 2000 rpm for 10 min. The plasma part was stored at -80°C in Eppendorf tubes for biochemical analyses.

Right ovarium tissues were fixed for 48 h in 10% formaldehyde solution. They were then passed through alcohol and xylene series and kept in paraffin in a stove before being embedded in paraffin blocks (Bancroft et al. 1994). Sections 5 μm in thickness were taken every 50 μm from the paraffin blocks using a microtome (Leica RM 2255, Leica Instruments, Nussloch, Germany) (Picut et al. 2015). The sections were randomly sampled at a proportion of 1:10. Forty sections (39.666 ± 2.159) were thus sampled from each animal using this method. The sections were stained with hematoxylin and eosin (H&E). Numbers of primordial follicles, early primary follicles, late primary follicles, secondary follicles, Graafian follicles and atretic follicles were counted in all areas of each section using a light microscope (Olympus BX51, Olympus Co., Tokyo, Japan) equipped with a digital camera (DP71 Olympus, Olympus Co., Tokyo, Japan) (Roshangar et al. 2014). Histopathological evaluations and scoring and photographing of the sections were carried out using the same microscope and digital camera.

Following routine tissue procedures for periodic acid–Schiff and Masson's trichrome staining, sections 5 μm in thickness were again taken using the same microtome. These were then stained using procedures appropriate to each stain (Bancroft et al. 1994). Analyses were performed on these sections with the help of the same light microscope and digital camera. Photographs were taken and transferred to a digital environment.

Evaluation of sections stained with hematoxylin-eosin, periodic acid–Schiff and Masson's trichrome

Sections stained with H&E were evaluated at $\times 20$ magnification under a light microscope.

Primordial follicle [definition = all primary oocytes are surrounded by a single layer consisting of flat follicular cells], early primary follicle [definition = all primary oocytes are

surrounded by a single layer made up of cuboidal follicular cells], late primary follicle or growing primary follicle (GPF) [definition = all primary oocytes are surrounded by a multi-layered follicle consisting of granulosa cells] secondary (antral) follicles [definition = an increased number of granulosa cells is observed. Spaces containing a clear fluid (liquor folliculi) then develop between these. These cavities then merge, giving rise to one large fluid-filled space, known as the antrum], Graafian follicle [definition = the follicle is relatively large in size. The antrum absorbs fluid and swells. Primary oocytes are located eccentrically and surrounded by corona radiata and cumulus cells] and atretic follicle [definition = the granulosa cells contain autophagic vacuoles and blobbing within the cytoplasm. Granulosa cells are divided from other neighboring cells (theca layer) by a discontinuous zona pellucida] (Ross and Pawlina 2011; Roshangar et al. 2014). Numbers were calculated in all areas on the sections sampled.

Ovarian tissue sections stained with H&E were scored at histopathological evaluation (0 = none, 1 = mild, 2 = moderate and 3 = intense). Scoring was performed with separate values, 0 (no finding), 1 (mild findings), 2 (moderate findings) or 3 (intensive findings) being given for each of the following: decrease in follicle cell dimensions, decrease in zona granulosa layer thickness, hyperchromasia in granulosa cells, decreased theca layer thickness, increased leukocyte infiltration in follicles and leukocyte infiltration in stroma.

Mitotic activity in follicle cells in sections stained with H&E was scored between 0 and 3. Scoring was performed under a light microscope, and assessment was performed by counting the numbers of cells in any of the phases of mitosis among granulosa cells in secondary (antral) follicles. An average number of 7–8 cells in phases of mitotic division were observed in sections from control group animals. Cell numbers of 7–8 or above in phases of mitotic division were therefore regarded as normal and scored as 0 (no decrease in mitotic activity). The presence of 5–6 cells in phases of mitotic division was scored 1 (a mild decrease in mitotic activity) and 3–4 cells in phases of mitotic division was scored 2 (a moderate decrease in mitotic activity), and 1–2 or no cells in phases of mitotic division was scored 3 (intense decrease in mitotic activity).

Zona pellucida, basal membrane and general histopathological ovarian evaluations were performed on sections stained with periodic acid–Schiff. Fibrosis was evaluated histopathologically on sections stained with Masson's trichrome.

TUNEL staining

The terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling assay (TUNEL) technique was employed for the assessment of apoptosis in ovarian tissues. The procedure outlined in the surgical and histological staining procedures section was adopted up to the paraffin block production stage. For TUNEL staining, the paraffin blocks were cut into 5- μm thick serial sections with the help a microtome (Leica RM 2255, Leica Instruments, Wetzlar

Germany). These were then exposed to routine histological deparaffinization procedures. Once the sections had been deparaffinized, they were stained using the TUNEL method via a cell death detection kit in accordance with the manufacturer's instructions (In Situ Cell Death Detection Kit POD, catalog number: 11684817910, Roche®, Mannheim, Germany) (Türedi et al. 2016).

Apoptosis evaluation with TUNEL-staining slides

Apoptosis analysis from the TUNEL-stained slides was performed with a light microscope (Olympus BX51; Olympus Co., Tokyo, Japan) at a magnification of $\times 400$. Numbers of TUNEL (+) cells observed in ovarian follicles, granulosa cells and the theca layer were recorded in five randomly selected areas in one section for each rat. One thousand cells were counted in each animal. Apoptosis was defined as the presence of brown nuclei (Bage et al. 2001). The apoptotic index (AI) was calculated using the formula $\text{AI} = \text{apoptotic cell nuclei number} / \text{total cell nuclei number counted} \times 100$ (Odacı and Özyılmaz 2015; Türedi et al. 2016). All counting procedures were performed blindly by one of the authors.

Biochemical analyses

Biochemical parameters in ovarian and blood tissue were studied in order to determine whether ovarian tissue was affected by EMF. Malondialdehyde (MDA), glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) levels were investigated as oxidative stress markers in ovarian tissue and blood specimens. 8-hydroxydeoxyguanosine (8-OHdG) levels were measured in order to determine DNA injury, and 3-nitrotyrosine (3-NT) and anti-Müllerian hormone (AMH) levels were measured to determine protein damage. In addition, total antioxidant status (TAS) and total oxidant status (TOS) were measured in serum, and the oxidative stress index (OSI), a predictor of antioxidant/pro-oxidant balance, was calculated from the ratio of TOS level to TAS level.

Determination of tissue MDA levels. MDA measurement was performed by modifying the method described by Mihara and Uchiyama (1978). This method is based on measuring the color of the molecule formed by MDA with thiobarbituric acid (TBA) in an acidic environment at an absorbance of 532 nm. Tissue homogenization buffer, 1% H_3PO_4 solution, TBA solution and standard solutions used in tissue MDA measurement were first prepared. In order to prepare the tissue homogenization buffer used in tissue MDA measurement [0.01 M phosphate buffer solution (PBS), pH 7.4], 10 PBS tablets (Medicano, 09-2051-100, Uppsala, Sweden) were dissolved in a beaker with approximately 900 mL distilled water. The pH value of the solution was then adjusted to 7.4 with a pH meter (Hanna Instrument (HI) 9321, Woonsocket, RI). The final volume of the solution with an adjusted pH value was then made up to 1 L. In order to prepare the 1% H_3PO_4 solution, 2.94 mL 85% H_3PO_4 (Sigma 04107, St. Louis, MO) was added to a quantity of distilled water, and the final volume was made up to 250 mL of distilled water. In order to prepare the TBA solution, 0.67 g TBA (Sigma T5500, St. Louis, MO)

was measured. Next, 50 mL distilled water and 50 mL acetic acid (Sigma, A6283, St. Louis, MO) were added and stirred with the assistance of a magnetic bar. For the preparation of standard solutions, 82.5 μL 1,1,3,3 tetramethoxypropane (Sigma, T1642, St. Louis, MO) was added to 0.01 M 50 mL HCl (Sigma 258148, St. Louis, MO) solution. The solution was then incubated for 1 h at 50°C. Various dilutions were made from the main stock prepared in this manner, and 40, 20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 nmol/mL standard solutions and blind specimens were prepared. Following preparation of the solutions, specimens of approximately 50 mg each were taken from each tissue. This tissue was then homogenized in 2 mL phosphate buffer (PBS, pH = 7.4) at 9500 rpm (4×10 s, 40°C) in an Ultra-Turrax homogenizer (model T25, Jane and Kunkel, Germany). Homogenates were centrifuged for 10 min at 4000 rpm. Supernatants obtained by centrifugation were thinned with PBS at ratio of 1:10, and MDA measurement was performed.

During measurement, 3 mL 1% H_3PO_4 was added to 500 μL of homogenate and mixed. To this was added 1 mL 0.672% TBA. After mixing, this was incubated for 60 min in a boiling water bath. At the end of that period, the tubes were left to cool at room temperature and were then centrifuged for 10 min at 4000 rpm, again at room temperature. Specimens 200 μL were taken from the resulting supernatant and placed into 96-well plates. Absorbances at a 532 nm wavelength were then read on a microplate reader spectrophotometer (Versamax, Molecular Devices, CA). An MDA standard chart was produced by plotting the absorbance results obtained against concentrations. Tissue MDA levels were calculated as nmol MDA/g wet tissue using this chart.

Determination of serum MDA levels. Rat serum specimens were stored at -80°C until biochemical analyses. Levels of MDA in serum specimens were evaluated using the thiobarbituric acid (TBA) reactive substance method developed by Yagi (1984). The red color resulting from the reaction between MDA, a product of lipid peroxidation, and TBA was measured spectrophotometrically. In order to remove water-soluble substances giving the same color by reacting with TBA, serum lipids and protein were broken down with the phosphotungstic acid/sulfuric acid system. The 0.084 N sulfuric acid (H_2SO_4), 10% phosphotungstic acid ($\text{H}_3(\text{W}_3\text{O}_{10})_4\cdot\text{H}_2\text{O}$), TBA solution and standard solutions used in serum measurement were first prepared. For the preparation of 0.084 N sulfuric acid (H_2SO_4) solution, 577 μL 97% H_2SO_4 (Sigma 320501, St. Louis, MO) and made up to 250 mL in volume with deionized water. For the preparation of 10% phosphotungstic acid ($\text{H}_3(\text{W}_3\text{O}_{10})_4\cdot\text{H}_2\text{O}$) solution, 5.55 g phosphotungstic acid (Sigma P4006, St. Louis, MO) was dissolved in 50 mL deionized water. For the preparation of TBA solution, 0.67 g TBA (Sigma T5500, St. Louis, MO) was weighed. Next, 50 mL distilled water and 50 mL acetic acid (Sigma, A6283, St. Louis, MO) were added and dissolved by stirring with a magnetic bar. Standard solutions were prepared by adding 82.5 μL 1,1,3,3 tetramethoxypropane (Sigma, T1642, St. Louis, MO) to 0.01 M 50 mL HCl (Sigma 258148, St. Louis, MO) solution. The solution was then incubated for 1 h at 50°C. Various dilutions were made from the main stock thus

prepared to produce 40, 20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 nmol/mL standard solutions and blinds.

During measurement, 150 μ L each of serum, 1200 μ L each of H_2SO_4 and 150 μ L each of phosphotungstic acid were added to experiment tubes. The tubes were then well mixed and left for 5 min at room temperature. The mixtures were then centrifuged for 10 min at 1500 g , and the upper phases were removed. Next, 2 mL each of distilled water was added to the residual parts and these were then vortexed until dissolution. In the following stage, 500 μ L each of TBA was added and incubated for 1 h at 100°C. Following incubation, the tubes were centrifuged for 10 min at 1000 g . Then, 200 μ L each was taken from the upper, transparent, part and placed into 96-well plates. Absorbances at a wavelength of 532 nm were read on a microplate reader spectrophotometer (Versamax, Molecular Devices, Sunnyvale, CA). An MDA standard chart was produced by plotting the absorbance results obtained against concentrations. Tissue MDA levels were calculated as nmol MDA/g wet tissue using this chart.

Determination of 3-NT, AMH, SOD, 8-OHdG, CAT and GSH levels in serum and tissue specimens. 3-NT levels were determined using a two-antibody sandwich ELISA kit (SunRed®, Cat No: 201-11-0020, Shanghai, China), AMH levels using a two-antibody sandwich ELISA kit (SunRed®, Cat No: 201-11-1246, Shanghai, China), SOD levels using a two-antibody sandwich ELISA kit (SunRed®, Cat No: 201-11-0169, Shanghai, China), 8-OHdG levels using a two-antibody sandwich ELISA kit (SunRed®, Cat No: 201-11-0032, Shanghai, China), CAT levels using a two-antibody sandwich ELISA kit (SunRed®, Cat No: 201-11-5106, Shanghai, China), and GSH levels using a two-antibody sandwich ELISA kit (SunRed®, Cat No: 201-11-5134, Shanghai, China), following the manufacturer's instructions. Absorbances of specimens were read at a wavelength of 450 nm on a microplate reader spectrophotometer (Versamax, Molecular Devices, Sunnyvale, CA).

Determination of TOS and TAS levels. TOS levels in serum and tissue specimens were determined using a colorimetric kit (Rel Assay Diagnostics, Cat No: RL0024, Gaziantep, Turkey) in line with the manufacturing company's recommendations. TOS assay is based on various oxidant species raising Fe^{+2} to Fe^{+3} , the resulting Fe^{+3} forming a colored complex with chromogen and the spectrophotometric measurement of that complex at 530 nm. The data obtained were calculated against the standard and total oxidant capacity was determined as μ mol H_2O_2 Equivalent (Eq)/L. TAS levels in serum and tissue specimens were determined using a colorimetric kit (Rel Assay Diagnostics, Cat No: RL0017, Gaziantep, Turkey) in line with the manufacturer's instructions. TAS assay is based on the antioxidants present in serum reducing dark green-blue ABTS reactive to a colorless state. The change in absorbance is observed spectrophotometrically at 660 nm. Total oxidant capacity in a specimen is determined as μ mol Trolox Eq/L by calculation against the standard.

Oxidative stress index calculation. Oxidative stress index (OSI) from ovarium tissues was calculated with the formula (Yulug et al. 2014):

$OSI = \text{total oxidant status (TOS)} (\mu\text{mol } H_2O_2 \text{ Eq/L}) / \text{total antioxidant status (TAS)} (\mu\text{mol Trolox Eq/L}) \times 100.$

Statistical analysis

Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) version 20.0 (SSPS Inc., Chicago, IL) software. All numerical data were expressed as mean $\pm$ standard error mean (SEM). Variables were first investigated to determine whether or not they were normally distributed. Those which were normally distributed were subjected to analysis of variance. The Student–Newman–Keuls test was performed post hoc. Non-normally distributed data were analyzed using the Kruskal–Wallis and Mann–Whitney tests. The chi-square test was used in the analysis of histological scoring results. $p < .05$ was regarded as statistically significant.

Results

Rat body and ovarian weight findings

Mean rat weights at the end of the study were 180.97 ± 2.85 g for the control group, 186.31 ± 2.86 g for the sham group and 159 ± 13.3001 g for the EMF group. No statistically significant difference was determined in body weights among the groups ($p > .05$). Mean ovary weights were 0.0713 ± 0.0066 g for the right ovarium and 0.0788 ± 0.0039 g for the left ovarium in the control group, 0.070 ± 0.0053 g for the right ovarium and 0.069 ± 0.0029 g for the left ovarium in the sham group and 0.0788 ± 0.0099 g for the right ovarium and 0.0713 ± 0.0058 g for the left ovarium. No statistically significance was observed between mean right and left ovarium weights for each group ($p = .680$; $p = .270$, respectively). Since there was no statistically significant difference in mean right and left ovarium weights in the groups, mean right ovarium weights were used in comparisons between the groups. No statistically significant difference was determined between the groups in terms of these right ovarium weights ($p = .059$).

EMF measurements

The electric field intensity threshold value determined for a single device in GSM-900 base station systems is 10.23 V/m (power intensity = 0.28 W/m^2) (TÜBİTAK-BİLTEN 2001; ICNIRP 2014). Electric field measurements from nine separate points in which the EMF group was exposed to an EMF effect were close to the determined values. On the basis of EMF measurements taken every day throughout the study, the mean intensity of the electric field measured from nine separate points on the internal floor of the EMF cage at the beginning of EMF application was 8.64 ± 1.13 V/m (power intensity = 0.198 W/m^2), and mean electric field intensity measured beneath the floor of the EMF cage was 7.31 ± 0.52 V/m (power intensity = 0.142 W/m^2). At the end of EMF application, mean intensity of the electric field at nine separate points on the internal floor of the EMF cage was 8.54 ± 0.98 V/m (power intensity = 0.193 W/m^2), and mean intensity of the electric field beneath the floor of the EMF cage was 7.36 ± 0.53 V/m (power intensity = 0.144 W/m^2) (Figure 1). The whole-body SAR was calculated (RadHaz SAR

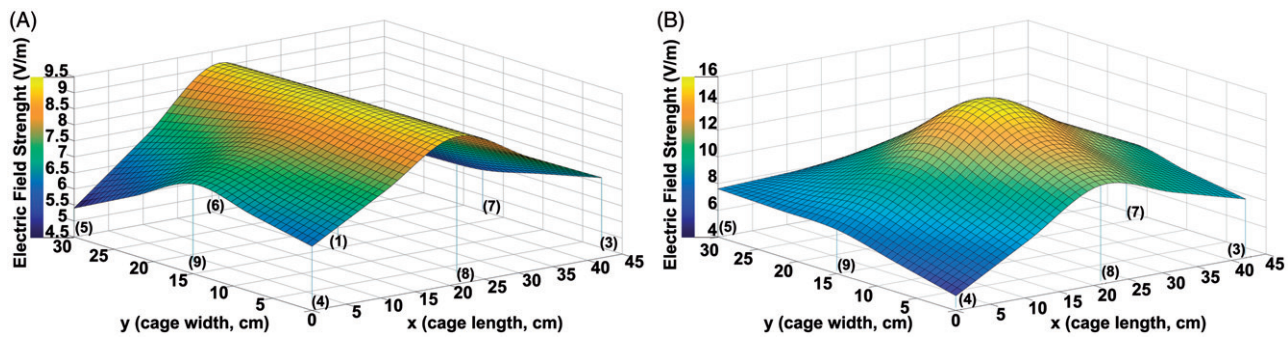


Figure 1. Positional distributions on the x–y plane of mean EMF intensities measured during EMF application from nine separate points on the top and floor of the EMF-application cage, with rats inside the cage. (A) Positional distribution on the x–y plane of EMF measurements on the floor of the EMF-application cage and (B) Positional distribution on the x–y plane of EMF measurements on the top of the EMF-application cage.

Equivalency Calculator, Version 1.0, © 2000 Richard Tell Associates, Inc., Colville, WA) at 0.0098 W/kg at the beginning of EMF exposure and 0.0096 W/kg at the end of EMF exposure.

EMF-cage temperature and humidity results

Temperature and humidity inside the cage were measured at the time the rats were placed inside the cage and during and at the end of application in order to determine changes during sham and EMF exposure. The mean internal temperature in the cage on the days on which EMF was applied was $18.256 \pm 0.28^\circ\text{C}$, while the mean humidity level was $46.04 \pm 1.009 \text{ g/m}^3$. Mean temperature during sham application days was $21.892 \pm 0.305^\circ\text{C}$, while mean humidity was $38.9 \pm 0.791 \text{ g/m}^3$. Statistical analysis of these findings revealed no significant difference between the sham and EMF groups in terms of temperature ($p > .05$) and humidity ($p > .05$).

Morphological findings

Follicle number findings. Follicle numbers were calculated at different stages in the control, EMF using sections obtained from right ovary tissues and stained with H&E (Table 1). No statistically significant differences were determined between the groups in terms of primordial follicle ($p = .370$), early primary follicle ($p = .803$), late primary follicle or GPF ($p = .113$) and Graafian follicle ($p = .051$) numbers (Table 1). Statistical comparison of secondary (antral) follicle numbers revealed that these decreased significantly in the EMF group compared to the control group ($p = .012$). However, there were no statistically significant differences in terms of secondary follicle numbers between the EMF and sham groups ($p = .141$), or between the control and sham groups ($p = .093$). No significant difference was determined between the groups in terms of atretic follicle numbers ($p = .314$) (Table 1).

Histopathological evaluation and scoring results. The zona granulosa, theca layer and stroma all had a normal morphological appearance in H&E stained sections from the control and sham groups (Figure 2(A–D)). No statistical finding that might elicit any pathological assessment at histopathological scoring was observed in either group (Table 2).

Table 1. Ovarian follicle numbers at various stages in the control, sham and EMF groups.

Ovarian follicle type	Control group	Sham group	EMF group
Primordial follicle	239.62 ± 27.35	241.37 ± 19.89	315.5 ± 40.86
Early primary follicle	88.625 ± 9.43	93 ± 20.07	83.25 ± 7.18
Late primary follicle or growing primary follicle	236.87 ± 18.57	203.25 ± 17.85	156.25 ± 28.17
Secondary (antral) follicle	194.37 ± 22.28	143.75 ± 19.32	$101.25^a \pm 22.53$
Graafian follicle	135.37 ± 29.58	163.12 ± 10.50	97.5 ± 19.04
Atretic follicle	17.37 ± 2.32	13.37 ± 2.07	17.25 ± 1.96

The data represent mean $\pm$ SEM.

The data were obtained from approximately 40 sections (39.666 ± 2.159) stained with H&E at $\times 20$ magnification under a light microscope.

^aSecondary follicle numbers in the EMF decreased significantly compared to the control group ($p = .012$).

In addition to the histopathological findings at evaluation of H&E stained sections from the EMF group (such as thinning in the zona granulosa and leukocyte infiltration) (Figure 2(E,F)), scoring of the findings revealed a moderate statistically significant decrease in the mitotic activity of follicle cells ($p = .001$). A mild significant decrease was also observed in follicle cell sizes in the EMF group ($p = .026$). A moderate significant decrease was observed in zone granulosa thickness in the EMF group ($p = .000$). A moderate significant increase in hyperchromasia was determined in granulosa cells in the EMF group ($p = .001$). A severe significant decrease was observed in the thickness of the theca layer in the EMF group ($p = .000$). A moderate significant increase was determined in leukocyte infiltration in follicles in the EMF group ($p = .022$) (Table 2).

Stroma analysis revealed a normal morphological appearance in the ovarian stroma in sections from the control and sham groups (Figure 2(A–D)) and a mild statistically significant increase in leukocyte infiltration in sections from the EMF group (Figure 2(E,F)) ($p = .024$). There was no significant variation among the groups in terms of edema and congestion findings (Table 2).

Histopathological analysis of sections stained with Masson's trichrome. Histopathological examination under light microscope of ovarian sections stained with Masson's trichrome revealed a normal morphological structure in stromal connective tissue from the control, sham and EMF groups. Analysis in terms of fibrosis revealed no significant variation among the groups. However, the presence of occasional impaired granulosa cells was noticeable in the EMF group (Figure 3(A–C)).

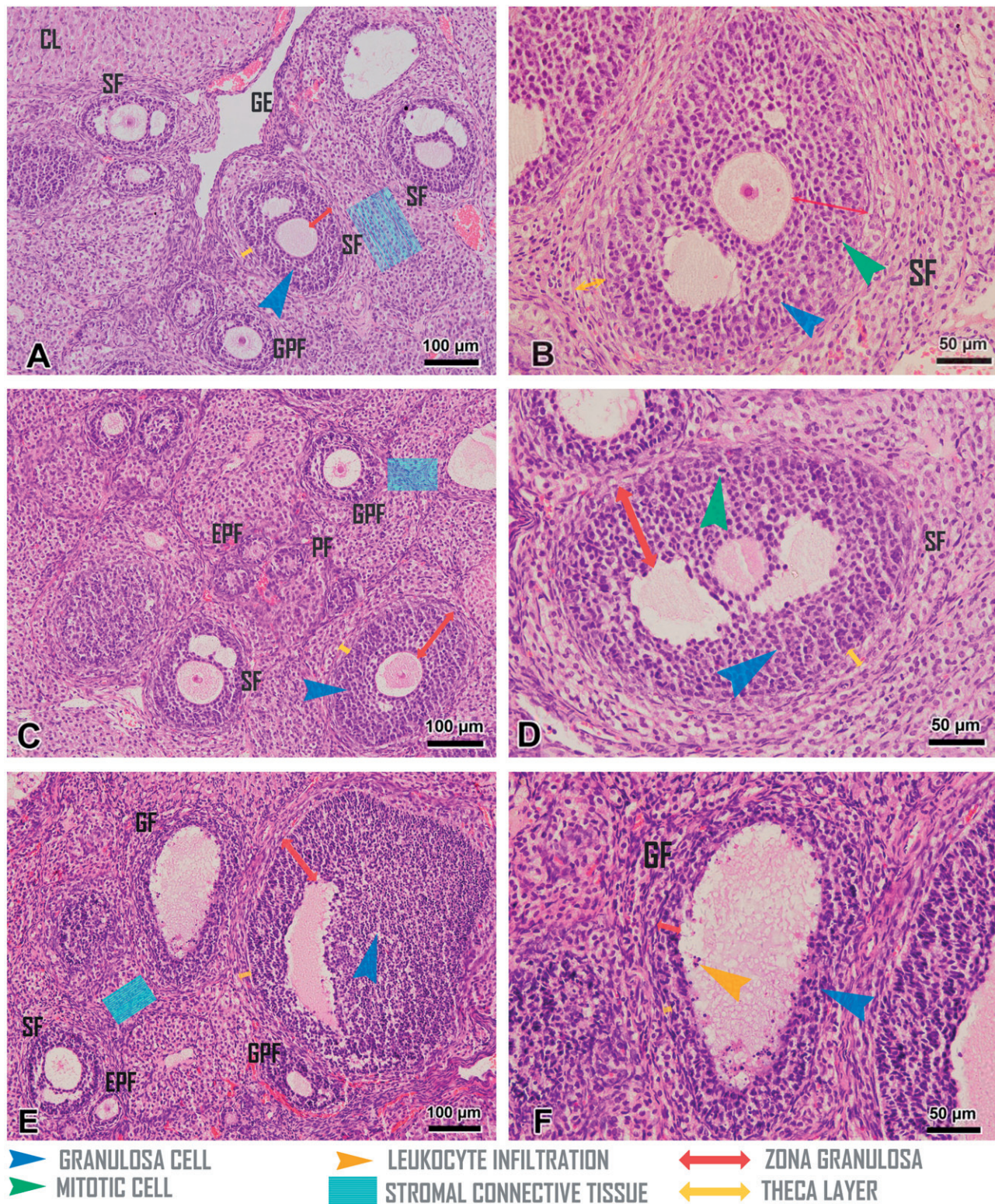


Figure 2. Microscopic images groups' ovarian tissues. Normal structure was observed in stroma (cyan rectangle), zona granulosa (two-way red arrow), theca layer (two-way yellow arrow), granulosa cells (blue arrow) and mitotic cells (green arrow) in sections from the control (A and B) and sham (C and D) groups. Diffuse leukocyte infiltration (yellow arrow) was observed in follicles and stroma from EMF group (E and F) sections (hematoxylin and eosin). Primordial follicle (PF), early primary follicle (EPF), late primary follicle or growing primary follicle (GPF), secondary (antral) follicle (SF), graafian follicle (GF), corpus luteum (CL), germinal epithelium (GE).

Histopathological analysis of sections stained with periodic acid–Schiff. Control group and sham group sections exhibited a normal appearance at histopathological examination of sections stained with periodic acid–Schiff. However,

thinning in the zona granulosa and impaired integrity in the zona pellucida were observed in sections from the EMF group compared to the control and sham groups (Figure 4(A–C)).

Table 2. Histopathological finding scoring results for rats from the control, sham and EMF groups (0 = normal, no finding; 1 = mild findings; 2 = moderate findings; 3 = a high level of findings).

Histopathological findings	Scoring		
	Control group	Sham group	EMF group
Decreased mitotic activity in follicle cells	0.12 ± 0.12	0.12 ± 0.12	2.25 ^a ± 0.36
Decrease in follicle cell dimensions	0 ± 0	0.25 ± 0.16	0.87 ^b ± 0.29
Hyperchromasia in granulosa cells	0.25 ± 0.16	0.5 ± 0.18	1.87 ^c ± 0.22
Decreased thickness in the zona granulosa layer	0 ± 0	0 ± 0	2.12 ^d ± 0.29
Decreased thickness in the theca layer	0.12 ± 0.12	0.25 ± 0.16	2.5 ^e ± 0.18
Increased leukocyte infiltration in follicles	0.5 ± 0.18	0.62 ± 0.18	1.87 ^f ± 0.35
Leukocyte infiltration in stroma	0.12 ± 0.12	0 ± 0	0.5 ^g ± 0.18

The data represent mean ± SEM. The data were obtained from approximately 40 sections (39.666 ± 2.159) stained with H&E at × 20 magnification under a light microscope and through random sampling from each ovary.

^aThe decrease in the mitotic activity score in the EMF group was significantly greater compared to the control and sham groups ($p = .001$).

^bThe decrease in follicle cell dimension score in the EMF group was significantly greater compared to the control and sham groups ($p = .026$).

^cThe decrease in the hyperchromasia score in granulosa cells in the EMF group was significantly greater compared to the control and sham groups ($p = .001$).

^dThe decrease in the zona granulosa layer score in the EMF group was significantly greater compared to the control and sham groups ($p = .000$).

^eThe decrease in the theca layer thickness score in the EMF group was significantly greater compared to the control and sham groups ($p = .000$).

^fThe increase in the leukocyte infiltration score in follicles in the EMF group was significantly greater compared to the control and sham groups ($p = .022$).

^gThe leukocyte infiltration score in the stroma in the EMF group increased compared to the control and sham groups ($p = .024$).

Results of apoptosis evaluation. No statistically significant difference was observed when apoptotic index (AI) values of follicles on sections from right ovarian tissues from rats in the control, EMF and sham groups were compared ($p = .21$). AI (%) was determined at 35.22 ± 4.41 for the control group, 42.52 ± 3.48 for the sham group and 47.27 ± 4.05 for the EMF group. However, more apoptotic cells were noted at histopathological examination under light microscope in the EMF group compared to the control and sham groups (Figure 5(A–C)).

Biochemical analyses results

Ovarian tissue biochemical analysis results. Left ovary tissues were used to elicit tissue biochemistry data. When the groups were compared in terms of tissue AMH values, these increased statistically significantly in the EMF and sham groups compared to the control group. However, the difference between the EMF and sham groups was not significant. In terms of tissue SOD levels, these also increased significantly in the EMF and sham groups compared to the control group. There was no significant difference between the EMF and sham groups. Tissue CAT levels increased statistically significantly in the EMF and sham groups compared to the control group. There was no significant difference between EMF and sham group values. EMF group 3-NT levels increased statistically significantly compared to those of the control group. No statistically significant difference was observed between the sham group and either the control or EMF groups. No statistically significant difference was observed between the groups in terms of tissue MDA ($p = .412$), GSH ($p = .493$) or 8-OHdG ($p = .796$) levels (Table 3).

Serum biochemical analysis results. Comparison of the groups' serum CAT and 8-OHdG levels revealed a statistically significant increase in the EMF and sham groups compared to the control group. No statistically significant difference

was determined between the EMF and sham groups. Serum TOS levels increased significantly in the EMF group compared to the sham and control groups. Serum OSI levels in the EMF group were significantly higher than those in the sham and control groups. There was no statistically significant difference between the sham and control groups in terms of serum TOS or OSI levels. No statistically significant difference was observed between the groups' serum AMH ($p = .151$), MDA ($p = .193$), SOD ($p = .457$), GSH ($p = .828$), 3-NT ($p = .151$) or TAS ($p = .095$) (Table 4).

Discussion

Although many studies have been performed, the effect of EMF on biological systems remains an important subject of research and debate. In addition to studies investigating the effects of EMF on numerous tissues, organs and systems, there have been others examining the effects on the reproductive system of EMF applied at different frequencies and for differing durations. One study reported shrinkage in oocyte nuclei, narrowing in the zona pellucida, several droplets in cytoplasm and dispersion of their organelles in rats exposed to a 3-mT (50Hz) EMF effect for 4 h a day over 4 months. That study reported frequent signs of apoptosis, including condensed nuclei, chromatin margination and nuclear membrane dilatation in granulosa and corona radiata cells from the experimental group rats. Primary morphological changes in the granulosa cells from the EMF-exposed group included retraction of granulosa cells, loss of microvilli and condensation or loss of mitochondrial cristae. The authors suggested that changes in oocytes indicated the cytotoxic effect of EMF and that changes occurring in granulosa cells coincide with the start of apoptosis in those cells. They also suggested that EMF exposure may interfere with the normal process of folliculogenesis. Roushangar and Rad (2007) studied the ovarian tissues of 2-d old mice pups

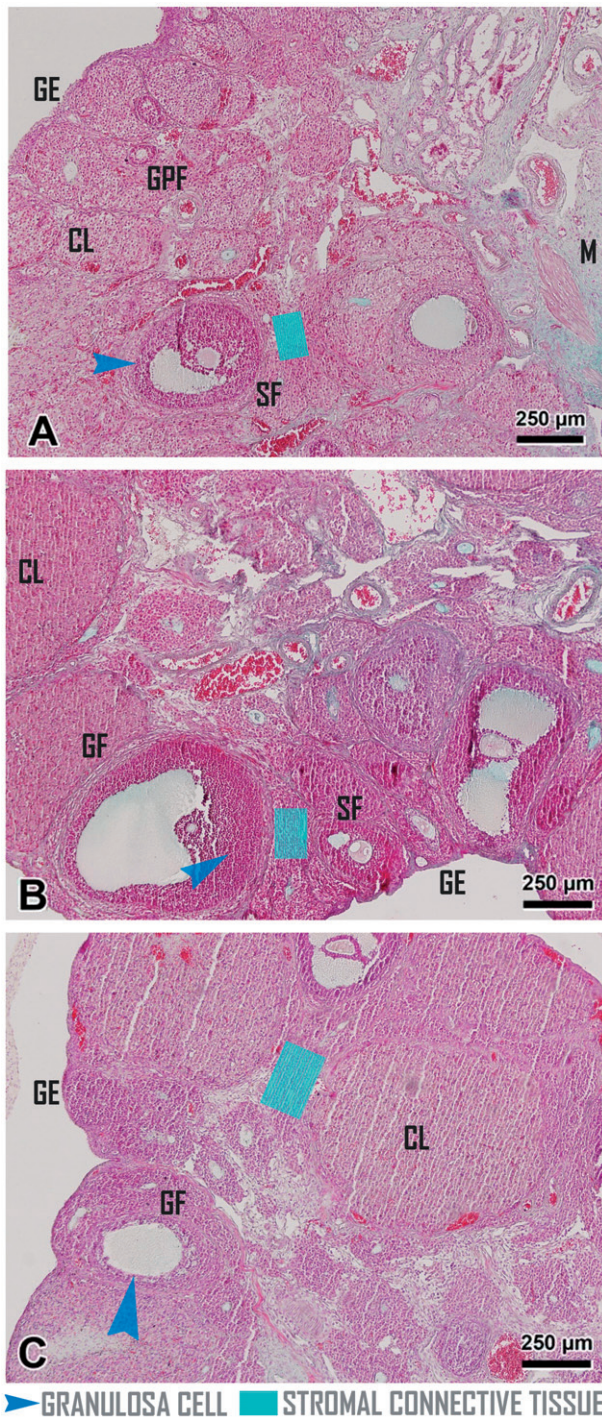


Figure 3. Normal structure was observed in the stromal connective tissue (cyan rectangle) and granulosa cells (blue arrow) in control (A) and sham (B) group sections. In EMF group sections, although the connective tissue in the stroma was regularly arranged, there were striking irregularities in granulosa cell (C) (Masson trichrome). Late primary follicle or growing primary follicle (GPF), secondary (antral) follicle (SF), graafian follicle (GF), corpus luteum (CL), germinal epithelium (GE), medulla (M).

obtained from dams exposed to the effect of 50-Hz EMF. They reported less developed primordial follicles and observed heterochromatic and shrunken nuclei in oocytes with vacuolated cytoplasm. In the adult groups, they also reported thinning in the zona pellucida and compromise of zona pellucida integrity, findings which are compatible with our own results (Roshangar et al. 2014). There was a

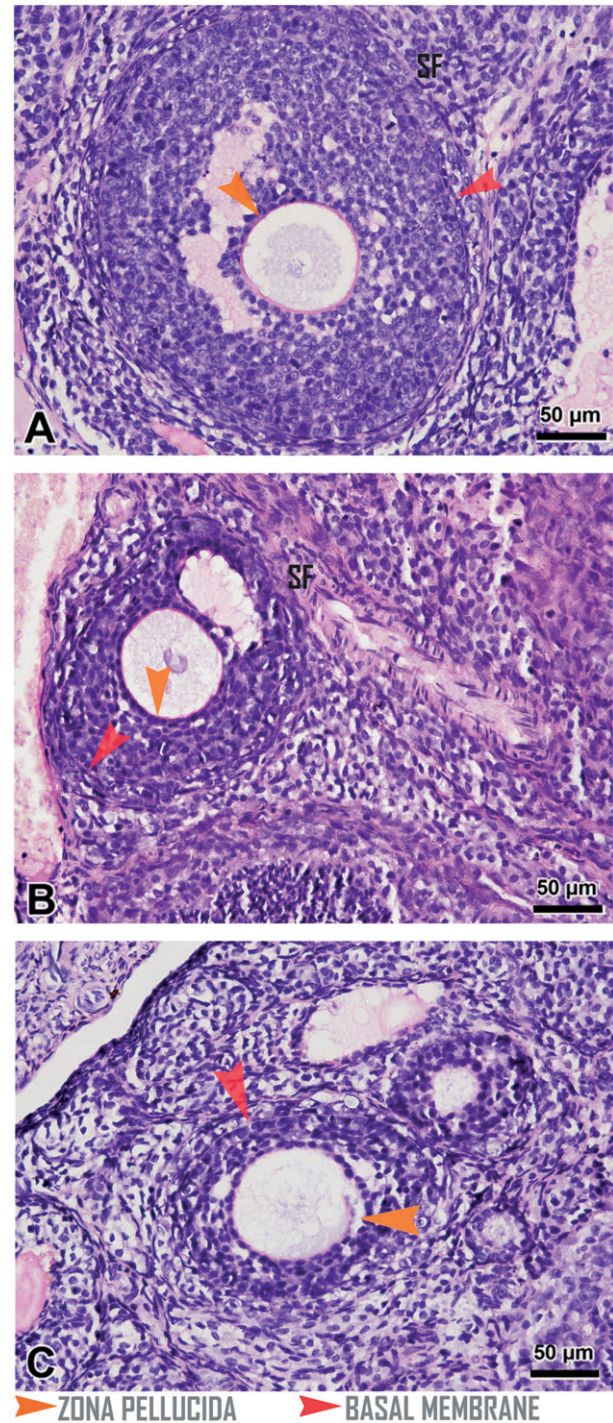


Figure 4. The zona pellucida (orange arrow) and basal membrane (red arrow) were normal thickness and structure in sections from the control (A) and sham (B) groups. In the EMF group (C), there was noticeable thinning in the zona pellucida and impaired integrity in the basal membrane (periodic acid–Schiff). Secondary (antral) follicle (SF).

significant, but moderate decrease in the zona granulosa layer in the EMF group in our study. Additionally, that study also reported decompositions in the granulosa layer, vacuolization, decomposition in oocyte nuclei, irregular nucleoli, follicular atresia and granulosa cells in the apoptotic phase (Roshangar et al. 2014).

Türedi et al. (2016) observed a decrease in primordial and tertiary follicle numbers, an increase in atretic follicles and atretic index, an increased damage in follicles,

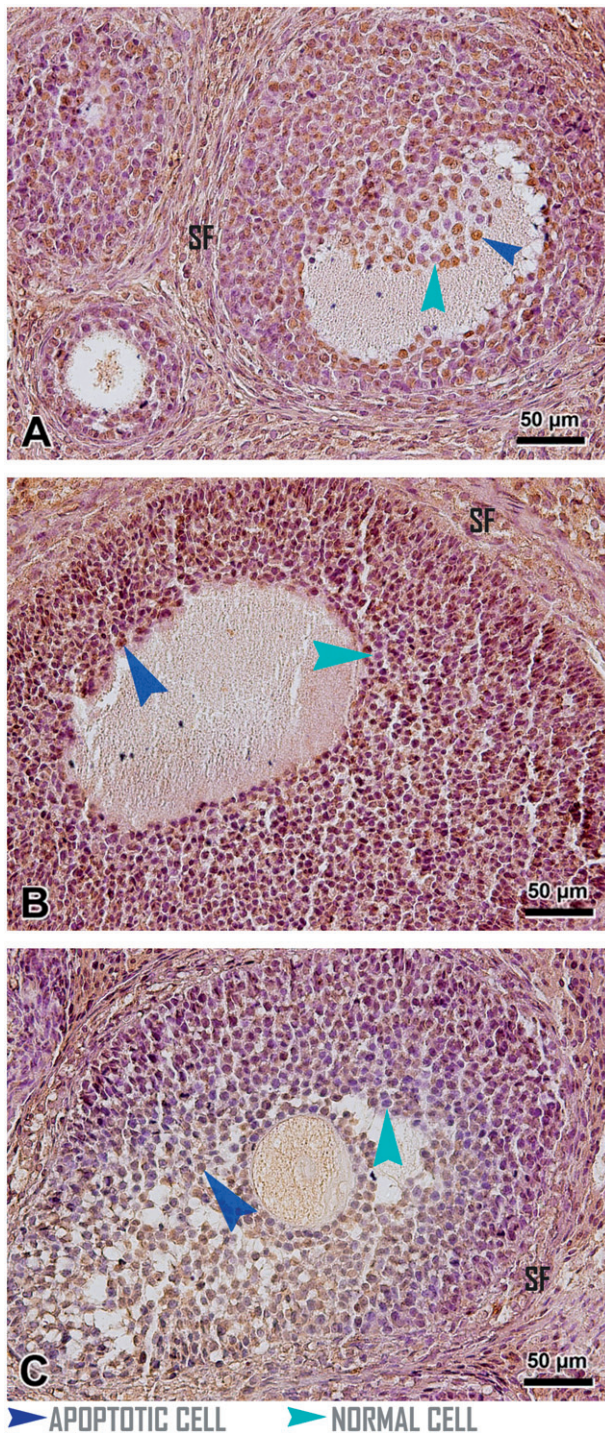


Figure 5. While very few apoptotic cells (dark blue arrow) were observed among normal cells (cyan arrow) in sections from the control (A) and sham (B) groups, there were noticeably more apoptotic cells in the EMF group sections (C) (TUNEL). Secondary (antral) follicle (SF).

vasocongestion, and albeit at a low level, fibrosis in stroma in ovarian tissues of rat pups exposed to the effect of 900-MHz EMF in the prenatal periods examined on postnatal day 34 (Türedi et al. 2016). In our study, we determined a significant decrease only in secondary follicle numbers and degenerations in follicle cells. Our results revealed no significant difference in other follicle type numbers or fibrosis. Our results and those of Türedi et al. (2016), therefore, differ from that perspective. This may be due to the fact that although

Table 3. Biochemical data from right ovary tissues from control, sham and EMF group rats.

Ovarian tissue biochemical markers	Control group	Sham group	EMF group
AMH (ng/mg protein)	5.27 ± 0.26	8.56 ^a ± 0.66	7.55 ^a ± 0.67
SOD (ng/mg protein)	14.97 ± 0.92	23.55 ^b ± 2.18	22.1 ^b ± 2.36
CAT (ng/mg protein)	46.47 ± 2.62	68.93 ^c ± 4.41	61.41 ^c ± 5.93
MDA (nmol/g tissue)	415.88 ± 35.94	449.68 ± 36.51	508.68 ± 72.64
GSH (mg/g tissue)	9.23 ± 0.22	9.50 ± 0.16	9.57 ± 0.23
3-NT (nmol/mg protein)	0.38 ± 0.01	0.48 ± 0.03	0.51 ^d ± 0.05
8-OHdG (ng/g tissue)	93.63 ± 2.45	95.7 ± 2.74	95.75 ± 3.32

The data represent mean ± SEM.

AMH: anti-Müllerian hormone; MDA: malondialdehyde; GSH: glutathione; CAT: catalase; SOD: superoxide dismutase; 8-OHdG: 8-hydroxydeoxyguanosine; 3-NT: 3-nitrotyrosine

^aEMF and sham group AMH values increased significantly compared to the control group ($p=0.00$).

^bEMF and sham group SOD values increased significantly compared to the control group ($p=0.00$).

^cEMF and sham group CAT values increased significantly compared to the control group ($p=0.00$).

^dEMF group 3-NT values increased significantly compared to the control group ($p=0.00$).

Table 4. Blood biochemistry results for control, sham and EMF group rats.

Blood biochemical markers	Control group	Sham group	EMF group
AMH (ng/mL)	5.21 ± 0.66	6.54 ± 0.12	6.11 ± 0.45
MDA (nmol/mL)	1.30 ± 0.10	1.26 ± 0.05	1.07 ± 0.11
SOD (ng/mL)	15.11 ± 0.64	16.26 ± 0.82	15.98 ± 0.45
GSH (mg/L)	577.15 ± 36.97	572.15 ± 7.04	556.31 ± 23.78
CAT (ng/mL)	48.17 ± 4.47	66.58 ± 1.34 ^a	59.1 ± 1.68 ^a
3-NT (nmol/L)	519.67 ± 25.66	572.45 ± 16.67	569.46 ± 18.38
8-OHdG (ng/mL)	4.25 ± 0.42	5.50 ± 0.07 ^b	5.01 ± 0.19 ^b
TOS (µmol H ₂ O ₂ Eq/L)	8.12 ± 1.22	8.14 ± 1.02	14.79 ± 1.47 ^c
TAS (µmol Trolox Eq/L)	0.76 ± 0.05	0.76 ± 0.05	0.82 ± 0.01
OSI	1.18 ± 0.31	0.88 ± 0.10	1.79 ± 0.16 ^d

The data represent mean ± SEM; AMH: anti-Müllerian hormone; MDA: malondialdehyde; GSH: glutathione; CAT: catalase; SOD: superoxide dismutase; 8-OHdG: 8-hydroxydeoxyguanosine; 3-NT: 3-nitrotyrosine; TAS: total antioxidant status; TOS: total oxidant status; OSI: oxidative stress index.

^aEMF and sham group CAT values increased significantly compared to the control group ($p=0.00$).

^bEMF and sham group 8-OHdG values increased significantly compared to the control group ($p=0.00$).

^cEMF group TOS values increased significantly compared to the control and sham groups ($p=0.00$).

^dEMF group OSI values increased significantly compared to the control and sham groups ($p=0.000$).

the EMF effect applied in both studies was one of 900 MHz, EMF exposure in our study occurred in middle and late adolescence, while in Türedi et al.'s (2016) study exposure was in the prenatal period.

Gul et al. (2009) placed a cell phone beneath a cage containing pregnant rats, in such a way as to be in direct contact with that cage, and exposed the rats to the effect of EMF for 12 h a day, with the cell phone in wait mode for 11 h and 45 min and in speak mode for 15 min. Similarly to our study findings, they reported no significant change when animals' body weights were compared. Female rats' right ovaries were examined on day 21, and a decrease in ovarian volume and total follicle numbers was observed in the group exposed to EMF (Gul et al. 2009). In our study, however, total follicle numbers were not investigated, numbers for each follicle type being calculated separately. According to our results, only secondary follicle numbers decreased in the EMF group compared to the control group, and we determined

no difference between the groups in terms of primordial follicle, primary follicle and Graafian follicle numbers. Bakacak et al. (2015) exposed 4-month-old rats to the effect of 900-MHz EMF for 15 min a day for 15 d. These were sacrificed at the end of EMF exposure during the estrus phase. They then counted primordial follicle numbers in five random sections from each ovary. The results from that study, in which primordial follicle numbers decreased in the EMF group compared to the control group, do not corroborate our own findings (Bakacak et al. 2015). No difference was determined between the EMF and control groups in our study. The main reasons for this may again be that the EMF exposure systems differed from one another, that Bakacak et al. (2015) sacrificed their rats in the estrus phase and the length of exposure to EMF. In addition, Bakacak et al. (2015) only performed counting in randomly selected ovarian tissue sections sampled at a rate of 1/10 based on a thickness of 50 μm once every 50 μm (Picut et al. 2015). The main reason for the variation between Bakacak et al.'s (2015) study results and ours is very probably the difference between counting methods.

Cecconi et al. (2000) applied 33-Hz and 50-Hz EMF to pre-antral mouse follicle culture for 5 d in their cell culture study. They reported no effect on follicle development up to the third day, but on the fifth day they observed decreased growth in subjects exposed to 33 Hz compared to the control group, while no difference was reported at 50 Hz. Severe impairment of antrum formation and decreased DNA synthesis in follicular granulosa cells with an impaired antrum and decreased estradiol release were reported at both frequencies (Cecconi et al. 2000). EMF causing decreased growth of preantral follicles and impairment of antrum formation in that study explains the decrease in secondary follicle numbers in our EMF group.

Long-term exposure to EMF has been reported to cause a decrease in antioxidant and an increase in TOS and OSI, leading to impairments of numerous organs and systems (Kunt et al. 2016). In our study, plasma TOS levels were higher in the EMF group than in the control and sham groups. No difference was determined among the groups in terms of plasma TAS levels. OSI values were also higher in the EMF group than in the control and sham groups. Çenesiz et al. (2011) applied 900 MHz EMF for 4 h a day over 20 d to one group and 1800 MHz EMF to another. They reported higher TOS values in both groups compared to the control group. TAS did not vary significantly between the groups (Çenesiz et al. 2011). In terms of TAS and TOS values, our findings are similar to those of Çenesiz et al. (2011).

In our study, CAT and SOD activity increased in tissue, while in serum only SOD activity increased. Various studies of reproductive and other systems have reported differing results in terms of SOD activity, irrespective of gender. Some studies have suggested that H_2O_2 inactivates SOD (Symonyan and Nalbandyan 1972). However, others have suggested that since CAT will react with the H_2O_2 that forms, levels of H_2O_2 will exhibit no inhibitory effect on SOD (Büyüksulu et al. 2006). In our study, free radicals increased because of EMF, and we think that this accounts for the increased activation of SOD and CAT.

Free radicals cause oxidative injury in proteins and amino acids (Stadtman and Levine 2003). Sypniewska et al. (2010) reported that exposure to a 35-GHz microwave and light caused the release of some proteins responsible for lysis in rat alveolar macrophages and an increase in 3-NT levels, and linked these changes to inflammation and oxidative stress (Sypniewska et al. 2010). Erdal et al. (2008) reported a significant increase in MDA and 3-NT values in liver tissues of female rats exposed to a magnetic field compared to female rats exposed to magnetic field and their own control groups among 8-week-old female rats exposed to a magnetic field for 4 h a day over 45 d (Erdal et al. 2008). These results are compatible with the findings of our study involving female rats.

No statistically significant change in tissue or serum MDA levels was determined in our study. Although some publications have observed an increase in MDA under the effect of EMF (Kerman and Senol 2012; Hajhosseini et al. 2013), no change has been determined in others. Uzar et al. (2011) exposed one group of rats to the effect of EMF from the pre-natal period until postnatal day 21, while another group was exposed to the effect of EMF until postnatal day 21 after birth of pups. Investigation of vertebral MDA levels revealed a significant increase in the group exposed to the effect of EMF in the pre- and post-natal periods, while no change was reported in the group subjected to postnatal exposure only. The authors suggested that this might be attributed to a greater effect of EMF in the embryonic period or to long-term EMF exposure increasing MDA levels (Uzar et al. 2011). Erdal et al. (2008) also suggested that the absence of a significant increase in MDA levels might be due to the length of EMF exposure (Erdal et al. 2008). Boder et al. (2015) reported that MDA levels in blood, kidney and brain of rats exposed to the effect of EMF increased compared to those of a control group, but that no change occurred in the liver. Based on the studies cited above, it may be suggested that MDA levels can vary depending on the tissue studied, length of exposure, SAR values, environmental factors and the EMF application apparatus (Boder et al. 2015). On the basis of this and other similar studies, we may conclude that MDA levels remained unchanged in our study due to the length of EMF or exposure or to organs being differently affected by EMF.

8-OHdG is a product of oxidative injury in DNA (Kasai and Nishimura 1984). While there was no difference in our study between the groups in terms of tissue 8-OHdG levels, serum 8-OHdG levels increased significantly in the EMF and sham groups. Lopucki et al. (2005) determined no difference between groups in human placental cotyledon 8-OHdG values exposed to a low-dose magnetic field (Lopucki et al. 2005). Other studies have reported an increase in 8-OHdG levels in tissue and serum (Yokus et al. 2005; Gürlü et al. 2014). On the basis of these studies, it may be concluded that the increase in serum 8-OHdG levels, while no change was observed in ovarian tissue 8-OHdG levels in our study may derive from the effect of EMF in other tissue not included in our study other than ovarian tissue. However, we possess no finding to support this hypothesis.

Our scan of the literature revealed no studies showing the effects of EMF on AMH levels. However, various studies have investigated AMH levels with ovarian follicle numbers and/or degeneration in rats (Ersoy et al. 2016). There was no significant difference between the groups in our study at the primordial and preantral follicle level. No significant difference was also observed between the groups in serum AMH levels. But AMH levels in tissue increased in the EMF group in our study compared to the control groups. In their study of preovulatory follicles, Kedem-Dickman et al. (2012) observed higher AMH levels in the follicular fluid of follicles with non-MII oocytes (immature oocytes) and atretic oocytes compared to those with MII oocytes (mature oocytes) in follicular fluid. They reported that AMH secretion plays a role in oocyte maturation that AMH inhibits oocyte meiosis and that AMH levels are therefore higher in immature oocytes than in MII oocytes. However, they also stated that impaired downregulation of AMH causes AMH levels in follicular fluid to rise and that the oocyte is exposed to atresia as a result of maintenance of meiosis inhibition. They attributed the high AMH in follicular fluids with atretic oocytes to this factor. We also think that oxidative stress caused by EMF may have compromised AMH downregulation in our study, and thus increased AMH levels. We also consider that this adversely affected oocyte maturation. However, we were unable to account for the increase in the sham group. We think that the histopathological findings in the EMF group ovarian tissue derived from damage caused by the oxygen radicals that formed. We also think that SOD and CAT levels in tissue increase to compensate for oxidative stress caused by EMF in ovarian tissue.

On the basis of our study findings, it may be concluded that exposure to a 900-MHz EMF in middle and late adolescence increases oxidative stress in the ovarium and leads to impairment of ovarian morphology and follicle quality and to decreases in mitotic activity and secondary follicle numbers.

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Disclosure statement

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