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ORIGINAL ARTICLE

The effects of electromagnetic fields on the number of ovarian primordial follicles: An experimental study

Murat Bakacak [a,*], Mehmet Sühha Bostancı [b], Rukset Attar [c], Özge Kizilkale Yıldırım [c], Gazi Yıldırım [c], Zeyneb Bakacak [d], Hamide Sayar [e], Agahan Han [f]

[a] *Department of Obstetrics and Gynecology, Kahramanmaras Sutcu Imam University, School of Medicine, Kahramanmaraş, Turkey*
[b] *Department of Obstetrics and Gynecology, Sakarya University Research and Education Hospital, Sakarya, Turkey*
[c] *Department of Obstetrics and Gynecology, Yeditepe University School of Medicine, İstanbul, Turkey*
[d] *Department of Obstetrics and Gynecology, Private Caka Vatan Hospital, Kahramanmaraş, Turkey*
[e] *Department of Pathology, Kahramanmaras Sutcu Imam University School of Medicine, Kahramanmaraş, Turkey*
[f] *Department of Obstetrics and Gynecology, Kanuni Sultan Suleyman Research and Education Hospital, İstanbul, Turkey*

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Abstract The aim of this study was to evaluate the effect of an electromagnetic field (EMF), generated close to the ovaries, on primordial follicles. A total of 16 rats were used in this study. The study group consisted of rats exposed to an EMF in the abdominal region for 15 min/d for 15 days. Both the study and control group were composed of eight rats. After the treatment period of 15 days, the ovaries of the rats were extracted, and sections of ovarian tissue were taken for histological evaluation. The independent samples *t* test was used to compare the two groups. In the study group, the means of the right and left ovarian follicle numbers were 34.00 ± 10.20 and 36.00 ± 10.53, respectively. The average total ovarian follicle number was 70.00 ± 19.03. In the control group, the means of the right and left ovarian follicle numbers were 78.50 ± 25.98 and 71.75 ± 29.66, respectively, and the average total ovarian follicle number was 150.25 ± 49.53. The comparisons of the means of the right and left ovarian

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* Corresponding author. Department of Obstetrics and Gynecology, Kahramanmaras Sutcu Imam Universitesi, Tip, Avsar Kampusu, Merkez, Kahramanmaras, Turkey.
E-mail address: muratbakacak46@gmail.com (M. Bakacak).

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follicle numbers and the means of the total ovarian follicle numbers between the study and control groups indicated that the study group had significantly fewer follicles ($p < 0.001$, $p = 0.011$, and $p = 0.002$, respectively). This study found a significant decrease in the number of ovarian follicles in rats exposed to an EMF. Further clinical studies are needed to reveal the effects of EMFs on ovarian reserve and infertility.

Introduction

Infertility is an important health problem that occurs in 15% of couples [1]. Knowing the status of the ovarian reserve is important for determining an infertility treatment method and for evaluating the probability of treatment success. The ovarian reserve is the amount of follicles in the ovary that can be ovulated in the future by the necessary stimulus [2,3]. Currently, a great number of tests can be performed to determine ovarian reserve [4]; however, histopathological examination of the ovary, which can generate more objective results, cannot practically be implemented, as the procedure is both invasive and decreases the ovarian reserve. Causes for a decrease in ovarian reserve include age, previous ovarian surgery, massive endometriosis, obesity, adhesions that cause anatomical defects in the pelvis, and courses of chemotherapy and radiotherapy [2,5]. In a study that examined the effect of a radiation field on ovary functions, ovarian failure was found in 68% of patients who had undergone abdominal radiotherapy involving both ovarian regions [6]. It is thought that the ever-increasing radiation and magnetic field exposure of humans in daily life will decrease ovarian reserve and increase infertility in the future.

An electromagnetic field (EMF) is an array of waves that arise with the gathering of electric and magnetic fields, which oscillate at a specific frequency and at definite intervals with each other. Mobile phones and wireless radiofrequency devices are the most common EMF sources that are used in close proximity to the human body. Many studies on the effects of mobile phones on human health have been conducted. In general, it has been reported that EMFs associated with the use of mobile phones cause sleep problems [7], weakness, headaches, loss of concentration [8], and an increased resting blood pressure [9]. Hardell et al. [10] reported that the use of mobile phones for $>$ 10 years increased the risk of acoustic neurinoma and glioma, with a higher risk in the half of the head corresponding to the side where the phone was used. That study also showed that tissues closer to the mobile phone were more greatly affected. Some studies on both the male and female reproductive systems have shown a negative effect of EMFs, while others have indicated no negative effect of EMFs. In a study of 52 male patients by Kilgallon et al. [11], a significant decrease in the sperm mobility of men who carried their mobile phone at the waist or gluteal area compared to those who carried the phone elsewhere or did not use a mobile phone was found. The results of that study support the hypothesis that tissues closer to EMF sources are more greatly affected.

The current study examines the effect of EMFs created by mobile phones positioned close to the ovaries on the number of ovarian primordial follicles (PFs) and the ovarian reserve. It is based on the hypothesis that tissues closer to an EMF source are more strongly affected [10,11].

Methods

Animals

Sixteen nulliparous and nonpregnant 4-month-old female Wistar-Hannover albino rats weighing 200—240 g were used in this study. The study conformed to the Helsinki Declaration and was conducted at the Yeditepe University Experimental Research Centre with the approval of the Yeditepe University Experimental Animal Ethics Committee. The rats were fed standard chow and tap water ad libitum and were kept at an ambient temperature of 22—26°C with 55—60% humidity and a 12 hour light-dark cycle.

Experimental protocol

The 16 animals were randomly allocated into two groups of eight; control group with no EMF exposure and study group with 900 MHz EMF exposure. The researchers performing the evaluations were blinded as to which rats were in which group.

No rats were anesthetized during the experiments. An EMF of 900 MHz was applied to the study group for 15 min/d for 15 days using the experimental exposure device. All applications were performed between 9:00 AM and 1:00 PM. All EMF applications were performed by the same researcher. No other mechanisms for EMF exposure were used. The rats in the experimental group were removed one by one from their cages. The left hand of the researcher grasped the rat at the neck and back and the right hand of the researcher applied the antenna device to the skin of the rat over the ovaries in the lower abdominal area (Fig. 1). A different researcher switched on the EMF exposure device to apply an EMF to the rat for 15 minutes. The same EMF application was used for all rats in the study group. The rats were put back into their cages after EMF application. The rats of the control group were taken one at a time from their cages in the same manner as the rats in the experimental group. As in the experimental group, the rats were only contacted by the antenna with no extra pressure applied directly to the abdomen. For the control

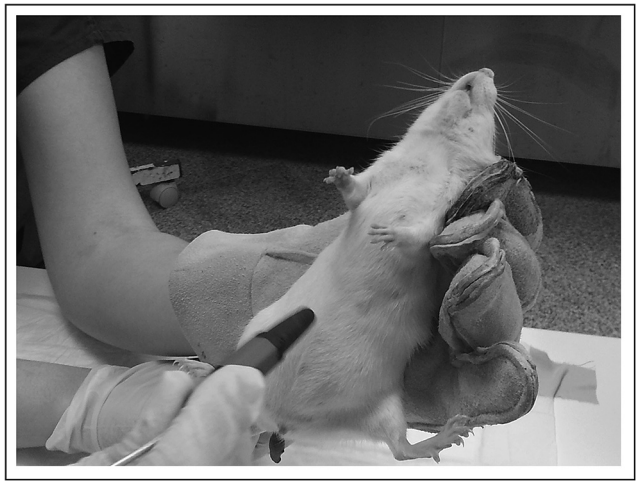

Figure 1. Electromagnetic field application method for experimental animals.

group, the device was not turned on; thus, no EMF was transmitted. After being held in this way for 15 minutes, the rats were put back into their cages. The study was completed at 15 days, and all animals were maintained until they began the estrous phase. The rats were then sacrificed, and the ovaries of both groups were extracted by dissection, weighed, and taken for evaluation.

Exposure device

An exposure device with a special antenna was used for generating the EMF (5 W peak output power and 1.04 mW/cm^2 power density), and the exposure emission was maintained at 900 MHz with a pulse repetition frequency of 217 Hz (Fig. 2). An animal experiment license is required to perform animal EMF exposure experiments in an unshielded environment at the frequencies used in this study, with the condition that performing the experiments in that unshielded environment will not cause any disruption to wireless communication. Therefore, the experiments were conducted in a Radio Frequency (RF)-shielded room and the devices were operated with an attenuation of 100 dB, which conforms to RF emission limits. The specific energy absorption rate (SAR) varied from 0.018 W/kg to 4 W/kg for the entire body. Thus, the heat effect generated by the device on the tissue was considered negligible. The power density and SAR measurements were performed in the Biophysics Laboratory of the Department of Biophysics (Yeditepe University, Faculty of Medicine, Istanbul, Turkey).

Figure 2. Electromagnetic field application device.

Estrous cycle

The body weights and daily phases of the estrous cycle were recorded before the experiment. After the last experimental day, the control and treated animals were maintained until they started the estrous phase. The estrous cycle phases were determined by observing a vaginal smear in the morning (08.00–10.00 AM.), as described by Zarrow et al. [12].

Histopathological examination

The extracted ovaries were fixed in 10% formalin solution and paraffin blocks were formed. As described in the literature, 5 mm sections were taken. The sections were enumerated according to the ovaries from which they were taken. One ovary from each rat was chosen randomly, and five sections were taken from that ovary and stained with haematoxylin and eosin [13,14]. The PFs in the sections were evaluated with an Olympus BX-50 (Olympus Optical Co, Ltd, Tokyo, Japan) microscope by a pathologist who was blinded to which group the sections were taken from (Fig. 3). The number of follicles in each section taken from the same ovary and the average follicle number of all five sections were determined.

Statistical analysis

SPSS version 21 (SPSS Inc., Chicago, IL, USA) program was used for data analysis. The normality of the data distribution was analyzed by the Shapiro-Wilk test and based on the coefficients of variation. Parametric methods were used for the analysis of the normally distributed variables, and nonparametric methods were used in the analysis of the nonnormally distributed variables. The independent samples *t* test was used for the comparison of the two groups. All quantitative values are presented in tables as mean ± standard deviation (SD). All categorical data are presented as numbers (*n*) and percentages (%). The data are presented with 95% confidence levels; and $p < 0.05$ indicates statistically significant differences.

Results

A total of 16 animals were included in the study, eight in the study group and eight in the control group. The mean weights of the rats were 222.5 ± 12.63 g and 220.88 ± 12.55 g in study and control group, respectively. No significant difference was found for mean rat weight between the study and control groups. In the control group, the mean right and left ovary weights were 74.3 mg and

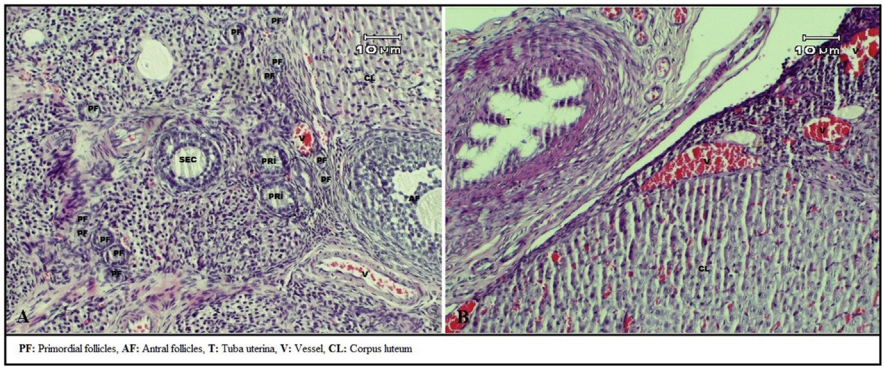

Figure 3. (A) Follicles in the ovarian sections taken from animals with no exposure to electromagnetic fields (EMFs) (×100 magnification). (B) Ovarian section (×100) from the subcortical region, which is expected to have the most primordial follicles, of animals exposed to EMF shows no primordial follicles.

71.8 mg, respectively. In the study group, these values were 72.1 mg and 69.5 mg, respectively. When combining the weights of the left and right ovaries, the mean ovary weights of the two groups were not significantly different ($p = 0.145$). For the experimental group, the average right and left ovarian PF numbers were 34.00 ± 10.20 and 36.00 ± 10.53, respectively, and the average of the total ovarian PF numbers was 70.00 ± 19.03. In the control group, the average right and left ovarian PF numbers were 78.50 ± 25.98 and 71.75 ± 29.66, respectively, and the average of the total ovarian PF numbers was 150.25 ± 49.53. The comparisons of the averages of the right and left ovarian PF numbers and the averages of the total PF numbers showed that the study group had significantly fewer PFs ($p < 0.001$, $p = 0.011$, and $p = 0.002$; respectively; Table 1, Fig. 4).

Discussion

With recent technological developments, people have become more exposed to EMFs in their daily lives. The biological effect of EMF exposure is the result of increased heat in the area of exposure or of energy absorption without heating. It is thought that the tissue damage caused by the EMFs of mobile phones is due to energy absorption rather than heating. However, studies on EMF-related tissue damage have produced conflicting results due to differences in the frequencies and widths of currents, magnetic flux densities, and exposure times used [15].

The influence of EMF exposure on the reproductive system has also been studied. Some contradictory results have been obtained in these studies, as a standardized methodology has not been used in the evaluation of the male and female reproductive systems. In addition to reports that EMF decreases fertility potential [16], sperm concentration, mobility, and seminiferous tubule diameter [17,18], and increases abnormal sperm morphology [19], there have also been reports that EMF does not affect the number of sperm in the testes or epididymis and does not alter sperm motility or morphology [20,21]. In previous animal experiments, it has been reported that EMFs decreased the number of follicles in the ovaries of the female reproductive system [22] and increased oocyte DNA damage [23], apoptosis, and oxidative stress in the endometrium and ovary [24,25]. Other meta-analyses and investigations have emphasized that EMFs have no negative effects on the female reproductive system [26,27].

There are a limited number of studies that have performed histopathological analyses of the effects of EMFs on the female reproductive system. One *in vitro* study

Table 1 Comparison of the mean number of follicles in the experimental and control groups.

	Experimental group ($n = 8$)	Control group ($n = 8$)	p[a]
Right ovarian PF average	34.00 ± 10.20	78.50 ± 25.98	<0.001
Left ovarian PF average	36.00 ± 10.53	71.75 ± 29.66	0.011
Total ovarian PF average	70.00 ± 19.03	150.25 ± 49.53	0.002

Quantitative data are presented as mean ± standard deviation values.
PF = primordial follicle.
[a] Independent *t* test.

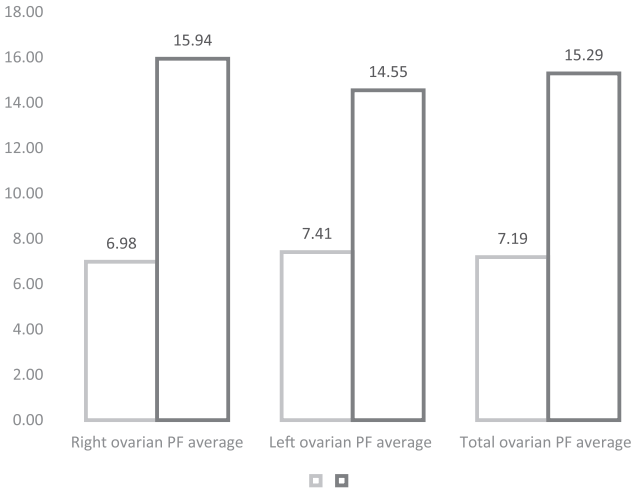

Figure 4. Distribution of mean numbers of follicles.

reported that 16 hours of EMF exposure damaged rat granulose cell DNA [19], and another study found that endometrial apoptosis and oxidative stress increased in animals with an EMF applied for 30 min/d for 30 days [21]. Research on the effects of EMFs on ovarian follicles has shown that oocyte nucleoli became smaller and deformed and the numbers of apoptotic bodies and autophagic vacuoles increased in granulose cells compared to the control group [28]. In a different study on the effects of EMFs applied to rats ($n = 43$), a mobile phone was placed just under the cages housing the experimental animals for 12 hours in total, including 11 hours and 45 minutes with the phone in standby mode and 15 minutes with the phone in the interactive mode. After 21 days, the right ovaries of all animals were removed to determine the ovary follicle numbers. For the histopathological analysis, sections with a 6 μm thickness were taken from the ovaries, and the follicles in the sections were counted. The results showed that the mean number of right ovary follicles in the group with EMF exposure was significantly lower than that of the control group ($p = 0.001$) [22].

Neither the EMF sources nor the frequencies of the EMFs were specified in the studies mentioned above. It has been suggested that the inconsistencies in the methods and the different durations and levels of EMF radiation used in these studies renders it impossible to make conclusions regarding the effects of mobile phones on tissues [26,27]. The use of experimental devices that reproduce the effect of mobile phones at a constant frequency rather than mobile phones themselves can provide more objective data. This is because frequency standardization is difficult to obtain in experiments performed with actual mobile phones, as conditions can vary; for example, an increase in frequency can occur due to a diminished battery charge, and a change in frequency can occur due to the conditions of the related base station. In the current study, the EMF was applied directly to the abdominal regions of the rats, as it was thought that the movement of rats inside the cage would affect the results obtained using a fixed EMF source.

In this study, the ovarian PF numbers of the rats exposed to EMF were significantly lower than those of the control group. This is the first reported study to analyze the direct effect of EMF application on the number of ovarian PFs in adult rats. Ominous effects of EMF exposure on the number of ovarian follicles and the ovarian reserve were observed in this animal study. Whether these effects alter fertility is not known. The limitations of the current study were as follows: (1) it was an animal experiment (although a human experiment would have been unethical); (2) ovarian follicle numbers could not be determined before the study due to technical difficulties; and (3) destruction and apoptosis were not analyzed in the extracted ovarian tissues. The small number of rats examined is another limitation of the current study, although the number was sufficient for statistical analysis. More studies that measure preintervention ovarian PF numbers in experimental objects randomized to both EMF exposure and control groups and have larger sample sizes are necessary.

In conclusion, the exposure of humans to EMFs is increasing with the widespread use of technologies, such as mobile phones and wireless communication. The non-standardization of EMF variables in previous studies has led to conflicting results. In the current study, a significant decrease in the number of ovarian follicles in rats exposed to EMFs was observed. Further clinical studies are needed to reveal the effect of EMFs on ovarian reserve and infertility.

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