CEOG Clinical and Experimental Obstetrics & Gynecology

Experimental Research

The effect of non-ionizing radiation on the ovarian reserves of female rats

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Summary

Objective: The authors aimed to investigate whether there was any effect of 1800 MHz GSM-modulated radio frequency radiation (RFR) as a source of non-ionizing radiation on the ovarian reserves of female rats by hematoxylin eosin staining under a light microscope, and also to evaluate the effect on the anti-Müllerian hormone (AMH) level of rats. *Design:* A prospective observational study. *Materials and Methods:* A total of 12 age-matched young adult female Wistar albino rats were divided into two groups. Group 1 (n=6) constituted the controls; group 2 (n=6) constituted the 1800 MHz exposed animals. RFR exposed group were kept ten cm away from the horn antenna to satisfy the near field condition. The control group was kept in the same setting without any RFR exposure. The exposure period was 20 minutes for five days/week for one month. *Results:* The results of this study showed that 1800 MHz RFR did not have a significant effect on the ratios of atretic follicles in rat ovary tissues when compared with the control group ($p > 0.05$). However, the authors detected statistically significantly higher AMH levels in RFR exposed groups ($p < 0.005$). *Conclusions:* 1800 MHz GSM-modulated RFR exposure in rats was found to have no adverse effect on ovarian reserves or follicles. The present authors' failure to detect any changes could be due to the limited duration of the RFR exposure or the limited number of subjects used in the study. AMH levels were significantly higher, which might be due to the aforementioned limitations in this study. There is a need for further experimental studies in which the effects of RFR emitted by cellular phones can be studied.

Key words: Radio frequency radiation; Rat; Ovarian reserve; Anti-Müllerian hormone.

Introduction

With developments in communication technology and the widening of opportunities offered by technology in this field, the use of mobile phones and the amount of radio and television broadcasting is rising with each passing day. Although these new technologies make life smooth for us, they also pave the way for possible health problems via dissemination of electromagnetic radiation (EMR). Since there are still many factors that remain unknown or poorly understood regarding the biological effects of electromagnetic waves, these factors have become the subject of recent scientific studies [1].

Some recent studies have indicated that the structure and functions of many enzymes and some cell organelles have been degraded by the effects of electromagnetic waves [2] and the risk of cancer has been shown to be increased after exposure to strong magnetic fields [3-5]. The World Health Organization (WHO) recently announced that radiation from cell phones can possibly cause brain cancer [6]. It has been revealed by the studies that the radiofrequency radiation emitted by standard cell phones can increase the rate of apoptosis in various tissue cells in the body and might be the result of harmful effects via oxidative stress [7, 8].

Women are born with a finite germinal reserve of resting primordial follicles. The establishment of the resting primordial follicle reserve begins early in fetal life and proceeds *via* a massive proliferative process that results in 7×10^6 potential oocytes at mid-gestation [9]. Apoptosis is responsible for the elimination of 85% of the potential oocyte population reached at mid-gestation, leading the developing ovary to contain just around 10^6 primordial follicles at birth [10]. The resting primordial follicle reserve continues to decline after birth, falling to around 400,000 oocytes when a woman enters puberty [10]. Several intracellular mechanisms responsible for apoptosis developing in normal and pathological situations in the prenatal and postnatal periods have been exhibited in the ovary [11-14]. While some genetic and molecular factors play an inductive

Revised manuscript accepted for publication February 10, 2016

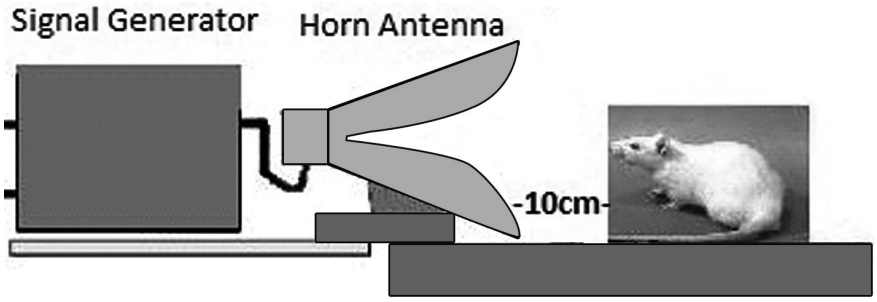

Figure 1. — The exposed rats (group 1) are kept ten cm away from the horn antenna to satisfy the near field condition.

role to this apoptosis, some lead to its' inhibition [15, 16]. Chemotherapy, radiotherapy, cigarette, and environmental chemicals increase the rate of death via apoptosis and thus cause the follicle reserves to run out more quickly [17]. Whereas there are many studies [18, 19] investigating the effects of electromagnetic waves causing ionization on ovary tissue in the literature, the number of studies investigating the effects of non-ionizing radiation is limited.

In the present experimental study, the ovarian reserve has been assessed by examination of the atretic follicles in the ovaries and the evaluation AMH levels in female Wistar albino rats, which were exposed to an 1800 MHz GSM-like radiofrequency electromagnetic field.

Materials and Methods

All experiments were performed on age-matched young adult female Wistar albino rats. The study conformed to the Helsinki Declaration. Procedures for using laboratory animals were approved by the Local Ethics Committee of Gazi University on the Use and Care of Animals Guidelines (Prot G.U.ET-09.037). Animals were housed in groups in Plexiglas cages kept in Gazi University Laboratory Animals Breeding and Experimental Research Center under well-controlled conditions of temperature at 22 ± 1°C, 45% humidity, under a 12-hour light/dark cycle and with free access to food and water.

Two groups were used in the experiment: group 1 (n=6): controls; group 2 (n=6): 1800 MHz exposed animals. The exposed group was kept ten cm away from the horn antenna to satisfy the near field condition (Figure 1). Rats in the group submitted as controls were used as 'sham' exposure animals. The control groups were kept in the same setting, without any RFR exposure. Animals were awake when they were exposed to RFR. The RFR or sham exposure periods were 20 minutes for all animals. The study was completed at one month, and all animals were maintained until they began the estrous phase. At the end of the study, the animals were anesthetized with ketamine 45 mg/kg and five mg/kg xylazine by intramuscular injection injection prior to decapitation. Blood samples were obtained for the determination of the AMH levels. The rats were then sacrificed, and the peritoneum was opened and ovaries of both groups were extracted by dissection and taken for evaluation.

While using mobile phones, users are generally exposed to RFR in the near field and the present authors aimed to simulate this situation in the present experiments. According to the ICNIRP 1998 Guidelines, the near field is the region where the distance from the radiating antenna is less than the wavelength of the radiated RFR [20]. At 1800 MHz the wavelength is about 16.6 cm. A synthesized signal generator was used for propagating the RF signal. A Horn antenna was used for application of RFR. Field strengths were controlled with a Narda EMR 300 and its appropriate probe during the exposures. Background RF level, to which controls were exposed, was measured at 0.265 V/m. The E field levels produced for 1800 MHz were 4.54 ± 0.41 V/m. The ICNIRP general public E field limits for these frequencies are 41.25 V/m and 58.34 V/m [20]. Since the E field levels used in this study are well below currently accepted limits, the exposure level used in this study can be considered non-thermal. Exposed E field levels were found to be higher than 0.265 V/m, which was the background E field. The signal generator and horn antenna exposures were performed in the Biophysics Laboratory of the Department of Biophysics (Gazi University, School of Medicine, Ankara, Turkey).

Oviductal tissue and fat were removed and ovaries were fixed for one night in 10% formalin solution, and paraffin blocks were made. As described in the literature, five 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 hematoxylin and eosin (HE) [21, 22]. The paraffin stained fractions were examined with a light microscope by a pathologist who was 'blinded' to which group the sections were taken from. The follicles observed in both ovary tissues were divided in seven groups as primordial, primary, secondary (preantral), tertiary (antral), early atretic, late atretic, and corpus luteum. The number of early or late atretic follicles has been found to be proportional to the total number of follicles. The percentages in control and study group have been compared.

For the quantitative determination of the AMH concentration in serum, an ELISA Kit for AMH was used. Its detection rate was 0.312-20 ng/ml.

Data were expressed as mean ± standard deviation ($\bar{x} \pm sd$) for each group. The Mann Whitney U Test was used to assess significance and $p < 0.05$ was considered to be statistically significant.

Results

All animals were age-matched young adult female Wistar albino rats. Normal development was been observed in animals during the experiment, and no deaths occurred. The average of the weights of rats used in the study was 238.6 ± 19.5 grams in the control group and 227.3 ± 16.5 grams in radiofrequency group. No meaningful statistical difference was found between two groups ($p = 0.302$). No statistically meaningful difference was also not found in the number of follicles between the control and radiofrequency groups ($p > 0.05$) (Table 1).

Table 1. — *The number of follicles in the control and radiofrequency groups.*

Follicle	Control group (n=6) Av ± SD	Median	Min-max	Radiofrequency group (n=6) Av ± SD	Median	Min-max	*p*
Primordial	2.5±0.7	2.5	1-4	3.8±3.6	3	0-9	1.000
Primary	2.8±1.9	1.3	0-6	2.7±1.6	2.5	0-5	0.518
Secondary	4.4±2.7	3	2-9	3.6±2.9	3.3	0-8	0.686
Tertiary	0.9±0.9	0.8	0-2	0.4±0.5	0.3	0-1	0.356
Corpus luteum	11.0±5.3	11.3	4-18	5.6±4.9	4.5	0-12	0.108

Table 2. — *The number of atresia in control and radiofrequency groups.*

	Control group (n=6) Av ± SD	Median	Min-max	Radiofrequency group (n=6) Av ± SD	Median	Min-max	*p*
Early atretic	3.2±1.0	3	2-5	4.4±3.7	4.5	0-10	0.514
Late atretic	8.4±7.1	7.3	2-19	9.3±6.6	9.3	2-19	0.809
Total atretic	11.6±7.4	9.8	5-22	13.8±9.2	11.5	3-25.5	0.574
Atretic/ T.follicle rate (%)	36.6± 23.2	32.6	13.9-66.7	47.5± 18.6	52.9	20.6-71.8	0.337

Table 3. — *Atretic/total follicular ratio in control and RFR exposed groups.*

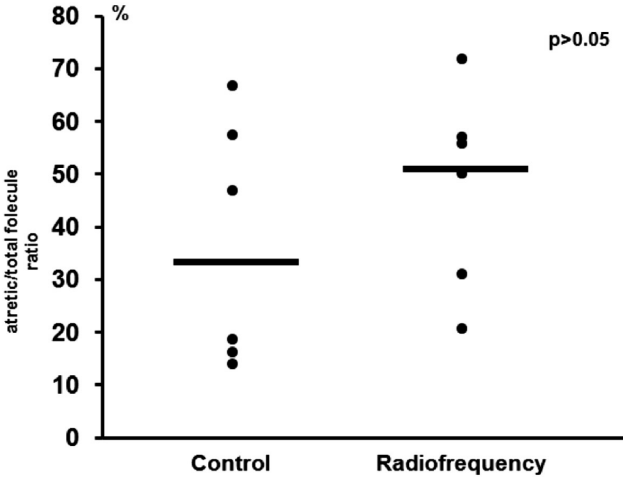

Table 4. — *AMH results in control and RFR exposed groups.*

Variable	Control	Radiofrequency	*p*
Number of examples (total 12)	6	6	
AMH ($\bar{x} \pm sd$)	1.16±0.70	5.49±4.55	0.009

When the ratios of early and late atretic follicles in all follicles were examined, it was found that the ratio of atretics in the control group was 0.366 ± 0.232 (median = 0.326) and the ratio of radiofrequency group was 0.415 ± 0.210 (median = 0.405). When two groups were compared, no meaningful difference was detected ($p = 0.423$) (Table 2).

No statistically meaningful difference was found in the number of atretic follicles in ovary and the proportion of atretic follicles to total follicles between the control and radiofrequency groups in this experimental study ($p > 0.05$) (Table 3).

The AMH levels of rats are listed in Table 4. As illustrated by the table, AMH levels were higher in RFR exposed group than in control group ($p < 0.005$).

Discussion

The use of cell phones, which are among the sources of radiofrequency electromagnetic energy, has rapidly become widespread. As we are exposed to RF waves spreading from cell phones, the studies about this subject have also increased rapidly. The effects of electromagnetic fields and the bio-effects of these waves on the reproduction system have been investigated by different studies in the literature [23-25] indicating that both the female and male reproduction systems are among the possible targets of non-ionizing radiation. However, no consensus has yet been developed from the results, and there is still a considerable need for well-designed studies on this subject.

There are numerous studies in the literature that show the adverse effect of radiofrequency waves produced by cell phones on endometrial tissue, granulose cells of the ovary, the quality of oocyte and embryo, the number of follicles, oocyte differentiation and folliculogenesis, risk of spontaneous abortion, and cardiac physiology of fetus in pregnancy [26-32].

Gul *et al.* also have found that the microwaves generated by mobile phones might decrease the number of follicles in rats [29]. Pregnant rats in the study group were exposed to mobile phones that were placed just under and in contact with the cages during the whole period of pregnancy. A mobile phone in a standby position for 11 hours and 45 minutes was turned on to speech position for 15 minutes every 12 hours. They found that the number of follicles in the exposed group was significantly lower than that in the control group, thus suggesting a toxic effect of RF-EMR *in utero* on pup ovaries ($p = 0.001$).

In a recent study, Bakacak *et al.* found a significant decrease in the number of ovarian follicles in rats exposed to an 1800 MHz EMF. The EMF was applied directly to the abdominal regions of the rats for 15 minutes/day for 15 days, as it was thought that the movement of rats inside the cage would affect the results obtained using a fixed EMF source [33]. Otherwise, it has been noted in some studies that different radiofrequency levels have no negative effect on the reproductive organs of rats or embryos, as seen in the present study [34-39].

Ogawa *et al.* observed no adverse effects of 1.95-GHz EMF exposure for 90 minutes/day in the morning on any reproductive and embryotoxic parameters, such as maternal body weight gain, number of live, dead or resorbed embryos, placental weights, sex ratios, weights or external, visceral or skeletal abnormalities of live fetuses [34].

Another study was performed to determine the effect of RF-EMR produced by cellular phones on baseline fetal heart rate, acceleration, and deceleration on non-stress test. Again, RF-EMR did not cause any demonstrable effect on fetal heart rate acceleration and deceleration on NST [35].

Elbetieha *et al.* investigated the prolonged exposure effect of 50 Hz magnetic field on the fertility of adult male and female mice. The authors found that this had no adverse effect on fertility and production in mice [36]. Another study showed that whole-body exposure to 2.14 GHz for 20 hours per day during gestation and lactation did not cause any adverse effects on pregnancy or the development of rats [37].

In a study by Sommer *et al.*, male and female mice were chronically exposed (life-long, 24 hours/day) to mobile phone communication electromagnetic fields at approximately 1966 MHz. According to Sommer *et al.*, the results of their study do not indicate any harmful effects on the histological, physiological, reproductive, and behavioral functions of long-term exposure of mice [38].

Aydin *et al.* showed that, the weights of the uterus and ovaries, progesterone levels, and estrogen levels were not significantly altered in adult Wistar female rats exposed continuously to a 50-Hz SLF-EMF for three months [40].

In the present study, measurable RF application was performed by using an RF signal generator and antenna system providing. Also, exposed groups were kept ten cm away from the horn antenna to satisfy the near field condition. The authors also chose the 20 minutes/day exposure because it is considered to be the mean exposure period of cellular phone use by most individuals. According to the present study, it was found that the exposure to radiofrequency radiation with 1800 MHz GSM for 20 minutes in a day has no meaningful effect on apoptosis in ovarian tissue of rats. This result might stem from the fact that the number of rats examined is small, although the number was sufficient for statistical analysis, and the time of daily and total RF application is short. Also in the present experimental conditions; the EMF was applied to the whole body of the rats for 20 minutes/day for a month in the cage; and exposed groups were kept ten cm away from the horn antenna. The limitations of the current study were as follows: it was an animal experiment (a human experiment would have been unethical) and ovarian follicle numbers could not be determined before the study due to technical difficulties.

The use of LH and FSH levels to evaluate ovarian reserve were measured in some studies. The marked reduction in FSH and LH levels may be associated with dysfunction of hypothalamic pituitary-gonadal axis that was shown by Al-Akhras *et al.* [41]. The present authors have compared the AMH levels of bloods taken from rats to evaluate the ovarian reserve differently. To the best of their knowledge, this is the first reported study to evaluate the effect of EMF application on the level of AMH state in adult rats.

AMH is a paracrine factor that is produced by granulosa cells of preantral and small antral follicles and suppresses initial follicle recruitment in the ovary [42]. In a study by Vural *et al.*, AMH and AFC were found to be the best ovarian reserve tests that can determine the total oocyte count retrieved [43]. The negative effects of chemotherapy and radiotherapy used in the treatment of cancer on AMH and ovarian reserve have been demonstrated in the literature. Serum AMH is a very convenient and sensitive indicator of follicular depletion and recovery in young women during and after chemotherapy [44].

In the literature, neonatal estrogen treatment and androgen administration stimulates AMH expression in the ovary [45, 46]. AMH is supposed to decrease in value according to the present authors' hypothesis, but AMH levels of 1800 MHz RFR-exposed rats showed a statistically significant increase in this study, which is difficult to explain. Perhaps this was due to the aforementioned limitations in the present study, and deserves further investigation.

Conclusion

EMF exposure is being reconsidered as new scientific information on radiation and health risks is produced. When it is considered that people from all ages are exposed to more RFR at close range as a result of carrying cell phones during the day, the present authors suggest that further studies should be conducted (including long-term exposure) to clarify many unknown aspects of the impact of electromagnetic radiation, and experimental designs randomized to both EMF exposure and control groups, and having larger sample sizes are necessary.

Acknowledgement

Animals for the project were supplied by the Fatih University Research Foundation, No: P53010911-1. Electromagnetic field measurement devices used in this study were supplied thanks to a grant from Gazi University Research Foundation, No: 31 / 2002-07.

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