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ORIGINAL ARTICLE

The effects of microwave emitted by cellular phones on ovarian follicles in rats

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Abstract
Objective The aim of this study was to investigate whether there were any toxic effects of microwaves of cellular phones on ovaries in rats.
Methods In this study, 82 female pups of rats, aged 21 days (43 in the study group and 39 in the control group) were used. Pregnant rats in the study group were exposed to mobile phones that were placed beneath the polypropylene cages during the whole period of pregnancy. The cage was free from all kinds of materials, which could affect electromagnetic fields. A mobile phone in a standby position for 11 h and 45 min was turned on to speech position for 15 min every 12 h and the battery was charged continuously. On the 21st day after the delivery, the female rat pups were killed and the right ovaries were removed. The volumes of the ovaries were measured and the number of follicles in every tenth section was counted.
Results The analysis revealed that in the study group, the number of follicles was lower than that in the control group. The decreased number of follicles in pups exposed to mobile phone microwaves suggest that intrauterine exposure has toxic effects on ovaries.
Conclusion We suggest that the microwaves of mobile phones might decrease the number of follicles in rats by several known and, no doubt, countless unknown mechanisms.

Keywords Follicles · Microwave of cellular phones · Rat

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Introduction

The expansive growth in mobile communication in recent years has resulted in an increasing exposure of the environment to weak radiofrequency (RF) electromagnetic fields (EMF). This has aroused a general interest in the possible effects of RF and microwave radiation (MWR) on human health. During the last decade, association has been suggested between chronic or long-term exposure to EMF and its toxic effects on reproduction [1, 2]. Calculation of the maximum temperature rise in the head from RF exposure during mobile phone use suggests that an increase of no more than about 0.10°C would be expected [3]. Thus, if there are some hard effects of low-level RF exposure on health, they are likely due to an increase in temperature [3, 4]. It was concluded that, although hazards from exposure to high RF fields were established, there have been no identified health hazards caused by low RF sources emitting fields, due to a significant temperature rise in tissues [5].

The misconception still persists that RF and MWR effects are solely the results of an increase in heat, contrary to the fact that a number of reported studies have demonstrated significant effects on various cellular activities in experimental systems under isothermal conditions [6]. An increased damage to macromolecules, by an increase in free radicals in cells, such as DNA, might be caused indirectly. EMF expositions may also modify the amount of cell

surface negative loadings. However, the magnitude of this effect is dependent on physical parameters and/or of the field applied. Specific absorption rate (SAR) is the rate of energy absorbed by a unit mass of the object and is usually expressed as W/kg. For 1°C temperature increase in human body, a power of 4 W/kg should be absorbed. It was identified that, on cellular level, modulated MWR already at low SAR levels below 10 W/kg changes the cell cycle, growth rates, enzyme activities, membrane structure and cellular transformation. [7–9].

Byus et al. [10] have reported a decrease in the activity of cyclic AMP-independent protein kinase in response to RF fields amplitude-modulated at extremely low frequencies (ELF). In biological systems, undesired effects, started or supported by EMF, trigger the cascade of events that end with adverse results [5]. Three mechanisms have been suggested to explain the effects of EMF on biological systems: magnetic induction, magneto mechanical effect and electronic interaction [2, 6]. It has been proved that microwave radiation causes changes in cell cycle and growth rates, enzymatic activities, structure of the cell membrane and cellular transformation [6–8]. It has also been showed that EMF affects the receptors on the cell surface [6]. In cellular aggregates obtained from tissues of animals, cells are separated by narrow fluid channels that take on special importance in signaling from cell to cell. These channels act as windows on the electrochemical environment surrounding each cell. These narrow fluid "gutters", typically not more than 150 Å wide, are also preferred pathways for intrinsic and environmental EMF, and they offer much lower electromagnetic impedance than cell membranes [2, 8]. Signals sent to the cells by RF radiation cause native proteins to improper fold. It results in the production of heat shock proteins by signaling the nucleus. EMF causes proteins in the cell membrane or free proteins in the cytoplasm to change in shape and disturbs their functions as receptors and enzymes [2]. In Bohr's study, it was pointed out that MWR could cause protein denaturalizing [11]. There has been an increasing concern that chronic or long-term exposure to EMF may cause adverse reproductive effects [1–4, 10, 12]. Reproductive effects of low energy of EMF are less well defined, and mechanisms responsible for effects on reproduction and development are not well understood [13].

In rats, undifferentiated gonads can be seen on approximately the 10th embryonic day as a part of the urogenital ridge, which forms from ventrolateral mesonephros and which are surrounded by coelomic epithelium. In mice ovaries, programmed cyst breakdown occurs at 20.5–22.5 days post-coitus, and at the end of this period only 33% of oocytes can reach the stage of primordial follicle. The mechanism of germ cell death has been poorly understood, but has been viewed as a random process, which can be exacerbated by nutritional deficits or by environmental factors [14, 15].

In this study, it was aimed to investigate whether there were any toxic effects of microwaves of cellular phones on the ovaries of rat pups.

Materials and methods

Materials

The approval for the study was provided by the ethical committee of Yuzuncu Yil University, Faculty of Medicine. A total of 60 female and 12 male Mus Musculus Swiss Albino-type, healthy, mature and reproductive rats were chosen. The rats were randomized as study (30 female, 6 male) and control (30 female, 6 male) groups. Every 15 female and 3 male rats were placed into polypropylene cages free from all kinds of materials, which could affect EMF. Water and standard pellet food were not restricted. Every morning all female rats were examined for the presence of vaginal plaque, which indicates coitus. Pregnant rats in the study group were exposed to cellular phones, the batteries of which were charged continuously. The phones were placed just under and in contact with the cage, for 11 h and 45 min in standby and 15 min in speech mode. The total amount of exposure time 12 h/day. Pregnant rats in the control group were exposed to cellular phones, the batteries of which were charged continuously. They were kept off in standby mode and placed just under and in contact with cage. Due to technical insufficiency, we could not measure the amount of microwave emitted by the cell phones. In this study 82 female pups of rats aged 21 days (43 in the study group and 39 in the control group) were used. On the 21st day after delivery, the female rat pups were killed and the right ovaries were removed.

The counting of ovarian follicles

Extracted ovaries fixed in bouin solution were placed in 10% formalin prior to the routine processing of the paraffin block. We obtained sections of 6 μm thickness. From the right ovaries of the rats, an average number of 12.1 ± 2.5 sections from the study group, and 12.5 ± 3.1 sections from the control group were obtained. Every tenth section was prepared as a slide and stained with hematoxylin and eosin (H&E) [15, 16]. As much as 43 slides from the study group and 39 slides from the control group were evaluated for the counting of follicles. Using a Nikon brand light microscope, the counting of follicles in the prepared sections was performed by a pathologist who was blinded to the study. The number of follicles in one ovary was calculated by counting the follicles in all the sections obtained from the same ovary.

Calculation of ovarian volume

To find the volume of ovarian sections, the images from Nikon TE 300 model microscope connected with a CCD (charge-coupled device) were recorded to power Mac 7500 model computer with the help of a computer-port. The area of ovarian sections was found by using the image J program. The volume of each tenth section was calculated by multiplying the area of an ovarian section by the thickness of the section (6 μm = 0.006 mm). The volume of each ovary was found by adding the volume of all sections obtained from the same ovary [15, 17].

Statistical analysis

The statistical analysis was done using SPSS 10.0 (Chicago, IL, USA) package program. Data in the study group were expressed as "mean ± standard deviation". The normal dispersion of groups was acquired by performing one-sample Kalmogonov Smirnov test ($P > 0.05$). The groups were evaluated according to Student's t unpaired independent test, since they had normal dispersion and were independent. The statistical significance was accepted as $P < 0.05$.

Results

The number of pups per delivery was found as an average of 4.8 ± 1.7 and 6.1 ± 1.3 in the study and control groups, respectively. There was a significant difference between the two groups in terms of the mean number of pups per delivery ($P = 0.001$). The average weight of pups in the study group and in the control group were 8.5 ± 1.3 g. (6–11.2) and 8.8 ± 1.2 g. (6.8–11.2), respectively. There was no statistically significant difference between the average weights of the two groups ($P = 0.282$).

The mean volume of the right ovaries of pups was found to be 0.5 ± 0.1mm^3 (0.3–0.7) in the study group and 0.6 ± 0.2mm^3 (0.1–1.6) in the control group. Concerning the ovarian volume, a statistically significant difference was found between the two groups (P = 0.005). In all sections obtained from the right ovary, all the follicles in every tenth section were counted and all of them were used in the calculation of follicle number for the right ovary of each rat. For one ovary, in all the sections obtained from every tenth section, the total number of follicles was averagely found as 475.4 ± 155.4 (186–798)/right ovary in the study group, and as 757.5 ± 275.9 (174–1333)/right ovary (Figs. 1, 2) in the control group. Statistically, when compared with the control group, the number of follicles was found to be significantly decreased in the study group ($P = 0.001$; Fig. 3). The number of follicles per 1 mm^3, which was calculated by the ratio of mean follicle number to the mean ovarian volume, was found to be 904.7 ± 312.7/mm^3 in the study group, and 1300.8 ± 395.8/mm^3 in the control group ($P = 0.005$).

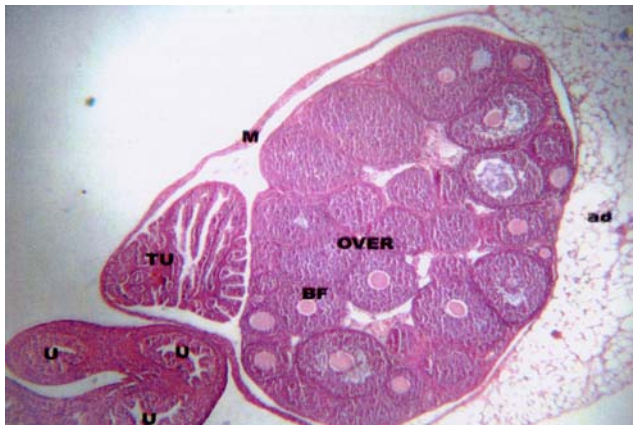

Fig. 1 View of follicles in an ovarian section from the study group. *BF* growing follicle, *TU* uterine tube, *U* fimbrial end, *M* mesothelium, *ad* perirenal adipose tissue. Hematoxylin-eosin ×4

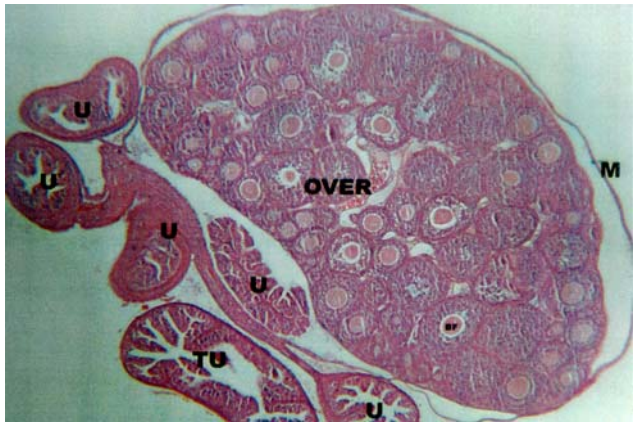

Fig. 2 View of follicles in an ovarian section from the control group. *BF* growing follicle, *M* mesothelium, *TU* uterine tube, *U* fimbrial end. Hematoxylin-eosin ×4)

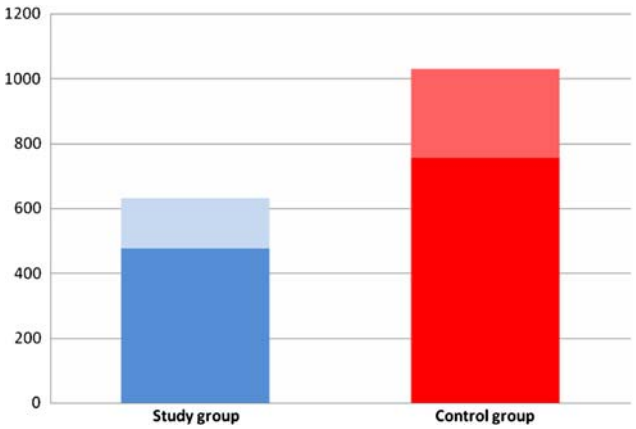

Fig. 3 The comparison of follicle numbers in right ovaries of pups in the study and control groups

Discussion

Several studies have been performed to investigate the effects of EMF on the reproductive system [18, 20]. Under the light of the previous results whether the exposure to EMF has any effects on reproduction and development has not exactly been defined yet [18–20]. It has been concluded that most of the effects observed were not due to an increased temperature in tissues. Chemical modulation on the cell surface resulting from poor EMF was demonstrated, and the poor signal at the beginning was shown to be enhanced by the binding of hormones, antibodies and neurotransmitters to the specific binding sites [21]. In one of the studies, some of the pups, which had been exposed to RF field during intra-uterine life were mated and the second generation pups were evaluated. No effect was observed in the pups of rats exposed to 915 MHz RF radiation. However, on exposure to 6 GHz RF radiation, there was developmental retardation in pups [22]. In our study, no significant difference was found in the mean weight of pups between the exposed and unexposed groups. Moreover, the number of follicles was found significantly decreased in the study group. The effects on reproduction has been evaluated on the basis of fertility rate of animals exposed to EMF, namely the number of pups per living delivery and the implantation and resorption rates [13, 22–24]. In our study, the number of living pups per delivery was 4.81 ± 1.74 among rats exposed to cellular phone MWR versus 6.13 ± 1.34 among unexposed rats. The difference between the two groups was statistically significant. No macroscopic anomaly was observed in other systems or organs. Our results are consistent with literature.

Elbetieha et al. [25] reported that after 90 days of exposure to a 50 Hz sinusoidal magnetic field, the fertility of mated mice was evaluated in order to investigate the effects of MF on it. A significant increase in the ovarian weight of the exposed mice was determined. This increase was attributed to the hypertrophy and hyperplasia in a specific tissue compartment in the ovary. In our study, ovarian weights were not measured. However, the volume of the sections was calculated and the mean ovarian volume was 0.54 ± 0.14 and 0.60 ± 0.24 mm^3 in the study and the control groups, respectively. The ovarian volume in the study group was found to be statistically lower than that in the control group ($P = 0.005$). There do not seem to be studies that evaluated the volume of ovaries other than ours.

In more technical studies investigating the effects of EMR on reproduction, the number of living pups per delivery and continuity of fertility were taken as reference parameters rather than histological examination [13, 23, 26, 27]. In the referred studies, it has been indicated that there is a decrease in these parameters. However, there have been no studies investigating the reproduction physiology histopathologically. For the first time in literature, by histological evaluation, this study questions whether electromagnetic radiation has any toxic effects on reproduction by causing changes in the number of ovarian follicles. We assume that the EMF from MWR emitted from cellular phones can cause a decrease in the number of ovarian follicles in rat pups exposed to cellular phone microwave radiation during their intrauterine life. Some known mechanisms such as apoptosis among follicles, hyperplasia in ovarian stroma and elongation mitosis time of cell. And no doubt countless unknown mechanisms may be causing this effect. We suggest that further studies are required to be done on this subject.

Conflict of interest statement None.

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