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Effect of a 20 kHz Sawtooth Magnetic Field Exposure on the Estrous Cycle in Mice

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Abstract Female mice post weaning were exposed to 20 kHz sawtooth electric and magnetic fields (EMF) with 6.25 μT peak intensity for 6 weeks. Estrous cycles were checked using vaginal smears over the last 10 days of the experimental period. The vaginal smears from EMF-exposed mice revealed an increase in the frequency of one or two phases persisting. The number of estrous cycles less than 1 was more in the EMF-exposed group than in the sham control group. Furthermore, in the EMF-exposed group, the duration of proestrous and metestrous stages of the estrous cycle was significantly increased compared with the control group. In conclusion, our results suggest that exposure to 20 kHz sawtooth EMF may affect normal cycling of the estrous cycle by disrupting the female reproductive endocrine physiology. We should not disregard the possible adverse reproductive effect of the 20 kHz sawtooth EMF generated under the occupational exposure situation in females.

Keywords: 20 kHz EMF, estrous cycle, mice

With an ever-increasing variety of technologies generating electric and magnetic fields (EMF), such as the production, distribution, and use of electric power, TV and radio broadcasting, and mobile telecommunications, concerns have been voiced in many countries about the possible adverse health effects of EMF. The predominant field frequency of human exposure corresponds to the 50–60 Hz range, which is in the range described as extremely low frequency (ELF), used for industrial and domestic electrical supply. The frequency range from 3 to 30 kHz is known as very low frequency (VLF). Human exposure to magnetic fields in this range is primarily from computer monitors or VDTs (video-display terminals), resulting from the approximately 20 kHz horizontal modulation of the electron beam [6]. ELF EMF exposure of rat granulosa cells induced DNA damage in the form of DNA single- and double-strand breaks and could increase the propensity for morphine-induced conditioned behaviors in rats [9, 14]. The possibility of adverse reproductive effects of exposure to EMF is also one of the important scientific debates [8, 19]. Epidemiological data of birth defects and miscarriages among pregnant office workers have led to concern that exposure to EMF might affect human reproduction [1, 7], but attempts to demonstrate such effects in human studies have generally been unsuccessful [2, 17, 24]. However, because of the allegation that there may be particular windows of frequency, wave shape, and intensity that may be deleterious, it is difficult to disregard low frequency EMF exposures as having no deleterious reproductive effects. Moreover, previous studies have focused exclusively on the possible reproductive toxicity resulting from maternal and fetal exposure during gestation [6, 10, 12, 13]. There have been fewer studies concerned with the reproductive risks resulting from the direct exposure of young female. In this study, we examined the effect of the EMF on the estrous cycles as a reproductive parameter in female mouse, under the usual environmental exposure of a 20 kHz sawtooth magnetic field, which is a frequency used in TV sets and personal computers, the necessaries of modern society [6], and the magnetic field strength of 6.25 μT peak intensity, which is the regulated exposure limit of the magnetic field for the public at 20 kHz [28].

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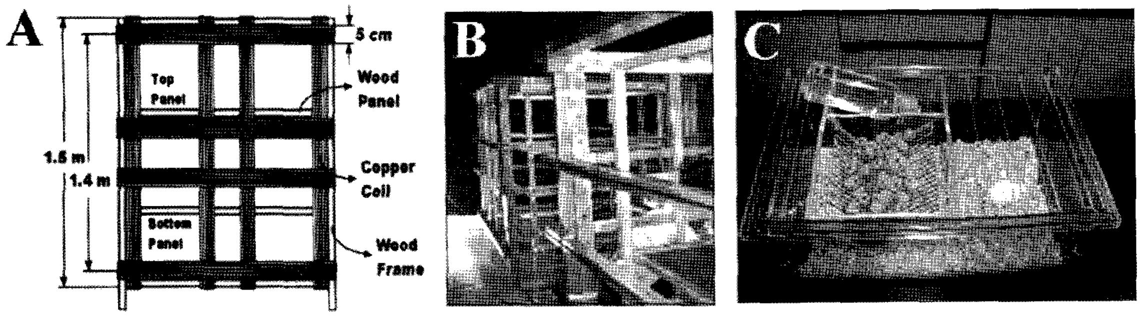

Fig. 1. The 2-axis magnetic field exposure equipment (20 kHz, 6.25 μT).
A. Schematic diagram of equipment. **B.** Pictures of the equipment. **C.** Type of cage.

MATERIALS AND METHODS

Design of the Magnetic Field Generation and Monitoring Equipment

The magnetic field generation and monitoring equipment were designed and constructed in collaboration with the EMF & Environment Research Team, Radio & Broadcasting Technology Lab, ETRI, Daejeon, Korea and previously described by Kim *et al.* [12]. Briefly, a two-axis magnetic field exposure equipment was designed to produce the magnetic field strength of 6.25 μT peak intensity. The exposure setup was made of wood in order to minimize the field perturbations, and the size was 1.5×1.5×1.5 m. The exposure setup consisted of four horizontal and four vertical wood frames on which the same number of turns on each coils were applied. Inside the frames, two wood panels (1×1 m) were placed and used as an exposure shelf where cages were positioned. The frequency was set to 20 kHz, which is the frequency of TV sets and personal computer monitors. A sawtooth current waveform was applied. A high voltage square wave generator was designed and constructed in order to supply and adjust input power (Vp) and the current waveform. For the magnetic field generation, coils of 1.2-mm-diameter enamel-coated copper wire were used. The maximum approved current of the coil was 3 A. Magnetic field strength and uniformity at two (top and bottom) panels in the exposure equipment were measured using a 3-axis magnetic field probe (HI-3637, Holaday Industries, Cedar Park, TX, U.S.A.). Each panel surface was divided into 25 regions (five horizontal and five vertical divisions) in order to estimate the field uniformity in the exposure chamber. The Vp was adjusted so that the magnetic field strength reached 6.25 μT peak at the center of the top panel. Measurement results of the magnetic field strength at the various positions in the exposure chamber were 6.17±0.16 μT at the top panel and 6.14±0.20 μT at the bottom panel. The field uniformity was 4–7% with respect to the center of the top panel. The Vp was 250 V and the output magnetic field waveform was the sawtooth wave of 20 kHz.

Animals and Exposure Protocol

Female ICR mice (SLC, Shizuoka, Japan), 21 days of age after weaning, were exposed to sham (control group, n=15) and 20 kHz sawtooth magnetic fields with 6.25 μT peak intensity (EMF group, n=15) for 8 h/day for 6 weeks. The sham-exposed animals were also kept on the wooden frames placed in a separate room. Five female mice were housed in each polycarbonate cage with a specially designed acrylic cover, watering bottle equipped with glass straw, and wood chip bedding (Fig. 1C). The distance that the animals were from the coils was 30 cm from the side panel and 35 cm from the top panel. Three cages were placed on top panel in the exposure chamber, and the cage positions were rotated every day during exposure to avoid minute differences in exposure conditions among the cages. Animals were maintained under controlled room temperature at 20±2°C, 45±5% humidity, and 12 h light/dark conditions. Standard rodent chow (Samtaco, Osan, Korea) and water were provided *ad libitum*. This study was conducted under guidelines for the use and care of laboratory animals, which was approved by the Institutional Animal Care and Use Committee of the Korea Institute of Radiological and Medical Sciences.

Check of Estrous Cycle

All animals were subjected to examination of the change of estrous cycle during the last 10 days of the experimental period, which is approximately 2 estrous cycles in mice. The phases of the estrous cycle were determined by observing the vaginal smear in the morning (09:00–11:00) as described by Rugh [23]. Vaginal smears are best obtained by means of an ordinary pipette, the tip of which has been flamed to a smooth, reduced aperture. A few drops of 0.9% sodium chloride solution are drawn into the pipette, introduced into the vagina, and then retracted into the pipette. The fluid is transferred to a slide and mounted under a coverslip with a trace of 1% methylene blue to add contrast and bring out the nuclei. Examination for cell type is carried out under low and then high power magnification, with reduced lighting. Cells may be nucleated or cornified

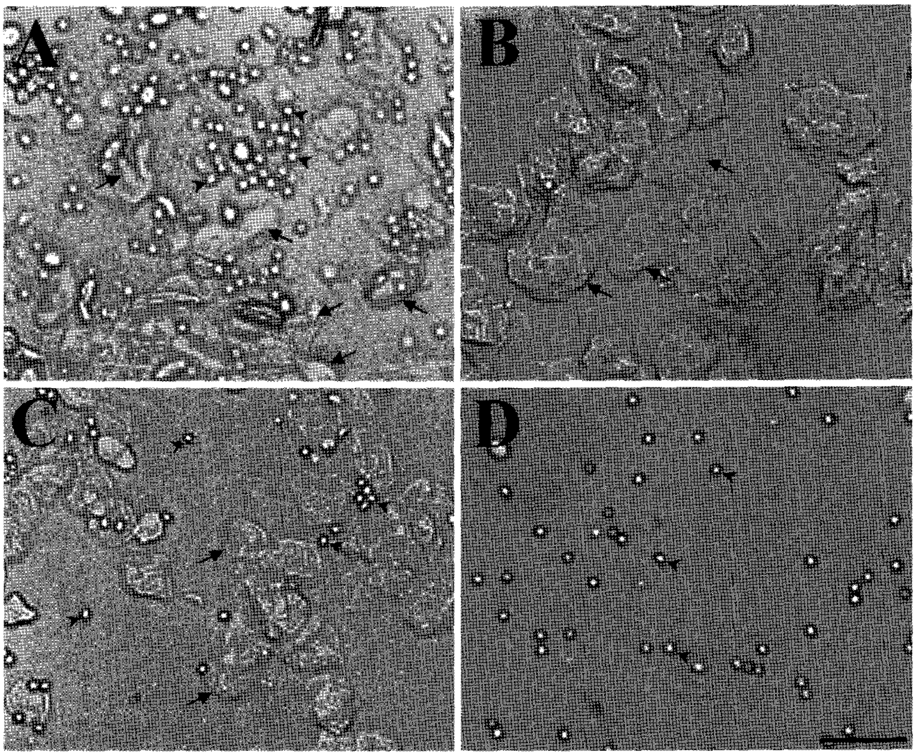

Fig. 2. Each stage of the estrous cycle of ICR mice.
A. Proestrous; **B.** Estrous; **C.** Metestrous; **D.** Diestrous. Arrows, epithelial cells; arrowheads, leukocytes. Bar=50 μm.

(old, non-nucleated) epithelial cells or leukocytes. The nucleated epithelium is generally oval or polygonal, with obvious nuclei that stain readily. As estrus approaches, there may also be some mucous present, easily identified by the proper stain. The phases through which the vaginal cells go represent parallel changes in the entire reproductive tract and are hence diagnostic. The major normal stages and their characteristics are as follows (Figs. 2A–2D): Proestrus, showing nucleated epithelial cells in approximately equal numbers; Estrus, showing large and squamous-type epithelial cells without nuclei; Metestrus, showing approximately equal numbers of leukocytes and epithelial cells, but the latter are large, folded, and with translucent nuclei; Diestrus, showing almost exclusively leukocytes.

Statistical Analysis

Blind test was conducted for assessments of the estrous cycle. To determine the total number of estrous cycles during 10 days of monitoring, each stage of the estrous cycle was counted as 0.25 cycle, and 4 consecutive stages of the estrous cycle was counted as 1 cycle. Data were subjected to Student's t-test to compare the number of estrous cycles and the duration of each phase of the estrous cycle between the control group and the EMF-exposed group. Chi-square test was used to compare the distribution of mice having the number of estrous cycle of at least 1 or less than 1 between the two groups. The P value of 0.05 or less was used as the criterion of statistical significance.

RESULTS

The background 50/60 Hz field due to room wiring and other electrical equipment was minimal. There is significant difference in the distribution of mice having the number of estrous cycle of at least 1 or less than 1 between the control and EMF group during the last 10 days of the experimental period (Table 1). The vaginal smears from EMF-exposed mice revealed a significant decrease in the mean number of estrous cycles when compared with the control group. In the EMF-exposed group, the frequency of one or two phases

Table 1. The distribution of mice having the number of estrous cycle of at least 1 or less than 1.

Treatment	(n)	Number of estrous cycles		P-value
		1	<1	
Control	(15)	13[a]	2	0.017*
EMF	(15)	7	8	

[a]Frequency.
*There is significant difference in the distribution of mice having the number of estrous cycle of at least 1 or less than 1 between the control and EMF group by Chi-square test at $p<0.05$.

Table 2. The number of estrous cycles and the duration of each phase of the estrous cycle.

Treatment	(n)	Mean number of estrous cycles[a]	Duration of each phase (days)[b]			
			Proestrous	Estrous	Metestrous	Diestrous
Control	(15)	1.28±0.38	1.63±0.76	2.47±2.00	2.84±2.29	1.15±0.49
EMF	(15)	0.83±0.44*	2.85±2.51*	3.75±2.93	4.41±2.78*	1.25±0.87

[a]The mean number of estrous cycles during 10 days of monitoring. Each stage of the estrous cycle was counted as 0.25 cycle, and 4 consecutive stages of the estrous cycle was counted as 1 cycle (Mean±SD).
[b]Duration of each phase (days) per estrous cycle was counted for 10 days of monitoring (Mean±SD).
*Significantly different from that of the control by Student t-test at $p<0.05$.

of estrous cycle persisting increased and the duration of proestrous and metestrous stage of the estrous cycle was significantly increased compared with the control group (Table 2).

DISCUSSION

The safety of human exposure to an ever-increasing number and diversity of EMF sources both at work and at home has clearly become a public health issue. In the present study, we observed the effect of a 20 kHz sawtooth magnetic field on the estrous cycle of female mice. Vaginal smears revealed statistically significant difference between the sham control and the exposed groups. EMF-exposed mice revealed an increase in the frequency of one or two phases persisting, and the mice having the number of estrous cycles less than 1 was more in the EMF-exposed group than in the control group. There was also significant decrease of the number of estrous cycles in the EMF-exposed group. Furthermore, the estrous cycle length of females exposed to EMF appeared to be longer than that of females of the control group. Similarly, there was a significant increase of the length of the estrous cycle of dairy cows exposed to 60 Hz EMF [3, 22]. ELF-EMF exposure reduces the capacity of the follicles to reach a developmental stage [4]. A longer estrous cycle may be a sign of reproductive impairment and correlates with a decrease in female fecundity [5]. However, Dawson *et al.* [6] failed to find evidence of disruption of the estrous cycle in female Wistar rats exposed to 10 kHz sinusoidal EMF at 0.2 mT. Because 20 kHz sawtooth EMF exposure resulted in significant changes in the estrous cycle in mice, species difference as well as the type and frequency of EMF should be considered for evaluation of the reproductive hazard of EMF exposure in animal experiments. Recently, however, Kim *et al.* [12] reported that exposure to a 20 kHz sawtooth magnetic field with 6.5 μT peak intensity does not inflict any adverse effect on fetuses of pregnant mice. Together, this suggests that the estrous cycle regulated by ovarian steroids is more sensitive to EMF exposure compared with fetal development and feto-maternal interaction and may be a major target of reproductive function impairment by EMF exposure in female.

Basically, the reproductive system is regulated by both neural and endocrine factors acting in the brain gonadal axis. Evidence from earlier studies in female rats had also been interpreted to indicate that neuroendocrine factors were primarily responsible for the loss of cyclicity [18, 26]. The changes in the pattern of the estrous cycle are closely associated with gonadal steroids, which in turn are controlled by the pituitary gonadotropins and hypothalamic gonadotropic releasing hormones. Thus, the changes of the estrous cycle observed in the present study may attribute to alterations at any level of the hypothalamic-pituitary-gonadal axis. Although still a matter of debate, exposure to EMF may suppress the synthesis of pineal hormone melatonin (MLT) in several species, in a fashion similar to light [11, 20, 21, 25, 27]. MLT acts in the mediobasal hypothalamus to modulate pulsatile luteinizing hormone releasing hormone (LHRH) secretion, triggering variations in the secretion of gonadotropins [15, 16]. This, in turn, may influence the production of gonadal steroids governing the estrous cycle in mice. Although we did not analyze changes in the hormonal profile of the mice following EMF exposure, alteration of the estrous cycle in EMF-exposed mice suggests a possible occurrence of endocrine disruption.

In summary, the exposure of 20 kHz sawtooth magnetic fields, which is the frequency of TV sets and personal computer monitors, showed the frequency decrease of the estrous cycle in exposed mice. It cannot be disregarded the possible adverse reproductive effect of the 20 kHz EMF under environmental exposure. Further elucidation of the alteration of the brain-gonadal axis following EMF exposure will be helpful for a comprehensive understanding of the toxic mechanism for EMF exposure.

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