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·*Original Article*·

Evaluation of spermatogenesis and fertility in F1 male rats after *in utero* and neonatal exposure to extremely low frequency electromagnetic fields

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Abstract

Aim: To determine whether *in utero* and neonatal exposure to a 60 Hz extremely low frequency electromagnetic field (EMF) results in spermatotoxicity and reproductive dysfunction in the F1 offspring of rats. **Methods:** Age-matched, pregnant Sprague–Dawley rats were exposed continuously (21 h/day) to a 60 Hz EMF at field strengths of 0 (sham control), 5, 83.3 or 500 μT from day 6 of gestation through to day 21 of lactation. The experimentally generated magnetic field was monitored continuously (uninterrupted monitoring over the period of the study) throughout the study. **Results:** No exposure-related changes were found in exposed or sham-exposed animals with respect to the anogenital distance, preputial separation, testis weight, testicular histology, sperm count, daily sperm production, sperm motility, sperm morphology and reproductive capacity of F1 offspring. **Conclusion:** Exposure of Sprague–Dawley rats to a 60 Hz EMF at field strengths of up to 500 μT from day 6 of gestation to day 21 of lactation did not produce any detectable alterations in offspring spermatogenesis and fertility. (*Asian J Androl 2005 Jun; 7: 189–194*)

Keywords: spermatogenesis; electromagnetic fields; prenatal exposure; postnatal exposure; rat

1 Introduction

Extremely low frequency (ELF, 50 and 60 Hz) electromagnetic fields (EMF) are associated with the production, transmission, and use of electricity; thus the potential for human exposure is very high [1]. Therefore, the possible adverse effects of ELF EMF on reproductive and developmental outcome have been extensively studied in both experiments involving animals and humans over the past several decades [2]. However, limited data have been published about these potential adverse effects [3–11]. Moreover, there have been conflicting findings regarding the alteration of spermatogenic and reproductive functions. A number of studies showed that exposure to ELF EMF did not induce any adverse effects on spermatogenesis and reproductive capacity in experimental animals and human [3-6]. In contrast, some studies conducted by other investigators showed clear damage to spermatogenesis [1, 7–11].

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Therefore, more careful and detailed studies need to be carried out to determine whether EMF exposure can induce adverse effects on spermatogenesis and reproductive capacity.

In view of the fact that embryo-fetuses and young, growing animals are more susceptible to the toxicity of xenobiotics, exposure to any xenobiotic during gestational and lactational stages of gonadal development may lead to permanent damage to the gonads. The present study was carried out on rats to investigate the potential adverse effects of *in utero* and neonatal exposure to 60 Hz EMF on the spermatogenesis and fertility of F1 male offspring.

2 Materials and methods

2.1 Animal husbandry and maintenance

Approximately 9-week-old, male and female specific-pathogen-free Sprague–Dawley rats (Orient Co., Seoul, Korea) were quarantined and acclimatized for 1 week before the experiment. Two female rats were placed into the cage of one male rat overnight. Vaginal smear was performed the next morning and this was designated as day 0 of pregnancy if sperm were detected. Forty pregnant females were then housed in a room maintained at a temperature of 23 ± 1°C, with good ventilation, a relative humidity of 50 % ± 10 % and artificial lighting from 08:00 am to 08:00 pm. Flow cytometric analysis of the effects of 50 Hz magnetic fields on mouse spermatogenesis. All animal rooms were constructed of non-metallic materials. Mated females were housed individually in clear polycarbonate cages with polycarbonate lids, and the cages were placed on racks designed and built specifically for EMF exposure. The standard laboratory rodent diet (Jeil Feed Co., Daejeon, Korea) and sterilized water were available *ad libitum*. This experiment was conducted in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International and animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals [12].

2.2 EMF exposure facility

EMF generation equipment was designed and constructed by the Korea Electrotechnology Research Institute as shown in Figure 1. The EMF was monitored continuously throughout the study by observing the current injected to the exposure system (this current is pro-

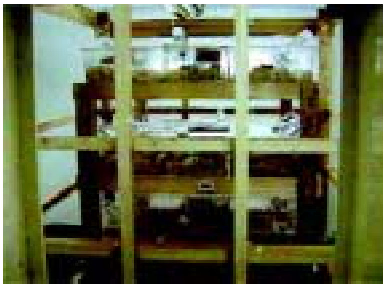

Figure 1. Picture of magnetic exposure facility.

portional to the EMF). The generator housing capacity was for approximately 50 rats. The field generator consisted of four square coils and a shelf system with three levels (top, middle and bottom) for the animals. Ten test animals were placed on each level. The spatial variation of the magnetic field (MF) was under 3 % within the animal testing area (1 m × 1 m × 1 m). Also, the temporal variation was negligible during the experimental period because high-performance automatic voltage regulation was adopted. The voltage fluctuation rate and harmonic rate of automatic voltage regulation using a power amplifier was nearly 0 %. The EMF exposure system is described in detail in a previous study [13].

2.3 Experimental design and EMF exposure

The study was conducted using four groups of rats; each group comprised 10 pregnant animals. Three groups were exposed to EMF and the fourth group served as a sham control. The sham-control female rats were handled in an identical manner to the EMF-exposed female rats. The field strength of 500 μT is the highest field strength reasonably attainable within an experimental exposure system and is approximately 2500 times higher than that to which humans are routinely exposed in normal residential environments. Most residential exposure is to magnetic fields that are less than 0.2 μT in intensity, although some areas in homes may exceed this intensity [14]. The field strength of 83.3 μT is the maximum exposure level recommended by WHO/ICNIRP (World Health Organization/International Commission for Non-Ionizing Radiation Protection) [15] and 5 μT is the expected maximal exposure level under a 765 kV Korean domestic electric cable. Based on these facts, the target MF strengths were selected as 5, 83.3 and 500 μT. The dosimetry measurement was done on each day of the experiment while the exposure of the rats to the EMF was taking place. Animals received sham exposure or exposure to 60 Hz EMF for 21 hours per day (12:00 am–9:00 am) from day 6 of gestation to day 21 of lactation. The study was blinded.

2.4 Examination of dams and F1 offspring

All dams were observed daily throughout gestation and lactation for mortality, morbundity, general appearance and behavior. Effects of EMF exposure on body weight change and parturition were also monitored. On day of delivery, the anogenital distance of male pups was measured using a caliper to determine the distance between the anus and the genital tubercle. On postnatal day 4, eight pups were selected (four males and four females, if possible) from each litter to produce comparable litter sizes, whenever possible. At weaning, two males and one female from each litter were randomly selected. Clinical signs and the day of preputial separation in male offspring were observed.

At 10 weeks old, one male rat from each litter was selected and was killed by carbon dioxide overdose. After external and internal macroscopic examinations, the testes were removed and weighed. Sperm analysis was conducted as has been previously described in other studies [16, 17]. In order to ascertain sperm head count, the left testis was homogenized and sonicated with 12 mL distilled water. The number of spermatids in the sperm suspension was counted under a light microscope with a Neubauer hemacytometer (Paulmarine, Germany). Step 17–19 spermatids survive in this homogenization and can be counted in a hemacytometer. In the rat, developing spermatids spend about 6.3 days in these steps [18, 19]. Thus, daily sperm production was calculated by dividing the number of spermatids determined, on a per-testis basis, by 6.3. To assess motility, the left caudal epididymis was minced in Hank's Balanced Salt Solution pH 7.2 (Sigma Chemical Co., St. Louis, MO, USA) containing 10 $mg \cdot mL^{-1}$ bovine serum albumin and maintained at 37 °C. Motility was observed using a microscope with a microwarm plate. Sperm morphology was also examined under the light microscope using sperm smears (sperm suspension containing 1 % eosin Y) collected from the left caudal epididymis. A total of 2000 sperm from each rat were examined for abnormalities in different regions of spermatozoa. The right testis was fixed with Bouin's solution for approximately 24 h, stored in 70 % ethyl alcohol for several days, embedded in paraffin, sectioned and stained with hematoxylin and eosin for histopathological examination. Transversal testicular sections were examined for testicular characteristics and the spermatogenic stages.

When the offspring reached maturity (11 weeks old), remaining one male and female from each litter were mated for 2 weeks (one male to one female, with brother-sister mating avoided) to evaluate their reproductive capability. Successful mating was ascertained by the presence of sperm in a vaginal smear and/or vaginal plug, and the 24 hours immediately following this was designated as day 0 of gestation. All females were subjected to cesarean section on day 15 of gestation. Based on the results, mating and fertility indices were calculated.

2.5 Statistical analyses

Statistical analyses were performed by comparing the exposed groups with the sham control group using SAS software (SAS Institute, NC, USA). Variables such as the number of live young, anogenital distance, the day of preputial separation, testes weight, sperm head count and daily sperm production were subjected to one-way analysis of variance (ANOVA) and the percentages of motile sperm and sperm abnormalities were analyzed by the Kruskal–Wallis nonparametric ANOVA [20]. If either of the tests showed a significant difference among the groups, the data were analyzed by the multiple comparison procedure of the Dunnett's post-hoc test [21]. The level of significance was taken as $P < 0.05$.

3 Results

As shown in Table 1, there were no statistically significant differences in the number of live young at birth, clinical signs, and body weight development of male offspring between exposed and sham-exposed groups. The anogenital distance of male offspring in the 5 μT group was slightly shorter (about 7 %) than that in the sham control group, but no significant difference was detected between the groups. No exposure-related effects were seen on the day of preputial separation; nor had the testis weight of male offspring been affected. On testicular histopathological examination, no exposure related changes were observed in the number and morphology of germ cells between the exposed and sham-exposed groups. As shown in Table 2, copulation and fertility indices in the exposed groups were similar to those in the sham control group. The sperm head count and daily sperm production of male offspring in the 500 μT group

Table 1. Effects of *in utero* and neonatal MF exposure on litter size, anogenital distance, preputial separation, testes weight and testicular histology of F1 offspring. Data in mean ± SD. –, No significant findings.

Parameters	Control group	5 μT group	83.3 μT group	500 μT group
Number of dams examined	10	10	10	10
Number of live young at birth	13.6 ± 3.1	13.3 ± 3.7	13.5 ± 2.5	14.1 ± 2.5
Anogenital distance (mm)	5.16 ± 0.17	4.81 ± 0.40	5.10 ± 0.35	5.07 ± 0.28
Preputial separation (day)	40.4 ± 0.6	40.5 ± 0.7	40.3 ± 0.6	40.4 ± 0.9
Testis weight (g)	3.14 ± 0.24	3.15 ± 0.22	3.22 ± 0.32	3.11 ± 0.22
Testicular histology	–	–	–	–

Table 2. Effects of *in utero* and neonatal magnetic field exposure on fertility and sperm parameters of F1 offspring. Data in mean ± SD. * Number of successful copulations/number of animals mated, + Number of impregnated animals/number of animals successfully copulated.

Parameters	Control group	5 μT group	83.3 μT group	500 μT group
Number of male rats examined	10	10	10	10
Copulation index (%)*	9/10 (90.0)	10/10 (100)	10/10 (100)	9/10 (90.0)
Fertility index (%)+	10/10 (100)	10/10 (100)	10/10 (100)	9/9 (100)
Sperm head count ($\times 10^6$/testis)	245.1 ± 19.7	244.5 ± 42.1	245.1 ± 21.7	226.2 ± 25.0
Daily sperm production ($\times 10^6$)	38.9 ± 4.3	38.8 ± 8.5	38.9 ± 4.5	35.9 ± 5.1
Sperm motility (%)	67.2 ± 8.6	68.8 ± 11.4	75.4 ± 7.4	66.7 ± 17.0
Sperm abnormality (%)	11.1 ± 4.9	10.3 ± 6.0	9.5 ± 3.5	11.2 ± 3.5
Small head (%)	1.6 ± 1.1	1.6 ± 1.3	0.8 ± 0.9	1.9 ± 1.8
Amorphous head (%)	0.2 ± 0.4	0.6 ± 0.7	0.2 ± 0.4	0.5 ± 0.7
Two heads/tails (%)	0.1 ± 0.2	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Excessive hook (%)	0.9 ± 0.9	0.6 ± 0.7	0.7 ± 1.0	0.7 ± 0.7
Blunt hook (%)	5.4 ± 2.3	3.8 ± 2.9	3.8 ± 2.0	4.6 ± 3.1
Folded tail (%)	0.1 ± 0.3	1.1 ± 1.2	1.1 ± 1.3	1.3 ± 1.3
Short tail (%)	0.2 ± 0.4	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2
No tail (%)	2.8 ± 1.4	2.8 ± 1.8	3.0 ± 1.8	2.2 ± 1.9

were slightly less (about 8 %) than controls, respectively, but the differences were not statistically significant when compared with the sham control group. The results of sperm motility and morphology in male offspring of the exposure groups did not differ from the sham control values.

4 Discussion

The present study was carried out in rats to determine the potential adverse effects of *in utero* and neonatal exposure to 60 Hz EMF on spermatogenesis and fertility of F1 male offspring. The results obtained in the present study clearly showed that *in utero* and neonatal exposure to 60 Hz at field strengths up to 500 μT did not cause any adverse effects on spermatogenesis and fertility in F1 male offspring.

The slight reduction of anogenital distance observed in the 5 μT group was considered to be an accidental finding, because this change was very slight and did not show a dose–response relationship. This interpretation was strengthened by the fact that there was no significant differences between the exposed and sham control groups with relation to preputial separation, and this is one of the sensitive indicators of male reproductive organ development. It was previously reported that evaluation of testicular sperm head counts seems to be a good indicator of spermatogenic damages and the number of testicular sperm heads corresponds to the number of elongate spermatids in the testis [16, 22]. In the present study, however, the slight decrease in the number of sperm heads observed in the 500 μT group is of doubtful toxicological significance, because this change did not exhibit a dose-response relationship and was unaccompanied by correlated testicular weight changes and histopathological findings. Histopathological examinations revealed that there were no exposure related adverse effects on the number and morphology of spermatogonia, spermatocytes, and spermatids were observed at any doses tested. The results of sperm motility observed in the three exposure groups also did not differ from the sham control values. Although various sperm abnormalities such as small head, amorphous head, two heads/tails, excessive hook, blunt hook, folded tail, short tail and no tail were observed in the both exposed and sham-exposed groups, there was no obvious difference in the incidence of abnormal sperm between the groups.

Conflicting observations have been reported regarding the potential toxic effects of EMF on spermatogenesis and reproduction in experimental animals and humans. Multigeneration reproductive toxicity study showed that continuous exposure of Spague-Dawley rats to 60 Hz magnetic fields has no significant adverse effects on adult reproductive capacity, developing fetus, and neonatal development in rats [3]. Lundsberg *et al.* [4] reported that human sub-fertility was not associated with occupational 0.3 µT exposure on morphology, motility, and sperm concentration among males. Kowalczuk *et al.* [5] also did not find dominant lethal mutation in the male germ cells of mice when they exposed to power frequency magnetic fields at 10 mT for the approximate period of spermatogenesis. Recently, Heredia-Rojas *et al.* [6] reported that 60 Hz and 2 mT magnetic field exposure did not affect meiotic chromosomes and morphological characteristics of male germ cells in mice. The lack of adverse effects of ELF EMF exposure on spermatogenesis and fertility observed in the present study is consistent with the results of abovementioned investigators [3–6]. On the contrary, De Vita *et al.* [8] reported that exposure to 50 Hz and 1.7 mT for 4 h caused a significant decrease in the number of elongated spermatids on day 28 after treatment. Al-Akhras *et al.* [7] reported that exposure of adult male rats to 50 Hz magnetic fields for 90 days had a significant effect on the fertility of females impregnated by the exposed males. Furuya *et al.* [9] suggested that long-term exposure to ELF magnetic fields (1.0 mT) had a possible effect on the proliferation and differentiation of spermatogonia. Recently, Ramadan *et al.* [10] also reported that exposure of fractionated doses of magnetic fields (20 mT) caused a significant decrease in sperm count, motility, and daily sperm production in mice. Most recently, Lee *et al.* [11] reported that continuous exposure to EMF (60 Hz, 0.5 mT) for 8 weeks caused an increased incidence of testicular germ cell death and this finding resulted from an increased incidence of germ cell apoptosis in mice. The apparent discrepancy among the studies might be due to differences in animals used, exposure period and intensity, environmental conditions, *etc*. Meanwhile, exposure of mice to static magnetic fields of 1.6 T, during a 30-day period, resulted in eversible changes in spermatogenic epithelium and in a considerable decrease in the number of mature germ cells [1]. According to the reports of Tablado *et al.* [23, 24], however, the motility and morphological characteristics of epididymal sperm were not affected after exposure to 0.7 T static magnetic fields in mice. These investigators also demonstrated in subsequent study that *in utero* exposure to 0.5–0.7 T static magnetic fields did not cause any adverse effects on the development of testis and epididymis in mice [25]. In view of our findings and the contradictory reports in the literature, it is necessary to conduct much wider studies under different experimental conditions, to help clarify the controversy concerning the possible spermatotoxic risk associated with magnetic field exposure.

Based on the results, it was concluded that *in utero* and neonatal exposure of Sprague–Dawley rats to 60 Hz EMF at field strengths up to 500 µT did not produce any detectable alterations in the offspring spermatogenesis and fertility.

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