

*Original Article*

## **The Effect of 17 Days Exposure to Static Magnetic Fields on the Hypothalamic-Pituitary-Gonadal Axis in the Male Rat**

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### **Abstract**

Power stations produce a range of magnetic fields more than 20 mT which are harmful to those working or living around them. Several investigators have reported an increased health risk due to exposure to electric and magnetic fields (EMF) at 50 and 60 Hz. Several studies have been reported especially with increased tumor incidence, effects on reproduction and development, and neural and behavioral changes.

This study evaluated the possible effect of static MFs 50 Hz on the secretion of Testosterone, LH and FSH hormones in male rats. Forty eight Wistar male rats (same range of age and weight) were randomly divided into four groups. Animals in group 1 were used as a sham exposure group. After one-week adaptation they were placed in exposure to three MFs for 40 minutes daily for 17 days. Group 2, 3 and 4 were exposed with 6, 12 and 24 mT static MFs at 50 Hz respectively. After experiments animals were killed and their bloods were collected in separated tubes and their serums were separated using a centrifuge with 3500 RPM for 15 min.

Hormones were measured using gamma counter equipment with RIA and IPMA methods. The results were analyzed by ANOVA statistical method. Our results show that testosterone, LH and FSH have not changed significantly ( $p < 0.05$ ) using these static MFs intensities. therefore it can be concluded that at least the static MFs used in this study (with these intensities and duration) can not affect the secretion of hypothalamic-pituitary-gonadal hormones.

**Keywords:** Static magnetic fields; Testosterone; LH and FSH.

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### **Introduction**

Exposure to electromagnetic fields (EMFs) is unavoidable for almost all citizens living in industrialized countries. Static magnetic fields (SMFs) can be encountered in a number of workplaces and situations (1). Electrification in developed countries has progressively increased the mean level of extremely low-frequency

electromagnetic fields (ELF-EMFs) to which populations are exposed; these human-made fields are substantially above the naturally occurring ambient electric and magnetic fields of  $\sim 10^{-4}$  Vm<sup>-1</sup> and  $\sim 10^{-13}$  T, respectively (2).

The number of investigations on the biological influence of EMFs has increased and has taken on new significance due to the growing societal concerns as to whether or not they are in fact harmful to humans (1).

One area that has been studied by a number of authors is the potential of EMFs to adversely

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affect reproduction (1). Studies have in most cases, evaluated the effects either on the female (reduction in fertility, placental resorption, miscarriages, and so on) or on embryo malformation (3). The possible effects of EMF exposure on male reproductive processes are few and experimental outcomes are quite different: reversible changes in spermatogenic epithelium (4), aberrations in rat spermatozoa (5, 6), and no effects on human fertility (7).

Forgacs et al (8) indicate a presumably direct effect of whole body MF exposure on the human chorionic gonadotropin-stimulated steroidogenic response of mouse leydig cells.

MF exposure can give rise to changes in neuroendocrine system function in a mammalian laboratory model (9). Suppression of melatonin in the blood and pineal of rat and hamster as a consequence of EMF exposure have been reported by several laboratories (10-15). Reduction in activity of N-acetyl transferase, the rate-limiting enzyme in the production of melatonin, has been reported in a number of these studies (10, 11). Melatonin has been demonstrated to inhibit the hypothalamic-pituitary-gonadal (HPG) axis in certain species of mammals (16).

There is also increasing concern that chronic or long-term exposure to low EMFs from many appliances, at home or workplace, may cause adverse reproductive effects (17). Using various mice as experimental models have attempted to elucidate the reproductive toxic effects of exposure to weak MFs, and the results have been found to be rather contradictory (18). A significant increase in malformations was found after gestational exposure to a pulsed MF (20 KHz) in C<sub>3</sub>H mice (18).

Al-Akhras et al has applied 25  $\mu$ T sinusoidal MF for 90 days on adult male and female rats and have found some adverse effects on fertility of male and female rats (19).

A lot of studies have been performed about biological effects of EMF exposure in mammals, for example, Okano et al (20) found that whole body exposure to SMF at 10 and 25 mT for 2-9 weeks suppressed and delayed blood pressure (BP) elevation in young, stroke resistant, spontaneously hypertensive rats (SHR). They have suggested in an other study that SMF (5 mT) may suppress and delay BP elevation via the

nitric oxide pathways and hormonal regulatory systems (21).

But data on the male reproductive system are limited. Thus, there would seem to be a lack of agreement between scientists on the possible negative effects of EMFs on male reproductive parameters. There is an obvious need for additional information about the effects of exposure on HPG axis. To this end, the present study investigates the effects of exposure to MFs on reproduction in adult male rats.

We have also some unpublished data on the effect of 2 hours daily SMF exposure to rats for 34 days which have altered the sperm shape and activity (22). We propose that these MFs can alter hormonal secretion such as HPG axis as well. In this study we have tested the effects of lower duration on HPG axis.

Some of previous studies measured rat mass, have reported controversial effects of EMF on the body mass (23-25). Some of investigators such as Marino et al. (23) Sandrey et al. (24) and Hilton and Phillips (25) reported increase, decrease and no difference between the weight of the control and electric or magnetic field treated rats, respectively depending on the time of the exposure.

## Experimental

### Materials and methods

A total of 48 male Wistar rats (obtained from Razi institute, Karaj, Iran. 2-3 months old and 130-150 g weight) were randomly divided into four groups. Animals in the group 1 were used as a sham exposure group. After a-week adaptation they were exposed to different (6, 12 and 24 mT) 50 Hz static MFs (SMFs) for 40 min daily for 17 days.

Rats were housed, four per cage, with free access to tap water and food. They maintained at approximately 25°C and 55% humidity with a dark/light cycle of 12/12 h. All procedures were performed in accordance to the guidelines of the university and National policies of animal for the care and use of laboratory animals, under an institutionally approved protocol.

Exposure cages were made of transparent Kaolin (resin) that each cage was equipped with top ventilation pores. Food and water were

withheld during the exposure period (40 min per day). The transparent cages and the open constructed coil systems allowed the animals to be easily viewed and to experience similar ambient conditions during both normal and exposure periods. In all experiments, the long axis of the cages was aligned with the direction of applied SMFs.

SMFs were designed and produced using homemade coil (Department of Medical Physics, Jundishapour Medical Sciences University of Ahwaz) which can generate static and pulsative MFs. Apparatus included electrical coil to 10000 turns with nucleus rectangular cubical dimensions 15×15 cm from pure iron in which the distance between the poles is adjustable. MFs were distinguished and measured using a gauss meter (Brockhause Messtechnik Company model 410) at different distances. At different points between and around the poles the MFs were measured by gauss meter. Distances of cages were determined and rats were moved freely in these homogeneous fields. In both sides between poles the SMFs intensities were  $6\pm 0.3$ ,  $12\pm 0.6$  and  $24\pm 1.2$  mT which 6 rats were in each cage during exposure of SMF. Each field was determined using a gauss meter in order to assure the constancy of the field. The current could be activated through a switch which was housed in a locked box together with the transformer and resistor. The constancy of the fields can be controlled by the variation of the amperage that was achieved by rheostat in the circuit of the system. The generated field did not cause any sound or any other manifestation that could be perceived by the rats or experimenters. Whole magnetic generator was fixed to a wooden frame box and the absence of significant vibrations was confirmed by acceleration measurements. Furthermore, the frame with coils mechanically separated from the animal cages. The coil design (number of turns, cross-section of conductor and rated current) was optimized in order to reduce joule-heating losses to the level which did not affect the temperature in the exposure area. Sham exposed rats were held in a similar non-energized system, the MF was equal to the background level ( $< 0.05$  mT).

Prior to exposure, animals were randomly assigned to one of the four exposure groups

( $n=12$ ). After 17 days exposure to the SMFs, they were anesthetized using ether, they have decapitated between 09:00 and 11:30 AM and their blood were immediately drawn into tubes without anticoagulant, allowed to clot, and then centrifuged to obtain the serum which was kept frozen under  $-20^{\circ}\text{C}$  until use. Hormones were measured using gamma counter equipment (Contron Company of Swiss) with RIA and IRMA methods (26).

50  $\mu\text{l}$  of serum was used for the measurement of testosterone by radioimmunoassay (Biosource Testo-RIA-CT Kit). In this assay, the cross-reactivity with dihydrotestosterone is less than 1%, and the minimal detectable concentration (M.D.C.) of testosterone was 0.44 ng/dl. The intra- and interassay variances (cv) were 4% and 8%, respectively.

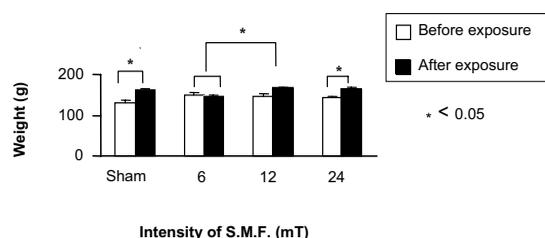
LH and FSH were measured using an IRMA method (DSL, Inc). In this assay, the cross-reactivity are non-detectable with others glycoprotein hormones (FSH, LH, TSH, HCG and  $\beta$ -hCG), M.D.C. of LH and FSH were 0.12 mIU/ml and 0.11 mIU/ml, respectively. The intra- and interassay variances of LH were 8% and 8%, and of FSH were 3% and 7%, respectively. Because specific rat's glycoprotein hormonal kit is expensive, we used human glycoprotein hormonal kits instead of animal kits. So routine cross-reactivity test with rat's hypophysis ( $n=15$ ) also have been done for majority of experiments, which showed there are no differences between rat's and human's kit in these circumstances.

### **Statistical analysis**

Results are expressed as mean $\pm$ S.E.M. One way analysis of variance (ANOVA) followed by, when appropriate, Tukey's test were used to determine the statistical significance of differences between means. A P-value of less than 0.05 was taken as statistical significance.

### **Results and Discussion**

Figure 1 has shown the animal weight changes due to exposure of different SMFs at the end of the exposures. As shown in Figure 1 only the weight of group 2, which exposed to 6 mT SMF were decreased significantly than 12 mT ( $P < 0.05$ ), and other groups did not change significantly.



**Figure 1.** The weight of animals changed after being exposed to magnetic fields. Animals were exposed to 6, 12 and 24 mT 40 min daily for 17 days. Exposed and sham-exposed rats were selected in the same range of weight (130-150 g) and no significant differences between their weights at the beginning of experiments. After 17 days exposure to MF, the weight of animals that were exposed to 6 mT decreased significantly in comparison with animals that were exposed to 12 mT ( $P < 0.05$ ), but other groups did not change significantly ( $N=12$ )

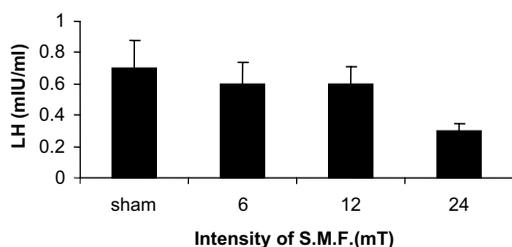
Glycoprotein hormones measurement must be done with rat special Kits. For this reason cross-reactivity test of human FSH and LH Kits have been done and correlation coefficient ( $r=0.89$ ) obtained which shows there are much more similarity between human and rat FSH and LH.

Measurement of hormones testosterone, LH or FSH in the exposed (6, 12 and 24 mT) and control groups have shown that none of the hormones were changed significantly in all groups (Figure 2-4).

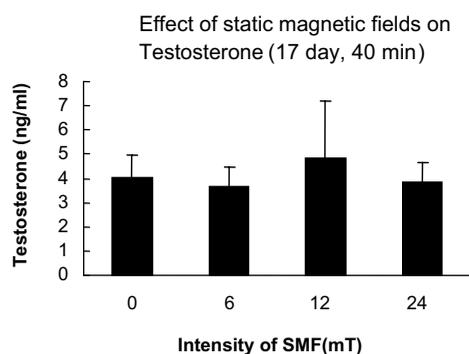
In relation to the hypophysis-gonadal axis hormones some controversial studies have been reported by different laboratories.

Free et al. (1981) have found that alterations have occurred in the secretion pattern of FSH in rats exposed to an 80 KV/m electric field for 20 to 56 days. They have also observed a reduction in plasma testosterone levels after 120 days of electric field exposure (27).

McGivern et al. (1990) have exposed pregnant Sprague-Dawley dams to a low-level, low-frequency pulsed EMF (15 Hz, 0.3 milli



**Figure 3.** The level of LH of animal groups that were exposed to 6, 12 and 24 mT for 40 min daily for 17 days. Non of sham-exposed or expose subjects LH level, have not changed significantly ( $N=12$ ).



**Figure 2.** The level of testosterone concentration of animal groups that were exposed to 6, 12 and 24 mT static MFs, 40 min daily for 17 days. Non exposed or sham-exposed subjects testosterone level have not changed significantly ( $N=12$ ).

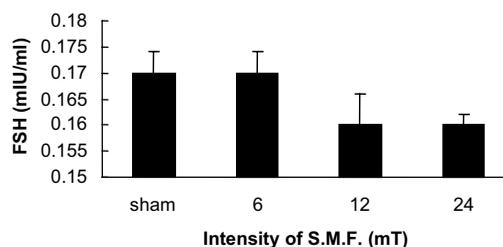
second duration, peak intensity 8 gauss) for 15 min twice a day from day 15<sup>th</sup> through day 20<sup>th</sup> of gestation (a period in development that is critical for sexual differentiation of the male rat brain). At 120 days of age, field-exposed male offspring had normal circulating levels of testosterone, LH and FSH hormones, as well as epididymal sperm counts (28).

Margonato et al. (1993) have done three-year investigation on electric field conducted on the biological effects of high intensity electric field exposure of rats for up to 18% of their life span, but they did not find any differences on LH, FSH and testosterone between exposed animals compared with sham-exposed rats (29).

Kato et al. (1994) reported that 6 weeks of nearly continuous exposure to circularly polarized 50 Hz magnetic fields did not change plasma testosterone levels in rats (30) which is in agreement to our data that we are presenting in this paper.

Margonato et al. (1995) did not find any magnetic field-induced morphologic and histological changes in tested rats after prolonged exposure to a 50 Hz magnetic field at 5 $\mu$ T (31).

Selmaoui et al. (1997) in the same research direction reported that LH and FSH secretion



**Figure 4.** FSH level did not change significantly using different intensities (6, 12 and 24 mT) of static magnetic field ( $N=12$ ).

was unaffected by the acute exposure to a 50 Hz linearly polarized magnetic fields of 10 $\mu$ T on the thirty-two young men, 20-30 years old (32).

Zecca et al. (1998) chose two groups of adult male Sprague-Dawley rats (64 rat each) and exposed them to EMF of two different field strength combinations: 5 $\mu$ T-1KV/m and 100  $\mu$ T-5KV/m for 8 months. They did not find any changes in serum LH concentration (33). The latter finding is in agreement with the report by kato et al. (1994) indicating of no change in plasma testosterone in rats exposed to MFs of similar strength. Indeed, testosterone release is mainly controlled by LH (30).

But Forgacs et al. (1998) have demonstrated that exposure to sinusoidal 50 Hz, 100 $\mu$ T MF increased the basal testosterone production in 48h primary mouse leydig cell culture, whereas the steroidogenic capacity to respond to HCG remained Unchanged (34).

In contrast Ozguner et al. (2002) observed that EMF stimulation resulted in leydig cell proliferation, increased testosterone level and testis weight, but a significant decrease in germ cell population has occurred (35).

Sert et al. (2002) have exposed male Wistar albino rats to a MF with intensity of 0.8 T, 3 h per day for 5 weeks, and found that testosterone levels altered (36).

In this study we have studied possible effect of a static 50 Hz MFs densities of 6, 12 and 24 mT exposure for 40 min daily for 17 days, on hormonal changes of the exposed and sham-exposed subjects. Our results show that testosterone, LH and FSH have not changed significantly, using static MFs, after 17 days. The weight of exposed group, with 6 mT decreased significantly in comparison with the group of 12 mT exposure ( $P < 0.05$ ), but the weights of other groups have not changed significantly.

Our data also show that at least the static MFs used in this study (with this intensities and duration) can not affect secretion of hormones.

This finding is in agreement with the finding described by Kato (30), Margonato (29, 31), Zecca (18) and Selmaoui (17). On the contrary, other investigators have shown that MF can affect the secretion of hormones. These controversies may be due to the type, duration and intensity of the EMF applied and the animals which have

been used in these studies.

In this study low intensity SMF (6 mT) decreased the animal weight significantly compared to moderate intensity SMF (12 mT) which is consistent with the work of Sandrey, et al. (9) but the weight of other groups which exposed to 12 or 24 mT did not affected at all (Figure 1).

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