



# The effect of prenatal exposure to 900-MHz electromagnetic field on the 21-old-day rat testicle

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## ABSTRACT

The aim of this study was to investigate the effect of exposure to a 900-MHz electromagnetic field (EMF) in the prenatal term on the 21-old-day rat testicle. Pregnant rats were divided into control (CG) and EMF (EMFG) groups. EMFG was exposed to 900-MHz EMF during days 13–21 of pregnancy. Newborn CG rats were obtained from the CG and newborn EMFG (NEMFG) rats from the EMFG. Testicles were extracted at postnatal day 21. Lipid peroxidation and DNA oxidation levels, apoptotic index and histopathological damage scores were compared. NEMFG rats exhibited irregularities in seminiferous tubule basal membrane and epithelium, immature germ cells in the lumen, and a decreased diameter in seminiferous tubules and thickness of epithelium. Apoptotic index, lipid peroxidation and DNA oxidation were higher in NEMFG rats than in NCG. 21-day-old rat testicles exposed to 900-MHz EMF in the prenatal term may be adversely affected, and this effect persists after birth.

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## 1. Introduction

Intensive use in daily life of devices with an electromagnetic field (EMF) effect, such as radios, televisions, computers and mobile phones, results in constant exposure to the effect of EMF. The fact that more than 80% of people in many countries use mobile phones has further increased concerns about the effect of EMFs associated with mobile phone use [1]. The effects of mobile phone use during pregnancy on the developing embryo/fetus have also attracted the interest of researchers. However, the kind of effect that EMF has or may have on the embryo and fetus is still the subject of debate.

A significant part of the studies performed maintain that the effect of EMF may affect embryo and fetus development and that this may compromise the normal development of vital organs [2–5]. Studies have also maintained that follicular capacity can decline in female rats exposed to the effect of EMF from fertilization to the implantation period, and that this may have an adverse impact on females' reproduction potential [6]. All these studies suggest that testis development in male offspring of female rats

exposed to the effect of EMF may be also compromised. The reason for this is testis growth beginning in approximately the second week of pregnancy in rats [7–9]. Studies have shown that the EMF in rats exposed to a low wave frequency (50 Hz) EMF during and after pregnancy increases germ cell death and gives rise to a high apoptotic index (AI) by inducing apoptosis in spermatogenic cells [10].

Our scan of the relevant sources revealed no studies of the effect of a 900-MHz EMF applied for 1 h between the 13th and 21st days of the prenatal period on the testes of young rats. The purpose of this study was therefore to investigate the effect of 900-MHz EMF applied during the prenatal period on the testes of 21-day-old rats using histopathological, morphometric and biochemical techniques.

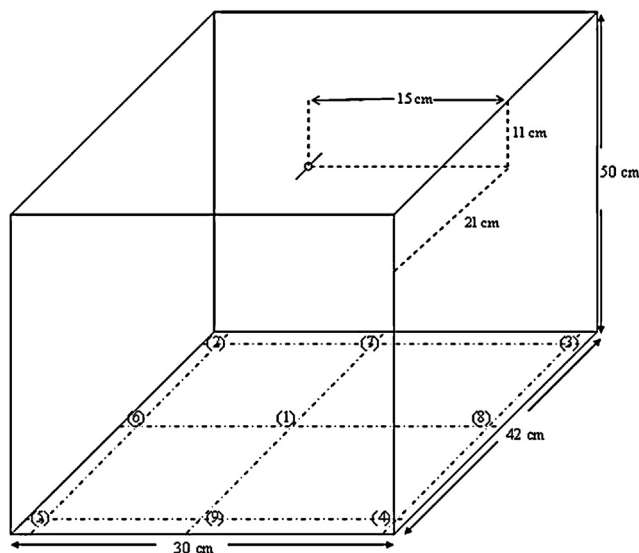
## 2. Materials and methods

### 2.1. Study design and animals

This was a randomized, controlled, nonblinded interventional animal study. Animal experiments and procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health, and was approved by the Karadeniz Technical University (KTU) Medical

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**Fig. 1.** Schematic representation of the dimensions of the Plexiglas jar used to expose rats to a 900 MHz EMF, the positions of the 9 points on the floor of the jar at which EMF intensity was measured and the location in the jar of the 15-cm copper semi-wave dipole antenna used to apply the EMF transmitted from the oscillator with a coaxial cable.

Faculty Local Animal Ethical Committee. A total of 40 rats were used in the study, 20 pregnant healthy Sprague Dawley females (6–8 weeks old, weighing 180–250 g), and 20 newborn males born to the pregnant rats. Rats were obtained from the KTU Surgery Research Center (KTUSRC). Rats were housed in the KTUSRC in standard plastic cages on sawdust bedding in an air-conditioned room at  $22 \pm 1^\circ\text{C}$  under controlled lighting (12 h light/12 h dark cycle). Standard rat chow and tap water were provided ad libitum.

## 2.2. Exposure to electromagnetic field

An ultra-high-frequency oscillator (1218-BV, Lockable Oscillator, 900–2000 MHz, General Radio Company, Concord, Massachusetts, USA, Serial No. 1483) fed with an uninterrupted power source (1267-B Regulated Power Supply, General Radio Company, Concord Massachusetts, USA, Serial No. 903) with output power of approximately 300 mW and a frequency adjusted to 900-MHz was used to establish a 900-MHz EMF. The oscillator was attached to a half-wave dipole antenna made from a  $1\text{ mm} \times 15\text{ cm}$  copper rod with the help of a coaxial cable. The antenna was installed into the central region, approximately 11 cm inside the open surface of a glass jar produced specially for the study made of Plexiglas and measuring  $30\text{ cm} \times 42\text{ cm} \times 52\text{ cm}$  (Fig. 1). Rats were placed inside this jar and exposed to 900-MHz EMF for 1 h.

## 2.3. Measurements of electromagnetic field intensity

Intensity of electrical field was measured using a wide-range measuring device with a measurement range of 100 kHz–2.5 GHz (Chauvin Arnoux CA43 Isotropic Electrical Field Intensity Meter). Positional averaging of intensity of electrical field measured while the jar was empty was 8.13 V/m beneath the jar (Fig. 2A) and 12.84 V/m on the floor (Fig. 2B), while when rats were inside the jar the field was 9.22 V/m beneath the jar (Fig. 2C) and 12.50 V/m on the floor (Fig. 2D) (Table 1). According to these measurements, rats were exposed to a mean electrical field intensity of 10 V/m inside the jar ( $0.265\text{ W/m}^2$ ). This value is regarded as equivalent to the intensity of electrical field that mobile phones in speak mode can create in their immediate surroundings (mean 1–10 V/m for variables such as mobile phone model, location, distance from base

**Table 1**

Results of the electric field strength measurement (V/m) at 9 different positions in the jar.

Position	With the jar empty (V/m)		With rats inside (V/m)	
	Beneath the jar	Jar floor	Beneath the jar	Jar floor
1	8.6	8.8	17.3	20
2	7.2	5.2	10.1	7
3	7	8.4	8.6	9
4	9.5	16.8	9.8	14.5
5	9.1	9.1	6.9	9
6	6	12.5	4.6	10
7	8.5	18.2	8.5	14
8	6.6	16	6.5	14
9	10.7	20.6	10.7	15
Mean $\pm$ SD	$8.13 \pm 1.53$	$12.84 \pm 5.28$	$9.22 \pm 3.59$	$12.50 \pm 4.06$

station and similar). These values are the limit values set out for a single source in the Global System for Mobile Communications (GSM)-900 base station systems [3–5,11].

## 2.4. Groups and experimental procedures

On the evening of the beginning of the study, each of the 20 female rats exhibiting two regular cycles was placed in the same cage as a male rat in order to mate. Pregnancy tests were performed the next day using vaginal smears. Female rats with sperm observed in their smear specimens were regarded as pregnant, and that day was recorded as day 0 of pregnancy. Six rats identified as pregnant were randomly divided into two groups of three rats each. The first group was adopted as the control group (CG), and no procedure was performed on these throughout pregnancy. The second group of mothers was adopted as the EMF group (EMFG). This group was placed inside the Plexiglas jar at the same time every day between the 13th and 21st days of pregnancy and exposed to EMF of 900-MHz for 1 h. The groups were kept in different cages in the same room during the experiment, except for during exposure to 900 MHz EMF. During EMF exposure, EMFG rats were separated from CG, and EMFG was exposed to EMF in the exposure room.

Each mother rat was placed into a separate cage before giving birth, and pups were left to feed naturally with their own mothers in the same cages. No procedure was performed on the new-born rats after birth. At the end of the experimental period, the rat pups from both groups of mothers were divided into males and females within their own groups. The study continued with 10 male rat pups each selected at random from the male rats from both groups. The new groups established were named newborn CG (NCG) and newborn EMFG (NEMFG). NCG consisted of newborn male pups from the CG rats, and NEMFG of newborn male pups from EMFG mother rats.

At the end of the study period (postnatal 21st day), all male rats were sacrificed on the same day by decapitation under deep anesthesia (Ketalar 50 mg/kg). Two cubic centiliter blood samples were taken from each rat and placed into EDTA tubes for biochemical parameter investigation. The animals' testes were then removed. Surrounding tissues were carefully removed, and right and left testes were weighed on a sensitive scale. After this stage, the study proceeded with the right testes. These were divided vertically into two. Half the testis was placed in Bouin's solution for histopathological examination. The other half was divided into two equal halves for biochemical analyses, placed into tubes (1.5 ml, Eppendorf, Hamburg, Germany) and stored at  $-80^\circ\text{C}$ .

## 2.5. Histological staining and analyses

The testes placed into Bouin's solution were kept for 3 days in a dry environment receiving no light. They were then subjected to routine histological examination procedures and embedded in

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