

Effect of whole-body 1800 MHz GSM-like microwave exposure on testicular steroidogenesis and histology in mice

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Abstract

The aim of our study was to evaluate the possible effects of whole-body 1800 MHz GSM-like microwave exposure on male reproduction. After repeated exposure of mice to microwaves at 0.018–0.023 W/kg whole-body specific energy absorption rate (SAR) an elevated serum testosterone level was measured, but no microwave exposure related histopathological alteration could be detected in the reproductive organs. The *in vitro* steroidogenic response of 48 h Leydig cell cultures obtained from exposed animals did not differ from the controls, suggesting that Leydig cells were not the primary targets of the applied microwave exposure or direct action of microwaves on Leydig cells was temporary only. In exposed animals the red blood cell count and volume of packed red cells were also increased. Further investigations are required to clarify the mechanism of action of the applied microwave exposure on male mice, as well as to establish the biological significance of the observed phenomena.

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1. Introduction

In the last decades the widespread use of electric devices and telecommunication equipments increased the electromagnetic radiation in our environment from 0 Hz up to 300 GHz. In this frequency range the non-ionizing radiation has too weak photon energy to break the atomic bonds. The microwaves (ranging from 300 MHz to 300 GHz) are increasingly present in our everyday life from microwave ovens to medical diathermy.

The most widely used GSM (global system for mobile communication) mobile phones operate also with microwave carrier frequencies in the near GHz range (850–1900 MHz). In addition, the GSM radiation contains extremely low frequency (ELF) components too, because the carrier signal is pulsed at 217 Hz with bursts of 576 μ s pulsewidth. Besides, there are two additional ELF pulsing of the signal: one at 8.34 Hz due to the time division multiple access (TDMA) mode, while another one at

2 Hz is present only in the energy-saving discontinuous transmission (DTX) mode [1].

Exposure to high density microwaves can cause detrimental effects on biological systems through thermal action [2,3]. With regard to localized heating and the susceptibility of individual tissues to heat, the central nervous system (CNS), the testis and the lens of the eye seem particularly sensitive [4]. Since all established biological and health effects of microwave exposure are related to thermal effect, safety guidelines do protect against excessive microwave heating. The microwave exposure can be quantified in terms of the amount of energy absorbed by a unit mass of the object. This is expressed as the specific absorption rate (SAR) with units of W/kg. The SAR value is independent from the duration (or repetition) of exposure. In tissue, SAR is proportional to the square of the internal electric field strength. Values of SAR depend on the incident field parameters (frequency, intensity, polarization, source–object configuration), the characteristics of the exposed body (size, internal and external geometry, dielectric properties of tissues) and ground effects and reflector effects of other objects in the field near the exposed body. Average SAR and SAR distribution can be computed or

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estimated from laboratory measurements. The exposure limits are calculated as mean of whole-body SAR, which are 0.4 W/kg for occupational exposure and 0.08 W/kg for the general public [4,5].

Below the safety levels thermal effect of microwaves on biological systems cannot be expected. However, there are many reports over the last three decades that microwave (or radio-frequency) exposure can exert non-thermal influences, at intensities well below those necessary to cause any detectable heating. In previous investigations it was found that non-thermal microwave (or high frequency) exposure is able to induce several changes at the level of DNA and protein molecules including the increase DNA single and double strand breaks in rat brain [6], induction of micronuclei in rat bone marrow [7], alteration of protein conformation without bulk heating [8] or induction of oxidative stress in rat brain tissue [9]. The molecular biological changes can evoke a number of alterations at the cellular level, such as increased Ca^{2+} efflux in cultures of human neuroblastoma cells [10], reduced melatonin secretion [11] or disturbed balance of chemicals in the dopamine-opiate system [12]. The consequences of the cellular changes may be manifested as different functional alterations at the level of organs and organism, like altered natural rhythms of the brain's electrical activity [13], increased permeability of the human blood–brain barrier [14,15] or promotion of lymphomas in transgenic mice [16]. Compared with continuous-wave (CW) radiation, pulsed microwave fields with the same average rate of energy deposition in tissues are generally more effective in producing a biological response, especially when there is a well-defined threshold that must be exceeded to elicit the effect [5]. Thus, it is quite possible that living organisms have a two-fold sensitivity to the extremely low frequency pulsed GSM signal [1].

Whether non-thermal microwave exposure affect reproductive ability is controversial; epidemiological data have been inconclusive and animal tests limited. Nakamura et al. [17] found that exposure to 2450 MHz CW microwave at 2 mW/cm^2 power density for 90 min decreased uteroplacental blood flow and increased progesterone and $\text{PGF}_{2\alpha}$ in pregnant rats. They suggest that the applied microwave exposure produce uteroplacental circulatory disturbances and ovarian and placental dysfunction during pregnancy, probably through non-thermal actions. In a later experiment the same authors did not find any non-thermal effects on uteroplacental blood flow, blood estradiol and progesterone, and splenic natural killer cell activity (NKCA) after exposure of pregnant rats to 915 MHz (CW) at 0.6 mW/cm^2 equal to a SAR of 0.4 W/kg [18]. Dasdag et al. [19] reported that the seminiferous tubule diameter in rat testes was decreased after only brief daily exposure at a whole-body SAR of 0.141 W/kg for a month to mobile phone radiation from commercially available GSM (890–915 MHz) phones. However, a subsequent study carried out to explore these results more thoroughly found that longer daily exposures to pulsed 800–915 MHz GSM microwave radiation at a whole-body average SAR of 0.52 W/kg had no effect on testicular function or structure in rats [20]. Magras and Xenos [21] reported a dramatic decrease in the fertility of mice exposed to extremely low level RF fields ($0.168\text{--}1.053 \text{ }\mu\text{W/cm}^2$) near the “antenna park of Thessaloniki” (almost 100 TV and FM

transmitter antennas on the Chortiatis mountain in Greece). They observed a progressive decrease in the number of newborns per dam, which ended in irreversible infertility. However, the study was undermined by the lack of a matched control group [22], and the possibility that the observed changes were the result of other stresses in the environment and/or seasonal changes in day length cannot be discounted [23].

The main goal of this study was to evaluate the possible effect of whole-body 1800 MHz GSM-like exposure (below the international SAR limit) on serum testosterone level, the in vitro steroidogenic capacity of cultured Leydig cells and the histology of reproductive organs in male mice. With the applied irradiation schedule we attempted to simulate the exposure of average mobile phone subscribers who regularly use the additional GSM-services (internet, multimedia, GPS-combined GSM navigation systems, etc.) in addition to the telephone conversations on workdays.

2. Materials and methods

2.1. Animals

Male NMRI mice (Charles-River, Hungary) weighing 35–41 g (9–10 week old) were kept in a room with a 12-h light:12-h dark photoperiod (lights on 7:00–19:00), temperature of 20–22 °C and relative humidity of 50–60%. The NMRI strain of mouse is commonly used in radiation and bioelectromagnetic research. In each experiment, 11–12 sham-exposed (control) and another 11–12 exposed mice were kept in plastic cages and allowed free access to standard laboratory pellets (Altromin®) and tap water. Five independent experiments were performed. The mice were kept and handled according to the guidelines of the Hungarian Law of Animal Care.

2.2. 1800 MHz GSM-like microwave exposure

The animals were exposed 10 times (over 2 weeks on workdays) and the duration of exposure was 2 h/day.

The field of mobile phones was simulated in a gigahertz transverse electromagnetic mode (GTEM) cell of 2 m height and $1.2 \text{ m} \times 1.2 \text{ m}$ of floor space (model S-GTEM; TKI-Network Ltd., Hungary), which is widely used for electromagnetic compatibility (EMC) tests [24]. The GTEM cell was positioned vertically therefore the E field strength was horizontal within the exposure area. The 1800 MHz radiation was produced by a mobile test phone (model NOKIA 7110; Nokia Mobile Phones Ltd.) connected to a Communication Test Set (model 4202S; Wavetek, Germany) and the signal was amplified by a power amplifier (model AS0204-17R; Milmega Ltd., UK).

The power was measured by a power meter (model HP 5247A; Hewlett-Packard, USA) and a radio-frequency (RF) spectrum analyzer (model U4941; Advantest, Japan) Fig. 1. The RF carrier signal was pulsed at 217 Hz with bursts of 576 μs pulsewidth and speech was not simulated (GSM-217 Hz signal).

During RF exposure 11–12 freely moving mice were kept in a pure (i.e. lacking any metallic fittings) plastic cage of $40 \text{ cm} \times 30 \text{ cm} \times 10 \text{ cm}$, which were placed over the absorbent material of the GTEM cell Fig. 2. The average power density was $100 \text{ }\mu\text{W/cm}^2$ (19.4 V/m) within the cage. Within the GTEM cell, the cage was positioned into that level where a given input power could generate the maximum field strength over the whole area of cage. According to the dimension of the GTEM cell and the position level of the cage, the required input power was to be calculated with the following equation:

$$E = \frac{(50 P_{\text{in}})^{1/2}}{h}$$

where E (V/m) is the field intensity at the given position, P_{in} (W) the input power and h (m) is the distance between the septum and the wall of the GTEM cell.

The distance from the septum at the given position was 0.85 m. A field uniformity of under than 15% was expected across the volume within the cage



Fig. 1. The experimental set-up for producing 1800 MHz GSM-like microwave radiation (Department of Non-ionizing Radiation, National “Frederic Joliot-Curie” Research Institute for Radiobiology and Radiohygiene, Budapest).

occupied by the animals. Field uniformity was confirmed by measurements made using a single axis (EF-1) E-field probe and an isotropic one (EP-33M) connected to the field meter (CA-43, Chauvin-Arnoux, France and PMM 8035, PMM S.r.l., Italy) via an optical cable. Both had calibration uncertainties of 1.5 dB. Measurements were made at 5 cm intervals in a horizontal plane across the volume occupied by the outer exposure box. The dominant field component was horizontal. The mean value of the field strength within the exposure volume was determined as 19.4 ± 2.8 V/m.

The whole-body specific energy absorption rate was estimated as 0.018–0.023 W/kg using data for a prolate spheroid model of a mouse [25]. The laboratory where the exposures have been performed has the Accreditation Certificate (No. NAT-1-0969/2002) complies with the criteria of EN ISO/IEC 17025:2001 Standard.

The sham-exposed control mice were kept in the same GTEM cell for the same time as exposed animals, however, without microwave exposure. On every workday the sham-exposed animals were placed for 2 h into the GTEM cell between 7:30 and 8:00 a.m. without microwave exposure, while the exposed animals were placed for 2 h into the same GTEM cell between 9:30 and 10:00 a.m. in the presence of 1800 MHz GSM-like microwave exposure.

Body weights were recorded on the first and the last day of the treatment. On the day of last treatment, immediately after the termination of sham-exposure (between 9:30 and 10:00) and exposure (between 11:30 and 12:00) mice were anesthetized with 60 mg/kg i.p. pentobarbital (Rhone-Poulenc Rorer, Vitry sur Seine, France), and blood samples were taken for hematology, serum chemistry and serum testosterone (T) determinations. After blood collection, the animals were overdosed with pentobarbital. Testicles, epididymes, adrenals, prostates,

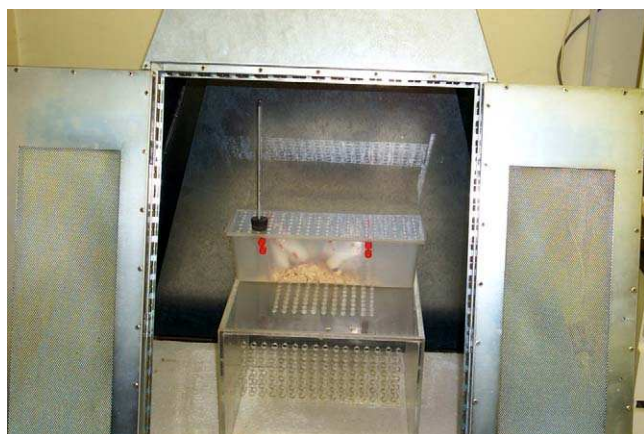


Fig. 2. The placement of mice in GTEM cell (radio-frequency shielded exposure chamber).

kidneys, hearts, livers and pituitary glands were removed for histology. One testicle of each animal was used for culture of Leydig cells.

2.3. Interstitial cell preparation and incubation

Interstitial cells were prepared and cultured from the right testicles of the animals. (The left testicles were used for histology.) For isolation of interstitial cells by mechanical dissociation without enzyme treatment the method of Stoklosowa [26] was used with some modification, as described earlier [27]. In each experiment, interstitial cells were isolated and collected in two blocks: one obtained from sham-exposed, and another one from the exposed animals. These mixtures of interstitial cells were diluted to 10^6 cells/ml in minimum essential medium (MEM; Gibco BRL) supplemented with 10% fetal bovine serum (FBS; Gibco BRL), 100 IU/ml penicillin and 100 µg/ml streptomycin (Sigma Chemical Co., St. Louis, MO). The cell suspensions were plated (with final volume of 500 µl/well) into sterile plastic 24-well plates (NuncloTM, Nunc, Roskilde, Denmark). Leydig cells were identified by the histochemical reaction for 3β-hydroxysteroid dehydrogenase (3β-HSD) enzyme [28,29]. In brief, cells were incubated for 3.5 h at 34 °C in the culture medium containing of 0.6 mmol/l dehydroisoandrosterone (DHA; Sigma), 0.6 mmol/l nicotinic acid adenine dinucleotide (NAD; Sigma), 0.2 mmol/l nitro blue tetrazolium (NBT; Sigma) and 7% propylene glycol (Sigma). The percentage of 3β-HSD positive cells was at least 70%. To assess viability prior to adding FBS to the culture medium, samples were taken and stained with trypan blue solution (Sigma) and the cells were counted in a hemocytometer. The interstitial cells were cultured in the presence or absence of 1, 10 or 100 mIU/ml human chorionic gonadotropin (hCG; Chorionin; Richter, Budapest, Hungary). The LH analog hCG was used to check the T response of the sham-exposed controls and to evaluate the possible effect of the whole-body microwave exposure on the steroidogenic capacity of Leydig cells in vitro. Cell cultures were maintained for 48 h at 34 °C under a humidified atmosphere of 95% air/5% CO₂. Following the incubation the aliquots of the culture medium were centrifuged and the supernatant was collected and frozen at –20 °C until testosterone (T) determination.

2.4. Quantification of testosterone

Determination of testosterone directly from aliquots of the culture media and blood sera was performed by enzyme linked immunosorbent assay (ELISA). The ELISA kits were purchased from Dialab (Austria). The samples were measured at 450 nm by an Anthos 2010 microplate reader and the data were evaluated by WinRead 2.3 computer software. The sensitivity of the T assay was 0.2 ng/ml and the inter- and intra-assay coefficient of variations were 3.9 and 6.2%, respectively. Cross-reactivity with 5α-dihydroxytestosterone was 10%.

2.5. Histological techniques

Testes (left), epididymes, adrenals, prostates, kidneys, hearts, livers and pituitary glands were fixed in buffered formol saline for histological examination. These tissues were dehydrated in ethanol, embedded in paraffin wax and stained with hematoxylin and eosin.

2.6. Hematology

Blood was taken from aorta, collected in plastic Eppendorf tubes containing K₃EDTA. White (WBC) and red (RBC) blood cells counts, mean cell volume (MCV), hemoglobine concentration (HGB), volume of packed red cells (VPRC) and platelets (PLT) were determined using the Animal Blood Counter Vet abcTM (Roche).

2.7. Serum chemistry

At sacrifice, free-flowing blood was collected in plastic Eppendorf tubes. After centrifugation, serum was removed and the samples were then analyzed for serum alkaline phosphatase, glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), cholesterol and triglycerides by an auto analyzer instrument (A.L. Instruments, France).

2.8. Statistics

All data were analyzed by two-way analysis of variance (ANOVA) using GraphPad Prism 3.01 computer software. A value of $p < 0.05$ was considered to be significant. Each experimental group has been passed over the normality test ($p > 0.1$). According to the Bartlett's test, the variances of experimental groups did not differ significantly ($p > 0.05$). In the statistical analysis of serum testosterone, blood variables and serum chemistry values, the factors were microwave exposure (sham-exposed or exposed) and experimental blocks (five independent experiments). The microwave exposure was a *fixed effect*, while the repetition of the experiment with different (randomly selected) subjects in the five independent experimental blocks resulted a *random effect*. In the statistical evaluation of the *in vitro* hCG stimulated testosterone response of Leydig cells, the factors were microwave exposure (sham-exposed or exposed), and *in vitro* hCG stimulus (four concentration points of hCG). To eliminate heterogeneity of variance among the data of experiments performed at different times, T levels of the culture media were expressed in per cent of sham-exposed, *in vitro* 100 mIU/mL hCG stimulated controls.

3. Results

3.1. Serum testosterone

In the 1800 MHz GSM-like microwave exposed mice two-way ANOVA revealed significantly elevated ($p < 0.05$) serum testosterone level (7.85 ± 1.08 ng/ml) compared with the sham-exposed controls (5.12 ± 0.79 ng/ml). Five independent experiments were performed, the total number of the samples was 56 (exposed) and 52 (sham-exposed).

3.2. Testosterone production of interstitial cell culture

The Leydig cells used in all experiments were viable ($>90\%$ viability; checked by trypan blue exclusion test) and steroidogenically active. In the cell cultures obtained from animals subjected to whole-body microwave exposure, two-way ANOVA demonstrated no significant change in the basal or *in vitro* hCG stimulated T response (Fig. 3) and initial cell yield also remained unaltered. No microwave exposure related alteration was observed by phase contrast microscopy in cell size, attachment and density of the 48 h semiconfluent monolayers.

3.3. Histology

No microwave exposure related histopathological alterations were found in testicles, epididymes, adrenals, prostates, kidneys, hearts, livers and pituitary glands.

3.4. Body weight

There was no significant difference in the body weights of exposed animals compared to the sham-exposed controls on the first and the last day of the exposure.

3.5. Hematology

The hematologic variables (mean $\pm$ S.E.M.) of microwave exposed and sham-exposed mice are summarized in Table 1. In the microwave exposed animals, two-way ANOVA revealed sig-

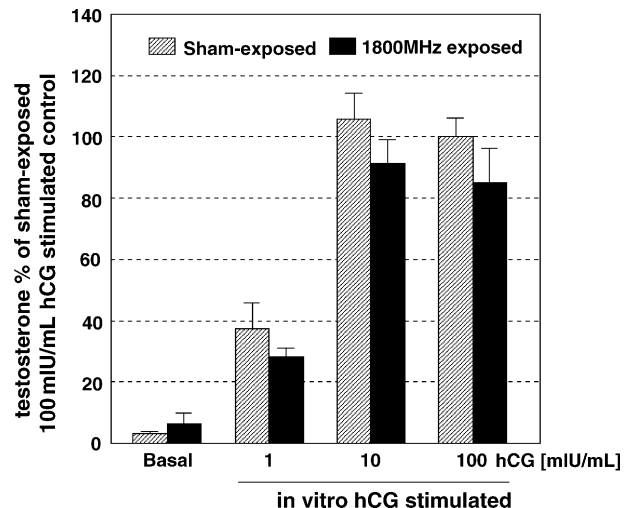


Fig. 3. Effect of whole-body 1800 MHz GSM-like microwave exposure on the basal or *in vitro* hCG stimulated testosterone response of 48 h mouse testicular interstitial (Leydig) cell culture. Bar values represent the mean ($\pm$ S.E.M.) testosterone production as per cent of sham-exposed, *in vitro* 100 mIU/mL hCG stimulated cells. Five independent experiments were performed, the number of replicates was 3–4 at each point per experiment. Two-way ANOVA demonstrated no significant change in the testosterone production of the cultures obtained from whole-body 1800 MHz exposed mice compared to the sham-exposed controls. The level of significance was set at $p < 0.05$.

nificantly higher ($p < 0.01$) red blood cell count (8.59 ± 0.10 T/l) and volume of packed red cells ($42.29 \pm 0.43\%$) compared with the sham-exposed controls (RBC: 8.12 ± 0.08 T/l; VPRC: $39.76 \pm 0.36\%$). The total number of the samples was 37 (exposed) and 36 (sham-exposed) obtained from five independent experiments.

3.6. Serum chemistry

The mean values ($\pm$ S.E.M.) of serum alkaline phosphatase, glutamic-oxaloacetic transaminase (SGOT), glutamic-pyruvic transaminase (SGPT), cholesterol and triglycerides for exposed and sham-exposed mice are shown in Table 2. Two-way ANOVA demonstrated no significant effects of microwave exposure on

Table 1
Mean values $\pm$ S.E.M. of blood variables

Blood variables	Sham-exposed	1800 MHz exposed
WBC (G/l)	4.54 ± 0.31 (36)	3.88 ± 0.27 (37)
RBC (T/l)	8.12 ± 0.08 (36)	$8.59 \pm 0.10^{**}$ (37)
MCV (fl)	49.00 ± 0.32 (36)	49.27 ± 0.40 (37)
HGB (g/l)	12.50 ± 0.10 (36)	12.70 ± 0.13 (37)
VPRC (%)	39.76 ± 0.36 (36)	$42.29 \pm 0.43^{**}$ (37)
PLT (G/l)	830.3 ± 43.43 (36)	879.7 ± 1.83 (37)

White blood cell counts (WBC), red blood cell counts (RBC), mean cell volume (MCV), hemoglobine concentration (HGB), volume of packed red cells (VPRC) and platelets (PLT) in sham-exposed and 1800 MHz GSM-like microwave exposed mice. () = total number of animals in five independent experiments. (The difference in the numbers of animals in Tables 1 and 2 arised from the very little amount of the available blood samples. In some cases there was not enough blood for hematology or the collected blood was coagulated.)

** $p < 0.01$ significant difference compared to the sham-exposed controls (two-way ANOVA).

Table 2
Mean values $\pm$ S.E.M. of serum chemistry

Serum chemistry	Sham-exposed	1800 MHz exposed
Alk. phosph. (U/l)	289.8 $\pm$ 12.57 (39)	279.1 $\pm$ 16.89 (42)
SGOT (U/l)	104.50 $\pm$ 6.21 (39)	123.7 $\pm$ 10.16 (43)
SGPT (U/l)	45.85 $\pm$ 2.29 (43)	47.48 $\pm$ 3.22 (47)
Cholesterol (mmol/l)	3.98 $\pm$ 0.11 (36)	4.19 $\pm$ 0.11 (41)
Triglycerides (mmol/l)	1.32 $\pm$ 0.06 (33)	1.19 $\pm$ 0.46 (40)

Alkaline phosphate (alk. phosph.), serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), cholesterol and triglycerides in sham-exposed and 1800 MHz GSM-like microwave exposed mice. () = total number of animals in five independent experiments. (The difference in the numbers of animals in Tables 1 and 2 arised from the very little amount of the available blood samples. In some cases there was not enough blood for hematology or the collected blood was coagulated.) Analyzing the data with two-way ANOVA, no statistical difference was found between the variables of exposed and sham-exposed animals.

these serum chemical parameters. The total number of measured samples was 40–47 (exposed) and 33–43 (sham-exposed) in five independent experiments.

4. Discussion

Our data obtained from five independent experiments show that repeated exposure to 1800 MHz GSM-like microwave radiation at 0.018–0.023 W/kg whole-body SAR value is able to elevate the serum testosterone level within the physiological range in male mice. To study a possible direct effect of the whole-body microwave exposure on testicular steroidogenesis, the *in vitro* testosterone production was also determined in 48 h Leydig cell cultures obtained from testicles of exposed mice. In toxicological experiments, *in vitro* measures of T production of Leydig cells seem to be more sensitive than *in vivo* indicators of testicular damage [30]. Determination of the LH or hCG stimulated T response of cells improves the sensitivity of this method. In present study, the unaltered *in vitro* steroidogenic capacity of Leydig cells obtained from microwave exposed mice suggest that Leydig cells were not the primary targets of the applied microwave exposure or the direct action was transient only and it was confined on the period of exposure. The mechanism of action of the applied microwave exposure on serum testosterone level cannot be discerned from present results. By contrast, in a previous study we found an elevated hCG stimulated T response of Leydig cell cultures obtained from whole-body 50 Hz magnetic field (MF) exposed mice, while the serum T level of the animals remained unaltered. In that experiment, we suggested a presumably direct effect of whole-body MF exposure on Leydig cells [31]. This hypothesis was supported by our previous *in vitro* data [32]. Although, the GSM-like microwave radiation applied in present study also contains a 217 Hz extremely low frequency (ELF) component, the site of action seems to be basically different from the main target(s) of the 50 Hz MF exposure.

Considering present results and the previous data on the effects of microwaves on central nervous system, the main targets of action are probably at the higher regulation level of the hypothalamic–pituitary–gonadal axis. The effect of GSM-like microwave exposure on serum testosterone level may be asso-

ciated with its possible effect on melatonin secretion of pineal gland. Some recent human data indicate that prolonged use of GSM mobile phones may lead to reduced melatonin production [11,33]. It is known that melatonin exerts an antigonadotrophic effect mainly at the level of the hypothalamus and pituitary [34–36] and decreased directly the testosterone secretion in Leydig cells too [37,38].

The non-thermal, pulsed microwave effects are connected with the transfer of information from irradiating field to the alive organism, through the latter's ability to "recognise" certain frequency of radiation, than with its ability to absorb energy from the field [39]. The intensity of radiation needed for this recognition is many orders of magnitude below even that currently associated with non-thermal effect [1].

At cellular level, one of the most critical target of the MF or microwave exposure in living systems the modification of cellular Ca^{2+} fluxes. It is known that Ca^{2+} -signalling has extreme sensitivity for coherent excitation by very low field energies within specific amplitude and frequency windows [40]. The level of the lowest published results for calcium ion efflux (in frog hearts) is 0.00015 W/kg (0.08 $\mu\text{W}/\text{cm}^2$) at 16 Hz modulated 240 MHz RF signal [41]. The mechanism of action of gonadotropin releasing hormone (GnRH) on the anterior pituitary gonadotropes has been studied in great detail and it is generally accepted that Ca^{2+} functions as a second messenger in the GnRH-activated signal transduction cascade [42–44].

The lack of any microwave related histopathological alterations in the investigated organs suggest that the applied GSM-like microwave exposure evoked only some functional changes in the irradiated mice. It may be hypothesized that the observed increase in serum testosterone levels was not due to an incidental non-specific stress. Previous investigators found that stress accompanied by a low serum testosterone level in men [45] and *in vitro* hCG stimulated androgenesis of Leydig cells obtained from stressed rats was also significantly reduced [46].

We recorded a slight, but statistically significant elevation of RBC and VPRC in microwave exposed mice. Although Friedman [47] in a small case-control study reported a possible microwave related polycythaemia among military radar workers, in contrast to our findings several previous investigators did not find any changes in most of the blood variables of non-thermal microwave irradiated animals [48–51]. The discrepancy between our data and the results of other reports may be due to the different experimental conditions and/or the various species (or strains) of animals used. Non-thermal effects have proved controversial, and independent attempts to replicate them have not always been successful. Such difficulties are not unexpected, however, because these effects depend on the state of the organism when it is exposed, particularly *in vivo*. The highly non-linear nature of living systems makes them hypersensitive to the prevailing conditions, and thus militates against the realisation of the identical conditions necessary for exact replication [1].

To clarify the mechanism and site of action of the applied GSM-like exposure on serum testosterone level additional determination of other endocrine parameters (e.g. FSH, LH and melatonin levels) is necessary. A following study with

head-only exposure may help to analyze the effects mainly at the level of CNS, while in vitro exposure of cell culture is the best solution to investigate a possible direct effect of microwaves on Leydig cells. Further experiments should be performed to assess the possible role of ELF components in the biological actions of GSM irradiation (microwave exposure in CW, TDMA or DTX mode, with or without speech simulation).

In summary, the present findings indicate that repeated whole-body 1800 MHz GSM-like microwave exposure below the maximum permissible exposure level recommended by ICNIRP is able to increase the serum testosterone level, RBC and VPRC in male mice within the physiological range. Further investigations are required to establish the biological significance and possible reproductive implications of these phenomena.

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