

The effect of 2.45 GHz non-ionizing radiation on the structure and ultrastructure of the testis in juvenile rats

Veronika Šimaiová¹, Viera Almášiová¹, Katarína Holovská¹,
Terézia Kisková², Františka Horváthová³, Zuzana Ševčíková⁴, Štefan Tóth⁵,
Adam Raček⁶, Enikő Račeková⁷, Katarína Beňová⁶, Petr Dvořák⁸ and Viera Cigánková¹

¹Department of Anatomy, Histology and Physiology, Institute of Histology and Embryology, University of Veterinary Medicine and Pharmacy in Kosice, ²Department of Animal Physiology, Institute of Biology and Ecology, Faculty of Sciences, ³Department of Anatomy, Faculty of Medicine, Pavol Jozef Safarik University in Kosice, ⁴Department of Pathological Anatomy and Pathological Physiology, Institute of Pathological Anatomy, University of Veterinary Medicine and Pharmacy in Kosice, ⁵Department of Histology and Embryology, Faculty of Medicine, Pavol Jozef Safarik University in Kosice, ⁶Department of Biology and Genetics, Institute of Biology, Zoology and Radiobiology, University of Veterinary Medicine and Pharmacy in Kosice, ⁷Institute of Neurobiology of Biomedical Research Center of Slovak Academy of Sciences, Kosice, Slovakia and ⁸Department of Gastronomy, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

Summary. Background. Nowadays, mobile devices that emit non-ionizing electromagnetic radiation (EMR) are predominantly used by juveniles and pubescents. The aim of the present study was to evaluate the effect of whole body pulsed EMR on the juvenile Wistar albino rat testis at a frequency of 2.45 GHz and mean power density of 2.8 mW/cm². Methods. The investigated animals (n=24) were divided into two control and two EMR groups (5 and 6 week old rats; 6 rats per group). Both EMR groups were irradiated continually for 3 weeks (2h/day) from postnatal days 14 and 21, respectively. Results. EMR caused an irregular shape of seminiferous tubules with desquamated immature germ cells in the lumen, a large number of empty spaces along the seminiferous epithelium and dilated and congested blood vessels in the interstitial tissue of the testis. The cytoplasm of Sertoli cells showed strong vacuolization and damaged organelles, with the cytoplasm full of different heterophagic and lipid vacuoles or the cytoplasm of spermatocytes with swollen mitochondria in both irradiated groups. A significant increase in the

total tubular area of seminiferous tubules was observed in both EMR groups compared with controls (P<0.001). A significant increase in the TUNEL-positive apoptotic nuclei (P<0.01) was accompanied by a significant rise in both Cu-Zn-SOD (P<0.01) and Mn-SOD (P<0.001) positive cells in the 6 week old experimental rats compared to control animals. Conclusion. Our results confirmed a harmful effect of non-ionizing radiation on the structure and ultrastructure of the juvenile rat testis.

Key words: Juvenile rats, Non-ionizing Radiation, Testis, Giant cells

Introduction

Microwave radiation or non-ionizing electromagnetic radiation (EMR) is a common part of daily life. It is employed not only for long distance data transmission but also in equipment used on a daily basis. Microwave ovens, cordless telephones, baby monitors, car alarms, bluetooth devices, wireless data networks and others cause a permanent indoor and outdoor exposure to radiation and may pose a threat to human health.

The level of energy of microwaves is far below the high frequency of X-rays and γ - rays, so it cannot break the covalent bonds in biological molecules. However,

Offprint requests to: D.V.M., Veronika Šimaiová, Department of Anatomy, Histology and Physiology, Institute of Histology and Embryology, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81, Košice, Slovak Republic. e-mail: nika.simaiova@gmail.com
DOI: 10.14670/HH-18-049

microwave radiation has sufficient energy that can cause molecular vibrations and rotations, with subsequent heat production within matter (Challis, 2005). The thermal effect, causing a rise in tissue temperature, results in important biological changes. Nonetheless, many experimental outcomes point to a minor role of changes induced through the thermal effect. These changes are rather the result of non-thermal mechanisms at the molecular level. The most frequently observed endpoints that have been observed in experiments are DNA damage and chromosomal instability (Lagroye et al., 2004; Paulraj and Behari, 2006; Yuriy et al., 2009; Kumar et al., 2010a), increased production of stress proteins (Kwee et al., 2001; Kim et al., 2006), altered cellular membrane properties (Kim et al., 1989; Gaber et al., 2005) and many others, in which the oxidative stress is considered the main mediator (Moustafa et al., 2001; Ilhan et al., 2004; Sokolovic et al., 2008; Kumar et al., 2010b; Kesari et al., 2011). Many experimental *in vivo* and *in vitro* studies revealed the negative influence of non-ionizing radiation on specific organ systems such as the cardiovascular system (Jauchem et al., 2000; Ozguner et al., 2005), neuroendocrine system (Sokolovic et al., 2008; Kesari et al., 2011; Bouji et al., 2012), immune system (Gatta et al., 2003; Johansson, 2009; Grigoriev et al., 2010), auditory apparatus (Oktay and Dasdag, 2006; Kaprana et al., 2011), eye (Akar et al., 2012), salivary glands (Sadetzki et al., 2008), liver (El-Bediwi et al., 2011) and kidney (Ingole and Ghosk, 2006; Gholampour et al., 2011; Khayyat, 2011).

A number of studies showed that non-ionizing radiation has a detrimental effect on the male reproductive system and has become another important environmental risk factor contributing to male infertility. This is mainly due to the high sensitivity of male gonads to various harmful agents, including physical factors. The most frequently observed phenomena are changes in the histological structure and ultrastructure of testis (Khaki et al., 2006; Khayyat, 2011; Koc et al., 2013; Luo et al., 2013; Odaci and Ozyilmaz, 2015), altered testosterone levels (Kumar et al., 2011; Bahaodini et al., 2015), as well as changes in qualitative or quantitative parameters of spermatozoa (Agarwal et al., 2009; Falzone et al., 2011; Lukac et al., 2011).

Tissues in younger or developing animals appear to be even more sensitive to the effects of microwave radiation because of their higher water content and subsequently different dielectric properties compared to the adult tissues (Peyman et al., 2001; Qaddi and Srifi, 2016). The steadily increasing trend of use of wireless devices by teenagers and children raises increased interest of researchers in the assessment of the influence of microwaves on growing and developing organisms, including the testis (Odaci et al., 2016; Saygin et al., 2016).

In scope of the current knowledge it is very important to focus not only on adult animals but also on juveniles. Thus, the aim of our study was to evaluate the effects of EMR on juvenile rat testes and the

reproductive outcome.

Materials and methods

Animals and experimental design

In this experiment, juvenile male Wistar albino rats ($n=24$) were used. The rats were bred in the animal facility of the Institute of Neurobiology, Slovak Academy of Sciences. They were housed in standard animal cages (two rats in each) under controlled temperature ($21\pm1^{\circ}\text{C}$) and subjected to a 12/12 h day light/darkness cycle with *ad libitum* access to food and water. The rats were randomly divided into two control and two experimental groups ($n=6/\text{group}$). Control groups consisted of control 5 week (C5w) and 6 week (C6w) old rats, which were sacrificed on the postnatal day 35 (P 35) and 42 (P 42), respectively. Experimental groups consisted of EMR irradiated 5 week old rats (EMR5w), exposed from the P14 continually for 3 weeks (2h/day) and 6 week old rats (EMR6w), exposed from the P21 continually for 3 weeks (2h/day). All experimental rats were exposed to whole-body pulsed non-ionizing electromagnetic radiation at a frequency of 2.45 GHz and mean power density of 2.8 mW/cm^2 in a purpose-designed chamber (Fig. 1). The uniformity of the electromagnetic field was monitored with a spectral analyser. Both irradiated and control rats were anesthetized by Isoflurane inhalation and then

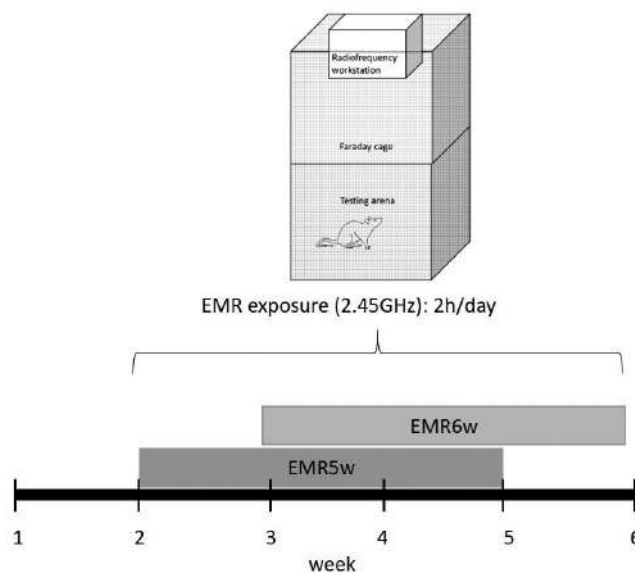


Fig. 1. Experimental scheme: 14- and 21-postnatal day old rats were exposed to the electromagnetic field (2.45 GHz and mean power density of 2.8 mW/cm^2) during three weeks for 2h per day. During the irradiation, the animals were placed in their home cages in an experimental shaded chamber capable of producing a homogenous field of electromagnetic radiation (EMR) and ensuring a free movement of experimental animals with minimum of stress. The chamber used the principle of Faraday cage.

Effect of non-ionizing radiation on the testis of juvenile rat

injected intraperitoneally with chloral hydrate. Deeply anesthetized rats were perfused transcardially with a solution of 4% paraformaldehyde in 0.1 M phosphate buffer. The experiment was performed at the Institute of Neurobiology of Biomedical Research Centre, Slovak Academy of Sciences. All ethical protocols and guidelines for animal handling and treatment were complied with and the experiments were supervised by the Ethical Committee for Animal Experiments at the Institute of Neurobiology, Slovak Academy of Sciences, Kosice, Slovakia and the State Veterinary and Food Administration of the Slovak Republic (No. Ro 2792/15-221/2).

Histopathological observation - light microscopy

Bioptic tissue samples were fixed in mDF (modified Davidson's fluid) solution (Latendresse et al., 2002) for 24 hours, dehydrated in ethanol and embedded in paraffin. After clearing in benzene, 5 μm thick tissue sections were stained with haematoxylin and eosin (H&E). The samples from the left-sided testis were processed for Durcupan™ ACM (Sigma-Aldrich Chemie GmbH, Germany) embedding. Semi-thin sections were stained with toluidine blue. Both were examined under a Zeiss Axio Lab A1 (Zeiss, Germany) light microscope and documented with a camera Axio Cam ERc 5 (Zeiss, Germany).

Morphometric analysis

Tissue samples (1×1×0.5 cm) from the right-sided testes were obtained from each animal and processed in the same way as those intended for histopathological observation. Seminiferous tubule cross sections were randomly chosen (50 tubules per group) and total tubular areas ($\times 10^4 \mu\text{m}^2$) were measured. Observations were made using a Nikon Labophot 2 light microscope (Nikon, Japan) at $\times 100$ magnification. The areas of tubules were determined with NIS-Elements Advanced Research 3.0 Programme (Nikon, Japan) using the functions Annotations and Measurements, Area.

Electron microscopy

Tissue samples up to 1 mm³ were obtained from 3 different parts (more superficial and deeper) of the left-sided testis of each control and experimental animal and fixed by immersion in 3% glutaraldehyde and postfixed in 1% osmium tetroxide (both in 0.1 M cacodylate buffer, pH 7.3). After dehydration in an ascending acetone series, they were transferred to propylene oxide for three hours and embedded in gelatine capsules by Durcupan™ ACM (Sigma-Aldrich Chemie GmbH, Germany). Sections of specimens were cut using an ultramicrotome LKB-Nova (LKB, Bromma, Sweden). Ultrathin sections were double contrasted with uranyl-acetate and lead citrate and examined under an electron microscope Tesla BS 500 (Tesla Brno, Czech Republic).

Detection of germ cell apoptosis

In situ Fluorescein TUNEL assay

Evaluation of apoptosis was accomplished using terminal deoxynucleotidyl-transferase-mediated deoxyuridine triphosphate *in situ* nick end labeling (TUNEL) in paraffin-embedded 3-5- μm thick sections and processed with the TUNEL method according to the manufacturer's instructions (*In situ* Cell Death Detection Kit, Fluorescein, Roche, Germany). After fluorescence staining, the sections were incubated with 10 mg/ml Hoechst 33342 (Sigma-Aldrich, St. Louis, MO, USA) in PBS for 5 min, washed three times in PBS, cover-slipped in mounting medium for fluorescence (H-1200, VECTASHIELD with DAPI, Vector Laboratories, Burlingame, CA, USA), and examined under a Leica DM2500 fluorescence microscope (Leica, Wetzlar, Germany) with ultraviolet and fluorescein isothiocyanate (FITC) filters. The total extent of apoptotic cell death (TUNEL positivity) in testicular tissue was evaluated in 10 randomly selected visual fields of the fluorescence microscope at a magnification of 400, based on the total number of TUNEL-positive cell nuclei, on exactly morphometrically measured area of seminiferous tubules of testis. The resulting evaluation as the so-called apoptotic cell death index expresses the average number of TUNEL-positive cell nuclei calculated on the 1 mm² tissue area of testis without interstitial tissue.

Immunohistochemical Cu-Zn-SOD and Mn-SOD analysis

For immunohistochemical detection the following antibodies were used: polyclonal antibody Cu-Zn-SOD (#ADI-SOD-100, Enzo Life Sciences, USA), polyclonal antibody Mn-SOD (#ADI-SOD-111, Enzo Life Sciences, USA). For detection of primary antibodies, a universal detection kit was employed (Cell & Tissue Staining Kit, HRP-DAB System, Rabbit Kit Cat. No. CTS005, R&D Systems, Abingdon, UK). After DAB chromogen 3, 3'-diaminobenzidine visualisation (Cell & Tissue Staining Kit, HRP-DAB System, Rabbit Kit Cat. No. CTS005, R&D Systems, Abingdon, UK), the sections were counterstained with haematoxylin and examined via Zeiss Axio Lab A1 (Zeiss, Germany) light microscope at a magnification of 100 and documented with a camera Axio Cam ERc 5 (Zeiss, Germany). Detection of SOD positive cells was counted in 50 seminiferous tubules/group with ImageTool program, version 2.00.

Statistical analysis

The statistical analysis was interpreted using a GraphPad InStat version 5.01 (GraphPad Software, San Diego, CA). For the evaluation of morphometrical analysis NIS-Elements Advanced Research 3.0 software was used. Results from morphometry and immunohistochemical analysis were assessed statistically using One-way ANOVA with the post hoc

Tukey multiple comparison test. The results are expressed as mean $\pm$ SD.

Results

Histopathological observation - light microscopy

The testicular tissue originating from the control groups exhibited normal structural features characteristic for peri-pubertal period, such as the regular shape of the seminiferous tubules, well developed seminiferous epithelium containing numerous maturing germ cells and including only a small number of elongated spermatids. The interstitial connective tissue housed a distinctive number of mature Leydig cells and typical blood vessels (Fig. 2A).

On the contrary, the testicular tissue obtained from both groups of irradiated animals revealed various diffuse degenerative changes. There was a certain amount of seminiferous tubules with irregular shape (Fig. 2B,C). Their lumens often contained small (Fig. 2B) or greater number (Fig. 2C) of desquamated immature germ cells. The seminiferous epithelium often contained many irregular empty spaces as the consequence of cellular desquamation (Fig. 2C). The basement membrane of several seminiferous tubules, which were often irregular in shape, showed an abnormal undulation and the tunica propria contained peritubular myoid cells. Dilated and congested blood vessels were often observed in the interstitial tissue. The interstitial Leydig cells appeared unchained (Fig. 2B,C).

Examination of the semi-thin sections stained with toluidine blue, which allowed more detailed observation of the seminiferous epithelium, revealed strong vacuolization of cytoplasm of Sertoli cells as well as all developmental stages of germ cells. The seminiferous epithelium of irradiated rat testis frequently contained dark necrotizing cells scattered between the other epithelial cells as well as giant multinucleated cells which were found in an adluminal boundary line. The incidence of irregular empty spaces between cells of seminiferous epithelium was also quite high (Fig. 3A,B).

Morphometric results

A significant increase in the total tubular area of seminiferous tubules was observed in both EMR exposed groups compared with the corresponding controls as shown in Table 1.

Table 1. Morphometric analysis of total tubular area ($\times 10^4 \mu\text{m}^2$) of seminiferous tubules.

C5w	EMR5w	C6w	EMR6w
30.36 $\pm$ 3.90	32.77 $\pm$ 4.68**	30.46 $\pm$ 4.54	35.10 $\pm$ 4.92***

Data are expressed as mean $\pm$ SD; ** $P < 0.01$ – significance of EMR5w vs C5w; *** $P < 0.001$ – significance of EMR6w vs C6w.

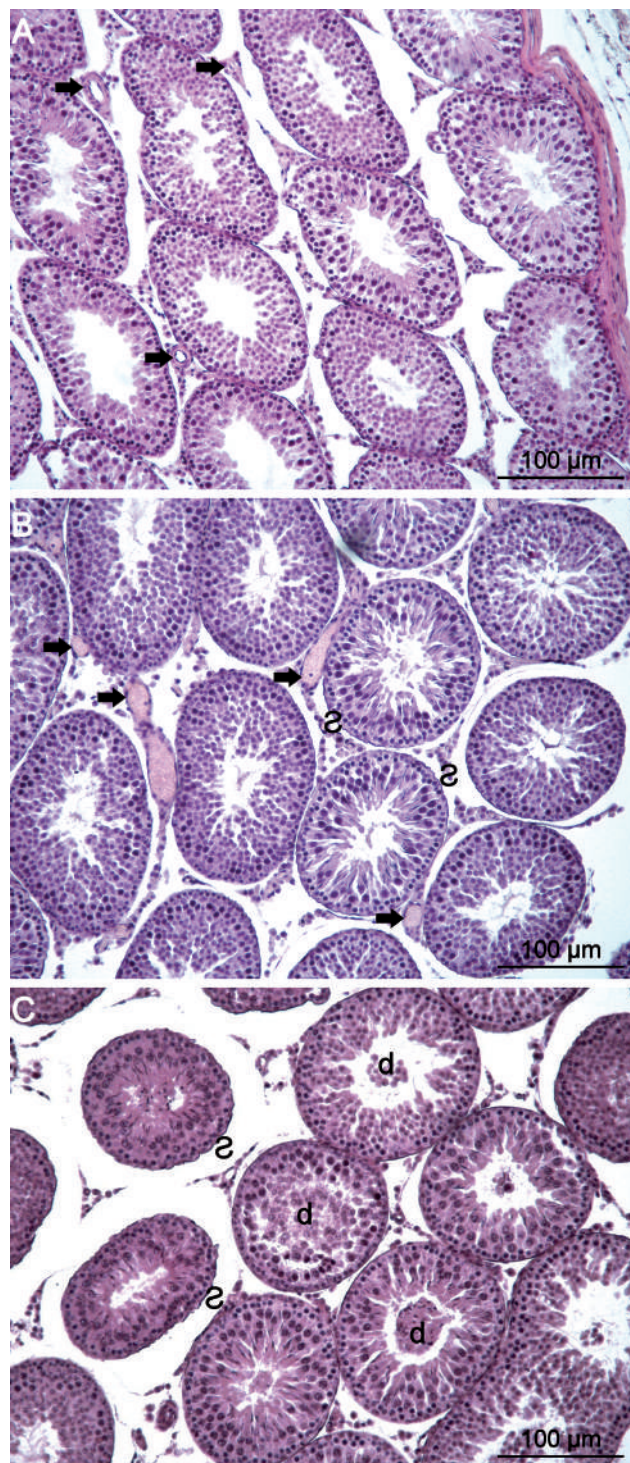


Fig. 2. Photomicrographs of histopathological changes in testicular tissue. Haematoxylin and eosin (H&E) staining. Testicular tissue of control rats with normal structure (A), small blood vessels in the interstitium (arrow). The testicular tissue of 5 week old experimental rats (B) with dilated and congested small blood vessels (arrow) in the interstitium and undulated basement membrane – Z Diffuse degenerative changes in the testicular tissue of 6 week old EMR (C) d: large groups of desquamated immature germ cells present in lumens of seminiferous tubules, Z: undulated basement membrane.

Electron microscopy

In both EMR groups, the radiation induced ultrastructural changes in different cell types of the seminiferous epithelium as well as in testicular interstitium. The majority of Sertoli supporting cells contained damaged organelles, such as swollen mitochondria, enlarged cisternae of the endoplasmic reticulum and plentiful lysosomes. The ultrastructure of nuclei was mostly normal. The cytoplasm often contained various heterophagic and lipid vacuoles. The tight junctions between the adjacent Sertoli cells were frequently affected. The spermatogonia possessed mostly unchanged nucleus, but their cytoplasm showed clear hydropic vacuolization. The spermatocytes and early spermatids had mostly typical nuclei, but their cytoplasm often contained swollen mitochondria and numerous electron-lucent vacuoles. Seminiferous epithelium contained many different empty spaces between the neighbouring germ cells and between germ and Sertoli cells (Figs. 4A,B, 5A,B). In both EMR groups, a higher proportion of pycnotic germ cells were seen. These cells were shrivelled with pycnotic nuclei. Their strongly electron-dense cytoplasm contained many small electron-lucent vacuoles and depleted organelles (Fig. 5B). Cellular particles of dead cells were often present in the form of heterophagic vacuoles within the cytoplasm of Sertoli cells (Figs. 4B, 5B). The basement membrane had constantly uneven thickness (Figs. 4A,B, 5A,B). Some seminiferous tubules were lined by highly undulated basement membrane with adjacent tunica propria (Figs. 4A, 5A). The fibrocytes, fibroblasts and

peritubular myoid cells often contained small electron-lucent vacuoles in their cytoplasm, although their nuclei were not altered (Figs. 4B, 5B,C). The majority of Leydig cells had mostly typical nuclei, but possessed constantly swollen mitochondria, poorly developed smooth endoplasmic reticulum and many electron-lucent vacuoles of different size in their cytoplasm (Figs. 4C, 5C). Some Leydig cells had damaged nuclei and their cytoplasm contained large electron-lucent vacuoles (Fig. 5C). The adjacent vascular elements had often impaired endothelial lining. The cytoplasm of endothelial cells was often vacuolated (Fig. 5C).

Evaluation of germ cell apoptosis

Apoptotic cell death marked by *In situ* TUNEL method in the testis of rats revealed no significant difference between apoptotic cell numbers in the 5 week old control rats (Fig. 6A) and the corresponding irradiated group (Fig. 6B). A significant increase in the TUNEL-positive nuclei was found in the 6 week old

Table 2. *In situ* Fluorescein TUNEL assay – Immunofluorescence analysis of apoptotic cell death.

C5w	EMR5w	C6w	EMR6w
10.60±5.19	10.10±5.41	8.00±4.22	21.22±9.92**

Data are expressed as mean ± SD; **P<0.01 – significance of EMR6w vs C6w.

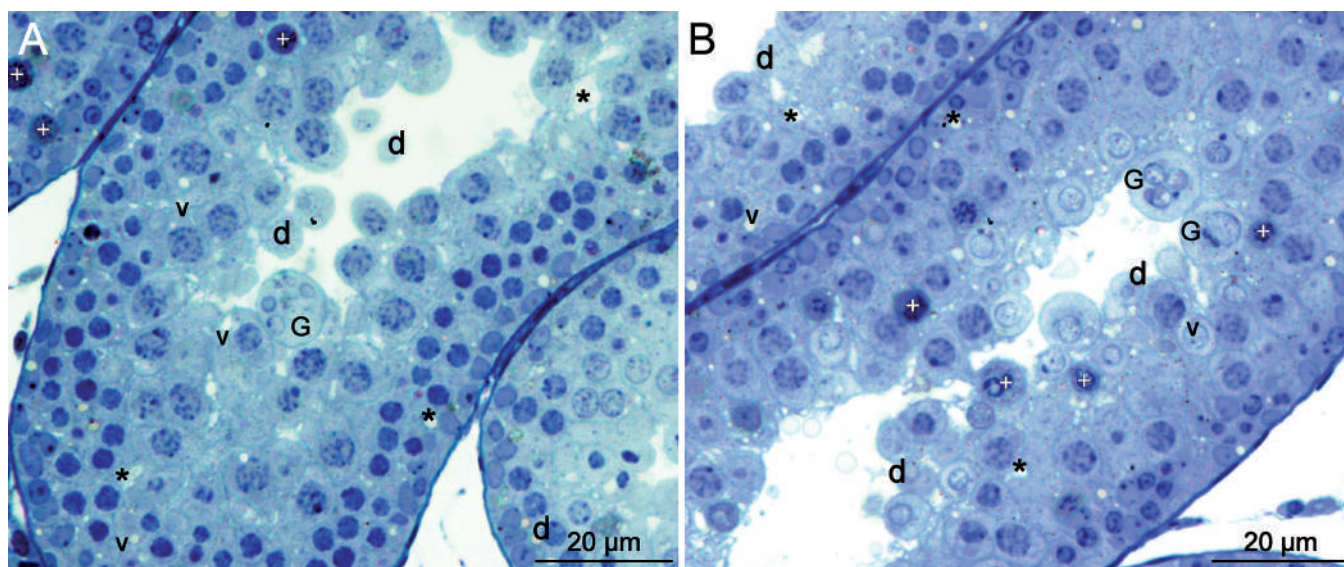


Fig. 3. Photomicrographs of the semi-thin sections. Toluidine blue staining. The testicular tissue of 5 week old EMR (A) with affected seminiferous epithelium. v: vacuoles in cytoplasm; d: desquamation of immature germ cells; +: cellular necrotization; *: presence of empty spaces between cells of the seminiferous epithelium; G: presence of multinucleated giant cells. The testicular tissue of 6 week old EMR rats (B) with affected seminiferous epithelium. V: vacuoles in the cytoplasm; d: desquamation of immature germ cells; +: cellular necrotization; *: presence of empty spaces between cells of the seminiferous epithelium; G: presence of multinucleated giant cells.

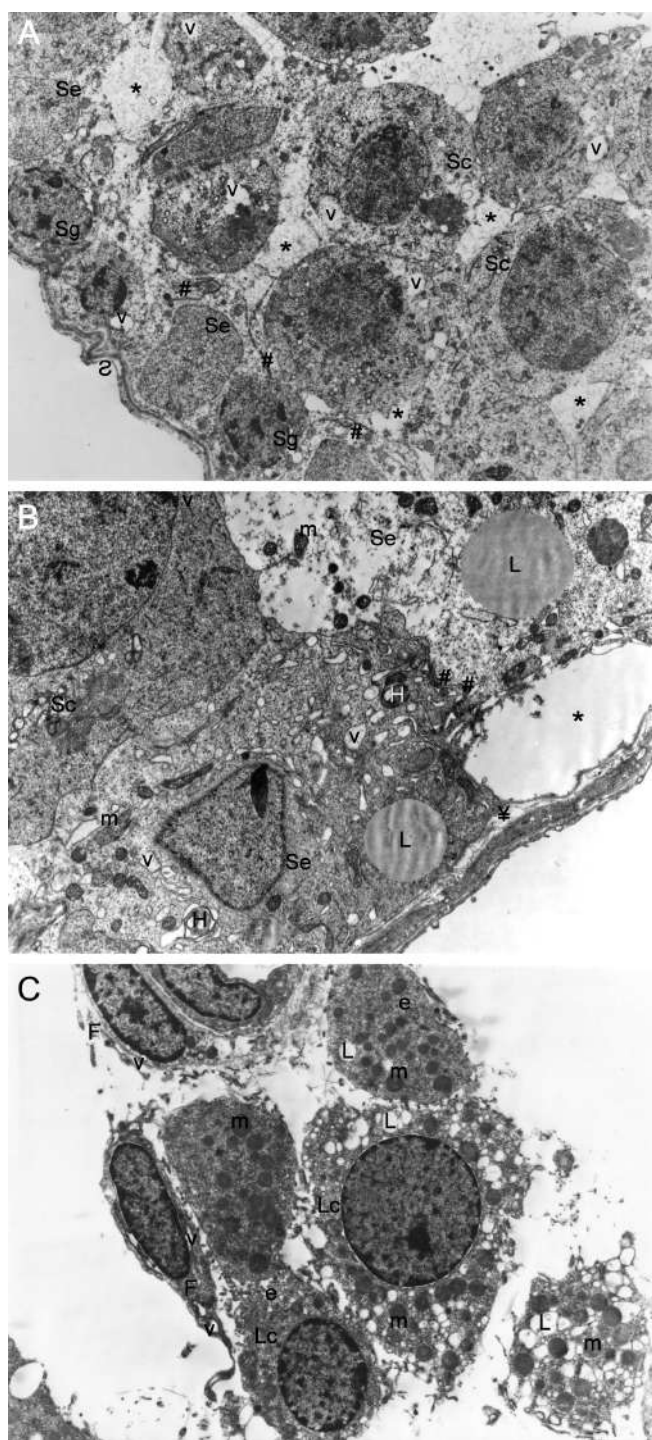


Fig. 4. Electron micrographs of the detail of seminiferous epithelium of 5 week old EMR rats with altered ultrastructure (A, B). Se: Sertoli cell; Sg: spermatogonium; Sc: spermatocyte; V: electron-lucent vacuoles; Z: undulated basement membrane; #: altered tight junction; m: swollen mitochondria; *: empty space; L: lipid vacuole; H: heterophagic vacuole; ¥: irregular basement membrane; Electron micrograph of the interstitium of 5 week old EMR rats (C) with altered ultrastructure. Lc: Leydig cell; F: fibroblasts and fibrocytes; e: smooth endoplasmic reticulum; V: electron-lucent vacuoles; L: lipid vacuoles; m: swollen mitochondria. A, C, x 2800; B, x 4200.

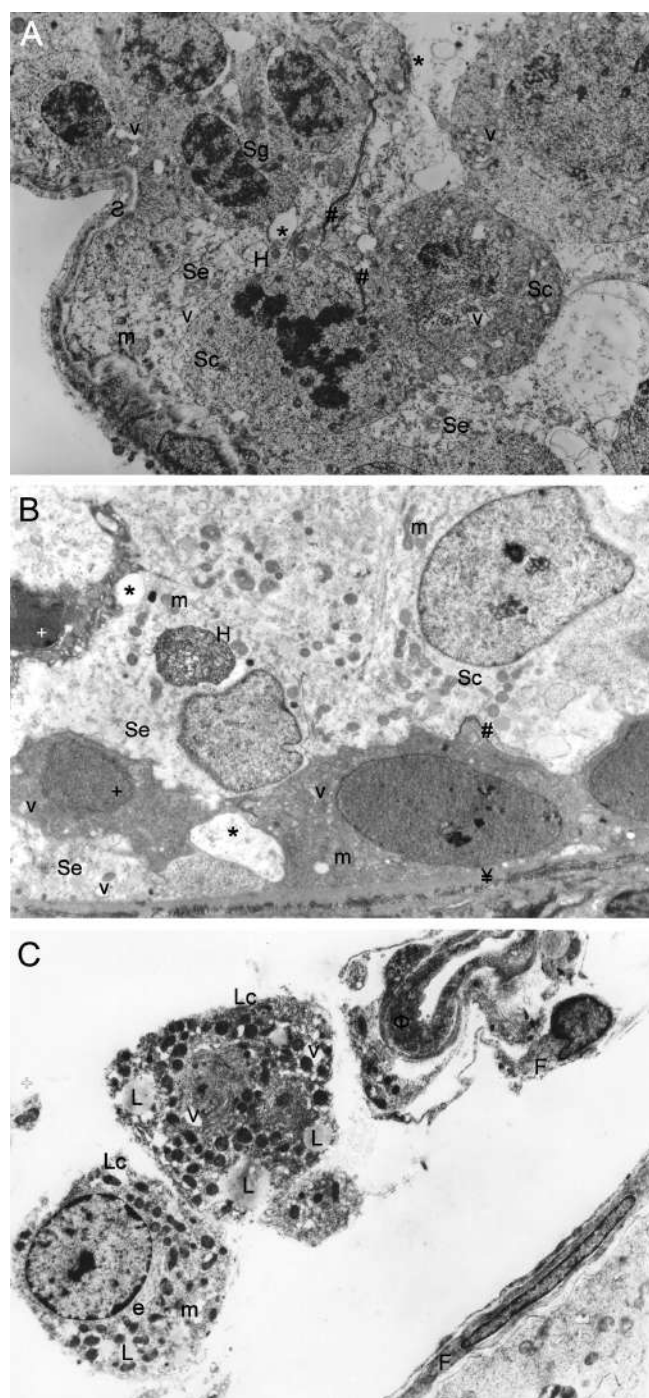


Fig. 5. Electron micrographs of the detail of seminiferous epithelium of 6 week old EMR rats with altered ultrastructure (A, B). Se: Sertoli cell; Sg: spermatogonium; Sc: spermatocyte; V: electron-lucent vacuoles; Z: undulated basement membrane; #: altered tight junction; m: swollen mitochondria; *: empty space; H: heterophagic vacuole; ¥: irregular basement membrane; +: pycnotic cell; Electron micrograph of the interstitium of 6 week old EMR rats (C) with altered ultrastructure. Lc: Leydig cell; F: fibroblasts and fibrocytes; e: smooth endoplasmic reticulum; V: large electron-lucent vacuoles; L: lipid vacuoles; m: swollen mitochondria; Φ: affected endothelium. A, C, x 2800; B, x 4200.

Effect of non-ionizing radiation on the testis of juvenile rat

EMR rats (Fig. 7B) compared to corresponding control group (Fig. 7A) (** $P < 0.01$), Table 2. The TUNEL-positive nuclei were localized mainly in the basal part of the seminiferous tubule epithelium (close to the basement membrane), especially in the large pachytene spermatocytes and in the formation of secondary

spermatocytes.

SODs detection

The colorimetric immunohistochemical results of SODs detection are shown in Table 3. We demonstrated

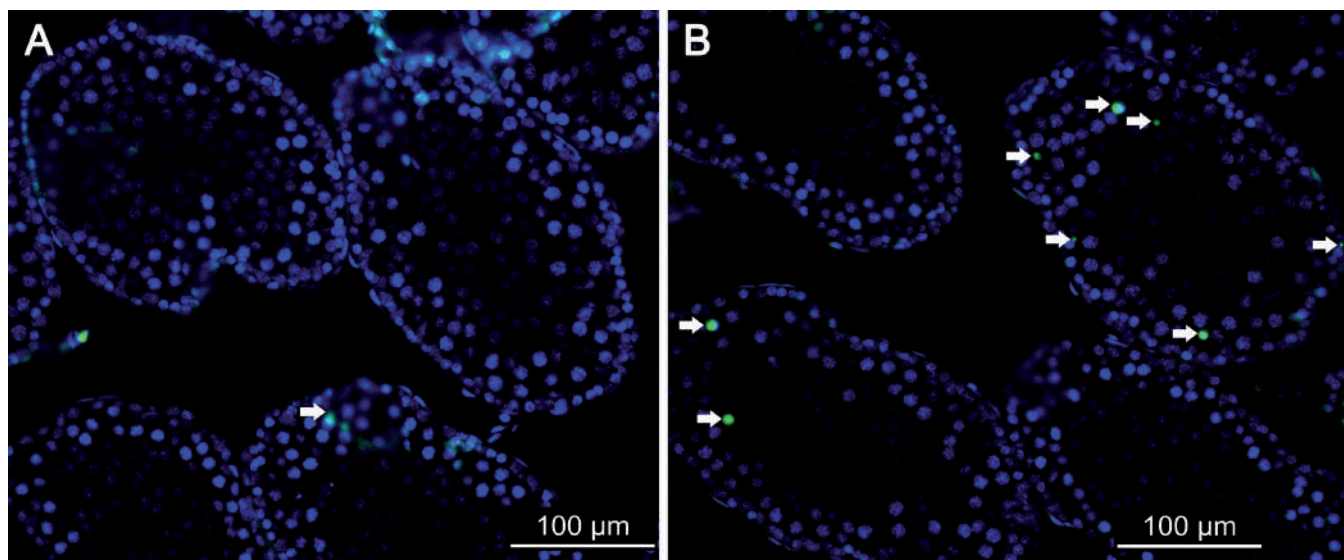


Fig. 6. Photomicrographs illustrating apoptotic level changes in the testes of rats. *In situ* TUNEL method. Testis of 5 week old control rats (A) with normal histological structure, TUNEL-positive nuclei (white arrow) in comparison with the testis of 5 week old EMR group (B) with multiple TUNEL-positive nuclei (white arrow).

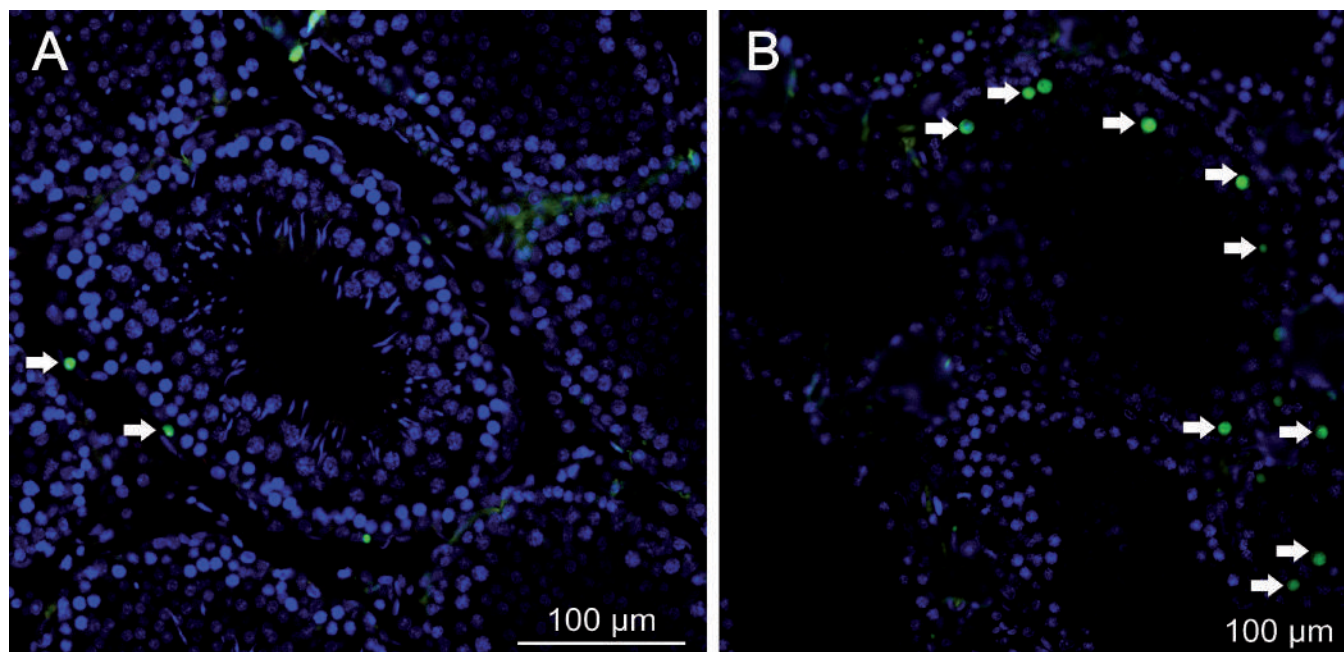


Fig. 7. Photomicrographs illustrating apoptotic level changes in the testes of rats. *In situ* TUNEL method. Testis of 6 week old control rats with normal structure (A) TUNEL-positive nuclei (white arrow) in comparison with the testis of 6 week old EMR group (B) with a significant increase in TUNEL-positive nuclei (white arrow).

that in 6 week old EMR rats both Cu-Zn-SOD (Fig. 9B) and Mn-SOD (Fig. 11B) were significantly increased compared to the corresponding controls (Figs. 9A, 11A) (**P<0.01 and ***P<0.001). Contrariwise, the 5 week old rats revealed no significant difference in Cu-Zn-SOD between control (Fig. 8A) and EMR (Fig. 8B) animals. Mn-SOD enzymes in the 5 week old rats were not in contrast between control (Fig. 10A) and EMR group (Fig. 10B). The Cu-Zn-SOD and Mn-SOD positive cells

Table 3. The presence of Cu-Zn-SOD and Mn-SOD enzymes in rat testis.

	C5w	EMR5w	C6w	EMR6w
Cu-Zn-SOD	58.30±12.45	67.00±14.28	60.60±14.63	91.30±21.38**
Mn-SOD	57.40±16.54	58.50±11.70	59.60±14.37	112.90±37.60**

Data are expressed as mean ± SD; **P<0.01 – significance of both Cu-Zn-SOD in EMR6w vs C6w and Mn-SOD in EMR6w vs C6w.

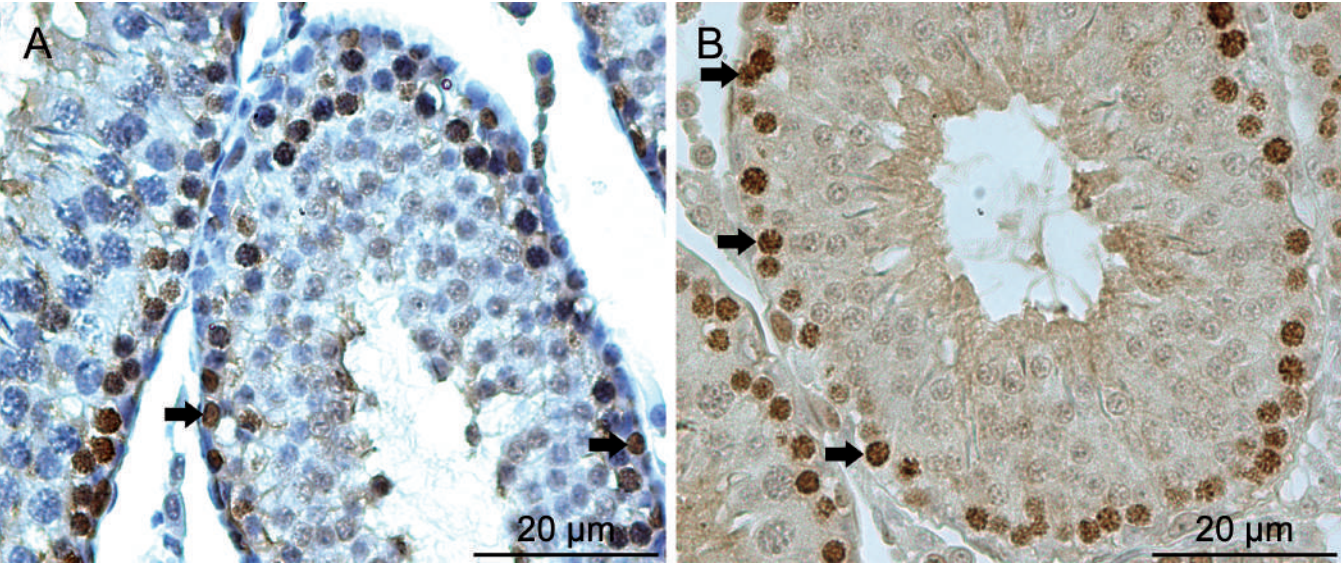


Fig. 8. Photomicrographs of the testes showing immunohistochemical detection and localization of Cu-Zn-SOD enzymes (arrow) in the testis of 5 week old control rats (A) compared to the testis of 5 week old EMR group (B).

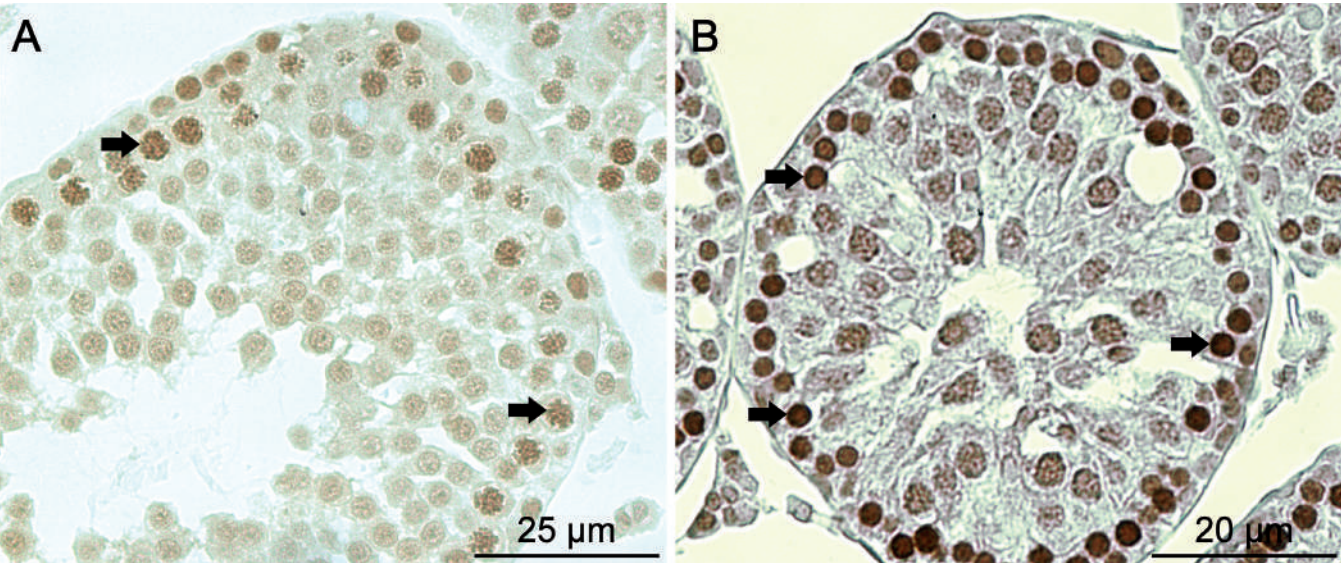


Fig 9. Photomicrographs of the testes showing immunohistochemical detection and localization of Cu-Zn-SOD enzymes (arrow) in the testis of 6 week old control rats (A), compared to testis of 6 week old EMR group (B).

Effect of non-ionizing radiation on the testis of juvenile rat

were mainly spermatogonial cells and spermatocytes, localized close to the basement membrane of seminiferous tubules of the testis.

Discussion

We investigated the effect of non-ionizing electromagnetic radiation on the rat testicular tissue in the peripubertal period of life. The frequency of 2.45

GHz and mean power density of 2.8 mW/cm² used in our study has been reported not to influence the body temperature of rats.

Histopathological examination of the H&E stained sections and semi-thin sections revealed various diffuse degenerative changes in the testicular parenchyma. Several seminiferous tubules were irregularly shaped and had undulated basement membrane, the seminiferous epithelium contained many empty spaces

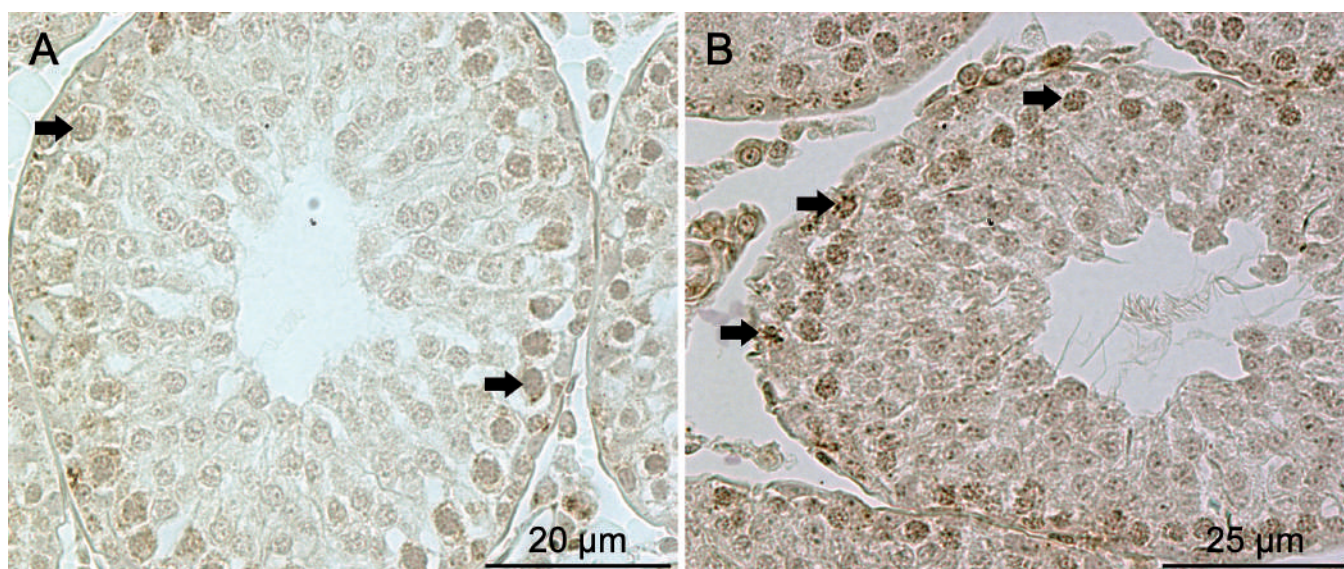


Fig. 10. Photomicrographs of the testes showing immunohistochemical detection and localization of Mn-SOD enzymes (arrow) in the testis of 5 week old control rats (A), compared to the testis of 5 week old EMR group (B).

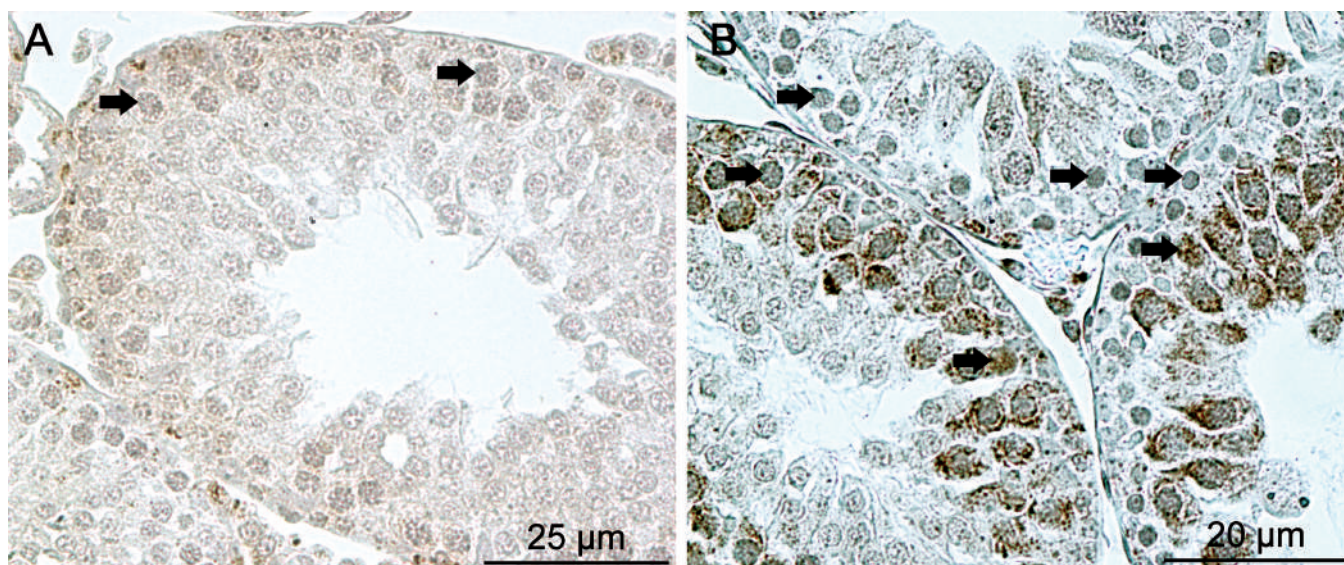


Fig. 11. Photomicrographs of the testes showing immunohistochemical detection and localization of Mn-SOD enzymes (arrow) in the testis of 6 week old control rats (A), compared to the testis of 6 week old EMR group (B).

as the consequence of cellular sloughing and their lumen often contained clumps of immature germ cells. Similar degenerative changes were described also in many studies that investigated the impact of microwaves, although with a focus on sexually mature individuals (Salama et al., 2010; El-Bediwi et al., 2011; Khayyat, 2011). The observation of semi-thin sections stained with toluidine blue revealed more detailed structural changes, such as the vacuolization of cytoplasm of germ cells and Sertoli cells, increased number of necrotizing germ cells as well as the presence of giant multinucleated cells in an adluminal boundary line of seminiferous epithelium. The cytoplasmic vacuolization as the sign of damage of organelles was also confirmed by electron microscopy as in our study as by Celik et al. (Celik et al., 2012), although the latter authors observed this phenomenon in adult rats. Light microscopy also showed an increased incidence of necrotization of germ cells in irradiated animals.

The adluminal boundary line of the seminiferous epithelium often revealed multinucleated giant cells. This phenomenon is related either to incomplete cytokinesis of normal cell division or to cell fusion. The presence of symplasts or multinucleated cells is known to be associated with the final common pathway of germinal cells degeneration in animals treated with different chemicals (Creasy et al., 2012; Spade et al., 2015) and ionizing radiation (Hussein et al., 2006). The formation of multinucleated giant cells is also regarded as a step of apoptosis of round spermatids (Murphy and Richburg, 2014). The most probable mechanism of pathogenesis of giant multinucleated cells is the impaired Sertoli cell maintenance of cytoskeletal bridge closure among groups of spermatids (Creasy et al., 2012).

The testicular interstitium showed congested and dilated capillaries in irradiated animals, which is a clear sign of activation of thermoregulatory mechanisms to dissipate heat from the organ. This is in agreement with the results of (Nassar, 2009) and (Celik et al., 2012) who also noted similar phenomenon. The altered testicular histology found in the present study, or in many other papers may be attributed to the increased testicular temperature or to the creation of the so-called hot spots in the testicles (Trosic et al., 2002).

Morphometric evaluation in both experimental groups of rats indicated a significant increase in total tubular area of seminiferous tubules. Our findings are supported by other researchers who revealed similar changes in testes and in other organs of rats after their exposure to electromagnetic radiation. Al-Damegh (2012) reported a significant increase in the diameter of seminiferous tubules in rats exposed to EMR emitted by a GSM cellular phone (900/1800/1900 MHz) compared to the normal testicular tissue architecture (Al-Damegh, 2012). Also electron microscopy showed a visible increase in the thickness of tunica propria and amount of collagen fibres in the rats exposed to cell phone. On the other hand, other authors described a significant

decrease in the diameter of seminiferous tubules in adult rabbit (Salama et al., 2010). Bahaodini et al. (2015) reported a decrease in the diameter of seminiferous tubules in rats after long-term exposure to low frequency EMR (Bahaodini et al., 2015).

Transmission electron microscopy revealed many ultrastructural changes in cells of germinal epithelium, basement membrane as well as in various constituents of the interstitium in the testicular tissue of irradiated animals. Sertoli cells contained affected organelles and their cytoplasm often stored heterophagic and lipid vacuoles as the characteristics proving their higher phagocytic activity due to increased necrotization of germ cells (Paniagua et al., 1985). The interruptions of intercellular connections between the adjacent Sertoli supporting cells are also in great consonance with the formation of empty spaces within the seminiferous epithelium and cellular desquamations that were observed in the present study. The spermatogonia, spermatocytes and round spermatids had constantly vacuolated cytoplasm as the result of degeneration of their organelles. Celik et al. (2012) found similar degenerative features (Celik et al., 2012).

The most serious degenerative feature, which was observed also by electron microscopy, was the increased necrotization of all three types of germ cells. Based on ultrastructural characteristics, the type of cellular necrotization was both apoptosis and necrosis, although necrosis was much more frequent. Despite the fact that necrosis was present in the seminiferous epithelium, no signs of inflammation were observed. This phenomenon is due to the intense phagocytic activity of Sertoli cells, which was also indicated by the presence of numerous heterophagic and lipid vacuoles within their cytoplasm. Both forms of cellular death were also described from the aspect of electron microscopy in adult mice (Nassar 2009). In the present study, the uneven thickness of the basement membrane was constantly observed, and some seminiferous tubules were even markedly undulated. Nassar (2009) and Celik et al. (2012) described similar ultrastructural lesions of the basement membrane (Celik et al., 2012; Nassar, 2009). Such histopathological changes may suggest altered testicular function because the interaction between constituents of the seminiferous epithelium and the peritubular compartment provided by a well-defined basement membrane is essential for maintenance of normal spermatogenesis.

The interstitial connective tissue components such as the fibrocytes, fibroblasts, peritubular myoid cells and endothelium often revealed numerous small electron-lucent vacuoles within their cytoplasm as the consequence of impaired organelles. The dilatation and congestion of the interstitial blood capillaries were also evident ultrastructurally. These findings are in agreement with Nassar (2009) and Celik et al. (2012), who also indicated congested blood capillaries and damaged blood vessels in the interstitium observed by electron microscopy (Nassar, 2009; Celik et al., 2012). The Leydig cells direct the spermatogenesis via the secretion

of testosterone which acts on the Sertoli and/or peritubular myoid cells to create an optimal environment. Although observation of Leydig interstitial cells by light microscopy failed to show any structural abnormalities, some degenerative changes were found at the level of their ultrastructure. They had typical ultrastructure of the nucleus, but constantly swollen mitochondria and wider cisternae of poorly developed smooth endoplasmic reticulum. Their cytoplasm often contained numerous lipid vacuoles. Some Leydig cells revealed severe damage of the nucleus and large lipid vacuoles in their cytoplasm. The impaired organelles as well as the presence of accumulated lipid droplets within the cytoplasm of Leydig cells indicated the disruption of their normal steroidogenesis. Similar ultrastructural changes were described in irradiated adult rats (Celik et al., 2012) and mice (Nassar, 2009). When evaluating the serum testosterone levels in irradiated animals, many authors have confirmed its significant changes (Kim et al., 2006; Yoon et al., 2007; Saygin et al., 2016).

Germ cell apoptosis serves as a mechanism for balancing the number of germ cells to support capacity of Sertoli cell (Print and Loveland, 2000). It occurs also after direct physical or chemical damage to germ cells (Richburg, 2000), as a response to damage to Sertoli cells. Several studies have reported higher incidence of apoptosis in the testis of laboratory animals after exposure to electromagnetic radiation (Lee et al., 2004; Kim et al., 2009; Saygin et al., 2011). We found that the intensity of the apoptotic cell death significantly increased in the 6 week old EMR rats compared to the corresponding control group. This is in agreement with Shokri et al. (2015) who revealed a significant increase in the number of TUNEL-labelled apoptotic cells in the group of 2.45 GHz Wi-fi exposed rat accompanied with an increased level of caspase-3.

The formation of reactive oxygen species (ROS) is a natural result of intracellular processes but may be triggered by environmental factors like electromagnetic radiation. Cells exposed to EMR *in vitro* (Wu et al., 2008; Yao et al., 2008a,b) and also *in vivo* (Lai and Singh, 2004; Oktem et al., 2005; Tkalec et al., 2007), can generate ROS and thereby elevate oxidative stress (Kazemi et al., 2015; Kumar et al., 2011). However, cells dispose of a defence mechanism that scavenges ROS. Antioxidants such as superoxide dismutase (SOD), catalase and glutathione peroxidase are a part of this mechanism. The role of SOD in clearing (removing) ROS is by catalysing dismutation of superoxid radical ($O_2^{\cdot -}$) to hydrogen peroxide, which is subsequently changed to water by catalase or glutathione peroxidase (Fridovich, 1975). We demonstrated that the detection of both Cu-Zn-SOD, the major cytoplasmatic antioxidant enzyme, and Mn-SOD, mitochondrial matrix enzyme, in 6 week old EMR rats was significantly increased compared to controls. Other authors investigated the activity of SOD enzymes, for example Koc et al. 2013, who detected significantly higher levels of SOD in the EMR (emitted by a mobile phone during calling)

exposed group of rats compared to EMR+ antioxidant group. Moustafa et al. (2001) concluded that acute exposure to EMR allowed to modulate the oxidative stress by reducing the activation of SOD and glutathione peroxidase (GPx) and enhancing lipid peroxidation, which are free radical scavengers (Moustafa et al., 2001). We assume that the EMR triggered the production of ROS that subsequently induced the expression of SOD enzymes. ROS, which were not sufficiently removed, could subsequently be involved in the formation of the observed morphological changes.

This paper shows that improved reproductive healthcare is of greatest importance for the future welfare of humankind. Nowadays children and adolescents often keep their wireless devices such as cell phones and laptops near their testicles, so attention should be paid to potential issues related to such practice. Young growing tissues are especially sensitive to the effects of many environmental factors, including microwave radiation. In our study, the effect of non – ionizing radiation on juvenile rats was investigated and it was revealed that the testes belong to the most radiosensitive organs.

Acknowledgements. The authors would like to thank to lab members, namely Mrs. Zlaťa Buričová and Ing. Lucia Takáčová for their technical cooperation by the preparation of specimens.

Funding: This work was supported by a grant schema of Slovak Republic VEGA 1/0214/15 and 1/0060/18.

Declaration of interest. The authors report no conflict of interests.

References

- Agarwal A., Desai N.R., Makker K., Varghese A., Mouradi R., Sabanegh E. and Sharma R. (2009). Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an *in vitro* pilot study. *Fertil. Steril.* 92, 1318-1325.
- Akar A., Karayigit M.O., Bolat D., Gültiken M.E., Yarim M and Castellani G. (2012). Effects of low level electromagnetic field exposure at 2.45 GHz on rat cornea. *Int. J. Radiat. Biol.* 4, 1-7.
- Al-Damegh M.A. (2012). Rat testicular impairment induced by electromagnetic radiation from a conventional cellular telephone and the protective effects of the antioxidants vitamins C and E. *Clinics* 67, 785-792.
- Bahaodini A., Owfard M., Tamadon A. and Jafari S.M. (2015). Low frequency electromagnetic fields long-term exposure effects on testicular histology, sperm quality and testosterone levels of male rats. *Asian Pac. J. Reprod.* 4, 195-200.
- Bouji M., Lecomte A., Hode Y., de Seze R. and Villegier A.S. (2012). Effects of 900 MHz radiofrequency on corticosterone, emotional memory and neuroinflammation in middle-aged rats. *Exp. Gerontol.* 47, 444-451.
- Celik S., Aridogan I.A., Izol V., Erdogan S., Polat S. and Doran S. (2012). An evaluation of the effects of long-term cell phone use on the testes via light and electron microscope analysis. *Urology* 79, 346-350.
- Creasy D., Bube A., de Rijk E., Kandori H., Kuwahara M., Masson R., Nolte T., Reams R., Regan K., Rehm S., Rogerson P. and Whitney

- K. (2012). Proliferative and nonproliferative lesions of the rat and mouse male reproductive system. *Toxicol. Pathol.* 40, 40s-121s.
- El-Bediwi A.B., El-kott A.F., Saad M. and Eid E. (2011). Effects of electromagnetic radiation produced by mobile phone on some visceral organs of rat. *J. Med. Sci.* 11, 256-260.
- Falzone N., Huyser C., Becker P., Leszczynski D. and Franken D.R. (2011). The effect of pulsed 900 MHz GSM mobile phone radiation on the acrosome reaction, head morphometry and zona binding of human spermatozoa. *Int. J. Androl.* 34, 20-26.
- Fridovich I. (1975). Superoxide dismutases. *Annu. Rev. Biochem.* 44, 147-159.
- Gaber M.H., Abd El Halim N. and Khalil W.A. (2005). Effect of microwave radiation on the biophysical properties of liposomes. *Bioelectromagnetics* 26, 194-200.
- Gatta L., Pinto R., Ubaldi V., Pace L., Galloni P., Lovisolo G.A., Marino C. and Pioli C. (2003). Effects of *in vivo* exposure to GSM – modulated 900 MHz radiation on mouse peripheral lymphocytes. *Radiat. Res.* 160, 600-605.
- Gholampour F., Owji S.M. and Javadifar T.S. (2011). Chronic exposure to extremely low frequency electromagnetic field induces mild renal damages in rats. *Int. J. Zool. Res.* 7, 393-400.
- Grigoriev Y.G., Grigoriev O.A., Merkulov A.V., Shafirkin A.V. and Vorobiov A.A. (2010). Autoimmune processes after long-term low level exposure to electromagnetic fields. *Biophysics* 55, 1046-1049.
- Hussein M.R., Abu-Dief E.E., Abou El-Ghait A.T., Adly M.A. and Abdelraheem M.H. (2006). Morphological evaluation of the radioprotective effects of melatonin against X-ray-induced early and acute testis damage in Albino rats: an animal model. *Int. J. Exp. Pathol.* 87, 237-250.
- Challis L.J. (2005). Mechanisms for interaction between RF fields and biological tissue. *Bioelectromagnetics (Suppl 7)*, 98-106.
- Ilhan A., Gurel A., Armutcu F., Kamisli S., Iraz M., Akyol O. and Ozen S. (2004). Ginkgo biloba prevents mobile phone –induced oxidative stress in rat brain. *Clin. Chim. Acta* 340, 153-162.
- Ingle I.V. and Ghosh S.K. (2006). Cell phone radiation and developing tissues in chick embryo – A light microscopic study of kidneys. *J. Anat. Soc. India* 55, 19-23.
- Jauchem J.R., Ryan K.L. and Frei M.R. (2000). Cardiovascular and thermal effects of microwave irradiation at 1 and/or 10 GHz in anesthetized rats. *Bioelectromagnetics* 21, 159-166.
- Johansson O. (2009). Disturbance of the immune system by electromagnetic fields – a potentially underlying cause for cellular damage and tissue repair reduction which could lead to disease and impairment. *Pathophysiology* 16, 157-177.
- Kapra A.E., Chimona T.S., Papadakis C.E., Velegrakis S.G., Vardiambasis I.O., Adamidis G. and Velegrakis G.A. (2011). Auditory brainstem response changes during exposure to GSM -900 radiation: An experimental study. *Audiol. Neurotol.* 16, 270-276.
- Kazemi E., Mortazavi S.M., Ali-Ghanbari A., Sharifzadeh S., Ranjbaran R., Mostafavi-Pour Z., Zal F. and Haghani M. (2015). Effect of 900 MHz Electromagnetic radiation on the induction of ROS in human peripheral blood mononuclear cells. *J. Biomed. Phys. Eng.* 5, 105-114.
- Kesari K.K., Kumar S. and Behari J. (2011). 900-MHz microwave radiation promotes oxidation in rat brain. *Electromagn. Biol. Med.* 30, 219-234.
- Khaki A.A., Tubbs R.S., Shoja M.M., Rad J.S., Khaki A., Farahani R.M., Zarrintan S. and Nag T.C. (2006). The effects of an electromagnetic field on the boundary tissue of the seminiferous tubules of the rat: a light and transmission electron microscope study. *Folia Morphol. (Warsz)*. 65, 188-194.
- Khayyat L.I. (2011). The histopathological effects of an electromagnetic field on the kidney and testis of mice. *Eurasia. J. Biosci.* 5, 103-109.
- Kim Y.A., Kim Y.V., Fomenko B.S., Holmuhamedov E.L. and Akoef I.G. (1989). Effects of microwave radiation on inducible ion transport of rat erythrocytes. In: *Charge and field effects in biosystems-2*. Allen M.J., Cleary S.F. and Hawkrige F.M. (eds). Boston, MA. Springer US. pp 223-231.
- Kim Y.K., Suarez J., Hu Y., McDonough P.M., Boer C., Dix D.J. and Dillmann W.H. (2006). Deletion of the inducible 70-kDa heat shock protein genes in mice impairs cardiac contractile function and calcium handling associated with hypertrophy. *Circulation* 113, 2589-2597.
- Kim Y.W., Kim H.S., Lee J.S., Kim Y.J., Lee S.K., Seo J.N., Jung K.C., Kim N. and Gimm Y.M. (2009). Effects of 60 Hz 14 microT magnetic field on the apoptosis of testicular germ cell in mice. *Bioelectromagnetics* 30, 66-72.
- Koc A., Unal D., Cimentepe E., Bayrak O., Karatas O.F., Yildirim M.E., Bayrak R. and Aydin M. (2013). The effects of antioxidants on testicular apoptosis and oxidative stress produced by cell phones. *Turk. J. Med. Sci.* 43, 131-137.
- Kumar S., Kesari K.K. and Behari J. (2010a). Evaluation of genotoxic effects in male Wistar rats following microwave exposure. *Indian J. Exp. Biol.* 48 586-592.
- Kumar S., Kesari K.K. and Behari J. (2010b). Influence of microwave exposure on fertility of male rats. *Fertil. Steril.* 95 1500-1502.
- Kumar S., Kesari K.K. and Behari J. (2011). The therapeutic effect of a pulsed electromagnetic field on the reproductive patterns of male Wistar rats exposed to a 2.45-GHz microwave field. *Clinics (Sao Paulo)*. 66, 1237-1245.
- Kwee S., Raskmark P. and Velizarov S. (2001). Changes in cellular proteins due to environmental non-ionizing radiation. Heat-shock proteins. *Electromagn. Biol. Med.* 20, 141-152.
- Lagroye I., Anane R., Wettring B.A., Moros E.G., Straube W.L., Laregina M., Niehoff M., Pickard W.F., Baty J. and Roti J.L. (2004). Measurement of DNA damage after acute exposure to pulsed-wave 2450 MHz microwaves in rat brain cells by two alkaline comet assay methods. *Int. J. Radiat. Biol.* 80, 11-20.
- Lai H. and Singh N.P. (2004). Magnetic-field-induced DNA strand breaks in brain cells of the rat. *Environ. Health Perspect.* 112, 687-694.
- Latendresse J.R., Warbritton A.R., Jonassen H. and Creasy D.M. (2002). Fixation of testes and eyes using a modified Davidson's fluid: Comparison with Bouin's fluid and conventional Davidson's fluid. *Toxicol. Pathol.* 30, 524-533.
- Lee J.S., Ahn S.S., Jung K.C., Kim Y.W. and Lee S.K. (2004). Effects of 60 Hz electromagnetic field exposure on testicular germ cell apoptosis in mice. *Asian. J. Androl.* 6, 29-34.
- Lukac N., Massanyi P., Roychoudhury S., Capcarova M., Tvrdá E., Knazicka Z., Kolesarova A. and Danko J. (2011). *In vitro* effects of radiofrequency electromagnetic waves on bovine spermatozoa motility. *J. Environ. Sci. Health A Tox. Hazard Subst. Environ. Eng.* 46, 1417-1423.
- Luo Y., Wang X., Chen Y., Xu S., Ding G. and Shi C. (2013). Effects of electromagnetic radiation on morphology and TGF- β 3 expression in mouse testicular tissue. *Toxicology* 310, 8-14.
- Moustafa Y.M., Moustafa R.M., Belacy A., Abou-El-Ela S.H. and Ali F.M. (2001). Effects of acute exposure to radiofrequency fields of cellular

Effect of non-ionizing radiation on the testis of juvenile rat

- phones on plasma lipid peroxide and antioxidase activities in human erythrocytes. *J. Pharm. Biomed. Anal.* 26, 605-608.
- Murphy C.J. and Richburg J.H. (2014). Implications of Sertoli cell induced germ cell apoptosis to testicular pathology. *Spermatogenesis* 4, e979110.
- Nassar S.A. (2009). Do microwaves of mobile phone affect the testicular tissue structure? *Egypt. J. Hosp. Med.* 37, 685-699.
- Odaci E., Hanci H., Yulug E., Turedi S., Aliyazicioglu Y., Kaya H. and Colakoglu S. (2016). Effects of prenatal exposure to a 900 MHz electromagnetic field on 60-day-old rat testis and epididymal sperm quality. *Biotech. Histochem.* 91, 9-19.
- Odaci E. and Ozyilmaz C. (2015). Exposure to a 900 MHz electromagnetic field for 1 hour a day over 30 days does change the histopathology and biochemistry of the rat testis. *Int. J. Radiat. Biol.* 91, 547-554.
- Oktay M.F. and Dasdag S. (2006). Effects of intensive and moderate cellular phone use on hearing function. *Electromagn. Biol. Med.* 25, 13-21.
- Oktem F., Ozguner F., Mollaoglu H., Koyu A. and Uz E. (2005). Oxidative damage in the kidney induced by 900-MHz-emitted mobile phone: protection by melatonin. *Arch. Med. Res.* 36, 350-355.
- Ozguner M., Koyu A., Cesur G., Ural M., Ozguner F., Gokcimen A. and Delibas N. (2005). Biological and morphological effects on the reproductive organ of rats after exposure to electromagnetic field. *Saudi Med. J.* 26, 405-410.
- Paniagua R., Amat P., Nistal M. and Martin A. (1985). Ultrastructural changes in Sertoli cells in ageing humans. *Int. J. Androl.* 8, 295-312.
- Paulraj R. and Behari J. (2006). Single strand DNA breaks in rat brain cells exposed to microwave radiation. *Mutat. Res.* 596, 76-80.
- Peyman A., Rezazadeh A.A. and Gabriel C. (2001). Changes in the dielectric properties of rat tissue as a function of age at microwave frequencies. *Phys. Med. Biol.* 46, 1617-1629.
- Print C.G. and Loveland K.L. (2000). Germ cell suicide: new insights into apoptosis during spermatogenesis. *BioEssays* 22, 423-430.
- Qaddi M.H. and Srifi M.N. (2016). Microwaves biological effects and dielectric properties of human tissues. *Mater. Res. Proc.* 1, 207-213.
- Richburg J.H. (2000). The relevance of spontaneous- and chemically-induced alterations in testicular germ cell apoptosis to toxicology. *Toxicol. Lett.* 112-113, 79-86.
- Sadetzki S., Chetrit A., Jarus-Hakak A.J., Cardis E., Deutch Y., Duvdevani S., Zultan A., Novikov I., Freedman L. and Wolf M. (2008). Cellular phone use and risk of benign and malignant parotid gland tumors: A nationwide case-control study. *Am. J. Epidemiol.* 167, 457-467.
- Salama N., Kishimoto T. and Kanayama H.O. (2010). Effects of exposure to a mobile phone on testicular function and structure in adult rabbit. *Int. J. Androl.* 33, 88-94.
- Saygin M., Asci H., Ozmen O., Cankara F.N., Dincoglu D. and Ilhan I. (2016). Impact of 2.45 GHz microwave radiation on the testicular inflammatory pathway biomarkers in young rats: The role of garlic acid. *Environ. Toxicol.* 31, 1771-1784.
- Saygin M., Caliskan S., Karahan N., Koyu A., Gumral N. and Uguz A. (2011). Testicular apoptosis and histopathological changes induced by a 2.45 GHz electromagnetic field. *Toxicol. Ind. Health.* 27, 455-463.
- Shokri S., Soltani A., Kazemi M., Sardari D. and Mofrad F.B. (2015). Effects of Wi-Fi (2.45 GHz) Exposure on apoptosis, sperm parameters and testicular histomorphometry in rats: A time course study. *Cell J.* 17, 322-331.
- Sokolovic D., Djindjic B., Nikolic J., Bjelakovic G., Pavlovic D., Kocic G., Krstic D., Cvetkovic T. and Pavlovic V. (2008). Melatonin reduces oxidative stress induced by chronic exposure of microwave radiation from mobile phones in rat brain. *J. Radiat. Res.* 49, 579-586.
- Spade D.J., Hall S.J., Wilson S. and Boekelheide K. (2015). Di-n-butyl phthalate induces multinucleated germ cells in the rat fetal testis through a nonproliferative mechanism. *Biol. Reprod.* 93, 110.
- Tkalec M., Malaric K. and Pevalek-Kozlina B. (2007). Exposure to radiofrequency radiation induces oxidative stress in duckweed *Lemna minor* L. *Sci. Total. Environ.* 388, 78-89.
- Trosic I., Busljeta I., Kasuba V. and Rozgaj R. (2002). Micronucleus induction after whole-body microwave irradiation of rats. *Mutat. Res.* 521, 73-79.
- Wu W., Yao K., Wang K.J., Lu D.Q., He J.L., Xu L.H. and Sun W.J. (2008). Blocking 1800 MHz mobile phone radiation-induced reactive oxygen species production and DNA damage in lens epithelial cells by noise magnetic fields. *Med. Sci.* 37, 34-38.
- Yao K., Wu W., Yu Y., Zeng Q., He J., Lu D. and Wang K. (2008a). Electromagnetic noise inhibits radiofrequency radiation-induced DNA damage and reactive oxygen species increase in human lens epithelial cells. *Mol. Vis.* 14, 964-969.
- Yao K., Wu W., Yu Y., Zeng Q., He J., Lu D. and Wang K. (2008b). Effect of superposed electromagnetic noise on DNA damage of lens epithelial cells induced by microwave radiation. *Invest. Ophthalmol. Vis. Sci.* 49, 2009-2015.
- Yoon Kim J., Tae Kim H., Hak Moon K. and Jin Shin H. (2007). Long-term exposure of rats to a 2.45 GHz electromagnetic field: Effects on reproductive function. *Korean J. Urol.* 48, 1308-1314.
- Yuri G., Shckorbatov Y.G., Pisinga V.N., Kolchigin N.N., Grabina V.A., Batrakov D.O. and Kalashnikov V.V. (2009). The influence of differently polarized microwave radiation on chromatin in human cells. *Int. J. Radiat. Biol.* 85, 322-329.

Accepted September 27, 2018