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Effect of electromagnetic irradiation produced by 3G mobile phone on male rat reproductive system in a simulated scenario

Sanjay Kumar, Jay Prakash Nirala, J Behari* & Paulraj R

Bioelectromagnetics Laboratory, School of Environmental Sciences
Jawaharlal Nehru University, New Delhi 110 067, India

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Reports of declining male fertility have renewed interest in assessing the role of electromagnetic fields (EMFs). Testicular function is particularly susceptible to the radiation emitted by EMFs. Significant decrease in sperm count, increase in the lipid peroxidation damage in sperm cells, reduction in seminiferous tubules and testicular weight and DNA damage were observed following exposure to EMF in male albino rats. The results suggest that mobile phone exposure adversely affects male fertility.

Keywords: Infertility, Malonaldehyde, Mobile phones, Seminiferous tubules, Sperm count, Testicular weight

Widespread uses of electromagnetic field emitting devices has escalated over the past decade and to many they are now an essential part of business, commerce, and society. Extensive use of such devices which emit or receive radiofrequency electromagnetic field (EMF) may cause adverse effects on human health. As the usage of cell phone has increased, it has also increased the concern regarding the harmful effects of its use on health. To protect public health, the World Health Organization (WHO) established the International EMF Project in 1996 to assess the scientific evidence of possible health effects of electromagnetic frequencies in the range of 30 Hz to 300 GHz. It is apparent that the wave frequency and, duration of exposure are the main factors which influence the hazardous effects of mobile phone radiation on the male reproductive system of male[1,2]. Electromagnetic field can have adverse effect on the reproduction system and could cause infertility[1-5]. Leydig's cells, seminiferous tubules and spermatozoa are the main targets of possible damages caused by EMF. Exposure to EMF may reduce testosterone biosynthesis, impairs spermatogenesis and damaging sperm DNA[6,7] Oxidative stress and hyperthermia are the main reason for infertility[8]. The diameter of the seminiferous tubule in the testis was reduced after EMF exposure at a specific absorption rate of 0.141 W/kg after a period of one month. It was also shown that the diameter of seminiferous tubules and mean height of the germinal epithelium of the rat testes were significantly decreased due to such exposure[9,10]. Subchronic exposure of rats affected testosterone production and markedly elevated malondialdehyde (MDA) in testis[1,2]. One of the bio-markers widely assayed in determination of oxidative damage in lipids is MDA and it is considered to be an important parameter for detection of damage to cell membrane[11]. Sperms are unique in structure and function and more susceptible to damage by lipid peroxidation (LPO)[12]. Sperm membrane is rich in polyunsaturated fatty acids that can undergo lipid peroxidation by exposure to EMF that can cause an increase in reactive oxidative species (ROS) in cells[13].

Infertile men with high seminal ROS levels have a lower percentage of motile sperm[14]. Overproduction of ROS and deficiency of antioxidants in cells cause an imbalance resulting in molecular oxidative damage (stress)[15], DNA damage, cell membrane lipid peroxidation, and amino acid modifications[16-18].

The effect of mobile phone frequency and 10-GHz exposure on reproductive pattern in male rats has been reported[1-4]. The subject of the present study is extended to include 3G mobile phone radiation (1900-2170 MHz) and to seek further support to previous findings, through the introduction of some additional parameters. It is planned to investigate biochemical changes that confirm the infertility pattern due to the

*Correspondent author
Telephone: 91-11-2593934
Fax 91-11-26717502
E-mail: jbehari@hotmail.com; nirala_prakash@yahoo.com

nonspecific stress caused by the EMF exposure. Effects and possible mechanism are presented in this communication.

Materials and Methods

Materials—Proteinase K, agarose, dithiothreitol, ethidium bromide and tetraethoxypropane were purchased from Sigma (St. Louis, MO, USA). The rest of the chemicals were purchased from Merck Chemicals (Mumbai, India).

Animals—Male Wistar rats (190±10 g; 70 days old) were used in the present study. The animals were maintained as per national guidelines and protocols, approved by the Institutional Animal Ethics Committee (IAEC-JNU/83/675-687; Code No. 12/2008) of Jawaharlal Nehru University, New Delhi. The animals were housed in clean polypropylene cages in a controlled temperature (25-27 ^{o}C with 40-50% RH) with constant 12:12 h L:D cycle (08.00–20.00 hrs light and the rest of the period dark). The animals were fed standardized normal diet (Tetragon Cheime Private Limited, Banguluru, India) and provided water *ad libitum*.

Animal exposure—The animals were divided into 2 groups of 6 each: sham and EMF-exposed. The experiments were repeated twice. The rats were placed in a Plexiglas cage ventilated with holes of 2 mm diameter. Exposure cages were made in such a way that a rat could be comfortably placed. A mobile phone was kept on a platform placed on the top of the exposure box housing with one animal at a time. The platform carrying the mobile phone was firmly tied with a string to a pulley, whose movement was controlled by a stepper motor (Fig. 1). The stepper motor was programmed to have an angular movement from 2 degree to 20 degree (moving at 2 degree per min). This system simulates a probability of the movement of a mobile phone near the head of an actual user in the 'talk mode'. The placing of the mobile phone antenna on the top of the animal cage ensured that the emission was mainly directed towards the animal. The frequency of the cell phone was fixed at 1910.5 MHz and kept in 'talk mode'. Animals were exposed for 60 days, two hours each day (6 days a week). Exposure was carried out in a blind manner. Immediately after the completion of the exposure, animals were sacrificed and assays on testicular parameters were carried out. All twelve samples were analysed for each parameter under blind condition.

Specific absorption rate (SAR)—Exposure was given by a 3G mobile phones (Nokia C5) having a time average specific absorption rate of 1.34 W/kg, as mentioned by the manufacturer. For calibration, the emitted power of mobile phones was measured by using a specially designed monopole probe connected to an Agilent fieldfox spectrum analyser (model-N9912A, Santa Clara, CA, United States). The average maximum and minimum emitted powers from the mobile phone were 1.41 and 0.113 mW respectively. Knowing the power density, the SAR was computed from the theoretical computation of Durney *et al*[19]. Correspondingly, the maximum and minimum SAR values were 0.28 and 0.0226 W/kg, respectively[19].

Testicular weight and sperm count—After the completion of the last exposure period, animals were sacrificed by cervical dislocation. Testes were dissected out, freed from adherent tissues and weighed up to nearest 0.001 g on a Mettler analytical balance (BL-220H, Shimadzu Corporation, Kyoto, Japan). Caput and cauda sections of each testis were cut into small pieces in 5 mL of warm saline or PBS solution to release sperm for 10 min. A sample was then diluted for sperm count using a hemocytometer [Neubauer improved double ruling (Fein-Optik, Blankenburg, Germany)]. Sperms were counted in 5

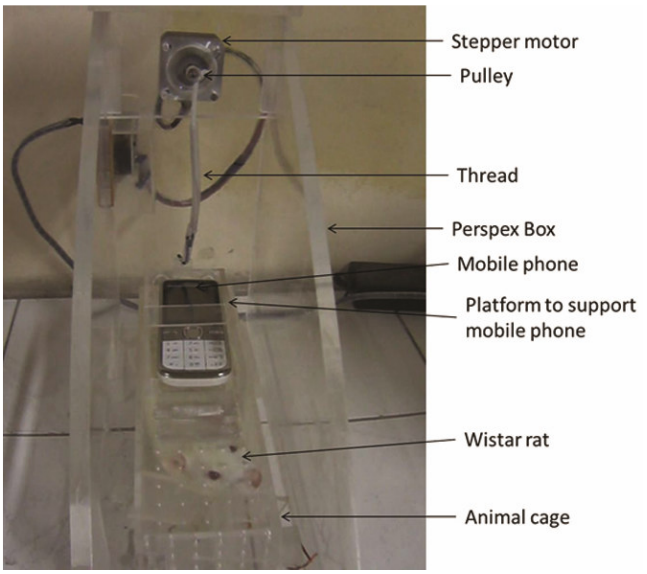

Fig. 1—Exposure set up of 3G mobile phone on Perspex animal holding cage (length: 55 cm, width: 17.5 cm, height: 15 cm). One end of platform supporting the mobile phone was tied to a string and the other end was fixed to the animal cage by a hinge. The string was attached to a pulley, movement of which was controlled by a stepper motor. The stepper motor was programmed to be controlled by a personal computer

squares of the slide and the average number was calculated. The hemocytometer was 0.1 mm deep, and had 25 large squares on it which represent an area of one square mm. The volume in 25 squares is 0.1 µl. Counts in 5 squares would consist of the sperm that settled out in 0.02 µL of the diluted sample. Therefore, the count was multiplied by 50,000 to determine the sperm concentration in 1.0 mL. To get the concentration of the original sperm sample, concentration of the diluted solution was multiplied by a dilution factor. Sperm counting was done according to WHO guidelines[20]. The following equation was used to convert the counts in five squares to concentration in 1 mL of the original sperm sample:

$$\text{Sperm count} = (\text{dilution factor})\,(\text{count in 5 squares})\,(5 \times 10^4)$$

By convention, sperm concentration is expressed in terms of number of sperm/mL.

Scanning microscopy of seminiferous tubules—Seminiferous tubules were cut out from a testis. The tubules were then cut into pieces, fixed into Karnovsky's fluid, post-fixed in osmium tetroxide for 2 h, and dehydrated with three sets of acetone dipping. After dehydration in the last portion of acetone, seminiferous tubules were dehydrated using a vacuum pump. Images of samples were taken using scanning electron microscope (Leo 435 VP) in the All India Institute of Medical Sciences (AIIMS), New Delhi. The diameter of seminiferous tubules was measured using the SiViewer software (Olympus Soft Imaging Solutions GmbH**,** Munster, Germany).

Malondialdehyde estimation—MDA in sperm (10^6 cells) was measured by monitoring the formation of thiobarbituric acid reactive substances (TBARS) using the method described by Buego and Austin[21]. The principle of the method is spectrophotometric measurement of the colour generated by the reaction of thiobarbituric acid (TBA) with MDA. Briefly, 2 mL of each sample treated with trichloroacetic acid (15% w/v) containing 1 mM EDTA was centrifuged at 1,000×g for 10 min. The supernatant was heated at 100 °C with an equal volume of TBA (0.7% w/v) for 20 min, and after cooling, the absorbance (532 nm) was measured using a spectrophotometer.

Detection of DNA strand brake using the Comet assay—In the present investigation, the comet assay (also referred to as single-cell gel electrophoresis) was used to determine DNA damage (DNA single strand breaks). Immediately after the last exposure period, one rat at a time was sacrificed and testis was removed. Seminiferous tubules were cut out from the testis and diced in phosphate-buffered saline (PBS) and kept at 37 °C in a water bath for 7–10 min. The spermatozoa released in PBS were collected by pipetting and centrifuged at 500×g for 5 min to obtain the spermatozoa. Assay was performed according to the method described by Codrington *et al*[22]. Fresh sperm samples (50 µL) were added to 500 µL of molten 0.5% low melting point grade agarose (suspended in phosphate-buffered saline, *p*H 7.4, at 42 °C). The mixture (60 µL) was pipetted out and poured onto a fully frosted slide and immediately covered with a cover glass (24×60 mm). The gel was allowed to solidify at 4 °C in the dark for 20 min. All subsequent steps were performed in the dark or under yellow light to prevent any additional DNA damage. These slides were immersed in an ice-cold lysing solution (2.5 M NaCl, 100 mM EDTA, and 10 mM Tris-HCl; final *p*H 10, containing 10% dimethylsulfoxide, 1% Triton X-100, and 40 mM dithiothreitol) and kept for 1–2 h at 4 °C. Slides were washed in distilled water for 5 min after the initial lysis. After lysing overnight, the slides were removed and washed in distilled water and then immersed in freshly prepared alkaline solution (1 mM EDTA and 0.05 M NaOH, *p*H 12.1) for 20 min. Slides were placed on the a gel tray submerged in Tris-borate-EDTA buffer (TBE) in horizontal electrophoresis unit. This tris-borate EDTa buffer level was kept at 2-3 mm above the slides. Electrophoresis was carried out at 14 V (0.7 V/cm) for 30 min. The slides were then removed from the electrophoresis apparatus and fixed in ice-cold 70% ethanol for 5 min and left vertical at room temperature to air dry. Slides were stained with 100 µL of ethidium bromide (20 µg/mL) and immediately analysed. Images were taken at 400 X magnifications using an Axio Cam MRm camera attached to a Carl Zeiss HBO 50 fluorescence microscope (Carl Zeiss HBO 50, Jena GmbH, Jena, Germany).

Comet scoring—Slides were assayed for DNA strand brakes. Twenty five cells were randomly analysed per slide for a total of 50 cells (two slides) per animal, and fluorescent images were scored for comet parameters. From each cell head and tail length (µm), intensity (%), and tail migration (µm) from the beginning of the nuclear area to the last five pixels of DNA perpendicular to the direction of migration at the leading edge were measured. The scoring of the comet

assay was done by using the Comet Assay IV Software (Perceptive Instrument Pvt Ltd, Suffolk, UK).

Histopathological examination—Testis samples were removed and fixed in 10% formalin. They were then embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Slides were examined by light microscopy through a 40X magnification.

Statistical analysis—Data are presented as mean±SE. Statistical analysis was performed using the two tailed Student's *t* test. A difference at $P<0.05$ was considered statistically significant.

Results

Sperm count—The total epididymal sperm count in the EMF exposed experimental animals differ statistically ($P< 0.0005$) from that of the sham group. In the exposed group, the average spermatocyte count (per mL) was $159.16\pm10.68\times10^6$ and in the sham group was $210.83\pm8.61\times10^6$. In comparison with sham exposed group, sperm motility of the EMF exposed group was not significantly different, although it is lower in irradiated rats.

Testicular weight—The testicular weight of EMF exposed group was 1.55±0.022 g whereas that of sham exposed group was 1.72±0.04 g. The weight in the EMF group was found to be significantly reduced by 9% ($P= 0.0005$). Reduction in the weight of rat testis was probably due to induced atrophy. The relative testicular weight shows an effect on the reproductive system.

MDA—MDA in sperm was measured by monitoring the formation of TBARS. The result shows a significant ($P<0.005$) increase in the testes of EMF exposed group (0.67±0.01) as compared with that of the sham-exposed group (0.42±0.03).

Diameter of seminiferous tubules—The seminiferous tubules appeared rounded or oval in their outlines and lined by germinal epithelium. The seminiferous tubules of the EMF exposed adult rats showed a marked reduction in diameter. The diameter of the EMF exposed animal was 157.62 ± 8.03 μm which was significantly reduced ($P=0.0002$) when compared with the sham control (181.49 ± 6.24 μm).

Comet assay—Figure 2 shows sample photographs of DNA comets of spermatozoa from sham exposed and EMF exposed rats. Significantly more tail migration took place in EMF exposed samples as compared to sham exposed control. Data of various parameters of comet measurements are presented in Table 1. They indicate that chronic exposure to mobile phone radiation increased DNA single strand breaks in spermatozoa of rat.

Histopathological examination—The testis is surrounded by a thick capsule called tunica albuginea. Leydig's cells are located in the interstitial tissue between the convoluted seminiferous tubules, which synthesize and secrete testosterone. Testes of EMF exposed rats exhibited no observable pathological alterations. Sham exposed and EMF exposed samples showed no significant histological difference in testicular sections stained with haematoxylin eosin (Fig. 3).

Discussion

Over the last decade, the exponential growth of mobile communications has been accompanied by a parallel increase in the level of environmental electromagnetic fields (EMF). As such, the continued expansion of mobile communications raises important questions because EMF has long been suspected to have biological effects.

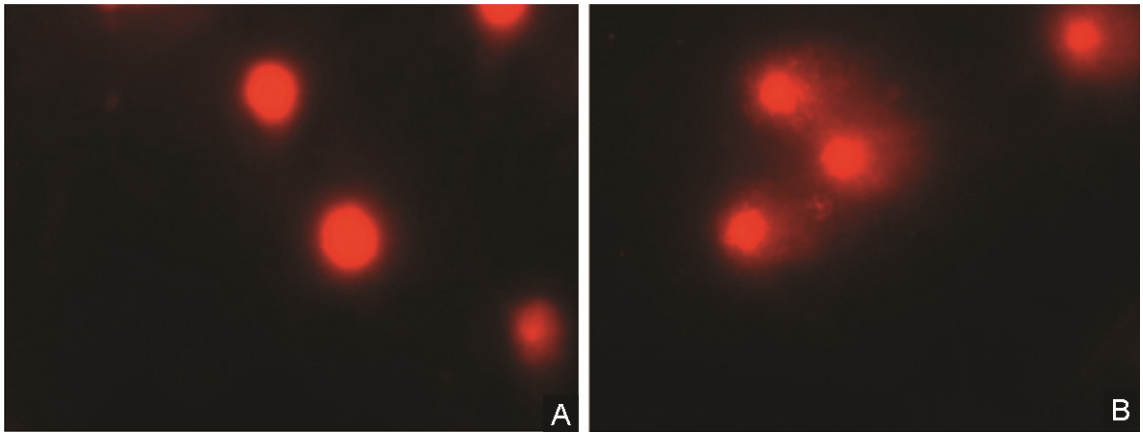

Fig. 2—Images of ethidium bromide–stained spermatozoa DNA obtained from rat testis of control group (A) and EMF exposed group (B). DNA damage level was determined by the increasing tail migration while DNA is seen to shift from the body of the head into the tail. (magnification 400x under fluorescence microscope).

Table 1—Sperm data by comet assay

[Values scored using Comet Score software are mean ± SD]

Parameters	Control	Exposed	*P* values
Comet length (px)	240.93 ± 70.29	392.93 ± 102.44	0.006
Comet height (px)	204.46 ± 46.6	259.3 ± 64.36	0.001
Comet area (px)	51717.8 ± 26849.41	103985.56 ± 48338.54	0.00003
Comet intensity	3666841.03 ± 2503584.94	6254537 ± 3682978.54	0.003
Comet mean intensity	69.07 ± 17.35	59.91 ± 16.25	0.03
Head diameter (px)	205.46 ± 46.58	254.9 ± 59.28	0.001
Head area (px)	51714.3 ± 26849.22	97356.77 ± 40297.53	0.00003
Head intensity	3423001.63 ± 2353990.11	4771510 ± 2866878.23	0.06
Head mean intensity	64.57 ± 17.17	47.45 ± 12.11	0.00007
%DNA in head	93.26 ± 4.91	77.57 ± 12.36	0.00006
Tail length (px)	39.96 ± 36.51	138.03 ± 57.84	0.00002
Tail area (px)	3.5 ± 13.69	6628.8 ± 31541.48	0.25
Tail intensity	243839.4 ± 243649.56	1483027 ± 1255060.51	0.0001
Tail mean intensity	237418.21 ± 247483.71	12842721.83 ± 1206822.34	0.0005
%DNA in tail	6.73 ± 4.91	22.42 ± 12.36	0.00006
Tail moment	2.75 ± 3.08	34.59 ± 45.02	0.0006
Olive moment	5.59 ± 4.72	34.13 ± 35.02	0.0001

Mammalian spermatogenesis is a complex process. Spermatogenesis occurs within a collection of U-shaped seminiferous tubules. The seminiferous tubules are lined by sertoli cells, which support germ cells in varying stages of development. In mammalian testes, germ cells expand clonally through many rounds of mitosis before undergoing the differentiation steps that result in mature spermatozoa. In the present study we have observed a reduction in sperm counts in rats after exposure to EMF.

Although a male partner produces sufficient numbers of spermatozoa to achieve fertilization, due to functional defects in these cells, conception is prevented. Fail to conceive in couples may be due to stress. Despite several reasons for defect in sperm cells, fragmentation of DNA has been associated with impaired fertilization. Ramadan *et al.*[23] reported a decrease in sperm count, motility and daily sperm production with marked testicular histopathological changes after magnetic field exposure.

Mobile phone exposure in males is linked to development of testicular abnormalities, atypical sperm, chromosomal aberrations and congenital defects in offspring[23]. Jung *et al.*[25] have found that EMF (including radiofrequency radiation RFF) could impair spermatogenesis in the testis, but only after excessive exposure. Dasdag *et al*.[26] have reported that mobile phone radiation affected testicular function through defects in male germ line. Aitken *et al.*[27-28] have shown that exposure to RFR lead a significant impact on the integrity of both mitochondrial and nuclear genomes in mice. It is thus evident that mobile phone radiation at a given power level is capable of inflicting damage to the germline.

The finding of an increase in MDA level in the present study seems to support the effect of mobile phone radiation on sperms. Lipid peroxidation may cause cellular injury to the spermatozoa due to an increase in sperm membrane fluidity[28]. Sperm membrane damage can be caused by an increase in reactive oxygen species, such as superoxide anion, hydroxyl radical and hydrogen peroxide produced by mobile phone radiation resulting in increased lipid peroxidation. Several authors reported that MDA and ROS levels increased and antioxidant levels were altered due to EMF exposure[28]. Moustafa *et al.*[29] showed that the lipid peroxide level in healthy adult male volunteers was significantly increased after 1, 2, and 4 h of exposure to radiofrequency fields. Malondialdehyde is a major oxidative degradation product of membrane and has been shown to be biologically active with hepatotoxic and genotoxic properties[30].

Results of the present study showed that mobile phone radiation did not cause any structural change in histopathological pattern in testicular organs and this suggests that the applied mobile phone exposure evoked only functional changes in the irradiated rats. Amara *et al.*[31] demonstrated effects of extremely-low

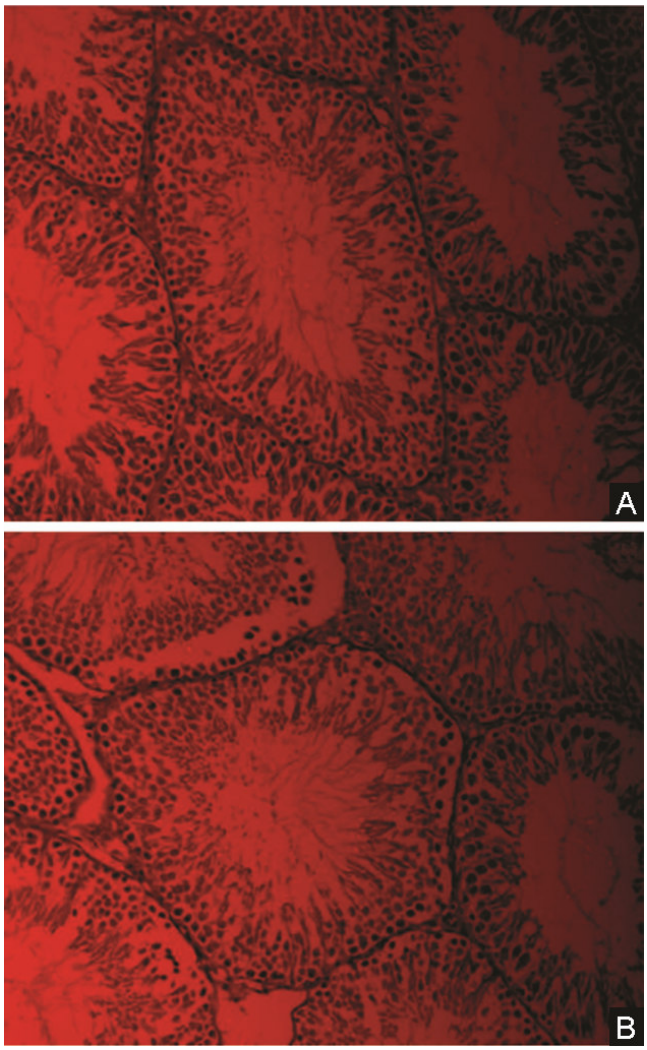

Fig. 3—Transverse section of rat testis. Histology of testis from sham exposed rat showed normal seminiferous tubule lined with stratified epithelium, composed of two major cells, the supporting cells (sertoli cells) and spermotogenic cells (A). No visible changes between the sham exposed and EMF-exposed groups were observed (B). [H&E, ??X]

frequency magnetic field (ELF-MF) in a histopathologic investigation of testicular tissue. Decreased spermatogenesis in some seminiferous tubules, congestion in blood vessels of the interstitium, and increase in interstitial edema and Sertoli cells were observed. Leydig cells were found to be normal in appearance.

The testis is known to be very sensitive to impact and injury. It is possible to some extent that when subjected to adverse conditions e.g. higher temperature or injury, testicular size can shrunk by competing against their intrinsic recovery properties of system and hormonal function through the use of externally administrated hormones or medicine. Testes may shrink or undergo atrophy during damage or hormonal-replacement therapy. In these cases, a loss in testicular volume corresponds with a loss of spermatogenesis. Handelsman and Staraj[32] have shown that reduced testicular sizes are related to advancing age, malnutrition, alcoholism, malignancy, chronic and terminal illness. Aweda *et al.*[33] showed that microwave affected the male reproduction by a consistent reduction in testis weight.

Among many biological targets, the DNA molecule has been frequently investigated with respect to potential damage caused by mobile phone radiation. It is an important consideration because of DNA's relevance for cell function, proliferation, viability and genetic material from one generation to the next generation. The ability to repair DNA lesions in cell is an ubiquitous defense mechanism that is essential for its survival and maintenance of its functions. Different cells have different repair maintenance mechanisms[33]. It was suggested that DNA repair pathways could be differently affected by EMF exposure[34,35]. Cells have their own set of antioxidant defence mechanisms to fight with free radical formation and to overcome the limit of damaging effects. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are the enzymatic defence systems of cells against oxygen radicals. Excessive production of free radicals specifically ROS, has been associated with a wide variety of clinical disorders[36-38] environmental stresses (such as UV, heat exposure, EMF exposure).

Exposure of a biological system to EMF cause a weak induced signal near the cellular boundary and lead to the penetration of energy from a coherent signal, with amplification derived from noise via stochastic phenomena[4]. Thus, the signal can circumvent the barrier height of the plasma membrane and penetrate inside the nucleus to cause DNA damage (e.g., strand breaks). This signal may also provide sufficient energy to cause overproduction of ROS[4]. Furthermore, mobile phone radiation might induce vasodilatation and increase the production of NO as stated by[39-41]. Moreover, stress could induce functional and structural lesions observed in this study. The main damage of increased ROS production can cause alterations in macromolecules such as polyunsaturated fatty acids in membrane lipids, vital proteins, and DNA[42-44]. Agarwal *et al.*[45] has reported similar results on human ejaculated sperms ,which provide support to these data.

Conclusion

This study demonstrates that exposure to mobile phone radiation can affect sperm functions via mechanisms that involve oxidative stress. Oxidative stress is the main cause of infertility in man.

Author's contribution

Sanjay Kumar did all the biological work and J. Behari was involved in the overall planning and designing of the experimental and contributed in supervision, revision and final approval. Jay Prakash Nirala was the designer of the experimental hardware setup. Dr Paulraj was involved in technical discussions. All the authors have read and approved the final manuscript.

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Declaration of interest

The authors have no conflicts of interest. They alone are responsible for the content and writing of the paper.

Reference

1 Kesari KK, Kumar S & Behari J, Mobile phone usage and male infertility in Wistar rats, *Indian J Exp Biol,* 48 (2010) 987.

2 Kesari KK, Kumar S & Behari J, Effects of radiofrequency electromagnetic waves exposure from cellular phone on reproductive pattern in male Wistar rats, *Appl Biochem Biotech*, 164 (2011) 546.

3 Kumar S, Kesari KK & Behari J, Evaluation of geno toxic effects in male wistar rats following microwave exposure, *Indian J Exp Biol*, 48 (2010) 586.

4 Kumar S, Kesari KK & Behari J, The influence of microwave exposure on male fertility, *Fertil Steril*, 95 (2011) 1500.

5 Nisbet HO, Nisbet C, Akar A, Cevik M & Karayigit MO, Effects of exposure to electromagnetic field (1.8/0.9 GHz) on testicular function and structure in growing rats, *Res Vet Sci*, (2012) (doi:10.1016/j.rvsc.2011.10.023).

6 La Vignera S, Condorelli RA, Vicari E, D'Agata R & Calogero AE, Effects of the exposure to mobile phones on male reproduction: a review of the literature, *J Andro*, 33 (3) (2012) 350.

7 Deepinder F, Makker K & Agarwal A, Cell phones and male infertility: dissecting the relationship, *Reprod Biomed Online*, 15 (2007) 266.

8 Dasdag S, Ketani MA, Akdag Z, Ersay AR, Sari I, Demirtas OC & Celik MS, Whole-body microwave exposure emitted by cellular phones and testicular function of rats, *Uro Res*, 27 (1999) 219.

9 Sarookhani MR, Asiabanha RM, Safari A, Zaroushani V & Ziaeiha M, The influence of 950 MHz magnetic field (mobile phone radiation) on sex organ and adrenal functions of male rabbits, *Afr J Biochem Res*, 5 (2011) 65.

10 Amara S, Abdelmelek H, Garrel C, Guiraud P, Douki T, Ravanat JL, Favier A, Sakly M & Rhouma KB, Effects of Subchronic Exposure to Static Magnetic Field on Testicular Function in Rats, *Arch of Med Res*, 37 (2006) 947.

11 Draper HH & Hadley M, Malondialdehyde determination as index of lipid peroxidation, *Method Enzymol*, 186 (1990) 421.

12 Alvarez JG, Touchstone JC, Blasco L & Storey BT, Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa. Superoxide dismutase as major enzyme protectant against oxygen toxicity, *J Androl,* 8 (1987) 338.

13 Gomez E, Irvine DS & Aitken RJ, Evaluation of a spectrophotometric assay for the measurement of malondialdehyde and 4-hydroxyalkenals in human spermatozoa: relationships with semen quality and sperm function, *Int J Androl*, 21 (1998) 81.

14 Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW & Behre HM, World Health Organization reference values for human semen characteristics, *Hum Reprod Update,* 16 (2010) 231.

15 Halliwell B & Gutteridge JM, *Free radicals in biology and medicine*, (Oxford University Press, New York) 2000.

16 Halliwell B & Gutteridge JM, *Free radicals in biology and medicine*, Vol. 1, 3rd edition (Oxford University Press, London) 1999.

17 Aitken RJ, De Iuliis GN, Finnie JM, Hedges A & McLachlan RI, Analysis of the relationships between oxidative stresses, DNA damage and sperm vitality in a patient population: development of diagnostic criteria, *Hum Reprod*, 25 (2010) 2415.

18 Aitken RJ & De Iuliis GN, On the possible origins of DNA damage in human Spermatozoa, *Mol Hum Reprod*, 16 (2010) 3.

19 Durney C H, Comparison of theoretical and experimental absorption of radiofrequency power, Final report to USAF under contract No. F41609-76-C-0025, University of Utah, Departments of electrical engineering and bioengineering Salt Lake city, Utah, December 1976.

20 World Health Organization (WHO), Establishing a dialogue on risks from electromagnetic fields, Radiation and environmental health, department of protection of the human environment. Geneva; Switzerland, (2002) 1.

21 Buege J A & Aust S D, Microsomal lipid peroxidation, *Methods Enzymol,* 52 (1978) 302.

22 Codrington AM, Hales BF & Robaire B, Spermiogenic germ cell phase- specific DNA damage following cyclophosphamide exposure, *J Androl*, 25 (2004) 354.

23 Ramadan LA, AbdAllah AR, Aly HA & Saadel Din AA, Testicular toxicity effects of magnetic field exposure and prophylactic role of coenzyme Q10 and L-carnitine in mice, *Pharmacol Res*, 46 (2002) 363.

24 Havas M, Biological effects of non-ionizing electromagnetic energy: a critical review of the reports by the US National Research Council and the US National Institute of Environmental Health Sciences as they relate to the broad realm of EMF bio effects, *Environ Rev*, (2000) 8:173.

25 Jung A & Schill WB, Male infertility. Current life style could be responsible for infertility, *MMW Fortschr Med*, 142 (2000) 31.

26 Aitken RJ, Bennetts LE, Sawyer D, Wiklendt AM & King BV, Impact of radio frequency electromagnetic radiation on DNA

integrity in the male germline*, Int J Androl*, 28 (2005) 171.

27 Aitken RJ, Krausz C & Buckingham DW, Relationship between biochemical markers for residual sperm cytoplasm, reactive oxygen species generation and the presence of leucocytes and precursor germ cell in human sperm suspensions, *Mol Reprod Dev*, 39 (1994) 268.

28 Dasdag S, Bilgin HM, Akdag MZ, Celik H & Aksen F, Effect of long term mobile phone exposure on oxidative-antioxidative processes and nitric oxide in rats, *Biotechnol Biotechnol Eq*, 22(4) (2008) 992.

29 Moustafa YM, Moustafa RM, Belacy A, Abou-El-Ela SH & Ali FM, Effects of acute exposure to the radiofrequency fields of cellular phones on plasma lipid peroxide and antioxidase activities in human erythrocytes, *J Pharmaceut Biomed*, 26 (2001) 605.

30 Koyu A, Naziroglu M, Ozguner F, Yilmaz R, Uz E & Cesur G, Caffeic acid phe ethyl ester modulates 1800 MHz microwave-induced oxidative stress in rat liver, *Electromag Biol Med*, 24 (2005) 135.

31 Amara S, Abdelmelek H, Garrel C, Guiraud P, Douki T, Ravanat JL, Favier A, Sakly M & Rhouma KB, Effects of subchronic exposure to static magnetic field on testicular function in rats, *Arch of Med Res,* 37 (2006) 947.

32 Handelman DJ & Staraj, Testicular size the effects of aging, malnutrition, and illness, *J Androl*, 6 (3) (1985) 144.

33 Aweda MA, Usikalu RM & Awobajo FO, Effects of exposure to 2.45 GHz microwave radiation on male rat reproductive system, *Int J Curr Research*, 2 (1) (2011) 120.

34 Schemezer P, Rajaee-Behbahani N, Risch A, Thiel S, Rittgen W, Drings P, Dienemann H, Kayser KW, Schulz V & Bartsch H, Rapid screening assay of mutagen sensitivity and DNA repair capacity in human peripheral blood lymphocytes, *Mutagenesis*, 16 (2001) 25.

35 Bahong W, Jiliang H, Lifen J, Deqiang L, Wei Z, Jianlin L & Hongping D, Studying the synergistic damage effects induced by 1.8 GHz radiofrequency field radiation (RFR) with four chemical mutagens on human lymphocyte DNA using comet assay *in vitro*, *Mutat Res*, 578 (2005) 149.

36 Delimaris J, Tsilimigaki S, Messini-Nicolaki N, Ziros E & Piperakis SM, Effects of pulsed electric fields on DNA of human lymphocytes, *Cell Biol Toxicol,* 22 (2006) 409.

37 Van Houten B, Woshner V& Santos JH, Role of mitochondrial DNA in toxic responses to oxidative stress, *DNA Repair (Amst)*, 5 (2006) 145.

38 Galli F, Piroddi M, Annetti C, Aisa C, Floridi E & Floridi A, Oxidative stress and reactive oxygen species, *Contrib Nephrol*, 149 (2005) 240.

39 Willcox JK, Ash SL & Catignani GL, Antioxidants and prevention of chronic disease, *Crit Rev Food Sci Nutr*, 44 (2004) 275.

40 Paredi P, Kharitonov SA, Hanazawa T & Barnes PJ, *Laryngoscope*, 111(1) (2001) 159.

41 Yariktas M, Doner F, Ozguner F, Gokalp O, Dogru H, Delibas N, Otolaryngology, *Head Neck Surg,* 132 *(* 2005) 713

42 Blokhina O, Virolainen E & Fagerstedt KV, Antioxidants, oxidative damage and oxygen deprivation stress: A review, *Ann Bot (Lon),* 91(2003) 179.

43 Liu C, duan W, Xu S, Chen C, He M, Zhang L, Yu Z & Zhou Z, Exposure to 1800 MHz radiofrequency electromagnetic radiation induces oxidative DNA base damage in a mouse spermatocyte-derived cell line, *Toxicoll Lett*, 218 (1) (2013) 2.

44 De lullis GN,Newey RJ,King BV & Aitkin RJ, Mobile phone exposure radiation induces reactive oxygen species production and DNA damage in a mouse spermatozoa *in vitro*, *PLoS One*, 4 (2009) e6446.

45 Agarwal A, Deepinder F, Sharma RK, Ranga G & Li J, Effect of cell phone usage n semen analysis in man atteneding infertility clinic :an observational study, *Fertil Steril*, 89 (2008) 124.