

Electromagnetic radiations on the functional potential of spermatozoa

Ranjitsingh A.J.A.*, Elizabeth Mathew M., Dhasarathan P. and Athinarayanan G.

Department of Biotechnology, Prathyusha Engineering College, Chennai-602025, INDIA

*ranjithsingh@prathyusha.edu.in

Abstract

The growing exposure to digital communication system and tools leads to radiation toxicity to the users. Unaware of the safety measures, even a kid at the age of one starts operating digital gadgets emitting radio frequency-electromagnetic radiations. Radiations from mobile phones, laptops, note pad, Wi-Fi or other devices are reported to be harmful beyond the permissible limit. So there is a growing concern for the overall health, reproductive and hormonal functions. Experimental studies were conducted by using a Wi-Fi network active laptop and live spermatozoa. The exposure of sperms to the source of EMF showed that the activity of the live sperms got reduced and mortality was observed depending on the exposure duration and the distance from EMF sources.

After 5h of exposure to RF-EMF source, the semen quality changed when compared with control. The vitality of sperm in the control was 95 ± 1.0 after 2 hr and it was reduced to 60 ± 1.5 percent at a distance of 1cm and 55 ± 2.2 percent at a distance of 10 cm. After 5 hour exposure, the vitality was reduced to 63 ± 1.86 at 1 cm distance and 70 ± 1.42 at 10 cm distance. The reduction in vitality of sperm after exposure to RF-EMF source for 2 hour was 35% at 1cm and 40% at 10cm distance.

Keywords: RF-EMF, digital devices, mobile phone, sperms, laptop, Wi-Fi.

Introduction

In this era of digitalization, most of people are exposed to electromagnetic radiations emitted from electronic devices. The rise in the use of mobile phones, laptops, wireless internet, access through Wi-Fi routers and modem in COVID-19 pandemic period may leave harmful effects on the fertility parameters. Exposure to electromagnetic radiation (EMR) from mobile phones was reported to affect human spermatozoa⁷. EMR induces scrotal hyperthermia and oxidative stress leading to the dysfunction of the sperms and infertility¹⁶. Electromagnetic pollution due to cell phone towers had induced deleterious changes in the human biochemical process and alterations in blood glutathione S transferase and total antioxidant activity in Jordanian people²⁹.

Electromagnetic radiations interfere with mitochondrial electron transport chain in spermatozoa and affect the sperm

function as reported earlier^{3,8,19,23,28,33}. The electromagnetic radiations caused oxidative stress in sperm functioning^{11,22}. Exposure of male rats to radiation emitted from Wi-Fi device had increased the serum 8-hydroxy -2 deoxyguanosine levels followed by DNA damage in germ cells^{2,10}. Maternal use of cell-phone during pregnancy reflected in shorter pregnancy duration and pre-term birth³⁶. The pregnant mother using mobile phone use for a long duration had increased the offspring behavioral problems, particularly hyperactivity/inattention.⁴

The radio frequency and electromagnetic field (RF-EMF) at a level of 850MHz – 2.4GHz gets absorbed by the human body⁵. RF-EMF exposure leads to breast cancer⁶. RF-EMF increases superoxide anions concentrations in the germ cells of males leading to oxidative stress and decrease in sperm motility.²¹ Prolonged use of laptop computers decreased the sperm vitality and count³¹. Wi-Fi radiation induced adverse effect on male reproductive system is extensively reviewed^{25,37}.

As there are several reports on the impact of Wi-Fi radiations on sperm quality, the present study is planned to analyze the impact of radiations from lap-top on live sperm incubated at different distance and duration.

Material and Methods

Incubation of spermatozoa near laptop connected to net setter: The present study was done on twenty five normozoospermic semen donor samples cryopreserved in a fertility clinic in South India. The sperm concentration was adjusted to 15 million/ml. Each prepared sperm suspension sample was aliquoted in 3 fractions and a drop of 500 ml was placed in 35 × 10 mm Petri dishes¹³. This was covered with 3 ml of ovoil (Vitrolife) to avoid evaporation. Fractions one were incubated near active laptop connected to net setter. Another fraction (control group) was incubated under similar conditions without the laptop. To induce the greatest possible effect, the laptop worked actively (uploading and downloading information) throughout the period of exposure.

The distance between the computer and each fraction was kept constant at 1cm and 10cm. The duration of exposure was 2 hours and 5 hours. The temperature near the laptop was kept at 26°C during the incubation time by an air conditioning system. The temperature on each medium drop was thoroughly controlled by a thermometer and recorded every 5 minutes. Unexposed aliquots were used as control and kept under identical temperature and conditions in another room away from other computers or electronic

devices. After the incubation period, sperm vitality, motility, oxidative stress and DNA fragmentation were determined on each aliquot.

Sperm vitality: The semen samples were mixed well. From this mixture a 50µl aliquot of semen was taken out and mixed with an equal volume of eosin-nigrosin suspension. For each suspension, smear was made on a glass slide and allowed to dry in air. The slides were examined immediately after drying, or later after mounting with a permanent non-aqueous mounting medium. The slides were examined with bright field optics at ×1000 magnification and oil immersion. The number of stained (dead) or unstained (vital) cells was tallied with the aid of a laboratory counter. Two hundred spermatozoa were evaluated in each replicate in order to achieve an acceptably low sampling error.

Scoring: The nigrosin provides a dark background that makes it easier to discern faintly stained spermatozoa with bright field optics; live spermatozoa have white heads and dead spermatozoa have heads that are stained red or dark pink. Spermatozoa with a faint pink head were assessed as alive. If the stain is limited to only a part of the neck region and the rest of the head area is unstained, this is considered a “leaky neck membrane”, not a sign of cell death and total membrane disintegration. These cells were assessed as alive:

$$\text{Vitality (\%)} = \frac{\text{Number of viable sperm}}{\text{Total number of sperm}} \times 100$$

Sperm motility studies: The semen sample exposed to the radiations from laptop was used for further assay. Sperm motility within semen was assessed at a stabilized temperature. The sperm sample was taken on sperm motility counter slides with a chamber of depth 20.7 µm. 6.5 µl semen samples were taken in the chamber covered with an 18 mm × 18 mm cover slip. The slide was examined with phase-contrast optics at ×200 or × 400 magnifications. Spermatozoa in an area at least 5 mm from the edge of the cover slip was counted to prevent observation of effects of drying on motility. The slide was systematically scanned to avoid repeatedly viewing the same area. The observation fields were changed often. The fields on the basis of the number of motile sperm seen were avoided (field choice was random).

Scoring in a given field was at a random instant. Before the spermatozoa to swim into the field or grid, scoring was done. The motility of all spermatozoa within a defined area of the field was assessed. To avoid overestimating, the number of motile spermatozoa scanning and count were done quickly. Initially the grid section was scanned for scoring progressive sperm [PR], next non-progressive [NP] spermatozoa were counted. These three categories of sperm movement were scored at one time and in larger areas of the grid. The number of spermatozoa in each motility category was tallied with the aid of a laboratory counter. About 200 spermatozoa in a total

of at least five fields in each replicate were evaluated in order to avoid sampling error.

The average percentage and difference between the two percentages for the most frequent motility grade (PR, NP) in the replicate wet preparations were calculated. Only intact spermatozoa (defined as having a head and a tail) are counted for sperm concentration assessment. Motile pinheads were counted. If no spermatozoa are observed in the replicate wet preparations, azoospermia was suspected:

$$\text{Progressive (PR) (\%)} = \frac{\text{Number of progressive sperm}}{\text{Total number of motile sperm}} \times 100$$

$$\text{Non progressive (NP) (\%)} = \frac{\text{Number of non-progressive sperm}}{\text{Total number of motile sperm}} \times 100$$

Detection of DNA fragmentation: Sperm DNA fragmentation was analyzed with halo sperm (Halotech). The SDF was determined from chromatin depression patterns using the halo sperm kit (Halotech DNA), a commercial variant of the sperm chromatin depression (SCD) test. For each experiment, 25 µl of diluted spermatozoa (10 × 10⁶ spermatozoa per ml) was added to a vial containing low melting point agarose and gently mixed. A small aliquot of the agarose – sperm mixture (10 µl) was then spread on pre-treated slide (provided in the kit), covered with a glass cover slip and placed in a refrigerator on a cold metallic plate for 5 minutes. After solidification, a first step including a controlled denaturation of DNA using an acid solution for 7 minutes at room temperature was performed.

Once the DNA was denatured in the putative DNA breaks, a controlled extraction of the nuclear proteins was produced. The DNA halos from the SCD test can be detected by Diff-Quick reagent. The degree of DNA dispersion was assessed by observing the relative halo size under bright field microscopy. A minimum of 500 spermatozoa per sample was evaluated. Sperms with large and medium sized halos are considered to be normal or non-fragmented sperm with small sized halo or no halo are considered to have significant DNA fragmentation. The percentages of DNA fragmentation were assessed by calculating DNA fragmentation index (DFI) and monitored by fluorescence and bright field microscopy respectively.

$$\text{DFI (DNA fragmentation Index)} = \frac{\text{Fragmented DNA}}{\text{Fragmented DNA} + \text{Non Fragmented DNA}}$$

$$\text{Sperm DNA fragmentation Index SDF (\%)} = \frac{\text{Number of spermatozoa with fragmented DNA}}{\text{No of spermatozoa counted}} \times 100$$

Reactive oxygen species estimation: Reactive oxygen species [ROs] released in the sperm sample exposed to RF-EMF source was estimated using chemiluminescence assay using luminol. Luminol gets oxidized in the presence of ROs. The luminescence generated by luminol was measured using luminometer (Luminometer, Vitl Life Science solutions). For the assay, luminol (5-amino, -2, 3- dihydro 1,4- phthalazinedione, Sigma- Aldrich) was prepared at a concentration 100mmol/L/L using DMSO (Dimethyl sulfoxide) and stored properly. From the stock solution, 5 mmol/L luminol was prepared freshly before the experiment. For measuring chemiluminescence, the semen samples exposed to radiations from laptop were liquified gently using plastic pipette and transferred to microfuge tube. To the semen sample, ten microliters of luminol were added and mixed well before loading in to illuminometer.

The assay was done in a chamber with reduced light exposure. Every one minute after the addition of luminol into semen sample, chemiluminescence was noted as relative light units/(RLU/Sec/10⁶ sperm) for 10 minutes. For negative control, phosphate buffered saline (PBS) was used. The positive control was prepared using 395µl PBS and 5 µl of 30 percent hydrogen peroxide (H₂O₂ O) mixed with 10 µl

of 5mM luminal. The ROS value less than 24 RLU/ Sec/10⁶ sperm is normal representing 87.2% of the ROS values of normal sperm parameters.

Statistics: The impacts of RF-EMF on motility, vitality, DNA fragmentation and ROS level were statistically analyzed with a significant test P < 0.05.

Results

In the present study, the impact of RF-EMF was emanated from a laptop connected to a Wi-Fi and working for 2- 5 hours upon the male gametes; spermatozoa was analyzed. To assess the quality of the sperms, the sperm vitality rate, progressive motility, non-progressive motility, DNA fragmentation index and ROS production in the semen samples were analyzed by exposing the sperm sample to a laptop connected to a net setter at a distance of 1 cm and 10 cm for a period of 2-5 h without disturbing. The semen sample kept at a distance of 1 cm showed a reduction in sperm vitality and progressive motility when compared with control group. Also the DNA fragmentation index (DFI) and ROS level in sperm were estimated after 2 and 5 h of exposure.

Table 1
Impact of RF-EMF exposure duration and distance on the semen characteristics,

Exposure duration (H)	Distance (cm)	Motility (%)		DNA fragmentation	vitality	ROs in term of chemiluminescence RLU/Sec/10 ⁶ sperm
		53.0 ± 1.5 (35.0)	31.0 ± 0.25 (-2.5)	16.0 ± 0.38 (-10.7)	60.0 ± 1.5 (35.0)	985 ± 15.0
2	1					
	10	55.0 ± 1.0 (33.0)	29.0 ± 1.0 (10.5)	10.8 ± 1.0 (-5.5)	55.0 ± 1.0 (40.0)	841 ± 14.0
5	1	45.0 ± 0.7 (43.0)	24.0 ± 1.5 (-5.5)	16.7 ± 0.85 (-11.4)	63.0 ± 1.70 (32.0)	1216 ± 23
	10	48.0 ± 0.90 (40.0)	26.0 ± 1.0 (-7.5)	15.5 ± 0.25 (-10.2)	70 ± 1.40 (25.0)	926. ± 17
CONTROL	1 cm	88.0 ± 1.0	18.5 ± 0.40	5.3 ± 0.40	95.0 ± 0.50	22.6 ± 1.8

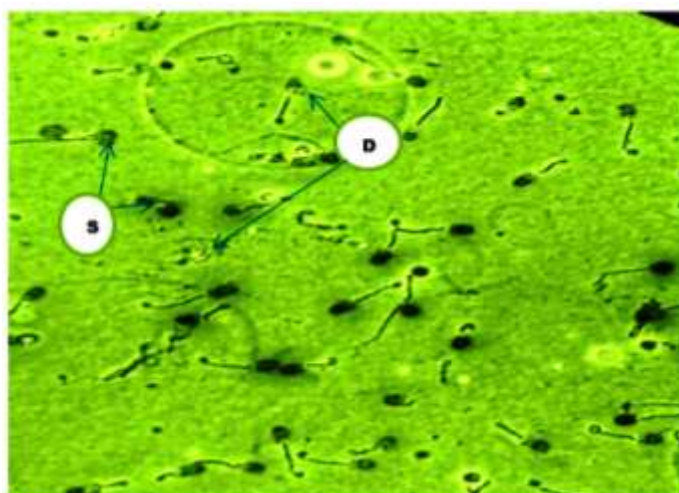


Fig. 1: DNA fragmentation (D), abnormal sperm(S) seen after exposure to EMF radiations

The deterioration of sperm quality (Table 1 and Fig. 1) varied according to the exposure duration. After 5h of exposure to RF-EMF source, the semen quality changed when compared with control. The vitality of sperm in the control was 95 ± 1.0 after 2 h and it was reduced to 60 ± 1.5 at a distance of 1cm and 55 ± 2.2 percent at a distance of 10 cm. After 5 hour exposure, the vitality was reduced to 63 ± 1.86 at 1 cm distance and 70 ± 1.42 at 5cm distance. The reduction in vitality of sperm after exposure to RF-EMF source for 2 hour was 35% at 1cm and 40% at 10cm distance. After 5 hour exposure, the reduction in vitality when compared to control was 32% and 25% respectively at 1 and 10 cm distance.

Discussion

The oxidative damages and accumulation of reactive oxygen species are reported to interfere with the vitality of the sperms as reported³³. In conformity with the damage to vitality, the RF-EMF radiations affected the motility of the sperm. The progressive motility of the sperm at 1 and 10 cm distance was significantly reduced after exposure to RF-EMF radiations from computer. The decrease in progressive motility was also correlated with exposure durations. When compared with control the reduction in sperm motility (PS) was 35%, 30%, 45% and 40% respectively at 1cm, 10cm distance after 2 h and 5 h.

The non-progressive motile sperm in the control group was $18.5 \pm 0.41\%$. The percentage of non- progressive motile sperm got significantly elevated (31.0 ± 1.5) at 1 cm distance after 2 hours and 26.0 ± 1.20 percent after 5 h duration. The percentage increases in non- motile sperm at 10 cm distance after 2 h and 5 h exposure duration were 10.5% and 7.5% respectively. The motility of the sperm is significantly affected due to the impact of RF-EMF radiations in different exposure duration and distance.

The integrity of the DNA inside the sperm cell decides the functional mechanism of sperms. There are several reports on RF-EMF and oxidative stress and DNA damage spermatozoa¹². DNA fragmentation index in the control sample was 5.3 ± 0.41 . In RF-EMF exposed sample for 2 hours at a distance of 1cm and 1cm, the DFI were 16.0 ± 0.38 and 10.8 ± 1.0 . After 5 hour exposure, the DFI were 16.7 ± 0.86 and 15.5 ± 0.25 at 1cm and 10 cm respectively.

It is well documented that the RF-EMF radiations from digital devices like mobile phones, laptop and Wi-Fi affect the sperm motility and progressiveness. Radiations from cell phone are found affecting sperm motility causing DNA fragmentation and alteration in gene expression^{1,32,38}. Electromagnetic radiations from smart phone (1800 –MHZ) and Wi Fi (2.45 GHZ) made an imbalance in antioxidants in semen and damaged sperm DNA³⁴. RFEMF exposure induces increased reactive oxygen species (ROS) production from the mitochondria leading to deleterious effect on sperms.^{19,35} In the present study also, the exposure of spermatozoa to the RF-EMF from laptop with Wi –Fi

functions affected its vitality, mobility and DNA integrity as reported¹⁸.

In the present observation, motility was less reduced in the semen sample kept at 10 cm when compared with 1 cm distantly located sperm sample. In the 5h exposed sperm samples, the impact of RF-EMF was high and the progressive motility, non-progressive motility and DFI were highly changed when compared with the sperm sample exposed to RF-EMF for 5h. It is reported that the RF-EMF emission from digital devices affects the reproductive physiology in the testes and the functional characteristics of sperm²⁴.

The radiation induces micronuclei formation, changes in protein kinases, anti-oxidative enzymes and disrupts genomic stability¹⁷. Radio frequency radiation (RFR) is reported to damage DNA and arrest cell cycle in the germ cells of male rats²⁰. The radiations from cell phone affected the semen quality and fertility in males²⁷. The exposure to radio frequency leads to proteotoxicity and cancer^{14,15,39}.

Reactive oxygen species generation: Reactive oxygen species (ROS) is a free radical generated in oxygen metabolism influencing positive or negative function in nearest stable biomolecules. The excessive generation of ROS leads to an imbalance in antioxidant potential and ends in oxidative stress. The oxidative stress in normal spermatozoa causes damages and dysfunction. The optimum level of ROS production by spermatozoa is beneficial. But excessive generation of ROS causes deleterious effects on the structure and function of spermatozoa and also changes in blood parameters²⁹. This is because of the fact that the poly unsaturated fatty acids (PUFA) present in the plasma membrane of spermatozoa undergo lipid peroxidation due to ROS attack.

As a result, the plasma membrane loses its integrity and DNA damage occurs^{9,26}. The electromagnetic radiation from mobile phones and Wi-Fi devices increased oxidative stress in uterus and made degenerative changes in endocrine functions including a reduction in plasma prolactin, progesterone and estrogen levels in pregnant rats³⁰.

Conclusion

The present study on the impact of radiations from a laptop upon the functional mechanism of spermatozoa confirmed that operating the digital devices without precautionary measures and distance interferes with fertilizable ability of the sperms that may end in infertile marriage. The present users of laptop or other digital devices must be cautious about the possible fertility problems from such devices.

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