

ЕКОЛОГІЯ ТА ОХОРОНА НАВКОЛИШНЬОГО СЕРЕДОВИЩА

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THE EFFECT OF ELECTROMAGNETIC RADIATION (EMR) FROM LAPTOP ON REPRODUCTIVE HORMONES, SPERM QUALITY AND PROSTATE SPECIFIC ANTIGEN OF MALE ALBINO RATS (*RATTUS NORVEGICUS*)

Humans in modern society are exposed to an ever-increasing number of electromagnetic fields (EMFs) generated from the production and supply of electricity, television sets, personal computer (PC), radio communication, and mobile communication, hence, it has become a public health issue. This study was conducted to evaluate the effects of electromagnetic radiation (EMR) emitted from new generation laptop computers on sperm quality and reproductive hormone of male albino rats. Male albino rats (10-12 weeks old) were exposed to RF-EMR from laptop computers which were grouped based on different exposure period (2 hours, 4 hours, 6 hours and 8 hours) for 4 weeks. The semen samples were obtained by caudal puncture of the epididymis from each participant for sperm quality analysis and blood was collected by cardiac puncture for hormonal analysis using the chemiluminescent microparticles immunoassay method. The analysis of variance was done for the hormonal concentration and sperm quality parameters to check for the significance difference at 5% level of significance. The Dunnett's multiple comparison test was done to test for significance comparison of radiation exposed groups and the control group. Exposure to laptop computer display unit was associated with significant reduction in sperm motility, sperm viability and sperm count ($P < 0.0001$), testosterone level ($P < 0.001$), follicle stimulating hormone level ($P < 0.01$). For the sperm morphology, there was no significant difference in the normal cells for the experimental setup, however, the appearance of abnormal cells in the exposed rats (2-8 hrs) were significant ($P \leq 0.001$). This study therefore showed that EMR from a charging laptop can significantly affect semen quality, male fertility and rendered instability of the male reproductive hormone with no effect on prostrate specific antigens.

Keywords: Radio-frequency electromagnetic radiation, computer display units, reproductive hormones, sperm quality, prostate specific antigen.

Problem statement. During the past several decades, many reports have suggested that the quality of semen in healthy men is declining (Rolland et al., 2013). In 2000, Swan et al. reported that there was a large annual decline in sperm concentration in European men (2.3%) and a smaller decline in US men (0.8%). The factors leading to changes in human semen quality may be very complex; such as environmental pollutants, seasonal variations and increased stress may be critical risk factors (Gollenberg et al., 2010; Pant et al., 2011; Zhang et al., 2013). Human exposure in modern society to electromagnetic fields (EMFs) comes from many sources, and situations are different in people's everyday lives. EMFs emanate from power lines, computer devices, televisions, radios, and telephones (Asghari et al., 2016). Extremely low frequency (ELF)-EMFs have 3 to 30 Hz frequencies and are generated from military communication. The EMFs to which humans are most frequently exposed are the 50 to 60 Hz super low frequency (SLF) EMFs generated from power cables for industrial and household electrical supplies and electronic goods. Very low frequency (VLF) EMFs with 3 to 30 kHz frequency are generated from PC monitors or TV sets. EMFs from TVs or PCs have a 6.25 μ T intensity with a 20 kHz frequency (Gye and Park, 2012). The use of computer has increased exponentially and become an important part of everyday life throughout the world. It has become an indispensable device in our daily life, offering flexibility and mobility to users.

A growing concern for their possible adverse effects on human health evokes a flurry of scientific activity to evaluate this dilemma. EMFs can have devastating effects on tissue with high concentrations of

electrons and ions. EMFs that cause changes in the behavior of cells and tissues alter the function of the cardiovascular system, and bone marrow (SCENIHR, 2015; Asghari et al., 2016). Under the influence of these fields, the balance of the CNS and the hormonal and respiratory systems become weak, resulting in decreased activity of the mentioned organs (Sobel et al., 1996; Harrington et al., 1997). Decline in male fertility has been attributed to the direct or indirect exposure to certain environmental factors such as RF-EMR (Agarwal et al., 2008; Otitoloju et al., 2010). Extremely low frequency magnetic fields have also been reported to initiate a number of biochemical and physiological alterations in biological systems of different species (Cuccurazzu et al., 2010; Volkow et al., 2011).

Many recent studies have raised questions regarding the safety of such RF-EMR exposure. Microwaves generated by mobile phones have been linked to several genetic defects (Aitken et al., 2005; Mailankot et al., 2009). According to the WHO's International Agency for Research on Cancer (IARC), RF-EMR fields have been classified as possibly carcinogenic to humans on the basis of an increased risk for brain glioma that some studies have associated with the use of wireless phones (Baan et al., 2011). Research also suggests that microwave radiation from mobile phones may induce chromosomal instability and also increased cancer risk (Sykes et al., 2001; Mashevich et al., 2003). Since radiation affects the biological materials posed by depositing energy at molecular levels, hence their stochastic and non stochastic effects might be important at sub-cellular levels (Meo et al., 2013). Hormones and receptors might be the prime targets for the worst possible health hazards to the users of the computers and their generations unborn. Therefore, the alterations caused by RF-EMR on human reproductive organs are plausible. Literatures abound on the hazards of mobile phone radiation on various organs; however, there is paucity of information on the hazards of laptop radiation on reproduction in male. This study is to ascertain the effect of computer laptop radiation on reproduction in male with a special focus on sperm quality, effects on male reproductive hormone and prostate specific antigen of male albino rats.

Materials and methods. Experimental and treatment setup. Thirty Albino male Wistar rats between the ages of 8-10 weeks, weighing from 120-200 g were housed in plastic cages in the animal house of the Department of Cell Biology and Genetics, Faculty of Science, University of Lagos, Nigeria, a well-ventilated room kept at $22\pm 2^{\circ}\text{C}$ with a 12-hr light-dark cycle. All animals had free access to a standard diet and water given ad libitum. The rats were divided into five groups of six animals each. Group I served as control and was exposed to no computer radiation, while groups II, III, IV and V were exposed to 2 hours, 4 hours, 6 hours and 8 hours laptop computer radiation (in power) for 28 days, respectively. Each rat was sacrificed by cervical dislocation. After dislocation the rat was dissected and the epididymis was punctured to collect sperm and blood was collected by cardiac puncture for hormonal analysis. Ethical approval for this study and procedures was obtained from the Animal Ethics Committee of Lagos University Teaching Hospital, University of Lagos.

Sperm Analysis. Total Sperm count. Sperm suspension was placed on the counting chamber which then spread under the cover slip by capillary action. The counting chamber was then mounted on the slide stage of the microscope and viewed at $\times 40$ magnification. A grid system divided the counting chamber into five major squares each containing 16 smaller boxes. The count included all the sperm cells within the five major squares using the top and right or left and bottom system of counting (Zaneveld and Polakoski, 1977; Verma and Chinoy, 2002).

Sperm Morphology. Sperm morphology was determined by the method of Rouge (2004). Microscope slides and nigrosin-eosin stains were pre-warmed to body temperature. A drop of stain was made onto the end of a slide and a small droplet of semen was pipette next to the stain. The edge of another slide was placed inside the drops of stain and semen, and the slide was rocked back and forth a few times to mix the sperm and stain. A second side was smeared across the surface of the first. The second slide was dried by waving it back and forth in the air. The slide was examined using a bright field microscope X 40 objective lens. Sperm head morphology was classified into normal and abnormal types. The abnormal spermatozoa were classified into two categories: (1) Spermatozoa with defective heads, and (2) Spermatozoa with defective tails.

Sperm Motility. A drop of the semen mixture was placed on a glass slide using 2 ml syringe, the preparation was then placed on a microscope. The motility of epididymal sperm was evaluated microscopically within 2–4 min of their isolation from the caudal epididymis and data were expressed as percentages of progressive motile, non-progressive motile and non-motile spermatozoa. The percentage of motility was evaluated visually at $\times 40$ magnification. Values were scored under progressive movement

sperm cells, non-progressive movement sperm cells and no movement or immotile sperm cells (Sonmez et al., 2007).

Sperm Viability. Epididymis sperm was squeezed onto a microscope slide and its smear was made, two drops of eosin-nigrosin stain were added to it and allowed drying. The slide was examined immediately after drying with bright field optics at $\times 1000$ magnification and oil immersion. The nigrosin provided a dark background that made it easier to discern faintly stained spermatozoa. With bright field optics, live spermatozoa had white heads and dead spermatozoa had heads that were stained red or dark pink. Spermatozoa with a faint pink head were assessed as alive. Stain limited to only a part of the neck region, and the rest of the head area unstained was considered a “leaky neck membrane”, not a sign of cell death and total membrane disintegration. These were classified as alive (Björndahl et al., 2003).

Hormonal Assay. Blood samples were collected by cardiac puncture of each male rat. The serum was separated out and was used to estimate the levels of total testosterone, luteinizing hormone (LH), Follicle stimulating hormone (FSH) and prostate specific antigen concentrations by Chemiluminescent Immunoassay (CLIA) (Basso et al., 2006).

Luteinizing Hormone (LH) Assay. 115 μL of serum was mixed with 6 μL of anti- β LH micro particles; the LH present in the sample bound to the anti- β LH micro particles. The solution was then washed with a wash buffer. 65 μL of anti- α LH was added to the resulting solution. 145 μL of hydrogen peroxide and 165 μL of sodium hydroxide was then added to the reaction mixture; the resulting chemiluminescent reaction was measured as relative light units (RLUs) (Oer et al., 2012). A direct relationship exists between the amount of LH in the sample (measured in mIU/mL) and the relative light units detected by the architect optical system. E.g if chemiluminescent reaction measures sample as 3 R.L.U.s, it will be calculated as $3 \text{ R.L.U.s} = \text{LH (miu/ml)}$, $\text{LH (miu/ml)} = 1/3 \text{ R.L.U.}$; $\text{LH} = 0.3 \text{ min/ml}$ (Oer et al., 2012).

Follicle Stimulating Hormone (FSH) Assay. 75 μL of serum was mixed with 65 μL anti- β FSH coated paramagnetic micro particles. Follicle stimulating hormone present in the sample bound to the anti- β FSH coated micro particles. The solution was then washed with a 2ml wash buffer, 75 μL anti- α FSH acridinium labelled conjugate was added. 145 μL of hydrogen peroxide and 165 μL sodium hydroxide was then added to the reaction mixture; the resulting chemiluminescent reaction was measured as relative light units (RLUs) (Scott, 2007). A direct relationship exists between the amount of follicle stimulating hormone (measured in mIU/mL) in the sample and the relative light units detected by the architect optical system. E.g if chemiluminescent reaction measures sample as 3 R.L.U.s, it will be calculated as $3 \text{ R.L.U.s} = \text{FSH (miu/ml)}$, $\text{FSH (miu/ml)} = 1/3 \text{ R.L.U.}$; $\text{FSH} = 0.3 \text{ min/ml}$ (Scott, 2007).

Testosterone Assay. 150 μL of serum was mixed with 65 μL of anti-testosterone, 75 μL testosterone acridinium and 100 μL testosterone assay diluents. The solution was washed with a 2ml wash buffer. 145 μL of hydrogen peroxide and 165 μL sodium hydroxide was then added to the reaction mixture; the resulting chemiluminescent reaction was measured as relative light units (RLUs) (Scott, 2007). An inverse relationship exists between the amount of testosterone (measured in ng/mL) in the sample and the relative light units detected by the architect optical system. If chemiluminescent reaction measures result as 0.04 R.L.U.s, $0.04 \text{ R.L.U.s} = 1/\text{amt of test}$, concentration of testosterone = 0.04 ng/ml (Scott, 2007).

Total Prostate Specific Antigen Assay. 100 μL of serum was mixed with 65 μL of anti-PSA micro particles. Prostate specific antigen present in the sample bound to the anti-PSA coated micro particles. The solution was washed with a 2ml wash buffer and 75 μL of anti-PSA acridinium was added to the solution. 145 μL of hydrogen peroxide and 165 μL of sodium hydroxide was then added to the reaction mixture; the resulting chemiluminescent reaction was measured as relative light units (RLUs) (Scott, 2007). A direct relationship exists between the amount of total prostate specific antigen (measured in ng/mL) in the sample and the relative light unit detected by the architect optical system (Scott, 2007).

Statistical Analysis. The obtained data were expressed as mean \pm standard error of mean. The analysis of variance for sperm morphology, sperm count and sperm motility, testosterone level, luteinizing hormone, and follicle stimulating hormone was performed using the Graph pad prism, version 7.01 ($p < 0.01, 0.05, 0.001$).

Results and discussion. Figure 1 shows that chronic exposure to RF EMR radiations showed a clear negative impact on the concentration parameters. Sperm samples from 2-hour to 8-hour groups ($P < 0.001$) exhibited a significant lower concentration as compared to the control group. It is evident that sperm counts of various radiation exposure hours with increasing time decreased significantly when compared with the control group. However, the sperm counts for the control and exposed rats were still within the standard range of sperm count for *Rattus norvegicus* (5.8 million/ml – 17.7 million/ml).

For the sperm morphology, in the control sperm samples were all normal (100%), there was no abnormality (fig. 2). Rats exposed to 4 hours of daily exposure had the highest percentage of abnormality (10%). Though, there was no significant difference in the normal cells for the experimental setup, the appearance of abnormal cells in the exposed rats (2 – 8 hrs) was significant ($P \leq 0.001$).

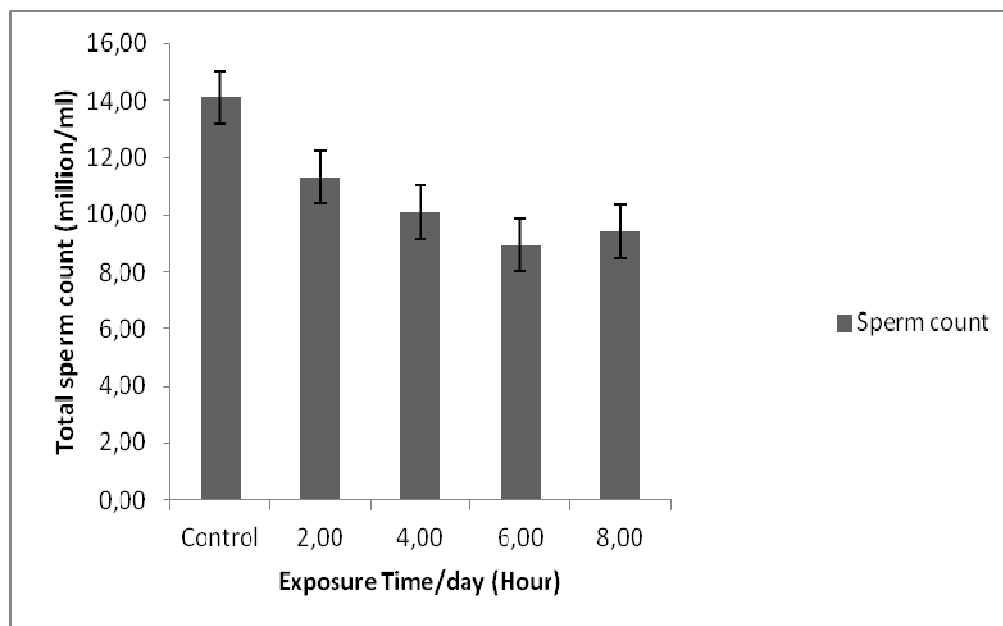


Fig. 1. The sperm count (million/ml) of albino rats exposed to radiation of varying hours for 28 days

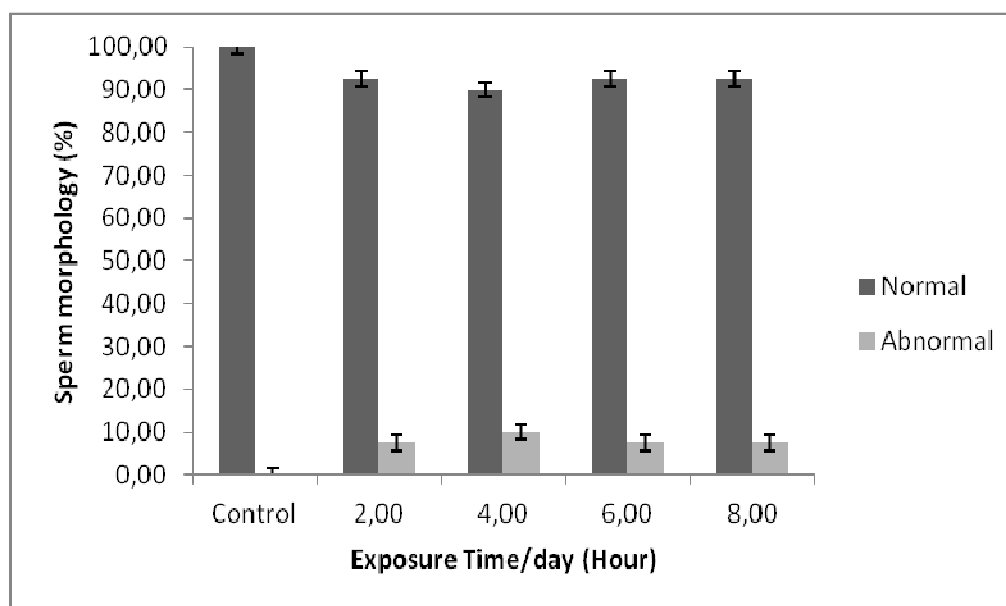


Fig. 2. The sperm morphology (%) of albino rats exposed to radiation of varying hours for 28 days

We also examined the proportion of the different sperm motility grades as shown in fig. 3. There was decrease in mean progressive sperm motility but an increase in the mean non-progressive motility and non-motile sperm cells of the radiation exposed group compared to the control group. For the control group all the sperm cells were motile with about 85 percent progressive sperms. The 6 hours and 8 hours per day of radiation exposure for 28 days caused significant changes in the sperm motility parameters.

The percentage rates of viable and non viable sperms cells of the exposed rats to computer radiation is shown in figure 4. There was decrease in mean viable sperm but an increase in the mean non-viable sperm cells of the radiation exposed group as compared to the control group. The percentage of the non-viable sperms increased with the hours of exposure while the percentage of viable sperms decreased with the exposure period. The percentage of the non viable was significant at 4-hour ($P \leq 0.001$), 6-hour ($P \leq 0.001$) and 8-hour ($P \leq 0.05$) daily exposure.

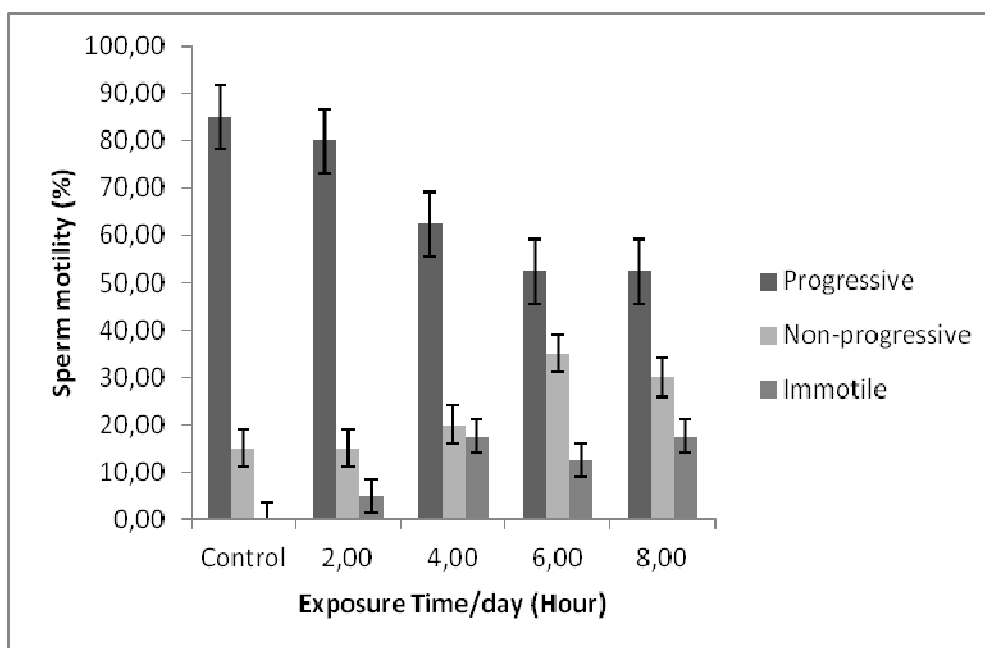


Fig. 3. The sperm motility (%) of albino rats exposed to radiation of varying hours for 28 days

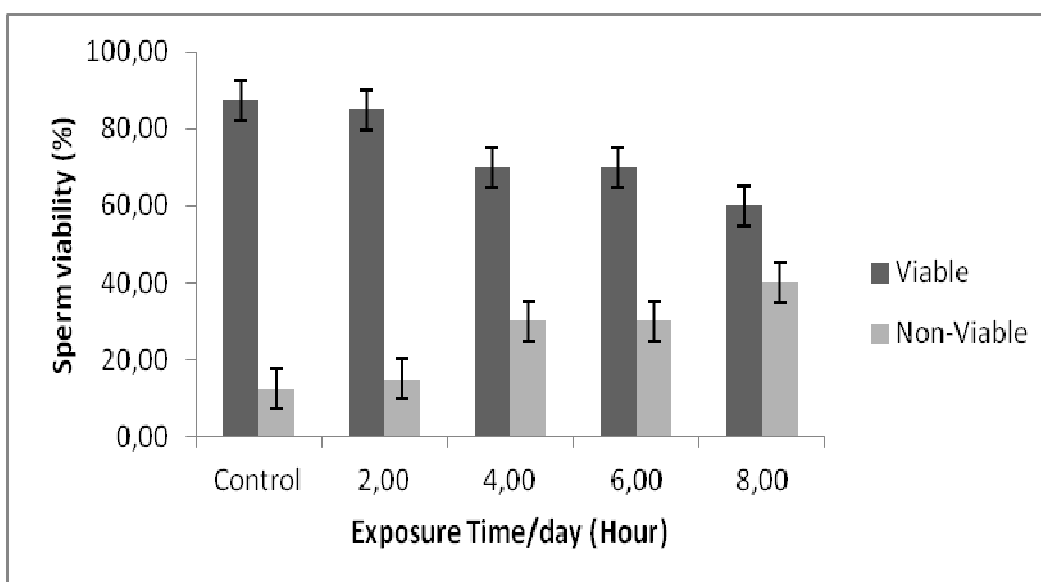


Fig. 4. The sperm viability (%) of albino rats exposed to radiation of varying hours for 28 days

The mean serum luteinizing hormone levels (LH) and follicle stimulating hormone (FSH) of rats exposed to computer radiation are shown in figure 5. The results show that luteinizing hormone level was not steady (increased and decreased) with different exposure periods compared to the control group. The LH level was the highest in rat exposed for 6 hours while it was the least for rats exposed for 8 hours. However, there were no significant differences in the control animals and exposed groups irrespective of the duration ($P > 0.05$). Figure 6 shows the levels of follicle stimulating hormone. FSH level decreased non-uniformly with increase in exposure period as compared to the control group. There were no significant differences in the FSH level of the control animals and exposed groups irrespective of the duration ($P > 0.05$).

The mean serum testosterone levels of control and exposed animals are shown in figure 6. There was relative decrease in mean serum testosterone level in radiation exposed groups as compared to control group. This reduction in testosterone level was significant in 6 and 8 hours of daily exposure ($P < 0.001$). The prostate specific antigen test showed no presence of prostate specific antigen in all the experimental animals.

In this study the exposure of rats to EMR from laptop display unit in power resulted in significant reduction in epididymal sperm concentration with increase in exposure hours after 28 days as compared to the control group. The decrease in sperm count in the radiation exposed group could be partly due to the induction of oxidative stress in the testes and the spermatozoa (Isojarvi et al., 2004). Relatively higher sperm morphological abnormalities observed as compared to the control group may be due to alterations in the process of sperm maturation, where sperm cells develop from damaged seminiferous tissue, which may have occurred as a result of the lipoperoxidative damage induced by radiation exposure on the testicular tissues (Yan et al., 2007). Notwithstanding the general paucity of information in this area, studies have highlighted the interesting finding that male infertility patients are frequently characterized by high levels of DNA damage to their spermatozoa (Lewis and Aitken, 2005).

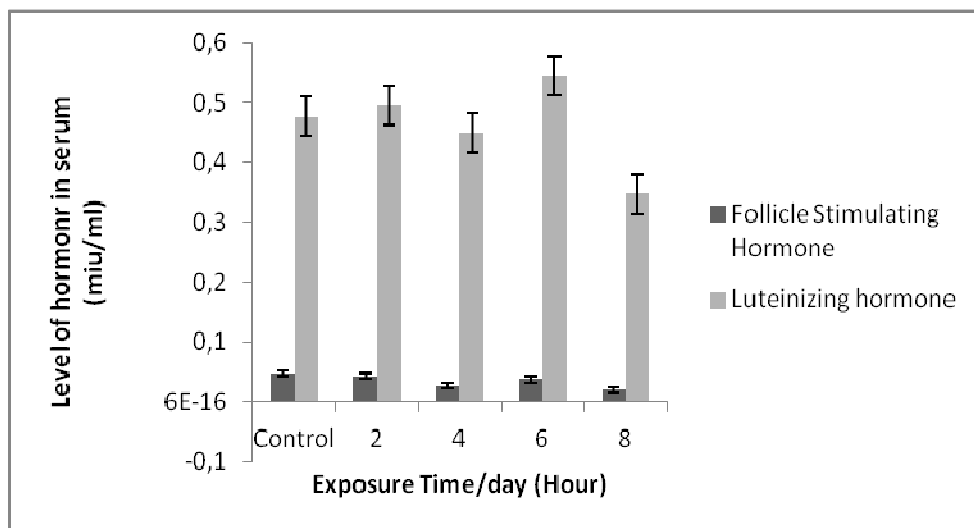


Fig. 5. The follicle stimulating hormone level (miu/ml) of albino rats exposed to radiation of varying hours for 28 days

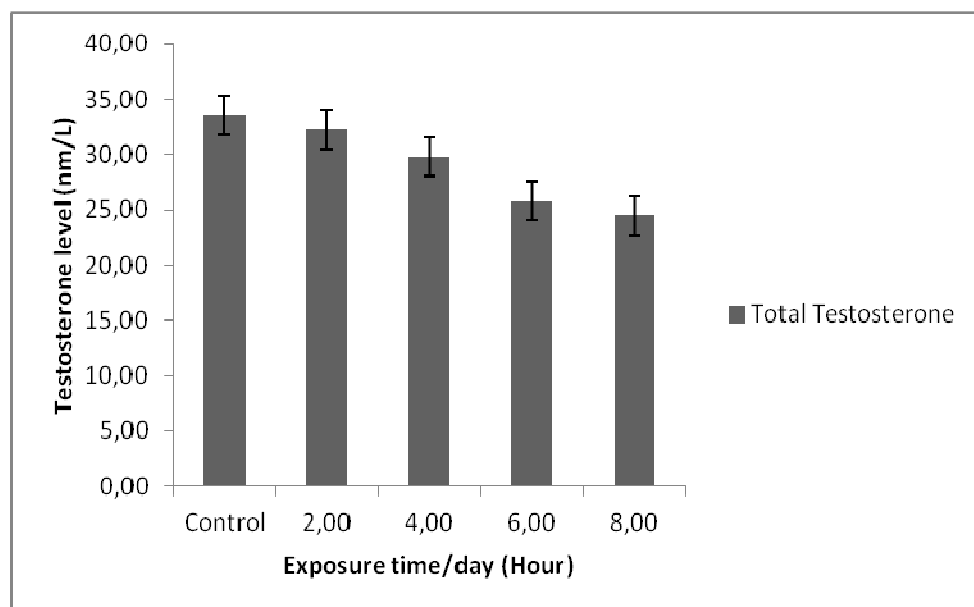


Fig. 6. The testosterone level (nm/L) of albino rats exposed to radiation of varying hours for 28 days

The reduced sperm motility seen in this study may be ascribed to magnetic and electromagnetic fields inducing oxidation of phospholipids, which are a major component in the sperm mitochondrial sheath (Tremellen, 2008). The present study shows a significant decrease in the progressive sperm motility in the groups exposed to radiation compared to the control group. This result corresponds with the previous findings by Conrado et al., (2012) who reported that the use of laptop computers connected to internet through Wi-Fi decreased human sperm motility. Adams et al., (2014) also reported decrease in sperm viability in their study of the effect of mobile telephone on sperm quality. Reduction in viability with increase in exposure hours may be a result of increased mitochondrial reactive oxygen specie (ROS)

production and DNA fragmentation in sperm. In a pilot study by De Iulii et al. (2009), human spermatozoa were found to respond to RF-EMR (at 1.8 GHz with a SAR of 27.5 W/kg) with a range of negative changes including dramatic declines in both sperm vitality and motility. This RF-EMR precipitated a state of oxidative stress leading to high levels of lipid peroxidation and a loss of sperm motility (Koppers et al., 2008). Therefore, these data highlight the particular vulnerability of human spermatozoa to oxidative attack.

It is known that testosterone is needed in very high quantities for maintenance of the reproductive tract. The lower serum testosterone concentration in the exposed group may be linked to the inhibition in secretion of pituitary gonadotropins (FSH and LH), which aid in testosterone biosynthesis or due to direct damage to Leydig cells (Oliva and Miraglia, 2009). Thus, atrophy of seminiferous tubules might be the cause for the decrease in the levels of testosterone observed in this study. In agreement with the current study, Gholampour et al. (2012) found that exposure to long-term extremely low frequency electromagnetic field caused reduction in the level of testosterone in testis in rats. Khayyat (2011) found that exposure of mice to EMF caused atrophy in the seminiferous tubules and Rajaei et al. (2009) mentioned that exposure to EMF for long periods could decrease the diameter of reproductive ducts. The significant decrease in testosterone serum level in EMF exposed group may be one of the factors that lead to the significant increase in LH serum level in the exposed group. The concentration of follicle stimulating hormone (FSH) decreased compared to the control group which could be a factor for decreased activation of seminiferous tubules. Studies conducted by Ozguner et al., (2005) on male rats showed that the diameter of seminiferous tubules and the mean height of the germinal epithelium were significantly decreased after 900 MHz EMF exposure (Ozguner et al., 2005).

LH is known to bind to receptors in Leydig cells and regulate gonadal function by promoting sex steroid production and gametogenesis (Warita et al., 2006). LH is a glycoprotein gonadotropin secreted by the anterior pituitary in response to gonadotropin-releasing hormone (GnRH). GnRH release from the hypothalamus into the portal circulation is episodic which in turn causes LH to be released in a series of secretory bursts, resulting in intermittently elevated LH concentrations in the blood (Bergendahl et al., 1996). LH release is driven mainly by the increase of Ca^{2+} . ELFEMF may modulate the cellular calcium regulatory mechanism which affects the affinity of calcium binding proteins such as calmodulins. In agreement with this result, Margonato et al. (1993) did not find any differences on LH and testosterone between exposed animals and control group's animals after exposure to high intensity electric field for up to 18% of their life span. Prostate-specific antigen (PSA) is a protein produced by normal prostate cells. This enzyme participates in the dissolution of the seminal fluid coagulum and plays an important role in fertility. This study shows that the exposure to radiation from laptop does not induce PSA (prostate specific antigen) for all the experimental groups (radiation exposed groups).

Conclusions. The present results clearly indicate that the exposure laptop computer at varying increasing hours for a period of 28 days while in power significantly changes the level of hormones (testosterone and follicle stimulating hormone) and sperm quality (sperm count, sperm viability and sperm motility). At present, it cannot be concluded whether this effect is induced by all laptop computers brands usage while not in power. It can be speculated that EMR from laptop computers may be the cause of sperm damage. These findings suggest that prolonged use of laptop computers on the laps or closer to a male user may decrease male fertility potential. Further research will be required to understand the long-term effects of laptops usage in man.

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ВПЛИВ ЕЛЕКТРОМАГНІТНОГО ВИПРОМІНЮВАННЯ (ЕМВ) НОУТБУКІВ НА СТАТЕВІ ГОРМОНИ, ЯКІСТЬ СПЕРМИ ТА ПРОСТАТ-СПЕЦИФІЧНИЙ АНТИГЕН САМЦІВ БЛИХ ЩУРИВ (RATTUS NORVEGICUS)

У сучасному суспільстві люди піддаються впливу постійно зростаючої кількості електромагнітних полів (ЕМП), які генеруються при виробництві та постачанні електроенергії, телевізорами, персональними комп'ютерами (ПК), радіозв'язком та мобільним зв'язком, тому це стало питанням охорони здоров'я населення. Проведено дослідження для оцінки впливу

електромагнітного випромінювання (ЕМВ) ноутбуків нового покоління на якість сперми та статевий гормон самців білих щурів. Самці білих щурів (віком 10–12 тижнів) піддавалися радіочастотному ЕМВ від ноутбуків і були згруповані відповідно до різного періоду впливу (2 години, 4 години, 6 годин і 8 годин) протягом 4 тижнів. Зразки сперми було отримано за допомогою каудальної пункції епідидимісу кожного піддослідного для аналізу якості сперми, за допомогою серцевої пункції було взято кров на гормональний аналіз із використанням методу хемілюмінесцентного імуноаналізу мікрочасток. Здійснено варіаційний аналіз для визначення концентрації гормонів і параметрів якості сперми з метою перевірки різниці значущості на рівні 5%. Проведено тест множинного порівняння Даннета для перевірки значущості груп, що піддаються опроміненню, та контрольної групи. Близькість до дисплею ноутбука була пов'язана зі значним зниженням рухливості, життєздатності та кількості сперматозоїдів ($P < 0,0001$), рівня тестостерону ($P < 0,001$) і рівня фолікулостимулюючого гормону ($P < 0,01$). У випадку морфології сперми не було суттєвих змін у нормальних клітинах в експериментальній моделі, однак кількість аномальних клітин в опромінених щурів (протягом 2-8 годин) була значною ($P < 0,001$). Таким чином, дослідження показало, що ЕМВ від ноутбука, що заряджається, може суттєво впливати на якість сперми, чоловічу фертильність і нестабільність чоловічого статевого гормону, проте не впливає на простат-специфічні антигени.

Ключові слова: радіочастотне електромагнітне випромінювання, комп'ютерний дисплей, статеві гормони, якість сперми, простат-специфічний антиген.

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