

Effects of the Exposure to Mobile Phones on Male Reproduction: A Review of the Literature

Minireview

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ABSTRACT: The use of mobile phones is now widespread. A great debate exists about the possible damage that the radiofrequency electromagnetic radiation (RF-EMR) emitted by mobile phones exerts on different organs and apparatuses. The aim of this article was to review the existing literature exploring the effects of RF-EMR on the male reproductive function in experimental animals and humans. Studies have been conducted in rats, mice, and rabbits using a similar design based upon mobile phone RF exposure for variable lengths of time. Together, the results of these studies have shown that RF-EMR decreases sperm count and motility and increases oxidative stress. In humans, 2 different experimental approaches have been followed:

one has explored the effects of RF-EMR directly on spermatozoa and the other has evaluated the sperm parameters in men using or not using mobile phones. The results showed that human spermatozoa exposed to RF-EMR have decreased motility, morphometric abnormalities, and increased oxidative stress, whereas men using mobile phones have decreased sperm concentration, decreased motility (particularly rapid progressive motility), normal morphology, and decreased viability. These abnormalities seem to be directly related to the duration of mobile phone use.

Key words: Sperm parameters, male infertility.
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Cellular phones operate using frequencies that differ by manufacturer and country, and concerns are growing about the possible negative effects of radiofrequency (RF) electromagnetic waves (EMW) emitted by these communication tools on human health. In particular, one of the biggest worries is that these RF-EMW may disturb testicular function and alter conventional and/or nonconventional sperm parameters.

A number of reports have suggested a possible link between cell phone use and decreased semen quality. For example, recently Agarwal et al (2008) suggested that the use of cellular phones adversely affected the quality of semen in 361 men attending an infertility clinic, and Fejes et al (2005) showed that the duration of cellular phone possession and the duration of daily transmission correlated negatively with semen quality in 371 men. These findings have been confirmed, although

in a smaller number of men (13 and 27, respectively) (Davoudi et al, 2002; Eroglu et al, 2006).

More commonly used cellular phones operate at a frequency of 850 to 1800 MHz; the radiant energy is absorbed by human body tissues and organs by aerial effect and/or coupling the RF signal and/or resonant absorption (D'Andrea et al, 1985). The specific absorption rate (SAR) defines the amount of RF energy absorbed into local tissues and represents a measure for evaluating the emission of transmitters located nearby the body. For cellular phones, SAR varies from 0.12 to 1.6 watts/kg of body weight.

Leydig cells, seminiferous tubules, and spermatozoa are the main targets of the damage caused by mobile phones on the male reproductive tract. In particular, cellular phone exposure reduces testosterone biosynthesis, impairs spermatogenesis, and damages sperm DNA. Scrotal hyperthermia and oxidative stress are the main mechanisms by which the damage is generated (Depinder et al, 2007). It is well known that testicular temperature is 2°C to 3°C lower than rectal temperature, and the optimal temperature for spermatogenesis is considered to be 35°C (Saikhun et al, 1998). From this point of view, the habit of keeping a mobile phone in the trouser pocket or the duration of its use may have an impact on possible generation of hyperthermia and oxidative stress as well.

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Table. Explanation of some technical acronyms found in the literature

Acronym	Explanation
W-CDMA	Wideband code division multiple access (W-CDMA) indicates a particular technology of multiple access to radio channel cellular networks of third generation (3G)
FOMA	Freedom of mobile stands for multimedia access (FOMA) is one of the 3G standards that uses W-CDMA transmission interface
SAR	Specific absorption rate (SAR) is a measure of the rate at which energy is absorbed by the body when exposed to a radiofrequency (RF) electromagnetic field; it is defined as the power absorbed per mass of tissue and is measured as W/kg
GSM	Global system for mobile communications (originally groupe spécial mobile; GSM) is a standard set developed by the European Telecommunications Standards Institute to describe technologies for second-generation digital cellular networks
Hz, GHz, and MHz	The Hertz (Hz) is the International System unit of frequency named after the German physicist Heinrich Rudolf Hertz, who made important contributions to the science of electromagnetism; Hz multiples are megahertz (MHz) = 10^6 Hz and gigahertz (GHz) = 10^9 Hz

Many animal studies have shown that EMW negatively interfere with the male reproductive system. However, similar studies are scant in men, and the results obtained in the experimental animal may only be translated to humans with caution. This review presents the main studies exploring the effects of mobile phones on the male reproductive system in various strains of experimental animals and in humans. The Table reports some acronyms used in mobile telephony.

Animal Studies

Studies on Male Sprague-Dawley Rats—One of the first studies on mobile phone exposure investigated the effects of exposure to RF electromagnetic radiation (EMR) on testicular and sperm function. To achieve this objective, rats were confined in Plexiglas cages specially designed for this study, and cellular phones were placed 0.5 cm under the cages (EMW with frequencies between 800 and 1800 MHz, such as those used by mobile phones, can penetrate tissue up to 2 cm). The experimental group was exposed to cellular phones activated for 20 min/d for 1 month, whereas the control rats were exposed to switched-off cellular phones placed beneath the cages for the same length of time. The results of this study showed no statistically significant difference between exposed and control rats as far as sperm count, morphology, lipid composition, malondialdehyde (MDA) concentration (an index of sperm plasma membrane lipid peroxidation), testicular histologic structure, p53 immune reactivity, and rectal temperature (Dasdag et al, 2003). By contrast, Yan et al (2007) reported a significantly higher incidence of cell death in spermatozoa collected from the epididymis in adult rats exposed to RF-EMR compared with unexposed rats. In addition, the former had abnormal clumping of spermatozoa that was not present in unexposed rats (Yan et al, 2007). This apparent discrepancy may be explained by the longer exposure to which the same strain of rats was exposed in this

latter study. Indeed, the experimental group was exposed to cellular phone emissions for two 3-hour periods/d for 18 weeks.

The effects of radiation exposure have also been evaluated in young developing male rats. Five-week-old rats were exposed to a 1.95-GHz wide-band code division multiple access signal, which is used for the freedom of mobile multimedia access, with a whole-body exposure for 5 h/d for 5 weeks, corresponding to the period of reproductive maturation in these rats. The whole-body average SAR was designed to be 0.4 W/kg. The control group received sham exposure. There were no differences in body weight gain or weights of the testis, epididymis, seminal vesicles, and prostate among the groups. The number of testicular and epididymal spermatozoa did not decrease in RF-EMR-exposed rats, and no abnormalities in sperm motility or morphology or the histologic appearance of the seminiferous tubules, including the stage of the spermatogenic cycle, were observed. Interestingly, the testicular sperm count increased significantly following exposure to the 0.4-W/kg SAR (Imai et al, 2011).

Lee et al (2010) examined the testicular histologic changes in rats exposed to an RF-EMR of 848.5 MHz for 12 weeks. The exposure schedule consisted of two 45-minute periods, separated by a 15-minute interval, with a whole-body mean SAR of 2.0 W/kg. The authors then investigated sperm counts in the cauda epididymis, MDA concentrations in the testes and epididymis, frequency of spermatogenesis stages, germ cell counts, and appearance of apoptotic cells in the testes. Finally, they performed p53, Bcl2, caspase 3, p21, and poly(adenosine diphosphate-ribose) polymerase immunoblotting of the testes in controls and exposed animals. On the basis of the results found, this study concluded that the subchronic exposure to 848.5 MHz did not have any detectable adverse effects on rat spermatogenesis (Lee et al, 2010).

Studies on adult and developing male Sprague-Dawley rats showed no substantial effects of RF-EMR

exposure except for a slightly increased sperm cell death rate.

Studies on Male Wistar Rats—Using adult male Wistar rats, Ribeiro et al (2007) reported that rats exposed to RF-EMR emitted by a global system for mobile communication (GSM) cellular phone (1835–1850 MHz) for 1 h/d for 11 weeks had similar testicular and epididymal weight, lipid peroxidation levels in these organs (evaluated by monitoring the formation of thiobarbituric acid [TBA] reactive substances after the reaction of TBA with MDA), serum total testosterone volume, and epididymal sperm count compared with unexposed control rats. In particular, rectal temperatures before and immediately after RF exposure were $36.9^{\circ}\text{C} \pm 0.4^{\circ}\text{C}$ and $37.1^{\circ}\text{C} \pm 0.3^{\circ}\text{C}$, respectively, in the control group and $36.9^{\circ}\text{C} \pm 0.4^{\circ}\text{C}$ and $37.0^{\circ}\text{C} \pm 0.3^{\circ}\text{C}$ in the experimental group. Absolute testes weight was 1.72 ± 0.08 g in the control group and 1.77 ± 0.17 g in the experimental group; absolute epididymal weight was 269 ± 19 mg in the control group and 265 ± 25 mg in the experimental group. Finally, the control group had $88 \pm 23 \times 10^6$ sperm/epididymal cauda and the experimental group showed $83 \pm 18 \times 10^6$ sperm/epididymal cauda.

Similarly, no effect on total sperm count was found in rats exposed to RF-EMR, emitted by an active GSM (0.9/1.8 GHz) mobile phone for 1 h/d for 4 weeks, compared with control rats that were exposed to a mobile phone without a battery for the same period. However, sperm motility decreased significantly in exposed rats. The average percent of motile sperm was $72.0\% \pm 8.7\%$ for controls and $43.1\% \pm 10.0\%$ in RF-EMR-exposed animals, a reduction of approximately 40%. RF-EMR-exposed rats also had significantly increased lipid peroxidation: endogenous MDA levels were approximately 8% in the testis and approximately 12% in the epididymis. A decreased glutathione content in testis (approximately 10%) and epididymis (approximately 24%) was also reported (Mailankot et al, 2009).

Kesari et al (2010) found a significantly decreased level of protein kinase C (an enzyme present in human sperm head, neck, and tail that is strongly associated with motility and the acrosomal reaction) and total sperm count along with increased apoptosis in adult rats exposed to RF-EMR in Plexiglas cages for 2 h/d for 5 weeks, with an SAR estimated to be 0.9 W/kg. Subsequently, these researchers investigated the production of free radicals following mobile phone exposure and the effects on fertility pattern using the same length of exposure and the same strains of rats. The levels of the antioxidant enzymes glutathione peroxidase and superoxide dismutase decreased, whereas the level of catalase increased significantly. MDA concentration increased significantly from 0.16 ± 0.01 vs 0.08 ± 0.01

TBA-reactive substances in experimental group and controls, respectively. Micronuclei evaluated as the ratio of polychromatic erythrocyte to normochromatic erythrocyte by flow cytometry was significantly lower in the mobile phone-exposed group (0.67 ± 0.15) as compared with the sham-exposed group (1.36 ± 0.07). Finally, histone kinase volume decreased significantly in exposed rats (3659.1 ± 1399.4 and 5374.9 ± 1366.9 P₃₂ counts/mg of protein, respectively, in the EMR-exposed and sham-exposed groups). A significant change in testicular sperm cell cycle of G₀–G₁ and G₂/M was recorded. Free radical production increased significantly (Kesari et al, 2011).

Finally, hypospermatogenesis was found in 3 of 16 male Wistar rats (18.7%) exposed to mobile phone radiation for 60 min/day (whole body) for 3 months, whereas another 3 rats (18.7%) had maturation arrest. In contrast, no spermatogenesis abnormalities were found in rats exposed to mobile phone radiation for 30 min/day for 3 months (Meo et al, 2011).

With some discrepancies, studies on Wistar male rats have shown that mobile phone exposure results in decreased sperm count and motility and increased oxidative stress.

Studies on Male Mice—A single study has been reported on mice. The experimental animals were exposed to 900-MHz RF-EMR at an SAR of approximately 90 mW/kg inside a waveguide for 12 h/d for 1 week, and the rate of DNA damage in spermatozoa of the caudal epididymal was assessed by quantitative polymerase chain reaction and alkaline and pulsed-field gel electrophoresis. The exposed mice were clearly normal, and sperm number, morphology, and vitality were not significantly affected. Gel electrophoresis revealed no evidence of increased single-stranded or double-stranded DNA breakage in spermatozoa taken from treated animals. However, a detailed analysis of DNA integrity using quantitative polymerase chain reaction revealed statistically significant damage in both the mitochondrial genome and the nuclear β -globin locus. This study suggested that although RF-EMR does not have a dramatic impact on male germ cell development, a significant genotoxic effect can be detected in epididymal spermatozoa (Aitken et al, 2005). However, it should be pointed out that the SAR used in this study was approximately 10-fold lower than that used in the study by Kesari et al (2010) in rats. The different experimental conditions and the different strains used may be partly responsible for the contrasting outcome. In fact, mice are much smaller than rats. Mice weigh approximately 30 to 50 g and have bodies that are 3 to 4 inches long with 3- to 4-inch tails. Rats, on the other hand, are far heavier and longer: they can weigh 10 times as much, averaging 450 to 650 g for

males, and have 9- to 11-inch long bodies and 7- to 9-inch tails.

Studies on Male Rabbits—Rabbits have also been used as an experimental model to evaluate the effect of mobile phone exposure on testicular function. In the study by Salama et al (2009), 30 individually caged, adult male New Zealand White rabbits, aged 20 weeks and weighing 3.15 to 3.25 kg, were used. They were randomly divided into 3 groups. The first one was the mobile phone group, with members individually placed in cages specifically designed (50 × 25 × 35 cm) for this study. These cages could accommodate plastic partitions according to the animals' dimensions (average, 30 × 16 × 18 cm) to restrict movement. Therefore, the animals rested throughout the period of the daily phone exposure with their genitalia opposing the antennae of the mobile phones, which were fixed to the cage bottoms. Mobile phones were conventional GSM handsets (900 MHz) that were turned to the standby position with a 2.92-V/m average strength of the electric field estimated at 0.5 cm away from the phone and 0.487 V/m at the most distant region inside the cage. The whole-body average SAR was 0.43 W/kg. Phone exposure was applied for 8 hours (9:00 AM–5:00 PM) daily for 12 weeks. Following this daily mobile phone exposure, the animals were returned to their individual standard cages (90 × 60 × 40 cm). Because of the restriction of animal movement and the possibility of stress-related outcome, 2 control groups were added for the measurement of fructose or citrate levels under stressful conditions. Animals of the first control group were the sham or stress controls (n = 11). They were placed in identical cages for 8 hours with the phones switched off. The animals in the second control group provided an additional control (n = 8) throughout the duration of the study and were housed in conventional cages provided by the animal room. In both control groups, the cages were positioned 7 m away from the phone group where the average strength of the electric field detected was equivalent to background radiation (0.18 V/m). Rectal temperature assessment was conducted for all animals in this study 2 times per week. The measurements were made both before and after phone exposure. A significant decline in both fructose concentrations (250 ± 8.4 mg% in the mobile phone group, 499 ± 7.3 mg% in stress controls, and 497 ± 4.1 mg% in ordinary controls) and number of motile spermatozoa (52% ± 2.3% in the phone group, 63% ± 2.0% in stress controls, and 73.4% ± 3.4% in ordinary controls) was observed in the phone group at the 10th week. However, no correlation was found between the 2 values. The stress control animals showed a similar but significantly less marked decline in motility. Citrate concentrations (one of the most important anions present in human

semen and the major regulator of ionized calcium levels in seminal plasma) and the other parameter studied did not differ significantly among groups (Salama et al, 2009).

Subsequently, these researchers, using a mobile phone emitting at 800 MHz, evaluated the longitudinal effect of RF-EMR on adult rabbits using a similar experimental design and protocol of exposure. Sperm analysis, sperm functional tests (viability, hypo-osmotic swelling, and acridine orange staining), histologic testicular sections, and serum total testosterone level were evaluated weekly. A decrease in the sperm concentration appeared after 6 weeks of exposure. This became statistically significant at week 8, compared with the 2 control groups (stress and ordinary) and the initial sperm count found in the phone group. Sperm motility was similar among the 3 groups until week 10, when it declined significantly, and thereafter in rabbits exposed to mobile phones and in the stress control group, with more significant decline in the phone group. Histologic examination also showed a significant decrease in the diameter of seminiferous tubules in the phone group vs that in the stress and ordinary controls. The other end points did not show any statistically significant differences (Salama et al, 2010). In conclusion, the 2 studies in rabbits conducted by the same group of researchers with the identical experimental design showed that RF-EMR exposure decreased sperm concentration and motility.

Human Studies

Human Spermatozoa Exposed to Mobile Phone Radiation In Vitro—A number of studies have attempted to elucidate the effects of cellular phone radiation on human sperm function using a direct approach that consisted of exposure of raw or selected spermatozoa to RF-EMR for a variable length of time. Eroglu et al (2006) exposed an aliquot of unprocessed raw spermatozoa to the RF-EMR emitted by an activated cellular phone (900 MHz), and another aliquot of the same ejaculate served as control. RF-EMR exposure caused a slight decrease in the rapid progressive and slow progressive sperm movement; by contrast, it increased the percentage of immotile spermatozoa (Eroglu et al, 2006). A similar in vitro experimental approach was conducted on semen samples from healthy donors (n = 23) and infertile patients (n = 9). After liquefaction, the semen samples were divided into 2 aliquots; an aliquot was exposed to a Sony Ericsson w300i cellular phone in talk mode for 1 hour. This phone emitted at 850 MHz with a maximum power <1 W and an SAR of 1.46 W/kg. This model had a loop shape and omnidirectional antenna placed on the top back of its handset. The distance between the phone antenna and each specimen

was kept at 2.5 cm. The second aliquot (unexposed) served as the control sample under identical conditions. Spermatozoa exposed to RF-EMR showed a significant decrease in sperm motility and viability, increase in radical oxygen species (ROS) production, and a reduced ROS total antioxidant capacity (TAC) score. The seminal plasma has an effective antioxidant system that can provide spermatozoa with a protective environment. The seminal plasma TAC is the sum of enzymatic (eg, superoxide dismutase, catalase, and glutathione peroxidase) and nonenzymatic (eg, ascorbate, urate, vitamin E, pyruvate, glutathione, taurine, and hypotaurine) antioxidants.

Levels of TAC and sperm DNA fragmentation in the exposed spermatozoa showed no significant differences compared with unexposed spermatozoa (Agarwal et al, 2009). These results suggested that RF-EMR emitted from cellular phones may increase the oxidative stress in human semen.

Different than the previous 2 *in vitro* studies exploring the direct effects of RF-EMR exposure on unselected spermatozoa, Falzone et al (2008) exposed density-purified spermatozoa to pulsed 900-MHz GSM mobile phone radiation at 2 SARs (2.0 and 5.7 W/kg) and compared the effects observed with controls over time. No effects of RF-EMR were found on sperm mitochondrial membrane potential, an early apoptotic event evaluated by flow cytometry following staining with JC-1, and on all sperm kinematic parameters (evaluated by computer-assisted sperm analysis) at an SAR of 2.0 W/kg. However, over time, the 2 kinematic parameters straight-line velocity and beat-cross frequency decreased significantly after exposure at an SAR of 5.7 W/kg (Falzone et al, 2008). Subsequently, using a similar approach, these researchers examined the effects of the radiation on the induction of apoptosis-related features in human spermatozoa. For this purpose, ejaculated, density-purified, highly motile human spermatozoa were exposed to mobile phones at SARs of 2.0 and 5.7 W/kg. At various times after exposure, flow cytometry was used to examine caspase 3 activity (caspase 3 is the major effector enzyme causing cell disruption during apoptosis; caspase 3 activity has been detected in the midpiece of ejaculated human sperm and has been shown to be significantly associated with low sperm motility or with decreased normal sperm concentration, motility, and morphology), phosphatidylserine externalization (phosphatidylserine translocation from the cytosol to the outer leaflet of the plasma membrane is an early apoptotic event), DNA fragmentation, and generation of ROS. RF-EMR had no statistically significant effect on any of the parameters studied (Falzone et al, 2010). Therefore, a stimulatory effect of mobile phone exposure on oxidative stress seemed to be

present only in unprocessed semen and not in density-purified spermatozoa. However, the lack of effect reported by Falzone et al (2011) may relate to different SARs. De Iuliis et al (2009) exposed purified human spermatozoa to RF-EMR tuned to 1.8 GHz and covering a range of SAR from 0.4 W/kg to 27.5 W/kg. Sperm motility and vitality decreased significantly, whereas the mitochondrial generation of ROS and DNA fragmentation increased significantly with increases in the SAR. In addition, highly significant relationships among SAR, oxidative DNA damage biomarker (8-hydroxy-2'-deoxyguanosine), and DNA fragmentation after RF-EMR exposure were also observed (De Iuliis et al, 2009).

Finally, Falzone et al (2011) evaluated sperm-fertilizing competence following exposure to RF-EMR. To accomplish this, highly motile human spermatozoa collected from 12 healthy, nonsmoking donors were exposed for 1 hour to 900-MHz mobile phone radiation at an SAR of 2.0 W/kg, and the acrosome reaction was evaluated at various intervals after exposure by using the viability probe (7-aminoactinomycin, a fluorescent chemical compound) to assess the acrosome reaction in live spermatozoa only. The acrosome was assessed with *Pisum sativum* agglutinin fluorescein isothiocyanate, and specimens were gated by light scatter properties (size and granularity) of spermatozoa and analyzed for dual-color fluorescence using flow cytometry. The radiation did not affect sperm acrosome reaction rate. Morphometric evaluation, appraised by computer-assisted sperm analysis, showed a significant decrease of the sperm head area and acrosome percentage of the head area among exposed compared with unexposed spermatozoa. The sperm competence to bind the zona pellucida following RF-EMR exposure decreased significantly compared with that of unexposed spermatozoa (Falzone et al, 2011). Therefore, the results of this study showed that although RF-EMR exposure does not seem to negatively affect the rate of the acrosome reaction, it significantly alters sperm morphometry and decreases the capability of spermatozoa to bind to the zona pellucida.

Together, *in vitro* studies suggested that following RF-EMR exposure, human spermatozoa show motility reduction, morphometric abnormalities, and increased oxidative stress. These alterations are somewhat dependent upon the SAR administered directly to spermatozoa.

Clinical Studies—One of the first clinical studies on the effects of RF-EMR on conventional sperm parameters was conducted in 52 men aged 18 to 35 years. The results of this study showed that men who carried a mobile phone in their hip pockets or on their belts had a lower sperm concentration than men who either did not carry a mobile phone or who stored it elsewhere in the

body (Kilgallon and Simmons, 2005). A much larger number of men ($n = 371$) was asked questions concerning cellular phone use habits, including possession, daily standby position, and daily transmission times before sperm analysis was performed. The results showed that the duration of possession and the daily transmission length correlated negatively with the percentage of rapid progressive motile spermatozoa and positively with the percentage of slow progressive motile spermatozoa. The low transmitter group of men had a significantly higher percentage of rapid progressive motile spermatozoa compared with high transmitters (Fejes et al, 2005).

Wdowiak et al (2007) examined the conventional sperm parameters of 304 men divided into 3 groups on the basis of their habits using mobile phones. One group ($n = 99$) did not use mobile phones, a second group ($n = 157$) used mobile phones sporadically for 1 to 2 years, and the third group ($n = 48$) regularly used mobile phones for more than 2 years. Analysis of the effect of RF-EMR exposure on sperm parameters revealed that an increase in the percentage of spermatozoa with abnormal morphology was associated with the duration of exposure to the radiation emitted by cellular phone. The results also confirmed a decrease in the percentage of spermatozoa with progressive motility in the semen that correlated with the frequency of mobile phone usage (Wdowiak et al, 2007). Similarly, Agarwal et al (2008) reported significantly lower sperm count, motility, and viability and normal morphology in 3 groups of men using cellular phones for variable lengths of time (<2 h/d, $2-4$ h/d, and >4 h/d), compared with men who did not use them.

Overall, clinical studies showed that cellular phone use is associated with decreased sperm concentration, decreased motility (particularly rapid progressive motility), normal morphology, and decreased viability. These abnormalities seem to be directly related to the duration of mobile phone use.

Conclusions

In aggregate, the literature has suggested that mobile phone use alters sperm parameters in both experimental animals and humans. Sperm motility and morphology seem to be the 2 parameters more frequently affected. There is evidence that mobile phone radiation results in increased oxidative stress, with subsequent sperm membrane lipid and DNA damage. These abnormalities seem to be directly related to the duration of mobile phone use. Nevertheless, more studies are necessary to provide stronger evidence that cellular phone use disturbs sperm and testicular function because the existing literature has several limitations. These include

dishomogeneity in terms of RF wavelength used, depth of penetration, and length of radiation exposure.

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