Published-Ahead-of-Print on July 28, 2011 by Journal of Andrology

Manuscript: J Androl 2011-014373 - revised

Effects of the exposure to mobile phones on male reproduction: a review of the literature

Running title: Cellular phone and male infertility

Sandro La Vignera, Rosita A. Condorelli, Enzo Vicari, Rosario D'Agata, Aldo E. Calogero

Section of Endocrinology, Andrology and Internal Medicine and Master in Andrological, Human Reproduction and Biotechnology Sciences, Department of Internal Medicine and Systemic Diseases, University of Catania, Catania, Italy

For submission to: Journal of Andrology

Keywords: mobile phone, sperm parameters, male infertility

Corresponding author: Dr. Sandro La Vignera, Section of Endocrinology, Andrology and Internal Medicine, Department of Internal Medicine and Systemic Diseases, University of Catania, Policlinico *"G. Rodolico"*, Via S. Sofia 78, Building 4, Room 2C19, 95123 Catania, Italy. FAX ++39-95-3781180; Email: sandrolavignera@email.it

Abstract

The use of mobile phones is now widespread. A great debate is going on about the possible damage that the radiofrequency electromagnetic radiation (RF-EMR) emitted by mobile phones exerts on different organs and apparatuses. 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 human beings. Studies on the experimental animals have been conducted in rats, mice, and rabbits using a similar design based upon mobile phone radiofrequency exposure for a variable length of time. Altogether the results of these studies show that RF-EMR decreases sperm count and motility, and increases the oxidative stress. In human beings, two 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 mobile phones. The results show 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, motility (particularly the rapid progressive one), normal morphology, and viability. These abnormalities seem to be directly related with the length of mobile phone use.

Introduction

Cellular phones operate using frequencies which differ from manufacturers and countries and concerns are growing about the possible negative effects of radio-frequency (RF) electromagnetic waves (EMW) emitted by these communication tools on the human health. In particular, one of the biggest worries is that they may disturb the testicular function and alter conventional and/or non conventional sperm parameters.

A number of reports suggest a possible link between cell phone use and decreased semen quality. For example, recently, Agarwal and colleagues suggested that the use of cellular phones adversely affects the quality of semen in 361 men attending an infertility clinic (Agarwal et al, 2008), Fejes and colleagues showed that the duration of cellular phone possession and the duration of daily transmission correlated negatively with semen quality in 371 men (Fejes et al, 2005). These findings have been confirmed, though in a lower number of men (13 and 27, respectively) (Davoudi et al, 2002; Erogul et al, 2006).

More commonly cellular phones operate at a frequency of 850-1800 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, impaires 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 2-3°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 the mobile phone in the trouser pocket or the duration of its use may have a major impact on possible generation of hyertermia and oxidative stress as well.

Many animal studies have shown that EMW negatively interfere with the male reproductive system. However, similar studies are scanty in men, and the results obtained in the experimental animal may be translated with caution to humans. This review presents the main studies exploring the effects to mobile

phones on male reproductive system in various strains of experimental animals and in the human beings. Table 1 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 the exposure to radiofrequency electromagnetic radiation (EF-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 (electromagnetic waves with frequencies between 800 and 1800 MHz, such as mobile phones, can penetrate tissue up to 2 cm). The experimental group was exposed to cellular phones activated 20 min/day 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 result 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 histological structure, p53 immune reactivity, and rectal temperature (Dasgad et al, 2003). By contrast, Yan and colleagues 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 which was not present in unexposed rats (Yan et al, 2007). This apparent discrepancy may be explained by the longer exposure 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/day for 18 weeks.

The effects of radiation exposure has also been evaluated in young developing male rats. Therefore, 5 week old rats were exposed to a 1.95-GHz wide-band code division multiple access (W-CDMA) signal, which is used for the freedom of mobile multimedia access (FOMA), with a whole-body exposure for 5 hours/day for 5 weeks, corresponding to the period of reproductive maturation in these rats. The whole-body average specific absorption rate (SAR) was designed to be 0.4 and 0.08 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-EMF exposed rats, and no abnormalities in sperm motility or morphology, and in the histological appearance of the seminiferous tubules, including the stage of the spermatogenic cycle, were

observed. Interestingly, the testicular sperm count increased significantly following the exposure to 0.4 SAR (Imai et al, 2011).

Lee and colleagues examined the testicular histological changes in rats exposed to a RF-EMR of 848.5MHz for 12 weeks. The exposure schedule consisted of two 45-min periods, separated by a 15-min interval, with a whole-body mean SAR of 2.0 W/kg. The authors then investigated sperm counts in the cauda epididymis, malondialdehyde concentrations in the testes and epididymis, frequency of spermatogenesis stages, germ cell counts, and appearance of apoptotic cells in the testes. Finally, they also performed p53, bcl-2, caspase 3, p21, and PARP 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 effect on rat spermatogenesis (Lee et al, 2010).

Studies on adult and developing male Sprague-Dawley rats show no substantial effects of RF-EMR exposure if not for a slightly increase sperm cell death.

Studies on male Wistar rats

Using adult male Wistar rats, Ribeiro and colleagues reported that rats exposed to EF-EMR emitted by a global system for mobile communication (GSM) cellular phone (1,835-1,850 MHz), for 1 hour/day 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, and the epididymal sperm count compared with unexposed control rats (Ribeiro et al, 2007). In particular, rectal temperature before and immediately after RF exposure was 36.9±0.4°C and 37.1±0.3°C in the control group, and 36.9±0.4°C and 37.0±0.3°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±23 x 10^6/epididymal cauda and the experimental group showed a value of 83±18 x 10^6/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 hour/day for 4 weeks, compared with control rats which 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±8.7% for controls and 43.1±10.0% in RF-EMR exposed animals; a reduction of ~40%. RF-EMR exposed rat had also significantly

increased lipid peroxidation, endogenous MDA levels were ~8% in the testis and ~12% in the epididymis. A decreased glutathione content in testis (~10%) and epididymis (~24%) was also reported (Mailankot et al, 2009).

Kesari and colleagues found a significant decreased protein kinase C (PKC) (an enzyme present in human sperm head, neck, and tail, strongly associated with motility and acrosomial reaction) and total sperm count along with increased apoptosis in adult rats exposed to RF-EMR in Plexiglas cages for 2 hours/day for 5 weeks, with a SAR estimated to be 0.9 W/kg (Kesari et al, 2010). Subsequently, these authors investigated the production of free radical 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, while catalase increased significantly. MDA increased significantly from 0.16±0.01 vs 0.08±0.01 TBARS (thiobarbituric acid-reactive substances), respectively in experimental group and controls. Micronuclei evaluated as polychromatic erythrocyte (PCE)/normochromatic erythrocyte (NCE) ratio by flow cytometry was significantly lower in mobile phone-exposed group (0.67±0.15) as compared with the sham-exposed group (1.36±0.07). Finally, histone kinase decreased significantly in exposed rats (3,659.1±1,399.4 and 5,374.9±1,366.9 P_{32} counts/mg protein, respectively, in the electromagnetic field-exposed group and in the sham exposed group). A significant change in testicular sperm cell cycle of G_0-G_1 and G_2/M was recorded. Free radical production increased significantly (Kesari et al, 2011).

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

Although with some discrepancy, studies on Wistar male rats showed that mobile phone exposure results in decreased sperm count and motility and increased oxidative stress.

Studies on mice

A single study has been reported on mice. The experimental animals were exposed to 900 MHz RF-EMR at a SAR of approximately 90 mW/kg inside a waveguide for 12 hours/day for 1 week, and the rate of DNA damage in spermatozoa of the caudal epididymal was assessed by quantitative PCR (qPCR) 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- or double-DNA strand breakage in spermatozoa taken from treated animals. However, a detailed analysis of DNA integrity using qPCR revealed statistically significant damage in both mitochondrial genome and the nuclear β-globin locus. This study suggests that while 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 is about 10-fold lower than that utilized in the study by Kesari and colleagues (2010) in rats. The different experimental conditions and the different strains used may take into account the contrasting outcome. In fact, mice are much smaller than rats. Mice weigh about 30-50 g and have bodies that are 3-4 inches long with 3-4 inch tail. Rats, on the other hand, are far heavier and longer: they can weigh 10-times as much, averaging 450-650 for males and have 9-11 inch long bodies and 7-9 inch tails.

Studies on rabbits

Rabbits have also been used as an experimental model to evaluate the effect of mobile phone exposure on the testicular function. In the study by Salama and colleagues (2009) a total of 30 adult male, individually caged White New Zealand rabbits, 20 weeks of age and weighing 3.15–3.25 kg, were used. They were randomly divided into three groups. The first one was the mobile phone (MP) group whose members were individually placed in cages specifically designed (50x25x35cm) for this study. These cages could accept placing plastic partitions according to the animal dimensions (30x16x18cm average), to restrict movement. Therefore, the animals rested throughout the period of the daily phone exposure with their genitalia opposing the antennas 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 2.92 V/m average strength of the electric field estimated at 0.5 cm away from the phone and 0.487V/m at the most distant region inside the cage. The whole-body average SAR was 0.43W/kg. Phone exposure was applied for 8 h (9:00 am–5:00 pm) daily for 12 weeks. Following this daily MP exposure, the animals were returned to their individual standard cages (90x60x40cm). Because of the restriction of animal movement and the possibility of stress-related outcome, two 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 h 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 meters 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 done for all animals in this study two times a week. The measurement was made both before and after phone exposure. A significant decline in both fructose concentrations (250±8.4 mg% in MP group, 499±7.3 mg% in stress controls, 497±4.1mg% in ordinary controls) and number of motile spermatozoa (52±2.3% in MP group, 63±2.0% in stress controls, 73.4±3.4m% in ordinary controls) was observed in the phone group at the 10th week. However, no correlation was found between the two values. The stress control animals showed a similar, but a 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 Authors, using a mobile phone emitting at 800 MHz, evaluated the longitudinal effect of EF-EMR on adult rabbits using similar experimental design and protocol of exposure. Sperm analysis, sperm functional tests (viability, hypo-osmotic swelling, and acridine orange staining), histological testicular sections, and serum total testosterone 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 two control groups (stress and ordinary) and to the initial sperm count found in the phone group of animals. Sperm motility was similar among the 3 groups until week 10 when it declined significantly, and thereafter in rabbits exposed to mobile phones and stress control group, with more significant decline in the phone group of animals. Histological examination showed also a significant decrease in the diameter of seminiferous tubules in the phone group vs. stress and ordinary controls. The other end-points taken into account did not show any statistically significant difference (Salama et al, 2010). In conclusion, the two studies in rabbits conducted by the same group of authors with the identical experimental design showed that RF-EMR exposure decreases 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 sperm function using a direct approach which consisted in the exposure of raw or selected spermatozoa to RF-EMR for a variable length of time. Erogul and colleagues exposed to the RF-EMR emitted by an activated cellular phone (900 MHz) for an aliquot of unprocessed raw spermatozoa, whereas 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 contrary, it increased the percentage of immotile spermatozoa (Erogul 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 two aliquots, an aliquot was exposed to a Sony Ericsson w300i cellular phone radiation in talk mode for 1 h. This phone emitted at 850 MHz with a maximum power <1 W and a SAR of 1.46 W/kg. This model had a loop-shape, omni-directional 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 a very effective antioxidant systems that can provide spermatozoa with a protective environment. The seminal plasma TAC is the sum of enzymatic (e.g., superoxide dismutase, catalase, and glutathione peroxidase) and nonenzymatic (e.g., ascorbate, urate, vitamin E, pyruvate, glutathione, taurine, and hypotaurine) antioxidants.

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

Differently from the previous two *in-vitro* studies exploring the direct effects of RF-EMR exposure on unselected spermatozoa, Falzone and colleagues exposed density-purified spermatozoa to pulsed 900 MHz GSM mobile phone radiation at two SAR levels (2.0 and 5.7 W/kg) and compared the effects observed with controls over time. No effects of RF-EMR was found on sperm mitochondrial membrane potential, an early apoptotic event evaluated by flow citometry following staining with JC-1, and on all sperm kinematic parameters (evaluated by computer assisted sperm analysis, CASA) at a SAR of 2.0 W/kg. However, over

time, the two kinematic parameters straight line velocity and beat-cross frequency decreased significantly after the exposure at SAR 5.7 W/kg (Falzone et al, 2008). Subsequently, using a similar approach, these authors 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 phone 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 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 seems to be present only in unprocessed semen and not in density-purified spermatozoa. However, the lack of effect reported by Falzone and colleagues (2011) may relate to the different amount of SAR exposure. In fact, De Iuliis and colleagues 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, while the mitochondrial generation of ROS and DNA fragmentation increased significantly with the augmentation of amount of SAR. In addition, highly significant relationships between SAR, oxidative DNA damage bio-marker (8-hydroxy-2'-deoxyguanosine), and DNA fragmentation after RF-EMR exposure were also observed (De Iuliis et al, 2009).

Finally, Falzone and colleagues evaluated the sperm fertilizing competence following exposure to RF-EMF. To accomplish this, highly motile human spermatozoa collected from 12 healthy, non-smoking donors, were exposed for 1 h to 900-MHz mobile phone radiation at a SAR of 2.0 W/kg and the acrosome reaction was evaluated at various intervals after exposure by using the viability probe [7-Aminoactinomycin (AAD) : a fluorescent chemical compound] to assess the acrosome reaction in live spermatozoa only. The acrosome was assessed with PSA-FITC and specimens were gated by light scatter properties (size and granularity) of spermatozoa and analysed for dual-colour fluorescence using flow cytometry. The radiation did not affect sperm acrosome reaction rate. Morphometric evaluation, appraised by CASA, 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 acrosome reaction, it alters significantly sperm morphometry and decreases the capability of spermatozoa to bind to the zona pellucida.

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

Clinical studies

One of the first clinical studies on the effects of EF-EMR on conventional sperm parameters was conducted in 52 men 18-35 years old. The results of this study showed that men who carried a mobile phone in their hip pocket or on their belt 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 lenght 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 to high transmitters (Fejes et al, 2005).

Wdowiak and colleagues examined the conventional sperm parameters of 304 men divided into three groups on the basis of their habit to use a mobile phone. One group (n=99) did not use mobile phones, a second group (n=157) used mobile phones sporadically for the period of 1-2 years, and the third group (n=48) used regularly mobile phone for more than 2 years. The analysis of the effect of RF-EMR exposure on sperm parameters revealed an increase in the percentage of spermatozoa with abnormal morphology is associated with the duration of exposure to the radiation emitted by cellular phone. The results confirmed also a decrease in the percentage of spermatozoa with progressive motility in the semen which correlates with the frequency of mobile phone usage (Wdowiak et al, 2007). Similarly, Agarwal and colleagues reported significantly lower sperm count, motility, viability, and normal morphology in three groups of men

using the cellular phone for a variable length of time [<2 hours/day, 2-4 hours/day, and >4 hours/day], compared with men who did not use it (Agarwal et al, 2008).

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

Conclusion

In aggregate, the literature suggests that mobile phone alters sperm parameters both in the experimental animal and in humans. Sperm motility and morphology seem the two parameters more frequently affected. There are evidence that mobile phone radiation results in an increased oxidative stress with consequent sperm membrane lipid and DNA damage. These abnormalities seem to be directly related with the length of mobile phone used. Nevertheless, more studies are necessary to provide stronger evidences that cellular phone use disturb sperm and testicular function since the existing literature has several limitation. These include dishomogeneity in terms of RF wavelength used, depth of penetration, and length of radiation exposure.

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Table 1. Explanation of some technical acronyms found in the text.

Acronym	Explanation
W-CMDA	The acronym W-CDMA (Wideband Code Division Multiple Access) indicates a particular technology of multiple access to radio channel cellular networks of third generation (3G).
FOMA	The FOMA (Freedom Of Mobile stands for Multimedia Access) is one of the 3G standards that uses W-CDMA transmission interface.
SAR	The specific absorption rate (SAR) is a measure of the rate at which energy is absorbed by the body when exposed to a radio frequency (RF) electromagnetic field. It is defined as the power absorbed per mass of tissue. It is measured by Watts per kilogram (W/kg).
GSM	GSM (Global System for Mobile Communications, originally Groupe Spécial Mobile) is a standard set developed by the European Telecommunications Standards Institute (ETSI) to describe technologies for second generation (or 2G) digital cellular networks.
Herz, GHz, and MHz	The Hertz (Hz) is the International System unit of frequency. Named after the German physicist Heinrich Rudolf Hertz who brought important contributions to science in the field of electromagnetism. Hz multiples are: Megahertz (MHz) = 10^6 Hz; and Gigahertz (GHz) = 10^9 Hz.