

Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study

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Objective: To evaluate effects of cellular phone radiofrequency electromagnetic waves (RF-EMW) during talk mode on unprocessed (neat) ejaculated human semen.

Design: Prospective pilot study.

Setting: Center for reproductive medicine laboratory in tertiary hospital setting.

Samples: Neat semen samples from normal healthy donors (n = 23) and infertile patients (n = 9).

Intervention(s): After liquefaction, neat semen samples were divided into two aliquots. One aliquot (experimental) from each patient was exposed to cellular phone radiation (in talk mode) for 1 h, and the second aliquot (unexposed) served as the control sample under identical conditions.

Main Outcome Measure(s): Evaluation of sperm parameters (motility, viability), reactive oxygen species (ROS), total antioxidant capacity (TAC) of semen, ROS-TAC score, and sperm DNA damage.

Result(s): Samples exposed to RF-EMW showed a significant decrease in sperm motility and viability, increase in ROS level, and decrease in ROS-TAC score. Levels of TAC and DNA damage showed no significant differences from the unexposed group.

Conclusion(s): Radiofrequency electromagnetic waves emitted from cell phones may lead to oxidative stress in human semen. We speculate that keeping the cell phone in a trouser pocket in talk mode may negatively affect spermatozoa and impair male fertility. (Fertil Steril® 2009;92:1318–25. ©2009 by American Society for Reproductive Medicine.)

Key Words: Cell phone radiation, radiofrequency electromagnetic waves, sperm, fertility, reactive oxygen species, oxidative stress, EMW

The tremendous development and use of mobile telecommunication services in the last decade has drastically increased the amount of radiofrequency electromagnetic wave (RF-EMW) exposure in our daily lives. As the use of cell phones has increased, so have concerns regarding the harmful effects of cell phone exposure on human health. As part of its charter to protect public health, the World Health Organization (WHO) established the International EMF Project in 1996 to assess the scientific evidence of possible health effects of electromagnetic frequencies in the range of 30 Hz to 300

GHz (1). Despite more than a decade of research in this field, the potential harmful effects of cell phone radiation remain controversial.

Recent epidemiologic (cross-sectional or prospective) studies have highlighted the role of cell phone exposure on sperm motility, morphology, and viability, suggesting a reduction in male fertilization potential (2–6). These studies examined the relationship of cell phone use and its effect on semen parameters and concluded that mobile phone use may cause a decrease in fertility (2–6). To conduct a scientifically robust epidemiologic study, a control group of people who are not using and have not used cell phones in the past is a necessity. However, enrolling a pool of such control subjects in today's culture is extremely difficult. An in vivo human exposure study to investigate the effects of cell phone radiation on semen parameters is not feasible, owing to ethical issues.

Various in vitro studies using animal models have consistently demonstrated oxidative stress in different tissues (kidney, endometrium, eye, testis, brain, myocardial tissue, and

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so on) in response to cell phone radiation (7–13). Studies have also shown potential beneficial effects of antioxidants, such as melatonin, vitamin C, and vitamin E, on oxidative stress status induced by RF-EMW in animals (7, 8, 12, 13). However, results of animal studies related to the effects of cell phone radiation on reproductive functions are conflicting (14–19). An animal model is not preferable for study purposes for several reasons, including the smaller dimensions of the testes, the nonpendulous scrotum, the free migration of the testes through the inguinal canal between the abdomen and the scrotum and the unavoidable exposure of the animal's entire body to RF-EMW at the time of the experiment (7, 20). Therefore, an in vitro model would be the most scientific way to assess the effects of cell phone exposure, allowing us to obtain reproducible results that can be replicated by in vivo studies. The World Health Organization's recent research agenda (2006) for studies on RF suggests that in vitro studies play a supporting role in health risk assessments and are critical to the optimal design of animal and epidemiology studies (21).

There are reports of exposure of human semen samples to cell phone radiation under in vitro conditions resulting in a decrease in sperm motility (neat semen) after 5 min (22). Other investigators found no effect of RF-EMW on mitochondrial membrane potential of spermatozoa and motility at a specific

absorbance rate (SAR) of 2 W/kg. However, they showed a decrease in straight-line velocity and beat-cross frequency at an SAR of 5.7 W/kg (23).

We hypothesized that cell phone radiation (talk mode) disturbs free radical metabolism in human semen by increasing free radical formation, by decreasing antioxidants, or by both mechanisms. In the present pilot study, our objective was to validate the results of several recent epidemiologic studies by establishing a cause-and-effect relationship between RF radiation emitted from a cell phone in talk mode and changes in semen parameters. We tested our hypothesis by examining the effects of RF-EMW on ROS levels, total antioxidant capacity, and DNA integrity of spermatozoa in unprocessed ejaculated human semen.

MATERIALS AND METHODS

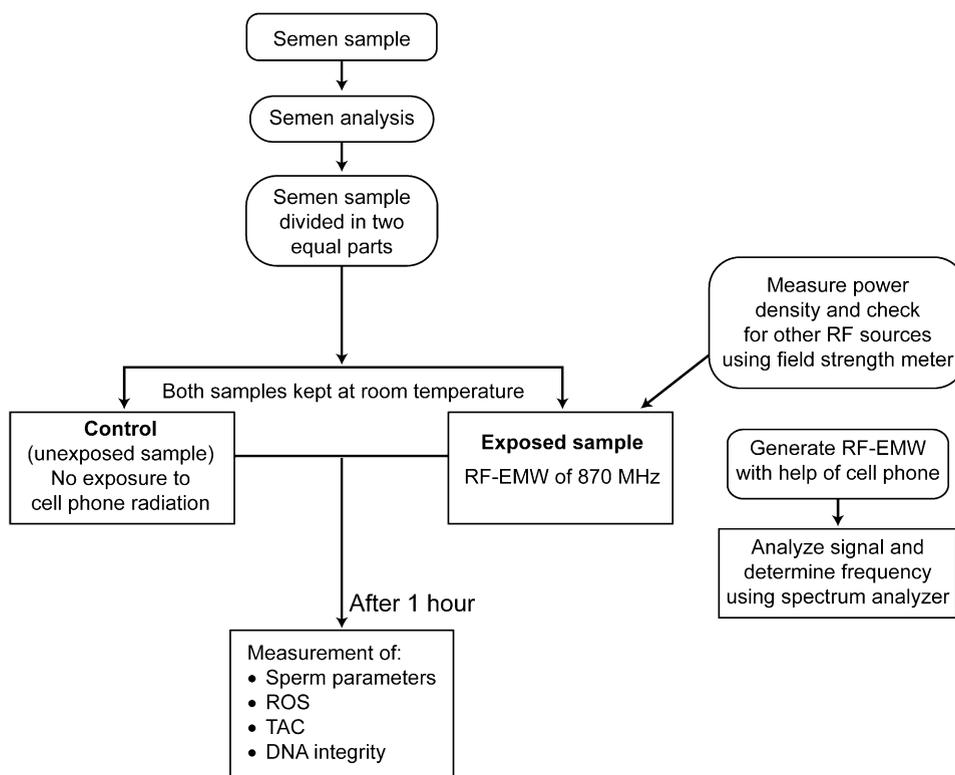
The study was approved by the Cleveland Clinic Institutional Review Board.

Subjects (Data Collection)

Semen samples were collected from 23 healthy donors and 9 patients presenting to the infertility clinic and referred to our lab. All specimens were collected by masturbation after an

FIGURE 1

Study design and set-up for the exposure of semen sample to RF-EMW. RF-EMW = radiofrequency electromagnetic waves; ROS = reactive oxygen species; TAC = total antioxidant capacity.



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abstinence period of 48–72 h and allowed to liquefy completely for 15–30 min at 37°C. Following liquefaction, each sample was divided into two aliquots: control group (sample not exposed, i.e. no exposure to cell phone) and exposed group (sample exposed to cell phone radiation).

Exposure of Semen Samples to Electromagnetic Waves

One aliquot of each divided semen sample was exposed to EMW emitted from a commercially available cellular telephone in talk mode (Sony Ericsson w300i; service provider AT&T; GSM-Global System for Mobile communications network; 850 MHz frequency; maximum power <1 W; SAR 1.46 W/kg). This phone model had a loop-shape, 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. In the United States the most common frequency is 850–900. Therefore, we decided to use this frequency in this pilot study. The duration of exposure was 60 min (Fig. 1). Unexposed (control) aliquots were kept under identical conditions but without RF-EMW exposure (6,7).

Power Density ($\mu\text{W}/\text{cm}^2$)

According to the International Commission for Non-Ionizing Radiation Protection (ICNIRP) and the Federal Communications Commission (FCC), the reference level for exposure of RF-EMW is peak power density. It is a commonly used term for characterizing an RF electromagnetic field (24, 25).

Power density was monitored during basal condition (no cell phone radiation) and experimental condition (cell phone in talk mode) in the laboratory throughout the experiment. Power density in the control condition was 0.01–0.1 $\mu\text{W}/\text{cm}^2$. Power density in the experimental condition (during cell phone in talk mode and at 2.5 cm from cell phone antenna) was 1–40 $\mu\text{W}/\text{cm}^2$.

Frequency and Temperature

The frequency emitted by the cell phone was confirmed with the help of a RF spectrum analyzer (Tektronix, Beaverton, OR). Both specimens (aliquots) were kept at room temperature to avoid the effect of temperature on ROS formation and semen parameters.

Semen Analysis

Immediately after exposure to cell phone radiation, both aliquots (control and exposed) were analyzed for sperm concentration, motility, and viability according to WHO guidelines (26).

ROS Measurement

Measurement of ROS in the exposed and unexposed aliquots was performed after 1 h by chemiluminescence assay using luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; Sigma Chemical Co., St Louis, MO). A 100-mmol/L stock solution of luminol was prepared in dimethyl sulfoxide. For the analysis, 10 μL of the working solution (5 mmol/L) was added to

400 μL of neat sperm sample. Chemiluminescence was measured for 15 min using a Berthold luminometer (Autolumat LB 953; Berthold, Bad-Wildbad, Germany). Results were expressed as $\times 10^6$ counted photons per minute (cpm)/ 20×10^6 sperm and as $\log(\text{ROS} + 0.001)$ (27), with the 0.001 constant chosen to achieve approximate normality for the ROS scale.

Total Antioxidant Assay (TAC) Measurement

The technique for total antioxidant (TAC) assay used in this study has been described previously (28).

This assay measures the combined antioxidant activities of all constituents, including vitamins, proteins, lipids, glutathione, uric acid, and so on. All samples were centrifuged at 1,000g for 10 min at 4°C. Clear seminal plasma was aliquoted and frozen at -70°C until the time of TAC assay. Seminal plasma total antioxidant measurements were performed using the antioxidant assay kit (Cat. no. 709001; Cayman Chemical, Ann Arbor, MI).

The principle of the assay is the ability of aqueous and lipid antioxidants in the seminal plasma specimens to inhibit the oxidation of 2,2'-azino-di-[3-ethylbenzthiazoline sulpho-nate] (ABTS) to ABTS^+ . Under the reaction conditions used, the antioxidants in the seminal plasma cause suppression of the absorbance at 750 nm proportional to their concentration. The capacity of the antioxidants present in the sample to prevent ABTS oxidation was compared with that of standard Trolox, a water-soluble tocopherol analogue. Results were reported as μmoles of Trolox equivalent.

ROS-TAC Score

The ROS-TAC score was calculated as described in our earlier study (29), although ROS in the present study was measured on a different scale, requiring the use of updated values for the mean and SD of ROS in the principal component standardization. The updated equation for standardized ROS is as follows:

$$\text{Standardized ROS} = [\log(\text{ROS} + 0.001) - (-2.0238)]/0.5151$$

For TAC, we used the earlier standardization:

$$\text{Standardized TAC} = (\text{TAC} - 1650.93)/532.22$$

With ROS and TAC negatively correlated, as in the earlier analysis, the original linear combination derived by the first principal component of standardized variables is once again the first principal component, even with original ROS measurements on a different scale. This first principal component, which accounts for the most variability among correlated variables, is as follows:

$$\text{Principal component} = (-0.707 \times \text{standardized ROS}) + (0.707 \times \text{standardized TAC})$$

As in earlier analyses, transformation of the ROS-TAC scores was done to ensure that the distribution of ROS-TAC scores had a mean of 50 and SD of 10.

ROS-TAC score = 50 + (principal component \times 10.629).

DNA damage

Sperm DNA fragmentation was evaluated using the terminal deoxynucleotidyl transferase-mediated fluorescein-dUTP nick-end labeling (TUNEL) assay kit (Apo-Direct; BD Biosciences Pharmingen, San Diego, CA) as described previously (30–32). Briefly, 1×10^6 spermatozoa were washed in phosphate-buffered saline (PBS), resuspended in 1% paraformaldehyde, and placed on ice for 30–60 min. Subsequently, spermatozoa were washed again and resuspended in 70% ice-cold ethanol.

Following a second wash in PBS to remove the ethanol, sperm pellet samples as well as the positive and negative control samples provided with the assay kit were resuspended in 50 μ L of the staining solution for 60 min at 37°C. The staining solution contained terminal deoxytransferase (TdT) enzyme, TdT reaction buffer, fluorescein-tagged deoxyuridine triphosphate nucleotides (FITC-dUTP), and distilled water. All cells were further washed in rinse buffer, resuspended in 0.5 mL propidium iodide/RNase solution, and incubated for 30 min in the dark at room temperature followed by flow cytometric analysis. Results of the TUNEL test were expressed as percentage DNA fragmentation (%DFI).

Statistical Analysis

Comparison of all parameters between the exposed and unexposed groups was done by using the Wilcoxon rank sum test. Analyses were performed using R version 2.3.1; *P* values of $< .05$ were considered to be significant. Statistical analysis was also performed separately in patient samples and donor samples. Summaries of analysis included mean and SD. Results of ROS values included median (25th and 75th percentiles), because SD was larger than the mean of ROS values.

RESULTS

Sperm Parameters

No significant difference was seen in sperm concentration between exposed and unexposed samples (58.87 ± 34.34 million/mL vs. 58.84 ± 35.20 million/mL).

Sperm motility was significantly lower in exposed samples compared with unexposed samples. Mean motility for exposed and unexposed samples was $48.62 \pm 17.36\%$ and $52.11 \pm 18.34\%$, respectively ($P=.003$). A significant difference was observed within donors ($P=.01$) but not in patient samples.

Sperm viability was significantly lower in exposed samples than in unexposed samples ($P<.001$). Mean viability for exposed and unexposed samples was $52.33 \pm 13.21\%$

and $58.97 \pm 14.81\%$, respectively. A significant difference was observed in donor samples ($P<.001$) but not in patient samples (Table 1).

Reactive Oxygen Species (ROS)

The ROS levels were significantly higher in exposed samples than in unexposed samples in all three groups. (overall: $P=.002$; donors: $P=.04$; patients: $P=.014$) (Table 1). Log (ROS + 0.001) values were significantly higher in the exposed group (overall: $P=.001$; donors: $P=.017$) and in patients ($P=.014$) (Table 1). The increase in both ROS value ($\times 10^6$ cpm/ 20×10^6 sperm) and log (ROS + 0.001) was significantly higher in infertile patients compared with the increase in these values in donors (Table 2). These values were counted by deducting the mean \pm SD value of exposed samples from the mean \pm SD value of unexposed samples (of patients and donor samples) (Table 1).

Total Antioxidant Capacity (TAC) and ROS-TAC Score

No significant difference was observed in TAC between exposed and unexposed samples. Overall, a significant decrease in ROS-TAC score was seen in exposed versus unexposed samples ($P=.032$) (Table 1). Exposed samples had a score of 46.29 ± 11.20 compared with 51.54 ± 13.37 for unexposed samples. However, the difference between ROS-TAC scores was not significant when comparing exposed and unexposed samples from donors and patients.

DNA Integrity

No significant differences in DNA integrity (%DFI) were seen between the exposed and unexposed groups ($7.80 \pm 6.62\%$ vs. $8.44 \pm 5.77\%$) (Table 1).

DISCUSSION

In the present study, we analyzed the cause-and-effect relationship between cell phone radiation (in talk mode) and decreases in semen parameters. Our results showed a significant increase in ROS production in exposed samples and a decrease in sperm motility, viability, and ROS-TAC score in exposed samples. No significant difference in DNA integrity and TAC levels between exposed and unexposed samples was found.

The most remarkable finding of the present study was an increase in ROS levels in RF-EMW-exposed semen samples. A plausible explanation for the ROS production is that it is due to stimulation of the spermatozoa's plasma membrane redox system by RF-EMW or the effect of EMW on leukocytes present in the neat semen.

Recently, Friedman et al. (33) showed that RF-EMW stimulate plasma membrane NADH oxidase in mammalian cells and cause production of ROS. This may be attributed to an increase in the activity of spermatozoal NADH oxidase after RF-EMW exposure. Aitken et al. (34–36) demonstrated that human spermatozoa possess a multiple plasma membrane redox system that shares similarities with transmembrane NADH oxidase. Activation of plasma membrane NADH

TABLE 1

Comparison of ROS, TAC, ROS-TAC score, sperm parameters, and DFI between exposed and unexposed samples from various groups.

Group	ROS (x10 ⁶ cpm/ 20 million sperm)		Log (ROS + 0.001)		TAC (mMoles/ Trolox equivalent)	
	Exp	NE	Exp	NE	Exp	NE
Overall	0.11 ± 0.21 0.013 (0.047, 0.1258)	0.06 ± 0.11 0.0075 (0.0017, 0.0387)	-1.72 ± 0.86	-1.97 ± 0.85	1.55 ± 0.38	1.68 ± 0.48
P value		.002		.001		.24
n	32		32		24	
Donors	0.06 ± 0.12 0.01 (0.0035, 0.022)	0.05 ± 0.10 0.007 (0.002, 0.0305)	-1.85 ± 0.78	-1.94 ± 0.80	1.53 ± 0.38	1.72 ± 0.52
P value		0.048		0.017		.08
n	23		23		16	
Patients	0.22 ± 0.33 0.02 (0.012, 0.293)	0.07 ± 0.15 0.008 (0, 0.062)	-1.37 ± 1.00	-2.03 ± 1.03	1.59 ± 0.41	1.52 ± 0.41
P value		0.14		.014		.74
n	9		9		8	

Note: ROS values are expressed as mean ± SD; median (25th, 75th percentiles). DFI = DNA fragmentation index; Exp = exposed; NE = non exposed; RF-EMW = radiofrequency electromagnetic waves; ROS = reactive oxygen species; TAC = total antioxidant capacity; TUNEL = terminal deoxynucleotidyl transferase-mediated fluorescence-dUTP nick-end labelling assay; P<0.05 was considered significant.

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oxidase may cause production of ROS (33). This can be detected by luminol-based chemiluminescence because luminol measures both intra- and extracellular ROS (27, 37).

Development of oxidative stress or disturbance in free radical metabolism by cell phone radiation has been demonstrated in a few animal studies. Chronic exposure to RF-EMW can decrease the activity of catalase, superoxidase dismutase (SOD), and glutathione peroxidase, and thus decrease total antioxidant capacity, but experimental studies designed to measure malonaldehyde level and SOD activity show conflicting results (7, 8, 13, 16–18, 38, 39).

Reactive oxygen species are produced continuously by spermatozoa, and they are neutralized by antioxidants present in the semen (29, 40). However, when ROS production exceeds the capacity of antioxidants, a state of oxidative stress is created. Previously, we demonstrated that ROS-TAC score is a more accurate measure of oxidative stress than ROS or TAC alone (29). The decrease in ROS-TAC seen in the present study suggests an increase in oxidative stress due to cell phone exposure. A decrease in sperm motility and viability is linked to concentration of superoxide anion in semen. When superoxide is produced extracellularly, it can oxidize membrane phospholipids and cause a decrease in viability (41). Short-term in vitro exposure to RF-EMW should not cause a decline in sperm concentration; however, chronic oxidative stress (in vivo examples: smoking, varicocele) may lead to a decrease in sperm count (40, 42).

Due to methodologic variations, interpretations of studies regarding DNA damage are complicated. Aitken et al. (15) demonstrated that exposure of mice to RF-EMW, 900 MHz, 12 h/day for 7 days led to damage to the mitochondrial genome and nuclear beta-globin locus of epididymal spermatozoa. In contrast, Stronati et al. (43) demonstrated no significant DNA damage in human lymphocytes exposed to RF-EMW at SAR of 1 and 2 W/kg for 24 h. Results of other studies are equally conflicting (44–52). Recent data suggest that RF-EMW may not have enough energy to cause DNA damage (46, 49, 51, 52). However, it may induce gene expression of proteins, including heat shock proteins (51, 53–55). In the present study, the sperm DNA integrity did not change in the EMW-exposed group compared with the unexposed control samples. The lack of any DNA damage may be explained by short-term exposure to cell phone radiation or the scavenging of free radicals by antioxidants in seminal plasma (29, 41, 56).

To assess the effect of EMW on sperm function, we used neat semen samples, which contain both mature and immature spermatozoa, unlike a recent study by Falzone et al. (23) who studied only mature sperm from the Percoll fraction. It has been suggested that free radical generating capacity may be higher in spermatozoa in the low-density region of the Percoll gradient (immature spermatozoa) compared with the capacity of sperm from the higher-density fraction (mature spermatozoa) (36). The present results show that the increase in seminal ROS values in donors and patients and the

TABLE 1

Continued.

Group	ROS-TAC score		Viability (%)		Motility (%)		TUNEL DFI (%)	
	Exp	NE	Exp	NE	Exp	NE	Exp	NE
Overall	46.29 ± 11.20	51.54 ± 13.37	52.33 ± 13.21	58.97 ± 14.81	48.62 ± 17.36	52.11 ± 18.34	7.80 ± 6.62	8.44 ± 5.77
<i>P</i> value	.032		< .01		.003		.62	
<i>n</i>	23		32		30		20	
Donors	48.63 ± 11.53	51.71 ± 13.75	53.52 ± 13.05	61.00 ± 13.71	50.60 ± 17.49	54.80 ± 17.61	8.21 ± 7.24	8.66 ± 6.45
<i>P</i> value	.14		< .01		.01		.78	
<i>n</i>	15		23		23		16	
Patients	41.91 ± 9.74	51.23 ± 13.54	48.43 ± 13.99	52.29 ± 17.41	43.56 ± 16.94	45.25 ± 19.42	6.16 ± 3.38	7.56 ± 1.24
<i>P</i> value	.15		.14		.36		.88	
<i>n</i>	8		9		7		4	

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increase in ROS levels in exposed samples from patients were significantly higher than the increase in ROS levels in donor samples. We therefore propose that immature and abnormal spermatozoa may be more susceptible to cell phone radiation. This may be explained by the fact that these patients already present with poor quality sperm in terms of both poor motility and abnormal morphology and presence of leukocytes. Poor sperm quality has been shown to generate higher levels of ROS. Therefore, excessive exposure to cell phone-emitted RF-EMW would be more likely to further deteriorate the sperm quality, even after this short exposure, in both mature as well as immature sperm to a larger extent, thereby increasing the likelihood of these patients being infertile.

This is a pilot study, and we acknowledge its limitations. One of them was that we did not measure seminal leukocyte counts. Semen volume also was a limiting factor in the number of samples that were available for measuring sperm parameters, ROS, TAC, and DNA damage.

The possibility that the higher ROS production in neat semen of the exposed group is due to the specific effects of

RF-EMW on leukocytes is a concern. Studies on immune-relevant cell lines regarding the effect of RF-EMW on free radical formation are equally conflicting. Various researchers have shown that RF-EMW has no effect on free radical release from immune-relevant cells (57–60). Many earlier studies have shown that a 50-Hz magnetic field at 1 mT induces free radical formation in phagocytes or monocytes (61, 62). In the present pilot study, we did not measure the magnetic field emitted by the cell phone battery.

The duration of RF-EMW exposure and experimental temperature during this pilot study were selected according to guidelines of EMW exposure in an *in vitro* experiment. Talk time on a cell phone differs from individual to individual, so deciding the duration for the experimental condition was a complicated matter. Recent *in vitro* studies on human sperm and human endothelial cell lines have used 1 h of *in vitro* exposure (55). A decline in ROS levels in semen with time at 37°C has been demonstrated (27). In a study by Esfandiari et al. (63), ROS levels were significantly higher in semen samples stored at a lower temperature (25°C vs. 37°C). According to the available guidelines, sensitivity

TABLE 2

Comparison of increase in ROS value between donor and patient groups.

	Donors (n = 23)	Patients (n = 9)	<i>P</i> value
Increase in ROS value (×10 ⁶ cpm/20 million sperm)	0.01 ± 0.03	0.15 ± 0.24	.022
Increase in log (ROS + 0.001) value	0.09 ± -0.21	0.66 ± 0.90	.019

Note: Increase in ROS value [or log (ROS + 0.001)] = mean ± SD value of exposed minus mean ± SD value of unexposed samples of patients as well as of donors. ROS = reactive oxygen species.

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of the experiment should be at the highest level to maximize the possibility of detecting any significant effect(s) of RF-EMW. To maximize the likelihood of observing the deleterious effects in the present pilot study, we chose an exposure time of 60 min at room temperature (1, 21, 64). The distance of 2.5 cm was selected to mimic the close proximity of the testis to a cell phone in a trouser pocket (on talk mode), e.g., while the man is talking on Bluetooth. Although we monitored the room temperature, we did not measure the temperature of semen samples after exposure; recent studies have shown that RF-EMW has no thermal effects at SAR <2 W/kg RF (19, 65, 66).

In conclusion, in this pilot study we have demonstrated that cell phone radiation causes oxidative stress in neat semen and leads to decreases in spermatozoa motility and viability. The fact that many men carry their cell phones in a trouser pocket (or clipped to their belts at the waist) while using Bluetooth is important. This technology exposes the testes to high-power-density cell phone radiation compared with the cell phone in standby mode. Based on our *in vitro* results, we can speculate that carrying a cell phone in a pocket may cause deterioration of sperm quality through oxidative stress. However, the phone and the male reproductive organs are separated by multiple tissue layers, so to extrapolate the effects seen under *in vitro* conditions to real-life conditions requires further studies, which currently are under way in our laboratory.

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