ANDROLOGY ORIGINAL ARTICLE

Use of laptop computers connected to internet through Wi-Fi decreases human sperm motility and increases sperm DNA fragmentation

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Objective: To evaluate the effects of laptop computers connected to local area networks wirelessly (Wi-Fi) on human spermatozoa.
Design: Prospective in vitro study.
Setting: Center for reproductive medicine.
Patient(s): Semen samples from 29 healthy donors.
Intervention(s): Motile sperm were selected by swim up. Each sperm suspension was divided into two aliquots. One sperm aliquot (experimental) from each patient was exposed to an internet-connected laptop by Wi-Fi for 4 hours, whereas the second aliquot (unexposed) was used as control, incubated under identical conditions without being exposed to the laptop.
Main Outcome Measure(s): Evaluation of sperm motility, viability, and DNA fragmentation.
Result(s): Donor sperm samples, mostly normozoospermic, exposed ex vivo during 4 hours to a wireless internet-connected laptop showed a significant decrease in progressive sperm motility and an increase in sperm DNA fragmentation. Levels of dead sperm showed no significant differences between the two groups.
Conclusion(s): To our knowledge, this is the first study to evaluate the direct impact of laptop use on human spermatozoa. Ex vivo exposure of human spermatozoa to a wireless internet-connected laptop decreased motility and induced DNA fragmentation by a nonthermal effect. We speculate that keeping a laptop connected wirelessly to the internet on the lap near the testes may result in decreased male fertility. Further in vitro and in vivo studies are needed to prove this contention. (Fertil Steril® 2012;■:■–■. ©2012 by American Society for Reproductive Medicine.)
Key Words: Laptop computer, Wi-Fi, sperm quality, fertility, sperm DNA fragmentation

In recent years, the use of portable computers (laptops, connected to local area networks wirelessly, also known as Wi-Fi) has increased dramatically. Laptops have become indispensable devices in our daily life, offering flexibility and mobility to users. People using Wi-Fi may be exposed to radio signals absorbing some of the transmitted energy in their bodies. Portable computers are commonly used on the lap (1–3), therefore exposing the genital area to radio frequency electromagnetic waves (RF-EMW) as well as high temperatures (3, 4).

Infertility is a common worldwide condition that affects more than 70 million couples of reproductive age (5). It has been suggested that male fertility has declined during the past several decades (6). Such decline has been attributed to the direct or indirect exposure to certain environmental factors such as RF-EMW (7).

Extremely low frequency magnetic fields can initiate a number of biochemical and physiological alterations in biological systems of different species (8–12). Many of these effects have been associated with free-radical production (13, 14). Free radicals are causative factors of oxidative damage of cellular structures and molecules such as lipids, proteins, and nucleic acids. Free radicals react with polyunsaturated fatty acids in cell membranes promoting a process called lipid peroxidation. In human spermatozoa the presence of unesterified polyunsaturated fatty acids is causally associated with the induction of reactive oxygen species (ROS) generation and lipid peroxidation (15). Damage may occur at the membrane level, leading to immotility and cell death, or at the DNA level. DNA integrity is essential to normal conception. Sperm DNA fragmentation has been associated with impaired fertilization, poor embryonic development, high rates of miscarriage, and increased incidence of morbidity in the offspring, including childhood cancer (16, 17). It has been proposed that genetic and

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environmental factors would be involved in the etiology of sperm DNA damage (18).

The RF-EMW from mobile phones may cause DNA damage (19), in addition to decreased motility and viability (20, 21). Increased levels of intracellular ROS (22) would be the cause of these deleterious effects.

Portable computers using Wi-Fi emit RF-EMW and are typically positioned close the male reproductive organs. Their potential negative effects on male germ cells have not been elucidated. To assess this potential association we used an in vitro model incubating human sperm in the presence of an active portable computer connected to the internet by Wi-Fi. Sperm viability, motility, and DNA fragmentation were the main study end points.

MATERIALS AND METHODS

Subjects

Use of these samples for research purposes was approved by the Institutional Ethics Committee of Nascentis Medicina Reproductiva, Córdoba, Argentina, and all participants gave written informed consent. Twenty-nine semen samples were collected by masturbation from healthy donors after 2–5 days of sexual abstinence. After liquefaction, sperm concentration and motility were determined by light microscopy, using a Makler chamber (Mid Atlantic Diagnostics Inc.). Sperm morphology was examined at ×1,000 oil immersion microscopy by strict criteria after staining with the Papanicolau method as previously described (23). Preparation and assessment were performed by a single experienced operator. Semen samples with more than 0.5 million/mL of peroxidase-positive leukocytes were discarded and not used in the study.

Motile spermatozoa were selected by swim up performed in modified human tubal fluid (HTF; Irvine Scientific) supplemented with 10% synthetic serum substitute (SSS; Irvine). Briefly, each sperm sample was diluted 1:1 with modified HTF and then centrifuged at 300 × *g* for 10 minutes. The supernatant was discarded and the pellet was gently layered with 1 mL of modified HTF/SSS and incubated at 37°C, at a 45° angle, for 1 hour. After the incubation period, the top 0.5 mL of the supernatant, which is enriched in the motile sperm, was withdrawn carefully and sperm concentration and motility were determined. The sperm concentration was adjusted to 10–20 million/mL with modified HTF/SSS.

Each sperm suspension sample was aliquoted in two fractions (A and B) and a drop of 400 μL was placed in 35 × 10 mm Petri dishes (Falcon 3001). This was covered with 3 mL of embryo oil (Irvine) to avoid evaporation. Fractions B were incubated under a laptop computer. Fractions A (control group) were incubated under similar conditions without the computer.

Incubation of Spermatozoa Under Laptop

For each sperm sample, one of the dishes (fraction B) was incubated at room temperature under a laptop computer (Toshiba Satellite M305D-S4829) connected to the internet wirelessly (Wi-Fi, frequency 2.4 GHz defined by IEEE 802.11b). To induce the greatest possible effect, the laptop worked actively (uploading and downloading information) throughout the period of exposure (24). To maximize the likelihood of observing deleterious effects the distance between the computer and each specimen was kept constant at 3 cm. This distance was the estimated distance between the computers resting on the lap and the testis/epididymis (Fig. 1B and C). The duration of exposure was 4 hours (Fig. 1A). The temperature under the laptop was kept at 25°C during the incubation time by an air conditioning system. The temperature on each medium drop was thoroughly controlled by an IVF Thermometer (Research Instruments) and recorded every 5 minutes. Unexposed aliquots (fraction A) were used as control and kept under identical temperature and conditions in another room away from any computers or electronic devices. After the incubation period, sperm vitality, motility, and DNA fragmentation were determined on each aliquot.

Vitality and Motility

Sperm vitality was evaluated by eosin stain according to specifications of the World Health Organization (25). Sperm motility was assessed microscopically using a Makler chamber (Mid Atlantic Diagnostics Inc.), and sperm movement was classified as progressive motility, nonprogressive motility, and immotility.

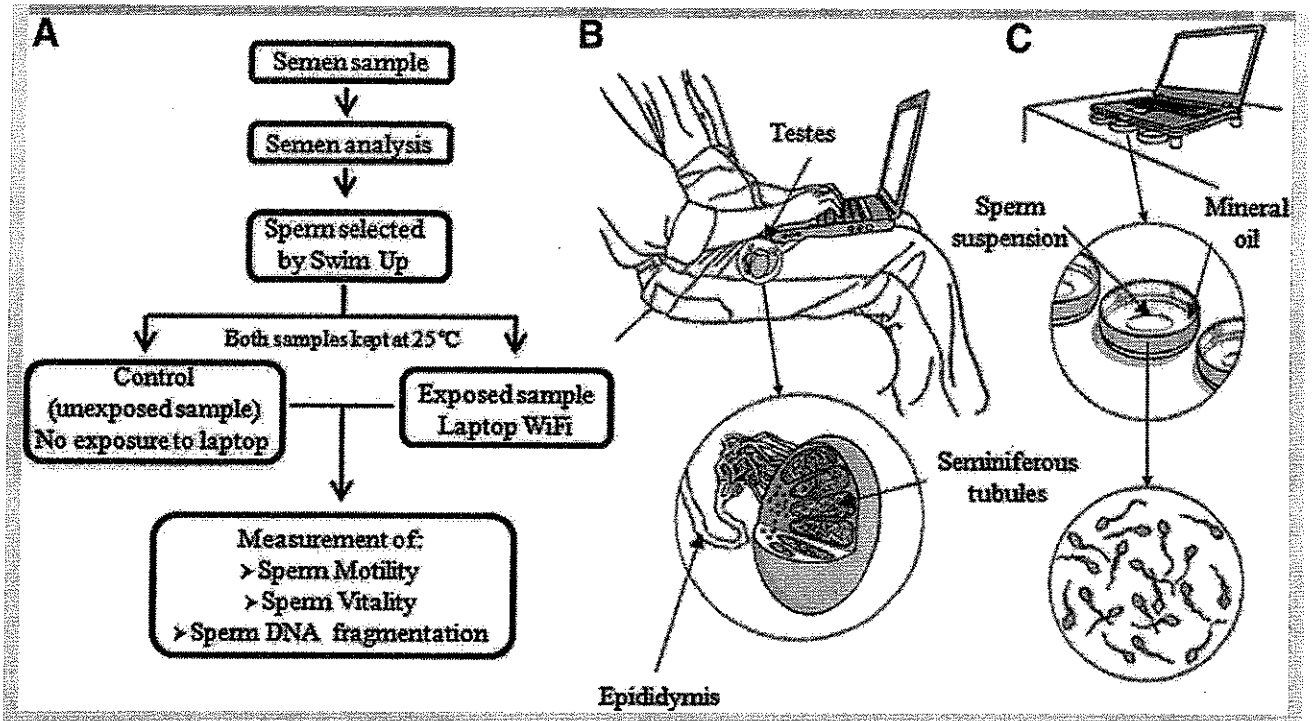
TUNEL Assay

Sperm DNA fragmentation was evaluated with TUNEL assay using the in situ cell death detection kit, fluorescein (Roche Diagnostics GmbH). The assay uses fluorescein-dUTP to label single and double DNA strand breaks according to manufacturer's instructions and was performed as previously described (26). Briefly, spermatozoa were fixed with paraformaldehyde (final concentration 2%, permeabilized with 0.1% Triton X-100) and incubated in the dark at 37°C for 1 hour in TUNEL reaction mixture containing 0.5 IU/μL of calf thymus terminal deoxynucleotidyl transferase and fluorescein-dUTP. Negative (omitting the enzyme terminal transferase) and positive (using deoxyribonuclease I, 1 U/mL for 20 minutes at room temperature) controls were included in each experiment. Mounting medium for fluorescence (Vectashield, Vector Laboratories) was added before the evaluation to prevent fluorescence quenching. A total of 500 cells were randomly analyzed per sample in a Zeiss Axioplan (Carl Zeiss MicroImaging) microscope with a ×1,000 oil immersion objective. Each sperm cell was classified as having intact DNA (no fluorescence) or fragmented DNA (green nuclear fluorescence). As expected, none of the cells showed fluorescent staining in the negative control, whereas 100% of the cells showed fragmentation in the positive control (treated with DNase). The results were expressed as percentage of sperm with fragmented DNA.

Power Density

A RF Field Strength Meter (Alphalab) was used to measure radiation under the experimental conditions. The RF Field Strength Meter detects the electric field of radio and microwaves from 0.5 MHz–3 GHz, and expresses the field strength

FIGURE 1



Study design and set-up for the exposure of human sperm to laptop. (A) Experimental design. (B) Schematic situation of the use of the laptop on the lap near the testes. (C) Diagram of the in vitro study. The Petri dishes were placed at 3 cm from a laptop computer. Each Petri dish contained a drop of 400 μL of sperm suspension in human tubal fluid/synthetic serum substitute (HTF/SSS) covered with mineral oil to prevent evaporation.

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as power density (0.001–2,000 $\mu W/cm^2$). The RF Field Strength Meter is directional and detects only the component of the electric field that has the same polarization as the long axis of the meter. To find the highest reading, the meter was located at the same distance as the Petri dishes and positioned vertically and horizontally, according to the manufacturer's specifications. The horizontal way showed higher reading and was recorded. Power density was monitored at the same distance of the Petri dishes, during basal condition (no exposure to laptop), laptop without Wi-Fi connection and laptop working in Wi-Fi mode throughout the experiment.

Statistical Analysis

Data were expressed as mean ± SD. The Mann-Whitney test was used to identify differences between two groups. $P<.05$ was considered statistically significant.

RESULTS

Donors' mean age was 34.1 ± 5.6 years (range 26–45 years). Semen parameters (volume, concentration, motility, vitality, and morphology) are presented in Supplemental Table 1 (available online). Many samples showed normozoospermia, whereas four samples showed low semen volume (LS6, LS13, LS27, and LS29) and three (LS15, LS16, and LS25) presented isolated teratozoospermia, according to World Health Organization reference values (25, 27).

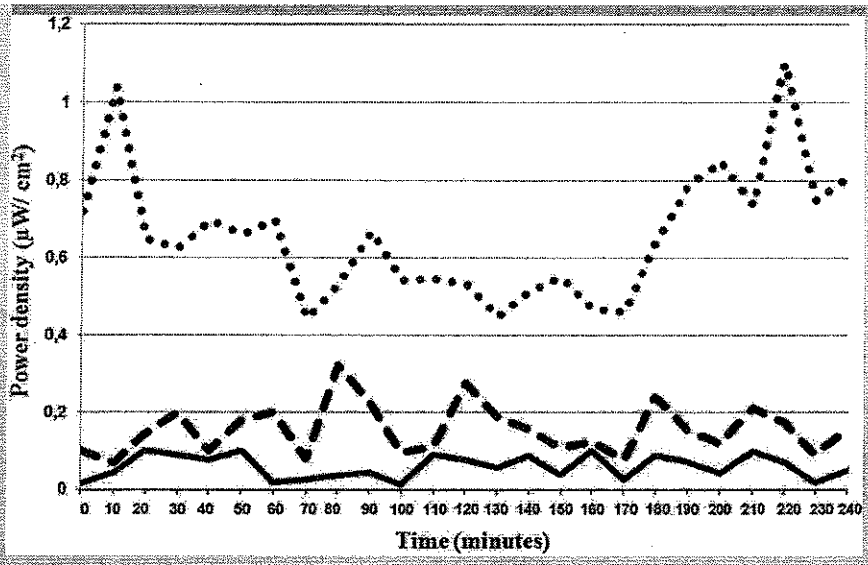
Room and under-the-laptop temperatures were monitored during the incubation time and kept at 25°C for both sperm fractions (A and B) by an air conditioning system (Supplemental Fig. 1, available online). The RF-EMW were recorded every 10 minutes in both groups throughout the experiment. The RF-EMW from a laptop working without Wi-Fi connection were checked in a pilot experiment (Fig. 2). The radiation from the computer operating with Wi-Fi was three or more times higher than without Wi-Fi and 7–15 times higher than basal conditions (not exposed to laptop).

Sperm parameters were evaluated after 4 hours of incubation of motile sperm selected by swim up and exposed to an active laptop computer under controlled temperature conditions. There were no differences in the percentage of viable sperm between the test and control groups (Fig. 3A). On the contrary, laptop exposure induced a significant decrease in sperm progressive motility with a concomitant increase in nonmotile sperm compared with the unexposed controls ($P<.05$). The percentage of nonprogressive sperm did not show statistically significant differences (Fig. 3B). Important, a significant increase in sperm DNA fragmentation was found in the fraction incubated under the computer compared with the control group (3.3 ± 6.0 vs. 8.3 ± 6.6; $P<.05$; Fig. 4A and B).

DISCUSSION

To our knowledge, this is the first study to examine the effect of portable computers on human spermatozoa in vitro. In the

FIGURE 2



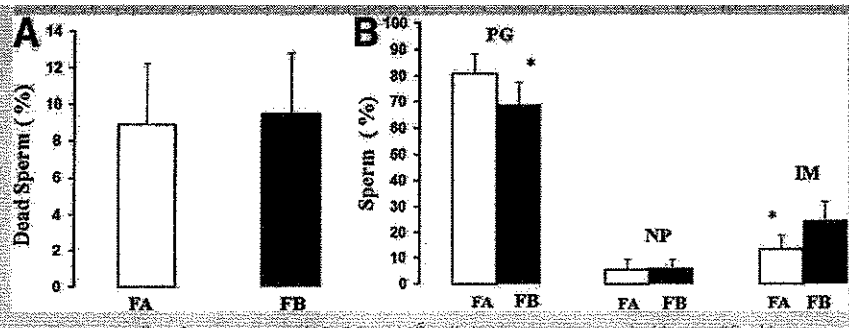
Variation of electromagnetic radiation (in microwatts per centimeter squared) during incubation time under the following experimental conditions: ●●● laptop connected to Wi- Fi, - - - laptop without connection to Wi-Fi, ——— no laptop (basal conditions). The radio frequency electromagnetic waves were 7–15 times higher under the laptop than in basal conditions. They were significantly decreased when the Wi-Fi was turned off compared with the laptop working with Wi-Fi.

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present study we demonstrate that laptop computers connected wirelessly to the internet decrease sperm quality by a nonthermal effect. We evaluated vitality, motility, and DNA fragmentation in sperm selected by swim up after incubation under a laptop connected to the internet by Wi-Fi. The results demonstrate a significant decrease in sperm progressive motility and a significantly higher proportion of sperm with DNA fragmentation when samples were incubated for 4 hours under the laptop. These differences were seen in comparison with aliquots of the same semen samples incubated under similar conditions but outside the proximity of any computer or electronic device.

Several lifestyle and environmental factors may adversely impact human health and, in particular, reproductive performance (18). Approximately 15% of the sexually active population is affected by clinical infertility and in 50% of the cases a male factor is involved, either as a primary problem or in combination with a problem in the female partner (28). In this regard it has been proposed that the increased use of certain new technologies may decrease fertility

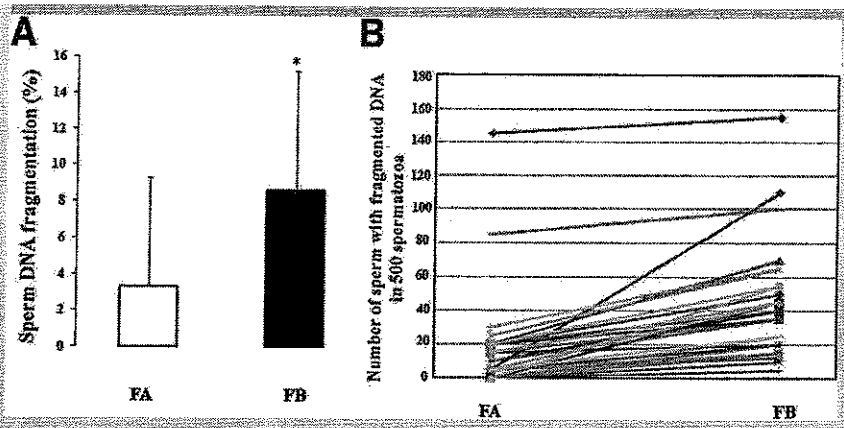
FIGURE 3



Laptop exposure and human sperm quality. Spermatozoa (10–20 × 10^6 cells/mL) were suspended in modified human tubal fluid/synthetic serum substitute (HTF/SSS) medium and incubated under a laptop computer connected to internet to Wi-Fi (FB). Another sperm aliquot was placed outside the reach of other computers or electronic devices (FA). Both groups were incubated for 4 hours at 25°C. (A) Percentage of dead sperm were not significantly different between the laptop exposed and unexposed groups and the unexposed cells (9.5% ± 3.3% vs. 8.9% ± 3.3%, $P>.05$). (B) Progressive sperm motility (PG) was significantly reduced in the group incubated under the laptop compared with that of control group (68.7% ± 8.8% to 80.9% ± 7.5%, $^*P<.01$). No difference was found in the percentage of nonprogressive (NP) spermatozoa between groups. Immotile sperm (IM) were significantly increased after laptop exposure (24.5% ± 7.6% vs. 13.6% ± 5.6%, $^*P<.01$).

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FIGURE 4



Laptop exposure and human sperm DNA fragmentation. Sperm suspensions were incubated under a laptop computer connected to the internet by Wi-Fi (FB) during 4 hours at 25°C. Aliquots of the same samples were placed outside of the reach of other computers or electronic devices, in a separate room (FA). (A) Sperm DNA fragmentation was increased after 4 hours of laptop exposure. In the test group, 8.6% ± 6.6% of the cells were fragmented, whereas only 3.3% ± 6.0% of the controls showed DNA fragmentation (*$P<.01$). (B) Plot of individual responses of sperm DNA fragmentation to laptop exposure. The number of sperm with fragmented DNA was evaluated in two aliquots of the same sample (500 cells/aliquot).

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potential by increasing long-term exposure to nonionizing radiation (18).

Laptop computer usage has increased significantly in recent years, especially in people of reproductive age. Frequently laptops are connected to the internet through Wi-Fi and commonly placed on the lap near the testes. Portable computers actively generate high temperatures that can increase the scrotal temperature and may produce deleterious effects on spermatogenesis (3, 29). In addition, laptop computers working by Wi-Fi are connected through RF-EMW (4, 24), which may damage spermatozoa in the male reproductive organs through microwave radiation.

To set up this study we first evaluated the radiation emitted from a laptop. The radiation varied during the test and depended on the flow of information between the computer and the network to which it was connected (Fig. 2).

Overall, however, the RF-EMW were 7–15 times higher under the laptop than under basal conditions (no laptop). Compared with the laptop working with Wi-Fi, RF-EMW were significantly decreased when the Wi-Fi was turned off.

It is well known that increased temperature may decrease sperm quality (30) and the use of portable computers on the lap increases scrotal temperature (3). Therefore to prevent confounding thermal effects, room and incubation temperatures were kept constant in both the unexposed and the under-the-laptop groups (Fig. 1) during the incubation time.

The first relevant finding of this study was a significant decrease in sperm progressive motility after exposure to the laptop. A plausible explanation for the impaired sperm motility could be magnetic and electromagnetic fields inducing oxidation of phospholipids, which are a major component in the sperm mitochondrial sheath (31). Several studies have shown that higher ROS values have detrimental effects on the motility of normal human spermatozoa (15, 32). Furthermore, it has been reported that infertile men with high seminal ROS levels have a lower percentage of motile sperm (27). This can be explained by a disturbance of the mitochondrial membrane potential, which causes high levels of ROS to be released into the cytoplasm, depleting the energy supply and affecting both sperm motility and kinetics (33, 34). Interestingly, in our study, sperm vitality was not different between the two experimental groups (Fig. 3).

Concerning spermatozoa, RF-EMW generated by mobile phones cause a decrease in their progressive movement, in both human and rat cells (21, 22, 35). In vitro human spermatozoa exposed to mobile phone radiation showed reduced sperm head area and decreased sperm binding to the zona pellucida (ZP) without an increase in acrosomal reaction compared with controls (36). High levels of sperm ROS (22, 37), as well as an increased percentage of sperm DNA fragmentation (37), have been reported after mobile phone exposure. However, other studies did not find any changes in DNA integrity or the induction of proapoptotic markers (22, 38). The lack of DNA damage observed in these studies might be explained by the shorter time of exposure to cell phone radiation or by the antioxidant effect of seminal plasma. The RF-EMW generated by mobile phones are similar to those generated by laptop computer and other mobile devices connected wirelessly to the internet (4).

In this regard we found that ex vivo exposure of human spermatozoa for 4 hours in the absence of seminal plasma induces DNA damage by a nonthermal effect (Fig. 4). This effect is similar to that observed by De Iuliis and co-workers (37) with sperm exposed to mobile phones in vitro. These investigators observed highly significant relationships between RF-EMW emitted by mobile phones, covering a range of specific absorption rates, the oxidative DNA damage marker, 8-hydroxy-2′-deoxyguanosine, and sperm DNA fragmentation. These changes were unrelated to thermal effects.

Research has shown negative consequences of electromagnetic fields on biological mechanisms. Genotoxic damage by 1.8 GHz in human fibroblast has been proposed as a direct

consequence of intermittent exposure to RF-EMW (39). After exposure to 2.45 GHz, alteration of gene expression was found in cultured human cells mediated by a nonthermal mechanism (40). Electromagnetic radiation from mobile phones induces activation of the extracellular signal-regulated kinases (ERK)-cascade thereby altering transcription and other key cellular processes (8). Chronic exposure to low intensity microwaves (2.45 and 16.5 GHz) causes statistically significant increase in DNA strand breaks in rat brain cells (41). A recent work showed that the exposure of oviducts to extremely low frequency electromagnetic field negatively affects early embryo development, causing a slowdown in the embryo cleavage rate (42).

In an in vivo study, it was demonstrated that acute exposure to radiofrequency fields of cellular phones may modulate the oxidative stress of free radicals by enhancing lipid peroxidation, and a decrease in the activity of the antioxidants, superoxide dismutase, and glutathione peroxidase, in erythrocytes from human volunteers (43). In addition, genotoxic effect on epididymal spermatozoa has been reported when mice were irradiated for 7 days at 12 hours per day (19).

As opposed to somatic cells, the spermatozoon is a highly specialized cell with a condensed DNA (packaged by protamines) and very small cytoplasmic area (44). During spermatogenesis, human spermatozoa may take up to 1 week to move from the seminiferous tubules in the testes to the cauda epididymis (45) and throughout this time they would be highly vulnerable to RF-EMW exposure (19, 37), especially from a source close to the testes and epididymis such as a Wi-Fi laptop computer.

Our data suggest that the use of a laptop computer wirelessly connected to the internet and positioned near the male reproductive organs may decrease human sperm quality. At present we do not know whether this effect is induced by all laptop computers connected by Wi-Fi to the internet or what use conditions heighten this effect. The mechanisms involved in mediating the decrease in sperm motility and DNA integrity also need further study. We speculate that RF-EMW from laptop computers wirelessly connected to the internet may be the cause of sperm damage. However, we cannot discard the possibility that damage to sperm is caused by the low radiation produced by the computer without internet connection. With the caveat that these data were obtained with sperm samples incubated in vitro, our findings suggest that prolonged use of portable computers sitting on the lap of a male user may decrease sperm fertility potential. The potential implications of these findings warrant this report and further basic and clinical investigation.

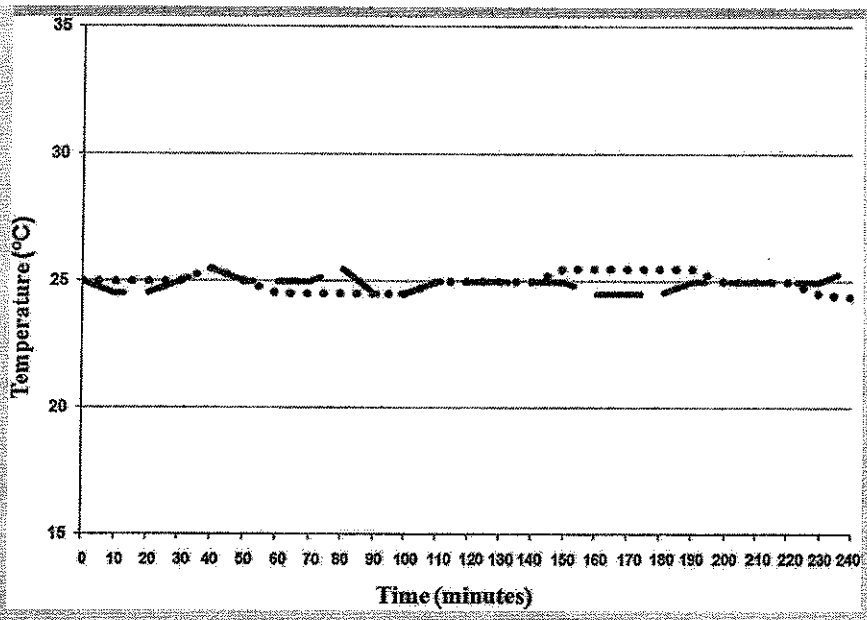
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SUPPLEMENTAL FIGURE 1



Incubation temperature in test (under the laptop; - - -) and control (unexposed; ● ● ● ●) groups. Room temperature was kept constant during incubation (4 hours) both in the control and the experimental groups.

Avendaño. Laptop usage and sperm quality. Fertil Steril 2012.

SUPPLEMENTAL TABLE 1

Basic semen parameters of study samples.

Semen parameter	Mean
Volume (mL)	2.8 ± 1.6[a]
Concentration (× 10^6 / mL)	111.0 ± 49.8
Vitality (%)	85.3 ± 4.6
Progressive motility (%)	60.2 ± 9.3
Morphology (%)	8.2 ± 4.7

[a] Mean ± SD.

Avendaño. Laptop usage and sperm quality. Fertil Steril 2012.