Effects of cellular phone emissions on sperm motility in rats

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Objective: To evaluate the effects of cellular phone emissions on rat sperm cells.
Design: Classic experimental.
Setting: Animal research laboratory.
Subjects: Sixteen 3-month-old male Sprague-Dawley rats, weighing 250–300 g.
Intervention(s): Rats in the experimental group were exposed to two 3-hour periods of daily cellular phone emissions for 18 weeks; sperm samples were then collected for evaluation.
Main Outcome Measure(s): Evaluation of sperm motility, sperm cell morphology, total sperm cell number, and mRNA levels for two cell surface adhesion proteins.
Result(s): Rats exposed to 6 hours of daily cellular phone emissions for 18 weeks exhibited a significantly higher incidence of sperm cell death than control group rats through chi-squared analysis. In addition, abnormal clumping of sperm cells was present in rats exposed to cellular phone emissions and was not present in control group rats.
Conclusion(s): These results suggest that carrying cell phones near reproductive organs could negatively affect male fertility. (Fertil Steril® 2007;88:957–64. ©2007 by American Society for Reproductive Medicine.)

Key Words: Cellular phones, male fertility, sperm motility, electromagnetic field, radiation effects, radiofrequency exposure, radiofrequency electromagnetic radiation

Cellular phone usage is increasing worldwide at an astonishing rate. The manufacturer of Nokia mobile phones estimated that more than 2 billion people would now be cell phone subscribers, based on 2004 growth rate trends (1). With this increase in popularity, concerns have arisen regarding human safety related to radiation emissions from cellular phones. Large doses of this radiofrequency electromagnetic radiation (RFEMR) have been related in previous studies to genetic defects, such as changes in the integrity of epididymal mitochondrial DNA (2), altered proto-oncogene c-fos (3) and protein kinase C expression (4), increased micronuclei formations (5, 6), increased chromosomal instability (7, 8), and changes in morphology, gene expression, and proliferation of fibroblasts (9). Research also suggests that RFEMR is related to sperm parameter deterioration (10) and to an increase in the risk of cancers through the changes in chromosomal stability (7–9).

However, current research on the effects of cellular phones on the human body is contradictory and inconclusive. Several researchers believe the source of many of the abnormalities found in laboratory tests is the combination of RFEMR and heat (10–13). According to some investigators, the effects of cellular phones are minimal (14–17) to nonexistent (18) when the factor of heat is eliminated (14–17) or not extreme (18).

Determining more definitively the effects of cellular phone use on male fertility is important, considering that men often carry cellular phones in their pockets, close to reproductive organs. The purpose of this study is, therefore, to clarify whether cell phones negatively affect sperm fertility, through evaluation of rats following exposure to phone emissions.

MATERIALS AND METHODS

Subjects

Sixteen 3-month-old male Sprague-Dawley rats, weighing 250–300 g, were the subjects of this research. For the care and use of laboratory animals, this study used the guidelines of the Biomedical Resource Center of the Medical College of Wisconsin. The Institutional Animal Care and Use Committee of the Medical College of Wisconsin approved the protocol. The rats were divided into two groups of eight rats each. One group received cell phone radiation exposure, and the other group acted as a control group.

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Materials

The four cell phones used in the study were Nokia 3588i (Keilalahdentie, Finland), which have a personal communications service code division multiple access (PCS CDMA) frequency band of 1.9 GHz (800 MHz digital and 800 MHz analog). These cell phones have three different modes: AMPS mode, CELL mode, and PCS mode. The various modes can be used based on signal reception, antenna use, and other factors associated with reception of different types of radiofrequency signals.

In AMPS mode, the specific absorbence rate (SAR) at a distance of 2.2 cm was measured to be 1.80 W/kg, and the power range was 0.0063–0.607 W. The SAR at a distance of 2.2 cm in CELL mode was 0.9 W/kg, and the power range was 0.00001–0.487 W. The SAR at 2.2 cm away in PCS mode was 1.18 W/kg, and the power range was 0.00001–0.335 W. The frequencies and specific modes of this phone fall within the cell phone radiation parameters set by the U.S. Federal Communications Commission (FCC) (19). Each cell phone was positioned 1 cm from the head of the rats, at equal distances between two rats in holding chambers.

Holding Units

Customized holding units (Fig. 1) and cell phone platforms were constructed for this study. The holding units for the rats consisted of 5.1 cm × 15.2 cm PVC tubes with holes for circulation, fitted with 0.59-liter clear plastic bottle tops on one end and common 7.6-cm bolts with nuts at the other end. As the rats grew larger during this study, new tubes were fashioned using 8.9-cm-diameter PVC pipes with holes for air circulation, 1-liter bottles, and 10.2-cm bolts with nuts. The holding units were plastic, because metal can absorb radiation energy.

The rats were acclimated to these holding units for 1 week before the beginning of testing by placing the units in the rat cages to allow the rats to become familiar with their smell and feel. After less than one day, the rats voluntarily entered the units to rest and sleep in them. By the end of the week, the rats would enter the holding units as soon as they saw them. Owing to this acclimation process, anesthesia was not required during the exposure time. Therefore, the rats did not have any ill effects or altered physiology from anesthesia, rendering the comparison to humans more relevant.

Methods

The experimental rat group was exposed to 3 hours of cell phone radiation, followed by a 30-minute rest period outside of the tubes and a second exposure for 3 more hours per day. During the 30-minute rest period, the rats were removed from the tubes and were free to walk around, eat and drink. The rats received this daily cell phone exposure for 18 weeks. The 8 rats in the control group were placed in identical tubes for the same amount of time as the experimental rats but without cell phone exposure.

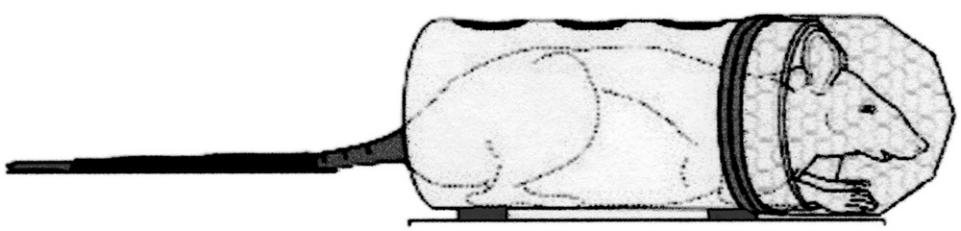
After week 18, the rats were killed for harvest of the tissues of interest. Incisions were made in the rat scrotums to dissect out the testicles and the epididymides. After transecting the proximal vas deferens, the sperm of each rat was allowed to passively flow into a Petri dish at 37°C in 2 mL HBSS for 10 minutes. Then 5-μL samples of sperm were moved from the dish into a microcell 50-μm chamber (Conception Technologies, San Diego, California) for evaluation of sperm motility and morphology. Total sperm counts and a molecular study were also completed.

Evaluation

To address the concern that the harmful effects of cell phones are due to heat given off by the phone rather than RFEMR, we took temperatures from both groups during a standard day of exposure. Temperatures of the rats were taken one day at the side of the face surface nearest the phone, using a Mini-Alarm thermometer with a probe (Fisher Scientific, Hampton, NH). These readings were taken approximately every 12 minutes during both of the 3-hour exposures. Final rectal temperatures were taken at the end of each of the two exposure times with a Big-Digit thermometer (Fisher Scientific). Because the rectal measurements irritated the animals, repeated measurements were not practical.

FIGURE 1

Rat in plastic PVC holding tube. The tube has a clear plastic bottle top and numerous air holes for adequate ventilation.



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Sperm Motility Analysis

The slides on which the sperm cells were counted were warmed to 37°C until the time of the analysis. The analysis was carried out at room temperature. An embryologist from the Reproductive Medicine Clinic performed a blind analysis of sperm motility using one epididymis of each rat. The embryologist was unaware of the purpose of the study when performing the counts.

The percentage of sperm motility was calculated using the number of live sperm cells over the total number of sperm cells (both motile and nonmotile), from two samples from one epididymis of each rat. All sperm cells that were not moving at all were considered to be nonmotile, while the rest, which displayed some movement, were considered to be motile.

Morphologic Analysis

Using the same epididymis from each rat, the morphology of the sperm cells was evaluated from two sperm samples per rat. One drop from the dish containing the sperm was placed on each slide, and immediately smeared and stained with Diff-Quik stain (Allegiance Scientific Products, McGaw Park, Illinois) to facilitate identification of morphologic differences between experimental and control groups.

The 32 slides (16 from the experimental group and 16 from the control group) were sent to the Department of Veterinary Pathobiology at the University of Missouri. There a professor specializing in deformities/mutations completed a blind assay of the slides.

All sperm cells were counted from each slide. The total number of cells per slide ranged from 70 to 128, with an average of 99 cells. Sperm cells were considered deformed if they were definitely headless, broken, or had bent tails and bodies which coiled back on themselves. Sperm cells that did not have hook-shaped heads and/or were not elongated were considered abnormal.

Sperm counts were done on a Nikon microscope (Nikon, Japan) at ×20 magnification using a 0.5-mm^2 counting area. Four locations on each slide were chosen for counting. The total number of sperm was counted first, followed by the number of abnormal sperm.

Total Sperm Counts

The other testicle and epididymis of each rat were flash frozen and a total sperm count was performed on the testicle. The testicles were thawed in saline, and the outer capsules were dissected away. The testicles were put into a glass Dounce homogenizer with 1 mL 0.9% saline/1% TX-100 and were homogenized in seven passes with the loose wand. An additional 1.5-mL buffer was added, and seven more passes were made with the tight wand. The volume was brought up to 10 mL with buffer, and two 11-μL samples were counted in a bright-line Neubauer hemocytometer.

The total sperm count reflects the number of sperm cells per mL of solution, as determined by the following method. Each sample was counted 4 times. Five diagonal squares were counted, which is a total of 0.2 mm^2 of the solution. We multiplied the result by 5 to get the total count in 1 mm^2 of solution and then multiplied that result by 10 to account for the dilution factor (of 10 mL). We multiplied that result by 10,000, to obtain the results in sperm cells/mL (per the instructions for the hemocytometer). The final numbers therefore reflect the first 5 diagonal square numbers multiplied by 5×10^5 cells.

Molecular Study

The remaining epididymides of the two groups were used for reverse-transcription polymerase chain reaction (RT-PCR) to assess the messenger (m) RNA levels of beta-actin (control) and two cell surface adhesion proteins, cadherin-1 (CAD-1) and interstitial cell adhesion molecule 1 (ICAM-1). Total RNA was extracted from each remaining epididymis using an SV Total RNA Isolation Kit (Promega, Madison, Wisconsin). The RNA was quantified by a 260/280 ratio using a Beckman Spectrophotometer (Fullerton, California).

The RT-PCR was performed using a SuperScript III One-Step RT-PCR Kit (Invitrogen, Carlsbad, California). Each reaction had 50 ng RNA with 0.5 μL of each specific forward and reverse primer (10 μmol/L). The primers used were as follows: beta-actin forward 3′-agccatgtacgtagccatcc-5′ and reverse 3′-ctctcagctgtggtggtgaa-5′; CAD-1 forward 3′-gggttgtctcagccaatgtt-5′ and reverse 3′-caccaacacacccagca tag-5′; and ICAM-1 forward 3′-aggtatccatccatcccaca-5′ and reverse 3′-gccacagttctcaaagcaca-5′. The reactions ran at 55°C for 30 minutes, 94°C for 2 minutes and 15 seconds, 55°C for 30 seconds, and 72°C for 1 minute for 40 cycles.

The DNA was run on a 1% agarose-gel-containing ethidium bromide in TAE buffer. A photo of the gel was taken on a Fotodyne 21 UV box (Fotodyne, Hartland, WI) with an Electrophoresis Photo Documentation Camera and Hood (Fisher Scientific). The photo was then quantitated in a Multi-Image Light Cabinet with AlphImager 2000 software (Alpha Innotech Corporation, San Leandro, CA).

RESULTS

Temperature

During the exposure times, the facial temperatures did not deviate by more than 1°C between experimental and control groups (Table 1). The mean initial temperature was 32.8°C in the experimental group and 33°C in the control group. During the first 3 hours, the average temperature was 33.3 ± 0.5°C in the experimental group, and 33.5 ± 1.1°C in the control group. The mean temperature during the last 3 hours was 32.6 ± 0.6°C in the experimental group, and 32.4 ± 0.8°C in the control group.

The rectal temperatures were measured before testing and at the 3-hour and 6-hour points. The results were similar in the two groups. After 3 hours, the mean experimental group temperature was 35.6°C, and the mean control group temperature was 36.4°C. After the full 6 hours of daily exposure, the

TABLE 1

Comparison of mean facial surface temperatures (in °C).

	First 3 hours			Last 3 hours	
Time (min)	Control	Experimental	Time (min)	Control	Experimental
0	33	32.8			
12	34.4	33.7	12	33	33
24	32.6	33.2	24	32	34
36	35.1	33.2	36	33	34
48	32.1	32.1	48	33	34
60	35	34.5	60	32	33
72	33.5	32.8	72	32	33
84	33.2	32.7	84	33	32
96	32.2	33.2	96	31	32
108	32	33.3	108	33	33
120	33.5	33.2	120	31	32
132	32.7	33.3	132	33	32
144	34	33.8	144	32	32
156	34.7	33.6	156	33	33
168	34.5	33.7	168	32	32
180	33.4	33.2	180	33	32
Mean for 3-hour period	33.5	33.3	Mean for 3-hour period	32.4	32.6
SD for 3-hour period	1.1	0.5	SD for 3-hour period	0.8	0.6

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mean value in both the experimental group and the control group was 35.8°C. None of the temperature differences between the two groups were statistically significant through paired *t* tests ($P>.05$).

Sperm Motility

Sperm motility was significantly different between the two groups in a chi-squared analysis ($N = 16$; $p<.05$). In the experimental group, a majority of the sperm cells were dead, with no motion and straight rigid tails (Fig. 2A). (Sperm cell death was determined through edema in the tails of the sperm, clumping of red and white blood cells to the body of the sperm, rigidity, and complete lack of movement.) In the control group, most of the sperm cells were alive, with constant active movement (Fig. 2B). The average percentage of live sperm in the experimental group was $44.88 \pm 20.66\%$, versus a mean of $70.93 \pm 12.94\%$ for the control group (Table 2).

Morphologic Study

The percentage of deformities for the experimental group was 34.3%, and the percentage of deformities for the control group was 32.1%. This difference in the occurrence of deformities between the two groups was not statistically significant ($P>.05$) through a paired *t* test.

However, whereas most of the live sperm cells in both groups appeared relatively normal, without severe abnormalities, sperm cells from the experimental samples frequently stuck together in large clumps. Sperm cells were considered to be forming a large cluster when 90% or more of the sperm cells were stuck together in one field under the microscope with ×20 magnification (Figs. 2C through 2E). In the experimental group, more than 80% of the experimental slides (13 of the 16 slides) had large clumps of sperm cells. The sperm cells in these clumps were able to do little more than squirm about, and they could not break free.

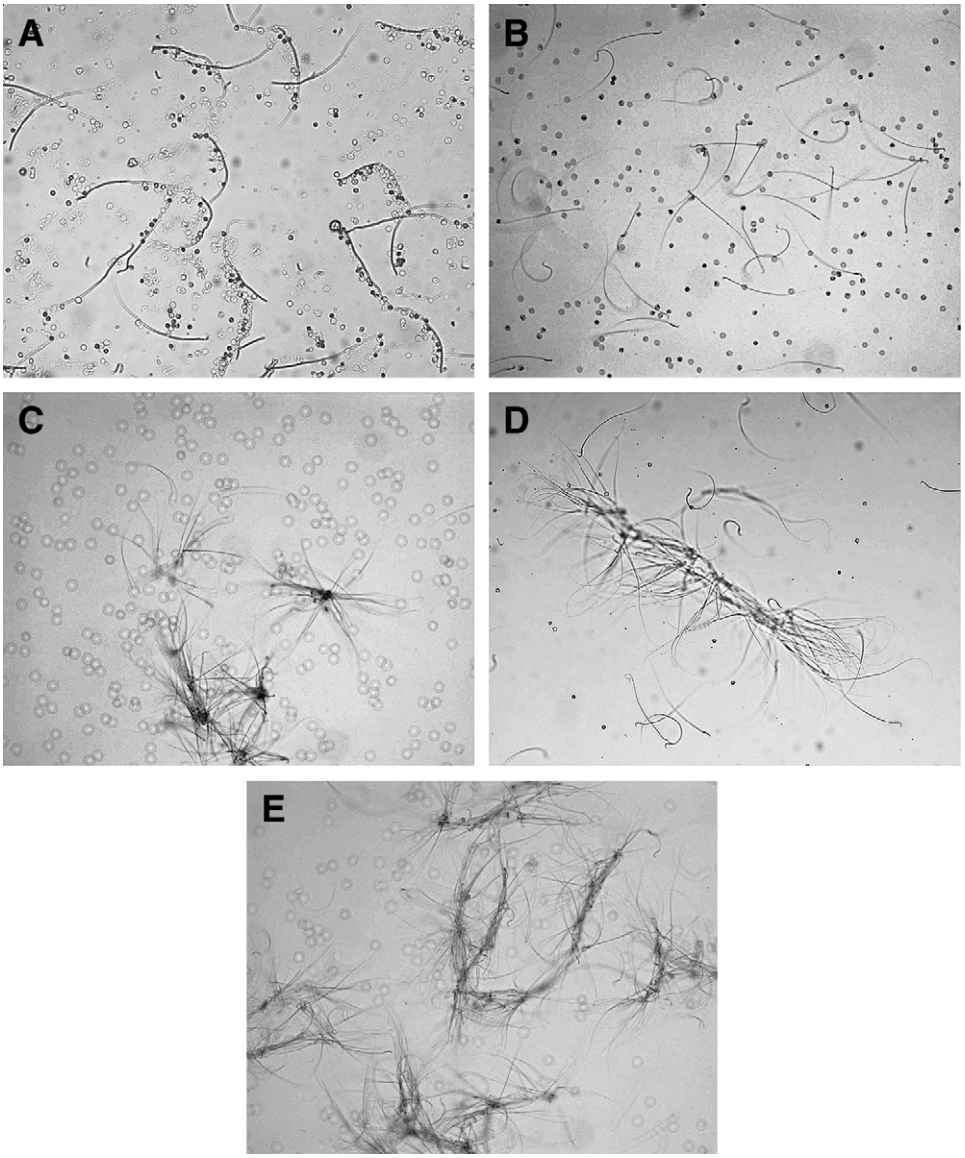
Three types of clumps were present. In type I, the sperm heads were closely stuck together to form an umbrella shape (Fig. 2C). In type II, the sperm cells were stuck together to form a small clump, with many small clumps stuck together to form a big grass-bundle shape (Fig. 2D). Type III clumps contained sperm cell tails stuck to the heads to form a ring shape (Fig. 2E). In addition to adhesion between sperm cells, many sperm cells in the experimental group were also limited in their mobility by the bonding of blood cells to them. Large clusters of sperm cells were not present in slides from the control group (Fig. 2B).

Total Sperm Count

The total sperm counts in the testicles did not statistically significantly differ between the experimental and the control groups, through a paired *t* test ($P>.05$; Table 3). The experimental group had a mean of $7.45 \times 10^7 \pm 1.03 \times 10^7$ sperm cells/mL, and the control group had a mean of $7.7 \times 10^7 \pm 8.11 \times 10^6$ sperm cells/mL.

FIGURE 2

Sperm samples at ×20 magnification. (**A**) Sperm cells from the experimental group, which appear dead. These cells are rigid with edema in the tails, giving them a wider appearance. Many blood cells are also sticking to the dead sperm cells. (**B**) Sperm sample from the control group. These individual sperm cells are evenly distributed across the slide without changes in gross morphology. These cells were actively moving. (**C**) Sperm sample from the experimental group, with sperm cells stuck together in type I clumps: Sperm heads are closely stuck together to form an umbrella shape. (**D**) Sperm sample from the experimental group, with sperm cells stuck together in type II clumps: Sperm cells are stuck together to form a small clump, and these small clumps are stuck together to form a big grass-bundle shape. (**E**) Sperm sample from the experimental group, with sperm cells stuck together in type III clumps: Sperm tails are stuck to the heads to form a ring shape. Some sperm cells in all types of clumps appeared to be alive, because there was some flagellar action, but they were unable to move individually.



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Molecular Study

The PCR analysis of the epididymides revealed an up-regulation of mRNA levels for the two cell surface adhesion proteins tested, CAD-1 and ICAM-1 (Fig. 3). The results for both cell surface adhesion proteins were statistically significant between experimental and control groups through paired *t* tests ($P<.001$; Table 4).

TABLE 2

Numbers and percentages of live and total sperm cells.

Rat #	Group	Trial #1 live (cells)	Trial #1 total (cells)	Live/total cells (%)	Trial #2 live (cells)	Trial #2 total (cells)	Live/total cells (%)	Average of two trials (%)
1	Experimental	8	42	19.05	10	44	22.73	20.89
2	Experimental	62	97	63.92	49	82	59.76	61.84
3	Experimental	24	69	34.78	27	77	35.06	34.92
4	Experimental	30	52	57.69	21	38	55.26	56.48
5	Experimental	19	36	52.78	22	36	61.11	56.94
6	Experimental	19	43	44.19	16	32	50.00	47.10
7	Experimental	66	90	73.33	56	85	65.88	69.61
8	Experimental	4	35	11.43	4	36	11.11	11.27
9	Control	40	62	64.52	44	65	67.69	66.11
10	Control	16	25	64.00	21	32	65.63	64.81
11	Control	26	28	92.86	13	14	92.86	92.86
12	Control	22	26	84.62	21	24	87.50	86.06
13	Control	27	39	69.23	24	32	75.00	72.12
14	Control	42	55	76.36	39	58	67.24	71.80
15	Control	80	156	51.28	83	138	60.14	55.71
16	Control	42	71	59.15	42	74	56.76	57.95

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TABLE 3

Total sperm counts from one testicle from each rat.

Rat #	Group	Mean
1	Experimental	7.49×10^7
2	Experimental	9.26×10^7
3	Experimental	8.13×10^7
4	Experimental	8.01×10^7
5	Experimental	5.85×10^7
6	Experimental	6.91×10^7
7	Experimental	7.05×10^7
8	Experimental	6.88×10^7
Experimental Group	Mean	7.45×10^7
	SD	1.03×10^7
9	Control	7.23×10^7
10	Control	7.08×10^7
11	Control	6.93×10^7
12	Control	7.71×10^7
13	Control	8.43×10^7
14	Control	8.29×10^7
15	Control	9.05×10^7
16	Control	6.86×10^7
Control Group	Mean	7.70×10^7
	SD	8.11×10^6

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DISCUSSION

As the data show, no significant differences emerged in the number of structural sperm mutations between the experimental and the control groups. The total sperm counts from the testes also were not significantly different between the two groups. However, the relative motility and appearance of the sperm from the epididymides in the experimental rat group differed from those of the control group.

The most striking abnormalities in the experimental group were significantly fewer motile sperm cells and numerous clumps of sperm cells. In these clumps, the heads of the sperm cells appeared to be sticking together. In the experimental group, an up-regulation was present in the mRNA levels of cell surface adhesion proteins CAD-1 and ICAM-1, which would create abnormal adhesion of the sperm cells. These proteins are normally present on the heads of sperm cells to facilitate the egg/sperm interaction during fertilization (20, 21), but the experimental group mRNA levels of these proteins were significantly higher ($P<.001$) than the control group levels. This up-regulation could explain the frequency of sperm clumping and the reduced number of motile sperm cells, owing to increased relative stickiness to each other. The adherent sperm cells would lose motility and eventually die. Further research should address this finding of sperm cell death and limited mobility and its underlying mechanism.

FIGURE 3

Representative RT-PCR reactions in a control group rat (**A**) and an experimental group rat (**B**). The first lanes on the left represent beta-actin for the internal control, the second lanes cadherin-1 (CAD-1), and the third lanes interstitial cell adhesion molecule 1 (ICAM-1). These gels show greater product formation in the experimental lanes than in the control lanes. Also, because the level of beta-actin is greater in the control group than in the experimental group, the CAD-1 and ICAM-1 differences are actually much greater when the beta-actin levels are normalized to each other.

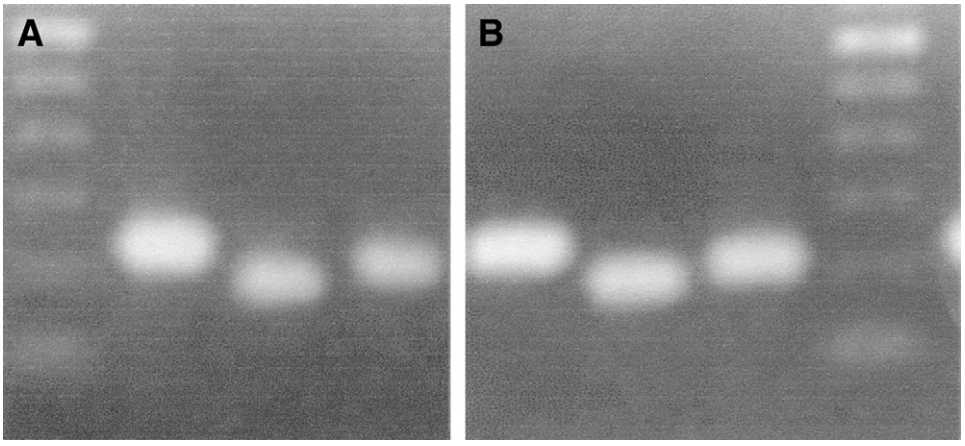

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TABLE 4

RT-PCR quantitation.

Rat #	Group	Beta-Actin (mean)	CAD-1 (mean)	ICAM-1 (mean)	Normalized #	CAD-1	ICAM-1
1	Exp.	120373	104702	110593	0.86	90043.72	95109.98
2	Exp.	123749	110202	113331	0.84	92569.68	95198.04
3	Exp.	120710	102370	90252	0.86	88038.2	77616.72
4	Exp.	113786	79488	73446	0.91	72334.08	66835.86
5	Exp.	87772	71445	73473	1.18	84305.1	86698.14
6	Exp.	101136	87741	90213	1.02	89495.82	92017.26
7	Exp.	105604	85936	84242	0.98	84217.28	82557.16
8	Exp.	94249	70235	64752	1.1	77258.5	71227.2
			Mean for experimental group			84782.798	83407.545
			SD for experimental group			6899.862	10811.081
9	Control	107114	75771	69352	0.97	73497.87	67271.44
10	Control	107103	70660	71546	0.97	68540.2	69399.62
11	Control	105318	66178	77885	0.98	64854.44	76327.3
12	Control	97302	69723	57388	1.06	73906.38	60831.28
13	Control	85456	57792	55567	1.21	69928.32	67236.07
14	Control	96327	62236	63362	1.07	66592.52	67797.34
15	Control	102334	59540	59759	1.01	60135.4	60356.59
16	Control	88434	51140	47576	1.17	59833.8	55663.92
Mean for all rats		103547.938	Mean for control group			67161.116	65610.445
			SD for control group			5397.177	6422.835

Note: The mean of the 16 beta-actin values was used to normalize the raw values of CAD-1 and ICAM-1 for a proper comparison between the experimental (Exp.) and control animals.

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Higher temperatures affect sperm maturity and motility (10–14). To ensure that the cellular phones did not increase the temperature of the rat through direct emissions from the running phone for hours, we rigorously measured temperature. Sensitive electronic temperature probes were placed adjacent to the rats' faces in the plastic tubes used in this study. After 3 hours of cellular phone exposure, mean face temperature of the experimental group did not differ from that of the control group, because of constant airflow through the tube. The rectal temperatures of both groups were virtually identical, even after the full 6 hours of exposure. The plastic wall of the tube and the wood-chip padding insulated against heat from cellular phone emissions. Furthermore, the epididymides were located within 16–18 cm from the phone without receiving any heat from the running phone. These factors eliminated mechanical heat influence from the phone, so only RFEMR affected the epididymides.

Male infertility is an increasing problem around the world. To address this problem, evaluating the possible side effects from use of new technology is critical. With over 2 billion people currently using cell phones, identifying the risks of cellular phone use is particularly crucial. Men should be aware that carrying cell phones in their pants pockets places them at risk of exposure to harmful microwaves, which could later hinder their ability to produce children.

Further study is necessary regarding the effects of long-term cellular phone usage on other tissues in the body as well, particularly the head and neck. Our current knowledge, combined with future experiments, will help to provide the general public with an improved awareness of the hazards of cellular phone use and the means for protecting itself.

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