Bioelectromagnetics 28:72–75 (2007)

Brief Communication

A Preliminary Study of Oscillating Electromagnetic Field Effects on Human Spermatozoon Motility

R. Iorio,[1] R. Scrimaglio,[3]* E. Rantucci,[3] S. Delle Monache,[1] A. Di Gaetano,[3] N. Finetti,[3] F. Francavilla,[2] R. Santucci,[2] E. Tettamanti,[3] and R. Colonna[1]

[1]*Department of Biomedical Sciences and Technology, University of L'Aquila, L'Aquila, Italy*
[2]*Department of Internal Medicine, Section of Andrology, University of L'Aquila, L'Aquila, Italy*
[3]*Department of Physics, University of L'Aquila, L'Aquila, Italy*

Some effects of extremely low frequency electromagnetic fields (ELF-EMFs) on human spermatozoa are reported. Significant increases in the values of the motility and of the other kinematic parameters have been observed when spermatozoa were exposed to an ELF-EMF with a square waveform of 5 mT amplitude and frequency of 50 Hz. By contrast, a 5 mT sine wave (50 Hz) and a 2.5 mT square wave (50 Hz) exposure did not produce any significant effect on sperm motility. The effects induced by ELF-EMF (50 Hz; 5 mT) during the first 3 h of exposure persisted for 21 h after the end of the treatment. These results indicate that ELF-EMF exposure can improve spermatozoa motility and that this effect depends on the field characteristics. Bioelectromagnetics 28:72–75, 2007.

Key words: ELF fields; square wave; sperm motility

INTRODUCTION

Recently, much attention has been paid to the influence of electromagnetic fields on cells and biological molecules. It is generally accepted that extremely low frequency electromagnetic fields (ELF-EMFs) can exert influences on biological functions of cells and tissues. These effects include bone healing [Basset, 1983], nerve regeneration [Kim et al., 2002], influences on cell calcium levels [Walleczek et al., 1999] and oncogene activation [Lagroye and Poncy, 1998]. On the contrary, the most part of studies concerning genotoxic effects were negative [Vijayalaxmi and Obe, 2005].

At present, there is little information regarding possible effects of ELF-EMFs on male reproductive system. In mammals, the application of ELF-EMFs at 50 Hz with intensities ranging from 1 mT up to 100 mT affected the proliferative/differentiative capacity of mouse spermatogonia [Furuya et al., 1998] while the ELF-EMF treatments did not induce clastogenic effects on human sperm chromosomes [Tateno et al., 1998]. With regard to sperm motility, an increase in the percentage of activated ejaculated sperm and a prolongation of their viability were shown in fish after in vitro sperm exposure to magnetic fields up to 100 mT [Formicki et al., 1990]. Furthermore, it has been observed that mice exposure to a constant intensity static magnetic field (0.7 T) for different periods of time did not produce any change in sperm motility [Tablado et al., 1996].

The aim of the present work was to investigate the effects on human spermatozoon motility of 50 Hz EMFs using sinusoidal and rectangular waveforms.

MATERIALS AND METHODS

The experimental apparatus includes: a waveform generator, a current amplifier, a solenoid and an incubator. The waveform generator was composed of a

Grant sponsor: Dipartimento di Fisica and Dipartimento di Scienze e Tecnologie Biomediche of Università degli Studi di L'Aquila (Italy)

*Correspondence to: R. Scrimaglio, Department of Physics, University of L'Aquila, L'Aquila 67100, Italy
E-mail: renato.scrimaglio@aquila.infn.it

Received for review 19 July 2005; revision received 8 June 2006

DOI 10.1002/bem.20278
Published online 3 October 2006 in Wiley InterScience (www.interscience.wiley.com).

PCI DAQ (Digital Acquisition PCI 6040E of National Instruments, Austin, TX, USA), placed in a personal computer, and the waveform editor software. In our experiments we employed a solenoid 200 mm long with a 160 mm diameter. The number of turns was 511 giving a resulting resistance of 5.5 Ω and a total inductance of 25 mH. A current generator Kepco BOP 72-6M (Kepco, Inc., Flushing, NY, USA) was employed to compensate the effects due to the induced current and the input current was continuously monitored by means of an oscilloscope (Tektronix 3054 digital oscilloscope, Tektronix, Inc., Beaverton, OR, USA) which measured the voltage across a 1 Ω (600 W) resistor. The Kepco BOP 72-6M has two bipolar control channels (voltage or current mode) selectable and individually controllable either from their front panel controls or by remote signals. In our case the input bipolar voltage signal (generated by means of the PCI DAQ 6040E) produces an output current signal with the required amplitude and waveshape. In order to verify the uniformity of the magnetic field in the solenoid core, some measurements were performed by using a gaussmeter (Gaussmeter model 912, RFL Industries, Inc., Boonton, NJ, USA). The maximum variation of the magnetic field was 2.4% within a cylindrical region (coaxial to the solenoid) 40 mm long and 10 mm diameter.

In order to control the temperature inside the solenoid a thermometric sensor (Fluke 51-II with bead probe, Fluke Corporation, Everett, WA, USA) was placed inside the coils during the experiments measuring a constant temperature of 37.0 ± 0.3 °C. The temperature was maintained at the indicated value by means of a fan (120 mm, 12 V, 100 m^3/h) placed in front of the coil, which was continuously operating during the experiment. The treated semen samples were located in the core of the solenoid. Treated and control samples were placed in a 10 ml sterile Falcon tubes (effective sensitive volume ranging from 300 to 500 μl) and maintained at 37 °C in two independent incubators (Steril-Cult 200, Forma Scientific, Marietta, OH, USA) with a humidified atmosphere containing 5% of CO_2. The characteristics of the magnetic fields used in this work were (a) a sinusoidal waveform with amplitude of 5 mT (I_{max} 1.4 A); (b) a square waveform with amplitude of 5 mT ($I_{max} = 1.4$ A); (c) a square waveform with amplitude of 2.5 mT ($I_{max} = 0.7$ A).

Semen samples, obtained from healthy normozoospermic donors after 3 days of sexual abstinence, were allowed to liquefy at room temperature [Rotem et al., 1992] for 30 min and then analyzed in terms of volume, pH, sperm concentration, motility, viability, and morphology according to World Health Organization guidelines of 1999. From the collected semen samples we chose the ones with a concentration greater than 20×10^6/ml and a progressive motility greater than 50%. The liquefied semen (1–3 ml) were loaded onto discontinuous gradient (Sil-Select) and centrifuged at 400*g* for 20 min at room temperature. The pellet obtained was diluted in 2.5 ml of Nutrient Mixture F-10 Ham [Ham, 1963], centrifuged at 300*g* for 10 min and then resuspended in 1 ml of fresh medium. For long culture experiments (24 h), the presence in the sperm preparation of other cells, particularly leucocytes, can further invalidate the life of spermatozoa [Saleh et al., 2002]. For this reason, before ELF-EMF treatment, semen samples cultured for 24 h were subjected to a swim-up method [Krausz et al., 1996]. Each sample was divided into two subsamples that were exposed (''treated'') or not (''control'') for 3 h to a uniform horizontal magnetic field at the center of a solenoid. During the experiments, a small part of the treated and the control samples was taken away and observed every 60 min from the beginning of the treatment.

Sperm concentration, motility and different characteristics were determined using ATS 20 system (JC Diffusion International, Gauville, France). This system was set up as follows: number of frames to analyze 25; number of frames/s 25; minimum track point for calculation of motility 2; minimum track point for calculation of velocity 8; maximum velocity 150; threshold velocity 10; pixel scale 1.351; cell size range (low) 4; cell size range (high) 20 and to control temperature the CASA system was equipped with a heated 37 °C stage. For sperm observation samples were allowed to settle for few seconds into a Makler chamber, previously precoated at 37 °C, and 100–200 spermatozoa per sample were screened. Each measure consisted of VCL (curvilinear velocity in μm/s), VSL (progressive velocity in μm/s), VAP (average path velocity in μm/s), LIN (linearity as a %), ALH (amplitude of lateral head displacement in μm), percentage of motile cells and percentage of ''rapid'' VAP (i.e., cells with a VAP greater than 25 μm/s). Each experiment was repeated three times over different semen samples and the results are expressed as means ± standard error at a given time point. The mean values and the variances obtained for the control and the treated samples at each time point were compared by using the ANOVA method and the Student–Newman–Keuls test. All tests were considered with a statistical significance at $P < 0.05$.

RESULTS AND DISCUSSION

In the first set of experiments sperm suspension was exposed to a sinusoidal magnetic field with an

amplitude of 5 mT and oscillating at a frequency of 50 Hz. At each time point investigated, no significant variation was observed neither in the sperm motility nor in the kinematic parameters evaluated with respect to the control (data not shown).

In the second set of experiments a two-phase square wave (50 Hz; 5 mT) with a rise time of 1.92 ± 0.04 ms was applied to sperm suspension. In this case, after 2 h of incubation from the beginning of the treatment, a significant increase in the percentage of motile spermatozoa and in the VAP > 25 μm/s was observed in the treated sample with respect to the control (Fig. 1a,c). Moreover, after 3 h of exposure to the magnetic field, the percentage of treated cells with VSL > 60 μm/s was significantly higher than that of the control (Fig. 1b). Hence, a significant increase in the LIN was also recorded during the ELF-EMF treatment, while the ALH and the VCL appeared to be unaffected by the electromagnetic treatment (data not shown). In order to examine a possible long-term effect of ELF-EMFs on spermatozoa motility, samples were cultured at 37 °C, 5% CO_2 for 21 h after the ELF-EMF treatment (3 h of exposure). As shown in Figure 2, the percentage of cells with a VAP > 25 μm/s and a VSL > 60 μm/s resulted significantly increased for the treated sample with respect to the control, but no significant variations was noticed in the percentage of motile cells. In order to investigate if 5 mT is the minimum intensity value of the square wave able to induce the observed effects on sperm motility, the semen samples were exposed to a square magnetic field of 2.5 mT (50 Hz). We point out that in this case the biphasic square wave (50 Hz; 2.5 mT) had a rise time of 2.00 ± 0.04 ms. No significant variations were observed in sperm motility, in percentage of cells with VAP > 25 μm/s and with a VSL > 60 μm/s of the treated sample with respect to control (data not shown), during the 3 h of incubation.

Therefore, the ELF-EMFs can affect the human sperm motility by increasing the percentage of motile spermatozoa and the correlated kinematic parameters, but these effects depend on both waveform and amplitude of the applied magnetic field. The sine and square waves are similar, since the amplitude (5 mT) and the frequency (50 Hz) are the same. However, the absence of effects on cells in the case of the sine wave requires a deeper analysis. In fact, the main differences between these two fields consist in the root-mean-square value and slope (field transient) of the waveform. Moreover, the ON-OFF transient of the square wave contains a number of frequencies which could have different effects on the cell with respect to a "simple" sinusoidal waveform at 50 Hz. It is also possible to hypothesize that the response of human spermatozoa to the external electromagnetic field depends on the

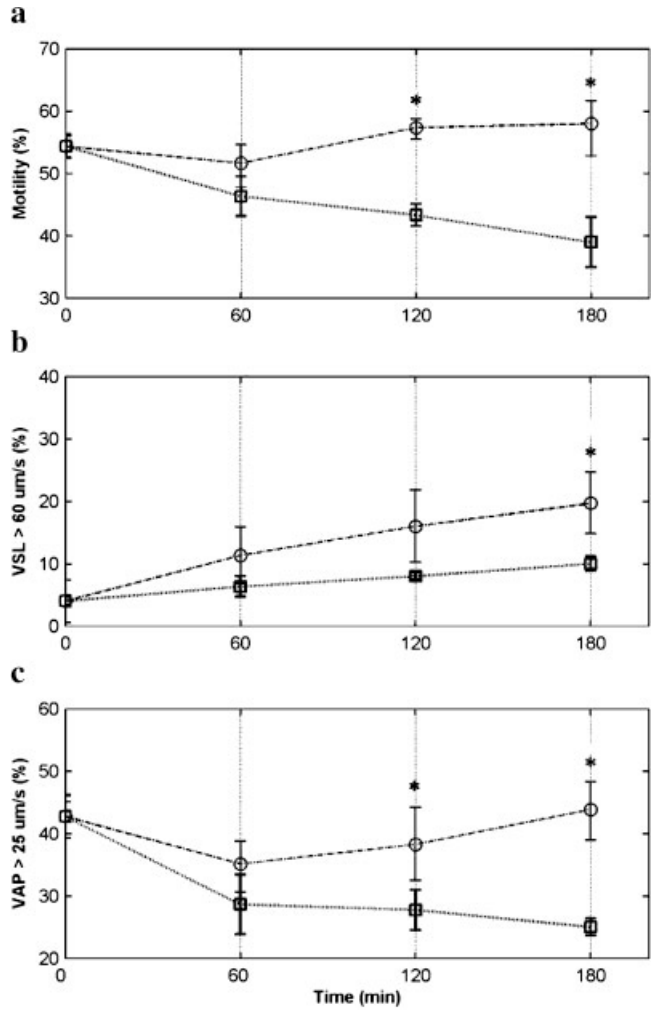

Fig. 1. Effects of a square magnetic field (5 mT; 50 Hz) on human sperm motility. Magnetic field was applied for 3 h. The treated (○) and untreated (□) samples were analyzed for the sperm motility (**a**), VSL (**b**), and VAP (**c**) every hour during the incubation time. Treated sample versus Control: $^*P < 0.05$ (one-way Anova, Student-Newman-Keuls test).

effective time during which the amplitude of the influencing signal exceeded a threshold value which is determined by the properties of the cell itself.

In view of the fact that the energy necessary for the sperm motility comes from ATP hydrolysis, it is possible to hypothesize an interaction between the electromagnetic field and the energy metabolism. There are two pathways for ATP production in mammalian sperm, glycolisis and mitochondrial respiration. Although oxidative phosphorylation is more efficient than glycolisis for ATP production, it has been suggested that most of the energy required for sperm movement is generated by glycolisis rather than oxidative phosphorilation [Mukai and Okuno, 2004]. In future investigations, it will be interesting to study

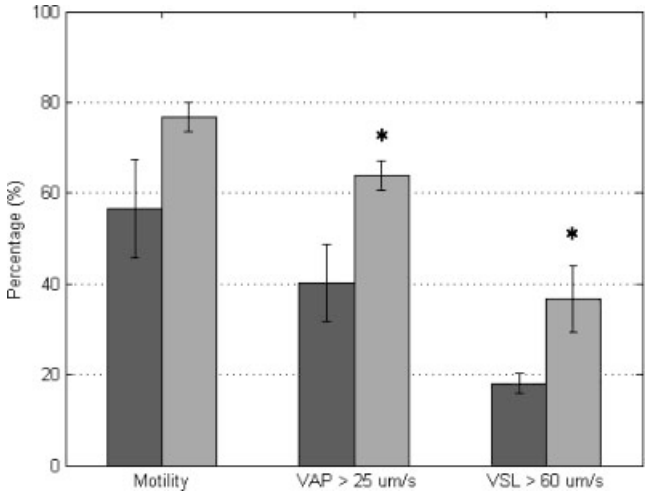

Fig. 2. Human sperm motility and kinematic parameters after 21 h of culture from the end of the square magnetic field (5 mT; 50 Hz) treatment. Spermatozoa exposed (Treated) or not exposed (Control) to the square magnetic field for 3 h were incubated for 21 h and analyzed for the sperm motility. Treated sample (gray columns); Control (black columns). Treated sample versus Control: $^{*}P < 0.05$ (one-way Anova, Student-Newman- Keuls test).

the relationship between the enhancement in the sperm motility characteristics and the energy metabolism with particular reference to glycolitic processes.

REFERENCES

Basset CAL. 1983. Beneficial effects of electromagnetic fields. J Cell Biochem 51:387–393.

Formicki K, Sobocinski A, Winnicki A. 1990. Motility of spermatozoa of Danube salmon (Hucho hucho L.), exposed to magnetic field prior to activation. Pol Arch Hydrobiol 37:439–447.

Furuya H, Aikawa H, Hagino T, Yoshida T, Sakabe K. 1998. Flow cytometric analysis of the effects of 50 Hz magnetic fields on mouse spermatogenesis. Nippon Eiseigaku Zasshi 53(2): 420–425.

Ham RG. 1963. An improved nutrient solution for diploid Chinese Hamster and human cell lines. Exp Cell Res 29:515–526.

Kim SS, Shin HJ, Eom DW, Huh JR, Woo Y, Kim H, Ryu SH, Suh PG, Kim MJ, Kim JY, Koo TW, Cho YH, Chung SM. 2002. Enhanced expression of neuronal nitric oxide synthase and phospholipase C-gamma1 in regenerating murine neuronal cells by pulsed electromagnetic field. Exp Mol Med 34(1): 53–59.

Krausz C, Bonaccorsi L, Luconi M. 1996. Two functional assays of sperm responsiveness to progesterone and their predictive values in in-vitro fertilization. Hum Reprod 11:1661–1667.

Lagroye I, Poncy JL. 1998. Influences of 50-Hz magnetic fields and ionizing radiation on c-jun and c-fos oncoproteins. Bioelectromagnetics 19(2):112–116.

Mukai C, Okuno M. 2004. Glycolysis plays a major role for adenosine triphosphate supplementation in mouse sperm flagellar movement. Biol Reprod 71(2):540–547.

Rotem R, Paz GF, Homonnai ZT, Kalina M, Lax J, Breitbart H, Naor Z. 1992. Ca2+-independent induction of acrosome reaction by protein kinase C in human sperm. Endocrinology 131: 2235–2243.

Saleh RA, Agarwal A, Kandirali E, Sharma RK, Thomas AJ, Nada EA, Evenson DP, Alvarez JG. 2002. Leukocytospermia is associated with increased reactive oxygen species production by human spermatozoa. Fertil Steril 78(6):1215–1224.

Tablado L, Perez-Sanchez F, Soler C. 1996. Is sperm motility maturation affected by static magnetic fields. Environ Health Perspect 104(11):1212–1216.

Tateno H, Iijima S, Nakanishi Y, Kamiguchi Y, Asaka A. 1998. No induction of chromosome aberrations in human spermatozoa exposed to extremely low frequency electromagnetic fields. Mutat Res 414(1–3):31–35.

Vijayalaxmi, Obe G. 2005. Controversial cytogenetic observations in mammalian somatic cells exposed to extremely low frequency electromagnetic radiation: A review and future research recommendations. Bioelectromagnetics 26(5): 412–430.

Walleczek J, Shiu EC, Hahn GM. 1999. Increase in radiation-induced HPRT gene mutation frequency after nonthermal exposure to nonionizing 60 Hz electromagnetic fields. Radiat Res 151(4):489–497.