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• *Short report*

Effects of combined magnetic fields on human sperm parameters

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Background: In previous investigations, it has been clarified that electromagnetic fields (ELF) can cause some changes in cellular behavior. The aim of this prospective study was to investigate the effect of magnetic field (MF) on human sperm parameters of motility, morphology, and viability. ***Materials and Methods:*** Semen samples were collected from 12 fertile men, and were allowed to liquefy for 15-30 min. Each sample was then divided into two aliquots. The experimental samples were placed in the ELF, while the control one was left intact. The applied fields were pulsed with distance of 6 m/ sec and effective intensity of 1mT and different frequencies of 10, 25 and 45 Hz at different time intervals. The constant field intensity was 1mT in all experiments. ***Results:*** In frequency of 10Hz, an increase in quick motility of sperm (1.8 times) occurred after 4h; however, slow motility was decreased by 40% after 2h. Also, the quick motility increased by 1.6 times in frequency 25 Hz after 4 h, while the MF had no effect on other sperm parameters. MF had no effect on any of sperm parameters in frequency of 40 Hz in 4 h. The stimulation ratio on the sperm viability was only significant at frequency of 10 Hz after 2 h after incubation. The sperm morphology was not influenced in any of the fields. ***Conclusion:*** This study reports the existence of certain frequency windows for the resonance of the effects of the MF on human spermatozoa. Rapid motility was significantly affected by the exposure of spermatozoa to MF, but sperm structural parameter had remained intact. **Iran. J. Radiat. Res., 2011; 9(3): 195-200**

Keywords: *Combined electromagnetic fields, spermatozoa parameters, semen, human.*

INTRODUCTION

Electromagnetic fields (EMFs), either natural or man-made, are found anywhere on earth. Natural fields are generated in thunderstorms. Electric field is a field that is created by differences in voltages and a magnetic field is a field that is created when electric current flows [1, 2]. General public are exposed to electromagnetic currents on a daily basis, and EMFs are more prevalent in modern lives. Using electricity, microwaves, cell phone, all result in exposure to EMF. Also, X-rays, MRI, and ultrasounds are used in medicine, which are other potential sources of EMFs [3].

Studies have been done to see the effect of EMF on fetal development and germ cells. In this regards, Chung *et al.* (2003) reported that EMF did not cause any major anomalies in rat fetuses [4]. Also, no significant differences were noticed in the number of corpus lutea, implantation, or dead fetuses. Later, Chung *et al.* (2005) found the same results when exposing mated female rats to magnetic fields [5]. The rats were divided into four groups of control, and three with magnetic exposures of 50, 833, and 5,000 mG for three weeks. The results again showed that there was no significant differences between the exposed and control fetuses. Also, no significant difference was observed in the sperm parameters, number of spermatid cells, and fertility potential of F1 males. Later, Al-Akhras *et al.* (2006) tested the effects of EMF on sex hormones and other parameters of 90 days old male rats [6]. Animals were exposed to magnetic field of 25μT given off by rectangular coils, 50 Hz. The authors found that there was no significant difference in the body and testis

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weight between the control and the exposed groups. However, there were differences in testicular sperm count, and levels of both testosterone and lutenizing hormones.

Only a few studies have examined the effects of EMF on human spermatozoa. In this regards, Tateno and co-workers (1998) exposed human sperm to magnetic field of 50 Hz to determine if there was an effect on DNA integrity [7]. The results showed that there was no increase in abnormalities of the chromosomal structure in exposed semen when compared with control. Recently, Lorio *et al.* (2007) examined the effects of extremely low frequency electromagnetic fields (ELF-EMF) on motility of human spermatozoa [8]. Their results showed that exposure of spermatozoa to ELF-EMF (50 Hz; 5 mT) significantly enhanced the motility. The effects which were induced by ELF-EMF during the first three hours of exposure lasted for 21 hours after the end of the treatment. The findings illustrated that ELF-EMF exposure can improve the sperm motion characteristics in vitro. Therefore, the recent studies on the effects of EMF on human spermatozoa demonstrate that some of the parameters, such as progressive motility may be enhanced during the early exposure. To further assess the role of ELF-EMF on reproductive cells of males, this prospective study was conducted to examine the effects of ELF on the sperm parameters of fresh ejaculates obtained from fertile men.

MATERIALS AND METHODS

Semen Samples

Twelve healthy fertile men were selected for this study. They were non-smokers and aged from 30 to 44 years. None of the subjects were under radiation or pharmaceutical treatments. The semen samples were collected after 2 to 3 days of abstinence.

Following 15-30 min of liquifation at 37ºC, each sample was analyzed according to WHO guideline [9]. Sperm specimens were obtained by ejaculation. A total of 200 spermatozoa were analyzed from each sample. Sperm count and motility evaluation were done using Makler chamber and light microscopy at 200X magnification. Progressive (rapid and slow), non-progressive and immotile spermatozoa were reported as a percentage. Sperm morphology (normal and abnormal) was evaluated using Geimsa staining. In addition, sperm viability was assessed by application of eosin-nigrosin (EN) staining. Briefly, 10 µl of fresh semen was mixed with 10 µl of EN for 30 seconds on a clean microscopic slide. Then, it was examined under light microscope for the percentage of vital (unstained) and dead (stained) spermatozoa.

After initial examination, the semen samples were immediately divided into two aliquots of control and experimental groups. The experimental samples were placed in the ELF, while the control ones were left inside an iron box/ sheild, which was set inside the incubator. This was done in order to keep the control samples away from magnetic field.

Exposure system

The experimental apparatus used in this study included a waveform generator, a current amplifier, a helmholtz coil, and an incubator. The waveform generator and current amplifier were designed and calibrated using ossiliscope (IWATSU, Japan).

In this experiment, two helmholtz coil with 100mm diameter and number of turns of 2×220 were employed, giving a resulting resistance of 20Ω and a total inductance of 20 mH. A current generator was employed to compensate. The effect was continuously monitored by means of an oscilloscope which measured the voltage across a 1Ω (600w) resistor. In our case, the input voltage signal produced an output current signal with the required amplitude and wave-shape. In order to verify the uniformity of magnetic field in the helmholtz core, some measurements were performed by using a gaussmeter (Holaday Ind., MN, USA). The

maximum variation of the magnetic field was 2% within a cylindrical region (coaxial to the coil) 50 mm long, 50mm diameter. To control the temperature inside the helmholtz coil, a thermometer sensor (TM916, Taiwan) was placed inside the coils during the experiments measuring a constant temperature of 37.0±0.1°C. The treated semen samples were located in the core of the coils. The characteristics of the magnetic fields used in this work were: a square waveform with magnetic field intensity effective B_{eff}=1mT and frequencies 10Hz, 25Hz and 40Hz plus parallel constant magnetic field with amplitude 1mT. The "stimulation ratio" was evaluated according to Kondo (1995) procedure [10], and the data are presented in tables 1-3.

Statistical analysis

Differences in the percentages of sperm parameters were compared between the control group and the experimental group by chi-square test. Differences were considered significant at $P<0.05$. All values are given as mean ± standard deviation (SD).

RESULTS

The results showed that seminal viscosity of each sample was within normal range. The mean of PH and semen volume was 7.3±1.1 and 3.5±0.57 ml, respectively. The mean of sperm count was 74.11±8.72×10^6. The findings also showed that the rates of sperm normal morphology and viability were 53.22±14.5% and 70.13±15.3%,

Table 1. Correlation of time with sperm parameters inside and outside of MF (frequency 10 Hertz).

Time (h)	Rapid motility	Slow motility	Non-prog. motility	Immotility	Normal morphology
0	[a]1±0	[c]1±0	1±0	1±0	1±0
1	[a]0.96±0.13	[c]1.14±0.14	0.98±0.21	1.10±0.24	0.98±0.03
2	[a]1.05±0.05	[d]0.69±0.11	0.97±0.20	1.09±0.16	1.07±0.15
4	[b]1.80±0.14	[d]0.62±0.12	0.75±0.19	0.84±0.16	0.96±0.08

The data are mean±SD. P<0.05.

Table 2. Correlation of time with sperm parameters inside and outside of MF (frequency 25 Hertz).

Time (h)	Rapid motility	Slow motility	Non-prog. motility	Immotility	Normal morphology
0	[a]1±0	1±0	[a]1±0	1±0	1±0
1	[a,b]0.82±0.18	1.14±0.21	[a,b]1.12±0.11	1.01±0.30	1.03±0.05
2	[b] 0.66±0.13	0.99±0.24	[a,c]0.88±0.10	1.28±0.96	0.99±0.01
4	[c]1.64±0.07	1.14±0.12	[c]0.80±0.08	1.00±0.10	0.99±0.02

The data are mean±SD.
Different superscript (a, b, c) in table are based on the factual condition with (<0.05).

Table 3. Correlation of time with sperm parameters inside and outside of MF (frequency 40 Hertz).

Time (h)	Rapid motility	Slow motility	Non-prog. motility	Immotility	Normal morphology
0	1±0	1±0	1±0	1±0	1±0
1	0.88±0.15	1.04±0.22	1.03±0.16	1.01±0.24	1.01±0.03
2	0.91±0.12	1.14±0.26	0.98±0.19	0.98±0.14	0.99±0.03
4	0.91±0.09	1.05±0.17	0.99±0.07	1.01±0.18	0.96±0.08

The data are mean±SD.

respectively. In addition, the rates of rapid and slow motilities were 22.10±1.7% and 48.32±4.2%, respectively. The rate of non-progressive motility was 15.15±12.7%, and of immotile spermatozoa was 14.19±3.0%.

The results also showed that both rapid sperm motility, as well as viability rates was increased inside and outside the MF at both 10 and 25Hz after 4h of exposure. However, the sperm morphology and non-progressive motility remained intact. As time proceeded, the rate of sperm morphology decreased slightly at all frequencies under investigation. The results also presented that the speed of each sperm parameter varied, but motion characteristic was more sensitive to low frequencies of 10 and 25Hz.

Table 1 shows the time correlation of sperm parameters both inside and outside MF with 10Hz. The finding showed that the difference between fast motility inside with outside MF was significant at 4h, when compared with other time intervals of 0, 1, and 2h. Therefore, MF at 10Hz did not influence the rapid sperm motility from 0 to 3 h after exposure, but it was significantly increased at 4h. Also, MF at 10Hz did not noticeably influence the sperm morphology, as well as non-progressive and immotile spermatozoa. The data also demonstrated that sperm viability was changed insignificantly at two different time periods of 2h and 4h after MF exposure. Sperm viability at frequency of 10 Hz slightly increased from 1.01±0 at 0h to 1.12±0.13 after 4h (figure 1).

In addition, sperm viability at frequency of 25 Hz insignificantly increased from 1.0±0 at 0h to 1.08±0.17 after 4h. As presented in figure 1, the parameter of sperm viability remained unchanged (1.0±0 at 0h and 0.97±0.04 at 4h) at 40 Hz. The findings also showed that at 25Hz, progressive motility increased, while non-progressive motility decreased significantly. Also, normal morphology remained unchanged at 25Hz. As the frequency increased to 40Hz, the most affected sperm parameter was rapid motility at 4h. This parameter achieved the highest rate of activity in frequency of 40Hz, when compared with lower frequencies of 10 and 25Hz.

DISCUSSION

In recent years, many research studies investigated the impact of EMFs exposure on reproduction and fertility potential [5-8].

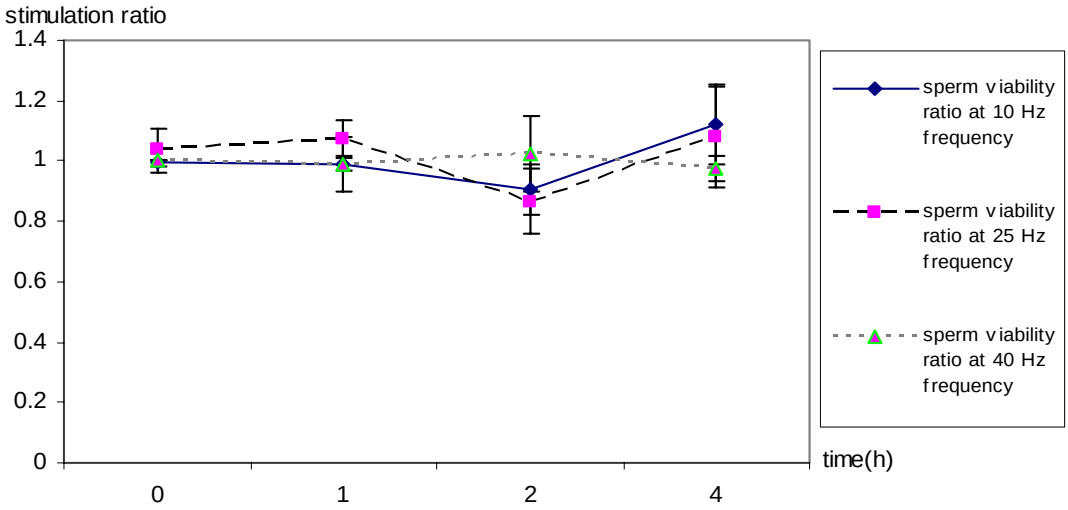

Figure 1. Time correlation with sperm viability inside and outside magnetic field at different frequency.

Mahmudsyah and Sudarti (2003) reported that even extremely low frequency EMFs exposure influenced the spermatogenesis of rats [11]. They concluded that all spermatgenic cells were reduced in numbers toward either the increasing intensity, or the longer time of exposure of EMFs. Others have shown variable effects of EMFs on spermatozoa activity after EMFs exposures [5, 8, 12].

In their study, Tablado *et al.* (1996) found that there was not any significant effect of EMF on sperm motility of albino mice [13]. On the contrary, Bernabo *et al.* (2007) observed that EMF does affect sperm motility of boar semen [14]. Together, the present study, as well as other studies, such as (Luo *et al.* 2006), suggest that care should be taken when using laboratory equipment for processing gametes *in-vitro* [15]. As EMFs is becoming more prevalent in today's work, laboratory, and home environments, precautions should be taken to avoid the EMFs, in order to improve the fertility potentials of gametes [16].

L'opuck *et al.* (2005) investigated the impact of MF with magnetic induction of 0.5mT and frequency 50Hz on sperm motility of ejaculates from 40 fertile men [17]. They chose frequency of 50Hz, because the MF which was inducted by electric devices in Europe is 50Hz. They assessed the sperm motility variables for duration of 2h. Their results indicated that rapid motility significantly increased following MF exposure. However, other types of sperm motility (slow, non-progressive) were reduced significantly after MF induction. Our study also showed similar outcomes, as sperm progressive motility was the only parameter that was influenced significantly at low frequency. This parameter was not affected at high frequency level of 40Hz. Our findings also indicated that MF may not damage the sperm structure or viability characteristics of human ejaculated spermatozoa. Therefore, human spermatozoa were very stable at structural level; while sensitive with their motion characteristics. However, Bernabo *et al.* (2007) reported that although EMF at extremely low frequency (50Hz and 1mT intensity) did not reduce sperm viability, but morphology was affected [14]. They exposed boar spermatozoa for 4.5h at 37C, in order to study sperm morphology, acrosomal integrity as well as viability. These types of variation should be further investigated and compared between human and other mammals [18].

In their study, Tateno and co-workers investigated the rate of chromosomal aberrations in human spermatozoa after exposure to low frequency EMFs [7]. Semen samples were exposed to EMFs at 50Hz, 20 mT for 2h at 37C under 5%Co2. They realized no significant elevation in chromosomal lesions of exposed spermatozoa during 2h of exposure to EMFs. Therefore, it was suggested that low frequency EMFs caused no harm to sperm chromosome of human if short exposure was applied.

In conclusion, both morphology and viability of human spermatozoa were not influenced by MF. However, rapid motility was significantly affected by exposure of spermatozoa to MF, especially in frequency 10HZ. This alteration may imply the risk of the reduction of sperm fertility in humans.

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