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Effect of Electromagnetic Wave Emitted from Mobile Phone on Some Reproductive Parameters in Adult Male Guinea Pigs*

Barış Atalay USLU[1]✉, Dide Kılıçalp KILIÇ[2], Fetih GÜLYÜZ[1], Yeter DEĞER[3], Ömer UÇAR[4]

1. Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Yuzuncu Yil University, Van
2. Faculty of Veterinary Medicine, Department of Physiology, Yuzuncu Yil University, Van
3. Faculty of Veterinary Medicine, Department of Biochemistry, Yuzuncu Yil University, Van
4. Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Ataturk University, Erzurum

Abstract: The effects electromagnetic wave (EMW) (900 MHz) emitted by mobile phones, for 60 days, upon the testis weight and epididymal sperm quality in male guinea pigs were investigated. Twelve healthy male guinea pigs were assigned randomly as the treatment (n=6) and control (n=6) groups. Treatment group (group EMW) was exposed to electromagnetic field of 900 MHz (217 Hz pulse rate, 2 W maximum peak power, SAR 0.95 w/kg) emitted by a mobile phone (used daily as; 20 min calling, then kept in 'standby' position for 23 h 40 min/day) for 20 min per day for 60 days. Control group (Group C) was maintained under similar conditions, with no radiation. Unlike the males in Group C kept in a separate room, those in exposed-group were subjected to electromagnetic radiation being operated at 900 MHz EMW field. There were not significant (P>0.05) differences between the groups based on the testicle weight (3.6 g vs. 4.4 g), sperm motility (61.7% vs. 70.0%), and abnormal sperm rate (5.8% vs. 3.7%) in treated and control males, respectively. However, there was a significantly (P<0.05) lower sperm number (549.6 x 10^6 sperm/ml) in Group EMW as compared to those in Group C (799.17 x 10^6 sperm/ml). According to the findings achieved, it was suggested that; i) the EMW radiation emitted by mobile phones (900 MHz) in daily use (for 20 min actively per day) up to 60 days unfavourably affected the testicle weight and epididymal sperm traits variably and at some levels, and ii) the number of spermatozoa was the most profoundly (adversely) affected sperm characteristics in male guinea pigs exposed to the radiation concerned.

Key words: Electromagnetic wave, Guinea pig, Sperm, Testis

Cep Telefonundan Yayılan Elektromanyetik Dalganın Erişkin Erkek Kobaylarda Bazı Üreme Parametreleri Üzerine Etkisi

Özet: Bu çalışmada, rutin kullanımdaki mobil telefondan yayılan 900 MHz'lik elektromanyetik alana (EMA) 60 gün süreyle maruz kalan erkek Gine domuzlarında testis ağırlığı ve epididimal sperm kalitesi araştırıldı. Oniki adet sağlıklı erkek kobay rastgele uygulama, EMA (n=6) ve kontrol, K (n=6) grubu olarak iki gruba ayrıldı. Uygulama grubu (Grup EMA), 60 gün süre ile her gün mobil bir telefonla yayılan (telefonu 20 dk çaldırma/konuşma modu, 23 saat 40 dk uyku/standby modu) 900 MHz'lik EMA'a (217 Hz pulse rate, 2 W maximum peak power, SAR 0.95 w/kg) maruz bırakıldı. Kontrol grubu (Grup K) benzer şartlar altında tutuldu, ancak radyasyona maruz bırakılmadı. Grup EMA ve K arasında, sırasıyla, testis ağırlığı (3.6 g ve 4.4 g), epididimal sperm motilitesi (%61.7 ve %70.0) ve anormal sperm oranı (%5.8 ve %3.7) bakımından anlamlı farklılıklar gözlenmedi (P>0.05). Bununla birlikte, Grup EMA'deki sperm sayısı (549.6 x 10^6 sperm/ml) Grup K'dekilerden (799.17 x 10^6 sperm/ml) önemli düzeyde (P<0.05) daha az idi. Elde edilen bulgulara göre; i) kobaylarda mobil telefonların 60 gün süreyle (günlük 20 dk aktif tarzda) kullanımına bağlı olarak yayılan EMA radyasyonunun (900 mHz) testis ağırlığını ve epididimal sperm kalite parametrelerini farklı ve belli düzeyde olumsuz yönde etkilediği, ve ii) anılan radyasyona bağlı olarak en olumsuz etkilenen kalite parametresinin sperm sayısı olduğu kanısına varıldı.

Anahtar kelimeler: Elektromanyetik dalga, Kobay, Sperm, Testis

✉ Barış Atalay USLU
Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Yuzuncu Yil University, Van,
e-posta: atalayuslu@hotmail.com

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INTRODUCTION

In the last two decades, the popularity of mobile phones and expansion of their service networks worldwide have dramatically increased the amount of electromagnetic wave (EMW) exposure in our daily lives. Hence, the concerns regarding their potential harmful effects on our own health increasingly draw more attention. Unlike the scientific arguments, the public concern is driven not only by the scientific facts but also by the anxiety of people, apart from their emotional, economic and political interests.

Having given the potentially harmful effects of EMW radiation on some biological systems, recent studies have dealt with the concerns regarding the safety of radio frequency (RF)-EMW exposure. For example, the microwaves emitted by mobile phones have been linked to several genetic defects (Pacini et al., 2002; Aitken et al., 2005; Tice et al., 2002). It has also been suggested that the microwave radiation may induce chromosomal instability and lead to increased risk of cancer (Sykes et al., 2001; Pacini et al., 2002; Mashevich et al., 2003; Agarwal 2007). The question as to whether these phones can lead to infertility became a debatable issue drawing increasingly more attention (Davoudi et al., 2002; Fejes et al., 2005; Kilgallon and Simmons 2005; Erogul et al., 2006; Agarwal 2007). However, the sequence of this infertility-related damage caused by the EMW exposure remains largely unclear (Agarwal et al., 2008).

Infertility affects approximately the 15 % of couples of reproductive age, with nearly half of the cases resulting from the male (Thonneau et al., 1998; Sharlip et al., 2002). A number of reports suggested a possible link between the use of mobile phones and infertility. Indeed, in a recent study (Agarwal et al., 2008), the use of phones adversely affected the semen quality (by decreasing the sperm count, motility, viability and morphology). Besides, this undesirable effect did not depend on the initial semen concentration, as to whether they were normo- (≥20 million sperm/ml) or oligozoospermic specimens (<20 million). Further, the sperm parameters reported therein were inferior when the phones used longer. Fejes et al. (2005) reported that the duration of exposure and daily transmission time were correlated negatively with the proportion of progressively motile sperm. Erogul et al. (2006) found lower sperm motility in men exposed to mobile phone for 5 min. Similarly, Davoudi et al. (2002) also found that using the phones for 6 h a day over 5 days decreased the proportion of motile sperm. Further, recently, decreased sperm concentration was reported in men carrying the phones close to the waist, as compared to controls or those carrying it elsewhere (Kilgallon and Simmons 2005). Therefore, it appears that the degree of undesirable effect of phones upon the sperm motility could be influenced by both the duration of use and by the type (place) of carriage.

As mentioned above, earlier studies have suggested a dose-dependent deterioration in semen quality and testicular tissue damage in men using the mobile phones. Therefore, the objective of the present study was to investigate the potentially harmful effects of the routine daily use of mobile phones emitting the EMWs upon the testicular organ and semen quality in male guinea pigs (as animal model) during 60 days of exposure.

MATERIALS and METHODS

Animals and Experimental Design

In the present study, totally 12 healthy adult male guinea pigs, aged 4-6 months and weighed around 650-750 g were used. The handling and maintenance of animals were carried out according to the recommendations of the Board of Ethics Committee, Yuzuncu Yil University, Van-TURKEY. Males were housed in cages 60x90x45 cm in size at the Experimental Research and Practice Unit for Laboratory Animals of the University. Following the

adaptation to a standard diet for two weeks, they were randomly divided into two groups, as follows:

1) Group C (n=6): Control animals were maintained under standard conditions, with no radiation.

2) Group EMW (n=6): Males were exposed to an electromagnetic field daily for two months.

Animals receiving daily radiation routinely were exposed to a mobile phone, being operated at a 900 MHz EMW field (217 Hz pulse rate, 2 W maximum peak power, SAR 0.95 w/kg), as applied 10 min after feeding every day (up to 60 days). The phone was used as, calling (to another phone number) for 20 min and then kept in 'standby' position almost all the day (for 23 h 40 min daily). The reasoning for employing 20 min full-power radiation was simply mimicking the routine use of mobile phones in our daily lives. The battery was charged daily (with full-power of electricity) on a regular basis. During the use, the phones were placed 0.5 cm under the cages. Animals in Groups C were kept in a separate room to avoid any risk of exposure to the EMW radiation.

Herein, since our main objective was to investigate only the routine effects of EMW exposure emitted by mobile phones in daily use, no device was used for the analysis of radiofrequency (RF) spectrum applied. Thus, we did not make any further attempt to measure the actual intensity of electromagnetic radiation (EMR), [specific absorption rate (SAR)] emitted from the full-power device used. Details of EMR provided herein were simply obtained from the User's Guide provided by the private manufacturer (details not given).

Sample Collection

Animals were sacrificed by decapitation under the light ether anaesthesia. Testicles and epididymes were removed and measurements of testis weight (in addition to body weight) were made routinely (by a lab scale, sensitive at ±1 mg) along with the evaluations of epididymal sperm concentration, motility and morphology routinely.

Epididymal Sperm Motility, Concentration and Morphology

At the end of 60 days of experimental period, animals were sacrificed by decapitation and, to obtain sperm counts, the entire epididymes from sexually mature males were kept in PBS and incubated at 37 ^{o}C for 60 min. Epididymal sperm motility, number (concentration) and abnormal sperm rates were determined, as given below.

The percentage of progressive motility was evaluated by a light microscope, using the method described by Sonmez et al. (2005). For this process, a slide was placed onto the microscope stage and, allowed to warm up to 37 °C using a hot plate. A couple of droplets of Tris buffer solution (Tris-hydroxymetyl aminomethane 3.63 g, glucose 0.50 g, citric acid 1.99 g and distilled water up to 100 ml) were then placed onto the slide. A tiny droplet of fluid (obtained from the left cauda epididymis using a pipette) was placed into the solution and mixed. The percentage of motility was evaluated at a magnification of 400 ×. Estimations were performed on three droplets of each sample. The average of five successive estimations was used as the final motility (%).

Sperm numbers (expressed as x10^6 sperm/ml) were counted by a modified method of Yokoi et al. (2003). Briefly, the epididymis was minced by scissors into 5 ml of physiological saline, placed in a rocker for 10 min, and incubated at room temperature for 2 min. The supernatant fluid was diluted at 1:100 with a fixative/staining solution (containing 5 g sodium bicarbonate, 1 ml of 35 % formalin and 25 mg eosin per 100 ml of distilled water). Total sperm number was determined with a haemocytometer. Approximately, a 1 ml of diluted sperm suspension was transferred into each counting chamber and allowed to settle down for 5 min for assessment under a light microscope at 200 × magnification.

For determination of the percentage of morphologically abnormal spermatozoa, the method described by Atessahin et al. (2006) was used. Briefly, a couple of droplets from Tris buffer solution were placed onto a clean, dry and pre-warmed slide. A tiny droplet of epididymal fluid and two droplets of Indian ink stain were put into the solution and mixed for one min. A thin slide of the stained sample was then prepared with the aid of another pre-warmed slide. The slide was left immediately to dry off in a clean dust-free environment. After the preparation, slides were assessed under a light microscope at a magnification of 400 ×. Four hundred sperm cells were examined on each slide and abnormalities of spermatozoa were expressed as the percentage (%).

Statistical Analysis

Data from the body weight, testes weight, sperm motility, sperm number and abnormal sperm rates were analysed by analysis of variance (Regression Analysis) using MINITAB statistical software programme (Minitab 1996). Differences of means (mean ±SEM) between the experimental groups were considered significant, as calculated by using the least significant differences (when $P<0.05$) (Ergün and Aktaş 2009).

RESULTS

The results of body weights, testicle weights, sperm numbers, sperm motilities and abnormal sperm rates following the EMW exposure in adult male guinea pigs are all summarised in Table 1. Body weights of animals were 706.2 and 692.5 g in Groups C and EMW, respectively ($P>0.05$).

Table 1: Routine effects of EMW exposure in daily use (for up to 60 days) upon the testicle weight, sperm motility, number and abnormalities in adult male guinea pigs

Tablo 1: Yetişkin erkek kobayların günlük kullanımdaki (60 gün süreyle) EMA'a maruz bırakılmasının testis ağırlığı, sperm motilitesi, sayısı ve anomalileri üzerine rutin etkileri

Parameters	Groups		Statistics		
	C (control) (n=6)	EM (phone) (n=6)	F-ratio	P value	Significance
Body weight, g	706.2±23.9	692.5±23.4	0.72	0.406	NS
Testes weight, g	4.428±0.219	3.618±0.310	0.01	0.930	NS
Sperm motility, %	70.00±1.29	61.67±3.33	0.01	0.921	NS
Sperm number, x10^6/ml	799.17^b±6.08	549.6±35.8^a	5.28	0.031	P<0.05
Abnormal sperm, %	3.667±0.333	5.833±0.477	0.66	0.426	NS

^{a,b} Means (±SEM) having different superscripts within the same row are significantly different ($P<0.05$). NS: not significant ($P>0.05$).
^{a,b} Aynı satırda farklı harf taşıyan ortalama değerler (±SEM) arasındaki fark önemlidir ($P<0.05$). NS: önemsiz ($P>0.05$).

Considering all the parameters studied, there were only numerical, non-significant ($P>0.05$) differences between the groups based on the testicle weights (3.6 g vs. 4.4 g), sperm motility (61.7% vs. 70.0%), and abnormal sperm rates (5.8% vs. 3.5%) in EMW-treated and control males, respectively. However, there was a significantly ($P<0.05$) lower sperm numbers (549.6±35.8 x10^6 sperm/ml) in Group EMW as compared to those in controls (799.2±6.1 x10^6 sperm/ml).

DISCUSSION

Herein, we investigated routine effects of potentially harmful radiation (emitted by mobile phone, operated at 900 MHz) upon the reproduction in male guinea pigs in daily use (20 min calling then kept in standby position afterwards every day) over a period of 60 days of exposure. In brief, the present findings indicated that; the number of spermatozoa

was markedly lower in males exposed to the EMW field in routine daily use for 60 days.

In this study, testis weights of guinea pig males in EMW-exposed group were considerably lower than those in controls. In the literature, the effects of RF-EMW are studied largely both in animals and human semen *in vitro*. Numerous studies indicated that EMWs decrease the size of testis. Indeed, a smaller diameter of seminiferous tubules (Dasdag et al., 1999; 2003a) was reported in rats following the exposure to radiations. Likewise, Ozguner et al. (2005; 2006) demonstrated a decrease not only in seminiferous tubular diameter but also in their epithelial thickness of rats exposed to RF-EMW of 869 to 894 MHz. These reports confirm the previous findings of Saunders and Kowalczuk (1981), who observed that the microwave radiation (50 mW/cm^2 at 2.45 GHz) for 30-40 min resulted in a marked degeneration in the seminiferous epithelium in mice. However, there also exist some controversial reports. Indeed, a recent study by Ribeiro et al. (2007) and follow up study by Dasdag et al. (2003b) could not find such an adverse effect of mobile phones with a lower frequency (1,835-1,850 MHz) on rat testis. These studies may indicate some degree of dose-dependency of testicular damage against the EMW radiation in lab animals.

Herein, we observed relatively lower values of sperm motility in EMW-exposed animals as compared to those in controls. In the literature, the EMW-generated damages upon the plasma membrane (Jones et al., 1979), DNA (Shen and Ong 2000), and mitochondria (Koppers et al., 2008) of sperm cells have already been reported. However, parameters studied were only the routine semen traits in control and the EMW-exposed males. Hence, it would be too speculative to make any further consideration for elucidating the actual mechanisms underlying the low motility observed in the EMW-treated males.

Total sperm concentrations were found to be markedly ($P<0.05$) lower in EMW-exposed group, as compared to those in controls. Similarly, in human, such marked adverse effect of EMW exposure upon the sperm traits (including the concentration) was observed, especially when the phones used for longer duration (Agarwal et al., 2008) or kept at a closer position (Kilgallon and Simmons 2005). Furthermore, herein, males exposed daily to the EMW had numerically higher rates of sperm abnormality and lower sperm motility as compared to those in controls. Indeed, the most striking sperm abnormalities in the exposure group were seen along with considerably lower motility and numerous clumps of sperm cells. In a controversial report, Dasdag et al. (2003a) observed that there was no spermatogenic apoptosis following the EMW exposure in male rats. However, Yan et al. (2007) observed that there was a rapid increase in the number of dead sperm, thus leading to a marked increase in the number of abnormal sperm following the exposure to the EMW for 6 h daily over 18 weeks. These findings presumably suggest that carrying mobile phones close to the testes could have adverse effects, more or less, upon the male fertility.

Regarding the exposure time and other co-factors, Otitoloju et al. (2010), studying the head abnormalities of sperm in mice caused by base stations, observed that there was 39-46% of abnormality following 6 months of exposure period. Such a high proportion of head abnormality might be due mainly to the long duration of that study, inevitably leading to the excessive EMW exposure well before the onset of pre-meiotic phase of spermatogenesis (Odeigah 1997; Otubanjo and Mosuro 2001). Besides, such disorders may also occur because of excessive (high-density) chemical exposure and genetic reasons (Odeigah 1997). In our study, with a shorter exposure time (2 months), the rate of abnormal sperm was relatively higher in EMW-exposed group than those in controls. Furthermore, previous reports have found a link between the large doses of EMW and genetic defects. Indeed, findings of several studies sug-

gested that EMW fields alter the proliferation rate of cells as well as rates of DNA, RNA, and protein synthesis (Goodman and Henderson 1988; Fitzsimmons et al., 1992). Additionally, Aitken et al. (2005) reported some EMW-related changes in the mitochondrial DNA of epididymal germline. Furthermore, Tice et al. (2002) observed that the exposure to the RF radiations causes increased micronuclei formations in human blood cells. Likewise, Mashevich et al. (2003) also stated that RF radiations resulted in increased chromosomal instability and DNA breakage in peripheral lymphocytes in human. However, epidemiological evidences from effects of RF-EMW on carcinogenesis remain controversial (Kundi et al., 2004; Lahkola et al., 2008). All these findings and reports may imply that the excessive time of EMW exposure may exert varying degrees of undesirable effects upon different cell types of the body in various species.

Collectively, present findings suggest that; i) the electromagnetic radiation emitted by mobile phones, as used routinely in our daily life (20 min calling per day, then kept nearby in standby position), was consistently harmful, more or less, for testicle weight and epididymal sperm traits studied, and ii) the number of spermatozoa was the most profoundly (adversely) affected sperm trait in adult male guinea pigs exposed daily to the EMW radiation (900 MHz field) over 60 days.

To highlight the uncertainty regarding the ongoing concerns about the safety of radiation emitted by mobile phones, the future comparative investigations are warranted upon the numerous parameters of body systems, not only for the reproductive system but also for others (such as skeletal, nervous, digestive, vasculatory, and excretory systems) of lab animals. These investigations concerned should also be conducted using different protocols, that may vary in; i) the placement (position) of mobile phones, ii) doses of EMW emittance, and iii) durations of exposure, to be applied in both genders of species including human, i.e. volunteers, if appropriate (provided that the ethical concerns are met). Undoubtedly, other co-factors such as; i) the life style, ii) occupational (job) history, and iii) the RF exposure to other sources such as radio towers, personal digital assistants (PDA), bluetooth devices and computers, should also be considered before more reliable conclusions could be drawn for human health.

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