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Effects of GSM-like radiofrequency irradiation during the oogenesis and spermiogenesis of *Xenopus laevis*

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ABSTRACT

We aimed to evaluate the effect of GSM-like radiofrequency electromagnetic radiation (RF-EMR) on the oogenesis, and spermiogenesis of *Xenopus laevis*, and so the development of the embryos obtained from Normal Females+Normal Males (i.e. "N(F)+N(M)"); Normal Females+RF-exposed Males (i.e. "N(F)+RF(M)"); RF-exposed Female+Normal Male (i.e. "RF(F)+N(M)"); and RF-exposed Female+RF-exposed Male (i.e. "RF(F)+RF(M)". Various, assessments were performed to determine potential teratogenic effects and mortality, body growth and behavior on first generation embryos. After exposing adults frogs of both sexes to 900 MHz RF-EMR (at 1.0 W/kg) for 8 h a day over a 5-week period, the embryos' specific energy absorption rate (SAR) was calculated.

In our present study (control group; 2.2% abnormal, 0.0% dead); with the N(F)+RF(M) combination, the long-term exposure of adult males to GSM-like radiation at 900 MHz (RF: 2 W) for 5 week/8 h/day resulted in normal, abnormal and dead embryo ratios of 88.3%, 3.3% and 8.3%, respectively ($p < 0.001$). In the RF(F)+N(M) combination, long-term exposure (5 week/8 h/day) of adult females led to normal, abnormal and dead embryo ratios of 76.7%, 11.7%, and 11.7%, respectively ($p < 0.001$). And in the RF(F)+RF(M) combination, long-term exposure (5 week/8 h/day) of both adult males and females led to normal, abnormal and dead embryo ratios of 73.3%, 11.7%, and 15%, respectively ($p < 0.001$).

With the exception RF(F)+RF(M) group ($p < 0.001$), no significant changes were observed on body growth (lengths) in comparison to the control group. It was also observed that the offspring of female adult *Xenopus exposed* to RF-EMR during oogenesis exhibited a more aggressive behavior compared to the control group. Cell phones radiation can thus lead to detrimental effects in humans' male and female reproductive cells.

1. Introduction

Nowadays, mobile phones are an inseparable part of daily life (Hamada et al., 2011), and used by everybody all around the globe (Mailankot et al., 2009; Grigor'ev, 2012). The number of cell phone subscriptions have reached 5 billion in 2011 (IARC), and 6 billion in 2014 (Mortazavi et al., 2014). RF-EMR exposure levels in populations worldwide have consequently increased (IARC, 2011).

In this context, there are growing concerns regarding the possible detrimental effects on health of RF electromagnetic waves (EMW) (Deepinder et al., 2007; Sepehrimanesh et al., 2014). The EMW emitted by mobile/cell phones are generally within the 0 Hz to 300 GHz frequency range (Boga et al., 2015). Most cell phones operate between frequencies of 850 and 1800 MHz (La Vignera et al., 2012). The legal limit for specific absorption rate (SAR) is currently accepted as 2.0 W per kilogram of body mass (ICNIRP, 1998), while most cell phones have an SAR of about 1.4 per kilogram of body mass (Agarwal et al., 2011, Adams et al., 2014). Human tissues absorb radiating energy through the aerial effect and/or the coupling of RF signal and/or resonant absorption (La Vignera et al., 2012). The World Health Organization's (WHO's) International Agency for Research on Cancer (IARC) describes RF-EMR as a form of radiation possibly carcinogenic (Group 2B) (IARC, 2011; Boga et al., 2015). Electromagnetic fields are also a kind of pollutants that can have negative effects on health and the environment (Lee and Yang, 2014). Environmentalists even describe EMFs as the "fourth type of pollution" after noise, water and air

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pollutions (Abdulrazzaq and Aziz, 2013). To date, there is still no consensus concerning the potentially harmful effects of radio-frequency electromagnetic radiation (RF-EMR) from mobile/cell phones (La Vignera et al., 2012). Although RF-EMR has been the subject of extensive animal and epidemiological studies for over a decade, there are still no conclusive evidence on how tissues, organs and overall health is affected by RF-EMRs that are emitted through mobile/cell phones (Leszczynski, 2004, Sepehrimanesh et al., 2014, Lee and Yang, 2014).

The various suggested detrimental effects associated with cell phone RF-EMR include cancer (Hardell et al., 2005; Little et al., 2012), higher blood pressure (Braune et al., 1998), and negative impacts on memory, learning, and stress (Aboul Ezz et al., 2013). Numerous RF-EMR studies indicate negative effects on male fertility: decreased spermatogenesis and disturbed reproductive hormone levels (Sepehrimanesh et al., 2014), decreased sperm quality (Adams et al., 2014), infertility (Deepinder et al., 2007), increased oxidative stress, decreased sperm count and motility, (Fejes et al., 2005; La Vignera et al., 2012; Mailankot et al., 2009), and the integrity of germ cells (Atasoy et al., 2013). Even if genotoxic effects on epididymal spermatozoa have limited effect on the development of germ cells in males, RF-EMR may also negatively affect spermatozoa at the epididymis (Aitken et al., 2005).

Amphibians are considered by many biologists to be excellent bioindicators of the health of an environment (Fort et al., 2000; Boğa et al., 2009; Balmori, 2006). The loss of global biodiversity, after analyzed populations of 5743 amphibian species in the world concluded that 1856 (%32) of them considered threatened of extinction proposed that amphibians together with other organism is a part of the global biodiversity crisis (Fort et al., 2000; Balmori, 2006, 2010; Boğa et al., 2009). Balmori (2006) proposed that electromagnetic pollution along with other environmental factors is a possible cause for decline and abnormalities of some wild amphibian populations exposed (Levengood, 1969; Balmori, 2006). Amphibians make external insemination and therefore, their eggs directly exposed to chemical and radiation (Balmori, 2006), the gastrula stages of their development appeared to be the most sensitive to such exposure (Levengood, 1969).

In this study, we used the Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX) technique) i.e. a screening assay used to determine and evaluate agents that are potentially toxic for human development (NTP, 2000). During the first 96 h of development, *Xenopus laevis* and human embryos are very similar, especially with respect to the development of their organs. In this context, FETAX provides an efficient method for determining and evaluating the teratogenic and toxic effects that different agents might have on human embryos (Boga Pekmezekmek et al., 2013; EPA, 1998b).

We here used this model system and exposed adult *Xenopus* to RF-EMR during their oogenesis and spermatogenesis stage in order to evaluate the effects on the offspring of these waves.

2. Materials and methods

The study methods were performed within the frame of the requirements listed in Standard Guide of the American Society for Testing Materials (ASTM, 2004).

2.1. Test substances

The human chorionic gonadotropin (hCG, Pregnyl, 5000IU) was provided by Organon (Turkey). The follicle-stimulating hormone (FSH) was provided by Serono (Turkey). The De Boers Tris (DBT) reagent and FETAX solution were both obtained from Sigma (USA). Embryos were maintained in FETAX solutions during FETAX test.

2.2. Assay methods and test organisms

Xenopus laevis (the African Clawed Frog) is a model organism largely used in animal studies, particularly in studies evaluating embryonic development. Breeding and embryos collection procedures were carried out according to ASTM 1439-98 (ASTM, 2004). The frogs were kept at 23 °C ($\pm$ 1 °C) in 95 × 60 × 44 cm aquaria, with 12 h light and 12 h dark (Boga Pekmezekmek et al., 2013), and the frogs were feed ad libitum.

2.3. In vitro fertilization

The fertilization of eggs was performed in vitro according to Lindi et al. (2001). In all the experiments, the females at a maximum dose of 35 IU three days before eggs were required. On the third days following this administration, hCG was injected to the females at a dose of approximately 700–1000 IU. After the latter injections, female *Xenopus laevis* laid their eggs on petri dishes. Shortly afterwards, suspensions of *Xenopus* sperm were applied to inseminate the eggs. The sperm suspensions were obtained by mincing *Xenopus laevis* tests in 1–2 mL of DBT (1.8 nM $CaCl_2$, 15 nM Tris–HCl, 119 nM NaCl; 7.5 pH); the DBT solution was kept cold while performing the mincing process. Two minutes after applying the sperm suspension, 10 mL of FETAX solution (96 mg/mL NaHCO3, 60 mg/mL $CaSO_4 \cdot 2H_2O$, 30 mg/mL KCl, 625 mg/L NaCl, 15 mg/mL $CaCl_2$, and 70 mg/mL MgSO4; 7.8–8.0 pH) was placed in all of the petri dishes. A few minutes afterwards identified the effectively inseminated eggs on the basis of the animal pole's orientation inside the eggs i.e. an upward orientation indicated successful insemination. Eggs. exhibiting non-uniform segmentation were excluded from the study. Using the Normal Tables, embryos between the midblastula and early gastrula (stage 8 to stage 11) were selected. The FETAX procedures were performed on these embryos (Boga Pekmezekmek et al., 2013; Nieuwkoop and Faber, 1994).

2.4. FETAX procedure

We determined the ratio of normal embryos according to ASTM (2004) Standard Guide; We separated adult Xenopus in control (3 females, 3 males) and assay groups (9 female, 9 male) and then performed IVF protocol to couples of *Xenopus laevis*. Previous studies have shown after inducing ovulation with human chorionic gonadotropin (hCG) stimulation seven weeks are necessary for repopulating the ovary with oocytes in stage VI. So, five to seven weeks are required for the oocytes' progress into the ovulatory stage (Keem et al., 1979; Smith,1955). For spermatogenesis, 36 days are required: cells spend four days in the leptotene, six days in the zygotene, twelve days in the pachytene, one day in the diplotene, one day in meiotic division, and 12 days in spermiogenesis (Kalt, 1976).

During repopulate ovary and sperm, assay groups were irradiated for 5 weeks with 900 MHz RF(2 W) during 8 h each day. During such irradiation, female and male were kept in different aquaria (390 × 550 × 350 mm) containing 3.5 cm water depth.

Step I: *Control group* – 3 Female and 3 male adult Xenopus maintained in aquarium without irradiation.
Step II: *Irradiated group.* 9 female and 9 male (each gender in different aquaria) irradiated with 900 MHz (2 W) during 5 week/8 h/day.
Step III: *Test*, made under three different combinations:

First combination – Control female and male Xenopus were applying IVF protocol and obtained embryos maintained in petri dish for 96 h.