Chapter

Effects of Electromagnetic Field on the Development of Chick Embryo: An In Vivo Study

Najam Siddiqi and Nasser Al Nazwani

Abstract

This study was conducted to explore the effects of electromagnetic waves on a developing chick embryo. The radiofrequency electromagnetic waves (RFW) emitted by different smart phones was measured by using a TriField meter. Chick fertilized eggs were placed in an egg incubator, divided into control and exposed groups. In the exposed group, a mobile phone was placed inside an incubator in call receiving mode, while in the control group, the mobile phone was not used. Studies were conducted at low and high exposure (dose) of RFW. Chick embryos were sacrificed at day 10 and day 15, and embryos were examined for mortality, gross malformation, weight, and length. Histology, electron microscopy, and Hsp 70 of liver were done for the high dose group. No mortality was observed in the low dose group; however, in the high dose group, the mortality was 14%, and deformities of the limbs and skin abnormalities were observed. Weight and length in the exposed groups were significantly lower than the control at higher dose. Histology and ultrastructure of liver revealed fatty infiltration, increase number of mitochondria, deformation, and disappearance of its cristae. Hsp 70 and mRNA levels were elevated in the exposed groups for high dose group.

Keywords: electromagnetic waves, mobile phone, chick embryo, liver, fatty change, mitochondria, ultrastructure, mortality, gross morphology, histology

1. Introduction

Cellular technology and broadband services are growing rapidly, resulting in a dense suffusion of nonionizing low radio frequency electromagnetic waves (RFW) in the atmosphere. This adds a new dimension to environmental pollution. Radiofrequency electromagnetic radiation is a form of energy used in wireless communication and emitted from mobile sets which can be absorbed into body tissues and converted into heat.

These waves were initially thought to be harmless to the humans; however, now scientific research has revealed that these waves may cause damage to the living cells. Smart phone emits radio waves while in use which include downloading data from the internet [1, 2]. Fetus and children are more radiosensitive than adults due to the presence of embryonic stem cells [3–7]. A child born in this era will start to be exposed to electromagnetic waves exposure as soon as he is born and will remain in this environment until he dies. Divan et al. reported behavioral problems in children who were exposed to prenatal and postnatal cell phone [8, 9]. This potential

hazard is more serious in developing countries, where the reported prevalence of mobile phone use by adolescents is more than 90%. Oman is ranked 10th in the world after Saudi Arabia, Russia, Kuwait, and Panama [10, 11]. Excessive use of mobile phones and base stations in the vicinity of residential areas are linked to symptoms such as headaches, sleep disturbances, lack of concentration, dizziness, memory loss, and increased risk of cancer were first reported as "Microwave sickness" in 1978 [2]. The International Agency for Research on Cancer, classified RFW as a "possible human carcinogen" which is published in "Agents Classified by the IARC Monographs", Volumes 1–109 (http://monographs.iarc.fr/ENG/Classification/Classifications Alpha Order.pdf). An increase incidence of thyroid cancer in South Korea and gliomas in Sweden were recently reported, and excessive use of mobile phones was held responsible to be a possible cause [12, 13]. Childhood leukemia in children is another disease due to exposer to RFW. Functions of the central nervous system [14], permeability of the blood brain barrier [15], and melatonin synthesis [16] are also affected.

To further study the effects of radio waves, the chick animal model has been used in the past. Laboratories have reported a high mortality of chicken embryos and malformations when exposed to electromagnetic waves emitted from mobile phones. The phenomenon was reported to be dose dependent [17–23]. The embryonic cells rapidly proliferate, differentiate, migrate, and suffer from apoptosis. During these processes, the cells generate electric currents which can be affected by the RFW [24]. This study was designed to observe the effects of electromagnetic field emitted by a mobile phone on rapidly proliferating cells in the developing chicken embryo.

1.1 Hypothesis

Rapidly proliferating and dividing cells are affected by RFW exposure, and this may be a dose dependent phenomenon.

2. Mobile phone RFW classification

The TriField meter revealed different intensities of electromagnetic waves from different mobile sets. The intensity of RFW was divided into four groups as follows: group 1: 0.01–0.1 mW/cm^2, group 2: 0.1–0.2 mW/cm^2, group 3: 0.2–0.1 mW/cm^2, and group 4: >1 mW/cm^2.

All the old mobile sets were placed in group 4 showing the highest levels of radiation (>1 mW/cm^2) which is recommended dangerous to health. Mostly, the smart phones were in groups 1 (0.01–0.1 mW/cm^2), 2 (0.1–0.2 mW/cm^2), and 3 (0.2–1 mW/cm^2), but few in group 4. It was further observed that downloading from the net using WiFi also results in high levels of radiations (**Figure 1**).

3. Low dose (total 200–300 minutes)

3.1 Methods

This is an animal experimental research study using "Cobb" (*Gallus gallus domesticus*) breed zero-day fertilized chicken eggs. These eggs were provided by Sohar Poultry Company S.A.O.G. (PO Box 2808, Ruwi, Postal code 112, Sultanate of Oman). 120 fertilized eggs were used for this experiment. 60 eggs were randomly divided into either the control or the exposed groups.

Figure 1.
TriField meter measuring the electromagnetic field exposure when the phone is receiving a call (the needle moving toward the right with increasing intensity of the electromagnetic waves) (a) group 1, (b) group 2, (c) group 3, (d) group 4, and (e) during downloading, the needle is hitting extreme right.

A 30-egg incubator Model EH-35, Sino-PFE Company, China was used which is equipped with temperature and humidity control and forced air ventilation; temperature was programed at 37° centigrade and humidity of 50–60% (**Figure 2a**). The experiment was run in batches of 30 eggs, due to the size of the incubator. The eggs were placed in egg holders which were programed to rotate 10 times/day. A mobile phone (power of 0.47 W/kg body and SAR 1.10 W/kg) and local service provider with 1800 MHz frequency were selected. A TriField Meter, model 100XE was used to confirm the emission of electromagnetic field of the mobile phone during the experiment (**Figure 2b**).

3.1.1 Exposed group

The selected mobile phone was placed in the center of the incubator in silent mode and vibration disabled. The farthest egg was within a radius of 16 cm. This

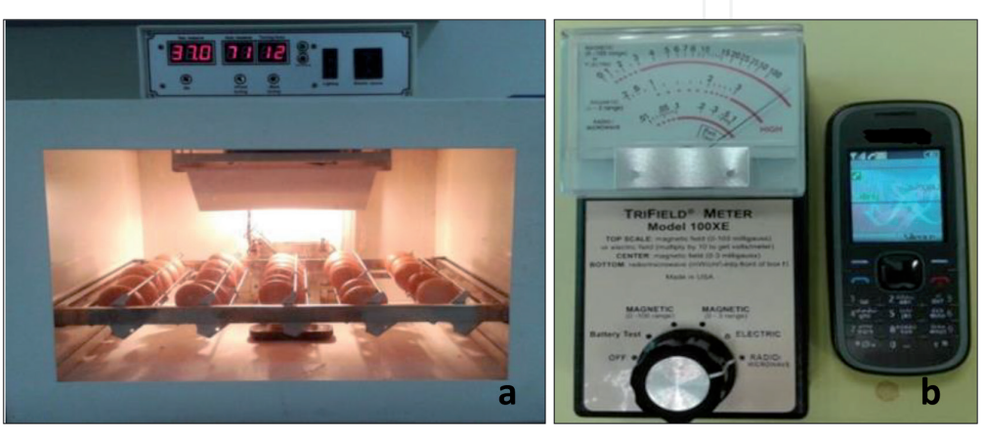

Figure 2.
(a) The egg incubator. (b) TriField meter showing high levels of electromagnetic waves transmitted from the mobile during call receiving (≥1 mW/cm²).

is important because the radius of single 1800 MHz frequency electromagnetic wavelength is approximately 16.5 cm [21].

The mobile phone placed inside the incubator was called from outside 4 times a day and each call lasted for 5 minutes. There was a gap of 4 hours in-between each call with a call free period during the night. Total exposure time/day was 20 minutes. To repeat the call on the same time every day, a ringing schedule was made, and call was recorded once the call was made. This prevented errors in making the calls. The experiment was conducted in batches of two with 30 eggs in each batch. A total of 10 embryos were scarified each at day 5 (exposure time 100 minutes), day 10 (exposure time 200 minutes), and day 15 (exposure time 300 minutes).

3.1.2 Control group

It was also conducted in batches of two. There was no mobile placed inside the incubator, and the eggs were not exposed to any electromagnetic field.

A total of 10 randomly selected eggs were removed from the incubator in both the groups at day 5, day 10, and day 15 each, the shells were removed, and the embryos were dissected from the membranes. Mortality of the embryos was observed by observing the movements in the limbs or beating of the heart. Hamburger and Hamilton developmental stages were used to assess the embryos for gross morphological abnormalities [25]. The embryos were washed in normal saline, blotted dry with tissue paper and the dependent variables, the weight, CR-length, and eye diameter were recorded.

A digital balance with a precision of 0.01 g (Universal Impex HA-3202) was used for measuring the weight, and a caliper (Mitutoyo Vernier calipers, Nanjing Sulang Trading Co., Ltd., China) was used to measure the length which was the length from the vertex to the tip of the coccyx and the maximum eye diameter.

For statistical analysis, student's t-test was used, and a p-value of <0.05 were considered as significant. All data were presented as the mean value ± SEM.

3.2 Results

Mortality was not observed among both the control and the exposed groups, and all 120 eggs revealed developing embryos.

3.2.1 Day 5

No morphological deformities were observed in both the groups. There was no significant difference between the average wet body weight in the exposed group (0.189 ± 0.035 g) and the control group (0.209 ± 0.031 g); ($t = 1.67$, $df = 28$, $p < 0.11$). The CR-length and eye diameter were not measured at day 5.

3.2.2 Day 10

Gross malformation was absent in both the groups. In the exposed group, small hemorrhages were apparent under the skin with areas of paler skin indicating reduced blood flow (**Figure 3b**). In the control group, the skin was pink in color throughout (**Figure 3a**). The average weight of the exposed group (1.572 ± 0.38 g) was significantly lower than the weight of the control group (2.331 ± 0.27 g), $t = 8.19$, $df = 48$, $p < 0.01$ (**Figure 5a**). This trend was also observed with the C-R length and eye diameter; all showing significant differences (**Figure 5b** and **c**).

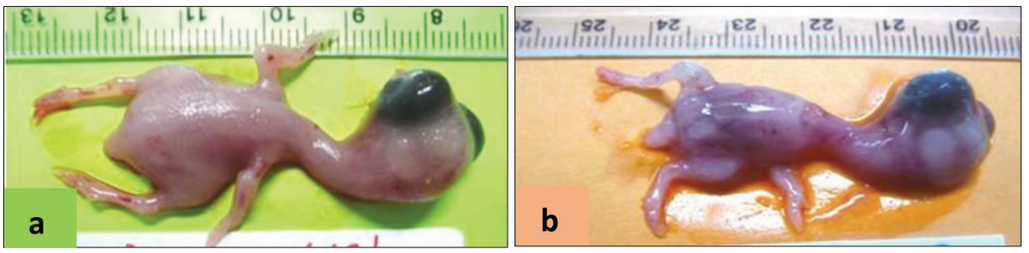

Figure 3.
Low dose: (a) day 10: control group showing normal embryo development. (b) Experimental group: embryos were smaller in size than the control group, and marked hemorrhagic areas could be seen under the skin alternating with pale areas.

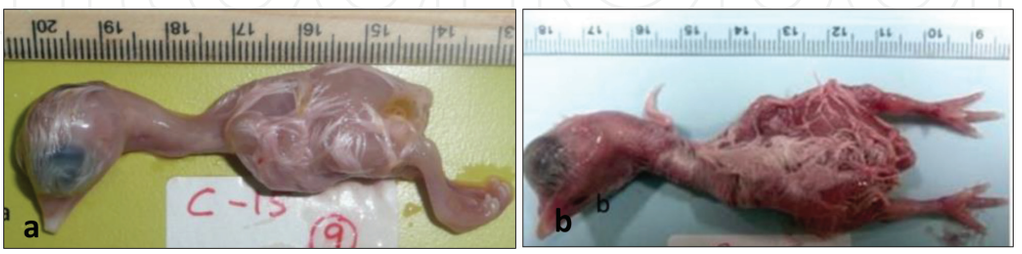

Figure 4.
Low dose: (a) day 15: control group showing well developed embryo with no anomalies or deformities. Skin pink in color, covered by feathers, well-formed upper and lower extremities and normal toes and eyes completely covered by eye lids. (b) Experimental group: no anomalies or deformities were observed and embryo showing same features as revealed in the control group.

3.2.3 Day 15

Gross appearance in both the groups revealed well-formed skin covered by white feathers and well-formed beak. The size of the wings and the limbs was increased, and toes can be seen well formed, middle toe the longest. The head size that was

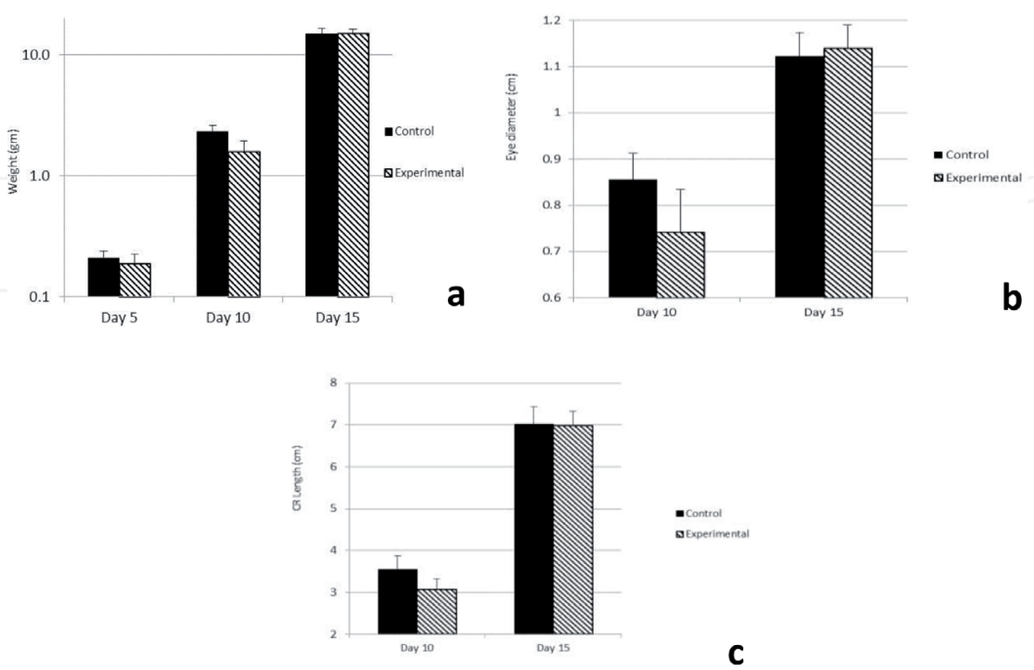

Figure 5.
(a) Wet body weight of the chick embryo: experimental and control groups at days 5, 10, and 15 showing significant different at day 10 ($p < 0.01$). (b) Eye diameter was significantly smaller in the exposed group than the control group at day 10 ($p < 0.01$). (c) C-R length of the chick embryo in the experimental group was significantly smaller than the control group at day 10 ($p < 0.01$).

much bigger as compared to the body before was now become smaller, which was in accordance with the normal development of the embryo. The eyes were now fully covered by eye lids. The skin no longer shows any hemorrhage areas, and blood vessels no longer visible under the skin (**Figure 4a** and **b**). No significant differences were observed in either average wet body weight, control (14.91 ± 1.73 g) and exposed group (14.82 ± 1.57 g), eye diameter, control (1.123 ± 0.051 cm and exposed 1.14 ± 0.05) and the average C-R lengths, control (7.013 ± 0.41 cm), and the exposed groups (6.978 ± 0.348 cm) (**Figure 5a–c**).

4. High dose (total 500–750 minutes)

4.1 Methods

A total of 20 fertilized eggs were incubated in the incubator with the mobile phone in silent mode with the vibration mode disabled. The distance of all the eggs from the mobile phone was maintained within one wavelength (approximately 16.5 cm) of the emitting 1800 MHz frequency electromagnetic waves [21]. The mobile phone was rung from another mobile phone for 5 minutes, 10 times daily with an exposure-free period in between the calls. No calls were made at night. The total daily exposure duration was 50 minutes in each 24 hours starting from day 1. The eggs were sacrificed at day 10 (maximum exposure time 500 minutes) and day 15 (total exposure time 750 minutes). For the control groups, another 20 eggs were placed in the incubator, and 10 eggs were sacrificed at day 10 and 15 each.

Control group's 20 eggs were incubated at the same conditions in the same incubator. The mobile phone was turned off, battery removed, and placed in the middle of the incubator. The embryos were examined just as in the experimental groups at days 10 and 15.

On the scheduled day of sacrifice, mortality, gross morphology, wet weight, and length of the embryos were measured. Liver was dissected and placed in 10% glutaraldehyde solution and stained by toluidine blue stain for histological and ultrastructural preparation. Another five specimens were fixed in RNAlater solution (Invitrogen) for heat shock protein 70 (HSP70) and messenger ribonucleic acid (mRNA).

4.2 Results

The results revealed 14% mortality in the exposed group. Gross morphology of the chick at day 15 showed deformities of the limbs, hemorrhages under the skin, lack of feathers, and few anterior abdominal wall defects (**Figure 6a** and **b**). The wet weight and head to toe length were significantly less in the exposed group at both day 10 and 15 (**Figure 7a** and **b**).

4.2.1 Histology: control group

At day 10, hepatocytes were seen with rounded central nucleus and nucleoli. They were lying in rows with spaces in-between to form the sinusoids. Central veins with few RBCs and portal areas were observed. At day 15, typical structure of the liver was apparent. Well-formed hepatic lobules formed by rows of hepatocytes and sinusoids lining with epithelium and large number of RBCs were clearly seen (**Figure 8a** and **b**).

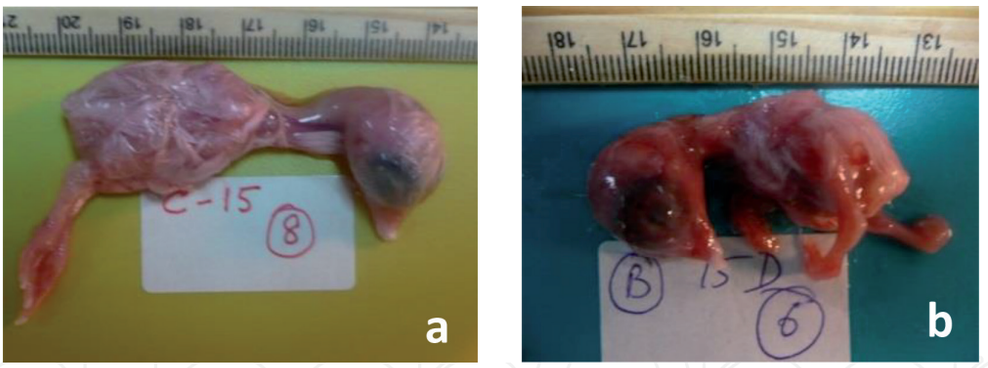

Figure 6.
High dose: (a) day 15: control group showing normal embryo development. (b) Experimental group: embryo was small in size, has marked deformities of the limbs, hemorrhages under the skin, and no growth of the feathers.

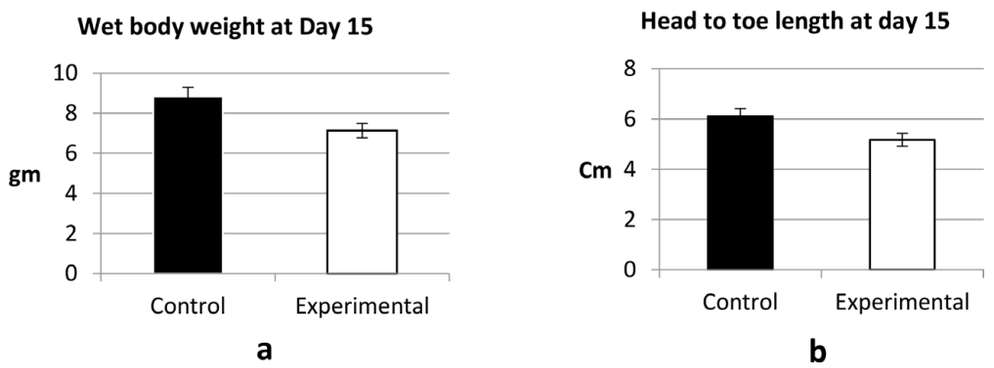

Figure 7.
High dose: (a) wet body weight of the chick embryo: experimental and control groups at days 15 showing significant different (8.84 vs. 7.13 g) (p = 0.29). (b) Head to toe length of the chick embryo in the experimental group was significantly smaller than the control group (6.11 vs. 5.17 cm) at day 15 (p = 0.25).

4.2.2 Exposed group

At day 10 and day 15, nucleus in many hepatocytes was not seen or pushed to the side and without prominent nucleolus. The hepatocytes were seen in rows with sinusoids in-between; however, marked infiltration of the fat vacuoles was observed in the cytoplasm of hepatocytes. The sinusoids were formed showing lining epithelial cells and RBCs. This signifies the beginning of fatty change (**Figure 8c** and **d**).

4.2.3 Electron microscopy: Control group

At day 10 and 15, the control group showed hepatocytes arranged in rows forming the hepatic lobules and sinusoids in-between the rows lined by simple squamous epithelium (**Figure 9a**). There was a big central rounded nucleus with central chromatin, surrounded by double layered nuclear membrane with pores. Cytoplasm contains many mitochondria with well-arranged cristae, rough endoplasmic reticulum, ribosomes, and some glycogen vacuoles. Sinusoids were formed lined by single layer of simple squamous epithelium, and few Kupffer cells were also found lining the sinusoidal wall. Spindle-shaped RBCs with oval nucleus were present in the sinusoids. The canaliculi were seen clearly in between the hepatocytes (**Figures 9a** and **10a**).

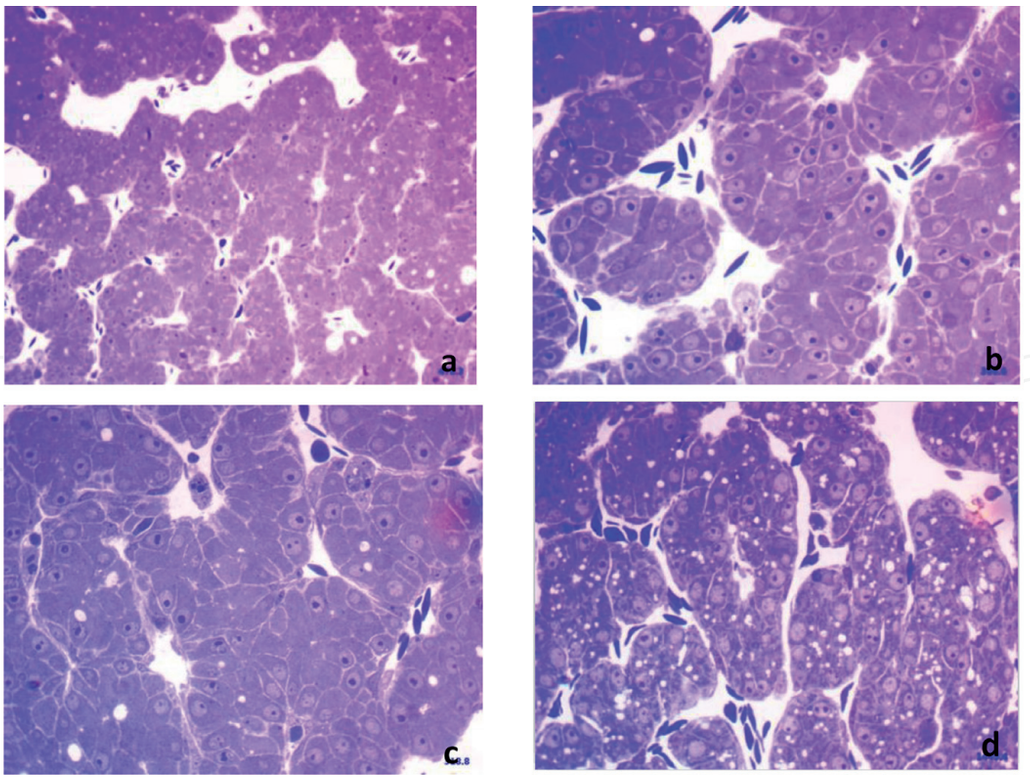

Figure 8.
Control group: (a) day 10 showing developing hepatocytes and sinusoids in between with RBCs and (b) day 15 showing well-formed hepatic lobules, normal hepatocytes arranged in rows, forming central vein, sinusoids lined by epithelial cells, and scattered well-formed RBCs inside the sinusoids. Exposed group: (c) day 10 showing infiltration of few lipid vacuoles in the hepatocytes and few necrotic hepatocytes and (d) day 15 showing marked infiltration of lipids causing necrosis of the hepatocytes.

4.2.4 Exposed group

At day 10, marked increase in the number of mitochondria can be observed (**Figure 9b**). Some of the mitochondria were swollen and surrounded by rough endoplasmic reticulum. The nucleus was in the center and circular in shape. Sinusoids were filled with red blood cells (RBC) and lined with simple squamous epithelium and Kupffer cells. At day 15, cytoplasm showed numerous lipid filled vacuoles

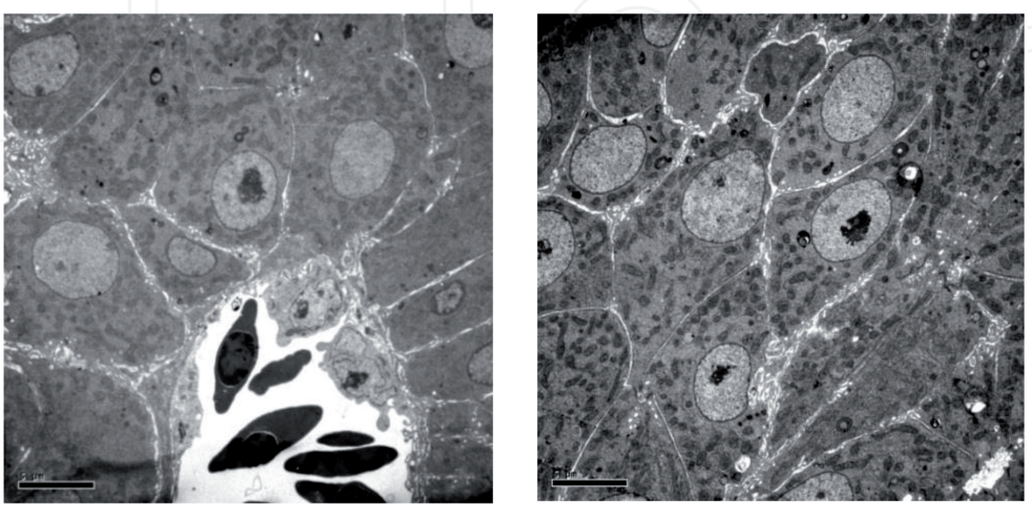

Figure 9.
Day 10, (a) in control group, polygonal hepatocytes arranged in row with large central round nucleus and showing sinusoids with multiple red blood cells, and canaliculi can also be seen in-between the hepatocytes. (b) Exposed group revealed marked proliferation of mitochondria and widening of the canaliculi.

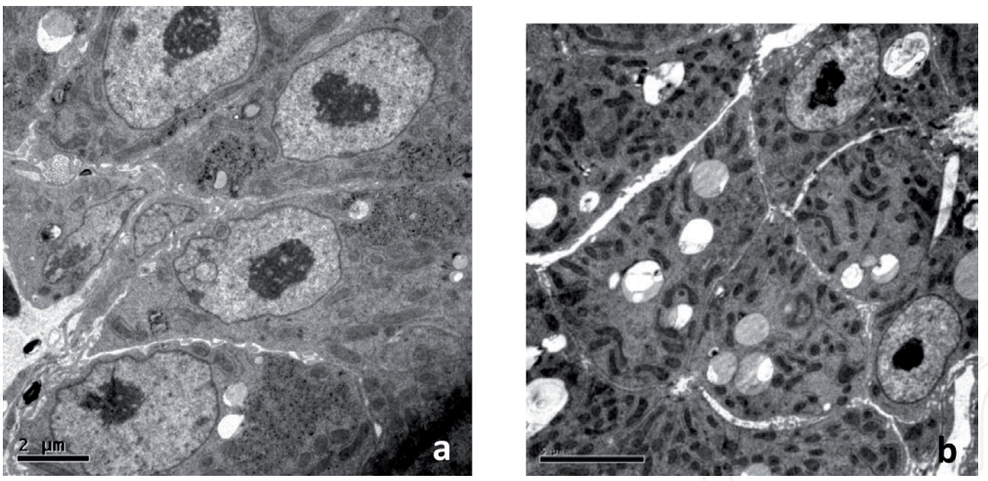

Figure 10.
Day 15, (a) control group showing hepatocytes arranged in rows, with few lipid vacuoles and normal looking canaliculi and (b) exposed group revealed mark infiltration of lipid vacuoles in the cytoplasm, and widening of the canaliculi was apparent.

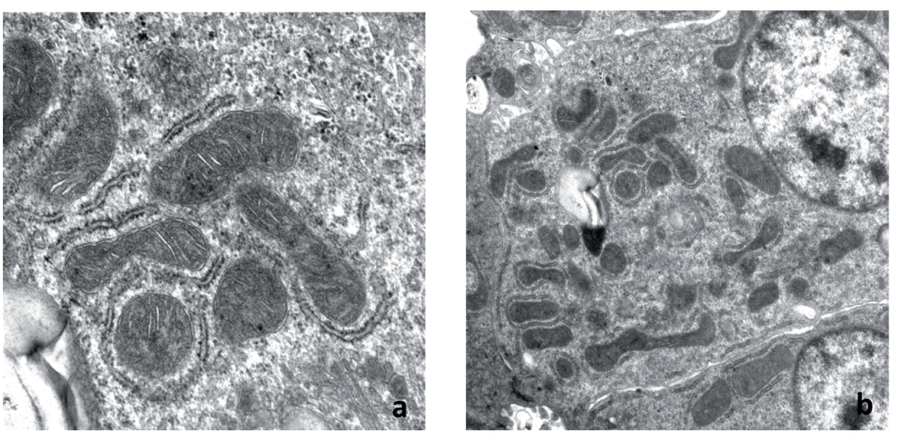

Figure 11.
(a and b) Day 15, exposed group: mitochondria showed change in shape, some become rounded, while others dumbbell shaped and surrounding by rough endoplasmic reticulum. Cristae were not clearly visible, and some areas of degeneration were apparent.

(**Figure 10b**). At day 15, mitochondria became electron dense, some were swollen, while others were elongated and dumbbell shaped. At places, mitochondria was degenerated and cristae invisible (**Figure 11a** and **b**). A prominent layer of rough endoplasmic layer around the mitochondria could be seen which was interrupted. Myelin-like figures can also be seen in the cytoplasm at day 15 in exposed groups. The canaliculi in-between the hepatocytes have been widened (**Figure 9b**).

4.3 Biochemical analysis

4.3.1 Heat shock protein 70

Homogenized sample using an automated homogenizer in a phosphate buffer at pH 7.6 was ultracentrifugated to separate contaminants and soluble proteins and cells. Messenger RNA (mRNA) was extracted from 40 tissue samples using the Qiagen RNeasy Mini Kit (QIAGEN, CA, USA) according to the manufacturer's instructions.

Next, DNAse treatment was given to the extracted mRNA using DNA-free™ DNA Removal Kit (Thermo fisher) according to the manufacturer's protocol. After which, cDNA was synthesized using *DNase treated RNA with the help of* High-Capacity cDNA Reverse Transcription Kit (Applied BioSystems, Austin, TX).

Quantitative RT-PCR was performed for the analysis of cDNA obtained from the 40 samples. TaqMan reagents are used to perform qRT-PCR according to manufacturer's protocol. The relative expression level of *hsp70* was calculated using the comparative delta Ct method by normalizing the cycle threshold values of *hsp70* with those of GAPDH.

Protein extraction was carried out through differential centrifugation to obtain separate subcellular fractions of protein from hepatocytes.

Extracted proteins were analyzed through SDS-PAGE and one step ELISA. HSP70—ab187399 Simple Step ELISA® Kit was used for the detection of HSP70 protein in the control and experimental samples.

4.3.2 Results

Different exposure durations displayed significantly different levels of HSP 70 mRNA compared to the normal. This increase in mRNA was 14% at day 10 and became 39% at day 15 (**Figure 12**). The amount of HSP70 protein has increased with longer exposure time although it did not prove strong correlation with the

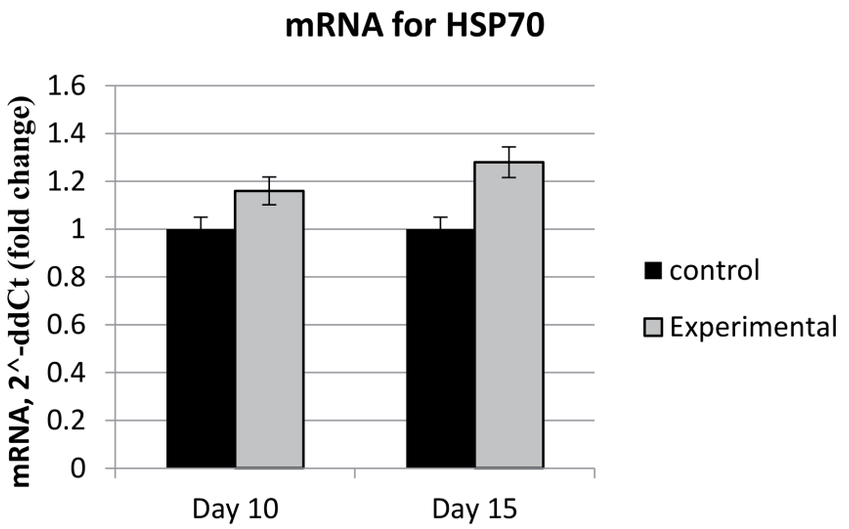

Figure 12.
mRNA for HSP70 for liver tissue at day 10 and day 15 in the control and experimental groups.

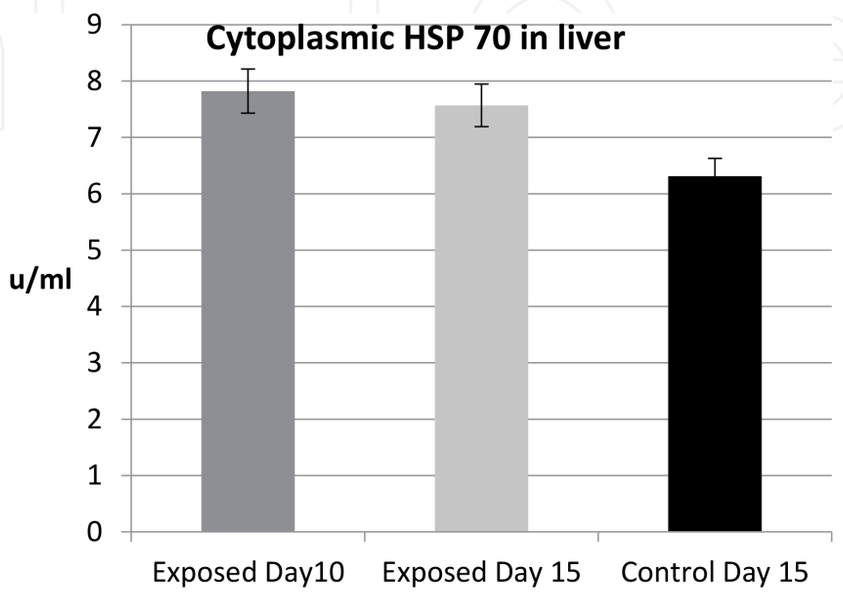

Figure 13.
ELISA graphs showing increase in the cytosolic HSP70 in the control and exposed groups in liver at day 10 and day 15.

expressed mRNA quantities (**Figure 13**). The total cellular protein represented by the cytoplasmic protein concentration was increased in treated samples than in untreated samples demonstrating cellular mechanism in attempting to express housekeeping genes and HSP70 for survival. Organs have shown differences. Our results indicate that HSP 70 mRNA is over expressed in an attempt to synthesize more of the HSP70 in order to resist electromagnetic waves irradiation.

5. Discussion

5.1 Measuring mobile phones' RFW strength

To confirm the emission of RFW from different mobile sets and to understand its strength, we decided to measure the RFW from different mobile sets using a TriField meter. It was found that the old mobile sets were emitting very high intensity of RFW which the TriField meter showing above 1 mW/cm^2. Absolute hazard thresholds have not been established yet; however, studies suggest that RFW above 0.1 mW/cm^2 may not be safe. Our study revealed that most of the smart mobile sets are emitting RFW above 0.1 mW/cm^2. According to our classification, only group 1 comes under 0.1 mW/cm^2; groups 2, 3 and 4 are all above this threshold.

5.2 Mortality

In the low dose groups (exposure time 20 minutes/day at 1800 MHz), no mortality was observed in either the control or experimental groups. Batellier et al. also reported a mortality rate of less than 1% when fertilized eggs were exposed to 900 MHz from day 7 to 14 [13].

In the higher dose group, the mortality rose to 14% in the exposed group, while no mortality was present in the control group. About 54% mortality was reported by Youbicier-Simo in the exposed group and 14% in the control group. He used continuous mobile phone exposure during the embryonic life of 21 days [14]. Higher mortality was also reported by Jyoti et al. in the exposed group. He highlighted that increased exposure duration and higher power (20 dBm) must have increased mortality in the exposed group [15].

5.3 Gross malformations

In the low dose group, the most significant finding in this study was growth retardation of the embryo at day 10 only; body weight, CR length, and eye diameter were all significantly decreased at day 10 in the exposure group. However, they were indistinguishable from the control group at both day 5 and day 15. No gross malformation was observed except subcutaneous hemorrhagic areas under the skin at day 10.

Cox et al. reported no malformations when he exposed the fertilized eggs to 50 Hz [16]. Al Qusdi et al. using 900–1800 MHz electromagnetic waves and by ringing 4 times for 15 minutes/day (60 minutes/day) reported significant increase in body weight and length at day 10, which could not be sustained at day 14 [17]. Gross malformation in the brain and retina was also reported; however, the incubator used in that study was not an egg incubator, which could have negative effects on the development of the embryo [17].

In high dose group in this study, 14% mortality, limb deformities, and changes in the skin feathers were observed. There was growth retardation of the embryo at both day 10 and day 15. Other studies also reported growth retardation [18]. Other malformations reported by Lahijani et al. such as spina bifida, monophthalmia,

microphthalmia, anophthalmia, exencephalic embryos, and embryos with asymmetrical faces, crossed or shorter beak, and gastroschisis were not found in this study [18]. In an extensive study done by Farrell et al. at five different laboratories and using 60 Hz magnetic field (pulsed and sinusoidal by a Tenma function generator) over 2500 chick embryos, did not show significant difference in the mortality for 48 hours; however, over the period of time, malformations were 6.8% in the exposed group compared to 1.8% in the control; most common anomaly reported was the neural tube defects [19].

Ubeda et al. observed in a study using 100 Hz and electromagnetic field intensity between 0.4 and 104 microTeslas (μT) that the chick embryo is sensitive to electromagnetic fields at extremely low frequency and intensity. He further stated that pulse shape may influence the development of embryo development [20].

Effects of electromagnetic waves on living cells are dose and duration dependent [21]. It is likely that growth retardation of the chick embryo in exposed groups was a result of interference in the proliferation of the multiplying embryonic cells due to the RFW exposure. Development of the embryo is a complicated process which includes cell proliferation, differentiation, relocation, and programed cell death. These events involve endogenous ionic currents and electric fields which could be disturbed by the RFW exposure. Growth retardation in the exposed group is most likely due to the adverse effects of RFW on the DNA. Production of heat shock proteins is stimulated to repair this damage. This also increases reactive oxygen species production and apoptosis [17, 22, 23]. DNA damage if not repaired would most likely result in cell death [23]. This cell self-repair depends on the intensity of the initial injury by the radio waves. Thus at day 10, the chick embryo cells proliferation and multiplication declined due to DNA damage by the RFW exposure, hence reducing the wet body weight of the embryo as compared to the control group. At day 15, the wet body weight, CR length, and eye diameter did not show significant difference from the control in low dose because the cells were able to repair the damage; however, in the high dose group, the damage could not be repaired and significant difference persisted at day 15.

5.4 Histology and electron microscopy

In this study, an increase number of mitochondria were observed in the exposed group at day 10 and day 15. It has been reported that oxidative stress induced by H_2O_2 leads to increase number of mitochondria and mtDNA in human lung [24]. At day 10 in the exposed group, some of the mitochondria were swollen. It has been reported that permeability of mitochondrial membrane is dependent on interaction between Ca^{+2} and reactive oxygen species (ROS) system. Increased sympathetic activity is considered a primary cause of REW-induced calcium influx into the mitochondria [26]. ROS when stimulated on inner surface of mitochondria will produce free O^{+2} which invade thiol protein and cause transition pores in the membrane to open thus increasing its permeability and causing mitochondria swelling [26]. Mitochondrial swelling was also observed by Atti [27] in rat hepatocytes in response to electromagnetic field exposure.

At day 15, in the exposed group, most of the mitochondria were elongated, dumbbell shaped, and in the process of degeneration. They were surrounded by fragments of rough endoplasmic reticulum and lots of free ribosomes. It was reported that mitochondria is the source of free radicals produced in response to electromagnetic wave exposure in human sperms [22]. It further reported potential causative mechanism of electron leakage from the mitochondrial electron transport chain which causes oxidative DNA damage.

The next most significant alteration in the hepatocytes was the increase number of lipid filled vacuoles in the exposed groups. It was reported that this marked

increase in the cytoplasmic vacuolation is due to disturbances in lipid inclusions and fat metabolisms in pathological conditions, blockage of gluconeogenesis due to free radicals changing the nature of lipids, protein groups, and cell damage [26]. It seems that rough endoplasmic reticulum surrounding the mitochondria is damaged and fragmented thus contributing in increase in fat droplets.

Electromagnetic waves caused fatty change in the hepatocytes of the developing chick embryos. In fatty liver, there is an increase in lipid droplets in the cytoplasm of the hepatocytes suggesting that cells are under oxidative stress when exposed to electromagnetic waves [28–30]. Lahijani et al. had similar results showing abnormal lipid droplets in the hepatocyte cytoplasm and pushing the nuclei to one side [26]. Similar results were reported in rats and rabbits [22, 31]. The breakdown of fat in the liver may be disrupted by radiation exposure, which may be similar to alcoholism, malnutrition, poisoning, and pregnancy. Fatty change is the beginning of injury to the hepatocytes, showing an increase in vacuoles filled with triglyceride fat, a sign of abnormal metabolism which may be due to the production of oxygen radicles species in the hepatocytes [29–31].

5.5 Biochemical test

Heat shock protein70 (HSP70) is a multifaceted chaperon protein that is conserved across species and is involved in many cellular metabolic processes. Its prime role as an anti-stress agent has been exploited heavily in an attempt to further clarify its mode of action and cellular distribution [33]. In this study, Hsp70 was also investigated for its role and degree of expression in stress conditions induced by electromagnetic waves. Previous studies have demonstrated the overexpression of Hsp70 mRNA under stress stimuli but have not elucidated the magnitude and the subcellular compartment localization of the protein [34, 35].

In this study, we observed significant increase in levels of mRNA and Hsp70 in the exposed groups at both day 10 and 15. This increase in mRNA was 14% at day 10 and became 39% at day 15 (**Figure 13**). Levels of mRNA and HSP70 in the liver tissue are demonstrating an important rule liver plays in combating cellular stress using HSP70 as one of the approaches to attenuate the stress stimuli (**Figure 12**).

Mobile phone radiation induces reactive oxygen species (ORS) and DNA damage as seen by increase in the HSP70 and its mRNA and which causes metabolic, immunological, and carcinogenic effects [2, 36–38]. ORS was also reported in human sperm, which affects genes, cell membrane function, and signal transduction [28, 39, 40]. Rao et al. recently provided new evidence supporting the theory that radio waves affect the plasma membrane [39]. Radio waves also induce NADH oxidase enzyme, which might play a key role in the various cellular adverse effects [36, 40]. Various cellular and physiological processes can be affected as a consequence of increased levels of free radicals, including gene expression, cell growth, apoptosis, and release of calcium from intracellular storage sites [32, 36, 41–49].

6. Conclusion

We conclude that even at a low dose of radio waves (20 minutes/day) emitted by mobile phones, the development of chick embryos as seen on the 10th day of incubation would be affected. However, the cells were able to repair the damage by day 15 and no significant difference was observed. At higher dose, the significant different persisted at day 15, and mortality was increased with morphological changes observed. Histological finding in the liver revealed fatty change which was confirmed by ultrastructural findings. The increase of HSP 70 and mRNA in the

exposed groups supported the assumption that RFW exposure has increased ORS which caused damage to the organelles and metabolism in the hepatocytes. These effects question the safety of mobile phones and their potential as a hazard to living cells. It is recommended that mobile phones should be used with caution.

Acknowledgements

I am grateful to Dr. Mubarak Pasha, Deputy Dean Administration for providing me with the laboratory equipment and tools to conduct this research. I am thankful to Dr. Mark Norrish, who at present is Associate Dean International at Coventry University, UK worked as Associate Professor at Oman Medical College in Oman, for his expertise in doing statistical analysis and Dr. John Mutusami, Head Department of Surgery, for his critical review and analysis. Special thanks to Dr. Azem Shalaby, Associate Professor at Department of Pathology, Dr. Irfan Ullah, Department of Pediatrics, Dr. Yahya Tamimi, Associate Professor of Biochemistry and College of Medicine and Health Sciences, Sultan Qaboos University for their strong collaboration in this research project. I am also in debt to our Dean, Dr. Salah Al Kusaiby, and Vice-Dean, Mohammad Al Shafaee, Department of Family Medicine, and Prof. ML Ajmani, Head of Department of Anatomy and Neurobiology, College of Medicine and Health Sciences, National University of Health Sciences, Oman, for their encouragement and endless support throughout this study. In the end I sincerely thank Mr. Mohammad Al Kindi, Ms. Fatima Al-Ghafri, Mr. Abdulrahman Al-Nabhani, Electron Microscopy Laboratory, Department of Pathology, and Ms. Zoya Sheikh, Biochemistry Department, Sultan Qaboos University, without their help I could never have completed this study. I am also thankful to all my MD students who chipped in from time to time and ensured continuous progress of this research. The first student who started this research was Dr. Syed M Saud, who also received 2nd prize in oral presentation at 8th GCC Medical Student's Conference at SQU, Muscat, Oman. Other students who participated were Dr. Ayesha Shafaq, Dr. Marwan Zaki, Dr. Afaf Al Farsi, Ms. Fatima Al Baloshi, Ms. Sakoon Said Alharthi, Mr. Mohammed Amir Rafe, Mr. Muhammad Hamza Kamal, Ms. Rana Hamood Al Maqbali, Ms. Reem Hamood Al Maqbali, and Ms. Sara Al Adawai.

Conflict of interest

There is no conflict of interest. This research was approved by the Ethical Research Board of the Medical College and supported by an internal grant.

Author details

Najam Siddiqi[1*] and Nasser Al Nazwani[2]

1 Department of Anatomy and Neurobiology, National University of Science and Technology, Sohar, Oman

2 Department of Biochemistry, National University of Science and Technology, Sohar, Oman

*Address all correspondence to: najamsiddiqi@nu.edu.om

References

[1] Samkange-Zeeb F, Blettner M. Emerging aspects of mobile phone use. Emerging Health Threats Journal. 2009;**2**:2-8

[2] Blake LB, Lai H. Biological effects from exposure to electromagnetic radiation emitted by cell tower base stations and other antenna arrays. Environmental Reviews. 2010;**18**:369-397

[3] Leitgeb N. Mobile phones: Are children at higher risk? Wiener Medizinische Wochenschrift. 2008;**158**(1-2):36-41

[4] Kheifets L, Repacholi M, Saunders R, Van deventer E. The sensitivity of children to electromagnetic fields. Pediatrics. 2005;**116**:303-313

[5] Maisch D. Children and mobile phones... is there a health risks? Journal of Australian College of Nutritional and Environmental Medicine. 2003;**22**:3-8

[6] Siddiqi N, Heming T. Effects of mobile phone radiofrequency waves (REW) on embryonic stem cells. The FASEB Journal. 2013;**27**:753.1

[7] Schüz J. Mobile phone use and exposures in children. Bioelectromagnetics Supplement. 2005;**7**:S45-S50

[8] Abramson MJ, Benke GP, Dimitriadis C, et al. Mobile telephone use in associated with changes in cognitive function in young adolescents. Bioelectromagnetics. 2009;**30**:678-686

[9] Divan HA, Kheifets L, Obel C, Olsen J. Prenatal and postnatal exposure to cell phone use and behavioral problems in children. Epidemiology. 2008;**19**(4):523-529

[10] World Health Statistics. World Health Organization, WHO Library Cataloguing-in-Publication Data; 2013

[11] Ngole I, Ghosh SK. Exposure to radio frequency radiation emitted by cell phone and mortality in chick embryo (*Gallus domesticus*). Biomedical Research. 2006;**17**:205-210

[12] Oh CM, Jung KW, Won YJ, Shin A, Kong HJ, Lee JS. Age-period-cohort analysis of thyroid cancer incidence in Korea. Cancer Research and Treatment. 2015;**47**(3):362-369

[13] Hardell L, cariberg M. Mobile phone and cordless phone use and the risk for glioma–Analysis of pooled case-control studies in Sweden, 1997-2003 and 2007-2009. Pathophysiology. 2015;**22**(1):1-13

[14] Maier R. Is CNS activity modified by pulsed electromagnetic fields? Biomedizinische Technik. Biomedical Engineering. 2001;**46**(1-2):18-23

[15] Franke H, Streckert J, Bitz A, Goeke J, Hansen V, Ringelstein EB, et al. Effects of universal mobile telecommunications system (UMTS) electromagnetic fields on the blood-brain barrier in vitro. Radiation Research. 2005;**164**(3):258-269

[16] Sukhotina I, Streckert JR, Bitz AK, Hansen VW, Lerchl A. A 1800 MHz electromagnetic field effects on melatonin release from isolated pineal glands. Journal of Pineal Research. 2006;**40**(1):86-91

[17] Zareen N, Khan MY, Minhas LA. Dose related shifts in the developmental progress of chick embryo exposed to mobile phone induced electromagnetic fields. Journal of Ayub Medical College. 2009;**21**(1):130-134

[18] Hamburger V, Hamilton HL. A series of normal stages in the development of the chick embryo. Developmental Dynamics. 1992;**195**(4):231-272

[19] Batellier F, Couty I, Picard D, Brillard JP. Effects of exposing chicken

eggs to a cell phone in "call" position over the entire incubation period. Theriogenology. 2008;**69**(6):737-745

[20] Youbicier-Simo BJ, Lebecq JC, Giaimis J, et al. Mortality of chicken embryos continuously exposed under GSM cell phone and validation of the effectiveness of a protective device. In: Proceeding of International Conference on Cell Tower Siting; Salzburg, Austria. 2000. pp. 233-235

[21] Jyoti KR, Bagai U. Effect of mobile phone frequency radiation on early development of chick embryo. International Journal of Science, Environment and Technology. 2014;**3**:1273-1280

[22] Cox CF, Brewer LJ, Raeman CH, Schryver CA, Child SZ. A test for eratology effects of power frequency magnetic fields on chick embryos. IEEE Transactions on Biomedical Engineering. 1993;**40**(7):605-610

[23] Al-Qudsi F, Azzouz S. Effects of electromagnetic radiation on chick embryo development. Life Sciences. 2012;**9**:983-991

[24] Lahijani MS, Farivar S, Khodaeian M. Effects of 50 Hz electromagnetic fields on the histology, apoptosis, and expression of c-Fos and β-catenin on the livers of preincubated white Leghorn chicken embryos. Electromagnetic Biology and Medicine. 2011;**30**(3):158-169

[25] Farrell JM, Litovitz TL, Penafiel M, Montrose CJ, Doinov P, Barber M, et al. The effect of pulsed and sinusoidal magnetic fields on the morphology of developing chick embryos. Bioelectromagnetics. 1997;**18**(6):431-438

[26] Ubeda A, Leal J, Trillo MA, et al. Pulse shape of magnetic fields influences chick embryogenesis. Journal of Anatomy. 1983;**137**:513-536

[27] De Iuliis GN, Newey RJ, King BV, Aitken RJ. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PLoS One. 2009;**4**(7):e6446

[28] Aitken RJ, Bennetts LE, Sawyer D, Wiklendt AM, King BV. Impact of radio frequency electromagnetic radiation on DNA integrity in the male germline. International Journal of Andrology. 2005;**28**(3):171-179

[29] Lee H-C, Yin P-H, Lu C-Y, Chi C-W, Wei Y-H. Increase of mitochondria and mitochondrial DNA in response to oxidative stress in human cells. The Biochemical Journal. 2000;**348**:425-432

[30] Lahijani MS, Tehrani DM, Sabouri E. Histopathology and ultrastructural studies on the effects of electromagnetic fields on the liver of pre-incubated white Leghorn chicken embryo. Electromagnetic Biology and Medicine. 2009;**28**:391-413

[31] Atti AA, Yehia MA. Histological, ultrastructural and immune-histochemical studies of the low frequency electromagnetic field effect on thymus, spleen and liver of albino swiss mice. Pakistan Journal of Biological Sciences. 2002;**5**(9):931-937

[32] De luliis GN, Newey RJ, King BV, John Aitken R. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PLoS One. 2009;**4**(7):e6446

[33] Angulo P. Nonalcoholic fatty liver disease. The New England Journal of Medicine. 2002;**346**:1221-1231

[34] Reddy JK, Rao MS. Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation. American Journal of Physiology. Gastrointestinal and Liver Physiology. 2006;**290**:852-858

[35] Ibrahim M, El-Ashry M, Ali E. The influence of 50 Hz magnetic field on liver function. Romanian Journal of Biophysics. 2008;**18**:113-122

[36] Tarantino P, Lanubile R, Abbro L, Dini L. Post-continuous whole body exposure of rabbits to 60 MHz electromagnetic fields: Effects on liver, spleen and brain. Radiation and Environmental Biophysics. 2005;**44**:51-59

[37] Brenu EW, Staines DR, Tajouri L, et al. Heat shock proteins and regulatory T cells. Autoimmune Disease. 2013;**813**:256

[38] Lantow M, Schuderer J, Hartwig C, Simkó M. Free radical release and HSP70 expression in two human immune-relevant cell lines after exposure to 1800 MHz radiofrequency radiation. Radiation Research. 2006;**165**(1):88-94

[39] Finka A, Mattoo RUH, Goloubinoff P. Meta-analysis of heat- and chemically upregulated chaperone genes in plant and human cells. Cell Stress & Chaperones. 2011;**16**:15-31

[40] Kiang JG, Gist ID, Tsokos GC. Regulation of heat shock protein 72 kDa and 90 kDa in human breast cancer MDA-MB-231 cells. Molecular and Cellular Biochemistry. 2000;**204**:169-178

[41] Spadaro JA, Bergstrom WH. In vivo and in vitro effects of a pulsed electromagnetic field on net calcium flux in rat calvarial bone. Calcified Tissue International. 2002;**70**(6):496-502

[42] Bonhomme-Faivre L, Marion S, Forestier F, Santini R, Auclair H. Effects of electromagnetic fields on the immune systems of occupationally exposed humans and mice. Archives of Environmental Health. 2003;**58**(11):712-717

[43] Shangcheng X, Zhou Z, Lei Z, Zhengping Y, et al. Exposure to 1800 MHz radiofrequency radiation induces oxidative damage to mitochondrial DNA in primary cultured neurons. Brain Research. 2010;**1311**:189

[44] Rao VS, Titushkin IA, Moros EG, Pickard WF, Thatte HS, Cho MR. Non-thermal effects of radiofrequency-field exposure on calcium dynamics in stem cell-derived neuronal cells: Elucidation of calcium pathways. Radiation Research. 2008;**169**(3):319-329

[45] Dasdag S, Akdag MZ. The link between radiofrequencies emitted from wireless technologies and oxidative stress. Journal of Chemical Neuroanatomy. 2016;**75**:85

[46] Verschaeve L, Heikkinen P, Verheyen G, Van Gorp U, et al. Investigation of co-genotoxic effects of radiofrequency electromagnetic fields in vivo. Radiation Research. 2006;**165**:598-607

[47] Donato A, Ceci P, Cannavo A, Tomei F, Naro F. Low power microwave interaction with phospholipase C and D signal transduction pathways in myogenic cells. Cell Biology International. 2004;**28**:683-688

[48] Markova E, Malmgren LO, Belyaev IY. Microwaves from mobile phones inhibit 53BP1 focus formation in human stem cells more strongly than in differentiated cells: Possible mechanistic link to cancer. Environmental Health Perspectives. 2010;**118**(3):394-399

[49] Desai N, Sharma R, Makker K, Sabanegh E, Agarwal A. Physiological and pathological levels of reactive oxygen species in neat semen of infertile men. Fertility and Sterility. 2009;**92**(5):1626-1631