ORIGINAL ARTICLE

GROWTH RETARDATION OF CHICK EMBRYO EXPOSED TO A LOW DOSE OF ELECTROMAGNETIC WAVES

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Background: The objectives of this study were to explore the effects of low dose of the non-ionizing (REW) emitted by a mobile phone on the development of chick embryo. **Methods:** one hundred and twenty chick fertilized eggs were equally divided into a control and an exposed group. Sixty fertilized eggs were placed in an egg incubator with a mobile phone (SAR US: 1.10W/kg (head) 0.47 W/kg body) in silent mode having vibration disable mode. Mobile was called for a total of 20 minutes in 24 hours. Twenty embryos each were sacrificed at day 5, 10 and 15, mortality, wet body weight, head to rump length, eye diameter and morphological changes were noted. The control group, 60 eggs were incubated in the same conditions, having removed the phone. **Results:** No mortality was noted. The experimental group exposed to REW showed subcutaneous haemorrhagic areas and significant growth retardation at day 10 as evidence by smaller eye diameter, wet weight and CR length than the control group. There were no significant growth differences at either day 5 or at day 15. **Conclusion:** Electromagnetic waves emitted from mobile phones even though for a very short duration of 20 minutes per day have affected the growth of the chick embryo at day 10 of incubation, Hence exposure of these waves are not 100% safe.

Keywords: electromagnetic waves, chick embryo, mobile phone, egg incubator, growth retardation

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INTRODUCTION

Cellular technology and broadband services are growing rapidly, resulting in exposure to high levels of non-ionizing low radio frequency electromagnetic waves (REW). This adds a new dimension to environmental pollution. In developing countries, this potential hazard exits where the use mobile phone technology is high. In some countries, the reported prevalence of mobile phone use by adolescents is more than 90% and the use in Oman is ranked 10th in the world[1,2] (after Saudi Arabia, Russia, Kuwait and Panama, WHO health statistics 2013).

Mobile phones use non-ionizing low radio frequency electromagnetic waves (REW) that causes DNA damage[3], affect genes, membrane function and signal transduction[4–6]. Functions of the central nervous system[7], permeability of the blood brain barrier[8] and melatonin synthesis[9] are also affected. REW exposure increases free-radical production which causes metabolic, immunological and carcinogenic effects.[10–13] 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[13], which are now linked to the base stations in the vicinity of residential areas and excessive use of mobile phones. Childhood leukaemia in children exposed to extremely low frequency (ELF) magnetic fields has already led to its inclusion as a "possible human carcinogen" by the International Agency for Research on Cancer, published in “Agents Classified by the IARC Monographs”, Volumes 1–109. (http://monographs.iarc.fr/ENG/Classification/ClassificationsAlphaOrder.pdf). Recently an increase incidence of thyroid cancer in South Korea and gliomas in Sweden have been reported which could be due to excessive use of mobile phones.[14,15]

To further study the effects of radio waves, animal models were used. Laboratories have reported a high mortality of chicken embryos and malformations when exposed to mobile phones and is also dose dependent.[16–24] During chick embryo development, embryonic cells are rapidly proliferating, differentiating, migrating and suffer from apoptosis. These cells generate electric currents which are affected by the REWs.[25] The study was done with the objective to observe the effects of very low duration of REW emitted by a mobile phone on rapidly proliferating stem cells in the developing chicken embryo.

MATERIAL AND METHODS

A mixed experimental design was used, with one between groups’ independent variable with two levels (emission of REW) and one within group independent variable with three levels (gestational time). There were five dependent variables; mortality, gross morphology, wet weight, crown-rump length (CR length) and eye diameter. 120 ‘Cobb’ (*Gallus gallus domesticus)* breed zero-day fertilized chicken eggs were acquired from Sohar Poultry Company S.A.O.G. (PO Box 2808, Ruwi, Postal code 112, Sultanate of Oman). These eggs were

kept at room temperature for 4–5 hours before placing them in the egg incubator.

A 30-egg incubator (Egg incubator Model EH-35, Sino-PFE Company, China) with automatic temperature, humidity control and forced air ventilation was used (Figure-1a.). It was also equipped with special egg holders with automatic egg rotation capability which was fixed at ten rotations per day. A popular mobile phone and service provider was selected with 1800 MHz frequency, power of 0.47 W/kg body and SAR 1.10 w/KG (head). A Tri Field Meter, model 100XE was used to detect the strength of REW of the mobile phone during the experiment (Figure-1b.). The eggs were randomly assigned to either the control or experimental condition, such that 60 eggs were at each condition. In the experimental REW exposure group, the mobile phone was switched on in silent mode with vibration disabled, placed in the centre of the incubator under the egg holders so that the farthest egg was within a radius of 16 cm[26] within one wavelength (approximately 16.5 cm) of the emitting 1800 MHz frequency electromagnetic waves[27]. The temperature was set at 37^{o} centigrade with a humidity of 50–60%. The experiment was run in batches of 30 eggs, due to the size of the incubator.

The mobile phone placed inside the incubator received a call from another mobile phone for 5 minutes, four times a day with an exposure-free period of 4 hours in between the calls. A schedule timing of ringing the mobile was made and it was repeated each day. Thus the total daily (24 hours) exposure duration was 20 minutes. Experimental group was run in two batches with 30 eggs in each batch. Within each batch of 30 eggs, 10 eggs were sacrificed at each; day 5 (exposure time 100 minutes), day 10 (exposure time 200 minutes) and day 15 (exposure time 300 minutes). In the control group was also run in two batches, but without mobile phone inside the incubator.

In both the groups, on the scheduled day of sacrifice, the 10 randomly selected eggs were removed from the incubator. A small hole was made in the shell and then a portion of the shell was carefully cut by scissors and removed. The embryo was dissected from the membranes and its survivability noted by either movements of the limbs or beating of the heart. Embryos were assessed for gross morphological abnormalities using the Hamburger and Hamilton developmental stages [28]. The embryo was then placed in a dish, washed in normal saline and blotted dry with tissue paper. The dependent variables were then recorded; weight, CR-length and eye diameter. Wet weight was recorded using a digital balance with precision of 0.01 gm (Universal Impex HA-3202). The length was taken from the vertex to the tip of the coccyx using a calliper (Mitutoyo Vernier callipers, Nanjing Sulang Trading Co., Ltd, China). The same calliper was used to record the eye diameter.

The student's t-test was used to detect any significant differences in the "means" of gross weights and lengths, and for the percentage survival of the embryos. Difference of a p-value of <0.05 were considered significant. All data are presented as the mean value±SEM.

RESULTS

At sacrifice, there was no mortality in any of the 120 eggs and all the eggs had been successfully fertilized. At day 5, development was assessed to be morphologically normal in both the control and experimental groups. There were no signs of congenital anomalies or deformity in the embryos. The average wet body weight in the experimental group (0.189±0.035 gm) was slightly less than the control group (0.209±0.031gm), however, this difference was not significant (t=1.67, df=28, p<0.11). (Figure-2). The eye diameter and CR-length were not measured at day 5. At day 10, assessment of gross morphology did not reveal any deformities or anomalies. In the control group the skin was typically pink in colour, homogenous in appearance and without any haemorrhagic areas under the skin (Figure-3a). However, in the experimental group, the skin showed small haemorrhagic areas alternating with pale areas, indicative of reduced blood flow (Figure-3b). The average wet weight in the experimental group (1.572±0.38 gm) was significantly lower than in the control group (2.331±0.27 gm), t=8.19, df=48, p<0.01 (Figure-2). The average eye diameter was significantly smaller in the experimental group (0.742±0.093 cm) than the control group (0.855±0.057 cm), t=4.90, df=45, p<0.01 (Figure-4a).The C-R length was significantly shorter in the experimental group (3.064±0.263 cm) than in the control group (3.543±0.32 cm), t=5.61, df=45, p<0.01 (Figure-4b).

At day 15, assessment of gross morphology showed, in line with expected embryonic development in both groups, that the skin was covered by clearly visible white feathers and the blood vessels were no longer visible under the skin. The beak was well formed and firm. The length of the wings and the limbs had increased and the toes were well developed, the middle toe being the longest. The head size had become smaller with respect to the body size and the eyes were fully covered by the lids. (Figure-5 a, b). The average wet body weight was similar for both groups; control (14.91±1.73 gm) and experimental group (14.82±1.57 gm), with no significant difference (Figure-2). There was no significant difference in the eye diameters (control: 1.123±0.051 cm and experimental 1.14±0.05 (Figure-5.) and the average C-R lengths in both groups; experimental (6.978±0.348 cm) and control (7.013±0.41 cm), (Figure-3 a, b.).

Figure-1 (a): The egg incubator, (b). Tri-Field meter showing high levels of electromagnetic waves transmitted from the mobile during call receiving (≥1mW/cm^2).

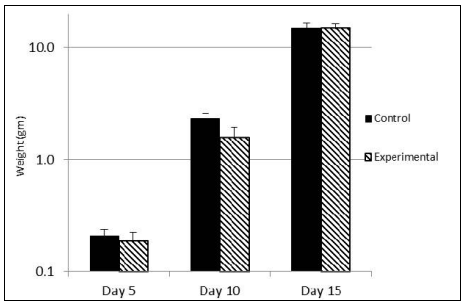
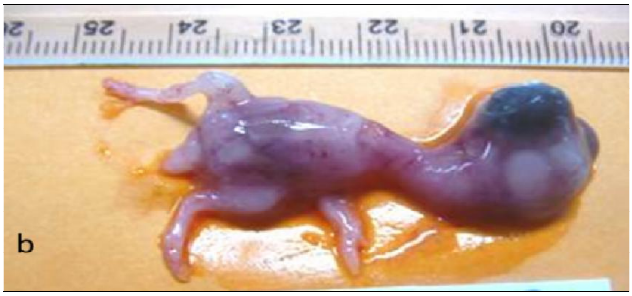


Figure-2: Wet body weight of the chick embryo: experimental and control groups at days 5, 10 and 15 showing significant different at day 10 (p<0.01).

Figure-3 (a): Day 10: Control group showing normal embryo development



(b). Experimental group: Embryos were smaller in size than the control group and marked haemorrhagic areas could be seen under the skin alternating with pale areas

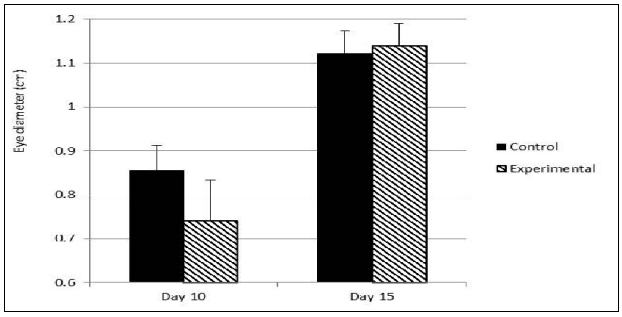
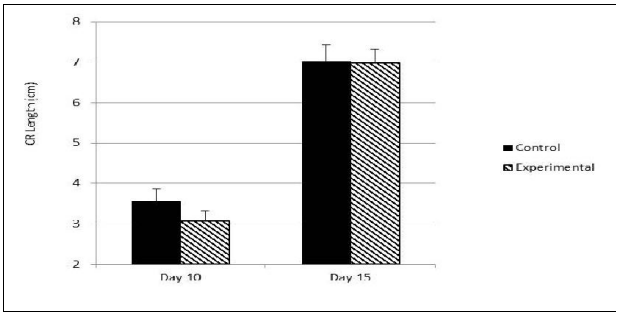


Figure-4 (a): Eye diameter of the chick embryo in the experimental group was significantly smaller than the control group at day 10 (p<0.01).



(b). C-R length of the chick embryo in the experimental group was significantly smaller than the control group at day 10 (p<0.01).

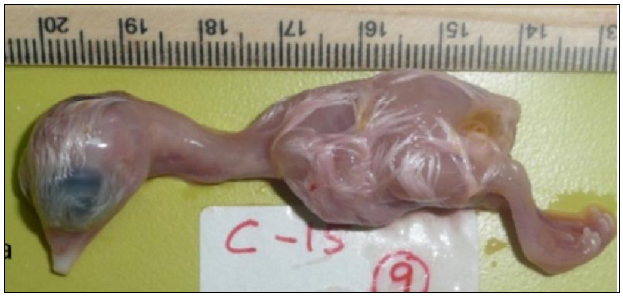


Figure-5 (a): At day 15, control, showing well developed embryo with no anomaly or deformity. Skin was covered by feathers, upper and lower extremities were well developed with normal toes and eyes completely covered by eye lids.

(b). Experimental: No anomaly or deformity was observed; embryo showing same features as observed in the control embryo.

DISCUSSION

It was interesting to note that in this study no mortality was observed in either the control or experimental groups, with an exposure time of only 20 minutes/day at 1800 MHz (very low dose). This is comparable with Batellier who reported a mortality rate of less than 1% from day 7 to 14 when fertilized eggs were exposed to 900 MHz.[29] Many other studies however, have reported a higher mortality in the radio wave exposed groups; Youbicier-Simo reporting a mortality of 54% in the exposed group and 14% in the control; where exposure was by continuous mobile phone activity during the embryonic life (21 days).[19] Jyoti *et al.* showed a higher mortality in the exposed group and reported that the increased exposure duration and higher power (20 dBm) had both influenced the mortality in the exposed groups.[24] Subcutaneous haemorrhagic areas in the exposed group, alternating with pale areas over the skin were noticed on day 10, similar to the effects reported by others.[23,30] Brain malformations, retinal thickness and bigger embryos at day 10 with a dose-duration of 60 minutes/day were also observed[23], however, the incubator used in her study was not an egg incubator. No malformation was noted in our study either in the exposed or in the control groups throughout the period studied with the dose duration of 20 minutes/day which is the smallest dose ever reported. Farrell *et al* reported the results of an extensive study where over 2500 chick embryos were evaluated using 60 Hz magnetic field (pulsed and sinusoidal by a Tenma function generator) at five different laboratories.[18] The eggs were exposed for 48 hours, showing no significant difference in the mortality of the embryo; however, malformations were 6.8% in the exposed group compared to 1.8% in the control, with the majority of the embryos exhibiting neural tube defects.[18] Other malformations such as spina bifida, mono-phthalmia, micro-ophthalmia, an-opthalmia and growth retardation were also reported.[25,30] Ubeda *et al.* using 100 Hz and electromagnetic field intensity between 0.4–104 microTeslas (μT) confirmed that the chick embryo is sensitive to electromagnetic fields at extremely low frequency and intensity and pulse shape may be a strong factor in determining slight or no modification of embryo development.[31] Exencephalic embryos, embryos with asymmetrical faces, an-ophthalmia, micro-phthalmia, crossed or shorter beak, gastroschesis and deformed hind limbs were also reported.[30] Cox *et. al.* reported no malformations when he exposed the fertilized eggs to 50 Hz.[17] The most significant finding in this study was the interaction between gestational age and REW exposure. This interaction presented as retarded growth of the embryo at day 10. 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. Similarly Al Qusdi *et al.* using 900–1800 MHz electromagnetic waves by ringing 4 times for 15 minutes daily (60 minutes/day) reported significant increase in body weight and length at day 10 which could not be sustained at day 14.[23] Electromagnetic waves effects on living cells are dose and duration dependent.[26] It is likely that the decreased wet body weight of the chick embryo in exposed group at day 10 was a result of interference in the multiplying of embryonic cells due to the REW exposure. Embryo development is a process which includes cell multiplication, proliferation, differentiation, relocation, and programed cell death. These events are carried out by endogenous ionic currents and electric fields and hence growth retardation at day 10 in the exposed group is most likely due to DNA damage, Reactive Oxygen Species production and apoptosis[30,32], REW exposure causes DNA damage which if not repaired would most likely result in cell death[3]. The cell has the power to repair itself when injured. This 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 decrease was due to REW injury; hence the wet body weight of the embryo was significantly less than the control group. At day 15, the wet body weight, CR length and eye diameter did not show significant difference from the control. It is likely that the DNA was repaired, and stress proteins along with other enzymes and an increase in calcium influx might have increased the proliferation of the cells in the exposed group. Different theories have been postulated regarding the effects of radio waves on the biology of living cells. Rao *et al.* recently provided new evidence supporting the theory that radio waves affect the plasma membrane.[33] Radio waves also induce NADH oxidase enzyme stimulation, which might play a key role in the various cellular adverse effects observed in in vitro studies. Increased levels of free radicals effects cellular physiology, gene expression, intracellular calcium release from storage sites, cell growth, and apoptosis.5,6,10,11,25,34,35 Radio wave effects on genes5 have also been reported resulting in signal transduction effects and alterations in membrane structure and function6, metabolic effects[10], and effects associated with free-radical production[11].

CONCLUSION

We conclude that even a low dose of radio waves (20 minutes/day) emitted by mobile phones affected the development of chick embryos as seen on the 10th day of incubation. This is most likely due to the effect of REW exposure on embryonic stem cells. This effect, questions the safety of mobile phones and their potential as a hazard to multiplying stem cells in developing embryos. It is recommended that mobile phones should be used with caution.

AUTHOR'S CONTRIBUTION

All the authors contributed equally.

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