Morphological Changes in Chick Embryos Development Exposed to Electromagnetic Radiation Emitted by Smart Mobile Phones

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In last decade, smart mobile phone devices has been public use and this has increased the concern about its potential effects on human body and embryo development. This study aimed to investigate the effect of electromagnetic radiation (EMR) emitted from smart mobile phone on chick embryo development. Fertile hen eggs were divided into three experimental groups of 30 eggs for each: control, sham and treated group. Treated group was exposed to EMR emitted by smart mobile phone during development period. The EMR was measured by a radiofrequency meter. At E7, E10 and E14 days of incubation embryos were collected at embryonic days and washed with normal saline. After that, embryos were weighted and photographed. In the present study, direct exposure (treated) and indirect exposure (sham) to EMR cause different congenital malformation phenotypes such as hemorrhagic, growth retardation, absence of neck or beak, limbs buds, brain malformation, beak malformation, decrease in feather formation, diminished pigmentation in iris and skin was not the typical pink color compared to the control. Furthermore, decreased growth parameters such as whole-body weight, whole-body length, forelimbs and hindlimbs, body mass index (BMI), eye weight and diameter were also observed. Furthermore, smart mobile phones found also to cause increased mortality rate during early and late developmental stages.

Introduction

Electromagnetic radiation (EMR) is emitted by many sources that we are exposed to in daily life such as radar, communication equipment, mobile phone base stations, high voltage lines, radio and television transmitters, substations and electrical equipment at home and work and many other electrical systems in the environment. When power is produced, transmitted or used, electrical devices produce fields around them called 'electric fields' and the association of certain elements in several devices results in the creation of both an electric and a magnetic component, called an electromagnetic field (EMF) [**1**]. Nowadays, wide field of (EMR) is emitted by communication equipment, mobile phone base stations, in addition to many electrical systems in the environment. more than three billion people across the world are exposed to EMR systematically in a daily basis [**2**]. Lifetime exposure to EMR is becoming the subject of significant investigation since it has the potential to cause substantial changes and deleterious effects in biological system [**3**]. Conflicting information is found in the medical literature; while some reports dismiss the assumed hazard associated with EMR. Various institutions including the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) have called for intense investigation of the effect of non-ionizing radiation (NIR) on human health in response to mounting body of research suggesting a link between EMR and a number of health risks, including reproductive dysfunction, cancer and central nervous system (CNS) disorders [**1**].

The biological effects of EMR can be classified as thermal and non-thermal. Thermal effects are related to the heat created by EMR in many areas. This mechanism happens via an alteration in temperature coming from radiofrequency (RF) fields. It is possible that each interaction between living tissues and RF field causes an energy transfer resulting in an increase in temperature. Skin and other superficial tissues usually absorb the non-thermal radiations emitted by smart mobile phones; this causes an insignificant increase in the temperature of the brain and other body organs [**4**]. Non-thermal mechanisms are not directly related to temperature change, alternatively it is slightly related to some other changes in tissues in association with the quantity of absorbed energy [**1,5,6**]. A significant part of several studies concerning EMF has investigated the non-thermal effects of RF on biological tissues [**1,7,8**].

Delgado and co-workers stimulated a great deal of interest among investigators working with EMF when they exposed fertilized chicken eggs to pulsed magnetic fields. They found dramatic effects; where the exposure to the 100-Hz and 1.2-μT field produced the greatest effects. There was a general inhibition of development. These defects include brain vesicles, auditory pit, neural tube, foregut, heart vessels and somites malformation; where the cephalic nervous system was the most sensitive and the heart was the least [**9,10**].

Lifetime Exposure to EMR is currently the issue of significant scientific investigation since it has the potential to the biological system. Therefore, the present study aimed to evaluate the possible effect of EMR emitted from smart mobile phone on chick embryo during early and late developmental stages in addition to hatchability rate in an attempt to estimate the potential hazards on human embryonic development.

Experimental

Material and methods

Fertilized chick eggs were obtained from local farm in Jeddah, Saudi Arabia. Eggs were weighted before incubation and average weight of eggs was 41.7 g.

Digital humidified egg incubators were purchased from Al-Hakeem Foundation model number (WQ). The present study used smart mobile phone was on Extended Global System for Mobile Communication (EGSM), 1300 MHz network and Specific energy Absorption Rate (SAR) of head and body was 1.18 W/kg (manufactory web). The EMR emitted by the smart mobile phone was measured by a commercially available radiofrequency meter (RF meter, Less EMF Inc, USA wavelength of 100MHz-8GHz, model number ED178S.

Experimental design

Total number of 72 eggs were divided equally into three groups (control, sham and treated). Eggs were incubated under identical standard conditions; 37.5ºC and suitable ventilation and humidity level between 65%-75% for three designated embryonic stages; E7, E10 and E14. Eggs were rotated automatically each 2 hours. Eggs were organized as square circumference having the On Silent mode smart mobile phone at the center of the square. To insure an equal EMR exposure level for each egg, fixed distance between the smart mobile phone and each egg was used (3.5 cm). For sham group, another incubator was used and placed next to the treated group incubator. RF meter was placed inside the incubator to measure RF intensity. For the treated group, the intensity of RF was red1 (180 mw/m²). While for the sham group, the amount of RF was yellow 1 (5.8 mw/m²), as shown in **Fig. 1**.

Regular phone calls are made from another mobile phone to the experiment smart mobile phone for the duration of 15 minutes every six hours daily.

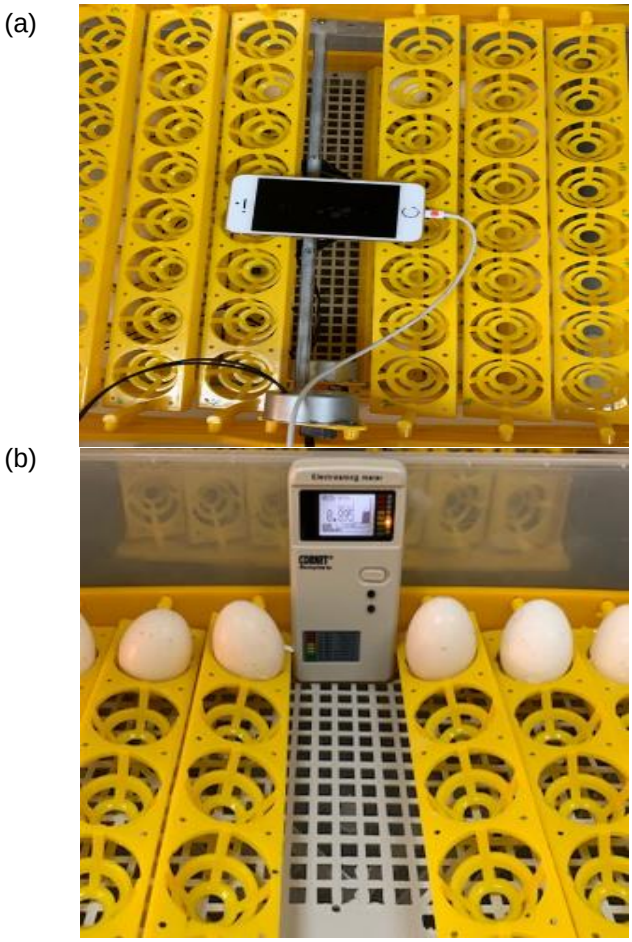

Fig. 1. Shows experimental designed. Top view of the place of mobile phone in the upper level of the incubator. The RF meter placed inside the incubator to check the intensity of radiation.

Sample collection and photography

Embryos were collected from all groups at the designated incubation days: E7, E10 and E14 and washed with normal saline, weighted than photographed using an iPhone Xs max camera (12 Megapixel) held on a tripod with a ruler as a scale when performing morphometrics for the photos. Eyes were photographed using a dissecting microscope (Olympus SZX10) which is shown in **Fig. 2**.

For hatchability experiment, 20 eggs were incubated in each group. The experiment was repeated two times.

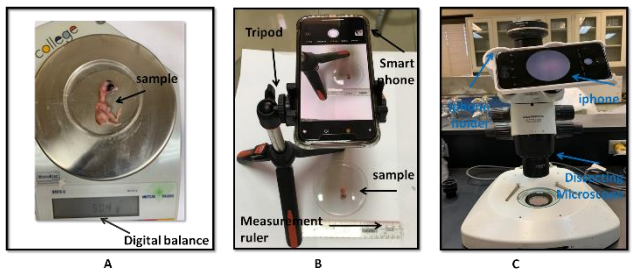

Fig. 2. Embryos weighing and photography methods. (A) Embryo weighing with digital balance. (B) Embryos photographed with iPhone camera with 12 Megapixel. (C) The method of photographing embryo eyes using the dissecting microscope with iPhone camera.

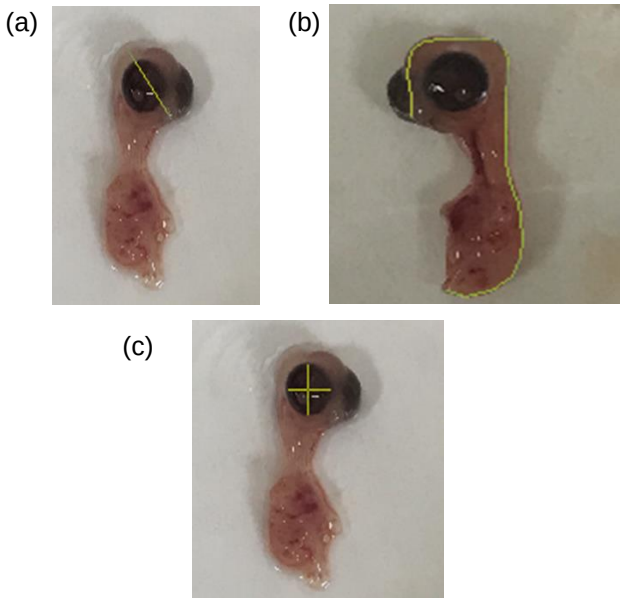

Fig. 3. Measurements taken for E7 chick embryos. (A) Head width. (B) Whole-body length. (C) Eye diameter

Morphometry

Measurements from photos of all embryos (control, sham and treated) were reported using CMEIAS Image tool software (http://cme.msu.edu/cmeias/ accessed on 2018). As **Fig. 3** showed the measurements taken from E7 embryos were for full embryonic body length, head diameter starting from the beak and eye diameter. Furthermore, the same measurements were taken for embryos of E10 and E14 days as well as beak length along the beak opening, neck length and fore and hind limb length as shown in **Fig. 4**. For embryos of E14, a scale was set in order to measure feather distribution (Low, Medium, High) as shown in **Fig. 5**. Body Mass Index (BMI) for each chick embryo was computed as follow:

$$\text{BMI}=\frac{Body\ weight\ (g)}{(Body\ length\ (cm))^2} \quad (11)$$

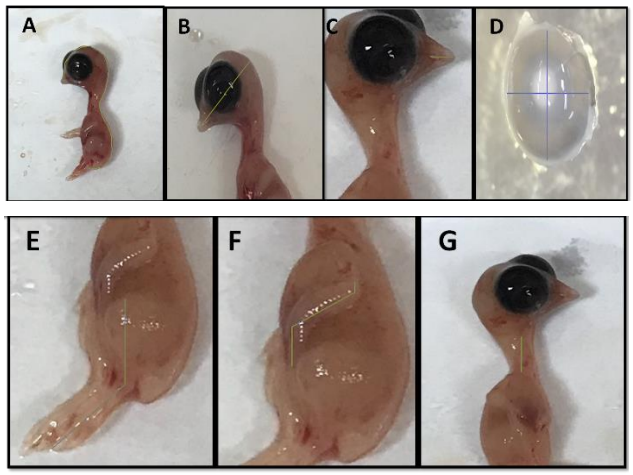

Fig. 4. Measurements taken for E10 and E14 chick embryos. (A) Whole-body length. (B) Head length. (C) Beak length. (D) Eye diameter. (E) Hindlimb length. (F) Forelimb length (G) Neck length.

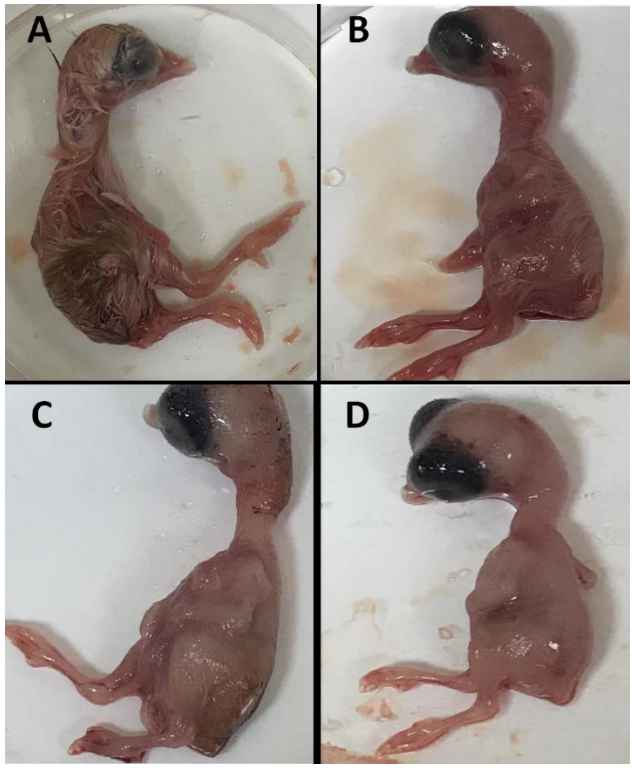

Fig. 5. Measurements method of feather distribution in E14 chick embryos. (A) High feather. (B) Medium feather. (C) Low feather. (D) Without feather.

Statistical analysis

Comparative analyses for control and experimental groups data were statistically analyzed using One-Way ANOVA and Nonparametric tests with SPSS 20 (IBM, USA) software. Statistical significance was assured when $p<0.02$. **Table 1.** The types and percentage of malformations were found in the three designated embryonic stages in control, sham and treated groups of chick embryos (n=30).

Table 1. The type and percentage of malformations were found in the three designated embryonic stages in control, sham and treated groups of chick embryos (n=30).

Embryonic age	Congenital malformation variation (%)							
	Treatment	MGR	FD	B	AH	BM	EM	FL	HL
E7	Control	10					16.6		
	Sham	56.25**		6.25			53.3**		
	Treated	68.42**					66.6**		
E10	Control	10.2		10.2	5.1		6.6	20	20
	Sham	62.6**		23.4	6.7	16.6	23.3	46.6**	50**
	Treated	66.5**		34.3*	9.3	12.5	33.5	56.6**	56.5**
E14	Control	10.6	12.5	3.1		3.1	3		
	Sham	32.3**	61.7**	35.3**	11.8		33.5**	33.3**	23.3
	Treated	44.5**	70**	36.1**	19.4	10	45**	43.3**	40**

Major growth retardation (MGR). Feather distribution (FD), Bleeding (B), Abdominal hernia (AH), Beak malformations (BM), Eye malformation (EM), Forelimb (FL) and Hindlimb (HL). Significance * $p< 0.02$, ** $p<0.01$.

Results and discussion

Morphological studies

The egg's weight before incubation in all egg showed no significant differences. In the control group, E7 embryos had a normal body and head masses with big eyes located on both sides of the head separated by the forebrain and the beak. There was a clear appearance of the beak and neck. This study had found that the EMR caused malformation in the sham E7 embryos such as growth retardation and bleeding. Moreover, the eyes, head, neck and beak appeared smaller than those in the control embryos. The limbs appeared as buds. While skin was not typically pink in color. One of the more significant findings to emerge from this study was that growth retardation and bleeding in the treated E7 embryos. The eyes and head were smaller compared to those in the control embryos with the absence of neck, beak and limb buds. Moreover, the eye pigmentation was low compared to the control. In some embryos the eye and limb buds were completely absent, and skin was not typically pink which is shown in **Fig. 6**.

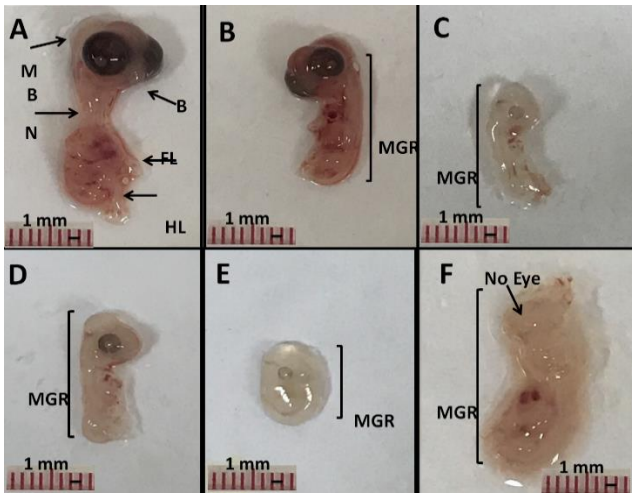

Fig. 6 Photographs of chick embryos E7 with iPhone camera with 12 Megapixel. Scale = 1mm.
Note: Mid brain (MB), Beak (B), Neck (N), Forelimb (FL), Hindlimb (HL) and Major growth retardation (MGR) and Small eye (SE)
(A) Control. (B) Sham had MGR, absence of the neck and beak, SE and limbs bud small. (C) Sham had MGR, absence of the neck and beak, very small eye with less pigmentation and limbs bud small. Skin was not typically pink in color. (D) Treated had MGR, absence of the neck and beak, small eye and limbs bud small, Skin was not typically pink in color. (E) Treated had MGR very small body, head and eye with less pigmentation and absent of limbs bud, Skin was not typically pink in color. (F) Treated had MGR with eye absent and limbs bud absent, Skin was not typically pink in color.

E10 control embryos had developed clear parts of the brain, where they had large and bulky eyes compared to the head size. The nictitating membrane covered the anterior most area scleral papillae and cornea. In addition, the tip of the upper beak had white scales with clear nasal opening and the limbs started to be elongate. It was noticeable that the three parts of forelimb became clear and the hindlimb toes are developed but were not separated yet. At least 9-10 rows of feather germs between upper eyelid and the dorsal midline were seen. In contrast, congenital malformations were seen in E10 embryos in sham and treated group. These malformations include subcutaneous bleeding, brain malformation, hernia, fragile and less pink skin, growth retardation, beak malformations such as small or absence of beak and neck. Almost all E10 sham and treated group demonstrated the absence in feather formation around the body. Furthermore, eyes were small with less pigmentation in some embryos. Additionally, in a number of fertilized eggs, embryonic growth was stopped, and the embryos were dead at the time of opening. The percentage of these congenital malformations was higher in treated group. One embryo was reported with no eye and another embryo had a different size for both eyes (one large and one small) in treated group. Also, there was one embryo with abnormal localization of the eye in the treated group which is shown in **Fig. 7**.

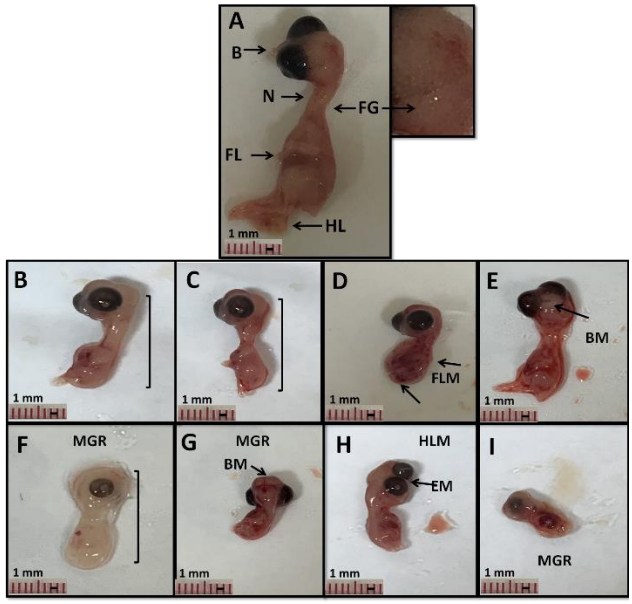

Fig. 7. Photographs of chick embryos E10 with iPhone camera with 12 Megapixel. Scale = 1mm. (A) Control [Note: Beak (B), Neck (N), Forelimb (FL), Hindlimb (HL), Feather germs (FG)] (B) Sham small embryo SE and small limb buds SLB, (C) Treated SE and SLB. (D) Sham with MGR. (E) Sham with BM. (F) Sham with MGR. (G) Treated with BM and MGR. (H) Treated with MGR and EM. (I) Treated with MGR.

Our results revealed that E14 control embryos developed eyelids normally. Eye size was proportional to the head size. As anticipated, the development of the eyelids, feather germs, hind limbs, wing, beak and feathers increased gradually compared to the control embryos of the E10. Even so the hindlimb didn't show the formation of the claws, the upper and lower beak had white scales. The body was covered with downy feather (soft and thin feathers). On the other hand, the defects seen in E14 embryos of sham and treated groups were growth retardation, subcutaneous bleeding, brain malformation, hernia, fragile skin, small or absent beak and neck malformation. Embryos had large and bulky eyes. The feather distribution was reduced or absent

comparing to the control. The growth of some embryos was stopped, and the embryos were dead. Some embryos had different size of limb such as one hind limb longer than the other one and shorter forelimb than that of the control group as shown in **Fig. 8**.

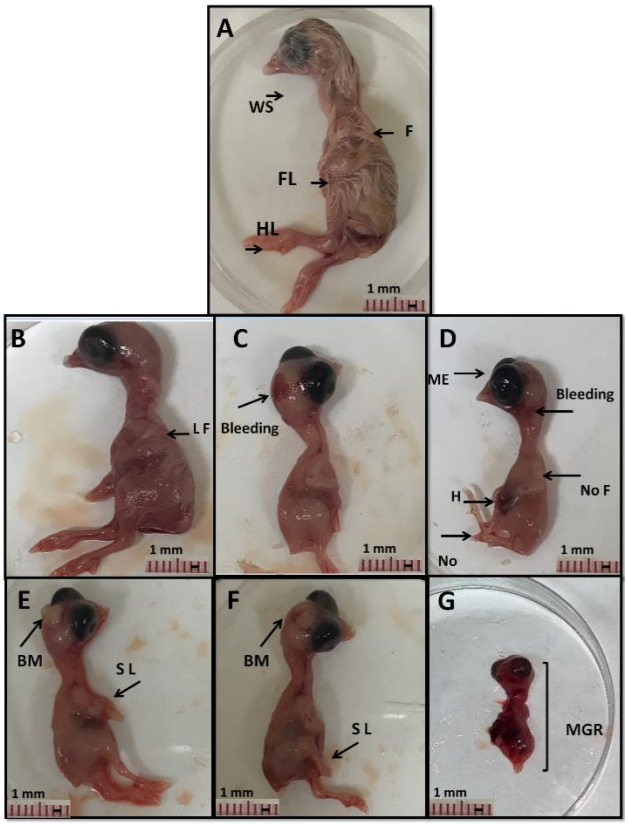

Fig. 8. Photographs of chick embryos E14 with iPhone camera with 12 Megapixel. Scale = 1mm.
(A) Control, (B) Sham with less feather distribution, (C) Sham with growth retardation and subcutaneous bleeding. (D) Sham with hernia, absent of feather distribution and small hindlimb. (E) Treated with brain malformation, small forelimb and beak. (F) Treated with small of beak brain malformation and different size of hindlimb. (G) Treated embryo with growth stopped and less eye pigmentation.
Note: Forelimb (FL), Hindlimb (HL), Less feather distribution (LF). White sclera (WS). Feather (F). Hernia (H). Small limbs (SL) and Major growth retardation (MGR), Forelimb malformation (FLM), Hindlimb malformation (HLM), Eye malformation (EM), Brain malformation (BM).

Morphometric studies

In the current study, the mean whole-body weight of chick embryos control group was 0.57 g, 1.73 g and 7.05 g for the E7, E10 and E14 respectively. Whereas, embryos in sham and treated groups showed a significant decrease in the whole-body weight in the same period of times compared to the control ($p<0.01$). The mean whole-body length of control group chick embryo were 21.75 mm, 32.70 mm and 53.36 mm in all mentioned incubation periods. While embryos in sham and treated groups showed a significant decrease in the whole-body length in the same incubation periods compared to the control ($p < 0.01$). With regarding to BMI, of control group chick embryo were 0.0267 g/cm, 0.0693 g/cm and 0.1403 g/cm in the E7, E10 and E14, respectively. Whereas, embryos in sham and treated groups showed a significant decrease in the BMI compared to the control ($p < 0.01$) in all incubation periods as shown in **Fig. 9**.

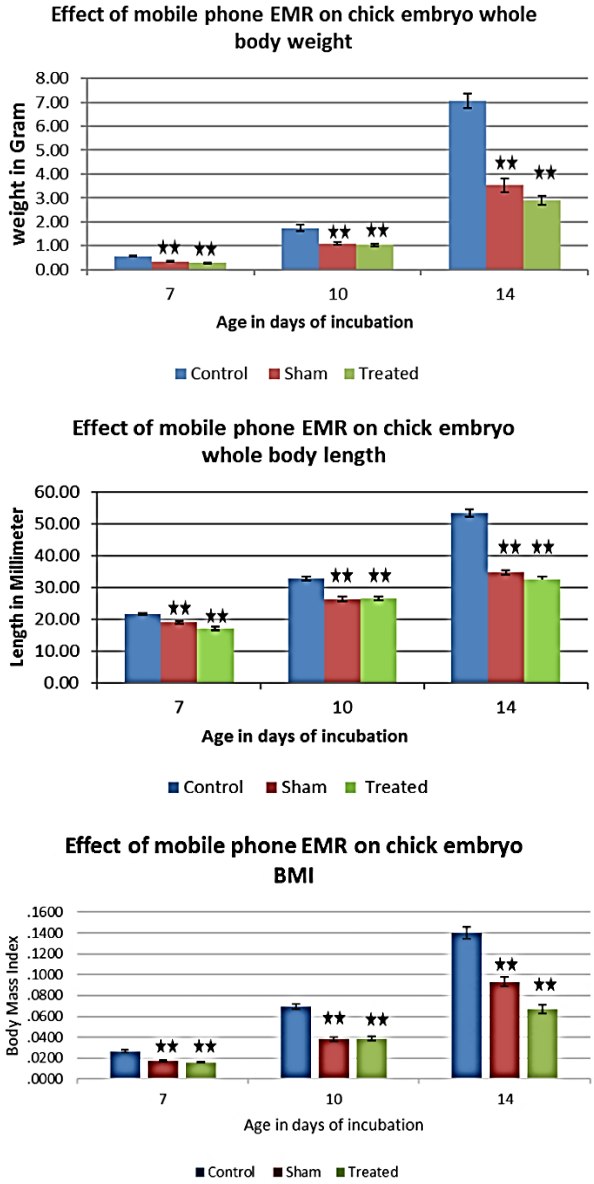

Fig. 9. Graph showing the effect of mobile phone electromagnetic radiations (EMR) on chick embryo whole-body weight, whole-body length and body index weight. Values are mean + SE taken from 30 samples for different incubation periods chick embryos for control, sham and treated groups. Significance ($^{*}p<0.02$), ($^{**}p<0.01$).

Follow-up analyses found that the mean of control group chick embryos head diameter was 9.15 mm, 14.04 mm and 21.10 mm in all incubation periods. Embryos in sham group showed a non-significant decrease in the head diameter E7 compared to the control, while treated group showed an increase. However, E10 and E14 embryos of sham and treated groups showed a significant decrease in head diameter compared to the control ($p<0.01$). The result showed that the means of chick embryos eye weights in

control group were 0.12 g, 0.34 g and 0.59 g in all incubation periods. Whereas, embryos in sham and treated groups showed a significant decrease in eye weight in the same incubation periods compared to the control (p<0.01). Moreover, the chick embryos eye diameter means in control group were 4.63 mm, 7.54 mm and 9.45 mm in E7, E10 and E14, respectively. Sham and treated embryos showed a significant decrease (p<0.01) in eye diameter in all incubation periods compared to the control which is shown in **Fig. 10**.

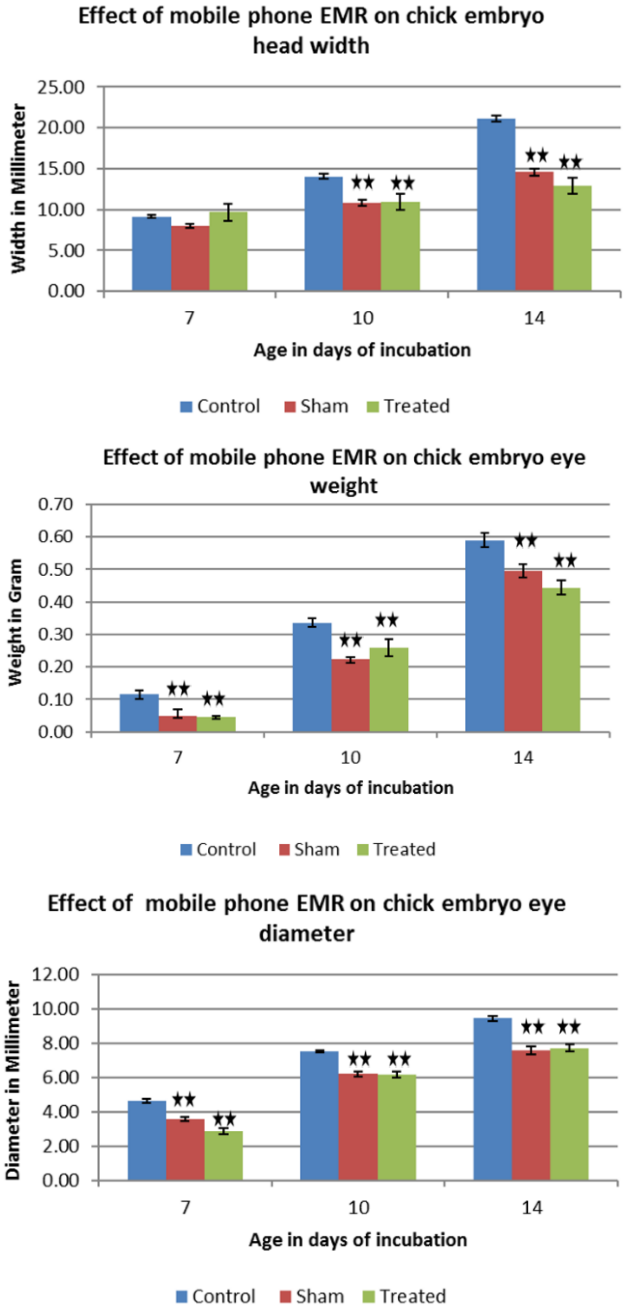

Fig. 10. Graph showing the effect of mobile phone electromagnetic radiations (EMR) on chick embryo head width, eye weight and eye diameter. Values are mean + SE taken from 30 samples for different incubation periods chick embryos for control, sham and treated groups. Significance ($^{*}p$<0.02), ($^{**}p$<0.01).

In the present study, the means forelimb length in control group chick embryos were 10.78 mm and 23.27 mm in the E10 and E14, respectively. Sham and treated embryos showed a significant decrease (p<0.01) in forelimb length in the E10 and E14 compared to the control. The means of chick embryos hindlimb length means in the control group were 14.89 mm and 34.46 in the E10 and E14, respectively. Moreover, embryos in sham and treated groups showed a significant decrease (p<0.01) in hindlimb length in E10 and E14 compared to the control. The means beak length in control group chick embryos were 3.49 mm and 6.83 mm in the E10 and E14, respectively. Moreover, sham and treated embryos showed a significant decrease (p<0.01) in beak length in E10 and E14 compared to the control. The means of control group chick embryos neck length were 4.51 mm and 8.74 mm in E10 and E14, respectively. In addition, sham and treated embryos showed a decrease in neck length in E10 and E14 compared to the control. Where the decrease was significant in E10 (p<0.02) and E14 (p<0.01) which is shown in **Fig. 11**.

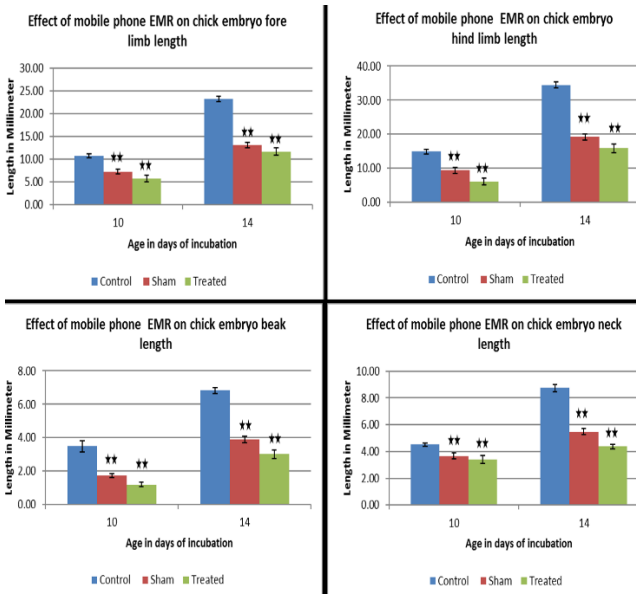

Fig. 11. Graph showing the effect of mobile phone electromagnetic radiations (EMR) on chick embryo fore limb length, hind limb length, beak length and neck length. Values are mean + SE taken from 30 samples for different incubation periods chick embryos for control, sham and treated groups. Significance ($^{*}p$<0.02), ($^{**}p$<0.01).

Hatchability

Based on the results, control chick embryo started hatching between E21 and E25, while sham and treated embryos started hatching on E24. In both sham and treated group three eggs hatched with herniated embryos and died immediately after hatching. The eggs that did not hatch had malformed embryos that stopped growing at different stages which is shown in **Fig. 12**. The results revealed that hatching rate in the control group was 100%, while it was 15% in both sham and treated groups.

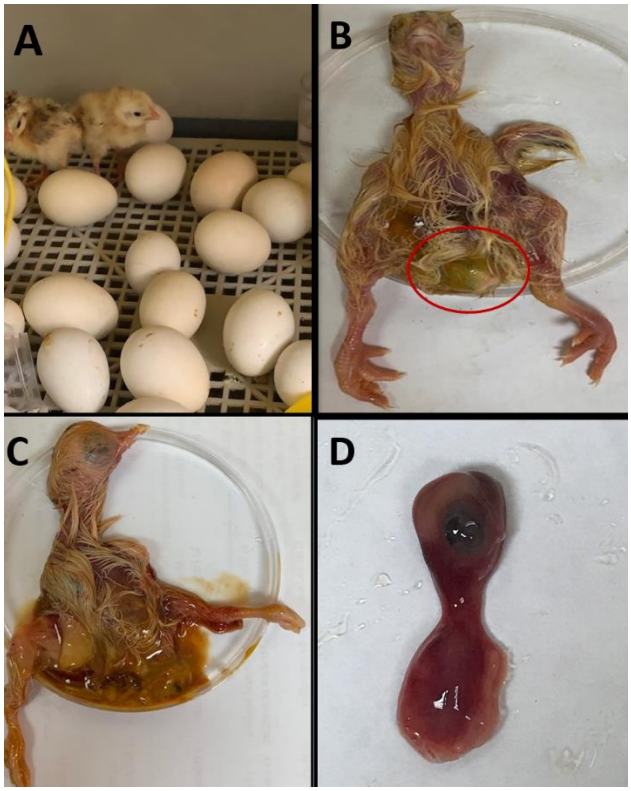

Fig. 12. Chick hatching for control, sham and treated groups.
(A) Control started hatching E21. (B) Sham with malformations seen after hatching E24. Note the hernia, it died immediately. (C) Treated with malformations seen after hatching E26. Note the hernia and small malformed leg. (D) Non-hatching eggs in treated group. Note malformed embryos that stopped growing at different stages.

Discussion

In the current study, chick embryos were used to investigate the possible teratogenic hazards of smart mobile phone electromagnetic radiation. Using the chick embryo as a model allowed us to expose all treated embryos to the same amount of EMR. The layers surrounding the embryo (eggshell, egg membranes and albumin) may have worked as partial barriers absorbing part of EMR. These barriers might be similar to body layers around the mammalian embryos.

The control embryos in this study showed growth parameters (whole-body length and whole-body weight) similar to the growth parameters reported by previous investigators [**12,13**].

The mentioned decreased growth parameters in sham and treated embryos, probably could be due to EMR interactions at cellular level and molecular level resulting in genotoxicity. This in turn might affect cell proliferation either by increasing or decreasing the proliferation rate and thus it plays an important role during early embryonic development. Similar observation was reported earlier by D'Silva *et al.,* (2017). A previous study reported a significant increase in body weight and length at the 10th day, which could not be sustained at day 14 using 900–1800 MHz electromagnetic waves and ringing 4 times for 15 minutes/day [**12**]. However, a study by Amer and co-worker described a decreased fetal weight in intrauterine exposure of rat and mouse animal models exposed to EMR ranging from 27.12 to 2450 MHz [**14**].

We suggest that the effect of EMR on growth parameters observed in all treated groups of embryos of E7, E10 and E14 could probably be due to difference in cellular responses to EMR at different embryological periods and the cells might be trying to rebalance their growth and differentiation rate to normal by activating various cellular stress response mechanisms [**12,15**]. EMR caused several craniofacial congenital malformations in E10 and E14 embryos of sham and treated groups, that included hemorrhagic areas under the skin, also the skin wasn't typically pink in color because of reduced blood flow. A similar observation was reported by [**16**].

The most obvious finding from this study was that the hatching rate was 15% in both sham and treated groups, while it was 100% in the control group. A study reported that the exposed groups to 1800 MHz EMR for100-200 minutes revealed no mortality at all, while at longer exposure for 500–750 minutes raised the mortality rate to 14% [**17**]. Higher mortality rate was also noticed by another study in the exposed group [**18**]. They highlighted that more exposure time and higher power might cause higher mortality rates in the exposed group. Another study reported a rate less than 1% when fertilized eggs were exposed to 900 MHz from E7 to E14 [**19**].

Development of the embryo is a complex process, which consist of cell proliferation, differentiation, migration and programmed cell death. These processes could be affected by endogenous ionic flows and electric fields which could be disturbed by the EMR exposure. Growth retardation in the exposed group is most likely due to the adverse effects of EMR on the DNA [**17**].

Conclusion

We concluded that at exposure to 1800 MHz EMR for 60 minutes per day emitted by smart mobile phones could affect the development of chick embryos as seen on the E7, E10 and E14.

Significant differences were persisted at all incubation periods with increased morphological changes and high mortality rate.

Acknowledgements

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Conflicts of interest

There are no conflicts to declare.

Data sharing and data accessibility

Research data are not shared.

Keywords

Mobile phone, congenital malformation, chicken embryo, morphological studies, electromagnetic radiation, morphometry.

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