DOSE RELATED SHIFTS IN THE DEVELOPMENTAL PROGRESS OF CHICK EMBRYOS EXPOSED TO MOBILE PHONE INDUCED ELECTROMAGNETIC FIELDS

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Background: The possible adverse effects of Electromagnetic Fields (EMFs) emitted from mobile phones present a major public concern today. Some studies indicate EMFs effects on genes, free radical production, immunological and carcinogenic effects. On the other hand there are studies which do not support the hypothesis of any biological impacts of EMFs. This study was designed to observe the effects of mobile phone induced EMFs on survival and general growth and development of chick embryo, investigating dose-response relationship if any. **Methods:** This was an experimental study in which developing chick embryos were exposed to different doses of mobile phone induced EMFs. For this purpose a mobile phone was placed in the incubator in the centre of fertilised eggs in silent ringing mode and was 'rung' upon from any other line or cell phone. After incubation for 10 or 15 days the eggs were opened and the developmental mile-stones of the surviving embryos were compared with the non exposed subgroup. **Results**: EMFs exposure significantly decreased the survivability of the chick embryos. The lower doses of EMFs caused growth retardation. However, this effect of growth retardation reallocated to partial growth enhancement on increasing the dose of EMFs and shifted over to definite growth enhancement on further raising the dose. **Conclusion**: There is an adverse effect of EMFs exposure on embryo survivability. Chick embryos developmental process is influenced by EMFs. However, these effects are variable depending upon the dose of EMFs exposure.

Keywords: EMFs, Chick embryo, Mobile phone

INTRODUCTION

The possible health hazards of mobile phone induced Electromagnetic Fields (EMFs) is no longer a new debate now. The 'interference phenomenon' of the mobile phone induced EMFs and those from the biological cellular activities, has been described as the potential cause of non thermal health effects. There are many studies which have contributed to the still unresolved safety issue of the EMFs. Many have reported the toxicological effects of EMFs, ranging from cognitive effects to genotoxity, and from skin ulcers to brain tumours, however there are contrasting studies in which EMFs have been utilised for therapeutic purposes.

The mobile phone operates upon radiofrequency EMFs, is held very close to human body, and requires base stations for its functionality. Hence the user's body is exposed to two sources of EMFs; one from the mobile phones itself and the other from the base stations usually implanted in residential areas. Obviously the dose of EMFs irradiation is different in different cases.

Keeping in view the casual implantation of the base stations in residential areas, and long talk times of the consumer this study was designed to investigate the dose dependant effects of mobile phone induced EMFs on the general growth and survivability of developing chick embryos.

MATERIALS AND METHODS

This Experimental study was carried out at the Department of Anatomy, College of Physicians and Surgeons Pakistan, Regional Centre, Islamabad. Fertilised chicken eggs of '*Desi*' breed were obtained from Poultry Research Institute of Punjab, Rawalpindi and were randomly divided into two main groups; Control A (n=60 eggs), and experimental B (n=120 eggs). Group A was further subdivided into 2 subgroups, Aa (n=30 eggs) sacrificed on day 10, and Ab (n=30 eggs) sacrificed on day 15 of incubation. The first day of incubation was taken as day one. Group B was subdivided into two series B1 and B2 based on the dosage of EMFs exposure. B1 (n=60 eggs), was exposed to15 minutes of mobile phone 'silent ringing' twice daily while series B2 (n=60 eggs) was exposed to 25 minutes of the same. Each series of the Group B, i.e., B1 and B2 were again divided into 2 subgroups labelled as 'a' and 'b' according to their day of sacrifice, each subgroup having 30 eggs. Subgroups B1a and B2a were sacrificed on day 10, while subgroup B1b and B2b were sacrificed on day 15 of incubation.

For the purpose of description the exposure levels were graded in ascending order as under:

- GRADE I: Low dose, less duration exposure level (for B1a subgroup)
- GRADE II: High dose, less duration exposure level (for B2a subgroup).

- GRADE III: Low dose, longer duration exposure level (for B1b subgroup)
- GRADE IV: High dose, longer duration exposure level (for B2b subgroup)

The exposure grading was one of the methodological limitations of the study. Grade II was high dose exposure till day 10 of embryonal life, while grade III was low dose exposure till day 15 of the embryonal life. It was difficult to mathematically grade the net exposed dose between these two exposure levels. However for the purpose of study the longer duration of exposure, i.e., till day 15 was taken as more intense intervention in the developmental process and considered as a higher dose level.

The eggs were arranged in double ring pattern on a double storey circular plate with 15 eggs on each plate. This plate system was placed in the incubator under standard incubation conditions. In the experimental group a mobile phone was placed in the centre of the lower plate on a small rectangular stand (Figure-1). This mobile was periodically rotated for maintaining almost equal levels of EMFs exposure in all directions through its built in antenna. The mobile phone was 'rung' upon (in silent and non-vibrant mode) from any other line or cell phone for the respective schedule of timings set for different subgroups. On the day of sacrifice the eggs were taken out of the incubator and broken open by conventional methods. The embryos were dissected out of their membranes and their survivability was noticed. The live embryos were processed further.

After a fixation time of 48 hours in 10% buffered formalin the embryos were taken out of the fixative, blotted dry on a piece of tissue paper and their lengths and weights were measured.

The length was taken from the vertex (highest point between the eye balls) and the tip of coccyx along the curvature of the spine. For this purpose a thread was stretched over the contours of the embryo between the above mentioned two points and the distance covered by the thread was measured by a scale.

The embryos were weighed using a precision digital balance with 0.001 gm readability.

Student's *t*-test was applied to detect any significant differences in means of the gross weights and lengths, and to the percentage survival of the embryos. A *p*-value of 0.05 or less was taken as significant.

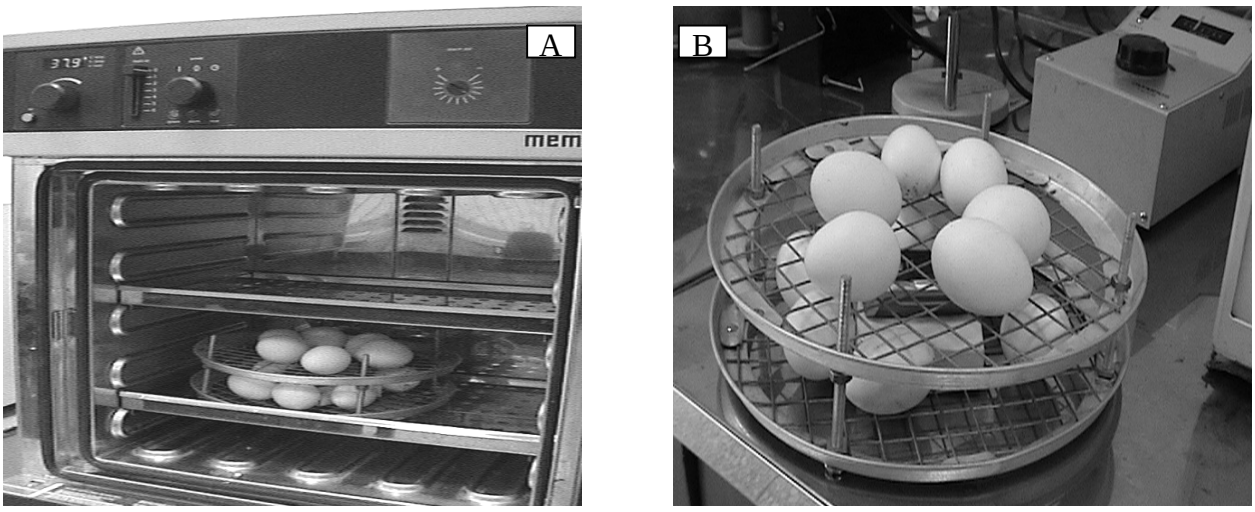

Figure-1: The exposure system

A: Double storey plate carrying fertilised chicken eggs kept in the incubator under standard conditions, with a mobile phone positioned in the centre of the lower storey. B: Magnified view of the exposure plate showing the position of the mobile phone. Note that all eggs are within a distance of one wavelength of the EMFs from the mobile phone.

RESULTS

The effect of EMFs on the survivability of chick embryos was evaluated by applying student's t test on the percentages of the dead embryos which were 3% in the control group A, and 21.06% in the treated group B. A *p*-value of < 0.001 indicated a significant increase in percentage of dead embryos in the Experimental group B as compared to control group A.

Assessment of growth parameters with respect to different exposure grades of EMFs:

Exposure Grade I:

The post fixed embryonal weights and lengths of the embryos exposed to Grade I were significantly less than those of the control embryos ($p<0.001$). This indicated a delayed growth in the treated subgroup as compared to the control one.

Exposure Grade II:
The post fixed weight of this subgroup was more than the control subgroup (p<0.001). The embryonal length was less than the control; however difference of mean lengths noticed was not significant. Hence, ignoring the non significant factors, this exposure level has caused a partial growth enhancement of the developing embryos i.e. with respect to the weight of the developing embryos only.

Exposure Grade III:
The post fixed weights and lengths of the treated subgroup were significantly more than the control embryos. These findings suggest a developmental stage more advanced than the exposure level II, where only the parameter of weight was significantly more than the control. (In this exposure level, however, the significance of weight difference was more (p<0.001) as compared to length difference (p<0.01).)

Exposure Grade IV:
Grossly the embryos were the most advanced in development as compared to the other exposure levels. The weights and lengths of embryos of this subgroup were significantly more than the controls with a p<0.001.

Table-1: Comparison of mean weights of treated and control groups by student's *t*-test

Exposure Grades	Treated subgroups Mean weight±SE (grams)	Control subgroups Mean weight±SE (grams)	*p* value
I	B1a (1.207±0.02)	Aa (1.394±0.02)	<0.001
II	B2a (1.513±0.02)	Aa (1.394±0.02)	0.001
III	B1b (9.563±0.285)	Ab (7.399±0.259)	0.001
IV	B2b 11.563±0.311	Ab (7.399±0.259)	<0.001

Table-2: Comparison of mean lengths of treated and control groups by student's *t*-test

Exposure Grades	Treated subgroups Mean length±SE (cm)	Control subgroups Mean length±SE (cm)	*p* value
I	B1a (4.129±050)	Aa (4.341±.032)	0.001
II	B2a (4.288±0.039)	Aa (4.341±.032)	>0.05
III	B1b (7.375±0.156)	Ab (6.845±0.098)	<0.01
IV	B2b (7.721±0.124)	Ab (6.845±0.098)	0.001

DISCUSSION

As noticed in the results the survivability of embryo worsened by the exposure to EMFs. However, these effects were not dose dependant. Most of the previous studies have also ruled out the dose dependant association of the radio frequency EMFs to their biological effects. However, Lai and Singh in their study earlier had exposed rats to 2450 MHz microwaves and demonstrated damage to DNA, which was dose dependant.[1]

The present study shows that in the surviving embryos, lower doses of EMFs caused growth retardation of the developing embryo. This effect of 'growth retardation' reallocated to 'partial' growth enhancement, on increasing the dose of EMFs and shifted over to 'definite' growth enhancement on further raising the dose.

GROWTH DEPRESSION:
Growth suppression of embryos exposed to EMFs has been reported by other researchers. Atli and Unlu have studied the effects of microwave frequency electromagnetic fields on the development of Drosophila melanogaster and have reported that EMF can cause developmental delay.[2]

Following are some of the factors which have been shown to cause growth retardation and may have contributed to the developmental delay noticed in this experiment.

- **Hormonal Stress:**

The EMFs are a source of stress induction in biologically active tissue.[3] Stress hormones although protective in the immediate aftermath of stress can promote damage when they are overproduced or not turned off. This wear-and-tear of the body has been called allostatic load.[4] Allostasis is the active process of maintaining stability, or homeostasis but allostatic load is the almost inevitable cost to the body of doing so. Intense or prolonged provocation of this response contributes to what have been called 'diseases of adaptation'. Recent research suggests that increased adrenocortical and sympathoadrenal responses are associated with small size at birth.[5]

- **Oxidative stress:**

EMFs also cause oxidative stress resulting in an increased likelihood of cell injury and cell death. Recent study on human volunteers exposed to EMFs for up to 4 hours showed statistically significant oxidative stress.[6] Same has been seen in animal studies.[7] Oxidative stress has been shown to cause delay in the embryonal development.[8]

- **Interference between EMFs and the biological electromagnetism:**

The sequences of developmental events are guided by endogenous ionic currents and electric fields. Disruption of these fields through the EMFs exposure can adversely affect these events[9] hindering the normal developmental process.

- **Genotoxicity:**

The genotoxic effect of the EMFs has come into discussion recently.[10] When cells encounter DNA damage, a cascade of signal transduction pathways activate cell cycle checkpoints leading to blocked cell cycle progression.[11] Such checkpoints have been reported to suppress cellular growth and in an active

process like embryogenesis these reactions could result in growth retardation.

REVERSAL OF DOSE-EFFECT RELATIONSHIP:

The delayed development induced by the EMFs in the project shifted to initially partial, and then definite growth enhancement with rising doses. There could be three possible explanations to this dose-effect reversal phenomenon, namely:

- **A pre-conditioning effect of EMFs:**

According to this effect any external influence at first manifests its damaging effect through stress proteins. Later, intracellular over production of these same proteins can render cytoprotection.[12] Hence the reversal of the growth retardation to growth stimulation may be explained on the same grounds.

- **Dose cumulating effect:**

EMFs have been scientifically and clinically shown and approved to be highly active 'medicines' for cells, animals and plants. So the prolonged exposure of EMFs may have had a cumulative effect leading to growth stimulation rather than depression as noticed with lower doses.

- **Role of genetics:**

It may have been a role of individual genetic make-up, that different flock of embryos (although belonging to the same species), had responded or suffered differently to the EMFs. Although this does not explain the dose related reversal of effects but may be a contributing factor in the diversity of the observed results.

GROWTH ENHANCEMENT:

The increase in growth noticed with higher doses can be explained as simple controlled growth activation or it can be debated as an uncontrolled preneoplastic manifestation. Three possible mechanisms involved could be a direct stimulatory effect, improved developmental environment or carcinogenic influences.

- **Direct stimulatory influence:**

Several different factors can cause embryonal growth activation as discussed under:

i. Growth factors

Recent studies suggest that EMFs exposure might function as soluble growth factors .[13] Roles of growth factors in early embryogenesis is also coming into picture. Insulin-like growth factor-I has been shown to stimulate DNA replication[14]; insulin-like growth factor II has a potential role in the endothelial-mesenchymal transition [15] while Fibroblast Growth Factor has been found to orchestrate gastrulation movements.[16]

ii. Increased cell proliferation

A study in 2006 suggested that EMFs had progressive stimulatory effects on both cellular proliferation and differentiation of mouse embryo limb bud.[17] Understandingly where such kind of effects can lead to advanced growth in developing embryos, they also hint the possibility of cancer transformation. Also this potential of EMFs has been utilized clinically for healing and repair of wounds and fractures.[18]

- **Improved environment:**

i. Cytoprotection/Role of Stress proteins

Intracellular over expression of heat shock proteins has been shown to render cytoprotection in many studies in which EMFs were used as stress inducers.[20] These proteins can render protection to the cells against other potential challenges of the embryonal life and render protection against cytotoxic and proteotoxic effects.[21] There could be a possibility that production and protective influence of stress proteins against the EMFs biological effects was dose dependant and required a specific threshold. This working mechanism has supportive evidence from the past in which prolonged heat stress leading to heat acclimation increases the basal Hsp72 level[22] and predisposed the Hsp molecular machinery to respond faster increasing the cytoprotection.[23]

ii. Improving hypoxia

Electromagnetic field exposure of chick embryos has been shown to protect against hypoxic insults.[24] In a reported study EMFs limited the area of necrosis after ischemic injury caused by permanent ligation of the left anterior descending artery of rats.[25] An improved hypoxia protection definitely is a better environmental condition for embryonal development.

iii. Increased metabolic processes

EMFs are said to activate metabolic processes, specifically the synthesis of factors controlling early embryonic development. In a study two cell mouse embryos were cultured in vitro. After exposure to EMFs, they acquired the ability to develop on their own and reached the stage of blastocyst without serum or growth factors in the culture.[26] These findings indicate that EMFs have a stimulating effect on the early development of embryos, increasing the resistance of embryos to unfavourable environmental conditions.

- **Carcinogenic influences:**

Findings suggest changes in polyamines, activation of c-myc and c-fos gene expression in cells exposed to electromagnetic fields.[19] Polyamines are compounds playing a role in both protein synthesis and cell differentiation through c-myc and c-fos gene activation. Thus EMFs affecting these proteins are affecting the mechanisms involved in cell proliferation and differentiation. These altered mechanisms also raise the probability of an uncontrolled neoplastic cellular proliferation induced by EMFs.

CONCLUSION

The results of this project suggest that the exposure of the developing chick embryo to EMFs decreases their survivability. Regarding the surviving embryos, different exposure doses exhibited different developmental patterns. Whereas the lower doses of EMFs exposure delayed the developmental process of the treated embryos, there was a partial and then definite growth enhancement on progressively increasing the exposure levels. The prevailing controversy regarding the health issues of EMFs may be evaluated in the light of dose related response diversities. Results of this project also demand focus of future studies on the tumorigenic tendency of EMFs.

It is suggested that till the emergence of more definite facts, use of EMFs devices including mobile phone should be limited to minimum.

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