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CELL PHONE INDUCED SPERMATIC & BIRTH DEFECTS ON ALBINO RAT

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ABSTRACT
The flooding of cell phone and towers in world market cause hazardous issue from public health to its future generation. This revengeful interaction between cell phone and human being is depends upon the time and frequency of exposure of electromagnetic waves, which emitted from cell phones. This radiation may cause genotoxic on exposed animals and also may affect to its future offspring. Here we give special attention on the defect of sperm cell on parent animal and gives birth defect to their offspring due to exposure of electromagnetic waves. This defect can be observed by using the male albino rats with the exposure of cell phone for a period of three months. The special cage was designed as mobile stand to keep mobile inside the cage. After termination period, the male animals were mated with female animals for observing birth defect. After gestation period, the delivery statuses of female animals were noted. The current result found that some congenital malformations were found in offspring e.g. infertility of parent animal, under weight of Offspring, Delivery of dead offspring, some physically changes in shape and size etc. This congenital character may be cause physically or mentally handicapped e.g. Change in mandible shape, Ear size, Skin pigmentation, Eye color, skeletal abnormalities etc on long term study. After mating period, the male animals were allowed for laperotomy e.g. sperm analysis. The sperm analysis was compared between the cell phone treated group and without cell phone treated group. The present finding concludes that there was significant damage (* P< 0.05) i. e. 67.60 % of sperm cell in cell phone treated group for three month study.

Keywords: Cell phone, Electromagnetic radiation, Special cage, congenital malformations and Sperm analysis.

INTRODUCTION

The prevalence of infertility among couples of reproductive age has been estimated as up to 15%, with a tendency to increase in recent decades. Infertility is defined as inability to conceive after a year of sexual intercourse without the use of contraceptives. In half of the cases the causative factor is the male. Males are exposed to the effect of various environmental factors which may decrease their reproductive capabilities by affecting semen Quality[1-6].

It may suggest that one of the reasons for the decrease in semen parameters is the effect of the surrounding environment. The male fertility may be manifested by a decrease in the amount of sperm cells, disorders in their motility, as well as structure. The causative agents may be chemical substances, ionizing radiation, stress, as well as electromagnetic waves[1, 7, 8].

The electromagnetic waves radiations, which are emitted from cellular phones, may affect biological systems by increasing free radicals that leads to enhance lipid Peroxidation (LP), and by changing the antioxidative activities of the liver, brain and sperm cells. The effect of these radiations on biological system is primarily due to an increase in temperature i.e. thermal[9], and some non-thermal effects have also been studied[10]. This radiation may affect spermatic cells by damaging to mitochondrial and nuclear genome in epididymal spermatozoa of mice[11], by interfering sperm motility and male fertility on long time exposure[12-15], by apoptic sperm cell death[16], by decreasing in diameter of seminiferous tubule[17, 18], weight of testicular organs (i.e., caput, cauda, and corpus), sperm count[19], and by destructing in Leydig

cells. Epididymal transit is the movement of sperm cell from head through body to tail of the epididymis. Sperm cells are stored in the caudal epididymis at the end of the Spermatogenesis, which can be influenced by Age, Diet, Drug, Temperature and successive ejaculation[20, 21].The normal cell of albino rat consists of hook shaped head, a thin neck, mid piece (Acrosome) and tail for whipping. It is only in rats and mice that the head of spermatozoa terminate in a distinct hook shape [22]. The objective of the biological evaluation is to find out the hazardous potential of cell phone on sperm cells which may affect infertility to parent animal or that may lead to birth defects to next generation. Here electromagnetic radiation exposure to Albino rat for period of three Months only. After three month study, the male animals were mated with fresh Female animal. The Delivery status of female animal was observed.

MATERIALS AND METHODS

Chemicals and reagent: Normal Saline, Methanol (HPLC grade, RFCL Ltd, India.), Eosin Dye (Lobachemie chemical).

Equipment: Cell phone, Special designed cage (such that a wooden box is fitted as close to the wall of cage as cell phone stand), Phase contrast Microscope (Nikon Eclipse E200, Japan).

Animal: Male albino rats of Wistar strain (12-16 weeks old and 125-150 gm of body weight) were collected from animal house. They were housed in special designed cage and provided with standard food pallets (prepared by Brook Bond India Limited) and water ad libitium. The experimental room was maintained 12 hour light-dark cycle (Automatically controlled), Room Temp up to 26 ± 2^{0} C and 45-55 % relative humidity till termination period. After 3-5 days acclimatization period, the animals were taken for experimental purposes. All animals were exposed only once for every experiment. The protocols for animal experimentation described in this study were approved by the Institutional Animal Ethical Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Design of Experiment:

***Exposure System*:** The rats were divided into two groups of six rats each. Group-1 as Control group (Kept without cell phone) and Group-2 as Treated group (Kept with cell phone) in special designed cage. The phone ring/missed call for 1 minute within regular interval of 5 minute for 12 hr/day. This Mobile exposure was started from 8 A. M. to 8 P.M. Similar experiment was performed with control animals without mobile ring. Both groups were provided with normal diet and water ad libitium under above specified laboratory condition for three month. After termination period, each male animal were placed for mating with fresh two female animals. Normally the gestation periods of female rats are 19-21 days. The Oestrus cycle testing can be done by vaginal smear preparation. After Oestrus testing, the male animals and female animals were separated. The female animals were kept for delivery. After delivery, the body statuses of offspring are observed. The male animals were allowed for sperm morphology assay.

Sperm morphology Assay: Both groups of animals were sacrificed. Epididymis was removed and minced in 1 ml of normal saline. The suspension was filtered through a nylon mesh into an eppendroff. To the filtrate, one drop of 1% Eosin dye was added and kept for 30 mints[23].Two or three drops of this were spreaded over the slide to fix the material. Slides were rinsed and screened for the sperm morphological abnormalities under a high power microscope. Thousands of sperms per group were screened and the sperm presenting the defect in shape and structure of either head or tail or both were considered as abnormal; and the data was presented as percentage incidence of total abnormalities.

Statistical Analysis: The data was expressed as Mean ± S.E. The Student-Newman-Keul's test was applied to evaluate the statistical significance of the data obtained, considering $P < 0.05$ as a limit of Significance.

RESULTS

There are different sperm abnormalities are found between the control and treated group viz. Coiled with Microcephali, Flagellum with ansa, Double Headed etc. There is significantly increase ($P > 0.05$) of sperm abnormalities up to 67.60 % in case of treated group for three month study as presented in Table-1. After the delivery of female rats, some birth abnormalities are found i.e. under wt of offspring, Dead offspring, Delay of delivery, infertility of male animals etc as presented in Table-2.

DISCUSSION

In this study, the amount and quality of the sperm was decreasing as the days of treatment with Eletromagnetic waves, which emitted from cell phone. The unidirectional progressive movement

(motility) decreased as the treatment prolongs. The percentage liveability indicated that the treatment adversely affected the liveability potential of the spermatozoa. The volume, motility and liveability are parameters in the determination of viability and fertility in the male animals. The results obtained from the study of the effect of cell phone on sperm cells are shown in Table-1. The sperm abnormalities are expressed as according to shape or structure compared to normal sperm such as Amorphous head, Hookless Sperm, Banana shaped, double headed, Coiled with microcephaly, Bent at Cephalocaudal region, Bent with projecting filaments, Microcephaly with tail defect and defective head with duplication of tail etc. After exposure of electromagnetic waves, it was observed that the use of cell phone has diminished effect on fertility potential up to 67.60 % for three month study of the male animal. This indicates that there is possibility of lower fertility rate of copulation or insemination [24]. After termination period, we found that the significant sperm abnormalities seen in cell phone treated groups as compared with control group by using the Student – Newman-Keuls test, where $P < 0.05$ as a limit of Significance. The electromagnetic radiation of cell phones may cause free radicals, which induces cell damage to spermatic cells due to reduction of histone kinase activity. The decrease in histone kinase activity may serve as a measure of the ability of an electromagnetic field to affect spermatogenesis. Above results indicate that electromagnetic field induces apoptosis and may be a major cause of infertility.

The reduction of spermatocytes in rats exposed to EMF suggests that prolonged exposure to low-level EMF may have negative effects on spermatogenesis that presumably deteriorates fertilization. These changes show that electromagnetic field radiation gives negative effects on spermatogenesis [25]. On the other hand, increased the level of CAT and decreased level of GPx, and SOD were also another key factor for infertility.

This type of study has some limits: the effects of the non-ionizing radiation emitted by cell phones depend on a number of factors besides the duration of transmission, e.g. the type of cell phone and the distance from the cell phone tower [26]. Here we examined only the duration of use, not specified to other variables, as this covered a fairly wide cross-section of males from the whole population. The function of the accessory glands was not examined. Their secretion may affect sperm motion, and also affect the functions in other ways.

In conclusion, the presented studies require continuation within a longer time span on a larger group of male rats and reversibility in electromagnetic field are needed in future.

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Table- 1: Evaluation of Sperm morphological parameters due to exposure of cell phone after 3 months i.e. Control Vs Treated group.

Parameters	**Control group (Without cell phone)**	**Treated group (With cell phone)**
Normal	76.66±0.8819	32.388 ± 1.790
Curved flagellum	10.66±0.6667	1.368±0.4629
Flagellum with ansa	7.33±0.3333	20.54±1.398 *
Bent at cephalocaudal region	2.66±0.3333	25.71±1.652
Amorphous	0.33±0.3333	6.24±0.9672
Double Headed	-	2.7±0.0 *
Multiple Abnormality	1.33±0.3333	7.34 ± 0.6931 *
Curved Flagellum	-	12.17±1.104
Hookless flagella	-	1.14±0.3405
Total Sperm Abnormality (%)	23.54	67.60 ± 1.790 *

***All values are expressed in Mean ± SE (Where N=6), Student-Newman-Keuls Test, * P < 0.05 Considered as limit of significance, when compared with control Group*.**

Table 2: After delivery of female animals, the following consequences of offspring were observed.

Male animals	Dt. of delivery	No Of offspring	Body wt. of Offspring (gm.)	Observation
A	a)26.12.09 b)26.12.09	(13) (8)	a) 6, 5, 6, 5, 6, 5, 5, 5, 6, 5, 5, 6, 5. b) 5, 6, 5, 5, 5, 5, 5, 6	Late delivery time & Under wt.
B	Not delivered	-	-	Infertility
C	a)27.12.09 b)26.12.09	(4) (6)	a) 7, 7, 7, 7 b)8, 8, 7, 8, 7, 7	Normal
D	Not delivered	-	-	Infertility
E	a) & b) 27.12.09	(17)	5, 5, 4, 4, 4, 4, 4, 5, 4, 5, 5, 4, 4, 4, 4, D, D.	Under wt. & 2 dead offspring born. After 2 days, three offspring were dead.
F	Death of male animal before mating.	-	-	Brain & liver Necropsy of male animal.

D- Dead offspring.

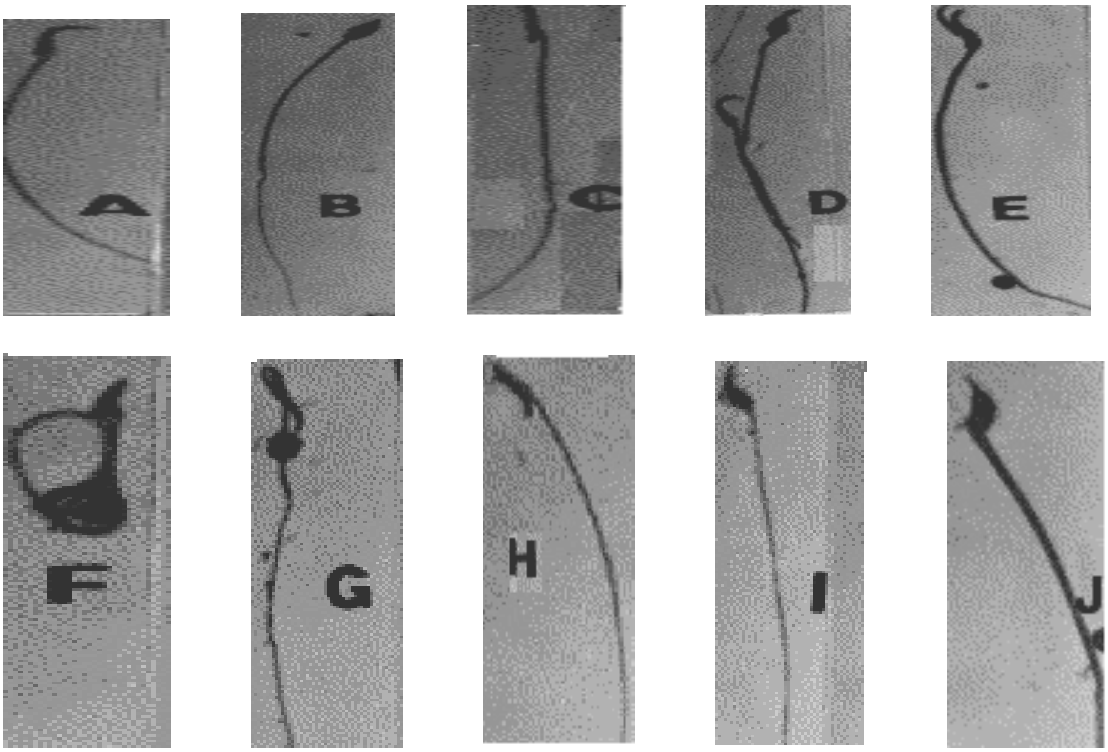

Figure 1: Different types of sperm abnormalities are as follows. A) Normal sperm;(B) Amorphous head;(C) Hook less;(D) Banana; (E) Double-headed;(F) Coiled with microcephaly; G) Bent at cephalocaudal junction;(H) Bent with projecting filaments;(I) Microcephaly with tail defect; and (J) Defective head with duplication of tail.

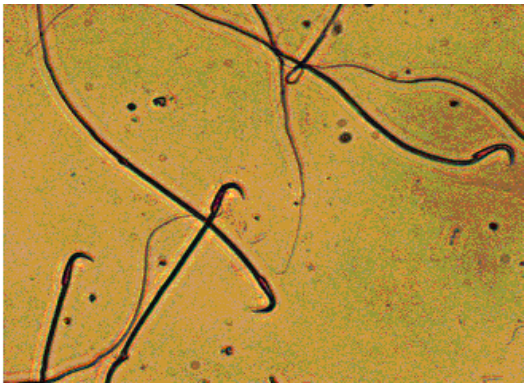

Fig-2:- Control Group (Slide-1) [Normal Sperm Cell]

Fig-3:- Control Group (Slide-2) [Normal Sperm Cell]

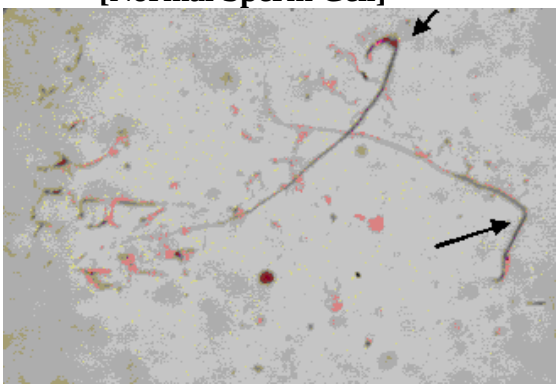

Fig-4:- Treated Group (Slide-1) [Bent at Cephalocaudal Region]

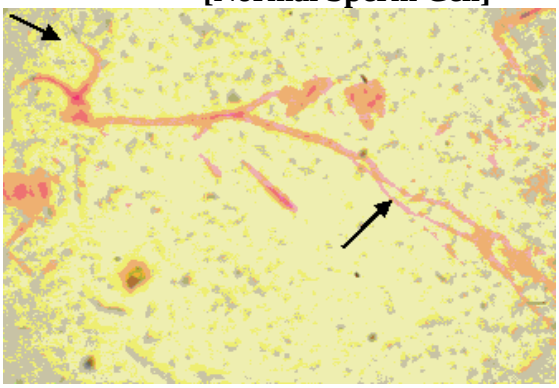

Fig-5:- Treated Group (Slide-2) [Duplication at head & tail]

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