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Impact of Radio Frequency Radiation Emitted from Cell Phone on Female Reproductive of Laboratory Animals

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(i) Introduction-

The Radio Frequency Radiation (RFR) is a sub set of electromagnetic fields covering the frequency range from 3KHz to 300GHz (Behari,2010,). In India, the use of cell phone initiated in 1994 and in 2001, 3.58 million subscribers were using cell phones in the country. Afterward, the number of subscribers increased drastically. 584.32 million and 1161.71 million subscribers were recorded during 2010 and 2019 respectively. Currently, India had more than 1.2 billion mobile users, of which about 750 million are using smartphone {Deloitte's 2022 Global TMT (Technology, Media and Entertainment, Telecom).

The technology has been introduced without fully understanding the nature of these radiation and prior detailed discussion within the scientific community. The potential exposure also developed, in the vicinity of fixed broadcast facilities often located in the residential area, school's hospitals etc with no attention to possible harmful; exposure to the occupants nearby if any.

(ii) Research Problem-

Due to decreased cost of mobile phone, the number of cell phone users have increased in the country drastically that has led to enormous increase in the level of RFR in the environment. It may be possible that these increased level of RFR in the environment may be adversely influencing the overall health of all living components of the environment with specific reference to animals and human beings. The reproduction is one of the most important physiological

functions of all animals and human beings as, it is necessary to continue the generation of the species on the planet. Studying the reproduction of animals is also very important to understand the various aspects of reproductive health of human being. There is no clarity in the existing literature on the possible adverse effect of the RFR emitted from cell phone on animal reproduction with special reference to female reproduction.

(iii) Solution Found

Therefore, it has been proposed to review the existing literature on the impact of RFR emitted from cell phone on reproduction of female animals with special reference to its mechanism of action. 12 following papers published between 2019-2023 have been identified using SCI indexed/Scopus/Science direct/Google scholar.

To evaluate the impact of radiation emitted from cell phone on developmental parameters Koohestani et al. (2019) conducted an experiment in which 16-18 days old pre-antral follicle (PF's) were isolated mechanically from the mice and were cultured for up to 12 days. PFs were divided into controlled and exposed groups. Human chorion gonadotropin was used for ovulation induction in culturing PF's. The parameters which were analysed are follicle size, survival, antral cavity formation, ovulation and oocyte maturation. Enzymatic antioxidants activities, Total Antioxidant Capacity (TAC) and Malondialdehyde (MDA) level were also measured.

The majority of the developmental parameters in the exposed PFs, were comparatively low to the controlled group. Similarly, the exposed PFs also showed reduced level of superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) activity in comparison to that of the unexposed group. On other side the mobile phone exposed PFs, had the levels of TAC low whereas MDA levels was significantly high in comparison to unexposed PFs samples. It's concluded that the developmental competence in mice PFs has been compromised by cell phone radiation because of increasing level of oxidative stress.

To understand steroidogenic activities of Pig myometrium the samples were collected during fetal peri-implantation period. Franczak et al. (2020) conducted an experiment therein; they took 100mg of porcine conceptuses in vitro and exposed them to 50Hz- 120Hz for 2-4hrs. They examine CYP17A1, HSD3B1, CYP19A3, HSD17B4 mRNA , related encoded protein and also steroid hormones. To determine the possible protective effect of progesterone, selected slices were treated with progesterone. It is concluded that mobile phone radiations altered the androgen synthesis and its release during the fetal peri-implantation from myometrium. The authors highlighted that EMF exposure frequency, duration and inclusion of progesterone in the culture determines secretion of androstenedione and testosterone. The authors finally concluded that EMF induces changes in optimal oestrogen concentration in intrauterine milieu resulting in disrupting the endometrial activity and decrease in receptivity of embryo for implantation. The author further concluded that the progesterone not only protects the uterine tissues from the radiation but also act as sensitizer in the EMF-treated endometrium.

Aburawi et al. (2021) undertook an experiment therein, albino female mice (12 in number) were grouped in to two groups i.e., unexposed and exposed group. Second group had 6 mice with ringing phone for 1hr/day, up to 90 days. Behavioural study using forced swimming maze and plus maze was carried out following histological examination of ovary and uterus. The authors reported that the unexposed group didn't show any changes in behaviour of the animal indicating that there is no impact of cell phone radiation on central nervous system (CNS). But on the other hand, exposed group showed abnormal histological features like abnormalities in inner mucosa, endometrium, lining of columnar epithelial cells of endometrium and in some glands near lamina propria, presence of large amount of cytoplasmic vacuoles in the corpus luteum and absence of oocyte inside follicle. In ovarian cortex decrease in count of primordial and primary follicle was seen. In addition to the above reduction in the number of glands in the stomach and atrophy of the uterine gland were also reported. Authors finally concluded that no behavioural and CNS function were affected due to cell phone exposure. But distinct histopathological changes were observed in the ovary and uterus after cell phone exposure.

Kim et al. (2021) studied the impact of EMF emitted from cell phone on placenta and pregnancy. Sprague-Dawley rats were used under this experiment and the animals were categorised into sham-exposed and RF-exposed category. Using 4W/kg specific absorption rate the animals were exposed to radiation at a whole-body from gestational day 1 to 19. Circulating levels of Corticotropin Releasing Hormones and Adrenocorticotropic hormone were measured. Simultaneously expression levels of placental 11β-hydroxysteroid dehydrogenase type 2 messenger RNA was also measured using RT-PCR technique. Histopathological examinations were carried out using haematoxylin and eosin staining for understanding the morphological changes in the placenta. The fetal parts of the placenta were measured using Zen 2.3 blue software. Cortisol levels in circulating maternal blood and in adrenal gland increased in RF-exposed group. The placental cortisol level and levels of *11β-HSD2* mRNA expression were stably maintained in the RF-exposed group. It has been reported that a significant increase of cortisol levels in circulating blood was noticed in exposed group during pregnancy however, no changes in the placental barrier was observed. The study concluded that though the exposure of RF-EMF emitted from cell phone for 8hr/day during pregnancy is protective to foetus because of the placental barrier but it is stressful to the pregnant rat.

Eftikhaar et al. (2021) conducted an experiment on 30 female wistar rats of 12-week aged with 300-400g body weight were used and divided into two groups. Every group was sub divided into two sub groups. Control group was further divided into two sub group. Exposed group was divided into base station exposure group and microwave oven exposed group. In microwave exposure group, ovary was replaced with meso ovarian cyst. In base station exposure group damage in follicle cells of ovary and secondary follicle with no oocyte and congestion were observed. Even degeneration of follicle cells, granulosa cells and no oocyte were also observed in exposed group.

To study the impact of EMR on full-body exposure of rat on the development of polycystic ovaries Mohammed et al. (2021) conducted an animal experimentation. The authors used 21 adult female rats divided into three groups of each having 7 rats i.e. control group, Estradial valerate (EV) group and EV+EMR group. Full body exposure to 150kHz for consecutive 8 weeks for EV+EMR group was undertaken. EV was orally administered to induce polycystic ovaries in both EV+EMR group and EV group and Estrous cycle was assessed. The authors concluded that 150kHz EMR has no such degenerative and morphological changes in developing follicles but an increase in number of developing follicle and a significant reduction in the size and number of follicular cysts was observed in exposed group.

Raouf and Girgis (2022) conducted an experiment investigating the impact of exposure to a 900-MHz EM-waves emitted from cell phones on nucleic acids contents, protein contents, kidney function (levels of urea and creatinine) and oxidative stress (malondialdehyde, MDA level) in rats at different stages of pregnancy. Thirty-two albino wistar rats were separated into 4 equal groups of eight animals in each group i.e., unexposed (controlled) group, pre-pregnancy group, during pregnancy group, during and after pregnancy group. New born & young rats from all groups were harvested for determination of various parameters. Level of DNA, RNA and protein were decreased significantly in exposed group in comparison to control group. Whereas no differences were reported in DNA, RNA and protein contents in pre-pregnancy EMW exposed rats. But a significantly increase in these parameters of all exposed groups, including young and new born rats of both during and after pregnancy was reported. No difference in kidney function and oxidative stress levels were observed in new born of all exposed groups in comparison to controlled. But these parameters increased significantly in young rats of both during and after pregnancy of exposed groups as well in all exposed groups compared to control. The authors finally concluded that EMW emitted from mobile phones adversely influenced the RNA, DNA and protein content, kidney function and oxidative stress of animals in exposed group.

To study the short-term exposure of EMF emitted from cell phone on adult and pregnant rabbits Tomruk et al. (2022) conducted an experiment therein, they used pregnant female New Zealand rabbits. The glutathione and hepatic glucose regulation dependent enzymes were biochemically analysed. RFR exposure with GSM 1800MHz having 14V/m with 2mW/kg of Specific Absorption Rate for 15 min/day for a week during pregnancy and also foetus development was investigated. The regulatory enzymes in the oxidative stage of phosphogluconate pathways to interpret the cytosolic

NADPH's biosynthesis in maintaining mitochondrial metabolism were analysed. Simultaneously the efficiency of maternal glutathione-dependent enzyme on both removal of metabolic disturbances during foetus development and pregnancy were also examined. In pregnant animals, GSH-dependent and hepatic glucose regulation including enzymes' capacity in short-term RFR exposure were also analysed . It was concluded that intrauterine exposure leads to cellular ROS-dependent disturbances in metabolic activity. Also, deficiency in the antioxidant levels of the exposed animal was reported.

Salameh et al. (2022) studied the impact of RF-EMF on the oxidative stress of liver and other related hepatic parameters of female rats at postnatal days. As the sign of pregnancy 4 female and 1 male rat were kept in a cage for examining the vaginal plug. (4 female pups/cages X 4cages/each post-natal age/each control or exposed group). Animals were kept in plexiglass cages, in a controlled chamber for 12h:12h at 22-degree Celsius temperature. The authors reported that the RF-EMF exposure led to elevation in oxidative stress, as shown in an increase in the level of MDA at PND-9 & PND-21, decrease in Catalase activity, decrease in PND-1 & PND-9 in mRNA expression and Catalase amounts. Even GPx activity was also decreased at PND-21 in exposed category. An elevation in cytoSOD at PND-9 & 21 and decrease in mitoSOD at PND-21 was reported in the exposed group in comparison to the control group. Significant elevation of glutathione peroxidase (GPx) level and mitoSOD activity were also found in all ages. Singnificant decrease in cytoSOD activity at PND-1, was observed whereas the parameter increased at PND-9 comparing to the non-exposed group. Moreover, at PND1, SOD1 mRNA expression increased and a decreased at PND21 and 9 was reported. The results of the study reported age-dependent effect of 24 h exposure of RF-EMF emitted from cell phone on oxidative stress, antioxidant level, its activity and expression in the female neonate and young rats which may be resulted in to adverse health effects at later stages.

Islam et al. (2023) studied the adverse effect of 4G phone radiation in the expression of immunogenic and vascular genes along with gross microscopic and biochemical alteration in chicken embryo. Sixty individuals with SAR of 1.4W/kg and the frequency of 2100MHz were exposed to RF-EMF in the incubator for 14 days, 60 min/night at 12cm distance. Haematoxylin and eosin staining was used for histopathological examination and Cresyl violet staining was used to examine the condition and numbers of neuron in the brain. The Expression of immunity genes (AVBD9, IL6) , VEGF-A and amniotic fluid's parameters were also examined. Data of the study clearly indicated that exposure to radiation leads to biochemical changes and alterations in expression of genes that adversely influence the development of embryo.

To study the impact of radiation on teratogenicity & on embryonic development of organisms Augustianath (2023) undertook an experiment on the of hen's fertilized eggs. The eggs were incubated in a digital humidified incubator. Exposed to radiation from 2G and 4G phones at regular ringing time intervals. Lower dose of call duration used 50 min/day whereas higher dose used was 90 min/day. The data showed that the hatchability of experimental groups i.e., 2G and 4G were decreased under both lower and higher exposure groups (40% and 55% at higher radiation exposure and 65% and 75% at lower radiation exposure). Similarly the teratogenic effects of radiation were reported on the chick morphology resulting as the non-retracted yolk sac, malformed legs and toes, macrocephaly, cross beak, disability in standing and balancing the body and variations in body weight, body length and beak length. The data of study shown that the radiation emitted from the cell phone poses potential threats to the organisms.

Drzewiecka et al. (2021) studied molecular mechanism of the harmful impact of the cell phone radiation reported during peri-implantation period. The authors studied the impact of short duration (2 h) of cell phone radiation on transcriptomic variation in the myometrium of pig during the peri-implantation using next-generation sequencing.

The authors observed that RF-EMF exposure effect the expression of 215 transcript active regions which is responsible with defence and immune responses. The authors concluded that RF-EMF emitted from cell phone alter the transcriptomic profile of the pig myometrium. This alteration in transcriptomic profile may result in compromising the immune system. The study also provided an insight information regarding mechanism of RF-EMF action in regulation of transcriptomic profile.

(iv) Conclusion –

The papers reviewed here clearly indicate that the RF-EMF emitted from cell phone adversely influence the histopathology of the ovary and uterus, damage the follicle cells, granulosa cells and also the oocyte (Aburawi et al 2021, koohestani et al 2019, eftikhaar et al 2021, mohammad et al 2021). Even the alteration in the androgen synthesis from the myometrium during the fetal peri-implantation period in the pig have been reported which is resulting in disrupting the endometrial function and decrease in receptiveness during embryonic implantation (Franczak et al 2020). So far, the mechanism of action of this radiation is concerned, the RF-EMF emitted from cell phone have been reported to alter the biochemical milieu including DNA, RNA and protein contents of the ovary and uterine cavity as well as disturbance in metabolic activity in cellular ROS dependent and deficiency in the intracellular anti-oxidant levels of the exposed animal (Rauf and Girgis 2022, Tomruk et all 2022). In-vitro studies also showed alteration of transcriptomic profile of pig's myometrium during peri-implantation period which may adversely affect the mechanism of oestrogen synthesis and its secretion. (Drozewiecka et al.2021). It may result in alteration of angiogenesis in the myometrium and changes the prolific potential and neoplastic myocytes transformed. (Drozewiecka et al.2021). Even the age dependent effect of these radiation on oxidative stress status, anti-oxidant enzyme levels and activities and expression in the female neonates & young rats may lead to adverse health effect during their later stage of life(Salameh et al 2022).

(v) Scope for further work-

It is clearly evident that the RF-EMF emitted from cell phone alter all the stage of reproduction in female animals. Since, the cell phone has been extensively used by the people of all economic status therefore, to find out the similar type of adverse effect in women if any, it is necessary to review the effect of RF-EMR emitted from cell phone on the reproduction of the women.

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