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The role of non-ionizing electromagnetic radiation on female fertility: A review

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ABSTRACT

With increasing technological developments, exposure to non-ionizing radiations has become unavoidable as people cannot escape from electromagnetic field sources, such as Wi-Fi, electric wires, microwave oven, radio, telecommunication, bluetooth devices, etc. These radiations can be associated with increased health problems of the users. This review aims to determine the effects of non-ionizing electromagnetic radiations on female fertility. To date, several *in vitro* and *in vivo* studies unveiled that exposure to non-ionizing radiations brings about harmful effects on oocytes, ovarian follicles, endometrial tissue, estrous cycle, reproductive endocrine hormones, developing embryo, and fetal development in animal models. Non-ionizing radiation also upsurges the free radical load in the uterus and ovary, which leads to inhibition of cell growth and DNA disruptions. In conclusion, non-ionizing electromagnetic radiations can cause alterations in both germ cells as well as in their nourishing environment and also affect other female reproductive parameters that might lead to infertility.

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Electromagnetic radiations; non-Ionizing radiations; ovarian follicles; reproduction; female fertility

1. Introduction

Electromagnetic radiation (EMR) is the combination of electric and magnetic fields having wavelike properties. These energy radiations are electromagnetic fields (EMFs); it is a natural phenomenon and could be generated by man-made sources. EMR can be categorized into non-ionizing radiation, which is considered less damaging, and ionizing radiation, which is potentially harmful to cells and DNA. X-rays, alpha particles, and gamma rays fall into the category of ionizing radiations. Ionizing radiations are more harmful as they cause DNA damage by breaking chemical bonds. On the other hand, non-ionizing radiations lack enough energy to break chemical bonds. Non-ionizing radiations could be further divided into the following types: extremely low frequency, intermediate frequency, hyper frequency, and static EMFs (Juutilainen 2005; Gye and Park 2012). EMR below 300 Hz come under extremely low frequency (ELF), generated by military equipment, home appliances, and railroads (Marci et al. 2018). Electrical goods and power lines generate extremely low frequency at 60 Hz (Kesari et al. 2018). Intermediate frequency EMFs are radiations that have frequencies ranging from 300 Hz to 10 MHz, generated by the computer display and industrial cables (Marci et al. 2018). AM radio transmissions produce intermediate frequency at 530–1600 KHz (Kesari et al. 2018). Hyper-frequency EMFs includes radiations with frequencies from 10 MHz to 3000 GHz, generated by FM radio (88–108 MHz), TV sets (50–700 MHz), mobile phones (850–

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2.4 GHz), laptops, Wi-Fi and microwave ovens (2.4 GHz), etc. (Marci et al. 2018; Kesari et al. 2018). Static EMFs are radiations with zero frequency produced by MRI and geomagnetism (Marci et al. 2018). Radiofrequency EMF (RF-EMF) is the energy transmission through radio waves that falls between intermediate frequency EMF and hyper-frequency EMF as it includes radiations with frequencies ranging from 100 kHz to 300 GHz (Kesari et al. 2018). Non-ionizing radiations also include part of the UV spectrum, visible light, and infrared rays (non-ionizing radiation-occupational safety and health Administration OSHA). An electric field is induced into the body when exposed to high levels of RF-EMF, and a force is exerted on free moving polar molecules, ions, and electrons following EMF gets transformed to kinetic energy. Consequently, these charged particles or molecules revolve and interact with more charged particles; subsequently, the kinetic energy would be converted into heat. Harmful effects can be triggered by this heating of the body. Dielectric disruption of cell membranes can be caused by the induced brief and strong electric field (ICNIRP 2020). The International Agency for Research on Cancer classified the radiofrequency radiation as 'a possible carcinogen' (in group 2B) to human (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2013).

The injurious effects of RFR exposure have been suggested by some studies *in vitro* on different cell types (D'Ambrosio et al. 2002; Ahmadi et al. 2016), whereas other studies identified no significant effects (Demirel et al. 2012; Khalil et al. 2014). In addition, the RFR emitted from mobile phones exerts thermal and non-thermal effects; also, insight exposure of RFR in the short-term and long-term may have an adversative effect on humans as well as animals (Singh et al. 2018). As elucidated by current literature, RFR radiated from RFR emitting devices are associated with a rise in various health-related problems, including sleep disturbances (Durusoy et al. 2017), increased chances of cancer (Gupta et al. 2020), headaches (Durusoy et al. 2017), and could cause adverse effects on male and female fertility (Gul et al. 2009; Gye and Park 2012; Roshangar et al. 2014; Ahmadi et al. 2016; Marci et al. 2018; Yadav et al. 2021; Negi and Singh 2021). The effects of EMF have been mainly classified as thermal (rise temperature in the range of 1°C) and non-thermal (rise in body temperature is below 1°C) (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2013). The thermal action involves a rise in local temperature in the radio frequency with an oscillatory current of the electric field (Belyaev 2005; Gye and Park 2012). Non-thermal action is understood by absorption of energy and alteration in the behavior of tissues, this mechanism involves no heat production (Asghari et al. 2016). The consequence of RF-EMF on the human body is evaluated via specific absorption rate (SAR) value. The SAR is a standard unit to measure the rate of energy absorption per unit mass of the body when exposed to RF-EMF, which is based on frequency, intensity, exposure duration, polarization, and position of electromagnetic device. The absorption rate would be higher if the position of the electromagnetic device is nearby (ICNIRP 2020). Penetration of RF-EMF into the body is based on the permittivity and conductivity of the tissues exposed. The lawful SAR of cell phones is restricted to 2.0 W/Kg by the International Commission on Non-Ionizing Radiation Protection, but the SAR level differs from nation to nation.

Yüksel et al. (2016) reported increased uterus lipid peroxidation levels and higher body temperature of newborn rats when pregnant rats and their newborns were exposed to EMF (900 MHz, 1800 MHz, and 2450 MHz). This shows that either thermal or non-thermal effects of EMF can cause damage to the female reproductive system (Yüksel et al. 2016). To investigate the effect of EMF on the genital system of females, different studies were conducted. Cecconi et al. (2000) identified that exposure to EMF could inhibit the formation of antral follicles in mammalian females. In addition, exposure to EMF in rat ovarian follicles caused degenerative changes in ovarian tissue and increased the number of apoptotic or necrotic cells in the follicles (Ahmadi et al. 2017). Whereas some studies depicted no significant differences in the structure, function, and mean weight of the ovaries (Bakacak et al. 2015; Alekperov et al. 2019).

A study was conducted by Oral et al. (2006) on rat endometrial tissue to examine apoptosis and oxidative stress induced by RF-EMF exposure, and they found increased levels of malondialdehyde (a lipid peroxidation index, used as a marker of oxidative stress) in endometrial tissue. Bcl-2,

caspase-3, caspase-8, and Bax were assessed immunohistochemically to evaluate apoptosis. Based on the results, it was evident that oxidative stress and endometrial apoptosis may be caused due to radiations from mobile phones (Oral et al. 2006). Furthermore, in the female reproductive system, signaling molecules like nitric oxide (NO) regulate ovulation processes and follicle development wherein, different isoforms of NO, such as endothelial nitric oxide synthase, neutral nitric oxide synthase, and inducible nitric oxide synthase, are expressed (Mitchell et al. 2004). An increase in NO levels may have harmful effects and cause disease like sepsis and endometriosis (Cinelli et al. 2020; Budani and Tiboni 2021). Burcu et al. (2020) reported increased inducible nitric oxide synthase levels in rats resulted from prenatal and postnatal exposure to EMF. Increased inducible nitric oxide synthase levels may affect ovarian physiology and fertility as the authors reported congestion, bleeding areas, vacuolization in granulosa cells, and degeneration of follicle structures in the ovarian tissue (Burcu et al. 2020). Shahin et al. (2017) conducted a study on mice, Mus musculus, wherein a significant decrease in follicle count, sex steroids, and ovarian steroidogenic proteins were observed in the mobile phone radiation exposed group. Degenerative changes and mobile phone-induced nitrosative and oxidative stress were also observed (Shahin et al. 2017). Hence, the effects of EMF on female reproductive system remained elusive, signifying the need to review the published literature related to EMF and its impact on female fertility. The purpose of this review is to assess the possible effects of non-ionizing radiation (extremely low-frequency EMF and radiofrequency radiation) on the female reproductive system and fertility.

2. Literature search and methodology

A computerized database literature search was carried out for the original research studies, which covered the effects of EMR exposure on the female reproductive parameters and fertility. The literature search was carried out through Google Scholar and PUBMED databases using the following keywords: 'electromagnetic fields or non-ionizing radiation or radiofrequency or electromagnetic radiation or mobile phone radiation' AND 'female reproduction or female fertility or infertility or female reproductive system'. Research articles published until 2020 were incorporated in the web search. Additionally, to explore the literature, several articles were taken from the cluster of Science sites. Only original research articles, guidelines, and a few review articles published in English which reported the effect of the EMFs on the female reproductive system were included. Both *in vitro* and *in vivo* studies were incorporated in the search. Studies encircling insufficient information and reporting the impact of EMF on other body organs were excluded. Two steps involved in study selection included: 1) Screening of the various research titles and their abstracts, excluding the search that failed to match with inclusion criteria. 2) The selected articles were then retrieved, reviewed, and information, such as animal strain, age, weight, exposure parameters (duration, frequency, SAR value, etc.) was extracted.

3. Effects of EMR on female fertility and reproductive system

There are limited studies when it comes to non-ionizing radiation's effect on the female reproductive system. The negative effects of EMR on the reproductive system have been analyzed by means of several *in vivo* and *in vitro* studies. Considering the bioethical and technical limitations associated with the procurement of oocytes, most of the available reports concerning the effect of EMR on its functions and female fertility are based on animal models. The existing scientific reports provide evidence for the effects of EMR exposure on the ovary, embryo, sex steroids, pregnancy, etc.

The widespread population is exposed to ineludible RF-EMR (radiofrequency electromagnetic radiation) on a daily basis, unaware of its hazardous effects on health. To evaluate the mechanism of RF-EMR action on the reproductive system of females, various studies were conducted. Diem et al. (2005) studied the effect of mobile phone radiations *in vitro* wherein EMR (1800 MHz) exposure induced DNA damage in rat granulosa cells and human diploid fibroblasts. Estrogen is essential for

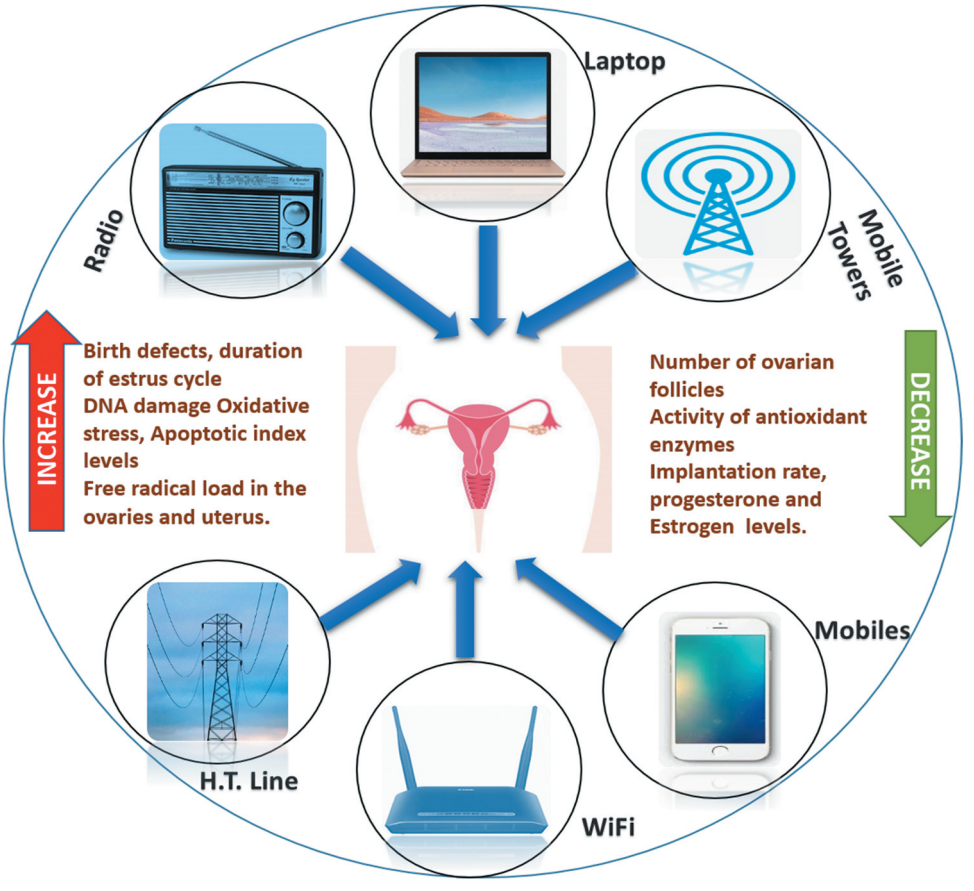


Figure 1. Possible effect of non-ionizing EMR on female reproductive health.

the regulation of fertility, and progesterone has a vital role in the regulation of estrous and menstrual cycles, but the studies observed alterations in the female sex hormones after EMR exposure, thus affecting fertility (Shahin et al. 2017; Burcu et al. 2020). A recent study on rat ovaries indicated no significant defects in the function and structure of the ovaries, but a decrease in the levels of serum sex hormones was observed (Alekperov et al. 2019). A study in dairy cows suggested that EMR affects complete estrus cycle duration and the luteal-phase duration in dairy cows, as indicated by Rodriguez et al. (2003). A significant increase in the total reactive oxygen species production was observed in the ovaries, uterus, and hypothalamus of the exposed group of mice (Figure 2) (Shahin et al. 2017). In accordance with the studies, non-ionizing radiations damage ovarian follicles, oocytes, and DNA, alter sex steroids, and increase the free radical load in the ovary and uterus that might lead to infertility (Figure 1). The findings of EMR studies *in vitro* are arranged in Table 1 and on animal models in Table 2. Impacts of electromagnetic radiation on the number of ovarian follicles are organized in Table 3, and Table 4 (Alekperov et al. 2019) showed its effects on the serum levels of sex hormones.

3.1. Effects on ovaries (ovarian follicles and oocytes)

EMR exposure has detrimental effects on the ovaries. It has been reported that exposure to EMR caused a significant decrease in the number of primary, preantral, antral, and preovulatory follicles as well as in corpus luteum and an increase in the number of atretic follicles (Figure 2)

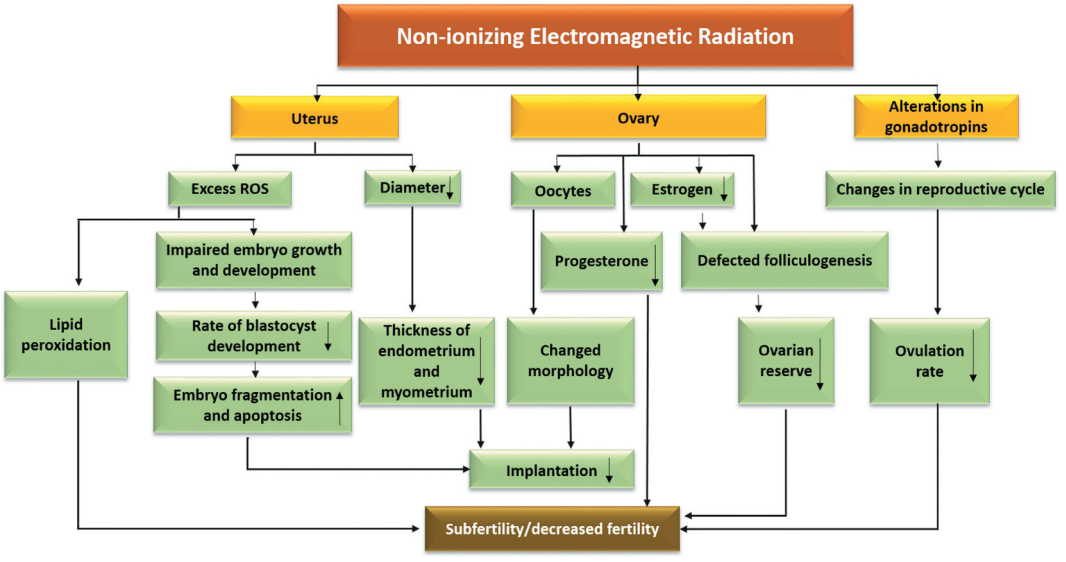


Figure 2. Possible mechanism of non-ionizing electromagnetic radiation action on the female reproductive system. Probable targets of non-ionizing EMR are the uterus, ovary, or by altering the gonadotropins. EMR exposure could cause a decrease in the uterus diameter and thickness of the endometrium and myometrium leading to decreased implantation rate and could be a factor for reduced fertility. ROS is also increased in the uterus due to non-ionizing radiations, which can result in a) oxidative damage and apoptosis in the uterus, b) lipid peroxidation, leading to impaired protein function (e.g. proteins involved in implantation), and c) impairment in growth and development of the embryo, which reduces the rate of blastocyst development and causes increased fragmentation and apoptosis in the embryo, ultimately leading to decreased implantation. Non-ionizing radiation also affects ovaries, changes the morphology of oocytes, and disrupts folliculogenesis resulting in decreased ovarian reserve, and induces alteration in the gonadotropin (FSH and LH) and sex steroid (estrogen and progesterone) levels, which affects the estrus/menstrual cycle causing a decrease in ovulation rate, contributing to subfertility or diminished fertility.

(Shahin et al. 2017). Roushangar and Rad (2007) tested apoptosis using TUNEL assay in the EMF exposed Wistar rats, and after exposure, they noticed, the number of apoptotic cells increased in the ovarian follicles. The authors also observed morphological changes in the oocytes and granulosa cells (Roushangar and Rad 2007). Another study, in this line, suggested that exposure to mobile telephony radiations induced cell death in egg chamber cells (nurse cells, follicle cells, and the oocytes) during early and mid-oogenesis (Panagopoulos et al. 2007). Khaki et al. (2016), with the help of transmission electron microscopy, showed separated theca layer of primary follicles and zona layer with irregular thickness. Isolated ovarian stroma with dilated blood vessels and distorted oocytes with shrunken nucleoli were also observed in the ovary of EMR exposed rats. A recent report in female albino mice suggested that exposure to mobile phone radiations produced damage in the ovary and uterus, as evident through histology (Aburawi et al. 2020). Similarly, deformed and shrunken nuclei in the oocytes were also reported in other studies (Figure 2) (Ahmadi et al. 2016, 2017), the authors reported an increase in apoptotic vacuoles in the granulosa cells (Ahmadi et al. 2017). An EMR of 50 Hz was found to disrupt the structure of ovarian stroma and cause alterations in the atresia of ovarian follicles (Ahmadi et al. 2016). Detrimental effects were reported on the implantation of fertilized ovum and formation of ovarian follicles that might lead to infertility (Ahmadi et al. 2016). Bakacak et al. (2015) observed a significant reduction in the number of primordial ovarian follicles in the EMF exposed rats. No significant difference was reported in the mean ovary and rat weights (Bakacak et al. 2015). Another study in rats depicted that long term exposure to EMR exerted harmful effects on oocytes and the ovarian follicles, thereby gonadal tissue and gametogenesis that could produce irreversible damage leading to infertility (Khaki et al. 2016). Later, Alekperov et al. (2019) showed similar effects of EMF on primordial follicles and the mean weight of ovaries. A study in mice

Table 1. In vitro studies on the effect of EMR on the female reproductive system.

Subject	Source	Exposure frequency/ SAR	Exposure Duration	Conclusion	References
Rat granulosa cell and human diploid fibroblasts	Exposure system	1800 MHz 1.2 or 2W/ kg	4, 16 and 24 hours; intermittent 5 min on/10 min off or continuous wave	DNA single-strand and double-strand breaks in cells.	Diem et al. (2005)
Mouse pre-antral follicles	Cell phone	Not mentioned	12 days 60 minutes/day	Significant decrease in maturation parameters and gelatinolytic activity.	Azimipour et al. (2020)
Mouse pre-antral follicles	Pulsed ELF–EMF exposure system	33 or 50 Hz Not mentioned	5 days	No significant effect was observed in the follicles exposed to 50 Hz, whereas 33 Hz exposure caused a reduction in the pre-antral follicles.	Cecconi et al. (2000)

Table 2. In vivo studies on the effect of EMR on the female reproductive system.

Subject	Source	Exposure frequency/ SAR	Exposure Duration	Conclusion	References
Female Sprague Dawley rats	EMF application system	900 MHz 0.01 W/kg	13-21 days 1 hour/day	Number of atretic follicles and apoptotic index levels increased.	Türedi et al. (2016)
Female Rats	Not mentioned	50 Hz Not mentioned	*8 weeks (3 weeks via intrauterine and 5 weeks after births) *13 weeks (3 weeks via intrauterine and 10 weeks after births)	Increased apoptotic cells in the ovarian follicles.	Ahmadi et al. (2017)
Female Wistar rats	A programmable generator of ELF EMF 435,321.001 (Thira-el) and a solenoid	30 Hz Not mentioned	10 weeks 2 hour/day	Decrease in FSH level in the proestrus phase and progesterone level in the estrus phase.	Alekperov et al. (2019)
Female Wistar albino rats	Exposure system	900 MHz 1800 MHz 2450 MHz 0.1 W/kg	60 min/day	Exposure of maternal uterus to EMF results in increased oxidative stress induced by EMF in the development of offspring.	Yüksel et al. (2016)
Drosophila melanogaster	Mobile phone	900 MHz Not mentioned	5 days 6 min/day	Decrease in reproductive capacity.	Panagopoulos et al. (2004)
Female Wistar albino rats	Exposure system	2450 MHz 0.1 W/kg	21 days 1 hour/day	Increased Oxidative stress index, total antioxidant status, and apoptotic cells.	Sangun et al. (2015)
Female BALB/c mice	EMF producing device	50 Hz Not mentioned	21 days 4 hour/day	Chromatin condensation to the nuclear margins and shrunken oocytes nuclei. Autophagic vacuoles around the nucleus of granulosa cells.	Roshangar et al. (2014)
Female Mus Musculus Swiss Albino rats	Cell phones	Not mentioned	12 hour/day	Decrease in the number of produced ovulatory follicles.	Gul et al. (2009)
Drosophila melanogaster	Cell phones	GSM 900 MHz DCS 1800 MHz 0.89 W/kg	6 days 6 min/day	Cell death was strongly induced in the egg chambers.	Panagopoulos et al. (2007)
Female rats	Not mentioned	50 Hz Not mentioned	*8 weeks (3 weeks via intrauterine and 5 weeks ectopic) *13 weeks (3 weeks via intrauterine and 10 weeks ectopic)	*Irregular arrangement of growing primary follicles with discrete theca layer in 8 weeks exposed group. *Destruction and disappearance of granulosa in the growing primary follicles in 13 weeks exposed group.	Ahmadi et al. (2016)

(*Continued*)

Table 2. (Continued).

Subject	Source	Exposure frequency/ SAR	Exposure Duration	Conclusion	References
Female Sprague–Dawley rats	Exposure system	50 Hz 3-mT Not mentioned	4 hour/day 9 weeks (prenatal period and postnatal period)	Increase in FSH level and decline in estradiol level.	Burcu et al. (2020)
Female Mus Musculus Swiss Albino mice	Mobile phone exposure set-up	1800 MHz Not mentioned	120 days 3 hour/day	Increased free radical load and reduced activity of antioxidant enzymes in ovary, hypothalamus, and uterus.	Shahin et al. (2017)
Female Wistar-Hannover albino rats	Exposure device	900 MHz 0.018 W/kg to 4 W/kg	15 days 15 min/day	Significant reduction in the number of ovarian follicles.	Bakacak et al. (2015)
Holstein cows	Exposure chamber	60 Hz Not mentioned	16 hour/day	Increased duration of the estrus cycle.	Rodriguez et al. (2003)
Female Wistar-albino rats	Exposure device	900 MHz 0.016 W/kg to 4 W/kg	30 days 30 min/day	Oxidative damage and Histopathologic changes in the endometrium.	Guney et al. (2007)

Table 3. Studies on the effect of EMR on the number of ovarian follicles (mean ± standard deviation values).

Subject	Source	Exposure frequency/SAR	Exposure Duration	Parameters evaluated	Control group	Experimental group			References
Female Wistar rats	A programmable generator of ELF EMF 435,321.001 (Thira-el) and a solenoid	30 Hz Not mentioned	10 weeks 2 hour/ day	Primordial follicles	55.72 ± 9.36	51.86 ± 9.12			Alekperov et al. (2019)
				Primary follicles	20.03 ± 3.23	18.72 ± 3.81			
				Secondary follicles	14.23 ± 3.49	14.03 ± 3.44			
				Antral follicles	12.08 ± 2.94	12.07 ± 2.96			
				Atretic follicles	7.97 ± 2.82	9.25 ± 3.05			
				Cystic follicles	1.98 ± 1.78	2.6 ± 1.86			
Female Sprague Dawley rats	EMF application system	900 MHz 0.01 W/kg	13-21 days 1 hour/ day	Primordial follicles	66.3 ± 10.3	36.5 ± 18.1			Türedi et al. (2016)
				Primary follicles	91.8 ± 33	71.15 ± 18.9			
				Secondary follicles	24.8 ± 12.1	37.3 ± 14.5			
				Tertiary follicles	7.5 ± 1.9	4 ± 2.2			
				Atretic follicles	16 ± 6.25	31.65 ± 6.5			
Female Wistar-Hannover albino rats	Exposure device	900 MHz 0.018 W/kg to 4 W/kg	15 days 15 min/ day	Primordial follicle average (right ovarian)	78.50 ± 25.98	34.00 ± 10.20			Bakacak et al. (2015)
				Primordial follicle average (left ovarian)	71.75 ± 29.66	36.00 ± 10.53			
				Primordial follicle average (total ovarian)	150.25 ± 49.53	70.00 ± 19.03			
						On dialing mode	On receiving mode	On stand-by mode	Shahin et al. (2017)
Female Mus Musculus Swiss Albino mice	Mobile phone exposure set-up	1800 MHz Not mentioned	120 days 3 hour/ day	Primary follicles	7.83 ± .601	4.50 ± .224	2.17 ± .307	1.833 ± .307	
				Preantral follicles	14.67 ± 1.382	8.17 ± .703	7.50 ± 1.056	5.00 ± .516	
				Antral follicles	8.34 ± .494	2.5 ± .224	1.84 ± .307	1.00 ± .307	
				Pre-ovulatory follicles	4.34 ± .422	1.67 ± .211	1.50 ± .224	0.50 ± .223	
				Atretic follicles	2.67 ± .334	10.18 ± 1.108	14.840 ± 1.276	22.170 ± 1.108	
				Total number of corpus luteum/ ovary	6.00 ± .365	2.84 ± .307	1.84 ± .307	0.834 ± .308	
				Total number of follicles/section (excluding atretic follicles)	35.17 ± 1.797	16.50 ± 1.408	12.84 ± .946	8.50 ± .764	

Table 4. Serum Levels of Sex Hormones in Rat of Experimental and Control Groups (M±SD).

Exposure parameters (Frequency and duration)	Subject	Hormones evaluated		Hormonal level in different Phases of estrous cycle			
				Proestrus phase	Estrus phase	Metestrus phase	Diestrus phase
30 Hz 10 weeks	Female Wistar rats	LH, mIU/ml	Control	25.58 ± 3.69	0.83 ± .10	0.47 ± .08	0.71 ± .09
			Experiment	24.99 ± 3.15	0.81 ± .10	0.48 ± .09	0.73 ± .09
		FSH, mIU/ml	Control	468.28 ± 21.74	1.10 ± .22	0.80 ± .06	0.81 ± .08
			Experiment	455.6 ± 22.12	1.02 ± .21	0.77 ± .10	0.81 ± .08
		Progesterone, ng/ml	Control	29.29 ± 4.24	8.95 ± 1.15	10.51 ± 1.92	8.09 ± 1.17
			Experiment	28.33 ± 3.96	8.33 ± 1.35	10.07 ± 1.84	7.98 ± 1.16
		Estradiol E2, pg/ml	Control	74.17 ± 5.31	18.14 ± 1.87	19.39 ± 2.46	25.20 ± 3.19
			Experiment	71.97 ± 4.76	18.79 ± 1.94	19.19 ± 2.61	25.78 ± 3.29

Reference: Alekperov et al. (2019).

found that mobile phone exposure exerts a deleterious effect on the hypothalamus, ovary, and uterus, affecting the ovarian and uterine activity adversely, eventually leading to ovarian and uterine dysfunction by increasing ROS production (Shahin et al. 2017). According to a recent *in vitro* study by Azimipour et al. (2020), mice pre-antral follicles, when treated with cell phone radiations for 60 minutes, showed a significant decrease in their diameter, survival rate, and antral formation rate on the 2nd-4th day of the culture period and on their ovulation rate after 12 days of the culture period (Figure 2). The gelatinolytic activity was also found to be decreased significantly in the exposed pre- antral follicles. Additionally, it was observed that gene expression of matrix metalloproteinase 2 and tissue inhibitors of metalloproteinase 1 showed a significant increase, whereas matrix metalloproteinase 9 and tissue inhibitors of metalloproteinase 2 expression was significantly reduced in cell phone exposed pre-antral follicles during the culture period, resulting in decreased developmental competence of pre-antral follicles in mice as matrix metalloproteinases and matrix metalloproteinase inhibitors are associated with follicular development in a coordinated and cell-specific manner (Bagavandoss 1998; Cooke et al. 1999; Azimipour et al. 2020). Studies with the help of comet assay showed RF-EMF exposure at a SAR value of 1.2 or 2 W/kg induces breaks in DNA strands in rat granulosa cells and human diploid fibroblasts (Diem et al. 2005). Some authors observed shrunken granulosa cells, thinning in zona granulosa, and theca layers in the EMR exposed rats (Okatan et al. 2018). Decreased mitotic activity in the follicles and a significant increase in the superoxide dismutase, catalase, and anti-Mullerian hormone levels were also reported in the ovarian tissue exposed to EMF (Okatan et al. 2018). One study involving different frequencies and duration reported an increase in apoptotic cell death in the egg chambers of Drosophila melanogaster when newly emerged flies were exposed (Sagioglou et al. 2016). According to a study, most of the *in vitro* grew oocytes obtained from ELF-EMF exposed follicles showed a reduction in their ability to attain meiotic maturation, and this suggests that EMF might reduce the reproductive potential of mammalian females (Cecconi et al. 2000). Exposure to high frequency EMFs are closely associated with toxicities of female gonads, as indicated by Xu et al. (2009).

3.2. Effects on endometrial tissue

EMR has an adverse effect on the endometrial tissue. According to a study conducted by Guney et al. (2007), 900 MHz EMR exposure in rats could decrease the endometrial activity of superoxide dismutase, glutathione peroxidase, and catalase and increase the endometrial levels nitric oxide and malondialdehyde. The authors observed histopathological changes in the endometrium and the presence of diffuse and severe apoptosis in the endometrial surface epithelial cells, stromal cells, and the glandular cells in the exposed group (Guney et al. 2007). Oral et al. (2006) also showed similar

effects of mobile phone radiation on rat endometrial tissue. It has been reported that exposure to 1800 MHz radiation caused a reduction in the uterus diameter as well as in the thickness of the endometrium and myometrium (Figure 2) (Shahin et al. 2017).

3.3. Effects on embryo and offspring

EMF impacts on embryo and offspring have been investigated by various groups of people. EMFs radiated from mobile phones and television were associated with the increased risk of embryo growth cessation during the first term of pregnancy (Han et al. 2010). In swine, 50 Hz ELF-EMF exposure 4 hours prior to ovulation resulted in decreased cleavage of fertilized eggs in the oviduct, suggesting that ELF-EMFs negatively affect embryo development (Bernabò et al. 2010). Borhani et al. (2011) conducted a study to evaluate the effects of EMF exposure on implantation and fetal development and found a significant decrease in the number of blastocysts and an increase in DNA fragmentation when mice were superovulated and mated following exposure (Figure 2). This also suggests that embryonic development could be affected by EMF exposure. It has been shown that prenatal exposure to 900 MHz EMF caused a decrease in the ovarian follicle amount, vacuolization, and degeneration of granulosa cells in rats (Türedi et al. 2016). A study by Roshangar et al. (2014) was conducted on pregnant BALB/c mice to evaluate the neonatal ovarian effect induced by EMF exposure. The authors reported irregularly arranged broken oocyte nests, irregular oocyte nucleus having condensed heterochromatin, and numerous vacuoles in the oocytes and thecal cells of neonates (Roshangar et al. 2014). According to the studies, maternal exposure to cell phone radiations could significantly decrease the number of ovarian follicles in the rat pups (Gul et al. 2009) and increase the oxidative stress in the maternal uterus during the development of offspring as well as in the growing rats (Figure 2) (Yüksel et al. 2016).

An increase in oxidative stress induces endometriosis, spontaneous abortion, and intrauterine growth restriction (Jackson et al. 2005; Gupta et al. 2007; Biri et al. 2007). Apart from these studies, a recent study on rats showed congestion, degeneration of follicle structures, and bleeding areas in the ovarian tissue on prenatal and postnatal exposure to EMF (Burcu et al. 2020).

The EMFs might have an association with pregnancy problems, such as low birth weight or preterm birth and infertility, as stated by some epidemiological studies (Lewis et al. 2016; Sadeghi et al. 2017; Esmailzadeh et al. 2019). Cao et al. (2006) reported a significant increase in spontaneous abortions and developmental defects in the offspring when mice were exposed to 50 Hz, 1.2 mT EMF during pregnancy. In contrast, Ohnishi et al. (2002) reported that exposure to a 50 Hz, sine wave EMF at 5.0 mT for nine weeks and two weeks before copulation had no effects on reproduction and fetal development in mice. Likewise, prenatal exposure to a 20 kHz saw tooth EMF at 6.5 μT for 8 hours per day inflicted no adverse effect on fetuses (Kim et al. 2004).

A significant reduction was observed in the fertility of female rats exposed to the magnetic field of 50 Hz for 90 days before mating, and a decrease in the living fetuses per litter and the mean number of implantations were also evident (Figure 2) (Al-Akhras et al. 2001). The authors observed that maternal exposure to mobile phone radiations for 10 minutes during pregnancy and after birth caused an increase in fetal and neonatal heart rate and a decrease in stroke volume and cardiac output (Rezk et al. 2008). An EMF of 900 MHz applied during the prenatal period was found to impair the follicular development process in female rat pups (Türedi et al. 2016). In contrast, according to a recent study, no developmental abnormalities in offspring have been observed on exposure to RF-EMF during and after pregnancy and lactation periods (Shirai et al. 2017). Similarly, exposure to Wi-Fi signals during the prenatal period did not cause any macroscopic abnormalities in the rat fetuses (Poulletier de Gannes et al. 2013).

3.4. Effects on hormones and estrus/menstrual cycle

EMR affect the key hormones regulating the menstrual/estrous cycle and pregnancy. Estrogen and progesterone are vital sex hormones in females. They are secreted from the ovaries and regulate the menstrual cycle and gonadotrophin secretion. Progesterone is required for implantation and estrogen for folliculogenesis (Lasley et al. 1975; Drummond and Findlay 1999; Al-Asmakh 2007). Prolactin is primarily produced in the pituitary gland, and melatonin is produced in the pineal gland. Both prolactin and melatonin affect implantation. A review of the literature performed by Tenorio et al. (2015) showed that treatment with melatonin or prolactin affects the processes responsible for the development and maintenance of pregnancy. Hence, alterations in their levels could harm fertility.

Yüksel et al. (2016) reported that long-term exposure to mobile phones and Wi-Fi-induced EMR caused a reduction in the plasma prolactin, progesterone, and estrogen levels in female rats. Similarly, a decrease in the levels of gonadotropins, progesterone, and estrogen, as well as in absolute and relative ovarian weights, was observed in the rats exposed to 50 Hz EMF (Figure 2) (Al-Akhras 2008). According to a study, prenatal and postnatal exposure to 2450 MHz EMF has no significant effect on serum FSH and E2 levels, whereas serum LH levels were increased in rat pups (Sangun et al. 2015). Likewise, the authors found no difference in the serum estradiol level or uterine wet mass of ovariectomized Sprague-Dawley rats exposed to 1,439 MHz TDMA EMF (Yamashita et al. 2010).

Aydin et al. (2009) observed that continuous exposure to 50 Hz EMF for three months did not affect the morphology and weight of ovaries and uterus, estrogen level, and progesterone level concerning the varying periods of the estrous cycle in the female Wistar rats. Some authors observed an increase in the duration of the estrus cycle, but no differences were detected in the plasma progesterone concentrations and total area under the progesterone curve in EMF exposed dairy cows (Burchard et al. 1998; Rodriguez et al. 2003). Likewise, extended estrus cycle (proestrus and metestrous stages) was also reported in mice exposed to a 20 kHz saw tooth EMF at 6.5 μT for six weeks, 8 hours/day after weaning (Figure 2) (Jung et al. 2007). In contrast, exposure to 10 kHz, 0.2 mT sine wave EMF had no effects on the estrous cycle of female Wistar rats (Dawson et al. 1998).

Alteration in the circulating melatonin and prolactin levels was observed in cows exposed to 60 Hz EMF for four weeks (16 hours/day) (Rodriguez et al. 2004). Some authors reported that RFR from mobile phones and base stations caused alterations in the prolactin but not progesterone levels, suggesting that RFR might have an impact on menstruation and pregnancy (Eskander et al. 2012).

4. Conclusion

According to the findings of this review, it can be concluded that non-ionizing EMRs radiated from cell phones, laptops, bluetooth devices, microwave ovens, or wireless networks might have detrimental effects on female fertility. Non-ionizing radiations can have destructive effects on ovary and uterus, affecting several reproduction parameters in females, such as folliculogenesis, oocyte morphology, and differentiation, hormones, reproductive cycle, and could lead to DNA damage. These effects may lead to subfertility and infertility due to increased free radical load and oxidative stress in the ovaries and uterus generated by these radiations. However, the effect of non-ionizing radiation on human gamete vis-à-vis female fertility is still poorly understood. Also, the findings of a whole-body EMR exposure in the same animal do not anticipate the results of a local EMR exposure. As a result, the outcomes of whole-body EMR exposure in animals cannot be directly compared to the outcomes of local EMR exposure in humans while using cell phones. Unfortunately, specific frequency and SAR value that causes impairment in the reproductive parameters and the exact mechanism of EMR action are not known so far. Moreover, further investigations are required to elucidate the role of EMR from mobile phones, Wi-Fi, and other

devices in female fertility. Lastly, it is the need of the hour to understand the exact mechanism of EMR action on female fertility, establish safe exposure limits, and overcome the radiation effects.

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Authors Contributions

Pooja Jangid written the manuscript, Umesh Rai analyzed and edited the manuscript, Radhey Shyam Sharma designed and edited the manuscript and Rajeev Singh conceptualized, designed, analyzed, written and supervised the manuscript.

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