



Impairment of Oogenesis and Folliculogenesis in Neonatal Rats after Maternal Exposure to Mobile Phones

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Abstract

Today's lifestyle has led to an increase in infertility. The study investigated the effects of cell phone radiation on oogenesis, folliculogenesis, and gestation stage. Also, its impact on neonates' ovarian hormones and their ovarian development during gestation was investigated. Fifty-four virgin female Wistar rats were randomly divided into three groups: the study group (conversation and stand-by mode, $n = 24$), the control group (stand-by mode, $n = 24$), and the sham (turn off, $n = 6$). The study and control groups were separated into 4 subgroups, including early stage (1st week), mid-stage (2nd week), late stage (3rd week), and all stages (3 weeks). The results showed that the concentration of plasma estrogen and progesterone, ovarian primordial follicle/primary oocyte, the number of primordial follicles, and nuclei diameters in the study and control subgroups decreased significantly in comparison with the sham group. The most significant reduction was observed in subgroups in which mothers were exposed to radiation for a long period during their pregnancy. Compared to the control and sham groups, the number of primordial follicle apoptosis markedly increased in the study subgroups. The pregnant rats showed significant effects in the entire stages of pregnancy, especially during the first stage (the first week) of development, which has not been reported previously. Exposure to cell phones during the different gestation stages probably decreases ovarian hormone secretion and may harm oogenesis. It also inversely increases the apoptosis of primordial follicles. Therefore, the gestational stages and duration of exposure to cell phone radiation affect the risk of ovarian harm.

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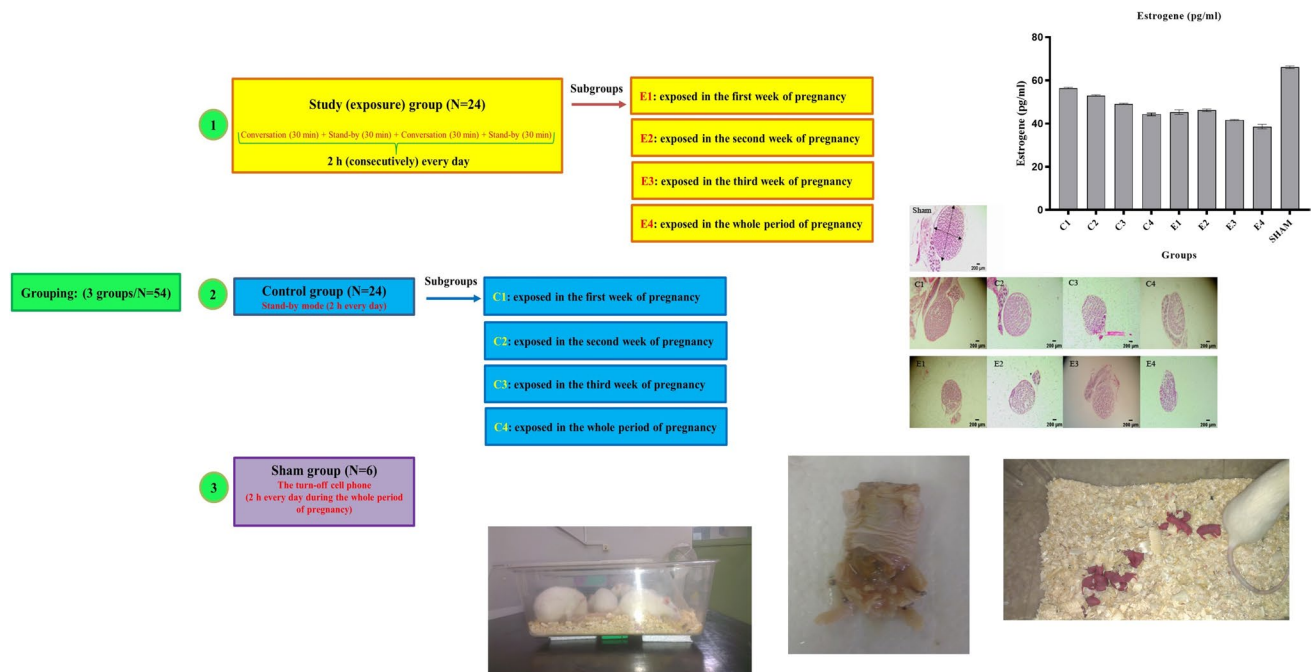
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Graphical Abstract



Keywords Cell phone · Primordial follicle · Oogenesis · Estrogen · Progesterone

Introduction

In recent decades, the electromagnetic field (EMF) in wireless communication has raised concerns as a potential risk factor [1]. Our lifestyle constantly exposes us to electromagnetic field (EMF) radiation, mainly produced by mobile phones. More than three billion people in more than 200 countries are consciously affected by the EMF of devices such as cordless phones, cell phones, and Wi-Fi [2]. In the last decade, a significant increase in EMFs emitted within the environment has been observed, and one of the major concerns for society is the proximity of these radiations to sensitive organs, such as the auditory system, brain, eyes, and testicles [2]. Infertility is one of the most common problems in society. Today, 15–30% of couples experience it and pay too much for its treatment every year [3, 4]. According to statistics, today's lifestyle, such as an increase in the age of marriage, strict and unplanned diets, excessive use of mobile phones, exposure to pollutants and smoke, and excessive caffeine consumption, has led to a decrease in the ovarian follicular reserve in females and a shortening of their reproductive life cycle [5–9]. Primordial follicles do not form after birth, and their number at birth will represent the total number of primordial follicles available to females during their reproductive life [10, 11]. In the ovaries of a 5-month-old human fetus, the numbers of oogonia

and primary oocytes reach their maximum, and more than 99% of them undergo atresia by the end of the reproductive age through a natural process; thus, the reserve of follicles decreases over time [12]. According to a study by Gul et al., in pregnant rats exposed to cell phone radiation, the number of follicles in their 21-day-old neonates was significantly reduced [13]. A study by Aburawi et al. showed that mice exposed to electromagnetic radiation from mobile phones had a reduction of the ovarian cortex in some primordial and primary follicles, an absence of oocytes in the follicle, and a large amount of vacuolated cytoplasm in the corpus luteum [14]. Khayat et al. investigated the effect of cell phone radio-frequency (RF) radiation during pregnancy on the expression of MMP-2 and MMP-9 after neonatal hypoxia–ischemia (HI) in rats. Their results showed that exposure to mobile phone RF radiation during pregnancy exacerbated HI damage in rat pups by increasing MMP-9 activity [15]. Moghadas et al. reported that exposure to mobile radiation significantly increased MDA (Malondialdehyde), and 8-OHdG (8-hydroxy-2'-deoxyguanosine) and decreased Total Antioxidant Capacity in the exposed rats [16]. Although studies have shown that 900 MHz EMF affects the ovary, they have not specified the adverse effects of the EMF on oogenesis and folliculogenesis during development separately on the first, second, and third week of gestation, and their ovaries at birth (natal day). Also, these studies have not answered

the important question, “Which weeks of the gestation period are most vulnerable to these fields?” In this regard, the objective of this study was to survey the effects of cell phone radiation on oogenesis, folliculogenesis, and gestation stages. We also investigated its impact on neonates’ ovarian hormones and their ovarian development during gestation.

Materials and Methods

Animals

Fifty-four three-month-old adult female Wistar rats (180–200 g) were used in this study. The animals were kept according to the conditions of light/dark cycle (12/12 h), relative humidity of 45–55%, and temperature of $24 \pm 2^\circ \text{C}$ with sufficient and free access to water and food in transparent cages in the animal sanctuary of Semnan University of Medical Sciences. All steps on how to work with animals were provided by the instructions of the Committee on Ethics and Protection of Laboratory Animals. Also, the study was reported under ARRIVE guidelines (<https://arriveguidelines.org>).

Study Design and Animal Groups

After confirmation of vaginal plug as gestational day zero, the rats were randomly divided into three main groups,

namely the study group (conversation and stand-by mode, $n = 24$), the control group (stand-by mode, $n = 24$), and the sham ($n = 6$). The study and control groups were divided into 4 subgroups, including the early stage (1st week), mid-stage (2nd week), late stage (3rd week), and all stages (3 weeks). These experimental subgroups (E1, E2, and E3) were exposed to cell phone radiation (Nokia X, 900MH) for 2 h (namely half-hour conversations and half-hour stand-by mode, consecutively) for 7 days every day but in the E4 subgroup for 21 days. Control subgroups were exposed to a turned-on cell phone without conversation for 2 h. The Sham group was placed in the vicinity of the turned-off phone for 2 h daily, as shown in Fig. 1. A Nokia-x cell phone ($\text{SAR}_{\text{Head}} = 1.3 \text{ W/Kg}$, and $\text{SAR}_{\text{Body}} = 1.44 \text{ W/Kg}$) was selected and placed under the cage to irradiate the animals. The rats were transferred to a Plexiglas cage without metal components. Special perforated plastic doors were used during irradiation to prevent animals from leaving.

Estrogen and Progesterone Measurements

In this study, the samples were immediately prepared and evaluated after birth (natal day) to minimize the effect of environmental factors on the study process. Therefore, on the natal day, a blood sample (0.2–0.3 mL) was taken from the heart of anesthetized neonatal mice to evaluate biochemical factors. Blood samples were poured into clean tubes and placed at laboratory temperature for coagulation for 30–60

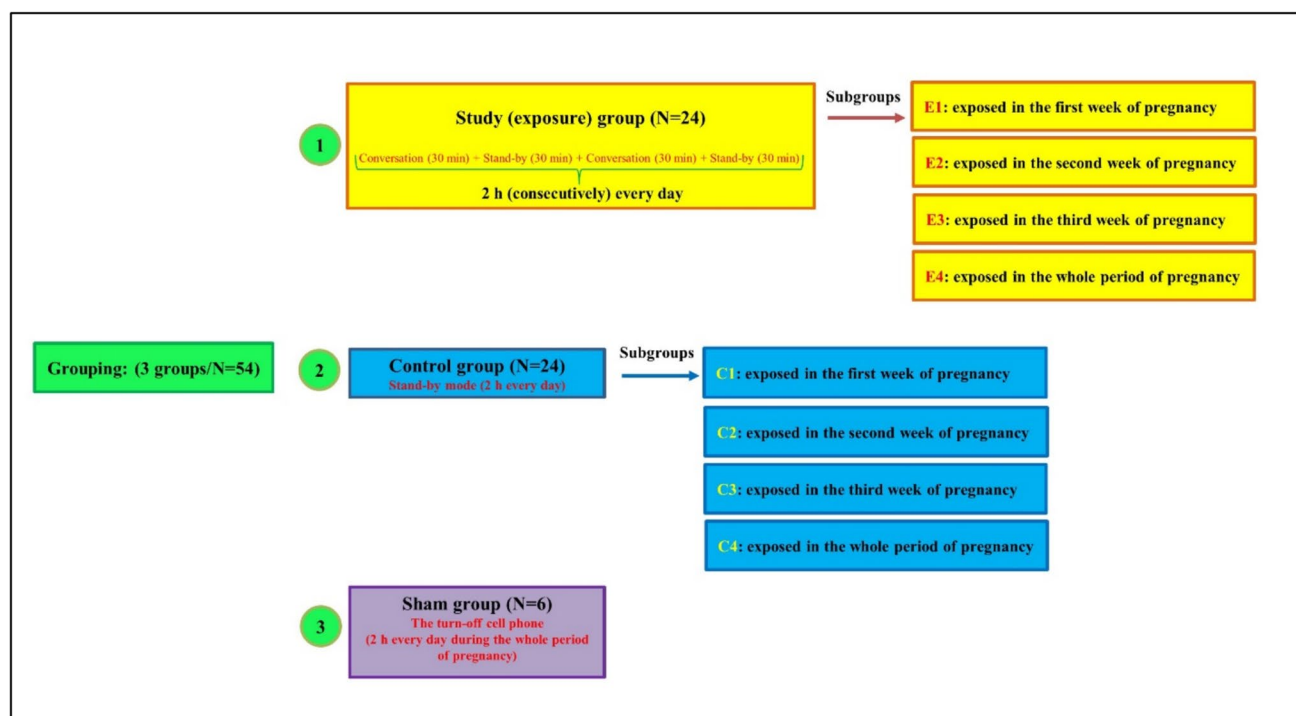


Fig. 1 Experimental design of the animal study. Group 1, Study (exposure) group; Group 2, Control group; Group 3, Sham group

min, and then centrifuged at 3000 rpm for 15 min. After separation, the sera were poured into appropriate microtubes and stored in a freezer at -70°C until tests were performed. Plasma estrogen and progesterone concentrations were measured by IMMULITE 2000 Estradiol and IMMULITE 2000 Progesterone methods, respectively.

Tissue Preparation and Histomorphometric Evaluation

The neonates were anesthetized with xylazine/ketamine, and blood samples were immediately taken from their hearts. Then, the abdomen was opened with a surgical scalpel blade, and the ovaries were removed and kept in 10% formalin for further tests. After stabilization of tissue samples, dehydration, clearing, and molding stages, 5-micron sections were prepared, and hematoxylin–eosin staining was conducted to evaluate the ovary and its primordial follicles and their contents, and TUNEL staining (TUNEL kit, Roche Diagnostic GmbH, Germany) was conducted to determine the apoptosis content of the follicles. This staining determines the extent of DNA degradation and fragmentation [17]. In this staining, the relevant kit was diluted 500 times, and TUNEL-Enzyme and TUNEL-Label solutions were used in 1: 9 ratios to stain positive control samples. For negative control samples, only the TUNEL-label solution was used. The bright green dots found in the tissues represent the apoptotic cells marked

during TUNEL staining. The nuclei of the cells in the tissue samples turned red, and the damaged cells in green were seen as TUNEL-positive [17]. Using an optical microscope equipped with a computer interface and the analysis software system, images were taken from the stained sections, and the histomorphometric changes of the samples were studied and evaluated using Motic Images Analyzer Plus 2 software.

Statistical Analysis

The data obtained from this study were analyzed as mean $\pm$ SEM for different groups using a one-way ANOVA test followed by a post hoc Tukey test (if data distribution was normal) and Mann–Whitney and Kruskal–Wallis nonparametric tests (if data distribution was not normal) by SPSS software version 16. $P \leq 0.05$ was considered a significant difference.

Results

Blood Estrogen and Progesterone Concentration

As shown in Fig. 2A, the concentration of estrogen in the plasma of all exposed subgroups was significantly decreased in the first, second, third, and entire weeks of gestation in comparison with the control subgroups ($P < 0.001$) and

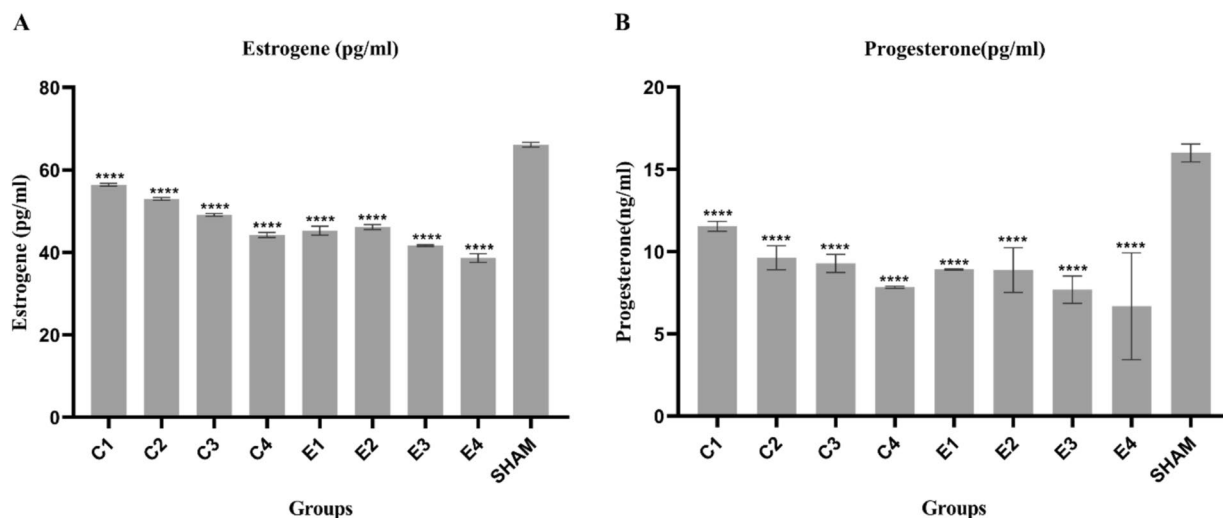


Fig. 2 The effect of cell phone radiation (900 MHz) on plasma (A) estrogen and (B) progesterone concentrations (Pg/mL) in neonatal rats. Estrogen levels in subgroups E1, E2, E3, and E4 reduced significantly compared to the control ($***P < 0.001$) and Sham ($****P < 0.0001$) groups. There is a significant difference between the Sham and other groups in estrogen and progesterone levels ($****P < 0.0001$). C1: Control subgroup exposed to standby-mode radiation pattern during the first week of pregnancy, C2: Control subgroup exposed to standby-mode radiation pattern during the second week of pregnancy, C3: Control subgroup exposed to standby-mode radiation

pattern during the third week of pregnancy, C4: Control subgroup exposed to standby-mode radiation pattern during the whole period of pregnancy, E1: Experimental subgroup with intermittent radiation pattern during the first week of pregnancy, E2: Experimental subgroup with intermittent radiation pattern during the second week of pregnancy, E3: Experimental subgroup with intermittent radiation pattern during the third week of pregnancy, E4: Experimental subgroup with intermittent radiation pattern during three weeks of pregnancy, Sham: The group exposed to the turn-off cell phone during the whole period of pregnancy

sham group ($P < 0.0001$). The progesterone concentration in all subgroups of the study and control groups was significantly decreased compared to the sham group ($P < 0.0001$). However, there was no significant difference in progesterone levels between the control and study subgroups (Fig. 2B).

Histomorphometric and Histopathological Evaluation of the Ovary

Ovary Diameters

Figure 3 shows a significant decrease in ovarian diameters in all the exposed subgroups compared to the sham group ($P \leq 0.0001$). There was a remarkable difference ($P \leq 0.0001$) between the sham group and subgroups of C3, C4, E1, E2, E3, and E4. The difference between the sham group and subgroups of C1 and C2 was $P < 0.01$ and $P < 0.001$, respectively (Fig. 3).

Primordial follicle

Figure 4 compares the number and diameter of primordial follicles in all experimental subgroups in the first, second, and third weeks and whole gestation (21 days). Overall, in the control and exposed groups (Fig. 4A), the diameter of

primordial follicles was significantly decreased compared with the sham group. As shown in Fig. 4B, the most significant effect in the number of primordial follicles was observed in the exposed subgroup of E4 ($P < 0.0001$). In the control group, the subgroup of C4 showed the most decrease in the number of primordial follicles compared to the sham group (Fig. 4B, $P < 0.01$). There was no difference between the sham group and the C1, C2, and C3 subgroups in the number of primordial follicles (Fig. 4B).

Primary oocytes

In the exposed group (study group), the E2, E3, and E4 subgroups showed that radiation from the cell phone caused a significant decrease in the diameter of the primary oocytes compared with the sham group, as shown in Fig. 5A. The most effect was observed in the E4 subgroup ($P < 0.0001$). However, there was no significant difference between the C1, C2, C3, C4, and E1 subgroups in the diameter of the primary oocyte compared with the sham group (Fig. 5A). There was a significant difference in the diameter of the primary oocyte between the C4 and E4 subgroups ($P < 0.001$). As shown in Fig. 5B, there was a remarkable difference ($P \leq 0.001$) between the sham group and subgroups of C4, E1, E2, E3, and E4 in the diameter of the primary oocyte nucleus.

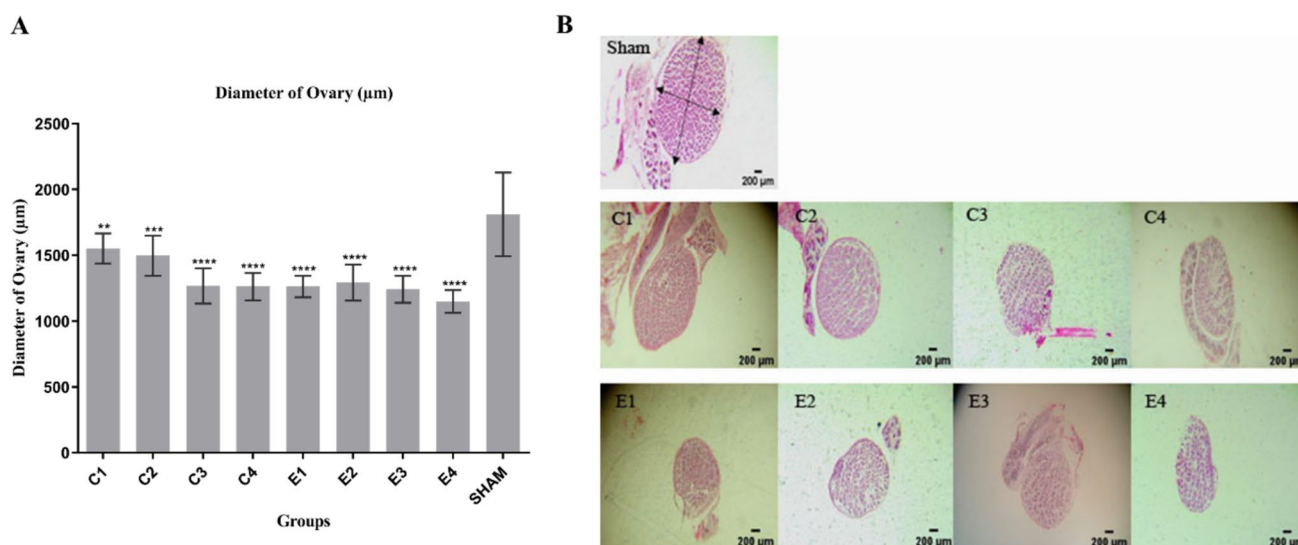


Fig. 3 The effect of cell phone radiation (900 MHz) on (A) mean ovarian diameter (μm) in neonatal rats and (B) light microscope image (H&E staining) of their ovary (the scale bar is 200 μm). The ovarian diameter significantly reduced in subgroups E1, E2, E3, and E4 compared to the Sham group (**** $P \leq 0.0001$). There is a significant difference between the Sham group and subgroups C3, C4, E1, E2, E3, and E4 (**** $P \leq 0.0001$). C1: Control subgroup exposed to standby-mode radiation pattern during the first week of pregnancy, C2: Control subgroup exposed to standby-mode radiation pattern during the second week of pregnancy, C3: Control subgroup exposed to standby-mode radiation pattern during the third week of pregnancy,

C4: Control subgroup exposed to standby-mode radiation pattern during the whole period of pregnancy, E1: Experimental subgroup with intermittent radiation pattern during the first week of pregnancy, E2: Experimental subgroup with intermittent radiation pattern during the second week of pregnancy, E3: Experimental subgroup with intermittent radiation pattern during the third week of pregnancy, E4: Experimental subgroup with intermittent radiation pattern during three weeks of pregnancy, Sham: The group exposed to the turn-off cell phone during the whole period of pregnancy. ** $p < 0.01$, *** $p < 0.001$, **** $p \leq 0.0001$ versus Sham group

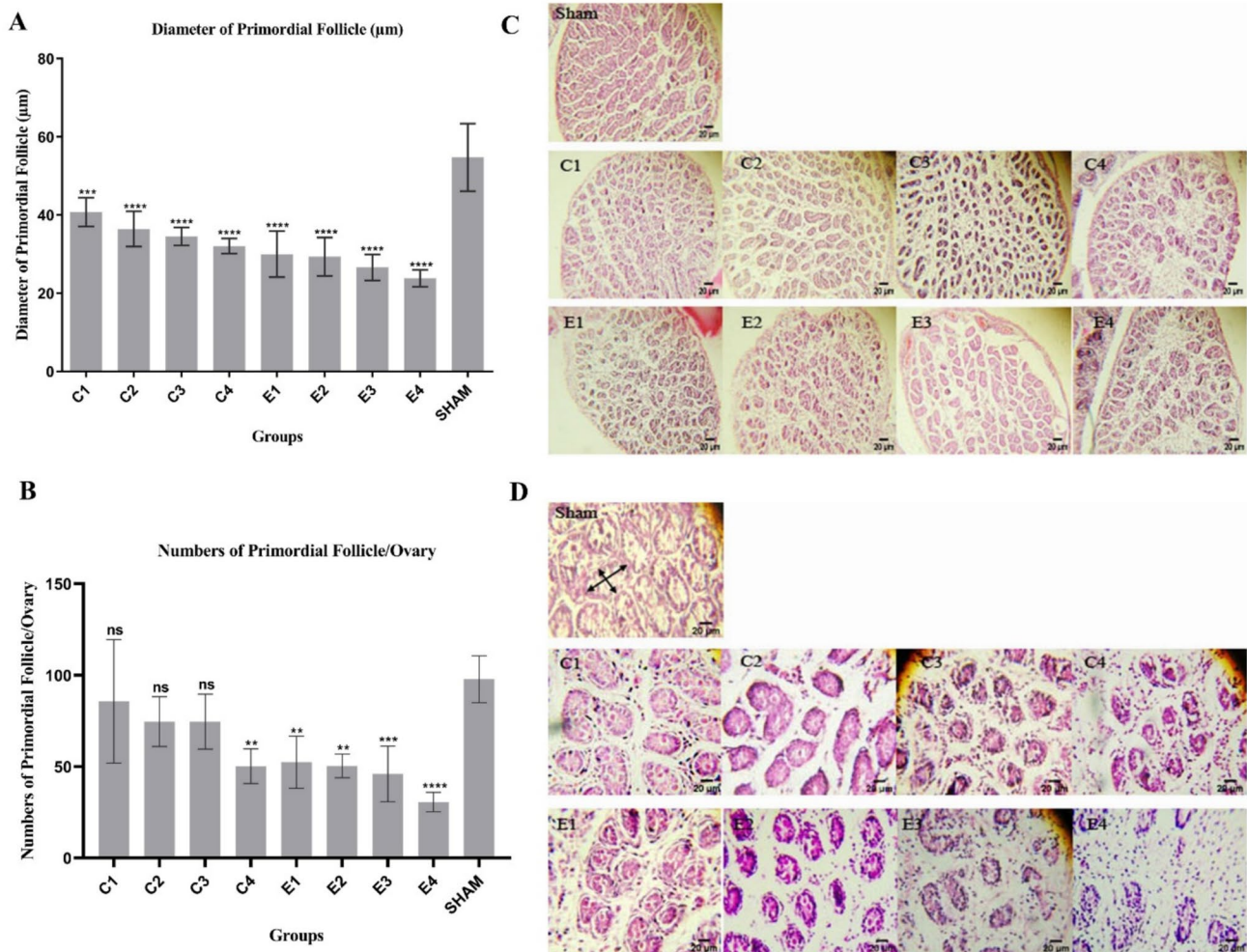


Fig. 4 The effect of cell phone radiation (900 MHz) on the number and the mean diameter of primordial follicles in the ovary of neonatal rats and a light microscope image of their ovary (the scale bar is 20 μm). The number (**A**) and the mean diameter (**B**) of primordial follicles in groups **E1**, **E2**, **E3**, and **E4** significantly reduced compared to Sham groups. A light microscopic image (H&E staining) of the ovary shows that the number (**C**) and the diameter (**D**) of primordial follicles are reduced in all subgroups. **C1**: Control subgroup exposed to standby-mode radiation pattern during the first week of pregnancy, **C2**: Control subgroup exposed to standby-mode radiation pattern during the second week of pregnancy, **C3**: Control subgroup exposed to

standby-mode radiation pattern during the third week of pregnancy, **C4**: Control subgroup exposed to standby-mode radiation pattern during the whole period of pregnancy, **E1**: Experimental subgroup with intermittent radiation pattern during the first week of pregnancy, **E2**: Experimental subgroup with intermittent radiation pattern during the second week of pregnancy, **E3**: Experimental subgroup with intermittent radiation pattern during the third week of pregnancy, **E4**: Experimental subgroup with intermittent radiation pattern during three weeks of pregnancy, Sham: The group exposed to the turn-off cell phone during the whole period of pregnancy. ns (not significant), ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ versus Sham group

Meanwhile, there was no significant difference between the C1, C2, and C3 subgroups in the diameter of the primary oocyte nucleus compared with the sham group (Fig. 5B).

Atretic follicles

Atretic follicles were examined in all groups. The exposed subgroups (E1, E2, E3, and E4) exhibited a significant increase in atretic follicles compared to the control and sham groups (Fig. 6A, $P \leq 0.0001$). Generally, the E4 subgroup

exposed for a longer time (21 days) had the highest number of atrophic follicles.

Discussion

Among the many lifestyle factors influencing infertility, mobile phone use has become important due to its dramatic increase in recent decades. The present study investigated the effects of cell phone exposure on neonatal rats' estrogen, progesterone, and ovarian follicles during the gestational

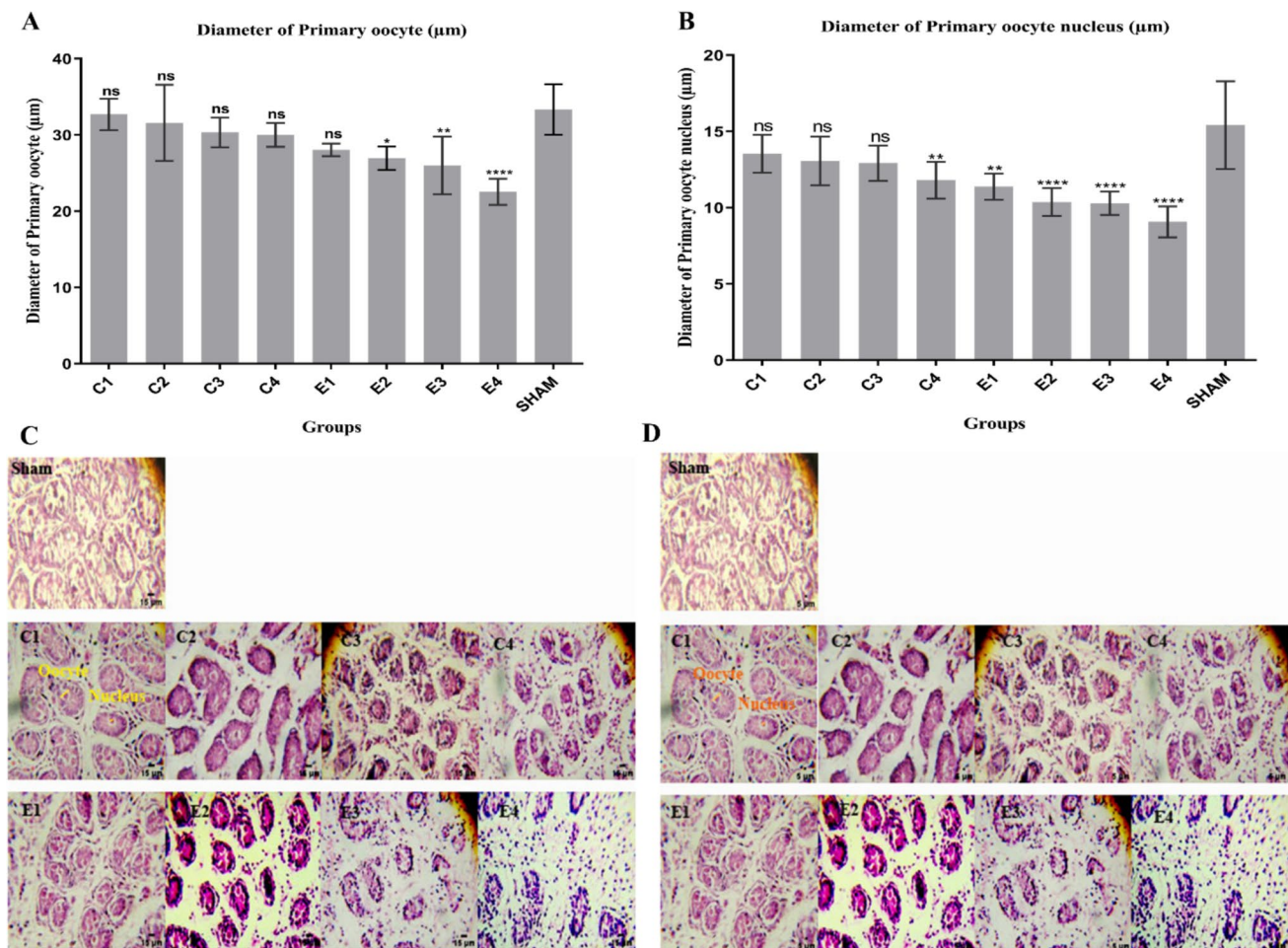


Fig. 5 The effect of cell phone radiation (900 MHz) on (A) the diameter of the primary oocyte (μm) and (B) the primary oocyte nucleus (μm) in neonatal rats. The diameter of the primary oocyte and nucleus in subgroups **E2**, **E3**, and **E4** had a significant decrease compared to the Sham group. Light microscopic image (H&E staining) shows that the diameter of (C) the primary oocyte (the scale bar is 15 μm) and (D) the primary oocyte nucleus (the scale bar is 5 μm) decreased in all groups. **C1**: Control subgroup exposed to standby-mode radiation pattern during the first week of pregnancy, **C2**: Control subgroup exposed to standby-mode radiation pattern during the second week of pregnancy, **C3**: Control subgroup exposed to

standby-mode radiation pattern during the third week of pregnancy, **C4**: Control subgroup exposed to standby-mode radiation pattern during the whole period of pregnancy, **E1**: Experimental subgroup with intermittent radiation pattern during the first week of pregnancy, **E2**: Experimental subgroup with intermittent radiation pattern during the second week of pregnancy, **E3**: Experimental subgroup with intermittent radiation pattern during the third week of pregnancy, **E4**: Experimental subgroup with intermittent radiation pattern during three weeks of pregnancy, Sham: The group exposed to the turn-off cell phone during the whole period of pregnancy. ns (not significant), ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ versus Sham group

stage. In this study, the samples were immediately prepared and evaluated after birth (natal day) to minimize the effect of environmental factors on the study process.

Our results showed that cell phone exposure during different weeks of pregnancy caused a significant decrease in serum estrogen and progesterone concentrations in neonatal rats. The concentration of estrogen in the plasma of all exposed subgroups (**E1**, **E2**, **E3**, and **E4**) was significantly decreased in comparison with the control subgroups ($P < 0.001$) and sham group ($P < 0.0001$). Also, the progesterone concentration in all subgroups (the study and control groups) was significantly decreased compared to the sham group

($P < 0.0001$). To date, there have been no reports on the effects of mobile phone exposure on serum estrogen and progesterone concentrations in neonatal rats immediately after birth (natal day). Our findings were consistent with those reported in other studies [18, 19]. Yüksel et al. reported that long-term exposure to EMR from mobile phones and Wi-Fi devices has been associated with decreased estrogen and progesterone levels in pregnant rats and their offspring [18]. Another study also showed that EMR exposure decreased serum estrogen and progesterone levels in pregnant rats, suggesting a progressive hormonal imbalance [19]. However, despite exposure to cell phones, some studies did not report

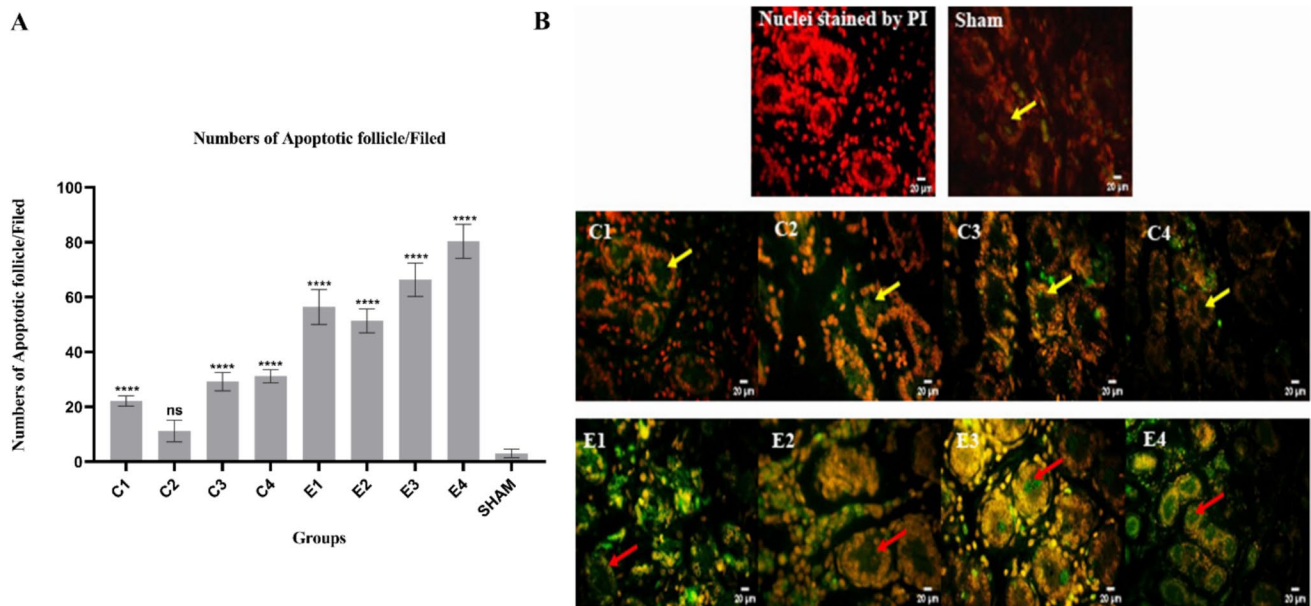


Fig. 6 The effect of cell phone radiation (900 MHz) on (A) the mean number of atretic follicles in neonatal rats and (B) the fluorescent microscope image of their ovary (the scale bar is 20 μ m). (A) The number of atretic follicles in subgroups E1, E2, E3, and E4 was significantly increased compared to the control and Sham groups (**** $P < 0.0001$). (B) Light microscopic image shows that the number of atretic follicles was increased in neonatal rats. C1: Control subgroup exposed to standby-mode radiation pattern during the first week of pregnancy, C2: Control subgroup exposed to standby-mode radiation pattern during the second week of pregnancy, C3: Control subgroup exposed to standby-mode radiation pattern during the

third week of pregnancy, C4: Control subgroup exposed to standby-mode radiation pattern during the whole period of pregnancy, E1: Experimental subgroup with intermittent radiation pattern during the first week of pregnancy, E2: Experimental subgroup with intermittent radiation pattern during the second week of pregnancy, E3: Experimental subgroup with intermittent radiation pattern during the third week of pregnancy, E4: Experimental subgroup with intermittent radiation pattern during three weeks of pregnancy, Sham: The group exposed to the turn-off cell phone during the whole period of pregnancy. ns (not significant), and **** $p < 0.0001$ versus the Sham group

any significant changes in reproductive hormones [20]. Hemayatkhah et al. (2010) reported that EMF induced by cell phones increased the estrogen, progesterone, LH, and FSH hormones [21]. These contradictions are probably due to the differences in the radiation dose, the type of samples, and the day of hormonal sampling. Most of these studies were conducted after birth and during puberty [20, 21]. Animal studies have reported that EMR exposure elevates oxidative stress markers, possibly contributing to hormonal imbalances [18]. The TRPV1 cation channels are suggested as potential pathways through which EMR exposure leads to mitochondrial dysfunction and increases the production of reactive oxygen species, affecting hormone levels [18, 19].

Our findings showed a reduction in the diameter of the ovaries of neonates born from rats exposed to 900 MHz cell phones at different stages of pregnancy. Ovarian diameters were significantly reduced in all exposed subgroups compared to the sham group ($P \leq 0.0001$). The difference between the Sham group and subgroups of C3, C4, E1, E2, E3, and E4 was ($P \leq 0.0001$). Meanwhile, the difference between the sham group and subgroups of C1 and C2 was $P < 0.01$ and $P < 0.001$, respectively (Fig. 3). Two studies on Balb/C mice reported that 940 MHz fields did not affect

ovarian weight and dimensions [21, 22]. In contrast, another study on pregnant adult rats showed that the same frequency increased the size and weight of ovaries and atretic follicles of neonates when they matured [23]. The results of these studies [21–23] were not consistent with our findings.

Other results of the present study indicated that mobile phone exposure significantly reduced the number and diameter of primordial follicles in neonates. Overall, the diameter of primordial follicles was significantly decreased in the control and exposed subgroups ($p \leq 0.001$) compared with the sham group. Indeed, the ovarian diameter decreased with increasing exposure time. The most effect in the number of primordial follicles was observed in the exposed subgroup of E4 (exposed to radiation for 21 days of gestation) ($P < 0.0001$). To answer the question “Which of the weeks of gestation did the EMF have the greatest detrimental effect?”, it should be said that the greatest decrease was observed in the third week of gestation. The results also showed that EMF can hurt folliculogenesis even in the first week of gestation.

Various studies investigated the effects of mobile phone exposure on primordial follicles, revealing significant impacts on both the number and diameter of these follicles [13, 24, 25]. A study by Bakacak et al. indicated a

significant decrease in the number of primordial follicles in rats exposed to EMF (900 MHz). They reported a reduction from an average of 150.25 follicles in the control group to 70.00 in the EMF group [24]. Also, Gul et al. showed a significant reduction in the number of ovarian follicles in 21-day-old rats exposed to cell phone radiation during the fetal period [13]. The study by Arslan et al. found that exposure to an EMF (900 MHz) significantly decreased the number and diameter of ovarian follicles in rats compared to control and carnosine-treated groups ($p < 0.05$) [25]. The results of these studies [13, 24, 25] were consistent with our findings. Our results showed that the number of follicles decreased with increasing exposure time.

This study also investigated the effect of cell phone radiation on the diameters of the primary oocytes and their nuclei in neonates. The results indicated that radiation from the cell phone caused a significant decrease in the diameter of the primary oocytes compared with the sham group, as shown in Fig. 5A. The highest effect was observed in the E4 subgroup ($P < 0.0001$). There was a significant difference in the diameter of the primary oocyte between the C4 and E4 subgroups ($P < 0.001$). A remarkable difference ($P \leq 0.001$) was also observed between the sham group and subgroups of C4, E1, E2, E3, and E4 in the diameter of the primary oocyte nucleus. We did not find any articles that specifically addressed the effect of cell phone radiation on the diameter of primary oocytes and their nuclei. However, Seify et al. investigated the impact of EMR on preimplantation embryo morphokinetics and blastocyst viability in mice. They observed a delay in embryo development, an increase in rates of fragmentation, and abnormal cleavage patterns in the experimental group compared to the control group, which may indicate underlying changes in oocyte and nuclear integrity [26]. Additionally, Arslan et al. reported significant irregularities in the structure of the oocyte and the surrounding granulosa cells, particularly in the EMF-treated groups [25].

Apoptosis is a profound process in various tissues that is necessary during the fetal period and maintains the homeostasis of multicellular organisms. In the ovary, the death of primordial follicles, before and after birth, is considered a natural process of ovarian development [27]. However, our results showed that cell phone radiation (900 MHz) increased the primordial follicle apoptosis. The atretic follicles in all subgroups of the study group were significantly increased compared to the control subgroups and the sham group ($P < 0.0001$). The most severe apoptosis was observed in neonatal rats exposed to conversation for a long time (E4, 21 days). The results were consistent with those reported in other studies [28–31]. A study by Türedi et al. found that prenatal exposure to continuous EMF (900 MHz) significantly increased apoptosis in primordial follicles of female rat pups [31]. The effect on

ovarian tissue is seen as cellular death in ovarian follicles (primordial, secondary, and Graafian) and an increase in atretic follicles [3–5, 9]. These effects depend on the distance between the EMF source and the organs [3], various frequencies, and the time of exposure [6].

Many studies seek to evaluate the potential of cell phone radiation and its adverse effects on human health [3]. However, most studies to date have investigated the effects of EMF on infertility in animals. The most limiting aspect of this type of investigation in human studies is the lack of control groups (pregnant women who do not use cell phones). On the other hand, in vitro studies on human granulosa cells may not be a suitable representative of the impact of EMFs because the reproductive organs are separated by several layers of tissue in humans. However, the results of the studies show that the effects of EMF exposure on health are due to increased temperature [4], disruption of hormone control pathways [5], energy absorption along with heating [6], changes in the value of the negative charge at the cell surface which depends on physical parameters [4], and oxidative stress in the endometrium and ovaries [7, 8].

Estrogen and progesterone prevent apoptosis of follicles by maintaining healthy follicles and their growth, respectively [32, 33]. One mechanism reported for the effects of EMF emitted from cell phones on the number of secondary and Graafian follicles and the increase in atretic follicles is impaired hormone control pathways, and possibly free radicals generated in ovarian tissue [21]. Studies have shown that since most of the body's volume is composed of water, radiation on the body can create free radicals [21, 34, 35]. The reaction between free radicals with macro and micro molecules results in the partial alteration or breaking of chemical bonds and may cause genetic changes. Changes in genetic activity stimulate biological and structural damage in the membrane and DNA of cells [34, 35] as well as ovarian tissue [21]. A study by Azimpour et al. [36] reported that cell phone exposure altered the growth and maturation rates of murine ovarian follicles by changing the expression of the MMP-2 and -9 genes.

These findings highlight the potential risks of exposure to mobile phone radiation during pregnancy and emphasize the importance of further research to understand the long-term effects on neonatal health. In addition, given that animal studies demonstrate that mobile phone radiation (EMF) negatively impacts reproductive health, the findings could translate into several practical public health recommendations to minimize risks, particularly for women of reproductive age. Hence, the World Health Organization (WHO) needs to gather evidence on the effects of EMF exposure on health outcomes to inform exposure guidelines and practical public health recommendations. For example, pregnancy guidelines should be prepared suggesting limited exposure, especially during early gestation.

Conclusion

This study examined the effects of cell phone exposure on estrogen, progesterone, and ovarian follicles in neonatal rats immediately after birth (natal day). Compared to the sham group, the results showed that cell phone radiation in both the study and control groups caused a decrease in the number and diameter of the primordial follicle, primary oocyte, its nucleus, and the amount of estrogen and progesterone hormones in neonatal rats during different and entire weeks of gestation. Also, these radiations cause an increase in apoptosis (atretic) of primordial follicles and a reduction in the diameter of the neonates' ovaries, which depends on the duration of radiation. Meanwhile, for the first time, the impact of mobile phones during different weeks of pregnancy was examined in this study. In sum, the most effects were observed in the third week of pregnancy. The results showed that the first week of pregnancy was also affected by cell phone effects. Although this study has investigated the effects of EMF on infertility in an animal model (rat), these findings can highlight the potential risks of exposure to mobile phone radiation during pregnancy on the future fertility of the fetus in the womb. However, further research is needed to establish a direct link between EMR exposure and infertility. Non-human primate models (monkeys) are recommended to achieve more reliable results.

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Author Contributions B.Y.: Conceptualization, funding acquisition, Project administration, review & editing. M.J.: Data analysis and interpretation, review & editing. Z.N.: Data analysis and interpretation, Preparation of figures, writing – review and editing. M.H.T.A.: Substantial contributions to the conception. M.A.: Project administration, Design of the work, data analysis and interpretation, writing – original draft.

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Availability of Data and Materials The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics Approval and Consent to Participate The approval (no.1179) for the study was provided by the Ethical Committee of Semnan University of Medical Sciences, Semnan, Iran, and all methods were performed in accordance with the relevant guidelines and regulations. Also, the study was reported in accordance with ARRIVE guidelines (<https://arriveguidelines.org>).

Consent for Publication Not applicable.

Competing interests The authors declare that they have no potential conflicts of interest.

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