Biological Effects of Exposure to a Radiofrequency Electromagnetic Field on the Placental Barrier in Pregnant Rats

Hye Sun Kim ,[1] Hyung-Do Choi ,[2] Jeong-Ki Pack ,[3] Nam Kim ,[4] and Young Hwan Ahn [1,5]*

[1]*Department of Neurosurgery, Ajou University School of Medicine, Suwon, Republic of Korea*
[2]*Radio Technology Research Department, Electronics and Telecommunications Research Institute, Daejeon, Republic of Korea*
[3]*Department of Radio Sciences and Engineering, College of Engineering, Chungnam National University, Daejeon, Republic of Korea*
[4]*School of Electrical and Computer Engineering, Chungbuk National University, Cheongju, Republic of Korea*
[5]*Neuroscience Graduate Program, Department of Biomedical Sciences, Graduate School of Ajou University, Suwon, Republic of Korea*

The placenta protects the fetus against excessive stress-associated maternal cortisol during pregnancy. We studied whether exposure to radiofrequency electromagnetic field (RF-EMF) radiation during pregnancy can cause changes in dams and their placentas. Pregnant Sprague–Dawley rats were divided into cage-control, sham-exposed, and RF-exposed groups. They were exposed to RF-EMF signals at a whole-body specific absorption rate of 4 W/kg for 8 h/day from gestational Day 1 to 19. Levels of cortisol in the blood, adrenal gland, and placenta were measured by enzyme-linked immunosorbent assay. Levels of adrenocorticotropic hormone and corticotropin-releasing hormone were monitored in maternal blood. Expression levels of placental 11β-hydroxysteroid dehydrogenase type 2 (*11β-HSD2*) messenger RNA (mRNA) were measured by reverse transcription polymerase chain reaction. Morphological changes in the placenta were analyzed using hematoxylin and eosin staining. Fetal parts of the placenta were measured using Zen 2.3 blue edition software. Maternal cortisol in circulating blood (RF: 230 ± 24.6 ng/ml and Sham: 156 ± 8.3 ng/ml) and the adrenal gland (RF: 58.3 ± 4.5 ng/ml and Sham: 30 ± 3.8 ng/ml) was significantly increased in the RF-exposed group ($P < 0.05$). Placental cortisol was stably maintained, and the level of placental *11β-HSD2* mRNA expression was not changed in the RF-exposed group. RF-EMF exposure during pregnancy caused a significant elevation of cortisol levels in circulating blood; however, no changes in the placental barrier were observed in pregnant rats. Bioelectromagnetics. 2021;42:191–199. © 2021 Bioelectromagnetics Society

Keywords: RF-EMF; pregnancy; hypothalamic–pituitary–adrenal axis; cortisol; placenta

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INTRODUCTION

Mobile communication is dramatically increasing globally, which has raised public concerns regarding possible adverse effects of radiofrequency electromagnetic field (RF-EMF) radiation, especially on vulnerable populations. In animal studies, the exposure of pregnant mice to whole-body radiofrequency radiation (RFR) from cell phones resulted in hyperactivity, impaired memory, and behavioral changes in the offspring [Divan et al., 2008; Aldad et al., 2012]. Etiological studies suggested an association between exposure to cellphone RFR and neurological dysfunction [Roosli, 2008; Tyler and Allan, 2014]. In an animal study with adolescent mice, increased levels of γ-aminobutyric acid and aspartate were observed after exposure to 1.8 GHz RFR at a brain-specific absorption rate (SAR) of 2.2 W/kg for 6 h/day for 28 days. The authors suggested that behavioral and neurological alterations could be a consequence of these changes [Zhang et al., 2017]. Exposure to RFR, such as wireless local area networks, so-called "wireless fidelity (Wi-Fi)," reportedly caused a significant increase in anxiety levels in male rats [Obajuluwa et al., 2017]. In a cohort study, prenatal exposure to cellphone RFR was associated with a significant increase in behavioral problems of emotion and hyperactivity around the age of school entry [Divan et al., 2008]. However, it is still unclear whether RF-EMF exposure during pregnancy is critically harmful to the fetus or not. The mechanisms of changes in fetal development following RF-EMF exposure are not yet fully understood.

Cortisol is a glucocorticoid steroid hormone, and the release of cortisol, which increases in response to stress, is controlled by the hypothalamic–pituitary–adrenal (HPA) axis. Adrenocorticotrophin hormone (ACTH) from the pituitary gland stimulates the adrenal cortex to secrete cortisol into the bloodstream. The placenta is an extra-embryonic organ that lies between maternal and fetal compartments and plays an important role in maternal–fetal exchanges of substances and hormones, including glucocorticoids [Lee et al., 2012; Pena et al., 2012; Togher et al., 2014; Zhu et al., 2019]. Glucocorticoids influence placental efficiency through changes in placental morphology, hormone synthesis, and transport physiology [Fowden et al., 2009].

In mammals, 10–20% of maternal cortisol passes to the fetus through the placenta, and 80–90% converts into an inert form during placental passage [Gitau et al., 1998]. This placental passage is controlled by 11β-hydroxysteroid dehydrogenase (11β-HSD2), which can convert active cortisol into inert cortisone. In the murine placenta, there is an abundant *11β-HSD2* expression in the labyrinth region of the fetal part of the placenta, which is consistent with its putative role in fetal development [Brown et al., 1996]. The activity of 11β-HSD2 is changed during pregnancy in response to fetal demands. Dysregulation of the maternal HPA axis or placental 11β-HSD2 allows greater transfer of glucocorticoid from the mother to the fetus [Sandman and Davis, 2012; Miranda and Sousa, 2018]. Increasing evidence supports the hypothesis that adult vulnerability to disease is programmed in fetal life [Pena et al., 2012; Togher et al., 2014; Zhu et al., 2019]. In animal studies, behavioral/anxiety phenotypes in the offspring were observed following exposure to excessive glucocorticoid during pregnancy, which was suggested as one of the key mechanisms of the risk of future diseases, such as schizophrenia, autism, attention deficit hyperactivity disorder (ADHD), and impaired cognitive development [Sandman and Davis, 2012; Miranda and Sousa, 2018]. Several studies have suggested that exposure to RFR as a stressor induced significant changes in stress hormones in adult animals under various RFR exposure conditions; however, the effects of gestational RFR exposure on maternal and placental stress responses have not yet been reported. The present study was carried out to evaluate the effects of exposure to RFR on both the maternal HPA axis and the placental barrier to predict any environmental changes related to fetal development.

MATERIALS AND METHODS

Whole-Body Radiofrequency Identification Exposure System

A reverberation chamber with a 915 MHz radiofrequency identification (RFID) signal source was used in this study [Kim et al., 2018]. Briefly, the whole-body exposure system (IRETEC, Anyang, ROK) with 915 MHz RFID was constructed using a reverberation chamber [Jung et al., 2008; Wu et al., 2010]. The internal dimensions of the reverberation chamber were $2.2 \times 2.1 \times 1.5$ m. A wooden table (1,400 mm long, 1,000 mm wide, and 700 mm high) was placed in the center of the chamber. Eight cages could be placed on the table without any change in field uniformity; each cage was $390 \times 235 \times 180$ mm. Field distribution and field uniformities in the reverberation chamber were directly measured using a three-axis isotropic probe (HI-6105; ETS-Lindgren, Cedar Park, TX). The whole-body averaged SAR of a rat model (Air Force Research Lab (AFRL), Dayton, OH) was calculated for the measured electric field strength using a commercial finite-difference time-domain tool (XFDTD version

6.5; Remcom, State College, PA) for the incident plane waves in six orthogonal directions with two polarizations. Although the field uniformity might be altered temporally, the average field uniformity during RF exposure could remain constant. To calculate the variation in SAR, we used a scaled rat model modified from a grown-up AFRL rat model that had 36 tissues, a mass of 339 g, and a voxel size of $0.39 \times 0.39 \times 0.42$ mm. An 11-bit digital personal identification number diode attenuator (Model 349; General Microwave, Farmingdale, NY) was used to control the output power level. The amplified RFID signal was then supplied to the chamber.

Animal Experiments

Twenty-one breeding pairs of Sprague–Dawley rats were obtained (Dae-Han Biolink, Seoul, ROK). The mass of female and male rats was 300 ± 10 g and 350 ± 10 g, respectively. The ambient temperature was maintained at $22 \, °C \pm 2 \, °C$, with a relative humidity of 50–60% and a 12:12-h light–dark cycle. Water and pelleted food (Dae-Han Biolink) were supplied ad libitum. After a 2-week quarantine period, 21 breeding pairs were mated in cages overnight. The presence of vaginal plugs in the morning signified pregnancy (gestational day 0, GD 0); male rats were removed from cages. Eighteen female rats with plugs, expected to be pregnant, were obtained on the same day and were assigned into three groups. Fourteen out of eighteen rats were assigned evenly into the sham-exposed and RF-exposed groups (seven per group), and the remaining four rats were assigned to the cage-control group. However, two out of seven rats placed in the sham-exposed group were finally found to be nonpregnant on the day of sacrifice. Therefore, the number of rats per group became as follows: cage-control (CTL, $n = 4$), sham-exposed (Sham, $n = 5$), and RF-exposed groups (RF, $n = 7$). Rats in the cage-control group were not placed in the reverberation chamber. Rats in the sham-exposed group were placed in the reverberation chamber but were not exposed to RF-EMF. Rats in the RF-exposed group were placed in the reverberation chamber and exposed to RF-EMF at a whole-body SAR of 4 W/kg 8 h/day from GD 1 to 19.

Sample Collection

On GD 20, dams were sacrificed using the CO_2 inhalation method. Maternal blood was collected in serum separating tubes (SSTs) (BD, Franklin Lakes, NJ) by direct cardiac puncture, centrifuged (2000 g, 10 min) to obtain the serum, and then stored at $-70 \, °C$ until the assay. The adrenal glands and pituitary glands of dams were harvested. The uterus was quickly removed, and fetuses were carefully separated from the placenta. Three placentas from 4 female rats in each group were randomly picked up, and the placental cortisol level of 12 placentas (4 per group) was analyzed. Placentas were dissected and prepared for histological sectioning. Placentas were fixed in 4% paraformaldehyde (Sigma, St. Louis, MO). The tissues were dissected and stored at $-70 \, °C$ until analysis. This investigation was carried out with the permission of the Ethics Committee (110405-25) on Animal Experiments of the Ajou University School of Medicine (Suwon, ROK).

Assessment of Pituitary Axis

The levels of cortisol were measured in maternal blood, adrenal gland, and placenta. Levels of pituitary ACTH and blood corticotrophin-releasing hormone (CRH) were determined. For this purpose, we conducted enzyme-linked immunosorbent assays using commercially available kits (Cusabio Biotech, Wuhan, China) according to the manufacturer's instructions. The detection ranges of the ACTH, CRH, and cortisol assays were 1.25–50 pg/ml, 1.25–20 ng/ml, and 0.049–200 ng/ml, respectively. Standards were assayed in duplicate and samples in triplicate. Absolute values are expressed as the arithmetic mean $\pm$ standard deviation (SD).

Histological and Morphometric Analysis

For histological analysis, 4% paraformaldehyde-fixed placentas were dehydrated through graded ethanol, cleared in xylene, and embedded in paraffin using a standard protocol. Paraffin-embedded placentas were sliced at a thickness of 4 µm. The slices were deparaffinized, hydrated through xylene alcohol, and stained with hematoxylin and eosin. We obtained bright field images using ZEISS Axio Scan.Z1 (Carl Zeiss Microscopy, Jena, Germany). After obtaining the images, we measured the surface area of the labyrinth and junctional regions. The sections taken from the part of the placenta closest to the center were used for the image analysis. Four sections (from different litters) per group were analyzed. Fetal parts of the placentas were measured using Zen 2.3 blue edition software (Carl Zeiss Microscopy).

Measurement of Messenger RNA (mRNA) Levels of 11β-HSD2

Total RNA was extracted using a PureLink RNA Mini Kit (Ambion, Life Technologies, Carlsbad, CA). Total RNA of the pineal gland was reverse-transcribed using the SuperScript III Reverse Transcriptase Kit (Invitrogen, Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. Quantitative reverse transcription polymerase chain reaction was performed using primers (Bioneer, Daejeon, ROK) designed to

detect placental *11β-HSD2* mRNA (forward primer: 5′-GGG TCT GGT TAA CAA TGC TGG-3′, reverse primer: 3′-TCG CGG AAA GTT ACC ACT GG-5′). Primers for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) were used in a separate reaction normalized to total complementary DNA content (forward primer: 5′-TCC CTC AAG ATT GTC-5′ reverse primer: 3′-AGA TCC ACA ACG GATA CAT-5′). Samples were amplified for 25 cycles using a denaturing temperature of 94 °C (30 s), an annealing temperature of 60 °C (30 s), and an extension temperature of 72 °C (30 s). Amplified products were resolved by electrophoresis on 1% agarose gels. Band intensities were analyzed using Quantity One 1-D analysis software, ver. 4.6.5 (Bio-Rad, Hercules, CA). Relative expression levels were normalized to the house-keeping gene GAPDH.

Statistical Analysis

The values of stress hormones, mRNA levels, and placental surface areas are shown as the mean ± SD. One-way analysis of variance (ANOVA) was used for statistical comparisons. *P*-values <0.05 were considered to indicate statistical significance. Values were analyzed using the statistical package SPSS ver. 23.00 (SPSS, Chicago, IL).

RESULTS

Effects of RF-EMF Exposure on the Maternal HPA Axis

The cortisol level was increased in maternal blood in the RF-exposed group compared with the cage-control and sham-exposed groups [RF ($n = 7$): 230 ± 24.6 ng/ml; Sham ($n = 5$): 156 ± 8.3 ng/ml; CTL ($n = 4$): 165 ± 8.6 ng/ml; $F(2,13) = 4.157$, $P = 0.040$; one-way ANOVA; Fig. 1A]. The adrenal cortisol level was significantly increased following RFR exposure [RF ($n = 7$): 58.3 ± 4.5 ng/ml; Sham ($n = 5$): 30 ± 3.8 ng/ml; CTL ($n = 4$): 33.7 ± 3.9 ng/ml; $F(2,13) = 29.2707$, $P = 0.00$; one-way ANOVA; Fig. 1B]. The blood CRH [$F(2,13) = 2.75$, $P = 0.103$;
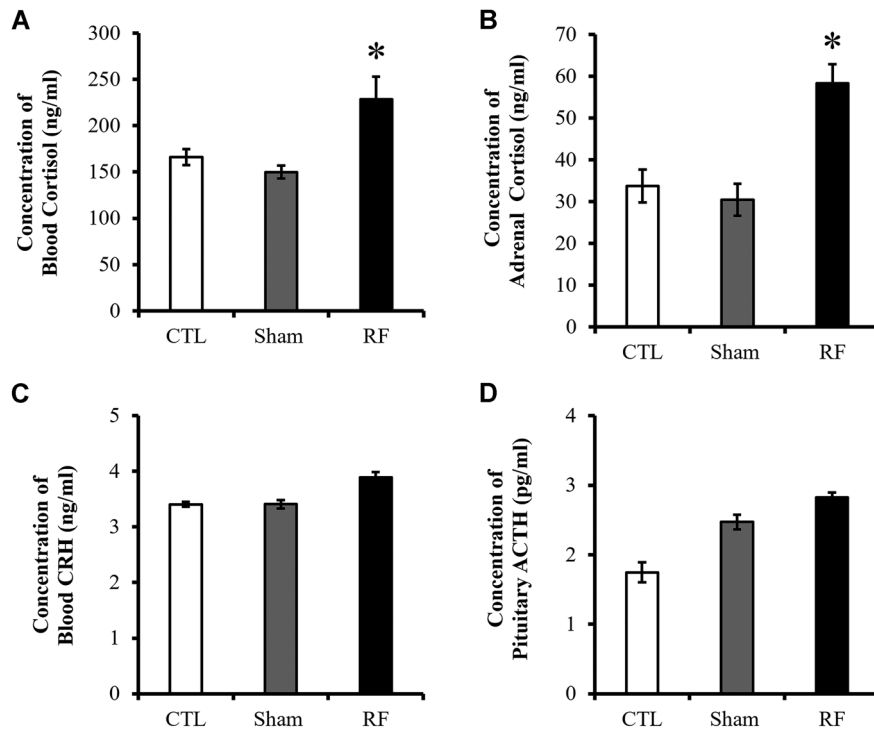

Fig. 1. Effects of exposure to RF on the concentrations of **(A)** adrenal cortisol, **(B)** blood cortisol, **(C)** blood corticotrophin-releasing hormone (CRH), and **(D)** pituitary adrenocorticotropic hormone (ACTH) in pregnant rats. Concentrations were measured using enzyme-linked immunosorbent assays. Cortisol levels in the adrenal gland (A) and blood (B) were significantly increased in the RF-exposed group compared with the sham-exposed group ($n = 4$–7, $P < 0.05$). The concentration of blood CRH (C) and pituitary ACTH (D) in the RF-exposed group was also increased compared with the sham-exposed group, but not significantly. Data are shown as the mean ± standard deviation for four cage-control, five sham-exposed groups, and seven RF-exposed rats. *Significance was set at $P < 0.05$. CTL = cage-control group; RF = RF-exposed group; Sham = sham-exposed group.

one-way ANOVA; Fig. 1C] and pituitary ACTH levels [$F(2,13) = 0.694$, $P = 0.517$; one-way ANOVA; Fig. 1D] were not significantly changed, although some increases were observed.

Effects of RF Exposure on Placental Morphometry

Hematoxylin and eosin staining can distinguish the border of the maternal decidua and fetal-derived junctional and labyrinth regions [Ain et al., 2006]. We carried out a morphometric analysis with the hematoxylin and eosin-stained sections to quantitatively measure the effect of RF exposure on the rate of change in the surface area in the fetal region, including the junctional region and labyrinth region.

The surface area of fetal parts in the cage-control ($n = 4$), sham-exposed ($n = 5$), and RF-exposed ($n = 7$) groups were $27,605,590 \pm 3,386,857$, $36,002,058 \pm 4,877,994$, and $35,146,821 \pm 4,831,159$ (μm^2), respectively [$F(2,13) = 4.562$, $P = 0.032$; one-way ANOVA; Fig. 2, Table 1]. The surface area of the junctional zone in the cage-control ($n = 4$), sham-exposed ($n = 5$), and RF-exposed ($n = 7$) groups were $6,317,028 \pm 895,062$, $8,663,881 \pm 2,379,078$, and $8,609,788 \pm 3,088,955$ (μm^2), respectively [$F(2,13) = 1.271$, $P = 0.313$; one-way ANOVA; Fig. 2, Table 1].

The surface area of the labyrinth zone in the cage-control ($n = 4$), sham-exposed ($n = 5$), and RF-exposed ($n = 7$) groups were $21,288,562 \pm 2,697,621$, $27,338,177 \pm 3,829,312$, and $26,537,032 \pm 3,442,171$ (μm^2), respectively [$F(2,13) = 4.089$, $P = 0.042$; one-way ANOVA; Fig. 2, Table 1]. Although significant differences in the surface area of the fetal parts and labyrinth zone were observed between cage-control and sham-exposed groups and between cage-control and RF-exposed groups, there was no significant difference in the surface area of the fetal parts, including the junctional zone and labyrinth zone, which was observed between sham-exposed and RF-exposed groups (>0.05).

Effects of RF Exposure on the Placental Barrier

The placental cortisol level was not significantly changed in the RF-exposed group compared with the cage-control and sham-exposed groups [RF ($n = 12$): 1.7 ± 0.06 ng/ml; Sham ($n = 12$): 1.6 ± 0.08 ng/ml; CTL ($n = 12$): 1.5 ± 0.03 ng/ml; $F(2,33) = 0.192$, $P = 0.827$; one-way ANOVA; Fig. 3B]. In addition, no significant changes in placental *11β-HSD2* mRNA levels were observed in the RF-exposed group compared with the cage-control and sham-exposed group (Fig. 3C).

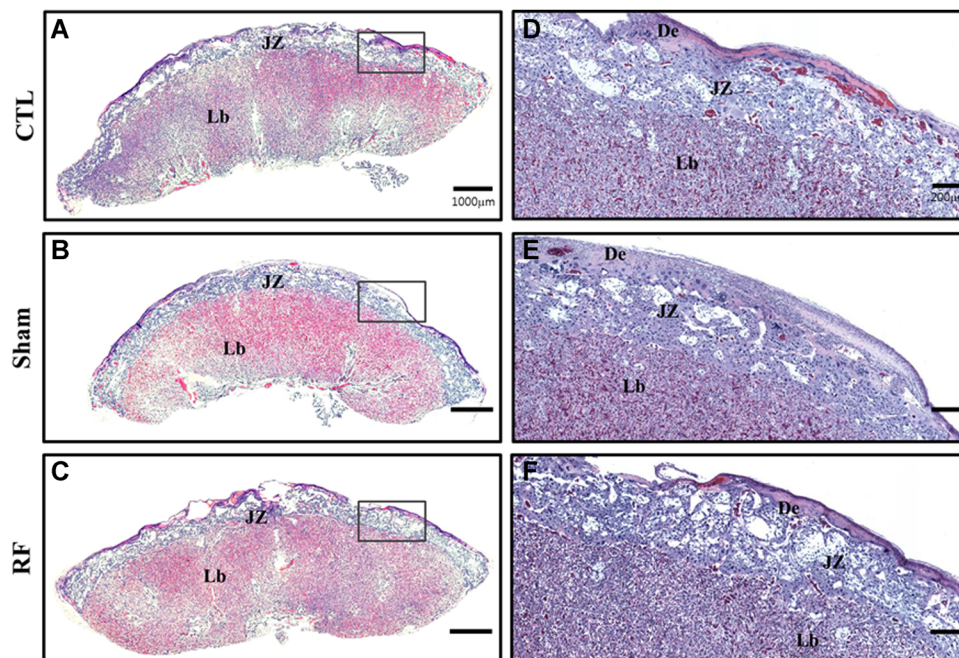Fig. 2. Histologic findings of the day-20 placenta from pregnant rats in each group (**A–F**). Low (A to C; scale bar = 1,000 μm) and high (D to F; scale bar = 200 μm) magnification views of the control, sham-exposed, and RF-exposed groups are shown. There was no significant change in the placental structure of fetal parts, including the junctional zone and labyrinth zone among groups. Placentas were stained with hematoxylin and eosin. CTL = cage-control group; De = decidua; JZ = junctional zone; Lb = labyrinth zone; RF = RF-exposed group; Sham = sham-exposed group.

TABLE 1. Mean Surface Area of the Junctional Zone and the Labyrinth Zone in the Fetal Parts of the Placenta

Location (μm^2)	CTL ($n = 4$)	Sham ($n = 5$)	RF ($n = 7$)	P-value
Fetal part	27,605,590 ± 3,386,857	36,002,058 ± 4,877,994	35,146,821 ± 4,831,159	0.032[a],* 0.769[b]
JZ	6,317,028 ± 895,062	8,663,881 ± 2,379,078	8,609,788 ± 3,088,955	0.313[a] 0.975[b]
Lb	21,288,562 ± 2,697,621	27,338,177 ± 3,829,312	26,537,032 ± 3,442,171	0.042[a],* 0.712[b]

The values are expressed as mean ± standard deviation. Statistical significance was determined by analysis of variance.
CTL = cage-control group; JZ = junctional zone; Lb = labyrinth zone; RF = RF-exposed group; Sham = sham-exposed group.
[a]From one-way analysis of variance test comparing values of CTL, sham-, and RF-exposed groups.
[b]From one-way analysis of variance test comparing values of sham- and RF-exposed groups.
*Statistical significance: $P < 0.05$.

DISCUSSION

The present study showed that exposure to RF during pregnancy caused a significant elevation of cortisol in the circulating blood and adrenal glands of pregnant rats. However, there were no notable changes in placental cortisol and the expression of placental *11β-HSD2* mRNA. The fetus may be exposed to RFR during maternal use of various devices emitting RF, such as cell phones. RFID is also a source of RF emission that can lead to more sporadic and unexpected exposures. We have studied the biological effects of RF exposure with an RFID exposure system [Kim et al., 2013, 2015]. In cohort studies, the relationship between prenatal and postnatal

Fig. 3. Effects of exposure to RF during pregnancy on the blood–placenta barrier. **(A)** No significant change in the level of placental blood cortisol was found in the RF-exposed group compared with the sham-exposed group. **(B)** No significant changes in the level of placental *11β-HSD2* mRNA were found in the RF-exposed group compared with the sham-exposed group. Data are shown as the mean ± standard deviation for four rats (cage-control group), five rats (sham-exposed group), and seven rats (RF-exposed group). *Significance was set at $P < 0.05$. *11β-HSD2* = 11β-hydroxysteroid dehydrogenase; CTL = cage-control group; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; mRNA = messenger RNA; RF = RF-exposed group; Sham = sham-exposed group.

exposure to cellphone use with behavioral problems in children was studied in the Danish National Birth Cohort [Divan et al., 2008, 2012]. They reported high odds ratios for behavioral problems, such as emotional problems and hyperactivity, at 7 years of age associated with maternal cellphone use during pregnancy [Divan et al., 2008, 2012]. Gestational stress is widely recognized as a potential contributor to the risks of ADHD in offspring [Linnet et al., 2003; Aguiar et al., 2010]. A cohort study reported that the risk of ADHD symptoms was increased in children with high blood lead levels who used cell phones [Byun et al., 2013]. They suggested that RF-EMF, as an environmental factor, may play a role in the development of ADHD. In children with ADHD, abnormal neurotransmitter function [Linnet et al., 2003] and HPA axis dysregulation [Aguiar et al., 2010] were observed. The neurobiological changes in children with ADHD appear to be similar to those associated with gestational exposure to stress [Buss et al., 2012; Pallares and Antonelli, 2015].

Several reports have focused on RF exposure as a stressor in animals [Bouji et al., 2012; Khirazova et al., 2012; Shahabi et al., 2018]. In animal studies, increased plasma corticosterone level [Bouji et al., 2012], plasma ACTH and cortisol levels [Shahabi et al., 2018], and glucocorticoid level [Khirazova et al., 2012] were observed in rats exposed to RFR from mobile phone signal. These results suggest that exposure to RF-EMF radiation may be stressful enough to induce the secretion of stress hormones.

The World Health Organization designated animal studies of RF exposure in vulnerable populations as high-priority research. However, few studies have examined the effects of RF-EMF exposure and the fetal environment during pregnancy [Nakamura et al., 1997; Nakamura et al., 2000]. In a rat study, virgin and pregnant (GD 9–11) rats were exposed to 2450 RF-EMF at an SAR of 1.8–2.2 W/kg for 90 min. They reported that blood levels of corticosterone and ACTH were increased in both virgin and pregnant rats after RF
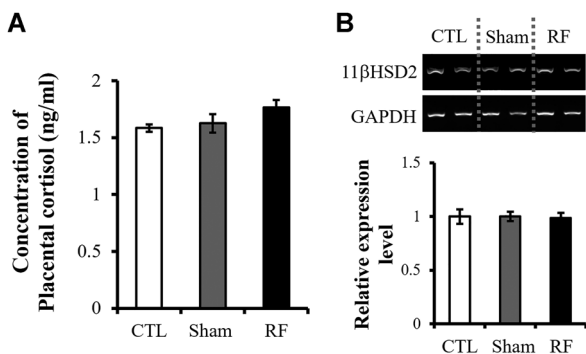
exposure [Nakamura et al., 1997]. In another study, virgin and pregnant rats were exposed to 2450 RF-EMF at a whole-body SAR of 0.36–0.44 W/kg for 90 min and the blood level of CRH was increased in both virgin and pregnant female rats [Nakamura et al., 2000]. In those two studies, all rats were restrained during RF exposure, and the status of the blood–placenta barrier was not further evaluated. We need to consider that restraint itself could be stressful enough to increase stress hormones such as cortisol even without RF exposure. For appropriate evaluation of the biological influences of RF exposure, we need to avoid restraint, as it can cause changes to stress hormones, especially in pregnant rats. Therefore, the present study was carried out with no restraint, and the maternal HPA axis and placental integrity were evaluated.

The disruption of cellular organization in junctional and labyrinth regions, along with concomitant changes in gene expression in the placenta, reflect defects in placental function. Gestational exposure to caffeine and 5-azacytidine during pregnancy in rats caused mismatches between the day of pregnancy and placental maturation in rats [Smith et al., 1987; Vlahovic et al., 1999], as did exposure to dexamethasone, a synthetic stress hormone in mice [Lee et al., 2012]. In the present study, we examined the structure of the junctional zone and the labyrinth zone through morphometry, but no significant changes were observed after RF exposure. We considered that our RF exposure conditions were not sufficient to induce any changes to the placental structure.

In addition to morphometric analysis, we measured 11β-HSD2 mRNA levels as an indicator of blood–placenta barrier function. Despite the beneficial effects of maternal cortisol, exposure of the fetus to excessive cortisol during pregnancy causes growth retardation [Calkins and Devaskar, 2011]. In particular, high levels of maternal glucocorticoids and downregulation of 11β-HSD2, regardless of their cause, may disrupt several essential developmental processes, including placental development [Seckl and Holmes, 2007]. In previous rat studies, inhibition of placental 11β-HSD2 mRNA using carbenoxolone and dexamethasone led to changes in the modulation of the HPA axis as was associated with increased stress- and anxiety-like behavior in pups [Welberg et al., 2000; Shoener et al., 2006]. In another rat study, decreased expression of placental 11β-HSD2 mRNA was found under chronic restraint stress during GD 11-20 [Mairesse et al., 2007]. In human studies, increased maternal anxiety was negatively correlated with placental 11β-HSD2 mRNA levels

[O'Donnell et al., 2012]. In our study, despite a significant increase in maternal cortisol levels, placental 11β-HSD2 mRNA was not changed in the RF-exposed group compared with the sham-exposed group. The current results suggest that although the serum cortisol levels became higher, stable expression of placental 11β-HSD2 mRNA might be important.

This study has several limitations. In this study, it might be ideal to consider both pregnancy condition and body mass in the SAR calculation. However, we did not have a pregnant rat model for SAR simulation at the start of the experiment, so we used an adult male rat model (339 g, AFLR) for simulation instead. Therefore, we could not take the pregnancy condition into account for SAR calculation. Instead, we considered the change of body mass in the SAR calculation. Female rats weighed about 300 g at the beginning of exposure to RF, and the average mass during RF exposure was about 330 g. And we did not measure rectal temperature in order to avoid procedure-related stress in pregnant rats. In our previous study, however, we reported no significant changes in body temperature during exposure to RF-EMF at an SAR of 4 W/kg in adult male rats. The body mass of male rats used in our previous study was heavier than those of the pregnant rats used in this study [Kim et al., 2020]. In our previous study, however, we reported no significant changes in body temperature during exposure to RF-EMF at an SAR of 4 W/kg in adult male rats.

Therefore, we considered that the maternal body temperature was unchanged during RF-EMF exposure. We did not measure the cortisol level in the fetus, as direct measurements in fetal tissues were practically impossible. Further studies are needed to understand the effect of exposure to RFR on fetal development during pregnancy. Placental function undergoes dramatic changes between stages during pregnancy [Woods et al., 2018]. In this study, we only examined the effect of exposure to RF on late gestational stages in rats. Therefore, we need to evaluate the influence of exposure to RFR during different gestational stages.

To the best of our knowledge, our study is the first to demonstrate the biological effects of exposure to RF-EMF on the placental barrier in pregnant rats. In summary, the current study indicated that the exposure to RF-EMF at an SAR of 4 W/kg for 8 h/day during pregnancy was sufficiently stressful to pregnant rats; however, the placental barrier protected the fetus from maternal stress.

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