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Rat fertility and embryo fetal development: Influence of exposure to the Wi-Fi signal

Florence Poulletier de Gannes[b,*], Bernard Billaudel[a], Emmanuelle Haro[a], Murielle Taxile[a], Laureline Le Montagner[a], Annabelle Hurtier[a], Saliha Ait Aissa[a], Hiroshi Masuda[a], Yann Percherancier[a], Gilles Ruffié[a,b], Philippe Dufour[d], Bernard Veyret[a,c], Isabelle Lagroye[a,c]

[a] University of Bordeaux, IMS laboratory, Bioelectronics group, ENSCBP, IPB, Pessac, France
[b] University of Bordeaux, IMS laboratory, IMS Transfert-A2M, ENSCBP, IPB, Pessac, France
[c] EPHE Bioelectromagnetics laboratory, ENSCBP, 33607 Pessac, France
[d] EVIC France, 33290 Blanquefort, France

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ABSTRACT

In recent decades, concern has been growing about decreasing fecundity and fertility in the human population. Exposure to non-ionizing electromagnetic fields (EMF), especially radiofrequency (RF) fields used in wireless communications has been suggested as a potential risk factor.

For the first time, we evaluated the effects of exposure to the 2450 MHz Wi-Fi signal (1 h/day, 6 days/week) on the reproductive system of male and female Wistar rats, pre-exposed to Wi-Fi during sexual maturation. Exposure lasted 3 weeks (males) or 2 weeks (females), then animals were mated and couples exposed for 3 more weeks. On the day before delivery, the fetuses were observed for lethality, abnormalities, and clinical signs. In our experiment, no deleterious effects of Wi-Fi exposure on rat male and female reproductive organs and fertility were observed for 1 h per days. No macroscopic abnormalities in fetuses were noted, even at the critical level of 4 W/kg.

1. Introduction

Reproduction is a critical function of the organism and involves two systems: the male and female genital organs. The consequences of reproductive organ damages can also be a cause of abnormal development in embryos. In recent decades, concern has been growing about decreasing fecundity and fertility in the human population [1]. Several studies indicated that semen quality may have decreased, associated with reduced fertility [2], but few potential factors have been identified. Exposures to non-ionizing EMF, especially RF fields used in mobile communications, have been suggested as a potential risk factor. The potential targets of mobile phone signals such as the sexual organs have focused some attention both in humans and animals, and related studies have yielded seemingly contradictory results (see reviews [3–6]).

Wireless fidelity (Wi-Fi) network involves short-range communication between an access point and many personal devices (e.g., computers, printers, gaming devices).

The popularity of portable devices supporting this technology working at 2450 MHz is continuously growing as it can be used worldwide at home, work, or near hotspots. In terms of health risks, few data are available on the effect of exposure to this type of signal and the main concern is focused on long-term exposure.

In the present project we investigated effects of Wi-Fi exposure on the rat reproductive system. Studies on fertility have to consider three groups: dams, males, and embryos. In this work, all three have been included: the infertility in dams and males, the lethality in pups, and the gross necropsy in all pups with external abnormalities or clinical signs. Free-moving male and female rats (before and during pregnancy) were either sham exposed or exposed to a Wi-Fi signal (2450 MHz) at exposure levels defined as whole-body specific absorption rates (SAR) of 0.08 and 4 W/kg, referring to the ICNIRP public exposure limit and critical level, respectively.

Abbreviations: CDMA, code division multiple access; EMF, electromagnetic fields; ICNIRP, international commission on non-ionizing radiation protection; RF, radiofrequency; SAR, specific absorption rate; WCDMA, wideband code division multiple access; Wi-Fi, wireless fidelity.

* Corresponding author. Tel.: +33 5 40 00 28 21; fax: +33 5 40 00 66 29.
E-mail address: florence.poulletier@ims-bordeaux.fr (F. Poulletier de Gannes).

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2. Materials and methods

2.1. Exposure conditions

The exposure system was a cubic reverberation chamber (150 cm × 150 cm × 150 cm), designed for whole-body exposure of free-moving rats to a Wi-Fi signal. The chamber was fitted with three mode stirrers for mode mixing. Six antennas powered by a 70-W amplifier, activated at random, and placed at the center of each side of the cube, provided uniform exposure of the animals [7]. The animal polypropylene cages were placed in a 40 cm × 40 cm × 40 cm volume at the centre of the chamber. Two whole-body SAR levels were tested on the rats: 0.08 and 4 W/kg. Sham-exposed animals were included in the protocol. All exposure conditions were coded and analyzed in a blind manner. The signal was Wi-Fi (2.45 GHz, IEEE 802.11b/g) based on a "dialog" between two PCs equipped with Wi-Fi cards (WG311v3 802.11 g; NETGEAR, San Jose, CA) and a IP traffic software (www.zti-telecom.com) which allows to fill the 16 channels randomly and which was set to 50% duty factor.

The three SAR levels were coded A–C, to insure that further biological analysis of all parameters was done blindly. A hybrid approach combining measurements and finite-difference time-domain simulations (FDTD) were used to characterize the incident power in the reverberation chamber and WBSAR in young rats [7]. The numerical rat model used in this study is the one segmented at the Brooks Air Force Base (San Antonio, TX). It is composed of 36 different tissues, weighs 374 g and its mesh size is 0.83 mm.

All procedures were performed in compliance according to national and European laws and regulations on animal welfare.

Thirty-six Wistar Han male and female rats were purchased (Janvier, France) at 6 and 7 weeks of age, respectively. Animals were housed in the animal facility under the following environmental conditions: 12 h dark–12 h light cycle, temperature 21 ± 1 °C, humidity 55–65%. They were fed ad libitum with food pellets (Standard Diet A04, SAFE, France) and water. On arrival to the facility, animals were housed for one week prior to the start of any experiment.

Ten-week-old male and female rats were mated after blind exposure to Wi-Fi during sexual maturation, either for 3 weeks (males) or 2 weeks (females) (one rat/cage). Couples were then further exposed to Wi-Fi for 3 weeks (one couple/cage). Exposure duration was 1 h per day and 6 days per week, which allowed to test for 3 exposure levels including sham exposure. Animals were exposed randomly between 9:00 a.m. and 5:00 p.m. After last exposure, animals were sacrificed, sexual organs and pups taken for analysis. Three independent experiments were necessary.

2.2. Body weights and food consumption

All through the experiment, body weights were recorded twice per week, and food consumption assessed over a 48-h period, once per week.

2.3. Fertility study in males

The males were weighed and sacrificed by intraperitoneal injection of sodium pentoparbital before macroscopic examination for any structural abnormalities or pathological changes with a particular attention paid to the organs of the reproductive system. Testes, epididymides, seminal glands and prostate were removed, weighed after dissection of fat and other contiguous tissues and then submitted to histological examination. Paired organs were weighed individually.

2.4. Fertility study in females

Between the 19th (P19) and 21st (P21) day of pregnancy, females were anaesthetized with ether for uterus and ovary removal and sacrificed by bleeding. Ovaries of each dam were first examined to determine the number of *corpora lutea* on each ovary and then submitted to a histological examination. The uterus removed from each dam was examined to determine (i) the number of live and dead fetuses per uterine horn, (ii) the number and location in each uterine horn of early and late resorption sites and (iii) the number and distribution of implantation sites on each uterine horn after coloring following the Salewski's method [8].

2.5. Statistical analysis

At each SAR level including sham, there were a total of 12 pairs of animals per group.

The Stat-Xact Kruskall–Wallis test (Stat-Xact software) among SAR groups was used against the sham-exposed group, only on continuous variables followed by post hoc tests. The body-weight time and food consumption profiles were analyzed using a two-way ANOVA (one independent and one related) based on the software at http://www.anastats.fr/. $p < 0.05$ was considered significant.

3. Results

All data were analyzed in a blind manner.

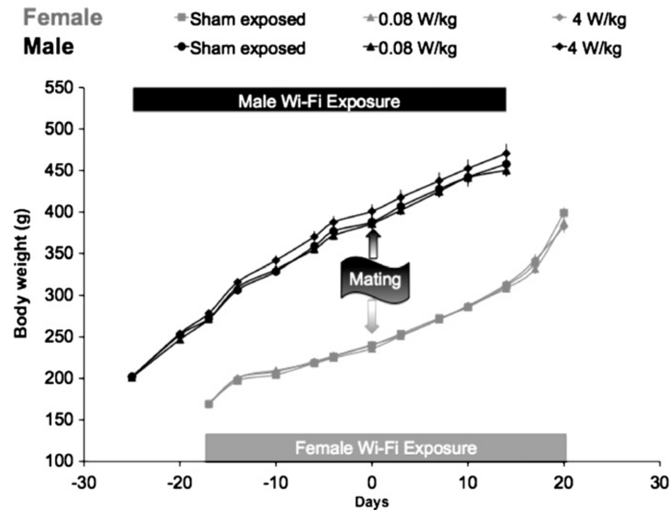

Fig. 1. Body-weight time course during Wi-Fi exposure. Animals were weighted twice a week. The mating date is indicated. Results are expressed as the mean ± SEM. $n = 12$ for males and females for each exposure conditions.

3.1. Body weight and food consumption

Male and female body weights were monitored, twice a week, during exposure to Wi-Fi for a total of 6 and 5 weeks, respectively. No significant differences were observed in males and females whatever the exposure conditions (Fig. 1).

Food consumption was very similar in males over the overall period of observation while in females it increased from post-mating D14 until post-mating D21, corresponding to the gestational period. No significant effect of exposure conditions was noticed (Fig. 2).

3.2. Male fertility

3.2.1. Gross necropsy

No macroscopic abnormalities were found in the males sacrificed at the end of the experiment except for the lack of the right kidney in one male in the 0.08 W/kg group. This finding was considered to be incidental and spontaneous in nature. Mean relative organ weight to body-weight ratios were similar among the groups for testes, epididymides, prostates and seminal glands (Table 1).

3.2.2. Histopathology

No microscopic lesions were found in the testes of all males at the end of the experiments. Some changes, common in the rat, were noted in (i) epididymides: focal inflammatory infiltrates with mononuclear cells (minimal to slight) in association with a slight to moderate edema in the interstitium at the upper part of the organ; (ii) prostate: edema (slight to moderate) in the interstitium of the prostate in association with inflammation; (iii) seminal vesicles: deposits of squamae, mononuclear cells and cell residues in the lumen. Numbers of males presenting these signs are shown in Fig. 3. No significant effect of Wi-Fi exposure was detected in the rat male sexual organs.

3.3. Pregnancy data

There were two non-pregnant females for a total of 36 animals: one in the 0.08 W/kg group and one in the 4 W/kg group.

Table 1
Mean relative organ weight to body weight ratios.

	Testes	Epididymides	Prostate	Seminal glands
0.08 W/kg	0.405 ± 0.0061	0.136 ± 0.0029	0.275 ± 0.0104	0.066 ± 0.0035
4 W/kg	0.367 ± 0.0080	0.127 ± 0.0022	0.242 ± 0.0176	0.065 ± 0.0037
Sham	0.391 ± 0.0071	0.136 ± 0.0031	0.256 ± 0.0153	0.063 ± 0.0033

Results are expressed as mean percentages ± SEM. $n = 12$ for all groups.

3.4. Ovary histology

No significant microscopic lesions were observed in ovaries of all females. Congestion noted in one and two animals from the 0.08 and 4 W/kg groups, respectively, was considered as incidental (data not shown).

3.5. Study of embryo-fetal development in caesarized females

The mean number of implantation sites, early, late and total resorptions, dead, live and total fetuses and post implantation loss were similar among the three exposure groups (Fig. 4).

No macroscopic abnormalities were seen by external examination of all fetuses.

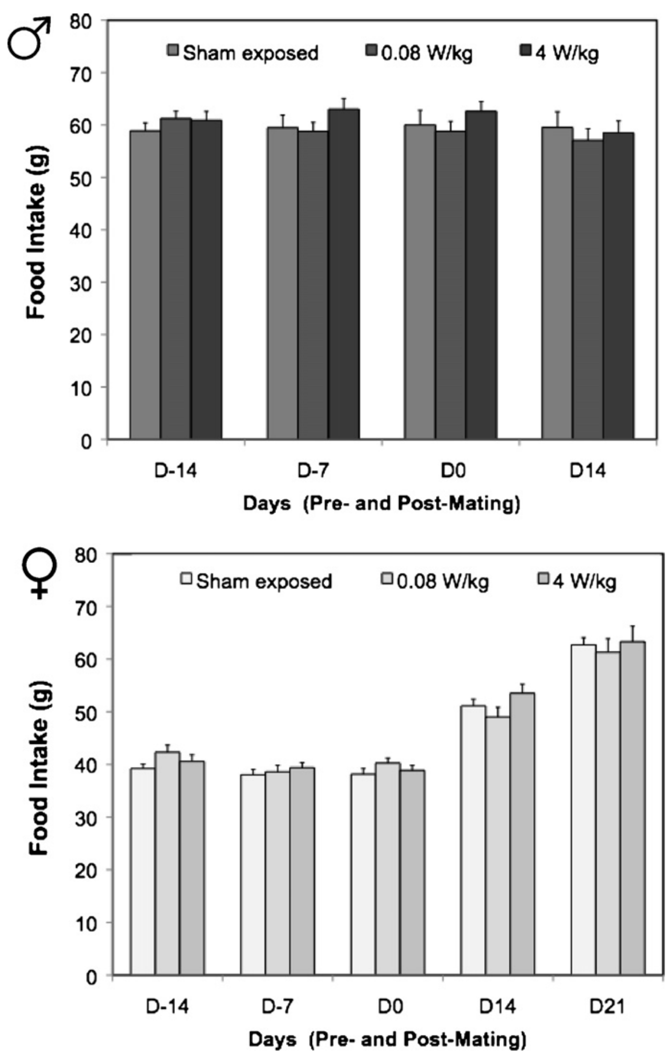

Fig. 2. Food consumption was estimated in males and females once per week over a 48 h period. D0 is the mating date. Results are expressed as the mean ± SEM. $n = 12$ for males and females for each exposure conditions.

Table 2
Mean percentage of males and females in caesarized females.

	♂	♀
0.08 W/kg	47.2 ± 4.7	52.8 ± 4.7
4 W/kg	49.5 ± 3.3	50.5 ± 3.3
Sham	39.4 ± 3.1	60.6 ± 3.1

Results are expressed as mean percentages ± SEM. $n = 11$ for 0.08 and 4 W/kg groups. $n = 12$ for the sham-exposed group.

The mean body weight of male and female fetuses was comparable in all exposure conditions (Fig. 5). No statistically significant difference could be detected in the sex ratio in the two exposed groups (0.08 and 4 W/kg) as compared to the sham-exposed group (Table 2).

4. Discussion and conclusion

To our knowledge, this work is the first to investigate the effect of Wi-Fi signal exposure on both sexual organs of rats and on reproduction. In our hands, no deleterious effects of Wi-Fi exposure on rat male organs were observed for 1 h per day, for 36 days. This present data are in line with recent reported animal experimental results demonstrating no adverse effects of mobile communication related RF-EMF on the testes. Rat whole-body exposure at 1950 MHz for 5 h per day, 7 days a week for 5 weeks (SARs of 0.08 and 0.4 W/kg) did not induce differences in body-weight gain or weight of the testes, epididymides, seminal vesicles, and prostate [9]. Low-level, whole body GSM-1800 MHz exposure (18–23 mW/kg), 2 h/day, 5 days/week for 2 weeks, had also no effect on the morphology of testes, epididymides and prostate in mice [10]. An exposure done using real mobile phones (a non-state-of-the-art exposure setup) did not induce changes in testicular and epididymal weights or sperm counts in adult rats [11]. Exposure at 848.5 MHz for 12 weeks (two 45-min exposures per day, separated by a 15-min interval) had no any observable adverse effects on rat spermatogenesis [12]. Two similar experiments were reported. In Sprague-Dawley rats exposed 20 min/day for 1 month at 0.52 W/kg whole-body, no significant effect was noted, even on testes [13]. When exposing Wistar rats to a GSM-900 MHz signal for 2 h per day, 7 days per week for 10 months, no significant effect was observed on the level of apoptosis in the testes [14]. In a study from Australia, sperm number, morphology, and vitality of testes were tested in mice exposed at 900 MHz in a waveguide, at 90 mW/kg, 12 h/day for 7 days without observation of abnormalities [15]. In rabbits, exposure at 900 MHz did not induce any significant changes in histological sections of prostatic complex, ampulla, and vesicular gland [16].

Nevertheless, others experiments have suggested links between RF-mobile phone exposure and male sexual organ toxicity. As an example, in a Turkish study, Wistar rats were whole-body exposed or sham-exposed using a mobile phone placed underneath the cages for 2 h per day for one month (0.14 W/kg whole body). Seminiferous tubular diameters of rat testes in the standby- and speech-mode groups were found to be lower than in the sham group. No significant effect was found on sperm morphology and counts but differences in the rectal temperatures of exposed rats

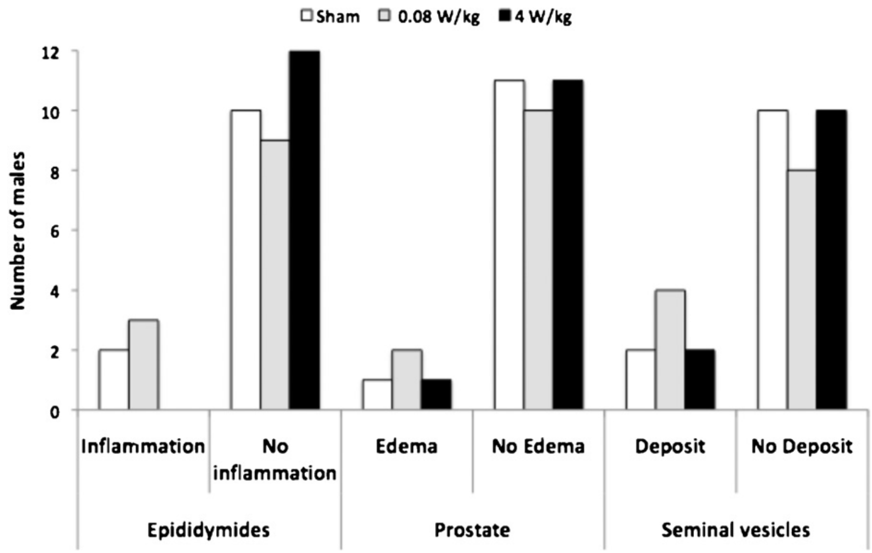

Fig. 3. Numbers of males presenting signs of inflammation in epididymides, edema in prostate or deposit in seminal vesicles are indicated for all exposure conditions.

were observed [17]. In another Turkish study, the diameter of the seminiferous tubules of rat testes decreased in exposed rats (30 min/day, 5 days/week for 4 weeks) [18]. The Indian authors of a recent study on Wistar rats exposed 2 h a day for 35 days (0.9 W/kg) to a commercial cell phone kept in the standby mode have observed overproduction of reactive oxygen species (ROS) that they have linked to potential fertilizing problem of spermatozoa [19,20]. RF from an active mobile phone (1 h per day for 28 days) negatively affected rat semen quality and may impair male fertility [21]. Male mice exposed to RF emitted from mobile phone base stations at a workplace complex and residential quarters had significantly and in dose-dependent manner more sperm head abnormalities compared to unexposed animals [22]. Rabbit histological testicular sections showed also a significant decrease in the diameter of seminiferous tubules in the exposed group (800 MHz, 8 h per day for 12 weeks, 0.43 W/kg) while testosterone concentration measurement did not show any differences [23]. Under the same exposure conditions, rabbit male sexual behaviour was significantly modified in the exposed group [24]. Unfortunately, most of the studies suggesting deleterious effects of RF fields exposure on male sexual organs used non-state-of-the-art exposure setups and low-quality exposure characterization (unknown SAR), thus making an accurate interpretation impossible.

Although the current study did not investigate sperm quality, the gestational outcome data did not suggest any impact on male fertility.

Studies on the impact of RF fields in the female genital system are still scarce. In our experiment, no harmful effects of Wi-Fi exposure were noticed in the female reproductive organs for 1 h per day, for 30 days. This is in contrast with previous published experiments, while exposure conditions were similar (≤1 h/day, 30 days). Histopathologic changes in the endometrium, as well as diffuse and severe apoptosis were present in the endometrial surface epithelial and glandular cells and the stromal cells. Diffuse eosinophilic leucocyte and lymphocyte infiltration were observed in the endometrial stroma of Wistar female rats exposed 30 min/day for 30 days to a GSM-900 signal [25]. The ovaries of female pups exposed in utero to a real mobile phone and removed 21 days after delivery revealed that the number of follicles was decreased in the exposed group [26]. The SAR was not specified.

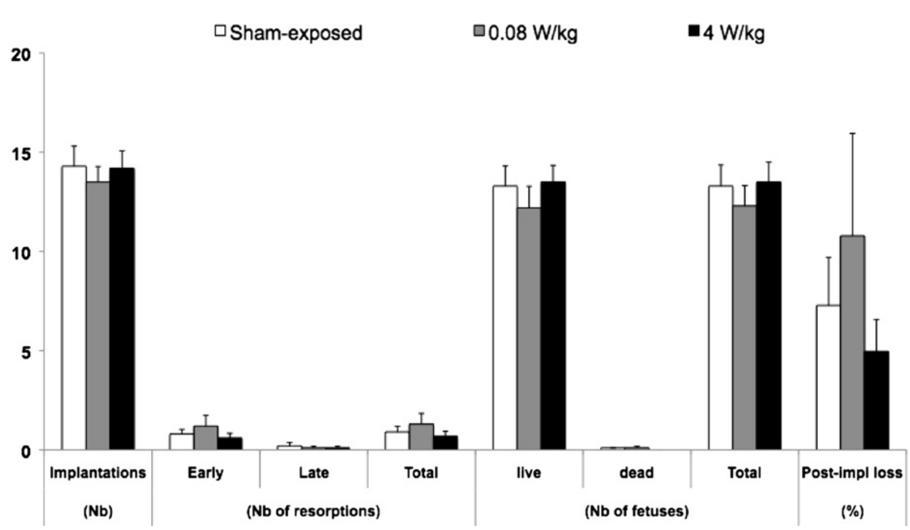

Fig. 4. Numbers of implantation sites, resorptions (early, late, and total), live, dead and total fetuses and percentages of post-implantation loss are given for all exposure conditions. Results are expressed as the mean ± SEM. $n = 11$ females for each exposed groups and $n = 12$ for sham-exposed condition.

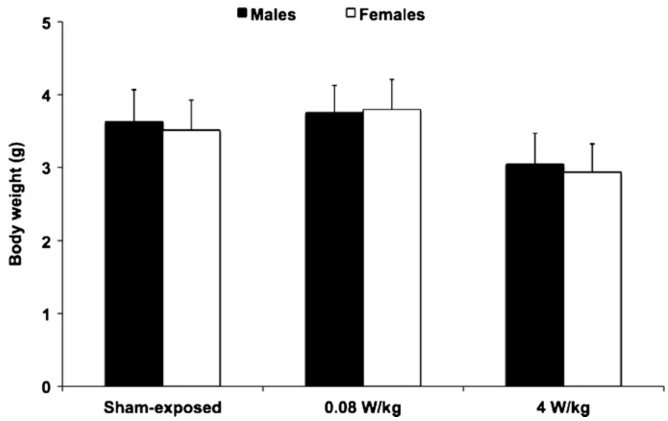

Fig. 5. Mean body weight of fetuses weighted on the day of caesarean section. Results are expressed as the mean ± SEM. n = 11 females for each exposed groups and n = 12 for sham-exposed condition.

In the present study, no abnormalities were observed in the pregnant females and the pups did not exhibit significant teratologic signs during prenatal development under all our exposure conditions, up to a whole body SAR of 4 W/kg. These results are in agreement with a recent study showing that whole-body exposure at 2.14 GHz for 20 h per day during gestation and lactation did not cause any adverse effects on pregnancy or the development of rats [27]. The effects of lifetime exposure to UMTS-1966 MHz fields on reproduction and development were also investigated in Germany over four generations of mice [28]. The WB SAR for adult animals was 0, 0.08, 0.4, and 1.3 W/kg, with a 24 h per day exposure over their lifetime. In this comprehensive study, no effect was found on pregnant females sacrificed on gestational day 18: number of fetuses, normal or malformed, per litter. In addition, no effect was observed on the number or development of pups. In South Korea, Lee et al. [29] exposed pregnant mice (WB SAR: 2.0 W/kg) to a CDMA signal or simultaneously to CDMA and WCDMA signals throughout the entire gestation period (P1–17). The mice were exposed for 45 min twice per day, with a 15-min interval in between. On P18 of gestation, fetuses were examined for teratological parameters. Neither type of exposure caused any observable adverse effect to mouse fetuses. All the above experiments are in line with reviews concluding that RF fields' exposure had no effect on the gestation and development of rats or mice [4,30,31]. Recently, our research group reported that there was no teratogenic effect caused by repeated exposure to the Wi-Fi signal even at the highest level of 4 W/kg [32].

In summary, our results provide complementary experimental evidence of an absence of effects of Wi-Fi exposure on male and female rat fertility at WB SAR values up to the ICNIRP critical level.

Conflict of interest

None.

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