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Effects of 5G frequencies on sperm parameters, mating success, and offspring outcomes in rats
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Abstract
Background and objectives
The advancement of 5G technology is highly anticipated due to its faster data transmission, yet its potential impact on male fertility remains unclear. This study evaluated the effects of 5G frequencies at 3.5 GHz and 24 GHz on sperm parameters, mating success, and offspring outcomes.
Methods
A total of 18 male Sprague Dawley rats (n=18) were divided into three groups: a Control group, a 3.5 GHz group, and a 24 GHz group (n=6). The Control group was sham-exposed. In contrast, the 3.5 GHz and 24 GHz groups were exposed to their respective frequencies for 7 h daily over 60 d. Following 46 d of exposure, a 1:1 mating procedure was conducted. Female rats (n=18) were monitored until gestation d 28 to assess pregnancy rates and birth metrics. Later, the male rats were sacrificed, and sperm quality was evaluated.
Results
Both exposed groups exhibited a significant decrease in sperm concentration and motility ($P<0.001$) compared to the Control group. The 24 GHz group had a lower pregnancy rate, whereas the 3.5 GHz group showed a lower live birth rate. Congenital anomalies were observed in the 3.5 GHz

group, whereas the 24 GHz group presented significantly lower birth weights (P< 0.001).
Interpretation and conclusions
The findings suggest prolonged exposure to 5G frequencies may have a negative impact on male fertility, pregnancy outcomes, and the birth metrics.
Keywords
Electromagnetic fields
Fertility
Pregnancy rates
Semen analysis
Wireless technology

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The rapid global rollout of 5G technology is driven by faster data transmission and lower latency.1 At the same time, its implementation has raised concerns regarding potential biological and health effects, particularly on reproductive health.2 While previous generations of wireless technology have been studied extensively, the latest 5G technology is creating new avenues for scientific exploration into its biological impact.3
Unlike its predecessors, 5G operates across low-band (700 MHz), mid-band (3.5 GHz), and high-band (24 GHz). Notably, the high band utilises millimetre waves (mmWaves) for the first time in wireless communication, setting a new benchmark for ultra-fast data transmission.4 However, the health impact of this cutting-edge technology remains debated due to limited understanding of its potential effects on public health.
Although 5G frequencies are classified as non-ionizing radiation,4 the increasing density of 5G base stations may lead to a greater potential for chronic exposure to higher-frequency radiofrequency electromagnetic fields (RF-EMF).2 While previous studies have explored the influence of RF-EMF on male fertility, the specific effects of 5G-related frequencies, especially on male reproductive health and its subsequent influence on pregnancy outcomes, remain largely unexplored.3,5
RF-EMF exposure can impair sperm quality, most consistently reducing motility and viability.6 It is well known that male reproductive health plays a crucial role in determining the success of conception and the overall health of offspring.7 Factors such as the pregnancy rate, live birth rate, sex ratio, and infant birth weight are key indicators of reproductive success and offspring well-being.8 However, limited studies have explored the potential effects of RF-EMF under 5G network exposure on male fertility and how this exposure might affect pregnancy outcomes in females mating with exposed males.
To address this gap, this study investigated the impact of 5G frequency exposure on sperm quality in male rats and its subsequent effects on mating outcomes. Key reproductive parameters, including pregnancy rate, live birth rate, sex ratio, and infant birth weight, were measured. By understanding these outcomes, this research aims to provide valuable insights into the broader discussion about the safety of 5G technology and its implications for reproductive health.
Methods
This study was undertaken by the department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lampur, Malaysia. This study was conducted from October 2023 until April 2025 following ethical

approval. The study was approved by the Institutional Review Board at Animal Ethics Committee UKM.

Animals

A total of 36 (N=36) Sprague Dawley rats consisting of 18 males and 18 females (?8 wk old, 200±50 g) were obtained from the animal unit. Male rats were obtained one month earlier to allow for their exposure to the 5G technology. All animals were housed in individually ventilated cages with food and water provided ad libitum, a 12-h light-dark cycle, and an ambient temperature of 22±5°C.

The male rats were randomly divided into three groups (n=6) in each group: a sham-exposed Control group and two exposure groups receiving 3.5 GHz and 24 GHz signals. The sample size was determined based on a Test Guideline OECD 407.9 The exposure procedure was conducted in the radiation room for 7 h daily over 60 d. No blinding was applied during the experimental procedures. All assessments were conducted with knowledge of the group assignments due to logistical constraints.

At the end of the experimental procedures, all animals were euthanized via intraperitoneal overdose (>0.1 mL/100g) of ketamine (3.34 mg/kg, Ilium, USA), xylazine (3.34 mg/kg, Ilium, USA), and zoletil-50 (1.66 mg/kg, Virbac, Australia) (KTX) cocktail. Death was confirmed by the absence of corneal reflex, loss of tail pinch reflex, forelimb, and hindlimb pedal reflex.

Exposure settings

The exposure model was designed to simulate realistic 5G environmental conditions10 using frequencies of 3.5 GHz (mid-band) and 24 GHz (high band) spectra allocated for 5G communication.10 Both frequencies were chosen to enable a comparative evaluation of their biological effects, as no prior study investigated pregnancy outcomes following paternal exposure at these frequencies.

The Control group was placed in the radiation room with the antenna or device used for the exposure set to inactive mode. The 3.5 GHz group was exposed using an omnidirectional microstrip antenna (Supplementary Fig. 1), operating at 22 dBm output power. The antenna provided by the Faculty of Electronics and Computer Technology and Engineering, Universiti Teknikal Malaysia Melaka. The 24 GHz exposed group utilized a 24 GHz Tuya WiFi Smart Human Presence Detector (Shenzhen, China), equipped with an LD2420 24G mmWave antenna capable of emitting a 24 GHz signal. This antenna operates at an 11 dBm output power and represents the higher end of the 5G technology spectrum, operating within the mmWaves radiation range.

Supplementary Fig. 1

The exposure device was positioned 20 cm from the animal cages (Supplementary Fig. 2), consistent with previous rodent studies.6 Additionally, this distance was also aligned with international safety guidelines from the United States Federal Communications Commission (US FCC) and International Commission on Non-Ionizing Radiation Protection (ICNIRP).11,12 Metal food holders were replaced with plastic containers to minimize electromagnetic interference, and a radiation meter, EXA Signal Analyzer N9010A (Keysight, USA), was placed inside the cage before exposure to verify the presence and strength of the signal.

Supplementary Fig. 2

Determination of the female oestrus cycle

Female rats’ oestrus cycle was monitored by vaginal smear analysis before the mating procedure. Vaginal lavage samples were collected between 7:00

AM and 10:00 AM.13 and examined under a field microscope, Olympus BX51, at 20x objective magnification to determine the stage of the oestrus cycle. The oestrus cycle is characterized by four phases: proestrus, oestrus, metestrus, and dioestrus (Fig. 1). These phases are identified based on the presence of three types of cells: nucleated epithelial cells (round and nucleated), leukocytes (small and round), and cornified keratinized cells (anucleate and irregular in shape). Female rats in the proestrus or oestrus phase were considered ready to mate.14

Fig. 1. The appearance of the cells in the vaginal cytology during the evaluation of the oestrous cycle under a light microscope, with objective magnification of 20X. (A) proestrus, (B) oestrus, (C) metestrus, (D) dioestrus. Here, K refers to keratinized cells; E, epithelial cells; L, leukocytes. The image was captured using Olympus BX51 (Olympus, Japan).

Mating procedure

The mating procedure begins at week 7 of exposure, one week before the end of the exposure period, to reduce the likelihood of recovery upon withdrawal of the exposure. Males and females cohabitated on a 1:1 basis, and daily vaginal swabs were collected and examined microscopically for the presence of sperm.13 The detection of a copulatory plug or sperm in the vaginal smears was considered gestation day 0,15 and all female rats were separated individually in monitoring cages until the delivery or for up to 30 d.

Evaluation of sperm parameters

After mating, male rats were euthanized, and their testes were harvested. The cauda epididymis was carefully separated and minced in 2 mL of pre-warmed phosphate-buffered saline for the sperm sample. The mixture was incubated at 37°C for 40 min, allowing the sperm to swim out for collection.

Sperm concentration

A drop of 10 µL of the sperm suspension was placed on a Makler chamber. Sperm concentration was counted as an average of five rows under 10x magnification.

Sperm motility

A drop of 10 ?L sperm suspension was placed on a microscope slide, and 200 spermatozoa were counted in duplicate at 40x magnification. Sperm motility was determined following the WHO guidelines, which are: A: rapidly progressive motile (RP), B: slow progressive (SP), C: non-progressive motile (NP), and D: immotile (IM).16 Motility was calculated as the percentage of total motile sperm (A + B+ C/total counted sperm) x 100.

Sperm viability

The hypoosmotic swelling test (HOST) was used to determine sperm viability. Sperm suspension was mixed with a hypo-osmotic solution (1:10), incubated at 37°C for 30 m. About 10 ?L of the mixture was applied to a microscope slide, smeared, air-dried at room temperature, and stained with a Diff-Quik Stain Kit (Epredia, USA) to improve sperm visibility under a bright-field microscope. Viable sperm cells were counted in duplicate under 40x magnification, examining 200 sperm cells per sample.

Pregnancy outcomes

All female rats were monitored throughout their gestation periods, and the gestation lengths for each animal were recorded. Mating index17 and pregnancy rate18 were calculated using the following formulas:

Birth metrics

Birth weight, live birth rate, and sex ratio of the pups: After 24 h post-natural delivery, each dam was assessed to record the total number of pups per litter, still births, and any observed anomalies. Furthermore, all the pups were weighed individually using a balance with 0.01 g sensitivity, and the live birth rate was determined by using the following formula.19 The sex ratio was also recorded after 24 h of delivery and calculated using the following formula:

All the pups and the dams were euthanized after the data collection.

Statistical analysis

All results were expressed as mean±standard deviation (mean±SD). One-way ANOVA was used to compare the significance of sperm parameters and the birth weight of pups. Kruskal-Wallis was used to analyze the gestation period. Statistical analysis was performed using GraphPad Prism 10 software (GraphPad Software, USA), with $P<0.05$ considered significant.

Results

Sperm parameters

The findings showed that the 3.5 GHz (119.2 x 106/mL ± 4.22, $P<0.001$) and 24 GHz (131.3 x 106/mL ± 8.55, $P<0.001$) exposed groups demonstrated a significant decrease in sperm concentration compared to the Control group (175.5 x 106/mL ± 2.31) (Fig. 2A). The differences between exposed groups were not statistically significant ($P=0.31$).

Fig. 2. Analysis of sperm parameters in each group. Data were generated using GraphPad Prism 10 software and presented as mean ± SEM. (A) sperm concentration in each group. ****Represents a significant different between 3.5 GHz and Control group with $P<0.0001$. ***Represents a significant different between 24 GHz and Control group with $P=0.0002$. ns, represent no significant difference between exposed groups. (B) Percentage of total motile sperm in each group. **Represents a significant different between 3.5 GHz and Control group with $P=0.0010$. ****Represents a significant different between 24 GHz and Control group with $P<0.0001$. (C) Percentage of viable sperm in each group. No significant difference was recorded.

The 3.5 GHz (43.6%±2.77, $P=0.001$) and 24 GHz (27.3%±7.58, $P<0.001$) exposed groups also showed a significant decrease in the percentage of sperm motility compared to the Control group (75.6%±3.12) (Fig. 2B) but no significant difference between the two exposed groups ($P=0.0822$). The percentage of sperm viability remained unchanged across the group (Fig. 2C).

Mating outcomes

Two females in the control group exhibited irregular oestrus cycles. However, the variability is a recognized biological phenomenon in rodents.20 Consequently, the Control group achieved a mating index of only 66.7%, whereas both exposed groups achieved a 100% mating index (Table I).

Table I. Mating index, pregnancy rate and the gestation period of female rats

Parameters	Control	3.5 GHz	24 GHz
No. of females placed with males	6	6	

6
No. of females mated
4
6
6
No. of females pregnant
4
6
5
Mating index (%)
66.7
100
100
Mating period (Days)
3.00±1.155
2.167±0.408
2.5±1.22
Pregnancy rate (%)
100
100
83.3
Gestation period (Days)
23±0
23±0
22±0.37
Data is presented as mean ± SD for the mating period and as median ± SEM for the gestation period. Mating index (%) = (No. of females mated/No. of females placed with males) × 100. Pregnancy rate (%) = (No. of pregnant female rats/Total successfully mated female rats) × 100
The mating period for the Control group recorded the highest (3.00±1.16 d), followed by the 24 GHz group (2.5±1.22 d), and the 3.5 GHz group (2.17±0.41 d), with no significant difference between the groups (P=0.50). Both the Control and the 3.5 GHz groups showed 100% pregnancy rates. However, the 24 GHz group had a reduced pregnancy rate of 83.3%, with one female rat failing to become pregnant despite the presence of sperm in the vaginal plug. The gestation period showed no significant difference across groups (P=0.05).
Birth metrics
Total number and gross anomalies of the delivered pups
The Control group generated 38 pups, the 3.5 GHz group produced 63 pups, and the 24 GHz group delivered 46 pups (Table II). No stillbirths or gross anomalies in the Control and 24 GHz-exposed groups. However, 10 pups from the 3.5 GHz group were stillborn with extensive anomalies, including the deformation of the head and body, and two pups also showed the presence of abdominal hematoma (Supplementary Fig. 3).
Supplementary Fig. 3
Table II. Birth metrics of the pups
Parameters
Control
3.5 GHz
24 GHz
No. of pups born
Live
38

53
46
Dead
0
10
0
Total
38
63
46
Live birth rate (%)
100
84.13
100
No. of live pups
Male
21
29
22
Female
17
24
14
Sex ratio
1.24
1.21
1.60
Weight at birth
7.312±0.74
7.210±0.60
6.707±0.49*
Data on weight at birth are presented as mean ± SD. Live birth rate (%) = (Number of live pups/Number of total pups born) × 100
Sex ratio = No. of live male pups/No. of live female pups (on postpartum Day 0)
* Represents a significant difference compared to the control group
Birth weight, live birth rate, and sex ratio of the pups
Live birth rate was 100% in Control and 24 GHz, but lower in 3.5 GHz (84.13%). Pup weight was significantly decreased in the 24 GHz-exposed group compared to the Control group (P< 0.001, 6.71 ± 0.49) (Table II). Meanwhile, the sex ratio (male: female) was recorded as 1.24 in the Control group, 1.21 in the 3.5 GHz group, and 1.60 in the 24 GHz group (Table II) with no statistically significant difference among the groups.
Discussion
Exposure to RF-EMF has been linked to impaired spermatogenesis,6 sperm DNA damage,21 and reduced testosterone levels.22 However, previous studies have focused primarily on conventional 3G and 4G technology. To the best of our knowledge, this is the first study to investigate the impact of the latest 5G technology, utilizing mid-frequency (3.5 GHz) and high-frequency (24 GHz) signals on sperm quality and pregnancy outcomes.
This study found a significant decrease in both sperm concentration and motility following exposure to 3.5 GHz and 24 GHz, consistent with previous studies.6 For 3.5 GHz, these effects may be mediated via two pathways: thermal effect, where absorption of microwave RF-EMF increases

testicular temperature23 and interrupts signalling that is crucial for spermatogenesis,24 thus impairing the sperm concentration. On the other hand, the non-thermal effect involves the development of testicular oxidative stress25 that can induce lipid peroxidation and apoptosis,26 ultimately causing sperm DNA damage. Testicular oxidative stress was also proven to cause dysregulation of intracellular Ca2+ signalling, changes in protein expression, and mitochondrial dysfunction.27 Since mitochondrial ATP production and membrane integrity are crucial for sperm movement, ROS-mediated mitochondrial injury could lead to reduced sperm motility. Although mmWaves radiation has shallower penetration than microwave frequencies, the biological effects may still extend beyond the skin. Localized heating with subsequent heat transfer, oxidative stress, and indirect cellular signalling disruptions could plausibly affect sperm concentration and motility.28 Even minimal penetration into biological tissues can induce thermal effects, leading to localized heating that alters the function of nearby organs, especially the testes.29
However, 3.5 GHz demonstrated a 100% pregnancy rate despite the reduced sperm quality, which is consistent with previous reports.30 The discrepancy between reduced sperm quality and unchanged pregnancy rates may be due to the high sperm production capacity in rodents.27 Thus, the functional reserve of sperm production can sustain conception despite increased cell apoptosis or DNA damage.27 Both exposed groups exhibited higher mating indices and shorter mating periods than the control group. The lower mating index and prolonged mating period in the Control group were attributable to irregular oestrus cycle in two females, suggesting that failure to mate may be due to the female rather than the male. In contrast, females paired with the 3.5 GHz and 24 GHz groups exhibited regular oestrous cycles, resulting in successful mating. To overcome this limitation, it is best to pair each male with multiple females to provide more accurate future assessments of male reproductive performance and minimize the influence of female cycle variability.
The 3.5 GHz exposed group exhibited severe gross abnormalities and stillbirths among the offspring. In contrast, the 24 GHz group showed a lower pregnancy rate and lower birth weight, suggesting that higher-frequency RF-EMF exposure may interfere with early embryonic development, leading to failed conception despite the presence of sperm31 or impair foetal growth.
These findings further insist that RF-EMF damages sperm DNA, leads to impaired embryonic development, and has adverse birth outcomes.30 Although sperm DNA damage was not assessed in this study, increased sperm DNA fragmentation has been reported in microwave studies.27,29 Besides, a recent mmWaves study at 35.5 GHz reported increased sperm DNA damage, markers of testicular oxidative stress, and depleted antioxidant defences.30 Future studies should correlate the testicular oxidative stress, sperm DNA fragmentation, and pregnancy outcomes to verify the underlying mechanisms.
There was an increase in the male-to-female ratio in the F1 offspring of the exposed group, which is consistent with a previous report.30 The mechanism remains unclear, but the disruption of reproductive hormones32 plays a key role in increasing the offspring sex ratio. The thermal effects or oxidative stress induced by RF-EMF exposure may differentially affect X- and Y-bearing spermatozoa, favouring Y-sperm survival or fertilizing capacity. Further mechanistic studies are required to clarify the underlying mechanisms.

Overall, the findings of the current study suggest a differential negative impact on pregnancy outcomes following mating with RF-EMF-exposed male partners. Future studies should also employ mating designs that vary exposure between partners and assess their effect on the pregnancy and offspring outcomes. Besides, a larger sample size would also be beneficial to identify subtle differences between the 3.5 GHz and 24 GHz exposure conditions. Blinding was not implemented in this study due to practical constraints in experimental execution, which may introduce observer bias in outcome assessment.

These findings may inform potential risks to male reproductive health from human exposure to RF-EMF–emitting devices at similar frequencies. However, translation to human reproductive health should be approached cautiously, as the real-world exposure scenarios are complex and remain challenging to replicate accurately in controlled experimental settings.

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Author contributions

AH, FHFJ, SFI, MHM: Designed the study; AH, AFZ, MFMR, NJ, SMAST, SV: Conducted the data acquisition, interpretation, analysis; ZZ, AAMB: design of the exposure setup and provided the antenna; AH: Manuscript writing; FHFJ, SFI, KO: Supervision, manuscript writing. All authors have read and approved the final printed version of the manuscript.

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Conflicts of Interest

None.

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation

The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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