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Effects of 5G radiofrequency electromagnetic radiation on indicators of vitality and DNA integrity of *in vitro* exposed boar semen

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Highlights

- 5G RF-EMR significantly increases the proportion of boar's spermatozoa with damaged DNA
- The proportion of spermatozoa with damaged DNA depends on the used frequency
- Sperm damaged cell membrane and/or low mitochondrial potential increases after 3500 MHz exposure

ABSTRACT

The effects of radiofrequency electromagnetic radiation (RF-EMR) on semen quality have been in the spotlight in recent years, though research results to date have been contradictory. The effects of RF-EMR amongst others depend upon frequency, and there is currently no literature concerning the influence of 5G frequencies on both DNA integrity and spermatozoa vitality in males. The aim of this study was to investigate the effect of 5G RF-EMR on sperm membrane integrity, mitochondrial potential, and DNA integrity of *in vitro* exposed semen of breeding boars. The study included semen samples of eight breeding boars of the Pietren breed and four breeding boars of the German Landrace breed, from 1.5 to 3.5 years in age. Freshly diluted semen of each boar was divided into a control ($n = 12$) and experimental group ($n = 12$). The samples of the experimental group were exposed for 2 hours to continuous RF-EMR at a single frequency (700 MHz, 2500 MHz and 3500 MHz) and an electromagnetic field strength of 10 V/m using a transverse gigahertz electromagnetic cell. Sperm DNA fragmentation was assessed using a Halomax® kit and sperm membrane integrity and mitochondrial potential was assessed using a PI/SYBR-14 LIVE/DEAD viability kit with JC-1. A significantly higher proportion of spermatozoa with DNA fragmentation was found in exposed semen samples for all frequencies compared to the control group. The highest DNA damage was recorded in semen samples exposed to 5G RF-EMR at 2500 MHz ($p < 0.01$) and 3500 MHz ($p < 0.05$) vs. control semen samples. A significantly higher proportion of spermatozoa with damaged cell membrane and good mitochondrial potential was recorded in semen samples exposed with 3500 MHz. *In vitro* exposure of breeding boar semen to 5G RF-EMR significantly increases the proportion of DNA fragmentation. The harmful effect of 5G RF-EMR on the proportion of spermatozoa with damaged DNA was frequency dependent. The 3500 MHz frequency displayed the most harmful effects due to significant impacts on DNA integrity and spermatozoa vitality indicators.

Introduction

The presence of different frequencies from radiofrequency electromagnetic radiation (RF-EMR) of anthropogenic origin, *i.e.*, radio communication systems, mobile phones, microwave ovens, and wireless communication system in the environment is known as electromagnetic smog or electrosmog [1]. Public concern about the impact of RF-EMR on health is controversial [2]. 5G technology is considered a potential health risk which requires further investigation and research regarding the possible health effects of electromagnetic field exposure [3]. Although the energy of RF-EMR is not sufficient to break chemical bonds, the results of numerous studies have shown that exposure to this energy at the frequency of 4G and earlier technology can cause various biological effects in different cell models. Previous studies on the effects of RF-EMF at frequencies from 900 MHz to 2.4 GHz on human and

animal sperm mainly investigated sperm motility, vitality and DNA damage under short-term conditions [4], [5]. For example, several (*in vitro*) studies showed that sperm motility, sperm viability and sperm-fertilizing ability were decreased as well as DNA fragmentation was enhanced in the human sperm after exposure at frequency of 850 MHz, 900 MHz and 2.45 GHz for one hour exposure [6], [7], [8].

Male fertility can be reduced by a range of environmental factors. Parameters that indicate reduced fertility can appear as a decreased sperm count, motility disorder or structural disorder. These changed sperm fertility parameters can be induced by biochemical factors, stress, ionising radiation, or electromagnetic waves [9]. Most research to date has focused on the harmful impact of RF-EMR on the male reproductive system, especially humans [10], while data on the effects of RF-EMR, especially the 5G assigned frequency, on the reproductive system of male domestic animals are scarce. In recent decades, pigs have become a common choice as animal models in biomedical research, due to their great similarity in physiological processes, *i.e.*, cellular mechanisms, with humans [11]. In addition, the pig genome is three times more similar to the human genome than the mouse genome, making pigs a more suitable model than rodents [12]. In this study, boar spermatozoa was selected as the model because of the high volume and sperm count that boar ejaculate (approx. 250 mL), enabling a large number of analyses [13]. Research on boar semen could also have practical applications, as large number of sows are fertilised with a single ejaculate, and electrosmog is a potential threat not only to human health but also to the health of domestic and wild animals.

Currently known data indicate that exposure to RF-EMR induces DNA damage and accumulation of reactive oxygen species (ROS) or oxidative stress due to increased levels of free radicals or superoxide anions [14]. An increase in superoxide anion concentration can cause a decrease in spermatozoa motility [15]. Some studies have shown that the RF-EMR will reduce the motility and vitality of spermatozoa cells, concurrently stimulating the formation of a DNA base adduct, which ultimately results in DNA fragmentation [14]. Formation of a biomarker for oxidative damage of DNA and DNA fragmentation are most likely co-correlated and ultimately linked to cell death [16].

Electron leakage from spermatozoa mitochondria is thought to be the main source of ROS in these cells [17]. Oxidative damage is induced when the production of ROS by the spermatozoa mitochondria exceeds the gamete's limited endogenous antioxidant defences, which are rapidly overwhelmed. Loss of fertilising potential and vitality is due to lipid peroxidation in the spermatozoa cell membrane [18]. ROS will also induce DNA damage in the spermatozoa nucleus and mitochondria and will do so at levels of oxidative stress that

are still compatible with the retention of motility and a capacity for fertilisation [16], [18], [19]. However, the potentially harmful effects of RF-EMRs at frequencies higher than 2500 MHz have not been previously investigated on quality and/or indicators of spermatozoa vitality of human or animal semen. Also, there are no data on the effects of RF-EMR on structural stability of spermatozoa chromatin *in vitro* exposed semen of breeding boars.

The hypothesis of the research is that there is a harmful effect of 5G RF-EMR on vitality and chromatin structural stability of spermatozoa and that the level of the effect depends on the frequency of radiation. Therefore, the aim of this study was to investigate the effects of 5G RF-EMR at three frequencies (700 MHz, 2500 MHz and 3500 MHz) on spermatozoa vitality indicators and DNA integrity on *in vitro* exposed semen of breeding boars.

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Section snippets

Boar management and sampling

The study was performed on semen samples of eight breeding boars of the Pietren breed and four breeding boars of the German Landrace breed, aged from 1.5 to 3.5 years. Semen was collected using manual fixation of the penis at a commercial centre for artificial insemination in Varaždin, Croatia (46°18'15"N, 16°20'16"E). Boars were kept in separate pens of approximately 12 m² (4 x 3 m). All pens were illuminated with natural light and the floors were covered with straw litter. Boars were fed

Spermatozoa DNA integrity and spermatozoa vitality indicators of boar semen

The results of sperm DNA integrity and spermatozoa vitality indicators are shown in Figure 2. and Figure 3. In the figures comparisons between samples exposed to 5G RF-EMR at 700 MHz, 2500 MHz or 3500 MHz and corresponding control samples (non-exposed) as well as between the total of all control and exposed samples regardless of frequency are shown.

In total, all exposed samples (all frequencies) in comparison with all control semen samples had a statistically significantly higher proportion of

Discussion

To date, many *in vivo* and *in vitro* studies in humans and animals have shown that RF-EMR exposure can alter homeostasis, endocrine function, reproductive function and foetal development [21], [22]. Furthermore, the results of numerous studies have indicated an association between cell phone use and a decrease in the number and motility of spermatozoa, spermatozoa with normal morphology and viability [14], [23], [24], [25]. In addition, the ROS generated by RF-EMR can have deleterious effects on

Conclusions

It can be concluded that the frequencies of 5G used in this study increase the proportion of spermatozoa with damaged DNA in the *in vitro* exposed sperm of breeding boars. The proportion of spermatozoa with damaged DNA was frequency-dependent. The 3500 MHz frequency showed the most detrimental effects due to significant effects on spermatozoa DNA integrity and vitality indicators. Our results suggest that the effects of 5G wireless communication technologies on sperm quality *in vitro* and *in vivo*

Declaration of Competing Interest

The authors declare no potential conflicts of interest.

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