Bundesamt
für Strahlenschutz

Spotlight on EMF Research

Spotlight on "The effect of 900-MHz radiofrequency electromagnetic fields during the adolescence on the histological structure of rat testis and its androgen and estrogen receptor localization" by Gur et al. in Int. J. Radiat. Res. (2021)

Category [radiofrequency, animal study]

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Competence Centre Electromagnetic Fields (KEMF)

1 Putting the paper into context by the BfS

There are concerns that radiofrequency electromagnetic fields (RF-EMF) might have a negative influence on male fertility. This issue has been addressed by many studies, but the overall results are inconsistent. As a potential mechanism of action oxidative stress is often cited in scientific studies and discussions. The term oxidative stress describes an imbalance between the production of reactive oxygen species (ROS), and the cellular antioxidative defense system. ROS are naturally produced during cellular energy production or by immune cells for pathogen defense, but also functions as a signal transducer. The level of ROS is normally controlled by antioxidative mechanisms, e.g. antioxidative enzymes. In this context it is important to distinguish between physiological oxidative stress (Eustress) that is necessary for cellular processes and harmful oxidative stress (Distress) between which there is no clearly defined boundary [2, 3].

2 Results and conclusions from the authors perspective

There is a controversial study situation about possible effects of RF-EMF on male fertility: some studies in the 900 MHz frequency range found effects on male reproductive health like hypospermatogenesis, or decreased motility, other studies found no negative effects on male reproductive health. In the present

study the effects of 900 MHz RF-EMF on adolescent rat testicular tissue were investigated by using histopathological and biochemical methods.

Eight male, 21 days old Sprague Dawley rats were randomly selected and put in one of three groups (control, sham-exposed, RF-exposed) and were exposed with 900 MHz RF-EMF for 1h/day (SAR: 0.01W/kg) for 25 days. The rats were sacrificed and their testes prepared for further analysis. For histopathological procedures, tissue samples were fixed, embedded in paraffin and 5µm slices stained with the H&E (Hematoxylin & Eosin) method. The sizes of the seminiferous tubules and seminiferous epithelium were determined microscopically. For immunohistochemistry the tissue sections were incubated with antibodies against the Androgen receptor (AR), Estrogen receptor alpha (ERα) and Estrogen receptor beta (Erß), followed by secondary antibodies and analyzed microscopically. Apoptotic cells were stained with a TUNEL detection kit and analyzed microscopically. For biochemical analysis the tissue samples were homogenized and the concentrations of the antioxidative enzymes Catalase (CAT) and Superoxiddismutase (SOD), and the amounts of the radical scavenger Glutathione (GSH) as well as Malondialdehyde (MDA), a marker for lipid peroxidation, were measured photochemically.

The histological analysis showed no differences in seminiferous tubule diameter, germinal epithelium thickness and apoptosis index in testicular tissue between the RF-exposed, sham-exposed and control groups. No changes were observed in the localization of the hormone receptors AR, ERα and Erß after exposure in comparison to the control group. In the biochemical analysis , the exposed group showed a significant increase in the level of MDA and CAT, but also a significant decrease in the level of GSH in comparison to the control group.

The results indicate that RF-EMF can cause oxidative stress in testicular tissue, but the damage caused by oxidative stress was too low to have an impact on the other endpoints investigated in this study. Although prolonged exposure to RF-EMF might lead to pathological disorders, the results of this study showed that exposure to 900 MHz RF-EMF during the adolescent period had no effect on the structure, development and differentiation of testicular tissue.

3 Comments by the BfS

The research question addressed in this study is of scientific interest, especially in consideration of the fact that the exposure was set within the recommended limit values. The authors mostly used adequate methods to answer the underlying question. However, there are some shortcomings: i) with only 8 animals the sample size is very small, ii) the experiments were not blinded, therefore a risk of bias cannot be ruled out, iii) no positive- or negative controls were included in the experiments (negative controls only used for the immunostainings), which is important to evaluate the measured effect sizes, especially in terms of oxidative stress [4] and iv) the exposure level is inaccurate. Due to the freedom of movement of the rats the individual exposure level could have been very different and valid data on whole-body absorption or local SAR in the testes cannot be derived from the data given. For the investigation of morphologic alterations of the testis tissue the authors used well established histological methods to measure the diameter of the seminiferous tubules and germinal epithelial thickness. Also, the usage of the TUNEL assay is a widely accepted method to quantify apoptotic cells. Overall, RF-EMF exposure did not lead to morphologic alterations or increased cell death. However, it must be taken into consideration that the methods used only allow a statement about the morphology, but not about the functionality of the sexual apparatus. The most important parameter of male fertility is sperm quality (motility, morphology, concentration), which was not investigated in this study [5].

The authors found no differences in hormone receptor localization between the control and the exposed group by optical analysis. To further support this statement a quantification of the data would have been helpful.

The authors also investigated, whether RF-EMF exposure leads to oxidative stress . To analyze this, they measured the concentration of antioxidative enzymes and lipid peroxidation by photochemical methods. The described methods are suitable to measure protein concentration, but per se not suitable to measure

oxidative stress. The expression and activity of antioxidative enzymes often increases in response to the production of electrophiles that activate the Nrf2 and KEAP1 transcription factors which regulate antioxidant enzyme genes [2]. But the same compounds are produced in metabolism, consumed in food, etc. So, while oxidative stress can result in an increase in antioxidant enzymes, they are not a reliable indicator. Furthermore, the authors measured the concentration of glutathione (GSH) at a single time point, but expression and activity of GSH are transient – in order to measure oxidative stress, a time course of GSH activity is necessary [6]. To investigate lipid peroxidation the authors measured MDA, which is known to be caused by many different reactions, not necessarily by oxidative stress [6]. Overall the measurements performed in this paper are not sufficient to draw conclusions about oxidative stress.

The underlying question of the study is of scientific interest and relevant for radiation protection. Unfortunately, some crucial criteria of scientific practice (blinding, positive/negative controls) are not met and the study lacks the investigation of important markers of male fertility. Due to the shortcomings the study does not contribute substantially to the state of the art in this field. However, the morphologic evaluation of tissue samples is adequate, and the authors discussed their results in a differentiated manner reflecting the large and heterogenous body of literature dealing with effects of RF-EMF on fertility in animal studies.

References

The first reference is always the manuscript at hand and the reference in the curly braces at the end of a reference {xx} correspond to a reference in the manuscript at hand and is consistent with the manuscripts reference style.

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