Bundesamt
für Strahlenschutz

Spotlight on EMF Research

Spotlight on “Expression levels of tam receptors and ligands in the testes of rats exposed to short and middle-term 2100 MHz radiofrequency radiation” by Katirci et al. in Bioelectromagnetics (2024)

Category [radiofrequency, animal study]

Spotlight - Mai/2025 no.1 (Eng)

Competence Centre for Electromagnetic Fields (KEMF)

1 Putting the paper into context by the BfS

A study published in 2017 triggered a large media response by reporting a significant decline in sperm counts among men between 1973 and 2011 in Western countries [2]. This decline was associated with multiple environmental and lifestyle influences. Radiofrequency electromagnetic fields (RF-EMF) from mobile phones were speculated to be one of the many possible causes. A recently published systematic review found low certainty of evidence for a decreased sperm count in rodents after exposure to high RF EMF levels [3]. The authors of the review recommended further investigations of this endpoint at exposure levels close to the limit values for humans. The continuing interest of the research community in this topic is exemplified by the present study.

2 Results and conclusions from the perspective of Katirci et al.

Several reports of negative health effects from exposure to RF-EMF, particularly from RF-EMF emitted by mobile phones, prompted the authors to evaluate its effects on the testis of exposed rats [1]. Their focus was on the TAM receptors Tyro3, Axl and Mer, which are members of the receptor tyrosine kinase family, and essential for several biological processes like survival, proliferation, regulation of the immune system and phagocytosis of apoptotic cells. Eight-week-old rats were split into five groups of ten animals each. The first group was a cage control group. Groups 2 to 4 represented the two sham-exposed and the two exposed groups, with one group exposed or sham-exposed for 1 week and the other group for 10 weeks. The restrained rats were exposed or sham-exposed to 2100 MHz RF-EMF for two hours per day, for one or ten weeks, at a whole-body specific absorption rate (wbSAR) of 0.16 W/kg and a mean SAR of 0.0347 W/kg for the testis. After one or ten weeks of exposure or sham-exposure, testicular tissue sections were stained with hematoxylin and eosin and analysed microscopically. The three TAM receptors, Tyro3, Axl and Mer, and their ligands, Gas6 and Pros1, were investigated by immunohistochemistry in the testes. In addition, cleaved caspase-3, a marker for apoptosis (i.e. programmed cell death), was examined by immunohistochemistry. The results of the immunohistochemical staining were assessed using the H-score that quantifies the expression level of a targeted protein. The H-score is determined by the percentage of positively stained cells and the intensity of the staining. At both exposure times, testicular tissue was histologically analysed using the Johnsen scoring system. This is a ten-point scoring system for quantifying the quality of spermatogenesis (development of sperm cells) based on cellular characteristics in the seminiferous tubules (the structures in the testicle where spermatogenesis occurs). A Johnsen score of 10 indicates maximum spermatogenesis activity, whereas a score of 1 indicates complete absence of germ cells [4].

The RF-EMF exposure did not cause any increase in rectal temperature. Sham-exposed rats showed normal testis morphology at both time points, whereas RF-EMF exposed rats showed a reduction in seminiferous epithelial thickness as well as a premature release of immature germ cells into the seminiferous tubule lumen. At both time points, the RF-EMF exposure groups showed a lower Johnsen score than the corresponding sham-exposed groups, but the difference was statistically significant only after ten weeks of exposure. The authors interpreted the results as a hint that RF-EMF exposure may cause apoptosis.

For immunohistochemical analysis, the developmental stages of seminiferous tubules were determined. During rat spermatogenesis, 14 stages of seminiferous tubules can be identified according to different developmental stages of germ cells they contain. TAM receptors and their ligands showed specific staining patterns, i.e. only expressed in specific cells or seminiferous tubule stages, but without statistically significant differences in the H-score between the groups. Cleaved caspase-3 staining was detected in round spermatids (precursors to sperm cells) of the VI-VIII tubule stages of seminiferous tubules in all groups. The H-score was higher in both exposed groups compared to the sham-exposed groups, but differences did not reach statistical significance. Round spermatids did not express Tyro3, Mer, Gas6 or Pros1, while Axl expression in this cell type was similar between sham-exposed and exposed groups. Therefore, the authors concluded that RF-EMF exposure may cause apoptosis in round spermatids independent of TAM receptors.

From their data, the authors conclude that RF-EMF exposure disrupts the normal histomorphology of the rat testis and may negatively affect TAM signalling and lead to an accumulation of apoptotic cells. However, the authors state that the impact on testicular function remains uncertain.

3 Comments by the BfS

The study addresses a relevant and current research topic at exposure levels close to human exposure limits. Although the authors give detailed descriptions of the animals and study design, the exposure characterisation is less detailed and the reported exposure levels can be challenged. At the given incident electric field strength, much lower wbSAR levels than reported can be expected for rats. Therefore, it remains unclear to what exposure level the testes were actually exposed to. Additional ambiguities relate to the assessment of the Johnsen score because there is no information given regarding the number of seminiferous tubules that were assessed per rat or how the seminiferous tubules were selected for analysis.

In their introduction, the authors refer to the paper by Deng et al. who describes the reported distribution of the expression of TAM receptors and their ligands in seminiferous tubules [5]. The results of the immunostainings in the present paper do not seem to match this report entirely. It would have been helpful to demonstrate the specificity of the staining using standard immunohistochemistry negative controls rather than just testing for nonspecific binding of the secondary antibody [6].

The results on the apoptosis marker cleaved caspase-3 as indicated by the H-score do not appear to be compatible with the immunohistochemical staining presented in the figures; those show high heterogeneity in staining intensity across seminiferous tubules stages in the sham group. However, the authors did not specify how they selected the seminiferous tubules for H-score evaluation. Moreover, the described H-score assessment is a method based on manual and subjective visual assessment and therefore susceptible to some level of bias and variability [7]. In general, apoptosis is an important and frequent process in the testis that can occur at any phase of spermatogenesis [8, 9]. In contrast, in this study, only one cell type stained positive for cleaved caspase-3 in exposed and sham-exposed groups. Notably, the cage control group showed only very weak staining overall. These inconsistencies are not sufficiently explained by the authors, and additional quantitative methods were not used to validate the expression of cleaved caspase-3 or TAM receptor and their ligands.

The research question underlying the study is of scientific interest and relevant to radiation protection. However, the critical points described above significantly reduce the study’s validity. For this reason, the study cannot contribute to the current state of scientific knowledge in this field.

References

[1] Katirci, E, Kirimlioglu, E, Oflamaz, AO, Hidisoglu, E, Cernomorcenco, A, Yargicoglu, P, Ozen, S, Demir, N. Expression levels of tam receptors and ligands in the testes of rats exposed to short and middle-term 2100 MHz radiofrequency radiation. *Bioelectromagnetics*. 2024; 45(5):235–248. DOI: https://doi.org/10.1002/bem.22504.

[2] Levine, H, Jorgensen, N, Martino-Andrade, A, Mendiola, J, Weksler-Derri, D, Jolles, M, Pinotti, R, Swan, SH. Temporal trends in sperm count: a systematic review and meta-regression analysis of samples collected globally in the 20th and 21st centuries. *Human Reproduction Update*. 2023; 29(2):157–176. DOI: https://doi.org/10.1093/humupd/dmac035.

[3] Cordelli, E, Ardoino, L, Benassi, B, Consales, C, Eleuteri, P, Marino, C, Sciortino, M, Villani, P, H. Brinkworth, M, Chen, G, P. McNamee, J, Wood, AW, Belackova, L, Verbeek, J, Pacchierotti, F. Effects of radiofrequency electromagnetic field (RF-EMF) exposure on male fertility: a systematic review of experimental studies on non-human mammals and human sperm in vitro. *Environment International*. 2024; 185:108509. DOI: https://doi.org/10.1016/j.envint.2024.108509.

[4] Johnsen, SG. Testicular biopsy score count – a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. *Hormones*. 1970; 1(1):2–25. DOI: https://doi.org/10.1159/000178170.

[5] Deng, T, Chen, Q, Han, D. The roles of TAM receptor tyrosine kinases in the mammalian testis and immunoprivileged sites. *Frontiers in Bioscience-Landmark*. 2016; 21(2):316–327. DOI: https://doi.org/10.2741/4390.

[6] Hewitt, SM, Baskin, DG, Frevert, CW, Stahl, WL, Rosa-Molinar, E. Controls for immunohistochemistry: the Histochemical Society's standards of practice for validation of immunohistochemical assays. *Journal of Histochemistry and Cytochemistry*. 2014; 62(10):693–697. DOI: https://doi.org/10.1369/0022155414545224.

[7] Meyerholz, DK, Beck, AP. Principles and approaches for reproducible scoring of tissue stains in research. *Laboratory Investigation*. 2018; 98(7):844–855. DOI: https://doi.org/10.1038/s41374-018-0057-0.

[8] Shaha, C, Tripathi, R, Mishra, DP. Male germ cell apoptosis: regulation and biology. *Philosophical Transactions of the Royal Society of London, Series B*. 2010; 365(1546):1501–1515. DOI: https://doi.org/10.1098/rstb.2009.0124.

[9] Aitken, RJ, Findlay, JK, Hutt, KJ, Kerr, JB. Apoptosis in the germ line. *Reproduction*. 2011; 141(2):139–150. DOI: https://doi.org/10.1530/REP-10-0232.

Impressum
Bundesamt für Strahlenschutz
Postfach 10 01 49
38201 Salzgitter

www.bfs.de

Tel.: +49 30 18333-0
Fax: +49 30 18333-1885
E-Mail: spotlight@bfs.de

Please always use the following URN when citing this document:
urn:nbn:de:0221-2025050952053

Spotlight - Mai/2025 no.1 (Eng)