Bundesamt
für Strahlenschutz

Spotlight on EMF Research

Spotlight on “2.45 Ghz microwave radiation induced oxidative stress: Role of inflammatory cytokines in regulating male fertility through estrogen receptor alpha in Gallus gallus domesticus” by Gupta et al. in Biophysical Research Communication (2022)

Category [radiofrequency, animal study]

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Competence Centre Electromagnetic Fields (KEMF)

1 Putting the paper into context by the BfS

2-3 Oxidative stress is often suggested as a possible mechanism of action of high frequency electromagnetic fields (HF-EMF) on male fertility. 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 (Disstress) between which there is no clearly defined boundary [2, 3].

2 Results and conclusions from the authors perspective

According to the working hypothesis of the authors, interaction of microwave (MW) radiation with living cells causes upregulation of reactive oxygen species which damages lipids, proteins and nucleic acids. This leads to an inflammatory response that modulates the expression of the estrogen receptor (ER), an important regulator of male fertility. In the study, activity of oxidative enzymes and expression of ERα was investigated in the testicular tissue of premature male chicken exposed to MW radiation.

14 days old male chickens (Gallus gallus domesticus) were divided into two groups with seven chickens in each group. The first group was exposed for 2h per day with 2.45 GHz of continuous MW radiation from a WiFi router, leading to an average whole-body SAR of 0.998 W/kg. The second group was sham exposed accordingly, but with the radiation device turned off. After 30 days the chickens were weighed and sacrificed. The testes were weighed and testis slices were stained with Hematoxylin and Eosin or immunostained with antibodies against the cytokines Interleukin-1ß (IL-1ß) and Interleukin-10 (IL-10). ERα expression was analyzed by Western blot. To analyze exposure induced oxidative stress the activity of antioxidative enzymes (Catalase (CAT), Superoxide-dismutase (SOD) and Glutathionesynthetase (GSH) and the concentration of H2O2 and Malondialdehyde (MDA) were measured by photochemical methods.

In the exposed group, morphometric examination of the testes revealed a statistically significant decrease of testicular weight, volume and gonadosomatic index (quantifier of the sexual maturity of animals). Further, histological staining demonstrated a substantial reduction in the diameter of seminiferous tubules in the exposed group as compared to the controls. In addition, the exposed testes showed a significant increase in IL-1ß and a decline in IL-10 immunoreactivity. In addition, compared with the control, the exposed tissue showed a significant increase in H2O2 and MDA and a lower expression of ERα. indicating a radiation-induced oxidative stress-regulated inflammatory response. According to the authors, the results suggest a stress-induced inflammatory response after exposure.

The authors conclude that the testes are vulnerable to free radical damage and becomes an easy target organ for MW exposure induced oxidative and inflammatory stress. Therefore, it is according to the authors evident that it may cause male infertility in chickens via downregulation of ERα in testis.

3 Comments by the BfS

The authors investigated effects of MW radiation on oxidative stress and male fertility. This topic is of high relevance for radiation protection, but the validity of results obtained results in chicken for humans is limited. Furthermore, the study does not meet essential quality criteria: it lacks positive and negative controls which are necessary to evaluate the size of an effect in the examined samples. The experiments were not blinded, which leads to a high risk of bias. The exposure calculation is not precise: the authors state that the router emits with 25dBm which is 0.312 W. There are 7 chickens with an approximate weight of 90g exposed. This means that a total power of 0.312 W has to be distributed over 630g of chicken tissue, which results in approximately 0.5 W/kg. This is half of what the authors state as exposure level. Furthermore, this very simplistic calculation does not consider that only a fraction of the emitted energy is actually absorbed by the chickens. Whether the given field strengths are correct, cannot be judged from the presented data. Therefore, further information (antennas used, as well as alignment and distance to the cage) would have been necessary. Furthermore the “sham-exposed” group was not really sham-exposed, because the device was completely switched off, so the control group has to be referred to as unexposed.

In terms of body weight and size of the testis the authors found significant differences between the - non-exposed and the exposed groups. Since positive and negative controls are lacking the actual effect size is unclear and could very well be due to the very small sample size (n= 7). Since the differences are very small in absolute numbers, all parameters analyzed could very well be within the normal physiological range.

It is a frequent dilemma that the markers selected to study oxidative stress are not specific and thus, not reliable: the activity of antioxidative enzymes often increases in response to the production of electrophiles that activate the Nrf2 and KEAP1 transcription factors [2], which regulate antioxidant enzyme genes. 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 vice versa not reliable indicators. Furthermore, measuring a single concentration of H2O2 is also not suitable to determine oxidative stress, because its concentration is transient – in order to make a reliable statement about oxidative stress, a concentration curve is necessary [4]. The level of MDA is influenced by many different processes aside from oxidative

stress, so this marker also lacks specificity [4]. Overall, the data do not allow conclusions about increased oxidative stress in the exposed group.

To show differences in ERα expression between the sham-exposed and exposed groups, a Western blot analysis was performed and the bands of ERα were quantified. However, the quantification of ERα was not done correctly. For a quantification of protein bands, it is a standard procedure to normalize the band of interest to the loading control (e.g. ß-Actin) in order to calculate differences in the loading of the gel. The authors also address this themselves and, therefore, show a complete gel to demonstrate that the samples applied contain equal amounts of ß-actin (without quantifying it). However, no ERα band at 64kDa is visible, so it could be a different gel which does not justify the author's conclusion.

Finally, significantly increased amounts of the pro-inflammatory cytokine IL-1ß and significantly lower amounts of anti-inflammatory IL-10 were observed in the exposed group compared to controls. The differences are statistically significant, but in absolute numbers the differences are rather small. The increased IL-1ß is produced by immune- and tissue cells due to tissue injury or infections, so the higher activation in the exposed group could have many different causes and is not necessarily due to MW exposure [5].

When evaluating the results in the context of the existing body of scientific literature, it is noticeable that almost exclusively studies are cited that confirm the authors results. Inclusion of studies that come to different conclusions would be desirable for a conclusive picture.

The underlying question of the study is of scientific interest and relevant for radiation protection. However, the study has too many methodological deficits to contribute to the state of the art.

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.

[1] Gupta, V. and R. Srivastava, 2.45 GHz microwave radiation induced oxidative stress: Role of inflammatory cytokines in regulating male fertility through estrogen receptor alpha in Gallus gallus domesticus. Biochem Biophys Res Commun, 2022. 629: p. 61-70. https://doi.org/10.1016/j.bbrc.2022.09.009.

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