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Effects of Sub chronic Exposure to EMF-RF on Sperm Parameters of Tilapia (*Oreochromis mossambicus*)

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ABSTRACT

Technological advancement in the communication industry has led to the production of modern devices capable of producing radiations. These radiations whether ionizing or non-ionizing have resulted in the formation of electro smog all over the world. The increase in electro smog in the environment is showing many negative effects like decrease in the sperm count, lipid peri-oxidation damage in sperm cell, reduction in seminiferous tubule and testicular weight, DNA damage, leukemia, low sperm count etc. in different organisms, still many technological advances are been established which may show a significant unwanted effect in the biology of organisms. This study aims at studying the sub chronic exposure of Tilapia (*Oreochromis mossambicus*) to EMF-RF having frequency of 2.3 GHz. Three groups were acclimatized to lab conditions. 45 numbers of fishes used for the study, 15 fishes were distributed in each group viz. control, group I with 1 hour/day exposure and group II with 2 hour/day exposure. Fishes were exposed to EMF-RF as per the experimental design. After the exposure some sperm parameters like milt volume, sperm count, sperm motility and duration were studied. The values from milt samples were tested statistically using descriptive statistics and single factor ANOVA. Sub chronic exposure of fishes to EMF-RF did show a significant change in the sperm parameters undertaken for the study. More similar studies are required to evaluate the harmful effects if any from the upcoming technologies.

Key words: *Sperm parameters, Sperm count, Sperm motility, EMF-RF.*

Introduction

Modern man aspires to be more productive in today's world. Huge technical advancements like the Internet, email, and most recently, mobile technology have all been made possible by our fast-paced existence the mobile phone. Our lives have become increasingly dependent on our mobile phones, and as societal expectations for maximum effectiveness rise, so do the technological capabilities of these devices. Bhat (2013). The effect of these gadgets on human health, more notably male fertility, is one facet of recent advancements in mobile phone technology that is frequently disregarded. Male fertility may be negatively impacted by recent advancements in cell phone technology, which may also be an increasing cause of male infertility. The fertilization capability of sperm and their motility is directly dependant; therefore sperm motility is most important factor in assessing fish semen quality Formicki *et al*. (2021). Fish spermatozoa are sensitive to a number of environmental variables, including temperature, ions, pH, and osmolarity, Alavi and Cosson (2005); Alavi and Cosson (2006); Alavi *et al.* (2007); Kirschvink (2001). Salmonids are most influenced by the K+ ion concentration.Billard (1986). Acipenseridae spermatozoa are immobile in seminal plasma (Ginzburg, 1972) and cyprinids are prima-

rily influenced by osmotic pressure. Cosson (2004). The sperm motility of sea trout was shown to be influenced by SMFs of 1 mT, 5 mT, and 10 mT and in time-varying MFs of the same intensity (50 Hz); they altered curvilinear velocity (VCL), rectilinear velocity (VSL), and average velocity (VAP). The exposure enhanced the duration of sperm motility to 12 days (288 h) in both static and time-varying fields, whereas the control sperm remained motile for just 3 days (72 h); in addition, the exposure increased the velocity Formicki *et al.* (2015). VCL, VSL, and VAP were among the metrics that an SMF enhanced in Danube salmon (*Hucho hucho* (L.). A 24 hour period of time in an MF of 1 mT resulted in 71.32% of eggs being fertilised by spermatozoa, compared to 58.23% in an MF of 5 mT, -59.99% in an MF of 10 mT, and 32.60% in the control Formicki *et al* (2015). The impact of the exposure persisted for several hours after the sperm sample was taken. Formicki *et al.* (2015); Nagler *et al.* (2000). The dispersion of genetic material in the heads of sea trout spermatozoa exposed to static and time-varying MFs was within the usual range, indicating that the applied fields had no impact on DNA fragmentation. Formicki *et al* (2015). This study aimed to determine the effect of EMF-RF on some sperm parameters in Mozambique tilapia (*Oreochromis mossambicus*).

Materials and Methods

Experimental design

Mature adult males (mean weight 171.56 ± 2.5 g and mean length 12.5 ± 2 cm (15 nos. for each group) were collected from a freshwater aquaculture farm. Control and experimental groups were kept in separate tanks. Male fishes of control and experimental group were kept separately in partitioned tanks with dechlorinated tap water for a period of 15 days with photoperiod of 12:12 light and dark period in lab conditions for acclimatization. Tank parameters were set at ideal conditions with temperature 27 ± 1 °C, oxygen 6.5 ± 0.3 mg/l and pH 7 ± 0.3. The fishes were fed with freeze dried tubifex worms (Hallofeed) two times a day.

Exposure to EMF-RF

Experimental groups I and II were exposed to Wi-Fi radiations (2.3 GHz) 100 mW for the period of 1 hour /day for 60 days and 2 hours/ day for 60 days.

Milt collection

Milt collection was carried out by using a mild squeezing approach at male fish's abdomen. After the milt had been given, it was collected using a pipette.

Sperm count

The sperm count was determined using a Neubauer cell hemocytometer. A small drop of diluted milt was placed on a clean glass slide and closed with the coverslip for microscopy. All experiments were carried out in triplicate. Within a few minutes, as the sperm became sedimented, the cells counts were observed at 100x magnification and calculated as spermatozoa × 10^6 per ml.

Sperm motility and duration

The milt was diluted with deionized water (1:100) at room temperature (31 °C). Approximately 20 µl of the activated sperm cells were transferred to a microscope slide (25 × 75 mm), covered with a coverslip (22 × 22 mm, 0.95–1.05 mm thickness), and examined at 200× magnification. Assessment of sperm motility was carried out after 10 s of dilution and sperm movement was observed within 3 min. Observations were made by the same observer from different angles, and repeated at least three times for each milt sample. During the observation, only the sperms that were actively moving in a progressive straight-line and forward direction were considered as a good one. Whereas, simply vibrating or turning on their axes were considered as immotile and low-quality spermatozoa. The motility period was measured using a stopwatch from the time of sperm activation until a phase of immobility of all the spermatozoa. The percentage of sperm motility was calculated using the following formula:

$$\text{Percentage of motile sperm} = \frac{\text{No. of motile sperm counted} \times 100}{\text{No. of sperm counted}}$$

Statistical Analysis

All the values of hormonal estimations were expressed in the terms of mean value ± standard error. Different groups were compared among each other using descriptive statistics and single factor ANOVA.

Results and Discussion

The results of the sperm parameters in *O.*

mossambicus exposed to EMF-RF (2.3GHz) are shown in Table 1. The results indicated that sperm parameters reduced when exposed to EMF-RF for 1 hour /day for 60 days and 2hours/ day for 60 days as compared to the control group.

Milt volume

The quantity of milt diminished with a significant ($p < 0.01$) decline in the 1 Hour /Day exposure group, followed by a further ($p < 0.01$) decrease in the 2 Hour/Day and control groups. The sequential decline of milt volume was recorded (Figure 1).

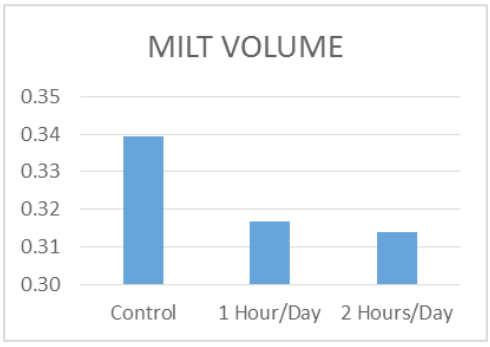

Fig. 1. Changes in milt volume of *O. mossambicus* (n =15) control and exposure groups

Sperm count

In this study, 1 hour /day and 2 hours/ day groups' exhibit strong significant ($p < 0.01$) decline of sperm count over the control group (Figure 2).

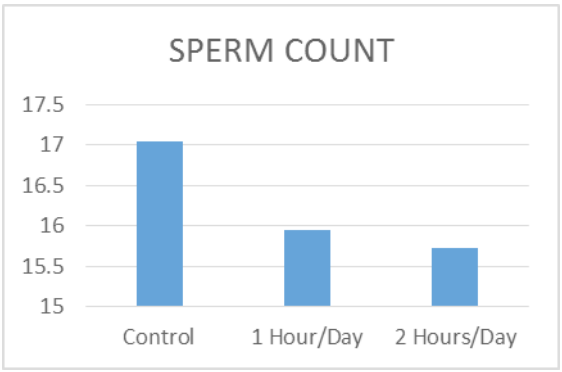

Fig. 2. Changes in sperm count of *O. mossambicus* (n =15) control and exposure groups

Sperm motility and duration

A significant decrease ($p < 0.01$) in the percentage and duration of sperm motility was found in 2 hours/day, followed by the 1 hour/day compared to the control (Figure 3 & Fig. 4).

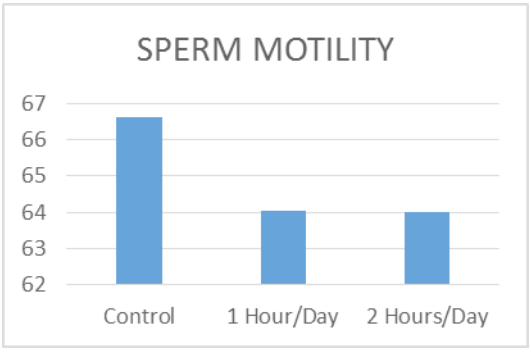

Fig. 3. Changes in sperm motility of *O. mossambicus* (n =15) control and exposure group

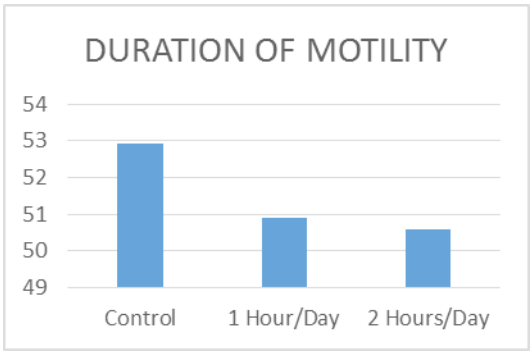

Fig. 4. Changes in sperm duration of *O. mossambicus* (n =15) control and exposure groups

Increasing development in the communication industry has led many researchers to take up the studies related with the effects of exposure of organisms including humans to the non-ionizing radiations emitted from the communication devices and also other sources like ground stations, satellites, antenna towers etc. Parameters like sperm count, sperm motility and duration etc. are of importance in the reproduction of the organisms. For example, when egg quality impact was considerable in facto-

Table 1. Changes sperm parameters in *O. mossambicus* (n =15) control and exposure groups.

Groups	Control	1 Hour/Day	2 Hours/Day
Milt volume (ml)	0.34	0.32	0.31
Sperm count ($\times 10^6$)	17.04 ± 0.08	15.95 ± 0.1	15.73 ± 0.1
Sperm motility (%)	66.62 ± 0.1	64.06 ± 0.3	64.00 ± 0.3
Duration of motility (Seconds)	52.91 ± 0.1	50.91 ± 0.3	50.58 ± 0.2

rial crosses of trout and seabass no sperm quality influence on fertilization was seen. Nagler *et al*. (2000); Saillant *et al*. (2001). However, one study demonstrated a considerable Carp fertilization variations caused by sperm in crosses employing either single males or hetero-sperm mixes where variation in fertilization was only explained by significant variations in sperm swimming velocity. Kaspar *et al*. (2008). The most recent evaluations unequivocally show that the majority of sperm traits contribute to the overall quality, although none of them are sufficiently integrative to fully define sperm's capacity to fertilize eggs. Bobe and Catherine (2010); Alavi (2008). Many studies are conducted on terrestrial animals, only a few of studies are conducted on the marine as well as freshwater dwelling animals. Unknowingly these organisms also get exposed to various sources like the submarine cables, SONAR, towers installed near the water bodies. Along with the lab studies conducted using model organisms, field studies are also required to fill the spaces in research on this problem.

The emission of radiofrequency is increasing in the environment. Organisms on the earth are getting exposed to the ever increasing sources of radio frequencies emitted especially from the cell phones, Wi-Fi's, ground stations are advancing in technology over last four decades. The result of this study shows a significant negative change in the sperm parameters after a long term exposure. The evaluation of sperm quality is crucial in aquaculture practice because it frequently determines its profitability because reproduction is a typical bottleneck in animal production. Additionally sperm quality estimation is a fundamental tool for biologists working with endangered species because successful cryopreservation for gene banks requires high-quality sperm Kowalski *et al*. (2019). It is suggested that long term exposure may affect the reproduction in many other organisms too. Since the communication industry is advancing with new technologies there is a need to conduct more studies.

Conflict of interest

We have no conflicts of interest to disclose.

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