



## Research Article

# New Electromagnetic Radiations Effects on Ultra Structure of Adult Bovine Sperm

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### Abstract

**Background:** The increase of electromagnetic radiations in the environment cause to growing public concern on the effects of them on human being health. The main purposes of many studies have recently been on these topics. Here we aimed to evaluate possible effects of electromagnetic radiations on ultra- structure of sperm.

**Method:** A healthy normal Holstein race bovine was selected for study. A total seminal fluid of a normal ejaculation obtained from normal ejaculation was divided equally into two sample groups. First control group (C1) and experimental group which exposed to electromagnetic radiations (E). At the end of test the ultra-structures of the sperms in all specimens was studied using transmission electron microscopy.

**Results:** Ultra structural changes were observed in the experimental groups comparing to control group(C1).

**Conclusion:** Our data suggest that, electromagnetic radiation have harmful effects on ultra-structure of bovine sperm and is a potential factor which can result in short term dysfunction of sperm in the various stages of fertilization. These effects may be observed later in life.

**Keywords:** Electromagnetic Radiations, Ultra structure, bovine Sperm

### Introduction

The existence of electromagnetic radiations in the environment is one of the main life-threatening factors. Today the use of these radiations has extremely gone up and so humans are involved in the pool of electromagnetic waves more and more every day (1, 2). Radars, satellites waves, cell phones and their various transreceiver stations, computers, power transporting lines, transformatory stations, towers of television senders, Copy machines, microwaves and many other electrical devises are sources of electromagnetic waves which have such a significant impact on environment that nobody can overlook (3, 4).

Now, supposing likely ultra-structural effects of these radiations on the sensitive bovine sperms, the resulting defects can interfere with the integrity of individual sperm cells. In this research GSM waves of 900 megahertz have been selected (because of similarity to cell phone waves) to radiate to sperms from a certain distance (5, 6). Then sperms have been examined by Transmission Electron Microscope. By this method, probable defects especially those on ultra-structure of cell membrane of sperms will become more sensible.

We looked forward results based on our hypothesis that electromagnetic radiations may influence ultra-structure of sperm cells and subsequently these changes may be a factor for infertility. We aimed to review methods of sperm collection and places in which sperms are collected, in case of positive findings of unfavorable effects of radiations on sperms.

### Method

A healthy normal Holstein race bovine has been selected for study and a total seminal fluid of a normal ejaculation obtained. 0.5 ml undiluted fluid were put in a medium containing Gluter Aldehyde 5.5% in Phosphate Buffer Saline (PH:7.2) to later examinations by electron microscope as primary control group. To increase in volume of seminal fluid a vitelline dilution medium containing 7% Glycerol, 22% vitelline, 2.2% Sodium Citrate, and antibiotics of Streptomycin and Penicillin is used. Then diluted semen has been packed by a bovine semen straw which packed about 2x10<sup>7</sup> sperms (4). After package of semen, 5 straw of 0.5 ml have been

selected. 5 straws directly exposed to electromagnetic radiations by 900 megahertz wave long for 5 min (an average dialogue time by phone). At the end of this stage we have two groups of samples:

First control group which not being radiated (C)

Experiment group which radiated (E)

To prepare samples for electron microscope, samples of groups C2 and E were centrifuged by 4000g for 4minutes to separate from dilution media followed by several rinsing by phosphate buffer saline. 5.5% Gluther Aldehyde and 1% Tetraoxide Osmium are used to primary and secondary fixations respectively. Samples were prepared to study with Electron Microscope through standard methods of dehydration, infiltration, molting, curing and trimming. Each specimen was later cut into 1 $\mu$ m sections by use of Leica Ultra cut. In some cases more thinner slices of 50-70 nm prepared following staining by toluidine blue. Final staining is done by PB citrate and uranyl asethate and was observed by use of Transmission Elctron Microscope (Carl Zeiss EM -900;Zeiss ,Germany) at 80 KV accelerating voltage.

## Results

Ultra-structural study of samples by Transmission Electron Microscope showed changes in structure of second control and experimental groups comparing to control group.

Ultra-structural changes by electromagnetic radiation in sperms of E group comparing to group C

Cytoplasmic membrane had several thicknesses and ruptures and in some areas twisting into interior of cell. Disconnection of capsule and multiform spaces were also seen (Figures A, E)

Cytoplasm showed non uniform pattern (Figures B,C and D)

Chromatin seems to be non uniform and heterogeneous (Figures B, H)

Microtubules surroundings of central tube had multi forms of oval, circular and polyhedral as well as their thickness and distances with each other (Figures F and G)

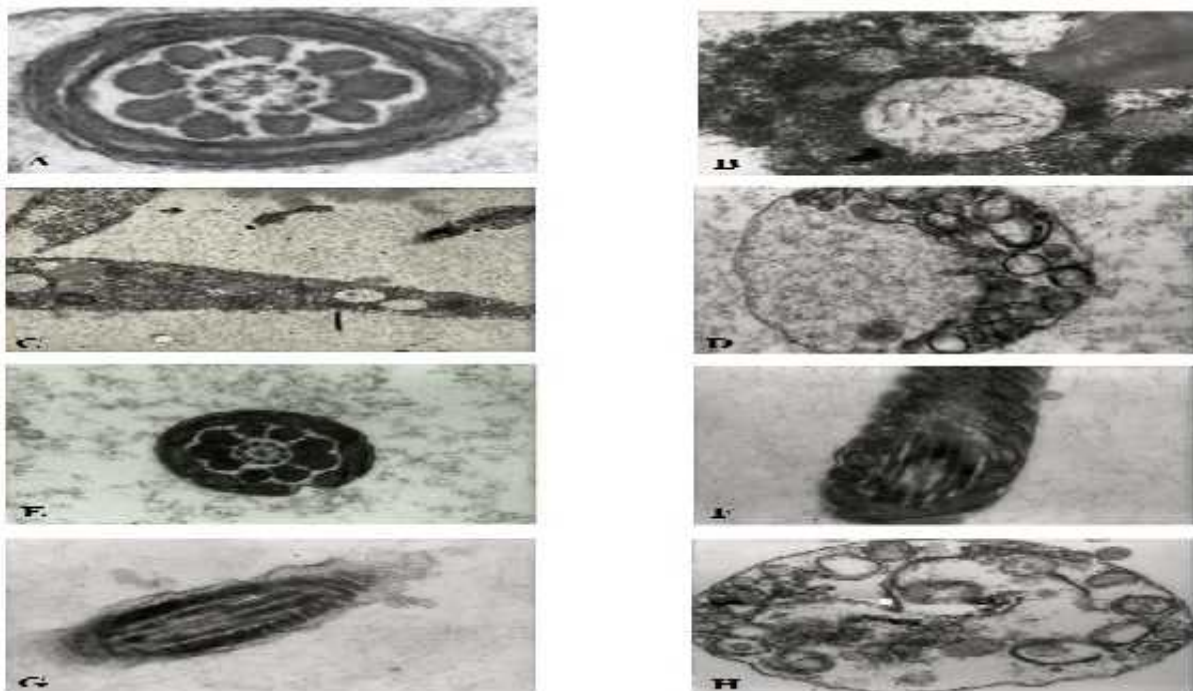
Acrosomal capsule had several interruptions. (Figure D)

Mitochondrial sheet was uneven in its thickness. (Figure H)

There were several spaces among capsule, membrane and inner elements. There were also some pyramidal bulgings to outer side (Figures B, D).

Terminal part of sperm tail are abnormal in horizontal plane (figure E)

there were obvious interruptions in communicating microfilaments of pivotal micro fibrils and central fiber in some areas (Figure F)



Figures: Ultra-structural study of samples by Transmission Electron Microscopy

## Discussion

Through the results of this study which are in accordance to studies of some previous researches (7-10), High increase in the use of communicating devices like cell phones through electromagnetic radiations has made public notifications to be attracted to discussing about divers effects of this waves (300KHz-300GHz) on living bodies and organisms. However, Essence of performing such this

study refers to researches done by Desai et al (11) and Mathie et al (12) and Ramundo et al (13) which investigated effects of the radiations on intra membrane structures like proteins and ion channels.

This study showed dis-compaction and non uniformity of intranuclear chromatin and its incongruity and heterogenic feature (figure H) that according to De Iuliis (14) and Aitken's (15) results can change the ultra structure of living tissues. This statement has been approved by Hong's studies as well (16, 17). He has reported precise effects of electromagnetic radiation on structure of chromatin and sperm of mice which our findings are in accordance to it. These changes can show the uniformity of harmful effects of electromagnetic radiations on ultra structure of live cells and DNA of male germ cells in homogenous animals (17). However there are occasionally differences between adjacent membranes in case of thickness, but our electro photographs showed over thickening of sperm cell membrane (Figures A, B, D and E).

The more detailed study of slides showed additional ruptures in cell membrane as well as departure of capsule in some area; inside twitching of membrane also was seen. In case of effect of cryopreservation, study showed following results: micro-ruptures in cell capsule, multi-shape appearances of central fiber surrounding fibers, irregular spaces among microtubules. Although Gravence got same results in a study on dimensions of bovine sperm changes through cryopreservation and cause of changes in his study differ from those of ours, but the approximately same results of both studies show fast and distinct reaction of sperm to environment factors.

Other findings of this study are disruptions seen in micro-filaments which connect pivotal micro-fibrils to central fiber. Also in some area there were pyramidal out-patchings toward outside as well as changes in mitochondrial sheath. These changes may result in failure in connecting ability of cells with each other thorough conclusions of Goldman (18).

Giving to lesser involvements of terminal part sperm tail in horizontal plane, we suggest that this part of bovine sperm is one of the resistant parts to electromagnetic waves. These results have been approved by Tablado et al and Ozguner (19, 20).

Finally we conclude that, electromagnetic radiation have harmful effects on ultra-structure of bovine sperm and is a potential factor which can result in short term dysfunction of sperm in the various stages of fertilization.

## References

1. Gye MC, Park CJ. Effect of electromagnetic field exposure on the reproductive system. *Clin Exp Reprod Med.* 2012 Mar;39(1):1-9.
2. Kumar S et al. influence of microwave exposure on fertility of male rates. *Fertil Steril* 2011;95(4),1500-2.
3. Jokela K, Puranen L, Gandhi O.P, " Radio Frequency currents induced in the human body for medium – frequency/high- frequency broadcast antennas *Health physics* , 66,1994.
4. Runa Emura, Tetsuya Takeuchi, Yasuo Nakaoka, and Terumasa Higashi. Analysis of Anisotropic Diamagnetic Susceptibility of a Bull Sperm *Bioelectromagnetics* 24:347-355 (2003)
5. Falzone, N., Huyser, C., Fourie, F., et al. (2008). In vitro effect of pulsed 900 MHz GSM radiation on mitochondrial membrane potential and motility of human spermatozoa. *Bioelectromagnetics*, 29(4), 268–276.
6. Falzone, N., Huyser, C., Fourie, F., et al. (2011). the effect of pulsed 900 MHz GSM radiation on the acrosome reaction, head morphometry and zona binding of human spermatozoa. *Int J Androl* 34(1); 20-26.
7. Ozlem Nisbet H, Nisbet C, Akar A, Cevik M, Karayigit MO. Effects of exposure to electromagnetic field (1.8/0.9 GHz) on testicular function and structure in growing rats. *Res Vet Sci.* 2012 Oct; 93(2):1001-5.
8. Thais Serzedello de Paula, Ricardo Pimenta Bertolla, Deborah Montagnini Spaine, Maria Adelaide Cunha, Nestor Schor, Effects of cryopreservation on sperm apoptotic deoxyribonucleic acid fragmentation in patients with oligozoospermia *Volume 86, Issue 3, September 2006, Pages 597-600*
9. K.R. Chohan, J.T. Griffin, D.T. Carrell. Evaluation of chromatin integrity in human sperm using acridine orange staining with different fixatives and after cryopreservation. *Andrologia*, 36 (5) (2004), pp. 321–326.
10. Kesari, K. K., Kumar, S., & Behari, J. (2011). Effects of radiofrequency electromagnetic waves exposure from cellular phone on reproductive pattern in male Wistar rats. *Applied Biochemistry and Biotechnology*, 164, 546–559.
11. Desai, N. R., Kesari, K. K., & Agarwal, A. (2009). Pathophysiology of cell phone radiation: Oxidative stress and carcinogenesis with focus on male reproductive system. *Reproductive Biology and Endocrinology*, 7, 114.
12. Mathie A, Kennard LE, Veale EL. Neuronal ion channels and their sensitivity to extremely low frequency weak electric field effects. *Radiat Protect Dosim* 2003; 106: 311-316.
13. Ramundo-Orlando A, Morbiducci U, Mossa G, D'Inzeo G. Effect of low frequency, low amplitude magnetic fields on the permeability of cationic liposomes entrapping carbonic anhydrase. I. Evidence for charged lipid involvement. *Bioelectromagnetics* 2000; 21: 491-498.
14. De Iuliis, G. N., Newey, R. J., King, B. V., et al. (2009). Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. *PLoS ONE*, 4(7), e6446.
15. Aitken RJ, Bennetts LE, Sawyer D, Wiklendt AM, King BV. Impact of radio frequency electromagnetic radiation on DNA integrity in the male germ line. *Int J Androl.* 2005; PMID: 15910543.
16. Hong R, Zhang Y, Liu Y, Weng EQ. Effects of extremely low frequency electromagnetic fields on DNA of testicular cells and sperm chromatin structure in mice. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi.* 2005 Dec;23(6):414-7.
17. MiKim YW, Kim HS, Lee JS, Kim YJ, Lee SK, Seo JN, Jung KC, Kim N, Gimm YM. Effects of 60 Hz 14 microT magnetic field on the apoptosis of testicular germ cell in mice. *Bioelectromagnetics.* 2009 Jan;30(1):66-72.
18. Goldman EE, Ellington JE, Foote RH. Reaction of fresh and frozen bull spermatozoa incubated with fresh and frozen bovine oviduct epithelial cells. *Reprod Nutr Dev.* 1998; 38:281–288.
19. Tablado L, Perez-Sanchez F, Nunez J, Nunez M, Soler C. Effects of exposure to static magnetic fields on the morphology and morphometry of mouse epididymal sperm. *Bioelectromagnetics* 1998; 19(6):377-83.
20. Ozguner, M., Koyu, A., Cesur, G., et al. (2005). Biological and morphological effects on the reproductive organs of rats after exposure to electromagnetic field. *Soudi Medical Journal*, 26(3), 405–410.