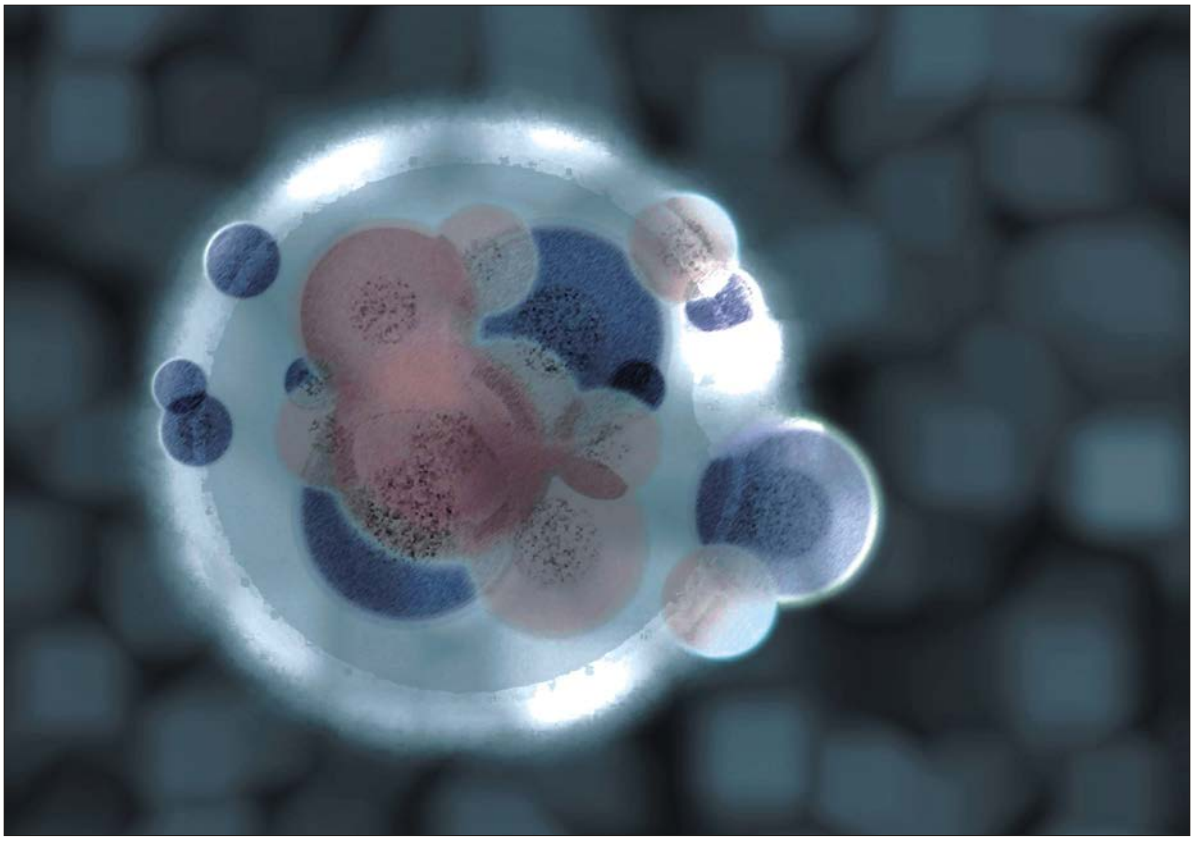

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Effect of Supplementation of Zinc on Count, Motility and *in vitro* Fertilization Capacity of Spermatozoa of Magnetic Field Exposed Rats

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Abstract: The aim of this study was to investigate the preventive effect of zinc on count, motility and fertilization capacity of rat spermatozoa that exposed to 1.5 Tesla magnetic fields. Thirty two adult male rats were subdivided randomly to 4 groups: group 1, serve as untreated controls; group 2, was exposed to the magnetic field for 30 min but received no additional treatment; groups 3 and 4, were exposed to a magnetic field for 30 min and received 200 and 500 ppm zinc sulfate oral daily, respectively. After 50 days all rats were killed and their epidydimises were removed. Then after incubation of sperm in the incubator within 37°C and 5% CO_2 for 1 h, the sperms count and motility were examined by an inverted microscope. For *in vitro* fertilization at first the sperm suspension of different groups of rats added to the freshly ovulated ova than combined sperm-oocyte suspension was incubated for 4-6 h. Sperm counts in 1 g of the epididymis were 2998.7±322.70 in group 1 and in groups 2, 3 and 4, 1022.9±128.66, 1978.4±457.79 and 2126.1±308.90, respectively. Therefore, group 2 has a significant lower sperm count in comparison with other groups ($p<0.05$). Sperm with progressive motility was 52.25±3.88 in group 1, 22.35±1.82 in the group 2, 49±26±1.66 in group 3 and 46.11±4.05 in group 4. Therefore, group 2 has a significant lower sperm motility in comparison with other groups ($p<0.05$). The same results were obtained in the case of pregnancy. The sperm count, motility and fertilization capacity may be preserving by zinc supplementation. Therefore, zinc might have the potential for usage for MRI patients as well as workers.

Key words: 1.5 Tesla magnetic fields, sperm count, sperm motility, Zn

INTRODUCTION

Infertility explains as inability of a couple to become pregnant after unprotected one year sexual intercourse. In the United States about 10% of couples are affected by infertility (Philippov *et al*., 1998). Both genders can affect by infertility. According to the American Society for Reproductive Medicine, around 33% of the time the diagnosis is due to female infertility, 33% of the time it is linked to male infertility and the rest 33% is due to a combination of factors from both partners (Philippov *et al*., 1998). A variety of stress factors such as microorganisms, hyperthermia, exposure to heavy metals (Ozawa *et al*., 2002) stressful stimuli such as prolonged immobilization (Almeida *et al*., 2000) and forced swimming (Mingoti *et al*., 2003; Saki *et al*., 2009) inhibit male reproductive functions and spermatogenesis. Magnetic Resonance Imaging (MRI) for medical diagnosis is one of the most important sources of static magnetic fields. Earlier study showed that a 30 min exposure to 1.5-T of static magnetic fields appears to have effect on spermatogenesis in mice (Chakeres and Vocht, 2005; Narra *et al*., 1996; Monfared *et al*., 2009). On the other hand, the gonads are the fastest growing tissues in the body, with zinc metalloenzymes essentially involved in nucleic acid and protein synthesis (Bedwal and Bahuguna, 1994). Zinc participates in mechanisms of the major metabolic pathways involving protein synthesis and turnover and it also has an important function in gene expression and embryogenesis (Aggett and Comerford, 1995). Zinc plays an important role in the physiology of spermatozoa, in sperm degeneration and in sperm membrane stabilization (Lewis-Jones *et al*., 1996). The present study was designed to investigate the protective effects of zinc supplementation on count, motility and *in vitro* fertilization capacity of spermatozoa of 1.5 Tesla magnetic field exposed rats.

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MATERIALS AND METHODS

Animals: This experimental study was performed in the physiology research center of Ahwaz Joundishapour University of Medical Sciences from March 2008 to August 2009. Total 32 adult male Wister rats with 3 months of age and weighing 210±10.6 g were used in this study. The animals were obtained from Laboratory Animals Care and Breeding Center of Ahwaz Joundishapour University of Medical Sciences, Ahwaz, Iran. The fertilizing ability of male mice was proven at the beginning of the experiment. All rats were randomly divided into four equal groups (n = 8); (1) control and (2) experimental groups. All animals were housed individually per cage under a 12 h light/dark cycle, 20±2°C temperature and 60-65% humidity-controlled room with food and water *ad libitum*. All procedures were approved by international guidelines and by the Institute Research Ethics and Animal Care and Use Committee of Ahwaz Joundishapour University of Medical Sciences. Every effort was made to minimize the number of animals used and their suffering.

Experimental setup: Thirty two adult male rats were subdivided randomly to 4 groups: group 1, serve as untreated controls; group 2 was exposed to an 1.5 Tesla magnetic field but received no additional treatment; groups 3 and 4, were exposed to 1.5 Tesla magnetic field but received 200 and 500 ppm zinc sulfate orally daily, respectively. After 50 days all rats were killed and their epidydimises were excised and weighed.

Static Magnetic Field (SMF) irradiation and grouping: Irradiation was carried out a 1.5 T, superconductive coil medical MRI unit of Ahwaz Joundishapour University of Medical Sciences. Rats in experimental groups were exposed in a perforated in 35×35 cm of the chamber to SMF in the core of MRI gantry at room temperature (24±1°C) for 30 min (Narra *et al*., 1996; Monfared *et al*., 2009).

Sperm motility analysis: Sperm motility of two study groups was determined using a Makler chamber. All counts were performed at 37°C in T6 media. The sperm motility was assessed and classified as progressive, no progressive. Initial sperm motility was manually assessed by a single individual in duplicate for each sample by evaluating 100 sperms. Total motility was defined as any movement of the sperm head and progressive motility was defined as the count of those spermatozoa that moved in a forward direction (Movassaghi *et al*., 2009).

Measurements of sperm count: Sperms were collected from the epididymis of each rat by flushing with the same volume (about 8 mL) of T6 medium. Collected samples were centrifuged at 100 g for 2 min and the precipitate portion was resuspended in 10 mL of fresh T6 medium. A fraction of suspension (100 μL) was mixed with an equal volume of 1% Trypan blue in the same medium and numbers of sperms were counted in four chambers of hemocytomere slide (Alvares and Story, 1984). The sperm number was expressed per milliliter of suspension.

Oocyte collection: Adult female Wistar rats that were between 10 to 12 weeks old were administered intraperitoneally with 10 IU Pregnant Mare Gonadotropine (PMG) serum for superovulation; this was followed 46-48 h later by the intraperitoneal administration of 10 IU Human Chorionic Gonadotropine (HCG). Mice were killed 12-14 h after HCG injection by cervical dislocation method. After disinfection with 70% alcohol and opening the abdomen wall, the Y shaped uterus, ovaries and oviducts were identified. The oviducts were excised as follows: clamping cornuas, dissecting the peritoneum and fat between ovary and tube and then cutting the fallopian tube from the proximal end and cumulus- oocyte complexes were collected in T6 medium. The granulosa cells of oocytes were removed by pipetting in T6 medium containing 80 IU mL^{-1} hyaluronidase and mature oocytes obtained and randomly divided into two groups (Malkov *et al*., 1998).

***In vitro* fertilization:** *In vitro* fertilization was carried out in drops of T6 medium added to 5 mg mL^{-1} BSA under mineral oil. A preincubated capacitated sperm suspension of different groups as mentioned above was gently added to the freshly ovulated ova which divided in four groups to give a final motile sperm concentration on 100000 ML^{-1}. The combined sperm-oocyte suspension was incubated for 4-6 h. The ova was then washed through several changes of a medium and finally incubated in drops of T6+5 mg mL^{-1} BSA under mineral oil. Fertilization was assessed by recording the number of 2 cell embryos 24-26 h after completion of fertilization *in vitro* (Saki *et al*., 2006).

Statistical analysis: Data are reported as the Mean±SD and percentage. The statistical significance of difference between the control and experimental groups was determined by the unpaired t-test. Differences between the means were considered to be significant when $p<0.05$ was achieved.

RESULTS

In every group of this study 8 adult male rats with 10 to 12 weeks old were used. Sperm count was 2998.7±322.70 in group 1 and in groups 2-4, the

Table 1: Sperm count in 1 g of the epididymis, different type of motility and fertilization capacity of sperm in groups of study

Groups of study	Sperm count (106)/ 1 g of epididymis	Progress motility	Non-progress motility	Immotile sperm	Fertilization capacity (%)
		----------(Mean±SD)----------			
Control group	2998.7±322.70	52.25±3.88	27.12±2.11	20.63±2.30	66.9
EMF	1022.9±128.66	22.35±1.82	28.42±5.9	49.23±3.17	20.3
EMF+200 ppm of Zn	1978.4±457.79	49.26±3.17	26.12±1.66	24.62±4.23	49.0
EMF+500 ppm of Zn	2126.1±308.90	46.11±4.05	24.12±2.43	30.57±2.36	55.4

1022.9±128.66, 1978.4±457.79 and 2126.1±308.90 values were observed, respectively (Table 1). Therefore, group 2 has a significant lower sperm count in comparison with other groups ($p<0.05$).

Table 1 shows that sperm with progressive motility was 52.25±3.88 in group 1, 22.35±1.82 in the group 2, 49±26±1.66 in group 3 and 46.11±4.05 in group 4. Therefore, group 2 has a significant lower sperm motility in comparison with other groups ($p<0.05$). In this regards no difference between other groups was encountered ($p>0.05$). In the term of non-progressive motility the data showed the values of 27.12±2.11 in group 1, 28.42±5.9 for group 2, 26.12±1.66 for group 3, 24.12 ±2.43 in group 4. Therefore, there was no significant difference between all groups ($p>0.05$).

The fertilization capacity of sperm observed values of 20.3% in group 2, 49% in group 3, 55.4% in group 4 comparing to 66.9% in group 1 (Table 1). The fertilization capacity of sperm of group 2 was significantly lower than other groups ($p>0.05$).

DISCUSSION

The present study was carried out in rats to determine the effect of administration of zinc on count, motility and fertilization capacity of spermatozoa of 1.5 Tesla magnetic field exposed rats. The results obtained in the present study clearly showed that a 1.5 Tesla magnetic field may reduce the number of sperm of rats. This finding is in agreement with the two earlier studies that reported the exposure to the 1.5 Tesla magnetic field caused a statistically significant reduction in testicular sperm on the 16th and 29th days after exposure (Narra *et al*., 1996; Monfared *et al*., 2009). This finding may be resulted from the increased incidence of testicular germ cell death. It previously reported that continuous exposure to 0.5 mili-Tesla for 8 weeks caused an increased incidence of testicular germ cell death and this finding resulted from an increased incidence of germ cell apoptosis in mice (Lee *et al*., 2004). In this study, we observed that exposure to 1.5 Tesla magnetic fields for 30 min a day caused a significant decrease in motility of sperm on day 50 after treatment. Previously, reported by Tablado *et al*. (1998) that sperm motility was not affected by 0.7 Tesla magnetic fields. The inconsistency between the above report and present study might be due to variation of field strengths. The reduced fertilization capacity of 1.5 Tesla magnetic field exposed rats is probably due to a decrease in the number and motility of sperm.

Bernabò *et al*. (2007) conducted a study to evaluate the effect of an acute exposure to a sinusoidal magnetic field (50 Hz, 1 mili-Tesla) on the ability of boar mature spermatozoa to acquire the fertilizing competence *in vitro*. As a consequence, they showed after 1 h of incubation magnetic field exposed cells displayed a reduced motility, a modest reactivity when co-incubated with solubilized zonae pellucidae and a reduction in oocyte penetrating ability. The present study observed that exposure to 1.5 Tesla magnetic fields for 30 min a day caused a significant decrease in motility of sperm on day 50 after treatment.

The protective effects of zinc supplementation on count, motility and fertilization capacity of spermatozoa of 1.5 Tesla magnetic field exposed rats investigated in the present study. Previous study has been shown that zinc is important for normal testicular development, maintenance of the germinal epithelium and motility (Favier, 1992) and also zinc administration minimized oxidative damage and reversed the impairment of spermatogenesis and testosterone production induced by cadmium in the rat testis (Amara *et al*., 2008).

CONCLUSION

As our knowledge this present study was evaluated the effect of administration of zinc on count, motility and fertilization capacity of spermatozoa of 1.5 Tesla magnetic fields exposed rats for first time. We observed that zinc treatment of 1.5 Tesla magnetic fields exposed rats significantly enhanced the sperm count, motility and fertilization capacity so zinc might have the potential for usage for Magnetic Resonance Imaging (MRI) patients as well as workers.

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