



Research Article

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## Effect of Supplementation of Zinc on Fertilization Capacity of Male Rats Exposed to Noise Stress

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### ABSTRACT

Due to harmful effects of noise stress on the fertility capacity and concentration level of sex hormones, it is trying to minimize the effects of stress. The aim of this study was to determine effects of zinc supplementation on fertilization capacity of male rats exposed to noise stress. In this experimental study 40 male Wistar rats weighing  $250 \pm 20$  grams were divided randomly into four equal groups ( $n=10$ ). In the first group or the control group, the rats were not exposed to noise stress. In the second group, the rats were exposed to noise with intensity of 90-120 dB and frequency of 300-350HZ and an ordinary one for 50 days at night times. In the groups 3&4, the rats were exposed to noise stress as mention above and received 300 and 500 ppm of zinc, respectively. After blood collection that was done in order to test hormones, in each group the male rats with the female rats of the same race was kept in a cage to perform mating. Every morning, female rats with positive vaginal plaque were removed from the cage and after 19 days they were killed and uterine horns of each rat were checked in order to count the live, the non- live and absorbed embryos. Data were analyzed using ANOVA, Tukey's and Duncan's tests and value of  $p < 0.05$  was considered as statistically significant. This study showed that the secretion of testosterone, luteinizing hormone (LH) and follicle-stimulating hormone significantly decreased in the male rats exposed to the noise stress compared with the three other groups ( $p < 0.05$ ). The number of the dead and absorbed fetuses significantly increased in rats exposed to the noise compared with the three other groups ( $p < 0.05$ ). In this study, In groups receiving the zinc as the amount of the hormones well as the number of live embryos obtained compared to the group receiving audio only, significantly increased ( $p < 0.05$ ). Due to the positive effects of zinc consumption in reducing the noise pollution damaging effects in the rat, it is recommended that other studies to be done in order to study the effect of prescription of zinc element on human samples.

**Keywords:** Noise stress, Testosterone, Luteinizing Hormone, Follicle-Stimulating Hormone , Zinc

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### INTRODUCTION

The stress is the natural reaction of the organism to external and internal stimuli that cause interruption of Homeostasis, resulting in the biophysiological and mental flow. In other words, stress is a nonspecific response of the body to any imposed force that may be the result of physical or psychological effects. Stress raises the body's immune response, such as the biological and psycho-social defense. There are several types of stress and they have negative effects on different parts of the body. Many studies are done on the impact of different stresses on sexual and reproductive hormone system, including the impact of heat stress and injectable hormones, which reduces the amount of testosterone and spermatogenesis (1).

Shifting Stress effect which means that rats are frequently moved from one room to another room, showed the increase of secretion of prolactin and growth hormone decreases,(2). Radiofrequency (RF) radiation induced stress is reportedly associated with increased sex cell death (3), and compulsory swim dramatically reduced the sperm production (4). The effect of heat stress on fertility causes impair in the spermatogenesis process, maturation of the ovule and oocyte development (5-9). There are evidences that the noise stress damages the cardiovascular, nervous, hearing, and endocrine systems (10-13). Even noise affects the insulin secretion morphology of testicular cells with the function of endocrine of testicles (14, 15). On the other hand, zinc concentration in organs such as the prostate, testis and seminal fluid is high and this reflects the role of zinc in the reproductive system that is strengthening the spermatogenesis, maturation of spermatozoa and maintaining germinal epithelium (16, 17). It is also an antioxidant element and has important and protective role against free radicals (18). According to the surveys, zinc element has an effective role in the number, motility, and fertilization capacity of spermatozoa (19).

### **Objectives**

The purpose of this study was investigating the protective effect of zinc as a vital component with close relation to the endocrine activity in order to reduce the effects of noise pollution on the level of testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and the fertility capacity of male rats.

### **MATERIALS AND METHODS**

A total of 40 male Wistar rats aged 70 days were obtained from the increasing and maintenance of laboratory animal's center of Jundishapur University, Ahvaz, Iran and after insurance of their weight ( $250 \pm 20$  g) they were divided randomly into four equal groups. Group I (control group) was not exposed to noise stress. Group II was exposed to the noise pollution with intensity of 120-90 dB and the frequency of 350-300 Hz for 50 days. The third group was exposed to the noise as same as group II and daily was given 200 ppm of zinc through drinking water, and the fourth group was exposed to same sound stress as group III and received 500 ppm zinc through drinking water daily. The rats were kept at  $25 \pm 2$  ° C temperature and light cycle of 12 hours of light and 12 hours of darkness (20).

#### **Stress method (exposing to noise)**

The cages of the study groups were placed in a room with dimensions of  $3 \times 4 \times 3$  meters which have been made soundproof with wood and acoustic components; the control group cage was transferred to a regular room. The experimental groups were divided into three groups. The first group was exposed to the noise. The second group was exposed to noise and received 200 ppm of zinc daily through drinking water. The third group was similar to the previous group, but received 500ppm of zinc daily through drinking water. In The room of the study groups a white noise machine, a sound producing device, was placed and set on the 19 o'clock on the frequency of 300-350 Hz and tendency of 90 to 120 dB (21), in a room, the for hours on, the device timer was set that after an hour of work and broadcast the sound by loudspeaker, worked off and restarted after a few minutes (from 15 to 60 minutes). This regulation can prevent any animal adaptation with stressful situations. It must to be noted that that the device change the frequency and the intensity from minimum to maximum in 2 to 3 minute period which help avoiding adaptation (22). To be insured about the amount and the intensity of the sound, a Noise level meter device was used. The device was turned on at 7pm and off at 7am and last for 50 days which is equivalent to the spermatogenesis period of rats (23). After this period, blood samples were taken from all the four groups of mice for study (20).

#### **Blood collection**

Blood samples were taken from the tail. For this purpose, after injection of 5 mg of xylazine -ketamine mixture (2.5 mg), the rats were tied and their tails were placed in a container with warm water in it and the tails were rubbed. After that the blood vessels became more distinct, blood samples were taken. Blood sample of each rat was collected in a tube containing the anticoagulant EDTA. After the completion of taking blood samples the samples were centrifuged by 3000 rpm for 15 to 12 minutes and separated blood serum transferred to another tube then concentration level of FSH, LH and Testosterone was measured by ELISA method and using test kits (Merk Company, Germany) (24, 25).

#### **Methods of measuring hormone level**

To measure the amount of testosterone hormone first, 25  $\mu$ L of the standard solution and the sample were poured into each cavity of the test kit plate and then 200  $\mu$ L of enzyme conjugate was added. After 10 seconds of mixing, it was incubated for 60 min at room temperature and then the cavities were emptied and washed three times to remove all the Sediments were washed thoroughly. In this phase, the 200  $\mu$ L of standard solution was added, after 15 min incubation 100  $\mu$ L of stop solution was added to stop the activities. Then  $450 \pm 10$  nm sorbent materials were added to each. After 10 minutes, the amount of each hormone was read by READER machine. To measure FSH levels, 25  $\mu$ L of calibrator (standard) and 25  $\mu$ L of the samples were poured into all cavities of test kit plate except a blank

hole, then 15 minutes of incubation and 100  $\mu\text{L}$  of conjugating enzyme added to all cavities except the BLANK hole after 30 minutes incubation the contents were discarded and washed with 200  $\mu\text{L}$  distilled water. Then 100  $\mu\text{L}$  of the substrate Solutions was added to each cavity and after 10 minutes each cavity was washed with 100  $\mu\text{L}$  stop solution. Measurement of LH and FSH levels were similar, with the difference that in the phase when calibrator and the sample were added cavity, incubation time was 10 minutes. The other difference was that adding conjugate was repeated 4 times with 300  $\mu\text{L}$  of distilled water and after 30 minutes and when color changing distinguished in the cavities it is read by reader machine.

#### **Mating and check the resulting embryos**

Each group of male rats was placed in a cage with female rats in a ratio of 2: 1 (two females and one male). Every day the female rats with positive vaginal plug were detected and have been isolated in a separate cage (16). After 19 days (of 21 days of the female pregnancy period) before the delivery is made, the rats were anesthetized using ether and then by dislocation of the spine (Cervical dislocation) were killed. Then the rats back marinated and the skin pulled up in the lower part of the abdomen and cut to right and left respectively, with scissors in a way that intra-abdominal organs were determined. Then, the uterus was removed and the fetus in each uterus was studied. To weight the fetus, a certain amount of water was poured into a beaker and it was weighed. Then a fetus was put in the water and it was weighed again, fetus weigh came out from the comparing between the two obtained numbers. The yellow spots with no fetus in it indicate the absorbed fetus.

**Data Analysis.** The data were analyzed using ANOVA and Tukey and Duncan tests and  $P < 0.05$  was considered as significant level of the test.

### **RESULTS**

The mean and standard deviation (SD) of testosterone hormone in the control group and the noise stress group were  $13.12 \pm 0.39$  and  $10.16 \pm 0.74$ , respectively; in addition, in the two groups receiving 200 ppm of zinc it was  $10.10 \pm 0.21$  and in the other group that received 500 ppm of zinc, it was  $11.09 \pm 0.47$ . The results showed that in all the three groups which exposed to noise stress the testosterone hormone levels significantly decreased in compare to control group ( $P < 0.03$ ). The testosterone levels in the two groups that receiving zinc increased in comparison to the group that was exposed to noise stress but did not receive zinc and differences were significant ( $P < 0.01$ ). Comparisons between the two groups which received zinc showed that the difference is not significant. In the control group and in the group exposed to noise stress and the groups that received 200ppm and 500ppm of zinc the mean and SD of LH hormone were  $23.59 \pm 1.27$ ,  $12.17 \pm 1.23$ ,  $17.37 \pm 1.68$  and  $21.09 \pm 1.97$ , respectively. The statistical analysis showed that in the three groups that were exposed to noise stress the hormone level significantly decreased ( $P < 0.004$ ) compared to control group. The LH hormone levels in the two groups which received zinc increased compared to the group that was exposed to noise stress, but did not receive zinc, the difference was significant ( $P < 0.002$ ). Comparison between the two groups which received zinc showed that the difference between the two groups is significant ( $P < 0.001$ ). The mean and SD of FSH hormone in the control group and the group that was exposed to noise stress were  $19.56 \pm 1.04$  and  $10.47 \pm 1.14$ , respectively. Also in the group which received 200 ppm of zinc it was  $11.35 \pm 1.01$  and in the group that received 5000 ppm of it was  $17.19 \pm 1.09$ , respectively. The results showed that in the three groups which were exposed to noise stress the LH hormone levels decreased significantly in comparison to the control group ( $P < 0.006$ ). The LH hormone levels in the two groups which received zinc were increased significantly compared to its level in group that were exposed to noise stress, but did not receive zinc ( $P < 0.01$ ). The comparison between the two groups showed that the difference between the two groups which received zinc was significant ( $P < 0.003$ ).

**Table1. Mean  $\pm$ SD of concentration of testosterone, FSH and LH in male rats**

Concentration of hormones Groups	Testosterone	LH	FSH
Control group	$15.12 \pm 0.39$	$23.59 \pm 1.27$	$19.56 \pm 1.04$
Exposed to noise stress	$7.16 \pm 0.74$	$12.17 \pm 1.23$	$10.47 \pm 1.14$
Exposed to noise stress +200ppm	$10.10 \pm 0.21$	$17.7 \pm 1.68$	$11.35 \pm 1.01$
exposed to noise stress +500ppm	$11.09 \pm 0.47$	$21.09 \pm 1.97$	$17.19 \pm 1.09$

The pregnancy rate in female who mated with male exposed to noise was significantly decreased in compared to control group (35% VS 85%) ( $P < 0.001$ ) and the fertilization capacity of male rat in groups that received zinc, significantly increased compared to that group that was exposed to noise stress, but did not receive zinc ( $P < 0.008$ ) (Table 2). The difference in fertility capacity between the two groups that received zinc, was significant and it was

higher in that group that received 500 ppm of zinc ( $P < 0.03$ ). The study results of the fetuses from the pregnancy on the 19th day of pregnancy period are as follows:

Comparison of the total number of live fetuses resulted from female rats' pregnancy by male rats in four groups in this study:

The mean and SD of the number of lived fetuses obtained from each rat in each uterus of control and exposed to the noise stress groups were  $6.4 \pm 0.5$  and  $3.1 \pm 0.3$ , respectively. Statistical analysis showed that the differences between two groups of study was significant ( $P < 0.001$ ).

The number obtained lived fetuses from females mated with male rats that received 200ppm zinc was  $4.7 \pm 0.4$  and in group that received 500 ppm of zinc, was  $5.3 \pm 0.5$ . The results showed that consuming zinc significantly increased the amount of live fetuses obtained in groups exposed to noise stress ( $P < 0.003$ ). There were no significant differences between the two groups that received zinc.

The mean and SD of number of dead fetuses from each female rat mated with male rats in the control and exposed to noise stress groups are respectively  $0.11 \pm 0.3$  and  $0.4 \pm 0.57$ . The number of dead fetuses significantly increased in uterus of female mated to male rat exposed to noise ( $P < 0.004$ ) but this difference was not significant between the groups received zinc and the control group.

The mean and SD of weight of fetuses resulting from mating of female rats with male rats in the control group was  $6.2 \pm 2.2$ , in exposed to noised stress was  $3.1 \pm 0.3$  and the groups those exposed to noise stress and received 200 ppm and 500 ppm of zinc were  $5.5 \pm 1.9$ ,  $5.9 \pm 1.6$  grams respectively.

The results of the study showed that the weight of fetuses resulting from mating of female rats with male rats exposed noise stress is reduced significantly compared to the control group ( $P < 0.01$ ); although, the difference of the weight of the fetuses resulting from mating of female rats with male rats received zinc compared to those in the control group is not significant.

The mean and SD of absorbed fetuses in female rats mated with the control group and experimental groups exposed to the noise stress were  $0.17 \pm 0.2$  and  $2.1 \pm 0.3$ , respectively. There was a significant difference between the control group and the group exposed to the noise stress ( $P < 0.003$ ). The difference between the groups that received two different concentrations of zinc and the control group was not significant.

**Table2. Results of the fetuses from the pregnancy in four groups of study**

	Control	Noise stress	200 ppm zn	500 ppm zn
Number of Male rats	10	10	10	10
Number of Female rats	20	20	20	20
Pregnancy rat	17(85%)	7(35%)	13(65%)	15(75%)
Total number of live fetuses	110	22	62	80
Fetuses per uterus (Mean $\pm$ SD)	( $6.4 \pm 0.5$ )	( $3.1 \pm 0.3$ )	( $4.7 \pm 0.7$ )	( $5.3 \pm 0.5$ )
Body weight (gram) (Mean $\pm$ SD)	$6.2 \pm 2.2$	$3.1 \pm 1.3$	$5.5 \pm 1.9$	$5.9 \pm 1.6$
Total number of dead fetuses	2	4	0	0
Dead fetuses per uterus (Mean $\pm$ SD)	$0.11 \pm 0.3$	$0.57 \pm 0.4$	$0.15 \pm 0.6$	0
Total number of absorb fetuses	3	15	2	3
Absorb fetuses per uterus (Mean $\pm$ SD)	$0.17 \pm 0.2$	$2.1 \pm 0.3$	$0.15 \pm 0.1$	$0.20 \pm 0.3$

## DISCUSSION

Results obtained from this study show that the amount of testosterone, FSH, LH hormones in the groups exposed to noise stress for 50 days with an intensity of 90 to 120 dB and frequency of 300-350 Hz decreased compared to control group in a significant way. A major part of sexual activity in men is controlled by the secretion of GnRH gonadotropin by hypothalamus. This hormone, in its turn stimulates the anterior pituitary gland to secrete of another

two hormones called, FSH, LH. The LH hormone or luteinizing hormone is the main stimulus for the secretion of testosterone from the testis and FSH hormone or follicle-stimulating hormone mainly stimulates spermatogenesis. Previous studies have shown that the use of the traffic noise with an intensity of 100 dB had a significant decrease in the level of testosterone in male albino rats ranges 200-250 grams. In this study, the histological study (histology) of Leydig cells also has been made and it has found that the cells are exposed to the noise their reactivity decrease extremely and this means that even if the LH amount decreases the testosterone levels again decrease (25). The similar results obtained in the present study could be for this reason. In another study conducted on Leydig cells, it was found that rats exposed to noise in chronic case Halts the maturation of gametes and testosterone reduction and on the one hand, to compensate for this increase in the number of Leydig cells with a compensatory mechanism to increase testicular steroid is done through testosterone failure (26). It is known that the hypothalamic - pituitary-adrenal axis is activated during stress and glucocorticoid secretion is reduced in Leydig cells. The decrease of testosterone level in this study by being exposed to the noise, which is considered as a kind of stress, is same with results obtained from the lack of transportation and LH hormone levels in this study were not changed but testosterone levels reduced as much as 82% (26). The results of this study about testosterone hormone are similar to the results the present study, but there is a difference in results of LH hormone. The Babino and Hsueh's research (1981) was compatible with the results of a previous study (27). Other studies have found that a reduction in testosterone levels is seen during any period of noise stress. These results are compatible with the results of the present study and through observing and the study of the sex cells, it turned out that the increased mortality in these cells, the cells are clearly visible (28-30). The results of this study in testosterone hormone are similar to the results of the present study. Studies show that a decrease in testosterone levels in rats exposed to noise stress also had a significant and obvious decrease in epididymal sperm count, (25). In addition, histological studies have identified that epididymal sperms in a group of rats who were chronically affected by noise, were agglutinated and the number of dead spermatozoa increased and maturation was blocked in sexual cells (30). In another study conducted in 1984, it has been revealed that the noise stress increases the amount of testosterone serum in the adult male Wistar rats which is not compatible to result of the present study. The study also has found that the chronic noise stress along with the light do not damage the function of the testes planning in rats. Probably the light caused this protective effect (31). But according to this study the noise reduces the amount of testosterone. In another study the increase in cortisol hormone in exposure to the noise stress with 40 dB tendency and frequency of 1100 Hz during 90 days has been proved. Considering that in industrialized societies usually a combination of pollution and stress, including air pollution, noise pollution, everyday stress, *etc.* increases cortisol, this study specifically and dedicatedly prove the increasing of cortisol due to the noise pollution (23). According to other research, the noise stress has an effect on LH hormone. According to the results of this research, which was done in 1984, the effect of acute noise stress on LH hormone is an increase in this hormone, but noise chronic stress effectively is a decrease in the hormone level at first, but after a while there was no change compared to normal. These studies were performed on the FSH hormone that the results of the hormone after being exposed to the sound are that FSH was not affected by stressing factor, the other hormones examined in this study were corticosterone and growth hormone and the results were similar to results of LH. In the case of LH and FSH, results are inconsistent with our results.

Which can be due to the time of the stress (15)?

On the other hand, several studies have been conducted on the role of zinc on spermatogenesis. In a study by Krishan *et al.* in 1998 about the protective role of zinc on spermatogenesis in rats it has been shown that the use of Asparaginase of zinc can protect spermatogonial cells against cell death induced by radiation (32). In another study conducted by Machian *et al.*, it has shown that the zinc consumption can prevent damage to the testicles and toxicity caused by chromium (33), or in another study conducted by Olivera *et al.* it showed that the zinc is a crucial element for maintaining properties of the sperm (18). In another study it has shown that zinc has an essential role in maintaining the health of sperm and it preserves the structure of the sperm and the chromatin of the core, increases the activity and forward moving of the sperm and its deficiency causes loss of impairment of acrosome reaction and fertilization of the Ovule (34). In another study it was found that zinc can reduce the harmful effects of electromagnetic radiation, so that after the administration of 200 and 500 ppm of zinc sulfate orally to rats, it was found that both the number and forward movement improved considerably (35). The results obtained from this study showed that the consumption of zinc by male rats significantly increases their fertility capacity compared to the rat group that exposed to sound. The number and weight of viable embryos derived from female rats mated with male rats that received zinc significantly increased compared to the group exposed to the sound. The study clearly showed that zinc consumption reduces the number of dead and even the absorbed fetuses. According to studies conducted in this field, another type of stress in the male rat, called forced swimming stress, has reduced the number of live fetuses and their weight and increased the number of dead and absorbed fetuses that is similar the results of the present study (19). Previous studies have shown the negative effects of stress hormones on sexual hormones, sexual behavior and also affects semen quality (35). In another study it was found that immobilization stress (non-moving) reduces the size and weight of the testes and similarly, impaired spermatogenesis and cell division (36). The results

of this study could be the reason related to the present research. In another study it was found that adult animals that are frequently exposed to immobility stress have low rates of fertilization and implantation and this state can be seen even in their offspring (37). Based on conducted studies this situation is probably happen when an ovule makes fertilization with a damaged and weak sperm, (35, 38, 39). Another important point is that infant mortality among those who were in stressful environment is far more and similarly, the absorption of the fetus before birth in the groups that exposed to stress, was obvious. If these results are confirmed in further studies the noise should be taken into account as a dangerous environmental pathogen factor. Because one of the important criteria in the survey of the development of a national is the rate of maternal and infant mortality it should also pay particular attention to this topic. One of the most important factors that could cause infant mortality is the low birth weight, which includes about 12 to 15 percent of births that their mortality risk in this period is three times more than the children with normal weight and also increases their vulnerability to the disease and its incidence. The present study suggests that the infants who grown and born in noisy environments are significantly less than those who grown and born in the natural environment. These results are compatible and similar to the results of another study conducted by Sarkaki (20). In another study conducted in 2000 the effect of noise pollution on pregnancy was assessed, and specified that the exposure of both sexes to the noise increased the risk of maternal death which is compatible with the present results. Similarly, the study found that the weight and the number of infants resulted from the group, which was exposed to the noise reduced compared to the control group. But these results are inconsistent with the present study (24). Another study conducted in the field of sound, have studied the impact of noise on the rate of twinning and it is known that twinning rates are higher in rats that were exposed to noise compared to mice that have grown in a quiet environment and that is a reason for the decrease in birth weight (20). This study has shown that the noise has an effect in the fertility rate, a percentage of twinning, maternal mortality, infant mortality, and low birth weight in mice.

Finally, it can be deduced that according to the very good effect of zinc consumption in reducing the detrimental effects of noise pollution in rats, it is suggested that in order to investigate the effect of zinc on human specimens, other studies to be designed.

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#### AUTHOR'S CONTRIBUTION

All authors have equally contributed for the design, conducting, and manuscript preparation of the study.

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