Effect of administration of vitamins C and E on fertilization capacity of rats exposed to noise stress

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Abstract

The aims of this study were to evaluate the effects of administration of Vitamins C and E on fertilization capacity in rats exposed to noise stress. 40 adult male rats were randomly divided into 5 equal groups. Group 1 as controls who were not exposed to noise and groups 2-5 exposed to noise with 90-120 dB intensity and 300-350 Hz frequency from 7 pm to 7 am everyday for 50 days. Group 2 exposed to noise and did not receive Vitamins. Group 3 received vitamin C, Group 4 received Vitamin E. Group 5 received Vitamins C and E concomitantly. After 50 days, serum Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) and testosterone were calculated. Then each rat was left with three female rats for mating. Pregnant females were sacrificed on the 19th day of pregnancy and evaluated for the presence and number of viable, dead and absorbed fetuses. The level of FSH, LH and testosterone significantly decreased in rats exposed to noise ($P < 0.05$). By administration of Vitamins in groups 3-5 we observed that the level of hormones significantly increased in compared to group 2 ($P < 0.05$). The fertilization capacity of male rats in groups 3-5 significantly increased in compared to group 2 ($P < 0.05$). There was significant difference between groups 1 and 2 in case of fertilization capacity ($P = 0.001$). The data in this study strongly suggests a negative role for noise stress on level of FSH, LH and testosterone level and also fertilization capacity of male rats. To complement the information it is suggested that this research be done on human samples.

Keywords: Antioxidants, infertility, mortality, pregnancy

Introduction

Infertility cases are about 20% due entirely to male factors, with an additional 30-40% of cases related to both male and female factors.[1,2] One of the most important aims is the evaluation of the infertile man to recognize reversible conditions, which are responsible for infertility. Exposure to chemical toxicants, stress, heat and cigarette smoking may cause infertility.[3,4] On the other hand, regarding to various effects of noise on different systems, the exact effect of this stress on fertility is yet to be elucidated. Geber had mentioned that the terato-genic action of noise is primarily the result of decreased utero-placental blood flow resulting in fetal hypoxia and increased secretion of maternal catecholamine.[5,6] and he noticed that in pregnant rats exposed to noise stress the litter size and the number of resorption per litter significantly have been reduced.[5] Previous study had shown that the stress like noise and forced swimming stress caused decreased in sperm count and motility and increased pre-implantation mortality, decreased litter size, embryo size and weight among the exposed offspring.[7-11] However, the data are inconsistent among the various experimental conditions.[12-16] As we know the antioxidants are the main defense against oxidative stress induced by free radicals. There are prevention antioxidants such as metal chelators and metal-binding proteins block the formation of new free radicals and scavenger antioxidants, which remove the free radicals that have already formed.[17] Oxidative stress may be limited by using chain-breaking antioxidants such as vitamin E and vitamin C as drug supplements.[18] More specifically speaking, these vitamins have been shown to have protective effect on testis and fertility.[4,19] This study was design to evaluate the effect of administration of vitamin C and vitamin E on fertilization capacity of rats exposed to noise stress.

Access this article online	
Quick Response Code:	Website: www.noiseandhealth.org
	DOI: 10.4103/1463-1741.112374

Methods

Animals

This experimental study was performed in the physiology research center from September 2010 to December 2010.

Thirty-two male rats of the Wistar strain weighing 220 ± 20 g were used for the experiments. The animals were acclimated to 22 ± 1°C and maintained under conditions of 12-h periods of light and dark, with free access to tap water and commercial rat food. All procedures were approved by international guidelines and by the Institute Research Ethics and Animal Care and use committee of Ahvaz Jundishapur University of Medical Sciences. Every effort was made to minimize the number of animals used and their suffering.

Experimental design

Forty adult male rats were randomly divided into 5 equal groups. Group 1 as control were not exposed to noise and groups 2-5 exposed to noise with 90-120 dB intensity and 300-350 Hz frequency from 7 pm to 7 am every day for 50 days. Group 2 exposed to noise and did not receive Vitamins. Group 3 received vitamin C (125 mg/kg/day), group 4 received vitamin E (75 mg/kg/day). Group 5 received Vitamins C and E concomitantly.[4] For this to be done, these groups were transported to the room which had dimensions of 3 × 4 × 3 meter and was lagged by wood and acoustic segments (anti-loud voice). In this room, the set of WHITE NOISE which produce noise was prepared at 19o'clock in the case of the frequency of 300-350Hz and intensity of 90-120dB.[20] and the set timer was arranged in a way that after 1 h of operation and producing noise by the speaker, it would turn off for a few minutes (From 15 min to 60 min), before it operates again. It has to be mentioned that, this would prevent animal's compatibility. The noise producing device changed the intensity and frequency of noise produced automatically in the district of minimum and maximum every 2-3 min, and this also aided in non-compatibility.[21] For evaluating the amount and intensity of noise, we used noise level meter and thus rate and intensity was controlled in this way.[22] Group 2 received vitamin C. Group 3 received vitamin E (75 mg/kg/day) Group 4 received Vitamins C and E concomitantly. After 50 days, blood sample was drawn from each rat's tail and sent to laboratory for serum Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) and testosterone measurement. These determinations were done with Elisa technique. After that time, each rat was left with three female rats for mating. Every morning, females with positive vaginal plaques were identified and separated. Pregnant females were killed on the 19th day of pregnancy by chloroform inhalation. Their uteri were then evaluated with regard to the presence and number of viable, dead and absorbed fetuses. To weigh the live embryos, a certain amount of water was poured and weighted then a fetus was spilled into the water. Finally, the weight difference of the two numbers, birth weight was achieved.

Statistical analysis

Data are reported as mean ± SD and percentage. The statistical significance of difference between the control and other groups was determined by ANOVA test for hormonal studies. Comparison of pregnancy rate and the number of dead/absorbed fetuses were determined by Chi-square test. Differences between the means were considered to be significant when $P < 0.05$ was achieved.

Results

The concentrations of FSH, LH and testosterone in plasma of male rates exposed/non-exposed to noise stress showed in Table 1. We observed that the levels of FSH in non-exposed to noise rats is 21.56 ± 1.07 and in exposed to noise rats is 10.48 ± 1.14. Statistical analysis showed that the difference between two groups was significant ($P < 0.05$). The concentrations of FSH in groups 3-5 were 18.12 ± 1.23, 17.46 ± 1.47 and 19.66 ± 1.92 respectively. The differences between groups 3 and5 were not significant ($P > 0.05$) but in compared to group 2 the differences were significant ($P < 0.05$).Levels of LH were 24.37 ± 1.27, 12.16 ± 1.23, 20.61 ± 1.07, 19.19 ± 1.11 and 18.90 ± 1.07 in groups 1 to 5 respectively. The level of LH in group 2 were lowest in compared to control and groups 3 to 5 ($P < 0.05$). The differences between groups 3 and 5 were not significant ($P < 0.05$). As shown in Table 1, the levels of testosterone in groups 3-5 significantly decreased in compared to control and groups 5 ($P < 0.05$). There was no statistical difference between the two groups (Control and group 5) in terms of testosterone level ($P = 0.07$).

As shown in Table 2, the pregnancy rate in female mated with control and noise exposed male was 21:24 (87.5%) and 6:24 (25%) respectively. Statistical analysis showed the significant differences between two groups ($P < 0.05$).Fertilization capacity of rats receiving Vitamins significantly increased in compared to non-treated group ($P < 0.05$). Present data showed that in the group treated concomitance with Vitamins C and E significantly increased in case of fertilization capacity ($P < 0.05$).The number of corpora lutea and also live fetuses in uterus of female mated with noise exposed males significantly decreased ($P < 0.05$).The body weight of fetus and number of dead fetus significantly decreased and resorption sites significantly increased in group 2 ($P > 0.05$).

Table 1: Plasma level of Follicle-stimulating hormone, Luteinizing hormone and testosterone hormones in different groups of study

Animal study	FSH (IU/I)	LH (IU/I)	Testosterone (*N*mol/l)
Variable			
Group 1 (control)	21.56±1.07	24.37±1.27	16.16±0.49
Group 2 (exposed to noise)	10.48±1.14[a]	12.16±1.23[a]	7.39±0.74[a]
Group 3 (noise+VitC)	18.12±1.23[b]	20.61±1.07[b]	8.56±0.77[a]
Group 4 (noise+VitE)	17.46±1.47[b]	19.19±1.11[b]	13.41±0.49[abc]
Group 5 (noise+VitC+VitE)	19.66±1.92[b]	18.90±1.07[b]	16.32±0.098[bcd]

[a]Significant in compared to control, [b]Significant in compared to group 2, [c]Significant in compared to group 3, [d]Significant in compared to group 4, FSH = Follicle-stimulating hormone, LH = Luteinizing hormone

Table 2: Fertilization capacity and subsequent off spring quality in different groups of study

Variable	Group 1 (control)	Group 2 (exposed to noise)	Group 3 (noise+VitC)	Group 4 (noise+VitE)	Group 5 (noise+VitC+Vit E)
Animal study					
Number of female rats who mated with male rat	24	24	24	24	24
Pregnant female (%)	21 (87.5)	6 (25)[a]	15 (62.5)[b]	16 (66.6)[b]	18 (75)[bcd]
Corpora lutea (total)	157	32	107	118	127
(Mean±SD) in rat	7.47±1.99	5.33±1.01[a]	7.13±1.15[b]	7.37±1.09[b]	7.05±1.21[b]
Live fetuses (total)	132	23	85	90	92
(Mean±SD) per rat	6.28±1.01	3. 83±0.9[a]	5.66±1.33[b]	6.28±1.01[b]	6.28±1.01[b]
Body weight (gram)	5.4±2.2	3.1±1.9[a]	4.1±1.7[b]	4.7±2.1[b]	5.2±1.09[bcd]
Dead fetuses (total)	5	5	4	4	4
(Mean±SD) per rat	0.23±0.01	0.83±1.09[a]	0.26±0.08[b]	0.25±0.02[b]	0.22±0.07[b]
Resorption sites (total)	7	10	6	5	7
(Mean±SD) per rat	0.33±0.01	1.66±1.01[a]	0.40±0.09[b]	0.31±0.04[b]	0. 38±0.07[b]

[a]Significant in compared to control, [b]Significant in compared to group 2, [c]Significant in compared to group 3, [d]Significant in compared to group 4

Discussion

Noise decreased serum testosterone level in our study, but supplementing rats with either vitamin C or E or the combination of both resulted in testosterone turning back to the normal range. This result is similar to a study by previous studies in which male albino rats were exposed to 100 dB of noise for 1 h and 3 h in acute group and daily 1 h exposure for 60 days, and 90 days in the chronic group. In that study, they could also show significant reduction in serum testosterone.[23,24] However, interestingly, acute noise stress of 80 dB increased the testosterone level. There is a decrease in testosterone secretion in male rats exposed to immobilization-induced stress, forced swimming stress, heat exposure-induces stress.[25-28] In this study, noise increased serum FSH levels. Adding vitamin E or Vitamins C and E to their regimen, noise exposed rats were protected from noise's effect on FSH level. On the other hand, vitamin C alone could not compensate for negative effect of noise stress.

Serum LH level was also affected negatively by noise to an extent that vitamin C or E could not reverse the effect. However, the combination of Vitamins C and E had a protective effect against noise stress. In another study by Kimmel *et al*., pregnant mice were exposed to 100 dB of noise on days 3-6, 7-10, or 11-14 of gestation.[12] Significantly increased resorption rates and decreased number of live fetuses per litter were observed in each of the treated groups of animals. We also reached the same results as the number of pregnant rats containing any number of dead and/or absorbed fetuses was higher in those which had mated with noise exposed male rats. In accordance with this finding Saki *et al*. (2010) previously reported that the forced swimming stress had a harmful effect on fertilization capacity of rat and subsequent offspring quality.[10] Moreover, in females mating with noise expose males who had received Vitamins either alone or in combination, this untoward occurrence was corrected. Nawrot and colleague[13] exposed mated female mice to either semi-continuous 126 dB low-frequency noise, intermittent 110 dB mid-frequency noise, or semi-continuous, very high frequency (18-20 kHz) 113 dB noise on days 1-6 or 6-15 of gestation. Significantly increased embryo and fetal mortality, decreased fetal weight, and decreased pregnancy rate were reported among the exposed animals. Noise's negative effect was also reported by cosa and co-worker.[14] They showed abnormalities in reproduction and a significant decrease in pregnancy rate and lethal effect in mice embryos exposed to high frequencies of noise Once again in our study and on assessing pregnancy rate, overall, there was not any significant difference; but when only rats in control group were compared with noise exposed rats the difference became meaningful. This is in line with previous results.

In a follow-up study, Nawrot *et al*.[29] exposed mated female mice to high-frequency 110 dB noise on days 6-15 of gestation. Decreased pregnancy rate and mean fetal weight and increased fetal mortality were observed among the exposed animals. In contrast, we did find noise stress to be predictable of increased occurrence of abnormal pregnancy outcome defined as fetal resorption and/or death. On the other hand, vitamin supplementation could potentially prevent them. One might translate this difference as more teratogenic or detrimental effect of noise stress on male fertility rather than on female reproductively.

Vitamin C neutralizes hydroxyl, superoxide, and hydrogen peroxide radicals and prevents sperm agglutination.[18] In addition, it also helps recycle vitamin E.[30] Lewis *et al*.[31] found vitamin C in reduced quantityin the seminal plasma of infertile men .In a study by Dawson *et al*.[32] vitamin C increased sperm counts *in vivo* in infertile malepatients. Akmal *et al*.[32] showed that vitamin C supplementation in infertile men might improve sperm count, sperm motility, and sperm morphology. Moving along with previous studies, we demonstrated that vitamin C had a protective effect on fertility rate and fetal abortion and death; however, and of interest was that its effect on FSH and LH was not significant.

In a randomized cross-over study, vitamin E improved sperm function as assessed by the zona binding test.[33] In our study,

vitamin E could avert the negative effect of noise regarding pregnancy rate and the rate of fetal death and abortion. Like vitamin C, it had no protective effect on the level of LH, but FSH level was improved. Likewise, combination of vitamin C and E made pregnancy rate and the rate of fetal abortion and death normal. Of importance was that the combination rendered FSH and LH levels normal. However, according to a literature review by Agarwal *et al*.[18] many studies have failed to examine the effect of antioxidants on a specific group of infertile patients with high oxidative stress. Lastly, we did not perform a histological evaluation, but taking into account the huge changes in hormonal milieu it may be conferred that noise would cause structural changes leading to abnormalities in FSH, LH and testosterone levels. Our data is in contrast with a study by Gunther[34] who examined the effect of noise on the fertility of 21 male Guinea pigs. Sounding of 110 dB lasted 3-5 h daily over a period of 22-31 days. Histological examination of the testicular tissue did not show any disorder of spermatogenesis. Nevertheless, he believed that the negative result was attributed to failings in the test arrangement. Sounding only lasted for a maximum of 31 days. However, the duration of spermatogenesis in Guinea pigs is about 40 days. Moreover, the sounding maximum was only 5 h per day, yet the time of recovery took 4-5 times longer every day. Consequently, the daily short-term depression of the neuroendrocrinium affected the gonadotropin secretion in similarity to the daily biorhythmic variation. Therefore the expected inhibition of fertility by the neuroendrocrinic way could not be demonstrated.[34]

Conclusion

The data in this study strongly suggests a negative role for noise stress on fertility of the male rats. These results, however, should be further assessed on humans (e.g., factory workers, those living near airports, etc.). It is also recommended that the effect of noise on testicular histology be evaluated in other studies.

Acknowledgments

This paper is issued from thesis of Ali Fathollahi and the financial support was provided by Ahvaz Jundishapur University of Medical Sciences.

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How to cite this article: Saki G, Jasemi M, Sarkaki AR, Fathollahi A. Effect of administration of vitamins C and E on fertilization capacity of rats exposed to noise stress. Noise Health 2013;15:194-8.

Source of Support: Ahvaz Jundishapur University of Medical Sciences, **Conflict of Interest:** None declared.