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Original Article

## Effect of noise stress on in-vivo fertilization capacity of male rats and subsequent offspring quality

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### Abstract

**Objective:** The present experimental study was designed to investigate the elucidative effect of 50 days of noise stress on fertilization capacity of rat and subsequent offspring quality.

**Material and Methods:** Two groups of 10 male rats were placed in two control and experimental groups for 50 days. In the experimental group, 90-120 dB sound intensity and frequency of 300-350 Hz of the device (daily at 7 pm to 7 am) was broadcasting. After this period, the male rats of both groups were exposed to the same breed female rats in a cage than 1:2 for mating. Every morning for seven days the female rats with positive vaginal plaque were separated and after 19 days were killed by cervical dislocation method then uterus and ovaries were examined for the number of corpora lutea, dead and live fetuses, embryo resorption, implantation sites and fetus weight. **Results:** Statistical studies showed that the noise stress significantly reduced the total number of embryos and alive embryos in the test group ( $p < 0.05$ ). As the number of dead embryos and embryos that are absorbed significantly are increased in rats which exposed to noise stress ( $p < 0.05$ ).

**Conclusion:** Results of this study have important implications for families attempting pregnancy. Stress pursuant to life events may have a negative impact on in vivo fertilization capacity of male rats and subsequent offspring quality.

**Key words:** Noise pollution; Stress; Embryo; Infertility

### Introduction

Stress is the natural reaction of the body against the effects of inside and outside stimuli that cause knock life balance, leading to flows of physiological and psychological stress. In other words, the stress is non-specific response to any body force imposed, which may result in mentally or physically effects. The body's stress response is sparking the defense as the defense of biological, psychological and social defense. There are different types of stress which have negative effects on the different body parts. Many studies have been conducted on the effect of stress on the sex hormonal system and reproductive system, including the impact of heat stress and hormone injection, which reduce testosterone and spermatogenesis levels<sup>1</sup>. The effect of movement stress, (meaning that animals over and over were carried from a room to another room) it has been

shown that prolactin secretion is high, GH is low and FSH & LH responds are more complex<sup>2</sup>. Previous study showed that the density stress (meaning that a large number of animals are kept in a cage) on leydig cell function was ineffective<sup>3</sup>. Radio radiation stress caused increases the dead germ cells<sup>4</sup>. In the forced swimming stress the spermatogenesis was significantly reduced<sup>5-7</sup>. It has been shown that heat stress disorders reproductive process in mammals such as spermatogenesis, maturation and development of oocytes<sup>8-9</sup>. Noise is as a kind of stress and harmful environmental factors and inevitable phenomena in the workplace and life. Contacting with noise or sound over standard can be a potential source of mental and physical disorders and injuries. The role of the noise as a stress on various ailments has been studied<sup>10</sup>. The noise is a factor not only at work rather at sleep and the rest of people, makes impairment whether

quantitative or the qualitative<sup>11-12</sup>. There are many evidences that the noise damages cardiovascular, neurological, hearing and endocrine systems<sup>13-16</sup>. The noise, affects insulin secretion<sup>17</sup>, morphology of cells in testes<sup>18</sup>, endocrine function of the testes and anterior pituitary<sup>19</sup>. The purpose of the present study was to determine whether noise stress applied to adult male rats may affect in vivo fertilization capacity and subsequent offspring quality.

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## Materials and Methods

This study was conducted from March 2009 to August 2010. A total 20 adult male Wistar rats, 3 months of age, weighing  $210 \pm 10.6$  g were purchased from Laboratory Animals Care and Breeding Center of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. The fertilizing ability of male mice was proven at the beginning of the experiment by selecting post first wave of spermatogenesis that mate and observed positive pregnancy<sup>20</sup>. All rats were randomly divided into two equal groups (n=15); 1) control and 2) experimental groups. All animals were housed individually per cage under a 12-h light/dark cycle,  $20 \pm 2$  °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 Ahvaz Jundishapur University of Medical Sciences<sup>11</sup>.

The test cage was placed within a room with dimensions of 3×4×3 m which has been insulated with wood and acoustic components (anti-noise) before, and control cage was transferred to the ordinary room. In the room with the test group, the sound producer device WHITE NOISE with the frequency of 300-350 Hz and intensity of 90-120 dB was placed for 7 pm<sup>21</sup>, and the device's timer was set to turn off for a few minutes (from 15 to 60 minutes) to work again after an hour of broadcasting the noise by loudspeaker. It causes to avoid making the animals compromise with any stressful situations. But it should be noticed that the machine in 2-3 minutes of periods, was automatically changed the intensity and the produced sound frequency in the range of minimum and maximum to help non-compliance<sup>22</sup>. To ensure the amount and intensity of sound, noise level meter device was used and these factors were controlled. Turning machine on and off was on 7 pm and 7 am on the next day respectively for 50 days (period of spermatogenesis in rats)<sup>11</sup>.

Adult control and stressed males were mated with sexually mature normal females presenting at least three regular cycles confirmed by the analysis of daily vaginal smears. Females in the *pro-oestrus* stage in the morning were mated with male rats overnight (*two females per one male*). The presence of spermatozoa in the vaginal smear on the next morning was indicative of copulation and was considered as dayzero of pregnancy. Autopsy was performed on day 20 of pregnancy when uterus and ovaries were examined for the number of corpora lutea, dead and live fetuses, and embryo resorption and implantation sites (total amount of live and dead fetuses plus embryo resorption). The rates of pre-implantation loss (corpora lutea minus implantation sites) and post-implantation loss (implantation sites minus live fetuses) were then determined<sup>7</sup>.

## Statistical analysis

Data are stated as a means  $\pm$  SD and percentage. The statistical significance of difference between the control and experimental groups was determined by the chi-square. Differences between the means were considered to be significant when  $P < 0.05$  was achieved.

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## Results

In this study 10 male rats were exposed to 90-120 dB sound intensity and frequency of 300-350 Hz by the white noise device (daily at 7 pm to 7 am). After this period, the male rats of both groups (exposed and non-exposed to noise stress) were mate with female mice.

As shown in table 1 the pregnancy rate in female mated with control and stressed male was 17/20 (85%) and 12/20 (60%) respectively. Statistical analysis showed the significant differences between two groups ( $P < 0.05$ ). Live fetuses per uterus (as estimate male fertility rate) of female mated with control and with stress males were  $8.88 \pm 0.4$  and  $5.83 \pm 0.2$  per uterus, respectively. Significantly difference was observed between two groups ( $P < 0.05$ ). The difference in weight of live fetuses of two groups of study was significant ( $P < 0.05$ ). A significant difference were observed in 158 and 98 points of uteruses of pregnant female by male rats of nonstress and stressed respectively. The difference was significant ( $P < 0.05$ ). The rate of pre-implantation loss in female rats mated with stressed male rats significantly increased ( $p < 0.05$ ).

**Table 1. Effect of 50 days of noise stress on fertilization capacity of rat and subsequent offspring quality**

Animal study	Control rats (n = 10)	Experimental groups (n = 10)
Variable		
Number of female rats mate with male rats	20	20
Pregnant females (%)	17/20 (85%)	12/20 (60%)
Corpora lutea	160	118
Live fetuses (Mean $\pm$ SD)	151(8.88 $\pm$ 0.4per uterus)	70 (5.83 $\pm$ 0.2 per uterus )
Body weight (gram)	6.2 $\pm$ 1.9	4.3 $\pm$ 1.2
Dead fetuses	2	8
Resorption sites	5	20
Implantation sites	158	98
Pre-implantation loss	2	20
Post-implantation loss	7	2

## Discussion

Today, noise pollution is known as one of the problems of human society so study of its effect on human life, it seems essential. Role of noise as a stress on various diseases has been studied and its effects on the body's hormones, pregnancy, abnormal delivery, premature birth and even neonatal weight and the number is pending. Since avoiding noise pollution during pregnancy is emphasized, its impact on the pregnancy closer to research in this study are presented. Our findings show that the number of live embryos and their weight compared with the group were not exposed to noise reduction, and the number of dead and absorbed embryos compared with the control group increase. It has been demonstrated that the forced swimming stress in male rats reduces the number of live embryos and their weight and increase the number of dead and absorbed embryos. So this is similar to the findings of this study<sup>7</sup>. Previous studies have shown the negative effects of stress on sex hormones, sexual behavior and also the quality of semen. Another study has indicated that stress of immobility caused to reduce the testes size and weight as well as impaired spermatogenesis and cell division<sup>23</sup>. The results can be reasons related to the present study. It has also been revealed that the adult animals exposed to intermittent immobilization stress have gotten low rate fertilization and implantation and even these cases can be seen in their children<sup>24</sup>. According to previously done research, these cases are probably because of fertilization an ovum with a damaged sperm<sup>23, 25-26</sup>. Other studies about the impact of stress on the levels of sex hormones have shown that the use

of voice traffic intensity 100 dB have been significantly reduced the levels of testosterone in male albino rats weighing 200-250 grams. According to histological studies of leydig cells, it has been demonstrated that the responsibility of these cells is extremely reduced by being exposed to noise and this makes that even if the LH levels rise, but the production of testosterone is reduced<sup>27</sup>. A study conducted on the leydig cells has reported that being exposed to state of chronic noise caused to stop maturation of germ cells and reduction of testosterone level. On the other hand, increasing the number of leydig cells with a compensatory mechanism to increase testicular steroid was occurred to compensate through the insufficiency of testosterone<sup>27</sup>. It has been demonstrated that during the stress the axis of hypothalamus - pituitary - adrenal is activated and reduce the secretion of Glycocorticoid by leydig cells<sup>28</sup>. There are some reports that decreasing of testosterone levels in rats exposed to noise is associated with significant reduction of epididymal sperm count<sup>27</sup>. Furthermore, during the histological studies, it has been revealed that the epididymal sperms in the group of rats which chronically affected by noise, were agglutinated and the number of dead sperms increased and the maturity of the germ cell was stopped<sup>29-30</sup>. One of the most important factors that can cause infant mortality is low weight that includes about 12 to 15 percent of births. The probability of mortality during this period is tripled and the amount of their vulnerability to disease increases than normal weight infants. This study shows that birth weight of babies that has grown in noisy environment significantly has been less than grown babies in a natural environment.

These findings are in agreement with the results of another study conducted by another researcher<sup>11</sup>.

Another study was performed on the role of noise pollution in the incidence of pregnancy. They indicated that noise pollution increase maternal mortality with reduction of vaginal delivery lead to maternal death that is consistent with the present results. In addition, it has been found that birth weight and number of infants in the group exposed to noise reduced compared to control group. But this result is inconsistent with the results of this study<sup>10</sup>. A study has shown the amount twin calving in rats that were exposed to noise was more than rats that have grown in a silent environment and this is a reason to lose weight of infants<sup>11</sup>.

This study has shown that noise influences on the fertility rate, twain calving, maternal mortality, infant mortality and birth weight of mice. These results show the importance of studying this environmental pathogen that is growing in the world today. Especially humans not only at work but also during sleep and relax, are exposed to this environmental factor.

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### Conclusion

Stressing spermatozoa during critical time points that fertilize oocyte and lead to embryo development can have a serious negative impact on pregnancy rates. Noise stress can decrease pregnancy rates during early and late embryonic development. Therefore, this result may point out the importance of the effect of stress in human in the field of sport profession and day to day life stress that further becomes a management tool for maximizing pregnancy success.

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