KMANPUB | Health Nexus Vol. 3 No. 1 (2025): 1-8

Article history:
Received 14 June 2024
Revised 20 August 2024
Accepted 01 September 2024
Published online 01 January 2025

Effects of Pre-Pregnancy Exercise on Anxiety Behaviors and Oxidative Stress Factors in Pregnant Mice

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A r t i c l e I n f o

Article type:
Original Research

How to cite this article:
Torabi, F., Eslami, Z., & Mohammadi, M. (2025). Effects of Pre-Pregnancy Exercise on Anxiety Behaviors and Oxidative Stress Factors in Pregnant Mice. *Health Nexus*, *3*(1), 1-8.
https://doi.org/10.61838/kman.hn.3.1.1

A B S T R A C T

The physical and psychological status of the mother and the environment she provides during pregnancy significantly influence fetal development and, subsequently, the health and adaptability of the child in the future. Therefore, the present study aimed to investigate the effect of pre-pregnancy swimming exercise on anxiety behaviors and oxidative stress factors in female mice during pregnancy. In this study, 26 NMRI mice, approximately 80-90 days old and weighing 25-35 grams, were selected. The female mice were randomly divided into two groups: 10 in the control group and 10 in the experimental group. The female mice in the experimental group underwent swimming exercise for 8 weeks before mating. The exercise method included two stages: an adaptation stage and a swimming exercise stage. During the adaptation period, to reduce stress caused by water, the animals were placed in shallow water (5 cm depth) for 10 minutes during the first week to adapt to the procedure. The swimming exercise consisted of three 10-minute swimming sessions with a 10-minute rest interval between each session per day. To evaluate anxiety behaviors, the open field test, dark/light box test, and elevated plus maze test were utilized. For assessing oxidative stress factors, the mice were anesthetized, and blood was collected from their hearts. The obtained data were analyzed using SPSS version 26 and one-way ANOVA. The results indicated that in the experimental group (swimming exercise), compared to the control group (no exercise), there was a significant reduction in anxiety behaviors, a significant increase in glutathione, and a significant decrease in malondialdehyde levels ($p \leq 0.05$). It appears that pre-pregnancy swimming exercise reduces anxiety behaviors, increases glutathione, and decreases malondialdehyde in pregnant female mice. Therefore, it is recommended that swimming exercise be performed before pregnancy to control pregnancy-related anxiety in female mice.

Keywords: *Swimming exercise, anxiety behaviors, oxidative stress, malondialdehyde, glutathione, pregnancy*

1. Introduction

In various societies, exercise is utilized as a means to prevent diseases, improve health, and enhance well-being. Due to its positive mental and physical effects, exercise plays a significant role in the rehabilitation and management of psychological and behavioral disorders. Anxiety symptoms during pregnancy can be associated with several

types of anxiety, including general anxiety, anxiety disorders, and pregnancy-related anxiety. Pregnancy-related anxiety and general anxiety can have negative consequences for the mother, such as nocturnal dyspnea, preterm birth, and postpartum depression (1). Pregnancy is the most sensitive period in a mother's life. The health and well-being of the mother have a direct impact on the life of the child. The fetus during this period is vulnerable and sensitive to various factors (2). Pregnancy is a critical period for fetal growth and development, and environmental factors influence both the fetus and the child (3). Sánchez-Polán et al. (2021) stated in a study that monitoring physical activity during pregnancy could be an effective approach to prevention and reducing anxiety and anxiety symptoms during pregnancy (4). Low levels of anxiety can serve as a physiological mechanism that stimulates and motivates an individual to cope with threatening situations. However, high levels of anxiety can negatively affect the autonomic nervous system and hormones, leading to undesirable outcomes and complications (5). Cancela-Carral et al. (2020) reported that regular physical exercise, including water-based exercises or a combination of land and water exercises during pregnancy, provides better control over maternal and neonatal weight and leads to improvements in cognitive (depression, quality of life, body image) and physical (back pain, fitness, mobility) levels in mothers (6). Physical activity during pregnancy is associated with reduced severity of pregnancy-related depression and anxiety, as well as reduced stress and improved quality of life (7). Stress in pregnant mothers is of great concern because any stress-inducing factor not only affects the mother but also impacts the fetus. Due to the connection between the fetus and the mother, physiological and hormonal changes in response to stress in the mother are transmitted to the fetus, causing fetal reactions (3). Stress during pregnancy increases maternal stress hormones and plasma concentrations, leading to changes in the uterine environment, programming of the developing fetus's endocrine, oxidative, and metabolic systems, and long-lasting detrimental effects in later life (8). Additionally, 35% of pregnant women exhibit signs of depression and stress during pregnancy; therefore, it was concluded that exercise has long-term effects on the health of offspring (9). Furthermore, stress experienced by the pregnant mother results in the constriction of the uterine arteries and reduced blood flow, leading to decreased nutrient and oxygen supply, ultimately affecting brain development. This can result in learning and memory disorders in the offspring, as well as changes in neurotransmitter activity, such as dopamine, serotonin, and norepinephrine, through excessive activation of the HPA axis (10).

Prenatal stress leads to increased depressive behaviors and elevated serum corticosterone levels, which, in turn, activate the immune system, reduce serotonin levels, and increase glutamate levels in a gender-dependent manner (11). Since the use of sedatives is limited due to their uncertain effects on the future child, exercise is used in various societies as a means to prevent diseases, improve health, and enhance well-being (4). Almalki et al. (2021) reported that maternal stress induces long-term behavioral changes in offspring (12). Harris et al. (2018) stated that exercise before pregnancy is essential and contributes to maximizing beneficial outcomes for childbirth (13, 14). Oxidative stress results from an imbalance between free radicals and antioxidants in the body, leading to an increase in free radicals. Free radicals are toxic products of oxygen metabolism and contain an unpaired electron. The excessive production of free radicals due to oxidative stress damages components such as DNA, proteins, and cell membranes, resulting in altered cell function or cell death. Free radicals play a crucial role in several biological processes, such as intracellular bacterial degradation. Free radicals also play a significant role in certain systematic cellular processes, including the regulation of homeostasis and a wide range of cellular functions. Free radicals are usually neutralized by the body's natural antioxidant system. If not effectively countered, free radicals can trigger a negative chain reaction in the body, damaging cell membranes (15). Glutathione is a non-protein thiol compound that plays a key role in cellular defense against oxidative stress. It is involved in cell proliferation, cytokine production, signal transduction, gene expression, and immune responses, with the majority present in its reduced form. Glutathione plays a primary role in defending against oxidative stress. Additionally, glutathione is present in most mammalian cells and participates in various functions, such as detoxification, protection against oxidants, maintaining proper protein thiol group status, and regulating cell proliferation (16).

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E-ISSN: 2981-2569

Rahimi et al. (2020) reported that exercise and physical activity reduce oxidative stress markers and increase glutathione levels in the brains of diabetic mice (17). The importance of this study lies in its potential to encourage women to engage in physical exercise, particularly swimming, before pregnancy. Considering the points mentioned and the fact that no research has been conducted on the effect of pre-pregnancy swimming exercise on anxiety behaviors and oxidative stress factors in pregnant mice, the present study aims to address the following questions: Can pre-pregnancy swimming exercise reduce anxiety during pregnancy? And can pre-pregnancy swimming exercise lead to changes in oxidative stress factors (malondialdehyde and glutathione) during pregnancy? Therefore, the necessity of this research is to explore whether mothers who engage in swimming exercise before pregnancy but cannot exercise during pregnancy will have offspring whose behaviors in response to stress and anxiety are affected. Consequently, this study aims to investigate the use of pre-pregnancy swimming exercise and its impact on anxiety behaviors and oxidative stress factors in pregnant female mice.

2. Methods and Materials

2.1. Study Design and Participants

This study employed an experimental research design. The subjects were 26 male and female NMRI laboratory rats, weighing between 22 and 25 grams, and approximately 80-90 days old. The rats were purchased from the Pasteur Institute of Iran and transferred to the laboratory animal facility at the Salari Institute of Cognitive and Behavioral Disorders. The rats were divided into control and experimental groups and participated in an 8-week program. The rats were housed in special plastic cages under standard conditions, with a temperature of 23 ± 1 degrees Celsius and a 12-hour light/dark cycle. Water and food were provided ad libitum. Each animal was tested only once and was removed after the experiment. Female rats were divided into two groups: Group 1, consisting of females that did not exercise, and Group 2, consisting of females that exercised. The male rats were kept separately until the day of mating. Female rats in the experimental group underwent swimming exercise for 8 weeks before mating, while the non-exercised animals were placed in a circular tank without water for a similar duration as the exercised animals. After the exercise sessions, one female and one male rat were placed in a cage for 48 hours for mating, with one male rat used for each female in each group. The presence of a vaginal plug the following morning was checked as an indicator of pregnancy. If a vaginal plug was observed, the first day of pregnancy was recorded, and the animal was transferred to a separate cage. The female rats did not exercise during pregnancy.

2.2. Exercise Program

Female rats in the experimental group underwent swimming exercise for 8 weeks before mating. The animals were placed in a circular tank (diameter: 80 cm, height: 30 cm) filled with water (temperature 23 ± 1 degrees Celsius). To prevent the animals from floating, four wave-making motors were placed at different angles in the tank. This method involved two stages: the adaptation stage and the swimming exercise stage. During the adaptation period, to reduce water-related stress, the animals were placed in shallow water (5 cm depth) for 10 minutes during the first week to adapt to the procedure. The swimming stage involved three 10-minute swimming sessions with a 10-minute rest interval between each session daily. The water depth gradually increased from 5 cm in the first week to 10 cm in the second week and 15 cm in the third week. The duration of swimming was also gradually increased from 20 minutes in the second week to 30 minutes per day starting in the third week. This exercise was performed daily between 12:00 and 16:00. After swimming, the animals were dried with a towel and warmed by an electric heater for 10-15 minutes.

2.3. Behavioral Anxiety Tests

Anxiety behaviors were assessed on days 8, 14, and 16 of pregnancy using the following tests:

Open Field Test: On the eighth day of pregnancy, pregnant animals were exposed to the open field test device. The open field test is used to assess anxiety and motor activity. This test consists of a wooden white box measuring 20×20×40 cm, with the floor divided into 16 equal square sections. The device also includes a central area (4 central squares) and a peripheral area (12 edge squares) next to the

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E-ISSN: 2981-2569

walls. A 100-watt lamp was positioned 90 cm above the device. A pregnant mouse was placed in the center of the box for 5 minutes, and the time spent in the central area (the four central squares) was recorded. A decrease in this measure was considered an anxiety-like behavior in the animal.

Light/Dark Box Test: On the 14th day of pregnancy, the animals were exposed to the light/dark box test device. This test consists of two compartments: a light compartment (30×27×27 cm) and a dark compartment (30×27×18 cm), connected by an open door (7.5×7.5 cm). The animal was placed in the center of the light compartment and allowed to explore freely in the device for 5 minutes. The time spent and the number of entries into the light compartment during this period were recorded as indicators of anxiety-like behavior.

Elevated Plus Maze: On the 16th day of pregnancy, anxiety was further assessed using the elevated plus maze. This device is made of wood and consists of four arms in the shape of a plus sign (+). The dimensions of the open and closed arms are 30×5 cm, with the closed arms having 15 cm high walls on both sides and at the end, while the open arms have a 1 cm high glass edge to prevent the mouse from falling. The four arms connect to a central area measuring 5×5 cm. The maze is elevated 50 cm above the ground. The mice were placed in the central area of the maze, facing an open arm. Adequate lighting was provided by a 100-watt lamp positioned 120 cm above the center of the maze. Entry into an open or closed arm was recorded when all four paws of the animal were in the respective arm. The number of entries and the time spent in the closed arms were considered indicators of anxiety. The more time the animal spent in the closed arms, the higher the level of anxiety.

2.4. Laboratory Measurements

After completing the behavioral tests, the mice were deeply anesthetized using ketamine (50 mg/kg) and xylazine (50 mg/kg). Blood was collected from the heart, and serum was separated by allowing the blood to stand on a cold surface for 15 minutes, followed by centrifugation at 3000 rpm for 10 minutes. The serum was collected in sterile microtubes and stored at -70 degrees Celsius for the measurement of malondialdehyde and glutathione.

2.5. Data Analysis

The findings of this study were analyzed using one-way ANOVA and SPSS software version 26, with a significance level of $p \leq 0.05$.

3. Findings and Results

Table 1 presents descriptive statistics, including mean and standard deviation, for anxiety behaviors and oxidative stress factors (malondialdehyde and glutathione) separately for the control and swimming exercise groups. As shown in the table, there is a significant difference between the two groups in all the studied variables as determined by one-way ANOVA ($p \leq 0.05$).

Table 1

Mean and Standard Deviation of Research Variables and ANOVA Results

Variable	Time/Count	Control (No Exercise)	Experimental (Exercise)	F	p
Open Field Anxiety Behavior Test	Time	88.50 ± 21.78	118.20 ± 18.91	10.602	0.004
Light/Dark Box Anxiety Behavior Test	Time	105.40 ± 16.08	123.60 ± 18.28	5.588	0.030
	Count	16.80 ± 5.33	24.60 ± 6.15	9.187	0.007
Elevated Plus Maze Anxiety Behavior Test	Time	66.90 ± 13.78	103.00 ± 24.26	16.744	0.001
	Count	12.40 ± 2.07	15.20 ± 2.35	8.018	0.011
Malondialdehyde		3.28 ± 0.76	2.50 ± 0.72	5.454	0.031
Glutathione		3.19 ± 1.03	4.58 ± 0.74	11.883	0.003

$p \leq 0.05$ is considered statistically significant.

Skewness is a measure of the asymmetry of the distribution of values. For a perfectly symmetrical distribution, skewness is zero. Positive skewness indicates a distribution with a longer tail on the right, while negative skewness indicates a distribution with a longer tail on the left. Generally, if skewness and kurtosis are within the range of -2 to 2, the data distribution is considered normal. Several samples showed non-normal distributions, indicating

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E-ISSN: 2981-2569

outliers. Therefore, the Mahalanobis test was used for normalization, and outlier data were removed to achieve a normal distribution.

Figure 1

Changes in Malondialdehyde between the Experimental (Swimming Exercise) and Control (No Exercise) Groups

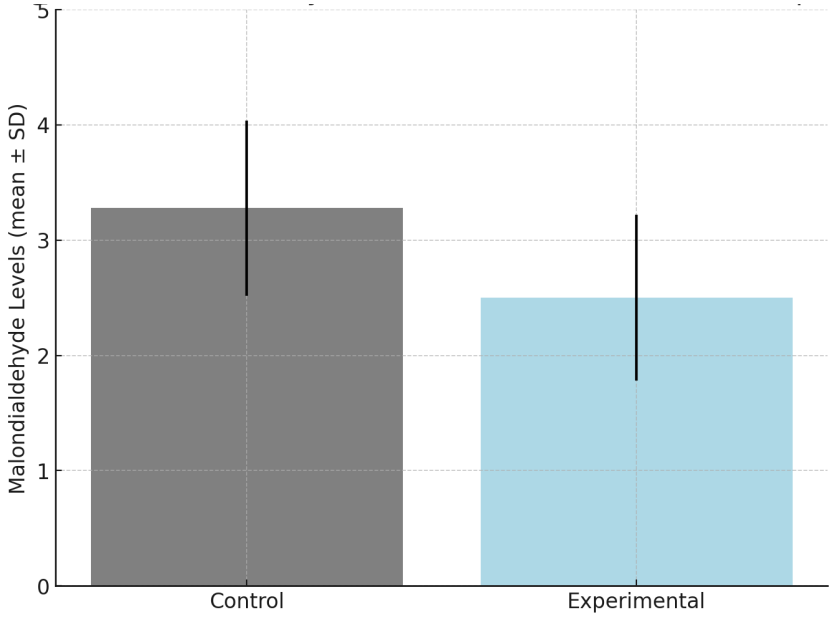
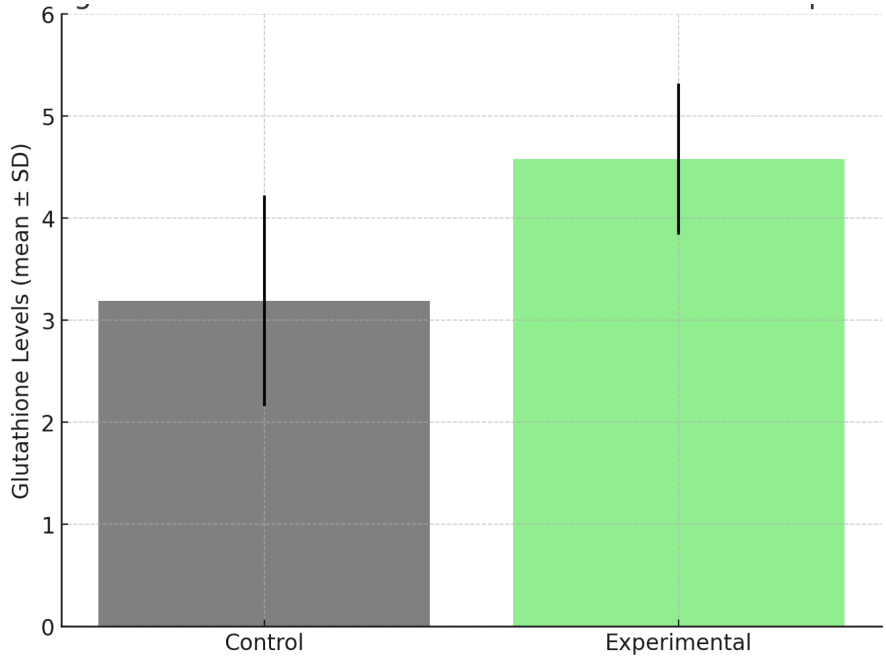


Figure 2

Changes in Glutathione between the Experimental (Swimming Exercise) and Control (No Exercise) Groups



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E-ISSN: 2981-2569

4. Discussion and Conclusion

The aim of the present study was to investigate the effects of pre-pregnancy physical activity on anxiety behaviors and oxidative stress factors (malondialdehyde and glutathione) in pregnant mice. The anxiety behavior parameters and oxidative stress factors in the experimental group that underwent the swimming exercise protocol showed significant improvements compared to the control group that did not undergo any intervention. According to the research findings, pre-pregnancy swimming exercise has a significant impact on the anxiety of pregnant female mice. These results are consistent with the prior findings (3, 6, 17, 18) but are not consistent with the findings of one study (7). Anxiety and stress are issues that pregnant women face throughout pregnancy, which can affect the fetus. On the other hand, pregnancy is accompanied by mental and emotional stress, which causes anxiety and stress in the pregnant mother. Regular aerobic exercise reduces oxidative stress and increases antioxidant capacity in the brain (18). Cai et al. (2020) stated in a study that physical activity during pregnancy can be associated with a reduced likelihood and severity of pregnancy-related depression and anxiety, as well as reduced stress and improved quality of life (19). Additionally, Sánchez-Polán et al. (2021) reported that monitoring physical activity during pregnancy could be a good preventive approach and reduce pregnancy-related anxiety and symptoms (4). Anxiety during pregnancy is a risk factor for both mothers and children and is associated with shorter pregnancy duration and adverse outcomes for fetal and child neurodevelopment (15). Mental health during pregnancy is one of the important issues that is increasingly receiving attention today. Studies have shown that from the beginning of pregnancy, the emotions and anxiety of pregnant mothers negatively impact the fetus (5). Research indicates that maternal stress is associated with preterm birth and low birth weight. Additionally, pregnancy-related stress is related to developmental delays and emotional disorders such as crying, low tolerance threshold, irritability, and poor social interactions (20). Enayati et al. (2020) reported that prenatal stress increases depressive behaviors, activates the immune system, reduces serotonin, and increases glutamate levels in a species-dependent manner (10). Stress can affect all systems of the mother's body. Given the significant interactions between exercise and maternal immune activity, swimming exercise can reduce anxiety and anxiety-like behaviors in offspring following maternal immune activation. According to the research findings, pre-pregnancy swimming exercise significantly impacts malondialdehyde and glutathione levels in pregnant female mice, which is consistent with the previous findings (8, 16). Exercise positively affects brain function, improving brain performance and inducing physiological and biochemical adaptations through various pathways. Aerobic exercises reduce oxidative stress and increase antioxidant capacity in the brain. Recent findings in studies on mice have shown that swimming exercise significantly reduces oxidative stress and anxiety-like behaviors in mice (18). Continuous exercise increases glutathione peroxidase activity and the level of reduced glutathione to prevent oxidative stress (21). Swimming exercise can be a potential treatment for behavioral and cognitive disorders and has anxiolytic properties (17). It also reduces oxidative stress markers and increases glutathione in the brains of diabetic mice (18). Exercise before pregnancy is essential and adds maximum beneficial effects to childbirth. Likewise, the possibility of epigenetic changes indicates a fundamental mechanism by which exercise improves maternal health (13). Therefore, the following recommendations are proposed:

- Conducting extensive studies on the national healthcare system to identify mothers at risk of oxidative stress through malondialdehyde testing during pregnancy and referring them to higher levels of treatment.
- Sport authorities should pay special attention to mental and emotional issues as one of the factors contributing to problems in the lives of pregnant women.

Authors' Contributions

F. T. conceptualized and designed the study, led data collection and analysis, and drafted and wrote the manuscript. Z. E. executed the animal experiments, including swimming exercise protocols and anxiety assessments, and collected and analyzed blood samples for oxidative stress markers. M. M. provided expertise in statistical analysis, contributed to interpreting results, and

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E-ISSN: 2981-2569

reviewed and edited the manuscript. All authors approved the final version of the manuscript.

Declaration

In order to correct and improve the academic writing of our paper, we have used the language model ChatGPT.

Transparency Statement

Data are available for research purposes upon reasonable request to the corresponding author.

Acknowledgments

We would like to express our gratitude to all individuals helped us to do the project.

Declaration of Interest

The authors report no conflict of interest.

Funding

According to the authors, this article has no financial support.

Ethics Considerations

The study placed a high emphasis on ethical considerations. Informed consent obtained from all participants, ensuring they are fully aware of the nature of the study and their role in it. Confidentiality strictly maintained, with data anonymized to protect individual privacy. The study adhered to the ethical guidelines for research with human subjects as outlined in the Declaration of Helsinki.

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