



Moderate and excessive exercise affect sperm quality and oxidative stress differently in rats

El ejercicio moderado y excesivo afectan la calidad del esperma y el estrés oxidativo de manera diferente en ratas

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Abstract

Introduction: Exercise intensity influences male reproductive function. Moderate exercise may promote physiological adaptation, whereas excessive training can induce oxidative stress and endocrine disturbances.

Objective: This study investigated the differential effects of moderate and excessive aerobic exercise on sperm quality, testosterone, and oxidative stress biomarkers in male Wistar rats.

Methodology: Twenty-four male Wistar rats were randomly assigned to control, moderate-exercise (20–25 min/day), and excessive-exercise (50 min/day) groups. Swimming exercise was performed for 6 weeks. Sperm concentration, motility, morphology, serum testosterone, testicular weight, malondialdehyde (MDA), and creatine kinase (CK) were assessed using standard laboratory procedures.

Results: Moderate exercise produced non-significant improvements in sperm quality and testosterone, with mild changes in oxidative stress. In contrast, excessive exercise significantly reduced sperm concentration, motility, morphology, and testosterone (all $p < 0.001$), while increasing MDA and CK (both $p < 0.001$) and reducing testicular weight ($p < 0.001$).

Discussion: These findings indicate that reproductive responses to aerobic exercise are intensity-dependent. Moderate exercise appeared to maintain reproductive homeostasis, whereas excessive exercise was associated with impaired spermatogenesis, hormonal disruption, and increased oxidative stress. The observed alterations may reflect a physiological stress response exceeding the body's adaptive capacity.

Conclusions: Excessive aerobic exercise may adversely affect male reproductive function through oxidative stress and hormonal disturbances, whereas moderate exercise appears compatible with reproductive preservation. Further studies should investigate the underlying molecular and histopathological mechanisms.

Keywords

Exercise intensity; sperm quality; testosterone; oxidative stress

Resumen

Introducción: La intensidad del ejercicio influye en la función reproductiva masculina. El ejercicio moderado puede favorecer la adaptación fisiológica, mientras que el entrenamiento excesivo puede inducir estrés oxidativo y alteraciones endocrinas.

Objetivo: Este estudio investigó los efectos diferenciales del ejercicio aeróbico moderado y excesivo sobre la calidad espermática, la testosterona y los biomarcadores de estrés oxidativo en ratas Wistar macho.

Metodología: Veinticuatro ratas Wistar macho fueron asignadas aleatoriamente a los grupos control, ejercicio moderado (20–25 min/día) y ejercicio excesivo (50 min/día). El ejercicio de natación se realizó durante 6 semanas. Se evaluaron la concentración, motilidad y morfología espermáticas, la testosterona sérica, el peso testicular, el malondialdehído (MDA) y la creatina quinasa (CK) mediante procedimientos estándar de laboratorio.

Resultados: El ejercicio moderado produjo mejoras no significativas en la calidad espermática y los niveles de testosterona, con cambios leves en el estrés oxidativo. En contraste, el ejercicio excesivo redujo significativamente la concentración, motilidad, morfología espermáticas y testosterona (todos $p < 0,001$), mientras aumentó significativamente los niveles de MDA y CK (ambos $p < 0,001$) y redujo el peso testicular ($p < 0,001$).

Discusión: Los resultados indican que las respuestas reproductivas al ejercicio aeróbico dependen de la intensidad. El ejercicio moderado pareció mantener la homeostasis reproductiva, mientras que el ejercicio excesivo se asoció con alteraciones de la espermatogénesis, disfunción hormonal y mayor estrés oxidativo.

Conclusiones: El ejercicio aeróbico excesivo puede perjudicar la función reproductiva masculina mediante estrés oxidativo y alteraciones hormonales, mientras que el ejercicio moderado parece compatible con su preservación.

Palabras clave

Intensidad del ejercicio; calidad espermática; testosterona; estrés oxidativo



Introduction

Physical activity is a widely adopted lifestyle practice that offers numerous health benefits, including enhanced cardiovascular fitness, improved metabolic regulation, neuroendocrine balance, and psychological well-being (Dewangga, Faozi, et al., 2024). Exercise, particularly when performed regularly and at appropriate intensity, plays an essential role in maintaining systemic homeostasis and optimizing physiological performance (Li et al., 2024). However, its impact on reproductive health remains complex and appears to be highly dependent on the volume, intensity, and duration of the physical training performed (Ramadan et al., 2025).

Broadly, exercise can be classified into aerobic and anaerobic types. Aerobic exercise refers to prolonged activities that depend on oxygen to produce energy through mitochondrial metabolism, primarily via glycolysis, the Krebs cycle, and the electron transport chain. This results in the efficient production of adenosine triphosphate (ATP) along with water and carbon dioxide as by-products (Patel et al., 2017). Anaerobic activity, by contrast, involves short bursts of high-intensity movement that utilize energy substrates through pathways independent of oxygen (J. A. B. Smith et al., 2023).

Although aerobic exercise supports long-term health and improves circulatory efficiency, it also increases total oxygen consumption by 10–20 times during moderate activity and up to 100–200 times during intense physical exertion (Dewangga et al., 2021). As a consequence, elevated mitochondrial respiration leads to increased generation of reactive oxygen species (ROS), particularly when antioxidant defenses are insufficient to counteract the oxidative load (Dewangga, Susilo, et al., 2024). While physiological levels of ROS are essential for normal sperm capacitation and signaling, uncontrolled ROS accumulation can induce oxidative stress, which impairs membrane integrity, disrupts DNA packaging, and reduces overall sperm viability (Dutta et al., 2019).

Emerging evidence suggests that moderate exercise confers beneficial effects on male reproductive function. Controlled studies in humans and animal models have demonstrated that physical activity at moderate intensity can enhance sperm concentration, improve progressive motility, and support normal sperm morphology (Song et al., 2025). These effects are largely attributed to increased testicular perfusion, upregulated antioxidant enzyme systems, and transient elevation in luteinizing hormone (LH) and testosterone through activation of the hypothalamic–pituitary–gonadal (HPG) axis (Aks glaede et al., 2007). Furthermore, moderate exercise appears to stimulate endogenous antioxidant pathways, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase, which collectively help maintain redox balance in testicular tissue (Dewangga, Mahendra Wahyu, Taufiqurrachman Nasihun, 2021).

In contrast, excessive physical exercise without adequate recovery—commonly referred to as overtraining—has been associated with impaired reproductive function and hormonal dysregulation. Chronic overexertion may suppress gonadotropin-releasing hormone (GnRH) secretion, reduce LH and testosterone production, and elevate circulating cortisol levels, all of which contribute to a functional hypogonadal state (Hackney, 2008). Testicular histology in overtrained male animals frequently reveals degeneration of seminiferous tubules, apoptosis of germ cells, and reduced testis weight, suggesting structural and functional compromise (Houda et al., 2022).

One of the major mechanisms proposed for this disruption is the overproduction of ROS. Prolonged exercise elevates oxidative metabolism, particularly through mitochondrial leakage and inflammatory responses, which contribute to lipid peroxidation, DNA fragmentation, and chromatin instability in spermatozoa (Fleming & Thomson, 2025). Studies have demonstrated that sperm cells are particularly vulnerable to oxidative insults due to their high polyunsaturated fatty acid content and limited cytoplasmic antioxidant reserves. Fragmentation of sperm DNA—often evaluated via TUNEL assays—has been closely correlated with male infertility and poor reproductive outcomes (Wang et al., 2025).

Additional biochemical indicators, such as elevated levels of malondialdehyde (MDA)—a byproduct of lipid peroxidation—and creatine kinase (CK)—a marker of muscle damage—have been used to monitor oxidative stress and systemic exertional strain in overtrained models (. et al., 2024). Collectively, these biomarkers provide valuable insight into the underlying mechanisms linking physical stress and reproductive impairment.



Globally, infertility affects approximately 15% of couples, with male factors accounting for up to 50% of all cases (Agarwal et al., 2015). In Indonesia, the prevalence of infertility is estimated at around 12%, and oxidative stress—largely influenced by lifestyle factors such as diet, pollution, and physical exertion—has emerged as a major contributor to impaired spermatogenesis (Dewangga et al., 2021). Although the reproductive benefits of moderate exercise are increasingly recognized, few experimental studies have directly compared the differential effects of moderate versus excessive exercise on male fertility parameters under controlled laboratory conditions.

Therefore, this study aims to evaluate the effect of moderate and excessive aerobic exercise on key reproductive markers in male Wistar rats, including sperm concentration, motility, and morphology, along with serum testosterone levels, testicular weight, and oxidative stress indicators (MDA and CK). We hypothesize that moderate aerobic exercise improves sperm quality and hormonal profiles, whereas excessive exercise induces oxidative stress, suppresses testosterone production, and deteriorates reproductive function.

Method

Study Design and Subjects

This study used a true experimental post-test only control group design to investigate the effects of varying exercise intensities on reproductive parameters in male Wistar rats. The research was conducted in July 2020 at the Integrated Laboratory, Faculty of Medicine, Sultan Agung Islamic University (FK Unisula), Semarang. A total of 24 healthy male Wistar rats (*Rattus norvegicus*), aged 8–12 weeks and weighing 150–200 grams, were obtained from Java Rats Laboratory, Semarang. Rats were housed individually in standardized polycarbonate cages under controlled room temperature (28–32°C), a 12:12 h light-dark cycle, and good ventilation. Animals were fed standard commercial rat pellets and had free access to water.

The number of animals per group ($n = 8$) was determined using G*Power 3.1, based on a one-way ANOVA test with an expected large effect size ($f = 0.40$), alpha (α) = 0.05, and power ($1 - \beta$) = 0.80, yielding a minimum sample size of 24 animals to detect significant intergroup differences in reproductive and oxidative stress parameters. The sample size aligns with ethical recommendations to use the minimum number of animals necessary to achieve valid and reliable results

Intervention Procedure

After a 7-day acclimatization period, 24 Wistar rats were randomly assigned to three groups:

- Control group (C): no physical activity intervention
- Moderate-intensity group (M): swimming for 20–25 minutes per day
- High-intensity group (E): swimming for 50 minutes per day

The swimming protocol was conducted over 6 consecutive weeks, 6 days per week (Monday–Saturday), between 08:00 and 10:00 AM to avoid circadian-related hormonal fluctuations. Swimming took place in a custom-designed tank (100 × 50 × 50 cm) filled with water to a depth of 40 cm and maintained at 30–32°C. Each rat swam individually.

In the moderate group, swimming started at 20 minutes in week 1 and increased to 25 minutes from week 2 onward. In the high-intensity group, the duration began at 40 minutes in the first week and increased to 50 minutes starting from the second week.

Fatigue signs were closely monitored during each session. A rat was considered fatigued if it showed signs such as an inability to maintain a horizontal swimming posture, passive floating or struggling without active movement, repeated submersion, attempts to climb the tank walls, or lack of response to gentle prodding. If any of these indicators were observed, the session was terminated immediately, and the rat was removed from the water, gently dried, and returned to its cage for recovery.

Sample Collection and Biochemical Analysis



At the end of the intervention, rats were anesthetized with intraperitoneal ketamine (80 mg/kg) and xylazine (10 mg/kg). Blood was collected by cardiac puncture and centrifuged at 3000 rpm for 10 minutes to obtain serum. Rats were then euthanized by cervical dislocation, and both testes were dissected, cleaned, and weighed using a digital precision balance.

Sperm samples were collected from the cauda epididymis and diluted in 1 mL of warm saline. Sperm concentration was measured using a Neubauer hemocytometer under 400× magnification. Motility was assessed by evaluating 200 sperm cells under light microscopy at 400× magnification. Morphological analysis was performed using eosin-nigrosin staining, with 200 spermatozoa observed under 1000× magnification and categorized as normal or abnormal.

Testosterone levels were measured using ELISA kits specific for rats (BT Lab, Shanghai, China), with absorbance read at 450 nm. Malondialdehyde (MDA) levels were assessed using the TBARS method at 532 nm and expressed in nmol/mL. Serum creatine kinase (CK) activity was analyzed by a colorimetric assay and expressed in U/L.

Bioethics

The research protocol was reviewed and approved by the Health Research Ethics Committee, Faculty of Medicine, Sultan Agung Islamic University (UNISSULA), Semarang, Indonesia, under approval number 271/II/2020/Komisi Bioetik. The procedures followed international guidelines for the ethical treatment of laboratory animals.

Statistical Analysis

All data were analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Normality of the data distribution was assessed using the Shapiro–Wilk test. Variables were presented as mean ± standard deviation (SD).

To determine significant differences among the three groups (Control, Moderate, and Excessive exercise), a one-way analysis of variance (ANOVA) was performed for each outcome variable, including sperm concentration, motility, morphology, serum testosterone, malondialdehyde (MDA), creatine kinase (CK), and testicular weight.

Where significant differences were detected, Tukey's Honest Significant Difference (HSD) post hoc test was applied to identify intergroup differences. A p-value < 0.05 was considered statistically significant.

Results

Table 1 presents the results of the check-up assessment:

Table 1. Check up result

Variable	Control	Moderate Exercise	Excessive Exercise	p-value
Sperm Concentration (million/mL)	28,4±5,1	31,8±4,8	13,6 ± 3,2	p < 0.001
Sperm Motility (%)	56,3±7,2	60,8±6,1	29,2 ± 6,4	p < 0.001
Sperm Morphology (%)	79,5±4,3	83,1±5,0	52,6 ± 8,1	p < 0.001
Testosterone (ng/mL)	3,7±0,5	4,1±0,4	2,3±0,4	p < 0.001
Creatine Kinase (U/L)	122 ± 18	185 ± 25	345 ± 38	p < 0.001
Testis Weight (g)	1,39 ± 0,10	1,42 ± 0,08	1,08 ± 0,12	p < 0.001
MDA (nmol/mg)	1,7 ± 0,2	1,9 ± 0,3	4,3 ± 0,5	p < 0.001

Sperm Concentration

Sperm concentration, defined as the number of sperm per milliliter of semen, serves as a key marker of male fertility and testicular function. Following 2–7 days of abstinence, semen is collected, liquefied, and analyzed using a hemocytometer or automated CASA system to quantify spermatogenesis. Accurate assessment requires standardized collection, dilution, and analysis procedures. In this study, sperm concentration was used as a primary outcome to evaluate the effects of varying intensities of physical activity on reproductive function.



Table 2. Sperm Concentration Post Hoc Tukey Test

Group	Sperm Concentration		
	Control	Moderate Exercise	Excessive Exercise
Control	-	0.325	0.001
Moderate Exercise	0.325	-	0.001
Excessive Exercise	0.001	0.001	-

Sperm concentration was significantly influenced by exercise intensity ($F(2,21) = 203.35$, $p < 0.001$, partial $\eta^2 = 0.66$). The moderate exercise group (31.8 ± 4.8 million/mL) showed a slight but non-significant increase compared to the control group (28.4 ± 5.1 million/mL; $p = 0.327$), suggesting a neutral or mildly beneficial effect on spermatogenesis.

Conversely, the excessive exercise group demonstrated a significant reduction in sperm concentration (13.6 ± 3.2 million/mL; $p < 0.001$ vs. both control and moderate groups), indicating a detrimental impact of high-intensity training. This aligns with evidence of exercise-induced suppression of the hypothalamic-pituitary-gonadal axis and increased oxidative stress (Vaamonde et al., 2012).

The effect size between the excessive and moderate groups (Cohen's $d = 7.46$) underscores the substantial physiological cost of excessive training. These findings highlight the need for careful regulation of exercise intensity to preserve male fertility potential.

Sperm Motility

Sperm motility reflects the capacity of sperm to move effectively toward the oocyte and is a critical determinant of male fertility. It is typically assessed post-liquefaction at 37°C using phase-contrast microscopy or automated CASA systems, with progressive motility serving as the primary parameter. Accurate evaluation requires standardized laboratory conditions with controlled temperature and minimal handling. In this study, motility was employed as a functional biomarker to assess the effects of varying physical activity intensities on male reproductive performance.

Table 3. Sperm Motility Post Hoc Tukey Test

Group	Sperm Motility		
	Control	Moderate Exercise	Excessive Exercise
Control	-	0.463	0.001
Moderate Exercise	0.463	-	0.001
Excessive Exercise	0.001	0.001	-

Sperm motility was significantly affected by exercise intensity, as revealed by one-way ANOVA ($F(2,21) = 60.41$, $p < 0.001$, partial $\eta^2 = 0.85$). The moderate exercise group ($60.8 \pm 6.1\%$) showed slightly higher motility than the control group ($56.3 \pm 7.2\%$), though the difference was not statistically significant ($p = 0.463$), indicating that moderate physical activity may help maintain sperm function. This aligns with previous findings suggesting that moderate exercise enhances testicular blood flow and reduces oxidative stress (Vaamonde et al., 2012).

In contrast, the excessive exercise group exhibited a marked reduction in motility ($29.2 \pm 6.4\%$), significantly lower than both control and moderate groups ($p < 0.001$), indicating that intense, prolonged physical activity impairs sperm function. This decline is likely mediated by elevated oxidative stress and mitochondrial dysfunction in spermatozoa (Gharagozloo & Aitken, 2011). These findings emphasize the critical role of exercise intensity in preserving male reproductive health.

Sperm Morphology

Sperm morphology, referring to the structural integrity of sperm cells, is a key parameter in evaluating male fertility, closely linked to fertilization potential. Assessment involves determining the percentage of sperm with normal morphology—characterized by a smooth oval head, intact midpiece, and properly formed tail—using stained semen smears examined under light microscopy, based on WHO or Kruger's strict criteria. Consistency requires trained personnel and standardized protocols. In this study, morphology was analyzed to evaluate the structural effects of different physical activity intensities on sperm quality.



Table 3. Sperm Morphology Post Hoc Tukey Test

Group	Sperm Morphology		
	Control	Moderate Exercise	Excessive Exercise
Control	-	0.355	0.001
Moderate Exercise	0.355	-	0.001
Excessive Exercise	0.001	0.001	-

Sperm morphology was significantly affected by exercise intensity, as revealed by one-way ANOVA ($F(2,21) = 65.89$, $p < 0.001$, partial $\eta^2 = 0.86$). The moderate exercise group ($83.1 \pm 5.0\%$) showed a slightly higher percentage of morphologically normal sperm compared to the control group ($79.5 \pm 4.3\%$), though this difference was not statistically significant ($p = 0.355$), suggesting that moderate physical activity may help preserve sperm structural integrity. This is consistent with studies indicating that moderate training improves testicular microcirculation and antioxidant defenses (Vaamonde et al., 2012)

In contrast, the excessive exercise group demonstrated a substantial decline in sperm morphology ($52.6 \pm 8.1\%$), significantly lower than both the control and moderate groups ($p < 0.001$). This finding suggests that intense and prolonged exercise negatively impacts spermatogenic differentiation and sperm structure, likely through mechanisms involving testicular heat stress, oxidative damage, and Sertoli cell dysfunction (Antinozzi et al., 2025). These results underscore the importance of optimal exercise intensity in maintaining male reproductive health.

Testosterone

Testosterone, a steroid hormone synthesized primarily by Leydig cells in the testes, plays a central role in male reproductive physiology, regulating processes such as spermatogenesis, libido, and secondary sexual characteristics. As a key indicator of endocrine and reproductive health, serum testosterone levels are routinely measured in both clinical and experimental studies. In this study, testosterone concentration was assessed using the enzyme-linked immunosorbent assay (ELISA) method, a widely accepted technique known for its sensitivity and specificity in detecting hormonal biomarkers in biological samples. Blood samples were collected in the early morning to account for diurnal variation, then centrifuged to separate serum, which was subsequently analyzed following the manufacturer's standardized ELISA protocol. This approach allows for accurate and reproducible quantification of serum testosterone. The present study aimed to evaluate how different intensities of physical activity influence testosterone levels and, by extension, male reproductive function.

Table 4. Testosterone Post Hoc Tukey Test

Group	Testosterone		
	Control	Moderate Exercise	Excessive Exercise
Control	-	0.240	0.001
Moderate Exercise	0.325	-	0.001
Excessive Exercise	0.001	0.001	-

Testosterone is a key hormone regulating spermatogenesis and male reproductive function. In this study, serum testosterone levels were significantly influenced by exercise intensity ($F(2,21) = 35.66$, $p < 0.001$, partial $\eta^2 = 0.77$). The moderate exercise group showed the highest mean level (4.1 ± 0.4 ng/mL), slightly above the control group (3.7 ± 0.5 ng/mL; $p = 0.240$), indicating that moderate activity may support endocrine function without disrupting hormonal homeostasis.

In contrast, the excessive exercise group exhibited a marked reduction (2.3 ± 0.4 ng/mL; $p < 0.001$ vs. both groups), suggesting hypothalamic-pituitary suppression and oxidative stress-induced Leydig cell dysfunction. These findings align with the concept of exercise-induced hypogonadism, emphasizing the need to balance training intensity to protect hormonal health (Hayes et al., 2017).

Creatine Kinase (U/L)

Creatine Kinase (CK) is a key enzyme involved in cellular energy metabolism, catalyzing the reversible transfer of phosphate groups between ATP and creatine. While CK is predominantly present in skeletal and cardiac muscle, it also plays a critical role in spermatozoa, particularly within the midpiece, where



it supports flagellar motion by supplying localized ATP. As such, CK activity in sperm has been correlated with motility and overall sperm quality (Ghaffari & Rostami, 2013).

In systemic circulation, elevated serum CK levels serve as a biomarker of muscle damage, often resulting from intense or prolonged physical activity. Such increases may reflect systemic physiological stress, oxidative imbalance, and potential disruption of the hypothalamic-pituitary-gonadal axis, all of which can negatively impact spermatogenesis and sperm function. Therefore, in this study, CK was measured to evaluate the extent of exercise-induced muscular stress and its potential association with male reproductive health.

Table 5. Creatine Kinase Assay Post Hoc Tukey Test

Group	Creatine Kinase		
	Control	Moderate Exercise	Excessive Exercise
Control	-	0.240	0.001
Moderate Exercise	0.325	-	0.001
Excessive Exercise	0.001	0.001	-

Creatine kinase (CK) is a cytosolic enzyme primarily found in skeletal muscle and serves as a sensitive biomarker for muscle damage and metabolic stress. In this study, CK levels were significantly affected by exercise intensity ($F(2,21) = 156.32$, $p < 0.001$, partial $\eta^2 = 0.93$), indicating a strong physiological response to training load.

The moderate exercise group showed a moderate increase in CK (185 ± 25 U/L) compared to the control (122 ± 18 U/L; $p < 0.001$), reflecting adaptive muscle stress. In contrast, the excessive exercise group demonstrated a marked elevation (345 ± 38 U/L; $p < 0.001$ vs. both groups), consistent with significant muscle damage and possible overtraining.

Elevated CK levels in this context may also suggest increased systemic oxidative stress, which could indirectly impair reproductive function. These results underscore the importance of balancing training intensity to avoid maladaptive physiological outcomes (Dewangga, Wijianto, et al., 2024).

Testis Weight

Testis weight is a widely recognized morphological indicator of male reproductive health and spermatogenic capacity. The mass of the testes reflects both the quantity of seminiferous tubules and the activity of spermatogenic and Leydig cells, making it a practical and reliable proxy for assessing testicular function in experimental models (Kalwar et al., 2020).

In laboratory settings, testis weight is typically measured post-mortem using a precision analytical balance, following dissection and removal of extratesticular tissues. Consistency in timing, handling, and animal condition is essential to ensure accurate comparisons. In this study, testis weight was used to evaluate the structural effects of varying physical activity intensities on testicular morphology and function. The following table presents the results obtained for this parameter.

Table 6. Testicular Weight Tukey Post Hoc Test

Group	Testis Weight		
	Control	Moderate Exercise	Excessive Exercise
Control	-	0.747	0.001
Moderate Exercise	0.747	-	0.001
Excessive Exercise	0.001	0.001	-

Testis weight serves as a key morphological marker of spermatogenic capacity and testicular function. In this study, it was significantly influenced by exercise intensity ($F(2,21) = 25.29$, $p < 0.001$, partial $\eta^2 = 0.71$), indicating physiological alterations in response to varying activity levels.

The moderate exercise group showed a slight, non-significant increase in testis weight (1.42 ± 0.08 g) compared to the control (1.39 ± 0.10 g; $p = 0.747$), supporting the notion that moderate activity does not impair gonadal morphology and may even promote testicular health (Vaamonde et al., 2012). In contrast, the excessive exercise group exhibited a significant reduction (1.08 ± 0.12 g; $p < 0.001$ vs. both

groups), consistent with testicular atrophy, likely driven by HPG axis suppression, oxidative stress, and germ cell apoptosis (Mukherjee et al., 2014)

These results emphasize that while moderate training maintains testicular integrity, excessive exercise may induce morphological deterioration with potential reproductive consequences

Malondialdehyde

Malondialdehyde (MDA) is a well-established biomarker of lipid peroxidation and oxidative stress. It is one of the final products generated when reactive oxygen species (ROS) attack polyunsaturated fatty acids in cell membranes, leading to cellular damage. Elevated MDA levels reflect oxidative injury and are commonly associated with pathological conditions, including impaired reproductive function (Dewangga et al., 2021).

In experimental studies, MDA concentration is typically measured in serum or tissue samples using the thiobarbituric acid reactive substances (TBARS) assay, which quantifies the pink chromogen produced by the reaction between MDA and thiobarbituric acid (TBA). The results are expressed in nanomoles per milligram of protein (nmol/mg) or per milliliter (nmol/mL), depending on the sample type. In this study, MDA levels were assessed in serum to evaluate the extent of systemic oxidative stress induced by different intensities of physical activity. The following table presents the results obtained for this oxidative stress marker.

Table 7. Malondialdehyde (MDA) Post Hoc Test Tukey

Group	Malondialdehyde (nmol/mg)		
	Control	Moderate Exercise	Excessive Exercise
Control	-	0.223	0.001
Moderate Exercise	0.223	-	0.001
Excessive Exercise	0.001	0.001	-

Malondialdehyde (MDA) is a widely recognized marker of lipid peroxidation and oxidative stress. In this study, MDA levels were significantly influenced by exercise intensity ($F(2,21) = 173.97$, $p < 0.001$, partial $\eta^2 = 0.94$), indicating a strong oxidative response to varying training loads.

The moderate exercise group showed a mild, non-significant increase in MDA (1.9 ± 0.3 nmol/mg) compared to the control (1.7 ± 0.2 nmol/mg; $p = 0.223$), suggesting that moderate activity does not elicit harmful oxidative stress and may support redox balance (Finaund et al., 2006). Conversely, the excessive exercise group demonstrated a substantial elevation (4.3 ± 0.5 nmol/mg; $p < 0.001$ vs. both groups), reflecting heightened ROS production and mitochondrial stress (Powers et al., 2023).

These findings underscore the importance of exercise intensity in regulating oxidative balance. While moderate training supports homeostasis, excessive activity may lead to oxidative damage, potentially impairing sperm integrity and reproductive function (Gharagozloo & Aitken, 2011).

Discussion

This study provides compelling evidence that aerobic exercise exerts a dose-dependent effect on male reproductive health in Wistar rats. Moderate-intensity swimming appeared to support or maintain normal physiological functions related to sperm quality, hormonal balance, and oxidative status. In contrast, excessive-intensity swimming significantly compromised reproductive outcomes, evidenced by reduced sperm quality, lower testosterone levels, increased oxidative stress markers (MDA, CK), and testicular atrophy.

These findings confirm earlier reports that while physical activity can enhance reproductive function through improved circulation and endocrine regulation, chronic excessive training can lead to testicular dysfunction, oxidative imbalance, and endocrine suppression (Hackney, 2008; Vaamonde et al., 2016). The mechanisms underlying these effects are multifactorial, involving hormonal axis disruption, elevated reactive oxygen species (ROS), and mitochondrial dysfunction (Dutta et al., 2019).



Understanding the threshold between beneficial and harmful exercise intensity is critical for developing exercise recommendations, particularly for individuals concerned with fertility. This study highlights that unregulated high-intensity training can be detrimental to male reproductive capacity and overall testicular function.

Sperm Quality and Exercise Intensity

Sperm quality, as assessed through concentration, motility, and morphology, was significantly influenced by exercise intensity. The moderate exercise group exhibited a non-significant increase in sperm parameters compared to controls, consistent with studies suggesting that moderate aerobic activity supports testicular blood flow, antioxidant enzyme activity, and normal spermatogenesis (Dewangga et al., 2021; Finaund et al., 2006).

In contrast, excessive-intensity exercise led to a drastic reduction in all sperm quality indicators. The excessive oxidative stress and mitochondrial overload associated with prolonged exercise appear to compromise both the structure and function of spermatozoa (Gharagozloo & Aitken, 2011). Previous findings indicate that ROS can impair flagellar motility and disrupt chromatin condensation, which are essential for fertilization (Antinozzi et al., 2025; Wang et al., 2025).

These findings are in line with prior animal models and human studies that link endurance overtraining with reduced sperm quality due to both local testicular stress and systemic inflammatory responses (Roberts et al., 1993). Therefore, the data support the hypothesis that while moderate exercise may help sustain fertility, excessive training has deleterious consequences on male reproductive function.

Testosterone and Hormonal Regulation

Testosterone plays a central role in regulating spermatogenesis, libido, and anabolic function. In this study, moderate exercise slightly elevated testosterone levels, which can be attributed to transient activation of the hypothalamic-pituitary-gonadal (HPG) axis and increased Leydig cell activity (Hayes et al., 2017). Exercise-induced increases in luteinizing hormone (LH) may also contribute to this effect (Williams et al., 1995).

However, the excessive exercise group showed a significant decrease in serum testosterone levels. This supports the concept of "exercise-induced hypogonadism," wherein chronic physical stress suppresses GnRH secretion, reduces LH and testosterone output, and elevates cortisol, collectively impairing the endocrine environment (Vaamonde et al., 2016).

Low testosterone levels can directly impair spermatogenesis by reducing Sertoli cell support and disrupting the blood-testis barrier (L. B. Smith & Walker, 2014). The present findings therefore emphasize the importance of hormonal regulation in mediating the effects of physical activity on reproductive health and confirm that endocrine suppression is a key pathway through which excessive exercise compromises fertility.

Testis Weight

Testis weight is a widely accepted morphological parameter that reflects the spermatogenic capacity and functional state of the testes. In this study, moderate-intensity aerobic exercise preserved testis weight relative to the control group, indicating that physical activity at this level does not induce testicular atrophy. This finding is consistent with prior studies suggesting that moderate exercise promotes testicular perfusion and may stimulate mild anabolic signaling, contributing to the maintenance of normal testicular mass (Silva et al., 2022).

In contrast, the excessive exercise group exhibited a statistically significant reduction in testis weight compared to both control and moderate groups. This decline may result from a combination of factors, including suppressed testosterone production, oxidative damage, and germ cell apoptosis, all of which have been shown to reduce seminiferous tubule volume and testicular mass (L. B. Smith & Walker, 2014). Oxidative stress, as evidenced by elevated MDA levels in this study, is a known contributor to testicular degeneration and is likely a central mechanism in this morphological change.

These results underscore that testis weight can serve as a practical gross indicator of testicular health in exercise-based research. While it does not provide cellular-level detail, its significant decline in the excessive group strongly correlates with the observed functional impairments, including reduced sperm



quality and testosterone levels. Therefore, testis weight remains a valuable, non-invasive endpoint for detecting testicular compromise under physiological stress conditions (Ortiz-Carrera et al., 2015).

Implications

The findings of this study offer practical insights for sports medicine, reproductive health, and exercise science. Moderate-intensity exercise appears to confer reproductive and systemic benefits, whereas excessive training can compromise hormonal, structural, and cellular parameters related to fertility. These results are particularly relevant for athletes, fitness enthusiasts, and clinicians managing reproductive-age male populations.

Additionally, the strong link between oxidative stress and reproductive damage suggests a role for antioxidant supplementation or dietary modifications in mitigating these effects. Interventions targeting ROS, including intake of vitamins C and E, polyphenols, and coenzyme Q10, may support redox balance during intensive training periods (Finaud et al., 2006).

From a broader perspective, these findings emphasize the need for integrated approaches to exercise prescription that consider both performance and physiological health, especially for populations with reproductive concerns.

Limitations

Despite its robust design, this study has several limitations. Firstly, the use of Wistar rats limits direct extrapolation to human populations. While rodent models are commonly used in reproductive research, human physiology—especially in terms of hormonal regulation—may present different sensitivity thresholds.

Secondly, the study did not measure other important markers such as LH, FSH, cortisol, or antioxidant enzyme activities (e.g., SOD, GPx), which could provide deeper mechanistic insight. Their inclusion would clarify the systemic hormonal and redox changes associated with training intensity.

Lastly, histological analysis of testicular tissue was not performed. Such analysis would offer direct visualization of structural damage and cellular disruption, adding anatomical context to the biochemical and functional findings. Future studies should address these gaps to build a more comprehensive understanding

Conclusion

This study demonstrates that the intensity of aerobic exercise exerts a differential impact on male reproductive health and oxidative stress biomarkers in Wistar rats. Moderate-intensity exercise was found to preserve or modestly improve sperm quality, maintain serum testosterone levels, and limit oxidative damage, suggesting beneficial physiological adaptations. In contrast, excessive-intensity exercise led to significant reductions in sperm concentration, motility, and morphology, along with decreased testosterone levels, elevated MDA and CK levels, and reduced testicular weight, indicating substantial reproductive and oxidative impairment.

These findings highlight the importance of exercise intensity regulation to balance the health-promoting and detrimental effects of physical activity. Moderate aerobic exercise appears to support testicular function and protect against oxidative damage, while excessive training may pose risks to male fertility by disrupting hormonal balance and enhancing oxidative stress. Further studies are warranted to explore the underlying mechanisms and to identify potential protective strategies—such as antioxidant interventions—that could mitigate exercise-induced reproductive dysfunction.

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