

The effects of moderate-intensity endurance exercise combined with ginger or Maca on sperm DNA fragmentation index and spermatogenesis in male rats



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ABSTRACT

Introduction: This study was conducted to survey the effects of moderate-intensity endurance training (MIET) combined with ginger (*Zingiber officinale*) or Maca (*Lepidium meyenii*) on sperm DNA fragmentation index (DFI), oxidative damage, and spermatogenesis in male rats.

Methods: Forty-two male rats (220 ± 20 g, 10 to 12 weeks) were randomly divided into the control, MIET, Maca (100 mg/kg body weight), ginger (100 mg/kg body weight via gavage), MIET+ Maca, and MIET+ ginger. MIET protocol for 8 weeks began at a speed of 15 m/min for 10 min, and the speed and time were gradually increased to 28 m/min for 60 min. Testosterone level in serum, along with malondialdehyde (MDA) and DFI in testis, were measured using detection kits. The stage of spermatogenesis was also assessed using the Johnson scoring method under a light microscope.

Results: Testosterone level in serum significantly increased in the ginger, MIET+ginger, and MIET+Maca groups compared to the control group ($P < 0.05$). MDA and DFI significantly decreased in the MIET+ginger, MIET+Maca, and ginger groups compared to the control group ($P < 0.05$). The numbers of Sertoli cells, Leydig cells, spermatogonia, and spermatocytes were significantly increased in the MIET+ginger and MIET+Maca groups compared with the control group ($P < 0.05$).

Conclusion: It can be concluded that moderate endurance exercise combined with ginger or Maca supplementation reduced MDA and DFA levels and increased serum testosterone levels and spermatogenesis. These findings emphasize that lifestyle modifications, including physical activity and natural antioxidants, are low-cost and non-pharmacological self-care strategies for the management of male fertility.

Keywords:

Moderate-intensity endurance exercise
Ginger
Maca
DFI
Spermatogenesis

Introduction

The increasing rate of infertility in young couples is recognized as a global concern that many societies

consider one of the major crises facing them. Statistics show that more than 15 percent of couples are infertile, 50 percent of which is due to male infertility. It has nu-

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merous causes, including genetic and environmental factors, physiological disorders, varicocele, urogenital tract infections, and sedentary lifestyles (Bakri et al., 2025). In this lifestyle, a person is physically inactive and engages in little or no physical movement and/or exercise. Male infertility and sedentary are positively associated (Pacey 2010; Xu et al., 2022). Sedentary can also increase inflammatory cytokines and free radicals in seminal plasma and cause oxidative stress, which are closely associated with reduced sperm quality and male infertility (Kurkowska et al., 2020; Szabó et al., 2023). Male infertility can occur due to sperm abnormalities, impaired testosterone production, dysfunction of the accessory glands, and hormonal disorders of the hypothalamic-pituitary-testicular pathway (Sengupta and Cho 2019). Sperm abnormalities can be due to changes in spermatogenesis and due to dysfunction of Leydig and Sertoli cells of the seminiferous tubules (Houda et al., 2021). Male infertility can be diagnosed by semen analysis, along with a detailed medical and sexual history and a complete physical examination (Barratt et al., 2017). Recently, DNA Fragmentation Index (DFI) and Malondialdehyde (MDA) are being performed as indicators to measure the amount of damage to DNA in sperm and cell membrane, respectively (Dorostghoal et al., 2017). The normality of DNA in sperm, in addition to the successful transmission of genes to offspring, is of great clinical importance in the diagnosis of male infertility. It is believed that abnormal DNA, in addition to abnormal sperm function, can lead to failed fertilization, poor embryo quality, failed implantation, spontaneous abortion, congenital abnormalities, and childhood diseases (Agarwal et al., 2019).

Today, the main approach to improving reproductive function in men and women is lifestyle modification, including not smoking, avoiding excessive alcohol consumption, avoiding exposure to radio waves and radiation, diet modification (avoiding fast food), exercise, and natural antioxidants. Extensive studies have shown that moderate-intensity endurance exercise, among others, can be used as one of the methods to improve male infertility (Hajizadeh Maleki et al., 2022; Wise et al., 2011; Zaňko et al., 2025). It is believed that moderate-intensity exercise enhances semen quality, hormonal balance, and metabolic health, and positively influences male fertility (Song et al., 2025; Zaňko et al., 2025). Moderate physical activity also reduces inflammation

and oxidative stress, enhances cardiovascular functioning, and contributes to oxygen and nutrient supply to the reproductive organs (Zaňko et al., 2025). While many studies have demonstrated that moderate-intensity endurance exercise improves semen quality, some reports have shown no effect or detrimental effects at high intensities or prolonged durations (Wise et al., 2011). Thus, consensus on the optimal exercise prescription for male fertility has not yet been fully established.

In addition to moderate exercise, in recent years, the widespread use of medicinal plants and their products has increased due to their very high antioxidant power and richness in flavonoids, availability, low side effects, and reasonable price (Fathi et al., 2021). Medicinal plants and their derivatives, including ginger (*Zingiber officinale* Roscoe) and Maca (*Lepidium meyenii*), are believed to have pharmacological properties, including antioxidant, anti-diabetic, anti-inflammatory, and androgenic (Ahmed Mustafa et al., 2021; Khwanes et al., 2022; Li et al., 2021; Luo et al., 2022; Rubio et al., 2011). Ginger is also a potent metabolic modulator that can improve reproductive dysfunction caused by ethanol (Akbari et al., 2017; Li et al., 2021). Ginger or Maca has a wide range of activities to improve reproductive function (Alsamawi et al., 2026). Ginger or Maca can inhibit inflammation and oxidative reactions in testicular and sperm (Alsamawi et al., 2026; Li et al., 2021). Ginger can also improve the levels of LH, FSH, and testosterone hormones as well as semen quality, and increase sperm fertility (Li et al., 2021). Despite numerous reports on the ameliorating effects of ginger and/or Maca, no report has yet reported the effects of these two when combined with moderate endurance exercise on parameters related to reproductive performance. Moreover, the increasing prevalence of male infertility highlights the importance of research on interventions to improve reproductive health, with a strong focus on physical activity and the use of herbal supplements. Therefore, this study was conducted to survey the effects of moderate-intensity endurance training (MIET) combined with ginger or Maca on sperm DFI, oxidative damage, and spermatogenesis in male rats.

Materials and Methods

Animals and Study design

All experimental procedures were performed in accordance with the Guide for the Care and Use of Labo-

ratory Animals and reported according to the ARRIVE Guidelines for Reporting Animal Research (Kilkenny et al., 2010). Forty-two adult male Wistar rats (220 ± 20 g, 10-12 weeks) were purchased from Razi Institute, Amol, Iran. After one week of acclimatization to the laboratory environment (free access to food and tap water, 12/12-hour light/dark cycle, 40% humidity, temperature 23°C), rats were randomly divided into six groups: Control, ginger (100 mg/kg body weight via gavage), Maca (100 mg/kg body weight via gavage), moderate-intensity endurance training (MIET), endurance exercise + ginger, and endurance exercise + Maca.

Supplement preparation and exercise protocol

The fresh ginger rhizome was purchased from a herbal market, Babolsar, Iran. Identification of the ginger plant was made by the herbalist of Shiraz University of Medical Sciences, and a voucher specimen was deposited in the university herbarium (voucher number: PM-948). It was dried in an oven at 40°C for 2 days, then ground into powder and sieved with a mesh (0.2mm). Maca supplementation as a Maca root extract was purchased from Bulk Supplement.com (Lot Number 2114806, Henderson, NV89011, USA). Ginger or Maca was administered daily after exercise at a dose of 100 mg/kg body weight by gavage (1 ml/rat) for 8 weeks (Hammadi 2026; Lentz et al., 2007). A MIET protocol was used in this study (Alsamawi et al., 2026). To familiarize the animals with the training protocol, the rats ran on a treadmill for 10 minutes at a speed of 10 meters per minute, 3 days per week. Animals started the MIET protocol for 8 weeks at a speed of 15 meters per minute for 10 minutes, and the speed and time gradually increased to 28 m/min for 60 minutes by the end of the third week, and remained unchanged until the end of the eighth week. A 5-minute warm-up and a 5-minute cool-down were performed at a speed of 10 meters per minute before each training session and at the end of each training session.

Sampling

All animals were anesthetized 48 hours after the last training session and after 12 hours of fasting by intraperitoneal injection of a combination of ketamine (80–100 mg/kg body weight) and xylazine (8–10 mg/kg body weight). Blood samples were taken from the heart and incubated at 37°C for 15 minutes. After incubation, the blood samples were centrifuged (3000 rpm, 10 minutes),

and the serum was used for testosterone measurement. Testicular and epididymal tissues were also immediately separated after blood collection and placed in a formalin bottle (10%) for histological measurements and a nitrogen tank for biochemical evaluations, respectively. Epididymal tissue extract was prepared for DFI and MDA assays.

Testosterone assay

Testosterone levels were assayed in serum by Zell Bio GmbH Rat Testosterone detection kit (Germany, CAT No. ZB-102591-R9648). ZellBio GmbH Rat Testosterone ELISA kit is based on the principle of competitive binding between testosterone in the test specimen and testosterone enzyme conjugate for a constant amount of anti-testosterone polyclonal antibody. In the first step, biotin-conjugated anti-testosterone antibody binds to the avidin-coated wells for 60 minutes at room temperature. During this incubation, a fixed amount of HRP-labeled testosterone competes with the endogenous testosterone in the standards and sample for a fixed number of binding sites of the specific testosterone antibody. Testosterone peroxidase conjugates immunologically bound to the well progressively decrease as the concentration of testosterone in the specimen increases. Unbound testosterone peroxidase conjugate is then removed, and the wells are washed. Next, a solution of TMB reagent is added and incubated at room temperature for 20 min, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450 nm. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. Zell Bio GmbH Rat Testosterone determines testosterone in the sample of rat serum or plasma. The detection range is 0.1–12 ng/ml, and the minimum detectable level of testosterone is 0.06 ng/ml.

MDA assay

The MDA assay kit (ZellBio GmbH, Germany, CAT No. ZB-MDA-96A) was used to measure the amount of oxidative damage in the epididymis (sperm). This kit uses the MDA-TBA adduct formed by the reaction of MDA and Thiobarbituric acid (TBA) under high temperature. MDA is measured in acidic media and heat ($90\text{--}100^{\circ}\text{C}$) calorimetrically at 535 (530–540 nm). The detection range is 0.78–50 μM . ZellBio GmbH MDA assay kit can determine MDA in samples with 0.1 μM

sensitivity.

Sperm DFI Assay

DFI was assessed by the Sperm DNA Fragmentation Assay (SDFA) kit (Rawon Sazeh Knowledge-Based Co. CAT No. SDFA-MR-48). This kit provides a rapid, easy, and reproducible method for distinguishing damaged DNA from intact DNA, without the need for complex laboratory equipment. The SDFA kit is based on the denaturation of DNA and the removal of proteins present in the nucleus of each sperm. In this case, normal sperm produce halos formed by DNA loops in the sperm head, which are absent in sperm with damaged DNA. In other words, sperm with fragmented DNA are unable to produce the characteristic halo of dispersed DNA loops. In addition to being a prognostic test for in vitro and in vivo fertilisation, DFI can also be a diagnostic tool for evaluating therapeutic interventions. Briefly, in the first step, the sperm sample was prepared: spermatozoa were drained from the caudal epididymis into a Petri dish containing PBS buffer and washed twice with PBS buffer. Then, a suspension containing 15 to 20 mL of sperm solution was prepared with PBS buffer. After that, 60 µL of prepared sperm from step 1 was added to an agarose tube, mixed well, and maintained at 37 °C (step 2). Then 15 - 20 µL of the sperm-agarose sample from step 2 was dropped onto a slide. The slide was kept on a precooled (5 minutes) Tray at 2 - 8 °C for 5 minutes (step 3). The slide was placed horizontally in the staining tray, and 8 to 10 mL of reagent-I was added to it so that it was completely immersed in the reagent for 7 minutes (step 4). Following step 4, the slides were placed horizontally in a staining tray and completely immersed in 8–10 mL of Reagent II for 20 minutes (step 5). They were then immersed in 8–10 mL of distilled water for 5 minutes. Subsequently, the smears were fixed and stained using the Wright staining method. Finally, the slides were air-dried, and 100–200 spermatozoa per smear were examined under a light microscope using a 40× objective. Tailed sperm were first identified, and their heads were evaluated as follows: A. Sperm with halo. B. Sperm without halo. C. Degraded sperm (sperm are smaller than normal size). The DFI (DNA Fragmentation Index) was calculated using the following formula: $DFI = [(Y + Z)/S] \times 100$, $S = X + Y + Z$. S: Number of sperm evaluated, X: Number of sperm with Non-fragmented DNA, Y: Number of sperm with fragmented DNA, and

Z: Degraded sperm.

Histological examination

Tissue sections were prepared by a pathologist who was unaware of the study process in the specialized pathology laboratory of Rouhani Hospital, Babol, Iran. After fixation, the tissues were embedded in liquid paraffin and then sectioned using a microtome at a thickness of 5 to 7 µm. The sections were placed on slides and stained with hematoxylin and eosin. Each slide contained three consecutive sections. Key parameters for the histology of the testis and spermatogenesis included the diameter of the seminiferous tubules, the thickness of the epithelial layer, the lumen diameter, the density and organization of spermatogenic cells, Leydig and Sertoli cells, and the presence of spermatids and mature sperm in the lumen of the seminiferous tubules, respectively. The diameter of the seminiferous tubules represents the overall size of the tubule, which can be affected by various factors. The thickness of the epithelial layer indicates the number and density of developing spermatogenic cells. An increase in thickness usually indicates improved spermatogenesis. The diameter of the lumen represents the space inside the tubule. A decrease in the diameter of the lumen and an increase in the thickness of the epithelial layer indicate increased sperm production. These variables were measured as parameters of histological examination of testicular tissue in five seminiferous tubules for each section using ImageJ software. Stages of spermatogenesis were examined under a light microscope (Bassler microscope, German, x 40) by assessing the density and organization of spermatogonia, spermatocytes, Leydig cells, and Sertoli cells in the lumen of the seminiferous tubules using the Johnson scoring system (Johnsen 1970). The Johnson scoring system evaluates the stages of spermatogenesis on a scale of one to ten. 1. neither germ cells and no Sertoli cells; 2. no germ cells; 3. only spermatogonia; 4. only a few spermatocytes; 5— many spermatocytes but no spermatozoa or spermatids; 6—only a few spermatids; 7. many spermatids but no spermatozoa; 8. only a few spermatozoa; 9. many spermatozoa but disorganized spermatogenesis; 10. spermatogenesis and tubules are observed completely and perfectly.

Statistical Analysis

Data analysis was performed using SPSS software

TABLE 1: The mean $\pm$ SD of testosterone in serum and MDA and DFI in epididymis. *** $P < 0.001$ and **** $P < 0.0001$ show a significant difference vs. the control group. # $P < 0.05$ and ### $P < 0.001$ show a significant difference vs. the Endurance Exercise-Maca group. \$\$\$ $P < 0.001$ show a significant difference vs. Endurance Exercise-Ginger group.

Group/Factor	Testosterone (nmol/L)	MDA ($\mu\text{mol/mL}$)	DFI (%)
Control	1.21 $\pm$ 0.14	1.84 $\pm$ 0.32	26.23 $\pm$ 2.87
Maca	1.46 $\pm$ 0.16#	1.51 $\pm$ 0.15###	23.32 $\pm$ 2.01###
Ginger	1.86 $\pm$ 0.21****\$\$\$	1.32 $\pm$ 0.26***	21.54 $\pm$ 2.21***\$\$\$
Endurance Exercise	1.42 $\pm$ 0.15####\$\$\$	1.42 $\pm$ 0.36\$\$\$	25.14 $\pm$ 1.33####\$\$\$
Endurance Exercise-Ginger	1.95 $\pm$ 0.16****	1.01 $\pm$ 0.34****	16.95 $\pm$ 2.18****
Endurance Exercise-Maca	1.76 $\pm$ 0.12****	1.10 $\pm$ 0.41****	17.65 $\pm$ 2.06****

(version 21). The Shapiro–Wilk test was used to assess the normality of data, and Levene’s test was applied to evaluate the homogeneity of variances. One-way analysis of variance (ANOVA) followed by the least significant difference (LSD) post hoc test was used to compare differences among the experimental groups. Two-way analysis of variance was used to examine the separate and interactive effects of moderate endurance exercise, ginger, and maca supplementation. Effect sizes were calculated using Cohen’s d for ANOVA models (Lenhard and Lenhard 2022). Ranges of interpretation for different effect sizes were 0-0.1: No effect size, 0.2-0.4: Small effect, 0.5-0.7: Intermediate effect, 0.87-1: Large effect, >1 : Very large effect. The significance level was set at $P < 0.05$, and the results were reported as mean $\pm$ standard deviation (SD).

Results

Biochemical results

The results of testosterone in serum and MDA and DFI in the epididymis are reported in Table 1. As shown, serum testosterone levels were significantly increased in the ginger ($p = 0.007$, $d = 3.642$), moderate endurance exercise-Maca ($p = 0.0001$, $d = 4.218$), and moderate endurance exercise-ginger ($p = 0.0001$, $d = 4.922$) groups compared to the control group (Table 1). Compared to the control group, the largest effect sizes were for the moderate endurance exercise-ginger ($d = 4.922$), moderate endurance exercise-Maca ($d = 4.218$), ginger ($d = 3.642$), Maca (F ($d = 1.663$), and moderate endurance exercise ($d = 1.447$) groups, respectively. The results of two-way ANOVA showed that the effect of Maca (F (1, 24) = 26.02, $p = 0.0001$) and the effect of moderate endurance

exercise (F (1, 24) = 19.44, $p = 0.0002$) were statistically significant, but the interaction effect (F (1, 24) = 0.6056, $p = 0.446$) was not significant. Moreover, the results of two-way ANOVA showed that the effect of ginger (F (1, 24) = 88.15, $p = 0.0001$) and the effect of moderate endurance exercise (F (1, 24) = 5.698, $p = 0.0252$) were statistically significant, but the interaction effect (F (1, 24) = 0.9117, $p = 0.349$) was not significant. The results of the one-way ANOVA analysis showed that serum testosterone levels increased significantly in the moderate endurance exercise- Maca ($p = 0.0001$), moderate endurance exercise- ginger ($p = 0.0001$), and ginger ($p = 0.0001$) groups compared to the control group. The results also showed that there was a significant difference between the moderate endurance exercise- Maca group, the Maca group ($p = 0.001$) and the moderate exercise group ($p = 0.004$). Moreover, a significant difference between the moderate endurance exercise group, the moderate endurance exercise-ginger group ($p = 0.0001$) and the ginger group (0.0001) was observed.

In addition, MDA levels were significantly reduced in the ginger ($p = 0.007$, $d = 1.784$), moderate endurance exercise-Maca ($p = 0.0001$, $d = 2.012$), and moderate endurance exercise-ginger ($p = 0.0001$, $d = 2.437$) groups compared to the control group (Table 1). Compared to the control group, the greatest changes and largest effect sizes were for the moderate endurance exercise-ginger ($d = 2.437$), moderate endurance exercise-Maca ($d = 2.012$), ginger ($d = 1.784$), Maca ($d = 1.321$), and moderate endurance exercise ($d = 1.313$) groups, respectively. The results of two-way ANOVA showed that the effect of Maca (F (1, 24) = 6.874, $p = 0.014$) and the effect of moderate endurance exercise (F (1, 24) = 11.32,

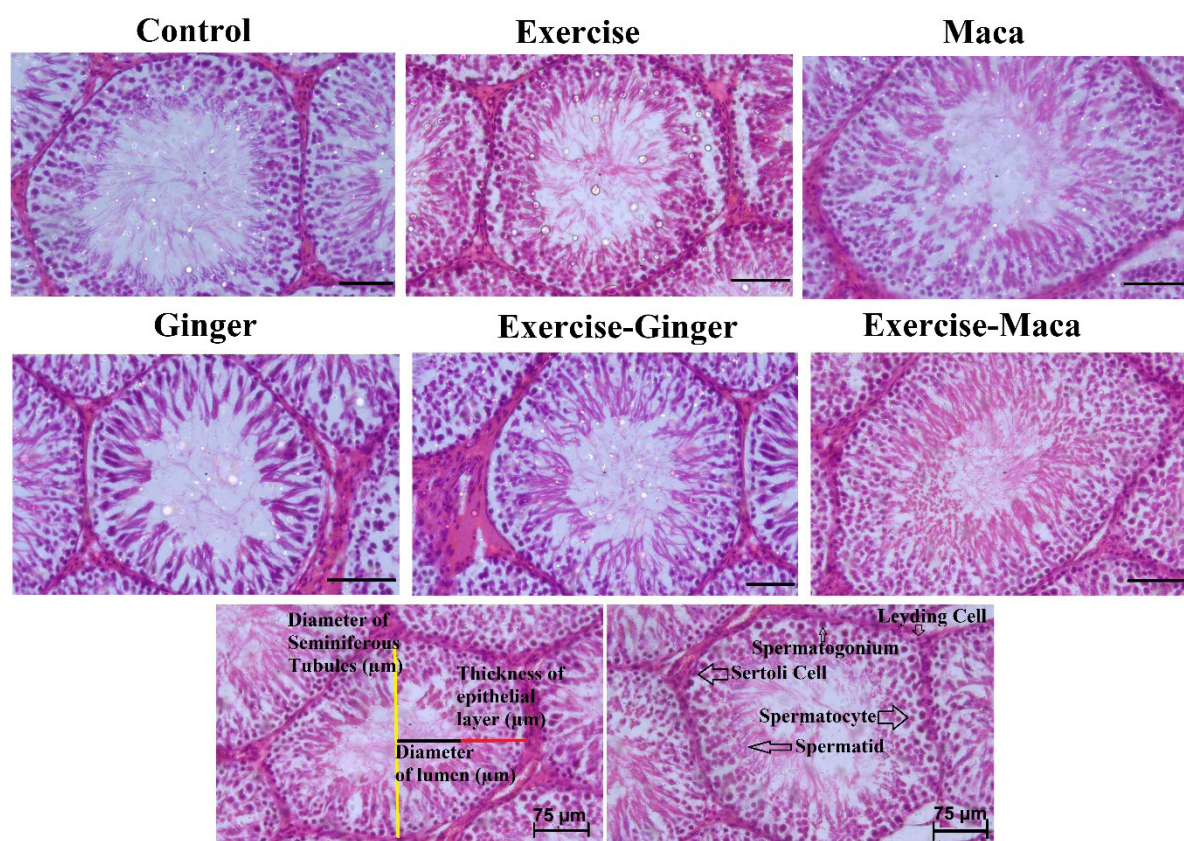


FIGURE 1. Histological examination with light microscopy (40× magnification) showed that moderate endurance exercise combined with ginger or Maca consumption for 8 weeks has an effective role in improving the reproductive status of testicular tissue in rats. The examination clearly showed that significant changes have occurred in the organization of the seminiferous tubule and in the number of germ cells and Sertoli cells in this tissue compared to the control group. The scale bar is 75 µm.

$p=0.0026$) were statistically significant, but the interaction effect ($F(1, 24) = 0.0002$, $p=0.986$) was not significant. Moreover, the results of two-way ANOVA showed that the effect of ginger ($F(1, 24) = 14.21$, $p=0.0009$) and the effect of moderate endurance exercise ($F(1, 24) = 0.9289$, $p=0.007$) were statistically significant, but the interaction effect ($F(1, 24) = 0.1931$, $p=0.6643$) was not significant. The results of one-way ANOVA analysis showed that MDA levels decreased significantly in the moderate endurance exercise- Maca ($p=0.0003$), moderate endurance exercise- ginger ($p=0.0001$), and ginger ($p=0.006$) groups compared to the control group. The results also showed that there was a significant difference between the moderate endurance exercise- Maca group and the Maca group ($p=0.025$). Moreover, a significant difference between the moderate endurance exercise- ginger group and the moderate endurance exercise group ($p=0.027$) was observed.

DFI levels were significantly reduced in the mod-

erate endurance exercise-Maca ($p=0.0001$, $d=2.012$) and moderate endurance exercise-ginger ($p=0.0001$, $d=2.437$) groups in comparison to the control group (Table 1). Compared to the control group, the largest effect sizes were for the moderate endurance exercise-ginger ($d=2.437$), moderate endurance exercise-Maca ($d=2.012$), ginger ($d=1.784$), Maca ($d=1.321$), and moderate endurance exercise ($d=1.313$) groups, respectively. The results of two-way ANOVA showed that the effect of Maca ($F(1, 24) = 41.15$, $p=0.0001$), the effect of moderate endurance exercise ($F(1, 24) = 17.39$, $p=0.0003$), and the interaction effect ($F(1, 24) = 7.98$, $p=0.009$) were statistically significant. Moreover, the results of two-way ANOVA showed that the effect of ginger ($F(1, 24) = 58.89$, $p=0.0001$), the effect of moderate endurance exercise ($F(1, 24) = 11.45$, $p=0.0025$), and the interaction effect ($F(1, 24) = 4.38$, $p=0.047$) were statistically significant. The results of one-way ANOVA analysis showed that DFI levels decreased significantly

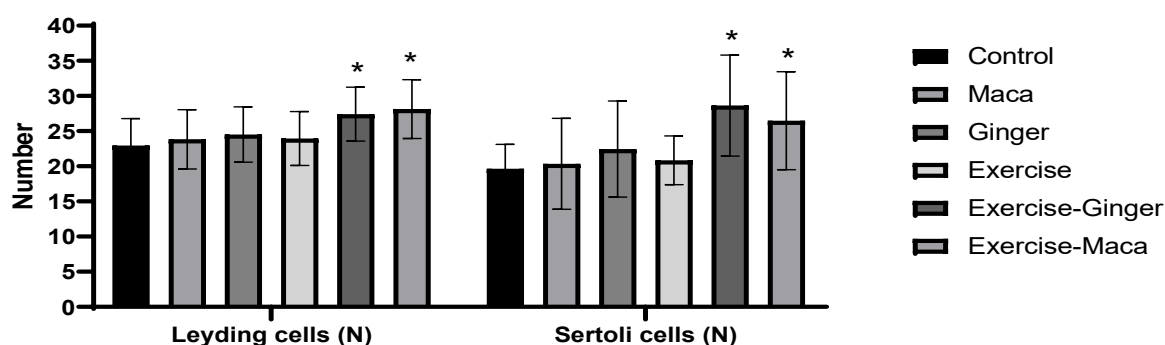


FIGURE 2. The means $\pm$ SD of the number of Leydig cells and Sertoli cells in the different groups. * $P < 0.05$ vs. the control group.

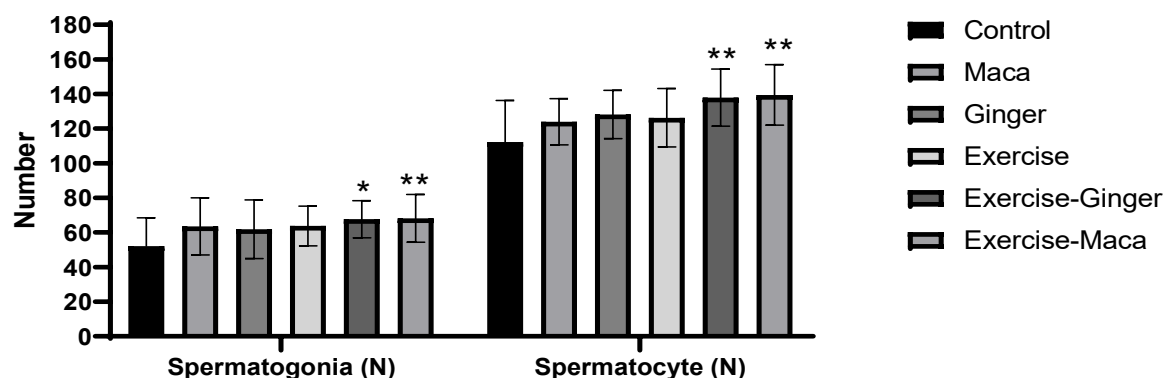


FIGURE 3. The means $\pm$ SD of the number of spermatogonia and spermatocytes in the different groups. * $P < 0.05$ and ** $P < 0.01$ vs. the control group.

in the moderate endurance exercise- Maca ($p=0.0001$), moderate endurance exercise- ginger ($p=0.0001$), ginger ($p=0.0006$), and Maca ($p=0.018$) groups compared to the control group. The results also showed that there was a significant difference between the moderate endurance exercise-Maca group, the Maca ($p=0.0001$) and moderate endurance exercise ($p=0.001$) groups. Moreover, a significant difference between the moderate endurance exercise-ginger group and the moderate endurance exercise group ($p=0.0001$) and the ginger group ($p=0.0007$) was observed.

Histological results

Histological examination showed that the number of Sertoli cells, Leydig cells, spermatogonia, and spermatocytes significantly increased in the moderate endurance exercise-ginger and moderate endurance exercise-Maca groups compared to other groups ($P < 0.05$). Although an increasing trend was observed in the num-

ber of spermatogonia and spermatocytes in the moderate endurance exercise, ginger and Maca groups compared to the control group, no significant differences were observed among them ($P > 0.05$) (Figures 1 and 2). The results showed a significant increase in the thickness of the epithelial layer and a decrease in the lumen diameter of the seminiferous tubules in the moderate endurance exercise- ginger- and moderate endurance exercise-Maca groups compared to the control group ($P < 0.05$, Figure 3). The diameter of the seminiferous tubules did not have a significant change in the moderate endurance exercise-Maca/ginger groups compared to the control group. While the the Johnson score had a significant change in these groups compared to the control group ($P > 0.05$, Figure 3 and 4).

Discussion

This study updates our understanding of the effects of interventions such as moderate-intensity endurance ex-

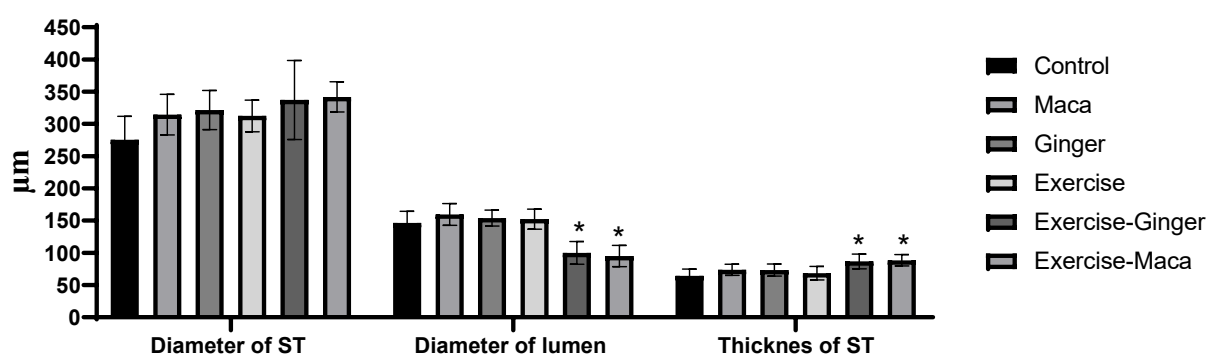


FIGURE 4. The means $\pm$ SD of the diameter of the seminiferous tubule (ST), the thickness of the epithelial layer of ST, and the diameter of the lumen of ST in the different groups. * $P < 0.05$ vs. the control group.

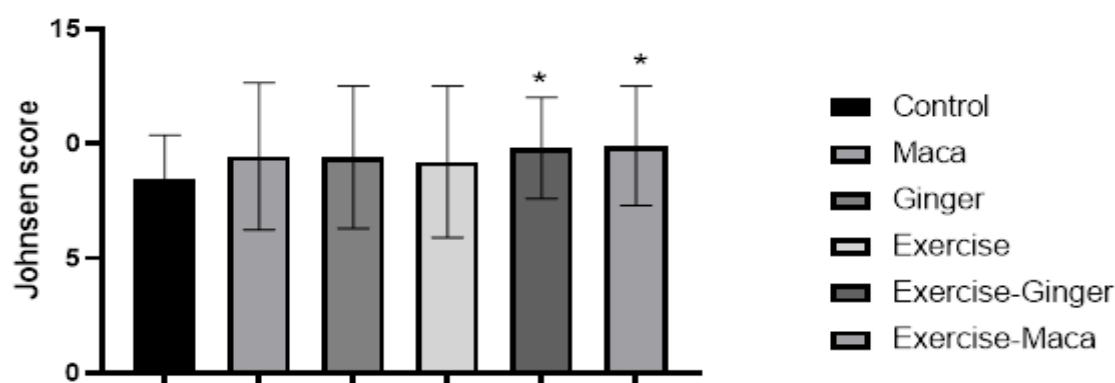


FIGURE 5. The means $\pm$ SD of the Johnsen score in the different groups. * $P < 0.05$ vs. the control group.

ercise, ginger, Maca supplementation, and the combination of exercise with Maca or ginger on testicular tissue activity, lipid peroxidation levels, sperm DNA integrity levels, and stages of spermatogenesis in sedentary rats. Our results showed that ginger supplementation alone and moderate-intensity endurance exercise combined with ginger or Maca supplementation for 8 weeks could increase testosterone and decrease MDA and DFI, which is in agreement with previous studies (Abdelkhalek et al., 2026; Alsamawi et al., 2026; Parsa et al., 2025; Xie et al., 2025). In line with our results, Alsamawi et al., (2026) reported that consuming ginger or Maca with endurance training could enhance the antioxidant system in testicular tissue and increase testosterone (Alsamawi et al., 2026). Abdelkhalek et al., (2026) reported that oral consumption of 60 mg/kg of body weight of Maca in nano-emulsion form twice a week for ten weeks could improve testosterone levels and reduce MDA (Ab-

delkhalek et al., 2026). Parsa et al., (2024) reported that feeding Japanese quail with ginger extracts (350 mg/kg) improved antioxidant status of the body, the concentration of testosterone, and spermatogenesis, followed by an increase in sperm production, fertility, and hatchability in quail eggs (Parsa et al., 2025). These results clearly indicate that the improvement of the cellular oxidative status (reduction in MDA) can be directly related to the increase in testosterone levels. The improvement of the cellular oxidative status is a relatively complex situation that occurs due to an imbalance in the pathways that lead to the production of free radicals and the mechanisms responsible for their clearance. The mechanisms responsible for the removal of free radicals are mainly the enzymatic and non-enzymatic antioxidant systems. These mechanisms are known as modifiable mechanisms that can be strengthened through the use of nutritional or pharmaceutical supplements and performing

moderate endurance exercise. Previous studies showed that ginger, Maca, and moderate endurance exercise could improve the enzymatic and non-enzymatic antioxidant systems (Li et al., 2021; Orhan et al., 2022; Xie et al., 2025). In our results, we observed that combining exercise with Maca or ginger, and ginger supplementation alone, improved the oxidative status of testicular tissue. This could be due to the anti-inflammatory and antioxidant effects of exercise and ginger supplementation. NF- κ B and Nrf2, which are involved in regulating oxidative stress and inflammation, could influence steroidogenesis and semen quality (Li et al., 2021; Xue et al., 2022). Ginger or moderate exercise could enhance Nrf2 signalling and inhibit NF- κ B signalling (Ahmed Mustafa et al., 2021; Fathi et al., 2021; Nemmar et al., 2018; Zhang et al., 2024). In addition to strengthening the antioxidant system, they could improve the process of steroidogenesis in testicular tissue and testosterone production, processes that have been well described by previous studies (Abdelkhalek et al., 2026; Alsamawi et al., 2026; Helalizadeh et al., 2020; Li et al., 2021; Ross et al., 2024). Li et al., (2021) showed that ginger increases StAR, P450scc and 17 β HSD expression, and directly increased steroidogenesis and the production of testosterone (Li et al., 2021). Banihani reported that ginger improves production of testosterone by enhancing the entry of cholesterol and recycling testosterone receptors, and inhibiting oxidative damage in the testes and the production of LH (Banihani 2019;2018). Ghareib et al., (2015) also indicated that ginger stimulates the cGMP pathway and enhances the production of NO, which increases blood flow in the testis (Ghareib et al., 2015). Mohammadi et al., (2014) showed that ginger extract increases the testosterone concentration and the number of spermatogonial and Sertoli cells in seminiferous tubules (Mohammadi et al., 2014). Moreover, ginger could ameliorate ethanol-induced testicular damage by improving homeostasis of the trace elements and total homocysteine (Akbari et al., 2017), the pituitary-gonadal axis (Li et al., 2021), inhibiting oxidative damage in the testis, and improving semen quality (Banihani 2019;2018). Based on these findings, it can be concluded that androgenic activity and strengthening of antioxidant systems by ginger and moderate endurance exercise can lead to improved steroidogenesis (testosterone production). This situation can also play an effective role in improving the tissue structure of the seminiferous tubules and

spermatogenesis (sperm production) of the testis, which we observed in our results.

Histological examination showed that moderate endurance exercise combined with Maca or ginger supplementation for 8 weeks played a significant role in improving the thickness of the epithelial layer, the diameter of the lumen of the seminiferous tubules, and the density and organization of spermatogenic cells along with Leydig cells and Sertoli cells in the lumen of the seminiferous tubules. An increase in thickness of the epithelial layer and a decrease in lumen diameter of the seminiferous tubules indicate an increased number and density of spermatogenic cells and improved spermatogenesis. In agreement with our results, Hammadi (2026) reported that ginger supplementation as an androgenic and fertility-promoting agent in a dose-dependent (100, 200, and 400 mg/kg) manner increased serum testosterone and LH levels, reduced abnormal sperm morphology, and improved organization and structure of the seminiferous tubules and sperm quality. Their histopathological examination showed that the spermatogenesis cycle was regular and sperm accumulation was observed in the lumen of the seminiferous tubules in all ginger groups (100, 200 and 400 mg/kg) (Hammadi 2026). Funmilola and Ayodeji (2025) reported that raw and boiled ginger improved sperm quality. Raw and boiled ginger also increased 3 β - and 17 β -hydroxysteroid dehydrogenase, lactate dehydrogenase, and glucose-6-phosphate dehydrogenase activities, as well as FSH, LH, and testosterone levels. In addition, both raw and boiled ginger improved organization and structure of the seminiferous tubule and increased Leydig cells and sperm cells while decreasing adipocyte size in the histological architecture of the testis, epididymis, and epididymal fat (Funmilola and Ayodeji 2025). Zhou et al., (2023) reported that Maca can improve spermatogenic function at normal physiological levels, or when the spermatogenic disorder is not severe enough (Zhou et al., 2023). Shukur and Hilal (2025) showed that moderate endurance exercise improved testosterone production and semen quality by enhancing antioxidant and micronutrient levels in obese men (Shukur and Hilal 2025). Akbari and Gaeini showed that moderate exercise training can be used as an effective strategy to reduce the harmful effects of mobile phone waves on semen quality (Akbari and Gaeini 2021). This effect could possibly be due to increased blood circulation in the genital areas

and changes in temperature and availability of antioxidants in this area (Akbari et al., 2019). Alsamawi et al., (2026) showed that moderate endurance exercise combined with Maca or ginger could improve testosterone production, spermatogenesis, and the organization and structure of the seminiferous tubule (Alsamawi et al., 2026). Ginger and Maca have a wide range of biological activities, including anti-inflammatory, antioxidant, anti-obesity, and sexual enhancement activities, due to the presence of bioactive compounds such as phytosterols, alkaloids, isothiocyanates, glucosinolates, macaene, and macaamides in maca (Rubio et al., 2011) and zingerone, 6-shogaol, [6], [8], and [10] gingerol in ginger (Alsamawi et al., 2026; Xue et al., 2022). These compounds can directly eliminate free radicals and indirectly increase the expression of antioxidant enzymes and inhibit oxidative chain reactions in testicular tissue and sperm (Li et al., 2021; Xue et al., 2022; Zhang et al., 2024). In addition, they can improve the level of gonadotropin hormones (LH and FSH) and, as a result, testosterone production and improve organization of spermatogenic cells in the seminiferous tubule and sperm quality (Akbari et al., 2017; Li et al., 2021). According to the results of these studies, it can be concluded that moderate endurance exercise combined with Maca or ginger may improve the organization and structure of the seminiferous tubule and increase the number of spermatogenic cells in the seminiferous tubule due to the enhancement of antioxidant systems and androgenic activity.

Our results showed that sperm DFI improved with ginger supplementation and moderate endurance exercise combined with ginger or Maca supplementation, which is in agreement with previous studies (Abdelkhalek et al., 2026; Alsamawi et al., 2026; Gill et al., 2019; Kurkowska et al., 2020; Parsa et al., 2025). Gill et al., (2019) reported that “there is an association between sedentary work and conventional semen characteristics, revealing a detrimental effect of seated work on sperm nuclear DNA integrity. A sedentary job doubled the risk of high levels of sperm DNA damage. The pathomechanism could be related to testicular heat stress resulting in sperm chromatin remodelling failure during spermiogenesis” (Gill et al., 2019). Kurkowska et al., (2020) reported that male infertility and sedentary are positively associated, with sedentary being associated with decreased sperm quality parameters and an increased risk of sperm DFI (Kurkowska et al., 2020). It

can also increase inflammatory cytokines and free radicals in seminal plasma and cause oxidative stress, which is closely associated with sperm DNA damage and reduced sperm quality (Kurkowska et al., 2020; Szabó et al., 2023). Pakpahan et al., (2025) showed that changes in MDA levels, as a byproduct of lipid peroxidation, can be associated with DNA fragmentation (Pakpahan et al., 2025). Moreover, Noegroho et al., (2022) reported that antioxidant supplementation can counteract against oxidative stress and improve the spermatogenesis process, reflected by a decrease in DFI, improvement of sperm parameters, and elevation of pregnancy rates confirmed by the included studies (Noegroho et al., 2022). Our results showed that MDA levels, as an indicator of sperm oxidative damage, decreased with ginger, exercise-ginger, and exercise-Maca, and there was a strong and positive correlation between MDA levels and DFI. In line with our results, Robertson et al., (2024) reported that sperm DNA fragmentation and its oxidative damage play a major role in male infertility, and although its relationship with the success of natural fertilization, implantation, and even the use of assisted reproductive technology (ART) has been partially determined (Robertson et al., 2024). Pakpahan et al., (2025) also reported that free radicals as agents of oxidative stress can damage the sperm plasma membrane and compromise DNA integrity, and there was a direct correlation between high DFI and poor sperm morphology, such that sperm with abnormal morphology had high DFI and vice versa. They also showed that head malformations had a stronger correlation with DFI compared with neck and tail malformations (Pakpahan et al., 2025). These results clearly indicate that high DFI is related to abnormal sperm morphology, and it can be a consequence of oxidative damage in testicular tissue and sperm (Pakpahan et al., 2025; Sengupta et al., 2024). Moreover, it seems logical and necessary to simultaneously perform routine seminological analyses and assess the oxidative damage and integrity status of sperm DNA during the diagnosis of male infertility.

Conclusion

It can be concluded that moderate endurance exercise combined with ginger or maca supplementation reduces MDA and DFA levels and increases serum testosterone levels. Improving antioxidant status and increasing testosterone production by ginger or maca supplementa-

tion and endurance exercise combined with ginger or maca supplementation can improve the organization of spermatogenic cells in the seminiferous tubules and increase spermatogenesis. These findings emphasize that lifestyle modifications, including physical activity, a proper diet, and the consumption of herbal products and natural antioxidants, are low-cost and non-pharmacological self-care strategies for the management of male infertility.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Ethics approval

All experimental procedures were performed in accordance with the ethical guidelines for the care of laboratory animals and approved by the Ethics Committee of the University of Mazandaran (Ethics Code: IR.UMZ.REC.1404.004).

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