Received: 29 October 2018 | Revised: 16 February 2019 | Accepted: 19 February 2019

DOI: 10.1111/and.13273

ORIGINAL ARTICLE

WILEY aNDROLOGIa First International Journal of Andrology

Effect of aerobic exercise, low-fat and high-fat diet on the testis tissue and sperm parameters in obese and nonobese mice model

Arezou Nematollahi[1,2] | Fatemeh Kazeminasab[3] | Marziyeh Tavalaee[2] | Sayed M. Marandi[3] | Kamran Ghaedi[4,5] | Mohammad N. Nazem[6] | Mohammad Hossein Nasr-Esfahani[2]

[1]ACECR Institute of Higher Education, Isfahan, Iran

[2]Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran

[3]Department of Exercise Physiology, Faculty of Sport Sciences, University of Isfahan, Isfahan, Iran

[4]Department of Cellular Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran

[5]Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran

[6]Department of Basic Sciences, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

Correspondence
Mohammad Hossein Nasr-Esfahani, Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran.
Email: mh.nasr-esfahani@royaninstitute.org

Funding information
Royan Institute

Abstract

Semen quality and male fertility depend on numerous factors such as age, environment, lifestyle, physical activity, genetic background and occupation. We aimed to access the effect of aerobic exercise, low- and high-fat diet on mice testis tissue, and sperm function. Obese and nonobese male mice C57BL/6 were exposed to high fat (Hf) or low fat (Lf) and/or activity (Exe: exercise or Sed: sedentary). Finally, testicular morphometric characteristics, sperm concentration and motility (light microscopy), sperm morphology (eosin/nigrosin dye), lipid peroxidation (BODIPY C11 Probe), chromatin (acridine orange and chromomycin A3 staining) were compared within obese groups (Hf/Exe, Lf/Exe, Lf/Sed, Hf/Sed) and nonobese groups (Hf/Exe, Lf/Exe, Lf/Sed, Hf/Sed). Both exercise and diet interventions did not show any alteration in testicular morphological characteristics, sperm morphology and DNA fragmentation within both obese and nonobese groups ($p > 0.05$). Exercise and/or diet resulted in a significant increase in sperm concentration and motility within both groups ($p < 0.05$). Exercise in both groups leads to high percentage of lipid peroxidation ($p < 0.05$). Exercise intervention significantly improved sperm protamine deficiency within obese group ($p < 0.05$). We concluded that exercise intervention was more effective than diet in improvement of sperm function within obese groups.

KEYWORDS
diet, DNA damage, exercise, lipid peroxidation, sperm parameters

1 | INTRODUCTION

Reproductive health is highly jeopardised by obesity which has a genetic, epigenetic and socio-environmental bases (Craig, Jenkins, Carrell, & Hotaling, 2017). Among social–environmental bases or lifestyle habits, overconsumption of high-energy diets that rich in sugars and saturated fats along with low physical activity could have negative feedback on male fertility potential. In addition, environmental pollutant can adversely affect various parameter of adipose tissue functions (Cardoso et al., 2017). Most of these compositions are lipophilic and can disrupt male reproductive function due to high lipid content of this tissue which in turn affect testis physiology especially testicular metabolism and germ cell development, and ultimately reduces sperm quality (Cardoso et al., 2017; Skakkebaek, Rajpert-De Meyts, & Main, 2001).

Previous studies showed that fatty acid composition of spermatozoa is associated with semen quality and male fertility potential.

Three types of natural fatty acids including saturated, monounsaturated and polyunsaturated are present in human body, and among them, polyunsaturated fatty acids cannot be synthesised and are obtained from diet. In this regard, it has been shown that mean of docosahexaenoic acid (DHA), as a polyunsaturated omega-3 fatty acid, was significantly lower in infertile men with astheno- and/or oligoasthenoteratozoospermia than normozoospermia (Khosrowbeygi & Zarghami, 2007). In addition, positive significant correlation exists between DHA and sperm parameters, and also a positive significant correlation has been reported between DHA and sperm DNA fragmentation (Andersen et al., 2016). Recently, González-Ravina et al., (2018) showed that DHA supplementation can improve sperm quality in asthenozoospermic men . Several studies demonstrated that high-energy diets are related to high production of reactive oxygen species (ROS), and consequently oxidation of lipid, DNA and proteins. Sperm cell due to low amount of cytoplasm and high level of unsaturated lipids is prone to ROS-induced DNA damage. Indeed, it has been shown saturated fatty acids (SFAs) play an important role in activation of Toll-like receptors (TLRs) involved in inducing inflammation, while polyunsaturated fatty acids (PUFAs) inhibit such an activation (Hwang, Kim, & Lee, 2016). Therefore, change in ratio of PUFA: SFA can induce activation of TLR4 receptor and induces a state of inflammation. In this regard, decreased PUFA: SFA ratio was associated with asthenozoospermia (Tavilani, Doosti, Abdi, Vaisiraygani, & Joshaghani, 2006) likely due to excessive ROS production. Excessive production of ROS is accounted as one of the main aetiologies of infertility (Keltz et al., 2010; Oliveira, Sousa, Silva, Monteiro, & Alves, 2017).

Energy control in testis is essential to promote normal spermatogenesis while obesity disrupts glucose and energy metabolism in this tissue (Oliveira et al., 2017). In addition to increase in white adipose tissue by exposure to obesogens, these compositions can enhance oestrogenic and adipogenic pathways. In this regard, Cardoso et al., (2017) stated that aromatase enzyme in the adipocytes increases and converts testosterone (T) to 17β-oestradiol (E2). Increased E2 stimulates a negative feedback at the hypothalamus–pituitary–testis axis and decreases secretion level of luteinising hormone (LH) and follicle-stimulating hormone (FSH). Low concentration of testosterone can disrupt normal spermatogenesis (Cardoso et al., 2017). In this regard, aromatase inhibitor which reduces adipocyte oestradiol production can improve semen parameters and fertility rate in obese infertile individuals (Schlegel, 2012).

Health promotion through exercise and physical activity, as an important lifestyle factor, has been recognised to delay some pathological situations such as stroke, myocardial infarction, diabetes and cancers (Gomes, Freitas, & Fardilha, 2015). An increasing number of research studies demonstrated the effect of exercise training in male reproductive system and suggested that moderate exercise has a beneficial effect on fertility potential, while acute (strenuous) physical activity such as professional cycling can have a deleterious effect on fertility potential (Hajizadeh Maleki & Tartibian, 2015; Vaamonde et al., 2009). In this regard, Palmer, Bakos, Owens, Setchell, & Lane, (2012) showed that diet and exercise could improve abnormal sperm physiology in mice with high-fat diet. In addition, unlike sperm concentration and morphology, a significant correlation was observed between prudent dietary patterns and sperm motility, while this correlation was not found between sperm parameters and a Western dietary pattern (Gaskins, Colaci, Mendiola, Swan, & Chavarro, 2012). According to the aforementioned literature, the aim of this study was to assess the impact of lifestyle (diet and exercise) on sperm quality, testicle morphometric characteristics and testis tissue in male mice C57BL/6.

2 | MATERIALS AND METHODS

This study received approval of the Institutional Review Board from the Royan Institute (NO: 95000212), and all the use and care of animals were in accordance with the Animal Ethics Committee of Royan Institute. All the mice were kept in a temperature-controlled (20–23°C) room, moisture of 50 ± 3% in special cages and a 12-hr light/12-hr dark photocycle. Food and water were provided ad libitum. In this study, in continuation of Kazeminasab et al., (2018) study, testis of 48 mice (male C57BL/6) belonging to eight groups was used. A protocol with two interventions (long-term high-fat diet and exercise and diet) were used according to Kazeminasab et al. (2018).

2.1 | First intervention

Initially, 48 mice were randomly assigned to one of the two diets for a period of 12 weeks: (a) low-fat diet (LF) (10% kcals from fat, 70% kcal from carbohydrate and 20% kcal from protein with 23% saturated, 30% monounsaturated and 47% polyunsaturated); (b) high-fat diet (HF) (45% kcals from fat, 35% kcal from carbohydrate, the 20% kcal from protein with 32% saturated, 35% monounsaturated and 33% polyunsaturated). Mice with high-fat diet displayed extreme states of obesity-based weighed gain (high fat vs. low fat: 32.22 ± 0.62 g vs. 23.1 ± 0.39 g, $p < 0.01$). The mean ratio of visceral fat to body weight was also significantly higher in HF compared to LF groups (5.32 ± 0.77% vs. 4.51 ± 0.75%) (Bagnol, Al-Shamma, Behan, Whelan, & Grottick, 2012). Therefore, mice in the first and second groups were termed nonobese (LF) and obese (HF) respectively. All animals had free access to water and food during the whole length of the protocol (Figure 1).

2.2 | Second intervention

After 12 weeks (at the beginning of the week 13), nonobese mice were randomly assigned to four groups with six mice in each group: (a) high-fat diet, exercise-trained (HF-E); (b) high-fat diet, sedentary (HF-S); (c) low-fat, exercise-trained (LF-E); and (d) low-fat diet, sedentary (LF-S). Also, obese mice were randomly divided into four groups with six mice in each group: (a) high-fat diet, exercise-trained (HF-E); (b) high-fat diet, sedentary (HF-S); (c) low-fat diet, exercise-trained (LF-E); and (d) low-fat diet, sedentary (LF-S) (Figure 1).

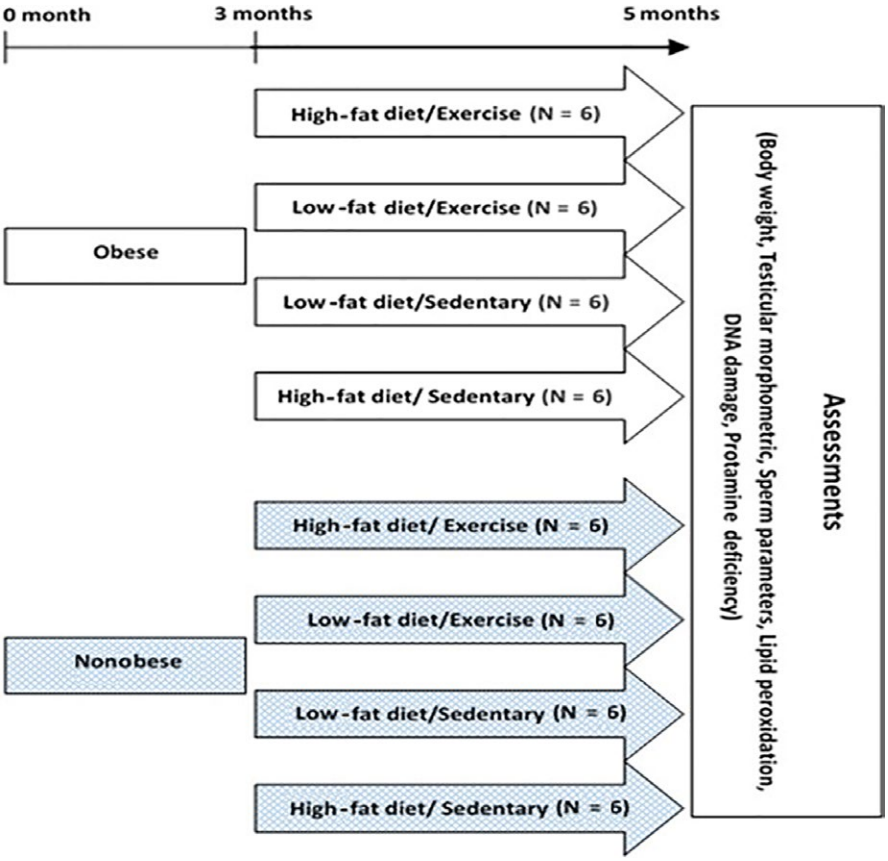

FIGURE 1 Experiment design of this study

The exercise training was as follows

Week 13: adaptation of mice, in exercise groups, began with by placing mice on the motor-driven treadmill beginning with 17 m/min for 15 min on day 1. This was increased daily to 17 m/min for 45 min.

Week 14–15: the mice in the motor-driven treadmill were subjected to exercise at a speed of 17 m/min for 45 min/day, 5 days/week, one session/day.

Week 16–17: the mice in the motor-driven treadmill were subjected to exercise at a speed of 19 m/min for 45 min/day, 5 days/week, one session/day.

Week 18–19: the mice in the motor-driven treadmill were subjected to exercise at a speed of 21 m/min for 45 min/day, 5 days/week, one session/day.

Week 20–21: the mice in the motor-driven treadmill were subjected to exercise at a speed of 23m/min for 45 min/day, 5 days/week, one session/day.

The angle of inclination was 0% gradient during the whole period of the study. This condition corresponded to the moderate intensity of about 65% of the maximal oxygen consumption (Powers et al., 1993). Untrained mice were not exposed to exercise session and stayed in their cages during the protocol. Untrained mice were not exposed to exercise session and stayed in their cages during the protocol.

For the current study, 24 hr after the last exercise session (at the end of 21 weeks), mice were intraperitoneally anaesthetised with a mixture of ketamine and xylazine, and then, left-side testicles and epididymides were dissected out. After dissection of testis and epididymides, testis was fixed in Bouin's fixative for haematoxylin staining and used for evaluation of testicular parameters such as Johnson's score, seminiferous tube diameter, spermatogenesis percentage, meiotic index, and seminiferous epithelium height. The left epididymides were divided into three distinct segments: caput, corpus and cauda. Cauda sections were placed in a Petri dish that contained 2 ml of sperm washing media (VitaSperm, Inoclon, Iran) + 10% albumin for 30 min. Then, the spermatozoa retrieved from the caudal epididymis were used for assessment of sperm parameters. Then, spermatozoa were washed with PBS for assessment of sperm lipid peroxidation, protamine deficiency and DNA fragmentation.

2.3 | Assessment of sperm parameters

Sperm concentration was assessed with a sperm counting chamber (Sperm meter; Sperm Processor, Aurangabad, India), and percentage of sperm motility was evaluated by light microscopy. For assessment of sperm concentration, 20 μl of sample was diluted with 20 μl distilled water, and then, 10 μl of this solution was transferred into a sperm counting chamber (Sperm meter; Sperm Processor, Aurangabad, India). Concentration of spermatozoa per ml was calculated. For assessment of sperm motility, 5 μl of sample was placed on a pre-warmed slide and percentage of motile spermatozoa was defined and reported.

Eosin/nigrosin staining was used for assessment of sperm morphology. Briefly, 20 µl of washed spermatozoa in phosphate-buffered saline (PBS) was mixed with 40 µl of eosin (Merck, Darmstadt, Germany) for 5 min. Then, 60 µl of nigrosin (Merck, Darmstadt, Germany) was added to this mixture. Then, two smears for each sample were prepared and 200 spermatozoa were counted under a light microscope. Percentage of abnormalities in head, neck and tail of spermatozoa were assessed, and data were reported as percentage of sperm abnormal morphology (Afiyani et al., 2014).

2.4 | Assessment of sperm lipid peroxidation

Percentage and intensity of sperm lipid peroxidation were assessed by BODIPY C11 Probe according to Aitken, Wingate, Iuliis, & McLaughlin, (2007). Briefly, BODIPY stain with a final concentration of 5 mM/DMSO was prepared and added to 2×10^6 washed spermatozoa with PBS buffer for 30 min. In addition, a positive control was considered for each sample by adding H_2O_2 to 2×10^6 washed spermatozoa in PBS buffer. Then, samples were centrifuged in PBS (500 *g* for 5 min) and percentage of lipid peroxidation were evaluated using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) (Aitken et al., 2007).

2.5 | Assessment of sperm protamine deficiency

Percentage of protamine deficiency in spermatozoa was assessed by chromomycin A3 staining according to Mohammadi, Hassani-Bafrani, Tavalaee, Dattilo, & Nasr-Esfahani, (2018). with minor modifications. Briefly, washed spermatozoon with PBS buffer from each sample was fixed with methanol: acid acetic (v/v) (Merck, Darmstadt, Germany) for 5 min at −4°C temperature. Then, slides were stained with 100 µl of 0.25 mg/ml chromomycin A3 solution (Sigma Chemical Co., St Louis, USA) for 1 hr, then were centrifuged in PBS (500 *g* for 5 min), air-dried and covered with coverslip. On each slide, 200 spermatozoa were counted using an Olympus fluorescent microscope (BX51, Tokyo, Japan) with the appropriate filters (460–470 nm). Spermatozoa with bright yellow staining (CMA3 positive or protamine-deficient spermatozoa) were distinguished from spermatozoa with dull yellow staining (CMA3 negative or normal protamine content), and percentage of protamine deficiency was reported for each sample (Mohammadi et al., 2018).

2.6 | Assessment of sperm DNA damage

Sperm DNA damage was assessed by acridine orange (AO) staining (Afiyani et al., 2014) with minor modifications. Two smears for each washed sperm sample were prepared and fixed with Carnoy's solution (methanol/acetic acid, 3:1) at −4°C temperature for 2 hr. After washing slides with PBS (1600 rpm for 5 min), slides were stained with 20 µl freshly prepared acridine orange stain 1% in distilled water, and then were added to a mixture of citrate phosphate buffer (80 ml citric acid 0.1 M + 5 ml NaH_2PO_4 0.3 M, pH = 2.5) for 90 min. Then, slides were washed with PBS (1600 rpm for 5 min) and evaluated by fluorescent microscope with 460-nm filter. For each sample, percentage of orange/red spermatozoa as sperm denatured DNA or damaged DNA were reported.

2.7 | Statistical analysis

All the collected data in the present study were analysed using the Statistical Package for the Social Sciences for Windows, version 23.0 (SPSS, Inc., Chicago, IL, USA). All the studied parameters showed normal distribution. Comparison of sperm parameters, lipid peroxidation and chromatin status was performed by two-way analysis of variance (ANOVA). Collected data were presented as mean ± standard error of mean (*SEM*), and $p < 0.05$ was considered to be significant.

3 | RESULTS

In this study, body weight, testicular morphometric characteristics, sperm parameters (concentration, motility and morphology), lipid peroxidation, DNA damage, and protamine deficiency were assessed and compared within obese groups (Hf/Exe, Lf/Exe, Lf/Sed, Hf/Sed) and nonobese groups (Hf/Exe, Lf/Exe, Lf/Sed, Hf/Sed).

3.1 | Effect of diet and/or exercise on body weight and testicular morphometric characteristics

In regard to body weight of the mice, there was no difference at the beginning of the first intervention (12-week HF and LF), while a significant difference was observed between LF-fed and HF-fed mice after five weeks of intervention (Kazeminasab et al., 2018).

The mean of testicular morphometric characteristics such as weight, length, width, thickness and volume of each testis was not significant within obese groups and nonobese groups ($p > 0.05$). In this study, Johnson's score, seminiferous tube diameter, percentage of spermatogenesis, meiotic index and seminiferous epithelium height were also evaluated, and any significant differences were not observed within both obese or nonobese groups (data not shown). Overall exercise and diet interventions had not significantly altered morphometric characteristics of both testes.

3.2 | Effect of diet and/or exercise on sperm parameters

In this study, exercise and/or diet interventions on sperm parameters such as sperm concentration, motility and morphology within obese and nonobese groups were assessed. As shown in Figure 2, means of sperm concentration were 36.33 ± 2.27, 37.17 ± 1.97, 33.00 ± 1.55 and 32.00 ± 1.41 within obese group (Hf/Exe, Lf/Exe, Lf/Sed, Hf/Sed) respectively. The exercise intervention with or without diet programme was significantly effective on sperm concentration within this group ($p = 0.036$). The means of sperm concentration were 37.00 ± 1.65, 41.80 ± 1.68, 33.40 ± 1.29 and 34.17 ± 1.60

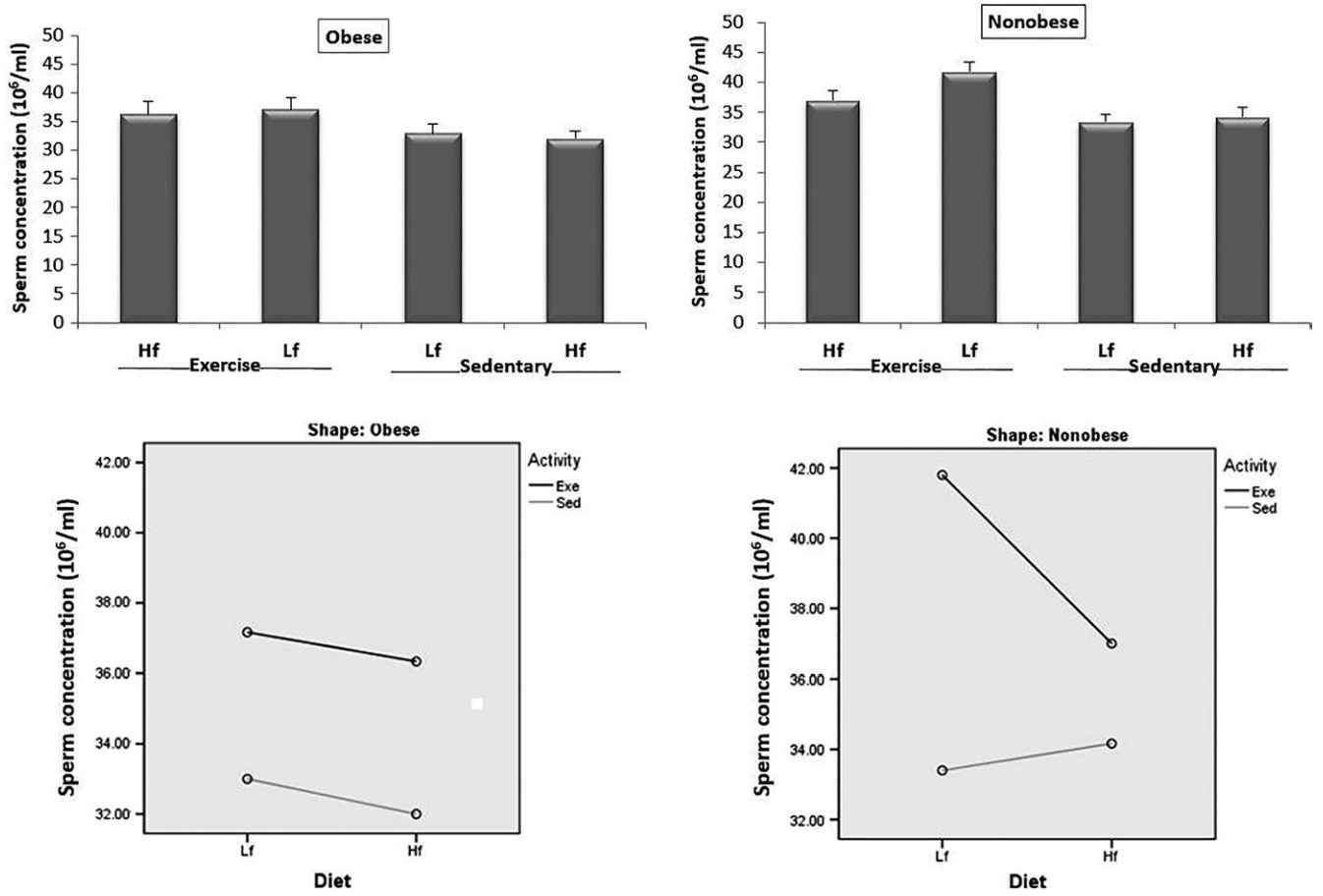

FIGURE 2 Comparison of the effect of exercise and diet on sperm concentration within obese and nonobese groups. Analysis of data by two-way ANOVA shows that intervention of exercise alone was significantly effective on sperm concentration within both obese (p = 0.036) and nonobese (p = 0.002) groups

within nonobese groups (Hf/Exe, Lf/Exe, Lf/Sed, Hf/Sed) respectively. Similar to obese group, exercise intervention was significantly effective on sperm concentration along with low-fat diet (p = 0.002). Collectively, two-way ANOVA shows that only exercise intervention was significantly effective in increase of sperm concentration within both obese and nonobese groups.

In regard to sperm motility, means of this parameter were 57.50 ± 4.14, 65.17 ± 2.72, 50.67 ± 2.70 and 44.40 ± 1.36 within obese group (Hf/Exe, Lf/Exe, Lf/Sed, Hf/Sed) respectively. Intervention of exercise (p < 0.001) and diet (p = 0.033) independent of each other were significantly effective on sperm motility in this group. Means of sperm motility were 58.00 ± 2.21, 64.60 ± 6.26, 62.60 ± 2.09 and 39.33 ± 3.17 within nonobese groups (Hf/Exe, Lf/Exe, Lf/Sed, Hf/Sed) respectively. In this group, both exercise (p = 0.011) and diet (p = 0.001) independently and dependently (p = 0.035, Figure 3) affect sperm motility. Therefore, both interventions are acting in paralleled and antagonistically. Collectively, two-way ANOVA shows that both exercise and diet interventions were significantly effective on sperm motility within both groups.

In this study, sperm abnormal morphology was assessed by eosin and nigrosin staining and results show that means of this parameter were 52.30 ± 2.26, 55.32 ± 2.52, 46.70 ± 2.06 and 55.85 ± 4.48 within obese group (Hf/Exe, Lf/Exe, Lf/Sed, Hf/Sed), and also 53.17 ± 2.90, 50.99 ± 2.69, 46.65 ± 1.58 and 50.88 ± 2.49 within nonobese groups (Hf/Exe, Lf/Exe, Lf/Sed, Hf/Sed) respectively. Two-way ANOVA shows that intervention of exercise and/or diet on sperm morphology within both groups was not statistically significant (p > 0.05).

3.3 | Effect of diet and/or exercise on sperm lipid peroxidation

In this study, level of sperm lipid peroxidation was assessed by BODIPY probe. The results of flow cytometry show that mean percentage of lipid peroxidation were 80.17 ± 3.37, 85.67 ± 2.52, 58.17 ± 4.61, and 57.40 ± 6.68 within obese group (Hf/Exe, Lf/Exe, Lf/Sed, Hf/Sed) respectively. The intervention of exercise (dependently and independently) significantly leads to high percentage of lipid peroxidation in this group (p < 0.001).

Mean percentage of sperm lipid peroxidation were 79.50 ± 6.81, 86.00 ± 4.23, 97.20 ± 0.86 and 92.50 ± 1.48 within nonobese groups (Hf/Exe, Lf/Exe, Lf/Sed, Hf/Sed) respectively, and unlike obese group, the intervention of exercise significantly leads to reduced level of lipid peroxidation within nonobese group (p = 0.004).

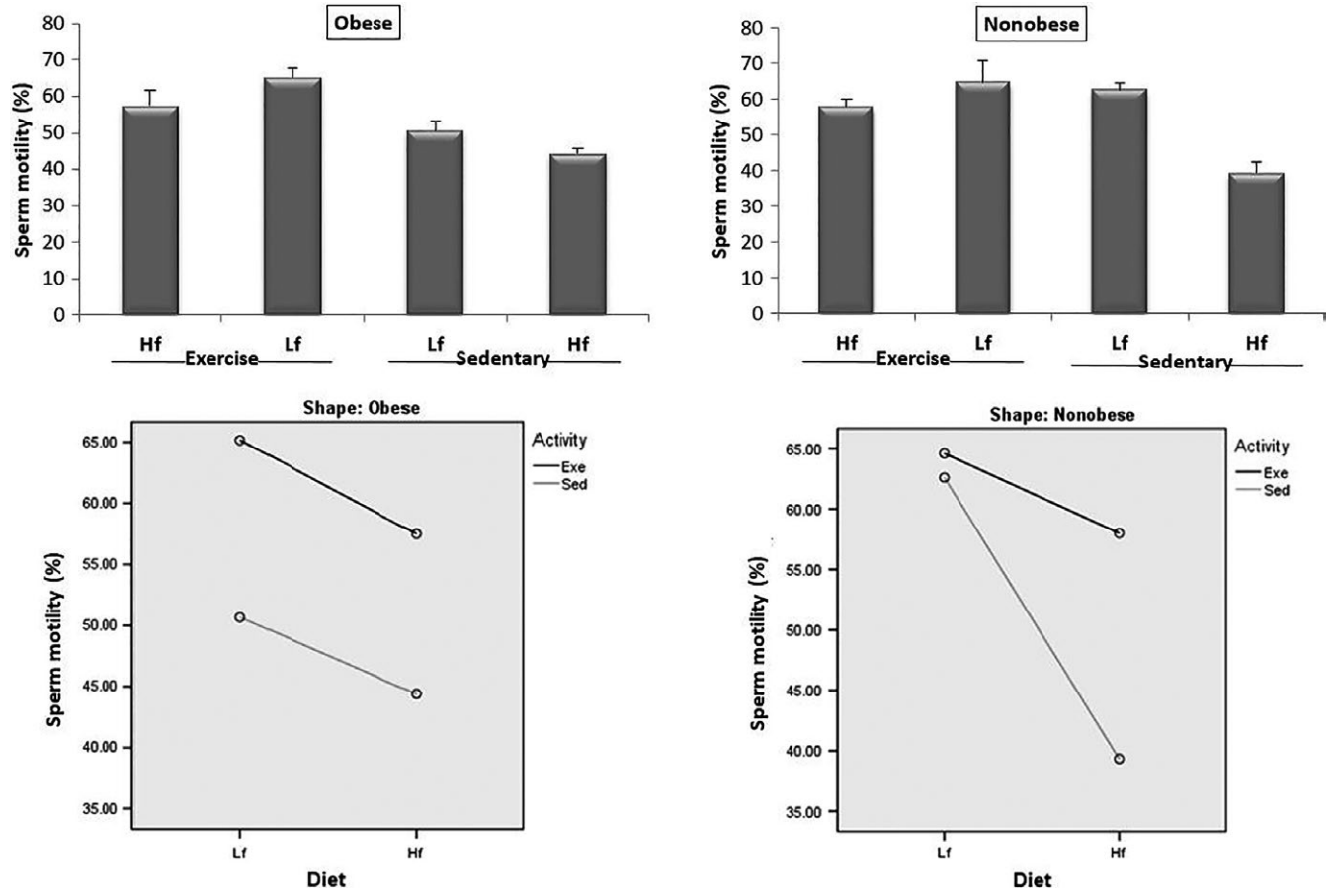

FIGURE 3 Comparison of the effect of exercise and diet on sperm motility within obese and nonobese groups. Analysis of data by two-way ANOVA shows that intervention of exercise ($p = 0.00$) and diet ($p = 0.033$) alone was significantly effective on sperm motility within obese group, while intervention of exercise ($p = 0.011$) and diet ($p = 0.001$) alone, and also combined intervention of exercise and diet ($p = 0.035$) were significantly effective on sperm motility in nonobese groups

Collectively, two-way ANOVA and reciprocal position exercise and sedentary show that only intervention of physical activity (exercise vs. sedentary) had a contradictory effect on lipid peroxidation between obese vs. nonobese groups (Figure 4).

3.4 | Effect of diet and/or exercise on sperm protamine deficiency

In this study, protamine deficiency in spermatozoa was assessed by CMA3 staining, and results show that means of protamine-deficient spermatozoa were 12.79 ± 1.68 (Hf/Exe), 12.77 ± 1.22 (Lf/Exe), 27.37 ± 3.28 (Lf/Sed) and 29.83 ± 2.95 (Hf/Sed) within obese groups. Analysis of data shows that exercise intervention alone was significantly ($p < 0.001$) effective on reduction of percentage protamine-deficient spermatozoa within obese groups. When this parameter was assessed within nonobese groups, 7.65 ± 1.30 (Hf/Exe), 9.69 ± 1.34 (Lf/Exe), 8.49 ± 1.34 (Lf/Sed), and 9.65 ± 1.50 (Hf/Sed), none of the interventions were not efficient in reduction of protamine deficiency, as values of this parameter were low in these groups. Collectively, these data show that exercise intervention alone can improve percentage of protamine deficiency within obese groups (Figure 5).

3.5 | Effect of diet and/or exercise on sperm DNA damage

In this study, DNA damage in spermatozoa was assessed by acridine orange staining, and results show that mean percentage of DNA-damaged spermatozoa were 11.81 ± 0.99 (Hf/Exe), 17.31 ± 2.48 (Lf/Exe), 16.81 ± 4.14 (Lf/Sed) and 18.72 ± 2.83 (Hf/Sed) within obese groups. Intervention of diet and exercise on mean percentage of sperm DNA damage was not significant within obese group.

Mean percentage of DNA damage within nonobese groups were 11.25 ± 1.61 (Hf/Exe), 15.22 ± 1.65 (Lf/Exe), 3.02 ± 0.60 (Lf/Sed) and 6.96 ± 0.38 (Hf/Sed). In this group, exercise alone ($p < 0.001$), and along with diet ($p = 0.006$) had an adverse effect on mean percentage of sperm DNA damage (Figure 6).

4 | DISCUSSION

In this study, we assessed the effect of aerobic exercise, low-fat and high-fat diet on the testis tissue and sperm parameters in obese and nonobese mice model. The results of the current study showed that

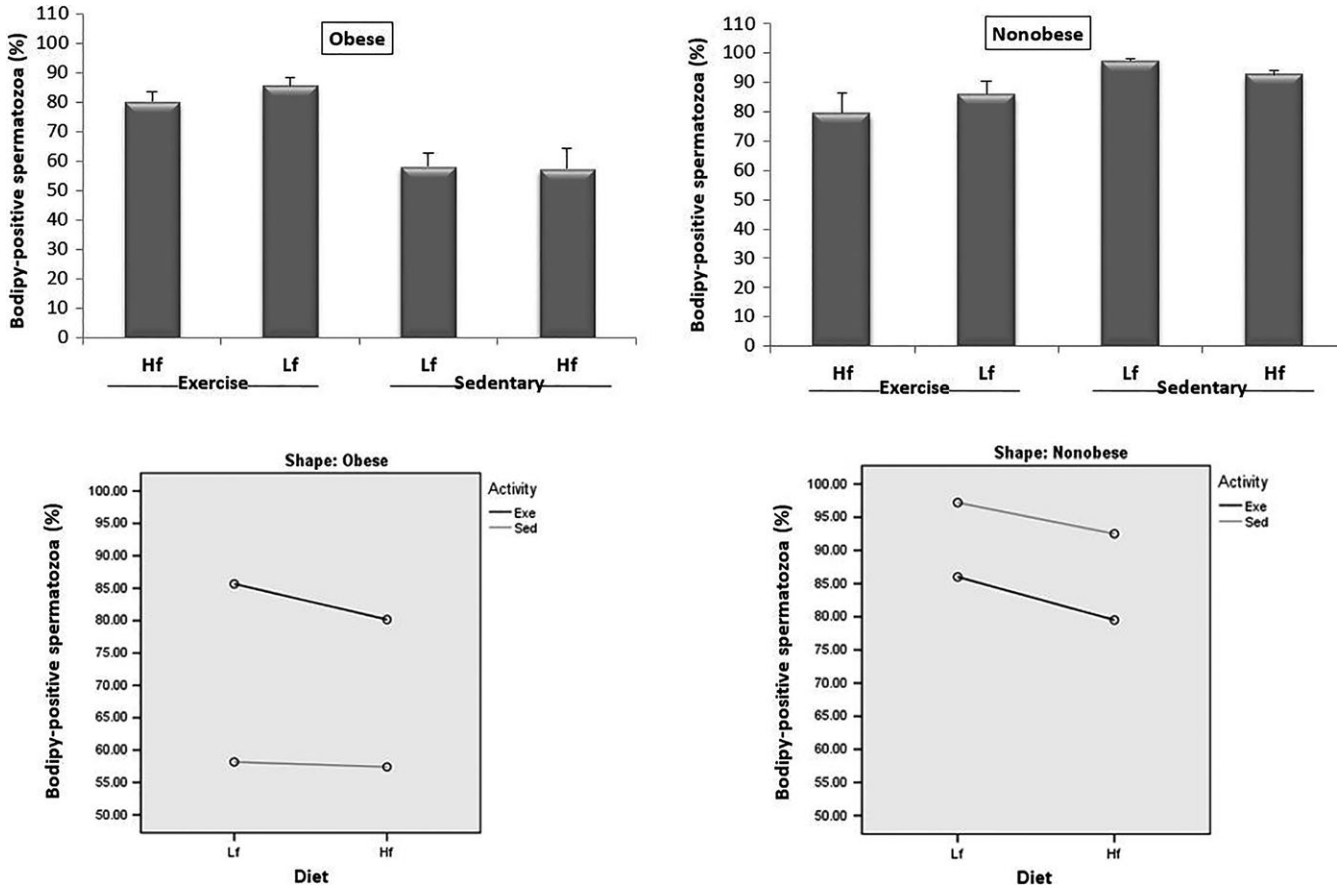

FIGURE 4 Comparison of the effect of exercise and diet on sperm lipid peroxidation within obese and nonobese groups. Analysis of data by two-way ANOVA shows that unlike nonobese group ($p = 0.004$), in obese group ($p < 0.001$) exercise with or without diet interval significantly leads to high percentage of lipid peroxidation in spermatozoa

diet and/or exercise interventions did not significantly alter body weight and testicular morphometric characteristics in both obese and nonobese groups. Only, diet was significantly effective on gaining body weight within nonobese groups, as previously they were on a low-fat diet. Our result is in of keeping with previous studies that have demonstrated obesity condition did not have harmful effect on testicular tissue and seminiferous tubules according to Johnson's score (Edmonds, Dallie, & Withyachumnarnkul, 1982; Vigueras-Villaseñor et al., 2011) but it has been shown that intake of high-fat diet for 120 days in rabbit could increase testicular fatty acids, cholesterol content, and change the composition and structure of sperm membrane (Diaz-Fontdevila & Bustos-Obregón, 1993), which alter the acrosome reaction kinetics. It is important to note previous studies have observed testicular tissue changes in super-obese rats (Yang et al., 2016).

Many studies demonstrating the negative impact of overweight and obesity on sperm parameters but conflicting results can be observed in literature. In this regard, some studies suggest a negative relationship between BMI and sperm parameters (Eisenberg et al., 2014; Keltz et al., 2010), while other studies have reported contradictory results (Gaskins et al., 2012; Kazeminasab et al., 2018; Yang et al., 2016). These contradictions are often contributed to multifactor nature of these phenotypes, which are difficult to account for in human studies due to the diverse genetic and social and environmental background. The result of the current study shows that exercise alone could significantly improve sperm concentration within both obese and nonobese groups. In relation to sperm motility, in obese groups, both diet and exercise independently while in nonobese groups, independently and dependently both interventions significantly improved sperm motility. Current data and previous studies clearly indicate the importance of diet and/or exercise for maintenance of sperm motility and concentration that are considered as the essential parameters for successful fertilisation and fertility. In this regard, Rosety et al., (2017) reported that short-term intervention of aerobic exercise could improve sperm quality in obese sedentary subjects.

Unlike sperm motility and concentration, intervention of diet and/or exercise had no effect on sperm morphology in both obese and nonobese model. In contrary to previous studies that showed high-fat diet can reduce seminiferous epithelium height, seminiferous tubule diameter and also the percentage of sperm normal morphology (Campos-Silva, Furriel, Costa, Sampaio, & Gregorio, (2015); Ibáñez et al., 2017), we did not observe such changes. The difference between these studies to a certain extent can be contributed duration of study interventions and type of exercise. Also unlike our result, Palmer et al. (2012) demonstrated that diet and/or exercise

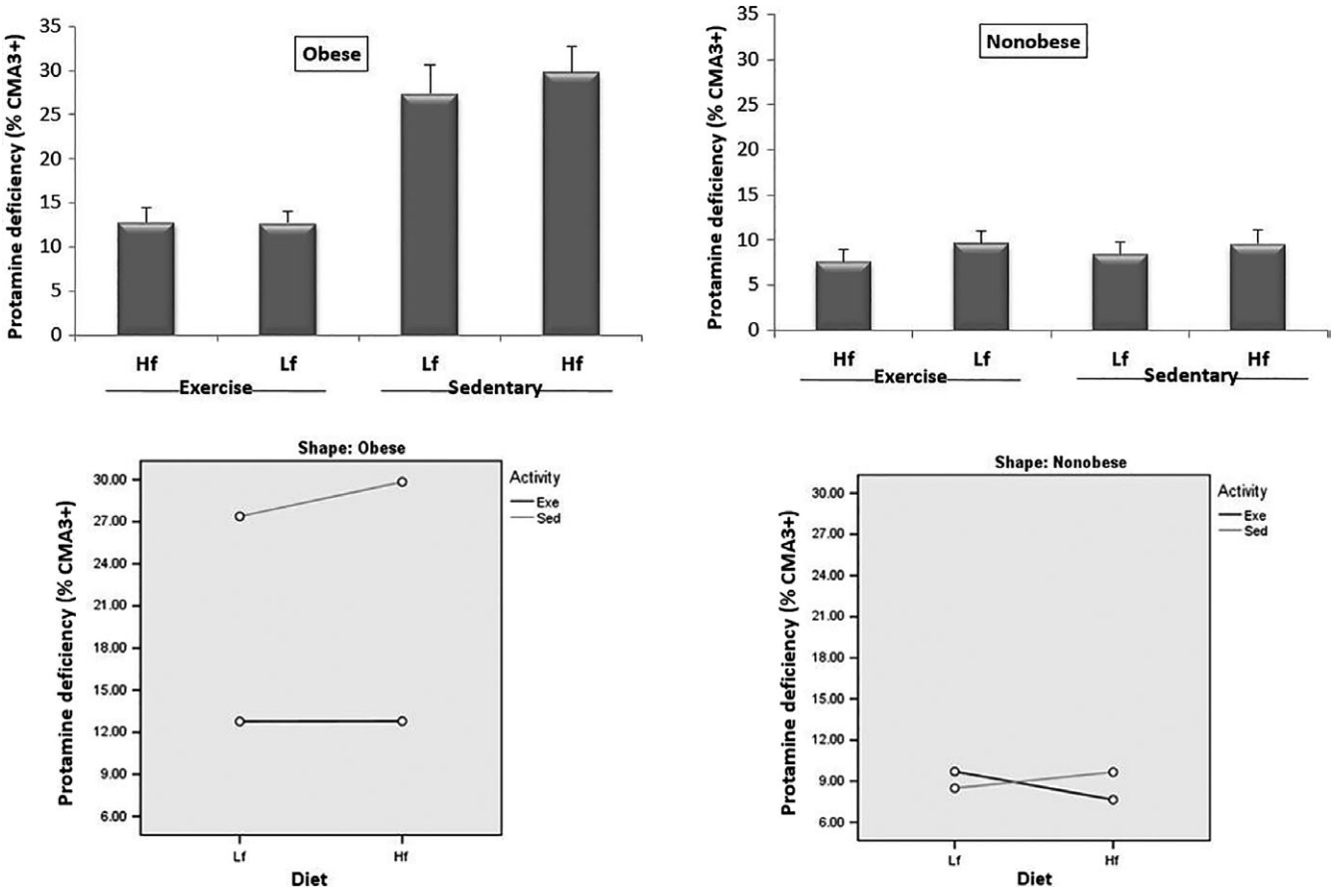

FIGURE 5 Comparison of the effect of exercise and diet on sperm protamine deficiency within obese and nonobese groups. Analysis of data by two-way ANOVA shows that exercise alone was significantly ($p < 0.001$) effective on reduction of percentage of protamine-deficient spermatozoa within obese groups, while diet and exercise were not efficient within nonobese groups

can improve the adverse morphology in obese mouse spermatozoa (Palmer et al., 2012). We concluded that although diet and/or exercise interventions have no effect on testicular morphometric characteristics (Dutra Gonçalves et al., 2017; Ghanayem, Bai, Kissling, Travlos, & Hoffler, 2010) in the obese and nonobese model, these interventions had effective impacts on sperm concentration and motility.

Oxidative stress is an important contributor to male infertility. Due to high level of cell division and mitochondrial oxygen consumption in testis and also relatively high level of unsaturated fatty acids in this tissue compared to other tissues, this tissue is more prone to oxidative stress (Asadi, Bahmani, Kheradmand, & Rafieian-Kopaei, 2017). On the other hand, oxygen pressure is low in seminiferous tube due to the presence of blood–testis barrier. In this regard, the result of the current study shows that the intervention of exercise significantly leads to the high percentage of lipid peroxidation within obese groups and DNA fragmentation within nonobese group. Indeed, muscle contractions during exercise lead to elevated levels of ROS production in skeletal muscle and other tissue (Powers & Jackson, 2008; Steinbacher & Eckl, 2015) and by-product of peripheral ROS may prone spermatozoa to higher level of lipid peroxidation (Zhao, Zhai, Liu, Wu, & Xu, 2014). But, if we consider exercise as a causative factor in inducing sperm lipid peroxidation, we could not explain why in nonobese group, in sedentary condition, the lipid peroxidation is not significantly different compared to mice that forced to exercise. Whether previous exposure to high fat level accounts for the difference remains to be clarified. But it is also important to note the exercise improved sperm count, motility and protamine content of spermatozoa but did not alter DNA fragmentation; therefore, the increased level of lipid peroxidation might not be considered to be pathological. In agreement with our conclusion, Palmer et al., (2012) demonstrated that intervention of exercise and/or diet could significantly improve fertilisation, blastocyst development and pregnancy rates in obese model by improving sperm motility, morphology, levels of sperm DNA damage and sperm mitochondrial ROS. One explanation for increased lipid peroxidation in obese mice and increased DNA fragmentation in nonobese mice with exercise could be due to a reduced oxygen tension in seminiferous tubule resulting in a state of hypoxia. In hypoxic condition, HIF-1α and then TNF-α increased which result in increased ROS production which upon reperfusion post-exercise could lead to increase sperm lipid peroxidation, which is within the normal limits as it does not affect sperm motility and other aforementioned sperm parameters (Ban, Ruthenborg, Cho, & Kim, 2014;

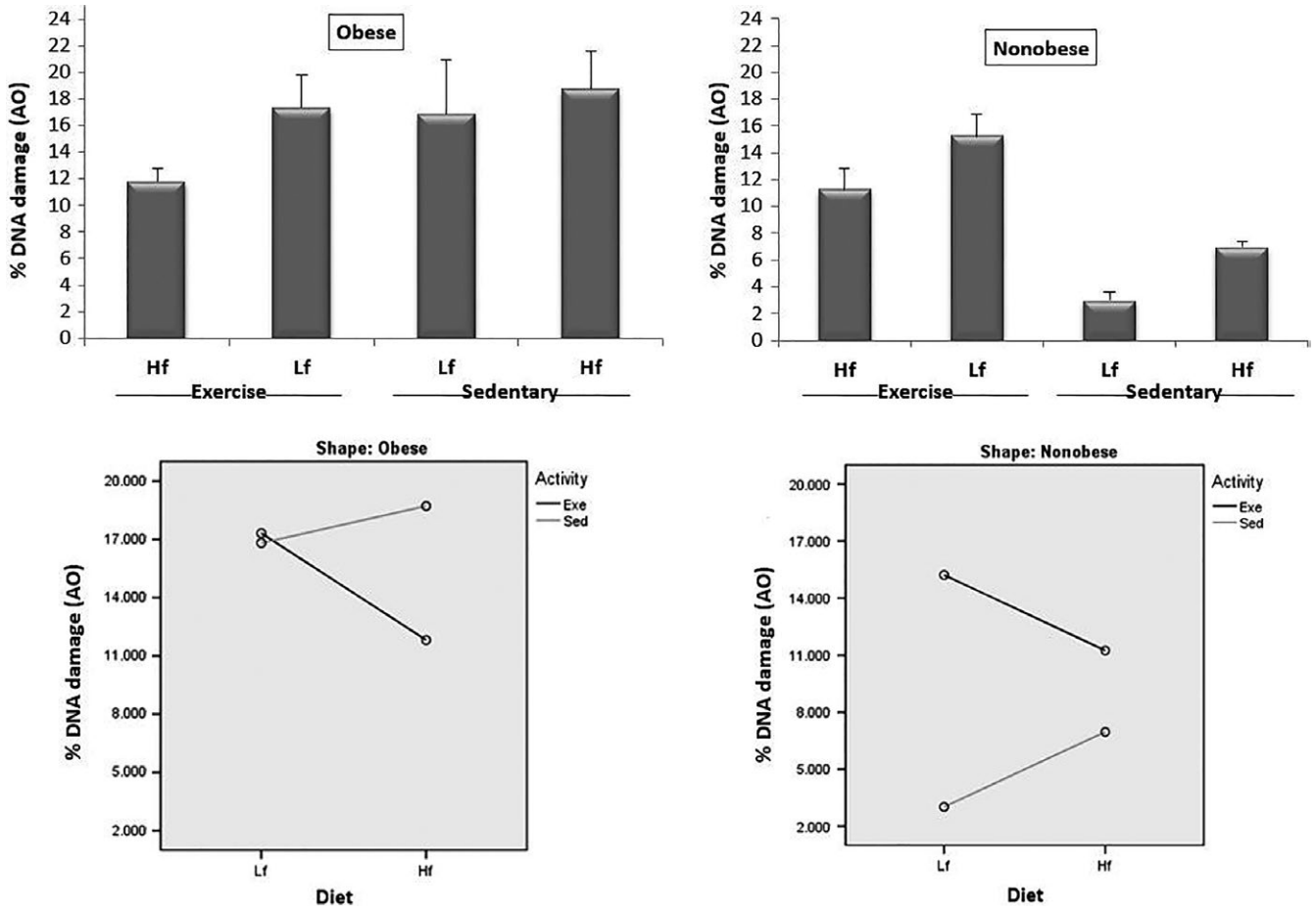

FIGURE 6 Comparison of the effect of exercise and diet on sperm DNA damage within obese and nonobese groups. Analysis of data by two-way ANOVA shows that only exercise alone was insignificantly efficient in reduction of sperm DNA damage in Ob/Hf/Exe group within obese groups. In relation to nonobese groups, exercise alone ($p < 0.001$), and along with diet ($p = 0.006$) had an adverse effect on mean percentage of sperm DNA damage

Ghandehari-Alavijeh, Zohrabi, Tavalaee, & Nasr-Esfahani, 2019). Indeed, it has been stated that in severe hypoxia concomitant phosphorylation of HIF-1α and P-53 together results in apoptosis, while in mild hypoxia, only HIF-1α is phosphorylated and results in cellular survival. Therefore, similar trend or mild hypoxia may take place during exercise and improved sperm parameters may be accounted by a similar phenomenon which requires future verification.

It is of note that in varicocele condition, reduced blood flow and increased haemolysis occurred, both of which result in iron deposition in testicular tissue. The consequence of this phenomenon is increased lipid peroxidation and DNA damage (Gholirad, Razi, & Hassani- Bafrani, 2017). It is possible that excessive exercise and obesity, similar to varicocele condition, lead to reduction in testicular blood flow and increase DNA damage. These possibilities need further verification.

The results of this study clearly show that both endurance exercise and diet interventions had no detrimental effect on histological parameters and sperm function within obese and nonobese groups. One of the advantages of the current study was the simultaneous assessment of both interventions of diet and/or exercise on obese and nonobese model; according to the two-way ANOVA, the exercise intervention was more effective than diet in improvement of sperm parameters and functional tests within obese groups.

ACKNOWLEDGEMENTS

This study was supported by Royan Institute, and we would like to express our gratitude to staff of Reproductive Biotechnology for their full support.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

ORCID

Marziyeh Tavalaee https://orcid.org/0000-0001-9954-964X

Mohammad Hossein Nasr-Esfahani https://orcid.org/0000-0003-1983-3435

REFERENCES

Afiyani, A. A., Deemeh, M. R., Tavalaee, M., Razi, M., Bahadorani, M., Shokrollahi, B., & Nasr-Esfahani, M. H. (2014). Evaluation of heat-shock protein A2 (HSPA2) in male rats before and after varicocele induction. *Molecular Reproduction and Development*, *81*(8), 766–776. https://doi.org/10.1002/mrd.22345.

Aitken, R. J., Wingate, J. K., De Iuliis, G. N., & McLaughlin, E. A.. (2007). Analysis of lipid peroxidation in human spermatozoa using BODIPY C11. *Molecular Human Reproduction*, *13*(4), 203–211. https://doi.org/10.1093/molehr/gal119.

Andersen, J. M., Rønning, P. O., Herning, H., Bekken, S. D., Haugen, T. B., & Witczak, O. (2016). Fatty acid composition of spermatozoa is associated with BMI and with semen quality. *Andrology*, *4*(5), 857–865. https://doi.org/10.1111/andr.12227

Asadi, N., Bahmani, M., Kheradmand, A., & Rafieian-Kopaei, M. (2017). The impact of oxidative stress on testicular function and the role of antioxidants in improving it: A review. *Journal of Clinical and Diagnostic Research for Doctors*, *11*(5), IE01-IE05. https://doi.org/10.7860/JCDR/2017/23927.9886.

Bagnol, D., Al-Shamma, H. A., Behan, D., Whelan, K., & Grottick, A. J. (2012). Diet-induced models of obesity (DIO) in rodents. *Current Protocols in Neuroscience*, *9*(9):1–13. https://doi.org/10.1002/0471142301.ns0938s59.

Ban, J. J., Ruthenborg, R. J., Cho, K. W., & Kim, J. W. (2014). Regulation of obesity and insulin resistance by hypoxia-inducible factors. *Hypoxia (Auckland, N.Z.)*, *2*, 171–183. https://doi.org/10.2147/HP.S68771.

Campos-Silva, P., Furriel, A., Costa, W. S., Sampaio, F. J., & Gregorio, B. M. (2015). Metabolic and testicular effects of the long-term administration of different high-fat diets in adult rats. *International Brazilian Journal of Urology*, *41*(3), 569–575. https://doi.org/10.1590/S1677-5538.

Cardoso, A. M., Alves, M. G., Mathur, P. P., Oliveira, P. F., Cavaco, J. E., & Rato, L. (2017). Obesogens and male fertility. *Obesity Reviews*, *18*(1), 109–125. https://doi.org/10.1111/obr.12469.

Craig, J. R., Jenkins, T. G., Carrell, D. T., & Hotaling, J. M. (2017). Obesity, male infertility, and the sperm epigenome. *Fertility Sterility*, *107*(4), 848–859. https://doi.org/10.1016/j.fertnstert. 2017.02.115.

Diaz-Fontdevila, M., & Bustos-Obregón, E. (1993). Cholesterol and polyunsaturated acid enriched diet: effect on kinetics of the acrosome reaction in rabbit spermatozoa. *Molecular Reproduction and Development*, *35*(2), 176–180. https://doi.org/10.1002/mrd.1080350211.

Dutra Gonçalves, G., Antunes Vieira, N., Rodrigues Vieira, H., Dias Valério, A., Munhoz Elóisa deLion Siervo, G., … Scantamburlo Alves Fernandes, G. (2017). Role of resistance physical exercise in preventing testicular damage caused by chronic ethanol consumption in UChB rats. *Microscopy Research and Technique*, *80*(4), 378–386. https://doi.org/10.1002/jemt.22806.

Edmonds, E. S., Dallie, S. K., & Withyachumnarnkul, B. (1982). Reproductive system of the obese male zucker rat. Reproductive capacity, artificial insemination and plasma testosterone levels. *Biology of Reproduction*, *27*(4), 891–897.

Eisenberg, M. L., Kim, S., Chen, Z., Sundaram, R., Schisterman, E. F., & Louis, G. M. (2014). The relationship between male BMI and waist circumference on semen quality: data from the LIFE study. *Human Reproduction*, *29*,193–200. https://doi.org/10.1093/humrep/deu322.

Gaskins, A. J., Colaci, D. S., Mendiola, J., Swan, S. H., & Chavarro, J. E. (2012). Dietary patterns and semen quality in young men. *Human Reproduction*, *27*(10), 2899–2907. https://doi.org/10.1093/humrep/des298.

Ghanayem, B. I., Bai, R., Kissling, G. E., Travlos, G., & Hoffler, U. (2010). Diet-induced obesity in male mice is associated with reduced fertility and potentiation of acrylamide-induced reproductive toxicity. *Biology of Reproduction*, *82*(1), 96–104. https://doi.org/10.1095/biolreprod.109.078915.

Ghandehari-Alavijeh, R., Zohrabi, D., Tavalaee, M., & Nasr-Esfahani, M. H. (2019). Association between expression of TNF- α, P53 and HIF1α with asthenozoospermia. *Andrologia*, *51*(2), e13189. https://doi.org/10.1111/and.13189.

Gholirad, S., Razi, M., & Hassani- Bafrani, H. (2017). Tracing of zinc and iron in experimentally induced varicocele: correlation with oxidative, nitrosative and carbonyl stress. *Andrologia*, *49*(6), e12687. https://doi.org/10.1111/and.12687.

Gomes, M., Freitas, M. J., & Fardilha, M. (2015). Physical activity, exercise, and mammalian testis function: Emerging preclinical protein biomarker and integrative biology insights. *OMICS*, *19*(9), 499–511. https://doi.org/10.1089/omi.2015.0065

González-Ravina, C., Aguirre-Lipperheide, M., Pinto, F., Martín-Lozano, D., Fernández-Sánchez, M., Blasco, V., & Candenas, L. (2018). Effect of dietary supplementation with a highly pure and concentrated docosahexaenoic acid (DHA) supplement on human sperm function. *Reprod Biol*, *18*(3), 282–288. https://doi.org/10.1016/j.repbio.2018.06.002

Hajizadeh Maleki, B., & Tartibian, B. (2015). Long-term low-to-intensive cycling training: Impact on semen parameters and seminal cytokines. *Clinical Journal of Sport Medicine*, *25*(6), 535–540. https://doi.org/10.1097/JSM.0000000000000122

Hwang, D. H., Kim, J. A., & Lee, J. Y. (2016). Mechanisms for the activation of Toll-like receptor 2/4 by saturated fatty acids and inhibitionby docosahexaenoic acid. *Eur J Pharmacol*, *785*, 24–35. https://doi.org/10.1016/j.ejphar. Review.

Ibáñez, C. A., Erthal, R. P., Ogo, F. M., Peres, M. N. C., Vieira, H. R., Conejo, C., … Palma-Rigo, K. (2017). A high fat diet during adolescence in male Rats negatively programs reproductive and metabolic function which is partially ameliorated by exercise. *Frontiers in Physiology*, *8*, 807. https://doi.org/10.3389/fphys.2017.00807

Kazeminasab, F., Marandi, S. M., Ghaedi, K., Safaeinejad, Z., Esfarjani, F., & Nasr-Esfahani, M. H. (2018). A comparative study on the effects of high-fat diet and endurance training on the PGC-1α-FNDC5/irisin pathway in obese and non-obese male C57BL/6 mice. *Applied Physiology, Nutrition, and Metabolism*, *43*(7), 651–662. https://doi.org/10.1139/apnm-2017-0614

Keltz, J., Zapantis, A., Jindal, S. K., Lieman, H. J., Santoro, N., & Polotsky, A. J. (2010). Overweight men: Clinical pregnancy after ART is decreased in IVF but not in ICSI cycles. *Journal of Assisted Reproduction and Genetics*, *27*(9–10), 539–544. https://doi.org/10.1007/s10815-010-9439-y

Khosrowbeygi, A., & Zarghami, N. (2007). Fatty acid composition of human spermatozoa and seminal plasma levels of oxidative stress biomarkers in subfertile males. *Prostaglandins Leukot Essent Fatty Acids*, *77*(2), 117–121.

Mohammadi, P., Hassani-Bafrani, H., Tavalaee, M., Dattilo, M., & Nasr-Esfahani, M. H. (2018). One-carbon cycle support rescues sperm damage in experimentally induced varicocele in rats. *BJU International*, *122*(3), 480–489. https://doi.org/10.1111/bju.14385

Oliveira, P. F., Sousa, M., Silva, B. M., Monteiro, M. P., & Alves, M. G. (2017). Obesity, energy balance and spermatogenesis. *Reproduction*, *153*(6), R173–R185. https://doi.org/10.1530/REP-17-0018.

Palmer, N. O., Bakos, H. W., Owens, J. A., Setchell, B. P., & Lane, M. (2012). Diet and exercise in an obese mouse fed a high-fat diet improve metabolic health and reverse perturbed sperm function. *American Journal of Physiology-Endocrinology and Metabolism*, *302*(7), E768–780. https://doi.org/10.1152/ajpendo.00401.2011.

Powers, S. K., Criswell, D., Lawler, J., Martin, D., Lieu, F. K., Ji, L. L., & Herb, R. A. (1993). Rigorous exercise training increases superoxide dismutase activity in ventricular myocardium. *American Journal of Physiology*, *265*(6), H2094–H2098. https://doi.org/10.1152/ajpheart.1993.265.6.H2094.

Powers, S. K., & Jackson, M. J. (2008). Exercise-induced oxidative stress: Cellular mechanisms and impact on muscle force production. *Physiological Reviews*, *88*(4), 1243–1276. https://doi.org/10.1152/physrev.00031.2007

Rosety, M. Á., Díaz, A. J., Rosety, J. M., Pery, M. T., Brenes-Martín, F., Bernardi, M., … Rosety, I. (2017). Exercise improved semen quality and reproductive hormone levels in sedentary obese adults. *Nutricion Hospitalaria Journal*, *34*(3), 603–607. https://doi.org/10.20960/nh.549.

Schlegel, P. N. (2012). Aromatase inhibitors for male infertility. *Fertility Sterility*, *98*(6), 1359–1362. https://doi.org/10.1016/j.fertnstert.2012.10.023

Skakkebaek, N. E., Rajpert-De Meyts, E., & Main, K. M. (2001). Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Human Reproduction*, *16*(5), 972–978.

Steinbacher, P., & Eckl, P. (2015). Impact of oxidative stress on exercising skeletal muscle. *Biomolecules*, *5*(2), 356–377. https://doi.org/10.3390/biom5020356

Tavilani, H., Doosti, M., Abdi, K., Vaisiraygani, A., & Joshaghani, H. R. (2006). Decreased polyunsaturated and increased saturated fatty acid concentration in spermatozoa from asthenozoospermic males as compared with normozoospermic males. *Andrologia.*, *38*(5), 173–178. https://doi.org/10.1111/j.1439-0272.2006.00735.x

Vaamonde, D., Da Silva-Grigoletto, M. E., García-Manso, J. M., Vaamonde-Lemos, R., Swanson, R. J., & Oehninger, S. C. (2009). Response of semen parameters to three training modalities. *Fertility and Sterility*, *92*(6), 1941–1946. https://doi.org/10.1016/ j.fertnstert.2008.09.010.

Vigueras-Villaseñor, R. M., Rojas-Castañeda, J. C., Chávez-Saldaña, M., Gutiérrez-Pérez, O., García-Cruz, M. E., Cuevas-Alpuche, O., & Zambrano, E. (2011). Alterations in the spermatic function generated by obesity in rats. *Acta Histochemica*, *113*(2), 214–220. https://doi.org/10.1016/j.acthis.2009.10.004.

Yang, Q., Zhao, F., Hu, L., Bai, R., Zhang, N., Yao, G., & Sun, Y. (2016). Effect of paternal overweight or obesity on IVF treatment outcomes and the possible mechanisms involved. *Scientific Reports*, *6*, 29787. https://doi.org/10.1038/srep29787.

Zhao, J., Zhai, L., Liu, Z., Wu, S., & Xu, L. (2014). Leptin level and oxidative stress contribute to obesity-induced low testosterone in murine testicular tissue. *Oxidative Medicine and Cellular Longevity*, *2014*, 190945. https://doi.org/10.1155/2014/190945.

How to cite this article: Nematollahi A, Kazeminasab F, Tavalaee M, et al. Effect of aerobic exercise, low-fat and high-fat diet on the testis tissue and sperm parameters in obese and nonobese mice model. *Andrologia*. 2019;e13273. https://doi.org/10.1111/and.13273