

Effects of Deep-Frying Sunflower Oil on Sperm Parameters in A Mouse Model: Do Probiotics Have A Protective Effect? ^[1]

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^[1] This research was made possible from the research project 2016-04048 of the Scientific Research Unit, Center of Excellence of Near East University

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Article ID: KVFD-2019-22063 Received: 14.02.2019 Accepted: 12.06.2019 Published Online: 13.06.2019

How to Cite This Article

Sayiner S, Gülmez N, Sabit Z, Gülmez M: Effects of deep-frying sunflower oil on sperm parameters in a mouse model: Do probiotics have a protective effect?. *Kafkas Univ Vet Fak Derg*, 25 (6): 857-863, 2019. DOI: 10.9775/kvfd.2019.22063

Abstract

The aim of this study was to investigate the effects of dietary sunflower oil and fried sunflower oil on reproductive sperm parameters, blood lipid profiles, lipid peroxidation and to additionally investigate the protective effects of probiotics. Five experimental groups were established using BALB/c type mice (n=8). A standard pellet mouse feed for the Control group was used. The pellet feed was fortified with sunflower oil (Oil Group), oil and probiotic (Oilpro), fried oil (Fryoil), fried oil and probiotic (Fryoilpro). At the end of the sixty-three-day feeding period, blood samples were collected via cardiac venipuncture, mice were euthanized and testis tissue samples were collected for analyses. Consumption of sunflower oil, natural or fried, decreased seminiferous tubule score (P<0.01) and increased plasma MDA levels (P<0.05) when compared with the control group. Nevertheless, the probiotic use appeared to prevent side effects. Probiotics also increased sperm motility (P<0.01). Prolonged exposure to high fat and fried oil diets would increase oxidative stress levels and have negative effects on fertility levels. Probiotic use may ameliorate such adverse effects. Additionally, this study model may lead to a better understanding of the effects of fast-food dietary habits on global fertility rates.

Keywords: Infertility, Probiotic, Frying oil, Sperm quality, Oxidative stress, Malondialdehyde

Derin Kızartılmış Ayçiçeği Yağının Bir Fare Modelinde Sperm Parametreleri Üzerindeki Etkileri: Probiyotiklerin Koruyucu Etkisi Var mı?

Öz

Çalışmamız, ayçiçek yağı ve kızartmış ayçiçek yağı eklenmiş diyetin reproduktif açıdan sperm parametreleri, kan lipid profili ve lipid peroksidasyonuna etkisi ve buna karşın probiyotik kullanımının koruyucu etkisini araştırmak amacıyla yapılmıştır. BALB/c tipi fareler kullanılarak 5 deney grubu oluşturuldu (n=8). Kontrol grubuna standart pellet yem verilirken diğer gruplara sırayla ayçiçek yağı (Oil), ayçiçek yağı ve probiyotik (Oilpro), kızartılmış yağ (Fryoil) ve kızartılmış yağ ve probiyotik (Fryoilpro) verildi. Altmış üç gün süren deneyin sonunda farelerden kardiyak venipünktür ile kan örnekleri alındı ve daha sonra ötenazi işlemi yapılarak testisler alınarak analizlerde kullanıldı. Kontrol grubu ile kıyaslandığında hem kızartılmış hem de kızartılmamış ayçiçek yağı seminifer tubul skorunu düşürdü (P<0.01) ve plazma MDA seviyesini artırdı (P<0.05). Ancak, probiyotik kullanımı bu olumsuz etkileri düzeltmede etkili bulundu. Probiyotikler sperm motilitesi üzerinde de olumlu etki gösterdi (P<0.01). Uzun süreli aşırı yağ ve kızartma yağı tüketimi oksidatif stresi artırarak fertilité üzerinde olumsuz etki yapabilir. Probiyotik kullanımı ise bu gibi olumsuz etkileri düzeltebilir. Yine, bu çalışma modeli fast-food diyet gibi kötü beslenme alışkanlıklarının olumsuz etkilerini anlamada önemli olabilir.

Anahtar sözcükler: İnfertilite, Probiyotik, Kızartma yağı, Sperm kalitesi, Oksidatif stres, Malondialdehit

INTRODUCTION

Infertility is a global problem, with approximately 50% of

infertility cases due to decreased sperm quality ^[1]. Various dietary factors and diet related obesity, increases the risk of male hypogonadism ^[2]. In practice, sunflower oil



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is principally used in the preparation of most popular fried foods [3]. It has been reported that intermittent and continuous frying using oils affect the physiological, histological and biochemical events, resulting in chemical changes in the oils [3–5]. In these studies, frying oils have been shown to cause increased reactive oxygen species (ROS) that can contribute to many pathological conditions [6,7]. Spermatozoa are very sensitive to oxidative stress and frying oils is likely to negatively affecting sperm production capacity, quality and number [8,9]. Insufficient antioxidant response to ROS causes lipid peroxidation (LPO) that results malondialdehyde (MDA) formation, a highly reactive molecule [10] and a predictable marker of plasma and tissue LPO [6].

Repeatedly heated edible oil can cause increase in ROS formation that leads to decreased radical scavenging and thereby oxidative stress [11,12]. To counteract this, it is essential to increase the level of antioxidant capacity. One of the latest concepts is the “gut-brain axis” [13]. Probiotics have been shown to enhance the body’s antioxidant capacity and strengthen the intestinal health and immune system, making it possible for the organism to cope with many negative metabolic activities [12,14]. They have positive effects on antioxidant properties and free radical metabolism [15]. Studies on mouse models have reported that probiotics have an positive effect on fertility [16,17]. Some poultry science studies have shown that the antioxidative capacities of probiotics can enhance the number and quality of spermatozoa [18,19]. However, the use of probiotics in livestock rearing is uncommon. Additionally, people nowadays prefer to cook their fries using vegetable oils and this has been shown to have detrimental effects, one of which is sperm quality.

Negative health effects of fried oil and positive health promotions of probiotics have been widely investigated, especially in high fat diet-induced obesity models [3,8]. However, so far, very little attention has been paid to determine whether edible sunflower oil, either raw or cooked, may reduce spermatozoon function and the possible protective effects of probiotics in human or animal models. In view of such findings, the aims of the present study were as follows: to investigate the effect of fried oil on sperm parameters, testicular tissue damage, to evaluate alteration in blood lipid profile and to investigate possible protective effect of use probiotics.

MATERIAL and METHODS

Animals

The experimental protocol of this study was approved by the Animal Experiments Ethics Committee of Near East University (Approval No: 2016/2-1). Animals were kept at a constant temperature ($22\pm 1^\circ\text{C}$) with 12 h light/dark cycle and housed in plastic cages.

Experimental Design

A total of 40 BALB/c mice at their period of sexual maturation (90 days old) were divided into 5 groups, 8 in each group ($n=8$). Group 1, as control group, received only a standard pellet diet. Group 2 (oil) received a standard diet supplemented with sunflower oil. Group 3 (Oilpro) was fed the standard diet supplemented with sunflower oil in conjunction with probiotic. Group 4 (Fryoil) received a standard pellet diet supplemented with deep-frying sunflower oil. Group 5 (FryOilpro) received a standard pellet diet supplemented with deep-frying sunflower oil in conjunction with probiotics. To prepare deep-frying sunflower oil, a commercial sunflower oil was heated three consecutive times to $+232^\circ\text{C}$ (smoke point). Feed parameters and other applications for each of the groups of animals used in the experiments are given in Table 1. Feed parameters were analysed by a commercially licenced laboratory (Safyem Ar-Ge Lab., Eskisehir, Turkey). The mice were allowed free access to their respective diets and water *ad libitum* for 63 days.

Preparation of Probiotic Drinking Water

A commercial probiotic in the form of 1 g water soluble powder in aluminium sachets was purchased from a local pharmacy. Each sachet contained 2×10^9 each *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum* and *Bifidobacterium lactis*. One sachet per adult person is the recommended daily dose. To confirm the number of live bacteria in each sachet, total content of the sachet was added to 100 mL sterile saline solution (0.9% NaCl, w/v) and gently mixed in a sterile Erlenmeyer flask. Total aerobic bacteria were counted from this water. The 10-fold increment serial dilution technique was conducted according to Miller and Wolin [20]. One mL of the homogenized suspension was then transferred into 9 mL of 0.9% saline solution (NaCl) and serially diluted

Table 1. Experimental groups ($n=8$) and feeding regimes used in the study

Groups	Feeds	Crude Protein (%)	Crude Fat (%)	Crude Cellulose (%)	Starch (%)	Ash (%)	Dry Matter (%)	Energy (Kcal/kg)
Control	Standard pellet diet	19.43	2.48	4.35	38.97	6.19	92.03	2789
Oil	Standard pellet diet + sunflower oil	17.51	13.77	4.65	34.37	5.63	93.11	3306
Oilpro	Standard pellet diet + sunflower oil + probiotic	17.51	13.77	4.65	34.37	5.63	93.11	3306
Fryoil	Standard pellet diet + deep - frying sunflower oil	17.21	14.1	4.99	33.94	5.64	93.22	3317
Fryoilpro	Standard pellet diet + deep - frying sunflower oil + probiotic	17.21	14.1	4.99	33.94	5.64	93.22	3317

from 10^{-1} to 10^{-8} by using the same saline solution tubes. From the last three diluted samples, 0.1 mL each was plated on the Trypticase soy agar (TSA, Merck, Germany) plates and the plates incubated at 37°C for 48 h. All the colonies grown on the plates were counted and results were expressed as \log_{10} colony forming units (CFU) per gram probiotic product. The total of 1.7×10^8 CFU/g live bacteria were detected in the probiotic source. After the count of CFU/g of probiotic product, the drinking water of mice was fortified by addition of powdered probiotic at a concentration of 1×10^7 CFU/mL live probiotic bacteria. The probiotic drinking water was refreshed at 3 d intervals during the experimental period, using one new sachet in each time. The probiotic used in the study was stored at room temperature during use as per manufacturers recommendations. All mice had access *ad libitum* to their water during the experimental period.

Body Weight Gain (WG), Feed Consumption, Energy Consumption and Consumed Energy Per g of WG

The mice were weighted at day 0 and at day 63. Body weight gain was obtained by subtracting the animal weight at day 63 from the initial weight (day 0). Feed consumption of each group of mice were followed by weekly measurements. Energy consumption and consumed energy per g of WG were calculated using consumed feed and the separate energy values of the feed mentioned in *Table 1*.

Collection of Blood, Semen and Tissue Samples

Biochemical tests were performed at the Diagnostic Laboratory of Animal Hospital, Near East University. Blood samples were collected by cardiac venipuncture into vacuum tubes containing K_2EDTA and clot activator tubes. Serum and plasma samples were obtained by centrifugation at 1500 g for 10 min at +4°C. The mice were euthanized via cervical dislocation technique. Testicles and epididymis were collected by performing laparotomy for better visualization of cauda epididymis and ductus deferens. The aforementioned anatomical structures were excised and placed in 5 mL warmed to 37°C Dulbecco's PBS. The excised organs were cut out in small pieces inside in the medium, and coated with mineral oil (FertiCult®). These were incubated for 20 min to allow the spermatozoa to diffuse through the Dulbecco's PBS medium. Immediately following this, the solutions were taken from the prepared suspension to prepare a microscopic slide for evaluation of the motility of the spermatozoa [21]. Epididymal fat was removed from each animal and weighed using a balance (ATX-224 [d=0.0001 g], Shimadzu, Kyoto, Japan). Testicles were also removed to measure lipid peroxidation levels. Samples were kept at -80°C until analysis.

Sperm Parameters

Sperm motility (%), concentration ($\times 10^6$ count/mL) and morphology (% abnormal) were evaluated using previously described methods [22,23]. To evaluate morphology the fixed

and dried smears were embedded with Giemsa stain for 5 min. All parameters were assessed under light microscope.

Histological Evaluation

Testicle tissue samples were fixed in 10% neutral formalin and then routinely processed for embedding in paraffin. 5 μ m sections of the paraffin tissue blocks were stained with Haematoxylin and Eosin. Histological sections were examined using a Leica DM500 light microscope coupled with a Leica Microsystem Framework integrated digital imaging analysis system (Leica Application Suit version 3.0 Serial 38132019 Leica ICC50 HD, Heerbrugg, Switzerland). Seminiferous tubules were scaled (50 tubules per animals) according to Johnsen's Tubular Biopsy Scores (JS) [24].

Blood Parameters

Total cholesterol (in mg/dL) and triglycerides (in mg/dL) concentrations were measured in serum samples (respectively TC Lot. 141617003, TG Lot. 141717003, Mindray, Shenzhen, China) using an automated chemistry analyser (BS120, Mindray, Shenzhen, China). Competitive ELISA test (Testosterone, DE1559, Lot. 29K126, Demeditec, Kiel, Germany) was performed to measure serum testosterone concentrations (in ng/mL). The tests were carried out in accordance with the manufacturer's directions. The washing steps of ELISA test were performed using an automated microtiter washer (MW-12A Microplate washer, Mindray, Shenzhen, China) and results were obtained using a microtiter plate reader at 450 nm (MR-96A Microplate reader, Mindray, Shenzhen, China).

Assessment of Lipid Peroxidation

MDA levels were measured in plasma and testicle samples in order to assess lipid peroxidation levels using commercially available assay kits (TBARS Assay Kit, Item No. 10009055, Batch No. 0510196 and 0502129, Cayman Chemicals, Michigan, USA). Plasma samples were directly treated. However, testicle samples were firstly homogenized. This was performed according to manufacturer's protocol using RIPA buffer (Item No. 10010263, Batch No. 0490889-1, Cayman Chemicals, Michigan, US) and a Dounce tissue grinder set (D8938, Lot. 3110, Sigma-Aldrich, Missouri, US) on ice. Following homogenization, samples were centrifuged at 1600g for 10 min at +4°C and supernatants were used for analysis. The principle measurement was based on the reaction with thiobarbituric acid (TBA) in boiling water for 60 min in acidic medium and measurement of the absorbance of the reaction mixture at 532 nm [25]. Absorbance were measured with a UV/VIS Spectrophotometer (Model T70, S/N:17-1814-01-0059, PG Instruments Ltd, UK). Plasma and tissue MDA concentrations were expressed as μ mol/L and μ mol MDA/g, respectively.

Statistical Analyses

Statistical analyses were carried out using GraphPad Prism

software (version 7.04, GraphPad Software, San Diego, CA, USA). All data were expressed as mean±standard deviation (±SD). Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. A difference of $P<0.05$ was considered statistically significant.

RESULTS

During 63 days, feed consumption was similar among all groups, with exception of the control group ($P<0.001$, Table 2). There was no difference between energy gain and energy gain rate to bodyweight gain. When the epididymal fat mass gains were examined, it was determined that Oil and Oilpro groups were significantly higher compared to the control group ($P<0.05$, Table 2). In addition, the FryOilpro group had lower epididymal fat mass compared to Oil group ($P<0.05$).

No statistically significant difference existed between the groups with regards to either sperm count or morphology (Table 2). However, sperm motility in Oilpro and Fryoilpro group were significantly higher than both Control and Oil groups ($P<0.01$). The results taken from Fryoilpro group demonstrated the highest motility rate in comparison to the other groups. Probiotic use had a positive effect on the sperm motility (Table 2).

Seminiferous tubules of the testicles were evaluated as Johnson score values (Table 2). The JS values of Control, Oilpro and Fryoilpro groups were higher than that of Oil and Fryoil groups. The values of Control were also better than that of Oil and Fryoil values ($P<0.001$). Also, the results of Oil were better than that of Fryoil ($P<0.05$). Both Oilpro and Fryoilpro were better than Fryoil on the JS values

($P<0.001$). The JS values of Oilpro and Fryoilpro were better than that of Oil JS values ($P<0.001$). The consumption of sunflower oil, as natural or fried, decreased tubule JS score when compared with normal diet used for feeding the Control group. Nevertheless, the probiotic use appeared to significantly prevent the effects of high fat and frying oil diets (Table 2).

Sperm morphology and testes tissue were histologically examined (Fig. 1). Abnormal sperm morphology was observed in both control (Fig 1.A) and experimental groups (Fig. 1.B-C). In the histologic evaluation of seminiferous tubules, normal structure was detected in control group (Fig. 1D). Nevertheless, degenerative changes and gaps have been observed in the seminiferous tubules of Fryoil group (Fig.1E). Ameliorative effects of probiotics observed that in Fryoilpro group (Fig.1F).

Triglycerides concentrations were only significantly lower in Oilpro group in comparison with the control group ($P<0.05$). In Oil group, plasma MDA levels, the end products of lipid peroxidation were significantly higher than control group ($P<0.05$). There was a significant difference in the MDA values of Oilpro and Fryoilpro versus Oil ($P<0.05$). However, the MDA values between Fryoil and Fryoilpro groups did not differ statistically ($P>0.05$). Serum concentrations of total cholesterol, triglycerides and testosterone are shown in Table 2. Plasma MDA and testes MDA levels are presented in Fig. 2.

DISCUSSION

Infertility is a worldwide problem and no definable cause can be found in 25% of infertile men^[26]. However, a relationship between increased ROS formation and decreased

Table 2. Feed consumption, weight and energy gain, epididymal fat increment and changes in sperm and biochemical parameters of the groups. Results are represented as \pm SD (n=8)

Parameters	Groups				
	Control	Oil	Oilpro	Fryoil	Fryoilpro
Feed consumption (g), 63 d	271±8.01	216±7.95 ^c	225±7.79 ^c	221±7.23 ^c	227±7.46 ^c
Energy consumption (KCal) 63 d	757	714	744	732	753
Consumed energy per g of WG	178	190	157	183	121
Weight gain (WG, g) 63 d	4.25±2.49	3.75±1.66	4.75±1.48	4.00±2.39	6.25±2.25
Epididymal fat (g)	0.36±0.05	0.55±0.08 ^c	0.49±0.18 ^a	0.45±0.21	0.43±0.13 ^a
Sperm count (x10 ⁶ count/mL)	19.49±6.96	19.75±6.65	19.62±11.17	13.12±5.16	16.81±9.65
Sperm morphology (% abnormal)	28.71±3.81	23.57±7.42	21.75±5.40	28.29±6.58	24.29±9.79
Sperm motility (%)	58.57±7.89	61.14±11.86	70.38±8.76 ^{be}	61.43±13.29 ^g	73.14±4.73 ^j
Seminiferous tubules scale (JS score)	8.40±0.35	7.78±0.43 ^c	8.39±0.34 ^f	7.61±0.27 ^{cd,h}	8.22±0.14 ^{fi}
Total cholesterol (mg/dL)	99.19±24.77	121.09±12.02	129.51±16.10 ^a	109.23±14.26	118.60±30.97
Triglycerides (mg/dL)	106.40±6.9	77.88±25.18	59.39±15.77 ^a	91.69±34.68	93.13±40.26
Testosterone (ng/mL)	2.70±3.69	2.96±4.14	3.38±3.55	2.30±1.74	3.74±3.10

^a $P<0.05$ versus Control; ^b $P<0.01$ versus Control; ^c $P<0.001$ versus Control; ^d $P<0.05$ versus Oil; ^e $P<0.01$ versus Oil; ^f $P<0.001$ versus Oil; ^g $P<0.01$ versus Oilpro; ^h $P<0.001$ versus Oilpro; ⁱ $P<0.001$ versus Fryoil

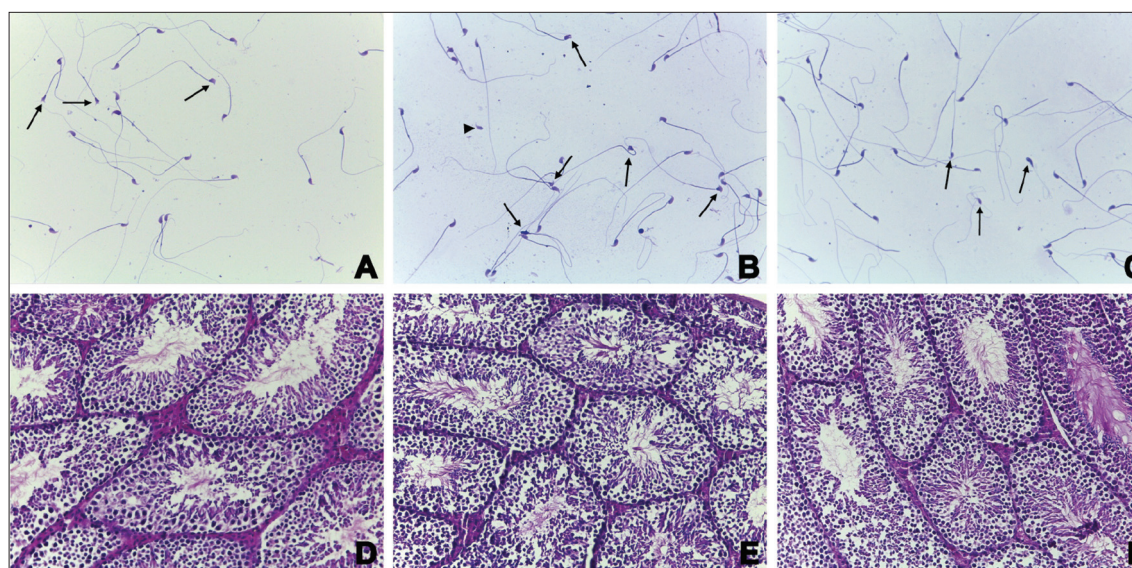


Fig 1. Representative images of sperm and testis tissue showing the effects of frying oil and probiotics. Sperm smear in control (A), Fryoil (B) and Fryoilpro (C) groups. Arrows indicate abnormal sperm, x400, Giemsa stain; Testis seminiferous tubules showed normal structure in control group (D), degenerative changes and gaps in the seminiferous tubules in Fryoil group (E) and ameliorative effects of probiotics observed that in Fryoilpro group (F). x200, H&E

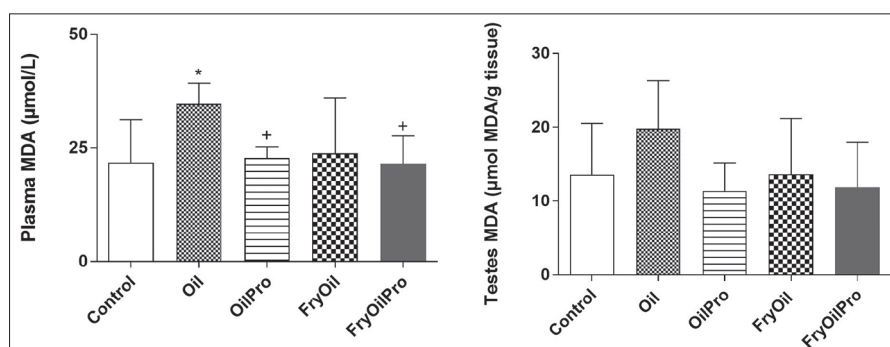


Fig 2. Plasma and testes MDA levels of the mice (\pm SD; n = 8). *P<0.05 compared to the "control"; +P<0.05 compared to "oil" (n = 8)

sperm motility has been determined [27]. High-fat diets are known to negatively affect the antioxidant capacity and increase fat-related oxidative stress and ROS levels. It is possible to decrease the oxidative damage by increasing antioxidant levels [15]. Recently, antioxidant properties of probiotics have been highlighted. Studies on the protective effects of probiotics on fertility in a high-fat diet are limited for both humans and animals [2,16,17]. In this study, the detrimental effects of deep-frying oil, even non-heat-treated sunflower oil, on the reproductive organs of male mice and the protective effect of probiotic use were investigated and evaluated. Free radicals play an important role in the pathophysiology of reproductive dysfunction in male animals and humans. It is known that sperm cell membranes are rich in polyunsaturated fatty acids and are very sensitive to damage from free radicals [28]. High fat-diet model studies have shown that sperm motility decreased, normal morphology has been impaired and antioxidant supplementation increased spermatogonium and Sertoli cell count [29]. Another study reported that high fat diet decreased testosterone level, impaired semen quality, caused atrophy and degeneration in seminiferous tubules,

and these effects were reversed with probiotic use [30]. In this study, it was determined that sperm motility increased significantly in probiotic fed groups. Sperm motility was better in the Fryoilpro group than that of Fryoil group (P<0.01). It has also been reported that high energy diets can cause mitochondrial dysfunction associated with overproduction of reactive oxygen species in testicular metabolism and that probiotics are a potential agent that can be used to eliminate these harmful effects [2,31]. Although the effects are not reflected in the statistics, abnormal sperm rates decreased in the Oil and Oilpro groups compared to the control group, also in the FryOilpro group compared to Fryoil and control groups (P<0.01). It has been observed that frying oil reduces the sperm concentration and the negative effect of the frying oil is reversed in the probiotic fed groups. Nevertheless, these results were not significantly reflected in the statistics.

Probiotics are recommended as an alternative to pharmacological products in many medical conditions, including modulation of obesity, which is often associated with poor semen quality. Dardmeh et al. [2] showed that *Lactobacillus*

rhamnosus PB01 has a positive effect on both weight loss and reproductive hormones, significantly improving sperm motility and kinematic parameters. Numerous studies have been published using various rodent species to investigate the effects of probiotics on health [16,32-35]. One study reported that probiotics improved seminiferous tubules with impaired morphology [36]. In this study, the negative effects of sunflower oil and fried sunflower oil on seminiferous tubules was demonstrated. These negative effects were ameliorated through the use of probiotics to the level of the control group. Additionally, sperm motility and seminiferous tubule scores increased in the probiotic high fat diet group.

An *in vivo* study conducted with rats, demonstrated that high fat diet did not alter cholesterol and triglyceride levels in rats. However, it significantly led to decreases in the testosterone levels and an increased fat mass of the epididymis [37]. Fernandez et al. [38] reported that sperm quality decreased and sperm counts were similar among groups of rats fed with high fat diet as sperm motility was impaired without any effect on other sperm parameters. Our study results showed similarities with those results. Our study showed that epididymal fat mass was significantly higher particularly in the Oil and Oilpro groups when compared with the control group ($P < 0.001$; $P < 0.05$). Epididymal fat mass was significantly higher in the FryOilpro than in the Oil group ($P < 0.05$), and the same trend was seen between Oil group and Oilpro group. The use of probiotics reduced the epididymal fat mass.

High fat diet decreases the levels of testosterone whilst increasing oxidative stress in rats [39]. Here we showed that the levels of testosterone did not change among groups. However, there were slight difference in MDA levels, an oxidative stress indicator between some groups. The levels of cholesterol in Oilpro group were significantly higher compared with control group. In the other groups, there were slight but not significant increases in cholesterol levels. It is thought that this situation may be related to the upregulation of cholesterol biosynthesis which is induced by increases in both lipid levels and the corresponding insulin expression [40]. In contrast to this, it is thought that the decrease in triglyceride levels in the experimental groups might be related to their metabolism. It is known that mice can affect lipid use and transport due to their higher metabolic rates compared with rats, hence this might be related to factors affecting insulin secretion stimulation [41]. Furthermore, excessive triglycerides do not accumulate in the liver of BALB/c mice fed with high fat diet and this may be associated with low fatty acid uptake [42]. Probiotics had no effect on both parameters among the experimental groups.

Diet and probiotics also had an effect on testosterone plasma levels. Although statistical differences were not significant, mean values were higher in probiotic fed group. This suggests that use of probiotics has positive effects on

fertility. These positive effects have been demonstrated in different *in vivo* studies conducted on different animal groups [8,14]. Additionally, MDA levels, an LPO indicator, in testes were slightly increased in Oil and Fryoil groups but not in probiotic groups. This is possibly be related to the duration of the experiment, and results may be significant over a longer period of time. Another finding suggests this may be associated with plasma MDA levels. These results were also similar with in an *in vivo* study conducted on rats [8,15]. It is thought that this type of diet would increase oxidative stress in the testis tissue in the long term and alter MDA values. However, the use of probiotics would be beneficial against these negative effects.

In conclusion, high fat diet, or high-energy diet in male mice, could result in negative effects caused by oxidants on reproduction even though mice were not subject to obesity. This was concluded from the study findings on sperm motility, seminiferous tubule score, MDA levels in plasma and testes. Results indicate the use of probiotics had potential to reverse the negative effects of deep-fried sunflower oil in mice. When the duration and conditions of the study are taken into consideration, it may be suggested that prolonged feeding time and direct gavage of the fat would increase oxidative stress level and negative effects on fertility. This study model could be important for understanding the effects of fast-food dietary habits on human reproductive health globally.

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