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Probiotic supplementation in men with asthenozoospermia: A randomized controlled trial

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ABSTRACT

Objective: To investigate the potential of oral probiotics to improve sperm motility and decrease DNA fragmentation in men diagnosed with asthenozoospermia.

Methods: Men diagnosed with asthenozoospermia, aged between 18 and 40 years, were randomly assigned to receive probiotic or placebo for 10 weeks. Sperm parameters (count, motility, and morphology) and seminal fluid biochemical markers were assessed using light microscopy and Diff-Quik staining. Intracellular reactive oxygen species levels were measured using the malondialdehyde (MDA) technique, while DNA fragmentation index (DFI) was evaluated through acidic aniline blue staining. Data from both groups were compared to determine the effects of probiotic supplementation.

Results: Sixteen men were included. The probiotic group ($n=8$) showed a significant increase in total sperm motility ($P<0.001$) and progressive motility ($P=0.003$) compared to the placebo group ($n=8$). Additionally, sperm count in the probiotic group was significantly higher than in the placebo group, although other sperm parameters did not show significant changes. Notably, levels of MDA ($P=0.027$) and DFI ($P=0.004$) were significantly reduced in the probiotic group, indicating a decrease in oxidative stress and DNA damage.

Conclusions: Probiotic supplementation effectively enhances sperm quality by mitigating oxidative stress and reducing DNA damage, thereby improving sperm motility in men with asthenozoospermia.

Study registration: The trial was registered with the Iranian Registry of Clinical Trials (IRCT20220119053769N1).

KEYWORDS: Sperm quality; Probiotics; Reactive oxygen species; Asthenozoospermia; Antioxidant; DNA damage; Infertility

affecting approximately one in six couples worldwide[1]. Clinically, infertility is defined as the failure to achieve pregnancy after 12 months of regular, unprotected sexual intercourse[2–4]. The global infertility rate among reproductive-age couples is estimated at 15%[4,5], with an average prevalence of 13.2% reported in Iran. Infertility can arise from female factors (50%), male factors (20%–30%), or a combination of both (20%–30%)[1,2]. According to the World Health Organization (WHO), the primary causes of

Key Points

Question: Can oral probiotic supplementation improve sperm motility and reduce oxidative stress and DNA damage in asthenozoospermic men?

Findings: In this randomized trial, 10-week FamiLact® probiotic supplementation significantly enhanced total and progressive sperm motility, increased sperm count, and reduced levels of seminal malondialdehyde (MDA) and DNA fragmentation index (DFI), compared to the control group.

Meaning: Probiotic supplementation may serve as a safe, non-invasive intervention to improve sperm quality by mitigating oxidative stress and DNA damage in men with asthenozoospermia.

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1. Introduction

Infertility is a significant and growing global health concern,

female infertility include ovulatory dysfunction (32%) and tubal damage (26%)[3]. In men, major etiologies include oligo-terato-asthenozoospermia (low sperm count with abnormal morphology and motility) (21%)[3], asthenozoospermia (reduced sperm motility) (17%)[3,4], teratozoospermia (sperm morphological abnormalities) (10%)[5,6] and azoospermia (absence of sperm) (9%)[3]. Sperm motility is crucial for migration through the female reproductive tract and successful fertilization. Impairments in motility, particularly in oligo-terato-asthenozoospermic and asthenozoospermic men, contribute to approximately 38% of male infertility cases[4] underscoring the need for targeted interventions. Asthenozoospermia is typically defined as <40% motile sperm and may result from multiple factors, including flagellar defects[7,8] elevated reactive oxygen species (ROS) and malondialdehyde (MDA) levels[9], or mitochondrial dysfunction[5]. Given that sperm mitochondria, located in the midpiece, generate adenosine triphosphate (ATP) essential for flagellar motion, mitochondrial damage can directly impair motility and fertility[5]. Beyond mitochondrial impairment, oxidative stress plays a pivotal role in male infertility[6]. Oxidative stress occurs when ROS production surpasses antioxidant defenses, leading to cellular damage[9,10]. In sperm, ROS are primarily generated through two mechanisms: the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system at the plasma membrane and the NADH-dependent oxidoreductase pathway within mitochondria[5,9]. Under physiological conditions, mitochondrial ROS generation is the dominant source. Although low ROS levels contribute to sperm maturation and antimicrobial defense[11,12], excessive ROS can induce DNA fragmentation[13–15], lipid peroxidation, and compromise sperm membrane integrity[6,14]. Sperm and seminal plasma possess endogenous antioxidant defenses—both enzymatic [*e.g.*, superoxide dismutase (SOD), glutathione peroxidase, catalase] and non-enzymatic (*e.g.*, vitamins C and E, glutathione, carnitine, and carotenoids)—that counteract oxidative stress. When this balance is disturbed, oxidative damage ensues, severely affecting sperm quality[9]. Numerous studies have recommended antioxidant supplementation—such as vitamins C, E, folate, zinc, selenium, carnitine, and carotenoids—to ameliorate oxidative stress-related damage in male infertility[12,16]. Recently, probiotics have emerged as a promising antioxidant strategy. Defined by the WHO as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host”[11,12,14], probiotics—particularly strains from the genera *Bifidobacterium* and *Lactobacillus*[17–19]—have demonstrated antioxidant properties in both *in vivo* and *in vitro* models. Animal studies have shown that probiotic administration increases antioxidant enzyme activity, while reducing nitric oxide and MDA levels[12]. Additionally, evidence suggests that high-fat diets can exacerbate oxidative stress and sperm damage, which may be mitigated by probiotic intervention[12,13]. Probiotic supplementation

has also been shown to reduce DNA fragmentation and structural abnormalities in sperm. Ross *et al* reported the clinical efficacy of antioxidant therapy in improving sperm quality and pregnancy outcomes in men with infertility[6]. Synbiotic formulations, which combine probiotics and prebiotics, may further enhance these benefits. One such product, FamiLact[®] (Zist Takhmir Co., Iran), contains a consortium of probiotic strains—including *Lactobacillus rhamnosus*, *Bifidobacterium lactis*, *Lactobacillus casei*, *Bifidobacterium breve*, *Lactobacillus acidophilus*, *Bifidobacterium longum*, *Lactobacillus plantarum*, *Bifidobacterium bifidum*, and *Streptococcus thermophilus*—along with fructooligosaccharides as a prebiotic. The prebiotic component promotes the survival and activity of probiotic strains within the gastrointestinal tract, offering synergistic effects beyond those of probiotics or prebiotics alone[2]. Considering the significant impact of asthenozoospermia on infertility and the potential of probiotics to mitigate oxidative stress, this study seeks to evaluate the influence of probiotic supplementation on sperm motility and seminal parameters—specifically morphology, concentration, and volume—in men with asthenozoospermia. Consequently, the results of this investigation may offer critical insights into an affordable and non-therapeutic strategy for enhancing male reproductive health.

2. Methods

2.1. Trial design

This study was designed as a parallel-group, randomized controlled clinical trial with a 1:1 allocation ratio. The intervention period lasted 10 weeks, with assessments conducted at baseline (T0) and at the end of the intervention (T1). The trial was registered with the Iranian Registry of Clinical Trials (IRCT20220119053769N1).

2.2. Participants

The study population consisted of men aged 18 to 40 years who presented to the infertility clinic of Beheshti Hospital in Kashan, Iran, with a diagnosis of asthenozoospermia, defined as progressive motility below 40% based on WHO criteria. Eligibility screening included a full reproductive history, clinical examination, and baseline semen analysis. Individuals were excluded if they were receiving antioxidant or hormonal medications, consumed probiotic-rich diets, were active smokers, or had varicocele, chromosomal abnormalities, or systemic illnesses that could influence semen parameters. Of the 40 men initially screened, 25 met the inclusion criteria; however, 9 either declined participation or discontinued follow-up, resulting in a final sample of 16 participants who were enrolled and randomized.

2.3. Interventions

Participants allocated to the intervention group received an oral probiotic supplement (FamiLact[®], 10⁹ CFU/day), containing multiple strains of *Lactobacillus* and *Bifidobacterium*, for a total duration of 10 weeks. The control group received no active supplement during the study period. All participants were instructed to maintain their usual diet and lifestyle habits and to avoid taking any additional supplements. Adherence and potential adverse events were monitored through weekly telephone contacts and monthly in-person visits. Semen samples were collected after a period of 2–5 days of abstinence and were analyzed within one hour of ejaculation.

2.4. Outcomes

The primary outcome of the study was progressive sperm motility assessed by light microscopy following WHO laboratory guidelines. Secondary outcomes included total motility, sperm concentration, and morphology assessed using Diff-Quik staining, as well as seminal markers of oxidative stress and DNA integrity. Oxidative stress was evaluated by measuring seminal MDA levels using the thiobarbituric acid reactive substances (TBARS) assay. DNA fragmentation index (DFI) was determined using acidic aniline blue staining, with dark-blue staining indicating chromatin immaturity or DNA damage. Additional semen characteristics—such as ejaculate volume, pH, viscosity, and macroscopic appearance—were recorded as supportive parameters.

2.5. Sample size

The sample size calculation was based on the primary endpoint of progressive motility. Previous randomized studies evaluating probiotic or synbiotic supplementation in infertile or asthenozoospermic men reported mean differences of 10%–12% in progressive motility with standard deviations of approximately 8%–10%. Using a two-sided alpha level of 0.05 and a statistical power of 80%, a minimum of 14 participants (7 per group) was required. To compensate for possible attrition, 16 participants were ultimately enrolled. Although the final sample size was modest, appropriate non-parametric statistical methods were used, and the limitations were addressed in the discussion.

2.6. Randomisation

Randomization was performed using a computer-generated list of random numbers to assign participants in a 1:1 ratio to the probiotic or control group.

2.7. Allocation concealment mechanism

Group assignments were placed in sequentially numbered, opaque,

sealed envelopes prepared by a researcher who was not involved in recruitment or outcome assessment, ensuring concealment until the moment of assignment.

2.8. Implementation

Participant enrollment was conducted by an investigator who was unaware of the allocation sequence. After confirming eligibility, the investigator opened the next envelope in sequence and assigned the participant to the corresponding study arm.

2.9. Blinding

Due to the nature of the intervention, blinding of participants was not feasible. However, all laboratory personnel responsible for evaluating semen parameters, MDA concentrations, and DFI measurements were blinded to group allocation to minimize assessment bias.

2.10. Statistical analysis

Data were analyzed using SPSS version 26 (IBM Corp., Armonk, NY, USA). Normality was assessed using the Shapiro–Wilk test. Within-group differences between baseline and post-intervention measurements were evaluated using the Wilcoxon signed-rank test. Between-group comparisons, including analysis of change scores ($\Delta = T1 - T0$), were performed using the Mann–Whitney *U* test or *t*-test. Normal distribution data were expressed as mean $\pm$ standard deviation (mean $\pm$ SD). Because most variables demonstrated non-normal distributions, results were expressed as median values with interquartile ranges (IQR). A two-tailed *P*-value of less than 0.05 was considered statistically significant.

2.11. Ethics statement

This study was approved by Ethical Committee of Kashan University of Medical Sciences (IR.KAUMS.MEDNT.REC.1401.40001), at the date of 01.06.2021. All study process was presented to participants and they were reassured about confidentiality of their records; they were requested to sign their written consent of participation in the study.

3. Results

3.1. Baseline demographics of participants

A total of sixteen men with asthenozoospermia completed the study and were included in the final analysis (*n*=8 probiotic; *n*=8 control). Baseline demographic, anthropometric, and semen characteristics were comparable between the two groups

Table 1. Baseline characteristics of participants in the probiotic and control groups.

Variables	Control (n=8)	Probiotic (n=8)	P-value
Age, years	32.6±5.8	35.4±4.7	0.32
Height, cm	177.62±3.96	175.00±9.64	<0.50
Weight, kg	87.50±14.22	83.87±13.38	0.61
BMI, kg/m ²	27.64±3.59	27.43±3.94	0.91
Total motility, %	20 (13–24)	21 (14–30)	0.84
Sperm count, ×10 ⁶ /mL	38 (24–50)	45 (30–65)	0.60
Morphology, %	2 (1–2.5)	2 (1.5–2.5)	0.87
Volume, mL	3.6 (3.0–4.4)	3.5 (2.8–4.8)	0.71
MDA, nmol/mL	0.082 (0.07–0.09)	0.078 (0.06–0.09)	0.62
DFI, %	26 (24–28)	26 (24–27)	0.90

Data are expressed as mean±SD or median (IQR). BMI: body mass index; MDA: malondialdehyde; DFI: DNA fragmentation index.

with no significant differences observed in sperm motility, concentration, morphology, semen volume, or oxidative stress markers (Table 1).

3.2. Motility parameters

After 10 weeks, the probiotic group demonstrated substantial improvements in motility indices. Total motility and progressive motility increased significantly relative to baseline, whereas the control group showed no meaningful changes. Analysis of change scores ($\Delta = T1 - T0$) confirmed significantly greater increases in both parameters in the probiotic group compared with controls (Figure 1 A, B).

3.3. Sperm count

Sperm concentration increased markedly in the probiotic

group ($\Delta +21 \times 10^6/\text{mL}$), while a decline was observed in controls ($\Delta -11 \times 10^6/\text{mL}$). The between-group Δ -difference was statistically significant (Figure 1C).

3.4. Morphology and semen volume

Sperm morphology and semen volume showed minimal within-group changes and no significant between-group Δ -differences, indicating no measurable effect of the intervention on these parameters (Figure 1 D, E).

3.5. Comparative analysis of baseline-to-post-intervention changes in semen parameters

The probiotic group showed significant gains in total motility, progressive motility, and sperm concentration compared with minimal or negative changes in the control group. Table 2 presents the post-intervention values and T1–T0 (Δ)-changes for semen parameters. Between-group Δ -comparisons confirmed that the increases in total motility (+13%), progressive motility (+9%), and sperm concentration ($+21 \times 10^6/\text{mL}$) were significantly greater in the probiotic group. In contrast, morphology and semen volume remained unchanged in both groups, indicating no treatment effect on these variables.

3.6. Seminal MDA concentration

The probiotic group exhibited a significant reduction in seminal MDA, reflecting a decrease in lipid peroxidation and oxidative stress. The control group demonstrated no change. Δ -analysis confirmed a significantly greater reduction in the probiotic group (Table 3; Figure 2).

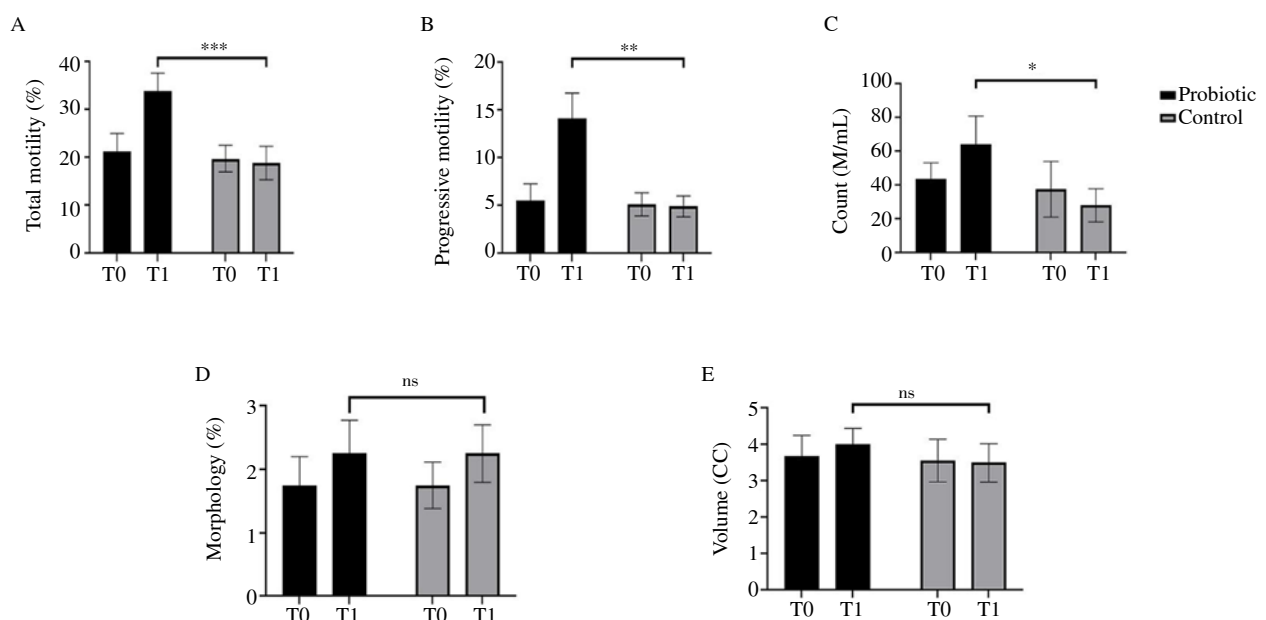


Figure 1. Effects of probiotic supplementation on semen parameters in asthenozoospermic men. A) Total motility; B) Progressive motility; C) Count; D) Morphology; E) Volume. T0: baseline; T1: the end of the intervention. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. ns: not significant.

Table 2. Post-intervention values and change scores ($\Delta = T1-T0$) for semen parameters.

Variables	Control T0	Control T1	Δ Control	Probiotic T0	Probiotic T1	Δ Probiotic	ΔP -value
Total motility, %	20 (13–24)	19 (12–23)	–1	21 (14–30)	34 (27–42)	+13	<0.001
Progressive motility, %	5 (2–8)	5 (2–7)	0	6 (3–10)	15 (10–18)	+9	0.003
Sperm count, $\times 10^6/\text{mL}$	38 (24–50)	27 (18–33)	–11	45 (30–65)	66 (42–89)	+21	0.02
Morphology, %	2.0 (1.0–2.5)	2.5 (2.0–3.5)	+0.5	2.0 (1.5–2.5)	3.0 (2.0–3.5)	+1	0.90
Semen volume, mL	3.6 (3.0–4.4)	3.5 (2.9–4.2)	–0.1	3.5 (2.8–4.8)	4.0 (3.2–5.0)	+0.5	0.38

Data are expressed as median (IQR). T0: baseline; T1: the end of the intervention.

Table 3. Changes ($\Delta = T1-T0$) in oxidative stress and DNA fragmentation.

Variables	Control T0	Control T1	Δ Control	Probiotic T0	Probiotic T1	Δ Probiotic	ΔP -value
MDA, nmol/mL	0.082 (0.07–0.09)	0.080 (0.07–0.09)	–0.002	0.078 (0.06–0.09)	0.065 (0.05–0.07)	–0.013	0.03
DFI, %	26 (24–28)	25 (23–28)	–1	26 (24–27)	22 (20–23)	–4	0.004

Data are expressed as median (IQR). MDA: malondialdehyde; DFI: DNA fragmentation index. T0: baseline; T1: the end of the intervention.

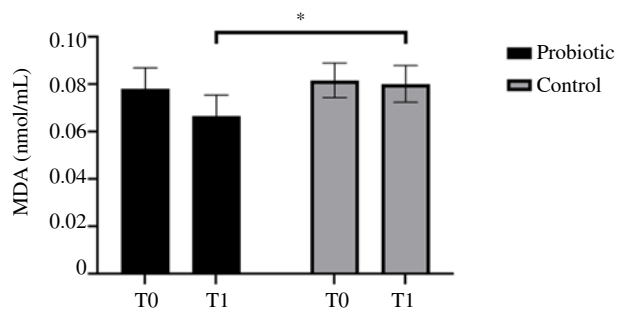


Figure 2. Reduction of seminal malondialdehyde (MDA) levels following probiotic supplementation. T0: baseline; T1: the end of the intervention. * $P < 0.05$.

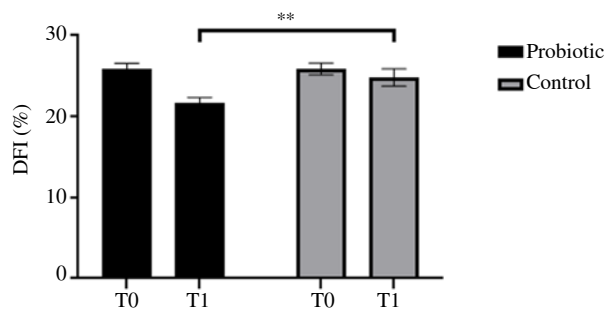


Figure 3. Decrease in sperm DNA fragmentation index (DFI) after probiotic supplementation. T0: baseline; T1: the end of the intervention. ** $P < 0.01$.

3.7. DFI

Probiotic supplementation resulted in a significant decrease in DFI, indicating improved sperm DNA integrity. The control group showed minimal change. The between-group Δ -difference was statistically significant (Table 3; Figure 3).

4. Discussion

Asthenozoospermia, a major cause of male infertility, is marked by reduced sperm motility. Enhancing motility and overall sperm quality is vital for natural conception and improving outcomes in

assisted reproductive techniques, including intracytoplasmic sperm injection (ICSI) and *in vitro* fertilization (IVF)[4,20]. This study investigated the effects of FamiLact[®] supplementation on sperm parameters in men with asthenozoospermia. Post-treatment analysis demonstrated that FamiLact[®] supplementation significantly enhanced total and progressive sperm motility and increased sperm count, whereas other seminal parameters were unchanged. Consistent with our study, another study shows that FamiLact[®] supplementation after 80 days at a dose (500 mg daily) improves semen parameters such as concentration, motility and abnormal morphology in men with idiopathic infertility. Based on this, synbiotic products or more precisely, FamiLact[®] can be considered as a safe and cost-effective treatment for the treatment of male infertility[2,21]. This study is similar to the present study both in terms of the type of probiotic supplement and in terms of the research result.

In a study, Valcarce *et al* used *Lactobacillus rhamnosus* CECT8361 and *Bifidobacterium longum* CECT7347 as probiotic strains in 9 patients with asthenozoospermia, where each volunteer took one daily capsule at a dose of 10^9 CFU per day. It did not affect parameters such as concentration, volume or pH, but it caused a significant improvement in sperm motility compared to the control group[4]. These probiotic strains are present in the FamiLact[®] supplement used in our study. It is also similar to the present study in terms of the dosage and its final result on people with asthenozoospermia. In a study conducted in 2020, by prescribing a probiotic supplement with *Lactobacillus* and *Bifidobacteria* species that was administered daily with 500 mg of probiotics for 10 weeks, sperm parameters including ejaculate volume, total sperm count, sperm concentration, and motility were significantly improved. Total sperm and live sperm significantly increased in the intervention group compared to the placebo group. Other parameters, such as normal morphology and one of the characteristics of sperm motility, did not have statistically significant changes[22]. Although the bacterial species, duration of use, and overall outcomes were similar to our study, one notable difference is that this study did not observe a significant change in one of the motility parameters. Maretti *et al* conducted a randomized clinical trial study consisting of 20 people receiving Flortec and 21 people receiving placebo (starch) for 6 months. They emphasized a significant increase in average progressive sperm motility as well

as concentration, normal morphology, total number and volume of semen[23]. Administration of Flortec to oligoasthenoteratospermia patients significantly improved sperm count and semen volume compared to a placebo group. Also, DFI was significantly reduced in the Flortech group, and Lee did not find any difference in the starch group[19]. Although the final results of this study were similar to the current study, there are differences with the current study in terms of the contents of the Flortec supplement and the time period (about twice the time of the current study).

Since the process of oxidative stress and excessive production of ROS leads to the damage of biological molecules in the body, and one of its reactions is lipid peroxidation, the final product of this reaction is the production of MDA. Therefore, in this study, we documented the changes in semen MDA as a document for intracellular ROS, and therefore measured the amount of semen MDA after consuming a period of FamiLact[®] supplementation, and the concentration of MDA in the FamiLact[®] group compared to the control group was significantly higher. It decreased, and as a result, intracellular ROS and free radical concentration decreased, and with the reduction of ROS, the amount of sperm DNA damage also decreased. Excessive production of ROS and abnormal sperm in semen and subsequent oxidative stress is one of the causes of infertility in men. Since antioxidants play an essential role in protecting cells against free radicals, it is likely that the decrease in the activity of antioxidants in the physiological system of the body is associated with a decrease in sperm quality[24]. In this regard, many previous studies have shown that lifestyle changes can significantly improve the quality and movement of semen. Antioxidant therapy is widely used to improve sperm quality, including sperm motility. This issue is exemplified by studies on antioxidants such as vitamin D3 and melatonin. For instance, vitamin D3 supplementation was studied as a strong antioxidant for treating asthenozoospermia[25]. In another study, Valcarce *et al* investigated melatonin, an antioxidant noted for its capacity-increasing properties. They have used total antioxidants and increased sperm quality in treated men[4]. Also, many studies have reported the effect of administration of oral antioxidants on sperm parameters. However, Piomboni *et al* also reported that selenium supplementation as an antioxidant had no effect on sperm parameters[26].

In recent years, it has been proven that probiotics have properties such as antiseptic agents against certain pathogens, antimicrobial activity, maintenance of tight junctions, modification of intestinal flora and metabolic activity. Probiotics also have a nutritional and anti-inflammatory effect on the mucosa. More importantly, the antioxidant properties of probiotics have been reported many times in different animal and human studies[24]. Some probiotics have been reported to increase the activity of antioxidant enzymes or modulate circulating oxidative stress, which protects cells from cancer-induced damage. These enzymes include glutathione S-transferase, glutathione, glutathione reductase, glutathione peroxidase (GPX), SOD and catalase. The production of bioactive peptides is considered

as an effective method of antioxidant activity in foods containing probiotic bacteria. These peptides obtained from hydrolyzed food proteins have antioxidant activities that can protect against the peroxidation of lipids or fatty acids. Also, some *Lactobacilli* have antioxidant activity and can reduce the risk of ROS accumulation during food consumption. Strong evidence for the antioxidant activity of *Lactobacillus* (*L.*) *plantarum*, *L. helveticus*, *L. acidophilus*, *L. fermentum*, *L. casei*, *Lactobacillus GG*, and several *Bifidobacteria* and foods containing these organisms have been conducted by studies[27]. Some of these mentioned bacteria correspond to the bacteria found in the femlock of the present study.

According to reports, high blood cholesterol or triglycerides cause testicular dysfunction and low quality of semen[27]. In fact, it can be concluded that high cholesterol and triglycerides stimulate the production of free radicals and reduce the antioxidant properties of intracellular antioxidants. And with the increase of fatty acids, especially unsaturated, it increases the probability of reaction with free radicals and causes lipid peroxidation. In a study in hyperlipidemic rats, the number of testicular cells, including spermatids and spermatocytes, decreased. One of the reasons for this decrease was due to increased oxidative stress. Therefore, according to our study, probiotics with a dose of 10^9 CFU were given to the male patients for 70 days. Probiotics reduced blood lipid levels by inhibiting hepatic cholesterol biosynthesis. In fact, bacteria produce short-chain fatty acids. These fatty acids cause cholesterol synthesis in the liver and distribution of cholesterol from plasma to the liver, or they affect cholesterol metabolism by absorbing cholesterol directly from the intestine[26]. Importantly, the *Lactobacillus* and *Bifidobacterium* strains contained in the FamiLact[®] supplement enhance total antioxidant capacity (TAC) and attenuate oxidative stress markers *via* multiple mechanisms, including the scavenging of ROS. Evidence from murine models indicates that probiotic administration can lower seminal free radical levels, thereby diminishing oxidative stress and subsequently reducing sperm DNA fragmentation[2].

In a study on six strains of *Lactobacillus acidophilus* and two strains of *Bifidobacterium longum*, all strains were able to protect lipids against oxidative stress and reduce MDA in semen[28]. In all the mentioned studies, bacterial strains similar to our study have been used and they almost improved sperm parameters and antioxidant capacity, but in our study, by assigning subjects to asthenozoospermic men, in addition to sperm parameters, the amount of MDA in the semen was measured, which is a proof of the presence of intracellular ROS. We also investigated the amount of sperm DNA damage that is related to the level of ROS. In addition, the higher content of unsaturated fatty acids on sperm also leads to an increase in mitochondrial reactive oxygen species (mROS), which leads to the loss of motility and DNA damage[4]. According to the results of the study conducted on FamiLact[®], a significant reduction in the DFI was detected in both FamiLact[®] and placebo recipients. In fact, the oxidative stress reaction causes

sperm DNA damage. Sperm DNA damage impairs the ability of sperm fertilization, implantation and embryo development, and subsequently reduces the rate of pregnancy and live birth. Therefore, by reducing the oxidative stress of seminal fluid, DNA damage of sperm is reduced[2]. In this regard, according to the present study, we observed a decrease in sperm DNA fragmentation in the FamiLact® group compared to the control group, and the decrease was significant. Also, there was no significant difference between the oxidative stress indices (in plasma and semen) and inflammatory factors (in plasma) between the two groups by prescribing probiotic supplements to oligoasthenoteratozoospermic individuals at the beginning of the study. However, at the end of the study, TAC and MDA levels of plasma and semen in the intervention group were significantly changed compared to placebo. On the other hand, the high content of polyunsaturated fatty acids (PUFA) in the sperm membrane increases mitochondrial ROS, which leads to loss of motility and DNA damage. This DNA damage causes a severe decrease in sperm motility. Based on the findings of the studies, it seems that probiotics reduce the oxidation of sperm membrane lipids by increasing the antioxidant power[22]. In previous reports, antioxidants such as vitamin E and *N*-acetylcysteine have been reported to improve the health of sperm plasma membrane and ultimately reduce DNA damage[29]. According to the studies, asthenozoospermic men were analyzed for DNA fragmentation after consuming two probiotic strains of *Lactobacillus rhamnosus* and *Bifidobacterium longum*[19,30]. It is quite clear that there is a relationship between poor motility and a high level of DNA damage. Also, after taking probiotics, a reduction in DNA fragmentation was observed compared to the control group[4]. Also, Maretti *et al* observed that DNA fragmentation was significantly reduced in the Flortek group, but no difference was found in the group receiving starch[23].

While the findings of this study provide valuable insight into the potential benefits of probiotic supplementation in asthenozoospermic men, some limitations should be considered. The sample size was relatively small, which may affect the generalizability of the findings. Additionally, the 10-week intervention period may not fully capture the long-term impact of probiotic supplementation on male fertility. While key parameters such as sperm motility, concentration, and oxidative stress markers were assessed, outcomes like hormonal profiles, pregnancy rates, and live births were not included. Further studies with larger populations and extended follow-up are recommended to validate and expand upon these results.

In conclusion, probiotic supplementation exerts a comprehensive protective effect on male fertility by enhancing sperm motility and count while reducing markers of oxidative stress and DNA fragmentation. These benefits are likely mediated through the attenuation of mitochondrial ROS production, preservation of membrane integrity, and mitigation of lipid peroxidation, as indicated by decreased seminal MDA levels. In addition, the anti-inflammatory and immunomodulatory actions of probiotic *Lactobacilli* may further

support sperm function by modulating the seminal microenvironment and signaling pathways associated with oxidative and inflammatory stress. Collectively, these findings highlight probiotics as a promising therapeutic approach for male infertility, particularly in patients with oxidative stress- and inflammation-driven sperm dysfunction.

Conflict of interest statement

The authors of the present study have no conflict of interest.

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Availability of data and material

The primary data for this study is available from the authors on direct request.

Authors' contributions

Ghazaleh Moshkdanian and Hamed Haddad Kashani were responsible for the study conception and design. Hassan Hassani Bafrani and Ghazaleh Moshkdanian performed data collection. Mina Toghraiee and Hamed Haddad Kashani prepared the first draft of the manuscript. Hamed Haddad Kashani and Zahra Ahaz made critical revisions to the paper for important intellectual content. All authors approved the final version of the manuscript draft.

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