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ORIGINAL ARTICLE



Probiotic effects on sperm parameters, oxidative stress index, inflammatory factors and sex hormones in infertile men

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ABSTRACT

Decreased sperm motility is one of the main causes of male infertility. The aim of this study was to evaluate the effects of probiotic supplementation on semen quality, seminal oxidative stress biomarkers, inflammatory factors and reproductive hormones. In this randomised, double-blind controlled clinical trial, 52 men with idiopathic oligoasthenoteratozoospermia attending a urology clinic, were randomly assigned to either an intervention or placebo ($n = 26$) group. This investigation was registered by the identification code of IRCT20141025019669N7 in the clinical trials registry of Iran. The Intervention group took 500 mg of Probiotics daily and the placebo group took a daily placebo for 10 weeks. Semen parameters, total antioxidant capacity, malondialdehyde, inflammatory factors and reproductive hormones were measured at baseline and at the end of the study. After the intervention, ejaculate volume, number, concentration and the percentage of motile sperm, total antioxidant capacity of plasma significantly increased and the concentration of plasma malondialdehyde and inflammatory markers significantly decreased in the intervention group. Probiotic supplementation in infertile men lead to a significant increase in sperm concentration and motility and a significant reduction in oxidative stress and inflammatory markers. Therefore, oral intake of probiotics has the potential to be one of the ways to deal with oxidative damage of sperm.

ARTICLE HISTORY

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KEYWORDS

Probiotic; fertility; semen analysis; asthenozoospermia; sex hormones

Introduction

According to the existing definitions, infertility is recognized as non-pregnancy after one year of unprotected sexual relations (Kukla, 2019). This disorder is one of the health problems that affect patients individually, socially and economically (Sarac & Koc, 2018). Fifty per cent of infertility problems related to male factors and male infertility is mainly due to a defect in spermatogenesis, which can be attributed to sperm dysfunction, reduced sperm count, sperm maturation, and sperm motility (Agarwal et al., 2015). The exact mechanism of the defect in sperm function in many cases is not known and these are called idiopathic causes. World Health Organisation (WHO) defined several subtypes of sperm malformation: asthenozoospermia, oligozoospermia, teratozoospermia, or their combinations. According to the WHO, the lower limit of normal sperm

motility is 32% of progressively motile sperm and 40% of total motile sperm (Dickey et al., 1999).

The human body system has a defensive system for dealing with free radicals called the antioxidant system (Forman et al., 2015). The imbalance between the produced free radical amounts and the antioxidant capacity causes oxidative stress (Bhattacharya, 2015). Reactive oxygen species (ROS) in physiological numbers are required for sperm motility, hyperactivation, capacitation, acrosome reaction and nuclear condensation. However, pathological levels of ROS can damage sperm function and reduce sperm motility, mainly through exhaustion of intracellular Adenosine triphosphate (ATP), and lipid peroxidation of the plasma membrane (Ayaz et al., 2018). In patients with infertility, the level of free radicals was significantly higher than that of healthy subjects and therefore, it seems that the

reproductive capacity has a significant relationship with the intake of dietary antioxidants (Bui et al., 2018).

Many studies have shown that in men with fertility problems, the level of inflammatory factors in plasma and semen are higher than in healthy people without infertility problems (Agarwal et al., 2018). Of course, it should be noted that appropriate levels of macrophages are essential for the proper functioning of sperm, but when the level of macrophage activity markers (such as neopterin) go up, they cause reproductive impairment (Mayerhofer et al., 2018). In the study of Sharkey et al. (2017), sperm motility was negatively correlated with the inflammatory factor of C-X-C motif chemokine ligand 8 (CXCL8) and the concentration of sperm.

Probiotics are well-documented as intestinal-based dietary bacteria that regulate the local immunity of the gastrointestinal system, and thus, they can activate metabolic pathways, as well as recover lost cell haemostasis and overall health (Rosenberg et al., 2016). Several mechanisms have been proposed for how probiotics work, including the elimination of pathogens as well as the production of inhibitors such as bactericides and organic acids (Alok et al., 2017). Extreme effects of probiotics as potent antioxidants have also been shown in numerous studies. In mice, for example, probiotics have an antioxidant effect to regulate the activity of free radicals, increase the activity of antioxidant enzymes, and also decrease the content of nitric oxide and malondialdehyde levels (Mishra et al., 2015). Lactic acid bacteria (LAB) and bifidobacteria are probiotics that are consumed more than other species, which have strong antioxidant properties. These metabolic antioxidant activities may be assigned to ROS scavenging, enzyme inhibition, and reduction activity or inhibition of ascorbate autoxidation in the intestine by neutralising free radicals (Ghoneim & Moselhy, 2016). Also, several randomized controlled trials have now shown that microbial modification by probiotics may improve gastrointestinal symptoms and inflammation in rheumatoid arthritis, ulcerative colitis, and multiple sclerosis (Saez-Lara et al., 2015). Therefore, the present study was designed to evaluate the efficacy of probiotic supplements on sperm quality, oxidative stress index, inflammatory factors, and sex hormones in men with idiopathic infertility.

Material and methods

Study design

This was a double-blind randomized controlled clinical trial which was approved by the Ethics Committee of

the Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (Code of Ethics Committee: IR.AJUMS.REC.1396.621). A total of 52 infertile men were recruited from the Department of Urology, Imam Khomeini hospital, Ahvaz Jundishapur University of Medical Sciences between April 2018 and March 2019. This trial was registered by the identification code of IRCT20141025019669N7 in the clinical trials registry of Iran. Despite the approval of the research project and its registration on the IRCT website, the study began shortly after the date due to the late preparation of supplements. This work was financially supported by supported by a Grant (Number: NRC-9618) from Vice-Chancellor for Research Affairs of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Inclusion and exclusion criteria

The Inclusion criteria were: (i) willing to cooperate; (ii) age range 20–45; (iii) oligoasthenoteratozoospermia of unknown origin (idiopathic) (Gottardo & Kliesch 2011); and (iv) normal levels of gonadotropins, testosterone and serum prolactin. Patients were excluded from the study if any of the following conditions existed: (i) there was a known cause of infertility (such as hormonal disorders, epididymal duct obstruction, and epididymo-orchitis), (ii) drugs or alcohol consumption; (iii) diabetes; (iv) kidney disease (creatinine more than doubled); (v) chronic liver disease (more than twice the normal transaminase); (vi) varicocele; (vii) infectious diseases with fever and leukocytosis characterized by chromosomal abnormalities; (viii) debilitating diseases sperm and sexual system such as varicocele; (ix) drugs that stimulate sexual system or interfere with sex hormones; (x) patients undergoing intracytoplasmic sperm injection (ICSI) due to sperm quality severe impairment and the presence of other causes of infertility; (xi) contact with pesticides, heavy metals and solvents; (xii) taking antioxidant supplements in the past three months; and (xiii) a body mass index (BMI) of 30 kg/m² or greater.

Subjects

At the beginning of the study, patients were randomized to receive either 500 mg of probiotic capsules daily (group 1), or a placebo (group 2) for 10 weeks. Patients were matched in terms of age, sex, and weight at the time of randomization. The coding was done by a third person, and the researcher and the patient were blind to the allocation. The capsules used in this study were prepared by the Zist Takhmir

Company, and the placebo capsules were completely similar to the probiotic appearance but containing only Maltodextrin. The combination of probiotic capsules includes: *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Streptococcus thermophiles*, Total viable count (TVC): 2×10^{11} colony forming units (CFU). Participants were interviewed face to face by trained professional nutritionists. All participants were asked to avoid using probiotic yogurt for the duration of the study due to the presence of lactobacilli and any other supplements to prevent inaccurate evaluation of the factors studied. In order to evaluate the diet of patients at the beginning and the end of the study, 3-day food recalls were completed through face-to-face interviews and telephone calls. The analysis of this questionnaire was done using Nutritionist IV (N4) nutritional software. Also, to evaluate the physical activity, we used the International Physical Activity Questionnaire (IPAQ). Data from the IPAQ were converted to metabolic equivalent-minutes/week using existing guidelines (Papathanasiou et al., 2009). Informed consent was obtained from these individuals to participate in the study.

Sample size calculation

To determine the sample size, we used the volume of the ejaculate (ml), before and after the probiotic with a prebiotic supplementation (Maretti & Cavallini, 2017). Where α (type-1 error) is 0.05, β (type-2 error) is 0.2, S_1 and S_2 are the variances of the volume of the ejaculate (ml), and Δ represent the different means of it. Thus, the power for detecting a difference between the two groups for various outcomes in the present study was 80%. The sample size required was 19 in each group. Considering the drop-in participants during the study, 26 people were considered for each group (i.e. a total of 52 infertile men).

Preparation of samples

After 3 days of sexual abstinence, semen samples were taken at the urology unit of Imam Khomeini hospital, Ahvaz Jundishapur University of Medical Sciences (Ahvaz, Iran). All semen allowed to liquefy at 37°C and then semen analysis was performed according to the World Health Organisation (WHO, 2010). After semen analysis, remnants of liquefied semen were immediately centrifuged at $300 \times g$ for 10 min. Also, venous blood samples were centrifuged at $3,000 \times g$ at

4°C for 10 minutes and serum was aspirated for hormone assays. The serum was stored at -80°C until further assays.

Biochemical analysis

A turbidimetric immunoassay was used for measurement of C-reactive protein (CRP) levels (BioSystems Co, Barcelona, Spain). Also, an enzyme-linked immunosorbent assay (ELISA) (DIAsource Co, Belgium) was used for determining serum levels of tumour necrosis factor α (TNF- α). Serum and seminal malondialdehyde (MDA) levels were measured by tiobarbituric acid method. Colorimetric method was used for analysing serum and seminal total antioxidant capacity (TAC) (Randox Laboratories Ltd, UK) (see Khosrowbeygi & Zarghami (2007)).

Reproductive hormones assay

Serum testosterone and prolactin were assayed using commercial radioimmunoassay kits. These commercial kits had been previously used with an inter-assay and intra-assay variation of less than 10%. The reference range for testosterone and Prolactin (PRL) is 10 to 35 nmol/l and 92 to 697 pmol/l, respectively. Luteinizing hormone (LH) was measured by immunochemiluminometric assay, in which intra-assay and interassay coefficients of variation were 3.4 and 3.8%, respectively. The normal LH range is 1.5 to 9.3 IU/l. Follicle-stimulating hormone (FSH) was also measured using immunochemiluminometric assay with an intra-assay and interassay coefficient of variation of 3.2 and 6.7%, respectively. The normal FSH range is 1.4 to 18.1 IU/l.

Statistical analysis

All data were presented as mean \pm standard deviation (SD). The distribution of the data was evaluated by the Kolmogorov–Smirnov test. Due to the normal distribution of variables, the independent sample t-test and the paired sample t-test were applied to analyses differences in variables between and within groups, respectively. Statistical computations were calculated using SPSS 16 for windows software (SPSS Inc., Chicago, IL, USA). $p < 0.05$ was considered statistically significant.

Results

Of the 63 patients who were ready to participate in this investigation, 11 did not meet the inclusion

Table 1. The comparison of baseline characteristics of the participants.

Characteristics	Probiotic (<i>n</i> = 25) (Mean ± SD)	Placebo (<i>n</i> = 25) (Mean ± SD)	<i>p</i> Value
Age (years)	32.23 ± 4.11	33.01 ± 3.91	*0.601
Duration of Marriage (years)	4.03 ± 1.11	4.29 ± 1.03	*0.317
Smoking history (number/percent)			
Never (%)	21 (84)	22 (88)	**0.801
Current smoker	4 (16)	3 (12)	
Education Status (number/percent)			
Less than high school	4 (16)	4 (16)	**0.703
High school diploma	11 (44)	10 (40)	
Bachelor's or higher	10 (40)	11 (44)	
Weight (kg)			
Base	87.17 ± 6.31	84.09 ± 8.41	0.129
End 12 weeks	86.45 ± 8.11	84.19 ± 7.8	0.225
BMI (kg/m ²)			
Base	27.23 ± 3.07	26.3 ± 2.11	0.17
End 10 weeks	27.01 ± 3.95	26.39 ± 1.95	0.303
Physical activity (met-h/week)			
Base	29.93 ± 4.25	29.18 ± 3.87	0.611
End 10 weeks	30.06 ± 4.14	29.23 ± 3.42	0.47

SD: Standard Deviation, %: Percent of patients in intervention or placebo group.

*Based on statistical analyses Independent samples *t* test.

**Based on statistical analyses Chi-squared test.

criteria. Therefore, 52 patients were recruited. However, one patient in the intervention group and one in the placebo group were excluded for personal reasons (Figure 1). The rate of cooperation and compliance of the patients in this study was 96.1 per cent. The mean age of the participants was 32.62 ± 4.01 years old. Patients did not report any serious adverse effects during the study related to the consumption of the probiotic capsule or placebo. There was no significant difference between groups in the baseline characteristics, physical activity and dietary intakes (Table 1). At the beginning of the study, participants did not have a statistically significant difference in sperm parameters that were used to evaluate sperm quality. These parameters included total sperm count, sperm concentration, and the percentage of progressive motile sperm, total motility, and sperm morphology (Table 2). However, at the end of the study, probiotic supplementation produced significant statistical changes. Ejaculate volume, total sperm count, sperm concentration, sperm total motility and live sperm significantly increased in the intervention group compared to the placebo group (Table 3). Other parameters such as normal morphology and one of the characteristics of sperm motility (grade c), did not have statistically significant changes (Table 2). Also, within groups analysis indicated that changes in the ejaculate volume, total sperm count, sperm concentration, sperm total motility and live sperm in the intervention group at the end of the study are statistically significant (Table 2).

At the beginning of the study, there was no significant difference between the oxidative stress indices (in the plasma and the semen) and inflammatory

factors (in the plasma) between the two groups. However, by the end of the study, plasma and semen TAC and MDA levels in the intervention group changed significantly compared to the placebo. Also, after 100 weeks of probiotic supplementation there was a reduction in CRP and TNF α (Table 3).

There was no significant difference in sex hormones between the two intervention and placebo groups. But probiotic supplementation after 10 weeks could increase testosterone and decrease serum FSH, LH and PRL, but these differences were not significant (Table 4).

Discussion

Excessive production of ROS by leukocytes and abnormal sperm in semen, and subsequent oxidative stress, is one of the reasons for infertility in men. Since antioxidants play a pivotal role in protecting cells against free radicals, it is likely that reducing the activity of the antioxidants in the body's physiological system is associated with a decrease in the quality of sperm (Majzoub & Agarwal, 2018). In recent years, probiotics have proven to have properties such as anti-infectious agents against certain pathogens, antimicrobial activity, maintaining tight connections, modifying intestinal flora and metabolic activity. Probiotics also have a nutritional and anti-inflammatory effect on mucus. The antioxidant properties of probiotics have been reported in various animal and human studies (Mishra et al., 2015).

According to our results, daily supplementation with 500 mg of probiotics for 10 weeks significantly improved sperm parameters. At the end of the study,

Table 2. Within- and between-group comparison of sperm quality parameters in baseline and after intervention in two groups.

Variables	Probiotic (n = 25) (Mean ± SD)	Placebo(n = 25) (Mean ± SD)	P1
Ejaculate volume (ml)			
Baseline	3.6 ± 0.91	3.73 ± 0.61	0.751
End	4.94 ± 0.63	3.85 ± 0.74	0.049
P2	0.041	0.801	
Total sperm count (10 ⁶ sperm/ejaculate)			
Baseline	57.6 ± 7.09	59.68 ± 8.03	0.358
End	79.04 ± 14.21	61.6 ± 8.47	0.002
P2	0.001	0.18	
Sperm concentration(*10 ⁶ /ml)			
Baseline	16.25 ± 4.5	16.78 ± 3.6	0.804
End	20.77 ± 6.49	16.83 ± 4.04	<0.001
P2	0.001	0.417	
Motility grade a + b (%)			
Baseline	19.59 ± 2.11	18.8 ± 2.19	0.705
End	26.73 ± 4.03	18.86 ± 3.08	<0.001
P2	<0.001	0.87	
Motility grade a (%)			
Baseline	3.68 ± 0.73	3.93 ± 0.89	0.69
End	7.21 ± 2.39	3.8 ± 1.13	0.001
P2	<0.001	0.805	
Motility grade b (%)			
Baseline	15.91 ± 4.75	14.87 ± 4.09	0.406
End	19.52 ± 7.11	15.06 ± 3.61	0.042
P2	0.03	0.603	
Motility grade c (%)			
Baseline	5.6 ± 2.01	7.51 ± 2.91	0.06
End	6.48 ± 2.23	8.17 ± 3.05	0.065
P2	0.061	0.103	
Motility grade d (%)			
Baseline	55.65 ± 9.11	56.15 ± 6.23	0.35
End	49.08 ± 7.21	55.74 ± 6.03	0.001
P2	0.01	0.316	
Motility grade a + b + c			
Baseline	25.19 ± 6.46	26.31 ± 7.01	0.541
End	33.21 ± 7.91	27.03 ± 7.45	0.043
P2	0.037	0.701	
Normal morphology %			
Baseline	9.17 ± 2.93	9.3 ± 3.01	0.45
End	10.23 ± 3.16	9.72 ± 3.25	0.09
P2	0.058	0.107	
Live sperm			
Baseline	52.37 ± 10.13	53.07 ± 9.35	0.58
End	62.43 ± 12.17	52.6 ± 8.9	0.013
P2	0.003	0.403	

SD: Standard Deviation.

P1: Comparing the mean of sperm quality parameters between two groups (The statistical analyses Independent samples t test).

P2: Comparing the mean of sperm quality parameters in each group at the baseline and end of the study (The statistical analyses Paired samples t-test).

ejaculate volume, total sperm count, sperm concentration, sperm total motility and live sperm significantly increased in the intervention group when compared to the placebo group. The bacteria used in this study were *Lactobacillus* and *bifidobacteria* species whose antioxidant properties were shown in various experiments (Saez-Lara et al., 2015). Sperm have many mitochondria to produce energy for motility which is required for successful fertility (Cassina et al., 2015). In the production of ATP, various oxidants are also produced. For example, the formation of superoxide in the electron transfer chain is the central source of ROS

Table 3. Within and between-group comparisons of the oxidative stress biomarkers and inflammatory factors from baseline to endpoint in two groups.

Variables	Probiotic (n = 25) Mean ± SD	Placebo (n = 25) Mean ± SD	P1
TAC (μmol/l)			
Baseline	1.67 ± 0.19	1.59 ± 0.21	0.12
End	2.33 ± 0.6	1.47 ± 0.2	<0.001
P2	<0.001	0.611	
MDA (μmol/l)			
Baseline	0.9 ± 0.11	0.95 ± 0.13	0.079
End	0.69 ± 0.07	0.91 ± 0.13	0.002
P2	0.003	0.109	
CRP (μM)			
Baseline	6.93 ± 2.11	6.85 ± 2.1	0.061
End	4.01 ± 1.09	6.45 ± 1.76	0.021
P2	0.001	0.413	
TNF α (μM)			
Baseline	11.28 ± 3.12	11.19 ± 3.39	0.65
End	8.85 ± 2.49	11 ± 3.01	0.01
P2	0.003	0.607	

SD: Standard Deviation.

P1: Comparing the mean of oxidative stress biomarkers and inflammatory factors between two groups (The statistical analyses Independent samples t test).

P2: Comparing the mean of oxidative stress biomarkers and inflammatory factors in each group at the baseline and end of the study (The statistical analyses Paired samples t-test).

Table 4. Within and between-group comparisons of sex hormones biomarkers from baseline to endpoint in two groups.

Variables	Probiotic (n = 25) Mean ± SD	Placebo (n = 25) Mean ± SD	P1
Testosterone (ng/ml)			
Baseline	14.61 ± 4.25	14.49 ± 4.91	0.44
End	16.58 ± 5.08	15 ± 4.11	0.081
P2	0.063	0.301	
FSH (ng/ml)			
Baseline	5.65 ± 1.27	5.6 ± 1.99	0.7
End	5.1 ± 2	5.43 ± 2.02	0.63
P2	0.21	0.244	
LH (ng/ml)			
Baseline	5.89 ± 1.75	6.01 ± 1.97	0.403
End	5.03 ± 1.14	5.86 ± 1.73	0.308
P2	0.109	0.58	
Prolactin (ng/ml)			
Baseline	366.5 ± 85.7	370.19 ± 79.91	0.19
End	359.01 ± 72.1	368.27 ± 74.09	0.1
P2	0.128	0.26	

SD: Standard Deviation.

P1: Comparing the mean of sex hormones between two groups (The statistical analyses Independent samples t test).

P2: Comparing the mean of sex hormones in each group at the baseline and end of the study (The statistical analyses Paired samples t-test).

in sperm. According to the scientific studies, specific probes for mitochondria-produced ROS (mROS) indicated that avoidable production caused membrane peroxidation and a reduction in motility. On the other hand, the high content of polyunsaturated fatty acids (PUFA) in the sperm membrane is also related to an increase in mROS again leading to motility loss and deoxyribonucleic acid (DNA) damage. This degradation of DNA causes a sharp decrease in sperm motility which is indicated by the DNA Fragmentation Index (Iommiello et al., 2015). Taking into account these

scientific facts, the antioxidant effects of bacteria can be justified and our results explained. In addition, oxidation PUFA in the sperm membrane produces malondialdehyde, which is an indicator of fat oxidation, and of several highly reactive α -b-unsaturated hydroxyaldehydes, such as 4-hydroxynonenal and 4-hydroxyhexanal. The production of these aldehydes in large quantities cause damage to sperm due to the reaction with amino acids and the production of DNA adducts (Agustina et al., 2018).

Based on our results, plasma and semen TAC and MDA levels in the intervention group changed significantly compared to the placebo after 10 weeks. Stimulating the host antioxidant system with probiotics can occur and increase antioxidant capacity (Mishra et al., 2015). Results of the study by A.N. Wang et al. (2009) demonstrated that dietary *Lactobacillus fermentum* supplementation could improve serum Superoxide dismutase (SOD) and Glutathione Peroxidase (GPx) and increase hepatic Catalase, compared to the control group in pigs. According to the findings of the studies, probiotics seem to reduce the oxidation of sperm membrane lipids by increasing antioxidant strength. Several signalling pathways have been proposed in various studies that mediated by probiotic bacteria (Wang et al., 2017). As noted in recent years, by regulating the pathway of Nrf2-Keap1-ARE signalling by probiotic bacteria, by increasing the antioxidant power, it increases the neutralization of reactive oxygen species. In this way, detoxification of ROS is performed by some genes that have been expressed by nuclear factor erythroid 2-related factor 2 (Nrf2) (Motohashi & Yamamoto, 2004). Furthermore, according to findings of Wang et al. (2017) probiotic *Bacillus amyloliquefaciens* SC06 regulated the Nrf2 expressions and improved the H₂O₂-induced IPEC-1 oxidative stress.

Animal studies have shown that inflammation of the reproductive system caused by pathogenic bacteria is reduced by receiving beneficial probiotics, which can help treat infertility (Sheldon et al., 2010). It appears that probiotic lactobacilli may produce a potential anti-inflammatory response, and hence, it can be speculated that they might play a therapeutic role in inflammation-induced infertility. According to the results of our study, plasma inflammatory factors (CRP & TNF α) levels significantly decreased in the intervention group after 10 weeks. Various scientific and efficient *in vitro* and *in vivo* models have been defined and used by researchers to evaluate the effect of anti-inflammatory properties of probiotics. Considering all the differences in the study methods,

the mechanisms underlying the useful effects of probiotics are imperfectly understood (Mann et al., 2014). The results of Talero et al. (2015) indicated that capsules with bifidobacteria, lactobacilli, and *Streptococcus thermophiles* decreased the TNF- α , Interleukin 1 beta (IL-1 β), Interleukin 6 (IL-6) production, and cyclooxygenase (COX)-2 expression, and increased IL-10 levels in colon tissue in mice exposed to 5, 10, and 15 cycles of dextran sulphate sodium (DSS). Also, probiotic administration reduced TNF- α and IL-6, and increased IL-10 serum levels. Wu et al. (2016) showed that *L. plantarum* may improve epithelial barrier function by reducing the expression of proinflammatory cytokines caused by enterotoxigenic *Escherichia coli*, perhaps through regulation of nuclear factor Kappa-B (NF-kB), and mitogen-activated protein kinase (MAPK) pathways.

The results of our study suggest that probiotic ability can make changes in sex hormones and these alterations may influence male reproductive activities. Serum testosterone levels were increased after daily consumption of 500 mg of probiotics. The study of Poutahidis et al. (2014) in mice that were treated with *Lactobacillus reuteri*, had increased seminiferous tubule cross-sectional profiles and increased spermatogenesis and Leydig cell numbers per testis when compared with the control group. Although the hormonal change was not significant in our study, it seems that the increase in testosterone levels is because of its direct result on Leydig cells and on testosterone production. Also, Probiotic supplement decreased the LH level. Testosterone secretion can reduce the LH level, because of negative feedback control, because testosterone has a direct influence on hypothalamus and production of Gonadotropin-releasing hormone. Finally, the LH secretion is reduced by the anterior part of pituitary. Also, testosterone, has a direct negative and weak feedback on the anterior pituitary and this feedback reduces LH secretion (Ng Tang Fui et al., 2017). There were no significant changes in the FSH concentration, in intervention groups receiving probiotic compared to control group. Several factors such as testis steroids, inhibin, activin and follistatin can exert feedback on FSH. These factors regulate FSH concentration due to central effects on Gonadotropin-releasing hormone, and the possible absence of any significant alteration in FSH level is the result of modifying effects of these factors (Das & Kumar, 2018).

Designing and conducting this study as a clinical trial and controlling confounding factors such as physical activity, dietary intake, and matching patients before entering the study are among the strengths of

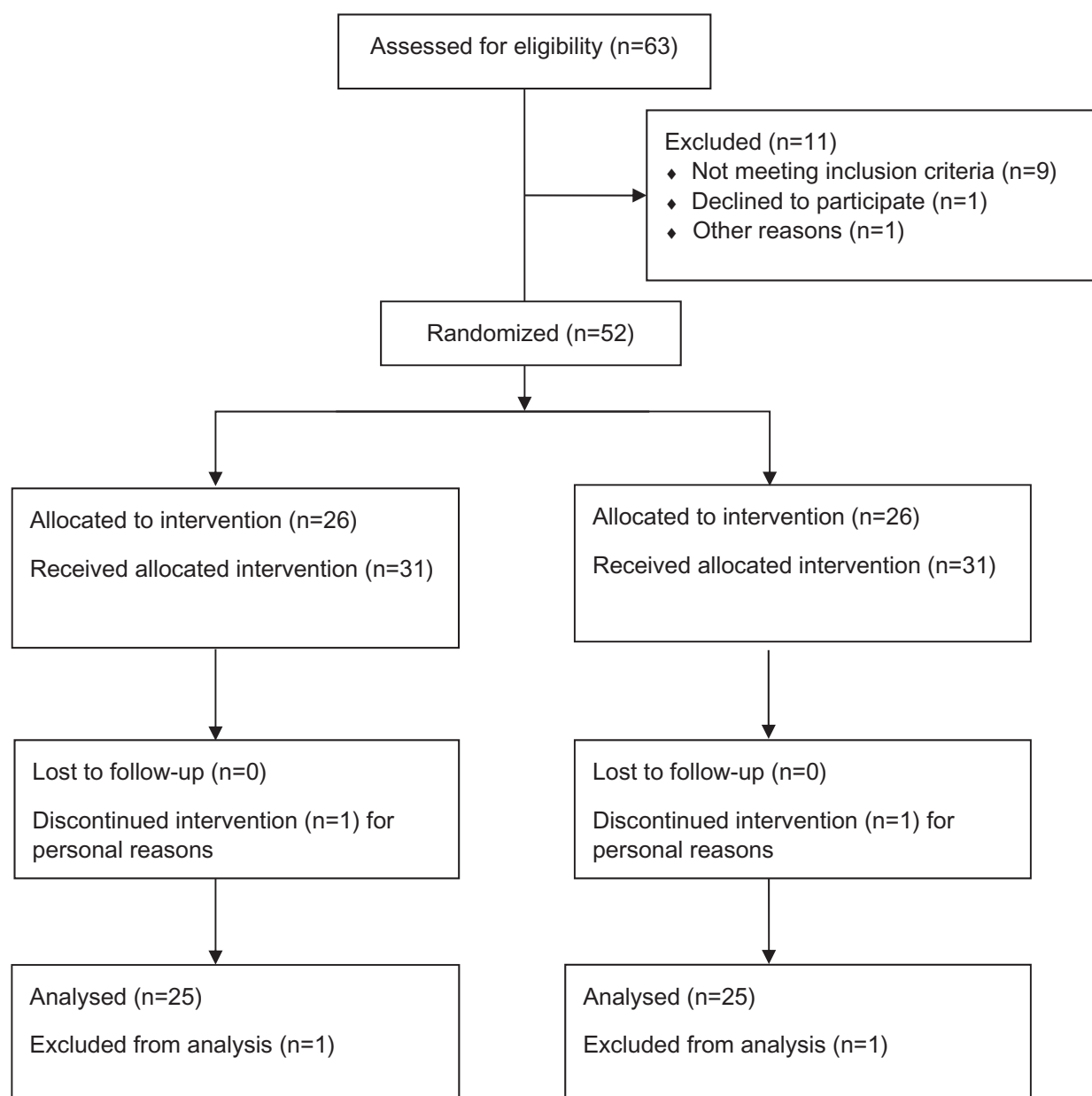


Figure 1. Flowchart of patients' recruitment for the clinical trial of Probiotic supplementation in infertile men.

this study. But not measuring some of the oxidative, antioxidant factors, and also DNA Fragmentation Index, are some weaknesses. For future investigations, it is recommended that different doses and strains of probiotics are examined.

In conclusion, the results of this study indicated that 10 weeks of treatment with probiotics can improve sperm function. After 10 weeks of treatment in oligoasthenoteratozoospermia men, mean count, concentration, and motility increased significantly compared with the placebo group. Based on our findings, medical therapy of oligoasthenoteratozoospermia with oral antioxidants can improve the quality of semen parameters.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Disclosure statement

There are no conflicts of interest.

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Affairs of Jundishapur University of Medical Sciences, Ahvaz, Iran.

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