Probiotic Effects on Sperm Parameters, Oxidative Stress index, Inflammatory Factors and Sex Hormones in Infertile Men

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Research

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Abstract

Background: Impaired sperm production, decreased sperm motility, and increased oxidative stress levels are the main causes of male infertility. To evaluate effects of supplementation with probiotic on the spermatogram, seminal oxidative stress biomarkers, inflammatory factors and reproductive hormones.

Methods: In this randomized, double-blind controlled clinical trial, 52 idiopathic asthenozoospermia men, attending urology clinic, were randomly assigned to one of intervention and placebo (n =26) groups. Participants in the intervention group took daily 500 mg Probiotic and those in the placebo group took daily placebo for 10 wk. Semen parameters, total antioxidant capacity, malondialdehyde, inflammatory factors, reproductive hormones, anthropometric and physical activity assessments were measured at the baseline and at the end of the study. Statistical analysis was performed using SPSS software.

Results: Out of 52 who participated in this study, 25 men in the intervention group and 25 men in the control group completed the protocol of the study. After the intervention, ejaculate volume, number, concentration and the percentage of motile sperm, total antioxidant capacity of plasma significantly increased in the intervention group and the concentration of plasma malondialdehyde and inflammatory markers significantly decreased in the intervention group.

Conclusion: Probiotic supplementation in infertile men, leading to significant increase in concentration and motility and significant reduce in oxidative stress and inflammatory markers. Possibly oral intake of probiotic has the potential to be one of the ways to deal with oxidative damage sperm of infertile men.

Introduction

According to the existing definitions, infertility is recognized as non-pregnancy after one year of unprotected sexual relation[1]. This disorder is one of the health problems that affects patients individually, socially and economically condition[2]. Fifty percent of infertility problems related to male factor. Male infertility is mainly due to a defect in spermatogenesis, which can be attributed to sperm dysfunctions, reduced sperm count, sperm maturation, and sperm motility[3]. The exact mechanism of the defect in sperm function in many cases is clearly not known that they are called idiopathic causes. World Health Organization (WHO) defined several subtypes of sperm malformation: asthenozoospermia, oligozoospermia, teratozoospermia or their combinations. In asthenozoospermia condition, the concentration of sperm, the ratio of morphology of normal sperm and the proportion of sperm motility is lower than the WHO values[4].

The human body system has a defensive system for dealing with free radicals called the antioxidant system[5]. The imbalance between the produced free radical amounts and the antioxidant capacity causes oxidative stress[6]. Reactive oxygen species (ROS) in physiological number is required for the sperm motility, hyper activation, capacitation, acrosome reaction and nuclear condensation. However, pathological levels of ROS can injury sperm's function and reduce sperm motility in mainly through exhaustion of intracellular Adenosine triphosphate (ATP) and lipid peroxidation of plasma membrane[7].

In patients with infertility, the level of free radicals was significantly higher than that of healthy subjects and then reproductive capacity and related mechanisms appear to be interrupted in low-antioxidant dietary[8].

The researchers concluded that in men with fertility problems, the level of inflammatory factors were high than healthy peoples in plasma and semen[9]. Of course, it should be noted that appropriate levels of macrophages are essential for the proper functioning of the sperm, but when inflammatory macrophages are more expressed, they cause reproductive impairment[10]. In the study of David J. Sharkey et al., the sperm motility was negatively correlated with the inflammatory factor of CXCL8 (C-X-C motif chemokine ligand 8) and the concentration of sperm[11].

Probiotics are well-documented as intestinal-based dietary bacteria that regulate the local immunity of the gastrointestinal system and thus have a comprehensive effect on metabolic pathways that can activate metabolic pathways, as well as recover lost cell hemostasis and overall health[12]. 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[13]. Extreme effects of probiotics as a potent antioxidant have also been proven in numerous studies. It has been shown in mice that 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[14]. Lactic acid bacteria (LAB) and bifidobacteria are the greatest quantity regularly used probiotics, which has 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 neutralizing free radicals[15]. 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[16]. According to the mentioned issues, the present study designed to evaluate the efficacy of probiotic supplement on sperm quality, oxidative stress index, inflammatory factors and sex hormones in men with idiopathic infertility.

Methods

Study Design

This study designed as a double blind randomized controlled clinical trial that approved with 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 who followed infertility treatment at department of urology, Imam Khomeini hospital, Ahvaz Jundishapur University of Medical Sciences between April 2018 and March 2019. This investigation was registered by the identification code of IRCT20141025019669N7 in 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: willing to cooperate, age range 20–45, oligosthenoterozospermia disease of unknown origin (idiopathic)[17], normal levels of gonadotropins, testosterone and serum prolactin, and oligospermia 5–20 million per ml sperm count, asthenospermia with mobility less than 50% (idiopathic). Patients were excluded from the study if any of the following conditions existed: There was a known cause of infertility (such as hormonal disorders, epididymal duct obstruction, and epididymoorchitis), drugs, alcohol consumption, diabetes, kidney disease (creatinine more than doubled), chronic liver disease (more than twice the normal transaminase), varicocele, infectious diseases with fever and leukocytosis characterized by chromosomal abnormalities, debilitating diseases sperm and sexual system such as varicocele, drugs that stimulate sexual system or interfere with sex hormones, patients undergoing ICSI due to sperm quality severe impairment and the presence of other causes of infertility, contact pesticides, heavy metals and solvents, taking antioxidant supplements in the past three months and a body mass index (BMI) of 30 kg/m2 or greater.

Subjects

At the beginning of the study, patients were randomized to group 1 who received 500 mg probiotic capsules daily and group 2 who took placebo for 10 weeks. The capsules used in this study were prepared by the Zist Takhmir Company, and the placebo capsules were completely similar to the probiotic appearance, using only Maltodextrin inside them. Each eligible patient received a randomization number, and were randomly divided into two groups of supplementation and placebo (n = 26). The investigator and patients were blinded to the treatment condition. The combination of probiotic capsules include: Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Lactobacillus acidophilus, Bifidobacterium breve, Bifidobacterium longum, Streptococcus thermophiles, TVC: 2 * 1011 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 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, 24-hour recall questionnaire, non-consecutive three-day, be 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[18].

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 in C. Maretti study[19]. Thus, with a sample size of 19 people in each group with probability 95% and a 99% confidence level can be ruled out to assume that the probiotic effect is

equal before and after the study. Considering the drop-in participants during the study, 26 people were considered for each group.

$N= [(Z1-\alpha/2 + Z1-\beta)\ 2\ (SD12 + SD22)] / \Delta 2$

$Z1-\alpha/2 = 2.58$, $Z1-\beta = 1.64$

$N = 19$

Preparation of samples

After 3 days of sexual abstinence, semen samples were taken at urology unit of Imam Khomeini hospital, Ahvaz Jundishapur University of Medical Sciences (Ahvaz, Iran). All semen was carried at 37 °C to liquefy. The samples were analyzed according to the World Health Organization[20]. Remnants of liquefied semen samples were immediately centrifuged at 300 × g for 10 min. Also, venous blood samples and centrifuged at 3,000 × g at 4 °C for 10 minutes and serum was aspirated out for hormone assays. The serum was stored at -80 C until further assays.

Laboratory Methods

Assessment of Sperm Motility

Motility assessment of sperms was performed according to WHO criteria[21]. Sperms were scored for motility evaluation expressed as grades a to d and progressive motility rate was calculated as the percentage of (a + b).

Biochemical analysis

Turbidimetric immunoassay was used for measuring of C-reactive protein (CRP) levels (BioSystems Co, Barcelona, Spain). Also, enzyme-linked immunosorbent assay (ELISA) (DIAsource Co, Belgium) was used for determining serum levels of tumor necrosis factor a (TNF-α). Serum and seminal malondialdehyde (MDA) levels were measured by tiobarbituric acid method. Colorimetric method was used for analyzing serum and seminal total antioxidant capacity (TAC) (Randox Laboratories Ltd, UK). This method has completely been explained by Khosrowbeygi et al[22].

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 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 to18.1 IU/l.

Statistical Analysis

All data were presented as mean ± SD. The distribution of the data was evaluated by the Kolmogorov–Smirnov test. Due to 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 patients did not achieve inclusion criteria. Then, 52 patients were recruited. Of the 52 patients enrolled in this study, one patient in the intervention group and one patient in the placebo group were excluded (Fig. 1).

The reason for their lack of cooperation was personal reasons. The rate of cooperation and compliance of the patients in this study was 96.1 percent. The mean age of the participants in this study was 32.62 ± 4.01 years old. Patients did not report any serious adverse effects during the study related to probiotic capsule or placebo consumption. There was no significant difference between groups in the baseline characteristics, physical activity and dietary intakes (Table 1).

Table 1

The comparison of baseline characteristics of the participants

Characteristicsa		Mean ± SD probiotic(n = 25)	Mean ± SD Placebo(n = 25)	Pv
Age(year)		32.23 ± 4.11	33.01 ± 3.91	∗0.601
Duration of Marriage (year)		4.03 ± 1.11	4.29 ± 1.03	∗0.317
Smoking history (number/percent)	Never smoker (%)	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/m2)	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
*Based on statistical analyzes Independent samples t test				
** Based on statistical analyzes Chi-squared test				

At the beginning of the study, patients 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 ($P > 0.05$, Table 2).

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

Variables		Mean ± SD probiotic(n = 25)	Mean ± SD Placebo(n = 25)	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	20.772 ± 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	

Variables		Mean ± SD probiotic(n = 25)	Mean ± SD Placebo(n = 25)	P1
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	
P1: Comparing the mean of sperm quality parameters between two groups (The statistical analyzes 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 analyzes Paired samples t-test)				

In this study, probiotic supplementation could produce significant statistical changes at the end of the study. Ejaculate volume, total sperm count, sperm concentration, sperm total motility and live sperm significantly increased in intervention group when compared to the placebo group ($p < 0.05$, Table 2). Other parameters such as normal morphology and one of the characteristics of sperm motility, grade c, did not have statistically significant changes ($P > 0.05$, 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 ($P > 0.05$, 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, in the end line, plasma and semen TAC and MDA levels in intervention group changed significantly compared to the placebo ($P < 0.05$). Also, after 10 week, probiotic supplementation reduced CRP and TNF α ($P < 0.05$, Table 3).

Table 3

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

Variables		Mean ± SD probiotic(n = 29)	Mean ± SD Placebo(n = 28)	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	
P1: Comparing the mean of oxidative stress biomarkers and inflammatory factors between two groups (The statistical analyzes 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 analyzes Paired samples t-test)				

There was no significant difference in sex hormones between the two intervention and placebo groups. But probiotic supplementation after 10 week, could increase testosterone and decrease serum FSH, LH and PRL, but these differences were not significant (P > 0.05, Table 4).

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

Variables		Mean ± SD probiotic(n = 25)	Mean ± SD Placebo(n = 25)	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
	Baseline	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	
P1: Comparing the mean of sex hormones between two groups (The statistical analyzes Independent samples t test)				
P2: Comparing the mean of sex hormones in each group at the baseline and end of the study (The statistical analyzes Paired samples t-test)				

Discussion

On the other hand, in recent years excessive production of ROS by leukocytes and abnormal sperm in semen has been produced, 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 cells[23].

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[14].

According to our results, daily supplementation with 500 mg of probiotics for 10 week significantly improved sperm parameters. In the end of the study, ejaculate volume, total sperm count, sperm concentration, sperm total motility and live sperm significantly increased in 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[16]. Sperm cells have many mitochondria, because the sperm motility, which is the main characteristic of fertility, energy is needed that is produced by mitochondria[24]. In the production of ATP in the cell, various oxidants are also produced. For example the formation of superoxide in the electron transfer chain is the central source of ROS in sperm. According to the scientific studies, specific probes for mitochondria-produced ROS (mROS) indicated that avoidable production caused in membrane peroxidation and reduction in motility. On the other hand, high content of poly unsaturated fatty acids (PUFA) in the sperm membrane is also related to an increase in mROS again leading to motility loss and DNA damage. This degradation of DNA causes a sharp decrease in sperm motility which is indicated by the DNA Fragmentation Index[25]. Taking into account these scientific facts, the antioxidant effects of bacteria can be justified and our results could be explained. In addition, oxidation PUFA in the sperm membrane produces malondialdehyde, which is an indicator of fat oxidation, and a several of highly reactive a, b-unsaturated hydroxyalkenals, such as 4-hydroxynonenal and 4-hydroxyhexanal. When these aldehydes are in high amounts, react with amino acids and nucleic acids to structure steady protein and DNA adducts, which damage sperm act[26].

Based on our results, in the end line, plasma and semen TAC and MDA levels in intervention group changed significantly compared to the placebo after 10 week. Stimulating the host antioxidant system with probiotics can occur and boost the activities of antioxidase well[14]. Results of Wang AN et al. study 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[27]. According to the findings of the studies and the mentioned facts, probiotics seem to reduce the oxidation of sperm membrane lipids by increasing antioxidant strength. Several signaling pathways have been proposed in various studies that mediated by probiotic bacteria[28]. As noted in recent years in scientific studies, by regulating the pathway of Nrf2-Keap1-ARE signaling by probiotic bacteria, it resists versus oxidative stress. In this way, detoxification of ROS are performed by some genes that have been expressed by Nrf2[29]. Furthermore, according to findings of Wang Y et al. probiotic Bacillus amyloliquefaciens SC06 regulated the Nrf2 expressions and improved the H_2O_2-induced IPEC-1 oxidative stress[30].

In animal studies, it has previously been observed that pathogenic bacteria that increase inflammation in the reproductive system that result in infertility disorder and, with administration of inflammatory reducing probiotics, might help in the restoration of fertility[31]. 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 the results of our study, plasma inflammatory factors (CRP & TNF α) levels significantly decreased in intervention group after 10 week.

Various scientific and efficient models have been defined and used by researchers to evaluate the effect anti-inflammatory effects of probiotics. They have done in vitro and in vivo studies. Considering all the differences in the study methods but anyway the mechanisms underlying the useful effects of probiotics last imperfect understood[32]. The results of Talero E et al. study indicated that capsules with bifidobacteria, lactobacilli, and Streptococcus thermophiles decreased the TNF-α, IL-1β, 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 sulfate sodium (DSS)[33]. Also, probiotic administration reduced TNF-α and IL-6, and increased IL-10 serum levels. Wu Y et al. showed that L. plantarum may improve epithelial barrier function by reducing the expression of proinflammatory cytokines caused by enterotoxigenic Escherichia coli, maybe through regulation of nuclear factor kappa-B (NF-kB), and mitogen-activated protein kinase (MAPK) pathways[34].

Results of our research are representative that probiotic ability to make changes in sex hormonal and these alterations may influence on male reproductive activities. Serum testosterone levels raised after daily consumption of 500 mg of probiotic. In the study of Theofilos Poutahidis et al. mice that treated with Lactobacillus reuteri, had increased seminiferous tubule cross-sectional profiles and increased spermatogenesis and Leydig cell numbers per testis when compared with control group[35]. Although this change was not significant in our study, but 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 LH level. Testosterone secretion can reduce LH level, because of negative feedback control, because testosterone has a direct influence on hypothalamus and production of Gonadotropin-releasing hormone. Finally, LH secretion is faired by anterior part of pituitary. Also, testosterone, has a direct negative and weak feedback effect on anterior of pituitary and this feedback reduce LH secretion[36]. There was no significant changes in FSH concentration, in intervention groups receiving probiotic compared to control group. Several factors such as testis steroids, inhibin, activin and folistatin are that exert feedback on FSH. These factors regulate FSH concentration due of central effects on Gonadotropin-releasing hormone, and possible absence of any significant alteration in FSH level is the result of modifying effects of these factors[37].

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 this study. But not measuring some of the oxidative, antioxidant factors and also DNA Fragmentation Index, an important index, are the weaknesses of the study. For feature investigations, recommended that different doses and strains of probiotics are recommended.

Conclusion

In conclusion, the results of this study indicated that 10 weeks of treatment with probiotic can improve sperm functions. After 10 weeks of treatment in oligoasthenoteratozoospermic men, mean count, concentration, and motility increased significantly compared with the placebo group. Based on our

findings, medical therapy of asthenoteratospermia with oral antioxidants can improve quality of semen parameters.

Abbreviations

ATP:Adenosine triphosphate, BMI:body mass index, CRP:C-reactive protein, CXCL8:C-X-C motif chemokine ligand 8, ELISA:Enzyme-Linked Immunosorbent Assay, FSH:Follicle-stimulating hormone, IPAQ:International Physical Activity Questionnaire, LAB:Lactic acid bacteria, LH:Luteinizing hormone, MDA:Malondialdehyde, ROS:Reactive oxygen species, TAC:Total Antioxidant Capacity, TNF-α:Tumor Necrosis Factor a, WHO:World Health Organization

Declarations

Acknowledgments

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Authors' contributions

BH, MK, EG, and HKH: designed the project; MK and HKH: developed the study; BH, MK, and MD: developed the laboratory measures; MK and HKH: contributed to the study design and developed the statistical approach; EG: contributed to the study design; BH, MK, and MD: conducted the trial and collected study data; HKH: prepared the manuscript; and all authors: reviewed manuscript drafts and read and approved the final version.

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Availability of data and material

The data analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

We confirm that any aspect of the work covered in this manuscript that has involved either human patient has been conducted with the ethical approval of all relevant bodies, in Ethical committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran by grant number of: NRC-9618. On the other hand, the protocol was registered in IRCT by number IRCT20141025019669N7 code. All the eligible and volunteered subjects had been written consent for supplementary care prior to research.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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Figures

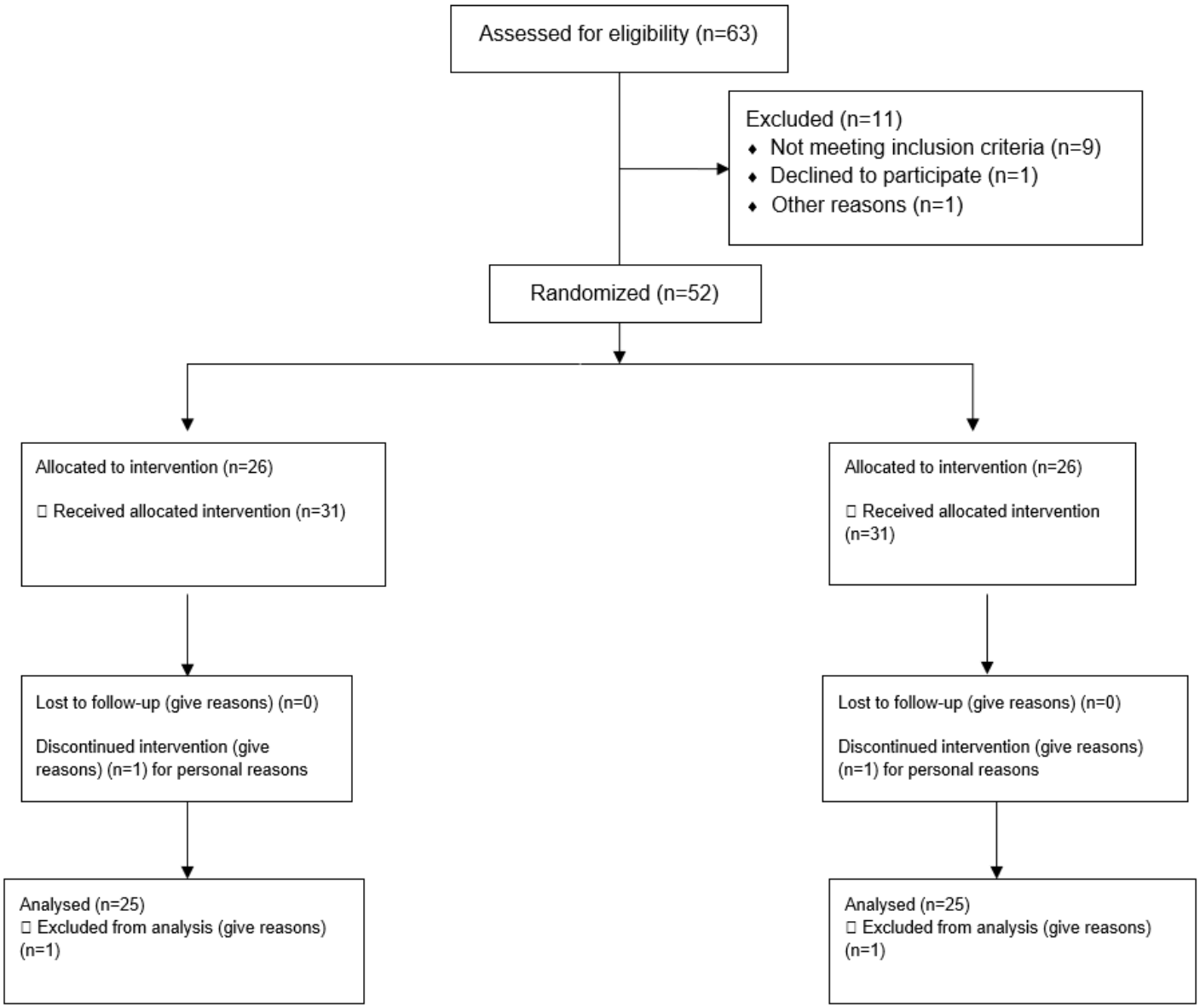

Figure 1

Flowchart of patients' enrolment

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