Original Article

Effects of probiotic supplementation on semen parameters after varicocelectomy: A randomized controlled trial

Mousa Asadi[1], Farshad Gholipour[2], Amirhossein Rahavian[3], Mohammad Javanbakht[1]

[1]Nephrology and Urology Research Center, Clinical Science Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran, [2]Isfahan Kidney Diseases Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [3]Andrology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Background: The use of probiotics in the treatment of infertility is a new area of research. In this study, our objective was to examine the efficacy of probiotic supplementation on semen parameters following varicocelectomy. **Materials and Methods:** We included infertile men in our study who were the candidates for subinguinal microscopic varicocelectomy. After the surgical procedure, the patients were randomly assigned into two groups: 38 individuals received probiotic supplementation (FamiLact®), while 40 individuals received a placebo for 3 months. We compared the preoperative semen parameters with the postoperative parameters to evaluate the effects of probiotic supplementation. **Results:** A total of 78 patients were included in the study. The two groups were similar in terms of age, body mass index, infertility period, and semen parameters at baseline ($P > 0.05$). A statistically significant difference was found in sperm concentration (33.7 ± 22.5 vs. 21.1 ± 16.1 × 10^6/mL, $P = 0.046$), and the percentage of sperms with normal morphology (15.0 ± 8.9 vs. 12.0 ± 11.5, $P = 0.016$) at 3 months favoring the probiotic group. Although the probiotic group exhibited higher values for semen volume and sperm motility at 3 months, the differences were not statistically significant ($P = 0.897$ and $P = 0.177$, respectively). **Conclusion:** Our study demonstrates that the short-term use of probiotics after varicocelectomy can provide additional benefits in improving semen parameters. Probiotic supplements are cost-effective and well tolerated, making them a suitable option for enhancing the outcomes of varicocelectomy.

Key words: Fertility agents, infertility, probiotic, semen analysis, sperm, varicocelectomy

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INTRODUCTION

Varicocele is a significant reversible condition that leads to male infertility.[1] It is estimated to affect approximately 35%–40% of individuals experiencing primary infertility and around 80% of those with secondary infertility.[2] The primary factors contributing to varicocele include elevated scrotal temperature, dysplastic testicular tissue, and oxidative stress.[3-5] Research has demonstrated that individuals with varicocele exhibit considerably elevated levels of reactive oxygen species and reduced antioxidant capacity in their seminal plasma.[5-7] Furthermore, there seems to be a correlation between higher intratesticular temperatures and elevated apoptosis.[8] The primary treatment for varicocele is varicocelectomy, although surgical intervention does not appear to affect the total antioxidant capacity.[9,10]

A recent meta-analysis conducted by Wang *et al*.[11] indicated that antioxidant consumption after varicocelectomy can improve seminal parameters. Recently, the positive impact of probiotics on semen indices has been investigated and their effectiveness has been validated.[12-14] Nevertheless, the specific mechanisms through which probiotics enhance male fertility remain a topic of discussion.[13] It seems that

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Address for correspondence: Dr. Mohammad Javanbakht, Nephrology and Urology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
E-mail: mhmjvbt81@gmail.com

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probiotics exert their influence by influencing hormone secretion, facilitating the scavenging of free radicals, and improving the microenvironment of the prostate.[15]

In an unpublished study conducted by the author, probiotics were found to have a greater impact compared to antioxidants in improving the semen parameters of patients with idiopathic oligoasthenoteratozoospermia, possibly due to their effects through various pathways. In this randomized clinical trial, our aim was to investigate the effect of probiotics on semen indices after varicocelectomy. To our knowledge, this is the first study designed to achieve this objective.

METHODS

Study design

Between September 2021 and March 2023, we conducted the present double-blind randomized clinical trial. The study received approval from the institutional ethics review board (IR.BMSU.BAQ.REC.1399.049), and written consent for the use of patients' data was obtained from each participant. All the stages of the study adhered to the principles outlined in the Declaration of Helsinki or its subsequent revisions. The current study has been registered at the IRCT.ir with the registration number IRCT20150420021869N4.

Study population

This prospective study included infertile male patients, aged 18 years or older, who had a left-sided varicocele. These individuals had been unable to conceive for at least 1 year and were scheduled to undergo subinguinal microscopic varicocelectomy.

The study excluded patients who had previously undergone surgery related to the genitourinary system, had a medical condition affecting fertility, had received fertility-related treatment in the past 3 months, had idiopathic infertility, and had a history of conditions such as cryptorchidism, testis tumor, trauma to the testis, mumps after puberty, metabolic disorders, or obstructive urogenital conditions. In addition, patients who adhered to a diet specifically designed to enhance fertility consumed extreme amounts of recreational drugs or had a positive HIV test were excluded from the study.

Data collection

Convenient sampling method was used. A standardized infertility evaluation was conducted for the patients enrolled in the study. The physical examination, including the application of the Valsalva maneuver, was conducted in a warm room with the patient in a standing position, following the protocol described by Hudson.[16]

The classification of varicoceles was determined using the guidelines recommended by the World Health Organization (WHO). Grade I varicoceles were defined as those that were palpable during the Valsalva maneuver. Grade II varicoceles were characterized by palpability at rest but not visibly apparent. Grade III varicoceles were classified as those that were both palpable and visibly apparent at rest.[17]

To determine the sample size for our study, we conducted a power analysis based on the findings of Wang *et al*. for sperm concentration after 3 months of intervention.[11] The effect size observed in the previous data was 9.7, with a standard deviation of 4.4. We set a significance level (α) of 0.05 for a type one error and aimed for a power of 0.8, corresponding to a type two error rate of 0.2. Using these parameters, we calculated the required sample size to be 10. However, we included 78 participants in the final analysis, which was far beyond the calculated sample size.

The allocation of participants in the study was conducted using the simple randomization method with the assistance of Excel 2020 software (Microsoft Corporation, Washington, USA). The randomization sequence was generated by our statistician using the "RANDBETWEEN (0;1000000)" function. Odd and even numbers were assigned to the intervention and control groups, respectively. Allocation concealment was maintained through the use of sealed envelopes, which contained group numbers indicating the assigned treatment group. The enrollment of participants was conducted by two urologists who were not aware of the allocation results. Two surgeons had an equal level of experience in performing microscopic varicocelectomy.

Out of the total participants, 45 individuals were assigned to receive oral synbiotic FamiLact® (manufactured by Zist Takhmir, Iran) two times a day for 3 months. A similar number of patients were allocated to the placebo group. FamiLact capsules consist of a combination of bacterial strains, including *Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus bulgaricus, Lactobacillus acidophilus, Bifidobacterium breve, Bifidobacterium longum*, and *Streptococcus thermophilus*, with each capsule containing 10^9 colony forming units of these strains. In addition, the capsules contain fructooligosaccharides, which serve as a prebiotic to support the growth and activity of these probiotic bacteria. The placebo drug had the same shape and color as FamiLact.

During the course of the study, a total of nine patients withdrew from the study, and three patients were lost to follow-up. The data analysis was conducted using the information from the remaining 78 patients, with 38 patients in the probiotic group and 40 patients in the placebo group.

The study flowchart illustrating the patient distribution and progress is depicted in Figure 1.

Semen analysis was performed upon the diagnosis of varicocele, and a second semen analysis was conducted 3 months after the surgery in both study groups. Computer‑assisted semen analysis (CASA) using medeaLAB CASA Version 4.1 (Germany) was performed within 1 h of sperm collection to analyze the semen samples. The semen samples were collected after 2–5 days of sexual abstinence. Semen parameters were evaluated following the guidelines outlined in the 5th edition of the WHO laboratory manual for the examination and processing of human semen,[17] including semen volume (mL), sperm concentration ($\times 10^6$/mL), sperm motility (%), and normal sperm morphology (%). The semen analysis was carried out by two experienced technicians in the andrology laboratory.

All patients underwent subinguinal varicocelectomy, which was performed using the microscope at ×10 magnification. A subinguinal incision of approximately 3 cm was made. After the subcutaneous fat was exposed, the spermatic cord was carefully grasped and lifted using a Babcock clamp. It was then placed on a Penrose drain for further manipulation or examination. The veins were carefully ligated while ensuring preservation of the lymphatic and arterial vessels.

The main focus of the study was to compare the various semen parameters between the groups, including semen volume, sperm concentration, sperm motility, and morphology. These measures served as the primary outcome measures in assessing the differences between the groups.

Statistical analysis

Descriptive statistics, such as mean (standard deviation), were used to summarize the data. The normality of the distribution was assessed using the Kolmogorov–Smirnov *Z*‑test. To compare the quantitative data between the groups, independent *t*‑tests or Mann–Whitney *U*‑tests were used. Wilcoxon test was employed to compare paired findings at baseline and after treatment within the groups. *P* value threshold of <0.05 was used to determine statistical significance in the study. All statistical analyses were conducted using the SPSS statistical software version 26.0 IBM SPSS statistics (Armonk, New York, USA).

RESULTS

A total of 78 patients were enrolled in the study. The baseline characteristics of the study participants were compared between the probiotic and placebo groups using an independent *t*‑test. No statistically significant differences were found between the groups for age, body mass

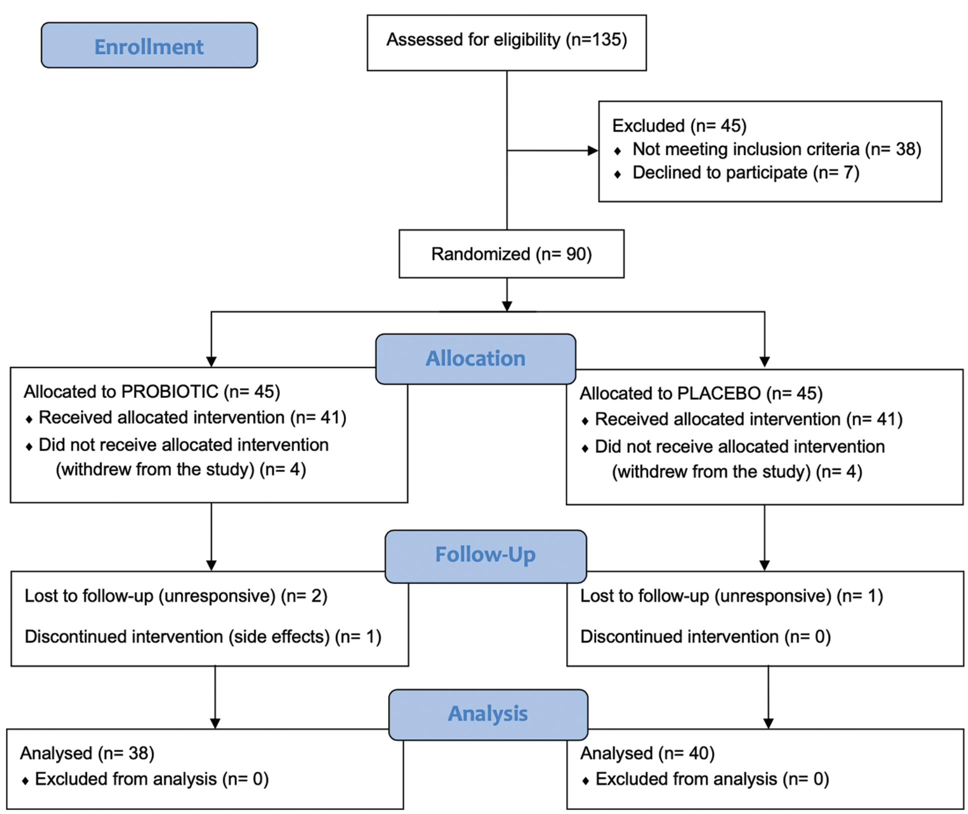

Figure 1: Study enrollment flowchart

index, and infertility period. The mean age was 31.9 (3.4) years in the probiotic group and 32.3 (4.5) years in the placebo group ($P = 0.070$). The mean body mass index was 25.1 (2.8) kg/m^2 in the probiotic group and 26.2 (2.8) kg/m^2 in the placebo group ($P = 0.174$). The mean infertility period was 28.2 (21.9) months in the probiotic group and 28.5 (22.0) months in the placebo group ($P = 0.113$).

Probiotic was well tolerated and only one patient in the case group discontinued the treatment because of flatulence. Table 1 presents the summary of semen parameters for the placebo and probiotic groups at baseline and 3 months postsurgery. Wilcoxon test and Mann–Whitney *U*-test were employed for the statistical analysis, evaluating within-group changes and between-group differences, respectively.

At baseline, there were no significant differences between the groups regarding any of the semen parameters ($P > 0.05$). Within-group analysis showed that in both groups, sperm concentration, sperm motility, and sperm with normal morphology increased statistically significantly compared to the baseline values ($P = 0.007$, $P = 0.007$, and $P = 0.026$, respectively).

At 3 months, sperm concentration was significantly different between the placebo (21.1 × 10^6/mL) and probiotic (33.7 × 10^6/mL) groups favoring the probiotic group ($P = 0.046$). Similarly, the probiotic group exhibited significantly higher sperm with normal morphology (15.0%) at 3 months compared to the control group (12.0%), ($P = 0.016$). Although the probiotic group showed higher values for semen volume and motile sperm at 3 months, the differences were not statistically significant ($P = 0.897$ and $P = 0.177$, respectively).

Moreover, the changes in semen parameters were calculated by subtracting the baseline values from the values after the intervention. Between-group analysis showed statistically significant differences between the groups regarding sperm concentration ($P = 0.049$) and normal morphology ($P = 0.038$) and nonsignificant differences regarding semen volume ($P = 0.741$) and normal sperm morphology ($P = 0.347$).

DISCUSSION

We observed that probiotic treatment had a positive impact on sperm concentration and morphology following varicocelectomy. Varicocelectomy, which is considered the standard treatment for varicocele, leads to significant improvements in semen parameters and reduced sperm DNA damage, convincing us to perform the surgery on patients.[6,18] However, it should be noted that not all patients undergoing varicocelectomy experience the same positive effects. For instance, Baazeem *et al*. conducted a study in 2011 that showed no significant increase in spontaneous pregnancy rates after varicocelectomy. Moreover, the study did find a decrease in sperm DNA fragmentation, reduced oxidative stress in the semen, and improvements in sperm concentration and motile sperm percentage.[4] A previous retrospective study involving 100 patients showed that varicocelectomy has the potential to alleviate persistent spermatic vein reflux and enhance semen indices in men with subfertility.[19] However, it remains unclear from the available data whether surgery can effectively counteract the effects of oxidation on sperm quality. Despite the improvements observed in objective parameters, the inconsistent clinical outcomes have led to the consideration of adjuvant therapies alongside varicocelectomy. In a recent meta-analysis, researchers

Table 1: Semen parameters presented as means (standard deviation) at baseline and 3 months after intervention with results of within- and between-group analyses

	Semen volume (mL)	Sperm concentration (×10^6/mL)	Motile sperm (×0.01)	Normal morphology (×0.01)
Placebo				
Baseline (a)	3.3 (1.5)	18.0 (11.0)	28.2 (24.6)	8.0 (6.9)
3 months (b)	3.4 (2.3)	21.1 (16.1)	34.2 (33.2)	12.0 (11.5)
Evolution (c)	0.1 (1.8)	3.0 (14.6)	6.1 (28.3)	3.9 (9.1)
Probiotic				
Baseline (d)	3.0 (1.4)	16.3 (11.4)	27.7 (24.3)	9.0 (5.7)
3 months (e)	3.9 (2.1)	33.7 (22.5)	43.0 (38.7)	15.0 (8.9)
Evolution (f)	0.9 (1.7)	17.3 (16.9)	15.4 (31.0)	6.1 (7.4)
Within-group analysis* (*P*)				
a versus b	0.109	0.007	0.007	0.026
d versus e	0.288	<0.001	<0.001	<0.001
Between-group analysis† (*P*)				
a versus d	0.694	0.734	0.968	0.741
b versus e	0.897	0.046	0.177	0.016
c versus f	0.741	0.049	0.347	0.038

*Wilcoxon test; †Mann–Whitney *U*-test

concluded that postoperative administration of antioxidant can effectively reduce oxidative stress and improve semen parameters.[11]

In recent years, probiotics have emerged as a potential treatment approach in various medical fields. Their minimal side effects and broad effects on different systems in the body make them an appealing option for treatment.[20] The literature suggests that probiotics have shown improvements in female fertility.[21] In 2017, Maretti and Cavallini incidentally discovered that patients with idiopathic oligoasthenoteratozoospermia who were taking probiotics for digestive issues experienced improvements in semen parameters.[15] Subsequently, further studies were conducted to investigate the effects of probiotics on semen parameters, which confirmed their effectiveness.[12-14] Nevertheless, there is ongoing debate regarding the exact mechanisms through which probiotics impact male fertility. The specific ways in which these beneficial bacteria enhance male reproductive health are not yet fully understood or agreed upon. It has been suggested that probiotics may regulate the pulsatile secretion of gonadotropins and promote fertility by interacting with kisspeptin.[22] Probiotics have the potential to reduce oxidative stress caused by free radicals.[23,24] In addition, probiotics may have a positive impact on prostatic microenvironment.[25]

In our study, the sperm concentration after 3 months of probiotic supplementation was found to be 12.6×10^6/mL higher than the control group. In a meta-analysis conducted by Wang *et al*.,[11] the sperm concentration after 3 months of antioxidant supplementation was found to be 9.7×10^6/mL higher than the control group. The difference in the percentage of motile sperm was also higher in our study compared to the reported values for antioxidants (15.4% vs. 5.4%). We assume that one of the reasons why probiotics exhibit greater efficacy than antioxidants in improving sperm concentration and motility is their ability to enhance semen parameters through multiple mechanisms, in addition to their antioxidant effects. However, the difference in the percentage of sperm with normal morphology in the present study was lower than the values reported in Wang's study for antioxidants (6% vs. 9.2%). It should be noted that without a head-to-head controlled trial, it is not possible to make a definitive statement regarding the comparative effectiveness of probiotics versus antioxidants in the postvaricocelectomy period.

To the best of our knowledge, this is the first study to examine the effects of probiotics after varicocelectomy. Probiotics are available at different price ranges and are generally affordable. Another notable characteristic is their minimal side effects, making them well tolerated by patients.

Our study had several limitations that should be considered. First, we focused solely on the impact of probiotics on semen analysis and did not assess other important factors such as hormonal profile, DNA fragmentation index, and antioxidant capacity of semen. Second, the duration of our study was relatively short, which prevented us from evaluating long-term outcomes such as fertility rates or the success of assisted reproductive methods. Third, we did not investigate the persistence of probiotic effects after discontinuation of treatment. Finally, it should be noted that the generalizability of our findings to all available probiotic products on the market may be limited, as there is variability among different products. Therefore, we recommend future research to conduct randomized controlled trials with longer follow-up periods, comparing the efficacy of probiotics with antioxidants and considering a broader range of outcomes, including fertility rates.

CONCLUSION

To summarize, our findings indicate that the short-term administration of probiotics following varicocelectomy can add extra benefit to varicocelectomy in improving sperm concentration and morphology. The affordability and favorable tolerability of probiotic supplements make them a suitable choice for enhancing the outcomes of varicocelectomy.

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Conflicts of interest
There are no conflicts of interest.

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