




The Effect of Probiotic Supplementation in Intrauterine Sperm Insemination Pregnancy Rate

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Received 2023 April 10; Revised 2023 May 07; Accepted 2023 May 09.

Abstract

Background: By using probiotic products, such as Lactovag, which contain high amounts of a safe and beneficial bacterial strain, the vaginal microbiome can be near healthy in infertile women, and as a consequence, the reproductive outcomes are improved.

Objectives: The present study was done to assess the effect of Lactovag on pregnancy outcomes in IUI cycles.

Methods: This single-blind clinical trial was conducted on 194 infertile women who underwent IUI in the infertility clinic of Yas Hospital between December 2021 and March 2022. In the intervention group, two weeks before IUI, Lactovage suppository was prescribed once per night, while in the control group, no extra interventions were done. All the patients underwent IUI in the same way. The primary outcomes of the study were biochemical and clinical pregnancy rates.

Results: The average age of women was 30.51 ± 4.6 years, ranging from 22 to 43 years. The average age of the women's partner was 34.89 ± 4.41 years, ranging from 24 to 49 years. There were no significant differences regarding the basic information of the two study groups. The most common infertility cause was polycystic ovary syndrome (40.7%). The biochemical and clinical pregnancy rate was 12.4% in the Lactovage group and 10.3% in the control group. Although the pregnancy rate improved with a Lactovage prescription, this difference was insignificant (P -value = 0.651).

Conclusions: While the pregnancy rate in women with probiotic therapy was higher, it was insignificant. Future randomized studies are needed to definitively examine probiotic therapy and establish its benefit in women candidates for IUI.

Keywords: Probiotics, Infertility, Insemination

1. Background

Infertility is a common condition, affecting about 15% of couples worldwide (1). Assistance with reproductive technology (ART) can considerably resolve this problem for these couples (2). One of the oldest methods of ART, which is still widely used, is intrauterine sperm insemination (IUI) (3).

The reproductive tract microbiome of a male or female can impact fertility functions such as implantation and establishment of pregnancy (4, 5). The reproductive tract microbiome is a combination of different microbes that live in the human body and have symbiotic relationships with their hosts (6).

The vaginal microbiota typically changes by hormonal changes throughout the menstrual cycle or even with exogenous hormone administration (7, 8).

A healthy vaginal community contains some beneficial microbial strains, of which *Lactobacillus* species are more predominant than the others, including *Acinetobacter*, *Pseudomonas*, *Comamonadaceae*, *Jonquetella*, *Fusobacterium*, *Bacteroidetes*, and *Prevotella* (9).

Lactobacillus species in a normal vaginal environment prevent the growth of pathogen bacteria, including bacterial vaginosis (BV) and *Gardnerella vaginalis*, by releasing bacteriocins (10). Also, lactobacilli acidify the vaginal pH through lactic acid secretion (11). In addition, various studies have shown that lactobacilli can increase the implantation rate by producing various substances such as lactic acid, hydrogen peroxide, bacteriocins, and antimicrobial toxic hydroxyl radicals (12).

Lactovag is a synbiotic (probiotic + prebiotic) formulation that contains high amounts (10^9 CFU)

of a safe and beneficial bacterial strain, “*Lactobacillus rhamnosus*.” Using probiotic products, such as Lactovag, which contains high amounts of a safe and beneficial bacterial strain, “*Lactobacillus rhamnosus*,” the vaginal microbiome can be near healthy in infertile women. As a consequence, the chance of pregnancy is improved. The therapeutic potential of probiotic therapy remains an exciting opportunity in IUI to improve the vaginal microbiome and pregnancy outcomes.

2. Objectives

The present study assessed the effect of Lactovag on IUI cycle pregnancy outcomes.

3. Methods

3.1. Study Design and Participants

This single-blind clinical trial was conducted on 194 infertile women who underwent IUI in the infertility clinic of Yas Hospital between December 2021 and March 2022. The inclusion criteria were infertile women older than 18 years and candidates for IUI (women with cervical or tubal abnormality, lack of ovulation, unknown or mixed infertility causes) and having a history of at least two previous unsuccessful IUI.

The exclusion criteria were women with bacterial vaginitis (related symptoms or signs in the woman’s complaint or physical examination) or unwilling to participate in the study.

The eligible participants were allocated to the study groups (intervention or control) using the random allocation rule. First, 97 letters A and 97 letters B are written on special papers not marked inside. Then all of them are placed in a bag, and for each patient, after obtaining informed consent, a paper is removed randomly and without replacement, and based on the letter written on it, the desired intervention is performed for the patient. This study is performed single-blind, and the gynecologist who does IUI does not know the patients’ group.

In the intervention group, two weeks before IUI, a Lactovag suppository capsule (Zisttakhmir pharmaceutical company, Iran) which included 109 CFU *Lactobacillus rhamnosus* and inulin, was prescribed once per night (total of 14 capsules), while in the control group, no extra interventions were done. All the patients underwent IUI in the same way. Furthermore, the patient’s monitoring was done for any possible complications

3.2. Sample Size Calculation

The sample size was calculated by considering the confidence limits of 95% and the error coefficient of 0.5%. Based on the sample size formula, 194 people (97 women in each group) were selected.

$$n = 2 \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta} \right)^2 \bar{p} \bar{q}}{(p_1 - p_2)^2}$$

Participants were asked to fill out a questionnaire about their demographic and obstetrics history, and their hormonal profile, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), and prolactin was investigated from the medical records.

The primary outcomes of the study were biochemical and clinical pregnancy rates. Biochemical pregnancy was defined as a positive β -hCG blood test after the second week of IUI. Clinical pregnancy was defined as pregnancy sac observation after the sixth week of IUI (13).

3.3. Statistical Analyses

All the statistical analyses were done using Statistical Package for the Social Sciences (SPSS) version 24.0. P-value < 0.05 was considered statistically significant. Data was presented using frequencies (%) for categorical variables and mean \pm standard deviation/standard error for continuous variables. The chi2 and independent-sample *t*-test were used to compare categorical and continuous data between the study groups. Also, logistic regression was applied to evaluate any association between study variables and pregnancy outcomes.

3.4. Ethical Approval

All participants accepted and signed informed consent. The study was conducted according to the principles of the Helsinki Declaration. This study was approved by the Ethics Committee of Tehran University of Medical Sciences ([IR.TUMS.MEDICINE.REC.1400.529](https://doi.org/10.22035/tjms.1400.529)). The study was registered as a clinical trial at the Iranian Registry of Clinical Trials ([IRCT20130808014301N3](https://doi.org/10.22035/tjms.1400.529)).

4. Results

One hundred ninety-four infertile women who were candidates for IUI were enrolled in this study. The average age of women was 30.51 ± 4.6 years, ranging from 22 to 43 years. The average age of the women’s partner was

Table 1. Comparison of the Basic Information of the Two Study Groups

Variables	Lactovag	Control	P-Value
Marriage duration (y)	6.4 ± 4.09	6.31 ± 3.1	0.867
Primary infertility (y)	3.36 ± 2.41	3.81 ± 2.20	0.179
Woman's age (y)	30.36 ± 4.72	30.66 ± 4.49	0.625
Women's partner age (y)	34.78 ± 4.51	34.99 ± 4.34	0.746
BMI (kg/m ²)	25.83 ± 3.6	25.93 ± 2.71	0.83
TSH (mIU/L)	2.63 ± 1.46	2.7 ± 1.62	0.738
FSH (mIU/mL)	5.96 ± 2.48	6.09 ± 2.37	0.707
LH (IU/mL)	6.66 ± 5.59	5.86 ± 3.59	0.239
PRL (ng/mL)	108.84 ± 18.20	97.26 ± 14.41	0.618

Abbreviations: BMI, body mass index; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PRL, prolactin.

Table 2. Multivariate Association Between Study Variables and Pregnancy Outcomes

Variables	Odds Ratio	95% Confidence Interval for the Odds Ratio		P-Value
Woman's age (y)	1.06	0.96	1.18	0.248
Primary infertility (y)	0.82	0.61	1.10	0.180
BMI (kg/m ²)	1.04	0.89	1.21	0.597
TSH (mIU/L)	1.07	0.79	1.45	0.653
FSH (mIU/mL)	0.89	0.70	1.12	0.305
LH (IU/mL)	1.03	0.92	1.15	0.619
PRL (ng/mL)	1.00	0.99	1.00	0.369
Lactovag usage	0.78	0.29	2.14	0.636

Abbreviations: BMI, body mass index; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PRL, prolactin.

34.89 ± 4.41 years, ranging from 24 to 49 years. The average duration of marriage of these people was 3.11 ± 2.71 years. There were no significant differences regarding the basic information of the two study groups (Table 1). The most common infertility cause was polycystic ovary syndrome (40.7%), followed by unknown infertility cause (20.1%), mixed infertility cause (17.5%), and tubal causes with a frequency of 7.2%. The biochemical and clinical pregnancy rate was 12.4% in the Lactovage group and 10.3% in the control group. Although the pregnancy rate improved with a Lactovage prescription, this difference was insignificant (P-value = 0.651) (Table 2).

5. Discussion

This study showed that the biochemical and clinical pregnancy rate was higher in women with Lactovage therapy, although this increase was insignificant.

In line with our study, Moreno et al. (14) demonstrated that the existence of an endometrial

microbiota pathological modification considered a non-Lactobacillus-dominated microbiota is associated with poor reproductive outcomes, including decreases in implantation and clinical pregnancy. Furthermore, the ongoing pregnancy and live birth rates were lower in those women, while these rates were not evaluated in our study.

Although recent evidence indicates that the loss of lactobacilli dominance causes infertility, preterm birth, maternal infections, and a higher risk of sexually transmitted diseases (15-17), probiotic supplements seem to restore the vaginal microbiota.

In this regard, a review study by Dai et al. (18) in 2015 in China showed that the use of probiotics and intestinal bacteria by affecting the metabolism of amino acids improves sexual performance, health, and fertility in both couples.

On the other hand, Van Ostrom et al. (19) study showed that the prevalence of BV was higher in infertile women

with tubal infertility causes, and probiotic therapy, besides BV elimination, can improve their fertility outcomes (20). In our study, tubal infertility causes were so rare that they might result in no significant findings.

A major strength of this study is that all participants underwent a similar protocol for IUI in a unit center with a similar technique. Our study had some limitations. First, the vaginal microbiome was not investigated with diagnostic tests, including PCR, before and after using Lactovag, and the pregnancy rate in the subjects was not investigated based on the microbiome status.

Further research must assess the proper dose, duration, and way to use (oral, vaginal, dried powder, or in suspension) Lactovag.

5.1. Conclusions

While the pregnancy rate in women with probiotic therapy was higher, it was insignificant. Future randomized studies are needed to definitively examine probiotic therapy and establish its benefit in women candidates for IUI.

Footnotes

Authors' Contribution: Z. R.: Design of the work; Z. R. and M. A.: Data gathering. E. F. and K. A.: Interpretation of data. E. F., KA, and M. A.: Drafting the manuscript; all authors approved the final version of the manuscript.

Clinical Trial Registration Code: The study registered as a clinical trial at the Iranian Registry of Clinical Trials [IRCT20130808014301N3](https://www.irct.ir/clinical-trials/IRCT20130808014301N3).

Conflict of Interests: Funding or research support: No; employment: No; personal financial interests: No; stocks or shares in companies: No; consultation fees: No; patents: No; personal or professional relations with organizations and individuals (parents and children, wife and husband, and family relationships): No; unpaid membership in a government or non-governmental organization: No; are you one of the editorial board members or a reviewer of this journal? Yes.

Ethical Approval: The study was conducted according to the principles of the Helsinki Declaration. This study was approved by the Ethics Committee of Tehran University of Medical Sciences, code: [IR.TUMS.MEDICINE.REC.1400.529](https://www.tums.ac.ir/ethics/IR.TUMS.MEDICINE.REC.1400.529).

Funding/Support: This study was supported and funded by the Tehran University of Medical Sciences and extracted

from a gynecologist resident thesis (the corresponding author) under the supervision of the first author.

Informed Consent: All participants accepted and signed the informed consent.

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