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Original Paper

Effect of Probiotics on Pregnancy Outcomes in Pregnant Women Receiving 17α-OHP Injection: A Randomized Clinical Trial

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ABSTRACT

Introduction: To date, few studies have evaluated the effects of probiotics in women at high risk of spontaneous preterm birth (PTB), which have presented contradictory results.

Objective: This study aims to investigate the efficacy of administrating probiotics together with 17α-hydroxyprogesterone (17α-OHP) on spontaneous PTB and related pregnancy outcomes in pregnant women at high risk for PTB.

Materials and Methods: This randomized clinical trial was conducted on 118 pregnant women at high risk for PTB (with a history of PTB or pregnancy termination in the second trimester) receiving 17α-OHP injection (250 mg, IM). They were assigned to probiotic group (n=58) and placebo group (n=60). The probiotic group received a 500 mg Lactofem bio-capsules orally and daily, containing *Lactobacillus acidophilus* 2×10^9 cfu/g, *Bifidobacterium bifidus* 2×10^9 cfu/g, *Lactobacillus rutri* 2×10^9 cfu/g, *Lactobacillus fermentum* 2×10^9 cfu/g, from the 16th to the 37th week of pregnancy. The placebo capsules contained starch powder. The obstetric outcomes included Preterm Premature Rupture of Membranes (PPROM), PTB and mode of delivery. The neonatal outcomes included anthropometric characteristics and Apgar score (at 1 and 5 minutes after birth). The obtained data were analyzed using t-test and chi-square test. The significance level was set at 0.05.

Results: The mean age of the participants was 30.27±7.56 and 28.93±7.32 years in the probiotic and control groups, respectively. Their mean body mass index (BMI) was 26.80±2.12 and 26.74±2.98 kg/m^2, respectively. Also, 8.62% and 15% of women in the probiotic and placebo groups had PTB before the 34th week of pregnancy, while 12.06% and 16.7% had PTB from the 34th to the 37th week of pregnancy, respectively. There were no significant differences between the two groups in these obstetric outcomes. After delivery, the newborn's weight was 2928.07±454.83 and 2879.16±348.27; head circumstance was 33.39±1.15 and 33.46±1.46; height was 49.58±1.30 and 49.93±1.45; Apgar score at 1 minute after birth was 8.7±0.6 and 8.6±0.5, and Apgar score at 5 minutes after birth was 9.8±0.6 and 8.9±0.8, respectively. There were no significant differences between the two groups in these neonatal outcomes.

Conclusion: The use of probiotic adjuvant in combination with 17α-OHP injection from the 16th to the 37th week of pregnancy cannot reduce the risk of spontaneous PTB or improve neonatal and obstetric outcomes in women at high risk for PTB.

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Highlights

- Probiotics, as living microorganisms, can provide health benefits when administered insufficient amounts.
- probiotic oral supplementation in pregnancy can affect on the risk of spontaneous preterm birth
- Administration of Probiotics adjuvant in combination with 17α-OHP injection did not reduce the risk of spontaneous preterm birth

Plain Language Summary

Probiotics, as live microorganisms, can have an impact on health. Some studies have confirmed the effect of probiotics on preventing spontaneous preterm labor. Given the increased likelihood of spontaneous preterm birth in some pregnant mothers, this study was conducted with the aim of investigating the efficacy of administrating probiotics together with 17α-hydroxyprogesterone (17α-OHP) on spontaneous preterm birth and related pregnancy outcomes in pregnant women at high risk for preterm birth. This study was conducted on 118 pregnant women at high risk for preterm birth (with a history of preterm birth or pregnancy termination in the second trimester) receiving 17α-OHP injection (250 mg, intramuscular injection [IM]). They were assigned to probiotic group (n=58) and placebo group (n=60). The results showed use of probiotic adjuvant in combination with 17α-OHP injection from the 16th to the 37th week of pregnancy cannot reduce the risk of spontaneous preterm delivery or improve neonatal and obstetric outcomes in women at high risk for preterm birth.

Introduction

Preterm Birth (PTB) is defined as birth before 37 weeks of gestation [1]. Up to 50% of PTBs are associated with maternal infection [2]. Genitourinary infections, including urinary tract infections, bacterial vaginosis and yeast vaginitis, annually affect about one billion women in the world. In recent years, genitourinary infections have been an important risk factor for PTB [3]. The most common route for urogenital pathogens that cause preterm labor is the ascending pathway [4]. Proteolytic enzymes act directly on the collagen of the cervix and fetal membranes and lead to early thinning of the cervix and its insufficiency, weakening of the fetal membranes, and subsequently Preterm premature rupture of membranes (PPROM) [5].

Probiotics, as living microorganisms, can provide health benefits to the host when administered in sufficient amounts. Probiotics displace and kill pathogens, and modulate the immune response by interfering with the inflammatory cascades that lead to PTB [6]. The mechanism of action of probiotics in the vagina is probably multifactorial. The production of lactic acid, bacteriocins, and hydrogen peroxide and the immune response modulation can be the possible mechanisms [7].

A cohort study in Norway reported a significant protective effect against spontaneous PTB in women who had a high intake of probiotic milk [8]. A review study comparing probiotics with placebo reported no statistically significant difference in gestational age at birth [9]. Another review study reported no significant finding that probiotics increased or decreased the incidence of PTB [10]. Regarding the effect of probiotic oral supplementation in pregnancy on the risk of PTB, no benefit or harm has been reported, and more studies are needed in this field. However, it has been proposed that the combination of *L. acidophilus* and Bifidobacterium bifidum probiotic species may be more useful in improving pregnancy outcomes [11]. Progesterone has been shown to suppress the contractions of the myometrium; therefore, one strategy is the use of supplemental progestogens, including Intramuscular (IM) injection of 17α-hydroxyprogesterone caproate (17α-OHP) in women with a singleton pregnancy and a history of singleton spontaneous PTB [12]. The 17α-OHP has been recommended to prevent PTB by the American College of Obstetricians and Gynecologists (ACOG) and the society for maternal-fetal medicine (SMFM). In August 2021, ACOG recommended that women with a singleton pregnancy and a history of spontaneous PTB should receive progesterone supplementation vaginally or by IM injection [13]. However, considering the consequences of PTB and the lack of theoretical agreement on the use of different drugs for the prevention of PTB, it is appropriate

to conduct further studies in this field. Due to the existing contradictions regarding the effects of probiotics in women at high risk of PTB and considering the benefits of probiotics (bacteriotherapy and immune regulation), this study aims to investigate the efficacy of adjuvant administration of probiotics on the spontaneous PTB and the related pregnancy outcomes in pregnant women at high risk for PTB receiving 17α-OHP.

Materials and Methods

This is a randomized clinical trial in compliance with the CONSORT guidelines [14]. The study population consists of all pregnant women referred to the gynecology clinics in Yasuj, Iran, from 2020 to 2022 who were receiving 17α-OHP injection (250 mg, intramuscular injection [IM]). Using the related formula, and by considering α=0.05, β=0.80, a 15% sample dropout rate, 20% cesarean rate in the probiotic group, and 46.7% cesarean rate in the control group according to the findings of Badehnoosh et al. [15], the sample size was determined to be 60 per group. First, 132 participants were assessed for eligibility, of whom 12 were excluded due to not meeting inclusion criteria and declined to participate. Finally, 120 patients were included. Inclusion criteria were a history of high-risk PTB (including a history of PTB or pregnancy termination at the second trimester), willingness to participate in the study, age 18-45 years, gestational age 16-24 weeks, no syphilis, gonorrhea or HIV, no elective or emergency cervical cerclage, and no maternal insulin-dependent diabetes mellitus, hypertension, lupus and clinical chorioamnionitis based on medical records. Exclusion criteria were unwillingness to continue participation in the study, failure to complete the treatment, taking drugs that affect the intestinal microbial flora (such as antibiotics), occurrence of any genital or urinary tract infection that required antibiotic treatment during the trial, having a fetus with congenital malformations and abnormal scan anomalies, or clinical chorioamnionitis.

Eligible participants were assigned to two groups: Group A: Lactofem capsule (containing *Lactobacillus acidophilus* 2×10^9 cfu/g, *Bifidobacterium bifidus* 2×10^9 cfu/g, *Lactobacillus rutri* 2×10^9 cfu/g, *Lactobacillus fermentum* 2×10^9 cfu/g; bio-capsule weight of 500 mg made by Zist Takhmir Company, Iran, administered orally and daily from the 16th to the 37th week of pregnancy) and Group B: Placebo capsules containing starch powder (not harmful during pregnancy) prepared by the medicinal plants laboratory, Yasuj University of Medical Sciences. Capsules in two groups were similar in shape and package. The randomization of patients was performed with a random allocation software using the block randomization method. To hide the treatment options, the list of treatments was placed in sealed and numbered envelopes (in a sequencing order). Participants and physicians were blinded to the allocation and were not aware of group allocation.

Socio-demographic and reproductive information including age, body mass index (BMI), educational level, occupation (housewife or employed), gravidity, history of abortion and parity were first recorded. All participants were informed that they could leave the study at any time. All participants followed the prenatal care and pregnancy outcome in Shahid Mofatteh gynecology clinic under the supervision of one gynecologist. Obstetric information included the history of PPROM, preterm labor (late and early) and mode of delivery (Instrumental, cesarean section, or normal vaginal delivery [NVD]). Neonatal information included weight, height and head circumference of the newborn and the Apgar score (1 and 5 minutes after birth). The side effects including fever, itching, diarrhea, vomiting, or other gastrointestinal symptoms were also recorded. PTB was considered as the primary outcome and pregnancy-related complications as the secondary outcome (Figure 1).

Data analysis was carried out in SPSS software, version 21 using descriptive statistics (frequency, percent, Mean±SD), chi-square test and independent t-test. Kolmogorov–Smirnov test was used to examine the normality of data distribution. The significance level was set at 0.05. There were no missing data. Therefore, no missing imputation technique was used.

Results

Two women from the probiotic group left the study because they were unwilling to continue the treatment. Therefore, the data of 118 women was analyzed. Their characteristics are presented in Table 1. There were no significant differences between the two groups in terms of age, BMI, educational level, occupation, gravidity, abortion, or parity. Table 2 presents the obstetric information for the two groups. As can be seen, 8.62% and 15% of women in the probiotic and placebo groups had PTB before the 34th week of pregnancy, while 12.06% and 16.7% had PTB from the 34th to the 37th week of pregnancy, respectively. Results also showed no significant differences between the two groups regarding PPROM or mode of delivery.

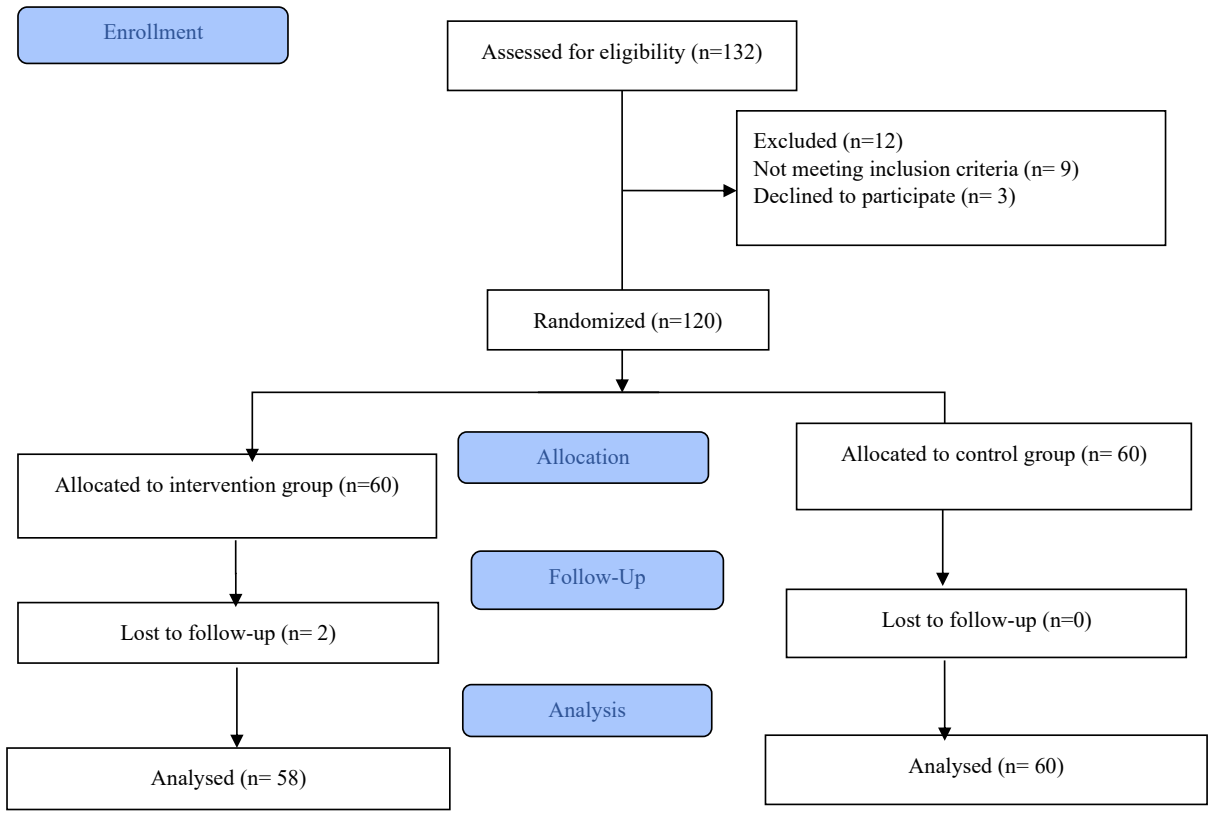

Figure 1. The CONSORT diagram of study

Table 1. Socio-demographic/reproductive characteristics of the two groups

Variables		Mean±SD/No. (%)		P
		Probiotic (n=58)	Control (n=60)	
Age (y)		30.27±7.56	28.93±7.32	0.32*
BMI (kg/m²)		26.80±2.12	26.74±2.98	0.89*
Occupation	Employed	25(43.1)	25(41.7)	0.89**
	Housewife	33(56.9)	35(52.3)	
Gravidity		1.62±1.10	1.50±1.21	0.57*
Parity		0.17±0.38	0.13±0.34	0.55*
Abortion		0.20±0.40	0.11±0.32	0.18*
Educational level	High school	2(3.4)	1(1.7)	0.32**
	Diploma	14(24.1)	18(30)	
	University	42(72.4)	42(68.3)	

BMI: Body mass index.

*Independent t-test, **Chi-square test

Table 2. Obstetric outcomes in two study groups

Variables		Mean±SD/No. (%)		P*
		Probiotic	Control	
Preterm labor (week)	<34	5(8.62)	9(15)	0.47
	34-37	7(12.06)	10(16.7)	0.52
PPROM		6(10.3)	8(13.3)	0.61
Delivery	NVD	32(55.2)	36(60)	0.54
	CS	25(43.1)	21(35)	
	Instrumental	1(1.7)	3(5)	

Abbreviation: PPROM: Preterm premature rupture of membranes, NVD:Normal vaginal delivery, CS: Cesarean section.

*Chi-square test

Neonatal characteristics of the two groups are presented in Table 3. Results showed that in the probiotic and control groups, the newborn's weight was 2928.07±454.83 and 2879.16±348.27; head circumstance was 33.39±1.15 and 33.46±1.46; height was 49.58±1.30 and 49.93±1.45; Apgar score at 1 minute was 8.7±0.6 and 8.6±0.5, and Apgar score at 5 minutes was 9.8±0.6 and 8.9±0.8 after birth, respectively. The results showed no significant differences in these variables between the two groups. Both groups were in the optimal range of Apgar score. No side effects were reported in any group.

Discussion

The results of this clinical trial showed that the efficacy of adjuvant administration of probiotics was not significantly different in preventing PTB in pregnant women at high risk for PTB than the 17α-OHP IM injection alone. Other pregnancy outcomes, including PPROM, mode of delivery, newborn weight, height, head circumference, and Apgar score at 1 and 5 minutes after birth, were not significantly different, either.

Probiotics have been reported as a preventive strategy for PTB [16]. The present study challenges previous studies on the effectiveness of starting using probiotics in the 16th week of pregnancy in preventing PTB and other maternal and neonatal outcomes. A previous study showed that probiotics containing lactobacilli were effective in treating bacterial vaginosis [17] and although it has not been established, the prevention of PTB, if present, is negative [18], because there is a connection between the use of probiotics and treatment of bacterial vaginosis which has a potential role in preventing PTB [19]. In a review study on the effects of prenatal probiotics on preventing PTB [10], probiotics were found to reduce the risk of genital tract infections (bacterial vaginosis) by 81%. However, there was insufficient evidence to determine whether probiotics reduced the incidence of PTB. No side effects after using the probiotics or prebiotics during pregnancy have been reported. However, a meta-analysis by Dugoua et al. [20] showed no differences in gestational age in the probiotic compared to the non-probiotic group.

Table 3. Neonatal outcomes in two study groups

Variables	Mean±SD		P*
	Probiotic	Control	
Newborn's weight (g)	2928.07±454.83	2879.16±348.27	0.51
Newborn's head circumference (cm)	33.39±1.15	33.46±1.46	0.62
Newborn's height (cm)	49.58±1.30	49.93±1.45	0.73
Apgar score at 1th min	8.7±0.6	8.6±0.5	0.35
Apgar score at 5th min	9.8±0.6	8.9±0.8	0. 40

*Independent t-test

Previous studies have shown no correlation between the label and the actual content of probiotic products in many cases [21, 22] and the main properties of some probiotic strains can be affected by industrial production processes, which can lead to their instability. Therefore, our results cannot be related to the commercial probiotic products. Based on prospective cohort studies, the use of fermented dairy products can be considered a valuable nutritional intervention for all pregnant women [23-25]. For example, consuming approximately three ounces of a fermented dairy product per day was associated with a significant reduction in the risk of spontaneous PTB [8]. The populations of these studies probably differ from the pregnant women in the present study in terms of ethnicity, genetics and immune system, since microbiota colonization varies by race/ethnicity and geographic location [26-30]. The women in our study were Iranian. The present study was designed to be clinically applicable in terms of gestational age at initiation of probiotic administration, as well as dosage and type of probiotics. Therefore, although our study failed to provide beneficial effects for probiotic supplementation during pregnancy compared to placebo, other microbial species or dosages may be effective. It is also possible that starting the intervention before pregnancy or continuing it for a longer period of time could affect the results. The consumption of probiotics in the present study was started in the 16th gestational week.

It is recommended that the protective effect of fermented dairy products, as well as the effect of their absence as a risk factor for PTB, be investigated to improve understanding of health outcomes during pregnancy and facilitate the implementation of effective health promotion strategies.

The strengths of this study included the use of rigorous and extensive inclusion and exclusion criteria, a large sample size with different dietary habits, and a wide range of probiotic product intake doses. However, there were some limitations/disadvantages. We did not assess women's adherence to the assigned intervention using stool analysis, as greater adherence may be associated with better outcomes. Also, women's dietary intake before and during pregnancy was not assessed.

Based on the results, it seems that the use of probiotics as adjuvants from the 16th to the 37th gestational week, along with 17α-OHP IM injection, does not reduce the risk of spontaneous PTB or improve other neonatal and maternal outcomes in pregnant women at high risk for PTB. However, further randomized clinical trials are needed to investigate the use of different species and doses of probiotics for a longer period to improve our understanding of the role of gut microbiota in pregnancy.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Yasuj University of Medical Sciences, Yasuj, Iran (Code: IR.YUMS.REC.1399.132) and was registered by the Iranian Registry of Clinical Trials (Code: IRCT20201108049300N1). Written informed consent was obtained from all patients. All procedures involving human participants performed in this study were in accordance with the ethical standards of the Helsinki Declaration and its later amendments.

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Authors' contributions

Data collection: Raziyeh Vanda, Mansoureh Moghadam-Sangcholi and Hossein Sadeghi; Data analysis: Fatemeh Bazarganipour; Draft preparation: Fatemeh Bazarganipour, Marcello Iriti and Seyed-Abdolvahab Taghavi; Supervision: Raziyeh Vanda and Fatemeh Bazarganipour; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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