

Research Article

Efficacy of Vaginal Probiotic Therapy in Modulating Fetomaternal Outcomes in Preterm Premature Rupture of Membranes

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ABSTRACT

Objectives: To evaluate the efficacy of adjunctive vaginal probiotics added to standard therapy in improving the fetomaternal outcomes among women presenting with preterm premature rupture of membranes (PPROM).

Study Design: Randomized controlled trial.

Place and Duration of Study: MCH, PIMS, Islamabad, from January 2024 to June 2024.

Methodology: A total of 100 women aged 18-40 years presenting with PPRM between 28+0 and 33+6 weeks of gestation were randomized into two equal groups. All women received standard PPRM management, while the intervention group additionally received a *Lactobacillus* vaginal capsule (containing $\geq 1 \times 10^9$ CFU) nightly for 10 days. The primary outcome was NICU admission, while the secondary maternal and neonatal outcomes included latency period, delivery within 7 days, chorioamnionitis, Nugent score, bacterial vaginosis, vaginal pH, inflammatory markers, birth weight, Apgar score, respiratory distress syndrome (RDS), early-onset sepsis, and neonatal mortality.

Results: NICU admission (the primary outcome) was significantly lower in the intervention group than in the control group (14% vs 40%, $p < 0.01$). The intervention group had a longer latency period ($p < 0.001$), lower delivery within 7 days ($p < 0.001$), decreased bacterial vaginosis ($p = 0.03$) and less chorioamnionitis ($p < 0.001$). Nugent score ($p < 0.001$) and WBC count ($p = 0.03$) also improved significantly. Birth weight and 5-minute Apgar score were also higher in the probiotic group ($p < 0.001$, for both). No significant differences were observed in RDS, early-onset sepsis, or neonatal mortality.

Conclusion: Vaginal probiotic therapy adjunctive to standard therapy was associated with improved fetomaternal outcomes including reduced NICU admission, prolonged latency, reduced chorioamnionitis and improved birth weight in women with PPRM.

Keywords: *Lactobacillus*, Neonatal Intensive Care Units, Pregnancy, Preterm Premature Rupture Of Membranes, Probiotics, Vaginal Microbiota.

INTRODUCTION

Preterm premature rupture of membranes (PPROM) is defined as spontaneous rupture of fetal membranes before the onset of labour at < 37 completed weeks of gestation and complicates about 3% of all pregnancies.^{1,2} PPRM is associated with about one-third of all cases of preterm birth, making it an important contributor to neonatal morbidity and mortality worldwide.^{2,3} This is particularly important in low- and middle-income countries (LMIC) where health-care budgets are constrained and neonatal intensive care resources are limited. The consequences of PPRM extend beyond

prematurity as the loss of the protective membrane barrier exposes the mother and fetus to ascending infection. PPRM is therefore associated with a variety of fetomaternal complications, including chorioamnionitis, placental abruption, cord compression, postpartum endometritis, and early-onset neonatal sepsis.^{2,4}

The vaginal microbiome has a key role in this pathway. The vaginal flora of healthy women of reproductive age is dominated by *Lactobacillus* species, which are responsible for producing lactic acid and keeping the vaginal pH below 4.5. These bacteria also produce hydrogen

peroxide and bacteriocins, and inhibit the growth and colonization of potential pathogens.⁵ Overgrowth of anaerobes and depletion of lactobacilli, as observed in bacterial vaginosis (BV) and other vaginal dysbioses, have been associated with ascending intrauterine infection, a pro-inflammatory cytokine environment, weakening of fetal membranes, and preterm birth. Women who develop PPRM often have dysbiotic vaginal flora, which can lead to intra-amniotic infection and subsequent membrane rupture.⁶

Standard management of PPRM comprises expectant care with latency antibiotics, antenatal corticosteroids (ACS) for fetal lung maturation, and close surveillance for infection. Delivery is planned at a predetermined gestation, or earlier if complications arise. Although latency antibiotics extend the duration of pregnancy and reduce chorioamnionitis, they are broad spectrum, fail to restore a lactobacilli-dominant flora and might worsen the vaginal ecosystem. They may also raise concerns about antimicrobial resistance and neonatal drug exposure. This raises the need for a biologically plausible strategy as an adjunct to the standard care that directly reconstitutes the protective vaginal microbiota.^{7,8}

Vaginal probiotics (VPs), which are typically preparations of *Lactobacillus* strains, can colonize the vagina, lower vaginal pH, and improve the treatment response in BV. They can therefore reduce recurrence of BV when used with or after antimicrobial therapy. The evidence for their ability to reduce preterm birth in general obstetric populations is, however, mixed, and most trials have addressed only prevention rather than women with established membrane rupture. The efficacy of VPs to prolong the latency period, lower maternal infectious morbidity, and improve neonatal outcomes in PPRM remains uncertain, and the data are scarce, especially from LMICs.^{9,10}

Admission to the neonatal intensive care unit (NICU) is a clinically meaningful neonatal outcome influenced by gestational age (GA) at birth, infectious burden, and respiratory complications, and contributes significantly to healthcare utilization and economic burden on families. This randomized, controlled trial was therefore planned to evaluate the efficacy of adjunctive vaginal probiotic therapy (VPT) in modulating fetomaternal outcomes in women with PPRM. The findings of this research may offer a simple adjunct to standard care for improving fetomaternal outcomes in women with PPRM.

METHODOLOGY

This randomized controlled trial was conducted at the MCH, PIMS, Islamabad, from January-2024 to June-2024. The protocol of the study was approved by the Institutional Review Board of the hospital. All participants gave written informed consent prior to their inclusion in the study.

Sample size was calculated by comparing two independent proportions. By taking a NICU admission rate of 40.8% in women who received probiotics and 77.9% in women who did not receive probiotics, with $\alpha = 0.05$ (two-sided) and 80% power, the calculated minimum sample size was 54 (27 per group).¹¹ However, to improve statistical power, and the capacity to assess secondary outcomes, a total of 100 women (50 per group) were enrolled.

A total of 100 women aged 18–40 years with a singleton pregnancy and PPRM confirmed between 28+0 and 33+6 weeks were enrolled. The confirmation of PPRM was made through pooling, nitrazine positivity or ferning on sterile speculum examination, further supported by ultrasonography.

The exclusion criteria consisted of women with clinical chorioamnionitis at presentation, established labour, major fetal anomaly, placenta praevia or abruption, cervical cerclage, antibiotic or probiotic use within two weeks and known hypersensitivity to the study product. Women with any indication for immediate delivery were also excluded.

All women received standard care including hospital admission, betamethasone 12 mg intramuscularly (two doses, 24 hours apart), latency antibiotics (as per institutional protocol), and expectant management to 36+0 weeks. This also included earlier delivery in cases of chorioamnionitis, fetal compromise, abruption, or labour.

Randomization was carried out using a computer-generated randomization sheet. The participating women were either enrolled in the intervention group (which additionally received a *Lactobacillus* vaginal capsule, $\geq 1 \times 10^9$ CFU, nightly for 10 days), or the control group (which received standard care alone).

The primary outcome was set as the rate of NICU admission. Maternal secondary outcomes included maternal Nugent score (Gram-stained high vaginal swab), BV (Nugent score ≥ 7), vaginal pH, C-reactive protein (CRP), and white blood cell (WBC) count at baseline and at follow-up (day 7 or before delivery, whichever was earlier), delivery within 7 days of

membrane rupture, latency period, clinical chorioamnionitis (Gibbs criteria), and adverse effects (vaginal irritation/itching).

Neonatal secondary outcomes were birth weight (BW), 5-minute Apgar score <7, respiratory distress syndrome (RDS), early-onset sepsis, and neonatal death within 28 days of birth.

Data were analyzed using SPSS version 26. Continuous variables were expressed as mean $\pm$ SD or median (IQR), as appropriate, while the categorical variables were shared as frequencies and percentages. The independent-samples t-test (or Mann–Whitney

U test) was used to compare continuous variables, while the chi-square test (or Fisher's exact test) was used for comparing variables. A p-value <0.05 was considered statistically significant.

RESULTS

The mean age of women was 28.53 ± 4.09 years which ranged from 18 to 37 years. The mean GA at the PPROM was 31.01 ± 1.37 weeks. The groupwise details of baseline demographics, obstetrics, vaginal, and laboratory characteristics were comparable between the groups as shown in Table-I.

Table-I: Baseline demographics, obstetrics, vaginal, and laboratory characteristics. n = 100

Variable	Intervention group (n=50)	Control group (n=50)	p-value
Age (Mean $\pm$ SD) years	27.96 ± 4.22	29.10 ± 3.91	0.16
GA at PPROM (Mean $\pm$ SD) weeks	30.92 ± 1.47	31.10 ± 1.28	0.52
Nugent score (Mean $\pm$ SD)	6.18 ± 1.98	5.87 ± 2.17	0.46
Vaginal pH (Mean $\pm$ SD)	5.33 ± 0.44	5.42 ± 0.51	0.30
C-Reactive protein (Mean $\pm$ SD) mg/L	11.08 ± 3.73	10.71 ± 4.47	0.65
White blood cells (Mean $\pm$ SD) $\times 10^9/L$	10.27 ± 1.96	10.32 ± 1.90	0.89

The primary outcome (NICU admission) occurred in a significantly higher number of neonates in the control group 20 (40.0%)

compared to the intervention group 7 (14%) ($p < 0.01$), as shown in Table-II.

Table-II: NICU admission rate. n = 100

Variable		Intervention group (n=50)	Control group (n=50)	p-value
NICU admission n (%)	Yes n (%)	7 (14)	20 (40)	$p < 0.01$
	No n (%)	43 (86)	30 (60)	

The secondary outcomes related to maternal clinical, vaginal and laboratory parameters demonstrated significantly longer mean latency period (9.82 ± 2.29 vs 6.73 ± 2.44 days, $p < 0.001$), lesser delivery within 7 days (44% vs 88%, $p < 0.001$), lesser incidence of clinical chorioamnionitis (38% vs 92%, $p < 0.001$), fewer women with persisted BV (23% vs 68%, $p < 0.03$), lower mean Nugent score (3.83 ± 1.77 vs 6.13 ± 1.97 , $p < 0.001$), and lower pH

(4.80 ± 0.35 vs 5.18 ± 0.48 , $p < 0.001$) in the intervention group compared to the control group. Similarly, WBC count was lower in the probiotic group (9.56 ± 1.85 vs $10.50 \pm 2.42 \times 10^9/L$, $p = 0.03$), whereas the difference in follow-up CRP was not statistically significant ($p = 0.05$). Vaginal irritation/itching was reported in 6 (12.0%) women in intervention group and none of the controls ($p = 0.03$). The details are shown in Table-III.

Table III. Maternal clinical, vaginal and laboratory outcomes. n = 100

Outcome variables	Intervention group (n=50)	Control group (n=50)	p-value
Latency period (Mean $\pm$ SD) days	9.82 ± 2.29	6.73 ± 2.44	< 0.001
Delivery within 7 days n (%)	22 (44)	44 (88)	< 0.001
Clinical chorioamnionitis n (%)	19 (38)	46 (92)	< 0.001
Nugent score at follow-up (Mean $\pm$ SD)	3.83 ± 1.77	6.13 ± 1.97	< 0.001
BV at follow-up n (%)	23 (46)	34 (68)	0.03
Vaginal irritation/itching n (%)	6 (12)	0 (0)	0.03

Laboratory findings at follow-up (Mean ± SD)	Vaginal pH	4.80 ± 0.35	5.18 ± 0.48	<0.001
	C-reactive protein mg/L	9.18 ± 3.64	11.13 ± 5.91	0.05
	WBC, follow-up (×10⁹/L)	9.56 ± 1.85	10.50 ± 2.42	0.03

Analysis of secondary neonatal outcomes showed significantly higher BW (1797 ± 325 vs 1495 ± 320, <0.001) and 5-min Apgar score (7.58 ± 0.95 vs 6.66 ± 1.12, p<0.001), while a significantly lower number of neonates with 5-

min Apgar <7 (12.0% vs 38.0%, p<0.01) in intervention group compared to the control group. However, no significant difference was recorded for RDS, early-onset sepsis (EOS) and neonatal mortality, as shown in Table-IV.

Table IV. Neonatal outcomes. n = 100

Neonatal outcomes	Intervention group (n=50)	Control group (n=50)	p-value
Birth weight (Mean ± SD) g	1797 ± 325	1495 ± 320	<0.001
5-min Apgar score (Mean ± SD)	7.58 ± 0.95	6.66 ± 1.12	<0.001
Neonates with 5-min Apgar <7 n (%)	6 (12.0%)	19 (38.0%)	<0.01
Respiratory Distress Syndrome n (%)	31 (62.0%)	36 (72.0%)	0.40
Early-onset sepsis n (%)	12 (24.0%)	8 (16.0%)	0.45
Neonatal death within 28 days n (%)	1 (2.0%)	2 (4.0%)	1

DISCUSSION

This randomized controlled trial evaluated the benefits of adding VPs to standard therapy on fetomaternal outcomes in women with PPROM. The primary outcome of the study showed significantly lower NICU admission in the intervention group than in the control group (14% vs 40%, p<0.01). Probiotic therapy also demonstrated a significantly longer latency period (9.82±2.29 vs 6.73±2.44 days, p<0.001), fewer deliveries within 7 days of membrane rupture (44% vs 88%, p<0.001), and lower clinical chorioamnionitis (38% vs 92%, p<0.001). At follow-up, the probiotic group had lower Nugent scores (p<0.001) and vaginal pH (p<0.001), with significantly fewer women having persistent BV (all p=0.03). WBC count was also lower in the intervention group (p=0.03), whereas no statistically significant difference was recorded regarding CRP (p=0.05). Among neonatal outcomes, BW and 5-minute Apgar score were significantly higher in the intervention group (both p<0.001). Similarly, Apgar score <7 was less frequent (12% vs 38%, p<0.01) among neonates in the group with VPs therapy. No significant differences were, however, observed in RDS, early-onset sepsis, or neonatal mortality. These findings regarding the proposed role of *Lactobacillus* in maintaining vaginal acidity and limiting pathogenic colonization are biologically plausible and are broadly consistent with previous evidence suggesting that VP supplementation may improve the microbiological environment and potentially influence the fetomaternal outcomes.

Moraes DV et al. enrolled women with PPROM between 24-36 weeks to conduct a pilot study on the role of vaginal *Lactobacillus rhamnosus* and *L. gasseri* in improving fetomaternal outcomes. The probiotic group showed a longer latency period and higher GA at delivery than the placebo group. Despite little variation in most other outcomes, these findings supported the VPs as an adjunct for prolonging pregnancy in PPROM.¹² Similarly, Daskalakis GJ et al. compared vaginal probiotics plus antibiotics with antibiotics alone in 106 women with PPROM. In this study conducted with Greek patients, the probiotic group had significantly higher GA at birth (35.49 vs 32.53 weeks), longer latency period (5.60 vs 2.48 weeks), and higher BW (2439 vs 2005 g), along with a lower NICU admission rate and shorter hospitalization period.¹³ A study conducted in Iran by Kahvazi F et al. involving 60 women, compared antibiotics alone with antibiotics plus VPs in women with PPROM. The VPs group showed significantly lower NICU admission, higher 1- and 5-minute Apgar scores, greater BW, and longer pregnancy duration (all P<0.05) than the antibiotics alone group.¹⁴

Continuing the evidence from LMICs, a study by Ibrahim FA et al. in Egypt evaluated the role of VPs plus standard antibiotic prophylaxis in women with PPROM. The women were followed through delivery and neonatal admission and the probiotic group showed higher GA (34.5 vs 32.0 weeks) at delivery and BW (2224 vs 1947 g), and lower NICU admission (24.3% vs 55.8%), suggesting potential neonatal benefit with this adjuvant therapy.¹⁵

The evidence in our local population came from Asif R et al., who compared VPs plus antibiotics with antibiotics plus placebo in a study of 230 women with PPRM. The probiotic group had significantly lower NICU admission (40.8% vs 77.9%), higher 1- and 5-minute Apgar scores (6.5 vs 5.4; 7.8 vs 6.6), and lower mortality (8.6% vs 20.0%) than the placebo group, supporting the role of probiotics in reducing infectious morbidity and prolonging the latency.¹¹

The biological plausibility leading to these consistent clinical benefits is also well explained by the researchers. Dysbiosis of the vaginal microbiome, marked by depletion of protective *Lactobacillus* species, has been linked to several obstetric complications, including PPRM. A recently published review by Sahu BK et al. described how *Lactobacillus* helps maintain a healthy vaginal ecosystem, limit pathogenic colonization, and reduce recurrent BV through lactic acid and hydrogen peroxide production, competitive adherence, and immune modulation. This mechanistic rationale supports the use of VPs as an adjunct in PPRM, while our findings provide direct clinical evidence of their benefits.¹⁶

These findings of these studies have also been corroborated by meta-analytic evidence. A meta-analysis by Baradwan S et al. comprising 4 RCTs found that the combination of VPs and antibiotics versus antibiotics alone, significantly prolonged the latency period ($p < 0.001$), reduced NICU admission and length of stay, and improved BW ($p = 0.002$) in PPRM. Neonatal sepsis and RDS were also less frequent with probiotics, however, this difference did not achieve statistical significance ($p > 0.05$).¹⁷

Adding to the large-scale evidence, Ardiani V et al. performed a meta-analysis of 3 RCTs on adjunctive use of VPs for PPRM. In this recently published analysis, probiotics modestly prolonged latency (MD 2.98 days; $p < 0.0001$) and reduced maternal infection (RR 0.43; $p = 0.005$), admission to the NICU (RR 0.59; $p < 0.0001$), and neonatal mortality (RR 0.38; $p = 0.01$). The authors, however, did not eliminate the risk of bias and confounding especially for NICU admission and mortality, and underscored the need for larger RCTs on the topic.¹⁸

Despite the methodological limitations present in some of these research works, the overall direction of evidence remains consistent with our findings, reinforcing the potential role of

VPs as a valuable adjunct in the management of PPRM.

The limitations of this research include its single-center design and a relatively small sample size, which may limit the generalizability of these findings and the ability to detect differences in uncommon neonatal outcomes. Moreover, long term neonatal outcomes and sustained effects on the vaginal microbiome were not assessed as part of the study protocol.

CONCLUSION

The findings of this study suggested that VPT adjunctive to the standard treatment was associated with a favorable vaginal microbiological profile, prolonged latency, reduced infectious morbidity, and improved neonatal outcomes in women with PPRM. VPT, therefore, may represent a simple adjunct to conventional PPRM care, offering a potential strategy to support vaginal microbiome balance and improve maternal and neonatal outcomes. Further multicenter studies with larger samples and longer neonatal follow-up are however warranted to confirm these findings.

Conflict of Interest: None.

Disclaimer: None.

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