

Vaginal Seeding and Maternal PPD in a Pakistani Cohort

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Abstract

Background: The postpartum period is a critical window for maternal mental health. Cesarean section (CS) disrupts the vertical transfer of the maternal microbiome and is associated with an increased risk of postpartum depression (PPD). While vaginal seeding is studied for infant microbiome restoration, its impact on the maternal microbiome and her mental health remains entirely unexplored.

Objective: To investigate the effect of vaginal seeding on the maternal gut microbiome and the incidence of PPD in a cohort of Pakistani women undergoing elective CS.

Study Design: A randomized, double-blind, placebo-controlled trial was conducted at Fatima Memorial Hospital. 150 women with term singleton pregnancies scheduled for elective CS were enrolled. Participants were randomized to have their infant and their own hands and face exposed to a gauze containing either maternal vaginal fluid (intervention, $n=75$) or sterile saline (placebo, $n=75$). The primary outcome was the change in maternal gut microbiome composition (16S rRNA sequencing) from baseline to 1-month postpartum. The secondary outcome was the score on the Edinburgh Postnatal Depression Scale (EPDS) at 3 months postpartum.

Results: 142 participants completed the study (71 per group). At 1-month postpartum, the intervention group showed a significantly different gut microbiome profile (PERMANOVA, $p=0.013$) with higher alpha-diversity (Shannon Index, $p=0.025$) and a greater relative abundance of *Lactobacillus* ($p=0.015$). At 3 months, the intervention group had a significantly lower incidence of PPD (EPDS ≥ 13 : 11.3% vs. 29.6%, $p=0.008$) and a lower mean EPDS score (7.1 ± 3.8 vs. 9.8 ± 4.9 , $p<0.001$). The change in *Lactobacillus* abundance mediated 32% of the intervention's effect on the EPDS score.

Conclusion: Vaginal seeding is associated with beneficial modulation of the maternal gut microbiome and a significantly reduced risk of PPD. This study is the first to reveal a potential maternal mental health benefit of this practice, positioning it as a dual-benefit intervention for the mother-infant dyad.

Keywords: Postpartum Depression, Vaginal Seeding, Microbiome, Gut-Brain Axis, Cesarean Section, Maternal Mental Health, EPDS, Pakistan.

Introduction

Postpartum depression (PPD) is a prevalent and debilitating condition, affecting approximately 20% of women in Pakistan, a rate higher than the global average [1]. The etiology is multifactorial, involving psychosocial, hormonal, and biological factors. Recently, the gut-brain axis has emerged as a critical pathway influencing mood and behavior [2]. The gut microbiome,

through the production of neurotransmitters, short-chain fatty acids, and immune modulation, can significantly impact brain function and emotional regulation [3].

The mode of delivery is a major determinant of the initial infant microbiome and also influences the maternal microbiome's postpartum recovery [4]. Cesarean section (CS) prevents the

natural transfer of maternal vaginal and fecal microbes to the newborn. This disruption has been linked to an increased risk of immune and metabolic disorders in infants [5] and is independently associated with a higher risk of PPD in mothers [6]. The practice of "vaginal seeding"—the deliberate exposure of a CS-born infant to maternal vaginal fluids—aims to restore the infant's microbiome [7]. However, the procedure inherently involves maternal auto-inoculation, as the mother handles the gauze. The effect of this exposure on the mother's gut microbiome and her subsequent mental health has never been investigated.

Pakistan has a rapidly rising CS rate, now exceeding 30% in urban centers like Lahore [8]. Concurrently, resources for managing perinatal mental health are scarce. A simple, low-cost intervention that could mitigate PPD risk would have significant public health implications.

We hypothesized that vaginal seeding would positively modulate the maternal gut microbiome and reduce the incidence and severity of PPD. This study aimed to test this hypothesis in a randomized controlled trial.

Methods

Study Design and Participants

This single-center, randomized, double-blind, placebo-controlled trial was conducted at the Department of Obstetrics & Gynaecology, Fatima Memorial Hospital, Lahore, from January to December 2023. Ethical approval was granted by the FMH Institutional Review Board (IRB/FMH/12/2022). Written, informed consent was obtained from all participants in Urdu, the local language.

Eligible women were aged 18-40 years, with a singleton pregnancy ≥ 37 weeks gestation, scheduled for an elective CS. Exclusion criteria were: (1) planned vaginal delivery; (2) requirement for intrapartum antibiotics; (3) known maternal HIV, HBV, HCV, or active HSV/HPV infection; (4) pre-existing diagnosis of major depressive disorder or other psychiatric illness; (5) use of antibiotics or probiotics within 2 weeks prior to delivery; and (6) major fetal anomaly.

Randomization and Blinding

A computer-generated randomization sequence (1:1 ratio) using block sizes of 4 and 6 was created by an independent statistician. Sequentially numbered, opaque, sealed envelopes containing the group assignment were prepared. The envelope was opened by a research midwife not involved in patient care or outcome assessment, who prepared the gauze accordingly. The operating surgeon, the patient, and the researchers collecting outcome data were blinded to the assignment.

Table 1: Baseline Demographic and Clinical Characteristics

| Characteristic | Intervention (n=71) | Placebo (n=71) | p-value |
|----------------------------------------|---------------------|----------------|---------|
| Maternal Age (years) | 28.4 \pm 4.1 | 27.9 \pm 3.8 | 0.45 |
| Pre-pregnancy BMI (kg/m ²) | 26.5 \pm 3.9 | 25.8 \pm 4.2 | 0.31 |
| Primiparous, n (%) | 25 (35.2%) | 28 (39.4%) | 0.60 |
| Gestational Age (weeks) | 38.5 \pm 0.7 | 38.6 \pm 0.6 | 0.35 |
| Baseline EPDS Score | 5.2 \pm 2.9 | 5.0 \pm 2.7 | 0.66 |
| Exclusive Breastfeeding at 1m, n (%) | 48 (67.6%) | 45 (63.4%) | 0.59 |

Intervention

One hour before CS, the research midwife inserted a sterile gauze (10cm x 10cm, 4-ply) moistened with 5mL of sterile saline into the posterior vaginal fornix.

Intervention Group: The gauze, saturated with vaginal fluid, was removed before surgery. The midwife wiped the newborn's lips, cheeks, and hands for 15 seconds. The mother then held the gauze and wiped her own hands and perioral area.

Placebo Group: An identical gauze soaked only in 5mL of sterile saline was used in the same manner.

All other care followed standard hospital protocols for CS.

Outcomes

Primary Outcome: Change in maternal gut microbiome composition from baseline (36-38 weeks gestation) to 1-month postpartum, assessed via 16S rRNA gene sequencing (V3-V4 region) on an Illumina MiSeq platform. Analysis focused on alpha-diversity (Shannon Index) and beta-diversity (Bray-Curtis dissimilarity, PERMANOVA).

Secondary Outcome: EPDS score at 3 months postpartum. The validated Urdu version of the EPDS was administered via telephone interview by a trained researcher blinded to the group allocation. An EPDS score of ≥ 13 indicated probable PPD.

Exploratory Analysis: Mediation analysis to test if changes in the gut microbiome mediated the effect on EPDS scores.

Sample Collection and Statistical Analysis

Maternal stool samples were collected at baseline and 1-month postpartum using home collection kits. DNA was extracted, sequenced, and analyzed using QIIME2. Based on a pilot study, a sample size of 142 (71/group) provided 80% power to detect a 0.3 difference in Shannon Index ($\alpha=0.05$). Statistical analyses were performed using SPSS v.26 and R v.4.1.0. Categorical data were compared using Chi-square tests, continuous data with t-tests or Mann-Whitney U tests. Microbiome data was analyzed with PERMANOVA and ANCOM-BC. Mediation was tested using the PROCESS macro. A p-value <0.05 was considered significant.

Results

Participant Characteristics

Of 187 women assessed, 150 were randomized. 142 completed the 3-month follow-up (71 in each group) (Figure 1). Baseline characteristics were similar between groups (Table 1).

Impact On Maternal Gut Microbiome

The gut microbiome composition significantly differed between groups at 1-month postpartum (PERMANOVA on Bray-Curtis, $R^2=0.018$, $p=0.013$) (Figure 2A). Alpha-diversity was higher in the intervention group (Shannon Index: 3.45 ± 0.51 vs. 3.21 ± 0.48 , $p=0.025$) (Figure 2B). Differential abundance analysis revealed a significantly higher relative abundance of the genus *Lactobacillus* in the intervention group (Log2 Fold Change = 1.8, $p=0.015$).

Impact on Postpartum Depression

At 3 months postpartum, the incidence of probable PPD (EPDS ≥ 13) was significantly lower in the intervention group (8/71, 11.3%) compared to the placebo group (21/71, 29.6%) ($\chi^2 = 7.12$, $p = 0.008$). The mean EPDS score was also significantly lower in the intervention group (7.1 ± 3.8 vs. 9.8 ± 4.9 , $t = -3.72$, $p < 0.001$) (Figure 3).

Mediation Analysis

The increase in *Lactobacillus* abundance at 1 month significantly mediated the relationship between the intervention and the lower EPDS score at 3 months (Indirect effect: $\beta = -0.61$, BootSE=0.29, 95% CI: -1.25 to -0.08). The mediation effect accounted for 32% of the total effect.

Safety

No serious adverse events were reported. Two infants in the intervention group developed mild, transient facial erythema that resolved within 24 hours without treatment.

Discussion

This is the first randomized trial to demonstrate that vaginal seeding exerts a significant beneficial effect on the maternal gut microbiome and mental health. In our Pakistani cohort, this simple intervention was associated with a 62% relative reduction in the risk of PPD at 3 months postpartum.

The findings are biologically plausible. The enrichment of *Lactobacillus*, a genus with known anti-inflammatory and gut-barrier strengthening properties, suggests that auto-inoculation helps restore a more resilient postpartum gut environment. This microbial shift likely influences the gut-brain axis, potentially by reducing systemic inflammation or increasing the production of neuroactive metabolites like GABA, which is synthesized by certain lactobacilli [3]. Our mediation analysis provides preliminary support for this mechanism [9- 10].

The clinical implications are substantial. In resource-constrained settings like Pakistan, where the CS rate is high and access to mental health services is limited, vaginal seeding could be a low-cost, empowering intervention that mothers can actively participate in. It shifts the paradigm of this practice from being solely infant-focused to a dual-benefit strategy for the mother-infant dyad.

Strengths and Limitations

Strengths include the randomized design, blinding, and use of objective microbiome metrics. Limitations include the single-center design, the 3-month follow-up for PPD, and the

lack of mechanistic data (e.g., inflammatory markers). The cultural context of Pakistan may limit generalizability to other populations.

Conclusion

Vaginal seeding following cesarean section is associated with a healthier maternal gut microbiome profile and a significantly reduced risk of postpartum depression. This novel finding positions vaginal seeding as a promising, simple intervention to safeguard maternal mental health in the vulnerable postpartum period. Further multi-center studies with longer follow-up are warranted to confirm these findings and elucidate the underlying mechanisms.

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Author Contributions

Dr. Aqsa Akram: Conceptualization, Methodology, Investigation, Writing – Original Draft, Supervision. Formal Analysis, Data Curation, Writing – Review & Editing. Investigation, Resources. Methodology, Writing – Review & Editing, Supervision.

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