European Journal of Obstetrics and Gynecology 310 (2025) 113976

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.journals.elsevier.com/european-journal-of-obstetrics-and-gynecology-and-reproductive-biology

Full length article

Probiotics in pregnancy and group B streptococcus colonization: A multicentric, randomized, placebo-controlled, double-blind study with a focus on vaginal microbioma

Daniela Menichini [a,*], Francesco De Seta [b], Salvatore Andrea Mastrolia [c,d], Irene Cetin [c], Anastasia Carafa [c], Susanna Santagni [e], Claudio Foschi [f,g], Matteo Cerboneschi [h], Serena Smeazzetto [h], Isabella Neri [a], Fabio Facchinetti [a]

[a] Obstetrics Unit, Mother–Infant Department, AOU of Modena, Modena, Italy
[b] Department of Obstetrics and Gynaecology, IRCCS San Raffaele Scientific Institute, University Vita and Salute, Milan, Italy
[c] Ospedale dei Bambini 'Vittore Buzzi', Milan, Italy
[d] Department of Obstetrics and Gynaecology, Ospedale Madonna delle Grazie, Matera, Italy
[e] Arcispedale Santa Maria Nuova Reggio Emilia – IRCCS, Reggio Emilia, Italy
[f] Microbiology Unit, IRCCS Azienda Ospedaliero – Universitaria of Bologna, Bologna, Italy
[g] Section of Microbiology, Department of Medical and Surgical Sciences, Alma Mater Studiorum – University of Bologna, Bologna, Italy
[h] NEXT Genomics Srl, Sesto Fiorentino, Florence, Italy

ARTICLE INFO

Keywords:
Probiotics
Pregnancy
Group B streptococcus
Microbiome

ABSTRACT

Objective: To evaluate the feasibility and effects of the use of probiotics in pregnancy, starting in the third trimester, on rectovaginal colonization of group B streptococcus (GBS) in women at low obstetric risk.
Methods: A multicentre, randomized, placebo-controlled, double-blind, parallel-group study was conducted in three tertiary hospitals in northern Italy and included low-risk pregnant women. The intervention consisted of oral administration of two capsules of probiotics or placebo from 30 weeks of pregnancy until 37 weeks of pregnancy. The primary outcome was GBS colonization, evaluated with rectovaginal swabs. In a subgroup, selected at random, changes in the vaginal microbiome after treatment administration were evaluated using 16S Metagenomic Sequencing Library Preparation sequencing and analysis.
Results: In total, 267 pregnant women were randomized to receive probiotics ($n = 133$) or placebo ($n = 134$). The two groups were similar at baseline. After treatment, no differences were found in the rates of positive rectovaginal swabs ($p = 0.24$) and antibiotic administration ($p = 0.27$). Only one case of postpartum fever (>38 °C) was found in the placebo group. Labour and delivery outcomes and neonatal outcomes were similar in both groups. Analysis of the vaginal microbiota showed that the relative abundance of *Lactobacillus* spp. was not modified significantly by the probiotics, but the relative abundance of *Gardnerella* spp. decreased significantly (3.6 ± 7.9 vs 5.5 ± 10.2; $p = 0.03$). Interestingly, the relative abundance of *Lactobacillus* spp. reduced significantly in women who subsequently presented with partial rupture of membranes (46.9 ± 43.6 vs 77.7 ± 24.9; $p = 0.02$).
Conclusion: Although the clinical outcomes were unaffected, administration of probiotics led to favourable changes in vaginal microbiota. It remains to be established how this effect could be translated into clinical advantage.

Introduction

Group B streptococcus (GBS), also known as *Streptococcus agalactiae*, is a pathogen present in the genital and rectal mucosa of approximately 25 % of healthy women. It is generally harmless and asymptomatic. However, during pregnancy, its presence in the urinary and vaginal tract at the time of delivery is the most important risk factor for neonatal infection, which can lead to neonatal early-onset sepsis (EOS), especially

* Corresponding author.
E-mail address: daniela.menichini91@gmail.com (D. Menichini).

https://doi.org/10.1016/j.ejogrb.2025.113976
Received 12 January 2025; Received in revised form 1 March 2025; Accepted 8 April 2025
Available online 11 April 2025

in the case of prolonged labour with partial rupture of membranes (PROM) [1].

In 98 % of cases, neonatal colonization is asymptomatic. The incidence of EOS is 1–3 ‰, with neonatal mortality of 50–60 % [2,3].

GBS is detected with universal culture screening at 35–37 weeks of gestation and, currently, positive women receive intrapartum antibiotic prophylaxis (IAP) in active labour to counteract neonatal GBS infections and reduce EOS [4]. Antibiotic prophylaxis is also effective when administered to women with risk factors for EOS (i.e. labour < 37 weeks, amniotic membrane rupture for 18 h, intrapartum temperature >38 °C) and unknown GBS status [5].

However, the widespread use of intrapartum antibiotics likely affects the biodiversity of maternal and neonatal microbiota, and is associated with mother and infant gut microbiota dysbiosis, a known risk factor for atopic diseases [6,7]. The vaginal microbiota has been recognized as a novel factor by which maternal stress and perturbations may contribute to reprogramming the developing brain of the offspring, predisposing individuals to neurodevelopmental disorders [8]. Moreover, IAP may decrease susceptibility to penicillin or ampicillin, the agents of choice for prevention of GBS-associated disease [9].

Several alternative strategies have been explored, including probiotic administration. A lactobacillus-dominant environment is thought to promote specific characteristics of the vaginal microenvironment, such as lower acidity and a reduction in biofilm formation, which may create conditions less favourable for disease-associated species such as *Gardnerella* spp. [10–12]. Consequently, probiotics have been investigated as a potential approach for the reduction of maternal GBS colonization and, ultimately, prevention of neonatal infections. A recent systematic review and *meta*-analysis conducted on the available evidence on probiotic supplementation in pregnancy concluded that probiotic administration in the third trimester of pregnancy was associated with reduced rectovaginal colonization of GBS at 35–37 weeks, and showed a safe perinatal profile [13]. However, the authors concluded that further research is needed to confirm the efficacy of probiotic administration in reducing the exposure of pregnant women to significant doses of antibiotics in labour. Also, it remains unclear which type of probiotic is the most effective, and the impact of its administration on the vaginal microbiome.

Rationale

A multicentre, randomized, placebo-controlled, double-blind, parallel-group study was conducted in women in the third trimester of pregnancy. The aim was to evaluate the feasibility and effect of the use of probiotics on rectovaginal colonization of GBS in women at low obstetric risk.

Methods

Study design

This study was approved by the Ethics Committee of Modena and Reggio Emilia on 24 September 2019 (Prot. N AOU0025949/19) and the Ethics Committee of Milan on 15 July 2020 (Prot. N 0019268), and was registered on Clinicaltrial.gov (NCT05156333).

The study was conducted at the University Hospital of Modena (coordinating centre), Children Hospital 'Vittore Buzzi' of Milan, and the Hospital of Santa Maria Nuova, Reggio Emilia.

Low-risk pregnant women at 30 weeks of gestation were included in the study. Women who had a positive urine culture for GBS, women who had previously had a newborn who suffered from GBS-related EOS, women who had used antibiotics in the month preceding enrolment, and women who were unable to understand the study and sign the informed consent form were excluded.

Written informed consent was collected at the time of enrolment after the women had been provided with all information regarding the study, risks and benefits, and the maintenance of privacy. The study procedures were conducted in accordance with the Declaration of Helsinki. Women were enrolled from October 2020 to July 2022.

Sample size calculation

The sample size calculation was based on a study conducted in the Emilia Romagna region by Berardi et al. [14] in which the rate of rectovaginal colonization of GBS varied between 28 % and 18 % in women who gave birth at term. Taking into account the heterogeneity of the population residing in the study area (African-Americans, Asians, Indians), the authors also referred to the colonization rates recommended by Campbell et al. [15]. In this study, GBS colonization ranged from 14.7 % in Asians to 36.5 % in African Americans.

Considering that few studies in the literature have evaluated the effect of probiotic administration on rectovaginal colonization of GBS, to estimate the effect, the authors referred to a study conducted in China on women who tested positive for rectovaginal colonization of GBS; in this study, administration of probiotics from 35–37 weeks of gestation until delivery led to a 42.8 % reduction in the rate of GBS colonization [16].

Therefore, in this study, it was hypothesized that Respecta leads to a 42.8 % reduction in colonization rate (from 28 % to 15.96 %).

The sample size was calculated using the Z test for the comparison of two independent proportions. With statistical power of 80 % and type I error set at 5 %, the calculated sample size was 186 subjects per arm (total 372 women).

Considering a maximum dropout rate of 10 %, 205 subjects were randomized per arm (total 410 women). The sample size was calculated using G*Power Version 3.1.9.4.

Randomization

Randomization (1:1) was performed at a central level using a computerized system. The investigators and the patients were blinded to the treatment assignment. The allocation sequence with alphanumeric codes and three-digit progressive numbers (i.e. MO_001, MI_001) was communicated by an independent central office within the coordinating site (Modena). The code corresponded to the number written on the packages to be administered, and the list of codes containing the assignment of the treatments was opened upon completion of the study (i.e. at the time of interim analysis).

Intervention

Women were assigned at random to one of two groups, and received two oral capsules containing a mixture of lactobacilli (Respecta) or two placebo capsules each day. Capsules were taken with a glass of water, preferably between meals, from 30 weeks of pregnancy until 37 weeks of pregnancy following rectovaginal swab screening.

Respecta is a class IIa medical device approved in Europe, studied and developed by Giellepi S.p.A. Health Science, Lissone, Italy. It is formulated in capsules containing 5×10^9 colony-forming units of probiotic blend (*Lactobacillus acidophilus* GLA-14, LMG S-29159 and *Lactobacillus rhamnosus* HN001, AGAL NM07/09514), in combination with bovine lactoferrin RCX (50 mg). The placebo capsules looked identical to the probiotics capsules, but contained maltodextrin (100 mg). The excipients were the same in both verum and placebo. The probiotics and placebo were provided by Giellepi S.p.A. Health Science.

Data collection

Data on demographics and socio-economic status at baseline were collected. Additionally, pregnancy, labour and delivery, and neonatal outcomes were collected from the hospital records following an anonymization procedure of mothers and newborns.

The following perinatal data were collected: delivery mode;

gestational age at delivery; Apgar scores at 1 and 5 min; length; birth weight; and gender.

Microbiome assay

At the time of enrolment (at 30 weeks of gestation) and at the end of treatment (at 37 weeks of gestation), a further vaginal swab was collected to evaluate whether vaginal colonization of the lactobacilli taken orally had occurred, evaluating both the vaginal microbiome and cytokine pattern.

This analysis was performed by the NextGenomics laboratory in Florence, using 16S Metagenomic Sequencing Library Preparation sequencing and analysis.

Next-generation sequencing experiments, comprising DNA extraction and primary bioinformatics analysis, were performed by NEXT Genomics S.R.L. (Sesto Fiorentino, Firenze, Italy). DNA extraction was performed with QIAamp Fast DNA Stool (Qiagen, Hilden, Germany), using an extraction negative control. The final yield and quality of extracted DNA were determined using a NanoDropOne spectrophotometer (Thermo Scientific, Waltham, MA, USA) and a Qubit Fluorometer 2.0 (Invitrogen Co., Carlsbad, CA, USA). 16S amplification was performed with primers – forward: 5′-CCTACGGGNGGCWGCAG-3′ and reverse: 5′-GACTACHVGGGTATCTAATCC-3′ (Klindworth et al., 2013) – which target the hypervariable V3 and V4 regions of the 16S rRNA gene. Each polymerase chain reaction was assembled according to Metagenomic Sequencing Library Preparation (Illumina, San Diego, CA, USA). A negative control was included in the workflow, which consisted of all reagents used during sample processing (16S amplification and library preparation) but did not contain sample to ensure no contamination. Libraries were quantified using a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA), and pooled to an equimolar amount of each index-tagged sample to a final concentration of 4 nM, including the Phix Control Library. Pooled samples were subject to cluster generation and sequenced on the MiSeq platform (Illumina) in a 2x250 paired-end format. The raw sequence files generated (fast files) underwent quality control analysis with FastQC. Following quality control, sequences were processed using QIIME2, which allowed for comprehensive analysis including de-noising, taxonomic assignment and diversity assessments, complementing initial quality control steps. The Metagenomics workflow classifies organisms using a database of 16S rRNA data. The classification is based on the Greengenes database (https://greengenes.lbl.gov/). The output of this workflow is a classification of reads at several taxonomic levels: kingdom, phylum, class, order, family, genus and species.

Statistical analysis

The collected data were inserted into Excel (Microsoft, Redmond, WA, USA) and analysed using R, following numerical coding of the questions and answers.

The primary and secondary endpoints were compared between the two treatment arms with a two-sided Z test for comparison of two independent commissions. Continuous data have been reported as mean ± standard deviation (SD). Categorical data have been reported as absolute and percentage frequencies. All probability values were two-tailed, and a p-value < 0.05 was considered to indicate significance. An interim analysis was planned when 50 % of the sample size was reached.

For microbiome data, taxonomy abundance between non-paired samples was analysed using non-parametric Mann–Whitney *U*-tests. Taxonomic differences between paired samples (same patient, before and after therapy) were established using the non-parametric Wilcoxon signed rank test.

Results

The Independent Data Monitoring Committee stopped the study for futility once the interim analysis data were available. Thus, a total of 267 pregnant women out of 410 planned were enrolled into the study.

Of the recruited subjects, 133 were allocated at random to the intervention group (probiotic group) and 134 were allocated to the control group (placebo group). Of these, 25 (9.3 %) were from the hospital in Reggio Emilia, 83 (31.0 %) were from the hospital in Milan, and 159 (59.7 %) were from the hospital in Modena.

Maternal baseline characteristics

The main maternal characteristics were similar in the two groups, and are reported in Table 1.

Rectovaginal swabs and use of antibiotics

The results of the rectovaginal swabs were unknown for 15 women. Of the other women, 15.7 % and 18.8 % of rectovaginal swabs tested positive in the placebo group and probiotic group, respectively (Table 2). Only one patient had postpartum fever (>38 °C) in the placebo group.

Pregnancy, labour and delivery

Table 3 reports the main pregnancy, labour and delivery outcomes.

The presence of at least one of the following medical conditions was similarly distributed between the two groups: gestational diabetes mellitus, pregnancy-induced hypertension, intrauterine growth restriction and pre-eclampsia.

The mean gestational age at delivery and the PROM rate did not differ significantly between the two groups. The time interval between PROM and delivery was also similar: 16.8 ± 15.1 h in the placebo group and 18.4 ± 16.7 h in the probiotic group ($p = 0.66$) (Table 3).

Regarding labour and delivery outcomes, there were no significant differences in the rate of induction of labour or caesarean section (Table 3). Operative vaginal deliveries were slightly more common in the probiotic group, but the difference was not significant ($p = 0.17$).

The most relevant result concerned maternal intrapartum fever ≥38 °C). There was only one case among the patients in the placebo group, in contrast to six cases in the probiotic group; this difference was significant ($p = 0.03$).

Table 1
Maternal baseline characteristics.

	Placebo group ($n = 134$)	Probiotic group ($n = 133$)	p-value
Mean maternal age (years), mean ± SD	33.2 ± 5.1	33.5 ± 5.2	0.72
Maternal age ≥ 35 years	52 (38.8)	52 (39.1)	0.69
Maternal age ≥ 40 years	14 (10.4)	20 (15.0)	0.13
Mean pre-pregnancy weight (kg), mean ± SD	71.5 ± 15.5	69.4 ± 15.0	0.28
Mean BMI (kg/m^2), mean ± SD	26.5 ± 5.7	25.7 ± 5.4	0.24
Pre-pregnancy BMI			0.27
Underweight	2 (1.5)	8 (6.0)	
Normal	62 (46.3)	56 (42.1)	
Overweight	30 (22.4)	35 (26.3)	
Obese	32 (23.8)	29 (21.8)	
Unknown	8 (5.9)	5 (3.7)	
Nulliparous	35 (26.1)	41 (30.8)	0.69

Data are n (%) unless otherwise indicated.
BMI, body mass index; SD, standard deviation.

Table 2
Rectovaginal swabs and use of antibiotics.

	Placebo group ($n = 134$)	Probiotic group ($n = 133$)	*p*-value
Positive rectovaginal swab	21 (15.7)	25 (18.8)	0.24
Unknown rectovaginal swab	8 (5.9)	7 (5.3)	0.40
Antibiotic administration	47 (35.1)	42 (31.6)	0.27

Data are *n* (%).

Table 3
Pregnancy, labour and delivery.

	Placebo group ($n = 134$)	Probiotic group ($n = 133$)	*p*-value
Medical conditions in pregnancy[aa]	41 (30.6)	39 (29.3)	0.85
Mean GA at delivery, mean ± SD	39.0 ± 1.6	39.1 ± 1.7	0.77
Preterm birth (GA < 37 weeks)	10 (7.5)	6 (4.5)	0.15
PROM	39 (29.1)	38 (28.6)	0.99
PROM-to-delivery interval (h), mean ± SD	16.8 ± 15.1	18.4 ± 16.7	0.66
Temperature ≥ 38 °C in labour	1 (0.7)	6 (4.5)	0.03
Labour			
Induced	19 (14.2)	27 (20.3)	0.37
Spontaneous	75 (55.9)	67 (50.3)	
Absent	40 (29.8)	39 (29.3)	
Delivery mode			0.48
Caesarean section	42 (31.3)	43 (32.3)	
Operative	5 (3.7)	10 (7.5)	
Vaginal	80 (59.7)	71 (53.4)	
Missing	7 (5.2)	9 (6.7)	

Data are *n* (%) unless otherwise indicated.
GA, gestational age; PROM, premature rupture of membranes; SD, standard deviation.
[a] At least one of the following: gestational diabetes mellitus, pregnancy-induced hypertension, intrauterine growth restriction and pre-eclampsia.

Neonatal outcomes

Mean birth weight (g) was similar between the groups: only 12 infants in the placebo group and six infants in the probiotic group had low birth weight ($p = 0.56$).

In the placebo group, seven newborns were in acidosis; of these, four had a 5-min Apgar score ≤7 and needed resuscitation. These infants were admitted to the neonatal intensive care unit (NICU) primarily for the management of acidosis and hypoglycaemia.

In the probiotic group, five newborns were in acidosis; of these, three had a 5-min Apgar score ≤7 and needed resuscitation. Ten infants in this group were admitted to the NICU, with reasons for admission including respiratory distress, hypoglycaemia and jaundice. Overall, the distribution of neonatal adverse outcomes was equal in the two study groups, and no sign ificant differences were observed (Table 4).

None of the newborns manifested early-onset GBS disease; only one case of fever was registered among those infants born to mothers who received placebo and required antibiotic therapy.

Vaginal microbiome

In total, 40 swabs were collected at baseline, and 36 women completed follow-up and provided a second sample at 35–37 weeks of gestation. Hence, a total of 72 samples (36 pre- and post-treatment) were analysed.

Overall, the vaginal microbiome at baseline was mainly dominated by *Lactobacillus* spp. (mean relative abundance 69.5 %), followed by *Enterococcus* spp. (4.2 %), *Gardnerella* spp. (3.6 %), *Streptococcus* spp. (2.7 %), *Atopobium* spp. (2.4 %) and *Staphylococcus* spp. (2.0 %). It is worth underlining that the level of lactobacilli varied considerably between women, ranging from a relative abundance of 0.8 % to 97.5 %.

Table 4
Neonatal outcomes.

	Placebo group ($n = 134$)	Probiotic group ($n = 133$)	*p*-value
Gender			0.15
Male	74 (55.4)	63 (47.4)	
Female	55 (41.0)	58 (43.6)	
missing	5 (3.7)	12 (9.0)	
Mean birth weight (g), mean ± SD	3222.6 ± 544.3	3219.3 ± 474.9	0.96
Low birth weight	12 (8.9)	8 (6.0)	0.56
5-min Apgar score ≤ 7	4 (2.9)	3 (2.5)	0.75
Need for resuscitation	4 (2.9)	3 (2.3)	0.32
Neonatal acidosis[a]	7 (5.2)	5 (3.7)	0.35
NICU admission	7 (5.2)	10 (7.5)	0.22
Neonatal antibiotics	1 (0.7)	0	0.15
Neonatal temperature ≥ 38 °C	1 (0.7)	0	0.15

Data are *n* (%) unless otherwise indicated.
SD, standard deviation; NICU, neonatal intensive care unit.
[a] Neonatal acidosis: umbilical cord pH ≤ 7.1 and base excess ≤-12 mmol.

Among lactobacilli, *L. crispatus* was the dominant species in most cases (52.7 %), followed by *L. iners* (25 %) and *L. gasseri* (16.6 %).

Data on the bacterial composition of the vaginal microbiome at genus level for each participant are available in Table S1 (see online supplementary material).

Fig. 1 shows the vaginal microbial composition before and after probiotic administration. The relative abundances of *Lactobacillus* spp. and *L. acidophilus* were not modified significantly by the use of probiotics [*Lactobacillus* spp. – mean relative abundance ± SD 66.0 ± 32.9 vs 58.2 ± 38.9 ($p = 0.36$); *L. acidophilus* – 0.82 ± 0.8 vs 0.83 ± 0.8 ($p = 0.7$]. This was also the case for *Streptococcus* spp. (3.8 ± 14.3 vs 4.6 ± 17.8; $p = 0.28$) and *S. agalactiae* (2.8 ± 10.9 vs 3.6 ± 14.2; $p = 0.7$).

On the contrary, a significant decrease in *Gardnerella* spp. (5.5 ± 10.2 vs 3.6 ± 7.9; $p = 0.03$) was observed in the probiotic group compared with the placebo group.

A significant increase in the ureaplasma level was noted in the placebo group (0.3 ± 1.5 vs 1.1 ± 4.6; $p = 0.001$) (Fig. 2). No variation in other taxa was noticed, including *Lactobacillus* spp. (73 ± 33.4 vs 70.6 ± 35.2) and *Streptococcus* spp. (1.6 ± 6.1 vs 0.1 ± 0.5) (Fig. 2).

Of note, the relative abundance of *Lactobacillus* spp. in post-treatment samples was significantly lower in women who subsequently presented with PROM (46.9 ± 43.6 vs 77.7 ± 24.9; $p = 0.02$). No other significant changes were noted in this group (e.g. *Gardnerella*, *Atopobium, Prevotella, Megasphaera* spp.).

Discussion

Principal findings

This study demonstrated that probiotic supplementation in low-risk women did not reduce GBS colonization prior to delivery, and did not influence the mode of delivery or neonatal outcomes.

Results

Previously, the authors published a *meta*-analysis which showed that probiotic administration during the third trimester of pregnancy was associated with reduced rectovaginal colonization of GBS at 35–37 weeks of gestation [13]. There are several explanations for these discrepant results. First and foremost, the mixture of probiotics utilized could have played a role. Indeed, most studies which reported a reduction in GBS colonization used a mixture of *L. rhamnosus* and *L. reuteri*, while *L. rhamnosus* associated with *L. acidophilus* and bovine lactoferrin was used in the present study.

A second source of variation could be due to the composition of the vaginal microbiome. Although *Lactobacillus* spp. were the main component of the flora in this study [17], the extent of its dominance

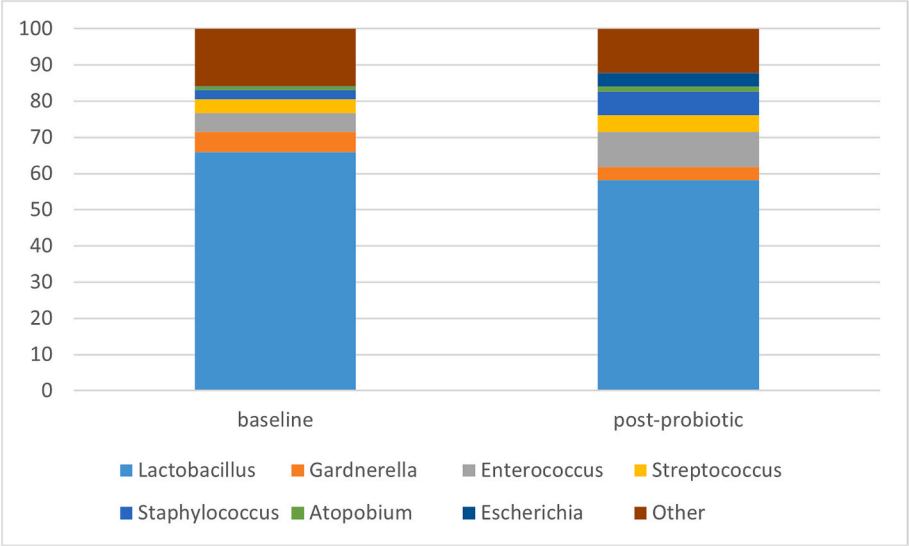

Fig. 1. Vaginal microbial composition in the probiotic group. Genus-level relative abundances. Only species with relative abundance >1 % in at least one group are reported, grouped and coloured by the corresponding genus.

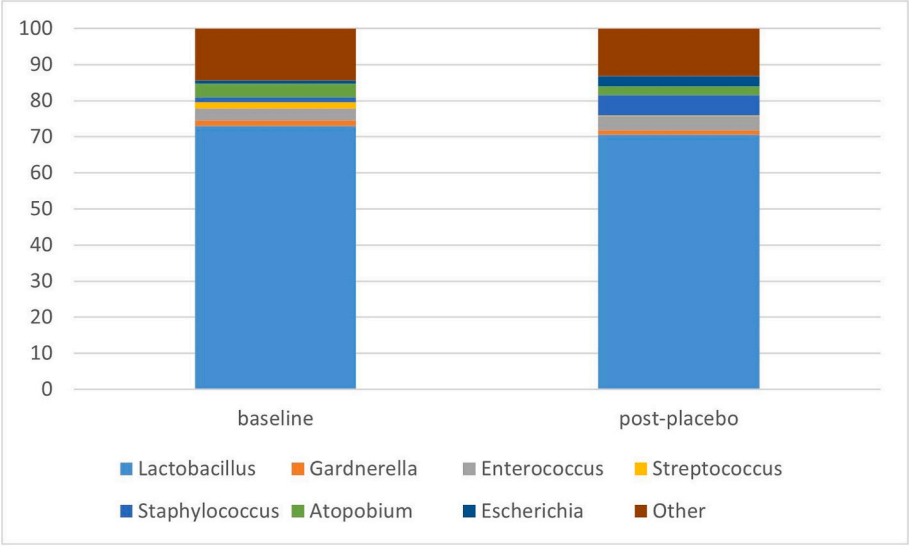

Fig. 2. Vaginal microbial composition in the placebo group. Genus-level relative abundances. Only species with relative abundance >1 % in at least one group are reported, grouped and coloured by the corresponding genus.

had great interindividual variation. It therefore seems not unlikely that an intervention aimed at enriching this bacterial genus may impact women differently in relation to the baseline composition of the vaginal microbiome.

In this context, *L. crispatus* was found to be the dominant *Lactobacillus* spp. in the analysed subset of women. This species is considered a hallmark of vaginal eubiosis, able to promote the maintenance of vaginal health, preventing the colonization and growth of adverse micro-organisms through various mechanisms [18]. Nevertheless, the microbiome of a significant proportion of subjects was enriched in *L. iners*, a 'transitional' species that often colonizes after perturbations of the vaginal environment and showing poor protective capabilities [19].

Clinical implications

Although the level of lactobacilli did not increase significantly following the administration of Respecta, it is worth underlining that use of the probiotic mixture led to a significant reduction in the relative abundance of *Gardnerella* spp. This result is particularly intriguing if one considers that *Gardnerella* spp. are anaerobic bacterial species involved in the pathogenesis of bacterial vaginosis, a condition of vaginal dysbiosis associated with adverse pregnancy outcomes [20]. *Gardnerella* spp. can produce various virulence factors – including sialidase, a hydrolase able to degrade local immunoglobulin A and vaginal mucins – likely contributing to the diminished viscosity of local secretion, resulting in increased vulnerability to pathogens [21]. Moreover, *Gardnerella* spp. have great ability to form biofilms that act as a scaffold to which other anaerobic species, such as *Atopobium vaginae* and *Prevotella* spp., can attach [22].

It is worth mentioning that a significant increase in the ureaplasma level was observed in the placebo group. Although these bacteria commonly colonize the urogenital tract of healthy subjects, being part of the normal commensal flora, they can be responsible for various adverse pregnancy outcomes, such as postpartum endometritis, chorioamnionitis, spontaneous abortion and premature birth [23].

Research implications

Other interesting results emerged when looking at the composition of the vaginal microbiome in women who subsequently presented with PROM. Indeed, as described previously, the presence of *Lactobacillus* spp. was linked to decreased risk of PROM [24]. Further studies are needed to understand the mechanisms underlying this protective effect.

Strengths and limitations

The strength of this study lies in its design, being one of the few double-blind, placebo-controlled trials exploring the impact of probiotic administration during pregnancy. Additionally, evaluation of the vaginal microbiome allowed the authors to capture the heterogeneity of vaginal flora, which may contribute to variability in responses to treatment.

A limitation of this study is that it was stopped before reaching the planned sample size. However, approximately 70 % of the sample was analysed, which provides a reasonable level of consistency to the findings. Furthermore, while the study did not demonstrate a significant reduction in GBS colonization, it highlights the challenges in rebalancing the vaginal microbiota, and suggests that factors such as treatment duration, probiotic composition and compliance may influence the outcomes. The findings emphasize the need for further research to better understand the complex interactions between probiotics and the vaginal microbiome.

Conclusions

The study findings suggest that administration of the tested probiotic in low-risk pregnancies did not reduce GBS colonization significantly. Several factors may explain the lack of effect, including: (i) potential issues with compliance among the participants; (ii) the possibility that a longer duration of administration may be necessary to observe a significant impact; and (iii) the composition of the lactobacillus mixture, as the antimicrobial effects of probiotics can be highly species- and strain-specific. Nevertheless, the probiotic mixture did show some biological effects, such as altering the vaginal microbiota. These results highlight the complexity of modulating the vaginal microbiome, and suggest that further research is needed to better understand the potential role of probiotics in this context.

Ethical approval and consent to participate

Ethical review and approval were waived for this study due to the observational nature of the study, in which data were anonymized. Informed consent was obtained from all subjects involved in the study.

Availability of data and materials

The datasets are available upon reasonable request from the corresponding author.

CRediT authorship contribution statement

Daniela Menichini: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Francesco De Seta:** Writing – review & editing, Methodology, Conceptualization. **Salvatore Andrea Mastrolia:** Writing – review & editing, Supervision, Resources, Investigation, Data curation. **Irene Cetin:** Writing – review & editing, Supervision, Resources, Conceptualization. **Anastasia Carafa:** Writing – review & editing, Resources, Data curation. **Susanna Santagni:** Writing – review & editing, Resources, Investigation. **Claudio Foschi:** Writing – original draft, Validation, Software, Methodology, Formal analysis, Data curation. **Matteo Cerboneschi:** Writing – review & editing, Validation, Software, Formal analysis. **Serena Smeazzetto:** Validation, Software, Formal analysis, Data curation. **Isabella Neri:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Fabio Facchinetti:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization.

Funding

This research received no external funding.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors wish to thank the residents working at the participating hospitals for their contributions to patient recruitment, sample storage and data collection.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejogrb.2025.113976.

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