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ORIGINAL RESEARCH ARTICLE

Cervical pessary and cerclage placement for preterm birth prevention and cervicovaginal microbiome changes

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Abstract

Introduction: Our objective was to compare the vaginal microbiome in low-risk and high-risk pregnant women and to explore a potential association between vaginal microbiome and preterm birth.

Material and methods: A pilot, consecutive, longitudinal, multicenter study was conducted in pregnant women at 18–22 weeks of gestation. Participants were assigned to one of three groups: control (normal cervix), pessary (cervical length ≤25 mm) and cerclage (cervical length ≤25 mm or history of preterm birth). Analysis and comparison of vaginal microbiota as a primary outcome was performed at inclusion and at 30 weeks of gestation, along with a follow-up of pregnancy and perinatal outcomes. We assessed the vaginal microbiome of pregnant women presenting a short cervix with that of pregnant women having a normal cervix, and compared the vaginal microbiome of women with a short cervix before and after placement of a cervical pessary or a cervical cerclage.

Abbreviations: CL, cervical length; CST, community state type; PCR, polymerase chain reaction; sPTB, spontaneous preterm birth; WG, weeks of gestation.

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Results: The microbiome of our control cohort was dominated by *Lactobacillus crispatus and inners*. Five community state types were identified and microbiome diversity did not change significantly over 10 weeks in controls. On the other hand, a short cervix was associated with a lower microbial load and higher microbial richness, and was not correlated with *Lactobacillus* relative abundance. After intervention, the cerclage group ($n = 19$) had a significant increase in microbial richness and a shift towards community state types driven by various bacterial species, including *Lactobacillus mulieris*, unidentified *Bifidobacterium* or *Enterococcus*. These changes were not significantly observed in the pessary ($n = 26$) and control ($n = 35$) groups. The cerclage group had more threatened preterm labor episodes and poorer outcomes than the control and pessary groups.

Conclusions: These findings indicate that a short cervix is associated with an altered vaginal microbiome community structure. The use of a cerclage for preterm birth prevention, as compared with a pessary, was associated with a microbial community harboring a relatively low abundance of *Lactobacillus*, with more threatened preterm labor episodes, and with poorer clinical outcomes.

KEYWORDS
16S rRNA gene, cerclage, cervical length, community state type, microbial community, microbial diversity, microbial richness, pessary, vaginal bacterial load

1 | INTRODUCTION

Spontaneous preterm birth (sPTB) remains the leading cause of neonatal morbidity and mortality worldwide.[1] Although improvements in neonatal care have led to higher survival rates,[2–6] perinatal outcomes can only be improved with a more accurate identification of pregnant women at risk of sPTB and appropriate interventions for prevention.[7]

Pregnant women with a short cervix (≤25 mm) before 24 weeks of gestation (WG) are at a high risk of sPTB.[8] Early identification of these pregnancies allows timely and targeted intervention with progesterone therapy, cervical cerclage or cervical pessary.[9] Recent studies have highlighted the influence of the vaginal microbiome composition on cervical length during pregnancy[10–12] and different susceptibilities to adverse outcomes when *Lactobacillus crispatus* is absent or present only at a low level in the vaginal microbiome and displaced by *Lactobacillus iners*, *Gardnerella vaginalis* or other bacterial species.[10,11,13–16] Similarly, recent data have shown the importance of vaginal bacterial load on determining preterm birth risk, which should be considered when interpreting study findings.[17]

The primary aim of our study was to assess the vaginal microbiome of pregnant women with a short cervix and compare it with that of pregnant women who had a normal cervix. A secondary aim was to compare the vaginal microbiome of women with a short cervix before and after placement of a cervical pessary or a cervical cerclage.

Key message

Our study suggests that a short cervix and cerclage intervention may be associated with preterm birth risk due to changes in the microbiome.

2 | MATERIAL AND METHODS

Between March 2016 and April 2018, an observational, longitudinal study was conducted in five hospitals across Spain (Hospital Universitari Vall d'Hebron, Hospital de Torrejón de Ardoz, Hospital Sant Joan de Reus, Hospital Materno-Infantil de Canarias and Hospital de Sant Pau de Barcelona) to examine the vaginal microbiome of pregnant women at risk of sPTB, having either cervical pessary or cervical cerclage intervention, vs low-risk patients. Cervical length (CL) was measured according to the Fetal Medicine Foundation guidelines at the routine second-trimester ultrasonography.

The study participants were assigned to one of three groups: control, pessary and cerclage. Women with a CL >25 mm were assigned to the control group, and women with a CL ≤25 mm were assigned either to the pessary or the cerclage group based on their obstetric history according to the hospital's protocol. Exclusion criteria were major fetal abnormalities, painful regular uterine contractions, active vaginal bleeding, ruptured membranes, placenta previa,

history of cone biopsy, previous or current progesterone treatment and any disorder or any medication, such as antibiotics, that might be associated with alterations in the vaginal microbiome.

Cervicovaginal swabs were collected from all participants at inclusion (between 18 and 22 WG) before the intervention (baseline, second trimester swab) and at around 30 WG (third-trimester swab).

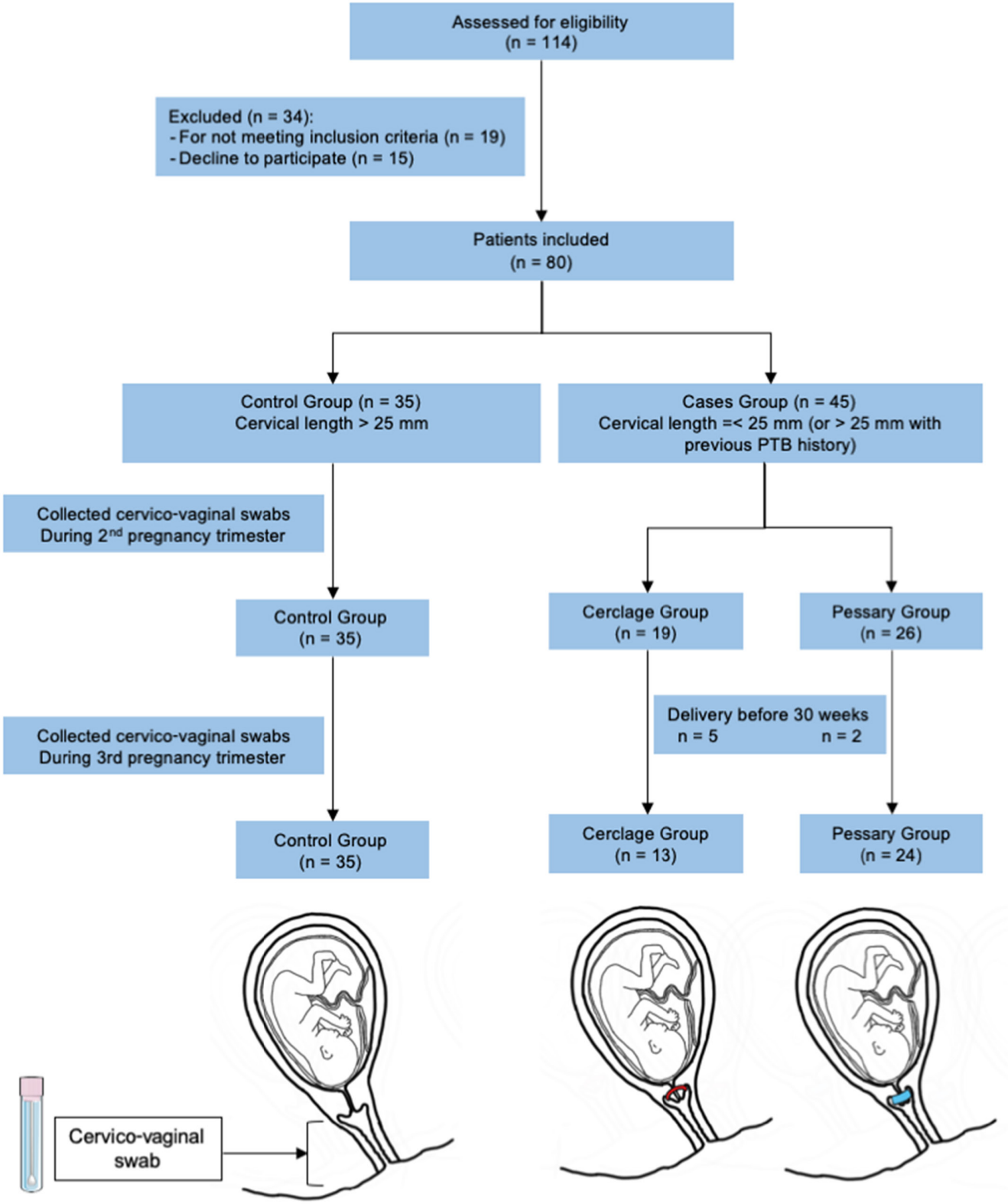


FIGURE 1 Flow chart of the study design.

One-size cervical pessaries (65×25×32 mm) for preventing sPTB were purchased from Dr Arabin GmbH & Co. A pessary of a different size was used only in patients who had problems with the normal size. Cervical cerclage was performed with a Mersilene band by the modified Shirodkar technique.[18]

Patients assigned to the control group were encouraged to follow a low-risk approach during pregnancy. Patients assigned to the pessary or cerclage groups were given detailed information on potential side effects, such as vaginal discharge and other symptoms. Placement of pessary and cerclage was guided by transvaginal ultrasound.[19,20] Pessaries and cerclages were removed at 37 WG. In this study, no patient received progesterone treatment for preterm birth prevention.

Indications for pessary/cerclage removal before term were: active vaginal bleeding, severe discomfort or persistent uterine contractions despite tocolytic treatment, suspected chorioamnionitis, and onset of labor.

The primary outcome was the comparison of the vaginal microbiome in pregnant women with a short cervix (cerclage or pessary groups) vs pregnant women with a normal cervix (control group). Secondary outcomes are shown in Table S1.

Cervicovaginal swabs were collected (Deltalab Amies; Copan Italia, LQ Amines) during a speculum examination. Swabs were stored at −80°C until processed. For profiling microbiome composition, genomic DNA was extracted from the swabs and the V4 region of the 16S rRNA gene was amplified and sequenced by polymerase chain reaction (PCR) following a previously described method.[21] Sequencing details are shown in Table S2. For microbial load determination, the V4 region of the 16S rRNA gene was amplified by real-time quantitative PCR (qPCR) following a previously described method.[22]

Differences in clinical variables were assessed using the SPSS 19 software. The chi-square test was used for categorical variables and the non-parametric Mann–Whitney *U*-test was used for continuous variables, with statistical significance set at $p < 0.05$. Microbial sequence data was analyzed using the QIIME 2 software. The alpha group significance plugin to test for differences in alpha-diversity was implemented in QIIME 2. Alteration of the microbiome community could also be appreciated at the alpha-diversity level, as calculated by the Chao1[21] and Shannon[22] indices. The Chao1 richness estimator gives weight to the low-abundant species, as it only

TABLE 1 Demographic characteristics of all three study groups[a]

	Control ($n = 35$)	Pessary ($n = 26$)	Cerclage ($n = 19$)	*p* value
Mean maternal age (years)	30.6 (5.8)	31.0 (6.2)	33.8 (5.1)	0.125
Pre-conception BMI (kg/m^2)	24.4 (4.4)	24.7 (4.9)	26.0 (5.3)	0.460
Ethnicity[b]				0.250
African American	3 (8.6)	1 (3.8)	2 (10.5)	
Asian	1 (2.8)	0	0	
Arab	0	3 (11.5)	0	
Caucasian	24 (68.6)	20 (76.9)	14 (73.7)	
Hindu	0	0	1 (5.3)	
Maghrebi	1 (2.8)	0	1 (5.3)	
South American	6 (17.1)	2 (7.7)	1 (5.3)	
Smoking status during pregnancy				0.689
No	16 (45.7)	15 (57.7)	10 (52.6)	
<5 cigarettes per day	16 (45.7)	6 (23.1)	6 (31.6)	
6–10 cigarettes per day	1 (2.8)	1 (3.8)	1 (5.3)	
11–20 cigarettes per day	2 (5.7)	3 (11.5)	2 (10.5)	
>20 cigarettes per day	0	1 (3.8)	0	
Assisted reproduction techniques	0	0	3 (15.8)	0.124
Obstetric history				
Parous with no previous preterm birth	15 (42.8%)	15 (57.6)	13 (68.4%)	0.176
Parous with a least one previous preterm birth	1 (2.8)	6 (23.1)	8 (42.1)	0.002[c]
Gestational age at inclusion (weeks)	20.4 (0.9)	20.8 (1.3)	20.6 (1.5)	0.100
Cervical length at inclusion (mm)	39.3 (6.9)	19.1 (4.0)	20.9 (3.9)	<0.0001[c]

[a]Continuous variables are shown as the median (standard deviation); categorical variables are shown as the number of cases (%).

[b]Race was self-reported.

[c]Statistical significance was set at $p < 0.05$.

accounts for singletons and doubletons, whereas the Shannon index accounts for both the abundance and evenness of the species present, and therefore gives weight to high-abundant species. The ANCOM plugin, embedded in QIIME 2, was used to test for differences in microbiota composition among groups according to treatment and/or time point. Community state types (CSTs) were assigned to each sample using hierarchical clustering with Euclidean distance metrics and complete linkage methods as described in the literature.[23]

to investigate the association between microbiome data and clinical variables, we used linear mixed models as implemented in the Multivariable Association with Linear Models (MaAsLin2) package (https://doi.org/10.1101/2021.01.20.427420). MaAsLin2 was set up with the following parameters: normalization = "TMM", transform = "LOG", correction = "BH", analysis_method = "LM", max_significance = 0.25 (default significance threshold), min_abundance = 0.0001, min_prevalence = 0.1. Age was added as a fixed effect. The participant identification number was added as a random effect. Results with a false discovery rate <0.05 were considered significant. A logistic regression model (statistics package, glm function) was used to predict the effect of *Lactobacillus* abundance and treatment type (pessary or cerclage) on timing of birth (term or preterm).

Differences in microbial load among groups were assessed using the non-parametric Wilcoxon signed-rank test (paired pairwise comparisons) and the Mann–Whitney *U*-test (unpaired pairwise comparisons). The Kruskal–Wallis test was used for continuous variables and the chi-square test for categorical variables.

TABLE 2 Obstetrical outcomes in all three study groups[a]

	Control ($n = 35$)	Pessary ($n = 26$)	Cerclage ($n = 19$)	*p* value
Cervical length after treatment (mm)	-	27.6 (6.2)	23.4 (4.6)	0.067
Cervical length at 30 weeks (mm)	38.9 (8.6)	26.8 (4.2)	16.3 (7.1)	0.001[b]
Symptoms at 30 weeks				0.01[b]
No	34 (97.1)	20 (76.9)	18 (94.7)	
Yes	1 (2.9)	6 (23.1)	1 (5.3)	
Cervical length at 37 weeks (mm)	-	16.7 (12.4)	14.0 (19.8)	0.347
Symptoms at 37 weeks				0.222
No	34 (97.1)	21 (76.9)	18 (94.7)	
Yes	1 (2.9)	5 (19.2)	1 (5.3)	
Threatened preterm labor episode	1 (2.9)	5 (19.2)	7 (36.8)	0.005[b]
Tocolytic treatment	1 (2.9)	5 (19.2)	7 (36.8)	0.005[b]
Corticosteroid therapy	2 (5.7)	7 (26.9)	10 (52.6)	0.001[b]
Duration of maternal hospital stay due to threatened preterm labor	1.0 (1.2)	1.7 (2.5)	4.8 (6.7)	0.045[b]
Chorioamnionitis	1 (2.9)	1 (3.8)	2 (10.5)	0.387
Bleeding during pregnancy	3 (8.6)	0	1 (5.3)	0.315
PPROM	1 (2.9)	3 (11.5)	2 (10.5)	0.377
Antepartum death	0	0	1 (5.3)	0.197
Spontaneous delivery <24 weeks	0	0	1 (5.3)	0.197
Spontaneous preterm birth <28 weeks	0	2 (7.7)	3 (15.8)	0.068
Spontaneous preterm birth <32 weeks	0	2 (7.7)	5 (26.3)	0.005[b]
Spontaneous preterm birth <34 weeks	0	3 (11.5)	6 (31.6)	0.002[b]
Spontaneous preterm birth <37 weeks	4 (11.4)	7 (26.9)	7 (36.8)	0.082
Any type of delivery <34 weeks	0	3 (11.5)	6 (31.6)	0.002[b]
Gestational age at delivery (weeks)	38.7 (1.8)	36.8 (3.8)	34.5 (5.9)	0.003[b]
Type of delivery				0.704
Vaginal	22 (62.9)	19 (73.1)	14 (73.7)	
Instrumental	5 (14.3)	2 (7.7)	1 (5.3)	
Elective Cesarean	3 (8.6)	1 (3.8)	0	
Emergency Cesarean	5 (14.3)	4 (15.4)	4 (21.0)	

[a]Continuous variables are shown as the median (standard deviation); categorical variables are shown as the number of cases (%).

[b]Statistical significance was set at $p < 0.05$.

TABLE 3 Neonatal outcomes among the three study groups[a]

	Control ($n = 35$)	Pessary ($n = 26$)	Cerclage ($n = 19$)	p value
Birthweight (grams)	3137.0 (383.1)	2800.7 (814.5)	2532.6 (1084.4)	0.158
Number of days admitted to NICU	0.5 (2.3)	6.1 (20.1)	11.9 (23.3)	0.056
Total days of neonatal admission	1.4 (2.5)	9.5 (24.0)	12.1 (26.9)	0.002[b]
Neonatal death	0	1 (3.8)	2 (10.5)	0.134
Birthweight <1500 g	0	1 (3.8)	6 (31.6)	0.03[b]
Birthweight <2500 g	2 (5.7)	11 (42.3)	8 (42.1)	0.001[b]
Necrotizing enterocolitis	0	0	2 (10.5)	0.031[b]
Intraventricular hemorrhage	0	1 (3.8)	2 (10.5)	0.262
Respiratory distress syndrome	0	2 (7.7)	3 (15.8)	0.058
Retinopathy	0	0	0	-
Treatment for sepsis	0	1 (3.8)	3 (15.8)	0.030[b]
Composite neonatal adverse outcomes	0	3 (11.5)	4 (21.0)	0.022[b]

[a]Continuous variables are shown as the median (standard deviation); categorical variables are shown as the number of cases (%).

[b]Statistical significance was set at $p < 0.05$.

P-values were subjected to multiple hypothesis testing correction using the Benjamini–Hochberg method with a false discovery rate threshold of 0.05. More details about the sequence analysis can be found in Table S2.

2.1 | Ethics statement

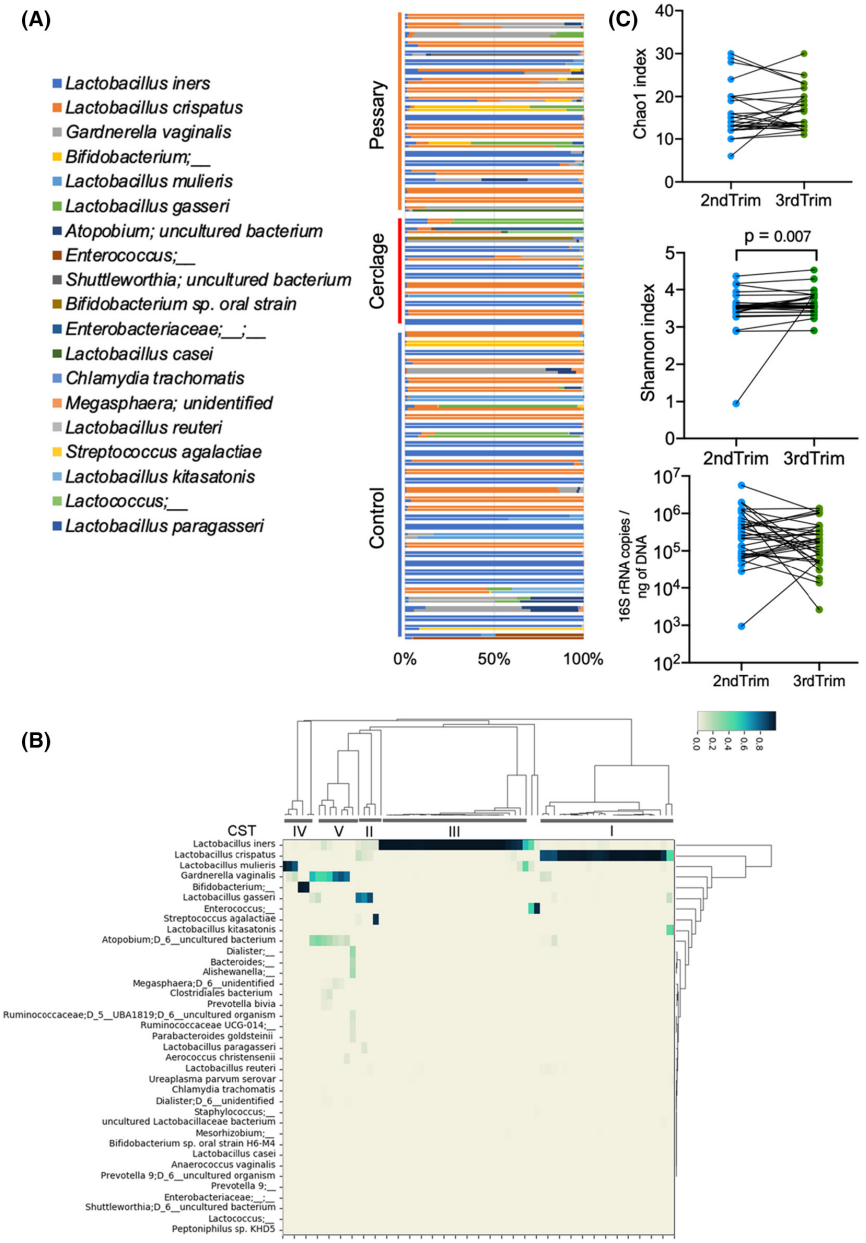
The protocol was approved by the local Ethics Committee of each participating hospital (registration number PR[AMI] 24/2016 and date of issue February 1, 2016). All participants provided their written informed consent.

3 | RESULTS

Among 114 screened pregnant women, 80 met the inclusion criteria (Figure 1): 35 women had CL >25 mm (control group) and 45 were considered to be at risk of sPTB (CL ≤25 mm or history of preterm birth) and were recommended to have either a pessary ($n = 26$) or a cerclage ($n = 19$). The 80 included pregnant women had a gestational age ranging from 18 to 22 weeks. Vaginal swab samples were collected at around 20 WG (baseline, second trimester swab) and 30 WG (third trimester swab), with a total of 152 swabs collected. One-size pessary (the recommended size) was used for all patients included. For the seven women who delivered before 30 WG, only one sample was collected (at baseline).

Demographics (Table 1) among groups were similar ($p > 0.05$). As expected, unfavorable obstetric history and short cervix (Table 1), with at least one previous sPTB, were more prevalent in the pessary and cerclage groups than in the control group ($p = 0.002$ and $p < 0.0001$, respectively). CL increased in the pessary and cerclage groups after intervention (Table 2); however, this increase was greater in the pessary group ($p = 0.001$). The cerclage group had more episodes of threatened preterm labor, greater need for treatment with tocolytics and corticosteroids, and longer maternal hospital stay due to threatened preterm labor ($p = 0.005$, $p = 0.001$ and $p = 0.045$, respectively, Table 2). The preterm birth rate before 32 weeks, 34 weeks and 37 weeks was higher in the cerclage group than in the control and pessary groups (26.3% vs. 0 vs. 7.7%, $p = 0.005$; 31.6% vs. 0 vs. 11.5%, $p = 0.002$; 36.8% vs. 11.4% vs. 26.9%, $p = 0.082$). Gestational age at delivery was also lower in the cerclage group than in the control and pessary groups (34.5 weeks vs. 36.8 weeks vs. 38.7 weeks, $p = 0.003$). Finally, the cerclage group had poorer perinatal outcomes (Table 3), such as more necrotizing enterocolitis, need for sepsis treatment and poorer composite neonatal outcomes; all differences were statistically significant ($p = 0.031$, $p = 0.03$ and $p = 0.022$, respectively).

The microbiome in pregnant women with a normal CL was represented by 10 genera that accounted for more than 99% of the total sequencing data. Among those, *Lactobacillus* (89%) was followed by *Gardnerella* (4.3%), *Bifidobacterium* (3.1%), and *Enterococcus* (0.5%). At the species level, *Lactobacillus iners* was the dominant species (46.3% on average), followed by *Lactobacillus crispatus* (33.3%), *Lactobacillus mulieris* (6.8%), *Gardnerella vaginalis* (4.8%), unknown *Bifidobacterium* (4%), unknown *Enterococcus* (1.8%) and *Lactobacillus gasseri* (1%) (Figure 2A). *L. iners* and *L. crispatus*, the two most abundant species, were exclusive of each other (rho = −0.56, $p = 6.8E\text{-}14$). Microbiome composition in the control group did not change significantly over the 10-week period at any specific taxonomic level. Alpha-diversity analysis indicated an increased diversity (according to the Shannon index of diversity) of the most abundant species. Furthermore, microbial load in the control group did not change significantly between the second and



third trimesters (Figure 2C). Over the 10-week follow-up period, only five participants had a shift in CSTs: one patient shifted from CST I to CST II, one patient from CST III to I, two patients from CST IV to III, and one patient from CST V to IV (Figure 2B).

Regarding the effect of a short cervix on the vaginal microbiome (Figure 3A), pregnant women with a CL ≤25 mm had a significantly lower number of 16S rRNA gene copies/ng ($p = 0.01$), suggesting that a longer cervix favors microbial growth. However, a short cervix was associated with a greater microbial richness ($p = 0.009$) and also with a trend towards a greater microbial evenness ($p = 0.15$) (Figure 3B). Diversity was not significantly correlated with CL, although there was a certain trend (rho = −0.2, $p = 0.075$). These differences in microbial load and alpha-diversity were not associated with differences in the microbial profile (ANCOM method), as the relative abundances of *L. iners* and *L. crispatus* were similar to those of the control group. A positive correlation between *Lactobacillus* relative abundance and CL was observed only for CL >25 mm (Spearman correlation test, rho = 0.43; $p = 0.004$); however, no such correlation was observed for CL ≤25 mm (Spearman correlation test, rho = 0.227, $p = 0.16$) (Figure 3C). *Lactobacillus* relative abundance was lower in the CL ≤25 group than in the CL >25 group (Mann–Whitney test, $p = 0.02$). Preterm birth was more prevalent in the cerclage group (logistic regression model, $p = 0.008$) but was not associated with any bacterial species, although a trend was found for *Lactobacillus mulieris* (logistic regression model, $p = 0.15$).

Regarding the effect of treatment on vaginal microbiome dynamics, microbial diversity analysis indicated an increased microbial richness ($p = 0.03$; Chao1 index, Wilcoxon test) and increased microbial load ($p = 0.018$, Wilcoxon test) at 30 WG in the cerclage group as compared with the control group (Figure 4A). These results were confirmed using multivariate association with linear models

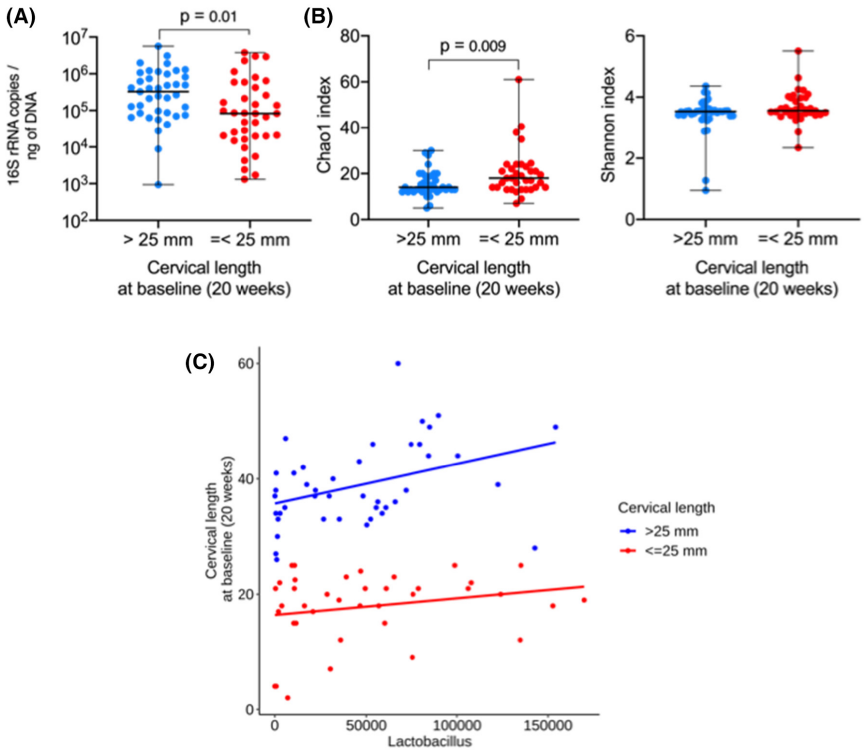


FIGURE 3 Association between cervical length and microbial load, microbial diversity and *Lactobacillus* relative abundance at 20 WG. (A) Microbial load as assessed by amplification of the 16S rRNA gene V4 region using quantitative PCR for cervical length (CL) >25 mm and CL ≤25 mm. (B) Microbial alpha-diversity as assessed by the Chao1 and Shannon indices on 16S sequence data for CL >25 mm and CL ≤25 mm (Chao1 index, $P = 0.009$; Shannon index, $P = 0.15$). (C) Correlation between CL and *Lactobacillus* relative abundance based on 16S rRNA sequence data (rho = 0.43, $P = 0.04$ for CL >25 mm).

(false discovery rate = 0.001 for bacterial load and false discovery rate = 0.02 for microbial richness). Microbial richness and microbial load did not differ significantly between the pessary and control groups. Interestingly, a negative correlation was observed between microbial richness and gestational age at delivery (rho = −0.24, $p = 0.03$; Figure 4B,C). Regarding CST analysis, the odds of shifting from CSTs I, II and V to CST IV were 15.1 times higher in the cerclage group than in the control group ($p = 0.005$) and 10.2 times higher in the cerclage group than in the pessary group ($p = 0.02$) (Figure 4D,E).

4 | DISCUSSION

Our study agrees with previous reports on microbiome composition in healthy pregnant women as being dominated by CST I (*L. crispatus*) and CST III (*L. iners*), with variations in CST IV (others) and CST V (*G. vaginalis*). These CSTs were relatively stable over time with few shifting from CST to another, but diversity increased slightly with gestational age. A short cervix was associated with a lower microbial load and a higher microbial richness, the latter being negatively associated with gestational age at delivery. Prevention of sPTB by cervical cerclage was associated with a microbial community harboring a relatively low abundance of *Lactobacillus*. Furthermore, the cerclage group had more threatened preterm labor episodes and poorer clinical outcomes than the other groups.

The vaginal microbiome of our control cohort was dominated by *Lactobacillus*, as reported by previous studies.[24,25] Indeed, lactobacilli are key players in the vaginal microbiome, probably through the production of lactic acid and the resulting decrease in pH.[12,26] Ramussen[26] reported a gradual decrease in *Lactobacillus* relative abundance from 24 WG until birth. Our data did not support this finding between 20 and 30 WG, but did agree with other studies on the stability of *Lactobacillus* relative abundance over all three trimesters.[27] However, this stability may be related to ethnicity, as suggested by other studies.[28] Our analysis identified five CSTs. CST I, II, III and V were dominated by *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus iners*, and *G. vaginalis*, respectively. CST IV was dominated by different microbial groups, such as *Lactobacillus mulieris*, unidentified *Bifidobacterium* or *Enterococcus*.[19] These differences may also be related to ethnicity.

Analysis of the groups at risk of sPTB due to a short CL showed that several variables, such as microbial load, *Lactobacillus* relative abundance and alpha-diversity indices, differed significantly from those of the group with normal CL. The fact that a short cervix is associated with low microbial load and high microbial richness suggests that a diverse vaginal microbiome may not favor the growth of usually dominant commensals, such as *Lactobacillus*. Regarding diversity, our results agree with a previous study reporting a higher microbial richness in women at risk of sPTB.[15] However, our results for microbial load seem to contradict those of Freitas et al.,[15] reported a higher microbial load in the group at risk of sPTB.

The cerclage group had a lower increase in CL and more threatened preterm labor episodes than the pessary group. In addition, at the vaginal microbiome level, the risk of shifting to a community with a lower relative abundance of *Lactobacillus* was higher in the cerclage group. These findings agree with a previous study comparing braided suture, such as Mersilene, which was used in our study, with monofilament; these authors observed that braided suture induced a persistent shift towards vaginal microbiome dysbiosis characterized by a reduced *Lactobacillus* spp. abundance and pathobiont

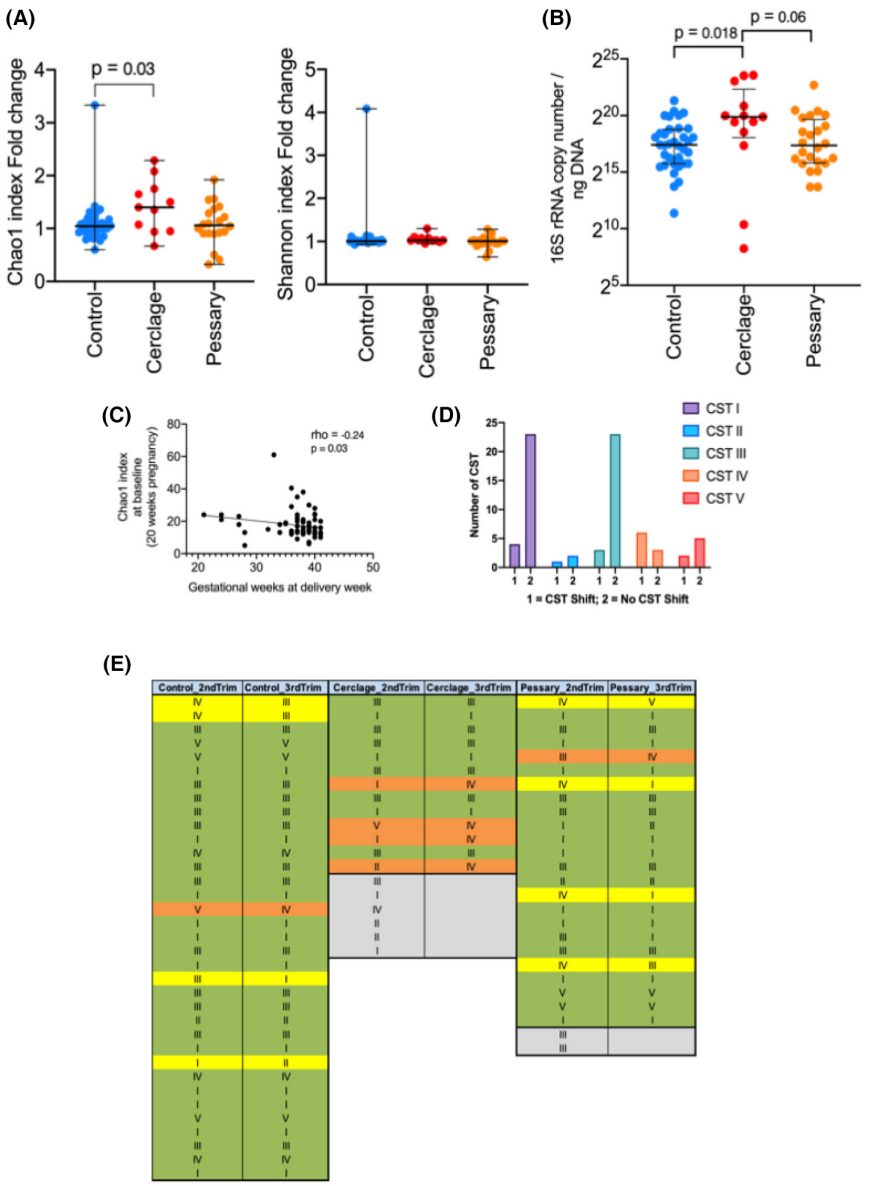


FIGURE 4 Microbial diversity, load and CST over time. (A) Evolution of microbial alpha-diversity according to the Chao1 and Shannon indices as fold-changes between the second and third trimesters. Only the cerclage group showed a significant increase in microbial richness. (B) Increase in microbial load at around 30 WG in the cerclage group vs the pessary group. (C) Chao1 index at baseline vs gestational age at delivery plot, showing a negative correlation (Spearman test, rho = −0.24, $P = 0.03$). (D) CST shift over time. The shift to CST IV contributed to the significance ($P = 0.01$). (E) CST (I, II, III, V) shift to CST IV was higher in the cerclage group than in the control or pessary groups (odds ratio 15, Wald test, $P = 0.005$) and pessary (odds ratio 10.2, Wald test, $P = 0.02$) groups. Gray cells highlight the missing samples owing to PTB before week 30; green cells indicate no shift in CST; yellow cells indicate shift to any CST; orange cells indicate shift from any CST to CST IV.

enrichment. Vaginal dysbiosis was associated with excretion of inflammatory cytokines and interstitial collagenase into cervicovaginal fluid and premature cervical remodeling. In comparison, monofilament suture had minimal impact on the vaginal microbiome and its interactions with the host.[10]

This study is the first study assessing the vaginal microbiome in pregnant women undergoing pessary or cerclage intervention. One of the main limitations of our study was the absence of randomization, which could lead to a bias such as selection bias, and could prevent the use of probability theory. The small size of each cohort, which may lead to type II errors, was another limitation of our study. Indeed, although the vaginal microbiome is less diverse than the microbiome of other body sites, its complexity is enhanced by the presence of CSTs. This hinders the identification of a microbiome profile associated with a specific intervention for preventing sPTB. Another limitation of our study was the use of a single variable region of the 16S rRNA gene to study microbiome composition. However, to the best of our knowledge, no other studies have investigated the vaginal microbiome in pessary carriers using multiple regions of the 16S rRNA gene.

The vaginal microbiome, which differs by ethnicity and vaginal microbial load in pregnancy, has not yet been stratified by ethnicity.[29] In our study, cerclage with Mersilene was associated with a microbial community harboring a relatively low abundance of *Lactobacillus*, more threatened preterm labor episodes and poorer clinical outcomes, as also found by some authors. Due to the limited cohort of our study, before extrapolating our results to other populations, further assessment is required using additional robust prospective randomized studies.

5 | CONCLUSION

Our findings indicate that a short cervix is associated with a lower microbial load and higher microbial richness as compared with a

normal cervix. In addition, microbial richness is negatively associated with gestational age at delivery. The use of a pessary for preterm birth prevention does not appear to have an adverse effect on the *Lactobacillus* relative abundance or microbial diversity. By contrast, cerclage with Mersilene was associated with a microbial community harboring a relatively low abundance of *Lactobacillus*, more threatened preterm labor episodes and poorer clinical outcomes.

AUTHOR CONTRIBUTIONS

MV was the principal clinical investigator. MV contributed to the study design, follow-up of patients and data collection, and prepared the first draft of both the protocol and article. MV is the guarantor for the paper. MG was the study coordinator, and contributed to the study design, patient follow-up and data collection, and prepared the protocol and article along with MV. AB and AF contributed to the study design and data collection. FY was the principal laboratory investigator. CM was the microbiome analysis coordinator and prepared the article along with MV and MG. FY, AE, ZX, OS, ZS and CB were part of the microbiome analysis team led by CM. The principal clinical investigators in the other hospitals were LV (Hospital Materno-Infantil de Canarias), AO (Hospital de Sant Pau), BM (Hospital de Reus) and MB (Hospital de Torrejón de Ardoz). EC reviewed both the protocol and the article. All authors had complete access to the data at the end of the trial and the decision to submit the article for publication was taken at a joint meeting, where the whole team reviewed and approved the final version. This final version was then submitted by the corresponding author.

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CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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