

Supplementary Materials

Large-scale characterisation of the pregnancy vaginal microbiome and sialidase activity in a low-risk Chinese population

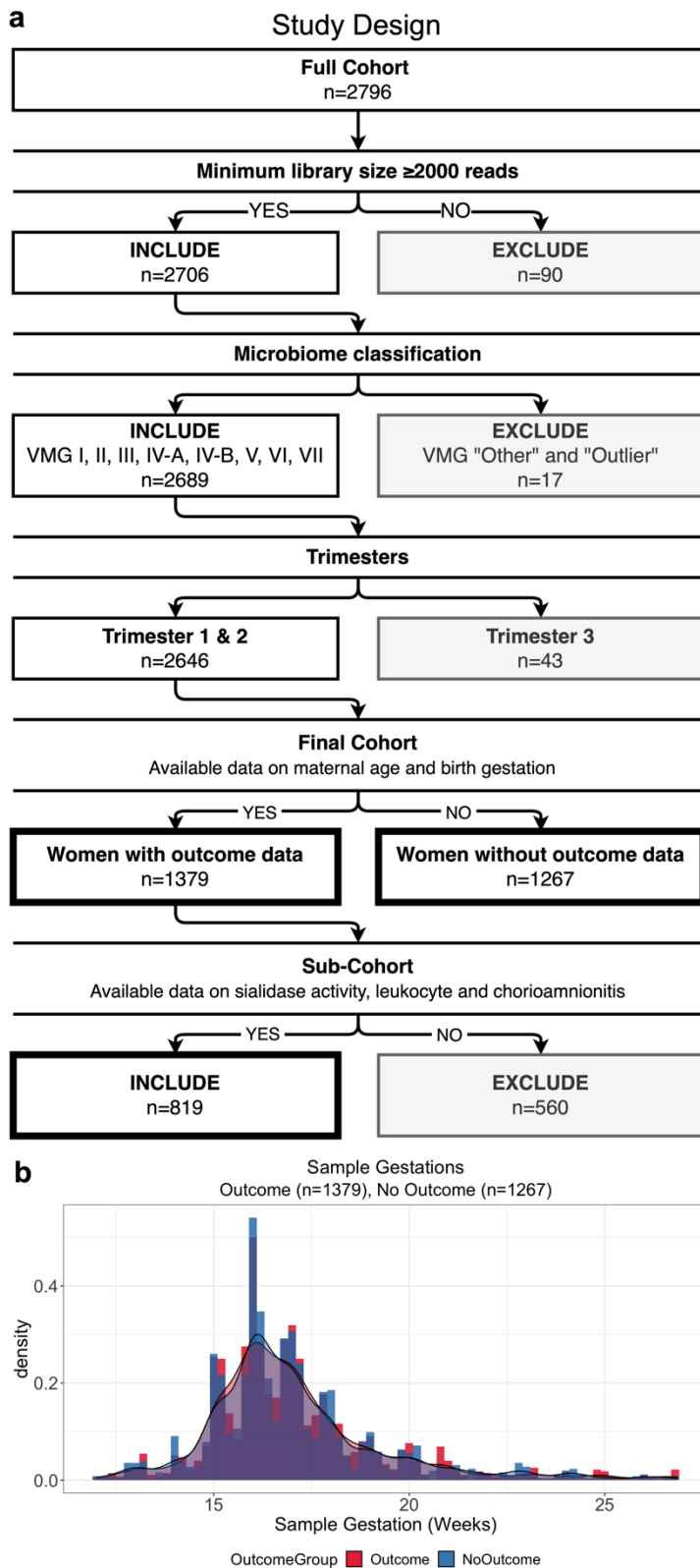
Sherrienne Ng^{1,2+}, Muxuan Chen^{3,4+}, Samit Kundu^{1,2}, Xuefei Wang⁵, Zuyi Zhou^{3,4}, Zhongdaixi Zheng⁶, Wei Qing^{3,4}, Huafang Sheng^{3,4}, Yan Wang⁵, Yan He^{3,4}, Phillip R. Bennett^{1,2}, David A. MacIntyre^{1,2*} and Hongwei Zhou^{3,4,7*}.

Contains:

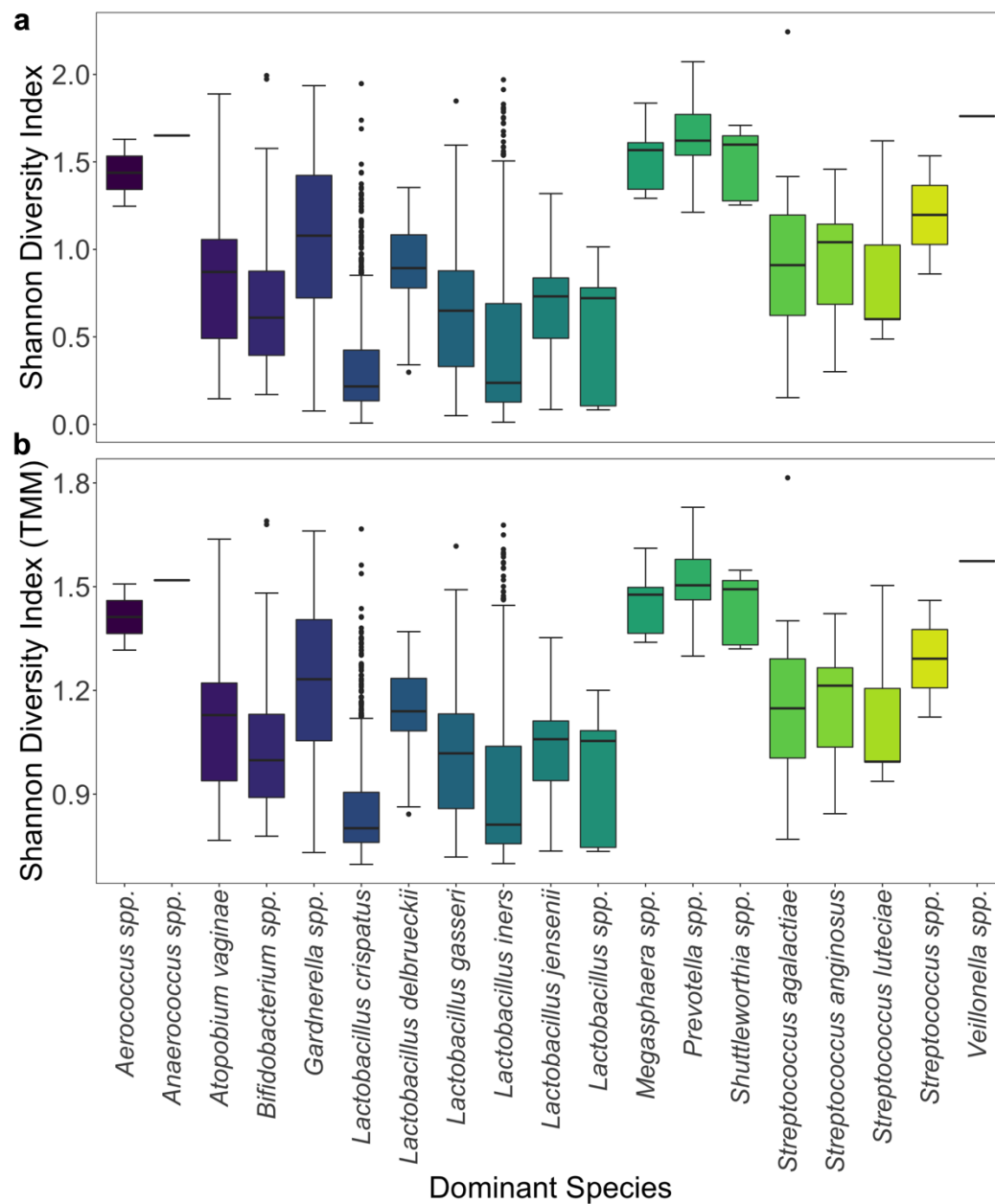
Supplementary Figures 1-7

Supplementary Tables 1-5

Supplementary Figures

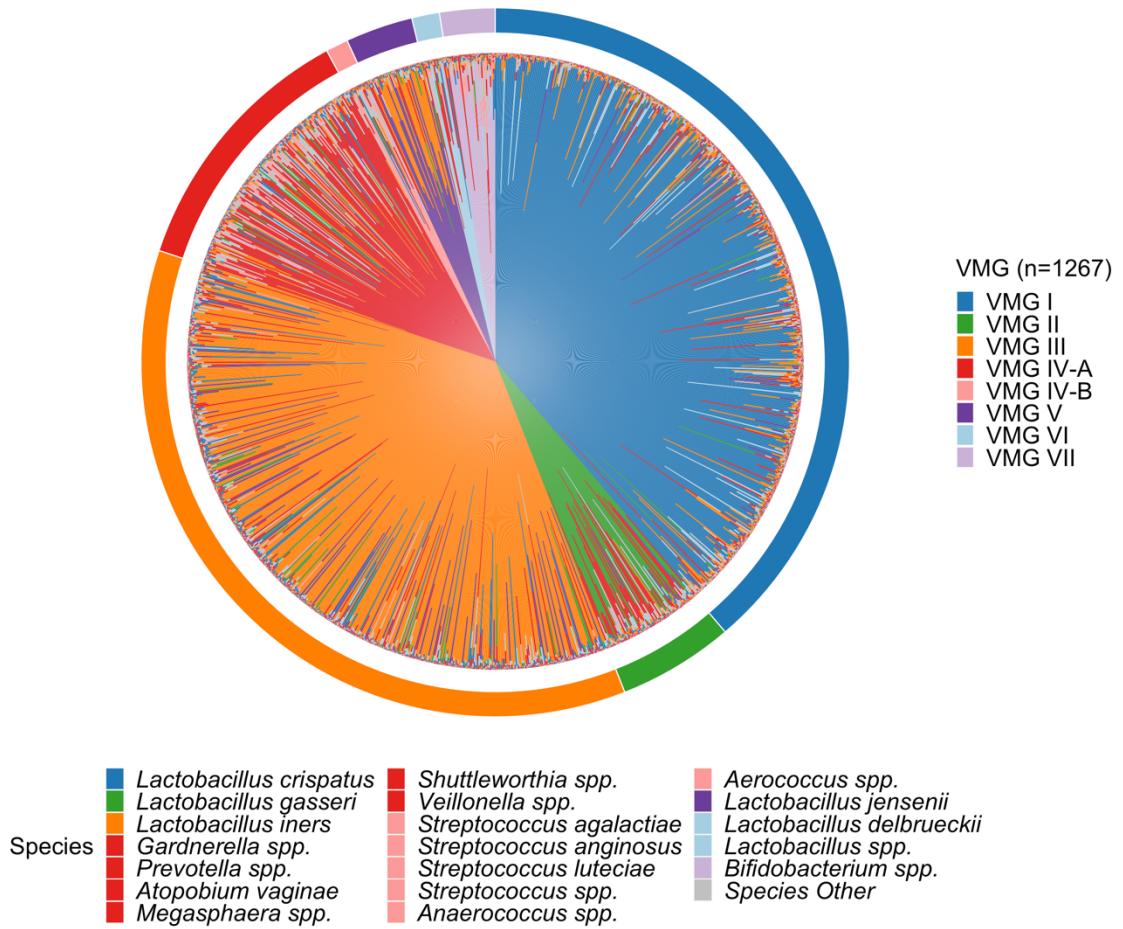


Supplementary Figure 1. (a) Study design of women included in final cohort analysis comparing women with and without outcome data and sub-cohort analysis of women with available maternal and neonatal data. (b) Density plot showing sample gestation of women with (red) and without (blue) outcome included in final cohort analysis.

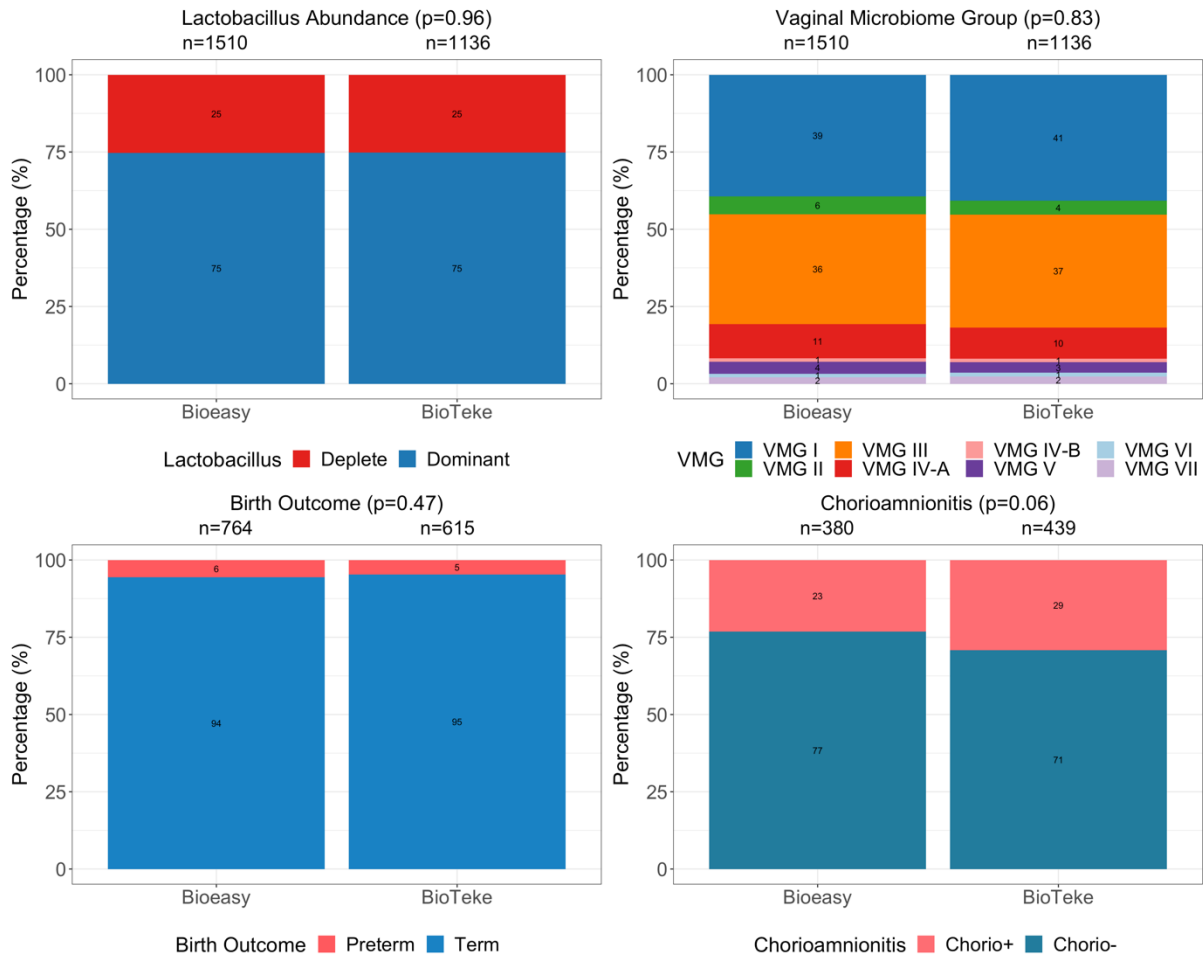


Supplementary Figure 2. Comparison of Shannon diversity index (a) before and (b) after trimmed mean of M-values (TMM) normalisation. The bounds of the box represent the first and third quartiles, centre line represents the median, and whiskers show min-to-max values.

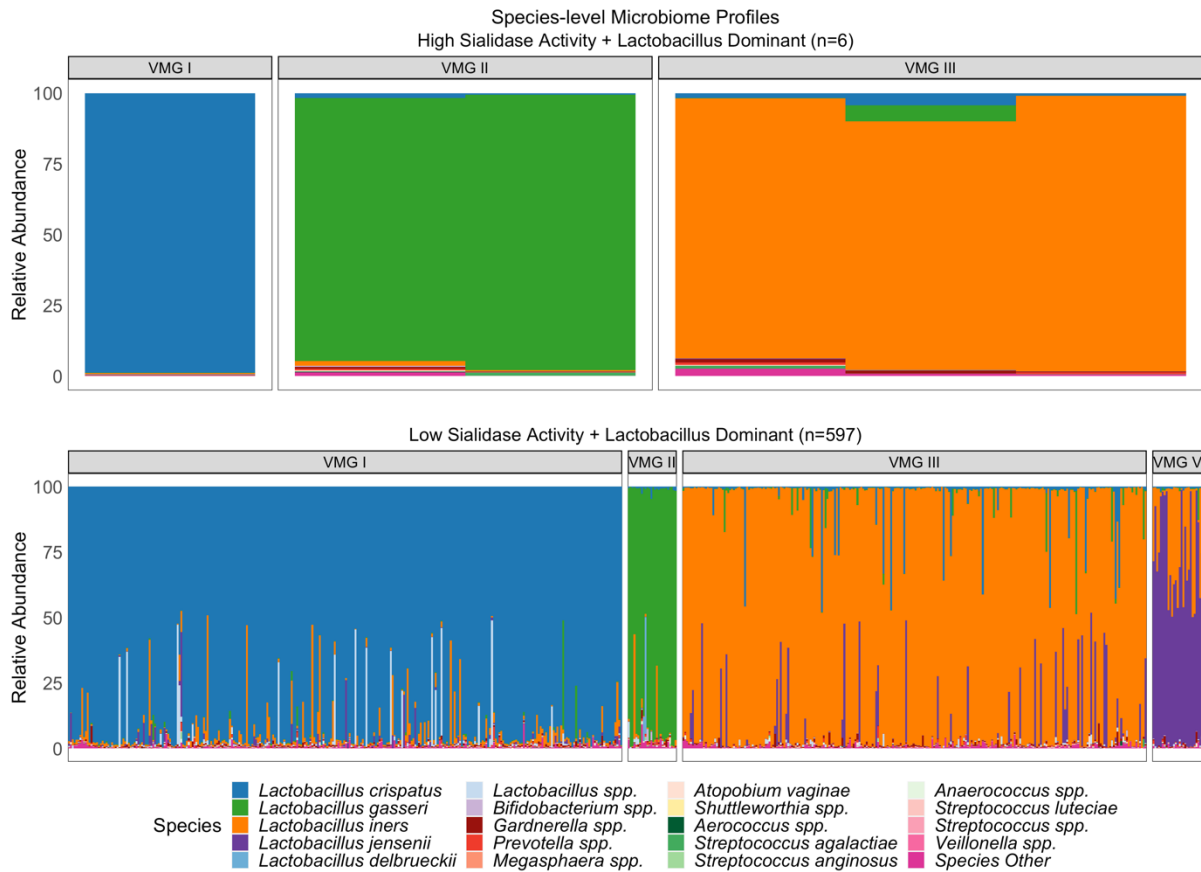
Vaginal Microbiome Groups



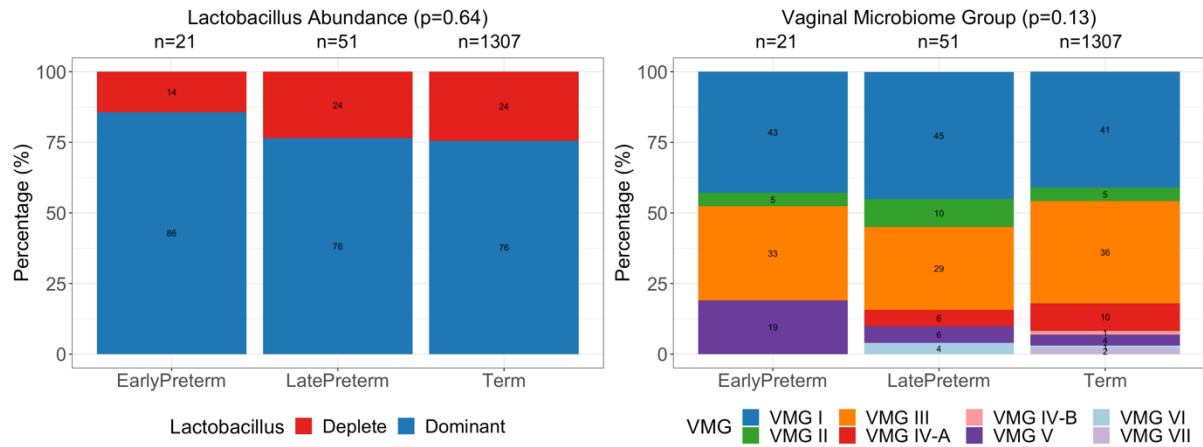
Supplementary Figure 3. Vaginal microbiome groups of women without outcome data. Radial plot showing vaginal microbiome groups (VMG) of women without outcome data. Species classified into VMG I (*L. crispatus* dominated, blue); II (*L. gasseri* dominated, green); III (*L. iners* dominated, orange); IV-A (BV-associated, red); IV-B (pathobionts, pink); V (*L. jensenii* dominated, purple); VI (Other *Lactobacillus*, light blue) and VII (*Bifidobacterium* dominated, light purple). Outcome data was defined as availability of maternal age and birth gestation.



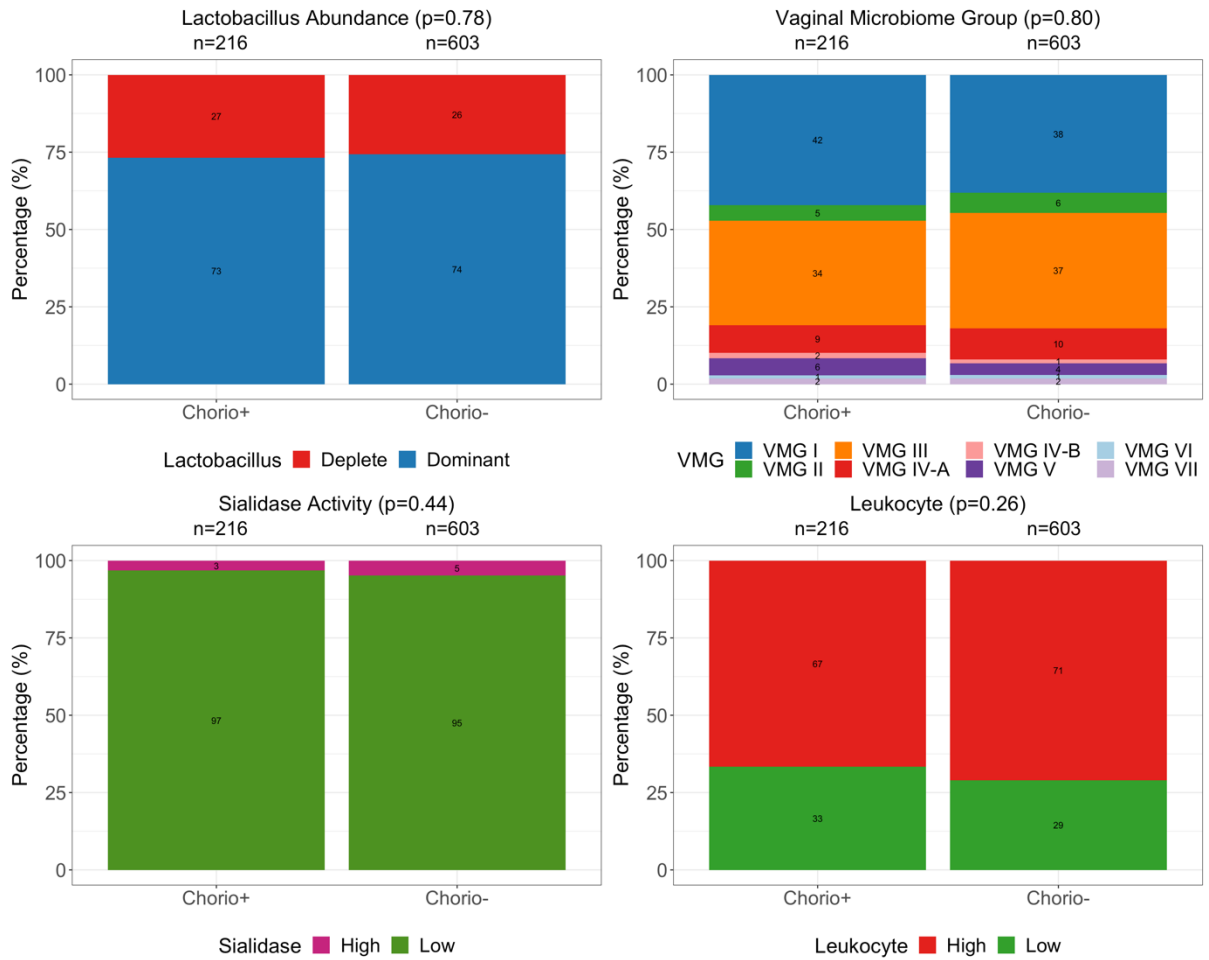
Supplementary Figure 4. Comparison of *Lactobacillus* abundance, VMG, birth outcome and chorioamnionitis proportions between samples extracted using manual (BioTeke) and automated (Bioeasy) DNA extraction kits. Statistical significance based on Fisher’s Exact Test.



Supplementary Figure 5. *Lactobacillus* dominant microbiome profiles of women with high and low sialidase activity. The microbiome profiles of women with *Lactobacillus* dominance and high or low sialidase activity dominated by Vaginal Microbiome Group (VMG) I (*L. crispatus*, blue), II (*L. gasseri*, green), III (*L. iners*, orange) and/or V (*L. jensenii*, purple).



Supplementary Figure 6. Microbiome composition across early preterm, late preterm and term groups based on *Lactobacillus* abundance and vaginal microbiome groups (VMG). Statistical significance based on Fisher's Exact Test.



Supplementary Figure 7. Proportions of *Lactobacillus* abundance, VMG, sialidase activity and leukocyte wet mounts based on chorioamnionitis presence (Chorio+) and absence (Chorio-). Statistical significance based on Fisher's Exact Test.

Supplementary Tables

Supplementary Table 1. Relative abundance and Shannon diversity index of dominant species groups.

Dominant Species Group	Relative Abundance*	Shannon Diversity*
<i>Aerococcus_spp.</i>	46.12 (45.17 - 47.07)	1.44 (1.34 - 1.53)
<i>Anaerococcus_spp.</i>	40.33 (40.33 - 40.33)	1.65 (1.65 - 1.65)
<i>Atopobium_vaginae</i>	64.46 (50.76 - 89.92)	0.87 (0.49 - 1.06)
<i>Bifidobacterium_spp.</i>	86.9 (73.01 - 92.94)	0.61 (0.39 - 0.88)
<i>Gardnerella_spp.</i>	58.25 (45.40 - 79.30)	1.08 (0.72 - 1.42)
<i>Lactobacillus_crispatus</i>	96.37 (90.35 - 97.97)	0.22 (0.13 - 0.42)
<i>Lactobacillus_delbrueckii</i>	71.08 (56.64 - 75.03)	0.89 (0.78 - 1.08)
<i>Lactobacillus_gasseri</i>	83.73 (63.89 - 93.95)	0.65 (0.33 - 0.88)
<i>Lactobacillus_iners</i>	96.19 (76.41 - 98.14)	0.24 (0.13 - 0.69)
<i>Lactobacillus_jensenii</i>	66.31 (56.38 - 89.41)	0.73 (0.49 - 0.84)
<i>Lactobacillus_spp.</i>	75.58 (54.30 - 98.53)	0.72 (0.11 - 0.78)
<i>Megasphaera_spp.</i>	35.92 (32.73 - 41.48)	1.57 (1.34 - 1.61)
<i>Prevotella_spp.</i>	39.48 (35.63 - 46.25)	1.62 (1.54 - 1.77)
<i>Shuttleworthia_spp.</i>	39.60 (33.78 - 50.54)	1.60 (1.28 - 1.65)
<i>Streptococcus_agalactiae</i>	75.90 (60.45 - 85.59)	0.91 (0.62 - 1.20)
<i>Streptococcus_anginosus</i>	69.50 (51.10 - 86.81)	1.04 (0.69 - 1.14)
<i>Streptococcus_luteciae</i>	87.11 (61.32 - 88.65)	0.60 (0.60 - 1.03)
<i>Streptococcus_spp.</i>	45.29 (42.93 - 47.65)	1.20 (1.03 - 1.37)
<i>Veillonella_spp.</i>	35.87 (35.87 - 35.87)	1.76 (1.76 - 1.76)

*Median (IQR)

Supplementary Table 2. Vaginal microbiome groups (VMG) of women with and without outcome data.

VMG	Women with available outcome data*	Women without available outcome data*
I (<i>L. crispatus</i> dominated)	567 (41.12%)	491 (38.75%)
II (<i>L. gasseri</i> dominated)	70 (5.08%)	67 (5.29%)
III (<i>L. iners</i> dominated)	495 (35.90%)	457 (36.07%)
IV-A (BV-associated)	130 (9.43%)	152 (12.00%)
IV-B (Non-BV taxa)	18 (1.31%)	13 (1.03%)
V (<i>L. jensenii</i> dominated)	57 (4.13%)	39 (3.08%)
VI (Other <i>Lactobacillus</i>)	16 (1.16%)	16 (1.26%)
VII (<i>Bifidobacterium</i> dominated)	26 (1.89%)	32 (2.53%)
Total	1379	1267

*number (%)

Supplementary Table 3. Relative abundance of *Lactobacillus*, *Bifidobacterium* and BV-type species in women with high or low sialidase activity.

Genus	High sialidase activity*	Low sialidase activity*	p-value**
Atopobium	1.14 (0.09 - 7.04)	0.09 (0.04 - 0.19)	p<0.01
Bifidobacterium	0.00 (0.00 - 0.03)	0.00 (0.00 - 0.00)	p>0.05
Clostridium	0.00 (0.00 - 0.02)	0.00 (0.00 - 0.00)	p<0.05
Gardnerella	37.99 (9.75 - 61.31)	0.34 (0.20 - 0.73)	p<0.01
Lactobacillus	29.28 (3.19 - 74.68)	98.44 (93.92 - 99.17)	p<0.01
Megasphaera	0.06 (0.00 - 11.62)	0.00 (0.00 - 0.06)	p<0.01
Prevotella	0.98 (0.06 - 10.14)	0.13 (0.05 - 0.33)	p<0.01

*Median (IQR)

**Mann-Whitney Test

Supplementary Table 4. Birth gestation of women based on *Lactobacillus* abundance, sialidase activity and leukocyte wet mount results. No difference in mean gestation at birth was detected between any of the assessed contrasts (Mann-Whitney test).

Lactobacillus Abundance		Sialidase Activity		Leukocyte Wet Mounts		Birth Gestation (Weeks)*
Deplete	Dominant	High	Low	Leuk+	Leuk-	
x		x		x		39.86 (39.29 - 40.50)
	x	x		x		39.14 (36.36 - 39.96)
x			x	x		39.43 (38.71 - 40.14)
	x		x	x		39.43 (38.71 - 40.14)
x		x			x	38.86 (38.25 - 39.32)
	x	x			x	39.64 (39.61 - 39.68)
x			x		x	39.29 (39.00 - 40.14)
	x		x		x	39.29 (38.57 - 40.14)

*Median (IQR)

Supplementary Table 5. Logistic regression analysis of women in subcohort (n=819) to determine contribution of *Lactobacillus* abundance, sialidase activity and leukocyte wet mount result to birth outcome.

Independent variable	Logistic regression coefficient	Standard error	p-value	Log-odds
Lactobacillus Abundance (Deplete = 1, Dominant = 0)	-0.09	0.37	0.81	0.92
Sialidase Activity (High = 1, Low = 0)	0.50	0.66	0.45	1.65
Leukocyte (High = 1, Low = 0)	-0.13	0.33	0.69	0.88