Supplemental data: Methods

Salivary peptide measurement. A final set of two scheduled MRM instrument methods including the isotopically labeled standards and encompassing a 10 minute window around the retention time of each peptides was performed using a 40 mm by 0.1 mm (Jupiter 5 micron, 300A) kasil fritted trap followed by a 200 mm by 0.1 mm (Jupiter 3 micron, 300A), self-packed analytical column coupled directly to an TSQ-Vantage (Thermo Fisher) via a nanoelectrospray source. Peptides were resolved using an aqueous to organic gradient flowing at 400 nl/min. Q1 peak width resolution was set to 0.7, collision gas pressure was 1 mTorr, and utilized an EZmethod cycle time of 3 seconds. For each run, saliva samples were prepared by precipitating the entire sample from each patient time point by addition of 1/3 volume of 100 percent w/v tricloroacetic acid (TCA). Pellets were washed twice ice cold acetone, dried briefly, were then resuspended with labeled peptides in 8 M urea 100 mM tris pH 8.5, reduced using TCEP [(tris(2-carboxyethyl)phosphine)], and then alkylated using iodoacetamide. They were then diluted back to 2 M urea and digested overnight with trypsin. Prior to injection, samples were subjected to C18 solid phase extraction cleanup and resuspended in a final volume of 20 µl of 0.1% formic acid. Four microliters of sample was loaded via autosampler and analyzed. The resulting RAW instrument files were imported into Skyline for peak-picking and quantitation.

16S rRNA amplicon sequencing of bacterial DNA.

The complete sequences of the primers were:

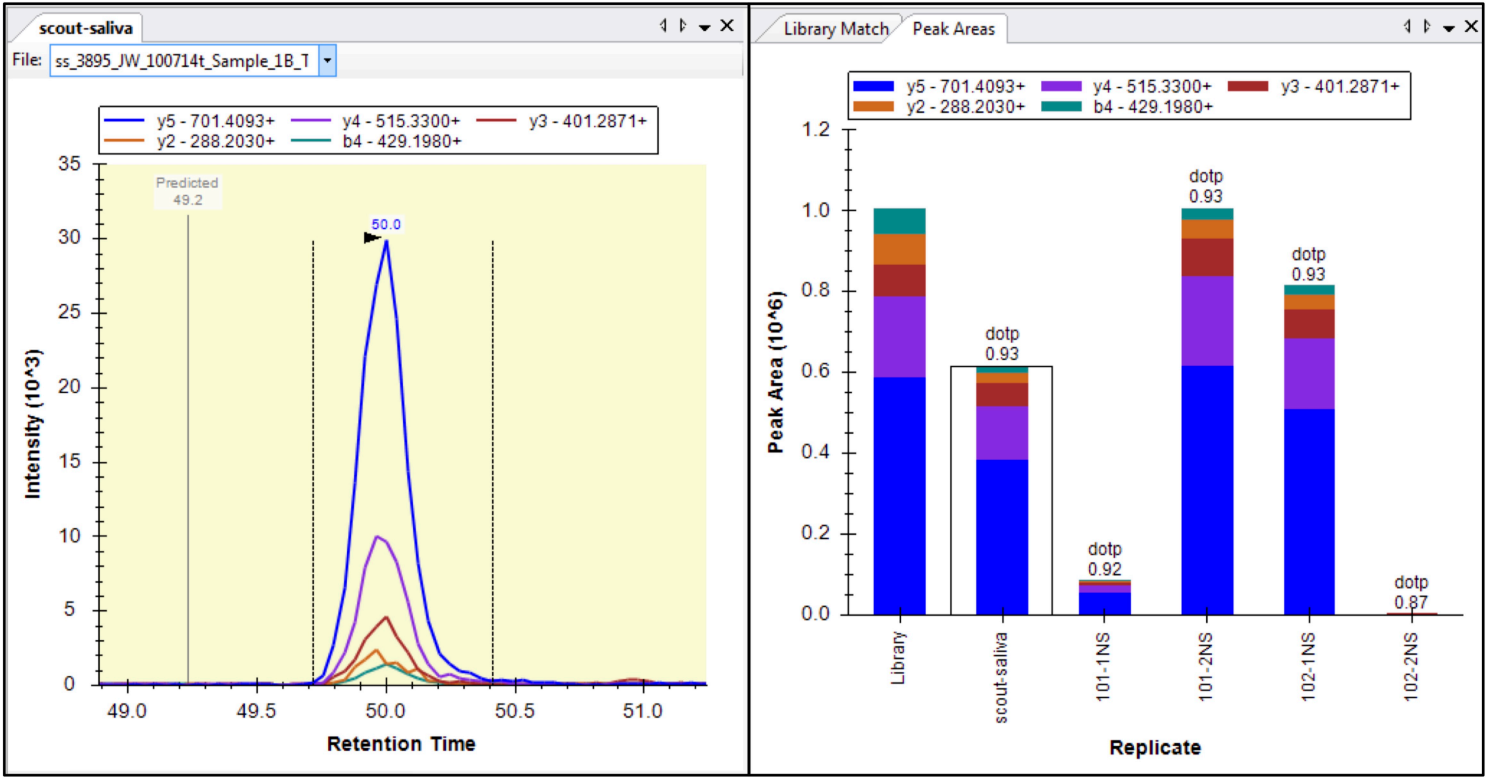
8F - 5’ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGAGTTTGATCCTGGCTCAG3’

BifidoF-5’ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGGGTTCGATTCTGGCTCAG3’

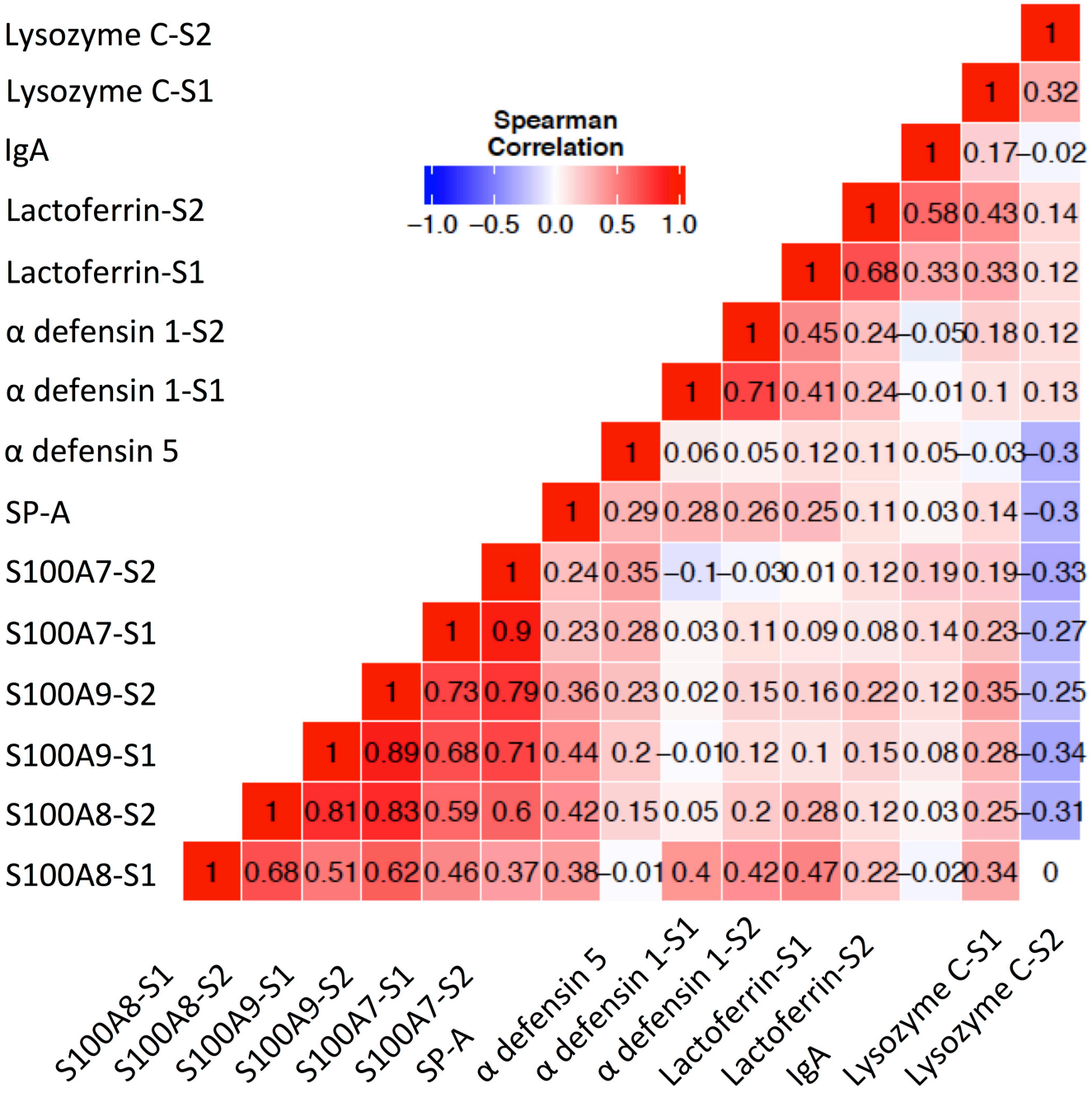
338R - 5’ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGCCTCCCGTAGGAGT3’

The thermal profile for the amplification of each sample had an initial denaturing step at 95°C for 3 minutes, followed by a cycling of denaturing of 95°C for 30 seconds, annealing at 55°C for 30 seconds and a 30 second extension at 72°C (25 cycles), a 5 minutes extension at 72°C and a final hold at 4°C. Each 16S amplicon was purified using the AMPure XP reagent (Beckman Coulter, Indianapolis, IN). In the next step, each sample was amplified using a limited cycle PCR program, adding Illumina sequencing adapters and dual-index barcodes (index 1(i7) and index 2(i5)) (Illumina, San Diego, CA) to the amplicon target. The thermal profile for the amplification of each sample had an initial denaturing step at 95°C for 3 minutes, followed by a denaturing cycle of 95°C for 30 seconds, annealing at 55°C for 30 seconds and a 30 second extension at 72°C (8 cycles), a 5 minute extension at 72°C and a final hold at 4°C. The final libraries were again purified using the AMPure XP reagent (Beckman Coulter), quantified and normalized prior to pooling. The DNA library pool was then denatured with NaOH, diluted with hybridization buffer and heat denatured before loading on the MiSeq reagent cartridge (Illumina) and on the MiSeq instrument (Illumina). Automated cluster generation and paired–end sequencing with dual reads were performed according to the manufacturer's instructions.

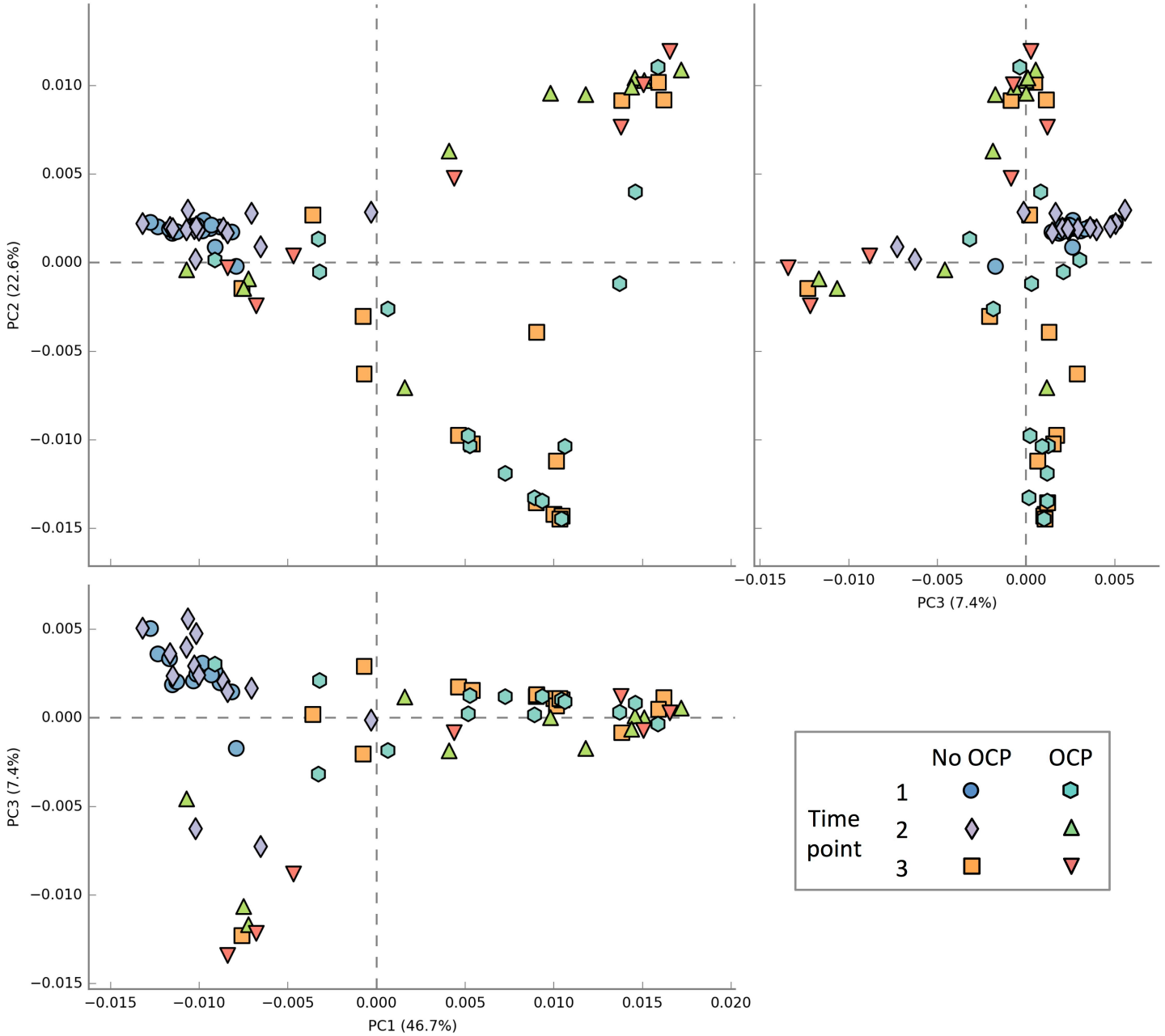
Supplemental Figure 1.



Supplemental Figure 2.



Supplemental Figure 3.



Supplemental Table 1. Maternal and neonatal characteristics with peptide data

	No OCP	OCP	P-value
Maternal Characteristics	N = 35	N = 30	
Age, median (quartiles)	25 (21, 30)	24 (21, 29)	0.79[1]
Race			
African American	26%	43%	0.4[2]
White	66%	53%	
More than one	6%	3%	
Unknown, not reported	3%	0%	
Ethnicity			0.91[2]
Hispanic or Latino	14%	13%	
Parity, median (quartiles)	2 (1, 3)	1 (1, 2)	0.2[1]
Gestation (multiple)	17%	23%	0.53[2]
Prenatal care	94%	100%	0.18[2]
Mode of delivery			0.35[2]
SVD	23%	33%	
Caesarian section	77%	67%	
Anesthesia			0.24[2]
Epidural	71%	87%	
General	14%	3%	
Hypertension	23%	37%	0.22[2]
Diabetes	3%	7%	0.47[2]
Tobacco use	14%	23%	0.35[2]
Premature rupture of membranes*	31%	20%	0.3[2]
Amniotic fluid			0.6[2]
Clear	86%	90%	
Bloody	14%	10%	
Antenatal steroids	89%	83%	0.54[2]
Neonatal characteristics			
Gestational age, median (quartiles)	29 (28, 30)	30 (28, 31)	0.23[1]
Birth weight (grams), median (quartiles)	1190 (950-1340)	1298 (1042, 1555)	0.18[1]
Male	40%	50%	0.42[2]
5 minute Apgar score, median (quartiles)	7 (7, 8)	7 (6, 8)%	0.56[1]
Surfactant	51%	67%	0.21[2]
Total days intubated	1 (0, 5)	1 (0, 3)	0.91[1]
Early onset bacteremia	3%	0%	0.35[2]
Late onset bacteremia	9%	3%	0.38[2]
Antimicrobial exposure, days, median (quartiles)	5 (3, 9)	3 (2, 8)	0.31[1]
Age at feeding initiation (days), median (quartiles)	2 (2, 2)	2 (2, 3)	0.77[1]
Initial feeding type			0.55[2]
Expressed breast milk	69%	67%	
Donor breast milk	31%	30%	
Formula	0%	3%	
Days to 100ml/kg/day enteral feeds, median (quartiles)	11 (10, 19)	11 (8, 13)	0.2[1]
Type of feeds at discharge			0.15[2]
Unfortified breast milk	6%	7%	
Fortified breast milk	37%	60%	
Formula only	57%	33%	
Necrotizing enterocolitis (≥stage 2)	3%, n = 1	7%, n = 2	0.76[2]
Days to discharge, median (quartiles)	50 (42, 68)	38 (30, 61)	**0.008**[1]

*[1]Wilcoxon rank test; [2]Pearson test, * >18 hours prior to delivery*

Supplemental Table 2: Peptide sequences

Protein target	*Peptide sequence*
Osteopontin	GDSVVYGLR
	ISHELDSASSEVN
Lysozyme	VLHDFGLDGYR
	DLTEWVDGCDF
Lysozyme C	WESGYNTR
	STDYGIFQINSR
Immunoglobulin A	SAVQGPPER
	QEPSQGTTTFAVTSILR
Lactoferrin	EDAIWNLLR
	FQLFGSPSGQK
Alpha-defensin 1	IPACIAGER
	YGTCIYQGR
Alpha-defensin 4	SSALQVSGSTR
Alpha-defensin 5	ESLSGVCEISGR
	QSGEDNQDLAISFAGNGLSALR
Alpha defensin 6	GANDQDFAVSFAEDASSSLR
	AYEADAQEQR
Beta defensin 4	SEFELDR
	ICGYGTAR
Cathelicidin (LL-37)	FALLGDFFR
	DFLR
	NLVPR
	AIDGINQR
Surfactant protein A	YDSNFQHPK
	LSAESTDER
Mannose-binding lectin	EEAFLGITDEK
	FFLTNGEIMTFEK
S100A7 (Psoriasin)	SIIGMIDMFHK
	GTNYLADVFEK
S100A8 (Calgranulin A)	ALNSIIDVYHK
	GADVWFK
S100A9 (Calgranulin B)	NIETIINTFHQYSVK
	VIEHIMEDLDTNADK
Peptidoglycan recognition protein 3	YIQPLLLK
	IGSSPSPAALSAAEGLISYAIQK
Peptidoglycan recognition protein 4	GHLSSSYVQPLLVK
	GYLTPNYLLVGHSDVAR
Polymeric immunoglobulin receptor	TDISMSDFENSR
	IIEGEPNLK

Supplemental Table 3. Salivary peptide variations by sample time (percent above detection limit).

	Time 1	**Time 2**	**P-value***
Peptide	N = 65	N = 65	
S100a7-S1	18 (8-44)	17 (8-27)	0.13
S100a7-S2	40 (5-92)	32 (5-70)	**0.049**
S100a8-S1	102 (40-315)	156 (45-395)	0.53
S100a8-S2	530 (184-1017)	533 (243-808)	0.29
S100a9-S1	1783 (503-3226)	1886 (744-2911)	0.89
S100a9-S2	267 (72-441)	268 (112-441)	0.79
Surfactant protein A	5 (0-7)	5 (0-6)	0.42
Alpha defensin 1-S1	1 (0-6)	1 (0-1)	0.39
Alpha defensin 5	1 (1-6)	1 (0-1)	**<0.001**
Lactoferrin-S1	14 (1-32)	34 (1-94)	**0.007**
Lactoferrin-S2	12 (7-20)	23 (13-61)	**<0.001**
Immunoglobulin A	10 (10-124)	97 (10-572)	**0.007**
Lysozyme C-S1	1191 (573-2146)	1869 (1168-2855)	**<0.001**
Lysozyme C-S2	0 (0-12)	10 (0-29)	**0.011**

*Median (quartiles), Time 1: 1-2 days of life; Time 2: 8-9 days of life. *Wilcoxon rank test*

Supplemental Table 4. Maternal and neonatal characteristics with microbiota data

	No OCP	OCP	P-value
Maternal Characteristics	N = 14	N = 14	
Age, median (quartiles)	28 (23, 31)	24 (21, 28)	0.14[1]
Race			
African American	21%	43%	0.32[2]
White	71%	50%	
More than one	0%	7%	
Unknown, not reported	7%	0%	
Ethnicity			0.54[2]
Hispanic or Latino	14%	7%	
Gestation (multiple)	7%	14%	0.54[2]
Prenatal care	100%	100%	
Mode of delivery			0.62[2]
SVD	14%	21%	
Caesarian section	86%	79%	
Anesthesia			0.65[2]
Epidural	71%	86%	
General	14%	7%	
Hypertension	21%	50%	0.12[2]
Diabetes	0%	14%	0.14[2]
Tobacco use	14%	29%	0.36[2]
Premature rupture of membranes*	43%	14%	0.094[2]
Amniotic fluid			0.3[2]
Clear	71%	93%	
Bloody	21%	7%	
Meconium	7%	0%	
Antenatal steroids	86%	86%	1[2]
Neonatal characteristics			
Gestational age, median (quartiles)	29 (28, 30)	28 (27, 31)	1[1]
Birth weight (grams), median (quartiles)	1240 (1035-1322)	1262 (770, 1428)	0.84[1]
Male	50%	64%	0.45[2]
5 minute Apgar score, median (quartiles)	8 (7, 8)	7 (6, 9)%	0.61[1]
Surfactant	43%	79%	0.053[2]
Total days intubated	1 (0, 2)	4 (1, 11)	0.03[1]
Early onset bacteremia	0%	0%	1[2]
Late onset bacteremia	7%, n = 1	0%	0.31[2]
Antimicrobial exposure, days, median (quartiles)	5 (3, 9)	3 (2, 8)	0.31[1]
Age at feeding initiation (days), median (quartiles)	2 (2, 2)	2 (2, 3)	0.11[1]
Initial feeding type			1[1]
Expressed breast milk	64%	64%	
Donor breast milk	31%	30%	
Formula	0%	3%	
Days to 100ml/kg/day enteral feeds, median (quartiles)	10 (9, 18)	12 (9, 17)	0.72[1]
Type of feeds at discharge			0.11[2]
Unfortified breast milk	14%	0%	
Fortified breast milk	36%	71%	
Formula only	50%	29%	
Necrotizing enterocolitis (≥stage 2)	7%, n = 1	8%, n = 1	0.59[2]
Days to discharge, median (quartiles)	49 (44, 60)	40 (29, 80)	0.23[1]

*[1]Wilcoxon rank test; [2]Pearson test, * >18 hours prior to delivery*