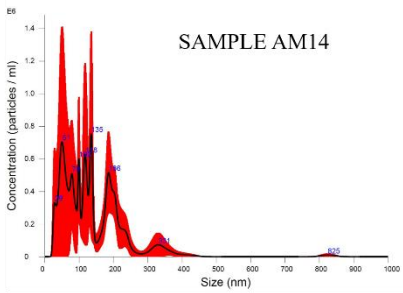
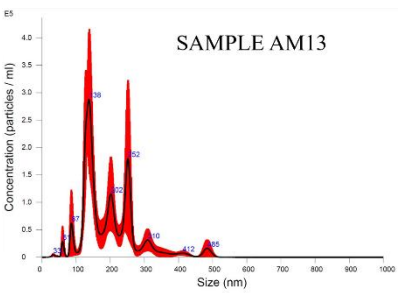
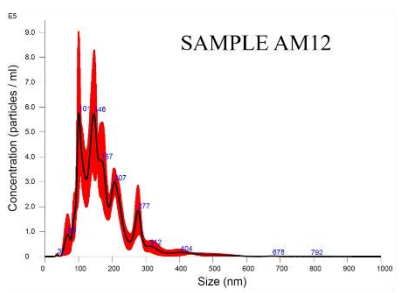
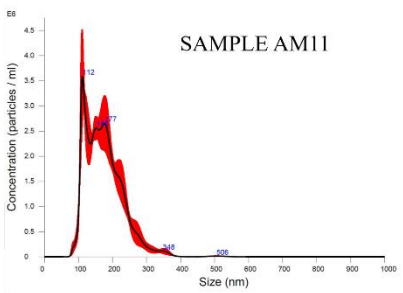
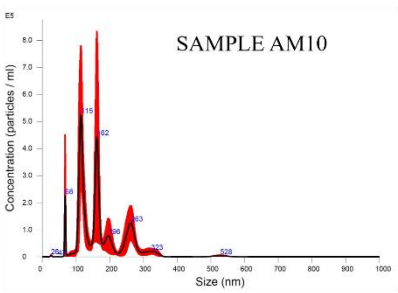
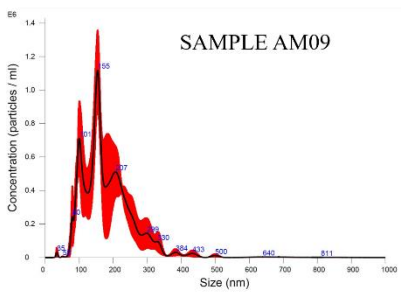
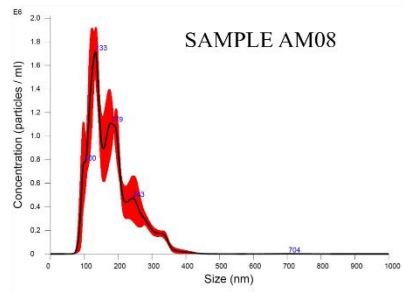
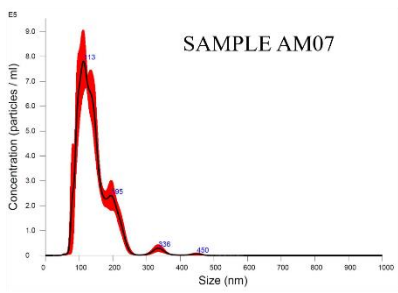
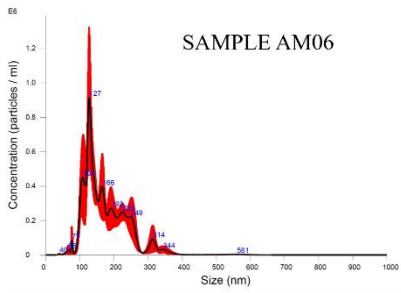
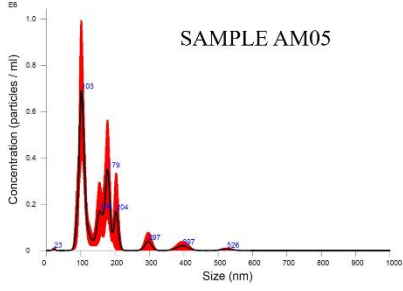
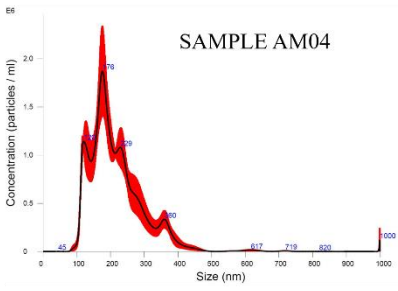
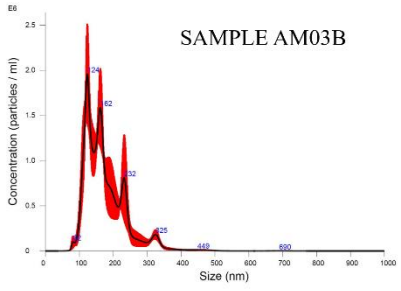
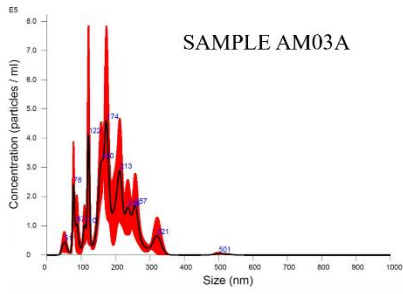
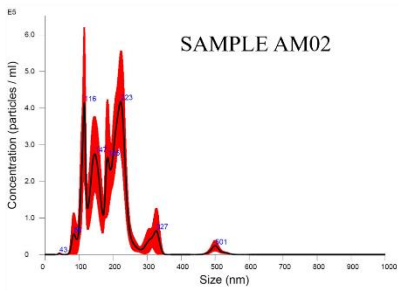
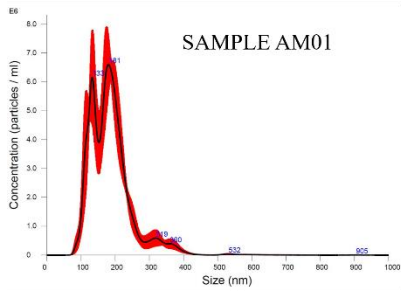


Fig. 1. ANCOM analysis of the differentially abundant taxa in the sample groups. In the analysis, the centered log ratio (clr) represents the effect size and W the number of times the 0-hypothesis has been rejected. Differentially abundant taxa are identified at the phylum and genus levels and marked as red dots in the volcano plots. The differentially abundant genera in the maternal feces (FE) vs. maternal feces-derived extracellular vesicles (FE EV) and amniotic fluid (AM) vs. amniotic fluid-derived extracellular vesicles (AM EV) comparisons are listed in Supplementary Table 8.



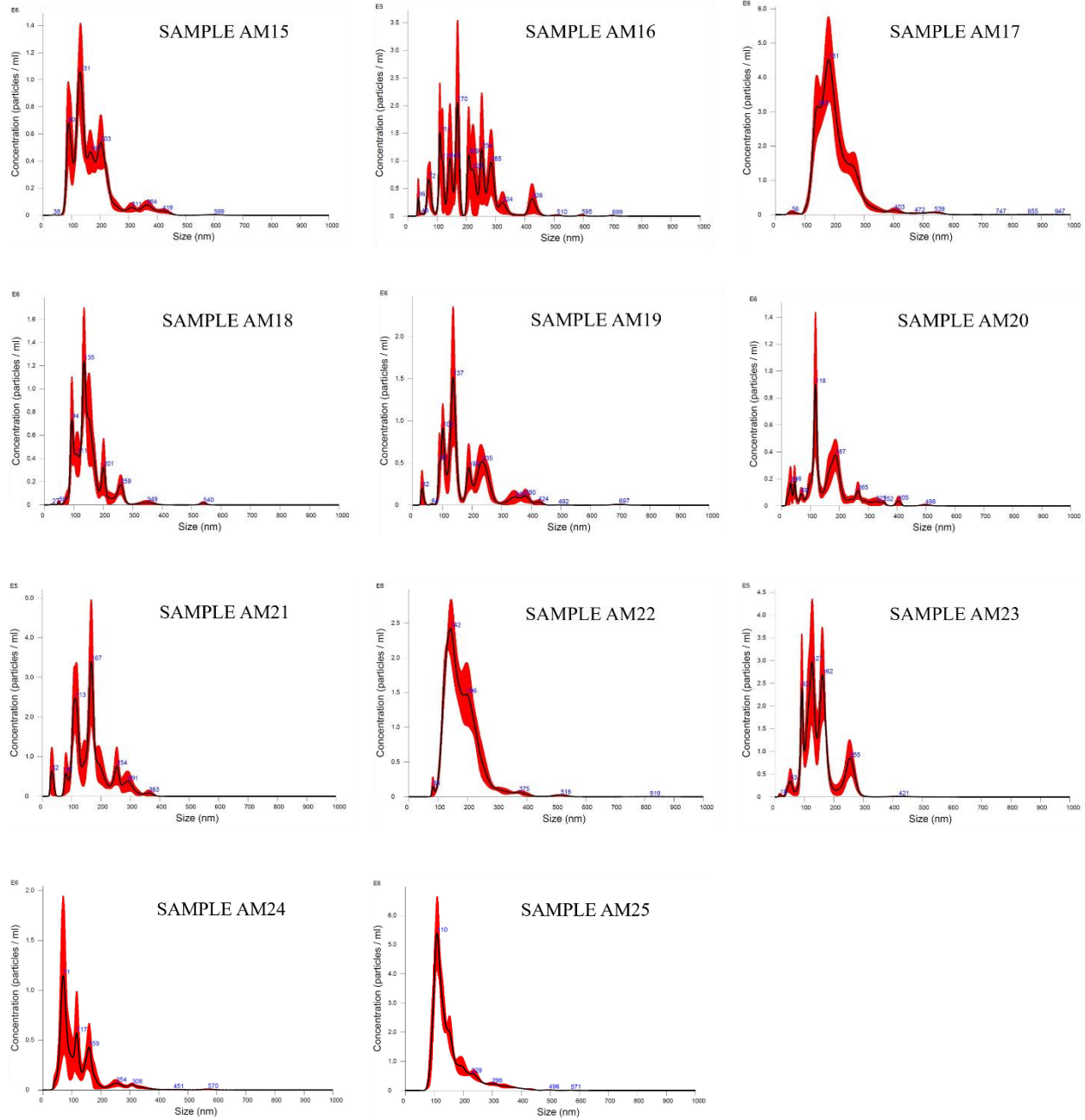
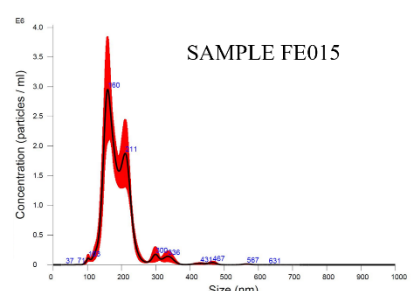
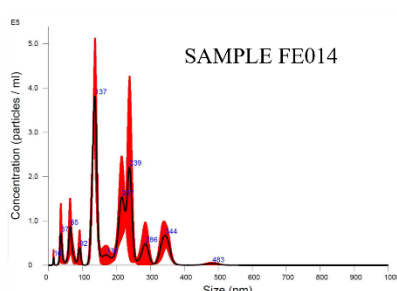
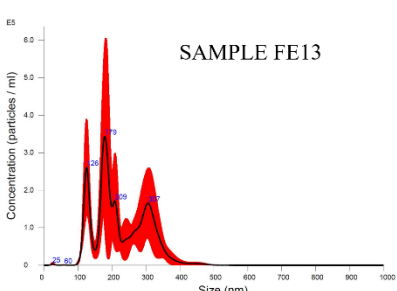
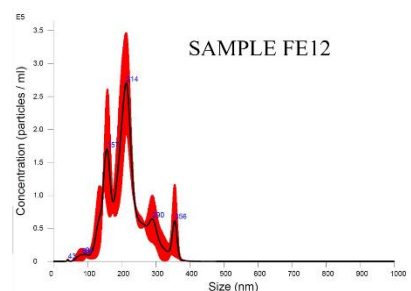
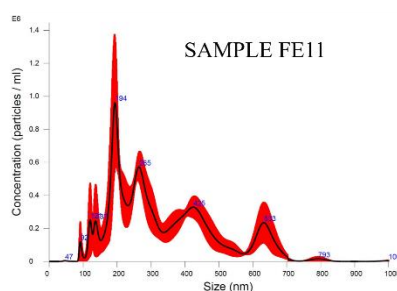
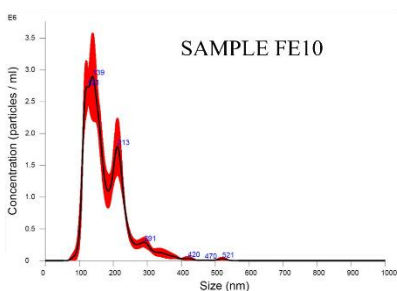
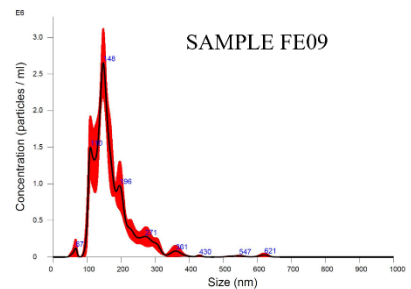
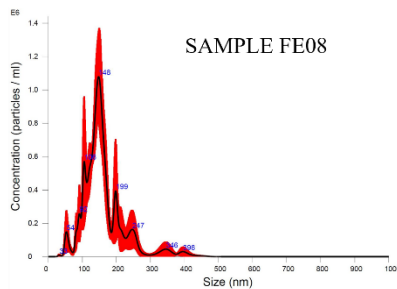
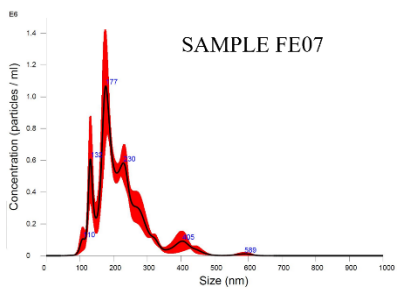
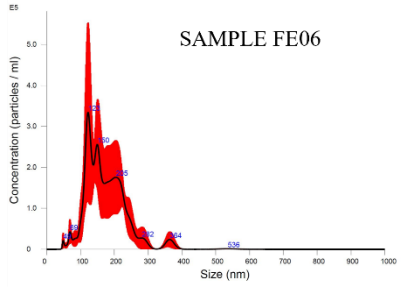
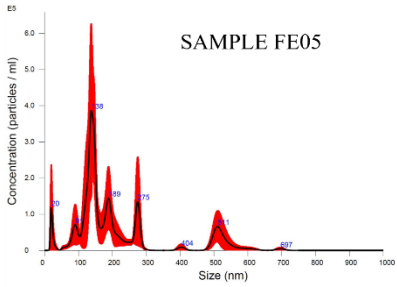
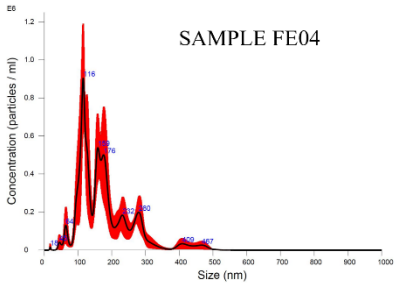
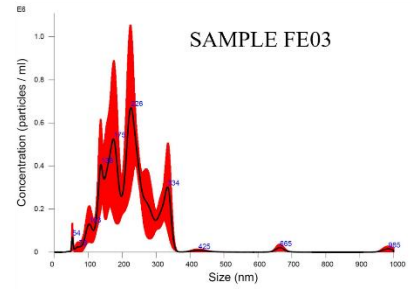
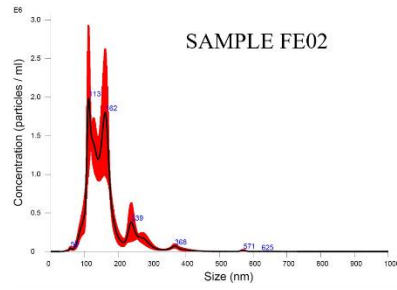
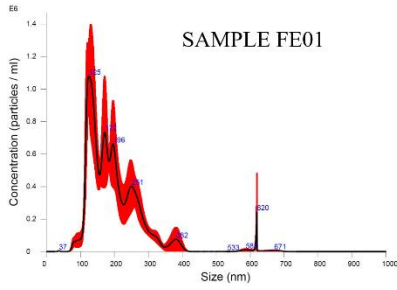


Fig. 2. Size distribution analysis of amniotic fluid-derived extracellular vesicles (EVs) by nanoparticle tracking analysis. Samples AM01 – AM25.



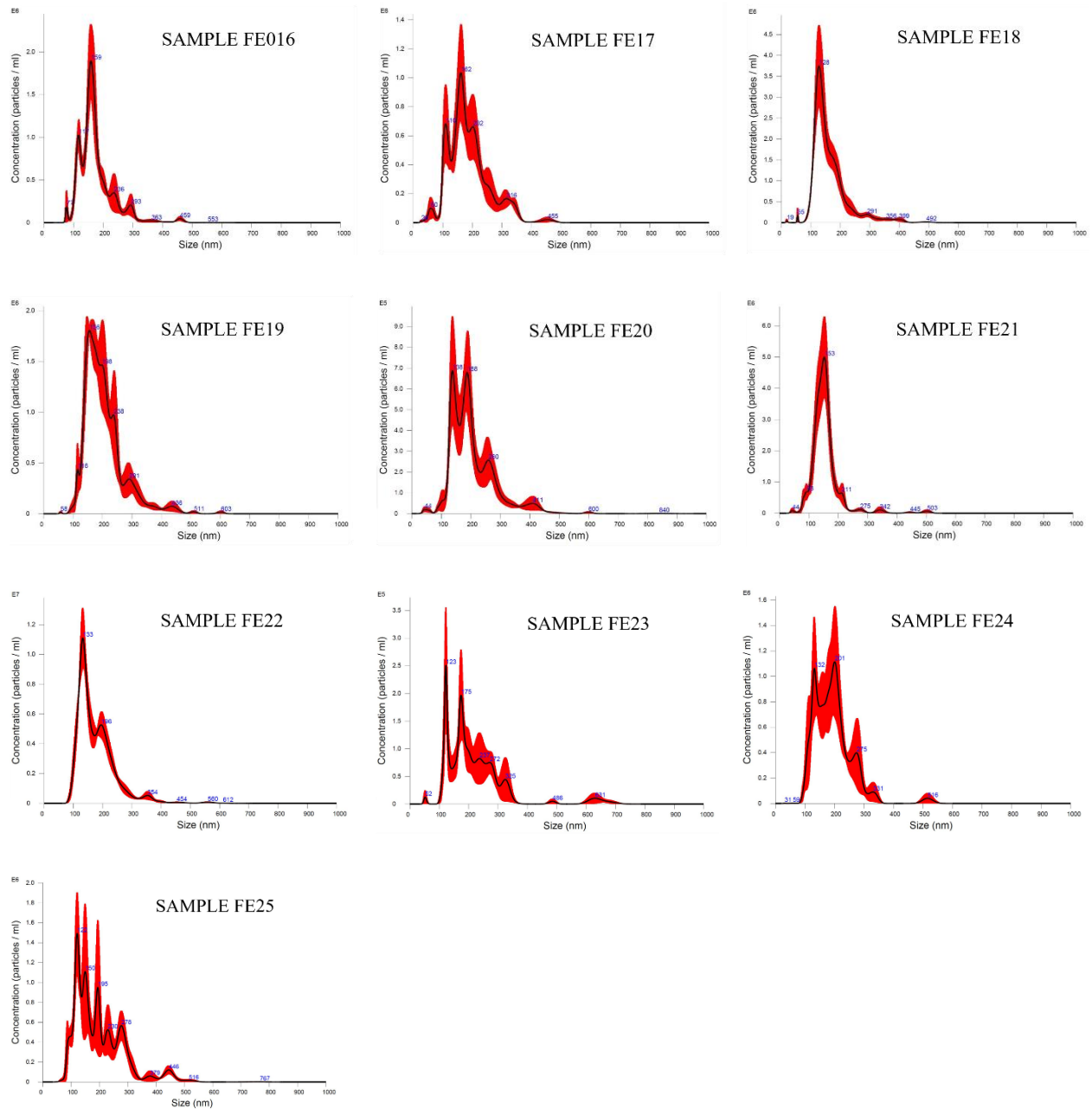


Fig. 3. Size distribution analysis of maternal feces-derived extracellular vesicles (EVs) by nanoparticle tracking analysis. Samples FE01 – FE25.

| Population characteristics | |
|---|-------------------------|
| <i>Maternal characteristics</i> | N=28^a |
| Maternal age (years) Mean (SD) | 31 (4.8) |
| Number of siblings Mean (SD) | 0.9 (0.9) |
| Maternal asthma N (%) | 2 (7.1) |
| Maternal allergy N (%) | 9 (32) |
| Gestational diabetes N (%) | 11 (39) |
| Smoking during pregnancy | 6 (21) |
| <i>S. agalactiae</i> screen positive ^b | 5 (18) |
| Antibiotics during pregnancy ^c | 4 (14) |
| Antibiotics during delivery ^d | 28 (100) |
| | |
| <i>Newborn characteristics</i> | |
| Female (%) | 13 (46) |
| Gestational age (weeks) Mean (SD) | 38.5 (1.5) |
| Birth weight (grams) Mean (SD) | 3420 (580) |
| Apgar 1 min Mean (SD) | 8.4 (1.3) |
| Apgar 5 min Mean (SD) | 8.8 (1.1) |
| Apgar 15 min Mean (SD) | 9.0 (0.7) |
| Antibiotics after birth N (%) ^f | 3 (11) |
| ^a Both fecal and amniotic fluid samples were available for 22 cases, fecal samples only for 3 cases and amniotic fluid samples only for 3 cases. ^b In 9 cases <i>Str. agalactiae</i> screening was not performed. ^c One mother had received pivmecillinam and one metronidazole. In two cases the antibiotic used was not recorded. ^d All except one received cefuroxime ^f Three infants received benzylpenicillin and tobramycin. | |

Table 1. Amniotic fluid and fecal samples: clinical data on the pregnant women.

| | AMNIOTIC FLUID | FECES |
|--|----------------|----------------|
| Number of protein identifications | 3526 | 8417 |
| Bacterial proteins | 340 | 7558 |
| Mean number of bacterial proteins per sample (range) | 30 (2-190) | 1333 (10-3671) |
| Human proteins | 3186 | 859 |
| Mean number of human proteins per sample (range) | 601 (71-2217) | 60 (4-707) |

Table 2. Proteins identified in the amniotic-fluid and feces-derived EVs.

| PROTEIN NAME | PHYLUM | HITS |
|--|----------------------------------|------|
| High-molecular weight cobalt-containing nitrile hydratase subunit alpha, H-Nhase, H-nitrilase, EC 4.2.1.84 | Actinobacteria | 1 |
| Flagellin | Firmicutes | 4 |
| Uncharacterized protein | Proteobacteria, Actinobacteria,, | 6 |
| tRNA (guanine-N(7)-)-methyltransferase | Proteobacteria | 1 |
| Enolase, EC 4.2.1.11 | Spirochaetes, Proteobacteria, | 3 |
| 30S ribosomal protein S8 | Proteobacteria | 1 |
| AAA-family ATPase | Actinobacteria | 2 |
| Tubulin_C domain-containing protein | Actinobacteria | 1 |
| Translation elongation factor EF-1 subunit alpha | Actinobacteria | 1 |
| 30S ribosomal protein S13 | Proteobacteria | 1 |
| 30S ribosomal protein S19 | Proteobacteria, Actinobacteria | 3 |
| Putative membrane protein YgcG | Actinobacteria | 1 |
| Pesticidal crystal protein Cry2Ab | Firmicutes | 1 |
| Fumarate reductase | Bacteroidetes | 2 |
| TonB-dependent receptor | Bacteroidetes | 1 |
| ATP-dependent zinc metalloprotease FtsH, | Actinobacteria | 1 |
| 2-isopropylmalate synthase, EC 2.3.3.13 | Firmicutes | 2 |
| Subtilisin BL, EC 3.4.21.62 | Firmicutes | 2 |
| M protease, EC 3.4.21.- | Firmicutes | 1 |
| Alkaline protease, EC 3.4.21.- | Firmicutes | 2 |
| Ribulose biphosphate carboxylase large chain, RuBisCO large | Cyanobacteria | 6 |
| OmpA-family protein | Proteobacteria, Bacteroidetes | 12 |
| DNA, contig: SP617 | Proteobacteria | 1 |
| Outer membrane protein OmpA-like peptidoglycan-associated | Proteobacteria | 1 |
| TIGR01841-family phasin | Proteobacteria | 1 |
| Phasin-family protein | Proteobacteria | 1 |
| DNA, contig: SP655 | Proteobacteria | 1 |
| TonB-linked outer membrane protein, SusC/RagA family | Proteobacteria, Bacteroidetes | 2 |
| MotA/TolQ/ExbB proton channel-family protein | Proteobacteria | 5 |
| Glutamate synthase [NADPH] large chain, EC 1.4.1.13 | proteobacteria | 1 |
| Glyceraldehyde-3-phosphate dehydrogenase, GAPDH, EC | Firmicutes | 6 |
| Porin | Bacteroidetes | 2 |
| DUF4988 domain-containing protein | Bacteroidetes | 1 |

Table 3. Bacterial protein identifications in the proteomic analyses of the amniotic fluid extracellular vesicles and maternal fecal extracellular vesicles.

| | PROTEIN NAME | PHYLUM | HITS |
|------|---|--|------|
| >80% | Contig40, whole genome shotgun sequence | Bacteroidetes | 1 |
| | IPTL-CTERM sorting domain-containing protein (Fragment) | Proteobacteria | 1 |
| ≥50% | tRNA (guanine-N(7)-)-methyltransferase | Proteobacteria | 1 |
| | Uncharacterized protein | Proteobacteria | 2 |
| >25% | Chaperone protein DnaK (HSP70) (Heat shock protein 70) | Thermotogae Chlorobi Chlorobi Cyanobacteria | 4 |
| | Glyceraldehyde-3-phosphate dehydrogenase 1 (GAPDH 1) | Firmicutes | 7 |
| | Uncharacterized protein (Fragment) | Actinobacteria Proteobacteria | 3 |
| | RRM domain-containing protein | Armatimonadetes | 1 |
| | Ubiquitin-like domain-containing protein | Proteobacteria | 1 |
| | AAA-family ATPase (Fragment) | Actinobacteria | 1 |

Table 4. Distribution of bacterial protein identifications in amniotic fluid EV samples.

| | PROTEIN NAME | PHYLUM | HITS |
|--|---|----------------|------|
| >70% | TonB-dependent receptor | Bacteroidetes | 17 |
| | SusC/RagA-family TonB-linked outer membrane protein | Bacteroidetes | 27 |
| | Uncharacterized protein | Bacteroidetes | 5 |
| | TonB-dependent receptor plug domain-containing protein | Bacteroidetes | 3 |
| | SusC protein | Bacteroidetes | 2 |
| | Flagellin | Firmicutes | 2 |
| | Tetratricopeptide repeat protein | Bacteroidetes | 19 |
| | Vitamin B12 transporter BtuB | Bacteroidetes | 1 |
| | Iron complex outer membrane receptor protein | Bacteroidetes | 1 |
| | TPR domain protein | Bacteroidetes | 1 |
| ≥60% | (Putative) Outer membrane protein | Bacteroidetes | 11 |
| | SusD/RagB-family nutrient-binding outer membrane lipoprotein | Bacteroidetes | 97 |
| | Outer membrane cobalamin receptor protein | Bacteroidetes | 4 |
| | Carboxypeptidase regulatory-like domain protein | Bacteroidetes | 3 |
| | Membrane protein | Bacteroidetes | 2 |
| | Uncharacterized protein | Bacteroidetes | 29 |
| >50% | High-molecular weight cobalt-containing nitrile hydratase subunit alpha (H-Nhase) (H-nitrilase) (EC 4.2.1.84) | Actinobacteria | 1 |
| | OmpA-family protein | Bacteroidetes | 25 |
| | Porin-family protein/Outer membrane beta-barrel domain protein | Bacteroidetes | 1 |
| | Cell surface protein | Bacteroidetes | 15 |
| | Conserved hypothetical exported protein | Bacteroidetes | 1 |
| | Hemin receptor | Bacteroidetes | 4 |
| | Uncharacterized protein | Bacteroidetes | 85 |
| ≥30% | Formate-tetrahydrofolate ligase (EC 6.3.4.3) | Firmicutes | 18 |
| | Ferrienterobactin receptor | Bacteroidetes | 3 |
| | OMP_b-brl domain-containing protein | Bacteroidetes | 18 |
| | NAD-specific glutamate dehydrogenase (NAD-GDH) | Bacteroidetes | 1 |
| | DNA, contig: SP617 | Proteobacteria | 1 |
| | STN domain-containing protein | Bacteroidetes | 2 |
| | Mucoicid inhibitor MuiA family protein | Bacteroidetes | 1 |
| | Ferritin (EC 1.16.3.2) | Bacteroidetes | 23 |
| | Major outer membrane lipoprotein Lpp | Proteobacteria | 32 |
| | Adenine deaminase (ADE) (EC 3.5.4.2) | Proteobacteria | 1 |
| | SLH domain-containing protein | Firmicutes | 2 |
| | Putative cell surface protein | Bacteroidetes | 3 |
| | MotA/TolQ/ExbB proton channel-family protein | Bacteroidetes | 13 |
| | DUF3869 domain-containing protein | Bacteroidetes | 21 |
| Collagen triple helix repeat (20 copies) | Bacteroidetes | 1 | |
| DUF4988 domain-containing protein | Bacteroidetes | 2 | |

Table 5. Distribution of bacterial protein identifications in the maternal fecal EV samples.

| | | Shannon Index | Observed features | Bray-Curtis dissimilarity |
|--------------|--------------|---------------|-------------------|---------------------------|
| Group 1 | Group 2 | p-value | p-value | p-value |
| AM (n=10) | AM_EV (n=24) | 0.186 | 0.576 | 0.001 |
| AM (n=10) | FE (n=22) | 0.713 | 0.002 | 0.001 |
| AM (n=10) | FE_EV (n=22) | 1.000 | 0.025 | 0.001 |
| AM_EV (n=24) | FE (n=22) | 0.003 | < 0.001 | 0.001 |
| AM_EV (n=24) | FE_EV (n=22) | 0.001 | 0.010 | 0.001 |
| FE (n=22) | FE_EV (n=22) | 1.000 | 0.150 | 0.001 |

Table 6. Pairwise results of the Wilcoxon rank sum test for Shannon Index, Observed features and

PERMANOVA for Bray-Curtis dissimilarity. The p-values for the Shannon Index and Observed features are adjusted using the Bonferroni correction.

| Maternal feces | | | |
|--|--------|------------------------------------|-----|
| Phylum (%) | | Genus (%) | |
| Bacteroidota | 53 | <i>Bacteroides</i> | 30 |
| Firmicutes | 44 | <i>Alistipes</i> | 13 |
| Proteobacteria | 1.3 | <i>Faecalibacterium</i> | 3.5 |
| Desulfobacterota | 1.2 | <i>Prevotella</i> | 3.4 |
| Unassigned | 0.44 | UCG-002 | 3.1 |
| Actinobacteriota | 0.30 | <i>Turicibacter</i> | 2.4 |
| Fusobacteriota | 0.24 | Erysipelotrichaceae_UCG-003 | 2.1 |
| Campilobacterota | 0.020 | <i>Barnesiella</i> | 2.1 |
| Verrucomicrobiota | 0.014 | Clostridia_UCG-014 | 1.9 |
| Spirochaetota | 0.0070 | <i>Blautia</i> | 1.8 |
| other | 0.0088 | other | 37 |
| Amniotic fluid | | | |
| Phylum (%) | | Genus (%) | |
| Firmicutes | 65 | <i>Peptoniphilus</i> | 33 |
| Bacteroidota | 21 | <i>Alistipes</i> | 8.2 |
| Unassigned | 6.6 | <i>Bacteroides</i> | 7.1 |
| Proteobacteria | 4.2 | <i>Unassigned</i> | 6.6 |
| Actinobacteriota | 2.4 | <i>Subdoligranulum</i> | 5.9 |
| Desulfobacterota | 0.68 | <i>Staphylococcus</i> | 5.8 |
| Myxococcota | 0.28 | <i>Prevotella</i> | 2.7 |
| Dependentiae | 0.15 | <i>Clostridium_sensu_stricto_1</i> | 2.4 |
| d__Bacteria | 0.14 | <i>Streptococcus</i> | 2.2 |
| Campilobacterota | 0.069 | f__Oscillospiraceae_NK4A214_group | 1.6 |
| other | 0.14 | Other | 25 |
| Feces-derived extracellular vesicles | | | |
| Phylum (%) | | Genus (%) | |
| Firmicutes | 70 | <i>Staphylococcus</i> | 31 |
| Bacteroidota | 14 | <i>Streptococcus</i> | 17 |
| Actinobacteriota | 7.5 | <i>Anaerococcus</i> | 8.1 |
| Proteobacteria | 5.6 | <i>Alloprevotella</i> | 4.5 |
| Deinococcota | 1.3 | <i>Bacteroides</i> | 3.0 |
| Fusobacteriota | 0.97 | <i>Porphyromonas</i> | 3.0 |
| Fibrobacterota | 0.064 | <i>Gemella</i> | 2.6 |
| WPS-2 | 0.057 | <i>Prevotella</i> | 2.1 |
| Acidobacteriota | 0.046 | <i>Lawsonella</i> | 2.1 |
| Myxococcota | 0.033 | <i>Williamsia</i> | 1.7 |
| other | 0.051 | other | 26 |
| Amniotic fluid-derived extracellular vesicles | | | |
| Phylum (%) | | Genus (%) | |
| Firmicutes | 71 | <i>Staphylococcus</i> | 40 |
| Bacteroidota | 14 | <i>Streptococcus</i> | 16 |
| Proteobacteria | 7.9 | <i>Alloprevotella</i> | 5.3 |
| Actinobacteriota | 5.7 | <i>Prevotella</i> | 3.7 |
| Deinococcota | 0.97 | <i>Porphyromonas</i> | 3.6 |
| Campilobacterota | 0.15 | <i>Neisseria</i> | 3.3 |
| Fibrobacterota | 0.12 | <i>Gemella</i> | 2.7 |
| WPS-2 | 0.11 | <i>Lawsonella</i> | 1.6 |
| Fusobacteriota | 0.066 | <i>Peptoniphilus</i> | 1.6 |
| Nitrospirota | 0.062 | <i>Dolosigranulum</i> | 1.5 |
| other | 0.054 | other | 20 |

Table 7. Relative abundance of the ten most abundant phyla and genera in each group of samples.

| FE vs. FE EV | | | | AM vs. AM EV | | | |
|------------------|----|---|-----|-----------------|----|-----------------------------|-----|
| Phylum | w | Genus | w | Phylum | w | Genus | w |
| Bacteroidota | 19 | <i>Staphylococcus</i> | 411 | Deinococcota | 20 | <i>Alloprevotella</i> | 359 |
| Actinobacteriota | 18 | <i>Porphyromonas</i> | 406 | | | <i>Bacteroides</i> | 358 |
| Deinococcota | 18 | <i>Alloprevotella</i> | 405 | | | <i>Alistipes</i> | 358 |
| Desulfobacterota | 16 | <i>Granulicatella</i> | 400 | | | <i>Dermacoccus</i> | 353 |
| Fusobacteriota | 15 | <i>Neisseria</i> | 400 | | | <i>Bradyrhizobium</i> | 353 |
| | | <i>Gemella</i> | 400 | | | <i>Vibrionimonas</i> | 353 |
| | | <i>Peptoniphilus</i> | 400 | | | <i>Gemella</i> | 350 |
| | | <i>Lawsonella</i> | 400 | | | <i>Porphyromonas</i> | 349 |
| | | <i>Finegoldia</i> | 397 | | | <i>Deinococcus</i> | 347 |
| | | <i>Anaerococcus</i> | 396 | | | <i>Granulicatella</i> | 346 |
| | | <i>Micrococcus</i> | 393 | | | <i>Dolosigranulum</i> | 346 |
| | | <i>Faecalibacterium</i> | 391 | | | <i>Neisseria</i> | 346 |
| | | <i>Dolosigranulum</i> | 390 | | | <i>Lawsonella</i> | 346 |
| | | <i>Eubacterium_coprostanoli</i> <i>genes_group</i> | 390 | | | <i>Williamsia</i> | 346 |
| | | <i>Alistipes</i> | 389 | | | <i>Streptococcus</i> | 345 |
| | | <i>Deinococcus</i> | 388 | | | <i>Staphylococcus</i> | 344 |
| | | <i>Williamsia</i> | 385 | | | <i>Veillonella</i> | 344 |
| | | <i>Bacteroides</i> | 385 | | | <i>Escherichia-Shigella</i> | 337 |
| | | <i>Streptococcus</i> | 384 | | | <i>Peptoniphilus</i> | 337 |
| | | <i>Oscillibacter</i> | 380 | | | <i>Prevotella</i> | 334 |
| | | <i>Veillonella</i> | 374 | | | | |
| | | <i>Ezakiella</i> | 374 | | | | |
| | | <i>Eremococcus</i> | 373 | | | | |
| | | <i>Barnesiella</i> | 367 | | | | |
| | | <i>Blautia</i> | 360 | | | | |
| | | <i>Parvimonas</i> | 356 | | | | |
| | | <i>Christensenellaceae_R-7_group</i> | 354 | | | | |
| | | <i>f__Ruminococcaceae_uncultured</i> | 353 | | | | |
| | | <i>Subdoligranulum</i> | 351 | | | | |
| | | <i>f__Lachnospiraceae</i> | 350 | | | | |
| | | <i>Colidextribacter</i> | 350 | | | | |
| FE vs. AM | | | | FE EV vs. AM EV | | | |
| phylum | w | genus | w | phylum | w | genus | w |
| Bacteroidota | 17 | <i>Dermacoccus</i> | 351 | Fusobacteriota | 16 | <i>Bacteroides</i> | 333 |
| Firmicutes | 17 | <i>Staphylococcus</i> | 348 | | | <i>Finegoldia</i> | 332 |
| | | <i>Bradyrhizobium</i> | 343 | | | <i>Fusobacterium</i> | 308 |
| | | <i>Faecalibacterium</i> | 329 | | | | |

Table 8. ANCOM analysis of differentially abundant taxa in the sample groups at the phylum and genus levels.