RESEARCH ARTICLE

Placentas delivered by pre-pregnant obese women have reduced abundance and diversity in the microbiome

Paula A. Benny1 | Fadhl M. Al-Akwaa2 | Corbin Dirx3 | Ryan J. Schlueter4 | Thomas K. Wolfgruber1 | Ingrid Y. Chern4 | Suzie Hoops5,6 | Dan Knights5,6 | Lana X. Garmire2
d
1Department of Epidemiology, University of Hawaii Cancer Center, Honolulu, HI, USA
2Department of Computational Medicine and Bioinformatics, North Campus Research Complex, University of Michigan, Ann Arbor, MI, USA
3University of Minnesota Genomics Center, University of Minnesota- Twin Cities, Minneapolis, MN, USA
4Department of Obstetrics and Gynecology, University of Hawaii, Honolulu, HI, USA
5BioTechnology Institute, College of Biological Sciences, University of Minnesota, Minneapolis, MN, USA
6Department of Computer Science and Engineering, University of Minnesota, Minneapolis, MN, USA

Abstract
Maternal pre-pregnancy obesity may have an impact on both maternal and fetal health. We examined the microbiome recovered from placentas in a multi-ethnic maternal pre-pregnancy obesity cohort, through an optimized microbiome protocol to enrich low bacterial biomass samples. We found that the microbiomes recovered from the placentas of obese pre-pregnant mothers are less abundant and less diverse when compared to those from mothers of normal pre-pregnancy weight. Microbiome richness also decreases from the maternal side to the fetal side, demonstrating heterogeneity by geolocation within the placenta. In summary, our study shows that the microbiomes recovered from the placentas are associated with pre-pregnancy obesity.

Importance: Maternal pre-pregnancy obesity may have an impact on both maternal and fetal health. The placenta is an important organ at the interface of the mother and fetus, and supplies nutrients to the fetus. We report that the microbiomes enriched from the placentas of obese pre-pregnant mothers are less abundant and less diverse when compared to those from mothers of normal pre-pregnancy weight. More over, the microbiomes also vary by geolocation within the placenta.

KEYWORDS
Microbiome, Obesity, Placenta, Pregnancy, 16S sequencing

Abbreviations: BMI, body mass index; DNA, deoxyribonucleic acid; HBV, hepatitis B virus; HIV, human immunodeficiency virus; NaOH, sodium hydroxide; OTU, operational taxonomic unit; PROM, preterm rupture of membranes; QC, quality control; QIIME, quantitative insights into microbial ecology; qPCR, quantitative polymerase chain reaction; rRNA, ribosomal ribonucleic acid; V4, fourth hypervariable region in 16S.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2021 The Authors. The FASEB Journal published by Wiley Periodicals LLC on behalf of Federation of American Societies for Experimental Biology.
1 | INTRODUCTION

The human microbiome is the collection of microorganisms that reside on or in human organ systems. A delicate relationship exists between the microbiome and the human body. When the balance is maintained, symbiosis, or a beneficial relationship between humans and these microorganisms is achieved. Whereas in dysbiosis, the imbalance of the human microbiome has been associated with several diseases and abnormalities, including pre-term birth. In particular, subjects who experienced pre-term labor had lesser Lactobacillus in their microbiome as compared to term gestation subjects. In addition, wider bacterial diversity was noted in pre-term pregnancies as compared to controls, including those associated with the vaginal microbiome such as Lactobacillus species and those associated with the oral microbiome such as Streptococcus thermophilus. Recent studies show that although placentas are unlikely to harbor microbes, they could possibly contain pathobionts.

We here conducted a study to determine if there is a relationship between maternal obesity status and microbiomes associated with placentas, from babies delivered by scheduled, non-labored cesarean sections. We divided the patients into cases and controls according to their pre-pregnancy weight: either pre-pregnant obese (BMI > 30) or normal-weighted (18.5 < BMI < 25). To account for the low bacterial biomass, we developed an optimized protocol to enrich the V4 region of bacterial 16S rRNA genes. Furthermore, we collected multiple placenta samples per patient, from the maternal, fetal and intermediate layers to examine the variations among them. The results show that microbiomes enriched in obese, and normal pre-pregnant weight women are different and could be biomarkers of maternal obesity.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Placenta samples were collected from pregnant mothers admitted for scheduled, non-labored, full-term cesarean section at ≥ 37 weeks gestation at Kapiolani Medical Center for Women and Children, Honolulu, Hawaii from November 2016 through September 2017. Such procedures minimized the introduction of other bacteria associated with vaginal births as well as bacterial contamination from air during births. The study was approved by the Western IRB board (WIRB Protocol 20151223). Women with preterm rupture of membranes (PROM), labor, multiple gestations, pre-gestational diabetes, hypertensive disorders, cigarette smokers, HIV, HBV, and chronic drug users were excluded from the study. Patients meeting inclusion criteria were identified from pre-admission medical records with pre-pregnancy BMI ≥ 30.0 (obese) or 18.5-25.0 (normal pre-pregnancy weights). Demographic and clinical characteristics were recorded, including maternal and paternal ages, maternal and paternal ethnicities, maternal pre-pregnancy BMI, pregnancy net weight gain, gestational age, parity, gravidity, and ethnicity. Placenta samples were obtained in areas equidistant from the cord insertion site and the placenta edge. Placenta samples were isolated (0.5 cm³) from the maternal, fetal, and intermediate areas using sterile surgical. Additionally, samples were obtained by waving airswabs in the air in the operating room, in the pathology lab where the placenta biopsies were collected, and in the research laboratory where extraction was carried out. Unopened airswabs were also used as a control.

2.2 | Extraction of genetic material

MOBIO PowerSoil DNA Kit (#12888-50) was used to extract DNA from placenta samples. Three hundred milligrams of placenta were homogenized, heated for 65°C and vortexed in a horizontal bead beater for 10 minutes. DNA was extracted from lysates by putting them through the MOBIO kit following the manufacturer’s protocol. Extracted DNA was quantified and quality control checked using NanoDrop.

2.3 | Bacterial DNA enrichment

Given the very low bacterial mass, an enrichment step was performed to remove host DNA contamination and improve 16S specific amplification. (NEBNext Microbiome DNA Enrichment Kit, # E2612L). Samples were enriched in sets of 8 for the optimal enrichment of bacterial DNA. Samples were processed in sets of 8 for two main reasons: (1) Technical limitations of the enrichment kit prevented high throughput processing, such that we could not perform the enrichment on all samples simultaneously; (2) We performed the enrichment on sets of 8, so that every sample was receiving approximately equal exposure time at each stage of the enrichment treatment. We intermixed obese and normal samples in each of the six batches and included airswab samples into batch # 5 and 6. DNAs were incubated with NEBNext magnetic beads for 15 minutes. Beads containing human host DNA were precipitated using a magnet, leaving microbial DNA in the supernatant.

2.4 | qPCR amplification

qPCR was performed to determine 16S counts within extracted samples. Isolated microbial DNA was amplified using primers to the hypervariable V4 region of the 16S rRNA gene, similar to others. Forward primer-

```mindterm
TCGTCGGCAGCGTCAGATGTATAAGAGACAGGAGACAGGG
```
BENNY ET AL.

TGCCAGCMGCCGCGGTAA. Reverse primer-GTCTCG
TGCGTCG GAGATGTGTAAGAGACACGGACTA
CHVG GGTWTCTAAT. PCR was performed using KAPA
HiFidelity Hot Start Polymerase; 95°C for 5 mins, 98°C for
20s, 55°C for 15s, 72°C for 1 minute for 25 cycles, 72°C
for 5 minutes. After 25 cycles of amplification, V4 specific
amplicons were observed by 2% agarose gels and Agilent
Bioanalyzer traces. V4 amplicon was detected at the ex-
pected size of 290bp. Samples were pooled, size-selected,
and denatured with NaOH, diluted to 8 PM in Illumina’s HT1
buffer, spiked with 15% PhiX, and heat denatured at 96°C for
2 minutes immediately prior to loading. A MiSeq600 cycle
v3 kit was used to sequence the samples, following the manu-
facturer’s protocol.

2.5 | Bioinformatics analysis

The 16S rRNA gene reads were analyzed using a robust bio-
informatics pipeline. Reads were stitched using PANDAseq
using 150 bp and 350 bp as the minimum and maximum
lengths of the assembled reads, respectively. Operational taxo-
nomic units (OTUs) were created by clustering the reads at 97%
identity using UCLUST. Representative sequences from each
OTU were aligned using PyNAST, and a phylogenetic tree
was inferred using FastTree v. 2.1.3 after applying the stand-
ard lane mask for 16S rRNA gene sequences, Pairwise UniFrac
distances were computed using QIIME. Permutation tests of
distance and principal coordinates analyses were performed
using the MicrobiomeAnalyst, a web-based tool for compre-
hensive exploratory analysis of microbiome data. Taxonomic
assignments were generated by the UCLUST consensus method
of QIIME 1.9, using the Greengenes 16S rRNA gene database
v. 13_8. We used the phyloseq R package to compute alpha
and beta diversity. We used SourceTracker (version 1.0.1) to
estimate the percentage of OTUs in placental samples whose
origin could be explained by their distribution in the airswabs. We
used Decontam to identify taxa microbiome that are more prevalent in airswabs than in placenta samples.

3 | RESULTS

3.1 | Demographic and clinical characteristics of the cohort

Our cohort consists of 44 women from three ethnic groups in-
cluding Caucasians, Asians, and Native Hawaiians, who un-
derwent scheduled full-term cesarean deliveries in Kapiolani
Medical Center for Women and Children, Honolulu, Hawaii
from November 2016 through September 2017. The patients
were included based on the inclusion and exclusion criteria
described earlier (Materials and Methods). In order to test

| TABLE 1 Clinical characteristics of the cohort |
|-----------------------------------------------|
| Variables | Obese (n = 26) | Non-obese (n = 18) | P value |
| Maternal age, years | 32.1(2.8) | 31.4(0.7) | .72 |
| Pre-pregnancy BMI, kg/m² | 34.1(5) | 21.8(1.2) | .0000001 |
| Gestational weight gain | 31.4(7.7) | 31.5(7.7) | .99 |
| Maternal Ethnicity | Caucasian = 5 | Caucasian = 7 | .29 |
| | Asian = 5 | Asian = 8 |
| | HPI = 13 | HPI = 3 |
| Parity | 0 | 2 | 2 | .01 |
| | 1 | 8 | 3 |
| | 2 | 2 | 6 |
| | >3 | 0 | 8 |

if there is a microbiome difference associated with mater-
nal pre-pregnancy obesity, the subjects were recruited into
two groups: normal pre-pregnant weight (18.5 < BMI < 25)
and pre-pregnant obese (BMI > 30) group. The patient de-
mographical and clinical characteristics are summarized in
Table 1. Maternal ages, gestational weight gain, and gesta-
tional age differences between the cases and controls are not
statistically significant, excluding the possibility of signifi-
cant confounding from these factors. Maternal pre-pregnant
obesity, however, is associated with increasing parity and
grading (P < .05). The variation in recruited cases vs con-
trols in each ethnic background reflects the multi-ethnic pop-
ulation demographics in Hawaii.

3.2 | Enrichment of the microbiome associated with placentas

Due to the low bacterial biomass, we developed a protocol to en-
rich the V4 region of bacterial 16S rRNA genes (see Materials
and Methods). Before sequencing, we first performed qPCR
to determine the 16S rRNA copy numbers within extracted
samples. As shown in Figure 1A, placenta samples contain
significantly more copies of 16S as compared to airswab or
water negative controls (Log10 transformed values: Placenta:
12.2; Airswab: 2.1; Water: 1.1). The significant difference (P
value < .05) of 16S transcript numbers between placentas and
airswab/water suggests that the enrichment protocol was suc-
ccessful. Furthermore, V4 amplicons post-PCR on the agarose
gel show the specific band of 290 bp—the expected size of
V4 amplicons, providing additional support for the success-
ful 16S amplification of microbiome associated with placenta
samples (Figure 1D). Upon positive confirmation from qPCR,
we implemented a bioinformatics analysis workflow following 16S sequencing (Supplementary Figure S2). As shown in Figure 1B, unenriched V4 samples yield much lower total reads (median: 68,468) as compared to enriched V4 samples (median: 516,479), confirming the success of the experimental protocol. We aligned the 16S sequencing reads using the Greengenes database. The enriched samples using V4 primers detected 57,468 ± 2,859 operational taxonomic units (OTUs), compared to 233 ± 36 OTUs from un-enriched samples (Figure 1C), again highlighting the strength of the enrichment step following DNA extraction.

3.3 Microbiomes associated with pre-pregnant obese women are less diverse than those of women of normal pre-pregnant weights

As many of the detected OTUs are unlikely to be of use when modeling the data, we removed OTUs that had low counts and variances. We removed 523 OTU features that had very few abundance levels (minimum counts) across samples and 3 OTUs that had low variance based on their interquartile ranges. As a result, a total of 26 taxa remained after the data filtering step (Figure 2A). Additionally, to check if the variation in data was driven by batch effects rather than by pre-pregnancy obesity status, we performed the principle component analysis of all samples (Figure 2B). We did not observe significant batch effects.

The heatmap of the microbiome associated with placentas of pre-pregnant obese mothers suggests the overall trend of less bacterial abundance and diversity, compared to that from the control group of normal pre-pregnant weights (Figure 2A). We then plot the Alpha diversity – Chao1 metric among samples (Figure 2C). Despite the variations, there appear to be higher diversities among the control samples compared to the cases. Moreover, the overall species richness, measured by alpha-diversity—Chao1 metric in the rarefaction curve, is less in pre-pregnant obese samples compared to control samples (t test, P value = 6.53E-05) across all read depths (Figure 2D). Next, we analyzed the taxonomic composition of the community through the direct quantitative comparison of relative abundance (Figure 2E). It is worth noting that control sample 66PI shows particularly high bacterial biomass compared to other control samples, possibly indicating an infection (Figure 2A). We thus excluded this sample from the following comparisons between cases and controls. The average relative abundance of Lactobacillus (Mann-Whitney U test, P value = .01) is significantly lower in obese samples compared to normal weight samples (Figure 2F), even though there are significant variations among individuals. Additionally, Haemophilus has a lower relative percentage in the obese group; however, the difference is not significant (P value = .24). Previously Haemophilus was observed to be less abundant in the salivary microbiome of obese subjects, compared to controls.23

Of note, as the UCLUST clustering method is known to call many OTUs, we used another popular method Dada226 which is more sensitive and specific to call OTUs than
UCLUST, and obtained similar alpha and beta diversity results.

3.4 Variability of the microbiome from the maternal to fetal side of the placenta

The placenta samples were collected from three different regions of the placenta: maternal side, intermediate layer, and fetal side. We next investigated the microbiome abundance and compositions among these three regions. The overall richness (measured by alpha-diversity) is lower in the fetal side, compared to the maternal ($P$ value = .01), and intermediate layer ($P$ value = .03), as shown in the rarefaction curves (Figure 3A). There is no significant difference between maternal and intermediate layers. All three placenta regions share most genus types, as expected (Figure 3B). Among them, Lactobacillus, the dominant taxa in all three layers, shows a trend of decreasing relative percentages from the maternal to fetal side (Figure 3B,C).
In this study, we sought to characterize the variations of the microbiome associated with the placentas of obese and non-obese women going through scheduled full-term cesarean deliveries. We found that pre-pregnant obese mothers have reduced bacterial diversity overall in placentas. Moreover, evidence also shows overall lower diversity of bacteria associated with the fetal side, compared to the maternal side. The microbiomes enriched from placenta samples are distinguishable from those in contamination controls. Our results demonstrate that reduced microbiome abundance and diversity in placentas, although in extremely low abundance, are identifiable features associated with maternal pre-pregnancy obesity.

The key characteristic of our study which makes it different from other studies is the enrichment process of the bacterial DNA. When we started analysing the 16S sequencing data from unenriched samples, very little OTUs were detected. We then used the NEBNext Microbiome DNA enrichment kit, a kit shown to be effective in enriching microbial DNA, allowing for a 100-fold enrichment of bacterial DNAs and successful detection of a large number of OTUs (Figure 1B,C). Our results demonstrate the usefulness of enrichment tools when testing samples that contain extremely low microbial biomass, such as the placenta.

Over all, the placenta microbiome associated with obese pre-pregnant women shows lower diversity compared to non-obese women, consistent with previous findings that also associate obesity with lower microbiome diversity. In the gut microbiome, it was reported that lower alpha diversity was associated with higher BMI and that low fecal microbial diversity was associated with higher body adiposity content. The oral microbiome was also less abundant in obese subjects, compared to normal-weight controls. Of note, other groups have also reported associations between the placental or pregnancy microbiome and other comorbidities such as gestational weight gain, gestational diabetes, diet, as well as earlier gestational age.

Excess gestational weight gain was described as a contributor to adverse pregnancy outcomes through adverse placental changes. Gestational diabetes and diet were reported to affect the maternal gut microbiome composition and normal metabolic functions and these changes which occurred during pregnancy persisted after birth and had lasting effects on offspring. In our study, the potential effects from gestational weight gain and pregnancy complications are minimized owing to the optimized experimental design and patient inclusion and exclusion criteria. As shown in Table 1, gestational weight gain between the cases and controls is not statistically significant. The women recruited in this study were all healthy without clinical complications such as gestational diabetes. Their diet could be different; however, these women were fasted before surgery, which minimized the confounding factors in the microbiome due to immediate dietary effects.

Another interesting finding is that Lactobacillus, a type of gram-positive bacteria, was significantly lower in placentas...
delivered from pre-pregnant obese women, as compared to normal weighted pre-pregnant controls. Lactobacillus, typically referred to as “good bacteria,” contributes to symbiosis in the digestive system where they convert sugar to lactic acid. It was postulated that Lactobacillus could transfer from the maternal gut to the placenta, possibly through hematogenous, enteric or enterohepatic transfer. While the exact mechanisms have not been fully elucidated, other groups have similarly shown an endometrial microbiome which was also enriched in Lactobacillus and further the reduction in Lactobacillus was associated with increased occurrence of endometriosis.

Our results show that the placenta microbiome differs from the maternal to fetal side, despite the small sample size. Several earlier studies have also revealed the geo-variations of the placental microbiome. It was reported that the basal plate which is in closest contact to the maternal uterine wall was dominated by Proteobacteria species, while the fetal membranes which were farthest away from the uterine wall were dominated by Firmicutes species. Villous tissue which is in between the basal plate and fetal membranes did not have a dominant species and contained a mixture of bacteria, confirming our observation of a microbiome gradient in the placenta. Furthermore, 16S in situ hybridization experiments confirmed the presence of bacteria primarily in villous parenchyma placental tissue and to a less extent in the chorion and maternal intervillous spaces. Since we observed more Lactobacillus in the maternal side compared to the fetal side, this may suggest that the origins of the placenta microbiome may be from the mother, possibly through circulation from the maternal gut.

5 | CONCLUSION

Using an enrichment protocol optimized for low bacterial biomass samples, we show that microbiome associated with placentas of pre-pregnant obese weighted mothers is less abundant and less diverse compared to the mothers of normal pre-pregnant weights.

ACKNOWLEDGMENTS

We would like to thank Dr. Vincent Young for giving helpful suggestions in preparing this manuscript. Dr. Lana X Garmire’s research is supported by grants K01ES025434 awarded by NIEHS through funds provided by the trans-NIH Big Data to Knowledge (BD2K) initiative (http://datascience.nih.gov/bd2k), P20 COBRE GM103457 awarded by NIH/NIGMS, R01 LM012373 awarded by NLM, and R01 HD084633 awarded by NICHD to LX Garmire. Funding was also provided in part by the Department of Obstetrics and Gynecology, University of Hawaii. 16S sequencing was performed by The University of Minnesota Genomics Center.

REFERENCES

1. Li X, Watanabe K, Kimura I. Gut microbiota dysbiosis drives and implies novel therapeutic strategies for diabetes mellitus and related metabolic diseases. Front Immunol. 2017;8:1882.
2. Antony KM, Ma J, Mitchell KB, Racusin DA, Versalovic J, Aagaard K. The preterm placental microbiome varies in association with excess maternal gestational weight gain. Am J Obstet Gynecol. 2015;212(653):e1-e16.
3. Prince AL, Ma J, Kannan PS, et al. The placental membrane microbiome is altered among subjects with spontaneous preterm birth with and without chorioamnionitis. Am J Obstet Gynecol. 2016;214:627 e1-e67 e16.
4. Doyle RM, Alber DG, Jones HE, et al. Term and preterm labour are associated with distinct microbial community structures in placental membranes which are independent of mode of delivery. Placenta. 2014;35:1099-1101.
5. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. Sci Transl Med. 2014;6:237ra65.
6. Lauder AP, Roche AM, Sherrill-Mix S, et al. Comparison of placenta samples with contamination controls does not provide evidence for a distinct placenta microbiota. Microbiome. 2016;4:29.
7. Zheng J, Xiao X, Zhang Q, Mao L, Yu M, Xu J. The placental microbiome varies in association with low birth weight in full-term neonates. Nutrients. 2015;7:6924-6937.
8. Chu DM, Ma J, Prince AL, Antony KM, Seferovic MD, Aagaard KM. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. Nat Med. 2017;23:314.
9. Jones HE, Harris KA, Azizia M, et al. Differing prevalence and diversity of bacterial species in fetal membranes from very preterm and term labor. PLoS ONE. 2009;4:e2805.
10. Leiby JS, McCormick K, Sherrill-Mix S, et al. Lack of detection of a human placenta microbiome in samples from preterm and term deliveries. Microbiome. 2018;6:196.
11. de Goffau MC, Lager S, Sovio U, et al. Human placenta has no microbiome but can contain potential pathogens. Nature. 2019;572:329-334. https://doi.org/10.1038/s41586-019-1451-5
12. Parnell LA, Briggs CM, Cao B, Delannoy-Bruno O, Schieffler AE, Mysoreskar IU. Microbial communities in placentas from term normal pregnancy exhibit spatially variable profiles. Sci Rep. 2017;7:11200.
13. Masella AP, Bartram AK, Truszkowski JM, Brown DG, Neufeld JD. PANDAseq: paired-end assembler for illumina sequences. BMC Bioinformatics. 2012;13:31.

14. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010;26:2460-2461.

15. Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics. 2010;26:266-267.

16. Price MN, Dehal PS, Arkin AP. FastTree 2—approximately maximum-likelihood trees for large alignments. PLoS ONE. 2010;5:e9490.

17. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7:335-336.

18. Dhariwal A, Chong J, Habib S, King IL, Agellon LB, Xia J. JD. PANDAseq: paired-end assembler for illumina sequences. Bioinformatics. 2010;26:266-267.

19. DeSantis TZ, Hugenholtz P, Larsen N, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol. 2006;72:5069-5072.

20. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE. 2013;8:e61217.

21. Knights D, Kuczynski J, Charlson ES, et al. Bayesian community-wide culture-independent microbial source tracking. Nat Methods. 2011;8:761-763.

22. Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome. 2018;6:226.

23. Wu Y, Chi X, Zhang Q, Chen F, Deng X. Characterization of the salivary microbiome in people with obesity. PeerJ. 2018;6:e4458.

24. Theoendel M, Jeraldo PR, Greenwood-Quaintance KE, et al. Comparison of microbial DNA enrichment tools for metagenomic whole genome sequencing. J Microbiol Methods. 2016;127:141-145.

25. Eisenhofer R, Minich JJ, Marotz C, Cooper A, Knight R, Weyrich LS. Contamination in low microbial biomass microbiome studies: issues and recommendations. Trends Microbiol. 2019;27:105-117.

26. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13:581.

27. Rogers GB, Carroll MP, Zain NM, et al. Complexity, temporal stability, and clinical correlates of airway bacterial community composition in primary ciliary dyskinesia. J Clin Microbiol. 2013;51:4029-4035.

28. Walker RW, Clemente JC, Peter I, Loos RJF. The prenatal gut microbiome: are we colonized with bacteria in utero? Pediatric Obesity. 2017;12:3-17.

29. Onderdonk AB, Delaney ML, DuBois AM, Allred EN, Leviton A. Detection of bacteria in placental tissues obtained from extremely low gestational age neonates. Am J Obstet Gynecol. 2008;198(1):110.e1-7.

30. Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell. 2012;3;150(3):470-480.

31. Chu DM, Antony KM, Ma J, et al. The early infant gut microbiome varies in association with a maternal high-fat diet. Genome Med. 2016;8:77.

32. Stanislawski MA, Dabelea D, Lange LA, Wagner BD, Lozupone CA. Gut microbiota phenotypes of obesity. NPJ Biofilms Microbiomes. 2019 Jul 1;5(1):18.

33. Le Chatelier E, Nielsen T, Qin J, et al. Richness of human gut microbiome correlates with metabolic markers. Nature. 2013 Aug 29;502(7464):541-546.

34. Liu Y, Ko EY, Wong KK, et al. Endometrial microbiota in infertile women with and without chronic endometritis as diagnosed using a quantitative and reference range-based method. Fertil Steril. 2019;112(707-717):e1.

35. Parnell LA, Briggs CM, Cao B, et al. Microbial communities in placentas from term normal pregnancy exhibit spatially variable profiles. Sci Rep. 2017;7:11200.

36. Collado M, Rautava S, Aakko J, et al. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. Sci Rep. 2016;6:23129.

37. Sefirovic MD, Pace RM, Carroll M, et al. Visualization of microbes by 16S in situ hybridization in term and preterm placentas without intraamniotic infection. Am J Obstet Gynecol. 2019;221(146):e1-e23.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

How to cite this article: Benny PA, Al-Akwaa FM, Dirkx C, et al. Placentas delivered by pre-pregnant obese women have reduced abundance and diversity in the microbiome. The FASEB Journal. 2021;35:e21524. https://doi.org/10.1096/fj.202002184RR