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## Bacterial DNA in Fetal Lung Samples May Be Explained by Sample Contamination

To the Editor:

We read with interest the work of Al Alam and colleagues (1) suggesting the existence of a fetal lung microbiome signature. As

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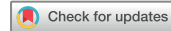
stated by the authors, the existence of a fetal microbiome remains a matter of debate. Collecting fetal material from human subjects during pregnancy is difficult, and therefore studies so far have mainly sampled placenta and amniotic fluid as proxies for the fetomaternal interface. Detection of microbial communities in these samples would support the existence of a fetal microbiome, which would open up the possibility of microbe-driven immune training commencing before birth.

In a recently published study, de Goffau and colleagues refuted the existence of a placental microbiome in 578 placental samples from both complicated and uncomplicated pregnancies, with the exception of *Streptococcus agalactiae*, which was found in ~5% of the samples (2). Using a rigorous approach that included analyses of extensive negative and positive controls, DNA-sequencing techniques, and the use of different DNA-isolation kits, they disproved previous claims of the existence of a placental microbiome (3) and suggested that contamination issues provide a convincing explanation for earlier findings.

Because the current study by Al Alam and colleagues (1), which describes microbial presence in 31 fetal/placental samples collected between 11 and 20 weeks of gestation after dilatation and curettage (D&C) and dilatation and evacuation (D&E) procedures, contradicts the results obtained by de Goffau and colleagues (2), we attempted to identify potential explanations for these discrepancies.

First, given the low biomass observed in fetal (lung) samples (1), the risk of contamination from nonbiological sources is high. Contamination may occur during sample collection, as both D&C and D&E procedures require passage of a medical instrument through the vagina, which is known to harbor a dense microbiota. Subsequent technical procedures can further contaminate samples, for example, by DNA-isolation reagents, well-to-well contamination, and the sequencing machine itself (2). Some contamination risks are unavoidable; however, to appropriately control for these risks, we would have expected more rigorous technical controls at various stages of the laboratory process, instead of the limited number of controls ( $n = 2$ ) obtained from only one part of the lab procedures of one of the two laboratories involved. It would have been even more important to control for contamination during sample collection, by collecting control samples from the medical equipment and the local environment before and during the D&C or D&E procedure; however, the authors do not refer to such controls.

The reported fetal lung profiles obtained by 16S ribosomal RNA amplicon sequencing are very biodiverse and include species previously described as part of the so-called “kitome” (i.e., contaminating DNA present in DNA-extraction reagents) (4), suggesting that the reported results can at least partly be explained by contamination. This notion is further supported by the reported discrepancy between 16S-based and whole-genome shotgun sequencing, with the latter showing no bacterial signature at all. In addition, the comparison between microbial DNA observed in samples versus controls (Figure 1B of Reference 1) suggests the possibility of vaginal cross-contamination, as highly abundant genera in the vaginal tract, *Lactobacillus* and *Gardnerella*, are also the most abundant genera reported in fetal samples.



## Reply to de Steenhuijsen Piters and Bogaert



From the Authors:

We appreciate the thoughtful letter from de Steenhuijsen Piters and Bogaert in response to our recent report (1). Our proof-of-concept study is the first demonstration that a microbiome signature can be identified in human fetal lung tissues. Newer next-generation culture-independent methodologies, however promising, present numerous challenges and questions, especially with regard to issues involving methodology. Hence, we appreciate the opportunity to address and expand upon our findings, and we welcome a scientific discussion to provide some clarifications or answers.

We concur with de Steenhuijsen Piters and Bogaert that collecting fetal material from human subjects is extremely challenging, and pregnancy complications such as miscarriage could be accompanied by local inflammation. Nevertheless, in our study, all samples were collected from assumed normal subjects after an elective abortion and after informed consent was obtained. No samples were derived from a miscarriage, and therefore the possibility of inflammation/bacterial translocation due to miscarriage or membrane rupture is extremely low.

Given the complication of host DNA contamination coupled with low microbial biomass, these samples were indeed challenging to work with, as alluded to by de Steenhuijsen Piters and Bogaert and others (2). At the time of sample collection, just as with any routine medical procedure, all instruments were sterilized before each procedure (dilatation and curettage or dilatation and evacuation) in each patient. This was the first study to sample both placenta and deep fetal tissue (intestine and lung), and we agree with the concerns about the likelihood of possible contamination from nonbiological sources in low-biomass samples such as these. The abortion material was extracted through the vaginal canal, which is not sterile, and some of the material could have come in contact with the vaginal flora. However, our data showed statistically distinct signatures in the lungs and placenta from the same subjects, suggesting that the DNA signature was specific and not just the result of detecting a contaminant from the vaginal canal. Samples from twin pairs in the cohort also had different microbiome signatures even though they passed through the same vaginal canal. Moreover, a recent study showed no differences in the microbiome signature in placenta, fetal membranes, or meconium between vaginal and Caesarian-section deliveries (3). In addition, a study from our group demonstrated the presence of abundant immune cell signatures in the fetal lung as early as 11

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In addition, the lack of information about the indications for the D&C or D&E procedures makes us question whether pregnancy complications, such as miscarriage, which is likely accompanied by local inflammation (5), bacterial translocation, and infection, might be at the basis of their findings. All of the above information is essential to assess the potential biological origin of the observed bacterial DNA in fetal samples.

In conclusion, although we do not dispute the possibility of the existence of a fetal lung microbiome signature, the study lacked robust controls during both sample collection and laboratory processes, giving rise to speculation about the validity of the reported findings. Therefore, we believe that the presented data insufficiently support the authors' conclusion that the human fetal lung harbors a microbiome signature. We do agree, however, that the road on which they have embarked—studying fetal tissues at early stages of pregnancy—is an interesting and important one, and therefore warrants more research. ■

**Author disclosures** are available with the text of this letter at [www.atsjournals.org](http://www.atsjournals.org).

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