We read with interest the work of 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.
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.

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