MICROBIOME

Comment on "The placenta harbors a unique microbiome"

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Aagaard *et al.* present "an initial snapshot of the human placental microbiome and reveal which organisms are present, what they are capable of doing, and how the placental community is likely structured" in their recent *Science Translational Medicine* article (*1*). They used a culture-independent genomic approach to determine the taxonomic classification of the placental microbiome. These sophisticated and expensive methodologies are ultimately based on sequence analysis of polymerase chain reaction amplicons from the bacterial 16*S* rRNA (ribosomal RNA) gene (*2*) and sheared DNA fragments [in the case of whole-genome shotgun sequencing (*3*)]. These methodologies, however, do not differentiate between living, dead, or ruptured bacteria; bacterial fragments; or even diluted bacterial contents, because all of these sources could generate the same positive signals. The authors therefore bolster their argument that they have identified living bacteria in the human placental parenchyma by referring to other studies that purport to identify intact bacteria in the placenta, most notably the work of Stout *et al.* (*4*), although it is unclear how completely intact, fully laid-out bacteria could be visualized in 5-μm tissue sections.

The hypothesis that the human placenta harbors living bacteria is a radical departure from currently accepted dogma and the many years of pathologic examination of human placentas by many clinicians and researchers. Although the presence of bacterial rDNA (ribosomal DNA) in amniotic fluid has been previously well described (*5*, *6*), Aagaard *et al.* made it a point to distinguish their results from these previous studies by excluding fetal surfaces from their specimens and excluding placentas from cases of clinical chorioamnionitis. Therefore, when stating that the placental parenchyma harbors bacteria, it is imperative to rule out all other explanations before such a conclusion is presented for publication. The authors certainly present a convincing argument for lack of exogenous contamination during the collection and processing of the placental specimens. However, the most obvious and simple control experiment was not presented in this paper: that is, performing the exact same microbiome analysis of maternal blood collected from an arm vein or other remote location, at or very close to the time of delivery.

No matter how careful these researchers may have been in the processing of the placental samples studied, they could not eliminate the large amounts of maternal blood that would necessarily have been present in the intervillous space of each and every specimen they studied. Therefore, the microbiome signals they measured were just as likely from the maternal blood as from the placenta. Without including the maternal blood control, there is no way to distinguish between these two sources. It is logical, in fact, to conclude that the maternal blood contained rDNA signals from bacterial breakdown products, or even a few bacteria secondary to a low-grade bacteremia, and therefore, the intervillous space of each of these placentas contained the same few bacteria or bacterial breakdown products.

Viewing the data presented by Aagaard *et al.* from this perspective makes great sense. The location with the greatest bacterial diversity in the human body is not the vagina or the gut, but the mouth, with more than 700 bacterial species identified (*7*). As with other such interfaces in our body, there is a constant battle between these bacteria, tissue integrity, and our immune systems that work to keep these bacteria from entering our bodies. Like other battle fields, there is much death and destruction, and the breakdown products of this conflict are swallowed, spit out, or in many cases, cleared in our circulatory systems. In the absence of the essential control experiment to evaluate the contribution of bacterial DNA fragments that were carried to the placenta via the mother's own blood flow into the placenta, no cogent conclusions can be made about the presence or absence of living bacteria that may or may not be harbored in the human placenta.

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