De-Discovery of the Placenta Microbiome

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Historically, the contents of the gravid uterus were thought to be sterile during healthy pregnancy. Pathogens are known to be able to cross the placenta and infect the developing fetus, but this was believed to be in disease states only. Challenging the idea of the sterile womb, several papers have reported detection of bacterial DNA in placenta,[1-3] amniotic fluid,[4] and even the developing fetus itself,[5] supporting the idea that a rich microbial community normally exists in utero. These surprising reports have received considerable attention in the lay press. However, a paper by Theis et al[6] in this issue of *AJOG* outlines how laboratory contamination provides a fully satisfactory alternative explanation. Several other groups have also contributed parallel data.[7-9] To be fair, the demonstration that contamination provides an alternative explanation does not prove that the colonized womb hypothesis is wrong—it just leaves us with no strong reason to believe it's right. However, data coming from another direction appears to conclusively dispel the idea of a placenta microbiome.

The story started in 2014, when the textbook view of the sterile womb was challenged by Aagaard et al,[1] who reported detection of bacterial DNA sequences in placenta samples. Multiple different types of bacteria were identified. Matching of community patterns suggested that the placenta microbiome most closely resembled the oral microbiome, suggesting a specific body site of origin. This was followed up by a subsequent paper from Aagaard investigating the placenta microbiome in preterm birth.[3] In addition, similar application of DNA sequence-based methods led to proposals for a microbiome of the amniotic fluid.[8] Still more recently, studies using murine models led to the proposal of a microbiome of the fetus itself.[5]

However, a complication in studies of samples with low amounts of bacteria, like those from placenta tissue, is the existence of low levels of bacterial DNA in commercial reagents and laboratory dust. The DNA-based methods can be highly sensitive, so that even a handful of bacterial DNA molecules may produce a signal. Several authors have made careful studies of this background,[10,11] which has come to be nicknamed the "kit-ome." A paper in 2016 (from my group and a large team at Penn) compared bacterial sequences in placenta samples to the contamination background, and found no clear distinction.[7] The study was carried out using 2 different kits for DNA purification, and in each case the placenta sample matched the contamination background, but the background was distinctive for each kit. In contrast, maternal samples from oral sites, which are known to have high bacterial loads, showed strong signals that were consistent regardless of the DNA purification kit used. Analysis using quantitative polymerase chain reaction (qPCR) to detect bacterial DNA also showed no differences in abundance of bacterial sequences between placenta samples and contamination controls.

Thies et al[6] provides particularly compelling data in favor of contamination as the origin of the placenta microbiome. They used qPCR, 2 different types of high-throughput sequencing, and bacterial culture to investigate placenta samples for a possible microbiome. To avoid complications due to contamination during vaginal delivery, the authors used only samples from cesarean deliveries. They found no support for the idea of a placenta microbiome. Again qPCR showed no evidence for a placenta microbiome over background, sequencing yielded no consistent difference from negative controls, and 19 of 20 samples showed no bacterial growth on analysis by culture. The 20th sample did grow 3 bacterial species, but these were not detected in data from either sequencing assay, consistent with contamination.

Two other papers have compared placenta colonization in samples from healthy term deliveries to those from preterm birth samples (one paper again from the team at Penn). Neither found any detectable placenta microbiome from healthy deliveries, but in some cases possible bacterial colonists were seen in samples from preterm deliveries associated with chorioamnionitis,[9,12] as expected from clinical experience.

Another recent study investigated bacterial and viral colonists in amniotic fluid from healthy term deliveries, and found no evidence for a microbiome or virome over the kit-ome background.[8]

As mentioned above, none of this proves that the placenta, or the womb generally, is sterile. Supporters could just say that their methods were superior to those used where background only was detected. There are always at least small differences in protocols run at different centers, which could be proposed as the reason for differential detection.

However, many decades of work with germ-free mammals argues strongly that the fetus is sterile.[13] It has been shown for many mammalian species, including humans, that it is possible to generate germ-free neonates by sterile cesarean delivery, followed by transfer into a sterile isolator. If the womb were colonized, wouldn't you expect the microbes present to grow out in the isolated neonate? Germ-free

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Received Nov. 13, 2018; accepted Nov. 13, 2018.

The author reports no conflict of interest

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0002-9378/free

https://doi.org/10.1016/j.ajog.2018.11.1093

Related article, page 267.

neonates have been generated for mice, rats, guinea pigs, rabbits, dogs, cats, pigs, lambs, calves, goats, baboons, chimpanzees, marmosets, and humans (reviewed by Perez-Munoz et al[13]). The human cases involved pregnancies in which infants were expected to have severe congenital immunodeficiencies, so as a precaution the neonates were delivered sterilely and sequestered in sterile isolators. There they remained sterile.[14,15]

What would allow the idea of the colonized womb to be preserved, given what seem to be conclusive data for sterility from gnotobiotic studies? It is hard to propose a workable model, even allowing ad hoc wild speculation. Maybe the placenta is colonized, but the fetus is not. This would require that all of the members of the bacterial community in the placenta remain sequestered there, and we know that many pathogens pass through the placenta and harm the fetus, including *Listeria monocytogenes*, *Treponema pallidum*, *Plasmodium falciparum*, *Toxoplasma gondii,* and many viruses including HIV, cytomegalovirus (CMV), and Zika, leaving this model unappealing. Maybe some special nutrient is available in the womb that allows bacteria to grow, but is missing after delivery; if so, supporters of the colonized womb hypothesis need to find this nutrient. Maybe delivery and exposure to oxygen results in induction of latent bacterial prophages, which replicate and kill their bacterial hosts. However, although prophages are common in bacterial genomes, they are not universal, and the placenta has been suggested to harbor many different bacterial species. Again, this explanation seems far-fetched. Maybe mammalian neonates produce a highly active antibacterial compound upon delivery that kills off the microbes derived from the womb; if so, it would be fantastic to isolate this molecule and reveal it to the world, as it would likely be therapeutically useful. Thus efforts to support the colonized womb hypothesis—despite evidence from gnotobiotic studies—require wild speculation that really does need experimental support.

In summary, DNA sequence-based evidence for bacterial colonization of the placenta, amniotic fluid, and fetus has been challenged by four papers that suggest instead that these results reflect reagent contamination. The proposal for a colonized womb is at odds with the observation that germ-free mammals can be generated readily by sterile cesarean delivery and transfer to a sterile isolator. If you wanted to design an experiment to test the sterile womb hypothesis, this seems to be about as good as you could do—remove the neonate from the womb in a sterile fashion, and see if it remains sterile. In fact, it does. At present, the sterile womb hypothesis seems to be by far the best fit to the data. ■

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