

# Infections linked to premature births more common than thought

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Previously unrecognized and unidentified infections of amniotic fluid may be a significant cause of premature birth, according to researchers at the Stanford University School of Medicine.

An analysis of amniotic fluid from women in preterm labor indicated that 15 percent of the fluid samples harbored bacteria or fungi - an increase of 50 percent over previous estimates. The heavier the burden of infection, the more likely the women were to deliver younger, sicker infants.

"If we could prevent these infections in the first place, or detect them sooner, we might one day be able to prevent some of these premature births," said research associate Dan DiGiulio, MD, who conducted the study in the laboratory of senior author David Relman, MD. About 12 percent of all births in this country are premature and the frequency of premature birth is increasing.

"To find that this amniotic compartment, which we have traditionally viewed as somewhat sacrosanct, is infected significantly more often than we thought is a little shocking," said Relman, professor of infectious disease and of microbiology and immunology. Previous analyses of the same samples using a different, more conventional method had concluded that only about 10 percent were infected.

DiGiulio and Relman collaborated with researchers at Wayne State University in Detroit, the Detroit Medical Center and the Eunice

Kennedy Shriver National Institute of Child Health and Human Development to conduct the research. DiGiulio is the first author of the study, which will be published in the Aug. 26 issue of *PLoS ONE*.

Relman received a 2006 Pioneer Award from the National Institutes of Health and is using the proceeds to characterize the microbial communities indigenous to humans and understand the roles of these communities in health and disease.

For this study, DiGiulio used a highly sensitive technique called polymerase chain reaction, or PCR, to track down micro-organisms in amniotic fluid samples collected from 166 women in preterm labor at the Detroit Medical Center between October 1998 and December 2002. Of these, 113 women went on to deliver their infants prematurely. Although not all these women were infected, such microbial invasion is thought to contribute significantly to the overall number of preterm births.

Previous efforts to conduct a census of bacteria, fungi and other living organisms in the fluid relied primarily on culturing, or growing, the invaders in the laboratory after withdrawing some of the liquid through a needle inserted into the amniotic cavity. But not every microbe is amenable to such a rough-and-tumble change of quarters.

"We've become very good at growing some bacterial species - what might be called the 'weeds' of the microbial world," said DiGiulio. "But many other species can't be cultured by current methods." In contrast, the PCR technique used by the researchers tracks down and copies small portions of DNA encoding a cellular component called ribosomal RNA that is shared by all living creatures - kind of like screening for "all people with fingers." Small differences among the fingerprints identify individual species.

In this way, the researchers cast a wide net and got the results to show for it. They used both PCR and laboratory cultures to determine that 25 of the 166 samples were infected with either bacteria or fungi. Seventeen bacterial and one fungal species were identified in the positive samples - far more than the 11 species found by conventional analysis - including some not previously suspected to play a role in preterm delivery. Furthermore, the investigation turned up a novel microorganism that may represent a previously unknown species.

One of the study's collaborators was Roberto Romero, MD, chief of the Perinatology Research Branch of the Eunice Kennedy Shriver National Institute of Child Health and Human Disease. Romero is also a professor of molecular obstetrics and genetics at Wayne State University. He is well-known for his research showing that infection is a common and important cause of preterm labor and delivery, and that affected fetuses may be predisposed to short- and long-term complications.

Infection of the amniotic fluid likely contributes to preterm delivery by causing an inflammatory response in the pregnant woman. It occurs most commonly either by microbes from the vagina that infiltrate the amniotic sac, or by microbes that travel through the mother's bloodstream from other parts of her body, such as the mouth. In fact, both gum disease and bacterial vaginosis have been shown to increase a woman's risk of delivering her baby prematurely.

DiGiulio and Relman found that all the laboring women whose samples were positive by either PCR or by culture delivered their infants prematurely. In addition, all those who were positive by both methods delivered their babies within one day of the initial fluid collection, and 68 percent of those that were positive by only one, but not the other, method delivered within the same time period.

They also found that 27 percent of samples from infected women who

delivered their infants before 25 weeks gestation were positive only by PCR; cultures from these samples falsely indicated they were uninfected. Because only about 30 percent of infants in this age group survive their early birth, it is extremely important to be able to accurately diagnose infection in these women.

Finally, as the burden of infection increased, the average gestational age of the newborns decreased. That is, a woman with many copies of bacterial or fungal rRNA, which suggests the presence of many bacterial or fungal cells, was likely to deliver a very young, very sick infant. Relman and DiGiulio are now collaborating with Romero's group on a much larger study of fluid collected during routine amniocenteses at about the 20th week of pregnancy. They hope to determine whether infections can be detected before the onset of preterm labor, which could lead to new prevention or treatment strategies.

"Although we know now that humans should respect and even cherish their microbial symbionts," said Relman, "this type of infection reflects a situation in which our microbial companions have overstepped their bounds. Something has gone awry with this balance."

Source: Stanford University

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