A few years ago, then-pregnant virologist Carolyn Coyne (above, purple shirt) wondered whether work she’d been doing with viruses was safe for her developing baby. She and Elizabeth Delorme-Axford (9 months along herself, above), and Yoel Sadovsky (next page photo) went on to unravel how the placenta protects the fetus.
A grad student looking for a dissertation project. A mentor/virologist with a sudden personal interest in the fetus. Said student coincidentally sitting in on a lecture given by a man who has dedicated his research life to women’s and fetal health. These are apparently the optimal preconditions for creating one of the first teams to begin sorting out how the placenta protects the fetus from viral infection.

Elizabeth Delorme-Axford (PhD ’13) was the student. (She is now a postdoctoral associate in microbiology and molecular genetics here at the University of Pittsburgh.) In 2008, while rotating through labs, she alighted in that of virologist Carolyn Coyne, PhD associate professor of microbiology and molecular genetics.

“I was pregnant, and no one knew,” says Coyne. “I was sitting under a tissue culture hood, purifying viruses. It was pretty early on in my pregnancy, and I thought, Should I be doing this?” She wondered whether the viruses she’d been working with might harm her developing baby. To Google she went.
Perhaps, Coyne thought, Delorme-Axford—known?!

Coyne lab after finishing her rotations. She was interested in helping her turn the unknown into the known. And she was.

“I’m really interested in women’s health,” says Delorme-Axford, “and I know that it’s not as studied as you might think it would be. But then I finished my rotation in Carolyn’s lab and moved on to my other graduate student rotations. On my third rotation, I was at the Eye and Ear Institute and saw a lecture given by Yoel …”

“Yoel” is Pitt’s Yoel Sadovsky, an MD, Elsie Hilliard Hillman Professor of Women’s Health Research, professor of obstetrics, gynecology, and reproductive sciences, as well as of microbiology and molecular genetics, and director of the Magee-Womens Research Institute. Delorme-Axford knew that Sadovsky worked with primary placental cells, or trophoblasts, which play an important role in embryo implantation and interaction with the uterus. These cells, she and Coyne thought, were likely to play a vital role in keeping viruses hosted by the mother from infecting the developing fetus. Delorme-Axford had returned to the Coyne lab after finishing her rotations. She wondered whether Sadovsky would be interested in lending a hand. And some of those placental trophoblasts.

He was. “He’s such a positive personality,” says Coyne.

Sadovsky does fairly beam, even when he talks about prospective bad outcomes or things not yet understood. Not because any of it is funny, but because bad things and lingering questions afford the opportunity to ferret out the unknown. Recently, he related the story of his collaboration with Coyne’s lab, beginning with the knowns:

“Viral infections … are one of the major insults during pregnancy. They can cause fetal death, small brains, abnormalities in the eyes, the ears, the heart. … Babies can be too small at birth [because of such infections], which causes complications later on.”

A gang of pathogens known collectively as TORCH (toxoplasma, “others,” rubella, cytomegalovirus, and herpes) is responsible for various congenital defects. Also about 4 million children, who contracted HIV in utero, have died of AIDS since the epidemic began. Yet, most viruses carried by the mother are thwarted, somehow, before they can cross the placenta and invade the fetus.

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Sadovsky’s advice took inspiration from another project under way in his lab. His work told him that placental cells produced all manner of vesicles (bubbly little organelles that play roles in metabolism, cellular transport, and enzyme storage, among other functions). Some of these vesicles, called exosomes, contain microRNAs, which help regulate gene expression. And, Sadovsky says, certain microRNAs are produced in great abundance only in the placenta and invade the fetus.

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As Carolyn says,” Sadovsky continues, “there is very little understood about how viruses cross the placenta. Some do, and some don’t. Some infect at delivery, others throughout pregnancy, and others, none of the above.”

So, equipped with Sadovsky’s primary placental cells, Coyne and Delorme-Axford went to work. (They’d started a study with immortalized placental cells bought from a lab supplier. But, Sadovsky conjectured, placental trophoblasts cultured in his lab would be much better suited for the work because they’re more closely related to placental cells in vivo.)

“This is what really launched this project,” Coyne says. “We work with these viruses that are highly infectious. They infect many human cell types. But when we tried to infect these [primary] placental cells, there was almost zero infection.

“I remember saying to Elizabeth, ‘This is really odd. These viruses infect lots of cells, and they infected the [other] placental cell lines, but not these primary cells [from Sadovsky].’

“Then I said to her, ‘Elizabeth, just go to the freezer and let’s see what other viruses we have.’” (Because Coyne and Delorme-Axford were exploring viral resistance rather than pathways of infection, they didn’t start off with viruses, like those in the TORCH series, that are typically capable of infecting a fetus.)

“We found that nothing we had on hand could infect these [primary] placental cells.”

Interesting? Sure. Entirely unexpected? Not really. But—and this is the big question—why was this the case?

Coyne also has an abiding interest in innate immunity, the essential immune system of individual cells that allows them to fight off infection. And one of the hallmarks of cellular innate immunity is the cell’s ability to spit out antiviral factors as a means of tripping up viral invaders.

“Wouldn’t it be cool if these placental cells just happened to release certain cytokines that are antiviral?” Coyne recalls thinking.

She then came up with a way to verify her suspicion. She asked Delorme-Axford to take the

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to sonication, a process involving sound waves (not the same type used in maternal-fetal ultrasound) that can destroy membranes, like those that are part of microRNA-carrying vesicles. When the trophoblast-conditioned medium was subjected to sonication, nonplacental cells introduced into it could no longer resist viruses. That’s because sonication destroyed the microRNA-laden exosomes that target the microRNAs to the cells, eliminating the antiviral protection conferred by the conditioned medium.

“If you depleted these little microvesicles from the medium, you completely lost the antiviral effect,” Coyne says. But, she adds, if you reintroduce lab-cloned microRNA to the mix, the effect is restored. These exosomes and their microRNAs, then, seemed to be the key to the placenta’s defense system.

MicroRNA was discovered in 1993. This class of noncoding RNAs is commonly understood as a source of gene regulation, assisting in the vital task of keeping cells healthy. Some microRNAs also help determine a cell’s fate. (A particular microRNA is responsible for turning certain cells into neurons, for example.) Benjamin tenOever, who is a PhD, professor of microbiology at Mount Sinai School of Medicine, an RNA expert, and a compatriot of Coyne’s, says the Pitt team has made a significant leap forward by discovering RNAs’ newfound role as an antiviral in fetal health.

“It’s an amazing body of work,” he says. “This is a very exciting new idea. These findings change the way in which we think about how the body can deal with viral infection.”

Yet having identified the placenta’s armor isn’t enough.

“We then spent a good amount of time figuring out what sort of mechanism is at work on the cell biology side,” Coyne says. “And I think the credit here goes to Elizabeth. As we started going through the process of thinking about what host cell pathways are involved, … she suggested autophagy.

Autophagy (from the Greek: auto “self” and phagein “to eat”) is the process by which cells degrade unnecessary or broken parts. When the process takes place in this milieu, it destroys the virus by shuttling the viral vesicles to the cell’s lysosome, which is full of enzymes that digest the bad stuff.

“So,” Sadovsky says, “if we take the microRNA, and we put it on the recipient cells, we not only cause resistance to viruses, but we also stimulate autophagy. And if we block autophagy, we block this resistance. We’re not sure if this is the only [process involved] or exactly how it works, but we are pretty sure that this is true.”

The resulting paper was published in the *Proceedings of the National Academy of Sciences* in July. And, before that, says Coyne, “I presented this for the first time (in 2012) to the American Society for Virology’s meeting, and it was a huge hit. I think that was because, to virologists, this was something they hadn’t thought about. Perhaps it required the aligning of the stars, getting the right people together at the intersection of several disciplines.”

Sadovsky likes to think of the placenta project as beginning to fill in a “black hole in biology.”

“I have to regretfully admit that the field of placental biology is fairly rudimentary,” he says. “Most people seem to think that whatever happens in pregnancy just happens, and then life starts. Nowadays, we know that many adverse things can occur to pregnant women that have adverse effects on the child’s early development and even into … health as adults.”

The Pitt team is aching to find out exactly what these very helpful microRNAs target: “Yoel is pursuing the target gene,” Coyne says. “The researchers are also identifying pathways leading to ramped up autophagy.

Along the way, they hope to find clues that will allow doctors to stymie the viruses that are able to slide past the placenta’s defenses and attack the fetus.

“With most infections, the patient does not know [that she is infected]. She may have a period called viremia in which the virus is systemic in the bloodstream,” Sadovsky says.

“We screen for viruses, but we don’t yet understand the mechanism by which they cross the placenta and infect the fetus; nor [do we] have an effective way to stop this.”

There may be more to the story. It’s possible that this collaboration will bear fruit outside the confines of the womb.

“Perhaps we can use the vesicles or the microRNAs from the placenta to bestow viral resistance outside of pregnancy. This could become a new paradigm for treating viruses in humans,” Sadovsky posits.

Beyond viral resistance, Coyne notes, there is a litany of human diseases—many of which fall under the umbrella of neurodegeneration—that are caused by defects in autophagy.

“These microRNAs could prove some therapeutic benefit just by being able to robustly induce autophagy,” she says.

Sara Cherry, an associate professor of microbiology at the University of Pennsylvania, says “the application of autophagy-inducers as potential therapeutics against viruses is real.” (She knows Coyne from another collaboration.)

It’s particularly impressive to her that the Pitt team has done this work without the aid of an animal model (“mouse placentas are very different” from humans, she says) and without much existing literature laying the groundwork. Before the researchers head down the road to novel therapeutics, they will lengthen the roster of pathogens they study.

“We’re adding other viruses and other nonviral pathogens that are [potentially dangerous in pregnancy],” such as *Listeria*, *E. coli*, and *Salmonella*, Coyne says.

Sadovsky is ready for that animal model. “We’re trying to create an in vivo model...