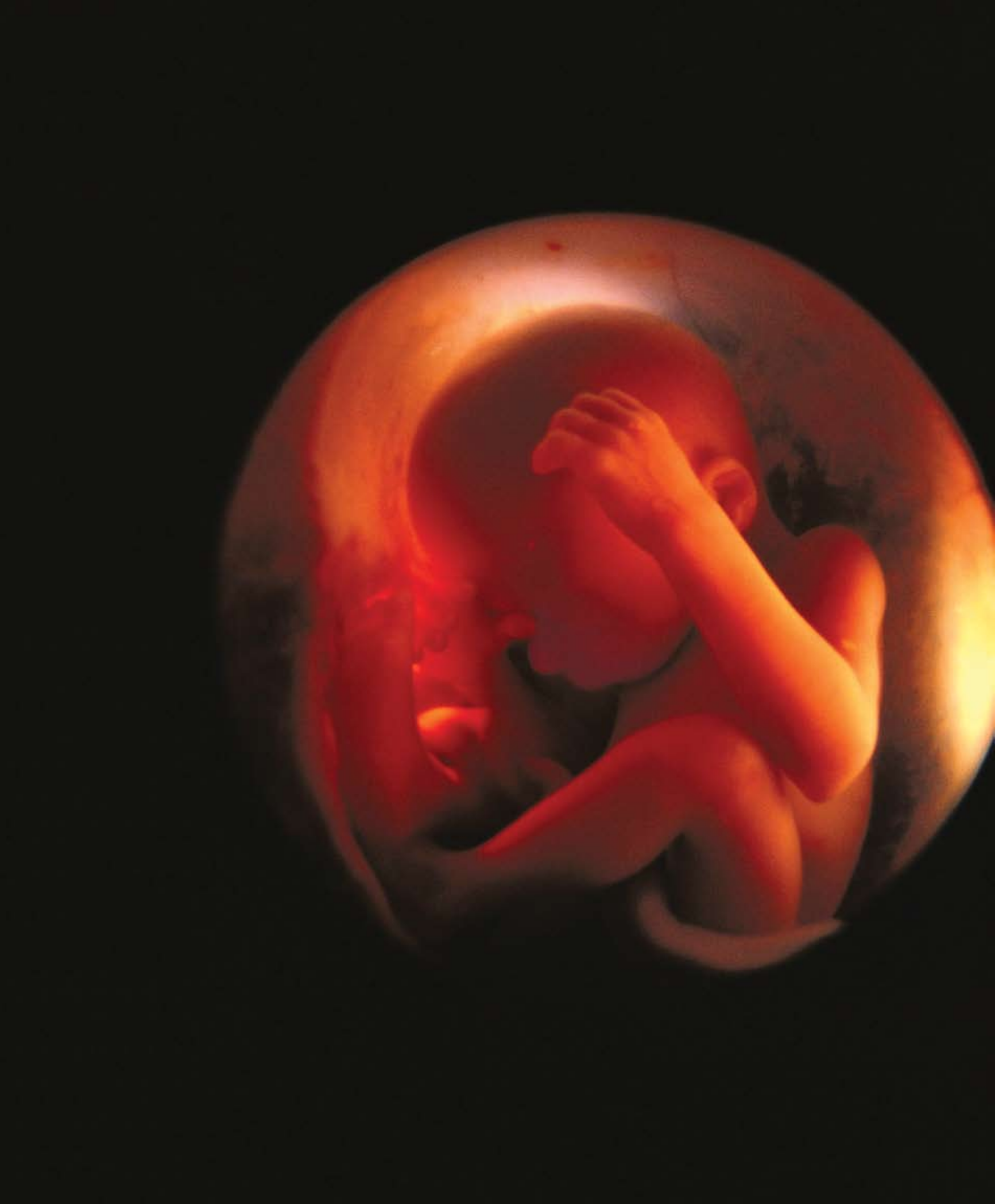


UNVEILING THE MOLECULAR MASTERPIECE AT THE BEGINNING OF LIFE | BY CHUCK STARESINIC

SHOWTIME

The ovaries of a fertile woman release an egg roughly once every 28 days. Among the billions of cells in her body, this one is an oddball. The largest cell in the body, it is actually visible with the naked eye. With only 23 chromosomes, it's also a halfling. It will begin to degenerate in about 24 hours unless it melds with a sperm cell—a much smaller and highly mobile chromosome-delivery mechanism—containing a complementary set of 23 chromosomes.

After fertilization, there is a short period of time when this speck of nascent human life is completely unmoored from the mother who will harbor it for the next nine months. The fertilized egg traverses the fallopian tube like a rudderless skiff on a lazy river. The journey can take a week, during which the cell divides repeatedly until it is a mass of a few hundred cells called a blastocyst. It arrives in the uterus untethered—in the womb but not yet of the womb. What happens next is critical. If it does not implant in the lining of the uterus, then there is no pregnancy; the little ball of cells will wither and fade, and nobody will ever know it existed.

Yoel Sadovsky assumes that every pregnancy involves insults or injuries to the placenta. What makes one pregnancy succeed, while another fails?

PHOTOGRAPHY | LENNART NILSSON

If it implants improperly, the pregnancy may later result in a spontaneous abortion or a fetus that ceases to grow at the normal rate and becomes a sick child.

For this condition, physicians use the phrase "intrauterine growth restriction," or IUGR. To expectant parents, it simply means that the fetus isn't growing as it should, and it is scary news. A pregnant woman may feel (justifiably, in a Darwinian sense) that the most important task she has ever tackled is to grow this baby. She does this mainly by maintaining her health and consuming extra calories and sufficient amounts of fat, sugars, protein, and other nutrients. A diagnosis of IUGR can therefore feel like a personal failure. However, the most common cause of IUGR is genetics, which are beyond the mother's control. The origin of the problem may go back to the earliest stages of development, even before implantation.


ROB KELLY

Sadovsky

Upon arriving in the uterus, the blastocyst is already divided into two distinct cell types. The inner mass of cells is meant to develop into the fetus. The entire outer layer is made up of cells called trophoblasts, which must form the placenta and the membranes that envelop a developing child. The trophoblasts are invasive cells, and that quality is key to anchoring the blastocyst in the uterus. Molecules extending outward from the lining of the uterus slow the movement of the blastocyst and draw it near, like a boat come to shore in the weeds. The trophoblasts that come into contact with the uterus invade the woman's tissue. The shape of the blastocyst changes from a sphere to an oblong mass. It seems to sprout tentacles that burrow, causing a small amount of maternal blood to be shed. The cells of the uterus both welcome the incursion and moderate it through the release of regulatory molecules.

Once firmly attached, these trophoblasts become the placenta. Four weeks later, it is a disk of tissue less than two centimeters in diameter. It will grow much larger, but even at that size the basic shape and structure of the mature placenta have been laid out. It is the fetus's extension into the mother and the interface between the two. Fetal blood will enter the placenta through arteries in the umbilical cord and flow into successively finer branches until it reaches tiny capillary loops that come into contact with maternal blood that pools in the placenta. The life-sustaining exchange of nutrients for waste products takes place through gossamer capillary walls. Rejuvenated blood flows back to the fetus via the umbilical vein; the mother's blood flows back to her through the uterine veins.

That's what happens when everything goes well. A placenta that functions improperly fails in subtle ways—transporting oxygen or nutrients insufficiently and stunting the growth of the fetus. An expectant mother may respond to a diagnosis of IUGR by second-guessing herself: *Did I take enough prenatal vitamins? Did I not eat enough protein back when the morning sickness was at its worst? Enough fish? Did too much fish give me mercury poisoning?* If only it were that straightforward.

Yoel Sadovsky is an obstetrician/gynecologist by training. He also is a bench scientist at the University of Pittsburgh School of Medicine who is after the very roots of the problem of fetal growth restriction. From the first week of his ob/gyn residency in 1986, his scientific interest has become progressively smaller (physically at least)—from the expectant woman in the exam room, down to the eight-cell blastocyst, to the genes that are responsible for successful implantation in the uterus.

Most of us think of the genome as a static entity, like an instruction manual. But genes change in that they switch on and off over time. For that reason, a snapshot of the genome is not enough to really understand genetics. Something approximating a movie or a light show would be better. The viewer would see great twisting ladders of DNA with broad sections that alternately glow, twinkle, project a searing light, then fall dark. If you paid close enough attention, you'd see patterns. Some genes that are far apart actually seem to be in dialogue—one lights up in response to another and then goes dark in response to a third. Other genes remain dark until outside influences—such as an injury or toxin—set them aglow.

Sadovsky is one of the leading scientific investigators uncovering what is behind this light show at the time of placental development. In the past decade, his lab has told us much of what we know about which genes light up in specific situations of fetal growth restriction, such as insufficient oxygen transport (hypoxia). Of equal importance to him are the genes that light up in pregnancies that result in normal-weight babies. In Sadovsky's view, every pregnancy may suffer damage. The light show is a series of actions and reactions—some with positive effects and others with negative—on the part of the mother and the fetus.

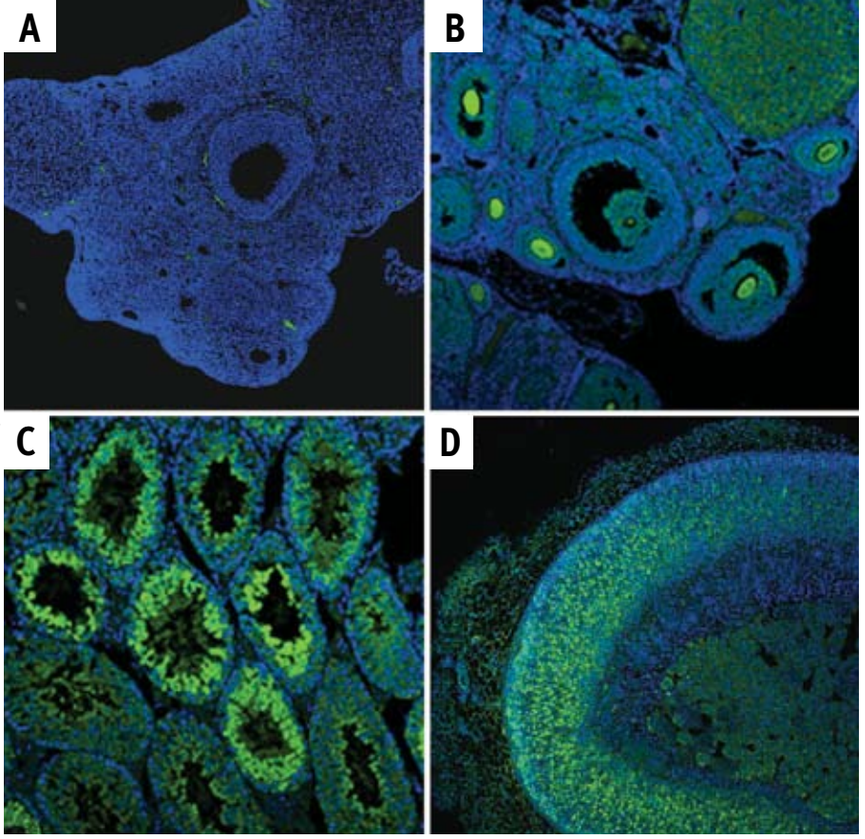
"Our assumption is that in every pregnancy, the placenta is exposed to different kinds of injuries or insults," Sadovsky says. "Insults can be different nutrients that the mother is eating. Insults can be a diminishing of the blood supply to the uterus. Insults can be different underlying diseases of the woman. In most of these pregnancies, the result is normal and the baby is alive and everything is fine. But in other pregnancies, the result becomes abnormal and, unfortunately, the range can be anything from just having a little bit of a small baby to having a baby who dies *in utero* or is developing inappropriately."

Sadovsky almost seems too big for the unexceptional, temporary office he occupies on the seventh and penultimate floor of the Magee-Womens Research Institute (MWRI) in Pittsburgh. He is moderately tall, with a boyish face and demeanor and wisps of brown curls. He reacts to others with a gentle sort of wonder and interest. He sits at a desk in a brown snowflake-patterned sweater. A round table and two thin chairs are tucked between him and the door with little room to spare. There is one window. With a bit of neck craning, it's possible to see the two narrow lanes of Craft Avenue below and, beyond, the red brick walls of Magee-Womens Hospital of UPMC. For a recent presentation, Sadovsky says he took a street map and drew a bridge linking the research institute and the hospital.

"I call it the 'Bridge over Craft Avenue,'" he says with a smile, liking the dramatic sound of his metaphorical creation. "That's what captures my job here. A virtual bridge of knowledge, education, and translation of discoveries to the bedside."

Since 2007, Sadovsky has been the scientific director of this institute and the Elsie Hilliard Hillman Professor of Women's and Infant's Health Research in Pitt's Department of Obstetrics, Gynecology and Reproductive Sciences. For the first time in his career, he does not provide direct care for patients. Instead, he devotes all of his time to laboratory research and the leadership of a cadre of scientists studying the health of women and infants at MWRI.

To the lay reader, Sadovsky's scientific papers—more than 80 since 1992—may as well be written in Sanskrit. He does not publish papers with titles like "Folic acid is associated with fewer birth defects." His papers bear titles such as, "Microarray-based identification



MOUILLET JF, YAN X, OU Q, JIN L, MUGLIA LJ, CRAWFORD PA, SADOVSKY Y. "DP103 DEFICIENCY AND REPRODUCTION." ENDOCRINOLOGY VOL. 149 NO. 5 2168-2175. © 2008, THE ENDOCRINE SOCIETY

In a 2008 paper in *Endocrinology*, Sadovsky and colleagues looked at a protein called DP103 to try to understand its role in reproduction. Stained green, DP103 was not detected in the control (A), which consisted of rabbit serum; it was quite visible in mouse ovaries (B), testes (C), and adrenal glands (D). To the researchers' surprise, mice engineered to lack this protein died very early in embryonic development, prior to implantation in the uterus, pointing to a critical, unknown role for DP103 in the blastocyst.

of differentially expressed genes in hypoxic term human trophoblasts and in placental villi of pregnancies with growth-restricted fetuses" (*Placenta*, 2005). Got that?

"What are the mechanisms that the placenta utilizes to respond to these types of injuries?" Sadovsky asks rhetorically. "Some proteins and some pathways we are pursuing actually enhance the ability of the placenta to withstand injury. Or some of them may actually potentiate injury. So, using samples from pregnant women and also using mouse systems, we are trying to understand the effect of the environment on placental gene expression. How do proteins within the placenta either accentuate the injury or mitigate the injury? The goal of this line of research is to identify pathways that we can potentially regulate to improve pregnancy outcomes. Some of these proteins that we are after can be regulated by drugs, so pharmacology is one way to do it. Another is molecular biology—regulation of gene expression by gene therapy, which is not yet here in pregnancy. But I think that a lot of the research we are pursuing right now may lay the groundwork for gene therapy in 10 or 20 years."

Sadovsky's scientific peers and colleagues say that they admire his implacable drive to take the most cutting edge-science available and look for ways to move it to real-world applications. For example, with colleagues at Washington University in St. Louis—his professional home from 1993 to 2007—Sadovsky became interested in a gene that was suspected to be involved in the release of the labor-inducing hormone prostaglandin at the onset of labor. He wrote a grant to study an animal model with this gene knocked out.

"He took the results of those animal studies and applied [them] specifically to humans," says Michael Nelson, one of Sadovsky's longtime scientific collaborators at Washington University. "He collected specimens from human pregnancies, including [placental tissues]" and studied gene expression and prostaglandin production and came to the conclusion that this gene was a key component of prostaglandin production in both premature and full-term labor. "Then he took it one step further," continues Nelson, explaining that this was when the drug celecoxib, which inhibits the expression of this same gene, had just gone on the market for arthritis treatment.

"The question was, could [celecoxib] be used safely in pregnancy for preterm labor? So he spearheaded the development of a protocol … . It was going to go to a randomized trial to show it was as safe as the other medications that were being used for preterm labor," says Nelson. Then the Vioxx scandal hit. Both drugs act on the same target, and it suddenly became a nonstarter, despite the fact that they are drastically different drugs.

"Celecoxib is still on the market and doing well," says Nelson. "It's just not yet been tested for preterm labor. There are some studies—one of the first ones was the one that Yoel spearheaded—but it's difficult to do clinical studies in pregnancy because of the concern for the mom and the fetus. The possibilities of adverse effects are untoward." Nelson expects studies like Sadovsky's, plus the slow accumulation of a preponderance of anecdotal evidence that the drug could be safe and effective in preterm labor, will eventually make another trial possible.

In the very first week of his residency, in 1986, Sadovsky was one of several new interns in a gaggle of doctors making rounds in the obstetrics and gynecology department at Washington University. He was 29 years old, with a crown of unruly curls and, in his own words, "a funny accent." (Born, raised, and educated from childhood through medical school in Jerusalem, Sadovsky had arrived in the United States less than a month earlier.)

The group was to cover each patient on the floor, with the interns presenting some patients and Nelson, a serious and meticulous man who had just recently been made attending physician, going over the management plans with the chief resident and the fellow. In front of one patient, Nelson made a comment about the success rate for women attempting to deliver a child vaginally after having previously birthed a child by caesarean section. But he got it wrong, and the curly-haired new guy with the funny accent knew it.

"He challenged that statement," says Nelson, who later checked the literature and found that his new intern was correct. "And it was in a very academic way; it wasn't like he was putting me down. But it was clear that he knew what he was talking about and that he had read the literature—even as an intern, which is pretty amazing, because there is so much to learn.

"In the first week that I worked with him, I knew he was one of the smartest people I'd ever seen in a residency," Nelson continues. "I said at that time, 'I want to work with this guy.'"

Today, Sadovsky seems slightly embarrassed that Nelson persists in telling this story after the 20 years that they have been close friends

and colleagues. Trying to duck the compliment, Sadovsky shrugs and says, "For him, it was unusual because he is a very knowledgeable person. He also has a very good memory."

After the four-year residency, Sadovsky undertook a fellowship in maternal-fetal medicine at the University of California, San Francisco, considered *the* place to train in women's reproductive health. There, he came under the tutelage of James Roberts, a well-known expert in high-risk pregnancies who ran the fellowship program.

For Sadovsky, who had just completed his clinical training, Roberts was a role model of what it meant to be a physician scientist. When Sadovsky stayed on at UCSF for an extra year to do research, Roberts made an interesting career move: He left UCSF for the University of Pittsburgh. It was 1992, and Pitt had taken the unusual step of creating a research institute dedicated to women's health. Roberts would become the first director of the Magee-Womens Research Institute.

Within a year of leaving UCSF for Pittsburgh, Roberts tried to recruit Sadovsky to his fledgling institute. At the same time, Nelson was recruiting him back to St. Louis. Sadovsky chose the latter, unknowingly delaying his arrival in Pittsburgh by 15 years.

Nelson recalls meeting up with Roberts at a grant review session that year.

"You won," said Roberts.

People in Pittsburgh, even within the University of Pittsburgh, may not be aware of what they have in MWRI. It's now a research powerhouse with a scientific focus that makes it unique in the nation. Established in 1992, it was the first independent research institute dedicated solely to the health of women and infants. Starting from almost nothing, it is now the largest such institute by far. Since 1992, it has brought more than $185 million in project funding to Pittsburgh from the National Institutes of Health and other sources.

The scores of investigators who do the scientific work at Magee have one thing in common, says Sadovsky: a scientific interest in the health of women and infants. Yet their academic homes in Pitt's schools of the health sciences are all over the departmental map: epidemiology, surgery, oncology, infectious diseases, psychiatry, pediatrics, pharmacology, and microbiology and molecular genetics, to name just a few. Their scientific tools, perspectives, and approaches are equally diverse. Having a group of scientists this large and diverse under one roof with one overarching purpose is unheard of in women's health.

As an independent research institute affiliated with the University of Pittsburgh, the institute alone is something special. In combination with its partner hospital across Craft Avenue, it presents opportunities for scientists that are otherwise hard to come by.

"It's the combination of strong research and strong clinical volumes side-by-side," that is particularly valuable, says W. Allen Hogge, professor and chair of Pitt's Department of Obstetrics, Gynecology and Reproductive Sciences. Magee is one of the highest-ranked and busiest women's hospitals in the nation, allowing investigators to easily plan joint projects that include both a basic science and clinical component. "There's also a huge database of biological samples [such as placental tissue], so it's one of the few places that all of the barriers that normally get in the way of research that involves clinical and basic science are taken away," Hogge adds. "The barrier is the width of Craft Avenue, and that's fairly unique. I don't know of another place in which the clinicians and investigators are as closely aligned geographically and scientifically."

Hogge's department, which includes many of the approximately 90 MWRI investigators, can be considered a boon to the institute, as well. "The department is far and away the most funded ob/gyn department in the country," he says, "with over $30 million of federal funding. There's nobody that comes close to that."

Of the position Sadovsky accepted at Magee, Nelson says, there's no job like it: "Magee-Womens Research Institute is a unique setup. There's no question in my mind."

Sadovsky is motivated by the opportunity he sees in Pittsburgh to change how scientists view the field of women's health, which he says has long been undervalued. "Many issues that occur in women were perceived as normal physiology," he says. "Like osteoporosis was considered normal physiology. Women after menopause are a little bit slower, have more fractures, and so forth, and this was considered normal. And a lot of disorders of pregnancy were just accepted as inevitable."

The field does not have the same prestige as, for example, neurobiology, immunology, or pathology. Young physicians considering the field may be told it's all about "catching babies" or doing hysterectomies. At a place like MWRI, Sadovsky says, it's an exciting opportunity to use the best tools that science has to offer in a historically neglected area.

Roberts has left a significant legacy for Sadovsky to build upon. He guided MWRI for 15 years, giving up the directorship in 2007 to concentrate on his own research on preeclampsia, a leading cause of preterm labor. A $31 million expansion in 2007 doubled the institute's research space. The 70,000-square-foot addition offers stunning views of Pittsburgh's hills and the Monongahela River, plus open rows of laboratory benches that run the full length of the building—independent scientists can literally take down the walls that separate them and expand or join their labs if they choose to do so.

Sadovsky is pursuing a commensurate expansion of core scientific capabilities at the institute. These include a histology core facility staffed with experts able to do detailed analysis of tissue structures, physiology, function, and hormone levels. This is how an incidental finding in something like a mouse with a gene knocked out can quickly turn into a meaningful discovery.

Sadovsky wants to recruit a cadre of mouse experts to populate the institute, too, because the mouse is such a fundamental tool for understanding physiology and the function of proteins. He envisions involving teams of researchers in computational biology from Pitt and neighboring Carnegie Mellon University.

The energy is attracting some existing Pitt labs to set up shop at Magee, as well. A proteomics core from the University of Pittsburgh Cancer Institute moved to MWRI in 2007.

"Proteomics is a cutting-edge, complex technology that deals with understanding protein structure as a means to make predictions of protein function in physiological conditions and diseases," Sadovsky says. "As you can imagine, both our institute and the cancer institute would focus on gynecological cancers. I think, together, building on technology such as proteomics, we can quickly go a long way."

Roberts, who once had qualms about leaving the leadership of MWRI to another scientist, admits that he has none now. In 2007, nearly 15 years after he ran into Michael Nelson and congratulated him on his recruitment of Sadovsky to Washington University with the words, "You won," Roberts bumped into Nelson at yet another professional meeting.

Nelson looked at him for a moment and said, "No. You won." ■

PHOTO/© LENNART NILSSON/ALBERT BONNIERS FORLAG AB, A CHILD IS BORN, DELL PUBLISHING COMPANY.

A NATIVITY STORY

AS ROBERTS LED RESEARCH ON PREECLAMPSIA, HE OVERSAW THE BIRTH OF AN INSTITUTE

BY CHUCK STARESINIC

James Roberts actually didn't like doing research. His heart was in the clinic. This may be surprising news for anyone who knows him as the founding scientific director of the Magee-Womens Research Institute (MWRI) and a University of Pittsburgh professor of obstetrics, gynecology, and reproductive sciences and of epidemiology. But that was in the late 1960s, during his residency in obstetrics and gynecology at the University of Michigan, and he felt that he didn't need any distractions from a job that was demanding enough—caring for the health of his patients.

That all changed during his maternal-fetal medicine fellowship at the University of California, San Francisco. He was given a research project and protected lab time.

"It was thrilling," he says now. For the first time, he was involved in uncovering mechanisms underlying physiology. In this case, they were looking at the myometrium—the muscle tissue that makes up most of the uterus—of rabbits and identifying the receptors on these muscle cells that were responsible for initiating contractions of the muscle. It led to publication in *Nature*, and the experience of discovery changed Roberts' direction.

He has now published more than 230 peer-reviewed papers, and his lab is the leading group in the country looking at mechanisms responsible for preeclampsia, a common pregnancy disorder that can cause preterm labor and lead to complications for the mother and child. This spring, a federally funded trial, led by Roberts and aimed at reducing the number of women who suffer from preeclampsia, reached its recruiting goal of 10,000 women.

The scientific work that makes this trial possible began more than 20 years ago, when Roberts and colleagues, including Pitt assistant professor Carl Hubel, submitted a grant proposal based on the hypothesis that a major mechanism for preeclampsia, which is marked by high blood pressure in the mother, could be found in the endothelium, the layer of specialized cells lining our blood vessels.

"Nobody had ever, to our knowledge, made this connection before," says Roberts. "But it was the kind of thing where, if you gave a talk on it, everybody left thinking it was obvious, as if we'd known it all along."

The group was funded with what the National Institutes of Health calls a program project grant—several interrelated grants rolled into one—which it's had for 20 years now. In that time, the investigators have identified cellular mechanisms and risk factors for preeclampsia, as well as potential interventions. Perhaps most notably, Roberts' group determined that although preeclampsia is a complex disorder linked to many factors, antioxidants might be able to prevent some women from developing it.

The clinical trial will help determine whether massive doses of nutrients like Vitamin A and E (exponentially higher doses than those found in prenatal vitamins) can stop preeclampsia in some women.

Roberts' energy has inspired the likes of Yoel Sadovsky, the institute's new scientific director. Roberts himself is at a loss to explain what he did for Sadovsky at UCSF in the early 1990s, back when he was in charge of the fellowship program. He mentored Sadovsky, but he says that mostly involved steering the promising fellow to labs where other investigators were doing things that interested him. But Sadovsky says that he was at his own moment of transition, and Roberts embodied the role of physician as scientist. It is fitting that Sadovsky took the reins of leadership at MWRI directly from Roberts in 2007. (See related story, p. 12.)

Last year, just as he handed over the directorship of MWRI, Roberts stepped aside so that Hubel could be the PI on the latest renewal of the program project grant. "It was a test for me: Could I stand not being the star?" says Roberts, who is still very much an active investigator in the project. "It felt great."

"When I was invited to take the job, I came here and found there was no institute except in the minds of a few people," Roberts says of the birth of MWRI. "I guess we had a little over a million dollars in grants when we started in 1992, and I think we had three basic scientists and maybe 20 or so clinical scientists. And by [2007], I think we were up over $100 million in committed grants and probably 30 or 40 basic scientists, with another 50 or 60 people doing other types of research—clinical, epidemiological, and so on. It's been very gratifying.

"But now," he adds with a laugh, "I'm enjoying the opportunity to actually read, and think, and teach." ■