

# It Takes More than Two

A Team Approach Spawns  
Reproductive-Biology Insights

by Krista Weidner



**Faculty members in the college's Center for Reproductive Biology and Health include, left to right, Ramesh Ramachandran, Troy Ott, Jon Oatley (seated), Francisco Diaz, Wansheng Liu, Joy Pate, and Paul Bartell.**

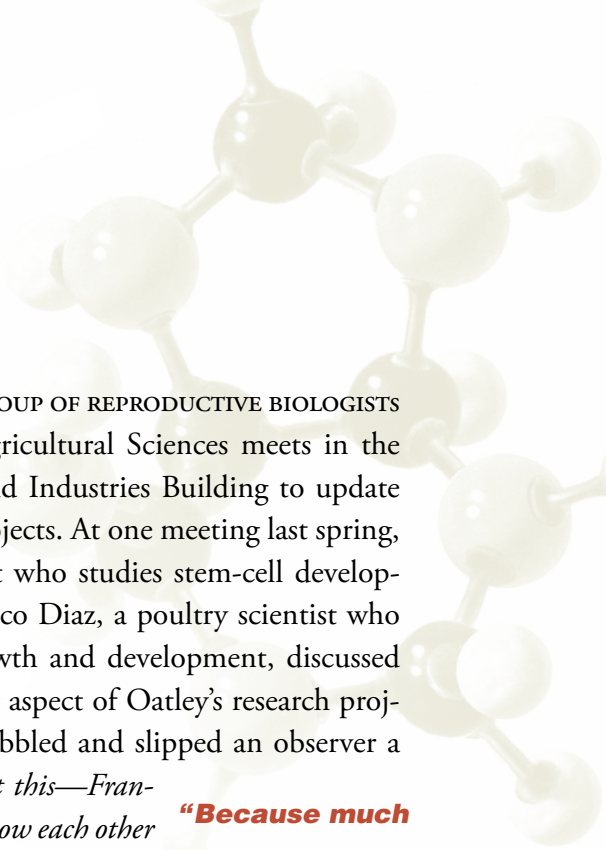


**E**VERY OTHER FRIDAY, A GROUP OF REPRODUCTIVE BIOLOGISTS from the College of Agricultural Sciences meets in the Agricultural Sciences and Industries Building to update one another on their projects. At one meeting last spring, Jon Oatley, an animal scientist who studies stem-cell development in the testes, and Francisco Diaz, a poultry scientist who researches oocyte (or egg) growth and development, discussed possible solutions to a puzzling aspect of Oatley's research project. Group leader Joy Pate scribbled and slipped an observer a

*note: What's great about this—Francisco and Jon didn't know each other a few months ago. Sounds like they are already collaborating—from totally different backgrounds.*

Pate's note sums up the nature of a new group of scientists in the college: six faculty members who were hired together as part of an initiative to create a center of excellence in basic reproductive-biology research. With the recent retirement of several long-standing faculty members, college administrators recognized a need to rebuild its research portfolio in basic animal and avian reproductive science. "Penn State has a legacy as a predominant institution in this research area," says Terry Etherton, head of the Department of Dairy and Animal Science. "John Almquist laid the groundwork in the

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1940s through the 1970s with his pioneering research in freezing of sperm and artificial insemination. We want to build on our strong tradition and take reproductive research into the next century.”

To that end, the college recruited and hired top researchers from across the country to build a complementary team in which each member focuses on a different aspect of reproductive biology. Together this team forms Penn State’s Center for Reproductive Biology and Health. “Because much of today’s life sciences research is conducted at the molecular level, we sought to recruit people who can ask and answer important and relevant questions in reproductive biology at that level,” says Robert Elkin, head of the Department of Poultry Science. “It’s exciting, the people we were able to recruit. They are top-notch scientists in their respective fields, and everyone has a niche.”

Reproductive physiologist Joy Pate, who arrived in February, was the last to come on board and was given the challenge of providing leadership to the group. “I remember, a couple of years ago, seeing the announcement that Penn State wanted to hire six reproductive biologists,” she says. “I was a professor at Ohio State at the time not looking to move, but I remember thinking, ‘What an exciting thing for the people who end up in that group.’ About two years later, I was asked to come and give a seminar and consider an endowed chair position. I met the group—some of the most enthusiastic people you will ever meet—and it was such a stimulating environment. I couldn’t pass it up, and here I am.”

Pate’s research area is the corpus luteum, or CL, the structure that forms on the ovary after ovulation. The CL produces progesterone—a critical hormone for establishing and sustaining pregnancy in mammals. If fertilization occurs and results in an embryo, the CL lives and sustains pregnancy by producing pro-

gesterone. On the other hand, if there is no fertilization during a cycle and thus no embryo, the CL dies. “We want to know what the difference is between a cycle when the CL dies and a cycle when there’s an embryo and the CL is maintained,” she says. “What drives it? What kills it? We know that somehow the embryo signals the CL, and the CL signals back. The CL needs the embryo to survive and vice versa.”

Pate uses corpus luteal tissue from dairy cattle to learn how immune cells are involved in whether the CL lives or dies. She has identified a unique type of immune cell, or lymphocyte, that interacts closely with other cells in the CL. Called gamma delta T cells, they are still somewhat of a mystery. “They’re unique

because they don’t follow the rules lymphocytes are supposed to follow,” Pate says. “They are the renegades of the lymphocyte world. We were surprised to find out those were the cells responding to luteal cells.

“And as if it weren’t weird enough that we found these gamma delta T cells,” she continues, “it turns out there are different cell subsets that have different functions. Some are inflammatory and can destroy the CL, while others are anti-inflammatory, or regulatory. We think that if these cells are inside the

CL when the CL is functional and there’s an embryo, the regulatory cells might dominate and maintain tissue homeostasis—the tissue is making progesterone and is happy. But if there is no embryo or the embryo dies, maybe the inflammatory cell type takes over and kills the tissue.” Pate recently received a USDA/NRI grant to continue studying gamma delta T cells and their interactions with the CL. “It’s opened up a whole new set of questions we didn’t even have a year ago,” she says.

Because the dairy cattle industry has seen a decline in fertility over the past 50 years, Pate hopes her basic research can be applied to improve fertility rates. If she and her colleagues can learn exact-

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ly how the CL is sustained, that knowledge can lead to more healthy, full-term births. Her research has implications for human health as well. Since the reproductive systems of cows and sheep are ideal models for those of humans, knowledge gained about animals could have benefits for human fertility.

Pate’s research has led her to ask questions that go beyond reproductive biology. For example, the way in which the fetus “invades” the uterus and the very rapid growth of the CL have similarities to cancer biology, so what she learns about those reproductive mechanisms could apply to tumor growth. In both reproductive tissues and tumors, rapid tissue growth demands a blood



supply. If the blood supply could be blocked, the tumor would be starved of oxygen and nutrients and it would die. “We keep our eye on what’s happening outside reproduction because what we learn could very well apply to other systems,” she says. “We strive to keep our work in context and look at the bigger picture.”

The corpus luteum is not the only part of the reproductive system responsible for maintaining pregnancy. Team member Troy Ott focuses his research on another critical aspect of female fertility: hormonal signaling between mother and embryo. Just as the CL is needed to sustain pregnancy, a “hormonal handshake” also needs to take place between the em-

bryo and the uterus for pregnancy to be established and continue. “The embryo first has to secrete some signals that the uterus has to receive, and if that takes place correctly, pregnancy is recognized, the mother changes her physiology, and the embryo grows,” Ott explains. “This hormonal handshake is a critical component in reproduction. We believe a lot of failed pregnancies did so because this signal was not sent or received properly or at the right time.”

Ott is interested in the immune accommodations that a mother’s body makes to allow a foreign body—an embryo—to continue to grow in her uterus. Because half the embryo is made up of the father’s genes, the mother’s immune

**In Joy Pate’s lab, postdoctoral researcher Kalidou Ndiaye examines a test tube that shows the separation of blood into three components—plasma (top), white blood cells (middle), and red blood cells (bottom). Ndiaye will isolate T-lymphocytes from the white cells and culture them with corpus luteum cells to look for interactions.**

system should reject it, he explains. “But, of course, that doesn’t happen. The uterus is a privileged site.” To find out why, Ott is looking at changes in gene expression in the uterus and circulating immune cells. When the embryo sends hormonal signals to the uterus, genes that cause the mother to recognize the embryo are turned on. Ott is working to



**Doctoral student Daniel Poole uses a magnetic cell separator on T-lymphocytes to pull out specific subsets called gamma delta lymphocytes.**

identify these genes and learn how they function to sustain pregnancy.

“We’re finding genes, and we’re getting some surprises,” he says. “For example, we focus on one particular gene family called Mx genes. We’ve known for a while that they have a role in fighting viral infections, but now we’re finding that they’re turned on in the uterus at very high levels in very early pregnancy. What are they doing there? They have other functions apart from the ones we knew about, and we’ll continue to do basic research to learn more about them.” In the meantime, because these genes are turned on so early in pregnancy, they can provide a way to test dairy cattle and sheep for pregnancy much earlier than current tests allow. Ott has patented a technology for using this family of genes to determine pregnancy status that has the potential to be a valuable reproduction-management tool for producers.

The Center for Reproductive Biology and Health also includes researchers who

look at male fertility. Jon Oatley is working with testicular stem cells, which are responsible for maintaining male fertility by producing new sperm. “I did the math once,” says Oatley, “and I figured out that on average, a human male produces 1,000 sperm with every heartbeat. If there were no stem cell population, that wouldn’t happen. Stem cells form the foundation from which sperm are produced continuously throughout a male’s lifetime.”

Oatley is looking at ways to manipulate sperm production with the goal of enhancing reproduction in dairy herds. Currently, the vast majority of cattle are bred through artificial insemination (AI), and there’s a finite amount of sperm available from genetically superior bulls. “Here’s the neat thing,” he says. “If we could remove stem cells from a bull’s testes, put them in culture, get them to grow, and then transplant them into the testes of a second male, the second male will produce the sperm of the first male.

Take that a step further and transfer those stem cells to ten bulls, and then instead of having one bull producing the sperm you want, you’d have ten. So you just expand

tenfold the amount of sperm available for AI. But the challenge is that stem cells are extremely rare. Only about one in every 3,000 cells in the testes is a stem cell.”

In the lab, Oatley has succeeded in removing testicular stem cells from mice and maintaining those cells in a culture dish for more than six months. Now he’s working on collecting stem cells from bull testes and growing them in culture. Those stem cells

survive only for a few weeks. “It’s a little more difficult than I thought, and we’re tweaking it,” Oatley says. “We are trying to perfect the environment in the culture dish by manipulating factors such as temperature, oxygen level, nutrients, and physical interaction with other cells. We’re getting closer every day. One of these days it will happen.”

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Oatley's research crosses over into the biomedical field through studies of genes that control stem cell populations. Research with mice shows that when certain genes important for stem-cell function are knocked out, the mouse becomes infertile. In humans, half of male infertility cases are due to a deletion in the Y chromosome, but for the other half, the cause of infertility is unknown, Oatley explains. For that unknown half, he hypothesizes that lack of sperm production could be due to mutations in stem cell genes. If that's the case, infertility could be corrected by removing the stem cells from the testes, fixing the mutation, and replacing the cells.

Oatley's colleague Wansheng Liu focuses on the Y chromosome, the chromosome responsible for male fertility. His long-term goal is to characterize the genes on the bovine Y chromosome that are essential for sperm production and male fertility and then develop a diagnostic test that cattle producers can use for bull fertility selection at an early age. "Bull fertility is a critical component of the bovine reproductive process," Liu explains. "But it hasn't been studied at a molecular level. We are the first ones to do this." Liu is working on a map of bovine Y gene markers that will show differences in the DNA sequences of different animals.

Currently, producers have to wait two to three years to determine whether a bull will be good for breeding. "But if we could do a DNA test when a bull calf is born, we can say, 'This young bull calf has the potential to become an excellent breeder,' or, 'This animal will be no good for breeding.' DNA markers can identify highly fertile, subfertile, or infertile sires at an early age, and this would significantly reduce breeding costs for sire selection."

**Suzanne Reding, a graduate assistant in Jon Oatley's lab, works to isolate stem cells from bull testes. The goal is to grow the stem cells in culture so they can be transplanted into another bull.**

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Appropriately, because Pennsylvania is one of the top egg-producing states in the nation, the college's Department of Poultry Science plays a crucial role in the reproductive biology initiative. "Milk and eggs are two of the major commodities produced by animals in Pennsylvania, and they are products of reproduction," says department head Elkin. "We want to continually improve the quality of these products. Our scientists are cutting edge, but we have practical applications in mind. We have a mandate to work on problems related to industry and the real world."

Poultry scientist Francisco Diaz is doing basic research on avian oocyte (or egg) development, which occurs in the ovarian follicle. In addition to the oocyte, an ovarian follicle contains other types of cells called somatic granulosa cells. Just as signaling is important in mammalian reproduction (as shown in Pate's and Ott's research), intercommunication between avian oocytes and

granulosa cells is necessary for the oocyte and follicle to develop. Diaz's research focuses on understanding the interactions between oocytes and granulosa cells that promote production of a fertile oocyte. He hopes the knowledge he gains can be used to treat infertility and develop better reproductive-management tools for poultry production and animal agriculture.

Another poultry researcher, Paul Bartell, is looking at how biological clocks in the brains of birds work to regulate daily and seasonal rhythms. Biological clocks in birds and other animals are regulated by multiple structures in the brain that communicate with one another to drive circadian (24-hour) and circannual (seasonal) activities. "The influence of biological clocks in timing reproduction has been well established, but we don't know exactly how biological-clock genes help regulate reproduction," he says. "We are trying to identify the signals from the biological clock that initiate reproductive development and maintain reproductive viability. For example, birds are sensitive to light. They need a certain amount of light during the day to initiate egg laying. But we've found that if birds have just a small amount of light



at exactly the right time, it will have the same effect on egg laying as a lot of light. So light falling at the proper phase of the internal rhythm is responsible for initiating reproduction.” What Bartell learns about how biological-clock genes regulate reproductive activity can lead to greater efficiency in the poultry industry.

Through all the reproductive biology group’s research runs a common thread: to share research-based knowledge with the goal of improving agricultural production and animal and human health. With cooperation as a priority, the six researchers featured here also work with faculty members who have had longer tenures in the college. Friday meetings include everyone who is working in some aspect of reproductive biology research, extension, and teaching.

In the same vein, the group plans to expand beyond the college. Researchers are planning for future collaboration with faculty from the College of Health and Human Development and the College of Medicine. “The Center for Reproductive Biology and Health has its roots in our college, but its branches will span the University,” says Ott. “While individual research is important, sharing knowledge benefits everybody and enhances everyone’s productivity. It’s like that saying ‘the rising tide raises all ships.’ We all work together and help each other, and that’s the recipe for success.”

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*Faculty referenced in this article include Paul Bartell, assistant professor of avian biology; Francisco Diaz, assistant professor of reproductive biology; Robert Elkin, professor and head of the Department of Poultry Science; Terry Etherton, distinguished professor of animal nutrition and head of the Department of Dairy and Animal Science; Wansheng Liu, associate professor of animal genomics; Jon Oatley, assistant professor of reproductive physiology; Troy Ott, associate professor of reproductive physiology; and Joy Pate, professor of reproductive physiology and C. Lee Rumberger and Family Chair in Agricultural Sciences.*

## John Almquist: A Fertile Mind



While faculty in the new Center for Reproductive Biology and Health are doing cutting-edge basic research at the cellular and molecular level, reproductive biology research in the college is far from new. John Almquist and his colleagues laid the groundwork through their research on artificial insemination beginning in the 1940s. Dedicated in 1949, the Dairy Breeding Research Center at Penn State was established to study artificial insemination and dairy cattle fertility. It was renamed in 1999 after John O. Almquist.

Almquist joined the college in 1944 to spearhead a dairy cattle breeding research program with the goal of defining future research needs in artificial insemination. One of his first experiments in 1946 involved coloring bull semen to make positive breed identifications easier. His first groundbreaking discoveries centered on adding antibiotics to diluted bull semen. Adding antibiotics controlled bacterial growth, reduced the early death of embryos, and increased fertility. These breakthroughs were universally adopted by artificial insemination associations.

In 1951, Almquist pioneered the use of milk as a medium to extend the life of bull semen. In 1954, he and his staff developed techniques for freezing bull semen in glass ampules, and by the 1960s, breeding associations had converted their entire inventories to frozen semen in ampules. Almquist’s discoveries helped breeding associations offer producers their choice of sires at much lower costs.

In the early 1970s, he developed new antibiotic combinations that controlled bacterial growth without compromising fertility. He also established that the thawing rate was much more important to sperm survival than the freezing rate. Almquist’s pioneering research in artificial insemination led to his receiving the Wolf Foundation Prize—the equivalent of the Nobel Prize for agricultural researchers—in 1981.

Other notable researchers at the center have included T. Y. Tanabe, Rupert Amann, Robert Flipse, Phil Senger, Dan Deaver, and Gary Killian. Collectively, the center’s scientists have made significant contributions in the areas of bull management, nutrition and behavior, sperm harvesting and preservation, sperm metabolism, methods of quantifying sperm-production rates, male endocrinology, estrous behavior, male fertility factors, characterization of oviductal environment, role of progesterone in maintenance of pregnancy, documentation of fertilization rate and embryonic mortality in normal and repeat-breeding cattle, and use of radiography to evaluate artificial insemination technique.