FERTILE MIND

BY TRISHA GURA

Jonathan Tilly defied decades of dogma by suggesting that women can make new eggs throughout their lives. Now some of his critics are taking a second look.

Jonathan Tilly likes to gauge the significance of his work by the hair on the backs of his arms. "Look at it standing up," he says, thrusting out his forearm on a mid-August afternoon. A reproductive biologist at Massachusetts General Hospital in Boston, Tilly was explaining a procedure to retrieve stem cells from the ovaries of a sterile woman. This experiment, he hopes, will help to quell criticism of his most controversial claim: that ovaries have the potential to make eggs indefinitely. This defies the long-held dogma that female mammals are born with all the oocytes (precursors to eggs) they will ever produce, a population that dwindles with age and is exhausted at menopause.

Tilly first challenged that doctrine in 2004, in a paper[1] suggesting that the oocytes in mouse ovaries are being replenished by stem cells. If properly understood, such cells could be harnessed to generate fresh eggs for women with fertility problems, or even achieve a goal Tilly has been pursuing for 25 years: delaying or halting menopause. "The hairs are still up," Tilly says. It "happens every time I think about that experiment".

He has since published a parade of headline-grabbing papers, culminating this year in a report[2] that he had isolated the elusive stem cells from human ovaries and coaxed them to develop into bona fide oocytes. But his work has been dogged by doubt. Some researchers question his methods and reasoning. Others have tried, and failed, to repeat his experiments. Tilly "always makes what I call 'big satellites', something tremendous in the sky," says molecular biologist Kui Liu at the University of Gothenburg in Sweden. "He exaggerates," Liu says, and produces a "big press release". "A few years later, people realize, 'Oh, not right'."

Tilly says he has weathered a lot of attacks. "When I made the decision to pursue this, it was out of pure excitement that we found something that could revolutionize the field. It never even crossed my mind that it would be so negative and so nasty. And it really is negative and nasty."

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But now the stand-off of mistrust, and sometimes open contempt, has taken a strange twist. Two of Tilly's most vociferous critics have become his collaborators: one serving on the board of advisers at his start-up company, OvaScience in Cambridge, Massachusetts; the other working directly with the stem cells that Tilly had isolated. "These cells are doing things *in vitro* that can really start to address scientific problems," says Evelyn Telfer, a reproductive biologist at the University of Edinburgh, UK, who was doubtful of Tilly's work in the past. "If we are really interested in the science ... then this is a great tool."

A COUNTING PROBLEM

The 'no new eggs' doctrine has a long history. In 1951, the influential anatomist Solly Zuckerman, at the University of Birmingham, UK, performed an in-depth analysis of evidence available at the time. He concluded that none of it effectively countered a proposal from the 1870s stating that female mammals stop producing oocytes after birth[3].

For the first 15 years of his career, Tilly focused mainly on programmed cell death, or apoptosis, and he was struck by the fact that no one had ever quantified the loss of eggs due to ovulation and natural oocyte death over time. So beginning around 1999, Tilly commandeered a microscope and mouse ovarian tissue in order to count the follicles, the cellular compartments in which oocytes develop, in mice at different ages. He found a mathematical imbalance: the number of degenerated follicles was three times higher than expected on the basis of the starting pool. If the mice were losing oocytes at this rate, their eggs should be depleted far sooner than they actually were. Something had to be replacing them, he concluded: stem cells were the likely culprit.

Few were willing to accept the idea. It took Tilly two years — and numerous rejections and revisions — to get the data published in *Nature*, in 2004. Controversy ensued over his methods as well as his conclusions. One critique said, for example, that it was "alarming" that Tilly used the rate of follicle disappearance in one mouse strain to calculate loss for another[4].

Tilly dropped most of his apoptosis work and steered his entire lab towards proving the existence and functionality of these stem cells. "You are sort of standing on the precipice wondering whether or not you should make the jump," he says. "Getting the 2004 paper published was for me the jump, because there was no turning back at that point."

A year later, Tilly reported that he had identified the source of these putative cells: bone marrow[5]. When he transplanted either marrow or blood from healthy mouse donors into sterile mice, the animals could produce cells that looked like oocytes. But he could not yet fertilize the resulting eggs and create embryos — the true test of an egg stem cell.

At least six groups challenged the bone-marrow finding. In one critique[6], a group led by Telfer wrote that none of Tilly's experiments had successfully been replicated, and that the results could be interpreted in other ways. Critics also asserted that Tilly was overreaching, particularly in media interviews.

In *The Boston Globe* in 2005, for example, Tilly is quoted as saying: "They're your own cells; you don't need anybody's approval. They go right into your blood supply and go right to your ovaries, where they mature into eggs." David Albertini, a reproductive biologist at the University of Kansas Medical Center in Kansas City, calls such claims outrageous: "A lot of us reproductive biologists feel that this is a frank travesty that has falsely raised the hopes of many women."

Tilly defended his comments and challenged his peers to go back to their labs and reproduce his experiments. Several did. In 2006, stem-cell biologist Amy Wagers at Harvard University in Cambridge, Massachusetts, and her collaborators stitched together the circulatory systems of two mice[7]. One, the donor, expressed green fluorescent protein (GFP) in its cells. The other did not. The scientists found that although green, glowing, blood-borne cells could infiltrate the ovaries of the recipient mice, these cells acted like blood cells, not oocytes.

Tilly, in response, performed a similar experiment, showing that mice sterilized by chemotherapy could give birth after a bone-marrow transplant[8]. But the babies did not express GFP, indicating that the eggs from which they were derived came from the recipient, not the donor. Tilly argued that the bone marrow either protected existing oocytes or revived oocyte formation, but critics argued that the chemotherapy probably didn't kill off all the recipient's oocytes in the first place.

SHANGHAI SURPRISE

With little independent replication of his work, Tilly was standing alone through much of the fray. Then, in 2009, Ji Wu at Shanghai Jiao Tong University in China and her colleagues reported that they had isolated from mice what she called "female germline stem cells" — not from bone marrow, but from ovarian tissue[9]. When her team transplanted the cells into chemotherapy-treated female mice, they developed into mature oocytes, then fertilizable eggs and, the clincher, healthy pups.

Although previously sceptical of the prospect, Telfer says that when she read Wu's paper in 2009 she paused, thinking "there must be something in this". She had met Tilly at the bar during a scientific meeting the year before and they talked about their differences. After Wu's paper, the two co-authored a commentary that articulated something like a truce[10]. "Although these findings do not establish that oogenesis occurs in adult females under physiological conditions," Tilly and Telfer wrote, "they strongly support the existence of [germline stem cells] in adult mouse ovaries. If equivalent cells can be found in human ovaries, stem-cell-based rejuvenation of the oocyte reserve in ovaries on the verge of failure may one day be realized."

Many set out to replicate Wu's results, including Tilly. And in February, he reported the isolation of what he called "oogonial stem cells" from human ovaries[2]. By injecting the cells into human ovarian tissue transplanted into mice, he was able to generate both follicles and what seemed to be mature oocytes (see *Nature* **483,** 16; 2012).

"So now we have two different labs, using conceptually a similar protocol, and both groups got confirmatory data," he says. "We felt at that point there should be no more debate."

But there was. Critics soon began pointing out a problem shared by both the teams' approaches. Each identify their respective stem cells using antibodies meant to bind a cell-surface protein, a common technique in cell biology. But the protein they target, called vasa, normally sits inside the cell, not on its surface. "There are a lot of people in the field struggling to understand how this can possibly work," says Patricia Hunt, a reproductive biologist at Washington State University in Pullman.

Tilly says that although mature oocytes do not express vasa on their surface, his cells — which are a cross between embryonic precursors and full-blown oocytes — do. Vasa, he says, becomes non-detectable on the cell surface as the cells mature into eggs. But, he adds, "We don't have any proof of that yet."

Liu in Sweden says that he initially believed Wu's paper when it came out. But his group could not repeat the technique. To bypass the cell-surface problem with vasa, Liu used an approach that tracks the protein inside the cells[11]. He was able to extract ovarian, vasa-expressing cells, but none of them underwent division — a major criterion for stem cells.

Wu contends that her cell-isolation technique is not easy to perform and invites scientists to come to her lab to learn it. She adds that Liu's group "didn't use our protocol of isolating the cells. So how to compare?"

In fact, Tilly says that his lab had trouble repeating Wu's protocol, too. Eventually, his team retrieved cells but "found consistent oocyte contamination". He had to modify the protocol to retrieve the mouse and human oogonial stem cells, and they differed in size from those Wu had isolated. Wu says that her cells and Tilly's are probably "subtypes" of each other and that there is still "a lot of work to do" to figure out exactly how they are related.

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Telfer, meanwhile, has begun to collaborate with Tilly. After going to Boston in 2011 to observe his human stem cells, she was impressed, and took a sample back to Scotland. Her team had worked out a culture system using mouse and cow tissues to grow egg precursor cells into fertilizable eggs entirely outside the body. With Tilly's cells she needed to adapt the technique for use in humans. "The first experiments blew me away, just blew me away," she says. Tilly's cells, she found, grew rapidly into oocyte-like structures. "I spent the whole night trying to find another explanation other than new follicles had formed," she says. "And I could not come up with one."

Telfer has applied for permission from the UK Human Fertilisation and Embryology Authority to attempt to fertilize the cells; such experiments are forbidden using US federal funding. If successful, the technique to make fertilizable human eggs outside the body could eventually be disseminated to fertility clinics throughout the world.

BOUNDARIES AND BACKLASH

The cells are also being used at OvaScience, which was founded in April 2011 and has secured US$48 million in venture capital. The company is exploiting Tilly's cells in several ways. One aim is to rejuvenate egg cells from older women by adding fresh cytoplasm and mitochondria. The research builds on a controversial experimental fertility technique in which egg cells are injected with cytoplasm from another woman's eggs. The OvaScience approach would use mitochondria extracted from the mother's own oogonial stem cells, which Tilly says would be healthier than those from an ageing mother's eggs, and should skirt some of the ethical and safety questions raised by using donor mitochondria. OvaScience plans to begin clinical trials this year in collaboration with two Boston-based fertility clinics.

Tilly's oogonial stem cells will also serve as a screening tool for new drugs that might block or boost egg production. Such drugs might help reverse infertility or even help delay or halt menopause. Albertini still worries that such claims inflate hopes but, like Telfer, he is trying to keep an open mind. The prospect of new models for screening fertility drugs convinced him to join OvaScience's scientific advisory board. "There are a lot of things that I know and I do that could be helpful to them," he says.

The company is swiftly moving forward, steered by the team that guided Sirtris, based in Cambridge, Massachusetts, a biotech firm focused on anti-ageing therapies. In fact, it was a collaboration between Tilly and Sirtris's founder, David Sinclair, at Harvard Medical School in Boston, that sparked the launch of OvaScience.

He and Tilly are "mutual admirers", Sinclair says, explaining that they joined forces in 2009 to explore the idea that egg quality declines with age because older eggs lack enough energy to support fertilization. Sinclair offers his anti-ageing expertise and his experience of controversy; some of the initial results on which Sirtris was founded could not be replicated and have been a source of contention in the field (see *Nature* **464,** 480–481; 2010).

"It is an interesting team that Jon and I make," Sinclair says, "because the two of us push the boundaries of science. And both of us have encountered backlashes in doing so."

Still, despite his characteristic gumption and ebullience, Tilly seems to be burdened by the continual sparring. Although he's shifted much of his time to studying oogonial stem cells in the ovary, he still maintains that bone-marrow stem cells might also create new eggs. His critics disagree, and even if they accept the existence of oogonial stem cells, they still question whether such cells normally function to produce new eggs.

"The data provided so far don't support this concept," says Albertini. Tilly maintains that these stem cells must be doing something in the body. But in exasperation, he is willing to concede that it may not matter in the clinic. "If you could take these cells outside the body, and get them to make a functional egg that can make a normal healthy baby, what do you care about the physiology?" ■

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