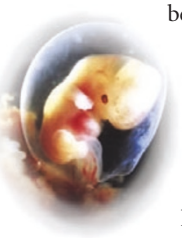
Skeptics demand duplication of controversial fertility claim

When Jonathan Tilly claimed in March 2004 that stem cells could replenish the supply of eggs in a mammalian ovary, experts in the field balked. But his claim in July that those stem cells may reside not in the ovary but in the bone marrow, and that they might circulate in blood, has shocked critics into downright disbelief.

Tilly's new work posits that stem cells in the bone marrow can travel through the blood to regenerate oocytes in a mouse ovary (*Cell* **122**, 303–315; 2005). His group at the Massachusetts General Hospital has found that mice with barren ovaries that received blood transfusions began producing new egg cells within days.



The study challenges the long-held view that mammals are born with a limited number of eggs that declines with age. Among experts, it has inspired, at best, cautious optimism and, at worst, vehement denial. But it is also challenging the field to reexamine one of its most sacred dogmas.

"There are so many inconsistencies between the first paper and this one, it makes it very difficult to believe in these findings," says David Albertini of the University of Kansas, referring to Tilly's earlier work, which suggested that the stem cells reside in the outer covering of ovaries (*Nature* **428**, 145–150; 2004). When the researchers could not isolate the stem cells from ovaries, Tilly says, their data led them to the ovarian blood supply, and then to the bone marrow.

The team transplanted blood from normal female mice engineered to produce green fluorescent protein to mice that had been sterilized by chemotherapy, a genetic mutation or both. In each case, the recipient ovaries gained fluorescent green egg cells. Bone marrow transplants also restored oocytes, although more slowly. But whether those eggs can be fertilized to produce fluorescent offspring is yet to be determined. That result would largely mollify the critics.

Tilly's work also hints that the ovary may alert the bone marrow when oocyte numbers run low. The expression of a gene marker used to track bone marrow stem cells fluctuates with the mouse's estrous cycle and disappears altogether when the ovaries are removed. The researchers suggest that ovaries send an unknown molecular signal to regulate egg development.

"Therapeutically, such a factor would be a gold mine to increase the oocyte stockpile or jumpstart new egg production," says Tilly, adding that if the work translates to humans, it could lead to ways to prolong women's fertility or delay menopause.

But talk of human implications frustrates critics who say the work must first be reproduced. "Relieving menopause is a long jump forward," says Jeffrey Chang, a reproductive endocrinologist at the University of California, San Diego. Chang says he appreciates the research, but "it's important not to over-interpret their work."

Kutluk Oktay, a fertility expert at Weill Cornell Medical Center in New York, says anecdotal evidence from his patients suggests Tilly's observations might hold true in people. But "people expect duplication of this study, and that may take someone else five years," Oktay says.

Baby steps

11 March 2004
First evidence that stem cells in mice replenish the egg supply of adult mouse ovaries. (*Nature* **428**, 145–150; 2004).

13 March 2004
First embryo from *in vitro* fertilization of an egg retrieved from a frozen-thawed ovarian tissue transplant patient; implanted embryo did not result in pregnancy. (*The Lancet* **363**, 837–840; 2004).

1 June 2004
Birth of a baby boy conceived through *in vitro* fertilization with sperm that had been frozen for 21 years. (*Hum. Reprod.* **19**, 1448–1449; 2004).

2 June 2004
Boston-based Extend Fertility freezes eggs for future use. Nearly 130 women have since banked eggs at about $15,000 each; birth rates hover around 20%.

7 June 2005
Woman who received a fresh ovarian tissue transplant from her identical twin sister delivers a girl. (*N. Engl. J. Med.* **353**, 58–63; 2005).

21 July 2005
Baby girl born to a woman who received a transplant of her own ovarian tissue, which was frozen before her treatment with sterilizing chemotherapy (*N. Engl. J. Med.* **353**, 318–321; 2005).

29 July 2005
Evidence that oocyte stem cells in mice reside in bone marrow and travel through blood. (*Cell* **122**, 303–315; 2005).

Because the work raises fundamental questions, five years is too long, experts say. But the work's controversial nature may make scientists reluctant to take on projects that confirm or counter Tilly's work, they note.

Tilly says it is "most revealing" that no one has published data refuting his hypothesis in the 18 months following the *Nature* paper. He says some colleagues set the burden of proof incredibly high, suggesting that he should have asked another lab to confirm his work in parallel. "If you are still critical," he says, "get into the lab and prove us wrong."

Kendall Powell, Denver

Maine company falls a-fowl for smuggling bird flu

As the virulent bird flu sweeping through Asia holds global attention, regulators in Maine are dealing with an avian virus closer to home. A Maine biotechnology company that makes vaccines for poultry diseases was in August fined $500,000 for smuggling a chicken flu virus into the US.

Vaccine manufacturer Maine Biological Labs in 1998 illegally imported the virus from Saudi Arabia so that it could develop a vaccine for a disease-plagued poultry farm in that country. According to court records, the following year the company used falsified documents to send 8,000 bottles of the newly created vaccine back to Saudi Arabia.

"If the virus got out, it could have decimated poultry populations and cost billions of dollars," says Keith Haffer, a vaccine consultant at Advantage Bio Consultants in South Dakota.

The smuggled virus was an H9N2 subtype, less virulent than the H5N1 virus that has claimed 57 lives in Asia since 2003. But low-virulence strains can rapidly mutate to become deadly. In 1983, the low-virulence H5N2 strain evolved into a highly pathogenic strain within six months in Pennsylvania, forcing authorities to kill 17 million birds at a cost of nearly $65 million.

These viruses can also occasionally be transmitted to people. An H9 virus infected two people in Hong Kong in 1999 and one in 2003, causing mild illness.

The Maine case came to light in 2002, when a whistleblower sparked a federal investigation that found widespread illegal activity at the lab. Investigators discovered that the company's employees had changed vaccine labels to minimize import costs for overseas clients and altered vaccine expiration dates. In July, four former executives were sentenced to fines up to $30,000 and a year in jail. The company is now under new management and says that it is in full compliance with the law.

To bring infectious agents into the country, companies are required to obtain permits from the US Department of Agriculture. The agency may deny permits for dangerous bugs not found in the US. In April 2005 it stipulated that certain agents can only be sent to those who are registered to receive them. But stopping someone from illegally importing a virus, says Suzan Holl, a spokeswoman for the agency, is "almost impossible."

Emily Singer, Boston