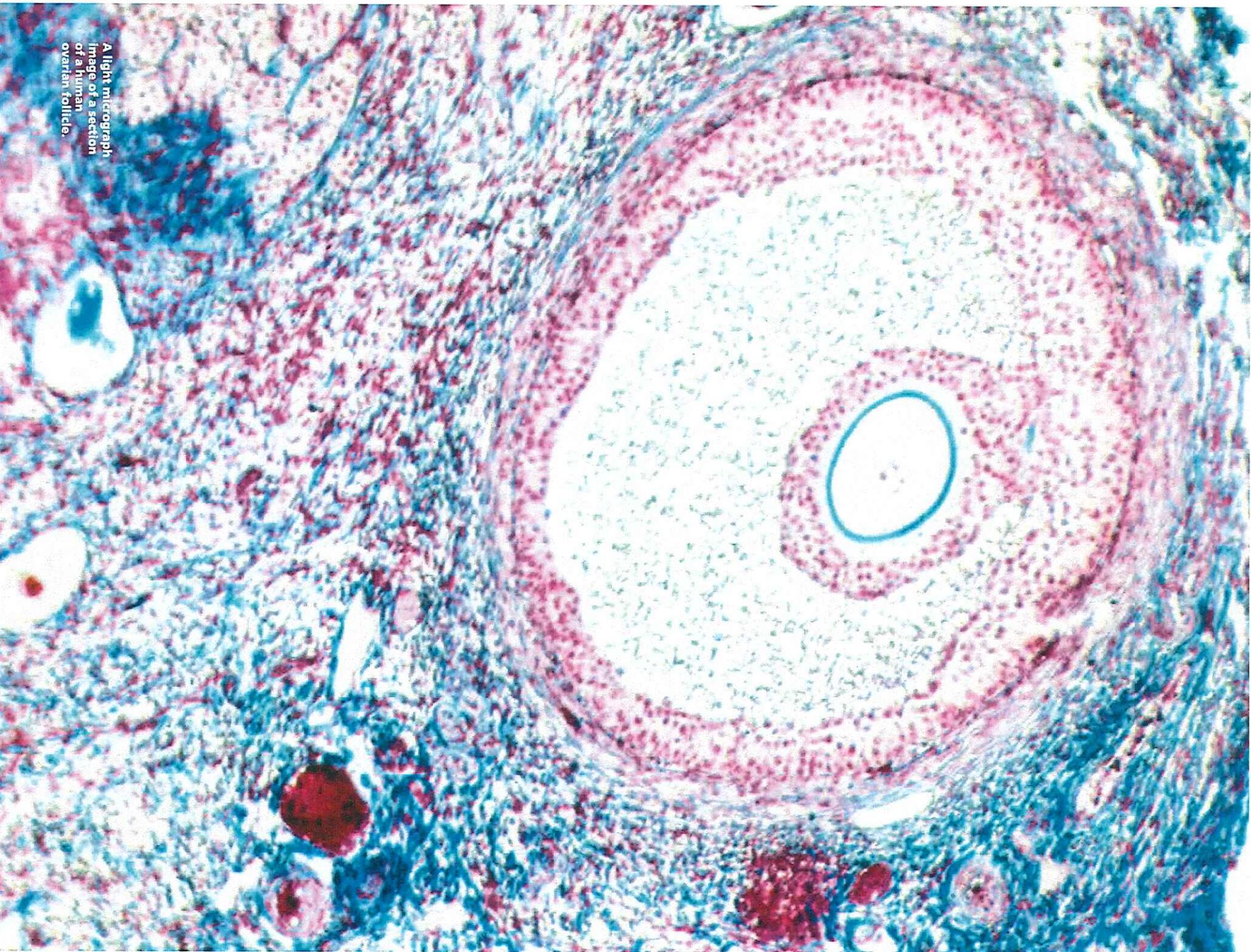


A light micrograph
of a section
of a human
ovarian follicle.



Young women diagnosed with cancer face yet another challenge: infertility. Experimental cryoresearch could restore their chance at motherhood.

BANKING ON HOPE

BY KATHERINE KORNEI

Tucked away in a brick warehouse complex in St. Paul, Minn., frozen in liquid nitrogen, sits Abby Bott's left ovary. Bott was only a college senior when doctors diagnosed her with Stage III colorectal cancer in 2009. They told her she needed aggressive chemotherapy and radiation to kill the cancer cells in her softball-sized tumor. However, the lifesaving regimen would also strike Bott's ovaries. She had always wanted children, yet if she survived her cancer, she would likely be infertile.

A cancer diagnosis is "one of the most existential crises you can imagine," says Teresa Woodruff, a reproductive scientist at Northwestern University. Given advances in treatments, cancer isn't necessarily a death sentence: 80 percent of children and adolescents diagnosed with cancer survive five years or more. But while chemotherapy and radiation are associated with temporary changes, such as hair loss and tissue swelling, the treatments can have an unseen, permanent effect: infertility due to irreparably damaged sperm or egg cells.

This intersection of oncology and fertility, known as oncofertility, has interested

Woodruff since the early 2000s, when she was the director of basic research at Northwestern's Robert H. Lurie Comprehensive Cancer Center. "I started asking folks about the effects of their cancer drugs on fertility," she says. That's when she realized that while young male patients were being offered the option of sperm banking, young female patients were being told to focus on surviving their disease because there were no such options for them. "A lot of the work we were doing in our lab could turn around this disparity," Woodruff says.

She had found her calling: providing cancer patients, particularly young girls and women like Bott, with fertility preservation options. Thus began what would become the Oncofertility Consortium.

Male fertility preservation is straightforward and immediate: Sperm donation and subsequent freezing, called cryopreservation, has been a widespread practice since the 1970s. For women, though, the process is more limited and more complicated. First, the female must be old enough to have mature eggs, called oocytes. These oocytes are available only in limited quantities at certain times of her monthly cycle, and must be surgically retrieved. The extracted oocytes can then be cryopreserved for later use or combined with sperm in a laboratory to create embryos via in vitro fertilization.

Researchers have been cryopreserving embryos for several decades and cryopreserving oocytes in the past several years, but some female cancer patients lack these options. Pre-pubertal girls can't yet biologically produce mature oocytes, and some sexually mature women, such as Bott, have aggressive cancers that must be treated immediately. "I definitely didn't have the [few weeks] to go through the preparation of all the hormones [for oocyte extraction]," Bott says. Instead, she'd need to preserve not her oocytes, but the tissues that produce them. And Woodruff and colleagues are pioneering an experimental procedure to do just this, allowing these girls and women with the fewest options for preserving their fertility to bank on the hope of becoming biological mothers.

A MILLION TO ZERO

Infant girls are born with two ovaries each containing about 1 million



Abby Bott, who now lives and works in the Washington, D.C., area, was diagnosed with Stage III colorectal cancer in 2009. To protect her chances of having biological children after aggressive chemotherapy, she had her left ovary removed and cryopreserved.

follicles, cell clusters in which single oocytes are nurtured and matured. These follicles naturally die over time: A girl entering puberty has only about 20 percent of the follicles she was born with. Once a female reaches sexual maturity, her body's hormones stimulate a monthly cycle in which about 400 follicles die and roughly 20

follicles begin to mature. But during that cycle, only one dominant follicle matures completely to release the oocyte contained within it. "We still don't really understand why one follicle begins the process of development," Woodruff says. Ultimately, "We start with a million, and we end with zero," she says.



Teresa Woodruff, the Oncofertility Consortium's director, instructs health professionals during her Oncofertility Summer Academy at Northwestern University. Northwestern and other institutions contribute to the consortium, which studies how to preserve the reproductive tissues of cancer survivors.

From birth until menopause, women possess immature follicles that can be harvested at any time and cryopreserved. If these follicles can then be thawed and reliably matured later, the oocytes can be extracted and combined with sperm to create embryos and, eventually, babies. However, stopping and restarting a woman's biological clock is experimental science. Early work by the Oncofertility Consortium showed that the freezing and thawing processes, for example, are apt to damage the particularly delicate

cellular structure of ovarian tissue and render the tissue unusable.

When the field of oncofertility research started heating up, several teams, including Woodruff's group, recognized the need to pool their expertise. In 2007, the group was officially named the Oncofertility Consortium, and, with funding from the National Institutes of Health, it brought the field together.

By 2009 the consortium was well-established, and Bott's nurse practitioner let her know that cryopreservation was an option.

About two weeks after she received her diagnosis, Bott lay on an operating table at Northwestern Memorial Hospital to have her left ovary removed. "I knew that this procedure was experimental at the time," Bott explains. "But it was my only option."

FREEZING FOR THE FUTURE

The Oregon National Primate Research Center (ONPRC), nestled next to a new housing development on the outskirts of Portland, might at first glance be mistaken for a suburban park with its large cedar trees,

The Ethics of Oncofertility

Cancer and fertility are intensely personal subjects that require people to make ethical decisions. Oncofertility is no different.

Laurie Zoloth, a professor of religious studies and a member of the faculty of bioethics and humanities at Northwestern University, has advised the Oncofertility Consortium since its inception. "Teresa [Woodruff] was interested from the very beginning about ethics questions," Zoloth says. "For her, the questions of ethics were just as important, fascinating and complex as the scientific questions."

Part of Zoloth's work concerns the complexities of deciding who should be permitted to preserve ovarian tissue — solely cancer patients, individuals with less life-threatening diseases or anyone who wishes to do so. She considers issues of justice and accessibility, because, as she wrote in the

American Journal of Bioethics, while "cancer and infertility are conditions that afflict all women equally and traverse class, race and ethnic lines, social and economic distinctions generally determine health care access."

Zoloth is also concerned about expanding the definition of "family" beyond biology, and she and other Oncofertility Consortium investigators have explored the issue of adoption. "People want babies that match them genetically," she says. "But what about adoption? Are people willing to take in children that have no families?" At any given time, there are roughly 500,000 children in American foster care. "That's a scandal," Zoloth says. And women who survive cancer are often ineligible to adopt children because agencies shy away from that lifetime risk of reoccurrence. Oncofertility Consortium team members have worked to convince adoption agencies that women who survive cancer, like Bott, should be allowed to adopt children. —K.K.

walkways and picnic benches. I enter the reflective glass doors of building No. 601 to pick up my visitor's badge and wait for Mary Zelinski.

Zelinski is a research associate professor in the ONPRC's Division of Reproductive and Developmental Sciences and a member of the Oncofertility Consortium. For the past seven years, she and her team have been studying how to cryopreserve and thaw primate ovarian tissue that is then transplanted back into the monkeys or cultured in the lab to mature its oocytes. "We use rhesus monkeys as a model for women because their reproductive cycles are identical to those of human females," she says. In her office, framed pictures of monkey infants share space with books on reproductive biology and fertility. She hands me scrubs, sanitary shoe covers and gloves. "Let's go to the lab."

It's nearly 90 degrees inside the lab, a long, narrow room lined with a bench of microscopes on one side and incubators on the other. Zelinski raises a petri dish holding what looks like a swollen pink seashell, no larger than a fingernail. It's an ovary from a sexually mature monkey. Zelinski uses a blade to slice off thin, translucent pieces of the ovary to expose the cortex, the

tissue that contains all the follicles. She places a slice of the cortex under a microscope and adjusts the focus and magnification until the follicles and the oocytes are visible.

A cancer patient like Bott who saves her ovarian tissue may wish to use it months or even years later. And since cryopreservation is vital to stopping all cellular reactions in the tissue, Zelinski and her team first had to develop methods for reliably freezing the delicate tissues. They added ethylene glycol and glycerol, which function like antifreeze, to the ovarian tissue samples to prevent cell-damaging ice crystals from forming. The tissue was then placed in liquid nitrogen, commonly used in cryopreservation, for long-term storage. "It freezes everything; it stops everything," Zelinski says.

But successful freezing is only one part of the battle. For it to be useful, cryopreserved ovarian tissue must thaw in a way that maintains its original cellular structure and function. Zelinski and her colleagues have found they can reliably thaw ovarian tissue by placing it in a series of solutions with lower concentrations of sucrose, a medical-grade table sugar. These solutions slowly and delicately draw out the antifreeze-like chemicals

without damaging the cells. "It took us a good three years to figure out these protocols," Zelinski says.

ONPRC researchers also have been investigating two ways of maturing follicles from cryopreserved, thawed tissue: transplanting strips of ovarian tissue back into a monkey's body, or growing the tissue in vitro by encapsulating individual follicles in a biomaterial that mimics an ovary.

In the first case, the scientists suture small pieces of ovarian tissue into a live monkey in places where there is ample blood flow — such as under the skin near the abdomen or arms — which promotes healthy follicle development. Ovarian tissue transplanted to these easily accessible locations lets researchers harvest mature eggs using just a needle as opposed to surgery. (Why not transplant onto the remaining ovary? Zelinski and her team are ultimately targeting the patient populations whose ovaries have atrophied due to radiation and chemotherapy.)

They've shown in work with monkeys that follicles in transplanted tissue resume their natural patterns of maturation within about three months.

Once the mature oocytes are extracted and combined with sperm

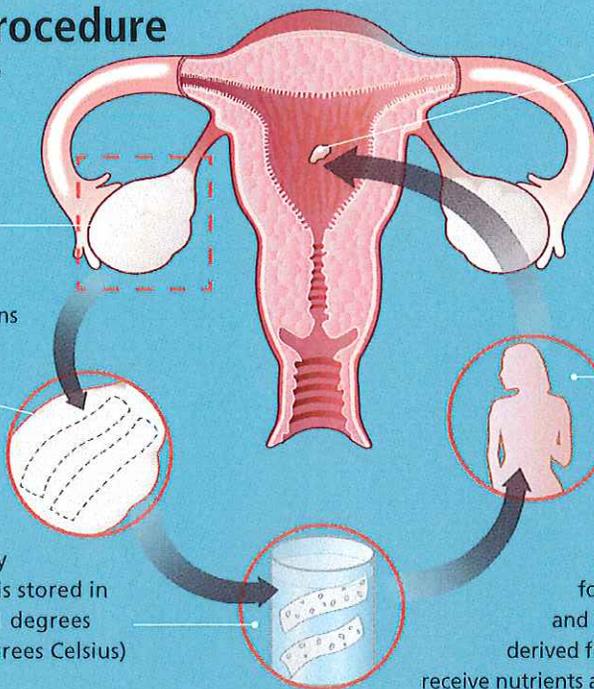
The Pioneering Procedure

Preserving a woman's reproductive tissues would involve multiple, complicated steps:

1 Laparoscopic surgery removes an ovary.

2 Tissue strips are cut from the outside of the ovary, which contains the highest density of oocyte-containing follicles.

3 Strips are exposed to ethylene glycol and glycerol, which act like antifreeze to prevent the formation of ice crystals and slow down any cellular reactions. The tissue is stored in liquid nitrogen at minus 321 degrees Fahrenheit (minus 196 degrees Celsius) for months or years.



5 Mature oocytes are extracted from the mature follicles and combined with sperm through in vitro fertilization. The embryo is placed into the patient's uterus or a surrogate.

4 When a patient is ready to use her tissue, the antifreeze-like solutions are drawn out of the strips. Next, follicles are matured to produce oocytes in one of two ways: The tissue is sutured into the patient's body in a place with ample blood flow, such as on a remaining ovary, within abdominal folds or under the skin of an arm; or individual immature follicles are extracted from the tissue and suspended in alginate, a biomaterial derived from brown algae. The follicles receive nutrients and hormones every few days.



A technician places ovarian tissue strips into a vial of preserving solution (left). The vial is then stored for safekeeping in a cryogenic tank (below).



in the laboratory, any resulting embryos are placed back into the monkey's uterus. In 2003, this technique helped Zelinski's team achieve the first live monkey birth using fresh (non-frozen) ovarian tissue that had been transplanted near a monkey's abdomen.

Since then, at least 23 women have become mothers using ovarian tissue that was cryopreserved, thawed and transplanted. In one of those cases, a 27-year-old woman in Belgium gave birth in 2014 after receiving a transplantation of her ovarian tissue she had cryopreserved during childhood. She was the first "really young patient who came back so many years later to try for a pregnancy," Zelinski says.

Now, Zelinski is studying how individual follicles can be matured outside a patient's body. This procedure is notably more challenging than suturing ovarian tissue onto existing live tissue, but it addresses a critical medical need: Ovarian tissue can harbor cancer cells, particularly if the patient has a blood-borne cancer such as leukemia or lymphoma. Reimplanting the tissue may reintroduce malignant cells. By maturing follicles in vitro, researchers can ensure patients don't receive potentially cancerous tissue.

When Oncofertility Consortium scientists first attempted to mature primate follicles — which are naturally three-dimensional — in two-dimensional petri dishes, the follicles flattened out and their oocytes collapsed and died. Seeing these discouraging results, Woodruff and colleague Lonnie Shea, a materials scientist, suggested suspending individual immature follicles in tiny beads of alginate, a substance derived

from brown algae and commonly used as an ice cream thickener. By encapsulating the follicles in the alginate and providing the growing cells with precise mixtures of nutrients and hormones, researchers at the ONPRC and elsewhere have been able to mimic the ovary's natural structure in the laboratory. Zelinski and her team have successfully matured follicles in alginate and are working to achieve a live primate birth using those oocytes.

LIFE, WITH OPTIONS

Bott had her cancerous tumor removed in March 2010 and then went through both chemotherapy and radiation. She was declared to be in remission, and she graduated from the University of Illinois at Urbana-Champaign that year with a degree in psychology. However, in 2012 Bott's doctors thought her cancer had returned, and she started treatment again. In 2014, Bott opted to have her uterus and remaining ovary removed to prevent potential recurrence.

The 29-year-old is now healthy and has started working again. Her ovarian tissue is still frozen in liquid nitrogen in St. Paul, waiting for science to catch up. "I'm really glad that the tissue is there," Bott says. "The fact that I have options makes a world of difference." **D**

Katherine Kornei is a freelance science writer based in Portland, Ore.

Major Moments in Assisted Human Reproduction

Infertility is as old as humankind, as is the use of herbs, remedies or rituals claimed to treat it. But the modern era of science-driven assisted human reproduction is less than a century old.

1953 First baby born using frozen sperm.

1978 Baby born via in vitro fertilization.

1980 Birth from a legal surrogate mother in America.

1984 Birth using a donated oocyte.

1984 Baby born as a result of a frozen embryo.

1990 Tests of "assisted hatching," a technique to help embryos attach to the uterine wall.

1992 Case of intracytoplasmic sperm injection, in which a single selected sperm is injected directly into an oocyte.

2003 Monkey birth from fresh, non-cryopreserved ovarian tissue.

2004 Human birth from cryopreserved ovarian tissue.

2015 "Stem-cell baby" born from an oocyte with mitochondria boosted using ovarian stem cells.

2016 Researchers find that when sperm enzymes fertilize the egg, there is a burst of naturally occurring zinc. These zinc "sparks" are brighter for healthier eggs, which could lead to more effective fertilization techniques.



