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Age is no barrier…

Advances in reproductive medicine hint that female fertility might be extended into late middle age and beyond. But will the methods be safe? And is society ready for this demographic shift? Kendall Powell investigates.

Benjamin Franklin said that the only certainties in this world are death and taxes. To these, women might add a third: the menopause. But this year, two separate developments have suggested that Franklin was right after all.

In March came a report indicating that ovaries contain stem cells that might be prompted to carry on making eggs throughout a woman's adult life[1]. Then, in October, Belgian doctors reported that a woman whose ovaries were damaged by cancer chemotherapy had given birth, possibly owing to a graft of her own preserved ovarian tissue that they had transplanted into her body[2]. Together, these results hinted that women might one day be able to delay starting their families until their forties or beyond.

The scientists working on these projects aim to develop methods to preserve the fertility of women who have been driven through a premature menopause by treatments for diseases such as cancer. But given that many women in industrialized societies want to delay reproduction while they further their careers, such methods are inevitably also going to be seen as tools for healthy women.

At the time of her first menstrual period, a woman's ovaries contain some 250,000 follicles, each of which has the potential to release an egg. But over her reproductive life, a woman will ovulate no more than 500 times, because her supply of eggs is progressively wiped out in a process called follicular atresia. In her late thirties, a woman's fertility dips sharply because of the declining quality of her remaining eggs and her failure to ovulate during many menstrual cycles. Eventually, by the age of about 50, she has so few follicles left that she can no longer respond to the hormones that stimulate follicular development and ovulation, resulting in the menopause.

The simplest way to extend a woman's reproductive life would be to freeze ovarian tissue or eggs until they are needed. More fundamentally, it might be possible to disrupt the molecular mechanisms behind follicular atresia. Alternatively, the stem cells in a woman's ovaries might be coaxed into replenishing her egg supply.

Cold storage

Although most fertility experts approve of giving women more options, opinions vary on the likely safety and efficacy of methods to delay childbearing. And there's even less consensus on whether society is ready for the consequences of such techniques. Some observers see a troubling future in which children will spend their entire adult lives caring for their elderly parents. But if we are living longer and healthier lives, say others, why restrict reproduction to our twenties and thirties?

Currently, the only established method of preserving a woman's fertility is to freeze embryos created by *in vitro* fertilization (IVF) for later transfer to the womb. Here, the odds of a successful pregnancy can approach 50% per attempt, for women younger than 35. But this option requires a woman to have a willing partner, or at least a sperm donor, to fertilize her eggs before freezing.

Men can store frozen sperm for later use. But until recently, no one would have suggested that women should freeze their eggs. The mature human egg is the largest cell in the body. As such, it is very sensitive to freezing, because ice crystals can turn its cytoplasm to mush upon thawing.

Recent advances in banking frozen eggs have been spurred in part by the Catholic church's view that embryos must be protected as living individuals. In predominantly Catholic Italy, embryo freezing is frowned upon, and the practice was banned in February this year. Realizing the need for an alternative, Eleonora Porcu and her team at the University of Bologna have developed a liquid — including a sugar and 1,2-propanediol, which acts as an antifreeze — that allows eggs to survive freezing.

Using her solution, and fertilizing eggs by injecting sperm, Porcu has improved the success rate from one birth per 100 frozen

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Improvements in freezing eggs and ovarian tissue (below) for reimplantation might one day allow grandparents to have more children of their own.

eggs to about one in five[3]. "Egg freezing is almost ready to be introduced into the clinical routine," she concludes. Indeed, the Boston-based firm Extend Fertility already offers egg banking through their US fertility clinics for prices that start at $12,500.

Banking on a baby

But Porcu and other experts stress that egg banking is not the female equivalent of storing frozen sperm. For a start, getting eggs is not a trivial procedure — the ovaries must be stimulated with hormones and the eggs then harvested with a surgical needle.

The limited number of eggs that can be frozen, compared with the enormous number of sperm in an ejaculate, also means that egg banking is a gamble with future fertility. Any woman who believes that frozen eggs represent "money in the bank" for later fertility is deluding herself, argues Zev Rosenwaks, who directs the Center for Reproductive Medicine and Infertility at Cornell University's Weill Medical College in New York. "It's, at best, a 20% shot," he says.

One way of improving the odds would be to freeze ovarian tissue. The tiny, immature eggs it contains should survive freezing and thawing with few problems. Once back in the body, the graft should begin to churn out eggs. Even if these have to be retrieved manually and fertilized by IVF, the odds of reproducing might still be reasonably good.

Researchers at Rosenwaks' Cornell centre, led by reproductive endocrinologist Kutluk Oktay, have been working along these lines. In March, they reported restoring the production of ovarian sex hormones in prematurely menopausal women, whose preserved tissue was transplanted under the skin of their forearm or abdomen.

From one of these patients, Oktay retrieved 20 eggs from the transplanted tissue and found eight that were suitable for IVF with her husband's sperm. Of these, one egg developed into a healthy-looking four-cell embryo[4] and was transplanted to the woman's uterus. Even though Oktay's procedure did not result in a pregnancy, it was an important proof of principle.

Experts are enthusiastic about applying the technique to women made infertile by cancer treatment, but are much more cautious about its use by healthy women seeking to delay reproduction. "We need to make sure we're not doing harm to these women," says Oktay. Given the need for surgery, and the unknown success rate, he suspects that the risks may outweigh the benefits for women with other options for conceiving. The American Society for Reproductive Medicine — a professional organization for fertility clinics — released guidelines on 18 October strongly suggesting that, for now, egg and ovarian tissue freezing should only be used on an experimental basis for helping chemotherapy patients.

In any case, if researchers can find a safe and subtle way of slowing a woman's reproductive clock, then the menopause could be delayed without surgery. There are two molecular puzzles that need to be solved if women's fertility is to be extended in this way. First, researchers need to slow the loss of immature eggs through follicular atresia. And second, they need to maintain eggs' cellular and genetic quality. Falling egg quality plays an important role in women's drop in fertility as they approach the menopause.

Stop the clock

Follicular atresia occurs through a mechanism of programmed cell death called apoptosis. At Harvard Medical School in Boston, Jonathan Tilly is trying to slow atresia. In 1999, his team made a mouse lacking a gene called *Bax*, which seems to control the rate of apoptosis. "Their ovaries kept ticking along well into old age, the mouse equivalent of 100 years old," Tilly says. The older mice could not get pregnant naturally, but through IVF had normal offspring[5].

But scientists do not know of any drug that could block *Bax* in the body. "Targeting *Bax* is not as easy as it sounds," Tilly says. So he is now working on a fatty molecule called sphingosine-1-phosphate (S1P), which acts upstream of *Bax* in the signalling pathway that regulates apoptosis. Tilly's team has shown that ovarian injections of S1P protect mice from a premature menopause caused by radiation[6,7].

The problem is that S1P regulates apoptosis throughout the body, and its widespread disruption might have life-threatening consequences. Apoptosis, for instance, is vital in the immune system. So to make S1P a safe way of preserving fertility, Tilly's team needs to deliver it specifically to the ovaries. They are currently testing a slow-release capsule inserted into the ovaries of rhesus monkeys.

Meanwhile, at Erasmus University in Rotterdam, the Netherlands, researchers led by Axel Themmen believe that they have identified an ovary-specific alternative to S1P. Anti-Müllerian hormone (AMH) directs male embryos to develop male reproductive organs. But women also make AMH, in cells that support egg development in ovarian follicles.

Female mice lacking AMH lose their folli-

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cles at three times the normal rate; so Themmen thinks that treatment with the hormone might extend a woman's fertility[8]. But more work must be done first to see the effects of AMH injections on lab animals.

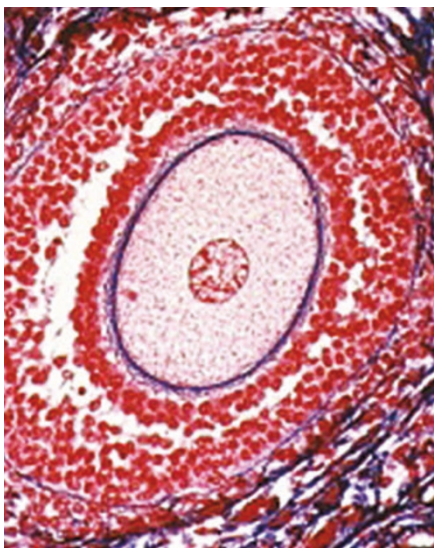
Even staving off follicular atresia will not solve the parallel problem of chromosomal damage in ageing eggs, which is associated with heightened rates of miscarriage and birth defects. Almost 80% of the eggs of women aged 40–45 show abnormal chromosome alignment[9].

This obstacle would be removed if older women could obtain a fresh batch of eggs. That's where another line of research in Tilly's lab comes in. In a paper published in *Nature* in March, his team presented evidence that adult mouse ovaries contain stem cells that divide to produce new ovarian follicles[1].

Looking more closely at follicular atresia, the team found that follicles were being lost at such a high rate that the supply ought to be exhausted by young adulthood. Second, they observed cells in the ovaries of adolescent mice that looked like the stem cells that exist in fetal females. They also treated female mice with a drug that kills such stem cells, and found that their follicles were rapidly depleted.

If human ovaries contain a similar population of stem cells, it might be possible to avert the menopause by boosting their division. But the idea that female mammals are born with a fixed supply of eggs is well established, and most experts want to see more evidence before accepting Tilly's ideas. "The claims Tilly makes are enormous and revolutionary, but it's been very seldom that one paper overturns a paradigm as deeply entrenched as this one," says Roger Gosden, scientific director of the Jones Institute for Reproductive Medicine at Eastern Virginia Medical School in Norfolk.

More women are delaying childbirth until later, when fertility begins to decline as developing eggs (below) are lost as a result of apoptosis.



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Tilly is tight-lipped about the details of his unpublished work, but feels confident that he will find evidence that women have ovarian stem cells. Oktay's study of his transplant patient also supports this idea. Oktay had originally estimated from the density of follicles in the transplanted tissue that the graft would function for about a year. More than 18 months later, the tissue continues to ovulate regularly — perhaps, Oktay suggests, because stem cells are replenishing the supply of follicles.

Tilly's paper will surely trigger a frenzy of activity as other researchers try to replicate and build from his findings. "The potential for stem-cell technology applications to reproduction are probably limitless," says Rosenwaks. But Tilly warns against trying to rush into the clinic. "We can't do this faster than we're doing it," he says.

Even so, Tilly's findings have given new urgency to the debate about the social implications of delaying menopause. Will older mothers be able to give their children the support that they need? Is this a fair question, given that elderly men can already become fathers? What are the implications for the demographics of society as a whole? Will it lead to a decline in fertility at the population level, as women eschew reproduction when they are most likely to conceive?

On the last point, reproductive biologists admit to concern. "Natural reproduction is still more efficient than anything we can do in the clinic," says Rosenwaks. "We should educate the public that the earlier, the better your chances, and the more likely you will have a healthy child."

Most fertility experts are unwilling to speculate on the wider consequences of extending women's reproductive lifespan. They argue that decisions about reproduction are a matter for individuals, and say that there should be no absolute upper age limit for motherhood. "I'm against any fixed line," says Gosden.

Outside of reproductive medicine, academics who have considered the issue wonder whether the focus on biological solutions is misguided. If the workplace was organized in ways that allowed women to have children in their twenties or early thirties without compromising their careers, they say, then the pressure to delay childbearing would be relaxed. "We're thinking about stopping the biological clock here, but why not work with the career clocks?" asks Claudia Goldin, an economist who studies women's employment issues at Harvard University. ■

Kendall Powell is a science writer based in Colorado.

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