EGG ENGINEERS

In a technical tour de force, Japanese researchers created eggs and sperm in the laboratory. Now, scientists have to determine how to use those cells safely — and ethically.

BY DAVID CYRANOSKI

Since last October, molecular biologist Katsuhiko Hayashi has received around a dozen e-mails from couples, most of them middle-aged, who are desperate for one thing: a baby. One menopausal woman from England offered to come to his laboratory at Kyoto University in Japan in the hope that he could help her to conceive a child. "That is my only wish," she wrote.

The requests started trickling in after Hayashi published the results of an experiment that he had assumed would be of interest mostly to developmental biologists[1]. Starting with the skin cells of mice *in vitro*, he created primordial germ cells (PGCs), which can develop into both sperm and eggs. To prove that these laboratory-grown versions were truly similar to naturally occurring PGCs, he used them to create eggs, then used those eggs to create live mice. He calls the live births a mere 'side effect' of the research, but that bench experiment became much more, because it raised the prospect of creating fertilizable eggs from the skin cells of infertile women. And it also suggested that men's skin cells could be used to create eggs, and that sperm could be generated from women's cells. (Indeed, after the research was published, the editor of a gay and lesbian magazine e-mailed Hayashi for more information.)

Despite the innovative nature of the research, the public attention surprised Hayashi and his senior professor, Mitinori Saitou. They have spent more than a decade piecing together the subtle details of mammalian gamete production and then recreating that process *in vitro* — all for the sake of science, not medicine. Their method now allows researchers to create unlimited PGCs, which were previously difficult to obtain, and this regular supply of treasured cells has helped to drive the study of mammalian reproduction. But as they push forward with the scientifically challenging transition from mice to monkeys and humans, they are setting the course for the future of infertility treatments — and perhaps even bolder experiments in reproduction. Scientists and the public are just starting to grapple with the associated ethical issues.

"It goes without saying that [they] really transformed the field in the mouse," says Amander Clark, a fertility expert at the University of California, Los Angeles. "Now, to avoid derailing the technology before it's had a chance to demonstrate its usefulness, we have to have conversations about the ethics of making gametes this way."

BACK TO THE BEGINNING

In the mouse, germ cells emerge just after the first week of embryonic development, as a group of around 40 PGCs[2]. This little cluster goes on to form the tens of thousands of eggs that female mice have at birth, and the millions of sperm cells that males produce every day, and it will pass on the mouse's entire genetic heritage. Saitou wanted to understand what signals direct these cells throughout their development.

Over the past decade, he has laboriously identified several genes — including *Stella*, *Blimp1* and *Prdm14* — that, when expressed in certain combinations and at certain times, play a crucial part in PGC development[3–5]. Using these genes as markers, he was able to select PGCs from among other cells and study what happens to them. In 2009, from experiments at the RIKEN Center for Developmental Biology in Kobe, Japan, he found that when culture conditions are right, adding a single ingredient — bone morphogenetic protein 4 (Bmp4) — with precise timing is enough to convert embryonic cells to PGCs[2]. To test this principle, he added high concentrations of Bmp4 to embryonic cells. Almost all of them turned into PGCs[2]. He and other scientists had expected the process to be more complicated.

Saitou's approach — meticulously following the natural process — was in stark contrast to work that others were doing, says Jacob Hanna, a stem-cell expert at the Weizmann Institute of Science in Rehovot, Israel. Many scientists try to create specific cell types *in vitro* by bombarding stem cells with signalling molecules and then picking through the resulting mixture of mature cells for the ones they want. But it is never clear by what process these cells are formed or how similar they are to the natural versions. Saitou's efforts to find out precisely what is needed to make germ cells, to get rid of superfluous signals and to note the exact timing of various molecules at work, impressed his colleagues. "There's a really beautiful hidden message in this work — that differentiation of cells [*in vitro*] is really not easy," says Hanna. Harry Moore, a stem-cell biologist at the University of Sheffield, UK, regards the careful recapitulation of germ-cell development as "a triumph".

Until 2009, Saitou's starting point had been cells taken from a live mouse epiblast — a cup-like collection of cells lining one end of the embryo that forms at the end of the first week of development, just before the PGCs emerge. But to truly master the process, Saitou wanted to start with readily available, cultured cells.

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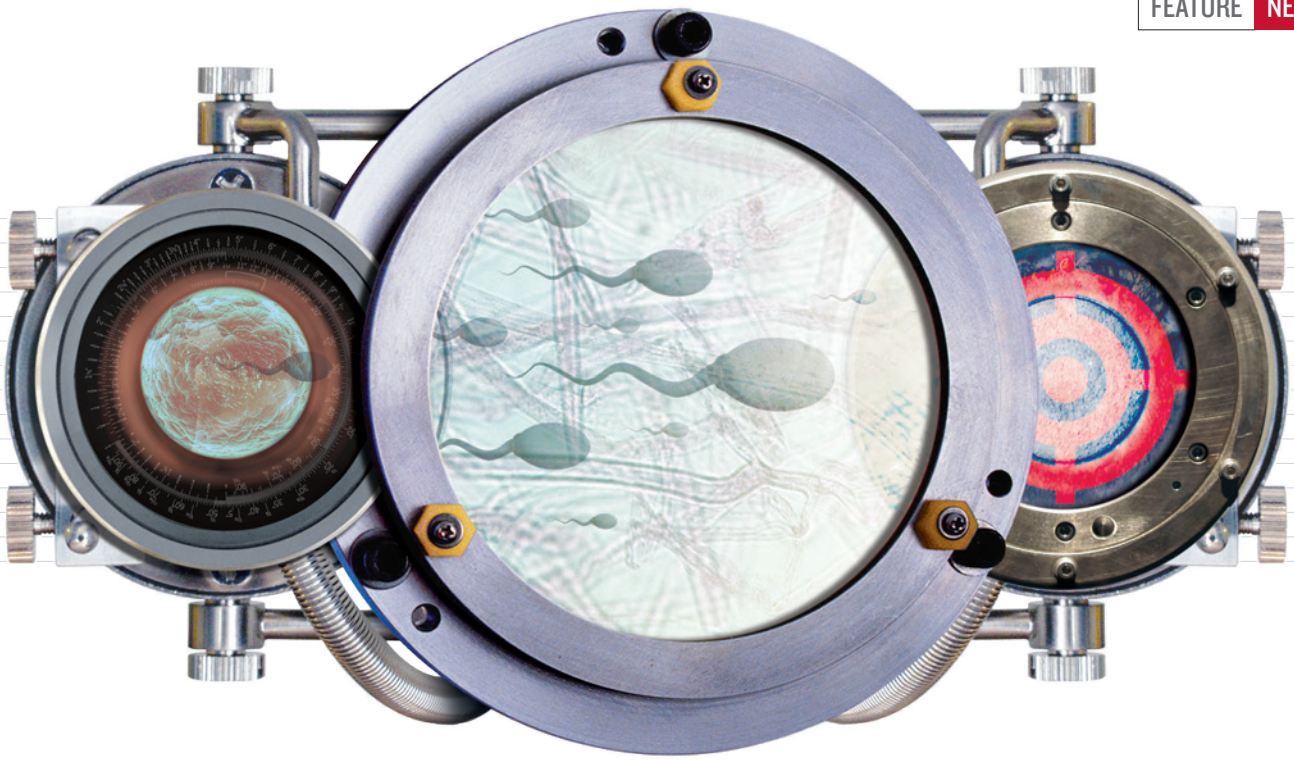


That was a project for Hayashi, who in 2009 had returned to Japan from the University of Cambridge, UK, where, like Saitou before him, he had completed a four-year stint in the laboratory of a pioneer in the field, Azim Surani. Surani speaks highly of the two scientists, saying that they "complement each other in temperament and in their style and approach to solving problems". Saitou is "systematic" and "single-minded about setting and accomplishing his objectives", whereas Hayashi "works more intuitively, and takes a broader view of the subject and has outwardly a more relaxed approach", he says. "Together they form a very strong team indeed."

Hayashi joined Saitou at Kyoto University, which he quickly found was different from Cambridge. There was much less time spent on theoretical discussions than Hayashi was used to; instead, one jumped into experiments. "In Japan we just do it. Sometimes that can be very inefficient, but sometimes it makes a huge success," he says.

Hayashi tried to use epiblast cells — Saitou's starting point — but instead of using extracted cells as Saitou did, he tried to culture them as a stable cell line that could produce PGCs. That did not work. Hayashi then drew on other research showing that one key regulatory molecule (activin A) and a growth factor (basic fibroblast growth factor) could convert cultured early embryonic stem cells into cells akin to epiblasts. That sparked the idea of using these two factors to induce embryonic stem cells to differentiate into epiblasts, and then to apply Saitou's previous formula to push these cells to become PGCs. The approach was successful[6].

To prove that these artificial PGCs were faithful copies, however, they had to be shown to develop into viable sperm and eggs. The process by which this happens is complicated and ill understood, so the team left the job to nature — Hayashi inserted the PGCs into the testes of mice that were incapable of producing their own sperm, and waited to see whether the cells would develop[6]. Saitou thought that it would work, but fretted. "It seemed like a 50/50 chance," he says. "We were excited and worried at the same time." But, on the third or fourth mouse, they found testes with thick, dark seminiferous tubules, stuffed with sperm. "It happened so properly. I knew they would generate pups," says Hayashi. The team injected these sperm into eggs and inserted the embryos into female mice. The result was fertile males and females[6] (see 'Making babies').

> "THEY ARE SETTING THE COURSE FOR THE FUTURE OF INFERTILITY TREATMENTS."

They repeated the experiment with induced pluripotent stem (iPS) cells — mature cells that have been reprogrammed to an embryo-like state. Again, the sperm were used to produce pups, proving that they were functional — a rare accomplishment in the field of stem-cell differentiation, where scientists often argue over whether the cells that they create are truly what they seem to be. "This is one of the few examples in the entire field of pluripotent-stem-cell research where a fully functional cell type has been unequivocally generated starting from a pluripotent stem cell in a dish," says Clark.

They expected eggs to be more complex, but last year, Hayashi made PGCs *in vitro* with cells from a mouse with normal colouring and then transferred them into the ovaries of an albino mouse[1]. The resulting eggs were fertilized *in vitro* and implanted into a surrogate. "I knew it had worked," he says, when he saw the pups' dark eyes pressing through their translucent eyelids.

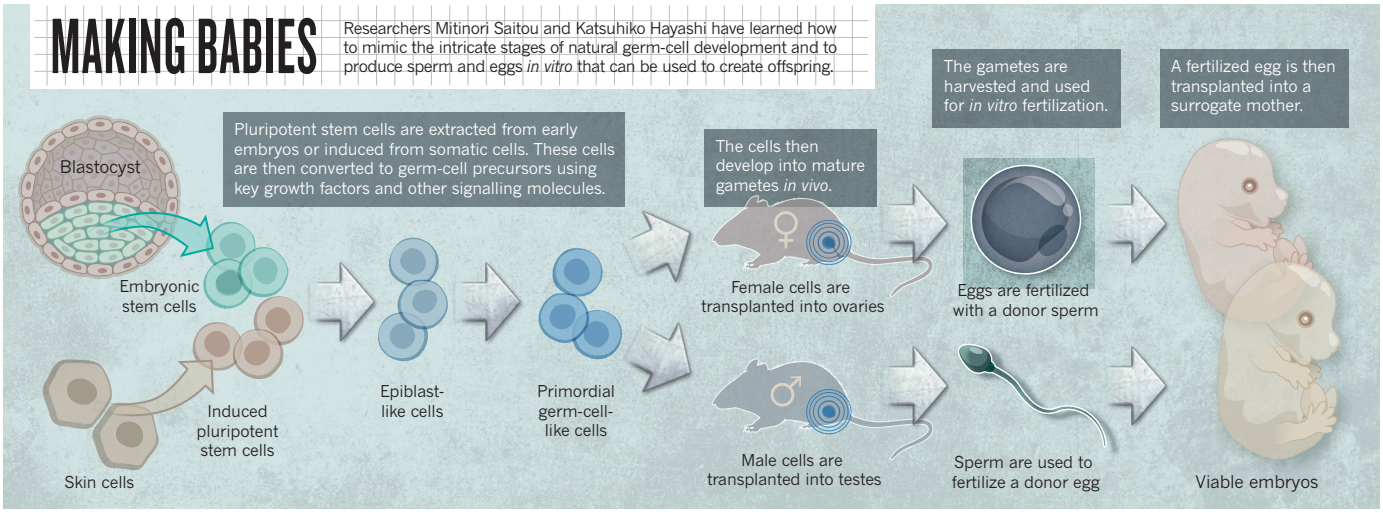
GERM-CELL BOUNTY

Other researchers have been able to replicate the process to generate laboratory-grown PGCs (although none contacted by *Nature* had used them to produce live animals). Artificial PGCs are of particular use to scientists who study epigenetics: the biochemical modifications to DNA that determine which genes are expressed. These modifications — most often the addition of methyl groups to individual DNA bases — in some instances carry a sort of historical record of what an organism has experienced (for example, exposure to foreign chemicals in the womb). In a similar way to how they work in other cells, epigenetic markers push PGCs to their fate during embryonic development, but PGCs are unique because when they develop into sperm and eggs, the epigenetic markers are erased. This allows the cells to create a new zygote that is capable of forming all cell types.

Faults in subtle epigenetic changes are expected to contribute to infertility and the emergence of disorders such as testicular cancer. Already, Surani's and Hanna's groups have used the artificial PGCs to investigate the role of individual enzymes in epigenetic regulation, which may one day show how the epigenetic networks are involved in disease.

Indeed, the *in vitro*-generated PGCs offer millions of cells for scientists to study, instead of the 40 or so that can be obtained by dissecting

ILLUSTRATION BY VIKTOR KOEN



early embryos, says Hanna. "This is a big deal because here we have these rare cells — PGCs — that are undergoing dramatic genome-wide epigenetic changes that we barely understand," he says. "The *in vitro* model has provided unprecedented accessibility to scientists," agrees Clark.

CLINICAL RELEVANCE

But Hayashi and Saitou have little to offer to the infertile couples begging for their help. Before this protocol can be used in the clinic, there are large wrinkles to be ironed out.

Saitou and Hayashi have found that although the offspring generated by their technique usually seem to be healthy and fertile, the PGCs that these offspring generate in turn are not completely 'normal'. The second-generation PGCs often produce eggs that are fragile, misshapen and sometimes dislodged from the complex of cells that supports them[1]. When fertilized, the eggs often divide into cells with three sets of chromosomes rather than the normal two, and the rate at which the artificial PGCs successfully produce offspring is only one-third of the rate for normal *in vitro* fertilization (IVF). Yi Zhang, who studies epigenetics at Harvard Medical School in Boston, Massachusetts, and who has been using Saitou's method, has also found that *in vitro* PGCs do not erase their previous epigenetic programming as well as naturally occurring PGCs. "We have to be aware that these are PGC-like cells and not PGCs," he says.

In addition, two major technical challenges remain. The first is working out how to make the PGCs convert to mature sperm and eggs without transplanting them back into testes or ovaries; Hayashi is trying to decipher the signals that ovaries and testes give to the PGCs that tell them to become eggs or sperm, which he could then add to artificial PGCs in culture to lead them through these stages.

But the most formidable challenge will be repeating the mouse PGC work in humans. The group has already started tweaking human iPS cells using the same genes that Saitou pinpointed as being important in mouse germ-cell development, but both Saitou and Hayashi know that human signalling networks are different from those in mice. Moreover, whereas Saitou had 'countless' numbers of live mouse embryos to dissect, the team has no access to human embryos. Instead, the researchers receive 20 monkey embryos per week from a nearby primate facility, under a grant of ¥1.2 billion (US$12 million) over five years. If all goes well, Hayashi says, they could repeat the mouse work in monkeys within 5–10 years; with small tweaks, this method could then be used to produce human PGCs shortly after.

But making PGCs for infertility treatment will still be a huge jump, and many scientists — Saitou included — are urging caution. Both iPS and embryonic stem cells frequently pick up chromosomal abnormalities, genetic mutations and epigenetic irregularities during culture. "There could be potentially far-reaching, multi-generational consequences if something went wrong in a subtle way," says Moore.

Proof that the technique is safe in monkeys would help to allay concerns. But how many healthy monkeys would need to be born before the method could be regarded as safe? And how many generations should be observed?

Eventually, human embryos will need to be made and tested, a process that will be slowed by restrictions on creating embryos for research. New, non-invasive imaging techniques will enable doctors to sort good from bad embryos with a high degree of accuracy[7]. Embryos that seem to be similar to normal IVF embryos could get the go-ahead for implantation into humans. This might happen with private funding or in countries with less-restrictive attitudes towards embryo research.

When the technology is ready, even more provocative reproductive feats might be possible. For instance, cells from a man's skin could theoretically be used to create eggs that are fertilized with a partner's sperm, then nurtured in the womb of a surrogate. Some doubt, however, that such a feat would ever be possible — the Hinxton Group, an international consortium of scientists that discusses stem-cell ethics and challenges, concluded that it would be difficult to get eggs from male XY cells and sperm from female XX cells. "The instructions that the female niche is supplying to the male cell do not coordinate with each other," says Clark, a member of the consortium.

Saitou used iPS cells from male mice to create sperm and from female mice to create eggs, but he says that the reverse should be possible. If so, eggs and sperm from the same mouse could be generated and used for fertilization, producing something never seen before: a mouse created by self-fertilization. Neither Hayashi nor Saitou is ready to try this. "We would only do this [in mice] if there were a good scientific reason," says Saitou. Right now he does not see one.

The two scientists already feel some pressure from patients and Japanese funding agencies to move forward. The technique could be a last hope for women who have had no luck with IVF, or for people who had cancer in childhood and have lost the ability to produce sperm or eggs. Hayashi warns those who write to him that a viable infertility treatment could be 10 or even 50 years in the future. "My impression is that it is very far away. I don't want to give people unfeasible hope," he says.

Patients see the end result — success in mice — and often ignore the years of painstaking work that led to such a technical tour de force. They do not realize that switching from mice to humans means starting again almost from scratch, says Hayashi. The human early embryo is so different from the mouse that it is almost "like starting over on a process that took more than ten years". ■

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