

# DDN NEWS

## SPECIAL REPORT

# Stem Cells

## THE NEXT GENERATIONS

Stem cells could be an answer for the problem of infertility in cases when *in-vitro* fertilization and other current answers to the issue are not feasible or not desirable—although many hurdles remain to making that option a reality.

### *Are stem cells key to fighting infertility?*

BY RANDALL C WILLIS

**F**ORTY YEARS AGO, on July 25, a dark-haired infant girl took her first breath, and in doing so, she transformed reproductive medicine. Test tubes aside, Louise Joy Brown was the first child successfully born from the revolutionary procedure known as *in-vitro* fertilization, or IVF.

Although Louise's parents were able to produce sperm and eggs, and her mother was able to carry the fetus to term, Leslie Brown had blocked fallopian tubes, which kept her from conceiving. Thus, the IVF treatment was an end-run on a physiological roadblock.

Other potential parents are not so lucky, however, being unable to produce sperm or eggs for any number of physiological or genetic reasons. In some cases, it may be because they are still children, facing a medical crisis like cancer that requires gonado-

toxic chemotherapy. (See also "The human face of infertility" on page 23)

For these people, the methods used to produce Louise Brown will not suffice. For these individuals, new and existing stem cell technologies may be necessary.

#### SEEKING STEM CELLS

The University of Pittsburgh's Kyle Orwig hopes to be at the vanguard of that effort, looking to develop the next generation of reproductive technologies to help reverse infertility.

"When I came to Pittsburgh in 2003, I would have described myself as a stem cell biologist and that I study the stem cells that are in the testes and make sperm," he says.

In a 2017 review, Go Nagamatsu and Katsuhiko Hayashi of Kyushu University explained germ cell formation as a two-step process and noted the origin of the stem cells at the heart of Orwig's studies.

"The first phase commences in primor-

dial germ cells (PGCs) that are specified at an early stage of embryogenesis," they explained. "PGCs migrate toward the gonad while proliferating and acquire an 'epigenetic ground state' upon settling in the gonad."

"In the second phase, however, differentiation becomes more complicated and interactive with surrounding somatic cells," they continued, highlighting the divergent pathways of male and female PGCs.

Entering the female gonad, PGCs undergo meiosis to form primary oocytes, which later mature with the onset of puberty.

"In the male gonad, on the other hand, PGCs are arrested at the G1 stage, and thereby become prospermatogonia," the authors wrote. "After birth, some of the prospermatogonia become spermatogonial stem cells (SSCs), which are crucial for sustaining spermatogenesis."

Orwig's initial efforts, at the University of Pennsylvania, involved transplantation

of SSCs into mice with a genetic defect that make them infertile. As the mouse testes were devoid of germ cells, he recounts, the transplanted cells engrafted robustly in the absence of competition.

He realized, however, that in the human males, genetic infertility would mean an absence of SSCs and therefore the need for donor cells. Under these circumstances, finding a sperm donor would be the easier route for IVF.

"The model that we settled on was people who become infertile because of a medical treatment like chemotherapy or radiation treatments for cancer," he explains. "Over the last 15 years, my lab has translated the stem cell transplantation technique to a monkey model of a young cancer survivor and showed that we could restore sperm production and fertilize eggs and make monkey embryos."

Despite the efforts of Orwig and others, moving SSCs into the IVF clinic has been elusive.

"Since the ability of SSCs to complete spermatogenesis *in vivo* after germ cell



"If you aren't suffering with infertility yourself, it may be hard to know what's going on inside other people," says Kyle Orwig of the University of Pittsburgh, who also serves as director of the Fertility Preservation Program at Magee-Women's Hospital. "I can guarantee you that there is a major psychological impact on the individual and on the couple. It is so easy for people in society or in science and medicine to say it is not a life-threatening disease, it doesn't affect very many people, so how important is it really?"

transplantation was first demonstrated, other strategies have been investigated such as autografting of testicular pieces and *in-vitro* maturation (IVM) up to the haploid stage," recounted Christine Wyns and colleagues at Cliniques Universitaires Saint-Luc and Université Catholique de Louvain in a recent review.

"In animals, resumption of spermatogenesis has been achieved in testicular grafts of fresh and cryopreserved ITT [immature testicular tissue] and following IVM, but in humans, none of these strategies have proved successful as yet."

The authors argued that this is mainly due to the lack of appropriate models for transplantation techniques, or gaps in our understanding of the prepubertal SSC niche and germ cell requirements for IVM differentiation.

The researchers noted that the extent of spermatogenesis depends on the number of SSCs transplanted, so because engraftment efficiency is low in non-human primates, several groups have explored opportunities to expand SSCs in culture.

"So far, no validated method was established for human SSC culture but SSC enrichment by cell sorting or differential plating was generally used before culture," they wrote.

In a 2016 review, Orwig and his colleague Kathrin Gassei added their own thoughts, describing the lack of methods to assay human spermatogenesis.

"Although transplantation to regenerate spermatogenesis with functional sperm and offspring is the gold standard assay for rodent SSCs, there is not equivalent assay of human SSCs," Gassei and Orwig wrote. "Molecular markers and

human-to-mouse xenotransplantation may be reasonable surrogate assays, but there is no gold standard that is universally agreed and deployed for human SSC experimentation. Perhaps *de-novo* testicular morphogenesis and/or decellularized testes can be developed into tools to assay complete human spermatogenesis."

Wyns and colleagues further suggested that such efforts in themselves raise potential clinical safety issues.

"Indeed, SSC transplantation in cancer patients requires techniques to exclude cancer cell contamination, because implantation of as few as 20 leukemic cells can result in cancer relapse," they explained. "Another important concern is the genetic integrity of propagated SSCs and their potential impact on offspring when transplanted."

Likewise, any conversation involving stem cells raises questions about potency.

Orwig hears many people express concern, but he looks to the lengthy history of testes transplant in animals and bone marrow transplant in humans.

"As long as you're dealing with adult tissue stem cells, the issues of potency, at least to me, are lower concern," he says.

Earlier this year, Ans van Pelt and colleagues at the University of Amsterdam decided to tackle the question of safety head on.

"To date, information on the possible tumorigenic potential of transplanted long-term *in vitro*-propagated SSCs has been limited," the authors suggested. "Studies have focused on the proof-of-concept that SSCT is able to restore fertility and generate offspring and have included analysis of the genet-

ic and epigenetic profile of generated spermatozoa and selected tissues from offspring... However, the long-term health effects and potential increased tumor incidence of cultured SSC transplanted recipients has largely been neglected."

To address this concern, the researchers transplanted *in vitro*-propagated SSCs into mice made infertile with busulfan, and then monitored those mice for 18 months to look for differences in tumorigenesis or mortality vs control.

Although malignancies occurred in both mouse populations, the incidence rates were not significantly different, and molecular characterization of the tumors suggested that none of them derived from transplanted SSCs. As well, mean survival after busulfan treatment was equivalent.

The researchers' effort helped fill a gap they felt existed in such experiments, suggesting that even though safety assessments are extremely important in preclinical studies of new reproductive techniques, they are rarely performed as standard procedures.

"Given the fact that the lifespan of mice is approximately 1.5 years, and that future human recipients have to live safely with their transplants through their entire life, there was a need for a large-scale study where mice were followed up during their full lifespan after transplantation of cultured SSCs," they wrote. "With the present study, to our knowledge, we are the first to confirm and strengthen the previously published data in a systematic way showing no increased tumor incidence after SSCT of *in vitro* propagated [germline stem] cells in mice."

Regardless of what we know

**"Since the ability of SSCs to complete spermatogenesis *in vivo* after germ cell transplantation was first demonstrated, other strategies have been investigated such as autografting of testicular pieces and *in-vitro* maturation (IVM) up to the haploid stage. In animals, resumption of spermatogenesis has been achieved in testicular grafts of fresh and cryopreserved ITT [immature testicular tissue] and following IVM, but in humans, none of these strategies have proved successful as yet."**

**Christine Wyns and colleagues at Cliniques Universitaires Saint-Luc and Université Catholique de Louvai**

from animal studies, however, at some point, it is necessary to determine the feasibility of SSC transplantation and other reproductive techniques in humans. In short, a mouse is not a monkey is not a human.

"I think it takes some guts to take the last step to the human clinic and that researchers and physicians are appropriately cautious," Orwig says. "However, humans are always that last and most relevant animal model that provide the critical evidence that advances the medical frontier."

He offers, as an example, the history of bone marrow transplantation. "The patients and physicians who did the first bone marrow transplants in the 1950s and 1960s were very brave in the face of frequently adverse outcomes," he says. "Bone marrow transplantation is now an established standard of care

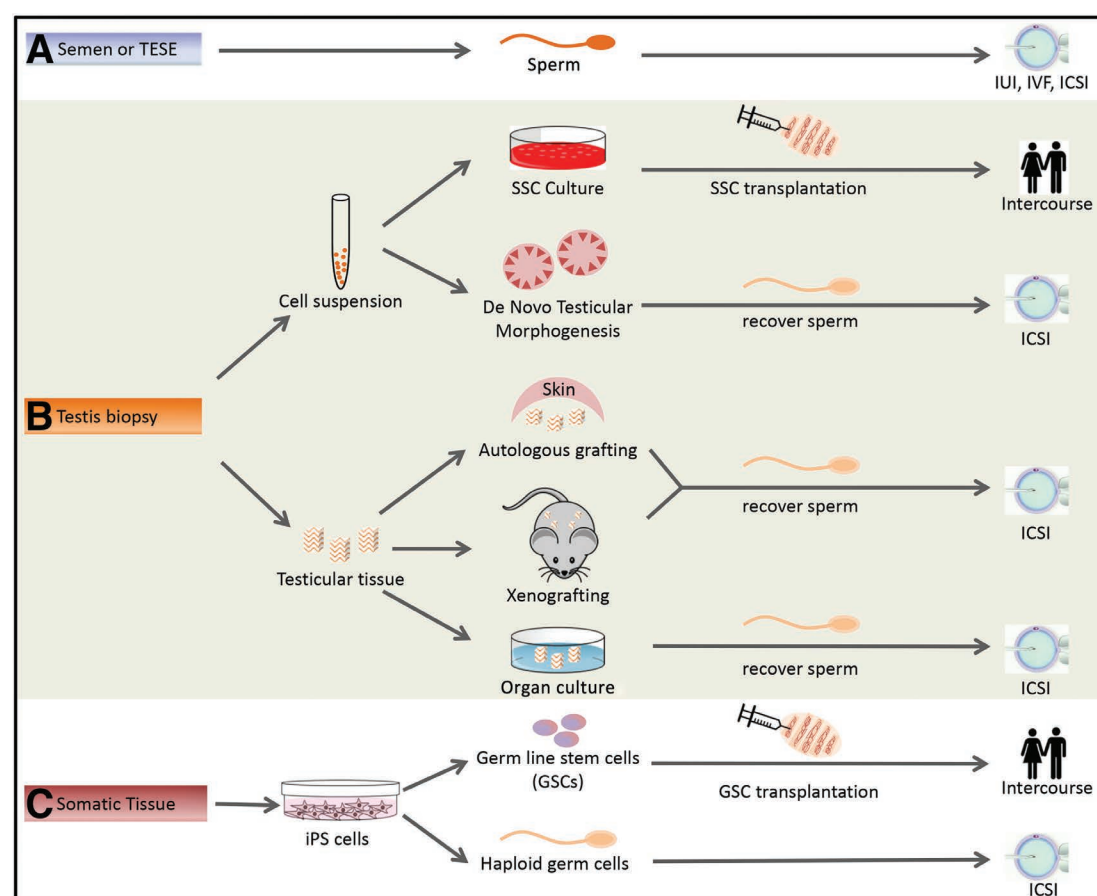
that saves lives."

With a similar future day in mind, his group has spent the past decade freezing testicular and ovarian tissue from children believing that they will someday be able to translate their technologies from the lab to the clinic and return fertility to those individuals. They've frozen tissues from about 250 kids to date.

#### FROM PATIENT TO PETRI?

Whereas SSCs offer hope to men, the two sexes are not on equal footing as women may not have the same opportunities in terms of oogonial stem cells (see the "Courting controversy" portion of this article beginning on page 25). And even though men may have the advantage, there may be situations when individual genomes make straightforward transplantation questionable.

Thus, researchers are starting



**MALE FERTILITY.** Using a variety of clinical and research approaches, researchers strive to return fertility to men. (Adapted from Gassei & Orwig. *Fert Steril.* 2016;105(2):256-266.) ICSI = intracytoplasmic sperm injection; IUI = intrauterine insemination; IVF = in vitro fertilization; SSC = spermatogonial stem cell; TESE = testicular sperm extraction

to explore other possible stem cell types.

"Imagine you have a young woman who is making this very difficult decision about having her ovaries removed because she has been diagnosed with having the BRCA mutation that is associated with having breast and ovarian cancer," says Amander Clark of UCLA's Eli and Edythe Broad Center for Reproductive Medicine and Stem Cell Research. "She has her ovaries removed, which means that unless she has undergone fertility preservation strategies—sometimes there's no time for that—she will have no option for having a biologically related child."

"Imagine if we could, when she's ready to have her family, take her skin cells, reprogram them back to iPSCs [induced pluripotent stem cells], correct the BRCA mutation [with gene editing] so she has no chance of passing that mutation on to her offspring, and then remake her germline again so that she can have children," Clark elaborates. "I think that would be a remarkable achievement if we could do it."

Clark suggests that we have already learned a significant part of this challenge from IVF.

"IVF begins with gametes," she explains. "So, for the couple coming in to overcome their infertility problems using IVF, embryos can be created and those embryos are evaluated by embryologists to pick the ones that would have the best chance of being able to make a baby."

"If we think about *in-vitro* gametogenesis, there is the same approach, at least at the end stage, for picking embryos," she enthuses.

"It is the step before that—what is highest quality gamete that can be made—that's the part that the field is still working on trying to achieve."

And that step is likely going to mean looking beyond the germ cells themselves.

Much as cancer is about more than the tumor, it's about the stromal microenvironment that surrounds it—gametogenesis ultimately is not just about the cells that become sperm and ova, but also about the tissues that surround them as well. Thus, the work with iPSCs will not just involve creating gametes but also producing tissues that support gametogenesis.

"When we make germline cells *in vitro*, we're very good at making early germline cells because that environment has been set up correctly," Clark explains. "To get them to complete gametogenesis, we have to figure out the new environment to put them in."

Thus, her lab is working toward culturing the cell mixtures into 3D organoid-like structures where the germline cells can receive the right instructions to differentiate through the next stages.

According to Clark, the proof of concept studies have already shown that *in-vitro* gametogenesis is possible in mice, work that Orwig is quick to point out was done in a single lab and has yet to be reproduced by other research groups in mice, let alone in other species.

As a next step, therefore, Clark and Orwig are collaborating to see if they can repeat those results in rhesus macaques, Old World monkeys much closer to humans both in terms of genomes and basic

reproductive physiology.

"Kyle and I have collaborative projects where we are working with macaque iPSCs to see if we can develop differentiation and transplantation approaches that can take an iPSC all the way through to a gamete," Clark continues, noting that initial efforts have focused on males.

"The rhesus macaque is a very important preclinical model," she presses. "If it turns out we are only able to make germline cells in rhesus macaque that are of poor quality and are not able to overcome infertility, I would say that this is a pretty big indicator that the technology is not ready yet for the human population."

"I am very excited about this technology," says Orwig. "If you think about it in the context of the patient, if I could turn skin cells into eggs or sperm, we wouldn't have to be exposing young patients to the risk of surgery to preserve their ovarian and testicular tissue before they start treatment. We could just wait until they grow up and use their skin cells."

At the same time, Orwig recognizes that IVF work and iPSC work are not equivalent, that the latter introduces potential risks unlikely to be found in the former, including concern about potency and tumorigenesis.

He also suspects that any work with iPSCs will lead to an entirely different regulatory framework than IVF, which currently does not require an IND because it fits within U.S. Food and Drug Administration (FDA) criteria of homologous use and minimal manipulation.

STEM CONTINUED ON PAGE 24

# THE HUMAN FACE OF INFERTILITY

**I**N A 2011-2015 STUDY of American families, the U.S. Centers for Disease Control and Prevention reported that 6.7 percent of married women aged 15 to 44 years were described as infertile, while 12.1 percent of all women across this age range experienced impaired fecundity.

This isn't just a "woman's problem," mind you, as 35 percent of couples with infertility challenges identified male and female factors, while eight percent of cases rested solely on male factors.

The statistics, however, don't describe the toll that infertility takes on individuals and couples.

Despite their research focus, both University of Pittsburgh's Kyle Orwig and UCLA's Amander Clark are never far from the all-too-human faces of infertility.

"If you aren't suffering with infertility yourself, it may be hard to know what's going on inside other people," says Orwig, who also serves as director of the Fertility Preservation Program at Magee-Women's Hospital. "I can guarantee you that there is a major psychological impact on the individual and on the couple."

"It is so easy for people in society or in science and medicine to say it is not a life-threatening disease, it doesn't affect very many people, so how important is it really?" he presses. "I hear what I would consider to be callous comments sometimes: You don't have the right to be fertile; there are kids waiting to be adopted, so why don't you just adopt?"

The disappointment at these attitudes is audible in his voice.

"You can't explain or understand what lives inside a person that just makes it that they just have to have a biological child, first of all," he explains. "And secondly, that adoption thing is not true."

"It is very hard to adopt a child," he continues. "That's particularly true in the cancer survivor scenario, where having a [potential] future cancer diagnosis, even if you're cured, is a mark against you because the thinking is that you might not be around when the child grows up."

Clark echoes Orwig's experiences even though her research is further from clinical

applications.

"I get emails on a weekly basis from couples who are asking me when will stem cell—iPSC, in particular—technology be available so that my partner and I can have a baby," she recounts. "Clearly this is a topic and a technology that is desired by members of our community."

That interest is a sign, even for those at the basic research stage, she continues, that this is something worthy of pursuit.

"It has potential to have a significant impact on the human population," she offers.

This opportunity to fulfill a fundamental human need is part of what makes Orwig so proud of the Fertility Preservation Program.

"Our program provides the most comprehensive menu of fertility preservation options that are available in a single integrated center anywhere in the world," he says. "We provide both standard of care as well as experimental options to women, men, girls and boys."

"When people are willing to travel across the world to pursue fertility preservation options that are here, it tells you how important the fertility issue is to people," he explains, recognizing the additional responsibility into which that faith and hope translates.

"Because we're freezing tissues for patients, it becomes a priority for our laboratory to responsibly develop the technologies that will allow patients to use those tissues in the future."

On the road to the human fertility clinic, Orwig relates, he is meeting all kinds of patients he never thought about before.

"This might be women who are already say in their late 30s or 40s before they try to have a child, so they have age-related infertility," he lists. "It could be kids who are transgender, who feel like they were born in the wrong body, so they take hormones to transition to the other gender. It can be people with genetic diseases."

These interactions have changed him and his mission.

"Our mission has expanded quite a bit from stem cell therapy to basically trying to develop solutions for patients with the most intractable cases of infertility." ■





**“If it turns out we are only able to make germline cells in rhesus macaque that are of poor quality and are not able to overcome infertility, I would say that this is a pretty big indicator that the technology is not ready yet for the human population,” says Amander Clark (right, with colleague Di Chen).**

CREDIT: AMANDER CLARK/UCLA

According to FDA guidance updated last year: “For cells or nonstructural tissues, minimal manipulation means that the processing of the HCT/P does not alter the relevant biological characteristics of cells or tissues.”

As well: “We generally consider an HCT/P to be for homologous use when it is used to repair, reconstruct, replace, or supplement:

- Recipient cells or tissues that are identical (e.g., skin for skin) to the donor cells or tissues, and perform one or more of the same basic functions in the recipient as the cells or tissues performed in the donor; or,

- Recipient cells or tissues that may not be identical to the donor’s cells or tissues, but that perform one or more of the same basic functions in the recipient as the cells or tissues performed in the donor.”

As Orwig explains, iPSCs clearly do not fall under minimal manipulation.

“You’re going to take the cells out, you’re going to keep them in culture for a long time, you’re going to differentiate them, you’re going select the ones that did the right thing, that’s a very extensive manipulation,” he counts off. “So, it is certain that some addi-

tional regulations will be necessary there as compared to the normal IVF clinic.”

While working toward the human clinic, Clark is also taking the opportunity provided by iPSCs to explore the cellular pathology of infertility, understanding the pathways involved in germline cell formation. Central to this effort has been her lab’s use of CRISPR gene editing to knock out genes they believed important to these processes.

“We’ve been able to publish now a number of new genes that are required for human germline development,” she recounts. “Some of these were a surprise because they were not known to function this way in other scientific models.”

She suggests that these surprises give us insight into some of what makes us uniquely human and serves as a warning about over-reliance on animal models. If you’re interested in human disease, she suggests, it is very important to study human cells.

“What iPSCs have enabled us to do is take samples from men and women who have been diagnosed with infertility and to be able to turn back the clock to a pluripotent stage to remake the germline cells, and to figure out what potentially could be going wrong,” Clark summarizes.

#### COURTING CONTROVERSY

Part of the reason behind the focus on male germline stem cells is the concept of the ovarian reserve.

“In most mammals, the pool of primary oocytes for life is fixed shortly after birth and gradually decreases during reproductive life,” explained Veronica Giorgione and colleagues at San Raffaele Hospital in a recent review. “The ‘ovarian reserve’ is thus established during the fetal period, when oogonia derived from PGCs promptly proliferate by mitosis before entering meiosis and differentiating into primary oocytes.”

This dogma has been challenged within the last 15 years, however, by researchers who suggest that there may be a female equivalent to the SSCs in the testes.

Although rare, it is argued, these oogonial germline stem cells (OGSCs) could be harvested from women for fertility preservation and then transplanted later to initiate neo-oogenesis.

In 2017, Justin St. John and colleagues at Monash University, Hudson Institute of Medical Research and OvaScience characterized the egg precursor cells (EPCs, aka OGSCs) from a mini-pig model using FACS, RNA sequencing and next-gen sequencing to look for cellular, nuclear and mitochondrial markers of potency.

“We have cultured isolated EPCs for one week, without passage, and observed that they were not dormant and were able to proliferate under *in vitro* conditions,” the authors wrote. “We then assessed the gene expression profiles of EPCs and found that they shared some key markers with porcine PGCs.”

They also noted a marker of pluripotency as well as several markers of cell proliferation and self-renewal, but the marker profile was not always in line with that of PGCs.

“Therefore, we suggest that these EPCs are undifferentiated multipotent lineage-specific oogonial cells, that could differentiate into oocytes or be dedifferentiated under the right conditions,” they wrote.

Not everyone is equally confident in the existence of OGSCs or the realistic possibility of neo-genesis.

“If there is a population of oogonial stem cells, it is not revealed by the disparate life histories of men and women,” Orwig offers.

“I won’t say that I don’t believe in oogonial stem cells, but I will say that I have substantial concerns,” he continues. “It doesn’t mean that the stem cells aren’t there, but that the environment of the ovary is not hospitable to their development after the fourth or fifth decade of life.”

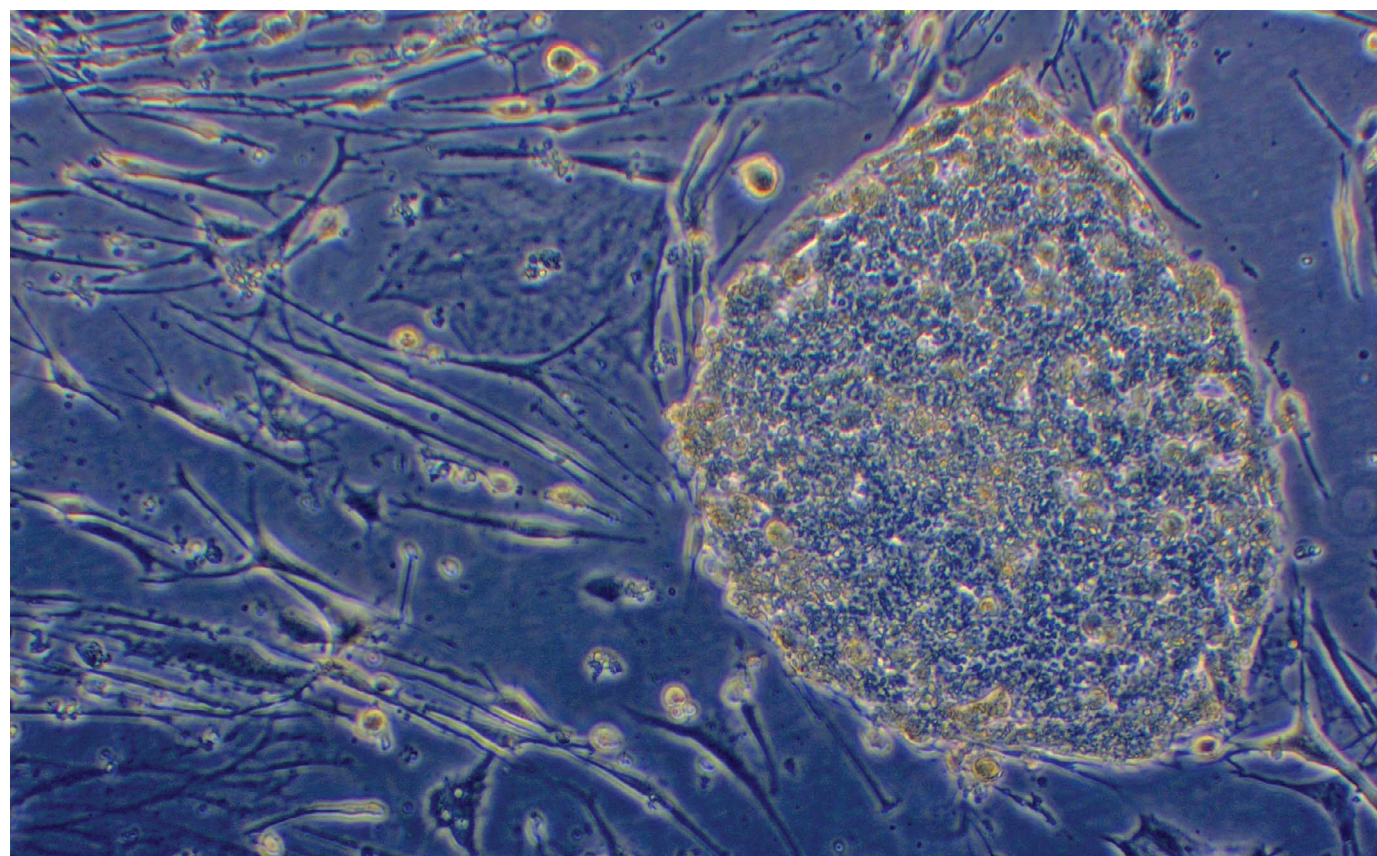
A similar thought was espoused in 2014 by University of Edinburgh’s Richard Anderson and colleagues.

“If neo-oogenesis does indeed exist, then the rate of new oocyte production must lessen with age in order for menopause to

**“Imagine you have a young woman who is making this very difficult decision about having her ovaries removed because she has been diagnosed with having the BRCA mutation that is associated with having breast and ovarian cancer. She has her ovaries removed, which means that unless she has undergone fertility preservation strategies—sometimes there’s no time for that—she will have no option for having a biologically related child. Imagine if we could, when she’s ready to have her family, take her skin cells, reprogram them back to iPSCs, correct the BRCA mutation [with gene editing] so she has no chance of passing that mutation on to her offspring, and then remake her germline again so that she can have children.”**

**Amander Clark of UCLA’s Eli and Edythe Broad Center for Reproductive Medicine and Stem Cell Research**





CREDIT: AMANDER CLARK/UCLA

**STEM CELL INSIGHTS.** By differentiating human iPSCs into germline cells and ultimately gametes, UCLA's Amander Clark hopes to better understand causes of infertility and possibly one day treat infertility.

occur," the authors wrote. "There may be two potential underlying mechanisms: failure of the fGSCs [female germline stem cells, aka OGSCs] to form oocytes or failure of the somatic environment to support oocyte development (or both). It is possible that fGSCs, like many other cells, undergo an aging process and thus lose their capacity to regenerate and differentiate."

The authors even went so far as to speculate on a distinction between what is possible and what is reproductively relevant.

"Although fGSCs may be isolatable from ovarian tissue and potentially able to form oocytes within follicles after various manipulations, this may occur only under experimental conditions and they may not have any relevance to the normal processes of ovarian function."

Clark is cautious and awaits more data from other models.

### BEYOND THE GERM

Doing a complete end-run on some of the ethical concerns about stem cells in the germline, other research groups are using stem cells to bolster the cells and tissues that are already in place.

In May, for example, Sonia Herraiz and colleagues at IVI Foundation, Igenomix and other institutes treated mice with chemotherapy to induce ovarian failure and then examined whether that failure could be reversed with infusions of bone marrow-derived stem cells (BMDSCs) versus controls of saline or peripheral blood mononuclear cells (PBMNCs).

They noted that whereas 75 percent of mice receiving BMDSCs recovered normal cyclicity, none of the control mice from either group did. Similarly, ovarian weight increased in the test mice compared with either control group. The ovarian tissue morphology of mice receiving BMDSCs also displayed more normal histology whereas those of the control group were more apoptotic.

The researchers then used human ovarian cortex from patients with poor ovarian response to create xenografts in ovariecto-

mized mice. They treated these mice as above to identify any effect of stem cells and noted significantly superior increases in follicular density and vascularization in the BMDSC group.

"BMDSC infusion rescued the remaining nondamaged oocytes in this highly gonadotoxic situation, allowing the birth of healthy pups as demonstrated by up to three consecutive pregnancies with an increased litter size even in the subfertile NOD/SCID strain," the authors added. "In contrast, pregnancy was not achieved in the PBMNC and control groups, supporting previous reports of complete infertility after administration of this standard gonadotoxic treatment."

"Our results raise the possibility that promoting ovarian angiogenesis by means of autologous BMDSC therapies could be an alternative to improve follicular development in aging women, in women treated with [chemotherapy] for cancer, and in [poor response] patients where the ovarian niche cannot maintain growth of their already limited follicular pool," they enthused in conclusion.

At the ENDO 2018 meeting in March, Ayman Al-Hendy and colleagues at Augusta University and University of Illinois, Chicago reported on their efforts to perform a similar experiment in women experiencing premature ovarian insufficiency. In a clinical trial that is ongoing, the researchers isolated autologous mesenchymal stem cells from bone marrow biopsies and injected them into one of the ovaries of each woman, using the second ovary as an internal control. They then monitored the women (two to date) for hormonal parameters as well as ultrasound and quality-of-life assessments.

Within months, both women resumed menses, demonstrated a marked reduction in their post-menopausal symptoms, and doubled their estrogen from pre-operative levels. Ultrasound imaging, meanwhile, showed that the treated ovary significantly increased in size compared to its untreated counterpart.

"While it is yet to be seen if conception is possible, these are all signs that ovarian func-

tion is returning and that stem cell therapy may be a promising approach to treat difficult cases of female fertility," Al-Hendy commented in a UIC blog post. "There is a lot we still don't know and we need to study this more in patients before making any conclusions, but the research is promising."

For their part, Guangdong Second Provincial General Hospital's Xiang-Hong Ou and colleagues attempted to reverse the natural decrease in oocyte quality that comes with aging, focusing their attention on mitochondrial dysfunction, which they suggested is a major contributing factor to IVF failure in older women.

"As the most prominent cell organelles in oocytes, mitochondria play pivotal functions and determine the developmental competence of oocytes," the authors explained. "With advanced maternal age in women, the most common aberrations in mitochondrial structure are mitochondrial swelling and cristae disruption."

Looking for an ideal and easily accessible mitochondrial source, the researchers harvested adipose-derived stem cells from mice, transferring those mitochondria into oocytes from an aged mouse.

Culturing the oocytes *in vitro*, the researchers noted that autologous mitochondrial transfer not only improved cell division, but also significantly reduced aneuploidy rates.

They then combined the transfer with intracytoplasmic sperm injection, doubling

the blastocyst rates *in vitro* over controls as well as dramatically increasing the number of pups born.

Although there remains a great distance between mice and humans, the researchers were enthusiastic that the "study may provide a promising strategy to increase oocyte quality and fertility in elder women."

For Orwig, mitochondrial transplantation is exciting but for a completely different application: women at risk of having children with mitochondrial diseases.

The method has been proven in animal models, he says, and there exists at least one child free of mitochondrial disease despite having older siblings with it.

Born in April 2016, the so-called three-parent baby was the result of transplanting the nucleus of the mother's egg into a denucleated donor egg and then fertilizing it with the father's sperm. In this case, the mother had the rare neurological condition Leigh syndrome.

New Hope Fertility Center's John Zhang and colleagues described the procedure in April 2017.

"In that context, mitochondrial therapy is interesting," Orwig says. "It has been approved in the United Kingdom. It is expressly not allowed in the United States."

He is hopeful that those restrictions will disappear, at some point, recalling that both the National Academy of Medicine and the FDA were in favor of approving mitochondrial replacement therapy.

"Then a law got put on the books that made it illegal," he continues. "In this regard, maybe people in the UK will lead the way and provide the evidence that will justify removing that law from the books."

### THE NEXT GENERATION

In the face of such potentially powerful technologies, Orwig believes the reproductive medicine clinic of the future is going to look different than it does today.

"I think that we're actually on the brink of a new era in reproductive medicine where we're going to be able to make eggs, make sperm for people who don't make them at all," he presses.

He points to a range of SSCs, which he feels are ready for the clinic, and touts ovarian tissue transplantation, which is already in the clinic and has produced more than 100 babies worldwide.

"Then you have the other extreme of very exciting technologies, such as iPSCs and *in vitro* germ cells, that may be earlier stage but could be very exciting when they get there," he enthuses.

Just as Louise Brown startled the world four decades ago, another swaddled infant may yet be on the horizon to delight not just his or her family, but maybe the world.

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**REVERSING MENOPAUSE?**  
By injecting autologous MSCs into ovaries, Ayman Al-Hendy and colleagues reversed the effects of premature ovarian insufficiency, rejuvenating ovarian function and reducing post-menopausal symptoms.