Fertility clinic meltdown: What happens when slumbering eggs are awakened early

The spindle apparatus is among the most elegant structures in a cell, quickly self-assembling from microtubules and grabbing and aligning chromosomes so that equal sets separate into the two daughter cells that result from a division. But can spindles in cells held at the brink of division in the suspended animation of the deep freeze at a fertility clinic survive being ripped from their slumber off-protocol, as happened the weekend of March 4 at the Pacific Fertility Clinic in San Francisco and University Hospitals Fertility Center in Cleveland?

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3-19-2018 Making_Liquid_Nitrogen_Ice_Cream_-_Sentosa_Singapore_-_16_Oct._2013-300x225
Liquid nitrogen, here used to make ice cream, not preserve eggs and embryos.

It was a stunning coincidence impacting the eggs or embryos of 500 couples on the west coast and 700 using the Ohio clinic. Liquid nitrogen ran low in a cryogenic device in San Francisco, and temperature fluctuations reportedly plagued the Cleveland facility.

The tragic events sent me back to developmental biology courses in grad school, and I read a bunch of technical papers and polled a few nerd friends. The experiments from the 1950s onward were controlled, and so my thoughts on the damage done in early March are hypothetical. I can't help but wonder what, exactly, happened to those eggs and embryos?

The media and the damage done

A news conference from one legal firm filing a class action lawsuit against the clinics was long on emotion and short on details. Here's a lawsuit from a different firm that provides a timeline of what the donors went through.

Media coverage lacked biological details too. WaPo mentions "damage to tissue" with a video proclaiming "the only way to tell the viability of the egg is to thaw and implant it." *Really?* Law firm websites parroted WaPo, not scientists (many experts in reproductive biology are PhDs, not MDs).

The New York Post and other outlets shared the sad story of Amber and Elliott Ash, who froze two embryos in 2003 after his cancer diagnosis. "The medical community calls it tissue. I like to think of it as my children," Amber said.

Carl Herbert, MD, head of the San Francisco clinic, was curiously optimistic on NPR. "The good news is, we have viable embryos — we've proven that from that tank." Viable enough to transfer?

Of oocytes and embryos

3-19-2018 sperm-on-5-day-300x300

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Days after fertilization, these tenacious sperm are still trying to gain entry.

Technically speaking, there's no such thing as an egg or ovum. Feminism aside, our gametes are oocytes until a sperm enters one and it magically then becomes a fertilized ovum. That's why scientific reports and reproductive health guidelines use "oocyte." Lawyers stick to the familiar. "*Has a freezer failure compromised your frozen eggs or embryos?"*

An oocyte packs in maximal nutrients through two divisions of meiosis. Before birth, a female has about a million oocytes stalled in the first meiotic division. Then, starting at puberty, a few oocytes awaken each month and continue meiosis, halting just before completion until a sperm comes along. If no sperm enters, the oocyte never finishes meiosis and leaves in the menstrual flow. If the oocyte is fertilized, its spindle apparatus ensures that the resulting fertilized egg has the right number of chromosomes from the female; similar division happened as the sperm formed.

When a cell isn't dividing, spindles break down. But as one cell splits to become 2, and then 2 become 4, and 4 become 8 as the cleavage divisions of the early embryo ensue, spindles form and vanish and reform to properly distribute the chromosomes. This time, in non-sex (somatic) cells, it's mitosis, not meiosis.

Because spindles are sensitive to temperature, the unexpected fertility clinic meltdowns are worrisome.

A brief history of cryopreservation

Efforts to freeze – or cryopreserve – eggs, sperm, and embryos have been ongoing for decades. The main challenge is to avoid formation of ice crystals, which can slice up cellular interiors.

3-19-2018 Sperm-20051108-150x150

Image not found or type unknown Sperm freeze easiest because they have little fluid – they’re not much more than a

bulbous DNA-stuffed head with a tail and a ring of empowering mitochondria. Sperm were first frozen in 1938, with the first human birth resulting in 1954.

Experiments unfurled on eggs a little later, using glycerol in the 1950s and the solvent DMSO (dimethyl sulfoxide) in the 1970s, in sea urchins, mice, hamsters, rabbits, sheep, and monkeys. Early efforts at gradual cooling wrecked the spindles of mouse, hamster, and rabbit eggs, leading to the wrong number of chromosomes (aneuploidy). The nuclei of some eggs even glommed together, ending up as “polyploidy digynics,” a form of parthenogenesis that doubles the female genome in a gamete. Might such developmental disasters appear “viable” if simply eyeballed, the chromosomes uncharted? Embryo pickers have told me, though, that they can tell a good embryo from an ill-fated one just by looking.

Various sugars, solvents, and antifreezes (glycerol, propylene glycol and ethylene glycol) have been used in egg and embryo preservation as “cryoprotectants.” They displace water while countering ice crystal formation and strengthening membranes, preserving fragile cellular insides and keeping the outsides intact as a freezing agent, like liquid nitrogen, is applied.

These chemicals have different roles. Sugars stay outside cells, drawing the water out of them by osmosis, while glycerol and ethylene glycol enter and prevent the cell from shriveling. In this way cells can dehydrate but maintain their three-dimensional shapes. It works because eggs and embryos are small; it wouldn’t work so well on a spleen, for example.

Slow cryopreservation, over two to three hours, with the cryoprotectants added one at a time as the cells gradually cooled, gave way to the much faster vitrifaction, which uses higher concentrations of cryoprotectants. It’s so fast that a glass-like consistency forms, not ice. Vitrification uses liquid nitrogen, plunging the temperature to -320.8 degrees Fahrenheit (-196 degrees Celsius).

3-19-2018 han-solo-carbonite-door-decal-300x250

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(Theatrical asides: Han Solo in *The Empire Strikes Back* was

flash-frozen in carbonite, which my husband Larry the chemist says does not exist. Larry brought liquid nitrogen home for our kids' parties – when poured on the floor it evaporates into an entertaining eerie steam. And famed baseball player Ted Williams' head is reportedly frozen at a cryogenics lab in Scottsdale, Arizona.)

Warming is precise and delicate. "It's basically the reverse process. The key factors that must be achieved on thawing are rehydration of the cell without blowing it up," embryo expert Barry Behr, PhD, told *Scientific American* in 2005. The cryoprotectants are gradually diluted out as the temperature slowly rises, and after a few hours at body temp, embryos can be implanted if they look okay – they've refilled the fluid-filled space at their centers and the cells are clear and not dark.

The first baby to develop from a frozen embryo was born in 1984. Births from vitrified human eggs that were then fertilized came in 1999. (A great review is here.) Usually 20 to 30 eggs are frozen, with 6 to 8 thawed for each IVF attempt.

From medical need to lifestyle choice

As with other assisted reproductive technologies, like IVF and preimplantation genetic diagnosis (PGD), the reasons for freezing eggs have evolved from medical need to lifestyle choice.

fert ovum x

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A human fertilized ovum. (Spike Walker, Wellcome Images)

In 2013, the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology published *Mature Oocyte Cryopreservation: A Guideline, which stated*"there are not yet sufficient data to recommend oocyte cryopreservation for the sole purpose of circumventing reproductive aging in healthy women because there are no data to support the safety, efficacy, ethics, emotional risks, and cost-effectiveness of oocyte cryopreservation for this indication."

In 2014 the American College of Obstetricians and Gynecologists (ACOG) published support of the 2013 statement.

At that time, egg freezing was being considered for women undergoing damaging treatments like chemo; had conditions like fragile X or XO (Turner's) syndrome that can include ovarian insufficiency; or who had their ovaries removed to prevent *BRCA*-related cancers. But by 2016, when ACOG declared egg cryopreservation "no longer considered experimental," the candidate pool expanded. And a vast economic opportunity presented itself to the egg and embryo freezers.

I perused a few relevant websites.

UCLA's Egg Freezing website first lists medical reasons and then *"fertility preservation for social or personal reasons to delay childbearing."*

The language at the Mayo Clinic website is uncharacteristically condescending: *"Your doctor can help you understand how egg freezing works,"* and then helpfully points out that freezing eggs doesn't require sperm. But the first entry in the list of reasons is: *"Egg freezing might be an option if you're not ready to become pregnant now but want to try to ensure your ability to get pregnant or have a biological child in the future."*

Blastocyst embryo x

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The early embryos that are frozen are balls of cells.

Prelude Fertility, which runs the facility in San Francisco, makes matters clear under a photo of a smiling, multiracial woman: *"Find that right person. Focus on your career. Finish your education. The age of your eggs (not you) is the number one cause of infertility. Freeze your eggs to preserve your option to build a family when you're ready."*

It isn't cheap. Egg freezing at Pacific Fertility costs $8,345 for the first retrieval and a year of storage; a second cycle is $6,995. And that's not counting charges for drugs, new patient consultations, lab tests, and continuing storage fees.

Back to the spindle

A paper from 2005 in *Human Reproduction* raises hope – after freezing and thawing, a spindle can reform. It's possible, because the tubulin protein pairs that build the microtubules that build the spindle naturally self-assemble.

For the study, conducted at the University of Bologna, 18 patients donated 110 oocytes. The cells were slowly cryopreserved using propylene glycol and sucrose, then thawed, as the researchers watched, capturing images with polarized light microscopy. Over the course of five hours, spindles reappeared in about three-quarters of the oocytes.

But tracking the return of the spindle, however elegantly, didn't go far enough. Does the spindle apportion a complete set of 23 chromosomes into the maturing egg? If not, a miscarriage or birth of a child with an extra or missing chromosome could result.

Emotions and lawsuits are running high right now, but I hope an opportunity to learn something about early development from the dual disasters isn't lost. Here's how.

blastocyst

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Collect data!

Pacific Fertility's website claims "the egg recovery rate after vitrification and later thawing is 83 percent, and fertilization rate is 84 percent." Is that so for the damaged material? How about constructing karyotypes (chromosome charts, which destroy the cell) for some of the retrieved eggs and embryos from patients who've stored several, leaving some aside in case things look good. If more eggs have other than 23 chromosomes, and more embryo cells have other than 46, compared to the numbers predicted based on the age of the mother when the cells were collected, then damage has indeed been done.

Perhaps what's learned can be used to improve the process of preserving eggs and embryos.

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