Studies to find better ways to preserve human eggs, ovarian tissue under way

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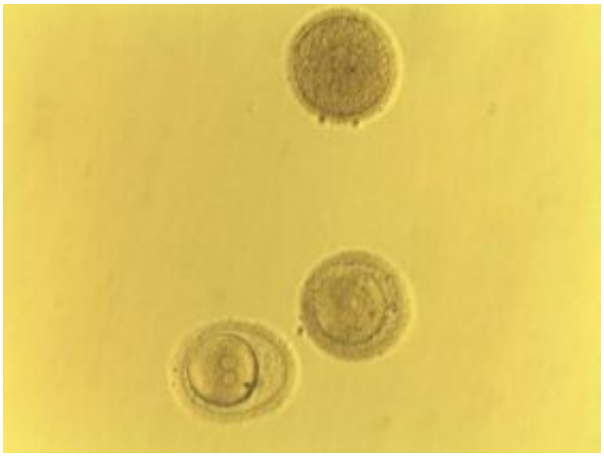

Three human eggs, donated to research because they were inadequate for fertilization, that have been vitrified then warmed. Credit: Medical College of Georgia

The goal is to make human eggs, ovarian tissue, blood vessels, even whole organs available when needed.

To get there, researchers are directly comparing slow-freezing techniques, used successfully for decades to preserve sperm and embryos, to a more rapid method of cryopreservation that transforms tissues into durable glass-like structures.

Phase I trials under way at the Medical College of Georgia are

comparing the two approaches in human ovarian tissue and eggs, or oocytes, as well as human-like cow ovarian tissue and eggs.

They start with reproductive tissues because young women with cancer produce a compelling need and are a good model for other tissues and organs.

“What we tell patients is that right now the standard of care for people who are going through cancer therapy is to use egg donors later on,” says Dr. Adelina M. Emmi, reproductive endocrinologist and medical director of MCG Reproductive Laboratories of Augusta.

Treatment for leukemia and cervical, ovarian, breast or other cancers often leaves women infertile because systemic chemotherapy and more focused radiation therapy, designed to kill rapidly spreading cancer cells, also can destroy dynamic reproductive tissue.

“I don’t think when you are faced with the reality that you may die, your fertility is the most important thing you are thinking or talking about, but there are a lot of women interested in talking about it,” says Dr. Emmi. She hopes her work with Dr. Ying C. Song, cryobiologist, will one day give her more to say.

They are collecting ovarian tissue from volunteers age 16 to 37 who need the tissue taken for some reason other than cancer, such as a hysterectomy for benign disease, says Dr. Song, MCG clinical associate professor at MCG and director of research for Augusta-based Xytex Research/Xytex International. Collaborators at the University of Texas Health Science Center and M.D. Anderson Cancer Center are doing the same.

With some of the tissue, they are using conventional cryopreservation. Chemicals to protect cells from the hazards of freezing are added before

taking tissue from the refrigerator temperature of 4 degrees Celsius to minus 80 degrees Celsius over two- and one-half hours. Later, liquid nitrogen takes it to minus 196 degrees

“You put it in a control-rate freezer that takes down the temperature one degree centigrade per minute so it drops the temperature very, very slowly,” says Dr. Song.

Slow cooling works well for simple tissue, such as sperm or even embryos, and for blood. “In blood, for example, conventional cryopreservation freezes the liquid part but not the cells inside. Liquid freezes and the water inside the cells moves out gradually so they dehydrate,” Dr. Song explains.

But, for more complex structures, such as a human egg or ovarian tissue, resulting ice formation can be destructive. “Ice crystals break up your inside organelles. That is what hurts eggs, which are very delicate,” he says.

“When you trigger ovulation with a hormone or naturally, you get the last separation of the chromosomes, from 46 to 23,” says Dr. Emmi. That separation enables a future baby to get half his chromosomes from mom and half from dad. Fragile spindles, which line up chromosomes for division, are easily broken during freezing so chromosomes can’t properly divide. Typically the resulting embryo dies. Plus, fertilization is unlikely since freezing often hardens the egg’s outer shell that sperm must penetrate.

“That is why we have tried to develop technology without freezing,” says Dr. Song, who has pioneered use of vitrification in blood vessels, cartilage and heart valves.

Vitrification, which takes tissue from room temperature to minus-100

degrees Celsius in 20 minutes, solidifies tissue into a clear, glass-like structure minus the opacity of ice cubes and frozen meats, a tell-tale sign of ice crystals within.

Dr. Song, whose research lab is in MCG's biotech incubator, has developed cryoprotectants that can be used safely in higher doses as well as agents to help protect tissue during the ultra-rapid process of de-vitrification.

"People use low concentrations of cryoprotection because they are toxic," he says. "The problem is, if you use lower concentrations, you cannot get true vitrification." The agents are needed to intercept water so it won't form ice. Interestingly if small ice crystals form during cooling, they can get larger during de-vitrification, which takes place in seconds.

"We developed a solution where we can warm up tissue in under five minutes and still get no ice formation," says Dr. Song, adding that ice formation aside, it is difficult to thaw rock-solid tissue at room temperature in a matter of seconds, meaning the current approach could have extremely limited use.

A study he published in March 2000 in Nature Biotechnology showed the approach he uses works well, at least in blood vessels. "Now we want to try this on eggs and ovarian tissue and see if we can develop a robust technology and improve outcomes," Dr. Song says.

Later, researchers will put ovarian tissue preserved both ways into mice to see if it survives and starts making proper connections.

"The reason for using ovaries is when you have cancer, if you need chemotherapy, you often don't have time to go through stimulation cycles to get oocytes," says Dr. Emmi. "You are concentrating on getting rid of cancer cells." Also, if a woman has breast cancer, for example,

hormones needed to induce ovulation could be problematic because many breast cancer cells have estrogen receptors.

As pieces of a puzzle come together, Dr. Song notes scientists already are developing methods to stimulate ovarian tissue to produce eggs outside the body, a process that could also make in vitro fertilization a lot more affordable. Others are looking for ways to ensure there are no cancer germ cells in salvaged tissue.

If all goes as hoped with this study, the next step will have Drs. Song and Emmi taking ovarian tissue from cancer patients, vitrifying it then, after they are sure it's cancer-free, re-implanting it when the woman is ready.

A concurrent phase I study is comparing standard cryopreservation to vitrification in eggs. They are using eggs from 60 women age 18-42 that would be discarded because they are not adequate for in vitro fertilization. They also are retrieving and maturing eggs from cow ovaries donated by a local slaughterhouse. Bull sperm will be used to test the viability of cow eggs afterward but human eggs will not be fertilized.

Standard cryopreservation has been tried and largely failed in human eggs, says Dr. Emmi, who believes some version of vitrification likely offers a better option for ovarian tissue and eggs. In fact, many in vitro fertilization programs, including the one she directs at MCG Health System, are moving toward vitrification, which also seems to work faster, better and cheaper in embryos.

They pursue the potential of egg preservation as well to really find out which is the best option: ovarian tissue or pure eggs. Also, a better way to preserve eggs – which last about 24 hours outside the body without preservation – would reduce the cost and logistical issues of coordinating donor eggs.

“The long-term goal is organ banking,” says Dr. Song, because even though there are insufficient numbers of donors for those on transplant lists today, another piece of the puzzle is developing techniques for growing organs. In fact, he is collaborating with scientists at Yale University and the Georgia Institute of Technology to regenerate blood vessels and pancreatic substitutes, noting studies published in Tissue Engineering and Cell Transplantation.

“Regenerative medicine will help supplement the shortage of organs in the future, and we need technology to preserve those we make.”

Source: Medical College of Georgia