# Drugs needed to preserve eggs for reproduction need to be given in stages

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This is Dr. Ali Eroglu from Medical College of Georgia. Credit: Phil Jones

Cryoprotectants needed to preserve eggs for reproduction need to be given in stages, albeit rapid ones, say scientists who have developed a mathematical model that predicts optimal time for loading and unloading these drugs.

Their studies in Rhesus monkey eggs, which are very similar to human eggs, show that a two-step process of easing into and out of the drugs needed to help protect eggs at subzero temperatures dramatically reduces the amount eggs contract and expand in the process.

These dramatic size shifts can literally rip an egg apart or, at the very least, reduce the chances it can be fertilized, says Dr. Ali Eroglu, reproductive biologist and cryobiologist in the Medical College of Georgia Schools of Medicine and Graduate Studies.

Scientists first looked at how fast the three most commonly used cryoprotectants - dimethylsulfoxide, ethylene glycol and propylene glycol - permeate monkey eggs. Faster permeability is better with these drugs which must be given at room temperature when their toxicity levels are high. With permeability rates in hand, MCG scientists used a mathematical model, developed in collaboration with Villanova University in Pennsylvania, to successfully predict optimal loading and removal times.

They found propylene glycol works best in monkeys. The drug penetrated the egg membrane faster and got out faster, Dr. Eroglu and his colleagues report in the April issue of *Molecular Reproduction & Development*.

All of the drugs worked best when used incrementally: putting some in the medium around the egg and a few minutes later adding a little more when it was time for cryopreservation and, conversely, transferring them to increasingly lower concentrations of the drugs when it was time for thawing.

While still less than 10 percent, success rates for egg preservation to help protect an endangered species or enable a cancer patient to retain fertility have improved in recent years as scientists learn more about how best to give and remove the drugs - including using more than one at a time - with the idea that they may work synergistically and hopefully with reduced toxicity.

Scientists also are taking important cues from critters with an innate ability to preserve themselves. Increasingly added to the mix of manmade cryoprotectants are sugars, which are used by a variety of species, such as brine shrimp and eight-legged tardigrades, a microscopic water-dweller also known as "water bears," to survive drought conditions. Scientists like Dr. Eroglu, are showing that these sugars, such as trehalose used by tardigrades, also can help humans, monkeys and other species that don't naturally produce

them.

With human egg preservation, the sugars currently are placed in the medium along with the cryoprotectants, where it appears to work some magic. Sugar can't permeate a cell membrane so high concentrations in the medium reduce the amount of fluid that moves inside the egg. Too much fluid causes the egg to swell and potentially burst. Almost paradoxically, because sugar thickens the medium, it helps put pressure on the cell to move cryoprotectants out when it's time.

Animals likely use sugars this way and to transform to a glasslike state. Those that naturally produce sugars have transporters that enable it to be outside and inside their cells, where it appears to afford additional protection. Dr. Eroglu has given mice eggs that added protection by injecting sugar in a process similar to how sperm can be injected for fertilization. He's found it dramatically reduces the temperature needed to reach the glasslike preserved state. In fact, he's shown trehalose can enable eggs to move into a glasslike state at -30 degrees Celsius compared to -80 C, -100 C or even colder temperatures required with only conventional cryoprotectants.

That means eggs can be stored and transported much more easily. "With conventional cryoprotectants, you have to store them in liquid nitrogen. With this, you can just put them in a freezer or transport them on dry ice," Dr. Eroglu says. Additionally, the more dramatic temperature shifts put additional stress on already stressed eggs.

Improved transportability could be a big plus if/when these preservation techniques are applied to organs, he says. About 40 percent of organs intended for transplantation are damaged before and during transportation and Dr. Eroglu theorizes the glasslike state that can preserve an egg for years could go a long way in reducing that.

One of his many goals is to make the protective powers of sugar more widely available by designing ones that can penetrate a cell or egg membrane.

Source: Medical College of Georgia