Advance achieved in dry preservation of mammalian sperm cells

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In a paper forthcoming in the November issue of the journal *Theriogenology*, a team of researchers from the University of North Carolina at Charlotte and the Smithsonian Conservation Biology Institute, announced the first successful drying and rehydration of domestic cat spermatozoa using a rapid microwave dehydration method.

The paper's authors, Jennifer Patrick and Gloria Elliott from UNC Charlotte, and Pierre Comizzoli from SCBI, show that the rehydrated spermatozoa have minimal DNA damage and are viable - they are able to produce embryos in vitro. Since the group had previously succeeded in producing viable dehydrated cat eggs, this finding shows the possibility of preserving feline reproductive cells in a dried state.

Far from being an esoteric accomplishment, the successful preservation of cat spermatozoa by dehydration is a potentially important step in addressing key issues involved in the reproductive biology of wild felids.

Many biologists and environmental scientists think that the biosphere is currently in the middle of a "sixth extinction" that may end in a vast reduction of the number of species on the planet and the collapse of vast ecosystems. There is a significant risk that in the near future, species key to the biological diversity of the planet may either go extinct or be so reduced in their genetic diversity that wild populations are not viable.

Science might be able to rapidly and successfully improve the status of small animal populations if more "libraries" of preserved eggs and sperm

are available. Scientists could simply use stocks of reproductive material, preserved in stable, dried form, re-hydrate them and create a population of viable embryos.

Why dried reproductive cells? The idea of preserving sperm, eggs and embryos for later use is not new, but generally the preferred preservation technique is for these materials to be frozen. Cryopreservation is a proven technology for preserving germ cells and embryos but there are problems with this approach, considering future uncertainty. Storage at freezing temperatures requires constant energy supplies, expensive technology and facilities, and complex upkeep operations- all difficult and costly things to continuously maintain over long periods of time, especially under occasionally adverse conditions.

Nature suggests another, perhaps more robust, solution: cellular stasis through dehydration. Plants, fungi and bacteria, do this commonly, putting their genetic material in spores, cysts, pollen and seeds, which keep it preserved for short periods - and also, sometimes, for much longer time scales - and allow it to be transported across distances as well.

Some animals that live in harsh deserts and other extreme environments—such as brine shrimp and tardigrades ("water bears")—have also developed the ability to put their biology into a state of dehydrated stasis, sometimes for long periods. They do this by producing and accumulating high concentrations of disaccharide sugars (like trehalose) in their cells, which replaces the water lost during dehydration and solidifies to a glass - really highly viscous liquid that stops chemical activity and immobilizes enzymes - an ambient-temperature freezing of cellular structures and activity, a molecular-level version of insects frozen in amber.

"When you are thinking about long-term preservation of organisms, you

aren't concerned with just electrical interruptions. Flooding and other weather events can require the relocation of samples under duress," Elliott notes. "Frozen samples aren't easily transportable whereas if your samples are stored as dry packets - just like dried fruits or any dry goods you have on a shelf - you can toss your collection in a bag and out the door you go. That's the concept - not only to keep the cost of storage low, but to make specimens easily transportable, facilitating the sharing or relocation of specimens."

The preservation method that Elliott's research team is investigating involves suspending cells in a dilute trehalose solution, and then concentrating it by removing the water with a gentle microwave-assisted heating process so that a trehalose glass forms, immobilizing biological molecules at ambient temperatures, similar to freezing.

Reproductive cells have previously been similarly dry-preserved with trehalose, using a freeze-drying technique, but the microwave-assisted method is faster and might allow for more extensive use of the technology.

"This allows us to get these preservation technologies into some low resource settings - third world countries such as developing nations,," Elliott said. "If you consider specimens for biodiversity research - those countries are not set up for that kind of collection and this method of preservation opens up that possibility."

The finding also expands the range of mammalian species whose germ cells can be successfully dry-preserved. Previous experiments have successfully dry-preserved sperm and egg cells in rats and mice, but the biology of rodent germ cells is significantly different from those of other mammals, including cats and humans. In rodents, the sperm cell is relatively simple and contains primarily just the male genetic material, while in cats and humans it also contains the centrosome, a cell structure

necessary for cellular division and the successful development of an embryo. Since the centrosome is vital to reproduction and since sperm are small cellular structures, cat sperm are potentially more challenging to preserve than rodent sperm.

"This is the first time this has ever been done with cat sperm, and cat sperm are closer to human sperm than are rodent sperm," Elliott notes.

"There has been a lot of work done on rats, but the rat is not necessarily a good model for centrosomal inheritance, which could affect fertility. This is why we believe the domestic cat model is a better model for humans than rodents, and this finding is significant," she said.

The reproductive success rate of the team's re-hydrated sperm was 6.5%, compared with a rate of 15% with fresh sperm, a reduction of viability, but still acceptable for preservation purposes. Rehydrated sperm were not motile, but that too was not critical for producing viable embryos.

"When we're drying and storing samples for the purpose of creating embryos, we don't have to have fully intact sperm as we will be doing intracytoplasmic sperm injections with the rehydrated samples," Elliott noted. "You don't have to have a tail, you don't have to have completely intact sperm heads - you are essentially injecting critical sperm components. The sperm heads don't have to be in fantastic shape but you do have to ensure that certain critical components are intact, including the centrosome."

Though the finding is a proof-of-concept, work remains to be done in developing and proving the technology. Elliott notes that it remains to be seen whether the dryness level currently achieved is high enough for long-term preservation without any refrigeration, and also whether further drying is possible. Once these conditions have been optimized, then testing needs to be done to ensure that the embryos can mature into

healthy kittens.

More information: Jennifer L. Patrick et al. Structural integrity and developmental potential of spermatozoa following microwave-assisted drying in the domestic cat model, *Theriogenology* (2017). DOI: 10.1016/j.theriogenology.2017.07.037

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