MedicalXpress

Study sheds light on infertility puzzle, could improve in vitro fertilization

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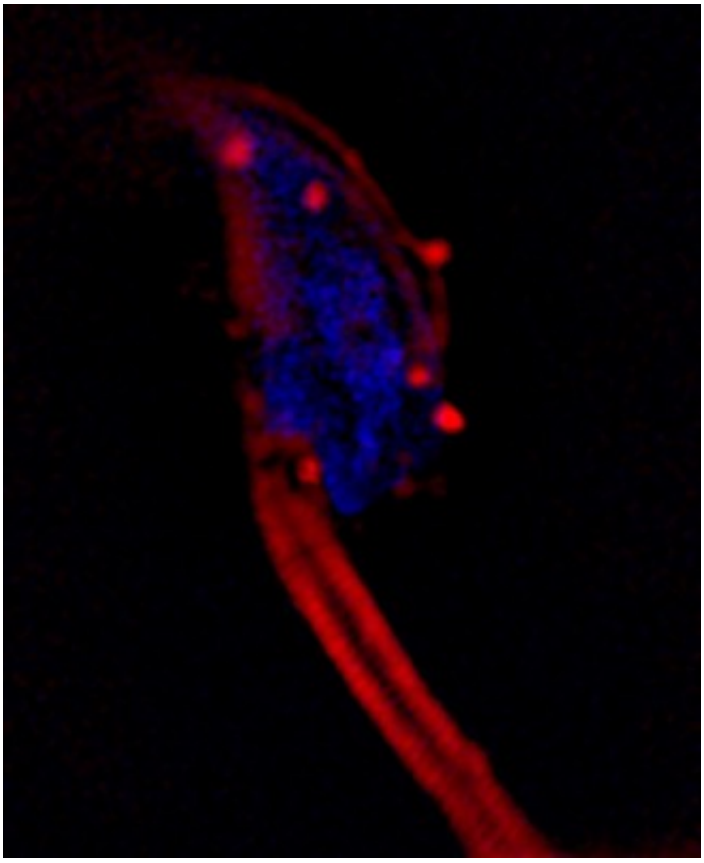

In studies of mice, the University of Delaware team detected tiny bodies which they named "oviductosomes" in fallopian tube fluids. These oviductosomes deliver a cargo of essential proteins, including a "calcium clearance pump," to the sperm before it fertilizes the egg. In this image, taken with a super-resolution microscope, you can see the oviductosomes, stained with a red dye, decorating the sperm's head and the midpiece of the sperm's tail, where they coalesce to give a continuous signal. Credit: University of Delaware/UD BioImaging Center--INBRE P20 GM103446

We don't know if a sperm actually experiences joy when it finally finds the egg, but it does wiggle excitedly.

Patricia A. Martin-DeLeon, a reproductive biologist at the University of Delaware, has witnessed this behavior many times in her studies of fertility in mice, the closest genetic model to humans (and with a much faster reproductive cycle).

It's what happens next in the fertilization process that DeLeon and her team have revealed for the first time, which could help couples struggling with infertility.

"There is communication between the sperm and the fallopian tube that helps prepare the sperm for its big push into the egg," says DeLeon, who is the Trustees Distinguished Professor of Biological Sciences at UD.

The research, supported by the National Institutes of Health-National Institute of Child Health and Human Development and the Delaware INBRE program, is published in the *Journal of Biological Chemistry*. It is one of the top most viewed articles published online this summer under the Membrane Biology affinity group, according to the editorial offices of the American Society for Biochemistry and Molecular Biology.

Understanding what happens in the fertilization process takes a little walk down biological memory lane, a reminder of nature's course that led to most of us.

Once the egg is released from an ovary and enters the Fallopian tube, the hair-like cilia that line this tiny tube sweep the egg toward the uterus. While in the tube, the egg will either meet the sperm and be fertilized, which must happen within a 12- to 24-hour window of time, or

fertilization will not occur and the egg will dissolve.

DeLeon and her UD team have identified particles in the secretions from the fallopian tube that help the sperm get ready for its all-important drive into the end zone. They termed these bodies "oviductosomes," from "oviduct," which is another name for the fallopian tube, and "exosomes," the teeny sacs found in all the body's fluids that help cells survive.

Hormones trigger the release of these oviductosomes, which are only 100 nanometers in diameter, or about 10 millionths of an inch wide. The tiny cargo-filled sacs then attach to the sperm like decorations on a Christmas tree before the sperm fuses with the egg.

Once these sacs are in place, they transfer proteins, including a "calcium clearance pump" (scientifically known as Plasma Membrane Ca2+-ATPase 4), to the sperm for use when the time is right.

"This calcium pump is required by the sperm just prior to fertilization, as well as in the early embryo," DeLeon says. "The sperm pumps out calcium and takes in hydrogen ions, which seems to give it that last push into the egg, and also is critical to starting the zygote's life."

Patricia A. Martin-DeLeon and her research team at the University of Delaware have discovered a communication between the fallopian tube and the sperm. Credit: Evan Krape/University of Delaware

Super-resolution microscope unveils process

DeLeon and her team made their discovery using the high-powered, three-dimensional super-resolution microscope that UD acquired in 2012. The technology, whose inventors won the Nobel Prize in 2014, can illuminate what's happening in a cell, right down to a single molecule.

The oviductosomes from a female mouse were pre-labeled with a fluorescent dye and incubated together with the sperm. Within an hour, the oviductosomes were fused to the sperm's surface. After two to three

hours, the oviductosomes continued to accumulate, primarily on the sperm's head and the midpiece of its tail.

Integrins, which are membrane receptors on both the sperm and the oviductosomes, helped to facilitate their bonding, along with fusion stalks on the sperm's surface.

"Discovery of these oviductosomes provides us with a window into the cargo being delivered by the female to the sperm," DeLeon says. "The implication is that we could improve IVF with this knowledge."

IVF, or in vitro fertilization, currently has a 32 percent success rate, and couples for whom the procedure fails face the puzzling, disappointing reality that they can't have a child of their own. Even when the process works, a high sperm-to-egg ratio is required, the opposite of what occurs in the body.

"We've shown that these oviductosomes are carrying critical molecules that include not only proteins, but also nucleic acids such as RNA and also lipids," DeLeon says. "That gives us hope they can be used as vehicles for improving fertility and the chances of producing healthy embryos and offspring."

Currently, DeLeon and her team are analyzing the protein-rich contents of this cargo to find out exactly what gives the sperm what it needs for its last push to penetrate the egg—always head first, tail out—to fertilize it.

As for the calcium clearance pump, the sperm needs to use it to reduce toxic calcium levels quickly. Otherwise, the sperm risks a buildup of nitric oxide, which can cause DNA damage.

"Our work may lead to the discovery of genes and gene products that

cause infertility," DeLeon says of the team's continuing research. "We may identify proteins required to improve the efficiency of IVF, and improve the outcome and health of the offspring. It's really another step in the direction of personalized medicine, since individuals carrying mutations of one of a variety of genes account for the largest group of infertile couples."

DeLeon has been making major contributions to reproductive science since she published her first scientific article—"Cannabis and Chromosomes," examining the impact of marijuana on embryonic cells—in The Lancet in 1969, as a master's student at the University of the West Indies in her native Jamaica.

The response to that paper was so dramatic, with requests for copies from scientists around the world, that DeLeon set her sights on a career in research and teaching and never looked back, continuing on for her doctorate at the University of Western Ontario. She joined the UD faculty in 1976.

More information: *Journal of Biological Chemistry*, www.jbc.org/content/early/2015 … M114.633156.abstract

Provided by University of Delaware