Mouse model may help explain and treat infertility

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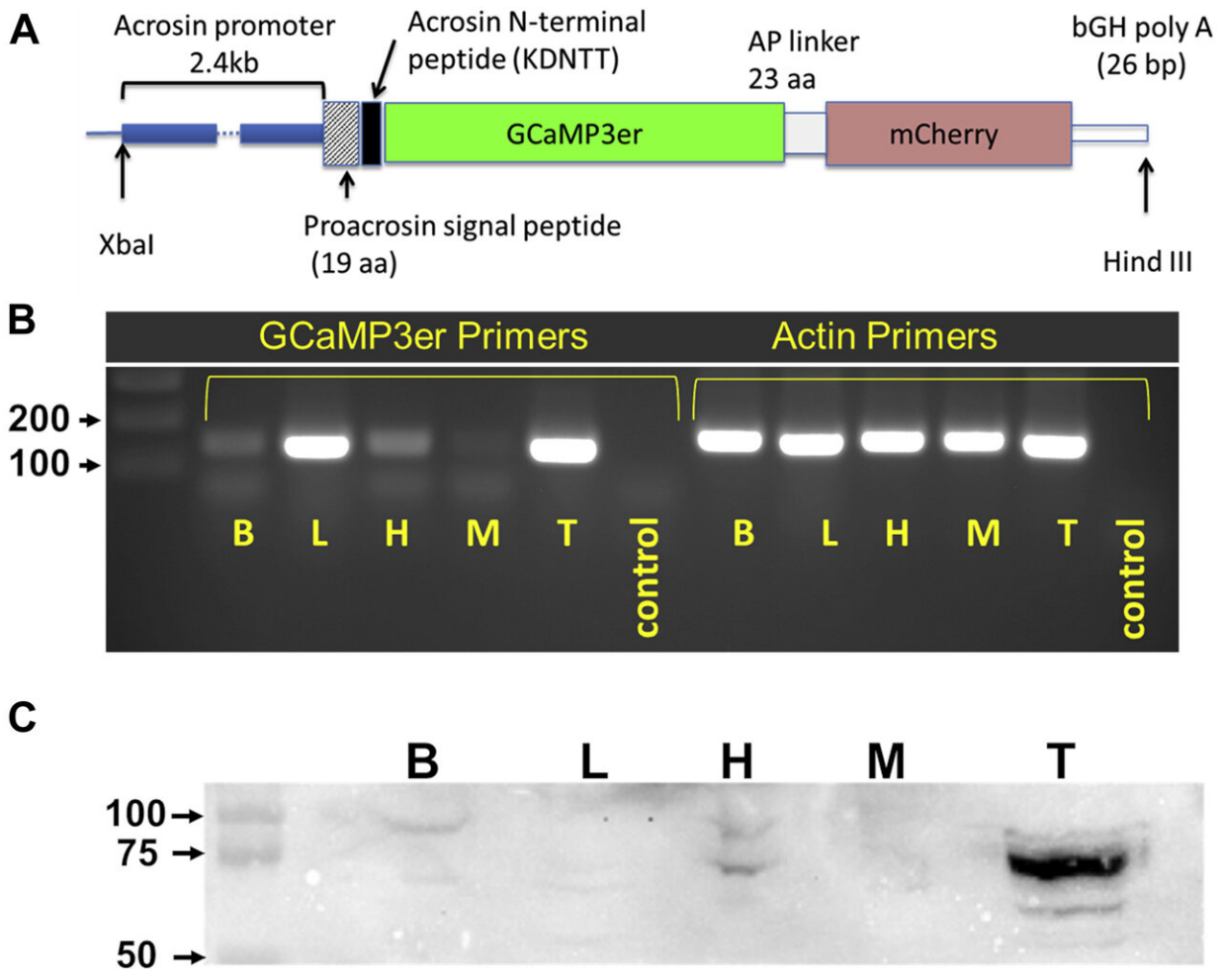

Design and validation of the mouse line expressing Acr-GCaMP3er-mCherry (Acrosome-targeted Sensor for Exocytosis [AcroSensE]). A, schematic of the construct. B, validation of Acr-GCaMP3-mCherry expression in the model using RT-PCR in various tissues (brain (B), liver (L), heart (H), muscle (M), testis (T), and negative control). Primer sets were designed to amplify 149 base pairs

between the proacrosin signal peptide and the GFP portion of the GCaMP3 (f':catggtcctgctggagttcgtg, r':ctggtcgagctggacgggcgacg). Actin was used as a positive control. C, immunoblot analysis of protein expression using anti-GFP confirmed high levels of expression of Acr-GCaMP3-mCherry in the testis at the predicted molecular weight of 75 kDa. Credit: *Journal of Biological Chemistry* (2022). DOI: 10.1016/j.jbc.2022.101868

Cornell researchers have created a genetically engineered mouse model that could shed light on the causes of human infertility and allow researchers to explore other areas of reproduction.

The work, published in the *Journal of Biological Chemistry*, and to be showcased in the *Journal of Visualized Experiments*, relied on a transgenic mouse model developed by researchers in the Baker Institute for Animal Health, part of the College of Veterinary Medicine.

"We have generated this mouse model to better understand sperm function, but it will also help us better understand mechanisms of infertility in humans," said Roy Cohen, a research assistant professor at the Baker Institute, who led the development of the mouse model. "Once you answer some of the basic questions in a model system and you obtain a better idea of how things work, then you have a starting point to tackle the complicated issue of human infertility."

Discovering how the sperm first begin the fertilization process could identify new targets for nonhormonal contraceptives or help couples who are struggling with their own fertility.

Experiments using the new model will be featured in a videotaped demonstration published in the *Journal of Visualized Experiments*. The journal recorded a demonstration of the experiments at the Baker

Institute on Nov. 6.

"This is a promising new research resource that we would love to share with other labs and really provide an advancement to the field in how to investigate one of the very first steps in fertilization," Cohen said.

Cohen and Dr. Alexander Travis, professor of reproductive biology at the Baker Institute and co-author of the research, sought to better understand a key step in the fertilization process. They focused on the acrosome—a vesicle in the head of the sperm that contains enzymes used to help penetrate the materials surrounding the egg. The release of these enzymes, through a process called exocytosis, is triggered by a change in calcium levels inside the sperm. How exactly the calcium changes and exocytosis happens, however, have become the source of scientific debate.

The traditional view, called the "acrosome reaction," argues that a calcium spike triggers the acrosome to expel its enzymes in an "all or nothing" manner, like popping a water balloon, when the sperm contact the egg's covering.

Many studies have called this theory into question, and a newer model suggests a more complex multi-step process: As the sperm passes through the jelly-like cloud of cells that surround the egg, the enzymes are released gradually, enabling the sperm to pass through the viscous environment, then the egg's covering, and eventually the egg membrane.

"Several laboratories showed that the situation is more complicated than this model of an explosive process when the sperm hits the covering of the egg," Travis said. "But if you look at all the textbooks, nobody pays attention to those studies, because people just like this simplified idea of the acrosome reaction."

Cohen and Travis designed their model with fluorescing markers that allowed them to visualize the calcium levels in the sperm. The proteins within the sperm glow red when inside the acrosome, while a green glow indicates a rise in calcium. This increase is needed to trigger exocytosis; then, when the acrosome's contents start to be released, this is observed by a loss of the red color.

By observing the interplay of red and green sensors in the sperm in real time, the researchers found that exocytosis happens in a gradual fashion within the sperm, further undermining the "popping a water balloon" model.

"The data we obtained helped document very clearly that this is a more complicated process than that old idea of the acrosome reaction," Travis said. "We're not the first who said there's a problem with the idea of the acrosome reaction. But now this mouse lets us separate and see these different steps in real time."

The research team will now focus on the role of specific calcium channels in human sperm and the way they regulate and trigger various processes, including acrosome exocytosis, Travis said.

"Many of the same molecules are found in different species, suggesting that they are important," Travis said. "But are they exactly the same channels in mice and humans? Are they regulated the same way? These are some of the questions we need to figure out."

Calcium channels, for example, are sensitive to different inhibitors, which could be the basis for developing human contraceptives. Travis, Cohen and their lab members are now performing research on human sperm toward this goal.

"We are actively engaged in not just working on the mouse but trying to

make a difference in human health as well," Travis said.

More information: Roy Cohen et al, A genetically targeted sensor reveals spatial and temporal dynamics of acrosomal calcium and sperm acrosome exocytosis, *Journal of Biological Chemistry* (2022). DOI: 10.1016/j.jbc.2022.101868

Roy Cohen et al, Real-Time Imaging of Acrosomal Calcium Dynamics and Exocytosis in Live Mouse Sperm, *Journal of Visualized Experiments* (2023). DOI: 10.3791/65962

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