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United States Department of Agriculture

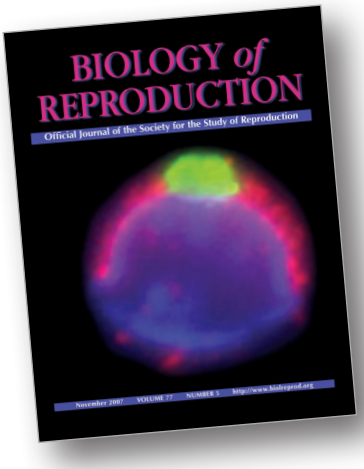
Cooperative State Research, Education, and Extension Service

National Research Initiative (NRI)

Research Increases Success of *In Vitro* Fertilization

by Stacy Kish, CSREES

In vitro fertilization of livestock is an expensive venture that is complicated by the occurrence of polyspermy, a lethal condition occurring when more than one sperm cell penetrates the egg coat. >>



Above: The research was featured on the cover of journal *Biology of Reproduction* Volume 77, Number 5.

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With funding from USDA's Cooperative State Research, Education, and Extension Service (CSREES), a team of scientists in Missouri determined how synthetic antibodies during *in vitro* fertilization can significantly reduce the occurrence of polyspermy, resulting in increased normal, single-sperm fertilization

During animal fertilization, the sperm cell penetrates the egg coat. The animal industry often uses in vitro fertilization, where an egg is fertilized outside the animal's womb, to ensure a higher rate of fertilization. During embryo transfer, losses by polyspermy in the cattle and pig industry cause financial losses and also reduce the success of embryo transfer technology.

Controlling animal reproduction is a critical step for the animal industry. The results from this project will provide substantial financial gain to the animal sector of U.S. agriculture from increased reproductive efficiency in livestock and distribution of new technology to commercial industries for animal fertilization.

Peter Sutovsky and colleagues at the University of Missouri–Columbia, in collaboration with the Academy of Sciences of the Czech Republic, Queen's University in Canada and Chungnam National University in Korea, examined a sperm cell's use of small particles, called proteasomes, which digest and weaken the egg coat during fertilization.

The scientists determined that a specific type of enzyme, called Ubiquitin C-terminal Hydrolase (UCH), regulates the ability of proteasomes to digest the egg coat.

The researchers identified two antibodies to UCHs, called UCHL1 and UCHL3, which recognize proteins in the pig sperm head and egg, respectively. The antibodies decreased the activity of sperm UCH and enhanced the activity of sperm proteasomes, increasing the occurrence of polyspermy in pig embryos. In contrast, the addition of synthetic UCH-enzymes during test tube fertilization significantly reduced the occurrence of polyspermy.

This study builds on the team's earlier research which revealed that a small protein, called ubiquitin, controls the destruction of sperm after fertilization and binds to abnormal sperm to prevent fertilization by an inferior individual. Altering the activity of ubiquitin-system proteins is a key to the management and reduction of polyspermy in embryos intended for *in vitro* fertilization.

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Synthetic UCH enzymes can substantially increase the percentage of embryos capable of normal development and implantation after embryo transfer.

This research provides additional evidence for the critical role that the ubiquitin-proteosome system plays during fertilization in livestock. This work will advance the understanding of how fertilization occurs, as well as demonstrate how manipulating the ubiquitin-proteosome system can improve the rate of *in vitro* fertilization to improve fertility in livestock.

CSREES funded this research project through the NRI Animal Reproduction program. Through federal funding and leadership for research, education and extension programs, CSREES focuses on investing in science and solving critical issues impacting people's daily lives and the nation's future. For more information, visit www.csrees.usda.gov. ■

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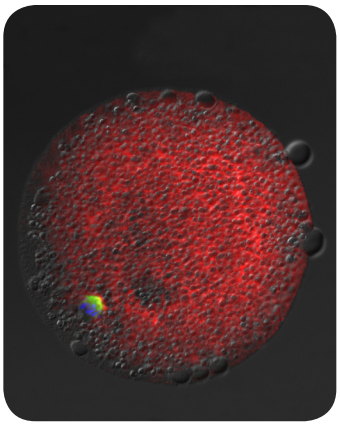
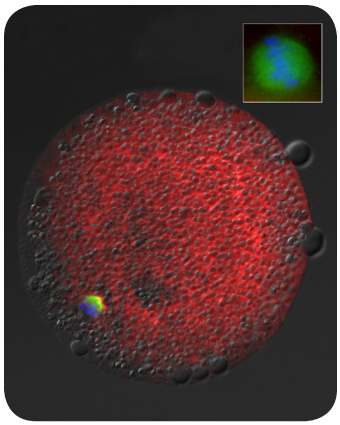
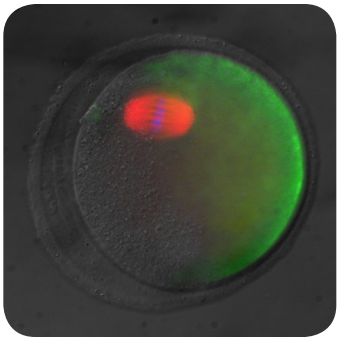




Right. Pig ovum labeled with antibodies recognizing two closely related deubiquitinating enzymes, the ubiqutin-C-terminal hydrolases UCHL1 **(near right)** (red labeling around the egg periphery) and UCHL3 **(far right)** (green labeling in the cleavage spindle). DNA of the egg chromosomes is counterstained blue.

Credit: P. Sutovsky



Right: Mouse ovum labeled with antibodies recognizing two closely related deubiquitinating enzymes, the ubiqutin-C-terminal hydrolases UCHL1 (red labeling around the egg periphery) and UCHL3 (green labeling in the cleavage spindle). DNA of the egg chromosomes is counterstained blue.

Credit: P. Sutovsky

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