# Researchers Generate Mature Egg Cells From Early Ovarian Follicles

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(PhysOrg.com) -- Researchers supported by the National Institutes of Health have for the first time activated mouse egg cells at the earliest stage of their development and brought them to maturity. In a related experiment, the researchers replicated the finding by also bringing human eggs to maturity in the laboratory.

Current infertility treatment techniques stimulate immature eggs so they develop to the stage at which the eggs can be fertilized, but these techniques work only on eggs at a comparatively late stage of development. These later-stage eggs are few in number and much more difficult to recover than the early-stage eggs used by the researchers in this study.

Using the new technique, the researchers brought dormant mouse eggs to full maturity within the laboratory. The eggs then were fertilized and transferred into female mice, which carried them to term.

The human eggs were not fertilized. The technique is still in its early stages, has not been sufficiently studied for human use and will require several more years of study.

According to the researchers, one day this technique could be used to treat female infertility, particularly forms of infertility in which the supply of available eggs is diminished or limited. Similarly, the technique could be combined with efforts to bank the ovarian tissue of women in need of cancer therapy that might cause infertility.

"The researchers have developed a promising new technique that may someday provide additional options for women seeking treatment for certain forms of infertility," said Alan E. Guttmacher, M.D., director of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), the NIH Institute that funded the study.

The findings appear online in the Proceedings of the National Academy of Sciences.

First author Jing Li conducted the research with Stanford University colleagues Yuan Cheng, Cynthia Klein and Aaron J.W. Hsueh; Kazuhiro Kawamura of Akita University; and Shuang Liu, Shu Liu and En-Kui Duan of the Chinese Academy of Sciences.

Immature egg cells are encased in structures known as ovarian follicles. At birth, most women have about 400,000 small, or primordial, follicles. Most of them remain dormant, with about 1,000 primordial follicles activated per month. After reaching the reproductive years, about 20-30 medium-size follicles are present at the beginning of the menstrual cycle, and, typically, only one follicle develops further and gives rise to the mature egg. Current fertility treatments focus on the comparatively small number of medium-size ovarian follicles already in the later stages of development.

An enzyme known as PTEN keeps the early follicles dormant until they are ready to be activated. In the current study, researchers bathed one of each pair of ovaries from three-day-old mice in a substance that erases the braking effect of PTEN together with a second substance, 740Y-P, to activate dormant follicles. After two days, the researchers saw early signs of activation in most follicles in the treated ovaries.

The researchers then transplanted pairs of ovaries into adult mice and gave the animals daily injections of follicle stimulating hormone

(FSH). Although FSH spurs activated egg cells to mature, it cannot activate dormant follicles. After two weeks, the ovaries treated with the PTEN blocker and 740Y-P were visibly larger and more than three times heavier than the untreated ovaries.

The treated ovaries also had up to six times more follicles in advanced stages of development than did the untreated ovaries, and a greater percentage of the treated ovaries contained egg cells that had reached maturity. The researchers then collected and fertilized the mature egg cells from the treated ovaries. From 118 two-cell embryos transferred into host mothers, 20 healthy mouse pups were born.

The researchers examined the same technique using primordial follicle-rich ovarian cortical tissues removed during the treatment of women with ovarian cancer. After treating sections of tissue with the same PTEN-blocking substance for 24 hours, the researchers transplanted the ovarian tissue into mice and gave the animals FSH injections every two days to stimulate egg development.

The sections of transplanted tissue each contained more than 50 primordial follicles. While 96 percent of follicles in the tissue had been dormant at the time it was transplanted, the researchers found that after six months, 89 percent of ovarian follicles in the treated tissue had begun to mature, compared with 40 percent in the untreated tissue. In addition, four times as many follicles had matured to advanced stages in the treated tissue. The researchers also confirmed the treated tissue contained 27 mature egg cells, compared with one in the untreated tissue.

"Although primordial follicles are far more numerous than later-stage follicles, they had been inaccessible for fertility treatments in their dormant state," said Dr. Hsueh, the article's senior author. "Activating them using this technique holds the promise of expanding the options for

women seeking treatment for infertility."

Dr. Hsueh said that this technique for stimulating dormant ovarian follicles, which he and his coworkers termed in vitro activation (IVA), ultimately could be used in treating infertility resulting from a reduced number of follicles, such as primary ovarian insufficiency (POI) [poi.nichd.nih.gov/], a disorder in which women have only a small number of follicles, which often fail to reach maturity. Similarly, for cancer patients about to undergo procedures that eliminate fertility, primordial follicles could be removed and frozen, then reactivated at a time when the woman is ready to have children.

The researchers plan to continue their research in animals, examining the safety of the PTEN blocker and other activating agents and testing the feasibility of auto-transplants, in which the stimulated ovarian tissue would be transplanted to a patient's arm or elsewhere in the body to mature. Such transplanted tissue would be easy to retrieve when the mature eggs are ready for fertilization.

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