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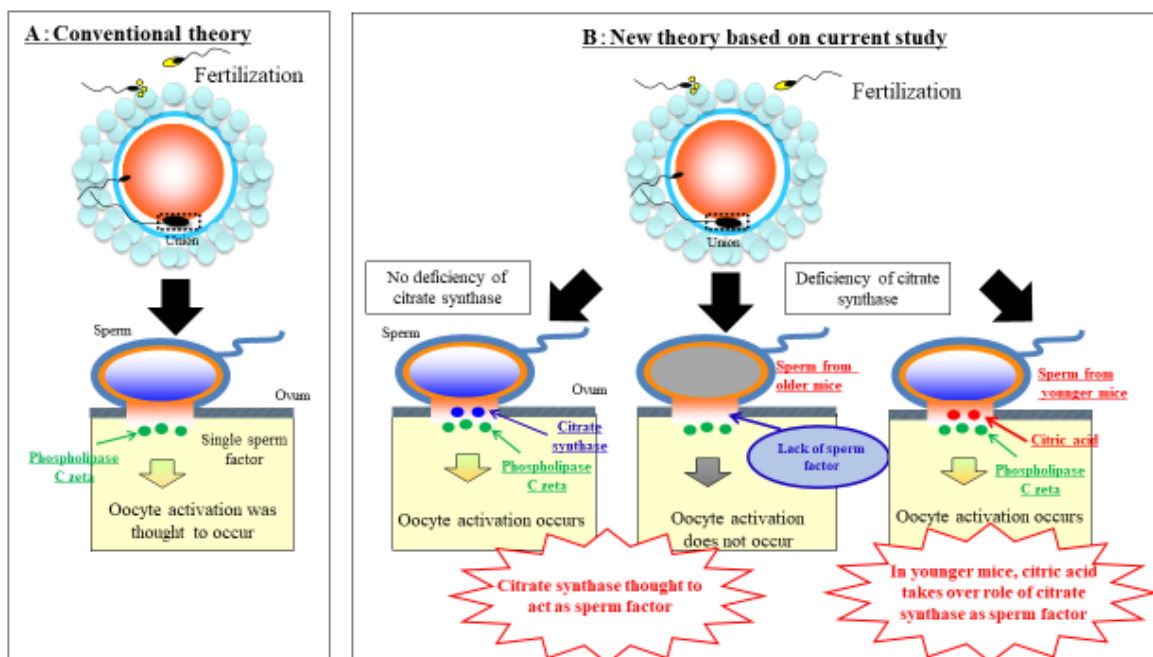
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**Discovery of an oocyte activation factor in sperm:
a potential major role for citric acid in sperm aging**

Tokyo, Japan, January 15, 2020 — The National Center for Child Health and Development's Reproductive Science Research Group (Satoshi Kasuyu, Director Kenji Miyato) discovered that citrate synthase contained in sperm works as a sperm factor to activate fertilized eggs by using a murine model. Moreover, their study revealed the possibility that male infertility occurs as a result of age-related attenuation of citrate synthase and its consequent inability to synthesize citric acid, which may play a large role in sperm aging. Sperm which appear normally motile may still be unable to activate fertilized eggs in the absence of a sufficient quantity of citric acid. As they progress to studies with human subjects, the researchers expect that the citric acid levels in sperm will be able to be used to assess the risk of male infertility.

The results of the study were published in the American pathology journal, *Laboratory Investigation*.

[Mechanism of oocyte activation]



[Key points]

- While it was previously believed that there was only one sperm factor responsible for oocyte activation, the present study revealed for the first time that citrate synthase may also function as a sperm factor.
- As citrate synthase becomes attenuated with age, the citric acid content of sperm decreases correspondingly, suggesting that citric acid is related to the aging of sperm and its inability to promote oocyte activation even if it shows normal motility.
- Further progress in this research is expected to result in applications for diagnosing and elucidating the causes of male infertility.

[Background and aims]

Infertility is not merely a problem of women; half the cases are due to reproductive anomalies in men, such as azoospermia, in which the testes are unable to produce sperm, and oligospermia, in which they produce too few. There are also cases in which sperm continue to be produced but are infertile and cases of male infertility which progress with age. Such cases are difficult to diagnose and have no known cause. The current study was therefore conducted with the aim of clarifying the causes of male infertility due to aging.

[Research summary]

An egg is fertilized when a sperm and egg unite. However, the union of a sperm and egg does not necessarily initiate embryological development in the fertilized egg. For cellular division to occur in a fertilized egg, i.e., for oocyte activation to occur, a chemical signal is required to indicate that the sperm has fused with the egg. This signal consists in increased calcium concentration and propagates through the ovum but first requires the transfer of a substance from the sperm to the ovum. This substance, known as the sperm factor, was until now thought to be phospholipase C zeta. However, research using newts showed that citrate synthase also acts as a sperm factor. The research group in the present study examined how this enzyme and the citric acid synthesized by it are related to oocyte activation in a mouse model.

Two forms of citrate synthase exist in mice, one of which was found in the present study to occur in large quantities in the sperm head. Therefore, male mice lacking the enzyme in the sperm head were first bred, then individuals with an age equivalent to about 30 human years were analyzed to reveal normal levels of one form of citrate synthase and correspondingly normal citric acid levels in the sperm head. However, in mice with an age equivalent to more than 30 human years, the function of citrate synthase showed marked attenuation with a corresponding decrease in citric acid levels.

Next, oocyte activation was assessed in the mouse sperm. In the younger mice (with an age equivalent to about 30 human years) with normal citric acid levels, the citric acid acted as a sperm factor in the absence of citrate synthase to trigger oocyte activation. However, sperm with lower levels of citric acid were found to be less likely to activate oocytes, thus leading to male infertility.

This study clarified that there are at least two sperm factors and furthermore, that if citrate synthase, the second sperm factor, is attenuated by age and the levels of citric acid synthesized in the sperm decrease, male infertility may result.

[Future outlook]

In the future, the NCCHD is planning research rolling male patients with infertility. Progress in this research is expected to result in a method of assessing the risk factors of male infertility based on measuring the citric acid content of sperm.

[Study information]

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About the National Center for Child Health and Development

The National Center for Child Health and Development (NCCHD) was established in 2002 by combining the National Okura Hospital and the National Children's Hospital. The NCCHD's philosophy emphasizes cooperation between its hospital and research wings to promote medical care and research aimed at fostering healthy future generations. The NCCHD is the largest hospital in Japan specializing in pediatrics, perinatal care, obstetrics, and maternal medicine and has 490 beds and an average of about 1000 daily outpatient visits. The NCCHD provides comprehensive and continuous "developmental care" encompassing every stage of life, from the fetus, neonate, infant, toddler, school-aged child, and adolescent to the adult, as well as future generations.

In addition, the center conducts research on elucidating the etiology and pathology of diseases and finding cures while also offering insights on building a society for healthy future generation