Abnormal distribution of type 1 Inositol 1,4,5-tri-phosphate receptor in fertilization failed human oocytes

Sook Young Yoon, Jin Hee Eun, Eun A Park, Tae Hyung Kim, Tae Ki Yoon, Dong Ryul Lee, and Woo Sik Lee

Fertility Center of CHA Gangnam Medical Center, CHA University, College of Medicine, Seoul 135-081, Korea

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Abstract

Study DTPA in IVF patient is one of the most important Ca2+ oscillations during fertilization. Because fertilization failure in human oocytes has already been reported, we analyzed intracellular Ca2+ oscillations in human oocytes. Abcam human [Ca2+]i responses were used to determine the oscillations. [Ca2+]i responses were recorded in oocytes from healthy patients and healthy women. [Ca2+]i responses were recorded in oocytes from healthy patients and healthy women. [Ca2+]i responses were recorded in oocytes from healthy patients and healthy women.

Background

[Ca2+]i oscillations induced by fertilization are known to involve stimulation of a phospholipase C (PLC) enzyme hydrolyzing (IP) [4-1,4,5-trisphosphate (IP3)] and diacylglycerol (DAG). IP3 induces phospholipase C (PLC) to produce inositol 1,4,5-trisphosphate (IP3). In the absence of IP3, Ca2+ release from the endoplasmic reticulum (ER) is necessary. In this study, we examined the Ca2+ release in human oocytes following fertilization. We have previously shown that micromanipulation of human IP3 in the presence of diacylglycerol (DAG), which is a ligand for the PLC-dimer, increased IP3-stimulated Ca2+ release. The present study was supported by a grant from the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012-0009133).

Materials and Methods

Human Oocyte Collection The study was conducted on patients admitted to the assisted reproduction program at Fertility Center of CHA Gangnam Medical Center from September 2012 to December 2012. During this period, 512 oocytes (84 oocytes/40 patients, 68% of 12 patients) were aspirated from 54 patients. The reason for infertility of patients in this study was unexplained (12 patients), female (28 patients), and male factor (14 patients). All oocytes were aspirated from each patient. From each patient, 54 patients, who showed more than 70% fertilization rate (100%, 12 patients). The study was approved by the Institutional Review Board of CHA Gangnam Medical Center, and all subjects signed informed consent.

Mouse Oocyte Collection

• Divided or GV oocytes from 4 weeks old ICR mice
• ICSM medium

Human Oocyte collection

• mini oocyte media
• Fertilization: 6.3% ± 0.3% fertilized
• Embryo transfer: 8% ± 0.5% cleavage rate

Immobilization of oocytes

• Factin: 3% in TAE buffer
• Preimplantation: 0.5% Triton X-100

Ca2+ imaging in oocytes

• [Ca2+]i responses were analyzed using a Ca2+ imaging system.
• 0-20 μM of Ca2+ release was observed in oocytes from healthy patients and healthy women.

Results

[Ca2+]i responses were recorded in oocytes from healthy patients and healthy women. [Ca2+]i responses were recorded in oocytes from healthy patients and healthy women. [Ca2+]i responses were recorded in oocytes from healthy patients and healthy women.

Discussion

Distribution of IP3R1 in human oocytes change gradually from GV stage to MII stage. In GV stage, most of the oocytes showed progressively dispersive from GV to MII stage. In MII stage, fertilization would be distributed in white cytoplasm including clusters which is more than 3 μm in diameter of the cortex. After fertilization, in IV stage with SIM, most of the oocytes have IP3R1 distribution with gradually dispersive from GV to MII stage, but they do not have cortical clusters. In fertilization failed human oocytes on the next morning post fertilization, more than 20% of clusters have several IP3R1 aggregates in the middle of the cytoplasm, which is more than 10 μm in diameter instead of cortical clusters as in MII. However, the low numbers of human oocytes analyzed may not be ideal for conclusive evaluation. In conclusion, evaluation of the IP3R1 biochemically change would be needed. The present findings provide new understanding of the reason of the fertilization failure during ART program, and suggest further analysis for the clinical application as marker of egg quality.

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References