REPRODUCTIVE ENDOCRINOLOGY AND INFERTILITY

Dynamic modulation of cytoskeleton during in vitro maturation in human oocytes

Shan Liu, MD, PhD; Yuan Li, MD, PhD; Huai L. Feng, PhD, HCLD; Jun H. Yan, MD, PhD; Mei Li, MD; Shui Y. Ma, MD; Zi J. Chen, MD, PhD

OBJECTIVE: To investigate the role of cytoskeleton in several important dynamic events during in vitro maturation of human oocytes.

STUDY DESIGN: Human germinal vesicle stage oocytes were divided randomly into control and study groups. After cultured for 24 hours, chromatin state and position, spindle formation and migration, cortical granules, and mitochondria distribution were evaluated.

RESULTS: In colchicine group, spindles did not form. Cortical granules migrated to the cortex but mitochondria maintained the peripheral distribution pattern in most of the oocytes. In cytochalasin B group, the migration of spindle and chromosomes to the cortex was prohibited. Microfilaments disruption influenced cortical granules migration but not redistribution of mitochondria.

CONCLUSION: Meiosis progression could not go beyond metaphase I stage when microtubule or microfilament polymerization was prohibited in human oocytes. The migration of cortical granules to the cortex and redistribution of mitochondria to the inner cytoplasm were mediated by microfilaments and microtubules, respectively.

Key words: cortical granule, microfilament, microtubule, mitochondria, spindle

Cite this article as: Liu S, Li Y, Feng HL, et al. Dynamic modulation of cytoskeleton during in vitro maturation in human oocytes. Am J Obstet Gynecol 2010;203:151.e1-7.

It has been well established that the maturation of human oocytes is comprised of nuclear maturation and cytoplasmic maturation.[1] Nuclear maturation refers to the resumption of meiosis, germinal vesicle breakdown (GVBD), chromatin condensation and redistribution, spindle assembly, and emission of the first polar body. Cytoplasmic maturation refers to the migration of organelles such as cortical granules (CG) and mitochondria and a series of biochemical changes in the cytoplasm. The well-orchestrated process of nuclear and cytoplasmic maturation ensures the normal fertilization and further development.

It has been revealed that the organization of the cytoskeleton, in particular microtubules and microfilaments, plays an important role in the regulation of these dynamic events. During the in vitro maturation (IVM) of horse oocytes, both the microfilament and microtubular elements of the cytoskeleton were seen to reorganize dramatically in a fashion that appeared to enable chromosomal alignment and segregation.[2] GVBD and meiotic spindle formation were not regulated by microfilaments, but the movement of chromosomes and spindle depended on a microfilament-mediated process in maturing mouse oocytes.[3-7] Similar results have been obtained in other species, including hamster,[8] bovine,[9] and pig.[10] To our knowledge, there have been few studies on the effects of microtubules and microfilaments on the meiosis process of human oocytes.

Microfilaments were essential for CG migration to the cortex during the maturation of porcine oocytes.[10,11] The same mechanism of CG migration also existed in mouse oocytes.[12] However, the role of microtubules and microfilaments played on CG migration during human oocytes maturation has not been available before the current study. And this is one of the intents of this study.

Oocyte maturation, fertilization, and early embryo development in porcine were associated with changes in active mitochondrial distribution and mitochondrial translocation was mediated by microtubules.[13,14] In mouse, microfilament depolymerization by cytochalasin could not affect central migration of mitochondria.[5] Our previous studies demonstrated that there were obvious changes in mitochondria distribution pattern during IVM of human oocytes.[15] However, the effects of cytoskeleton on mitochondria redistribution during human oocytes maturation have not been clarified yet.

In the current study, we aimed to investigate the dynamic modulation of cytoskeleton during IVM of human oocytes, including the condensation and movement of chromosomes, formation and migration of spindle to the cortical region, and redistribution of CG and mitochondria.

From the Center for Reproductive Medicine, Shandong Provincial Hospital, Shandong University, Key Laboratory of Reproductive Medicine (Drs Liu, Y. Li, Yan, M. Li, Ma, and Chen), Shandong Province, Jinan, China; and the Center for Human Reproduction, North Shore University Hospital, New York University School of Medicine (Dr Feng), Manhasset, NY.

Received Nov. 22, 2009; revised Jan. 8, 2010; accepted May 5, 2010.

Reprints: Zi J. Chen, MD, PHD and Yuan Li, MD, PHD, 324 Jing5wei7 Rd., Jinan, 250021, China. chenzijiang@hotmail.com and sdliyuan@hotmail.com.

This work was supported by the National Natural Science Foundation of China (Grant nos: 30772328, 30973170).

0002-9378/$36.00
© 2010 Mosby, Inc. All rights reserved.
doi: 10.1016/j.ajog.2010.05.011

MATERIALS AND METHODS

Ovarian stimulation and collection of germinal vesicle (GV) phase oocytes

GV oocytes were collected from patients undergoing intracytoplasmic sperm injection (ICSI) treatment for male factors or combined with oviduct infertility from December 2007 to November 2008 at Center for Reproductive Medicine, Shandong Provincial Hospital, Shandong University. Ovarian stimulation was performed using standard protocols in our center, including downregulation of the pituitary gland with a gonadotrophin-releasing hormone agonist (GnRHa; Decapeptyl; Ferring, Kiel, Germany), followed by ovarian stimulation with exogenous follicle stimulating hormone (FSH; Gonal-F; Serono Laboratories, Aubonne, Switzerland). Ultrasound examination was carried out during the process. When 2-3 follicles of 18-20 mm in diameter were observed, and 17β-oestradiol concentrations reached 200 pg/mL per follicle more than 14 mm in diameter, ultrasound-guided follicle drilling and aspiration were performed 36 hours after the administration of 10,000 IU human chorionic gonadotrophin (HCG; Profasi; Serono Laboratories, Aubonne, Switzerland).

Ovarian stimulation protocols generated predominantly mature metaphase II (MII) oocytes. However, GV stage oocytes were frequently obtained, which, when cultured in appropriate medium, showed a high incidence of resumption and completion of maturation.[16,17] In the current study, those GV oocytes from ICSI cycles were collected after informed, written consent. The study was approved by the ethics committee at Shandong Provincial Hospital, Shandong University. In all, 207 patients in 220 cycles donated their GV oocytes. The age of the patients was 28.7 ± 1.9 years (mean ± SD; range, 25–34 years).

In vitro maturation

GV oocytes were cultured for 24-48 hours at 37°C under conditions of 5% CO_2 in air. The maturation medium adopted in the current study was similar to previous study with slight modifications.[18] It was composed of commercially available G-2 medium (Vitrolife, Kungsbacka, Sweden), supplemented with 10% (v/v) human serum albumin (HSA; Vitrolife), 50 IU/mL penicillin (Sigma-Aldrich, St. Louis, MO), 50 μg/mL streptomycin (Sigma-Aldrich), 0.15 IU/mL HCG, 0.075 IU/mL recombinant FSH, 10 ng/mL epidermal growth factor (EGF; Sigma-Aldrich), and 10 μg/mL oestradiol (Sigma-Aldrich). The extrusion of the first polar body was used as the criterion for nuclear maturation.

TABLE 1

Effects of colchicine and CB on the meiotic process during maturation of human oocytes

		Developmental stages		
Groups	No. of oocytes	GV, n (%)	MI, n (%)	MII, n (%)
Control	76	12 (15.79)	12 (15.79)	52 (68.42)
Colchicine 10 μM	99	22 (22.22)	77 (77.78)[a]	0
CB 10 μM	96	10 (10.42)	80 (83.33)[a]	6 (6.25)

CB, cytochalasin B; *GV*, germinal vesicle; *MI*, metaphase I stage; *MII*, metaphase II stage.

[a] The meiosis process was arrested in MI phase in most of the oocytes treated by colchicine or CB. Values within the same column differ significantly from control group ($P < .05$).

Liu. Dynamic modulation of cytoskeleton during in vitro maturation in human oocytes. Am J Obstet Gynecol 2010.

Treatments

IVM was performed once GV oocytes were collected. They were divided randomly into control and study groups. More than 50 IVM procedures were performed for each group. Oocytes in control group were cultured in drug-free maturation medium for 24-48 hours. Oocytes in study group were cultured in maturation medium containing 10 μM microtubule polymerization inhibitor, colchicine (Sigma-Aldrich), or 10 μM microfilament polymerization inhibitor, cytochalasin B (CB; Sigma-Aldrich) for 48 hours. Chemicals of this concentration can disrupt cytoskeleton polymerization specifically without doing harm to oocytes.[5,10-12] Then the chromatin state and position, spindle formation and migration, CG migration, and mitochondria redistribution during IVM of human oocytes were evaluated.

Immunofluorescence staining of microtubules and microfilaments

Oocytes were fixed with 2% (w/v) paraformaldehyde in phosphate-buffered saline (PBS) for 10 minutes at 37°C. Then they were washed in PBS containing 5 mg/mL HSA for 3 times at 37°C, each for 5 minutes. The oocytes were extracted in PBS containing 5 mg/mL HSA and 0.1% (v/v) Triton X-100 for 45 minutes at 37°C, followed by 3 washes in PBS. For the immunostaining of microtubules, the oocytes were incubated in a mouse monoclonal antibody against α-tubulin (T-5168; Sigma-Aldrich) diluted by PBS (1:100) for 90 minutes at 37°C. Then they were washed with PBS and incubated in fluorescein isothiocyanate (FITC)-conjugated goat-anti-mouse Ig G (F-4018; Sigma-Aldrich) diluted with PBS (1:300) for 60 minutes at 37°C. Microfilaments labeling was performed by incubation of permeabilized oocytes with 1 μg/mL FITC-phalloidin (P-5282; Sigma-Aldrich) for 60 minutes at 37°C. Chromosomes were stained by exposing the oocytes to 10 μg/mL Hoechst 33342 for 10 minutes.

Immunofluorescence staining of CG and mitochondria

For the detection of CG, oocytes were first exposed to acidic Tyrode's solution to remove the zona pellucida. Then they were fixed and permeabilized as described previously. After being washed in PBS, the oocytes were stained with 10 μg/mL FITC-labeled Lens Culinaris Agglutinin (LCA) (L-9262; Sigma-Aldrich) in PBS for 30 minutes. For the staining of mitochondria, zona intact oocytes were used. Oocytes were stained by 0.5 μM MitoTracker Green FM (Molecular Probes, Eugene, OR) for 30 minuts at 37°C. After 3 washes in PBS, they were fixed as described previously. Chromosomes were stained using the same method as mentioned previously. Finally, the oocytes were mounted on a glass slide with a drop of antifade medium (P-36934; Invitrogen, Carlsbad, CA) and

Download English Version:

https://daneshyari.com/en/article/3435662

Download Persian Version:

https://daneshyari.com/article/3435662

Daneshyari.com