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LS-13-P-2721 Investigation Of Mitochondrial Activity And Cytoskeleton Organization In 3 Pronuclear Oocytes After Intracytoplasmic Sperm Injection

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Digyny, is the presence of a third pronucleus due to failure of the extrusion of the second polar body (1). The oocyte mitochondria have critical roles such as production of ATP or regulation of Ca+2 homeostasis during oocyte maturation, fertilization and subsequent developmental processes (2,3). The cytoskeleton of the oocyte has regulatory function in meiotic spindle formation, chromosome segregation, pronuclear apposition and cytokinesis (4, 5). Mitochondrial membrane potential, distribution of F-actin and gamma-tubulin, and ultrastructure of the 3 pronuclear oocytes were investigated.
Oocytes from patients who were engaged to assisted reproduction program were collected and after ICSI process oocytes with 3 pronuclei were selected for the study. Informed consents were taken from all patients. Some oocytes were fixed by %4 paraformaldehyde and treated by anti-gamma tubulin and FITC – phalloidin antibodies for immunoflourescent study. For ultrastructural evaluation, oocytes were fixed with %1 glutaraldehyde and embedded in Epon 812. Sections were investigated by Jeol 1011 transmission electron microscope. Mitochondrial membrane potential was evaluated in fresh oocytes stained with JC-1 by fluorescent microscope.
Mitochondrial membrane potential of oocytes with three pronuclei was found to be comparable to normal zygotes (Figure 1). Gamma – tubulin were stained predominantly at the subplasmalemmal domain and microfilaments were localized at the cortical, but not perinuclear areas (Figure 2). Either partial or no cytoplasmic halo were detected. Large vacuoles were seen in cortical regions of oocyte cytoplasm. Ruptured and dilated ER cisternae were detected in some oocytes. Lipofuscin granules, degenerated mitochondria and multilamellar bodies were also seen in the ooplasm (Figure 3 and 4).
These findings suggest that mitochondrial membrane potential has no direct influence on extrusion of the second polar body. In our study quality of the oocytes with 3 pronuclei were in poor condition and most of them were showing evidences of aging. Aging features, triggered by various conditions including culture media and in vitro environment, might have caused failure in microtubule organization and microfilament distribution and such a disruption associated with the dynamics of the cytoskeleton may play a role in the formation of 3 pronuclei.

References:

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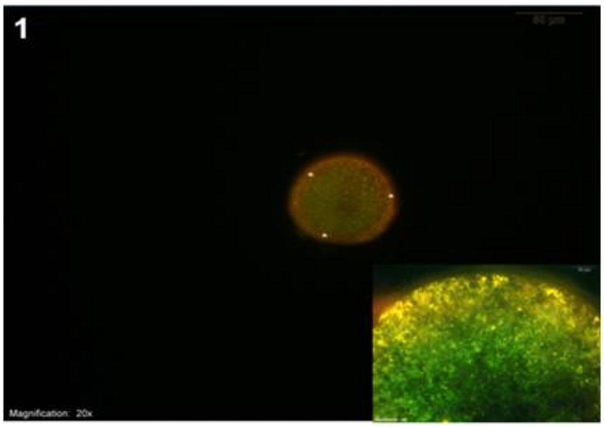

Fig. 1: Fig.1: Mitochondrial membrane potential. JC-1 aggregate accumulation shows yellow-orange high membrane potential (asterisk).

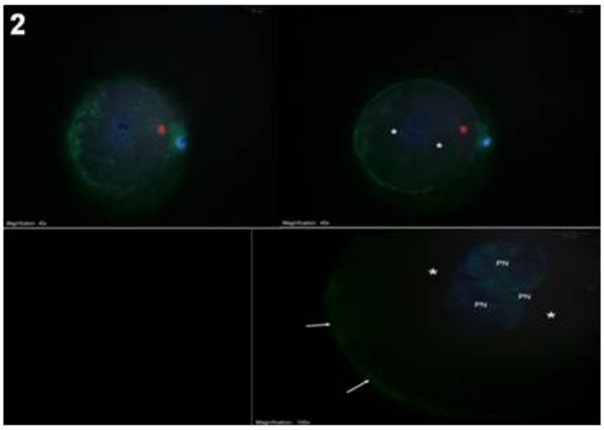

Fig. 2: Fig. 2: FITC-phalloidin labeled actin filaments (green). Actin filaments located surface of the 3PN oocyte (arrows). No staining around the pronuclei (asterisk). DAPI: blue, PN: pronucleus

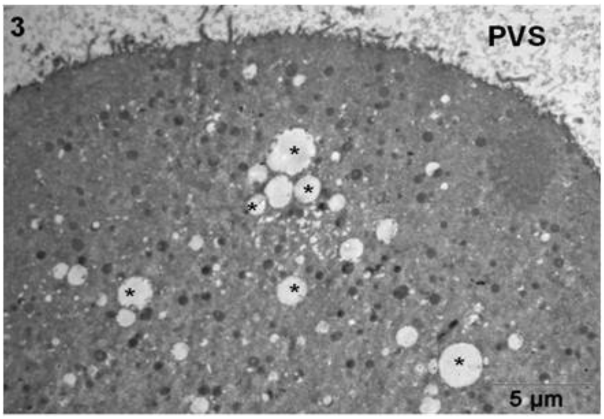

Fig. 3: Fig. 3: Dilated SER cisternae (asterisk) detected at the cortical region of the oocytes. PVS: perivitellin space

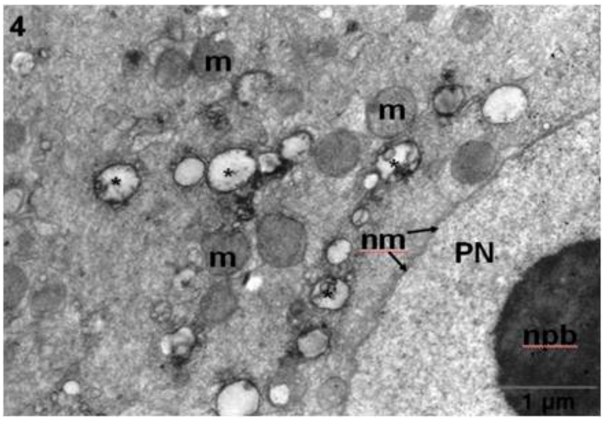

Fig. 4: Fig. 4: Vacuolated mitochondria (asterisk) M: mitochondria, nm: nuclear membrane, npb: nuclear precursor body, PN: pronucleus