American Society for Reproductive Medicine （71st Annual Meeting）
Baltimore（USA）、2015.10.17-21

Microfilaments play critical roles in mitochondrial traffic in porcine GV oocyte

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Objective: Oocyte maturation requires a variety of ATP-dependent reactions, such as germinal vesicle breakdown, spindle formation, and polar body extrusion, which is required for fertilization. Mitochondria are accordingly expected to be localized to subcellular sites of ATP utilization. Although cytoskeleton-dependent traffic for mitochondria has been studied extensively in somatic cells, the mechanism of mitochondrial dynamics in mammalian oocytes remains obscure. This study describes dynamic aspects of mitochondria in porcine oocytes at germinal vesicle stage.

Design: Basic research study

Materials and Methods: Mitochondria in donor oocytes were stained with MitoTracker-Orange (MTO). Donor oocytes were centrifuged at 10,000 x g and 37ºC for 15 min. Mitochondria-enriched ooplasm were micropunctured and injected into either central or subcortical area of recipient oocytes. Mitochondria-injected oocytes were cultured with or without colcemid, cytochalasin B or cytochalasin D. The image of mitochondrial dynamics in the recipient oocytes was captured every 15 min using a confocal microscopy for 15 hours, and analyzed quantitatively with ImageJ. Relative area and distribution of fluorescent mitochondria in recipient oocytes were calculated on the basis of their initial values at 0 h of culture. Total of 227 mitochondria-injected oocytes were observed.

Results: Mitochondria injected centrally moved unidirectionally to subcortical area and those injected subcortically moved along plasma membranes. The area occupied with MTO fluorescence increased significantly during 15 hours. The area occupied with MTO fluorescence at both sites of injection was not affected by colcemid, but inhibited strongly by cytochalasin B and cytochalasin D.

Cnclusions: This study shows that central mitochondria move from central to subcortical area and those in subcortical area move along plasma membranes, and suggests that microfilaments play critical role in mitochondrial traffic in porcine GV oocyte. The method established in this study may permit studies of the pathophysiology of intracellular traffic of mitochondria and other organelles in oocytes from patients with infertility.

Support: None