QUANTITATION OF CELLULAR VOLUME IN MATURE MOUSE OOCYTE AS DEMONSTRATED BY LSM

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To study the osmotic response is to measure the cellular volume since any osmotic shock is paralleled by the volume changes resulted from respective water movement across the cytoplasmic membrane. Therefore investigation of the cell osmotic behaviour requires the approach for cellular volume quantitation. Oocyte was supposed to have ball shape [1]. Spherical modeling based on simple mathematical relationship allowed cellular volume to be estimated. More adequate approach of laser microtomography represents direct method of volume measuring employed for single blastomere of multicellular mouse embryo [2]. In the current study this technology was applied for single oocyte (Fig.1).

Oocyte volume was determined with laser scanning microscopy (LSM) followed by three-dimensional reconstruction (3-DR). The keeping of the intact volume of mouse oocyte was based on freeze-drying technique. After cryofixation in liquid propane and subsequent low-temperature dehydration the cell was immediately immersed in the Epon medium. The monolayer of freeze-dried embedded oocytes was examined with a laser scanning microscope (Leica DM2500, Germany) in the mode of transmitted light. Using 532-nm laser a gallery of optical slices of mouse oocyte was collected in Z-direction with deepness step of 1 µm thick. 3-DR was performed in the 3ds max medium.

Our data indicate that cell volumetric modeling based on spherical extrapolation is not applicable even to the simple system of oocyte. The direct measurement of cell volume by LSM quantitation (Fig.1B) gives volume value 13% less than indirect approach using mean diameter (Fig.1A). Presence of polar body, lack of exact spherical shape and spreading the oocyte during long-term incubation can be the reason for mistake in determining the egg volume.

Figure 1: Picture of the oocyte cross-section (A) and 3-DR image of the same cell (B); V-cell volume; pbh-polar body hall; pL-picoliter; ZP-zona pellucida.