Human oocyte ultrastructural changes after vitrification

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Vitrification currently offers interesting perspectives in the field of oocyte cryopreservation, being judged more tolerable than slow cooling. Vitrification is also claimed to be a relatively easy, effective and low cost procedure [1-3]. The recent improvement of this technique allowed higher survival rate, lower oocyte abnormalities (in terms of meiotic spindle integrity and chromosome alignment) and faster spindle post-warming recovery, compared to slow cooling [4-6].

However, nowadays experimental and clinical results are controversial and lacking of consistent morphological data. Indeed, light and electron microscopy studies are considered a key tool in the evaluation of the effects of cryopreservation of ovarian tissues [7,8].

To this aim, immature (germinal vesicle -GV- stage and metaphase I -MI- stage, i.e. after the GV breakdown) and mature (metaphase II -MII- stage) oocytes were obtained from consenting IVF patients and vitrified according to the Cryotop method [1,2], using ethylene glycol and dimethylsulfoxide as cryoprotectants. After warming, the oocytes were fixed with glutaraldehyde and processed for light and transmission electron microscopy observations, as previously reported [7,8].

All vitrified/warmed oocytes appeared rounded in sections, with a homogeneous cytoplasm, an intact oolemma and a continuous zona pellucida. Immature oocytes showed characteristic organelles such as dense, rounded mitochondria, isolated smooth endoplasmic reticulum (SER) membranes, dictyosomes belonging to the Golgi system, small mitochondria-vesicle complexes and scattered cortical granules (CGs), the latter found in subplasmalemmal areas or located deeply in the cytoplasm. In some cases, minute vacuoles were also present in the inner cytoplasm, particularly around the nucleus of the GV-stage oocytes. Mature oocytes showed mitochondria-SER aggregates in the cytoplasm and a rim - often discontinuous - of CGs just beneath the oolemma. A reduced number of microvilli was also sporadically found in these oocytes. Meiotic spindles were also observed in favorable sections. Isolated vacuoles of different sizes were sometimes detected, mostly in the cortical cytoplasm, irrespective of the maturational stage of the oocyte. The general morphology and the organelle distribution of immature and mature oocytes is represented in figure 1.

In conclusion, the oocytes subjected to the above method of vitrification show good overall preservation. However, the alteration in CG and microvillus patterns in mature oocytes, or the oocyte vacuolization suggests the need of further ultrastructural studies on human vitrified oocytes, to determine the actual safety and efficiency of this technique.

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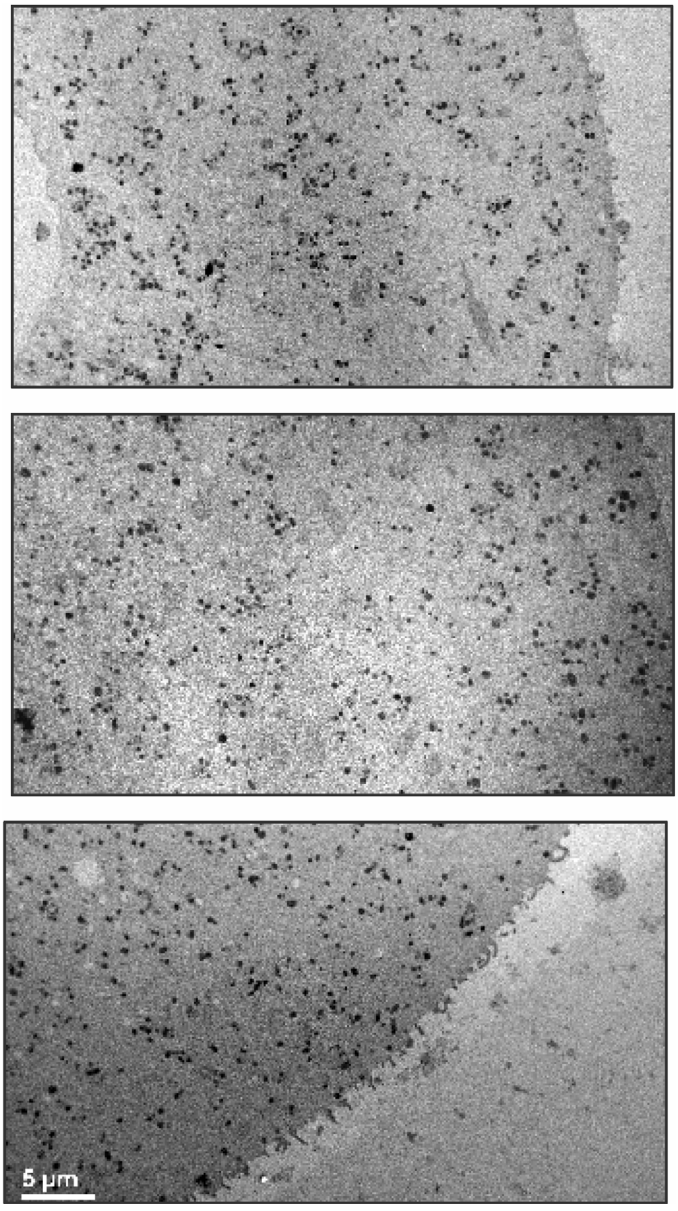

Figure 1. From top to bottom: general morphology and organelles microtopography are shown by Transmission Electron Microscopy (TEM) in GV, MI and MII human oocytes after vitrification. Bar=5 µm.