

Ultrastructural features of human metaphase II oocytes subjected to slow freezing or vitrification in an IVF program: a comparative analysis

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During the past two decades important advances have been made in the field of assisted reproduction by using oocyte cryopreservation. However, mature (metaphase II) oocytes are very susceptible to cryodamage. In order to contribute to the identification of a cryopreservation protocol with minimal side effects on the oocyte structure and function, we evaluated and compared the subcellular features of human oocytes cryopreserved either with slow (controlled rate) freezing or vitrification (ultra-rapid freezing). Supernumerary human metaphase II oocytes were donated by consenting patients enrolled in an IVF program. The age of these women ranged from 27 to 32 years old. The eggs were cryopreserved using slow freezing with 1.5M propanediol + 0.2M sucrose concentration or a closed vitrification system (Cryotip Irvine Scientific CA). Fresh oocytes were used as controls. Samples were fixed and prepared for light and transmission electron microscopy (LM and TEM) observations. By LM, all the oocytes were generally rounded, 90-100 microns in diameter, with regular ooplasm showing uniform distribution of organelles. By TEM, mitochondria-smooth endoplasmic reticulum (M-SER) aggregates were the most common structures found in all the oocytes fixed or cryopreserved within 3-4 hours after the retrieval. M-SER aggregates appeared instead partially replaced by mitochondria-vesicle complexes when oocytes were maintained in culture for a prolonged period of time. A slight to moderate vacuolization was found in the cytoplasm of the oocytes subjected to slow freezing. Slight microvacuolization was also found in vitrified oocytes, whereas vacuoles were almost completely absent in fresh controls. Amount and density of cortical granules (CGs) appeared abnormally reduced in all cryopreserved oocytes, irrespective of the protocol applied. In conclusion, it has been evidenced that prolonged stay in culture induces an intracellular membrane "recycling" in the oocytes, that causes the transformation of slender, anastomosed SER tubules into rounded vesicles surrounded by mitochondria, whose role is still uncertain. In addition, even though all cryopreservation protocols studied ensured a good overall preservation of the oocyte, vacuolization appears as a recurrent form of cell damage. This happens both during slow freezing and, at a lesser extent, during vitrification using a closed device. In addition, premature CG exocytosis was observed in both groups.

Keywords: Oocyte, cryopreservation, ultrastructure, human.