Ultrastructure of human oocytes subjected to different protocols of assisted reproduction

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INTRODUCTION: Oocyte cryopreservation protocols have not been fully optimized yet and overall clinical success remains suboptimal. Further experimental and clinical studies seem thus necessary in order to better understand the effects on human oocytes of all factors associated with freezing and to tailor the best protocol for human oocyte cryopreservation. Our aims were to evaluate and compare the ultrastructure of human mature oocytes frozen-thawed (F/T) with either slow freezing or vitrification, with both closed and open systems.

MATERIAL AND METHODS: The oocytes, obtained from consenting IVF patients, were fixed at sampling (fresh controls) and after freeze/thawing. Fresh and F/T oocytes were processed for light and transmission electron microscopy (LM and TEM) observations.

RESULTS: By LM, both fresh and F/T oocytes were generally rounded, 90-100 microns in diameter, provided with an ooplasm showing a uniform distribution of organelles and surrounded by an intact zona pellucida. By TEM, slight to moderate vacuolization was found in the cytoplasm of F/T oocytes subjected to slow freezing. The oocytes vitrified with a closed device also might show slight vacuolization. On the contrary, vacuoles were, in general, only occasionally detected in F/T oocytes after vitrification with an open device, and in fresh controls as well. Amount and density of cortical granules (CGs) appeared abnormally reduced in F/T oocytes, irrespective of the protocol applied (slow freezing or vitrification).

DISCUSSION: In conclusion, a) the cryopreservation protocols currently in use, ensure a good overall preservation of the oocyte; b) however, vacuolization appears persistently as a recurrent form of cell damage during slow freezing and, at a lesser extent, during vitrification using a closed device; the quasi absence of vacuoles seems instead the most relevant marker of quality in oocytes vitrified with an open device; c) premature CG exocytosis during cryopreservation seems a non-specific, ubiquitous phenomenon occurring during freeze/thawing, suggesting the appropriateness of the use of ICSI as the preferred insemination method after cryopreservation.

KEY WORDS: oocyte, cryopreservation, human, ultrastructure