Occurrence of vacuolization in human mature oocytes subjected to slow cooling

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Ooplasm vacuolization is a sign of cryoinjury. In this study human mature oocytes, obtained from consenting IVF patients, were cryopreserved by slow cooling using PrOH and sucrose as cryoprotectants. Oocytes were fixed at sampling (controls) and at different time intervals during thawing (1st, 2nd and 3rd passage in the thawing solutions: groups A, B and C, respectively). These oocytes were then observed by light and transmission electron microscopy (LM and TEM), to determine at what stage of the procedure the vacuoles may form and/or increase in number.

By LM, both fresh and cryopreserved oocytes were rounded, 90-100 μm in diameter, provided with a homogenous ooplasm and surrounded by a continuous zona pellucida. Areas in which staining and matter consistency appeared reduced, identified as vacuoles, were only occasionally detected in the ooplasm of fresh controls. On the contrary, vacuoles of different sizes and shapes were numerous in the cytoplasm of the cryopreserved oocytes belonging to all experimental groups (A, B, C). Morphometric analysis revealed that the differences between fresh control and cryopreserved samples were statistically significant ($P<0.05$). In particular, the mean number ± SD of vacuoles larger than 0.5 μm in diameter per 100 μm^2 was 1.06±0.18 (control), 7.2±1.5 (group A), 17.05±5.5 (group B), 9.5±5.2 (group C). Thus, vacuoles appeared to increase during thawing, reaching a maximum amount in group B (difference between A and B was statistically significant, $P=0.033$), and then undergoing a partial recovery at the end of the thawing process (difference between A and C was not statistically significant, $P=0.5$). By TEM vacuoles appeared as empty spaces lined by a membrane often interrupted.

These data: 1) evidence that vacuolization may be a recurrent form of cell damage during slow cooling; 2) reveal that vacuoles primarily form during freezing but may further increase in number at thawing, particularly during the PrOH step-wise dilution.

Key words
Oocyte, cryopreservation, electron microscopy, human