

## Mechanism of Meiotic Spindle Disappearance and Regeneration in Oocyte Vitrification.

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## Abstract

As assisted reproductive technologies (ART) become the standard of care for many infertile individuals, there has been a growing demand for the technology to cryopreserve oocytes. A cryopreservation method, called vitrification, promises high rates of oocyte survival, fertilization and development. However, a potential problem with oocyte vitrification is the transient disappearance of the meiotic spindle during freezing. Our goal is to understand the mechanisms behind the meiotic spindle disappearance and reappearance during vitrification. This understanding is essential to develop safer and more effective vitrification procedures. To gain insight into the mechanisms, we examined the integrity of the spindle microtubules, and three centrosome components, NEDD1, pericentrin and  $\gamma$ -tubulin, in mouse oocytes at various steps during vitrification and thawing. The distribution of these components was assessed by immunostaining using specific antibodies. We found that the spindle microtubules started to disappear gradually in the vitrification solutions, because of reduced temperatures and exposure to cryoprotectants, and became absent after exposure to liquid nitrogen. Regeneration of the spindle microtubules initiated during the thawing process, first in an excessive manner, generating a wide spindle and ectopic microtubule asters. However, the spindle was adjusted after the 37°C incubation for normal size and shape, suggesting a dynamic nature of spindle regeneration. For centrosome components, staining of pericentrin, but not NEDD1 and  $\gamma$ -tubulin, was observed largely throughout the vitrification and thawing process. Thus, pericentrin appears more resilient to the vitrification process, suggesting that the assembly of the centrosome components to enable microtubule repolymerization is similar during thawing and normal meiosis.

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