OPTIMIZING CRYOPROTECTANT EQUILIBRATION DURING CYROPRESERVATION OF INDIVIDUAL SECONDARY FOLLICLES

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Abstract Body

Cryopreservation of preantral follicles and their development in vitro is an experimental fertility preservation therapy for prepubertal cancer patients who cannot undergo ovarian transplantation due to the risk of re-transmitting malignant cells. Cryopreservation of individual macaque secondary follicles damages transzonal projections (TZPs) leading to decreased follicle survival. In order to determine whether differences in the volume change of the oocyte relative to the whole follicle are damaging TZPs during cryopreservation, secondary follicles were mechanically isolated from ovaries of adult rhesus monkeys (n=3). Healthy follicles (n=214) were divided into groups consisting of equilibration with: 0.5xPBS, 1xPBS, 2xPBS and 5xPBS at 4C, 25C and 37C or with cyroprotectants (CPAs): 15% glycerol, 15% ethylene glycol, 15% glycerol+15% ethylene glycol, and 1M sucrose at 25C. After fixation in isosmotic 4% paraformaldehyde, TZPs were immunolocalized, evaluated with confocal microscopy and quantified via staining intensity translated into a % TZP score. A low TZP score indicates fewer attached TZPs representing more damage. No difference in % TPZs was noted between loading and unloading, indicating TZP damage occurs during loading. Equilibration as a function of temperature suggests that shrinkage is causing TZP damage. A 3D model of a secondary follicle was created and used to capture mass transport, toxicity, and intracellular connections during the equilibration phase of cryopreservation. An inverse correlation between maximum oocyte-to-granulosa distance and normalized TZP score suggested that TZP damage is related to volumetric changes in the follicle. Experimental data and cell-based modeling both show that differing diffusion rates and inter-tissue mobility between granulosa cell layers and the oocyte correlates with TZP damage. Mechanical stress leading to TZP breakage rather than CPA toxicity underlies follicular damage during equilibration. This model will be useful to computationally determine optimum CPA loading protocols for cryopreservation of individual follicles.

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