Improving Trehalose Uptake and Release via Cold-responsive Nanoparticles to Promote Dehydration Tolerance of Cumulus-Oocyte Complexes during Microwave-assisted Drying

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As global demand for fertility preservation rises, developing dry-preservation strategies for simpler and more economical long-term storage at non-freezing temperature becomes increasingly appealing. The crucial step in stably maintaining viable gametes at a dried state involves an incorporation of trehalose, a non-permeable disaccharide known to shield anhydrobiotic organisms against dehydration stress. Our previous work demonstrated that cold-responsive nanoparticles (CRNPs) can transport trehalose into cat cumulus-oocyte complexes (COCs) within a 4-hour co-incubation at 38.5°C, followed by a cold-triggered release at 4°C for 15 min. Building on this foundation, this study aimed to (1) optimize the uptake and release of CRNPs to allow safe incorporation of more intracellular protectants, and (2) examine the effect of increased trehalose uptake on membrane and DNA integrity of oocyte after drying and rehydration. COCs were incubated with CRNPs in culture media with different concentrations of bovine serum albumin (BSA) and for different duration. Removing BSA from the culture medium and extending CRNP exposure time from 4 hours to overnight improved CRNP uptake by up to 8-fold. Additionally, an alternative cold treatment at 14 °C for 15 min effectively triggered the release of trehalose without compromising COC competency. Under these enhanced trehalose-delivery conditions, we assessed the effect of intracellular trehalose on desiccation tolerance of COCs. COCs were co-incubated with either blank CRNPs or trehalose-encapsulated CRNPs, followed by cold treatment. COCs then were placed in 0.3 M trehalose and dehydrated via microwave-assisted drying for 0, 5, 10, or 15 min (approximately corresponding to removal of 0%, 35%, 74%, and 96% of water, respectively). Dehydrated COCs were immediately rehydrated in culture medium for 30 min before evaluating membrane (propidium iodide staining; N = 153) and DNA (TUNEL assay; N = 104) integrity. After 10 min of drying, percentages of oocytes with intact membrane significantly increased ($P < 0.05$) from 29% (blank control) to 63% in the presence of intracellular trehalose. No significant advantages were observed at other time points. DNA integrity was maintained in the majority of oocytes (≥ 93%) across all drying times, irrespective of trehalose delivery. Collectively, results suggest that intracellular delivery of trehalose via CRNPs enhances the desiccation tolerance of COCs and contributes to the improved preservation of oocyte membrane integrity during partial dehydration. Our findings offer valuable insights into developing effective dry-preservation strategies to meet the growing demand for fertility preservation.