

ADVANCES IN THE FUNDAMENTAL CRYOBIOLOGY OF MAMMALIAN OOCYTES

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ABSTRACT

Having effective means to cryopreserve mammalian oocytes could increase the efficiency of managing populations of laboratory animals, increase the effectiveness of breeding programs for livestock, and improve the means by which assisted reproductive therapy is applied to human patients. Unfortunately, for most mammals oocyte cryopreservation suffers from inefficiencies. The work completed in this dissertation was directed at advancing our knowledge of the fundamental cryobiological properties of oocytes from cows, pigs, and humans. The first series of experiments was designed to determine the likelihood of damage to the metaphase II spindle from osmotic stress. Increasing levels of hypotonic and hypertonic stress resulted in an increased proportion of oocytes displaying a damaged spindle as assessed by immunocytochemical staining. Human oocytes appeared more sensitive to hypertonic stress compared to oocytes from cows and pigs. Hypotonic stress caused more damage to cow oocytes compared to human and pig oocytes. Pig oocytes were also shown to lose in vitro developmental potential, and the proportion damaged was greater compared to the proportion showing damage to the spindle. The permeability of mature human oocytes to ethylene glycol and water was also determined. It was shown that the permeability was temperature dependent. The results from the osmotic tolerance and membrane permeability studies for human oocytes was used to develop a theoretically-optimized procedure for vitrifying human oocytes in standard 0.25 cc straws.