

Public Abstract

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Title:Advances in the Fundamental Cryobiology of Mammalian Oocytes

Having an effective means to cryopreserve mammalian oocytes could contribute to improvements in the management of laboratory animals and domestic livestock, and could also increase the effectiveness of therapy in the field of reproductive medicine. Unfortunately, to date, attempts to cryopreserve oocytes from most mammals have met with limited success. The work undertaken in this dissertation was designed to develop a better understanding of some of the fundamental cryobiological characteristics of oocytes from pigs, cows, and humans. The damaging effects of osmotic stress on the metaphase II spindle of oocytes from these three taxonomic groups were determined. The results showed that oocytes from all three groups exhibited damage to the spindle in direct proportion to the level of osmotic stress applied in the treatments. Human oocytes showed a lower tolerance to hypertonic conditions compared to oocytes from cows and pigs. On the contrary, oocytes from cows were more sensitive to hypotonic conditions compared to the oocytes from humans and pigs. Furthermore, the effect of osmotic stress on the developmental potential of oocytes from pigs was characterized. The sensitivity as measured by the effect on developmental potential was greater under hypertonic stress in comparison to the sensitivity as measured by disruption of the meiotic spindle. The permeability of mature human oocytes to ethylene glycol and water in the presence of ethylene glycol was also examined. The results demonstrated that the oocytes were less permeable to ethylene glycol in comparison to propylene glycol and dimethylsulfoxide as reported in previous studies. Finally, a theoretically-optimized procedure for vitrifying mature human oocytes using standard 0.25 cc freezing straws was developed based upon the osmotic tolerance and membrane permeability characteristics determined from the initial studies. The results suggest that a method which incorporates a 4-step cryoprotectant addition and a 2-step cryoprotectant removal procedure will allow the cells to be loaded with sufficient ethylene glycol to maintain a vitreous state during cooling and warming, yet only result in 10 percent of the cells losing viability due to osmotic perturbations. The theoretically-optimized procedure is very similar to a procedure developed for cattle oocytes using an empirical approach which allowed a high proportion of oocytes to maintain in vitro developmental potential. With improvements in human oocyte cryopreservation, serious challenges to increasing the effectiveness of the therapy applied in assisted reproductive medicine can be overcome.