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Effect of different cryoprotectant agents on spermatogenesis efficiency in cryopreserved and grafted neonatal mouse testicular tissue

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Restoration of male fertility associated with use of the cryopreserved testicular tissue would be a significant advance in human and animal assisted reproductive technology. The purpose of this study was to test the effects of four different cryoprotectant agents (CPA) on spermatogenesis and steroidogenesis in cryopreserved and allotransplanted neonatal mouse testicular tissue. Hank's balanced salt solution (HBSS) with 5% fetal bovine serum including either 0.7 M dimethyl sulfoxide (DMSO), 0.7 M propylene glycol (PrOH), 0.7 M ethylene glycol (EG), or glycerol was used as the cryoprotectant solution. Donor testes were collected and dissected from neonatal pups of CD-1 mice (one day old). Freezing and seeding of the testicular whole tissues was performed using an automated controlled-rate freezer. Four fresh (non-frozen) or frozen-thawed pieces of testes were subcutaneously grafted onto the hind flank of each castrated male NCr nude recipient mouse and harvested after 3 months. Fresh neonatal testes grafts recovered from transplant sites had the most advanced rate of spermatogenesis with elongated spermatid and spermatozoa in 46.6% of seminiferous tubules and had higher levels of serum testosterone compared to all other frozen-thawed-graft groups ($p<0.05$). Fresh grafts and frozen-thawed grafts in the DMSO group had the highest rate of tissue survival compared to PrOH, EG, and glycerol after harvesting ($p>0.05$). The most effective CPA for the freezing and thawing of neonatal mouse testes was DMSO in comparison with EG ($p<0.05$) in both pre-grafted and post-grafted tissues based on histopathological evaluation. Likewise, the highest level of serum testosterone was obtained from the DMSO CPA group compared to all other cryoprotectants evaluated ($p<0.05$). The typical damage observed in the frozen-thawed grafts included disruption of the interstitial stroma, intercellular connection ruptures, and detachment of spermatogonia from the basement membrane. These findings indicate that neonatal mouse testes were most effectively preserved when frozen with HBSS medium with DMSO and that the type of CPA is a significant factor to obtain the most advanced stages of spermatogenesis and steroidogenesis after cryopreservation, thawing, and transplantation of neonatal mouse testes.

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