Submission Summary

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Paper Title
Mitigation of freeze-thaw-induced damage in prepubertal testicular tissue using liposome-based freezing medium

Abstract
Cryopreservation of testicular tissue holds significant promise in the field of fertility preservation, particularly for prepubertal boys diagnosed with cancer. However, prepubertal testicular tissue cryopreservation is still considered to be in the experimental stage. Thus, the development of an efficient cryopreservation protocol is imperative. The freeze-thaw process-induced damage to the testicular cells is primarily caused by the loss of membrane lipids. We hypothesize that preventing the loss of membrane lipids during the freeze-thaw process could improve the cryosurvival of prepubertal testicular tissue. Hence, the present study was undertaken to assess the efficacy of prepubertal testicular tissue cryopreservation using a membrane lipid-rich liposome-based medium. The liposomes with a size of 205 nm, polydispersity index of 0.5, and a zeta potential of -59.90 were prepared and used in the study. Surface electron microscopic analysis of the liposomes revealed uniform size and texture. Prepubertal Swiss albino mouse testicular tissues were cryopreserved using the slow freezing method in a freezing medium comprising 5% DMSO and 30% FCS in the DMEM/F12 medium, with or without the addition of liposomes. Upon thawing, the viability, DNA damage, apoptosis, and oxidative stress of the tissues were assessed. The liposome-based medium significantly enhanced cell viability ($p<0.05$), while concurrently reducing DNA damage ($p<0.05$) and apoptosis ($p<0.05$) in the testicular tissue. Furthermore, a significant decrease in malondialdehyde ($p<0.05$) and glutathione levels ($p<0.05$) were observed. These findings suggest that the liposome-based medium holds substantial translational value in the cryopreservation of testicular tissue from prepubertal boys undergoing chemotherapy.

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