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Development of a Cryopreservation Protocol for Type A Spermatogonia

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The aim of this study was to develop a cryopreservation protocol for type A spermatogonia. Testes from 5- to 7-month-old calves were collected, and type A spermatogonia were isolated using two-step enzymatic digestion and Percoll separation. Cells were resuspended in minimum essential medium (MEM) supplemented with 1% bovine serum albumin (BSA) in a final concentration of 6 x 10^6 per mL, and the effects of different cryoprotectants and freezing protocols were tested. Cells frozen/thawed in medium containing 10% fetal calf serum (FCS) and 1.4 M glycerol or dimethyl sulfoxide (DMSO) had a significantly (P <.05) higher percentage of living cells compared to medium with only FCS, whereas DMSO gave a significantly better cell survival rate than glycerol did. An increase in the concentration of FCS in the DMSO-based medium to 20% had no effect on survival after freezing and thawing. Furthermore, inclusion of 0.07, 0.14, or 0.21 M sucrose in DMSO-based medium resulted in a significant improvement of cell survival, cell proliferation in culture, and colonization efficiency in recipient testes. A controlled slow-freezing rate (1°C/min) resulted in significantly (P <.05) more viable cells than fast (5°C/min) freezing. However, noncontrolled-rate freezing, with a comparably low cooling rate, gave even better results than the controlled-rate slow freezing. Cryopreservation in MEM-based medium containing 10% FCS, 10% DMSO, and 0.07 M sucrose using a non-controlled-rate freezing protocol appeared to be a simple and effective way to preserve type A spermatogonia, with a high yield of almost 70% living cells after thawing. Frozen/thawed spermatogonia survived in culture and retained the ability to proliferate as determined by colorimetric and bromodeoxyuridine incorporation assays. To test whether the stem cells among the A spermatogonia retained their ability to colonize the testis of a recipient mouse, bovine spermatogonia were transplanted. This resulted in colonization 2-3 months after transplantation. In conclusion, for the first time, a method specific for cryopreservation of type A spermatogonia, including spermatogonial stem cells was developed, which allows long-term preservation of these cells without apparent harmful effects to their function.

Key words: Freezing, spermatogonial stem cells, transplantation

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