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A New Protocol for Effective Cryopreservation of Human Embryonic Stem Cells by a Minimum Volume Cooling Method

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Recently, human embryonic stem (hES) cells have become very important resources for ES cell basic research, cell replacement therapy, and other medical applications; thus, efficient cryopreservation methods for these cells are needed. This study examined whether a newly developed minimum volume cooling (MVC) vitrification method, which was tested through cryopreservation of sensitive bovine oocytes, can be used for freezing hES cells. Feeder-free cultured hES cell (MB03) colonies were mechanically dissected into several small clumps following enzymatic treatment. We compared the freezing efficiency of a slow-cooling method using a cryo-module (0.4-0.6C/min, 20-30 clumps/vial) and MVC vitrification using a modified 0.5-ml French mini-straw designated as a MVC straw (>20,000° C/min, 10 clumps/straw). After thawing, *in vitro* survival of hES cell clumps was higher for MVC-vitrified cells (80.8%, 97/120) than for slow-cooled cells (38.2%, 39/102). Further, the proliferation rate of surviving MVC-vitrified cells was similar to that of control hES cells from 2 weeks after thawing. In addition, vitrified-thawed hES cells demonstrated a normal karyotype, were positively immunostained for surface marker antibodies (AP, SSEA-4 and TRA-1-60) and the Oct-4 antibody, and could differentiate into all three embryonic germ layer cells *in vitro*. This result demonstrates that hES cell clumps can be successfully cryopreserved by a newly developed MVC vitrification method without loss of human cell characteristics

Key words) *hES cell, Vitrification, Minimum volume cooling method, MVC straw, In vitro survival*