B-11 Vitrification of Mouse Blastocyst Using Cryoloop

Youm HW[1], Kim SK[1], Song SJ[1], Park YS[1], Koong MK[2] and Kang IS[2]

Laboratory of Reproductive Biology & Infertility[1], Department of OB/GYN[2], Samsung Cheil Hospital and Women's Healthcare Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Objective: The aim of this study is to compare the efficiency of a method for the cryopreservation of mouse blastocyst.

Methods: 2-cell stage of mouse embryos were obtained and cultured to blastocyst stage in T6 medium supplemented with 10% fetal bovine serum. Morphologically normal blastocysts were collected and randomly divided to one control and three experimental groups. In control, blastocysts were cultured *in vitro* continuously for an additional two days. In experiment 1, blastocysts were exposed to vitrification solution (ethylene glycol) only without cryopreservation (exposure only group). In experiment 2 and experiment 3, blastocysts were cryopreserved by slow-freezing procedure with glycerol (slow-freezing group) or by vitrification procedure using cryoloop (cryoloop group), respectively. Frozen blastocysts were thawed and cultured for additional two days. Twenty four hours after thawing, some blastocysts were fixed and stained with Hoechst 33342 (bisbenzimide) and the number of nuclei in each blastocysts were counted to confirm the survival of blastocysts in experimental groups.

Results: Survival rate and hatching rate of the blastocysts in slow-freezing group (24h: 72.4% and 66.0%, 48h: 63.2% and 64.6%) was significantly lower (X^2-test $p<0.05$) than those of control group (24h: 93.4% and 86.0%, 48h: 88.5% and 90.7%). However, the survival rate and hatching rate of the blastocysts in cryoloop group (24h: 84.1% and 84.1%, 48h 79.3% and 87.7%) was well compared with those in the control group. The mean (±SD) cell number of blastocyst in the exposure only group (89.2±11.5) and cryoloop (89.0±11.0) groups, except slow-freezing group (79.0±10.0), were not significantly different from that of control group (93.1±13.9) 24h after thawing (Student's t-test).

Conclusion: This study demonstrates that higher survival rate of vitrified-thawed mouse blastocyst can be obtained using cryoloop as the embryo container at freezing rather than slow-freezing procedure. The results of this study suggest that vitrification using cryoloop (with ethylene glycol) may be a preferable procedure for mouse blastocyst cryopreservation and could be applied to the human blastocyst cryopreservation.

B-12 배양액내 GM-CSF의 첨가가 생쥐 및 인간 수정란의 배발생에 미치는 영향

을지병원 의과학연구소[1], 을지의대 산부인과[2], 생리학교실[3]

김동훈[1] · 고덕성[1] · 김묘경[1] · 이회창[1] · 박원일[2] · 권혁찬[2] · 이호준[1,3]

목 적: 본 연구는 cytokine의 일종인 GM-CSF를 배양액에 첨가하여 생쥐 수정란의 배발달을 살펴