## P-23 Modification of Cryopreservation Method for Biopsied Embryos in PGD Treatments

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**Objectives:** The aim of this study was to evaluate the optimal cryopreservation method using model system for human biopsied embryos in PGD treatment.

**Materials and Methods:** Mouse two cell embryos were collected from ICR female mice and cultured for 16~18 hours. Blastomere of 6~10 cell stage embryo was biopsied, and the biopsied embryos were incubated for 3 hours. The biopsied embryos were frozen with 1,2-propanediol (PROH) as a cryoprotectant in choline based medium (CJ2) or phosphate buffered saline (PBS) by slow freezing protocol of automatic cell freezer. In the human model, 6~10 cell embryos developed from 3PN were biopsied, and the embryos were frozen at D0 (3 hours after biopsy) or D1 (24 hours after biopsy). After rapid thawing, the mouse embryos were cultured to blastocyst stage. The frozen human embryos were thawed at the same process as mouse model and cultured for 24 hours. The survival, further development and blastocyst formation rate were examined in each group.

**Results:** The survival rate of mouse frozen-thawed biopsied embryos in CJ2 vs PBS (87.2% vs 81.3%) was not significantly different. But, the blastocyst formation rate of mouse frozen-thawed biopsied embryos in CJ2 (67.6%) was significantly ($p<0.05$) higher than in PBS (46.2%). The survival rate of D0 and D1 human biopsied embryos in CJ2 (73.9% and 82.4%) was significantly ($p<0.05$) higher than that in PBS (25.0% and 36.4%). The further developmental rate of human biopsied embryos in CJ2 (69.6 %) was higher than that in PBS (50.0%).

**Conclusions:** Our result shows that efficacy of cryopreservation of biopsied embryos could be improved by CJ2 medium in mouse and human model. The successful cryopreservation of biopsied embryos is useful to increase the chance of normal pregnancy for couples of PGD treatment with FISH and PCR.

## P-24 Development of Effective Cryopreservation Method for Mouse Oocytes

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**Objective:** The purpose of this study was to evaluate the efficacy and effect of various cryopreservation method on the survival and the cytoskeletal stability of metaphase II mouse oocyte.

**Materials and Methods:** Mouse ovulated oocytes (metaphase II) were collected and cryopreserved by a modified slow-freezing method with 1.5 M propanediol (PrOH)- 0.1M sucrose or by vitrification protocol using cryo loop and EM grids with 40% ethylene glycol-0.6 M sucrose. Four hours after thawing, intact oocytes were fixed, and stained spindles with fluoroscein isothiocyanate (FITC) conjugated antibody against to α-tubulin and chromosomes with propidium iodide (PI). Spindle morphology was classified as follows: normal (barrel-shaped), slightly abnormal and abnormal (multipolar or absent).

**Results:** After thawing, survival rate of the oocytes in vitrification group (62.7%) was significantly ($p<0.01$) higher than that of slow-freezing group (24.4%). Vitrification with cryo loop showed significantly higher survival rate than that with EM grids (67.7% vs 53.5%, $p<0.05$). Normal spindle and chromosome configurations of oocytes after thawing between two vitrification group was not significantly different.

**Conclusion**: For mouse ovulated oocytes, vitrification with cryo loop may be a preferable procedure comparing to slow-freezing methods. Further study should be needed to investigate the developmental competency of frozen-thawed mouse oocytes

## P-25 Glycerol와 DMSO을 이용한 생쥐 고환조직의 동결-융해 후 미세구조의 변화 관찰

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**Background & Objectives:** 동결보호제인 glycerol과 DMSO가 동결보존 시 정세관의 형태에 미치는 효과를 비교하기 위하여 광학 및 전자현미경을 이용하여 관찰하였다.

**Method:** 생쥐의 정세관을 20% glycerol 혹은 DMSO를 동결보호제로 사용하고 computer controlled freezing program을 사용하여 동결하였다. 동결보존한 생쥐의 정세관을 37℃ water bath에서 융해 후 광학 및 전자현미경적 관찰을 위하여 4% glutaraldehyde에 고정하였다. 탈수, 치환과정을 거쳐 광학현미경 sample은 파라핀을 포매제로 사용하여 7 μm의 두께로 H-E staining을 실시하고, 전자현미경 sample은 Epon을 포매제로 사용하여 70 nm의 두께로 uranyl acetate와 lead citrate를 이용한 double staining을 실시하여 각각 관찰하였다.

**Results:** 동결을 시행하지 않은 정상 대조군의 생쥐 정세관에서는 정조 세포 (germ cell)와 Sertoli 세포에서 정상적인 세포소기관을 관찰할 수 있었고, 세포사이의 공간을 관찰할 수 없었다. 그러나 동결-융해한 정세관에서는 다수의 mitochondria 파괴, 세포질의 공포 그리고 세포와 세포사이의 공간이 존재하는 것을 관찰할 수 있었다. 일부 세포의 경우, 세포 소기관이 파괴되어 생성된 듯한 다각형의 빈 공간을 관찰할 수 있었다. Glycerol과 DMSO에 의한 동결보호 효과를 비교하였을 때 두 실험군간의 세포형태 변화는 유사하였다. 즉, 두 실험군에서 세포미세구조의 파괴, 정조세포의 기저막 이탈, lysis된 세포 그리고 세포간의 결합파괴 등이 모두 관찰되었다. 그러나 glycerol군에 비해 DMSO군의 조직에서 세포간 결합과 세포미세구조의 보존상태가 비교적 양호하며 lysis된 세포수도 적었다.

**Conclusions:** 정세관의 동결보존 시 세포소기관, 세포간의 결합 그리고 세포의 파괴가 나타났다. 이와 같은 현상을 극복하기 위해 사용하는 동결보호제인 glycerol과 DMSO의 효과를 비교하였을 경우