P-15 Gene Expression of Heat-shock Protein (HSP 70.3) from Mouse Blastocysts Vitrified at Different Stages of Development In Vitro

Lee SE[1], Lee JH[1], Jung YJ[1], Lee JH[1], Choi KW[1], Lee YB[2], Lee SJ[2]

[1]IVF Lab, [2]MDplus LSI, Mirae and Heemang Infertility Clinic

Background & Objectives: In vitro development of mammalian embryo is affected from various environmental stresses exposed, such as pH, heat and osmolarity. Freezing and thawing processes of embryos include not only thermal shock but also rapid osmotic change. So the frozen-thawed embryos have lower development in vitro culture to retarded or ceased development. The purpose of this study was to measure the development into blastocysts of mouse embryos vitrified at different stages of development and their expression patterns of major stress-induced gene HSP 70.3 by RT-PCR.

Method: Two-cell embryos were collected from superovulated 6 week-old ICR mice at post hCG 46 hrs. The embryos of every experimental group were vitrified at different time of culture, 2-cell at 48 hr, 8-cell at 72 hr and blastocysts at 95~100 hr post hCG. After thawing, vitrified embryos were cultured upto expanded blastocysts additively. In vivo and in vitro control blastocysts were collected at post hCG 100~105 hr. RT-PCR confirmed by mouse specific β-action expression and HSP 70.3 expression was analyzed by RT-PCR and densitometry.

Results: Developments into blastocysts were 55.3%, 56.9% and 80.7% respectively from the vitrified embryos at 2-cell, 8-cell and blastocyst stages. In vitro development of blastocysts from 2 cell embryos without vitrification was 88.6%. The expression of HSP 70.3 was significantly high in blastocysts vitrified at 8 cell stage compared with in vivo and in vitro control and other experimental groups. All of other groups showed similar expression in amount.

Conclusions: HSP 70.3 is a heat shock protein, a major stress gene, playing an important role for the prevention of cellular damages from external stress. The fact that all stages of embryos except blastocysts vitrified at 8 cell stage showed the similar expressions of HSP70.3 compared with in vivo blastocysts might suggest that they are recovered from stress after the source of stress is removed. Such higher expression from blastocysts vitrified at 8 cell stage is supposed to due to an abnormal control at on set of zygotic gene expressions for development and differentiation.

P-16 생쥐 포배기 배아를 이용한 효과적인 완만동결법의 비교 연구

김지선 · 송상진 · 홍지영 · 김수경 · 최수진 · 천강우 · 변혜경

성균관대학교 의과대학 삼성제일병원 생식생물학 및 불임연구실

Background & Objectives: 생쥐 포배기 배아의 동결, 융해 후 생존률을 높이기 위하여 동결 시작온