P-10 *In Vitro/In Vivo* Development of Vitrified Immature Mouse Oocytes

Sepill Park, Eun Young Kim, Hwa Kyung Nam, Keum Sil Lee, Sae Young Park San Hyun Yoon*, Kil Saeng Chung and Jin Ho Lim***

*Maria Infertility Medical Institute (마리아기초의학연구소), *Maria Infertility Clinic, Seoul, **College of Animal Husbandry, Kon-Kuk University*

This study was carried out to investigate *in vitro/in vivo* development of vitrified-thawed immature mouse oocytes. Immature mouse oocytes were vitrified with EFS40 (40% ethylene glycol, 18% ficoll and 0.5 M sucrose). Thawed oocytes were matured for 16 hr *in vitro*. Matured oocytes with the first polar body were fertilized with the concentration of $1\text{-}2\times10^6$/ml of epididymal sperm. After fertilzation, cleavage ($\geq$2-cell) and *in vitro/in vivo* development rates were examined. The results were summarized as follows: *in vitro* maturation rate of immature mouse oocytes in the vitrified-thawed group was similar to that in the exposed group (67.5%) and control group (66.3%), but cleavage rate of vitrified-thawed oocytes (64.9%) and blastocyst formation rate (59.0%) were significantly different compared to those of the exposed group (83.7 and 74.7%) and the control group (90.7 and 83.7%) ($p<0.05$). However, when the blastocysts derived from vitrified-thawed immature mouse oocytes were transferred to pseudopregnant mouse, total implantation (31.3%) was slightly lower than that in control (40.8%), but live fetus formation rate (66.7%) was slightly higher than that in the control group (58.1%), there was not significantly different. Therefore, when *in vitro* produced blastocyts were transferred into recipients, although the *in vitro* development of vitrified-thawed oocytes was decreased, live fetus formation rate was similar to that of the control group. The present results indicate that immature mouse oocytes can be frozen successfully by vitrification with EFS40.

P-11 Establishment of In Vitro 3-Dimensional Culture System of Mouse Endometrial Cells
I. Cytohistological Study on Mouse Endometrium

Hwa Kyung Nam, Eun Young Kim, Keum Sil Lee, Sae Young Park, San Hyun Yoon*, Sepill Park and Jin Ho Lim*

*Maria Infertility Medical Institute (마리아기초의학연구소), *Maria Infertility Clinic, Seoul*

This study was designed to identify the ultrastructural changes of mouse endometrium during periimplantation period and obtain the fundamental information for the establishment of 3-dimensional culture system of mouse endometrial cells in vitro. The used female ICR mice (6 wks) were conducted on pregnant. The biopsies were obtained from whole uterus at cycle days 0 (D0) and 4 (D4) (the day of detection of the vaginal plug designated as day -1). The biopsied ma-

terials were fixed 2.5% glutaraldehyde and 1% osmium tetroxide. Subsequently, they were dehydrated and embedded in Epon. After the embedded biopsies were sectioned and stained, they were observed under light, transmission and scanning electron microscopy (LM, TEM and SEM). The results obtained in these experiments were summarized as follows. 1) For LM, the biopsied materials at D4 (secretory phase) were appeared that the stromal layer was extended by increased connective tissues and the endometrial glands and vessel were fully developed compared with D0 (proliferative phase). 2) For TEM, the mouse endometrium was consisted of 3-layers, a simple polarized columnar epithelial cells, basement membrane and stromal cells. At D4, the distribution of microvilli, endoplasmic reticulum, Golgi body, lipid and glycogen deposits, secretory granules and surface area were increased. 3) For SEM, the degree of folding and microvilli of surface of mouse epithelial cells was became more and more, and at D4 the appearance of pinopodes as a specific marker of uterine receptivity was found. Therefore, these results indicated that mouse endometrium was dramatic morphological changes during periimplantation period and will give support to the establishment of in vitro 3-dimensional culture system of mouse endometrial cells.

P-12 Fast and Efficient One-Step Dilution Method of Vitrified Bovine IVM/IVF/IVC Blastocysts

Keum Sil Lee, Eun Young Kim, Hwa Kyung Nam, Sae Young Park, San Hyun Yoon*, Sepill Park and Jin Ho Lim*

Maria Infertility Medical Institute (마리아기초의학연구소),
**Maria Infertility Clinic*

This study was to find the easier and more effective dilution technique of vitrified bovine IVM/IVF/IVC blastocysts for the field trial. For vitrification, in vitro produced blastocysts were exposed in glycerol (G) and ethylene glycol (EG) mixture and 10% FBS add in mDPBS (10% (v/v) G for 5 min, 10% G plus 20% (v/v) EG for 5 min, and 25% G plus 25% EG (v/v) for 30 sec), placed in nitrogen vapor for 3 min, and plunged into LN_2. At thawing, straw was placed in air for 10 sec, in water of 25℃ until all ice had disappeared. And then they were placed in 25℃ and 36℃ water according to the different dilution time treatment groups. 1) When the *in vitro* survival of vitrified bovine blastocysts according to different dilution time was examined, the results of the 1 min dilution group (≥re-expanded: 86.6, hatched: 56.6%) were higher than those of other treatment groups (2 min; 93.7, 37.5%, 2.5 min; 76.4, 29.4%, 3 min; 88.8, 36.1% and 3.5 min; 83.7, 8.1%). 2) When *in vitro* survival of the vitrified groups according to different developmental stage was examined using the 1 min dilution method at thawing, the results of expanded blastocyst (56.3%) and early hatching blastocyst (57.1%) were significantly higher than that of early blastocyst (36.6%) ($p<0.05$). 3) Also, when the *in vitro* survival of the vitrified groups according to different developmental age was investigated, the results of day 7 (60.0%) and day 8 blastocysts (60.0%) were significantly higher than those of day 9 blastocysts (29.4%) ($p<0.05$). Therefore, these results demonstrated that 1 min dilution method at thawing is suitable for the field trial of the vitrified day 7 or day 8 bovine expanded/ early hatching blastocysts.