Optimization of In Vitro Culture System of Mouse Preantral Follicles

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This study was to establish in vitro culture system of mouse preantral follicles and to obtain higher in vitro development rates and production of live young. Preantral follicles were obtained from 12-day-old FI mouse (C57BL×CBA) by enzymatical methods. Oocyte–granulosa cell complexes (OGCs) of preantral follicles were loaded on Transwell-COL insert and cultured in a MEM supplemented with 5% FBS, 100 mIU/ml FSH and 100 mIU/ml hMG for IVG. IVM was performed in a MEM supplemented 1.5 IU/ml hCG for 18 hrs and IVF was carried out in M16 medium. Embryos were cultured in modified M16 medium supplemented 10% FBS for 4 days. The effect of the OGCs size on the nuclear/cytoplasmic maturation was significantly higher in 120–150 μm (MII: 33.0%, ≥2-cell: 36.7%, ≥morula: 20.9%) than in 70–110 μm (MII: 12.2%, ≥2-cell: 10.2%, ≥morula: 4.8%) (p<0.001). In period of the IVG days, the rate of ≥2-cell was significantly higher in 10 days (38.2%) than in 12 days (20.0%) (p<0.01). In period of IVF time, 9 hrs (≥2-cell: 31.5%, ≥morula: 14.3%) indicated significantly higher cytoplasmic maturation rate than 4 hrs (≥2-cell: 17.5%, ≥morula: 4.8%) and 7 hrs (≥2-cell: 20.4%, ≥morula: 6.1%) (p<0.01). However, there was no difference in cytoplasmic maturation between co-cultured preantral follicle (≥morula: 17.4%) and preantral follicle cultured in M16 (≥morula: 17.4%). 22 morula and blastocysts produced in above optimal condition were transferred to uterus of 2 pseudopregnant recipients, 1 recipient was pregnant and then born 1 live young. This result demonstrates that in vitro culture system of preantral follicles can be used efficiently as another method to supply mouse oocyte.

Key Words: Mouse preantral follicle, In vitro growth, In vitro fertilization, Co-culture