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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 F1 mouse (C57BL×CBA) by enzymatical methods. Oocyte-granulosa cell complexes (OGCs) of preantral follicles were loaded on Transwell-COL insert and cultured in αMEM supplemented with 5% FBS, 100 mIU/ml FSH and 100 mIU/ml hMG for IVG. IVM was performed in αMEM 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 ㎛ (MII: 33.0%, ≥2-cell: 36.7%, ≥morula: 20.9%) than in 70-110 ㎛ (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***