Establishment of Basic System for Manipulating Preantral Follicles

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Biotechnology has been developed from the basic knowledge of biology, medicine and animal science, thus multidisciplinary approach is absolutely required for efficient developing biotechnical methodologies and materials. The final end-products derived from biotechnology contribute to improving human welfare, which are related to discovering novel drugs, inventing medical materials and devices, and developing novel technologies to overcome various catastrophic diseases. We have attempted to develop materials and growth factor delivery system to mature preantral (primordial, primary and secondary) follicles in mammals. The researchers workingfor gamete biotechnology and tissue engineering actively involves this research association. To date, the relationship between retrieval method and follicle stage, and in vitro growth of follicles and intrafollicular oocytes were elucidated. The objective of this study is to establish a basic manipulation system of preantral follicles. Randomized, prospective study using animal model was conducted and, as experimental model animal, two-week-old female F1 (C57BL6/DBA2) mice were employed. Preantral follicles of primary, early secondary and late secondary follicles were retrieved by either a mechanical or an enzymatic method. The collected follicles were cultured in vitro for various durations and in vitro-growth of preantral follicles were sequentially monitored. The maturation of intrafollicular oocytes retrieved from cultured follicles was further assessed and the fertilizability of the oocytes matured was monitored after in vitro-insemination with epididymal semen. As results, a mechanical method retrieved more preantral follicles than an enzymatic method. Regardless of retrieval methods, the number of early secondary follicles was larger than that of primary and late secondary follicles. Retrieval of primary follicle by an enzymatic method was not possible. The follicles cultured in vitro commonly underwent a step-by-step growth consisting of the follicular, diffuse, pseudoantral and degenerative stages, but the retrieval method greatly influenced the follicular growth: to reach the pseudoantral stage, primary, early secondary and late secondary follicles retrieved by the mechanical method required 11, 10 and 7 days, respectively. In the case of the early and secondary follicles retrieved from an enzymatic method, however, 9 and 6 days were necessary, respectively. When intrafollicular oocytes collected from pseudoantral follicles were cultured in vitro, various durations were required for final maturation (developed to metaphase II stage) of oocytes. The optimal maturation time of follicular oocytes was 11 days, 7~9 days and 7 days for primary, early secondary and late secondary follicles, respectively. When microscopic observation was made for cultured oocytes,

general decrease in oocyte diameter was detected in oocytes derived from all categories of follicles compared with in in vivo-derived oocytes. The enzymatic retrieval typically reduced zona thickness of oocytes compared with in vivo-ovulated oocytes, while the mechanical method did not reduce. Pronuclear formation after parthenogenetic activation was possible in all mature oocytes derived from in vitro-cultured follicles of different categories.

In conclusion, preantral follicles underwent step-by-step growth in vitro and retrieval method greatly affected in vitro-growth of preantral follicles and follicular oocytes. The results of this study positively confirmed the validity of the research on the development of follicle culture system for future medicine.