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Antrum Formation and Growth of Mouse Pre-antral Follicles Cultured in Two Different Culture Media without Hormones

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Mouse follicles require the addition of gonadotropins (Gns) to complete maturation and ovulation of oocyte and antrum formation of follicles *in vitro*. However, we tried examination of *in vitro* growth of mouse pre-antral follicles in medium without Gns and physiological factors. And also, pre-antral follicles were isolated from ovaries by mechanical method. Our present studies were conducted to evaluate on the growth of follicles and intra-follicular oocytes and antrum formation *in vitro* of mouse pre-antral follicles in two different media. Pre-antral follicles (91-120 ㎛) were isolated mechanically by fine 30G needles not using enzymes from ovary of 3-6 weeks old female ICR mice. Isolated pre-antral follicles were cultured in 20 ㎕ droplets of TCM (n=17; follicles: 107.8 ±1.58 ㎛; oocytes: 59.9±1.2 ㎛) or MEM (n=12; follicles: 109.3±2.53 ㎛; oocytes: 55.4±1.6 ㎛) under mineral oil on the 60 mm culture dish. All experimental media was supplemented with 10% FBS but without Gns and/or physiological factors. Pre-antral follicles were individually cultured in drops for 8 days. Antrum formation and growth of pre-antral follicles and intra-follicular oocytes were evaluated using a precalibrated ocular micrometer at ×200 magnifications during *in vitro* culture. Results between different groups were analyzed using combination of Student's t-test and Chi-square, and considered statistically significant when $P<0.05$. Antrum formation of pre-antral follicles had started in two culture media on day-2. On day-8, antrum formation had occurred in 58.3% (7/12) of pre-antral follicles cultured in MEM, but only in 23.5% (4/17) of those cultured in TCM ($P=0.0364$). Growth of pre-antral follicles and intra-follicular oocytes were observed on day-4 and -8. On day-4, follicular diameters was similar ($P=0.1338$) in TCM (119.4±2.58 ㎛) and MEM (125.4±4.52 ㎛). However, on day-8, diameters of pre-antral follicles cultured in MEM (168.9±17.29 ㎛) was significantly ($P=0.0248$) bigger than that in TCM (126.7±4.28 ㎛). On day-4 and -8, diameters of intra-follicular oocytes were similar TCM (67.1±1.3 and 72.4±0.9 ㎛) and MEM (65.2±1.7 and 73.3±1.5 ㎛), respectively. We can conform that medium not supplemented with Gns and/or physiological factors can be used for *in vitro* antrum formation and growth of mouse pre-antral follicles and intra-follicular oocytes. In conclusion, MEM supplemented with FBS can be used for growth *in vitro* of mouse pre-antral follicles isolated mechanically.

(Key words) ***Mouse pre-antral follicles and intra-follicular oocytes, Antrum formation, Growth, Culture media***