

Effects of FSH and LH on Maturation of Bovine Preantral Follicle

D. J. Kim, H. J. Chung, S. J. Uhm, H. T. Lee, K. S. Chung,
Animal Resources Research Center, Konkuk University,

The culture of preantral follicles has important biotechnological implications through its potential to produce the large quantity of oocytes for embryo production, transgenesis research, conservation of rare breed, and a potential source of ovarian genetic material. The present study was conducted to establish the optimal conditions of *in vitro* culture for intact bovine preantral follicles; and to examine the developmental ability of oocytes derived from the *in vitro*-grown preantral follicles; and to investigate the effects of various concentrations of FSH and LH on these processes. Bovine preantral follicles ($150 \pm 1.2 \mu\text{m}$), surrounded by theca cell, were isolated enzymatically and mechanically from ovarian cortical slides in Leibovitz L-15 medium containing 1 mg/ml collagens and 0.2 mg/ml DNase I and cultured for 25 days in the presence of different concentrations of bovine FSH and LH in α MEM medium with insulin, transferrin, and selenite. The survival was tested by Trypan Blue and Hematoxylin. The survival and growth rates of follicles were higher in FSH treatment groups than these in control ($P < 0.001$), but there were no significant differences between the LH treatment groups and the control. In 25 days, the survival and growth rates of follicles in FSH and LH treatment group (50%, $300 \pm 1.0 \mu\text{m}$) were higher than in FSH treatment group (40%, $244 \pm 0.5 \mu\text{m}$) and the control group (25%, $160 \pm 1.0 \mu\text{m}$). Fifty-five percent of healthy antral follicles were obtained, and 60% of the oocytes complete meiotic maturation to the metaphase II stage. Twenty-two percent of the mature oocytes underwent cleavage, and 9% developed to the blastocyst stage. In this study, *in vitro*-grown oocytes ($111 \pm 1.5 \mu\text{m}$), under our culture conditions, were not equivalent in size to the *in vivo*-grown oocytes ($130 \pm 1.3 \mu\text{m}$). Therefore, these results suggest that bovine preantral follicles with intact theca cell can grow to the antral stage in 25 days, and that oocytes from those follicles can acquire the meiotic competence and normally undergo fertilization and development to the blastocyst stage. However, the developmental capacity of *in vitro*-grown oocytes is presumably not comparable to those of the *in vivo* counterparts

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