P-0133

## Production of Transgenic Granulosa Cells after Retrovirus Vector Injection into Follicle in Mouse

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Recently, production of transgenic animal by nuclear transfer has been known as a useful method. The production of cloned offspring derived from nuclear transfer depends upon a variety of factors such as species, donor cells type and cell cycle, and source of recipient ova. Therefore, we attempted a different transgenic methods using follicular granulosa cells (GCs). In general, ovulated GCs undergoes lutenization and transformation in vitro which might defective effects on developmental potential. In order to avoid the GCs transformation in in vitro culture system, we introduced a direct injection of retrovirus into the follicles and then collected them mechanically from ovaries of 6-8 week-old ICR mice. Retrovirus vector constructed with pLN  $\beta$  EGFP was injected into the follicles. The follicles are cultured in  $\alpha$ -MEM supplemented with human FSH, LH and ITS in Costar Transwell dish for 4 days. Survival rate of virus injected follicles was 52.1% (12/23) and expression rate of EGFP gene was 33.3% (4/12). In this study, we found GCs performed transgenesis in our culture system. In addition, the GCs in follicle may be developed in vivo like environment rather than in vitro environment. Thus, the use of GCs as donor cells may be useful in the nuclear transfer for cloning of genetic modification. Therefore, these results suggest that follicular GCs can be transfected by viral vector during folliculogenesis in vitro.

(Key words) granulosa cells, in vitro culture, retroviral vactor, transgenesis, mouse