세포를 이용한 난소재조합 기술을 확립하고자 수행하였다.

Method: 생후 1일자 생쥐의 난소 조직을 효소 처리하여 난자와 체세포로 분리하고, 분리된 각각의 세포에 FAM labeled siRNA를 transfection하였다. 그 후 transfected ovarian cell은 calcium alginate를 이용해 encapsulation켜서 난소 조직을 다시 형성하도록 한 후 4일 동안 배양하여 조직학적 관찰을 하였다. 특정유전자에 대한 siRNA는 long dsRNA에 RNase III를 처리하여 제작하였고, 유전자의 knock down은 real-time PCR로 확인하였다. 난소는 in vivo control, sham control, TFO (transfected oocyte - somatic cell), TFS (oocyte - transfected somatic cell)로 나누어 비교 분석하였다.

Results: Sham 그룹에서 원시 난포의 형성을 관찰할 수 있었으나 난포의 발달속도가 in vivo control에 비해 다소 지연되는 것을 관찰할 수 있었다. 체세포만을 transfection한 TFS 그룹은 난자들이 sham 그룹보다 커져 있었고, 1차 난포와 흡사한 난포도 관찰할 수 있었으며, 입방형 과립세포도 관찰할 수 있었다. 반면에 난자만을 transfection한 TFO 그룹은 난포의 형성이 현저히 적었고, 형성된 난포의 상태도 매우 불규칙하였다.

Conclusions: 난포발달과정에 관여하는 특정 유전자의 시간적-공간적으로 특이한 기능을 연구하기 위해서 사용할 수 있는 기법으로 ovarian cell에서 RNAi를 통해 목적하는 유전자의 발현을 변화시킨 후, 난소를 재건할 수 있는 기반이 마련되었다는 것에 큰 의미가 있다고 사료된다. 앞으로 이러한 난소재조합 기술이 확립되면 primordial-primary follicle transition의 기작을 연구하는데 유용하게 사용될 것으로 기대한다.

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P-6 Follicular Development of Transplanted Ovarian Tssues in the Mouse Model

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Background & Objectives: The objective of this study was to determine whether ovarian function would be restored following subcutaneous autologous ovarian transplantation in the mouse model.

Method: Four-week old ICR mice (n=7) were ovariectomized at right ovary. These ovarian tissues were autologously transplanted at dorsal subcutaneous site as left ovary was intact. Four mice were stimulated with PMSG and 3 mice were non-stimulated as a control. The ovarian grafts were collected for histological examination around 8 weeks after transplantation. The number of follicles in intact ovary and the transplanted ovarian tissue was counted and examined for morphological appearance in paraffin embedded sections. The PMSG and PMSG-hCG primed oocytes were recovered from the transplanted mice. The

number of oocytes in intact ovary and the transplanted ovarian tissues was counted.

Results: The proportion of antral follicles was lower in transplanted than in control ovary (57/329 (17.3%) versus 63/282 (22.3%)), but there was no significant difference. Especially, the proportion of PMSG primed antral follicles was significantly lower in transplanted than in control ovary (31/190 (16.3%) versus 34/144 (23.6%)). And the rates of primary and secondary follicles were increased after transplantation indicating early follicular growth. The recovered oocytes from PMSG-hCG primed transplanted ovary were higher than only PMSG primed one (35.3% versus 20.0%).

Conclusions: Our results suggest that subcutaneous auto-transplantation of ovarian tissues is feasible in the mouse model. Primordial follicles in subcutaneous ovarian graft retain their developmental potential and can reach antral follicle. In addition, PMSG-hCG primed GV (Germinal vesicle) oocytes in subcutaneous ovarian graft can mature to MII stage. In conclusions, this mouse model would be useful for human clinical trial.

P-7 Analysis of DNA Fragmentation in Bovine Cloned Embryos Activated with Different Activation Protocols

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Background & Objectives: This study was performed to investigate whether different activation protocols affect in vitro development and the incidence of apoptosis in bovine cloned embryos.

Method: Matured bovine oocytes were enucleated and reconstructed by ear fibroblast cells. The fused embryos were chemically activated using 5 μM ionomycin for 5 min followed by 4 hr culture in i) 1.9 mM 6-dimethylaminopurine (6-DMAP), ii) 10 μg/ml cycloheximide (CH) plus 5 μg/ml cytochalasin B (CCB) or iii) 10 μg/ml CH. Activated embryos were cultured at 39℃ in a humidified atmosphere of 5% CO_2 air. Day 7 control or NT blastocysts in each group were stained by TUNEL for the analysis of DNA-fragmented nuclei and with propidium iodide for determination of the total number of cells.

Results: Different activation protocols did not affect the rate of blastocyst formation (6-DMAP = 15.9%, CH plus CCB = 13.5%, CH = 17.0%). Total cell number of cloned blastocysts were significantly higher after activation with 6-DMAP (119.6±31.6, 60~145) or CH (121.9±31.0, 64~160) than that with CH plus CCB (87.8±35.7, 28~140). However, a significant increase in apoptotic index was observed in cloned embryos activated with CH plus CCB (9.7%) compared with DMAP (7.9%) or CH (8.0%) ($p<0.05$).

Conclusions: These results indicated that the CH or 6-DMAP treatment could be efficient method not only to improve developmental rate but also to produce good quality cloned embryo.