by nuclear transfer using somatic cell (bovine fetal fibroblasts)-derived cell lines in HanWoo. As donor nuclei, bovine fetal fibroblast cells, from the fetus approximately 60 to 70 day old, were used. The cell lines have been maintained in culture for at least 6 passages. These fibroblast cells were confirmed normal by karyotyping. Cells were induced into quiescence by serum deprivation for 3-5 days before nuclear transfer. In vitro matured oocytes were enucleated after 18~20 h of maturation, and enucleation was confirmed under UV light after 10 min of incubation with 5 mg/ml Hoechst 33342. The reconstituted embryos were placed into the fusion chamber (3.2 mm gap; BTX 453, USA) in a solution containing 0.26 M mannitol and aligned manually. Two DC pulses (1 s apart) of 1.8 kV/cm for 15 μs were used to fuse the cells. After the electrical pulse, embryos were exposed to 5 μM ionomycin for 4 min, washed with Hepes-buffered TALP medium for 5 min, and cultured in 1.9 mM 6-dimethylaminopurine (6-DMAP) for 3-4 h. Activated embryos were cultured in modified CR1aa (or TALP). On Day 5, embryos were transferred to fresh CR1aa (or TALP) + 10% fetal bovine serum (FBS). On Day 7, embryo development was recorded. Embryos that developed to the blastocyst stages were transferred into recipient cows on Day 6 to 7 of estrous cycle (estrus=0).

The reconstituted embryos were successfully fused (65.4%; 121/185), cleaved (77.7%; 94/121), and developed to blastocysts (23.4%; 22/94). Eleven blastocysts were transferred into 5 recipients. Two recipients were pregnant, confirmed with ultrasonography at Day 50 to 60, and both of them are ongoing their pregnancy. These results show that the cell derived from HanWoo somatic cell lines can be reprogrammed by nuclear transfer and develop subsequently in vivo as well as in vitro.

O-3 The Isolation of IL-1β Induced Genes in Rat Ovary and the Construction of the cDNA Library

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The process of ovulation comprises oocytes maturation, cumulus expansion and follicle rupture triggered by a surge of LH released from the pituitary gland. By the way we have not be able to understand the whole ovulatory process until now. So it is presumed that cytokines especially IL-1β may play an intermediary role in the ovulatory process. Although the relevance of IL-1β to ovulatory process remains uncertain, several lines of evidences support such possibility. The aim of this study is to confirm that IL-1β is an established mediator of ovulation. To do this we have utilized the technique 'Suppression Subtraction Hybridization' which has been developed for the generation of subtracted cDNA libraries. This techniques combines normalization and subtraction in single procedures and used selectively amplify target cDNA fragments (differentially expressed) and simultaneously suppressed non target cDNA amplification. So we performed this procedure and determined the efficiency of the subtraction process. After that the cDNA products were inserted into a plasmid based vector system. Individual colonies were isolated and placed into the

wells of plates and were frozen at -80C and next the individual clones were sequenced. So we got 341 sequencing data which included 75 known genes, 181 homologous genes, 73 novel genes and 12 unclassified genes. And next following the identification of the full length sequences, a homology search will be performed to determine how closely related the whole sequences is to the already cloned StAR gene. and we will try to determine which cell types express the gene.

O-4 The Regulation of LIF Gene Expression by Interleukin-1 in the Mouse Peri-implantation Embryos and Uterine Endometrial Cells

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Cytokines have a critical role in embryo implantation. Leukemia inhibitory factor (LIF) is one of these cytokines and it has been shown to be a pleiotropic molecule involved in different activities in various tissues and cell types. LIF mRNA expression is regulated by interleukin-1 (IL-1) in human synovial fibroblasts, lung fibroblasts, and decidual cells. In this study, the effects of IL-1 on LIF transcription were examined in mouse peri-implantation embryos and endometrial cells. The endometrial cells were prepared at 24, 48, 72, 96 and 120 hour post-hCG, and the culture was performed with Ham's F-10 containing IL-1β (500 pg/ml) or IL-1ra (60 ng/ml). The embryos were cultured from 2-cell to blastocyst in KSOM containing IL-1β or IL-1ra. LIF mRNA was detected in morula and blastocyst, and in the endometrial cells of day 1 and 4 of pregnancy *in vivo* and *in vitro* cultured with medium alone. LIF transcripts were first detected at 8-cell stage and the amount of LIF mRNA was shown a tendency to increase in blastocyst treated with IL-1β. LIF mRNA was detected from day 1 to 5 of pregnancy, and the amount of LIF mRNA was abundant in day 4 of pregnancy than others, in the endometrial cells treated with IL-1β. In contrast, LIF mRNA was reduced in blastocyst and the endometrial cells of day 4 of pregnancy in the case of treatment of IL-1ra. In addition, LIF mRNA was not detected in the endometrial cells of day 1 of pregnancy. These results suggest that LIF mRNA expression is controled by IL-1 in the embryos and endometrial cells at the time of implantation.