B-1 Nuclear Reprogramming in Bovine Oocytes Following Somatic Cell Nuclear Transfer

Shin MR, Park SW, Cui XS and Kim NH

Department of Animal Sciences, Chungbuk National University, Cheong Ju, Chungbuk, Korea

Objective: Limited information is available during nuclear reprogramming in nuclear transferred embryos. In this study we determined DNA synthesis and nuclear remodeling following somatic cell nuclear transfer in bovine oocytes.

Method: Matured bovine oocytes were enucleated by aspirating the frist polar body and metaphase chromatin using a beveled pipette. Bovine fibroblast cells were fused into enucleated oocyte by electrical stimulation. Reconstructed oocytes were activated with ionomycine and 6-dimethylaminopurin, and then were cultured in CR1aa medium. The organization of nuclear and microtubules was observed using laser-scanning confocal microscopy. DNA synthesis was labeled with 5-Bromo-2'-Deoxyuridine.

Results: At 1 hour after fusion, microtubule aster was seen near the transferred nucleus in most bovine oocytes. Most of fibroblast nuclei remodeled to premature chromosome condensation (PCC) and to the two masses of chromosome in reconstructed oocytes. Microtubular spindle was seen around condensed chromosome. Gamma-tubulin was detected in the vicinity of condensed chromosome, suggesting this is a transient spindle. The spindle seperated nucleus into two masses of chromatin which developed to two pronuclear like structures. Two pronuclear like structures were than apposed by microtubular aster and formed one syngamy like nuclear structure at 15 h following nuclear transfer. At 17 to 18 h after fusion, two centrosomes were seen near the nucleus, which nucleates micrtubules for two cell cleavage. DNA synthesis was initially detected in reconstructed bovine oocytes at 3 h after fusion and heavily detected in reconstructed bovine oocytes at 6 h after fusion.

Conclusion: These results suggested introduction of foreign centrosome during nuclear transfer, which appeared to give an important role for somatic cell nuclear reprogramming, and DNA synthesis started in nucleus at 3 h following nuclear transfer.

B-2 Establishment and Maintenance of Embryonic Stem Cell and Embryonic Germ Cell in Human and Mouse

Park JH, Kim SJ, Yoon HS and Roh SI

Infertility Research Center, MizMedi Hospital, Seoul, Korea

Introduction: Embryonic stem (ES) cells and embryonic germ (EG) cells have an ability to remain undifferentiated and proliferate indefinitely *in vitro* while they have a potency to differentiate all three embryonic germ layers. ES cells are originated from inner cell mass of blastocyst, whereas EG cells are originated from primordial germ cells. ES cells and EG cells were established in almost of mammalian

species. In human, three groups report the establishment of ES or EG cells in the world.

Obsective: In this report, we will present basic research about methods for establishment and maintenance of ES and EG cells.

Material and Methods:

1) The methods for the isolation of inner cell mass from blastocyst: immunosurgery and picking the out-growth mass by using micropipette. Also, for the isolation of embryonic germ cells, gonadal ridge and mesenteries of nine weeks old human fetus were mechanically disaggregated.

2) Media condition:

	Human ES cell	Human EG cell	Mouse ES cell
DMEM	High-glucose	High-glucose	High-glucose
FBS	20%	20%	15%
Sodium pyruvate	No	1 mM	No
Glutamine	2 mM	2 mM	2 mM
Nonessenceial amino acid	1%	1%	1%
β-mercaptoethanol	0.1 mM	0.1 mM	0.1 mM
Fibroblast growth factor	4 ng	1 ng	No
Leukemia Inhibitory factor	2000 U	1000 U	1000 U
Forskolin	No	10 μM	No

3) Conformation of established cell line: Immunohistochemcal staining - alkaline phosphatase, SSEA-1, SSEA-3, and SSEA-4

4) Transplantation method: Teratoma formation in nude mice

Results:

1) Maintaining of undifferentiated state proliferation of mouse ES cells dependent on not feeder cells but presence of leukemia inhibitory factor (LIF), whereas feeder cells were indispensable for culture of human ES and EG cells.

2) Cytokines were essential for culture of mouse ES cells, EG cells and human EG cells. Especially, LIF is known as a key factor that induced the proliferation of ES and EG cells. But, exceptionally human ES cell culture does not need LIF.

3) For the culture of EG cell, forskolin, stem cell factor, and basic fibroblast growth factor must be supplemented in culture medium.

Conclusion: Recently, stem cell study was focused on *in vitro* guided-differentiation, for the research of developmental biology in mammals and clinical applications using transplantation therapy. However, it is not clear that how the stem cell proliferate indefinitely, how the stem cell maintain the state of undifferentiation and how the stem cell interact with feeder cells. According to these reasons, the basic study for the maintenance of stem cell is important for the advanced research.