P-15 Parthenogenetic Mouse Embryonic Stem (mES) Cells have Similar Characteristics to in vitro Fertilization mES Cells

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Objective: This study was to compare the characteristics of parthenogenetic mES (P-mES) cells and in vitro fertilization mES cells.

Materials and Methods: Mouse oocytes were recovered from superovulated 4wks hybrid F1 (C57BL/6xCBA/N) female mice. The oocytes were treated with 7% ethanol for 5 min and 5 μg/ml cytochalasin-B for 4 h. For IVF, the oocytes were inseminated with epididymal sperm of hybrid F1 male mice (1×10^6/ml). IVF and parthenogenetic embryos were cultured in M16 medium for 4 days. Cell number count in blastocysts was carried out differential labelling using propidium iodide (red) and bisbenzimide (blue). To establish mES cells, blastocysts in IVF and parthenogenetic groups were treated immunosurgery and recovered ICMs were cultured in LIF added DMEM culture medium. To identify mES cells, the surface markers alkaline phosphatase, SSEA1, 3, 4 and Oct4 staining in replated ICM colonies were examined. Also, the number of chromosome was checked in P-mES and mES.

Results: In vitro development rates were blastocysts derived from parthenogenetic group (14.5%) lower than IVF group (68.0%). And, cell numbers of ICM of parthenogenetic blastocysts (12.1) were lower than those of IVF blastocysts (23.0). Three ICM colony recovered from parthenogenetic 9 blastocysts and 1 ICM colony recovered from IVF 26 blastocysts were sub-cultured, continuously replated during 20 passage and 11 passage culture duration without differentiation. Using surface markers staining, alkaline phosphatase, SSEA1, 3, 4 and Oct4 in P-mES and mES colony were examined, Sub-cultured two groups colonies were strong positively stained by alkaline phosphatase. and SSEA1 staining, and negatively stained by SSEA3, 4 staining. Also, the number of chromosome was normal in ES colony from two groups.

Conclusion: This study suggested that P-mES cell can be successfully established and that those cell lines have similar characteristics to IVF mES cells.

P-16 In vitro Neural Cell Differentiation Derived from Human Embryonic Stem Cells: II. Generation of Specific Neurons from Neural Progentor Cells Treated with BDNF and PDGF

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Objective: This study was to investigate generation of the specific neural cell in vitro from the neurosphere derived from human embryonic stem (hES, MB03) cells.

Materials and Methods: For neural progenitor cell formation derived from hES cells, we produced

embryo bodies (EB: for 5 days, without mitogen) from hES cells and then neurospheres (for 7~10 days, 20 ng/ml of bFGF added N2 medium) from EB. And then for the differentiation into neuronal cells, neurospheres were cultured in N2 medium (without bFGF), supplemented with brain derived neurotrophic factor (BDNF, 5 ng/ml) or platelet derived growth factor (PDGF, 20 ng/ml) for 1 or 2 weeks. Specific neuronal cell differentiation was identified with immunocytochemistry using glutamate (1:1000; Sigma), enzyme tyrosine hydroxylase (1:1000; Sigma), serotonin (1:1000; Sigma) and GABA (1:1000; Sigma) antibodies.

Results: In the presence of BDNF or PDGF, most of neural cells derived from hES cells were differentiated into glutamatic and GABA neuron in vitro. But, we confirmed that there are a few serotonin and tyrosine hydroxylase positive neuron in the same culture environment.

Conclusion: This result suggested that most of neural cells derived from hES cells were in vitro differentiated into glutamatic and GABA neuron in the presence of BDNF or PDGF.

P-17 In vitro Development of Somatic Cell Nuclear Transferred Bovine Embryos Following Vitrification-thawing of Enucleated Oocytes at Matured (MII) Stages

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Objective: This study was to evaluate the in vitro survival of vitrified-thawed bovine enucleated MII (eMII) oocytes by minimun volume cooling (MVC) method and their in vitro development after somatic cell nuclear transfer (SONT).

Materials and Methods: Bovine oocytes were recovered from slaughtered bovine ovary and matured in TCM-199 supplemented with 10% FBS. After incubation for 20 h in IVM medium, recipient oocytes were stained using 5 μg/ml Hoechst and their 1st polar body and MII plate were removed by enucleation micropipette under UV filter. eMII oocytes were subjected to activation before (pre-activation group) or after (post-activation group) vitrification in 5 μM ionomycine added CR1aa medium for 5 min. For vitrification, eMII oocytes were pretreated in EG10 for 5 min, exposed in EG30 for 30 sec. Thawing was taken by 4-step procedures [1.0 M sucrose (MS), 0.5 MS, 0.25 MS, and 0.125 MS added PBS, for 1 min per each step] at 37℃. Survived eMII oocytes were subjected to nuclear transfer with cultured adult bovine ear cells. Reconstrusted oocytes were cultured in 10 μg/ml of cycloheximide and 2.5 μg/ml of cytochalasin D added CR1aa medium for 1 h, and then in 10 μg/ml of cycloheximide added CR1aa medium for 4 h. Subsequently, the reconstructed oocytes were washed three times and incubated in CRIaa medium for 48 h. The cleaved embryos were then selected and further cultured on cumulus-cell monolayer drop in CRIaa supplemented with 10% FBS for 7 days.

Results: Survival rates of bovine vitrified-thawed eMII oocytes in pre-activation and post-activation groups were 88% and 80%, respectively. Nuclear transferred eMII oocytes in post-ativation group indicated