agonist or GnRH antagonist. Production of estradiol and progesterone during culture was determined by EIA. Apoptosis of cultured granulosa cells was examined by DNA laddering.

Results: While estradiol production was significantly decreased in only 100 ng/ml of GnRH agonist treatment group compared to control group, progesterone production was significantly decreased in both 10 ng/ml and 100 ng/ml of GnRH agonist groups. However, there was no significant difference in estradiol and progesterone production between the GnRH antagonist groups and control group. Apoptotic DNA laddering was increased by GnRH agonist, but not by GnRH antagonist.

Conclusions: The results may indicate that the steroidogenesis is more impaired in GnRH agonist treatment than that in GnRH antagonist treatment. GnRH agonist may more induce apoptosis than GnRH antagonist in rat granulosa cells.

O-4 Effects of Activation Timing on the Fertilization and in-vitro Development of Porcine Round Spermatid Injectied (ROSI) Embryos

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Background & Objectives: During fertilization, the nuclear envelop breakdown (NEBD) of the male gamete within the oocyte cytoplasm is prerequisite for the decondensation of the male gamete nucleus and subsequently for male pronuclear (PN) formation. In ROSI procedure, but, the cytoplasm of round spermatid impairs or delays the exposure of the round spermatid nuclear membrane to the oocyte cytoplasmic factors that induce NEBD. In addition, the capability of mammalian oocyte to induce NEBD of male gamete disappears within a few hours after oocyte activation. So, prolonged exposure of a round spermatid to a non-activated oocyte till NEBD and oocyte activation at this time of NEBD may be more efficient for successful fertilization and early development of ROSI embryos. On the other hand, if round spermatid nuclei were left in non-activated oocyte for an extended period of time, normal fertilization might be failed due to premature chromosome condensation (PCC) of spermatid chromosomes. Therefore, this study was undertaken to evaluate the optimal exposure time for peak NEBD rate after injection and to investigate the effect of oocyte activation timing on the PN formation and in-vitro development of porcine.

Method: ROSI embryos. The round spermatids were injected into matured oocytes at 40~44 h of in-vitro maturation. Injected eggs were fixed at 0.5, 1, 2, 3, 4 h before activation, and NEBD state was examined. The three groups of oocytes were activated before and after injection of spermatid using single direct current pulse (100V/mm, 50 μsec): group 1) at 2 h before injection (pre-AC), group 2) within 0.5 h after injection (immediate-AC), group 3) at 2 hr after injection (post-AC). Activated eggs were cultured in NCSU-23 + 4 mg/ml BSA for 7~8 days for blastocyst development. And, during in-vitro culture, PN formation was evaluated at 15~18 h after injection.

Results: The proportion of oocytes with NEBD significantly increased in groups with over 2 hours exposure time ($p<0.05$). The proportion of eggs with PCC rate at 4 hours increased significantly compared to that others (25.0% vs 0~13.3%) post injection ($p<0.05$). Normal fertilization and development rate to the blastocyst stage were significantly higher in post (61.8% and 19.7%) activation group compared to that of pre (32.4% and 10.9%)or immediate (39.4% and 9.1%) activation groups ($p<0.05$).

Conclusions: The optimal exposure time of peak NEBD rate is 2~3 h after injection. And, activation in 2~3 hours after round spermatid injection improved the normal fertilization and early embryo development rate.

O-5 Effect of the Some Neural Inducing Factors on Neural Differentiation of Human Embryonic Stem Cells

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Background & Objectives: Embryonic stem cell has pluripotent and self-renewal characteristics, so it is expected that used for clinical application as a source of a cell therapy for variable degenerative diseases. Because ES cells have unlimited proliferation capacity, neural progenitor or terminally differentiated neural cells derived from ES cells might be used for alternative neural cell source. In this study, we test some inducing factors for finding of optimal condition of neural differentiation of human ES cells in vitro.

Method: Human ES cell lines, SNUhES-1, SNUhES-2, SNUhES-3, were used for neural differentiation. Undifferentiated ES cells were cultured on a feeder layer of mitomycin-C treated mouse embryonic fibroblasts. ES cell colonies were mechanically separated to proper size containing about 500~800 cells at 5 days after the transfer. These dissociated colonies were transferred into bacterialogical culture dish containing ES cell culture medium without bFGF for embryoid body (EB) formation. After the 4-day culture, EBs were cultured for 8~9 days on gelatin and laminine coated culture dishes or cover slips in EB media containing N2 supplements, 20% SR or FBS, with or without the differentiation factors, bFGF, LIF, RA, β-NGF, for neural progenitor cell enrichment. Whole or some parts of nestin-enriched EB forming the neural progenitor network were separated mechanically and cultured for one day in suspension condition to form the neurosphere-like structure. They were transferred onto fibronectin coated culture dishes and cultured with additional differentiation factors, RA, β-NGF, EGF, PDGF, each during 2~3 weeks. Nestin enriched EBs and further differentiated cells from human ES Cells were confirmed by flowcytometry, RT-PCR and Immunofluorescence using specific Abs against variable markers of neural progenitor (nestin), early or mature neuron (N-CAM, neurofilament, betaIII tubulin), glial cell (GFAP) and Fluorescence conjugated secondary Abs.

Results: ES cells derived neural progenitors expressed the nestin were more increased by bFGF and LIF addition in EB media containing N2 supplements than control culture condition. Nestin mRNA expressions were increased time dependently but after the 8-day culture, they were decreased rapidly. After the neural