P-28 Expression of Ids and OUT mRNA in the Preimplantation Mouse Embryos and Uterus

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The helix-loop-helix (HLH) protein class of transcription factors are important regulators of cellular proliferation and differentiation in a number of cell types. The Id proteins are a family of related mammalian HLH protein, which are hypothesized to act negative regulator of DNA-binding bHLH proteins. We investigated into existence of Ids and OUT mRNA in the preimplantation mouse oocytes, embryos and uterus, uterus about estrus cycle.

Id 1, 2 & OUT just existed in the blastocyst stage embryo, and Id 4 not existed in eggs. Id 3 expressed in the GV stage oocyte, after 4-cell stage embryos. Expression of Ids and OUT mRNA in the reproductive cycles uterus analyzed that Id 1 entirely expressed in the reproductive cycle uterus, and Id 3 & OUT expressed in the other cycles uterus except the estrus cycle, and Id 4 slightly expressed in the others cycle except the diestrus cycle, but Id 2 only expressed in the metestrus cycle. Compared to the mouse implantation site and inter-implantation site, Id 1, 3 dramatic expressed, these genes increased each 1.79 fold, 1.75 fold in the implantation site. Id 2 was variable expressed in the samples. These results suggest that Id and OUT may act functional regulator in the preimplantation mouse embryos and uterus and Ids and OUT may influence by sex hormone.

P-29 Bovine Eggs Fertilized using Male Haploid Somatic Cell Derived from Sequential Nuclear Transfer without Sperm

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Objective: In this study, we evaluated whether bovine eggs reconstructed using male haploid somatic cell derived from sequential nuclear transfer without sperm can be normally developed *in vitro*.

Materials and Methods: Bovine GV oocytes were recovered from slaughtered bovine ovary and matured in TCM-199 supplemented with 10% FBS. Twenty-two hours after IVM, recipient oocytes were stained using 5 μg/ml Hoechst and their 1st polar body (PB) and MII plate were removed by enucleation micropipette under UV filter. Then, G0/G1 stage bovine male ear skin cells were introduced into enucleated recipient oocytes. Reconstructed eggs were fused to enucleated oocytes by electric pulse and then chemically activated. Eighteen hours after activation, each nucleus of the constituted eggs containing 2 sets of chromosomes from somatic cells was again direct injected into normal MII oocytes. Reconstructed eggs

were activated by chemical activation method. And morphological characteristics of developed eggs were observed under phase-contrast microscope.

Results: In the results, 70 (42.2%) of 166 donor cell and recipient oocytes units were fused. After the first of the nuclear transfer, 10 (18.9%) of 53 recovered embryos developed to 2 pronuclei with a haploid karyotype after activation. After the sequential nucleus transfer of 20 haploid pronuclei, 5 of 20 eggs were recovered, and 2 eggs with 2 sets of chromosomes were normally extruded 2nd PB.

Conclusions: This result suggested that bovine eggs reconstructed using male haploid somatic cell derived from sequential nuclear transfer can be fertilized without sperm and that the advantage of this technique is for men who can not produce sperm in human IVF-ET program.

P-30 녹용 (Cervi Pantotrichum Cornu) 추출물이 인간 정자 운동성에 미치는 영향

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This study was carried out to determine the effect of extract of Cervi pantotrichum cornu on human sperm motility. Four different types of media were prepared such as plain Ham's F-10 medium (control medium), control medium containing 0.3% bovine serum albumin (medium A), control medium containing the extract of Cervi pantotrichum cornu aqua-acupuncture medium (medium B) and medium B containing 0.3% bovine serum albumin (medium C). Human semen were washed and divided into 4 fractions and sperm were cultured in those medium for up to 72 hours at 37℃ in a humidified atmosphere of 5% CO_2 in air. A total twenty eight semen samples including 14 normospermia and 14 asthenospermia were used for this study. In normospermia group, motility of control medium and medium A, B and C were 4.1%, 1.3%, 64.5% and 77.1%, respectively after 24 hours of incubation, and were 0.0%, 0.0%, 8.8% and 44.9%, respectively after 48 hours of incubation. In asthenospermia group, motility of control medium and medium A, B and C were 2.0%, 2.2%, 58.3% and 85.1%, respectively after 24 hours of incubation, and decreased to 0.0%, 0.2%, 5.8% and 29.6%, respectively after 48 hours of incubation. In both groups, highest sperm motility was observed in medium C group compared with other media. Furthermore motile sperm were found in medium C after 72 hours of incubation while no motile sperm was observed in the other media. Therefore it could be concluded that the extract of Cervi pantotrichum cornu affects on the human sperm motility.