## P21 Directed differentiation of human embryonic stem cells to pancreatic endoderm

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Objectives: Recent studies demonstrate the successful differentiation into various cell types from human embryonic stem cells (hESCs), but few reports have shown the potential of hESCs to differentiate into pancreatic lineages. In this study, we examine if hESCs can be efficiently directed into pancreatic endoderm that gives rise to pancreatic cell type expressing insulin, together with C-peptide and proinsulin.

Materials and Methods: To direct differentiation into pancreatic endodermal fates, human EBs (hEBs) were cultured in the presence of 20% fetal bovine serum for the first 4 days and the serum-treated hEBs were then treated with 10 ~ 100 ng/ml activin A and/or 10 μM retinoic acid in serum-free conditions for the days indicated. RT-PCR and immunostaining analysis were performed to analyze gene and protein expressions, associated with each germ layer, pancreatic progenitors and insulin-producing cells.

Results: Sequential treatment of Serum/Activin/RA enhances the population of pancreatic endoderm during EB formation. Almost all of the $Pdx1^+$ cells derived from hESCs at the end of EB formation coexpress Nestin, but afterward these cells become heterogeneous and eventually only the cells expressing both Pdx1 and Nestin are involved in the formation of islet-like aggregates. Upon transplantation, the pancreatic endoderm derived from hESCs gives rise to the cell type that expresses insulin, together with C-peptide and proinsulin.

Conclusions: Our results suggest that the pancreatic endoderm can be derived from hESCs in defined culture conditions, corresponding to fundamental concepts of pancreatic early development, and provide additional evidence of the Nestin expression in human pancreatic progenitors. These findings are expected to facilitate further investigations for purification of transplantable islet progenitors and generation of high amount of mature insulin-producing cells *in vitro*.

**Key words:** human embryonic stem cells, endoderm, pancreas, Pdx1, differentiation

## P22 A comparison of frozen-thawed human sperm viability, motility index and morphology with slow and vitrification

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Objectives: This study was to investigate the effect of cryopreservation by slow and vitrification methods on the sperm viability, motility and morphology of frozen-thawed human sperm.

Materials and Methods : Semen specimens were obtained from men who presented for semen analysis. Whole ejaculated semen was centrifuged at 300 × g for 5min and the diluted in a test yolk buffer(TYB, Irvine Scientific, USA). Diluted semen shared 2 cryo vial(Cryogenic vial, Corning, Canada). One cryo vial was cooled from 25℃ to 4℃ by ramp rate of −0.5℃. After a hold time of 30 seconds at 4℃, gradually reduce the temperature to −80℃ at a rate of −10℃/min. After 10 minutes of temperature equilibration, transfer the cryo vial into liquid nitrogen tank and store until thawing.

The other cryo vial was suspend 5Cm above liquid nitrogen horizontally on metal platform in uncirculated liquid nitrogen vapor for 10 minutes. After freezing plunge into liquid nitrogen tank.

Remove the cryo vial from liquid nitrogen, hold at room temperature for 30 seconds, and put into a 37℃ water bath for 5 minutes.

Sperm stained by eosin-nigrosin and Diff-Quik method were observed under the inverted microscope. Sperm motility and morphology by sperm analysis imaging system(SAIS, Medical supply Co, Korea).

Results : After rapid freezing and thawing, sperm viability was lower (60 ± 2.2%) than slow freezing method (62 ± 2.1%) and sperm morphology was higher (46 ± 7.7%) than that (44 ± 8.3). But there was no significantly. Sperm motility index was significantly higher(MOT: 47.40 ± 20.06%, VCL: 38.12 ± 15.58 ㎛/s, VSL: 28.19 ± 14.10 ㎛/s, VAP: 33.64 ± 15.15 ㎛/s and HYP: 2.77 ± 2.71%) than slow freezing and thawing (MOT: 43.39 ± 18.79%, VCL:33.91 ± 13.50 ㎛/s, VSL: 19.98 ± 10.88 VAP: 24.60 ± 11.72 ㎛/s and HYP: 1.33 ± 1.57%; $P<0.05$).

On the other hand, significant difference were not observed MAD, WOB, DNC and DNM by slow and vitrification.

Conclusions : Vitrification method is a useful method of preserving human sperm and has the advantage of time effectiveness, simplicity, cost reduction, and no need for devices such as programmable freezers.

**Key words:** cryopreservation, vitrification, viability, morphology, sperm