

**P-8**      **Enhancement of Re-closure Capacity by the Intra-amniotic Injection of Human Embryonic Stem Cells in Surgically Induced Spinal Open Neural Tube Defects in Chick Embryos**

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**Background & Objectives:** To evaluate the potential of the stem cell therapy as a method for prenatal management of spinal open neural tube defect (ONTD), the influence of embryonic stem cells injected into the amniotic cavity on the re-closure capacity of spinal ONTD was investigated.

**Method:** Spinal neural tube was incised open for a length of 6 somites using chick embryos of Hamburger and Hamilton stage 18 or 19. Embryos were divided into three groups: control group (no injection), vehicle group (injection of glucose in PBS), human embryonic stem (hES) cell group (injection of 20,000 hES cells with green fluorescence protein (GFP) in vehicle).

**Results:** On 3, 5, and 7 days after neural tube incision and immediate intra-amniotic injection, ONTDs were significantly more re-closed in the hES cell group than in the control and vehicle groups. On light and fluorescence microscopic examinations, hES cells were not found in the re-closed area but were present at the area on the process of re-closure, covering ONTDs.

**Conclusions:** Intra-amniotic injection of hES cells enhances re-closure capacity of surgically induced ONTDs in chick embryos. The hES cells do not incorporate themselves into the neural tube but do help re-closure.

**P-9**      **Calving Production from Hanwoo (Korean Cattle) IVM/IVF/IVC Blastocysts: Direct Transfer of Vitrified and Quick One-Step Diluted Hanwoo Blastocysts**

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**Background & Objectives:** This study was to examine whether vitrified Hanwoo (Korean cattle) IVM/IVF/IVC blastocysts can survive in vitro/in vivo by a quick one-step dilution method and these embryos result in live births.

**Method:** Blastocysts produced in vitro were vitrified by serial exposure to glycerol (G) and/or ethylene glycol (EG) mixtures of 10% (v/v) G for 5 min, 10% G plus 20% EG (v/v) for 5 min, and 25% G plus 25%

EG (v/v) for 30 sec. The blastocysts were then loaded in straw, placed in cold nitrogen vapor for 3 min and plunged into LN2 (-196). One-step dilution within the straw was done in 25 and/or 36 water baths for different times (from 1 min to 3.5 min).

**Results:** In vitro survival of vitrified embryos was 76.9~93.5% after the one-step dilution. However, 48 h after thawing, the in vitro development rates in the beyond hatching or hatched state in 1 min dilution group (the quick one-step dilution method; 73.3 and 56.7%, respectively) were better than those of the other treatment groups (55.6~61.3% and 8.1~36.1%, respectively). Direct transfer of quick one-step diluted embryos into recipient cows resulted in an overall pregnancy incidence of 33.3% (12/36). The good pregnancy incidence was obtained when the recipients estrus cycle was one day earlier than the age of the transferred embryos (53.3 vs. 25.0~27.3%), irrespective of synchronization methods or condition of the corpus luteum. We obtained nine offspring from 12 pregnant cows.

**Conclusions:** Therefore, quick one-step dilution and direct transfer of vitrified bovine IVM/IVF/IVC blastocysts could be applied as an efficient method for the field trials.

## P-10                    중대 뇌동맥 폐색 뇌졸중 (Focal Ischemia) 동물 모델 쥐에 대한 인간 배아줄기세포 이식 효과

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**Background & Objectives:** 혈관 폐색에 의한 인지 및 기억장애 동물모델에서 뇌졸중 치료제로써 인간배아줄기 세포의 신경세포 보호효과 및 기억력증진에 미치는 효과를 조사하고자 실시하였다.

**Method:** 중대 뇌동맥 폐색 (MCA)에 의한 쥐의 동물모델은 Sprague Dawley계 흰 쥐 (260~300 g)의 국소 중대뇌동맥을 일시적으로 폐색시켜 만들었다. 이 연구 (미국 국립보건원에 등록된 MB03세포)에 사용된 인간배아줄기세포는  $3 \times 10^4$  cells/cm<sup>2</sup> 밀도의 배양접시 내에서 4일 동안 embryoid bodies (EBs)의 형성을 유도하고 추가적으로 4일 동안 RA ( $10^{-6}$  M, sigma)에 노출하였다. 분리된 세포들은 0.1% gelatin coated dish에 평판 배양하고 14일 동안 N2 배양액에서 분화시켰다. 줄기세포이식은 입체정위 수술장치를 이용하여 각 실험군의 쥐 뇌의 내측 중격위치에 주입하였다. 이렇게 만들어진 실험군은 수중미로학습, 방사성 미로학습을 이용하여 인간배아줄기세포의 인지 및 기억증진의 기억장애의 기능회복의 효능을 검토하였다.

**Results:** 수중 미로 학습의 획득시행에서 중풍 유발군이 모의 시술군에 비해 도피대에 도달하는데 소요되는 시간이 유의하게 증가하였으며, 인간배아줄기세포 처치가 학습획득수행에 현저한 증진효과를 보여주었다. 수중 미로 학습의 검사시행에서 중풍 유발군이 파지검사에서 모의 시술군에 비해 도피대를 제거하고 도피대 영역에 머무르는 정도가 증가하였으며 공간기억력에 대한 증진효과를 보였다. 또 각 군의 조직을 UV로 관찰한 결과 신경세포의 생성이 증가되었음을 보여주었다.

**Conclusions:** 따라서, 본 연구는 신경세포로 분화된 인간배아줄기세포 이식은 혈관 폐색 동물모델에서의 인지 및 기억력 개선 치료에 이용될 수 있음을 나타낸다.