P-16 Pregnancy of Somatic Cell Nuclear Transferred Hanwoo (Korean Cattle) Blastocysts Vitrified by Minimum Volume Cooling Method

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Objective: This study was to test whether the Hanwoo blastocysts derived from nuclear transfer (NT) with Hanwoo adult ear cell can be successfully cryopreserved by minimum volume cooling (MVC) method.

Materials and Methods: For the vitrification, day 7 or 8 NT blastocysts were pretreatd in 10% ethylene glycol for 5~10 min, exposed in EG30 for 30 sec, each embryo was individually put on the inner wall of 0.25 ml straw, and then straw was directly plunged into LN_2. Thawing was taken by 4-step procedure (1.0 M sucrose (MS), 0.5 MS, 0.25 MS, and 0.125 MS) at 37℃. Survived NT embryos were transferred into uterus of Hanwoo recipients.

Results: After thawing, in vitro survival rate (88.9%) of NT group was not significantly different with that of control group (85.4%). After transfer of survived embryos (10, 2/each) in NT group, 4 of 5 total recipients did not return to the subsequent estrus cycle at 30 days, and pregnancy was monitored by ultrasound sonography. One recipient cow is ongoing pregnancy on 6 month.

Conclusions: It is concluded that the somatic cell NT Hanwoo blastocysts can be successfully cryopreserved by simple and efficient MVC method and that these embryos have in vivo developmental potentiality.

P-17 The Expression of Peptidyglycine-amidating Monooxygenase (PAM) in the Placenta of Pregnant Women

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Objective: High levels of oxytocin mRNA expression have been detected in human uterine tissue. Oxytocin precursors are present in much higher concentrations in the uterus than oxytocin itself. This implies that the enzymes necessary for conversion of oxytocin precursors to oxytocin might be missing or not active. The last step in the synthesis of oxytocin is the alpha amidation of the terminal glycine via the PAM. PAM is an enzyme needed for the synthesis of many peptides. The placenta produces many peptides that require amidation, including oxytocin. The purpose of the present study was to determine the presence of PAM in the human placenta, its variation with gestational age and cellular location within the placenta.

Materials and Methods: Tissues from full-term vaginal delivery (n=7) and preterm cesarean delivery (n=5) were obtained. Tissues were either frozen on dry ice or fixed in 10% formalin, paraffin embedded and sectioned. The frozen tissue was homogenized, extracted and Western blotted. A polyclonal antibody to recombinant PAM (Unigene Inc.) was used to probe for PAM both on the Western blots and for ICC (Immunocytochemistry) using the Vectastain ABC method. The ICC slides were blinded and reviewed by two examiners who scored the intensity of staining on a scale of 1 (no stain) to 5 (highest intensity). Western blots were quantified by densitometry. ICC data were analyzed by nonparametrics analysis while Western blots were compared by non-paired *t*-test.

Results: A marked decrease in an immunoband of PAM was detected by Western blot at full-term vaginal delivery compared to preterm cesarean delivery (1.980.75 vs 4.590.34, $p<0.05$). In addition, the staining for PAM was located primarily in the region of the cytotrophoblast.

Conclusions: Three main results may be drawn from this study: (1) PAM is detected in the placenta; (2) it decreased at the time of delivery; and (3) it is located primarily in the cytotrophoblast. In conclusion, the decrease in PAM suggests that certain amidated peptides are diminished at the end of pregnancy in the placenta. The diminished peptide synthesis may contribute to events important in the delivery mechanism.

P-18 연령 및 회수되는 난자의 수가 임신, 유산, 및 다태임신에 미치는 영향

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목 적: 시험관 아기시술에 있어서 환자를 연령 및 회수되는 난자의 수에 따라 10개 미만과 10개 이상의 두 그룹으로 분류하여 임신율, 다태임신율 및 유산율을 조사함으로서 어느 군에서 이식되는 난자의 수를 줄일 수 있는지 알아보고자 조사하였다.

대상 및 방법: 2001년 4월부터 동년 8월까지 프라우메디 병원 불임클리닉을 방문하여 체외수정 시술을 받은 94 cycle을 대상으로 하였다. 환자를 연령 및 회수되는 난자의 수에 따라 임신율 다태임신율, 유산율을 조사하였다. 한편, 수정란의 이식은 난자 회수 후 3일 오후에 실시하였으며 이식되는 배아의 수는 5개 이하로 제한하였다.

결 과: 연령에 따른 임신율, 다태임신율 및 유산율은 30세 이하에서 임신율은 15/24 (62.5%)였으며, 이중 다태임신은 7/15 (46.7%), 유산은 4/15 (26.7%)였다. 31~35세에서 임신율은 15/35 (42.9%)였으며 이중 다태임신은 5/15 (33.3%), 유산은 2/15 (13.3%)였다. 36~40세에서 임신율은 13/27 (48.2%) 이었으며, 이중 다태임신은 2/13 (15.38%), 유산은 3/13 (23.08%) 이었다. 41세 이상에서 임신율은 5/8 (62.5%) 이었으나, 다태임신은 없었으며 유산은 4/5 (80.0%)로 높게 나타났다.

또한 연령에 관계없이 회수되는 난자의 수가 10개 미만인 경우와 10개 이상인 경우로 분류해 볼 경우 임신율은 각각 23/50 (46.0%), 23/44 (52.27%)였으며, 다태임신율은 각각 1/23 (4.35%), 11/23 (47.87%) 이었으며, 유산율은 각각 9/23 (39.1%), 1/23 (4.35%)으로 나타나 회수되는 난자의 수가 10개 이상의 군에서는 유산율이 유의하게 감소한 반면, 다태임신율이 유의하게 증가되는 경향을 나타내었다.

한편, 30세 이하이고 회수되는 난자의 수가 10개 미만인 경우 임신율은 3/8 (37.5%), 다태임신은 없