

질소에 침지하여 동결보관을 실시하였다. 용해는 PBS + 20% hFF + 0.5M sucrose, PBS + 20% hFF + 0.25M sucrose에 각각 5분간 침지하였다가 G2.2에 6시간 동안 추가배양을 실시한 다음 Re-expansion 유무를 확인하고 이식하였다. 배아이식을 위한 환자의 준비는 생리가 규칙적인 경우 luteal support를 하지 않고 배란 확인 후 5일째에 배아이식을 하였고, 불규칙적인 여성의 경우 Estradiol valerate를 경구 투여하여 자궁내막을 준비하고 luteal support는 progesterone in oil (50 mg/day)을 투여하거나 gel type의 progesterone (90 mg)을 질강내에 투여하여 5일째에 배아이식을 시행하였다.

**Results:** 총 21 case에서 배아생존율은 89.9% (71/79)였으며, 이중 Re-expansion rate는 69.0% (49/71), case당 이식되는 난자의 수는 2.9 (61/21)개, 착상율은 29.5% (8/61), Fertilization rate는 61.9% (13/21)이었으며, 이중 2 case에서 유산되어 ongoing rate는 84.61% (11/13)이었다. Case 별로 2~4개의 배아가 이식되었으며, 2 case에서 3태가 관찰되었다.

**Conclusions:** 이상의 결과로 미루어볼 때 임신률과 착상율 및 ongoing rate는 일반적인 시험관아기시술에 비해 떨어지지 않으며, 본 연구에 공시된 환자들이 시험관아기시술에서 이미 1~2회의 실패한 경험을 가진 환자임을 고려해볼 때 배아의 효율적 이용뿐만 아니라 환자들의 경비절감 및 정신적 부담을 덜어주는 데 크게 기여할 것으로 사료되어진다.

## P-16 Improved Hatching of Blastocyst in Korean Native Cattle after Addition of Hypoxanthine, Aminopterin and Thymidine (HAT) in Media at Day 5

JC Han<sup>1,2</sup>, HS Lee<sup>2</sup>, HD Park<sup>1</sup>

<sup>1</sup>Department of Biotechnology, Daegu University, Kyoungbuk, <sup>2</sup>ELLE OB/GYN, Daegu, Korea

**Objective:** The blastocyst stage has been associated with exceptionally high implantation rates. Good quality blastocyst is a fundamental requirement for the accurate implantation. The hatching of blastocyst of the zona pellucida for the accurate implantation is an essential step, which has to occur prior to uterine implantation. The aim of this study was to evaluate the effect of HAT on hatching of blastocyst in Korean native cattle.

**Materials and methods:** Cumulus oocyte complexes were matured in TCM-199 with pyruvate 0.2 mM, FSH 0.01 IU/ml and 10% FBS for 24 hr. Matured oocytes (not observed) were inseminated with frozen-thawed sperm in Fer-TALP supplemented with BSA 6 mg/ml for 24hr. Fertilized oocytes (not observed) were cultured in YS medium (Heo et al., 1996) with NEAA 10 ml/l and 20 % hFF for 24 hr. Two cell embryos were cultured to blastocyst stage in YS medium with NEAA 10 ml/l, EAA 5 ml/l, 20% hFF and 10% FBS (IVC medium) for 24 hr. On day 5, 369 embryos were divided among one of five media: i) control (IVC medium); ii) H (IVC medium with H 400  $\mu$ M); iii) A (IVC medium with A 1.6  $\mu$ M); iv) T (IVC medium with T 64  $\mu$ M) and v) HAT (IVC medium with HAT). Results (BL, HBL and HBL/BL) were compared between the groups using Chi-square.

**Results:** The BL and HBL rates were higher ( $p < 0.05$ ) in control (43.8%, 15.6%) and HAT (33.3%, 24.0%) than in H (14.7%, 2.7%), A (15.1%, 3.8%) and T (14.3%, 0%). The rate of HBL per BL was

significantly higher ( $p < 0.05$ ) in HAT (71.9%) than that in any other groups (0~35.7%).

Groups	No. of used Embryos	BL (%) <sup>*</sup>	HBL (%) <sup>**</sup>	HBL/BL (%)
Control	96	42 (43.8) <sup>a</sup>	15 (15.6) <sup>a</sup>	15/42 (35.7) <sup>a</sup>
H	75	11 (14.7) <sup>b</sup>	2 ( 2.7) <sup>b</sup>	2/11 (18.2) <sup>a</sup>
A	53	8 (15.1) <sup>b</sup>	2 ( 3.8) <sup>b</sup>	2/8 (25.0) <sup>a</sup>
T	49	7 (14.3) <sup>b</sup>	0 ( 0.0) <sup>b</sup>	0/7 ( 0.0) <sup>a</sup>
HAT	96	32 (33.3) <sup>a</sup>	23 (24.0) <sup>a</sup>	23/32 (71.9) <sup>b</sup>

Values within the same column with different superscripts differ significantly ( $p < 0.05$ ).  
<sup>\*</sup>: blastocyst, <sup>\*\*</sup>: hatched blastocyst

**Conclusions:** These results suggested that HAT (added on day 5) increase the hatching of bovine blastocyst. We are still studying the effects of different addition time and concentration of HAT on embryonic developmental capacity in Korean native cattle.

## P-17 Genetic Screening for Male Factor Infertility and Transmission of Y Chromosome Microdeletion

HS Lee, HW Choi<sup>1</sup>, MH Moon<sup>1</sup>, YS Park<sup>1</sup>, YS Lee<sup>2</sup>, JT Seo<sup>2</sup>,  
 JY Kim<sup>3</sup>, MK Koong<sup>3</sup>, JH Jun<sup>1</sup>

*Laboratory of Reproductive Biology and Infertility, Samsung Cheil Hospital<sup>1</sup>,  
 Department of Urology<sup>2</sup>, Department of Ob/Gyn<sup>3</sup>, Sungkyunkwan University  
 School of Medicine, Seoul, Korea*

**Background & Objectives:** The genetic screening of microdeletions of the Y chromosome is a useful diagnostic analysis for the work-up of male factor infertility. We evaluated the relationship between the type of Y chromosome microdeletion and clinical phenotypes of male factor infertility. The transmission of Y chromosome microdeletion from a severe oligozoospermic father to his offsprings through intracytoplasmic sperm injection (ICSI) was examined.

**Method:** During 1997-2003, genomic DNA was extracted from peripheral blood of 725 men with idiopathic azoospermia or oligozoospermia, attending the Infertility Clinic at Samsung Cheil Hospital. The azoospermia factor (AZF) regions, AZFa, b, c, and Sry on Y chromosome were amplified using a polymerase chain reaction (PCR). And we analyzed the DAZL mutation (T54A) on chromosome 3 in 290 samples. To test the transmission of Y chromosome microdeletion, genomic DNA was extracted and analyzed from twin male infants, who had a father with the microdeletion.

**Results:** Microdeletions of AZF on Y chromosome were detected in 8.0% (58/725) of subjected samples. We could not find any deletion of Sry and mutation of DAZL in our samples. The incidences of microdeletion type were 6.9% (4/58) in AZFa, 6.9% (4/58) in AZFb and 60.3% (35/58) in AZFc, 20.7% (12/58)