using a beveled pipette (30 mm in diameter) in PBS containing 0.1% BSA and 7.5 μg/ml cytochalasin B. Nuclear transfer of transfected bovine fetal fibroblasts into the enucleated oocytes was accomplished by membrane to membrane method. Fused eggs were activated by treatment of Ca inophore and 6-DMAP. Reconstructed bovine eggs were cocultured with bovine oviduct epithelial cells in 500 μl of CR1aa containing 10% FBS for 8 days. All eggs were cultured at 39℃ in an atmosphere of 5% CO_2 in air. We examined the reconstructed bovine embryos developed in vitro culture system to determine the inetegration of EPO by PCR. Out of 203 bovine eggs reconstructed by nuclear transfer 167 (58.2%) embryos were cleaved, and among cleaved embryos, 19 (11.2%) embryos developed to the blastocyst stage. Of these blastocysts, 100% integration of EPO gene in 19 embryos was determined by PCR. Therefore, this result indicates that bovine oocytes reconstructed by nuclear transfer from transfected bovine fetal fibroblasts can successfully develop to the blastocyst stage. Futhermore, this novel procedures presumably be a attractive method to produce efficiently the transgenic cattles in the future.

P-3 Production of Transgenic Bovine Embryo by Microinjection of Retroviral Vector into Perivitelline Space

J. Y. Yoon[1], S. J. Uhm[1], K. H. Chung[1], S. H. Kim[1], B. S. Kwon[1], Y. T. Heo[1], T. Kim[2], H. T. Lee[1] and K. S. Chung[1]

[1]Animal Resource Research Center, Kon Kuk University, Seoul
[2]Department of Physiology, Catholic University of Taegu-Hyosung, Taegu, Korea

A critical requirement for integration of retroviruses, other than HIV and possibly related lentiviruses, is the breakdown of the nuclear envelope during mitosis. Nuclear envelope is reforming immediately after cell division, thereby permitting the translocation of the retroviral preintegration complex into the nucleus and enabling integration to proceed. In the oocyte, during metaphase II (M II) of the second meiosis, the nuclear envelope is also absent and the oocyte remains in M II arrest for a much longer period of time compared with M-phase in a somatic cell. Thus, this study was investigated the possibility for the production of transgenic bovine embryos by microinjection of retroviral vector (LNC-EPO and LNβ-EGFP) into perivitelline space. We injected retroviral vectors into the perivitelline space of in vitro matured bovine oocytes of M II stage. These bovine oocytes were co-cultured with sperm (1 x 10^6/ml) treated by Percoll gradient for 42~44 hr. Only fertilized embryos were co-cultured with bovine oviduct epithelial cells in 500 μl of CRI aa containing 10% FBS for 8 days. Finally, embryos developed in vitro were examined to evaluate the integration of EGFP and EPO by PCR and the expression of EGFP gene in the blastocyst stage by fluoresence emission. Among embryos microinjected with retroviral vectors, 52.8 (EGFP) and 45.9% (EPO) were cleaved and then 17.3 (EGFP) and 22% (EPO) were developed to the blastocyst stage. Integration of EGFP (386 bp) and EPO (702 bp) in these blastocyst stage were 21.1 and 22.2% by PCR analysis. The green fluorescence was emitted in all EGFP gene integrated blastocysts. Therefore, we demonstrated that reverse-transcribed gene

transfer at M II stage oocyte might be able to be adopted to produce the transgenic bovine embryos. In addition, these blastocysts will be transferred into recipients for the production of transgenic cow.

P-4 A New Method of Sperm Preparation at Testicular Sperm Extraction Intracytoplasmic Sperm Injection (TESE-ICSI) Cycle: Simple, Effective and Rapid Method

경북대학교병원 산부인과학교실 · 대구대학교 축산학과*

박기상 · 이택후 · 송해범* · 전상식

Objectives: Although ICSI is an effective clinical treatment for male patients, new types of technical difficulties have arisen, because of extremely small number of spermatozoa are handled in this procedure. The recovery of spermatozoa from TESE samples for the use of ICSI procedure is very difficult. The aim of this study was to attempt to recover the spermatozoa easily from TESE samples using a 3% polyvinylpyrrolidone (PVP) droplet.

Materiala and Methods: In TESE samples, excess tissue and viscous mass were removed and centrifuged (for 5 min at 1500 rpm). The pellet was resuspended with 10 μl human follicular fluid (hFF). Spermatozoa were recovered from the bottom of the 3% PVP droplet. Frozen samples were thawed 30 min before ICSI (Percoll was not used for washing). Spermatozoa were recovered from the bottom of the 3% PVP droplet.

Resilts: Results were presented in the following tables.

Table 1. Spermatozoa Recovery Rate

	TESE samples		
	Fresh	Frozen	Total
# TESE-ICSI cycles	5	3	8
Spermatozoa recovery rate	5 (100)	3 (100)	8 (100)

Table 2. Fertilization Rate

No. of	TESE samples		
	Fresh	Frozen	Total
TESE-ICSI cycles	5	3	8
Oocytes injected (/ cycle)	43 (8.6)	27 (9)	70 (8.8)
Fertilizes oocytes			
per cycles	5 (100)	2 (66.7)	7 (87.5)
per oocytes	29 (67.4)	11 (40.7)	40 (57.1)