O-13 Systems for Production of Calves from Hanwoo (Korean Cattle) IVM/IVF/IVC Blastocyst

II. Simple, Efficient and Successful Vitrification of Hanwoo Blastocyst

Eun Young Kim, Deog Im Kim*, Noh Hyung Park*, Yoo Seok Weon*, Hwa Kyung Nam, Keum Sil Lee, Sae Young Park, San Hyun Yoon, Sepill Park, Kil Saeng Chung*** and Jin Ho Lim***

Maria Infertility Medical Institute (마리아기초의학연구소),
**Hanwoo Improvement Center, NLCF* ***Maria Infertility Clinic, Seoul,*
****College of Animal Husbandry, Kon-Kuk University*

This study demonstrates that higher survival of vitrified-warmed bovine IVM/IVF/IVC blastocysts can be obtained using electron microscope (EM) grid as an embryo container at freezing, rather than plastic straw. In vitro produced day 7 bovine blastocysts after IVF were vitrified with EFS40 freezing solution, which is consisted of 40% ethylene glycol, 18% ficoll, 0.3 M sucrose and 10% FBS added m-DPBS and their post-survival after thawing was compared when two types of embryo containers (EM grid and straw) were used at freezing. Embryo survival in vitro was assessed as re-expanded and hatched rates at 24 hr and 48 hr after thawing, respectively. When the effect of exposure in vitrification solution and chilling injury from the freezing procedure on in vitro produced expanded blastocysts were examined, embryo survival in the exposure group (100.0, 73.3%) was not different compared with that in the control group (100.0, 84.4%). After vitrification, the hatched rate of the EM grid group (67.8%) at 48 hr after thawing was significanlty higher than that of the straw group (53.3%) ($p<0.05$). Fast developed embryos (expanded blastocyst and early hatching blastocyst stage) were indicated the better resistance to freezing than delayed one (early blastocyst stage), irrespective of embryo containers (early; 57.1 and 24.4%, expanded; 84.7 and 60.6%, early hatching; 91.7 and 80.0%) ($p<0.001$). Especially, in expanded and early hatching blastocysts, embryo survival of the vitrification-EM grid group (67.8, 95.0%) was significantly higher than that of the vitrification-straw group (53.0, 65.0%) at 48 hr post thawing, respectively ($p<0.05$, $p<0.001$). Therefore, this study presents the usability of EM grid as an advanced freezing technology for the simple, efficient, successful vitrification of bovine IVM/IVF/IVC blastocysts.

O-14 Advanced Culture System of Human Primordial Germ Cell on the Feeder Cell Secreting Murine Recombinant LIF

J.H. Park, S.J. Kim, B.R. Do, I.K. Jeon, S.I. Roh and H.S. Yoon

Infertility Research Center, Miz Medi (Young-Dong Jeil) Hospital, Seoul, Korea

Embryonic germ (EG) cells as like embryonic stem (ES) cells have the ability to remain undifferentiated and proliferate indefinitely *in vitro* while maintaining the potential to differentiate into derivates of all three embryonic germ layers. EG cells have several morphological characteri-

stics such as small cytoplasm, large nucleus, and prominent nucleoli. In long term culture of ES and EG cells, LIF (leukemia inhibitory factor) promotes the proliferation and suppresses the differentiation of ES and EG cells. To develop stem cell culture system, we cultured STO-SNL2 cells (transfected with plasmid containing the murine LIF gene) as a feeder cell in DMEM-high glucose supplemented with 15% FBS. To stop the proliferation of feeder cell, mitomycin C was treated, and we conformed LIF secretion from STO-SNL2 cells by western blotting and RT-PCR. Finally, gonadal ridges and mesentries (PGCs isolated from 9 weeks human fetus) were mechanically disaggregated and then incubated in 0.5% trypsin-5.3 mM EDTA. PGCs initially cultured and subsequently passaged on STO-SNL2 feeder cell. Feeder cell duplicated every 48 hrs and had 80% plating efficiency after thawing. To find optimal mitomycin C concentration, serial diluted mitomycin C was treated and then cultured for 7 days. 10 μg/ml of mitomycin C was optimal concentration to stop feeder cell proliferation. Secreted LIF proteins were confirmed by western blotting. Also, RT-PCR products of LIF mRNA was exactly the same as the expected sizes on the base of murine LIF gene sequence transcripts. Gonadal ridges and mesentries were cultured on feeder cell for 7~15 days in the presence of forksolin, human basic fibroblast growth factor (hbFGF) and secreted LIF. PGCs gave rise to large compacted multicelluar colonies resembling those of pluripotent stem cells termed embryonic germ cells. Most cells within the colonies were alkaline phosphatase positive. These results showed that the murine recombinant LIF can replace the human LIF in human PGCs culture. So, this advanced system can be used for human PGCs culture.

O-15 돼지 단위발생란의 생산 및 아미노산 대사능

한경대학교 동물생명자원학과, [1]서울대학교 수의과대학

정연길 · 박용습 · 노상호 · 황우석[1] · 윤종택

최근 복제돼지의 생산에 관한 연구가 활발하게 진행되고 있으나 후기배로의 발생율은 매우 저조한 실정이다. 본 연구에서는 돼지 체외성숙난자을 이용하여 인위적 활성화조건의 확립과 배 발육률을 검토하였고, 단위발생 상실배 및 배반포의 아미노산 대사능을 측정하였다.

도축돈 유래 난자를 44시간 체외성숙배양한 후 (FSH 및 LH 첨가 배양액에서 22시간, 호르몬이 제거된 배양액에서 22시간 배양) 난구세포를 제거하였다. 실험 1에서는 5 μM ionomycin (Ca), 7% ethanol (ET), 직류펄스 (DC, 1.2 kv/cm, 50 μs)를 각각 또는 복합처리하여 활성화를 유도하였고, 실험 2에서는 실험 1의 결과, 가장 적합한 것으로 나타난 DC의 최적 통전시간을 확립하기 위하여 통전시간을 각각 5, 10, 30, 50, 70 및 90 μs로 나누어 실험을 실시하였다. 단위발생란의 배양은 NCSU-23 배양액을 이용하였다. 실험 3에서는 활성화 자극 후 5일째 상실배와 6일째 배반포를 필수아미노산 + 비필수아미노산 및 0.1% PVA가 첨가된 NCSU-23 배양액 30 μl drop에 각 drop 당 46~154개씩 넣어 12시간 배양하여 배양액 내 유리아미노산 농도를 측정하였다 (Biochrom20™, Pharmacia, UK).

난자를 활성화할 경우 Ca, ET 보다 DC의 활성화율이 유의적으로 높았으며 ($p<0.001$), Ca, ET 및 DC를 복합처리할 경우에도 활성화율의 상승은 나타나지 않았다. 전기자극시간