embryos which developed in vitro in the NCSU 23 medium were cultured in the BSA free NCSU 23 medium supplemented with 20% fetal calf serum, the incidence of hatching or hatched out was significantly increased as compared to the control groups. However, addition of amino acids, vitamins or insulin to the NCSU 23 medium did not enhance the early morula developing to the hatched embryos. When either in vivo or IVM/IVF derived 1- to 2- cell stage embryos were cultured 4 days in the NCSU 23 and an additional 4 days in the NCSU 23 supplemented in the fetal calf serum, the percentages of hatched blastocysts were significantly higher than the control groups. These results suggested that dual culture conditions are required to optimize in vitro culture system for the development of the porcine embryos in vitro.

P-15

Development of Porcine IVM/IVF Produced Embryos to the Hatching Blastocyst *In Vitro* as Affected by Amino Acids and Fetal Bovine Serum

Maria Infertility Medical Institute, Seoul,
Maria OB/GYN[1], Seoul
College of Animal Husbandry[2], Kon-Kuk University

Uhm Sang Jun, Kim Eun Young, Kim Myo Kyung, Yoon San Hyun[1], Park Sepill, Chung Kil Saeng[2] and Lim Jin Ho[1]

The objective of this study was to test the effect of the amino acids and fetal bovine serum (FBS) to the blastocyst and hatching developments of 2-4 cell embryos produced by porcine in vitro matured-fertilized oocytes. In experiment 1, the development of 2-cell embryos on NCSU medium (0.4% BSA) at 36 hr after in vitro fertilization (IVF) examined according to the time course. The formation of blastocoel revealed at 96 hr after culture of 2-cell embryos. In experiment 2, the zona thickness (16.3 ± 2.34, 11.1 ± 2.89, 5.2 ± 2.61), embryo size (155.8 ± 7.0, 174.9 ± 10.5, 235.7 ± 21.3) and cell number (13.1 ± 3.44, 22.6 ± 6.37, 57.4 ± 10.60) investigated at various blastocysts (early, middle, expand) at 7 day after IVF. In experiment 3, the blastocyst and hatching development of 2-4 cell embryos at 50 hr after IVF tested on NCSU medium supplemented with the amino acids (2% BME amino acids and 1% MEM non-essential amino acids), FBS (10%) and BSA (0.4%). The embryos cultured in NCSU medium developed to blastocyst when added the amino acids, FBS or BSA. Especially, the hatching development of embryos obtained on the NCSU medium containing the amino acids or FBS. However, the morula and blastocyst of embyos cultured for 3 days on NCSU (0.4% BSA) could be develop to the hatching stage as transfer on NCSU (10% FBS). Therefore, these experiments suggest that the hatching development of porcine in vitro matured-fertilized embryos affects by amino acids and FBS.

P-16

Effects of Epidermal Growth Factor (EGF) on Mouse IVF Embryo Development and Their Cell Number

Maria Infertility Medical Institute, Seoul,
Maria OB/GYN[1], Seoul,
College of Animal Husbandry[2], Kon-Kuk University

Kim Eun Young, Uhm Sang Jun, Kim Myo Kyung, Yoon San Hyun[1], Park Sepill, Chung Kil Saeng[2] and Lim Jin Ho[1]

Control of growth and differentiation during mammalian embryogenesis may be regulated by growth factors. The objective of this study was to determine the effect of EGF on the preimplantation development of mouse IVF embryos and their ICM and TE cell number, and to examine the expression of EGF-R protein on

embryonic development by indirect immunofluorescence. The results obtained in these experiments were summarized as follows; Group culture (5 embryos/ 25 $\mu\ell$) showed more improved development rate to blastocyst than singly culture. This inferior development of singly cultured 2-cell embryos improved by the addition of EGF. Especially, 2-cell embryos cultured singly in 10 ng/ml of EGF (62.4%) indicated significant difference in development to blastocyst compared with contol group (47.9%). Also, cell number of ICM and TE by differential labelling showed the increased pattern in the EGF treatment group. The stimulating effect of EGF with the development level was significantly increased after 4-cell stage ($p<0.05$). ICM proportion also showed the increased pattern with the developmental level in the EGF treatment group. In addition, expression of EGF-R by indirect immunofluorescence detected after 4-cell stage. Therefore, EGF could stimulate preimplantation mouse embryo development by binding with expressed EGF-R after 4-cell stage and produce the more increased ICM and TE cell number of blastocyst.

P-17

Toxicity of Bovine Oviductal Fluid(BOF) on the Development of Mouse Embryos *In Vitro*

Department of Biology, Seoul Women's University

Younghee Lee, Euna Park and Haekwon Kim

To investigate the role of oviductal environment during early mammalian development, we examined the effects of bovine oviductal fluid(BOF) on the development of mouse 2-cell embryos *in vitro*. All of embryos cultured in medium containing 5% or more of BOF underwent degeneratation after 48 hr, whereas 60% of embryos cultured in the absence of BOF developed to morulae. When BOF was heated at 65℃ for 30 min and then added to the culture medium, the embryotoxic effect of BOF was not removed at all. However, heating of BOF at 90℃ for 30 min resulted in partial loss of the embryotoxicity. When BOF heated at 65℃ for 30 min was further treated with 0.1% chymotrypsin for 1 hr or overnight and then added to the culture medium, 66% and 82%, respectively, of mouse 2-cell embryos developed to blastulae after 72 hr culture. None of embryos cultured with untreated BOF developed but degenerated. The addition of an anti- oxidant, including glutathione(GSH), to the culture medium removed the embryotoxic effect of BOF so that 85% of embryos developed to morulae in the presence of 10 mM of GSH after 48 hr culture. From these results, it is suggested that bovine oviductal fluid contains a protein-like factor(s) which becomes embryotoxic in exposure to the air, probably via oxidation reaction.

P-18

Bovine Oviductal Fluid Do Not Support the Outgrowth of Mouse Blasotcyst *In Vitro*.

Department of Biology, Seoul Women's University

Younghee Lee, Jungwon Ahn, Eunjin Kim and Haekwon Kim

Except several primates including human, tubal implantation has not been reported to occur in mammals. By using an *in vitro* model system wherein the trophoblast cells of mouse embryos attach to and outgrow on tissue culture plates precoated with FBS, the ability of bovine oviductal fluid to support the outgrowth of