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

blastocysts was examined. When mouse 2-cell embryos were cultured onto the dishes that were precoated with 2 mg/ml of FBS, about 60 % of those embryos attached to culture dishes and were shown to outgrow. In contrast, embryos that were cultured onto the dishes precoated with 2 mg/ml of BOF did not attach and outgrow at all. Moreover, all of mouse blastocysts underwent degeneration when they were cultured in medium containing 5 mg/ml of BOF during 48 hr culture. When BOF was pretreated with 0.1 % chymotrypsin and then added to culture medium, it promoted outgrowth of blastocysts onto FBS-coated culture dishes. Addition of glutathione (GSH) at 10 mM conc. into the culture medium gave a silmilar result, while 1 mM or lower conc. was not effective. Neither pretreatment of BOF with chymotrpysin nor inclusion of GSH to the culture medium, however, supported outgrowth of mouse blastocysts unless culture dishes were precoated with FBS. Based upon these results, it is concluded that bovine oviductal fluid does not inhibit outgrowth of mouse blastocysts but lacks outgrowth-promoting activity *in vitro*.

Group 4, discussion : 15:00~15:30

P-19

인체의 난관 수종액(Hydrosalpingeal fluid)이 생쥐 배아 발달에 미치는 영향

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최근 난관수종을 갖는 불임환자에서 체외수정시술시 임신율이 감소된다는 보고들이 있다. 그 기전으로는 난관수종에 고여 있는 난관수종액은 자궁강 내로 유입되어 자궁내막에 변화를 일으킴으로써 착상을 방해하거나, 난관수종액 자체가 수정란의 발달 및 착상을 방해한다는 설이 있으나 아직 규명되지 않았다. 그리고 난관 수종액이 배아에 미치는 영향에 대해서는 아직 보고된 바 없다. 따라서 본 연구는 난관수종액이 첨가된 배양액에서 생쥐 배아를 키워 그 발달과정을 관찰함으로써, 난관수종액 자체가 수정란의 발달에 어떤 영향을 미치는가를 알아보고자 하였다.

대상 및 방법 : 난관수종액은 수술을 받는 난관수종을 갖는 10명의 환자로 부터 채취되어 -20 C에 보관하였고, 자궁내막 조직은 내막검사에 의해 얻어 Ham's F10 배양액에서 배양시켰다. 난관수종액이 0%,10%,20%,50%로 첨가된 T6 배양액을 사용하여 tissue culture dish에서 (실험1), 단층의 자궁내막 세포가 깔려있는 dish에서 각각 80개의 2세포기 생쥐 배아를 6일간 배양시킴으로써 그 발달과정을 관찰하였다 (실험2).

결과 : 실험1에서 포배기 (blastocyst)까지 발달한 비율은 난관수종액이 0%,10%,20%,50%가 첨가된 배양액에서 각각 70%, 75%, 70%, 59% 로 50%가 첨가된 배양액에서 통계적으로 의의있게 낮았으며($p<0.05$), hatching 되는 율은 각각 70%, 65%, 60%, 46% 로 역시 50%가 첨가된 배양액에서 낮게 나타났으나, outgrowth 되는 율은 50%, 44%, 48%, 36%로 통계적인 차이가 없는 것으로 나타났다. 실험2에서는 자궁내막 세포가 있을 때 난관수종액의 배아에 미치는 영향이 달라지는 지의 여부를 알기 위해 시행하였던 실험으로, 그 결과 역시 실험1과 비슷한 경향을 나타내었다. 즉 포배기까지 발달된 비율은 각각 82%, 75%, 65%, 57% 으로 50%의 난관수종액이 첨가된 배양액에서 의미있게 낮았으며, hatching 되는 율은 78%, 68%, 63%, 60% 로 낮아지는 경향을 보이기는 하였으나 통계적인 차이는 보이지 않았다. 단층의 자궁내막 세포에 착상되는 율은 64%, 55%, 58%, 58% 으로 통계적으로 유의한 차이는 없었다.

결론 : 본 연구에 의하면 난관수종액은 생쥐 배아의 포배기 발달 및 hatching 에 나쁜 영향을 주나, 이미 배아가 발달된 후 outgrowth 나 착상에는 영향을 주지 않음을 알 수 있었다. 따라서 체외수정 시술시 난관수종을 갖는 환자에게서 나타나는 임신율의 감소는 난관수종액이 배아의 발달을 저해시키는 것이 하나의 원인이 되는 것으로 시사된다.