120 Effects of coculture between early stage embryos and the cell of extra sexual organ on embryo development. K. Koike, H. Saito, T.Saito, M.Hiroi, Dept. Obstet. and Gynec., Yamagata Univ. Sch. Med., Yamagata.

[purpose] We examined the effect of the coculture between embryos and the cell of extra sexual organ, Vero cell. [method] Vero cells were put in an organ culture dish at the concentration of $2x10^4$dish^1 or $4x10^4$/dish^2. To examine embryo growth, 2 cell stage mouse embryos were cultured for 72 hours, and at every 24 hours, embryo growth was observed. Uneven cleaved human embryo were cultured with Vero cells. [result] SCEs of embryos cultured at 2 and 8 cell stage showed no significant difference between control and coculture groups. Meanwhile 24 hours after culturing, the rate of embryo growing into 8 cell stage and more showed no difference among three groups. At 48 hours culture, the hatching rate of 1 coculture group was higher than that of the control ($p<0.01$). At 72 hours after culture, the rate of hatched embryos was higher in coculture groups when compared with the control ($p<0.001$). All human embryos with coculture developed into blastocyst and more, meanwhile control did not reach blastocyst stage. [conclusion] Coculture with cells of extra-sexual organ enhanced embryo development. And mild demaged embryos could recover normal development by coculture.

121 The biological active substance produced by early embryo in preimplantation state. S.Nakagawa, A.Ida, M.Shigeta, K.Koyama, S.Isojima, Dept.Obst.and Gynec.,Hyogo Medical College, Hyogo.

To clarify the biological active substance produced by early embryo, we examined suppressive factors to mixed lymphocyte reaction (MLR) in supernatants of in vitro fertilization and embryo replacement (IVF-ER). After oocyte and sperm were incubated for 18 hours, the medium were replaced by fresh medium and followed another 24 hours incubation. The suppresive activity of IVF-ER supernatant were examined in MLR. 60.1% (51/88) of fertilized ovum medium showed inhibiting activity to MLR at over than 20% suppressive indes, on the other hand, none of the 9 ovum which failed to fertilize showed such suppressive activity. The suppressive activity was demonstrated to be dose responsiveness by the addition of fertilized ovum medium to MLR, however it was not related to embryo cell stages. Biochemical analysis of the suppressive factor elucidated that the substance was low molecular weight (smaller than MW 5000), heat stable, trypsin resistant and interestingly extractable by chloroform/methanol treatment to organic fraction with keeping activity. These data suggest that fertilized early embryo produce MLR suppressive substance and this substance seems to be not protein but glycolipid or phospholipid.

122 Effect of oxidative stress on the whole mouse embryo culture. T.TOKURA,Y.NODA,K.NARIMOTO,T.MORI, Dept.Gynec. and Obst., Faculty of Medicine,Kyoto Univ.,Kyoto.

Previous studies in our laboratory have shown that SOD, a scavenger of superoxide radicals, promotes mouse early embryo development in vitro. In this study, we analyzed the effects of SOD on the development after the implantation stage. Mouse blastocysts obtained from superovulated ICR female were cultured in the medium (α-MEM + 10%FCS + nucleosides) with various concentrations of SOD (2 to 500 μg/ml) in an atmosphere of 5% CO_2 in air at 37°C. Morphological observation was performed until Day 4 of culture, and the culture efficacy was compared with that of the control without SOD. At low SOD concentrations of 5 μg/ml or less, the two cell layers and egg cylinder formation rates showed no significant difference from those of the control. However, at high SOD concentrations of 10 μg/ml or more, both rates decreased significantly. These results indicate that SOD at high concentrations shows adverse effects on the embryo development after the implantation stage. Taking into consideration the fact that superoxide radicals are involved in cell differentiation, SOD at high concentrations might decrease the superoxide radicals in the inner cell mass (ICM), resulting in the degeneration of ICM during differentiation.