P9 Immediate early genes, Mcl-1 and IEX-1, associate each other and modulate apoptosis signaling in the ovary

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Objectives: IEX-1 (immediate early response gene X-1) is a stress- and growth-induced gene up-regulated under various conditions including growth factors, cytokines, and irradiation. The functional role of IEX-1 in the circumstances aforementioned is not clear yet. In the present study we have identified IEX-1 as a Mcl-1-interacting protein and investigated the possible roles of IEX-1 and Mcl-1 in their apoptosis signaling pathways in the ovary.

Materials and Methods: We have screened 3×10^6 independent clones of human ovarian cDNA library using Mcl-1 as the bait. The interacting clones were identified by direct sequencings of DNA isolated from yeast and transformed to *E. coli*. Cell viability assay, FACS analysis, and immunoblot were performed using the ovarian cell line SK-OV-3 following transfection of different plasmid DNAs.

Results: Using the yeast two-hybrid screening system, we identified that IEX-1 interacts with an anti-apoptotic protein, Mcl-1 (myeloid cell leukemia-1). Their interaction in the ovarian cells was further confirmed by immunoprecipitation followed by Western blotting. IEX-1 induces apoptosis in human ovarian cells and efficiently prevents Mcl-1-mediated survival of the cells.

Conclusions: Our data demonstrate that IEX-1 is a novel ovarian apoptotic protein likely to play important role in the survival of ovarian follicles.

Key words: Mcl-1, IEX-1, immediate early response, apoptosis, ovary

P10 An evaluation of the effectivity of the two-step (consecutive) embryo transfer method in Assisted reproductive technologies.

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Objectives: The concept of the receptivity induction mechanism of the implantation of embryo has been studied. The two-step embryo transfer method has been designed applying the concept. The aim of this study was to evaluate the efficacy of the two-step embryo transfer method.

Materials and Methods: Experiment 1: Seventy-six cases, 96 cycles who underwent two-step embryo transfer (group 1) were compared with 107 cases, 137 cycles who underwent blastocyst transfer (group 2). The cancellation rate, the number of transferred embryos, pregnancy rate, implantation rate and multiple pregnancy rate were calculated.

Experiment 2: Twenty one cycles and patients in the Group 1 with previous implantation failure even transferring high grade blastocysts were categorized to Group 3. Sixty seven cycles, 54 patients with recurrent IVF failure (more than 4 times) in Group 1 were categorized to Group 4. Twenty one cycles, 18 patients over forty years old in group 1 were categorized to Group 5. The pregnancy rate and implantation rate were estimated in each group.

Results: Experiment 1: There was no difference in age, the number of oocytes retrieved and endometrial thickness between groups 1 and 2. The number of previous IVF-ET attempts were significantly higher in the group 1 (5.5 vs. 4.0; $P<0.01$) and total number of transferred embryo were significantly higher in the group 1 (2.5 vs. 1.7; $P<0.01$). ET cancellation rate was significantly lower in the group 1 (0.0% vs. 28.5%; $P<0.01$). There was no difference in the pregnancy rate and the multiple pregnancy rate between the two groups. The implantation rate was significantly higher in group 2 (38.0%) than in group 1 (24.8%) ($P<0.05$).

Experiment 2: The pregnancy rate and the implantation rate were 38.1% (8/21) and 20.0% (11/55) in the group 3, 41.8% (28/67) and 22.6% (37/164) in the group 4, 28.6% (6/21) and 13.7% (7/51) in the group 5 respectively.

Conclusions: Two-step embryo transfer resulted in a significantly lower cancellation rate compared with blastocyst transfer. There was no difference in the multiple pregnancy rates regardless of the increased number of transferred embryos. Therefore the two-step embryo transfer method may benefit patients who seem to fail to achieve pregnancy due to a lack of endometrial receptivity.

Key words: two-step (consecutive) embryo transfer, blastocyst, *in vitro* fertilization, implantation window