

expanding blastocysts.

Blastocysts were cryopreserved during controlled-rate freeze cycle (-2°C/min to -7°C, manual seeding at -7°C, -0.3°C/min to -38°C, and -196°C LN<sub>2</sub>) following the stepwise addition of cryoprotectant (0.55M and 1M glycerol + 0.2M sucrose in DPBS containing 20% hFF, respectively). Blastocysts were thawed quickly followed by stepwise removal of cryoprotectant (0.55M glycerol + 0.4M sucrose, 0.44M, 0.33M, 0.22M, 0.11M and 0.00M glycerol + 0.2M sucrose in DPBS containing 20% hFF, respectively) and cocultured for 2 hrs or 18 hrs with cumulus cells. Blastocyst transfers were routinely performed using a Tomcat catheter into the intrauterus from 4- to 4.5 days following ovulation. The results obtained from this retrospective study are as follows.

There are several advantages in cases of transferring human embryos to the uterus at the blastocyst stage; better synchronization of the uterine endometrium and embryo is possible to select better quality embryos with implantation potential for transfer and to reduce the risk of a multiple pregnancy by the selection of only one or two better quality embryos. Therefore, these data also suggest that freezing at the blastocyst stage for a high cumulative success rate is a reliable method in IVF-ET technology.

## **17 The direct effect of epidermal growth factor (EGF) on nuclear maturation and EGFR expression in human oocytes**

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The present study was accomplished 1) to examine the direct effect of the EGF on the nuclear maturation of the human oocytes without cumulus cells, and 2) to examine the existence of EGF receptor (EGFR) in the oocytes. The human immature oocytes were collected from patients undergoing ovulation induction. The GV-stage oocytes were completely denuded cumulus cells and corona cells and cultured in the TCM 199 medium with or without EGF (50 ng/ml). After 36 hours of culture, maturation rate was evaluated by the first polar body extrusion. The maturation rate was 75% (13/17) and 35.7% (5/14) in the presence and absence of EGF, respectively ( $p < 0.05$ ). There was a positive effect of EGF on the maturation of the denuded immature oocytes. The existence of EGFR transcripts and proteins in the GV oocytes were determined by reverse transcription-polymerase chain reaction and immunocytochemistry, respectively. EGFR transcripts and proteins were detected in the oocytes. The results of the present study indicate that EGF can react directly through the EGFR in the oocytes to promote the nuclear maturation of the oocytes.