

Oocyte freezing

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Our efforts have focused on establishing an effective IVM system for human oocytes since the late 1980s, and recently, we developed a new ART technology for enhancing implantation and pregnancy rates. These newly developed technologies mainly consist of vitrification and in vitro-maturation (IVM) of the human immature oocytes. These technologies greatly contribute to establishing an oocyte bank.

IVM is a recently developed culture technique for supporting the growth of immature oocytes [germinal vesicle (GV) to telophase-I (T-I) stage] and has become increasingly important in ART methods. The establishment of an IVM program can yield many advantages. Patients suffering from congenital or postnatal reproductive disorders, such as premature ovarian failure or polycystic ovarian syndrome (PCOS), can achieve pregnancies by transfer of viable embryos derived from IVM and in vitro fertilization (IVF) systems. Furthermore, application of IVM for the long-term preservation of mammalian gametes, embryos, and tissues allow us to establish oocyte banks. Oocyte banks will help restore fertility in women with various catastrophic diseases such as malignant cancer. The establishment of oocyte banks can alleviate certain ethical concern regarding embryo freezing. The disposal of excess oocytes can be avoided, and the surplus cryopreserved oocytes will remain viable until their future use.

We recently employed a "vitrification" method for developing cryopreservation technique of oocytes. Vitrification is a convenient and practical method, which does not require expensive equipments. Also, intracellular ice crystal is not produced by vitrification, and it may decrease various biological changes in oocyte cytoplasm including cryoinjuries of oocytes. In our protocol, use of the EM grid for oocyte transfer may help rapid heat conduction from the outside into the oocyte and relatively short protocol prevents oocytes from damages by solution effect of the cryoprotectant. But, the disadvantage of vitrification using grid is that oocytes with the vitrification solution directly come in contact with liquid nitrogen during cooling and storage. To protect the risk of the possible transmission of viral pathogens to embryos vitrified and stored in contaminated LN₂, we are currently conducting experiments to develop effective vitrification method by using the straw.

Immature oocytes retrieved from unstimulated PCOS patients were vitrified using grid method and the embryos were transferred into the patients. A total of 301 oocytes were retrieved from 14 cycles and morphological normality of oocytes after vitrification was 83% (249/301). Among them, 68% of the oocytes matured in vitro and 68% of the matured oocytes were normally developed to the pronuclear stage after IVF. Cleavage rate was 90%. We transferred the embryos derived from vitrified oocytes to uterus of the patients at 3 days after IVF, without successful implantation yet. However, we are continuously attempting to use the cryopreserved oocytes

for the clinical treatment and anticipate the positive results.

We also vitrified mature oocytes retrieved from stimulated IVF cycles. Thirty-four patients agreed to undergo frozen-oocyte-derived embryo transfer procedure after failing the fresh cycle IVF-ET. Of the 872 oocyte-cumulus complexes (25.6 ± 11.9) retrieved from 34 stimulated patients, 394 oocytes were used for fresh IVF-ET cycles and the remaining 474 oocytes-cumulus complexes were cryopreserved using same grid method. A morphological survival rate of the oocytes was 68.6% (325/474). Of those, 61% (198/325) was metaphase II oocytes with morphologically normal cytoplasm suitable for ICSI procedure. Fertilization rate of these vitrified/thawed oocytes was 71.7% (142/198), and cleavage rate on day 2 was 95% (135/142). At the time of embryo transfer, six patients did not go through ET procedure because of the fertilization failure or poor embryo quality. A total of 125 cleaving embryos from vitrified oocytes were transferred to the remaining 28 patients and 6 patients achieved clinical pregnancies. The pregnancy and implantation rate of vitrified IVF-ET were 21.4% (6/28) and 6.4% (8/125), respectively. All pregnancies resulted in the delivery of healthy babies. These data clearly demonstrated that both immature and mature oocytes could be vitrified for long-term preservation.

Vitrification combined with IVM is certainly a reliable technique that has a potential for an effective long-term preservation method for human oocytes. All of these research and cautious clinical application will markedly contribute to developing human reproductive medicine, yielding a highly efficient ART program.