

## Clinical Application of Human Oocyte Cryopreservation

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Oocyte freezing remains a valuable and attractive technique in the range of assisted reproductive technologies available at present. Although achieving pregnancies using oocyte cryopreservation in conjunction with IVF, we have faced the negative effects of cryopreservation on the integrity of several of the oocytes' unique features involved in normal fertilization and embryonic development, such as premature cortical granule exocytosis leading zona hardening, increased parthenogenetic activation and damage to the cytoskeletal elements of the oocytes, in particular disruption of the meiotic spindle, led to a period of dormancy regarding research on the procedure. The main biophysical factor affecting human oocyte survival is the intracellular ice formation that generally pierces the membrane causing cell lysis and breaks the meiotic spindle inducing chromosome aneuploidy. Because the human oocyte has a large quantity of water in their cytoplasm, it is very difficult to avoid ice crystal formation by slow freezing and thawing protocol. 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.

Our efforts have focused on establishing an effective in vitro maturation (IVM) system for human oocytes [germinal vesicle (GV) to telophase-I (T-I) stage] since the late 1980s, and we developed recently a new important ART technology for enhancing implantation and pregnancy rates of infertility patients with selected causes. 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, the long-term preservation of mammalian gametes allow us to establish oocyte banks by combine with IVM system. By these techniques, the disposal of excess oocytes can be avoided, and the surplus cryopreserved oocytes will remain viable until their future use. All of these research and cautious clinical application will markedly contribute to developing human reproductive medicine, yielding a highly efficient ART program.