



Efficiencies of oocyte vitrification, fertilization, embryo development and implantation: a preliminary report II

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Objective: Previously, we reported successful oocyte vitrification and embryo development following oocyte warming. In this report, we aim to further assess the efficacies of oocyte vitrification, in vitro development and implantation following embryo transfer

Design: Prospectively designed pilot trials and retrospective data analysis

Setting: Private fertility center

Materials and methods:

Oocytes used for the validation trials of oocyte freezing were donated from the patients underwent for IUI treatments. When the patients have more than 3 large follicles, the excessive follicles were removed by ultrasound-guided aspirations. The oocytes used for egg banking and subsequent embryo transfer were recovered from patients following standard long or short stimulation protocols.

The cumulus cells surrounding oocytes were stripped off, matured oocytes at MII stage were selected for vitrification. For validation trials, the sibling oocytes were evenly divided into treatments of a control group (fresh oocytes) and a vitrification group (frozen-thaw oocytes). For oocyte banking patients, the oocytes were vitrified and stored in liquid nitrogen tank for several months up to 2 years. The frozen-thaw oocytes were fertilized by ICSI. The embryos were cultured in commercial IVF media with 6% CO₂, 5% O₂, and 89% N₂ at 37 °C for 5 days. The embryo development was recorded. In embryo transfer cycles, good quality day 5 embryos were selected for transferring into recipients in natural cycles. Pregnancy outcomes were recorded.

Percentage data were analyzed by Fisher's exact test. P value <0.05 is considered statistically significant.

Results:

Table 1. Comparison of embryo development following vitrification-warming up among sibling oocytes

| Groups | Replicates | # of oocytes | # of survivals of thawing | # of 2PNs | # of blastocysts | |
|---------------|------------|--------------|---------------------------|-----------------|------------------------|----------------------|
| | | | | | # of total blastocysts | # of 4AA blastocysts |
| Control | 5 | 13 | N/A | 13(100%) | 9(69%) | 7(54%) |
| Vitrification | 5 | 14 | 14 (100%) | 13(93%) | 7(50%) | 1(7%) |
| P value | | | | >0.05 | >0.05 | 0.04* |

Table 2. Pregnancy outcomes of embryo transfer cycles following frozen-thawing of oocytes

| # of patients for egg freezing | 10 | Percentage |
|---------------------------------|----------|------------|
| # of frozen oocytes | 62 | |
| # of thawed oocytes | 62 | |
| # of survived oocytes | 54 | 87 |
| # of fertilized oocytes | 47 | 87 |
| # of 6-8 cells at day 3 | 30 | 64 |
| # of blastocysts at day 5 | 15 | 32 |
| # of AA blastocysts | 6 | 13 |
| # of patients transferred | 7 | |
| # of embryos per transfer | 2.1 | |
| # of HCG positives | 6 | 86 |
| # of fetal heart beat positives | 4 | 57 |

Conclusions:

Oocyte cryopreservation can be potentially applied to the following clinical situations:

1. Preserve fertility of female cancer survivors.
2. Oocyte banking for delaying ages of childbearing.
3. Quarantine oocytes in oocyte donor program.
4. Use for an alternative to embryo cryopreservation due to ethical consideration.

Our preliminary data showed high survival rates of oocytes following procedure of vitrification and warming up. Although total blastocyst developmental rates had no difference between fresh oocytes and frozen-thaw oocytes, less high quality blastocysts were produced in frozen-thaw oocytes. However, comparable pregnancy outcomes were achieved in the embryo transfer cycles with frozen-thaw oocytes.

More clinical data were required to validate the feasibility of large scale program for offering oocyte freezing to perspective patients.