

Direct comparison of in vitro development of the embryos derived from vitrified oocytes vs. freshly collected oocytes in humans

Rania Baydoun BSc, Bin Wang PhD, and Samuel Soliman MD FRCSC

Newlife Fertility Center

Objective: To assess the efficacies of oocyte vitrification and in vitro developmental competency following warming and fertilization

Design: Prospectively designed experiment

Setting: Private fertility center

Materials and methods:

The patients undergoing IUI treatments were hormonally stimulated. When the patients have more than 3 large follicles, the oocytes from excessive follicles were removed by intra-vaginally ultrasound-guided aspirations. The recovered oocytes were either discarded or donated to research consented by patients. The cumulus cells surrounding oocytes were stripped off through enzyme digestion and pipetting, matured oocytes were selected for the trails. The sibling oocytes were evenly divided into two treatments of a control group and a vitrification group.

- For the control group, the oocytes were fertilized by ICSI method with donated frozen-thaw sperm.
- For the vitrification group, the oocytes were frozen with vitrification method (Cryo-top) and warmed up after immersing in liquid nitrogen for about one hour.

Briefly, the oocytes were equilibrated in ES for 5 minutes, and then placed in VS for 5 seconds. Loading of the oocytes onto a Cryo-top straw and subsequent plunging into liquid nitrogen were completed within 15 seconds. The oocytes were warmed up by placing the tip of the Cryotop straw in TS for 1minutes at 37 °C, followed by washing the oocytes in DS, 3 minutes; WS1, 5 minutes; WS2, 5 minutes. Recovered oocytes were cultured in a sequential medium +10% SPS for 30 minutes before an ICSI procedure. The oocytes from the control group and vitrification group were cultured in sequential media+10% SPS, 6% CO₂, 5% O₂, 37 °C for 5 days. Some of the embryos derived from the vitrification group were biopsied at 3 days of culture and 5 days of culture respectively for aneuploidy analysis by Fluorescent in Situ Hybridization. The embryo development was recorded and the data were analysed by Fisher' exact test.

Results:

Data with * are statistically different (p<0.05)

Groups	Replicate s	MII oocyte s	Method of freezing	No. of survivals after thawing	2 PNs	Day 3 development		Day 5 development	
						No. of >5 cells	No. of 8 cells(1)	No. of blastocysts	No. of 4AA
Control	5	13	N/A	N/A	13	13(100%)	11 (85%)	9(69%)	7 (54%)
Vitrificatio n	5	14	Vitrificatio n	14	13	12(86%)	3 (21%)	7(50%)	1 (7%)
P value							* P=0.0018		*P=0.04

Two day 3 embryos at 8 cell stage were biopsied for FISH analysis. The biopsied embryos were left for further culture. At day 5, two blastocysts developed from the biopsied embryos were fixed and subjected to FISH analysis of whole embryonic cells.

The embryo #1 showed 2n karyotype of chromosomes X, Y, 15 and 17 at day 3; however, the blastocyst # 2 had 65 cells with a mosaic pattern of karyotypes containing 60% of 2n cells. The embryos #2 showed 2n karyotype of chromosomes X, X, 15 and 17 at day 3, similarly, the blastocyst #2 had 111 cells with a mosaic pattern of karyotypes containing 65% of 2n cells.

Conclusions

Although oocyte cryopreservation has been successful employed in a number of clinics across the world, the direct comparison of development between the embryos derived from fresh oocytes and vitrified oocytes were rarely reported due to limited availability of the donated oocytes for research. In our experiment, we demonstrated a high survival rate of oocytes following warming up of vitrified oocytes, as well as a high fertilization rate. However, the quality of embryos at day 3 and day 5 according to morphology assessment is poorer compared with that of the embryos derived from fresh oocytes. Preliminary FISH analysis showed that embryos derived from vitrified oocytes had normally karyotypes at day3, however substantially mosaic patterns of karyotypes at day 5 were observed. We have successfully obtained full term live births with the vitrified donated oocytes in clinical cycles (data not shown), which indicated that lower quality of blastocysts from vitrified oocytes could survive to term.