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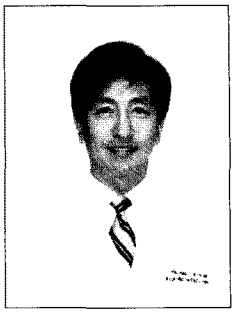

2001-present	Medical Director
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1996-2001	Director
	Assisted Reproductive Technology, Naval Medical Center, San Diego, California
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1987-1988	Intern
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	Loyola University Stritch School of Medicine, Maywood, Illinois
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Oocyte Freezing using Vitrification: Our Experience with fertile population

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Successful cryopreservation of human oocytes may be one of the ways to overcome the age related decline of ovarian function of women who desire future pregnancies. With continuous improvement in knowledge and technique of cryobiology, successful pregnancy rate from frozen embryo transfer has risen steadily over the last two decades. Embryo cryopreservation has become a routine part of modern ART practices. However, advances in oocyte cryopreservation technology are still slow and controversial despite intense public interest.

Although the progress is intermittent and frustrating at times, more centers around the world are reporting improved techniques and successful outcomes (pregnancies) for the last 3 years. One of the promising areas of research is a flash freezing method or vitrification. Several publications have documented improved pregnancy rates with slow freezing of oocytes. In this abstract, we are sharing our experiences with the CHA-vitrification method in cryopreservation of human oocytes among fertile population.

In order to test the efficiency of CHA-vitrification technique on human oocytes, we chose fertile population to eliminate confounding variables of unsuccessful pregnancies

Design: IRB approved prospective clinical study

Study population: Post-tubal ligation patients with proven prior child bearing

Inclusion criteria: Age less than 36 without intervening medical problem, Basal follicle counts greater than 9, BMI less than 30, No severe male factors

MATERIALS AND METHODS

Patients underwent routine ovulation induction protocol as in IVF. Ovulation was triggered with hCG when at least 2 follicles measured 18 mm in diameter. All retrieved eggs were cryopreserved using CHA-vitrification protocol for 6 months (adopted from local sperm banks' policy). After thawing, only the mature (MII) oocytes were fertilized by ICSI

Oocytes vitrification was performed in two steps process. Oocytes were first equilibrated in cryoprotectant solution containing 1.5 M ethylene glycol at 37° C for 2.5 min followed by introducing vitrification solution containing 5.5 M ethylene glycol and 1M sucrose for 20 second at room temperature. Oocytes were then placed on the electron microscope (EM) grid and immediately plunged to the liquid nitrogen. A total of 412 oocytes from 20 patients (20 egg retrieval procedures) were cryopreserved using CHA-vitrification technique.

Thawing is performed according to five-step protocol using 1.0 M, 0.5 M, 0.25 M, 0.125 M, 0 M of

sucrose solutions. The EM grids were transferred sequentially to the thawing solutions at 2.5 min intervals at 37℃.

The oocytes were fertilized by ICSI and fertilized embryos were transferred to the uterus 3days, 5days or 6days after thawing.

Egg freezing Study (Navy Pts)										
Pts	Age	# eggs	# Thaw	# Surv	# Fert	Transfer	# Blast refrozen	Pregnant	Wks delivery	Remarks
A	35	19	19	7	3/6	3, D3	0	No		
B	30	15	15	12	5/7	4, D3	0	Y, single	Full term	F: 7lbs 9oz
C	27	24	24	18	8/15	3, D5	0	Y, triple	32 wks	2F,1M:3"7",2"7",4'4"
D	30	22	22	14	8/10	2, D5	0	Y, single	Full term	M: 10'15"
E	28	42	32	30	15/19	2, D5	0	Y, single	39 wks	F: 8lbs 8oz
F	26	66	32	30	22/25	3, D5	6	Y, triple	31 wks	2M,1F: 3'10",3'9",3'3"
G	30	31	31	26	13/13	3, D5	0	Y, single	Full term	M: 8'8"
H	34	29	29	21	14/15	2, D5	5	Y, single	Ongoing	
I	26	17	17	13	10/12	3, D5	0	Y, single	Ongoing	
J	30	20	20	19	17/19	2, D5	7	Y, single		Miscarried at 6 wks
K	30	8	8	6	2/4	2, D3	0	Y, single	Ongoing	
L	34	24	24	22	15/15	3, D5	4	No		
M	34	12	12	6	3/5	3, D3	0	Y, triple	Ongoing	
N	32	16	16	13	8/9	3, D6				

RESULTS

From 20 patients, a total of 412 oocytes were retrieved. Of those, 13 patients came back to utilize their 285 vitrified oocytes after 6 moths of waiting period. Remaining 7 patients are still waiting to satisfy their 6 month waiting term. 224 out of 285 oocytes survived thawing (79%). Fertilization rate and cleavage rate were 82% (135/165) and 88% (119/135) respectively. Average number of transferred embryos was 2.7 ± 0.5. 18 Surplus embryos were refrozen for future use. Eleven of thirteen patients (84.6%) became pregnant, and implantation rate based on ultrasound exam was 48.6% (17/35). So far, six patients delivered ten healthy babies (two sets of triplet and four singletons). One patient encountered miscarriage at 7 weeks gestation. Four patients are currently on going in their 2nd and 3rd trimesters. Egg to baby ratio was 6.4%. In other words, it took 15.7 frozen eggs to produce one baby after going through CHA-vitrification protocol.

CONCLUSION

Our study shows highly efficient CHA-vitrification method in maintaining oocyte viability and functional integrity. Complete report of our current study will be available by the end of 2006. It is our goal to continuously improve and optimize this technology so that this would eventually become a part of treatment options in future ART practices.