Oocyte and Ovarian Tissue Vitrification for Restoration of Reproductive Function
“Efficiency and the Limit”

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Disclosure information: Nothing to declare
VITRIFICATION

For:

Keeping the precious life of Human: oocyte/embryo

With no damages, no risks

Until the day of the Patients’ dream will come true
Brief History of Human Clinical Vitrification

First IVF-Blastocyst (Cattle) (1991)
First Cattle IVM Oocyte (1992)
Porcine Oocyte (Most difficult cell) (1998)

First Pregnancy: Oocyte : Hand made Cryotop (1999)
Cryotop Method (original solution) (2000)
First Oocytes Vitrification Baby in USA, Latin America, Europe (2003,4)

Cryotop II Version (85%Survival) (2004)
Cryotop III Version (90% Survival) (2008)

CryoTec Method CDM (non serum) (2012)
100% survival oocyte, PN Zygote, 4cell, Blastocyst (-to now)

(>2,500,000 cases in 75 countries for 16 years)

Oocyte 90,000 Babies, Embryo 1,000,000 Babies
specially constructed fine polypropylene strip attached to a plastic handle (Cryotop method). In the second experiment, the Cryotop method, which had yielded the best results, was used to vitrify human oocytes. Out of 64 vitrified oocytes, 58 (91%) exhibited normal morphology after warming. After intracytoplasmic sperm injection, 52 became fertilized, and 32 (50%) developed to the blastocyst stage in vitro. Analysis by fluorescence in-situ hybridization of five blastocysts showed that all were normal diploid embryos. Twenty-nine embryo transfers with a mean number of 2.2 embryos per transfer on days 2 and 5 resulted in 12 initial pregnancies, seven healthy babies and three ongoing pregnancies. The results suggest that vitrification using the Cryotop is the most efficient method for human oocyte cryopreservation.

Keywords: birth, bovine, Cryotop, human, pregnancy, vitrification
Survival and Pregnancy rate of Vitrified Oocytes

(95%) Surv.

(41%) Preg.

(Kuwayama, RBMOnline 2005)
Real Meaning of 90% Survival

90% survival for Normal Oocytes of General patients

0% survival for Sensitive Oocytes of Cancer/Old patients

90% Survival
The Cryotec Method

Vitrification (13min)

Warming (10min)
Survival Rates after Vitrification by Cryotec method (2012)

- **Oocyte**: 100% (n=725)
- **4-8cells**: 100% (n=2012)
- **Blastocyst**: 100% (n=1478)
Human Oocyte Bank for Unmarried Blood Cancer Women

Nagi-chan (16 years old, High School 2nd)

Malignant lymphoma 2001.05

Future Mother (First Oocyte Bank) 2001.09

Medical interview 2001.10

OPU/Oocytes Vitrification 2001.11

Bone Marrow Transplantation 2005.03

Graduate High school 2005.04

School of Nursing 2008.04

- Good Nurse!

2001- 356 patients

with 1,638 oocytes (4.6/P)

2014.8.7♂ 3295g
Ovarian Transplantation Between Monozygotic Twins Discordant for Premature Ovarian Failure:
Dr. Sherman Silber, 2007
Ovarian tissue transplantation (Silber 2005)

Ovarian tissue vitrification for extra tissue
① Ovarian cortex slicer

1cm thickness
Protocol of ovarian tissues vitrification

1. **Ovary**
   - 1mm x 1cm x 1cm
   - Ovarian tissue

2. **ES, 25min**

3. **VS, 15min**

4. **Cryotissue**
   - Ovarian tissue

5. **Vitrification**

6. **LN$_2$**

7. **Survival assay**

8. **Thawing**
   - TS(37°C), 1min

9. **Transplantation**
   - WS, 10min
   - DS, 5min
Vitrification of ovarian tissue

Vitrified ovarian tissue: Translucent

Cooling rate 17,000°C/min, Warming rate 32,000°C/min
Visual Aspect of Ovarian Tissue during Cooling in LN2

- **M; Milky**
  (Ice Formation; = Not Vitrified)

- **I; Intermediate**
  = Partially Vitrified.

- **T; Translucent**
  = Vitrified
New Strategy of fertility preservation for cancer patients: 2013 ~ ”Vitrification of GV oocytes and Ovarian tissue”

1. Oocyte collected from follicle
2. Resected ovary
3. Cryopreservation
4. Vitrified ovarian cortical tissue
Ovariectomy by Single port laparoscopic Surgery
(By Dr.Kikuchi)
(A) Immature (GV) oocytes (n=35)
366 oocytes (10.5 oocytes /patient)

(B) Ovarian tissues (n=39)
344 tissues (8.8 slices/patient)
Re-transplantation by Two port laparoscopic Surgery (by Dr. Kikuchi)
“Closed” Ovarian Tissue Vitrification
Porcine growing oocytes of the preantral follicles in ovarian tissues before and after vitrification.
Take home message

1. Non-invasive Clinical Oocytes Vitrification has been established.

2. Based on vitrification, Oocytes Bank has been spread world wide.

3. >50% cancer patients can not have babies by oocytes vitrification.

4. **Oocyte + Ovarian Tissue** vitrification may be a real method.

5. Ovarian Vitrification needs Closed (not for oocyte vitrification)
Conclusions

To raise more possibility of having babies to cancer women, combination of vitrifications of GV oocytes and ovarian tissues can be more effective than conventional oocytes vitrification.

Based on huge experiences of oocytes vitrification for 15 years, 100% survival vitrification method was developed. This leads better opportunity in fertility as a whole, for not only cancer patients but also healthy women and common infertile patients.
Thank you very much!

To
My new friends in ISFP.

From Masa