The 5th World Congress of the ISFP
Vienna, AUSTRIA
2017.11.18

Session 10 Storing ovarian tissue

Debate- Slow freezing vs vitrification

Vitrification

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COI disclosure

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St. Marianna University School of Medicine, Kanagawa, Japan

In conjunction with subject announcement, there are not the companies in the COI relations that I should disclose.

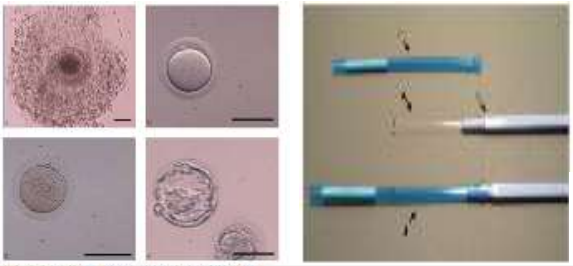
Ovarian tissue cryopreservation

Highly efficient vitrification method for cryopreservation of human oocytes

Masashige Kuwayama (PhD) is currently the Scientific Director of Kato Ladies' Clinic (Tokyo, Japan), the world's largest IVF unit. In 1986, he began work in the field of embryology with Dr Hanada. They developed assisted reproduction techniques (IVM, IVF, vitrification, embryo culture, ES cell) and established a bovine embryo mass production system as the leader of a National Project in Japan in 1990. He obtained the first calves after oocyte vitrification, IVF, in-vitro culture and blastocyst transfer in 1992. He moved to human IVF in 1999, developed the Cryotop vitrification method for human oocytes and established the first human oocyte bank in 2001. The first babies following oocyte vitrification in USA and Japan were obtained by his group using the Cryotop method. He is also interested in rejuvenescence of old defective oocytes, and obtained the first calf from old infertile cattle with germinal vesicle transfer in 2002.

Dr Masashige Kuwayama

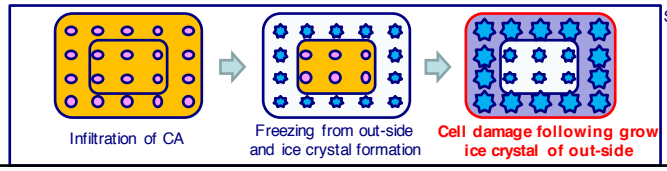
Kuwayama et al: Reproductive Bio Medicine Online 2005



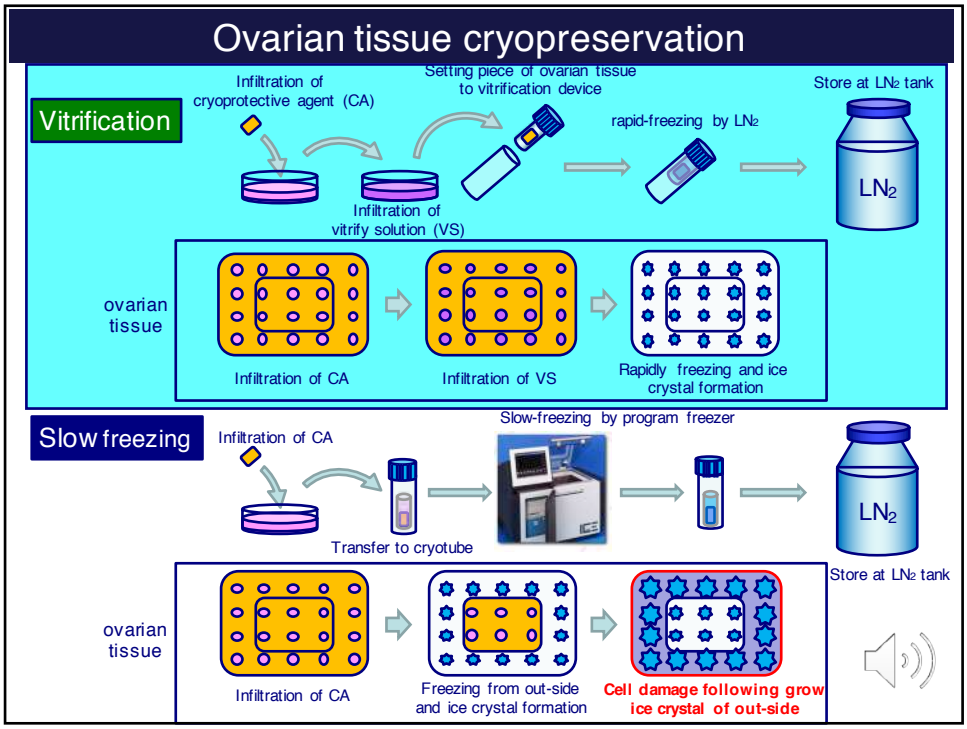
Ovarian tissue cryopreservation

Vitrification

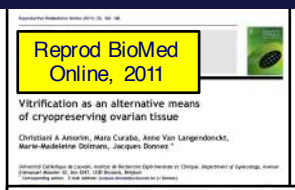
Slow freezing



Ovarian tissue cryopreservation



Slow freezing vs vitrification



Final considerations

As previously stated, vitrification appears to offer a quick, easy and inexpensive means of cryopreserving ovarian tissue that does not require special equipment, and studies on human and different animal species have shown promising results. There is nevertheless room for improvement as

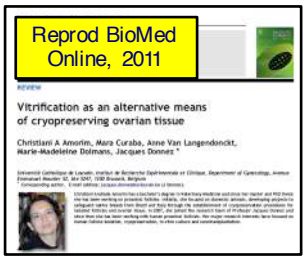
Table 1 Comparison of vitrification and slow freezing for cryopreservation of ovarian tissue.

Characteristics	Vitrification	Slow-freezing
Direct contact with liquid nitrogen	Yes	No
Ice formation	No	Yes
Time	Fast (minutes)[a]	Slow (hours)
CPA equilibration	Yes	Yes
CPA concentration	High (over 40%)	Low (10–15%)
Sample size (human)	Up to 5 × 1 × 1 mm[b]	Up to 2 × 4 × 12 mm[c]
Cooling rates (°C/min)	15,000–30,000	0.15–0.30
Cost	Protocol-dependent (usually inexpensive)	Equipment-dependent (usually expensive)
Special equipment	No	Yes
Technical expertise	Risky	Simple
Routinely applied for cryopreservation of human ovarian tissue	No	Yes

Adapted from Moore and Bonilla (2006).
[a]Considering just one cryocycle.[b]Li et al. (2007) and Huang et al. (2008).[c]Donnez et al. (2004).

Slow freezing vs vitrification

Reprod BioMed Online, 2011



Vitrification as an alternative means of cryopreserving ovarian tissue

Christiani A Amorim, Mara Curaba, Anne Van Langendonckt, Marie-Madeleine Dolmans, Jacques Donnez

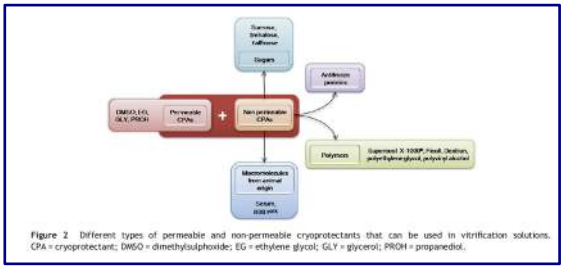


Figure 2 Different types of permeable and non-permeable cryoprotectants that can be used in vitrification solutions. CPA = cryoprotectant; DMSO = dimethylsulphoxide; EG = ethylene glycol; GLY = glycerol; PROH = propanediol.

Suitable Vitirification solutions?

- ✓ Toxicity
- ✓ Feasibility

Penetration of the entire tissue

- ✓ Requires good training in the beginning
- ✓ Thinner is the best
- ✓ Risk in technical expertise

Human Reproduction 2009

human reproduction

ORIGINAL ARTICLE *Reproductive biology*

Vitrification versus controlled-rate freezing in cryopreservation of human ovarian tissue

Victoria Keros[1,†], Susanna Xella[2,†], Kjell Hultenby[3], Karin Pettersson[1], Maryam Sheikhi[1], Annibale Volpe[2], Julius Hreinsson[1,4,†], and Outi Hovatta[1,5,†]

[1]Department of Clinical Science, Technology and Intervention, Division of Obstetrics and Gynaecology, Karolinska Institutet, Karolinska University Hospital Huddinge, K 57, SE 141 86 Stockholm, Sweden [2]Fertility Unit, Mother-Infant Department, Institute of Obstetrics and Gynaecology, University of Modena and Reggio Emilia, 41100 Modena, Italy [3]Department of Laboratory Medicine, Karolinska University Hospital Huddinge, SE 141 86 Stockholm, Sweden [4]Department of Women's and Children's Health, Uppsala University, SE 751 85 Uppsala, Sweden

[5]Correspondence address. Tel: +46-8-58583858; Fax: +46-8-5858-575, E-mail: Outi.Hovatta@ki.se

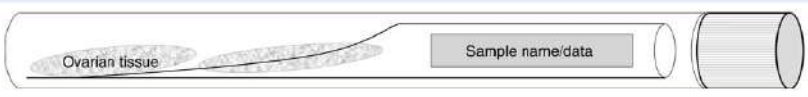


Figure 2 Schematic representation of the device used for vitrification of human ovarian tissue. Pieces of ovarian cortical tissue are placed on a 0.5 ml hand-cut insemination cryostraw and enclosed in a 5.0 ml Nunc cryotube for sto[illegible] nitrogen.

Ovarian tissue vitrification

Human Reproduction 2009

human reproduction ORIGINAL ARTICLE *Reproductive biology*

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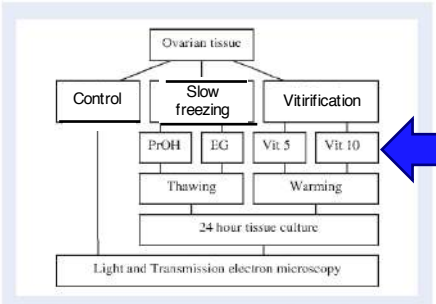
Vitrification

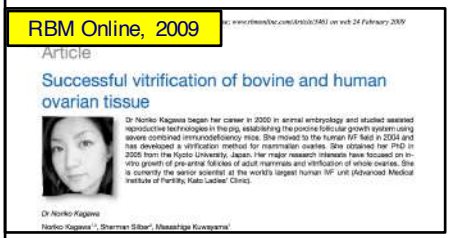


EG
+
DMSO
+
PrOH
+
Sucrose

- ✓ The ovarian stroma was significantly better preserved after vitrification than after slow freezing.
- ✓ On the other hand, the follicles were similarly preservaed after all freezing methods.

Ovarian tissue vitrification

RBM Online, 2009



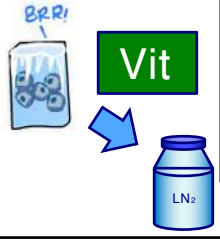
Article

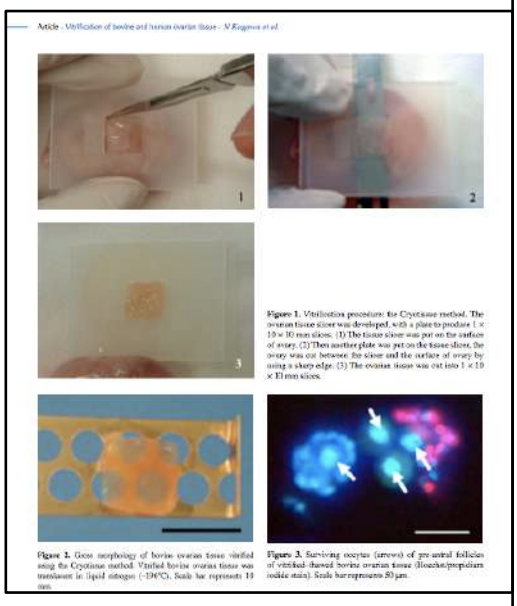
Successful vitrification of bovine and human ovarian tissue

Dr Noriko Kagawa began her career in 2000 in animal embryology and studied assisted reproductive technologies in the pig, establishing the porcine follicular growth system using severe combined immunodeficiency mice. She moved to the human IVF field in 2004 and has developed a vitrification method for mammalian ovaries. She obtained her PhD in 2005 from the Kyoto University, Japan. Her major research interests have focused on in-vitro growth of pre-antral follicles of adult mammals and vitrification of whole ovaries. She is currently the senior scientist at the world's largest human IVF unit (Advanced Medical Institute of Fertility, Kato Ladies' Clinic).

Dr Noriko Kagawa

Noriko Kagawa[1,2], Sherman Silber[2], Masashige Kuwayama[1]





Article · Vitrification of bovine and human ovarian tissue · N Kagawa et al.

Human Reproduction 2013

Reprod. Advance Access published February 20, 2013

GINAL ARTICLE *Infertility*

Morphological and functional preservation of pre-antral follicles after vitrification of macaque ovarian tissue in a closed system

A. Y. Ting[1], R. R. Yeoman[1], J. R. Campos[1,2], M. S. Lawson[1], S. F. Mullen[3], G. M. Fahy[4], and M. B. Zelinski[1,*]

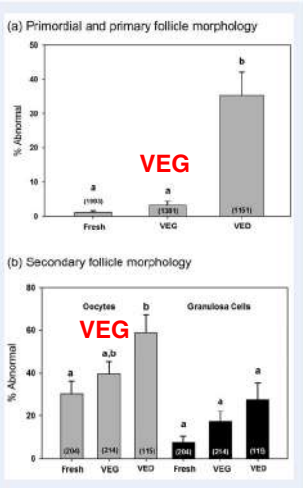


Figure 4 Macaque follicle morphology after vitrification. Percentages (mean ± SEM, n = 4) of abnormal primordial and primary follicles (a) as well as abnormal oocyte and granulosa cells found in

VEG :
25% EG+ 25% Glycerol + 0.2% PVP + 0.2% PVA + 0.4% PG

VED :
25% EG+ 25% DMSO + 0.2% P + 0.2% PVA + 0.4% PG

Figure 1 The vitrification straw and examples of the process used for macaque ovarian tissue. A high-security tissue straw (diameter = 6 mm, length = 65 mm) before (a) and after (b) VS loading and heat seal. During cooling in LN_2 vapor, VS with sufficient concentrations of CPAs vitrified (c), whereas VS with inadequate CPA formed ice crystals (d). During the two-step warming procedure, immediately after samples are plunged into a warm water bath, VS with sufficient CPAs did not show devitrification (e), whereas previously vitrified VS with inadequate CPA formed ice during warming (f). Rapid cooling (directly into LN_2) and warming (directly into a water bath) caused extensive fracture (c and e, inserts).

Slow freezing vs vitrification

JARG, 2015

FERTILITY PRESERVATION

Impact of the cryopreservation technique and vascular bed on ovarian tissue transplantation in cynomolgus monkeys

M. M. Dolmans[1,4] · M. M. Binda[1] · S. Jacobs[2] · J. P. Dehou
J. Ambroise[5] · J. Donnez[6] · C. A. Amorim[1]

10% DMSO+26% EG+2.5%+PVP +1M sucrose

Methods of OTC

Fresh ovarian tissue

Slow freezing

Vitrification

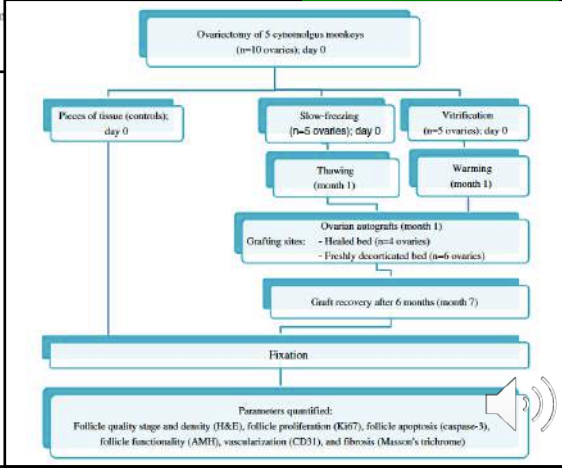
VS

Grafting sites

A healed bed

Freshly decorted bed

VS



Slow freezing vs vitrification

JARG, 2015

J Assist Reprod Genet (2015) 32:1251–1262
DOI 10.1007/s10815-015-0542-y

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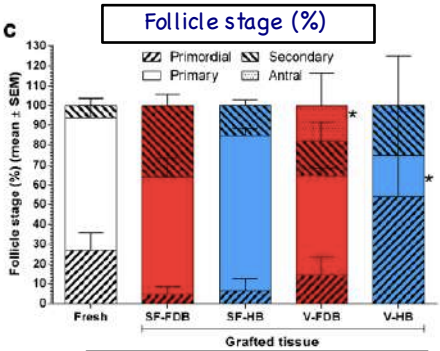
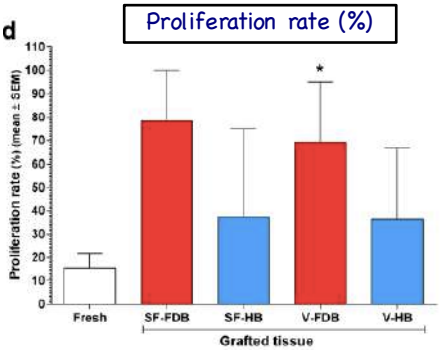
Follicle stage (%)



Proliferation rate (%)



vitrified tissue grafted to a freshly decorticated vascular bed

vitrified tissue grafted to a freshly decorticated vascular bed

Slow freezing vs vitrification

JARG, 2015

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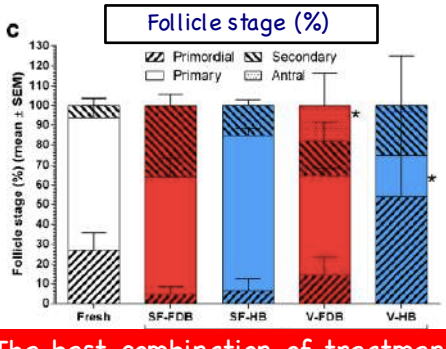
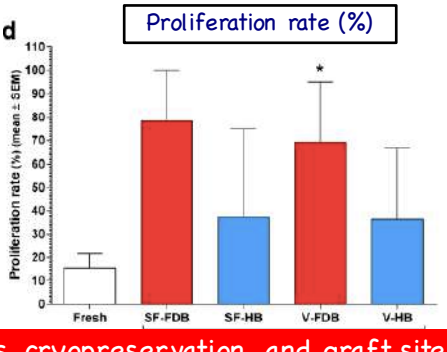


Proliferation rate (%)



The best combination of treatments, cryopreservation, and graft site preparation was vitrification and grafting to the freshly decorte bed.

Slow freezing vs vitrification

Fertility and Sterility, 2016

ORIGINAL ARTICLES: FERTILITY PRESERVATION

Improving ovarian tissue cryopreservation for oncologic patients: slow freezing versus vitrification, effect of different procedures and devices

Sonia Herraiz, Ph.D.,[a,b] Edurne Novella-Maestre, Ph.D.,[a,b,c] Beatriz Rodríguez, B.Sc.,[a,b,d] César Díaz, M.D.,[a,b,d] María Sánchez-Serrano, Ph.D., M.D.,[e] Vicente Mirabet, Ph.D.,[f] and Antonio Pellicer, Ph.D., M.D.[a,b,d]

SF VS Vit

SF: ethyl vinyl acetate bag

VT1: VS1 and metaric grids

VT2: VS2 and metaric grids

VT3: VS1 and ethyl vinyl acetate bag

VT4: VS2 and ethyl vinyl acetate bag

VS1: 20% EG+20% DMSO+0.5M sucros...

VS2: 10% EG+10% DMSO+10% PrOH+10%PVP

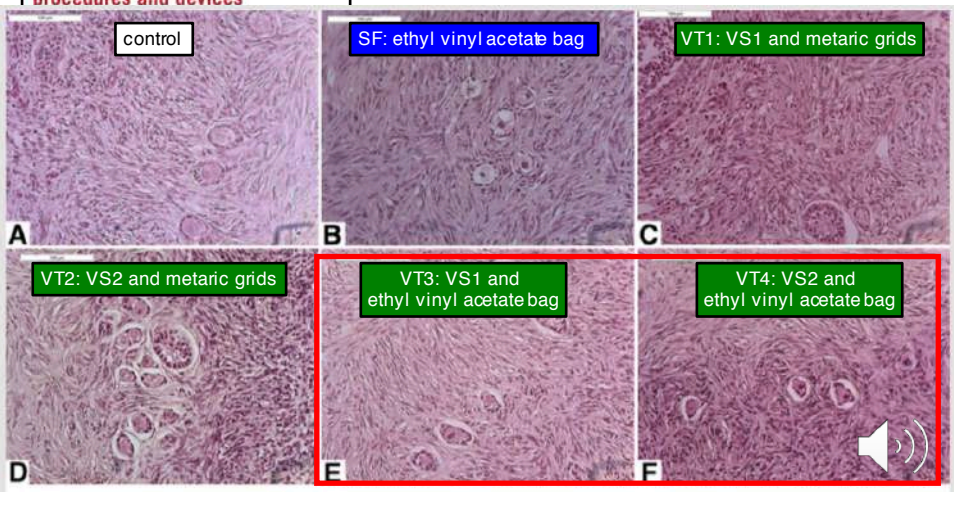
Slow freezing vs vitrification

Fertility and Sterility, 2016

Improving ovarian tissue cryopreservation for oncologic patients: slow freezing versus vitrification, effect of different procedures and devices

Histologic evaluation and follicular population

In vitro study



Slow freezing vs vitrification

Fertility and Sterility, 2016

Improving ovarian tissue cryopr… patient… vitrification… proced…

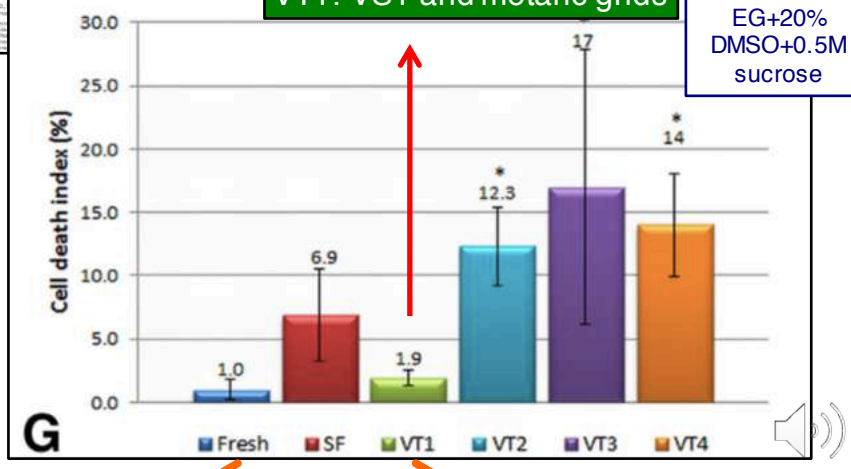
Cell death

Histologic evaluation and follicular population

In vitro study

VT1: VS1 and metaric grids

VS1: 20% EG+20% DMSO+0.5M sucrose



Slow freezing vs vitrification

Fertility and Sterility, 2016

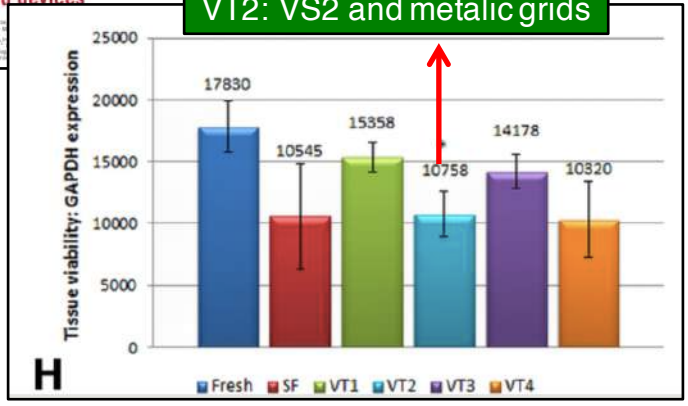
Improving ovarian tissue cr… pa… vi… procedures and devices

Tissue viability

Histologic evaluation and follicular population

In vitro study

VT2: VS2 and metalic grids



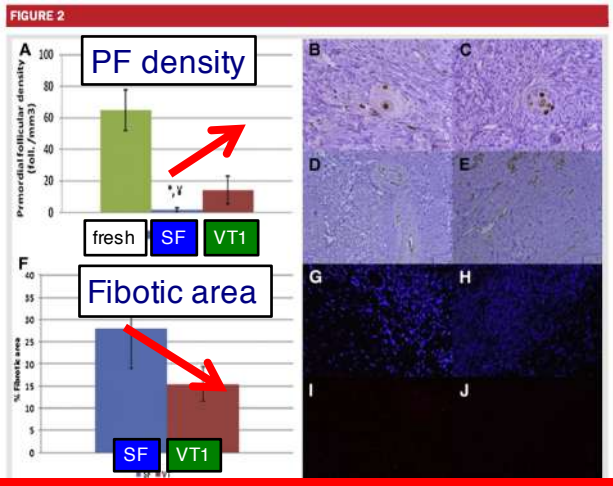
The combination of a metallic grid with the VT1 solution obtained significantly better results than the other experimental groups.

Slow freezing vs vitrification

Fertility and Sterility, 2016

Improving ovarian tissue cryopreservation for oncologic patients: slow freezing versus vitrification, effect of different procedures and devices

In vivo study

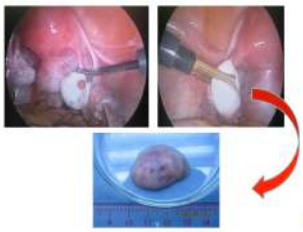


Human ovarian tissue using EG, DMSO, sucrose, and SSS does not affect the normal morphology of oocytes, follicles, and blood vessel distribution after transplantation. Ovarian tissue vitrification offers greater efficiency than slow freezing.

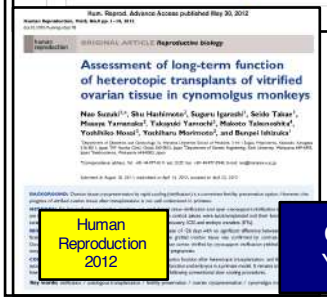
Ovarian tissue vitrification
Pre-clinical study: since 2006

2006～

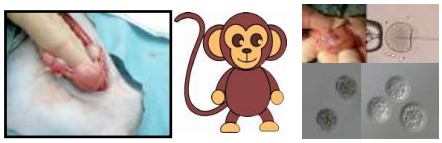
2009～



5.64 M (35% v/v) EG + 5% PVP + 0.5 M sucrose
5min



Human Reproduction 2012



Collaborative research with Dr. Shu Hashimoto and Dr. Yoshiharu Morimoto (IVF Namba Clinic, Osaka, Japan)

Ovarian tissue vitrification & TP: 2010.1-2017.10

Disease	Pts OTC	Pts OTC	Pts TP
Breast Cancer (BC)	64		3
SLE	4		
Uterine cervical cancer (SCC)	2		
Ewing sarcoma	1	**87**	
Leukemia	5		
Malignant Lymphoma	4		
MDS	3		
others	4		
POI		193	153
Total		280	176

Pts.: Number of patients, OTC: ovarian tissue cryopreservation, TP: transplantation

Ovarian tissue vitrification: application to the new treatment for the POI patient

Patient	1	2	3	4	5	6	7
Onset of POI	23y	33y	38y	29y	31y	28y	38y
AMH	4.9 pMol	0.04-0.11 ng/ml	<0.01 ng/ml	<0.01 ng/ml	<0.16 ng/ml	<0.1 ng/ml	<0.01 ng/ml
Age when IVA was done	25y	33y	40y	31y	36y	30y	40y
Pregnancy outcome	Live birth 2012.12	Live birth 2014.5	Chemical abortion	Abortion	Abortion	Live birth 2017.5	Abortion 2017.5

Three live-births were achieved using vitrified-thawed ovarian tissue.

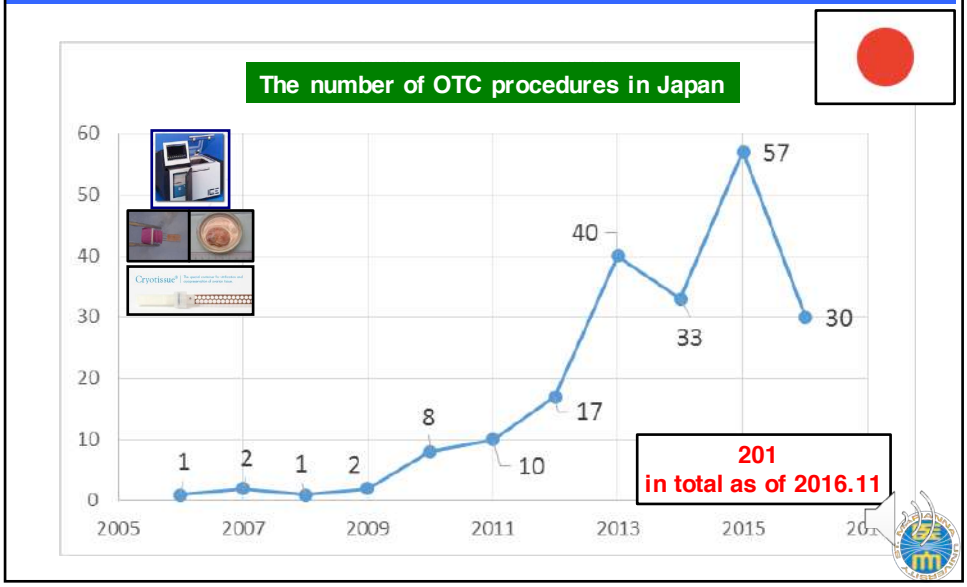
Kawamura K, Suzuki N et al: PNAS 2013

Ova Cryo Device Type M

Suzuki N, et al. Human Reproduction 2015

Research Grant Program on the survey for children and child care promotion on the Ministry of Health, Labor and Welfare in Japan

Research about the effectiveness of Oncofertility treatments for the CAYA cancer patients
By Dr. Seido Takae(St. Marianna University) and Nao Suzuki (PI)



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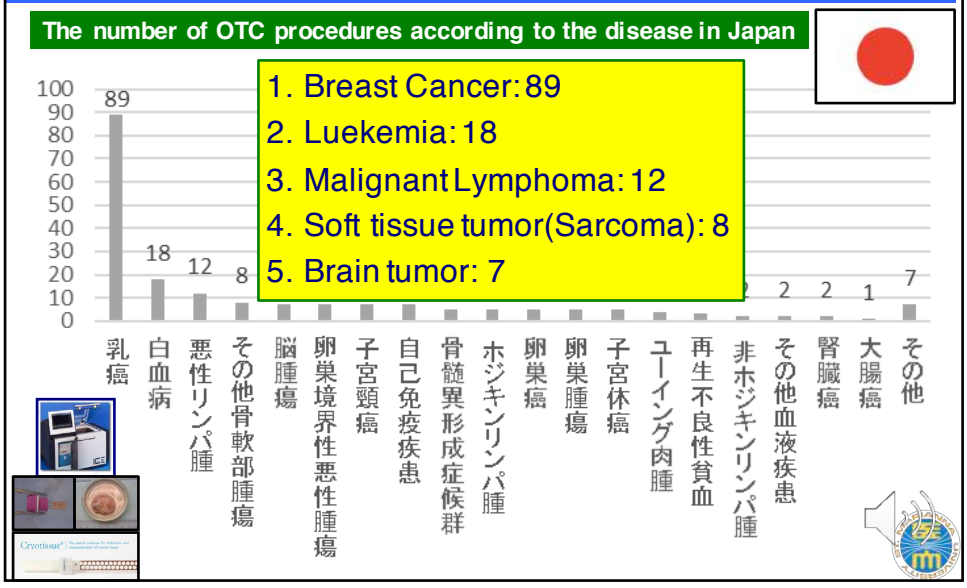


Patients with breast cancer who underwent ovarian tissue transplantaion

Breast Cancer（Luminal type): 39yo (at the time of a diagnosis)

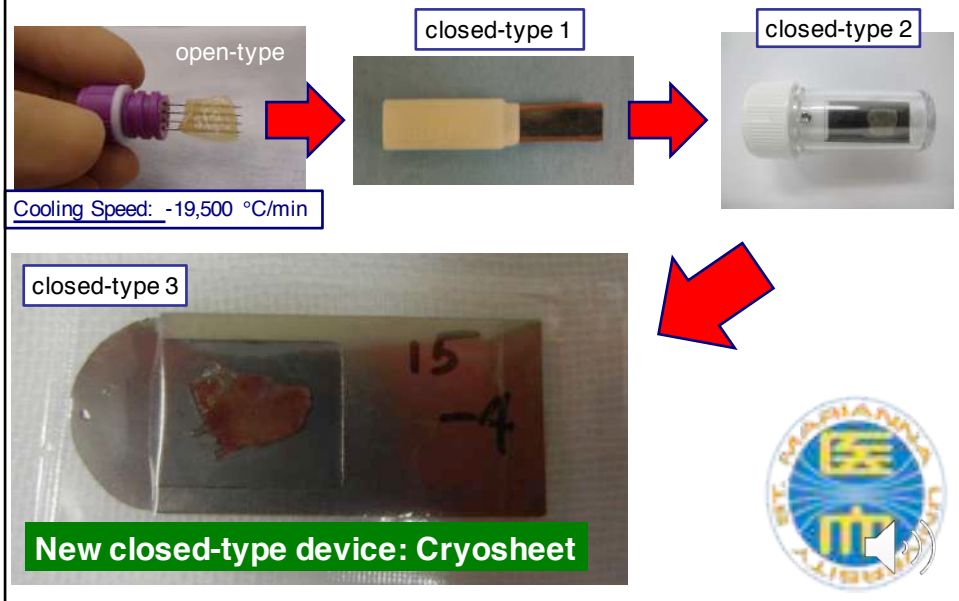
0G0P unmarried
＜FH＞ n.p.

201X.7: Diagnosis of breast cancer cT2N1M0 cStageIIB Luminal type
201X.9: Marriage、**ovayectomy and OTC,** AMH: 28.4pMol/L

201X.10～: **NAC(EC→DTX)**→cPR
201X+1.3 Mastectomy + armpit lymph node dissection
ycT2N1M0 ; ycStageIIB, Luminal type
201X+1.4～ Hormonal therapy (TAM+GnRHa : TAM: until 201X+5.2）

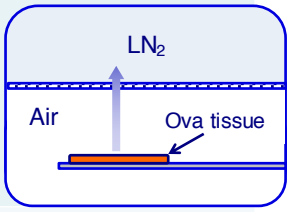
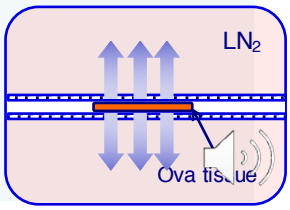
201X+5.9:44yo, Laproscopic ovarian tissue transplantation
【pre-surgery】FSH: 59.8 mIU/ml, E2: <25 pg/ml, AMH: <0.01ng/ml
【pst-surgery】**Menstrual cycle improvement (pre** : 85.0±19.7 days intervals→post : 41.6±17.4days intervals), **AMH: 0.02ng/ml**, egg collection: two times but no eggs

Ovarian Tissue Vitirification: New closed-type device: Cryosheet

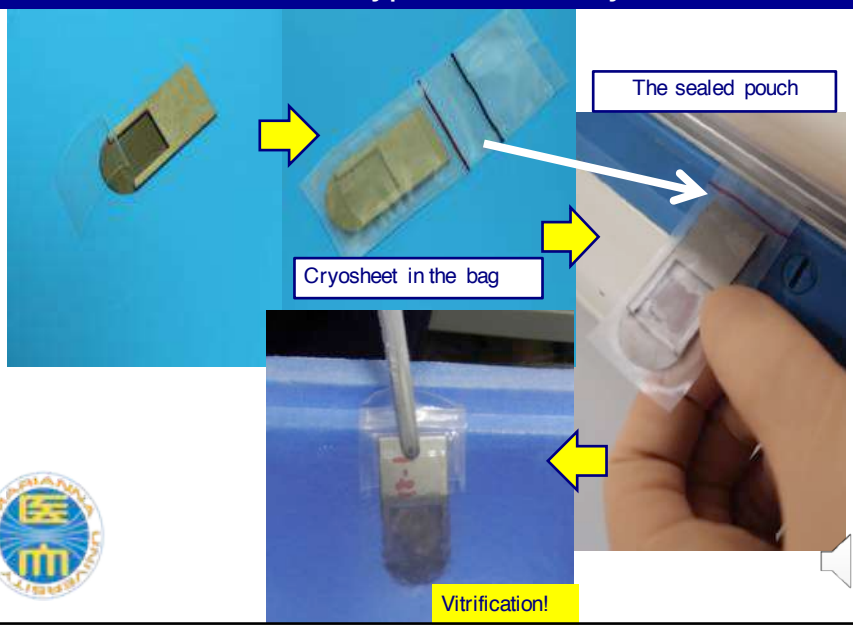


Ovarian Tissue Vitirification: New closed-type device: Cryosheet

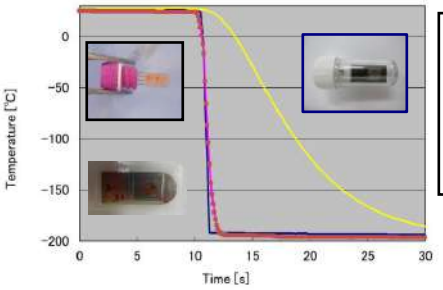
～Improving the cooling speed～

	Problem and improvement	
 Conventional device (closed-type 2)	The distance between ovarian tissue and liquid nitrogen is too great, therefore freezing is slow (<1,000°C/minute)	
 Improved device (closed-type 3: Cryosheet)	Freezing could be speeded up by making the distance between ovarian tissue and liquid nitrogen shorter.	

Ovarian Tissue Vitirification: New closed-type device: Cryosheet

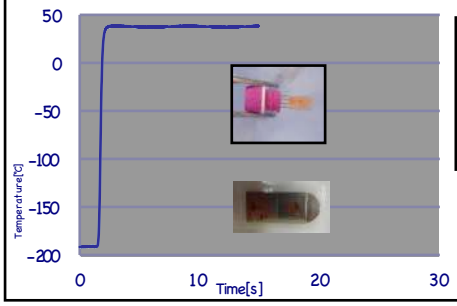


Ovarian Tissue Vitirification: New closed-type device: Cryosheet



Cooling Speed

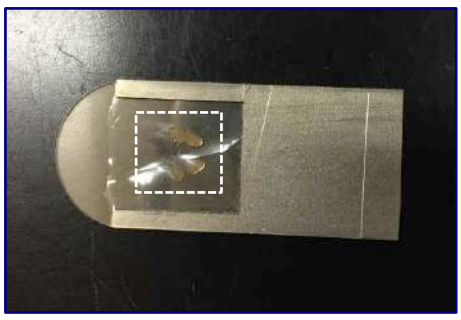
- Cryo Device TypeM:-19,500 °C/min
- Closed-type 2：-13,00°C/min
- Closed-type 3 (Cryosheet)：-12,000°C/min

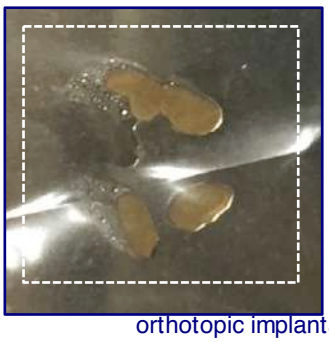


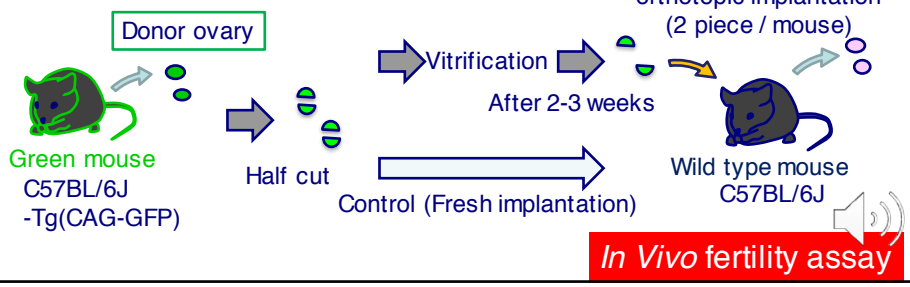
Heating Speed

- Cryo Device TypeM: 42,000 °C/min
- Closed-type 3 (Cryosheet)：42,000°C/min

Ovarian Tissue Vitirification: New closed-type device: Cryosheet

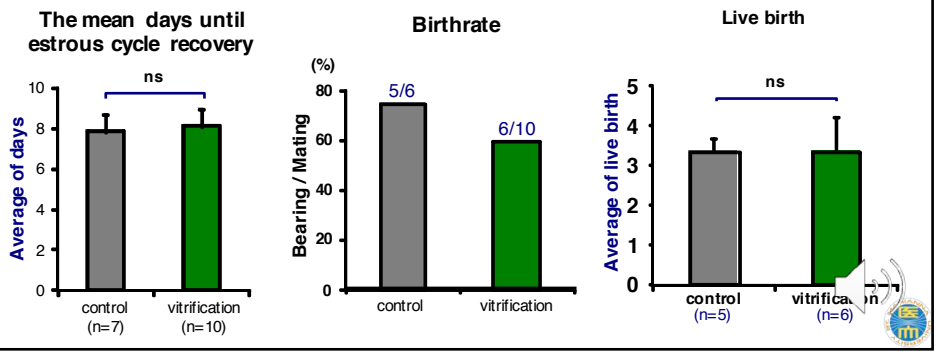






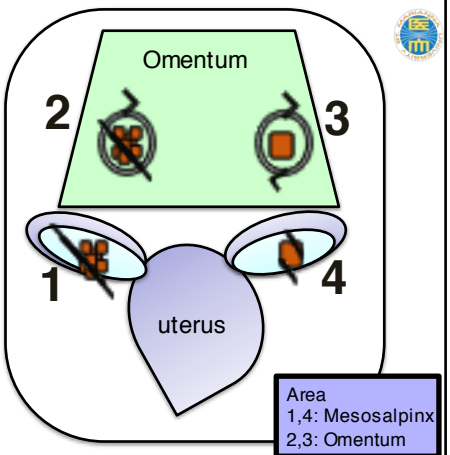
Ovarian Tissue Vitirification: New closed-type device: Cryosheet

Results : *In Vivo* fertility assay



Ovarian Tissue Vitirification: New closed-type device: Cryosheet

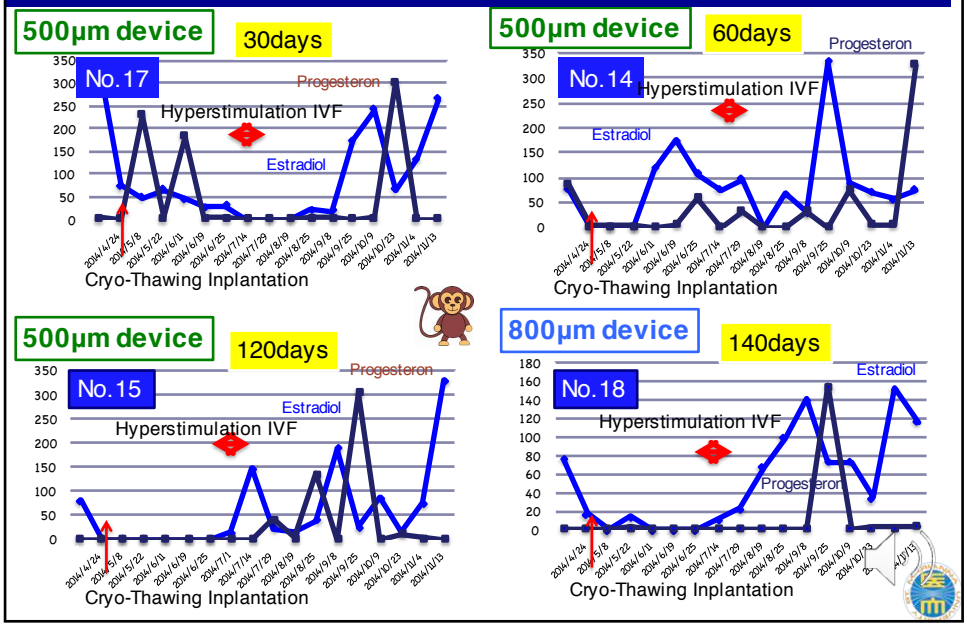
cynomolgus monkeys: n=6



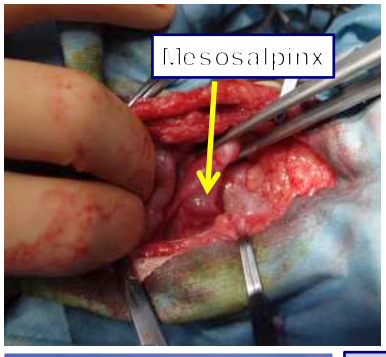
Type M-II Device
- ✓ 500μm Titanium device
- ✓ 800μm Titanium device

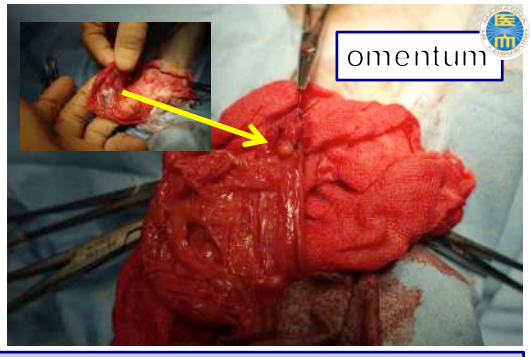
Size and Shape
- ✓ Small Pieces 1,2: Right side:
- ✓ Sheet 2,3: Left side:

Ovarian Tissue Vitirification: New closed-type device: Cryosheet



Ovarian Tissue Vitirification: New closed-type device: Cryosheet





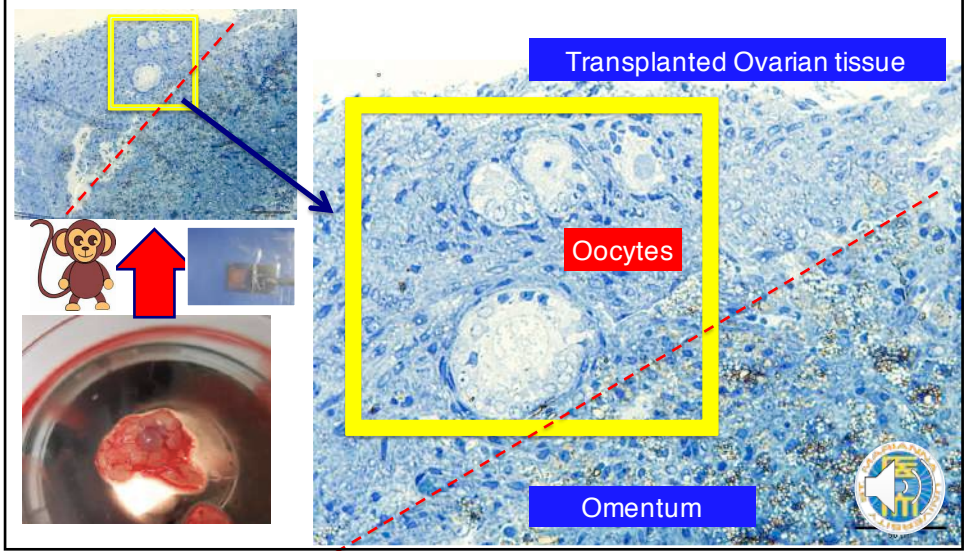
Retrieved Oocytes

Mesosalpinx :Oocyte Number=11
Omentum :Oocyte Number＝5

Sheet ovarian tissue :Oocyte Number=8
Pieces ovarian tissue :Oocyte Number=8

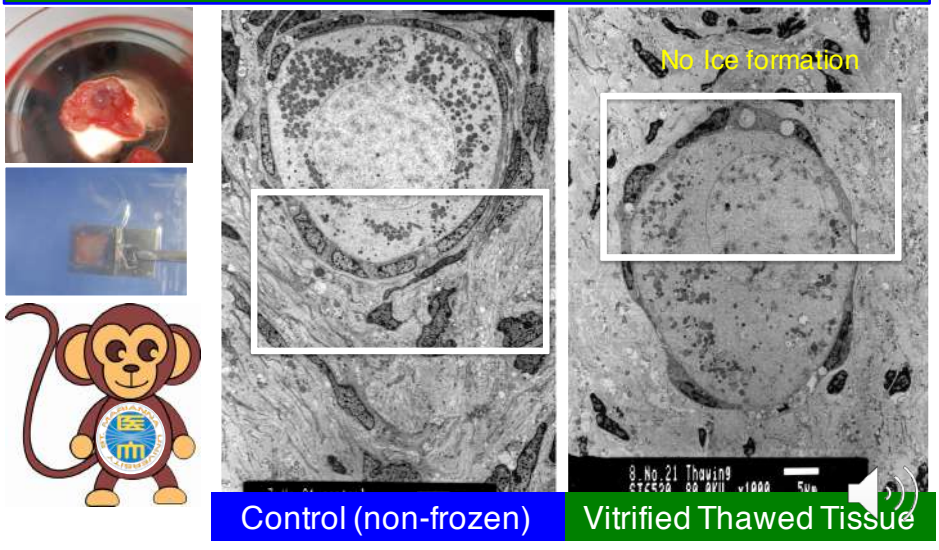
Ovarian Tissue Vitirification: New closed-type device: Cryosheet

No18 Omentum :Gill's Haematoxylin stained

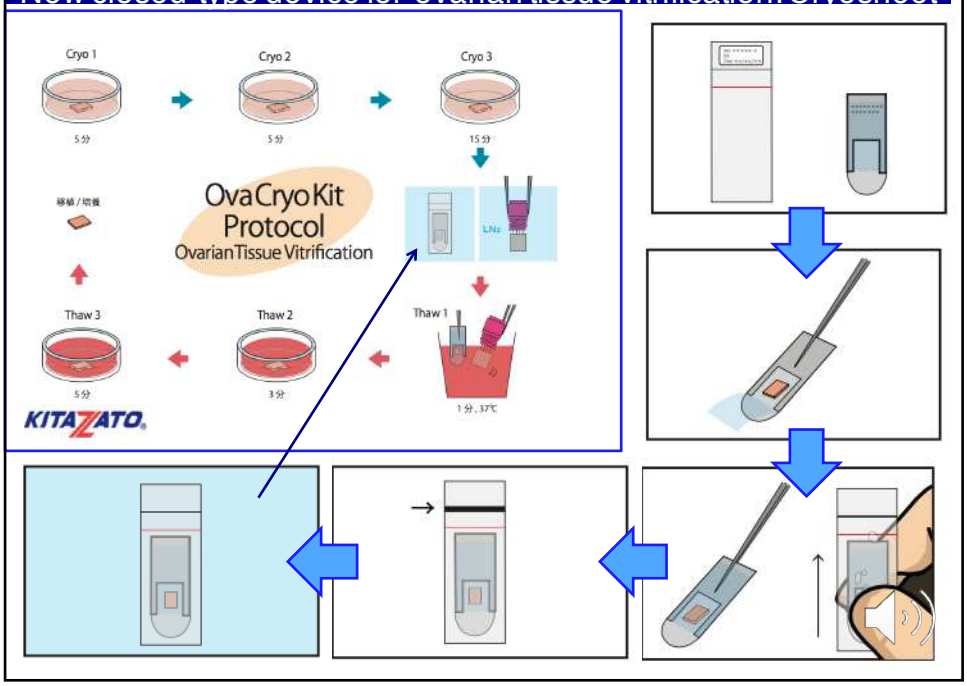


Ovarian Tissue Vitirification: New closed-type device: Cryosheet

No21 Omentum : Transmission-electron microscopy



New closed-type device for ovarian tissue vitrification: Cryosheet



Human ovarian tissue cryopreservation: Sf vs Vit

Donor case 1 : 39years African American/ Hart Attack
Brooklyn Hospital Center
Donor case 2 : 23years White/ Unknown
Brookhaven Memorial Hospital
Donor case 3 : 31years White/ intracranial bleeding
NYC Health Hospitals Jacobi

Sf

Slow freezing

Slow Freezing Solution
MEMα+Sucrose+Dimethyl sulfoxide+Human Serum Albumin

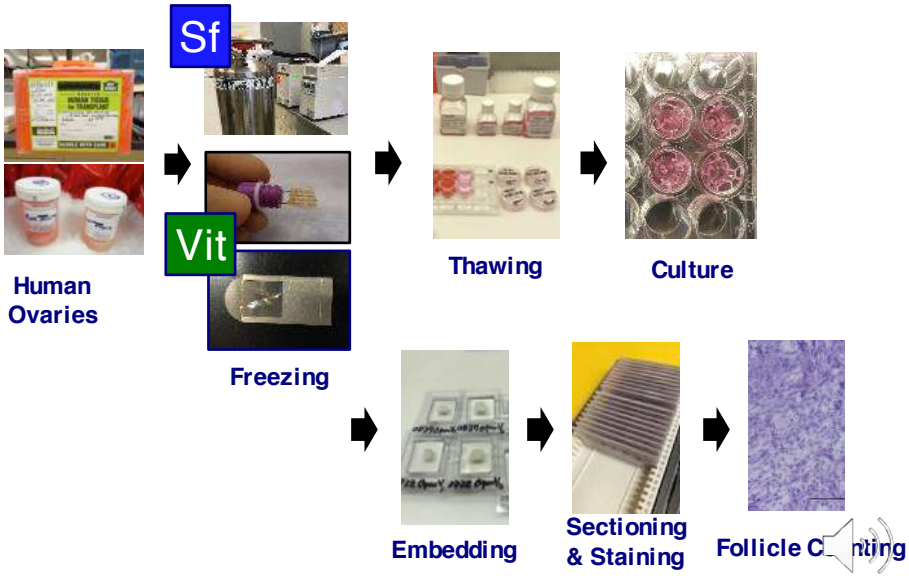
Vitrification

Vit

Vitrification Solution
H199 +Sucrose+Ethylene Glycol +Polyvinylpyrrolidone+Synthetic Serum Substitute

Human ovarian tissue cryopreservation: Sf vs Vit

Summary of Study Design



Human ovarian tissue cryopreservation: OVf vs CVit

Vit

vs

Figure 3. Immunohistochemically stained histological sections from cryopreserved human ovaries using open and closed VF devices. **a,** γH2AX negative pdf; **b,** γH2AX positive pdf; **c,** γH2AX negative pyf; **d,** γH2AX positive pyf; **e,** AC3 negative pdf; **f,** AC3 positive pdf; **g,** AC3 negative pyf; **h,** AC3 positive pyf. (***Pdf***, primordial follicle; ***Pyf***, primary follicle)

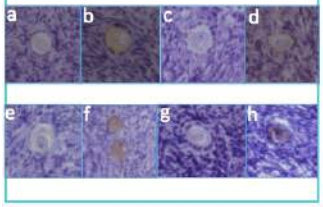


Table 1. Comparison of primordial (pdf) and primary (pyf) follicle densities, oocyte DNA double strand breaks (by γH2AX) and apoptotic cell death pathway activation (AC3) in open and closed VF.

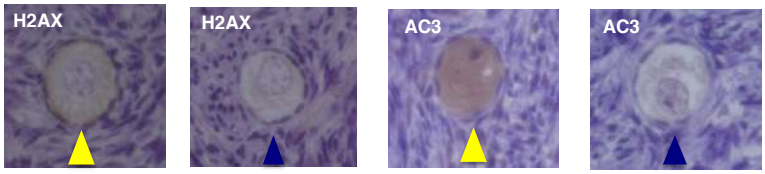
Variables	OV (0h)	OV (24h)	OV (96h)	CV (0h)	CV (24h)	CV (96h)	p-value OV (0h) vs. CV (0h)	p-value OV (24h) vs. CV (24h)	p-value OV (96h) vs. CV (96h)
Pdf Density (/mm²)	62±12.1	56.6±7.5	63.3±16.9	74.6±13.6	73.3±11.7	37.5±3.3	0.26	0.07	0.25
γH2AX+ Pdf (%)	31.6±5.7	30±16.3	35.5±2.8	40.1±7.6	27.3±2.4	20±9.5	0.41	0.88	0.27
AC3+ Pdf (%)	12.8±3.1	14.7±8.2	25±0.5	21.3±5.9	4.3±2	6.6±2.8	0.27	0.29	*0.03*
Pyf Density (/mm²)	6.6±1.5	6±1.7	1.3±1.1	6.6±1.1	4.6±0.5	6.6±1.5	1	0.42	0.15
γH2AX+ Pyf (%)	30±16.3	6.6±5.7	5±4.3	33.3±15.2	5±4.3	5±4.3	0.63	0.42	1
AC3+ Pyf (%)	26±15.3	0	2.5±2.1	12.4±6.1	1.6±1.4	5±4.3	0.34	0.42	0.74

Human ovarian tissue cryopreservation: Sf vs Vit

Impact of Vitrification vs SF on Primordial Follicle Density, DNA DSBs and Apoptosis

γH2AX positive : DNA double-strand-breaks
AC3 positive : Apoptotic follicles

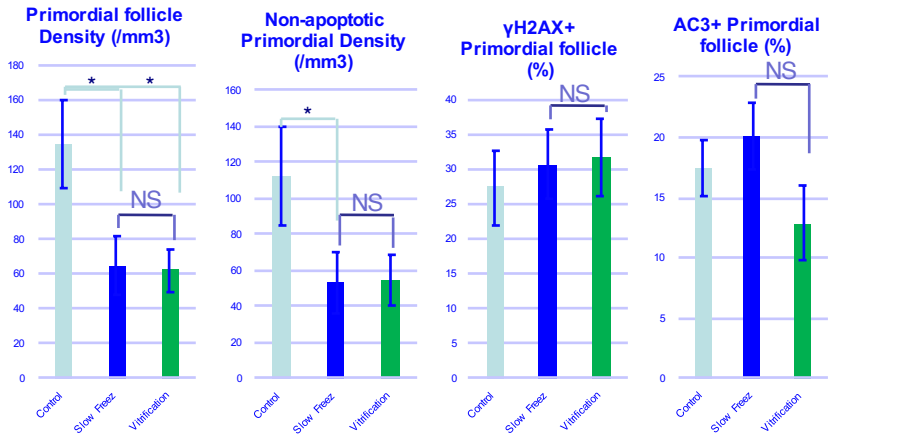
Positive ▲ Negative ▲



	Control	Slow Freezing	Vitrification	Control vs. SF	Control vs. VF	SF vs. VF
Primordial follicle Density (/mm3)	134.6±25	64±17	62±12.1	0.02	0.04	0.77
γH2AX+ Primordial follide (%)	27.3±5.4	30.6±4.9	31.6±5.7	0.46	0.39	0.42
AC3+ Primordial follicle (%)	17.5±2.3	20.1±2.7	12.8±3.1	0.03	0.12	0.06
Non-apoptotic Primordial follicle density (/mm3)	112.6±27.5	52.5±17.2	54.9±14	0.02	0.54	.63

Human ovarian tissue cryopreservation: Sf vs Vit

Impact of Vitrification vs SF on Primordial Follicle Density, DNA DSBs and Apoptosis



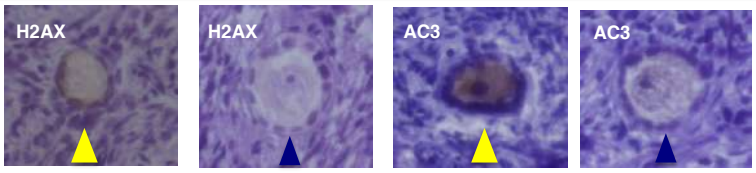
* : Significant
NS: Non Significant

Human ovarian tissue cryopreservation: Sf vs Vit

Impact of Vitrification vs SF on Primary Follicle Density, DNA DSBs and Apoptosis

γH2AX positive : DNA double-strand-breaks
AC3 positive : Apoptotic follicles

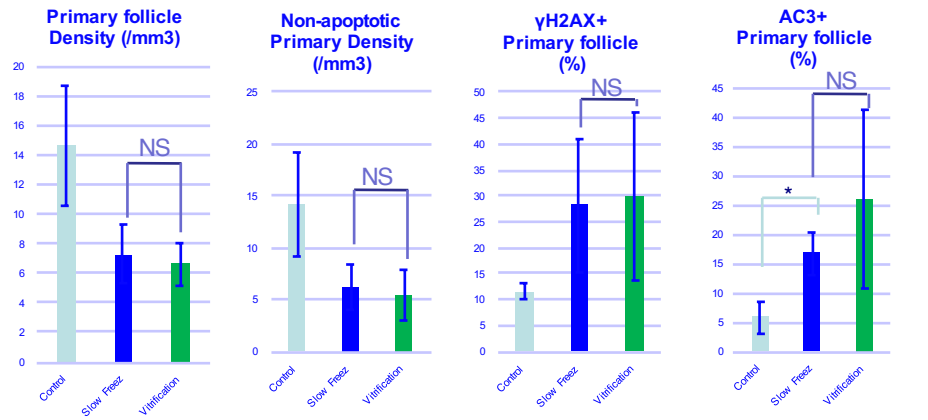
Positive ▲ Negative ▲



	Control	Slow Freezing	Vitrification	Control vs. SF	Control vs. VF	SF vs. VF
Primary follicle Density (/mm3)	14.6±4.1	7.3±2	6.6±1.5	0.09	0.12	0.42
γH2AX+ Primary follide (%)	11.6±1.4	28.3±12.8	30±16.3	0.34	0.4	0.83
AC3+ Primary follicle (%)	5.8±2.6	16.8±3.7	26±15.3	0.03	0.3	0.56
Non-apoptotic Primary follicle density (/mm3)	14.1±5	6.2±2.3	5.5±2.4	0.1	0.08	.16

Human ovarian tissue cryopreservation: Sf vs Vit

Impact of Vitrification vs SF on Primary Follicle Density, DNA DSBs and Apoptosis

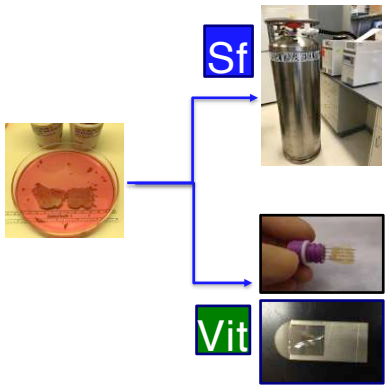


* : Significant
NS: Non Significant

Human ovarian tissue cryopreservation: Sf vs Vit

Summary and Conclusion
Impact of Vitrification vs SF on Follicle Density, DNA DSBs and Apoptosis

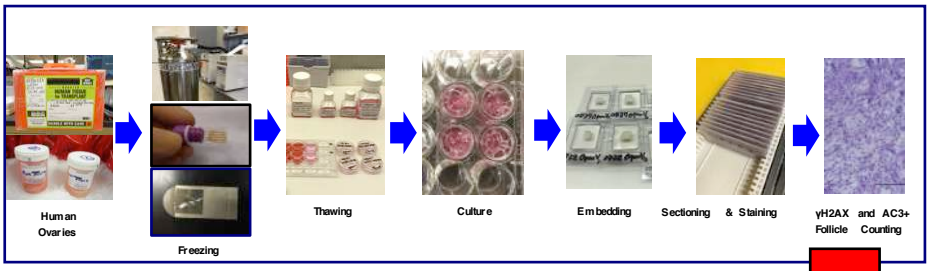
- ✓ **Similar follicle density**
- ✓ **Similar DNA DSB Breaks**
- ✓ **Similar apoptotic activation**



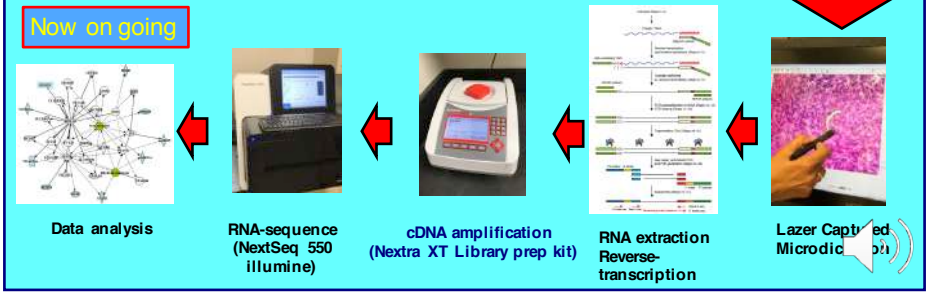
Based on histological and immunohistochemical evaluation, Sf and Vit methods appear to be comparable.

Human ovarian tissue cryopreservation: Sf vs Vit

A Collaborative research with New York Medical Collage and Yale University to improve the ovarian tissue cryopreservation method



Now on going



Slow freezing vs vitrification

Scientific Reports, 2017

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Vitrification versus slow freezing for human ovarian tissue cryopreservation: a systematic review and meta-anlaysis

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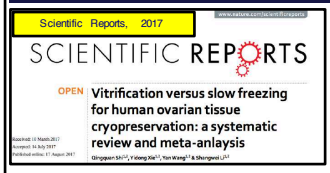
Qingquan Shi[1,2], Yidong Xie[1,2], Yan Wang[1,2] & Shangwei Li[1,2]

Sf vs Vit

Slow freezing vs vitrification



95% CI: 0.97 [0.74-1.28]

Sf Vit

Study or Subgroup	Vitrification Events	Total	Slow freezing Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Amorim et al. 2012	122	204	50	83	8.0%	0.98 [0.58, 1.65]
Chang et al.2011	143	238	18	30	5.9%	1.00 [0.46, 2.18]
Fabbri et al. 2016	225	410	127	232	9.7%	1.01 [0.73, 1.39]
Gandolfi etal. 2006	128	328	149	222	9.5%	0.31 [0.22, 0.45]
Herraiz et al. 2014	421	765	125	220	9.9%	0.93 [0.69, 1.26]
Huang et al. 2008	278	326	250	297	8.7%	1.09 [0.70, 1.69]
Keros et al. 2009	60	126	84	153	8.4%	0.75 [0.47, 1.20]
Li et al. 2007	65	81	69	95	6.4%	1.53 [0.75, 3.11]
Sanfilippo et al. 2015	392	482	293	374	9.6%	1.20 [0.86, 1.69]
Wang et al. 2008	150	182	75	90	6.7%	0.94 [0.48, 1.84]
Xiao et al. 2010	475	582	136	178	9.0%	1.37 [0.91, 2.05]
Xiao et al. 2013	100	139	80	132	8.1%	1.67 [1.00, 2.77]
Total (95% CI)		**3863**		**2106**	**100.0%**	**0.98 [0.74, 1.28]**
Total events	2559		1456			

Heterogeneity: Tau² = 0.17; Chi² = 49.97, df = 11 (P < 0.00001); I² = 78%
Test for overall effect: Z = 0.17 (P = 0.86)

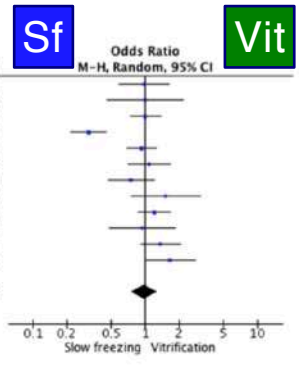


Figure 2. Random effect model of odds ratio with 95% CIs of the proportion of intact primordial follicles: slow freezing versus vitrification.

Slow freezing vs vitrification

Scientific Reports, 2017

SCIENTIFIC REPORTS

OPEN Vitrification versus slow freezing for human ovarian tissue cryopreservation: a systematic review and meta-anlaysis

95% CI: 0.71 [0.62-0.80]

Vit

Sf

Study or Subgroup	Vitrification Events	Total	Slow freezing Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Amorim et al. 2012	6	36	8	20	3.1%	0.42 [0.17, 1.03]
Chang et al.2011	109	240	18	30	9.6%	0.76 [0.55, 1.05]
Herraiz et al. 2014	116	350	66	160	27.3%	0.80 [0.63, 1.02]
Sanfilippo et al. 2015	13	64	9	29	3.7%	0.65 [0.32, 1.36]
Xiao et al. 2010	167	597	78	184	35.9%	0.66 [0.53, 0.82]
Xiao et al. 2013	48	149	67	145	20.4%	0.70 [0.52, 0.93]
Total (95% CI)		**1436**		**568**	**100.0%**	**0.71 [0.62, 0.80]**
Total events	459		246			

Heterogeneity: Chi² = 3.05, df = 5 (P = 0.69); I² = 0%
Test for overall effect: Z = 5.38 (P < 0.00001)

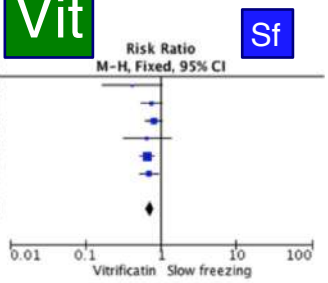


Figure 3. Fixed model of risk ratio with 95% CIs of the DNA fragmentation in primordial follicles: vitrification versus slow freezing.

Slow freezing vs vitrification

Scientific Reports, 2017

SCIENTIFIC REPORTS

OPEN Vitrification versus slow freezing for human ovarian tissue cryopreservation: a systematic review and meta-anlaysis

95% CI: 1.69 [1.47-1.94]

Sf

Vit

Study or Subgroup	Vitrification Events	Total	Slow freezing Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
4.1.1 equilibration for 10min						
Chang et al.2011	181	300	138	300	15.5%	1.31 [1.13, 1.53]
Keros et al. 2009	6971	7708	6530	19144	18.9%	2.65 [2.60, 2.71]
Wang et al. 2008	55	100	40	100	10.3%	1.38 [1.02, 1.85]
Xiao et al. 2010	318	600	58	200	12.6%	1.83 [1.45, 2.30]
Subtotal (95% CI)		**8708**		**19744**	**57.3%**	**1.73 [1.12, 2.69]**
Total events	7525		6766			
Heterogeneity: Tau² = 0.19; Chi² = 107.57, df = 3 (P < 0.00001); I² = 97%						
Test for overall effect: Z = 2.45 (P = 0.01)						
4.1.2 equilibration for 5min						
Chang et al.2011	141	300	138	300	14.8%	1.02 [0.86, 1.21]
Keros et al. 2009	6983	7619	6530	19144	18.9%	2.69 [2.63, 2.74]
Wang et al. 2008	39	100	40	100	8.9%	0.97 [0.69, 1.37]
Subtotal (95% CI)		**8019**		**19544**	**42.7%**	**1.40 [0.64, 3.08]**
Total events	7163		6708			
Heterogeneity: Tau² = 0.47; Chi² = 153.74, df = 2 (P < 0.00001); I² = 99%						
Test for overall effect: Z = 0.84 (P = 0.40)						
Total (95% CI)		**16727**		**39288**	**100.0%**	**1.69 [1.47, 1.94]**
Total events	14688		13474			
Heterogeneity: Tau² = 0.03; Chi² = 262.31, df = 6 (P < 0.00001); I² = 98%						
Test for overall effect: Z = 7.29 (P < 0.00001)						
Test for subgroup differences: Chi² = 0.21, df = 1 (P = 0.64), I² = 0%						

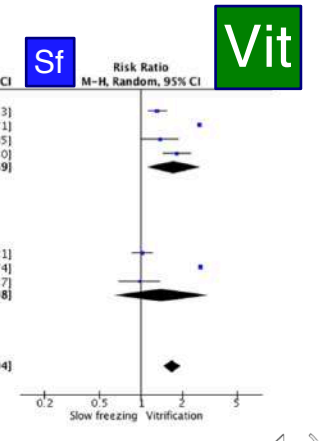


Figure 4. Random effect model of risk ratio with 95% CIs of the proportion of normal stromal cells: slow freezing versus vitrification.

Slow freezing vs vitrification

Scientific Reports, 2017

SCIENTIFIC REPORTS

OPEN Vitrification versus slow freezing for human ovarian tissue cryopreservation: a systematic review and meta-anlaysis

95% CI: 3.44 [-5.09-11.98]

Sf Vit

Study or Subgroup	Vitrification Mean	SD	Total	Slow freezing Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Fabbri et al. 2016	3.84	5.36	410	4.12	5.65	232	33.3%	-0.28 [-1.17, 0.61]
Herraiz et al. 2014	15.3	3.8	756	4.4	0.7	220	33.4%	10.90 [10.61, 11.19]
Oktem et al. 2011	0.97	0.1	728	1.27	0.1	953	33.4%	-0.30 [-0.31, -0.29]
Total (95% CI)			**1894**			**1405**	**100.0%**	**3.44 [-5.09, 11.98]**

Heterogeneity: $Tau^2 = 56.86$; $Chi^2 = 5874.85$, df = 2 ($P < 0.00001$); $I^2 = 100\%$

Test for overall effect: $Z = 0.79$ ($P = 0.43$)

Mean Difference IV, Random, 95% CI

-100 -50 0 50 100

Slow freezing Vitrificatipon

Figure 5. Random effect model of the mean difference with 95% CIs of the primordial follicle density: slow freezing versus vitrification.

Slow freezing vs vitrification

Scientific Reports, 2017

www.nature.com/scientificreports

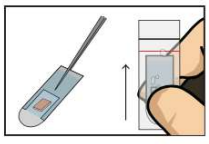
SCIENTIFIC REPORTS

OPEN Vitrification versus slow freezing for human ovarian tissue cryopreservation: a systematic review and meta-anlaysis

Received: 10 March 2017
Accepted: 14 July 2017

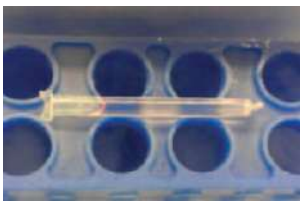
less primordial follicular DNA strand breaks and better preservation of stromal cells.

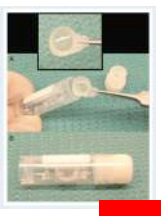
Ova Cryo Device Type M



Future: Ovarian tissue vitrification

Device





?

PrOH
EG
DMSO
PVP
Sucrose
Glycerol
PVA
Trehalose

Solution

Vitrification

Ovarian tissue cryopreservation

"Vitrificaion of human ovarian tissue??

PROS	CONS
1. Excellent survival of ovarian stroma and blood vessels	
2. A fast method	
3. No particular apparatuses needed	
4. Also feasible clinically	
5. Excellent survival in tissue culture	

Dr. Hovatta, World Congress on Fertility Preservation 2009 in Belgium

Ovarian tissue cryopreservation

"Vitrificaion of human ovarian tissue??

PROS	CONS
1. Excellent survival of ovarian stroma and blood vessels	1. No live births reported so far
2. A fast method	2. Requires good training in the beginning
3. No particular apparatuses needed	3. Concentrations and osmolarities and the speed of the procedure are of extremely high importance
4. Also feasible clinically	
5. Excellent survival in tissue culture	4.Toxic influence on the tissue is possible if less trained personnel

Dr. Hovatta, World Congress on Fertility Preservation 2009 in Belgium

Ovarian tissue cryopreservation

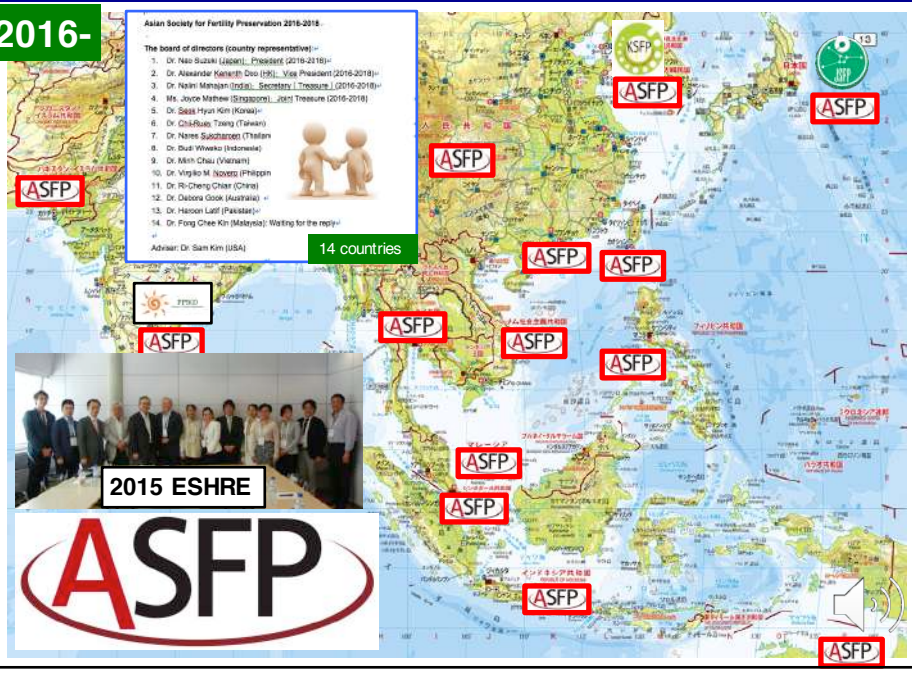
"Vitrificaion of human ovarian tissue??

PROS	CONS
1. Excellent survival of ovarian stroma and blood vessels	1. No live births reported so far(at that time)
2. A fast method	2. Requires good training in the beginning
3. No particular apparatuses needed	3. Concentrations and osmolarities and the speed of the procedure are of extremely high importance
4. Also feasible clinically	
5. Excellent survival in tissue culture	4.Toxic influence on the tissue is possible if less trained personnel

Dr. Hovatta, World Congress on Fertility Preservation 2009 in Belgium

ASFP (Asian Society for Fertility Preservation): 2016-

2016-



The 1st Congress of ASFP; 2016.11.18-19
Ho Chi Minh City, Vietnam

ASFP

The 1st Workshop and Symposium of ASFP; 2017.9.23-24. Shanghai, China

Hands on seminar: Ovarian tissue vitrification

At St. Marianna University, Japan:
w/member of Seoul Natl Univ.
2014.2.10-13

In India: Fertility Preservation Society (India)
New Deli, India
2014.9.7-8

At St. Marianna University, Japan:
w/ Dr. Kemar, Dr. Mila And Dr. Kock
2014.11

In Indonesia: Reproductive Medicine Society
Medan, Indonesia
2015.2.3

Hands on seminar and Ovariectomy and ovarian tissue vitrification for 2 young cancer patients.
Almaty, Republic of Kazakhstan
2015.5.1-2

At St. Marianna University, Japan:
w/ Dr. Novero's team and Dr. Lee
2015.8.15

Hands on seminar: Ovarian tissue vitrification

In Philippines: OVARIAN TISSUE CRYOPRESERVATION POST GRADUATE COURSE AND WORKSHOP.
w/ Member of Dr. Novero's team
Manila, Philippines
2016.2.24

At St. Marianna University, Japan:
w/ Member of Dr. Sirayapiwat and Ms. Numchaisrika
2016.3.16

ASPIRE2016,
Jakarta, Indonesia
2016.4.7

Ovariectomy and ovarian tissue vitrification for 2 POI patients.
Shanghai, China.
2016.11.14

At St. Marianna University, Japan:
w/ Professor Lee's te[illegible] from China
2016.9.27

"PROS of ovarian tissue vitrification are a fast method and no particular apparatuses needed." It is easily handled for anyone at anywhere in anytime"

Take home message

- Based on several data, Sf and Vit methods appear to be comparable.
- Ovarian tissue vitrification may be a promising new fertility preservation method for young cancer patients.

- ✓ Excellent survival of ovarian stroma and blood vessels
- ✓ A fast method
- ✓ No particular apparatuses needed
- ✓ Also feasible clinically
- ✓ Excellent survival in tissue culture
- ✓ **It is easily handled for anyone at anywhere in anytime!!**