

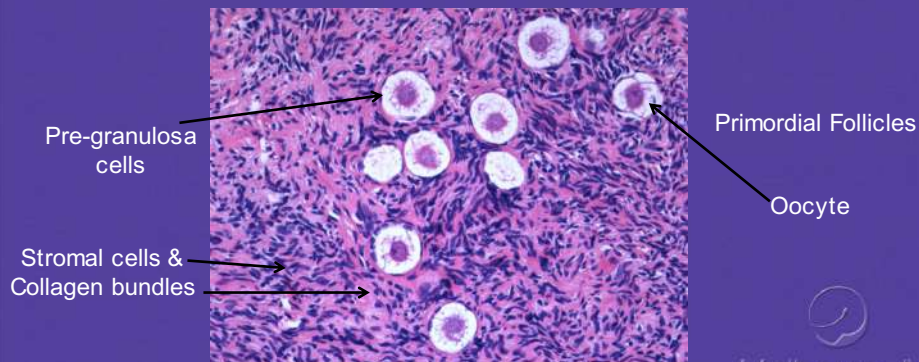
Cryopreservation of Ovarian Tissue Slow Freezing

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Aims of Cryopreservation of Human Ovarian Cortex

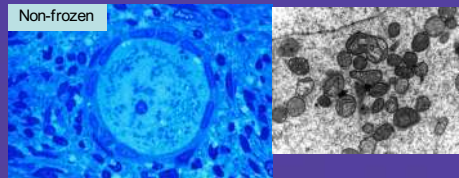
- Preserve multiple cell types
- Preserve intra & extra cellular organisation



Cryopreservation of Ovarian Cortex

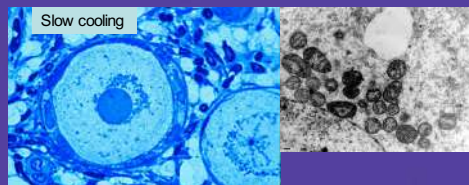
Slow control rate freezing

3 dehydration regimens, DMSO, EG, PROH



Gook 1999, 2003

PROH +sucrose dehydration: 90 min



MelbourneIVF
Excellence in fertility care

Functional Preservation

Xenografting
Clinical evidence

MelbourneIVF
Excellence in fertility care

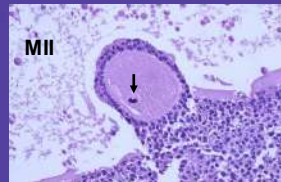
Xenografting



Multiple healthy follicles



Gook 2001



MII oocytes (diameter 116 μm)

Human OT frozen with PROH+ sucrose



Reproducible follicle development in human slow frozen ovarian tissue

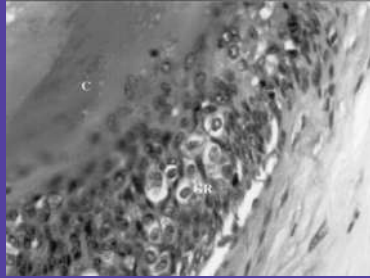
Patient	Cancer	Age	Number (per graft)			★ = M II
			Total	Large Antral	Max Diameter	
A	ALL	18	21 (2.6)	1 (0.1)	2	★
B	ALL	18	137 (8.6)	29 (1.8)	5	
C	AML	18	22 (2.7)	0	1	
D	Spinal Tumour	18	11 (1.8)	6 (1)	3.5	★
E	Osteo	20	9 (1.5)	2 (0.3)	4	★★★
F	Osteo	20	194 (9.7)	9 (0.4)	5	★★★★★
G	Lymph	22	5 (0.8)	4 (0.7)	3	
H	Breast	21	13 (2.2)	1 (0.2)	2.6	
J	Breast	31	36 (2.6)	6 (0.4)	6	★
Total			448 (5.0)	58 (0.6)		

Frozen with PROH + Sucrose



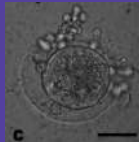
Functional Preservation with Slow Freezing using **DMSO**

Kim. Xenotransplantation of human ovarian tissue. Fertil Steril 2002.



Corpus luteum

Kim. Assessment of the integrity of human oocytes retrieved from cryopreserved ovarian tissue after xenotransplantation Human Reproduction 2005



MII oocyte



Unequivocal evidence of successful cryopreservation of primordial follicles

Slow frozen with PROH



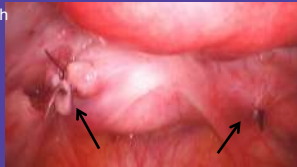
Size 1x1x3-4 mm



15 slices per suture

25 yo Ovarian granulosa cell tumor bilateral oophorectomy

1 suture each L&R pelvic side wall



1 suture each L&R abdominal wall



Second graft evidence of activity in first graft L&R abdominal wall



Stem 2013 fertility care

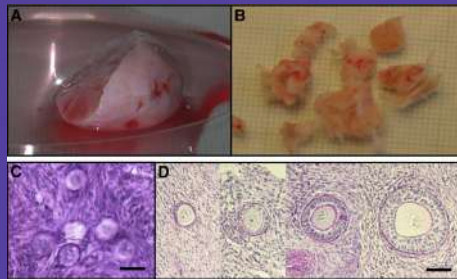
Similar evidence of preservation of primordial follicles

Pregnancy following bilateral oophorectomy and orthotopic grafting of ovarian tissue frozen using **DMSO**

Donnez 2012

Birth of twins following bilateral oophorectomy and heterotopic grafting of ovarian tissue frozen using **EG**

Kristensen 2017

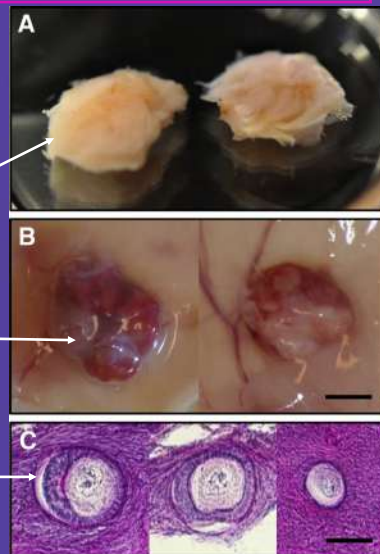


Refreezing of ovarian tissue

23yo Mucinous Cystadenocarcinoma
Frozen with EG → grafted
Birth → remove tissue

Tissue re frozen with EG
→ Xenograft for 4 weeks

Secondary follicles
Kristensen 2017



Clinical Evidence of Reproducible Preservation



Preservation of Function with Slow Freezing: Longevity of Function

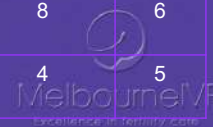
	Cryoprotectants	Patients	Graft Function (years)
Jensen 2015	1.5 EG + 0.1 suc	2	≥10
		9	≥5
Liebenthron 2015	1.4 DMSO	1	7
Stern 2011	1.5 PROH + 0.1 suc	1	5.4
Silber 2015	1.5 PROH + 0.2 suc	1	3.2

Resulting in pregnancy



Preservation of Function with Slow Freezing: estimated total 125 Births

	Cryoprotectants	Patients	No. Pt with functioning graft	Pregnancies	Births
Meirow 2015	1.5 DMSO + 0.1 suc	20	20	14	10
Donnez 2013	1.5 DMSO + 0.1 suc	13	10	6	6
Demeestere 2015	1.5 DMSO + 0.1 suc	10	9	5	4
Pellicer 2013	1.5 DMSO + 0.1 suc	18	17	4	2
Liebenthron 2015	1.4 DMSO	68	43	16	12
Jensen 2015	1.5 EG + 0.1 suc	41	41	24	14
Silber 2010, 2015	1.5 PROH + 0.2 suc	11	11	8	6
Stern 2011,2013	1.5 PROH + 0.1 suc	19	16	4	5



Successful Grafting: Independent of Size



Size
1x1x3 mm



15 slices per
suture

Stem 2013



D

Silber. Fertility after ovary transplantation
Fertil Steril 2010



Andersen
2012
10x10x1mm



Comparison of outcomes from tissue grafted at hetero and orthotopic site

	Heterotopic	Orthotopic
Pt with attempted OPU	14	9
OPUs	67	53
OPU eggs=0	31	20
Mature oocytes	42	43
Fertilisation	37 (74%)	29 (67%)
No. of Day 2 embryos	33 (89%)	25 (86%)
No. Embryos FZ	19	10
No. Embryos transferred	10 (4 thawed)	16 (2 thawed)
Births	3	2
Biochem Pregnancies	1	3
Clinical Pregnancies	2	2

Slow frozen with PROH

MelbourneIVF
Excellence in fertility care
Gook

Prepubertal Ovarian Tissue



grafting into peritoneal cavity of female SCID mouse with hormone stimulation resulting in follicle development

Luyckx 2013

MelbourneIVF
Excellence in fertility care

Birth from slow frozen peri-pubertal ovarian tissue

- Ovarian tissue cryopreserved with DMSO at age 13
- Age 23 tissue grafted (residual ovary, peritoneal bursa, abdominal subcutaneous)
- Menstruation at 5 months post graft
- Follicular development all sites
- Regular cycles for 2 years
- Spontaneous pregnancy and live birth

Demeestere 2015



9 yo β Thalassaemia to have BMT
1 ovary removed and slow frozen Leeds
Age 23 Menopausal
4 pieces grafted to ovary + 1 piece to uterus Copenhagen
Function 3 months post grafting
IVF cycles

Leeds 2017



Safety



Safety: No impact of long storage duration on function

- Demeestere 2015, Callejo 2013
Tissue in storage for **10** years subsequently thawed and transplanted resulting in a birth.
- Stern 2013
Tissue in storage for **9** years subsequently thawed and transplanted resulting in two births.
- Jensen 2015
Tissue in storage for **8** years subsequently thawed and transplanted resulting in two births.



Safety: Risk of cross contamination



Reduced risk of cross contamination of vials
in vapour storage



Residual cryoprotectant concentration in **human** ovarian tissue after warming

	Slow freezing DMSO (mg/g)	Vitrification DMSO (mg/g)	Vitrification EG (mg/g)
After cryopreservation	70	130	112
After first warm solution		73	63
Second solution	0.25	37	33
Completion of warm procedure	0.39	9.8	9.8

8x4x1mm
Slow frozen using 1.5 M DMSO
(Isachenko 2012)

10x10x1
Vitrification using 2.8M DMSO +
3.6M EG
(Kitazato Cryotissue kit)

Nakamura & Kyono 2017



Summary

- Evidence of successful cryopreservation of primordial follicles
 - Functionally- xenografting, heterotopic grafting
- Reproducible / robust technology
 - Numerous births in multiple clinics
 - Longevity of function
- Graft outcome
 - Number Pregnancy ~ 125
- Safety
 - No impact of long-term storage
 - Vapor storage reduces potential cross contamination



Acknowledgements

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