Oocyte cryopreservation: which oocyte and which method

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Which oocyte ?

Why cryopreserve oocytes ?

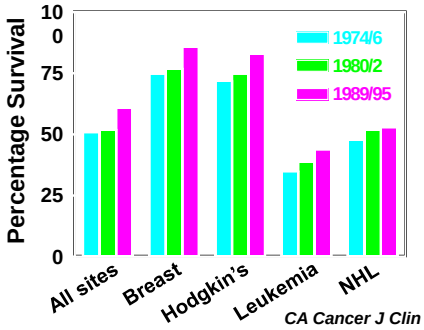
- Urgent need for fertility preservation
- Alternative to embryo cryopreservation
- Streamline oocyte donation
- “Social” egg freezing

Developmental stages

- Primordial follicles
- *Immature oocytes (developing follicle)*
- *Mature oocytes*

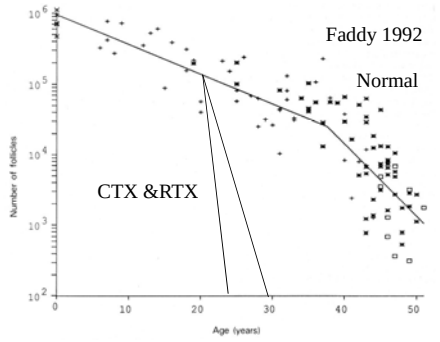
Cancer Survivor Trends

~ trends in 5-year US cancer survival rates ~

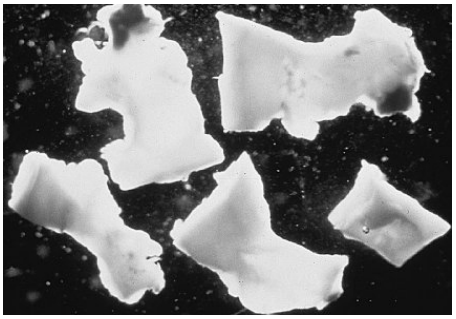


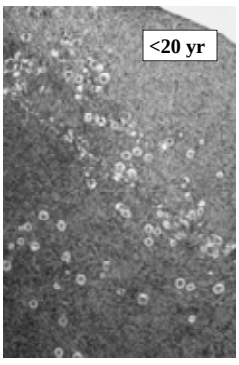
CA Cancer J Clin 50:1, 2000

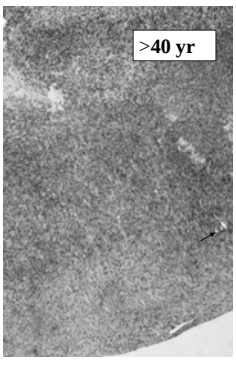
Reduction in the Follicular Population Associated with Aging in the Female



Ovarian cortex – primordial follicles

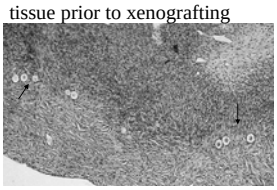


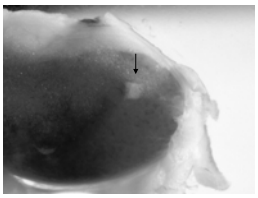




Xenografting

Primordial follicles present in tissue prior to xenografting

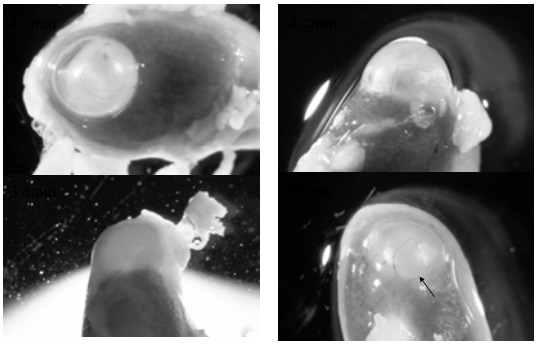




Size of cryopreserved tissue xenografted under renal capsule

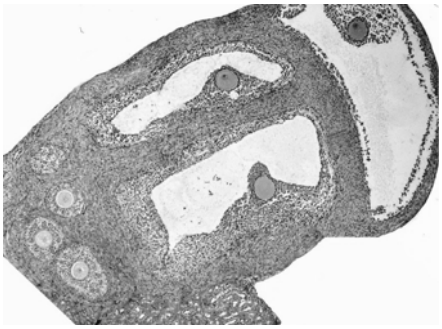
~0.5 x 0.5 x 1 mm

Antral follicle development in human cryopreserved ovarian tissue following xenografting ; 4 patients.

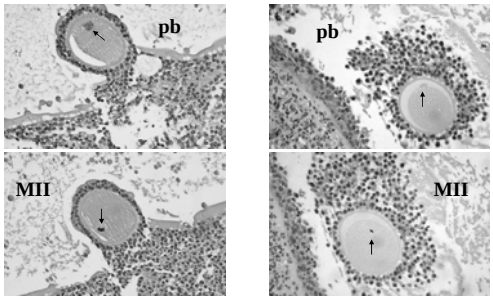


Gook et al 2005

Multiple growing follicles in xenograft after cryopreservation



Mature oocytes within large antral follicles following hCG.



Gook et al 2003

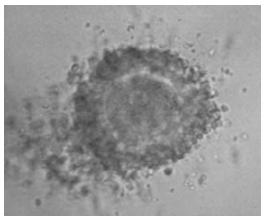
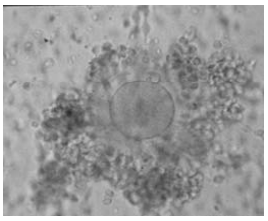
Cryopreservation of ovarian cortex

- Small number of reports of pregnancy/birth following autografting
- ? Future prospect of In Vitro Growth (IVG)
  - A two-step serum-free culture system supports development of human oocytes from primordial follicles in the presence of activin
  Telfer et al *Human Reproduction (2008)*

Developmental stages

- *Primordial follicles*
- Immature oocytes (developing follicle)
- *Mature oocytes*

Small antral follicles - immature oocytes (IVM)

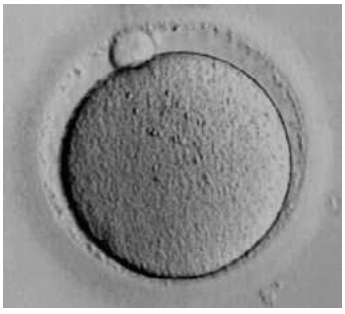



Pregnancies and live birth following vitrification – *Chian et al 2008*

Developmental stages

- *Primordial follicles*
- *Immature oocytes (developing follicle)*
- Mature oocytes

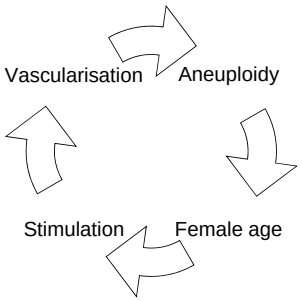
Mature oocyte cryopreservation



Hundreds of babies born

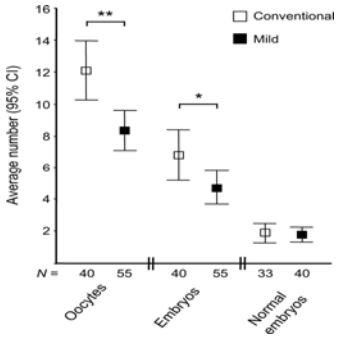
Which mature oocyte?

Mature oocyte quality - considerations



Ovarian stimulation
- more oocytes to freeze

Oocyte and embryo yield and embryos successfully biopsied and diagnosed by fluorescent in-situ hybridization (FISH) as chromosomally normal on the basis of FISH results from one cell following conventional and mild stimulation



Baart, E. B. et al. Hum. Reprod. 2007 22:980-988; doi:10.1093/humrep/del484

Human Reproduction

Oocyte selection ?

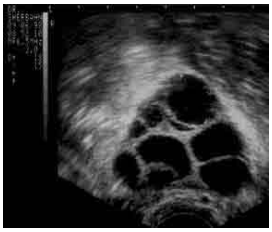
Selective vitrification of euploid oocytes

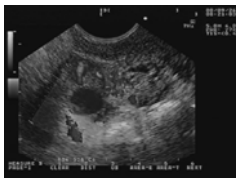
- *Sher et al 2008*
- Donor oocytes
- Polar body biopsy and analysis by CGH
- Vitrification

- Increased developmental potential in warmed euploid vs aneuploid oocytes

Oocyte improvement ?

?? Inadequate vascularisation





Female age

Day 2 SET – By Female Age

	Fresh embryos		Thawed embryos	
Age	SETs	FH	SETs	FH
< 36	833	31.7%	1006	24.6%
36-39	391	19.7%	502	18.3%
>39	507	10.7%	572	10.3%

Which method ?

Ovarian tissue cryopreservation

- Extended period of dehydration (ca 90 mins) may be required prior to slow cooling
  Gook et al 1999
- Vitrification

Human oocyte cryopreservation

early successes with slow cooling in 1980's (DMSO) not reproducible

Chen, 1986

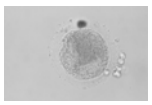
Van Uem, 1987

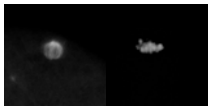
Oocyte cryopreservation - challenges

- Survival (large cell)
- Temperature sensitive spindle (aneuploidy)
- Cortical granule discharge

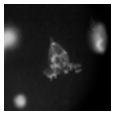
Slow cooled human oocytes – 1.5M $PrOH_2$/ 0.1M sucrose (Gook et al, 1993)

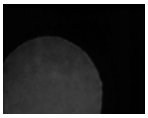
~50% survival





Normal spindle and chromatin

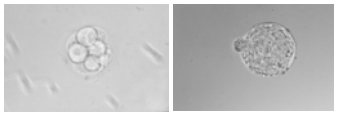




Normal cortical granules

Nomal fertilisation and embryo development

Gook et al, 1994



Gook et al, 1995

First birth following oocyte cryopreservation using 1.5 M propanediol and 0.1 M sucrose.

Porcu et al (1997)
Fertil Steril, 68 , 724

Summary of reported clinical outcomes from oocytes cryopreserved in 1.5M Propanediol/0.1M sucrose

- 4027 oocytes thawed
- 51% thawed oocytes survived
- 54% of injected oocytes fertilised
- 85% of embryos cleaved normally
- 10% of transferred embryos implanted

Attrition rate

- 100 thawed
- 51 survive
- 27.5 fertilise
- 23.4 develop
- 2.3 implantations (FH) – per 100 thawed oocytes

? Optimal cryopreservation

Suggested improvements to methodology

- Use of increased sucrose concentrations (increase dehydration prior to freezing)
- Substitution of choline for sodium (reduce 'solution effect')
- Vitrification

Optimal dehydration ?

Variation in Membrane Hydraulic Permeability of Human Oocytes

Membrane hydraulic permeability Lp (µm/atm/min) measured in individual oocytes at 20° C

Oocyte	1	2	3	4	5	6	7	8
Lp	0.32	0.6	1.09	0.56	0.16	0.51	0.23	0.8

Hunter et al, 1992

Summary of reported results using elevated sucrose methods

	0.1M suc	0.2M suc	0.3M suc
No. thawed	**4027**	**1451**	**7595**
Survival	**51%**	**71%**	**73%**
Fertilisation	**54%**	**80%**	**73%**
Development	**85%**	**93%**	**90%**
Implantation	**10%**	**17%**	**6%**
FH's/100 thawed oocytes	**2.3**	**9.0**	**2.9**

Cryopreserved biopsied embryos : impact of increased dehydration

		Embryo survival	Blastomere survival
Non biopsied	0.1M sucrose	78.3%	70.3%
Biopsied	0.1M sucrose	43.7%	46.0%
Biopsied	0.2M sucrose	74.6%	66.8%

Jericho et al, Hum Rep, 18, 568-71 (2003)

Impact of increasing dehydration on cryosurvival of slow cooled day 2 embryos

	Embryo survival	Fully intact	Blastomere survival
0.1M sucrose	78.5%	54.6%	74.1%
0.2M sucrose	92.6%	80.5%	91.1%

Edgar et al, submitted

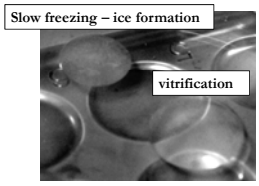
Differential sucrose concentration during dehydration and rehydration

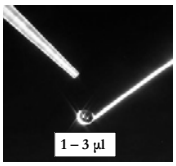
- higher during initial post thaw rehydration steps

Summary of results using Na depletion (choline substitution) methods

	0.1M suc	0.2M suc	0.3M suc
No. thawed	127	815	890
Survival	52%	60%	70%
Fertilisation	56%	66%	72%
Implantation	21%	11%	13%
FH's/100 thawed oocytes	6.1	3.7	5.8

Vitrification





Kuwayama method – 2.7M EG + 2.1M DMSO +0.5M sucrose

Summary of reported results using vitrification (Kuwayama method)

No. warmed	1454
Survival	93/83%*
Fertilisation	87%
Development	93%
Implantation	16%
FH's/100 thawed oocytes	12.0/10.7

OOCYTE VITRIFICATION

Permeating Cryoprotectant	5M EG	2.7M EG + 2.1M DMSO	2.7M EG + 2.0M PROH
Sucrose	1.0M	0.5M	0.5M
Survival (no. of thawed oocytes)	75% (838)	83% # (1454)	80% (395)
Fertilisation	74%	87%	70%
Cleavage	94%	93%	53%
Embryos per 100 thawed oocytes	52	76	30
Implantation rate	10%	16%	13%
Implantations per 100 thawed oocytes	5.2	10.7	3.8

Efficiency of assisted reproduction is dependent on:

- Which patients we apply it to
- The quality of the oocytes
- The ability to apply selection criteria to the available biological material

Cobo et al (2008)

- **From oocytes vitrified using Kuwayama method**
- Pregnancy rate : 65%
- Implantation rate: 41%

Cobo et al (2008)

- **231 oocytes warmed**
- **224 survived (97%)**
- **171 fertilised (76%)**
- **23 transfers / 49 embryos (38 blastocysts)**
- **15 pregnancies (11 ongoing) / 20 sacs**
- **Therefore, 8.7 implantations (sacs) per 100 warmed oocytes (20/231)**
- **Mean female age: 26.7**

The clinical efficiency of oocyte cryopreservation

Edgar & Gook, *Reprod Biomed Online (2007)*

Gook & Edgar, *Hum Reprod Update (2007)*

Comparison with

Clinical efficiency of using cryopreserved embryos

Embryo cryopreservation

- 100 oocytes
- 65-70 embryos
- 40-45 suitable for cryopreservation
- 30-35 survive
- 5-10 implant

- Therefore, 5-10 implantations per 100 oocytes

ART in Australia - 2006

- 190,613 oocytes were collected

- 6,060 fetal hearts were detected from transfer of fresh embryos

- Therefore, only 3% of oocytes resulted in a fresh implantation

Conclusions

- Oocytes can be cryopreserved at all developmental stages

- Both slow cooling and vitrification may be appropriate for oocyte cryopreservation

- Many factors must be considered when assessing the clinical efficiency of oocyte cryopreservation

ACKNOWLEDGEMENT

Dr Debra Gook