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Cryopreservation of human embryos: the embryo or the procedure?

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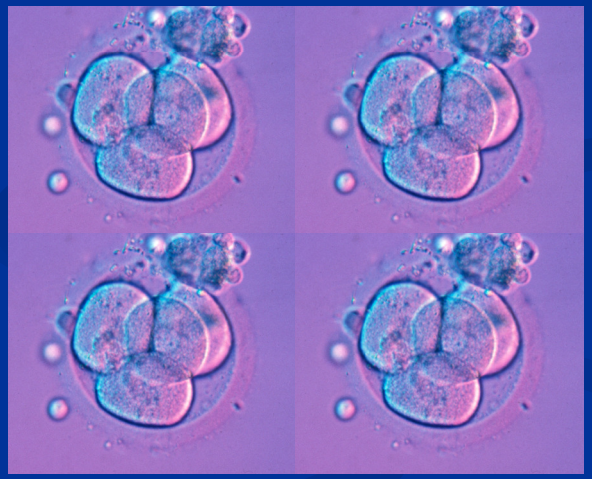
Why do we need to cryopreserve human embryos?

1. Optimal ART requires embryo selection
2. Embryo selection requires multiple oocytes/embryos



3. Transferring multiple embryos is contraindicated

vs

Consequently

4. Responsible ART requires embryo cryopreservation

12 month period of ET's (day 2) at Melbourne IVF

- 60% Single Embryo Transfer
- 77% Single Embryo Transfer in women <36
- 1345 babies in total
- 618 from thawed embryos (46%)
- 80% of all women giving birth from a fresh cycle have stored embryos

Factors impacting on the clinical outcome from embryo cryopreservation

- Characteristics (quality) of embryos prior to freezing
- Biological consequences of freezing/thawing
- Efficiency of methodology

Is there a significant difference in the outcome from fresh and cryopreserved embryos?

SET's in women under 36

Embryos	No of SET's	Implantation Rate
Fresh	2524	31.1% *
Cryopreserved	3020	24.1% *

* $p < 0.001$

Is this difference due to differences in the embryos or the impact of cryopreservation?

Importance of developmental rate on day 2 (42 hpi)

Fresh vs *Equivalent* Stored Embryos

	Embryos transferred	FH's	Implantation Rate
4 cells* Fresh	1567	260	16.6%
4 cells* Thawed Intact	794	134	16.9%
2 cells* Fresh	899	58	6.5%
2 cells* Thawed Intact	401	29	7.2%

* 40 – 42 hpi

Edgar et al (2000) Human Reproduction 15, 175

Implantation rates from SET's in women under 36

No of cells	Fresh	Cryopreserved
< 4	11.0% (91)	10.6% (94)
4	31.6% * (748)	28.6% * (807)
> 4	15.3% (59)	16.4% (213)

* Not significant

Additional markers of embryo quality

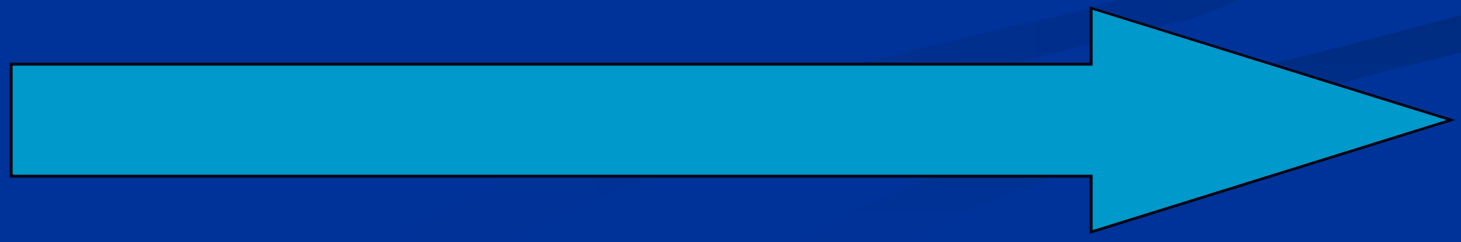
Early events

23/24hr post-insemination

2 pronuclei (PN)

1 cell embryo (Syngamy/NEBD)

2 cell embryo (early cleavage/ EC)



Implantation rates in fresh SET's (n)

Women <36

23/24 hpi	I.R.
EC	35.7% (325)
NEBD	28.8% (400)
PN	19.5% (215)

Embryo morphology/fragmentation

Embryo Grade	Fragmentation
1	0%
2	1-10%
3	11-30%
4&5	>30%

Implantation rates in fresh SET's (n)

Women <36

Grade	I.R.
1	31.5% (276)
2	31.2% (484)
3	21.3% (183)

4 cell embryo/ EC/ Grade 1 Implantation rate (no of SET's)

	All ages	< 36
Fresh	34.9% (567)	42.5% (334)
Cryopreserved	36.4% (66)	45.2% (31)

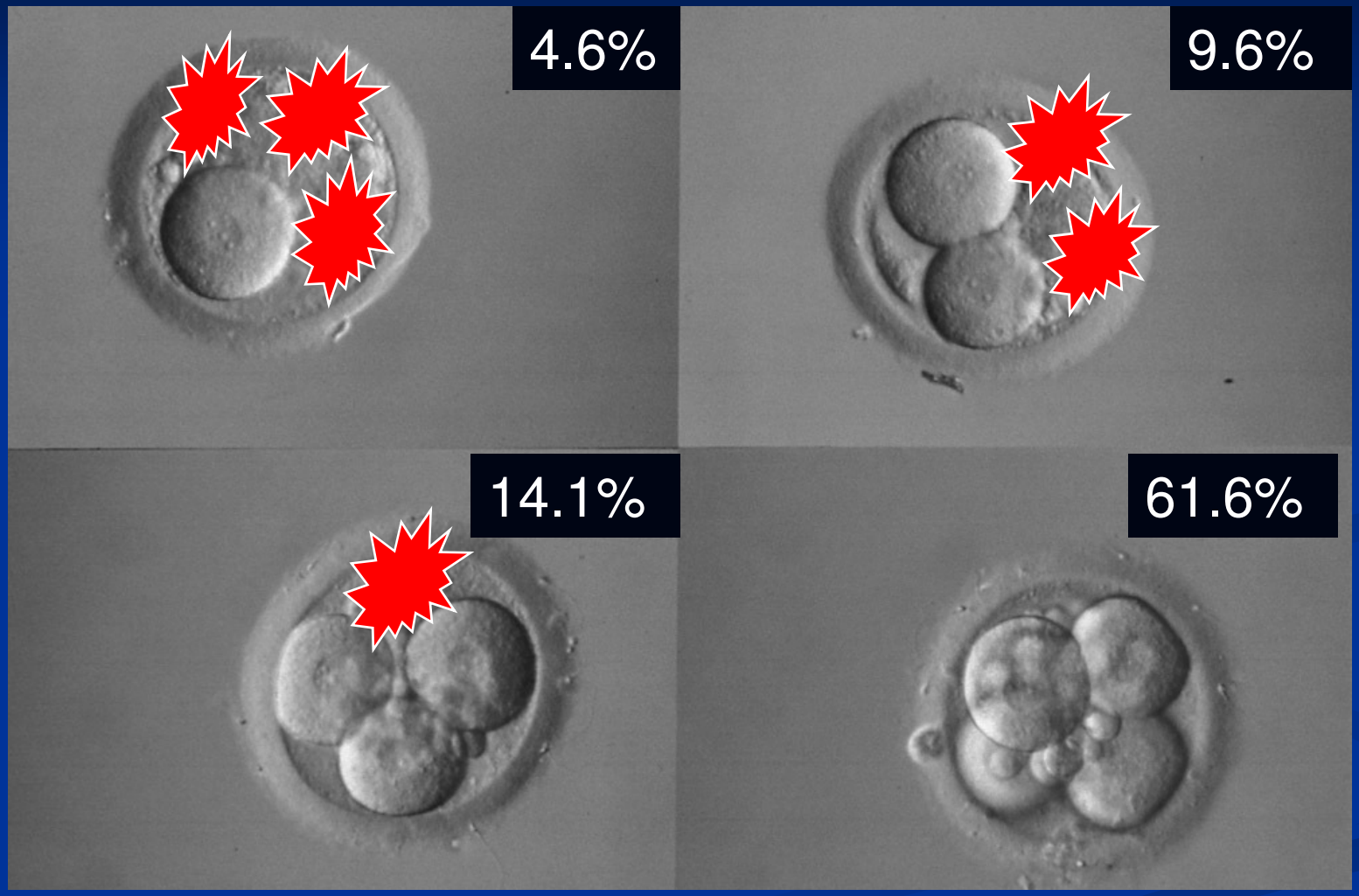
Conclusions

1. Embryo quality before freezing is strongly associated with post thaw implantation potential
2. Thawed embryos can have similar implantation potential to **EQUIVALENT** fresh embryos

Possible biological consequences of embryo cryopreservation

- Cell loss
- Arrested/compromised development
- Altered function/metabolism

Cell loss (1.5M $PrOH_2$ + 0.1M sucrose)



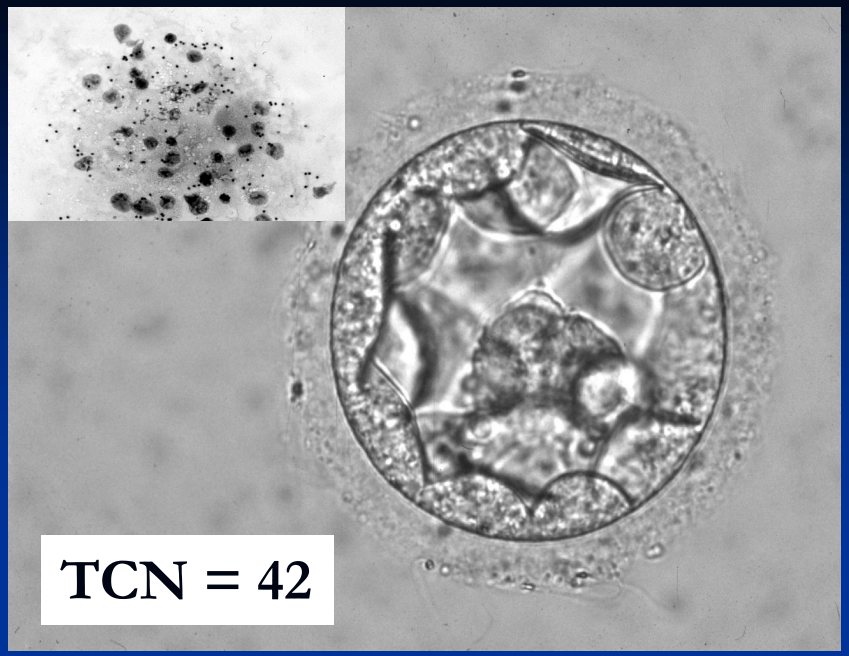
* + 10.1% with no surviving blastomeres

Biological impact of blastomere loss

Does blastomere loss in stored embryos :

- Impair preimplantation development ?
- Result in reduced cell numbers at the blastocyst stage ?

Surplus cryopreserved day 2 embryos - thawed and cultured to the blastocyst stage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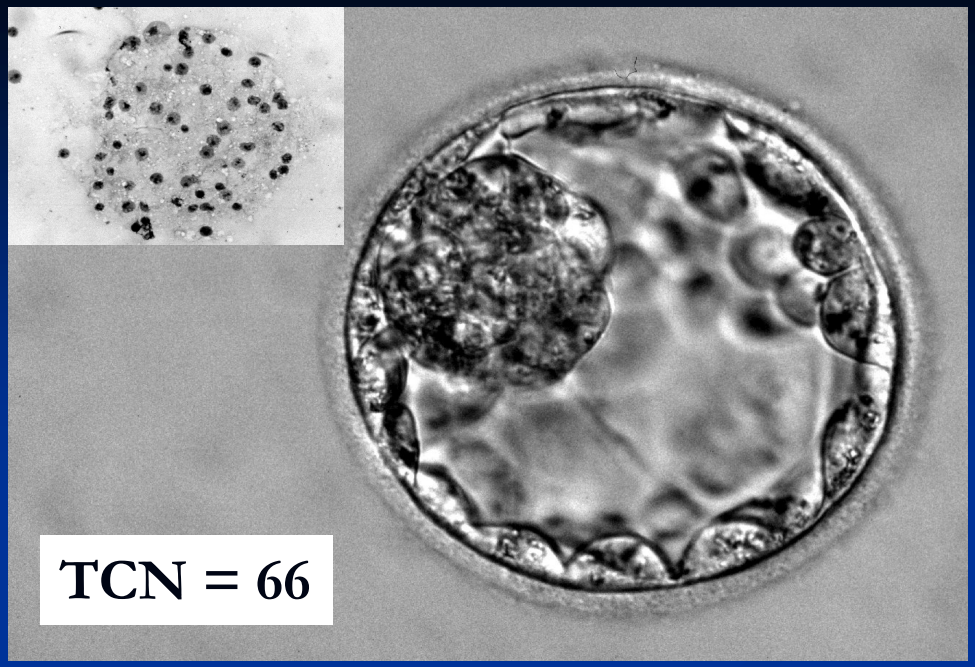
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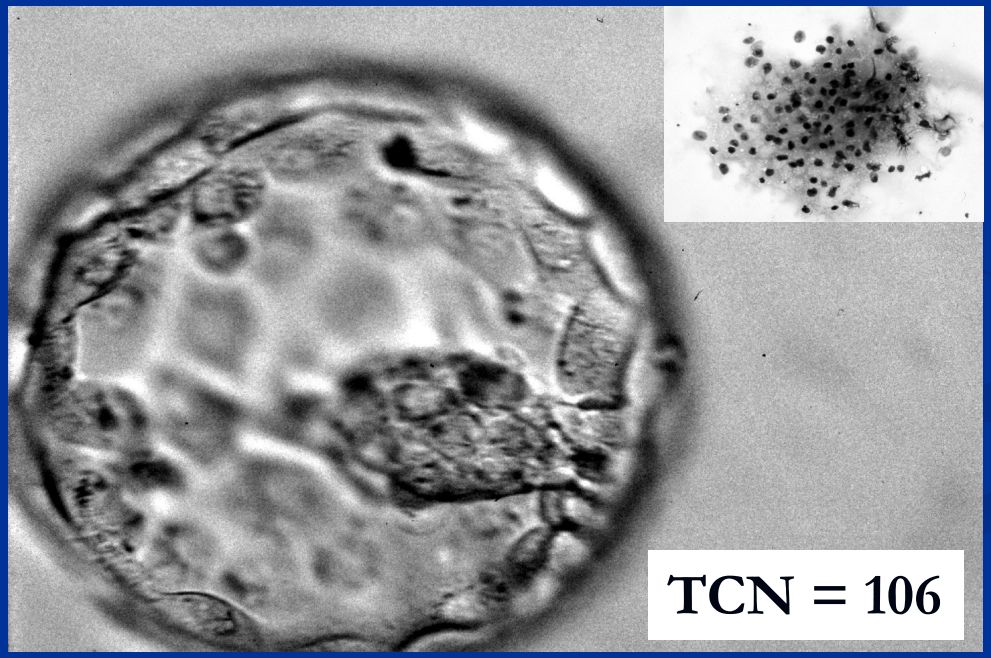

TCN = 106

Development of Intact and Partially Intact Thawed Cleavage Stage Embryos In Vitro

	Development to blastocyst	Mean cell number in blastocysts
Intact	92/225 (40.9%) [a]	58.4 [b]
Partial	41/167 (24.6%) [a]	45.0 [b]

[a] $p < 0.01$ [b] $p < 0.05$

Archer et al, Hum Rep, 18, 1669-73 (2003)

Clinical significance of blastomere loss

Outcome from SCETs in relation to survival (4 cell embryos) Women <36

Prefreeze blastomeres	Post thaw blastomeres	SCETs	FHs	Implantation rate
4	4	722	179	24.8%
4	3	146	40	27.4%
4	2	92	8	8.7%

Edgar et al, Rep BioMed Online, 14, 718-23 (2007)

Conclusions

1. Embryo quality before freezing is strongly associated with post thaw implantation potential
2. Thawed embryos can have similar implantation potential to **EQUIVALENT** fresh embryos
3. Blastomere loss can reduce implantation potential

Post thaw resumption of mitosis

Outcome from SCETs in relation to resumption of mitosis

Blastomere survival	Resumption of mitosis	SCETs	FHs	Implantation rate
4 of 4	YES	641	165	25.7%
4 of 4	NO	81	14	17.3%
3 of 4	YES	113	34	30.1%
3 of 4	NO	33	6	18.2%
2 of 4	YES	68	7	10.3%
2 of 4	NO	24	1	4.2%

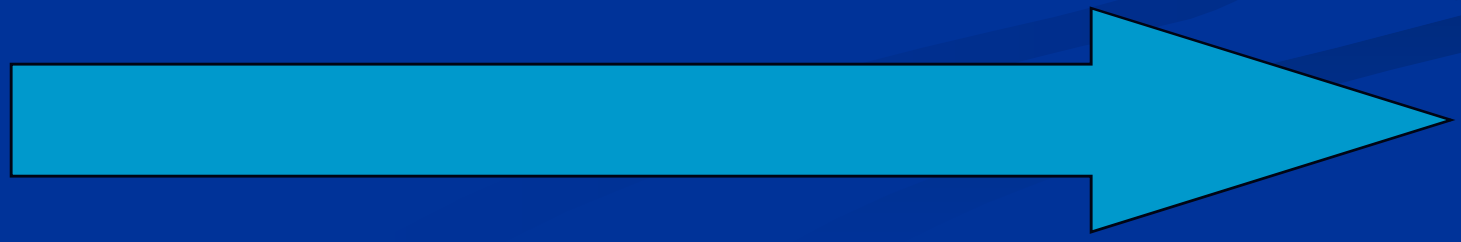
Early events

23/24hr post-insemination

2 pronuclei (PN)

1 cell embryo (Syngamy/NEBD)

2 cell embryo (early cleavage/ EC)



Implantation rates in fresh SET's (n)

Women <36

23/24 hpi	I.R.
EC	35.7% (325)
NEBD	28.8% (400)
PN	19.5% (215)

Post thaw resumption of mitosis in relation to timing of syngamy/first cleavage

23/24 hpi	Post thaw resumption of mitosis (n)
EC	92% (287)
NEBD	86% (652)
PN	70% (852)

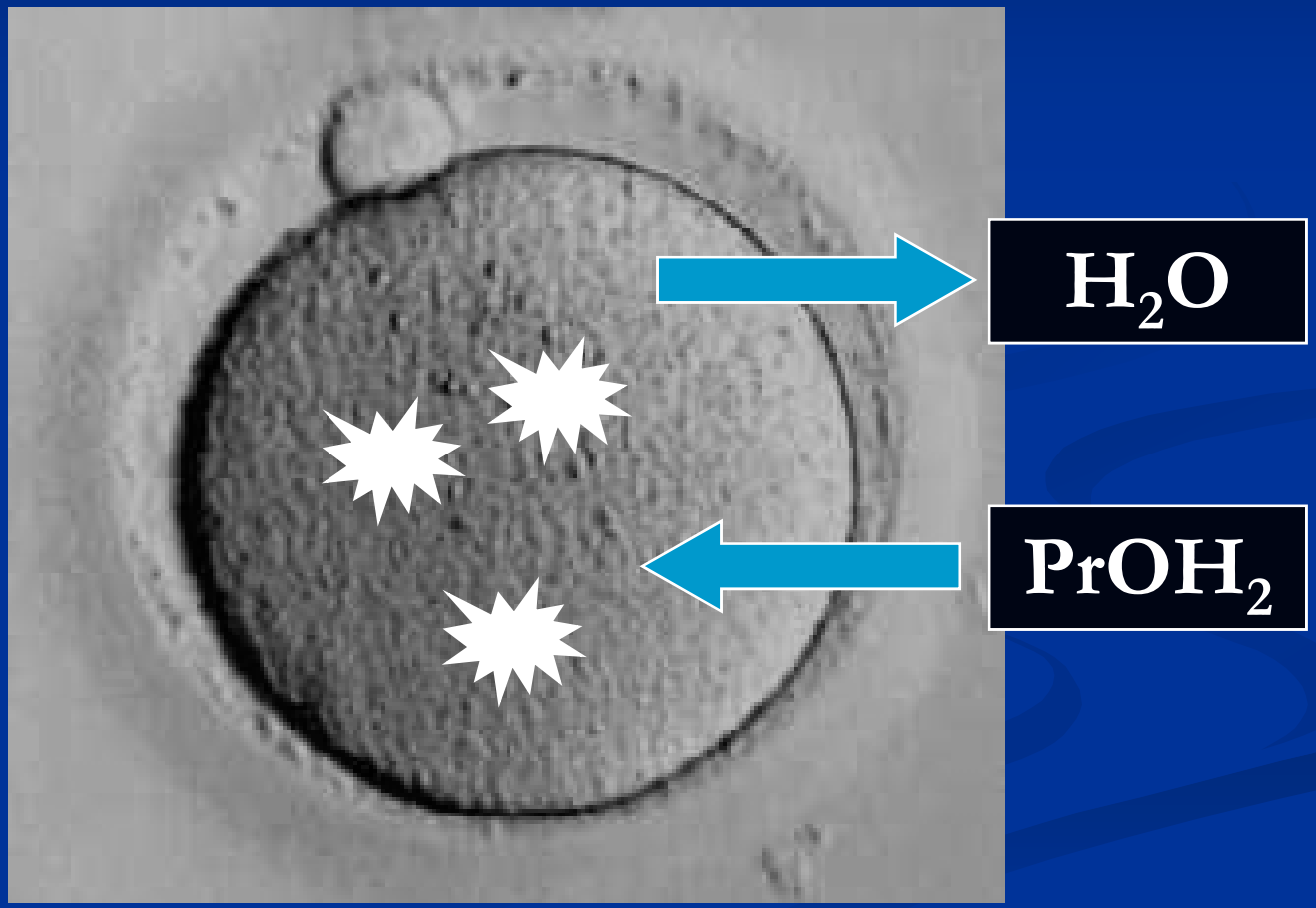
The embryo or the procedure ?

- The embryo +++
- The procedure ???

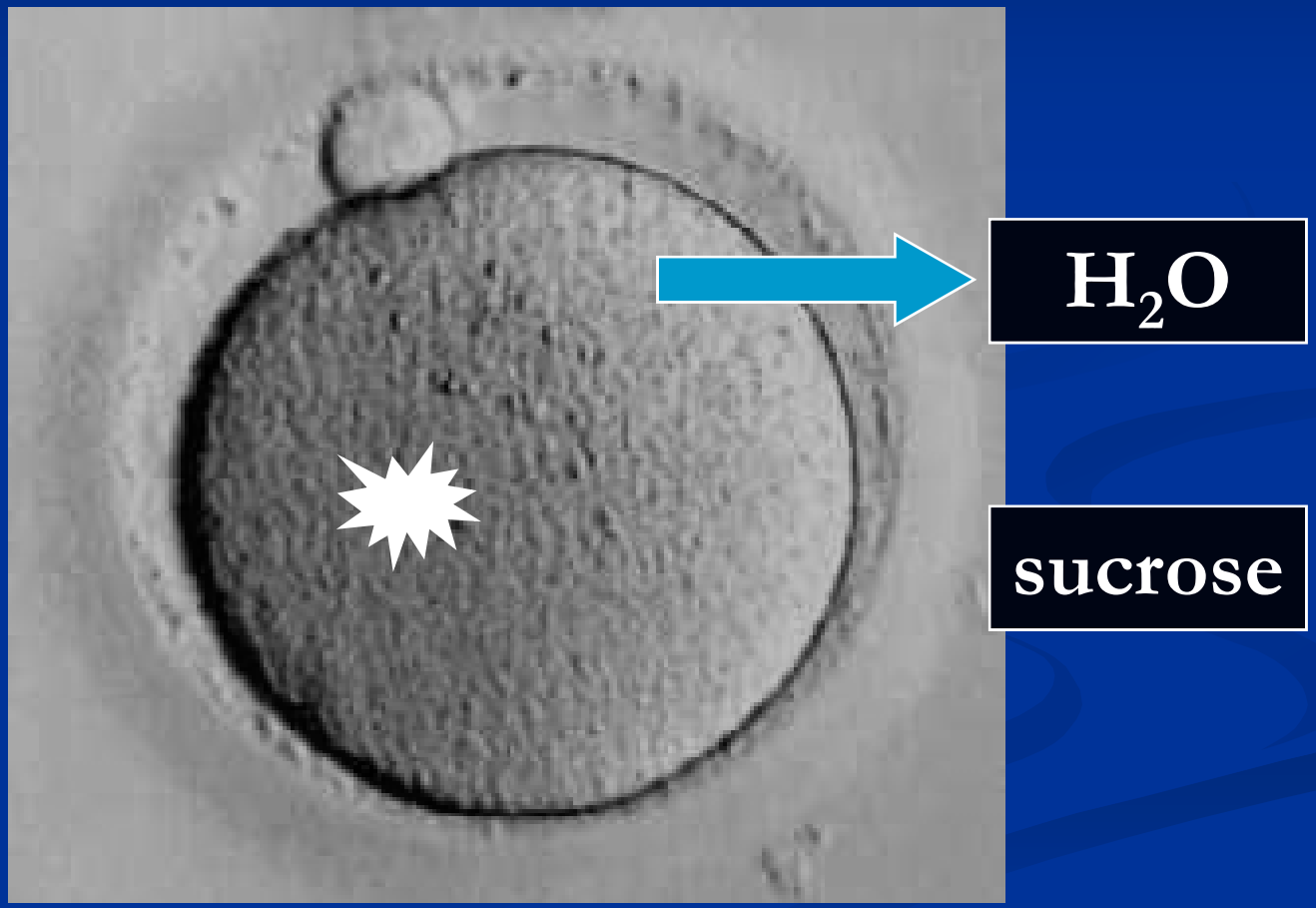
Optimal outcomes from embryo cryopreservation

Standard dehydration for slow cooling

Permeating cryoprotectant (1.5M)



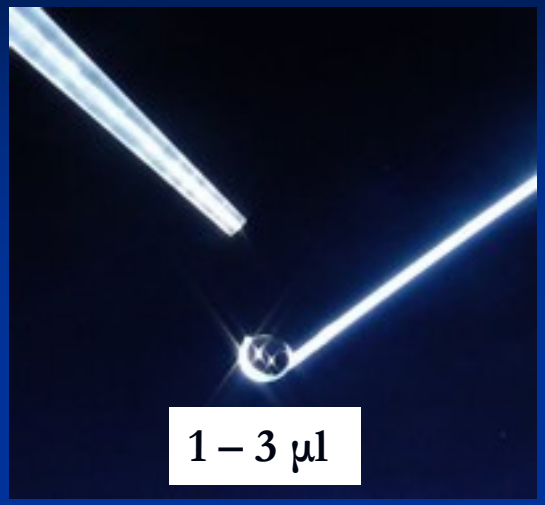
Non-permeating cryoprotectant (0.1M)



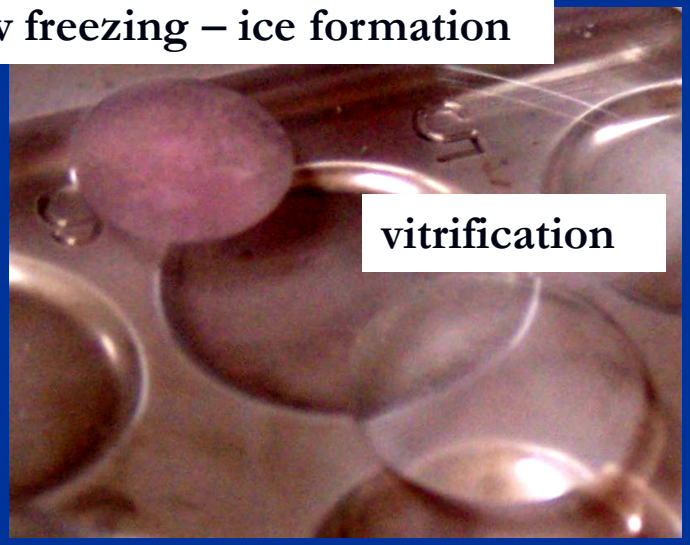
Differential sucrose concentration during dehydration and rehydration

- higher (0.2M) during initial post thaw rehydration steps

Vitrification



Slow freezing – ice formation



Very high concentrations of cryoprotectant

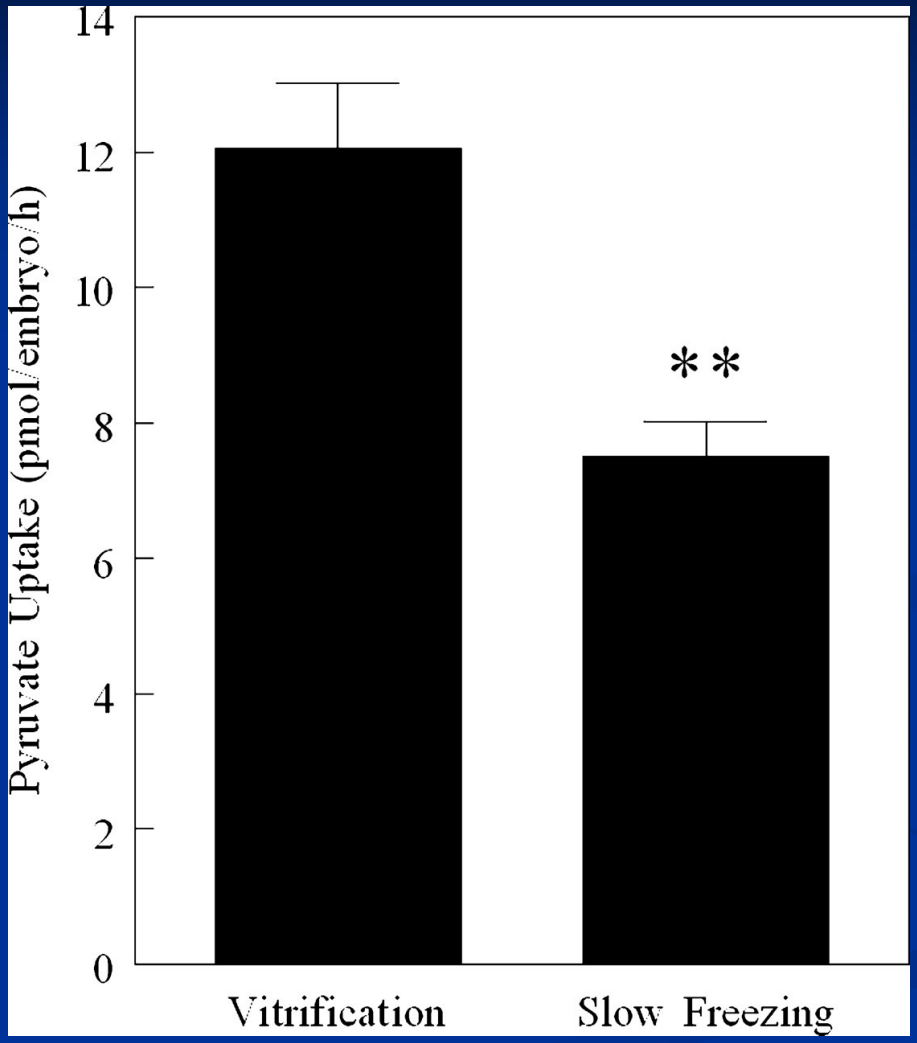
Table II. Outcomes of vitrification and slow freezing

Day 3 embryos – Balaban et al., 2008

	Vitrification	Slow freezing		*P*-value
Cryosurvival (%)	222/234 (94.8)	206/232 (88.7)		0.02
Embryos with 100% blastomere survival (%)	173/234 (73.9)	106/232 (45.7)		<0.01

Metabolic consequences ??

Pyruvate uptake by cryosurvived embryos



Balaban, B. et al. Hum. Reprod. 2008 23:1976-1982; doi:10.1093/humrep/den222

Human Reproduction

? Optimal slow freezing

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

Increased dehydration (0.2M sucrose) prior to slow cooling

- Mature oocytes
- Biopsied embryos

- Further elevation of sucrose (0.3M) during initial rehydration

Cryopreserved biopsied embryos : impact of modified method

Embryos	Method	Embryo survival (>50%)	Blastomere survival
Non biopsied	Standard	78.3%	70.3%
Biopsied	Standard	43.7%	46.0%
Biopsied	Modified	74.6%	66.8%

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

Increased dehydration of non biopsied day 2 embryos ??

Edgar et al, RepBioMed Online (2009)

Single Step Freeze Method used at Melbourne IVF

- Embryos dehydrated in a single step using 1.5M PROH plus 0.1M Sucrose prior to slow cooling

- Embryos thawed and rehydrated using a 3-step method with decreasing concentration of sucrose

0.5M sucrose ⟹ 0.2M sucrose ⟹ 0M sucrose

Modified Freeze Method

- Elevated sucrose concentration (0.2M) during dehydration and slow cooling

Embryo Survival I

	0.1 M Sucrose	0.2 M Sucrose
Embryos Thawed	474	471
Surviving embryos (≥ 50% of cells)	372	436
Embryo Survival	78.5% *	92.6% *

* $p<0.02$

Embryo Survival II

	0.1 M Sucrose	0.2 M Sucrose
Embryos Thawed	474	471
Fully Intact (100%)	259 (54.6%)[a]	379 (80.4%)[a]
50%-99% Intact cells	113 (23.8%)	57 (12.1%)
<50% intact	102 (21.5%)[b]	35 (7.4%)[b]

a : $p<0.001$ b: $p<0.001$

Blastomere Survival

	0.1 M Sucrose	0.2 M Sucrose
Embryos Thawed	474	471
Total Number of B;astomeres Thawed	1918	1870
Total Number of Surviving Blastomeres	1421 (74.1%)*	1704 (91.1%)*

* $p<0.001$

Resumption Of Mitosis

	0.1M	0.2M
Surviving cells	1421	1704
No of cells after overnight culture	2159	2560
% Increase in cells	51.9%*	50.2%*

* Not Significant

Clinical outcomes (< 36yrs)

	0.1 M Sucrose	0.2M Sucrose
Embryos Thawed	183	217
Embryos Transferred	139	193
FH	32	48
IR/Embryo Transferred	23.1%	24.8%
IR/ Embryo Thawed	17.5%	22.1%

Conclusions

1. Embryo quality before freezing is strongly associated with post thaw implantation potential
2. Thawed embryos can have similar implantation potential to **EQUIVALENT** fresh embryos
3. Blastomere loss can reduce implantation potential
4. Optimal procedures can minimise blastomere loss

Cryopreservation of human embryos: the embryo or the procedure?

BOTH