Current status of embryo cryopreservation

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Embryo cryopreservation

- Embryo cryopreservation has always played a central role in assisted reproductive treatment.

- The use of frozen embryo transfer has resulted over than 60,000 health births in USA - SART

- Maximizing effectiveness of the IVF cycle.

Cryoinjury

- Extracellular & Intracellular Ice Formation
- Toxicity of cryoprotectant
- Osmotic swelling
- Fracture

Questions for cryopreservation

- Methods

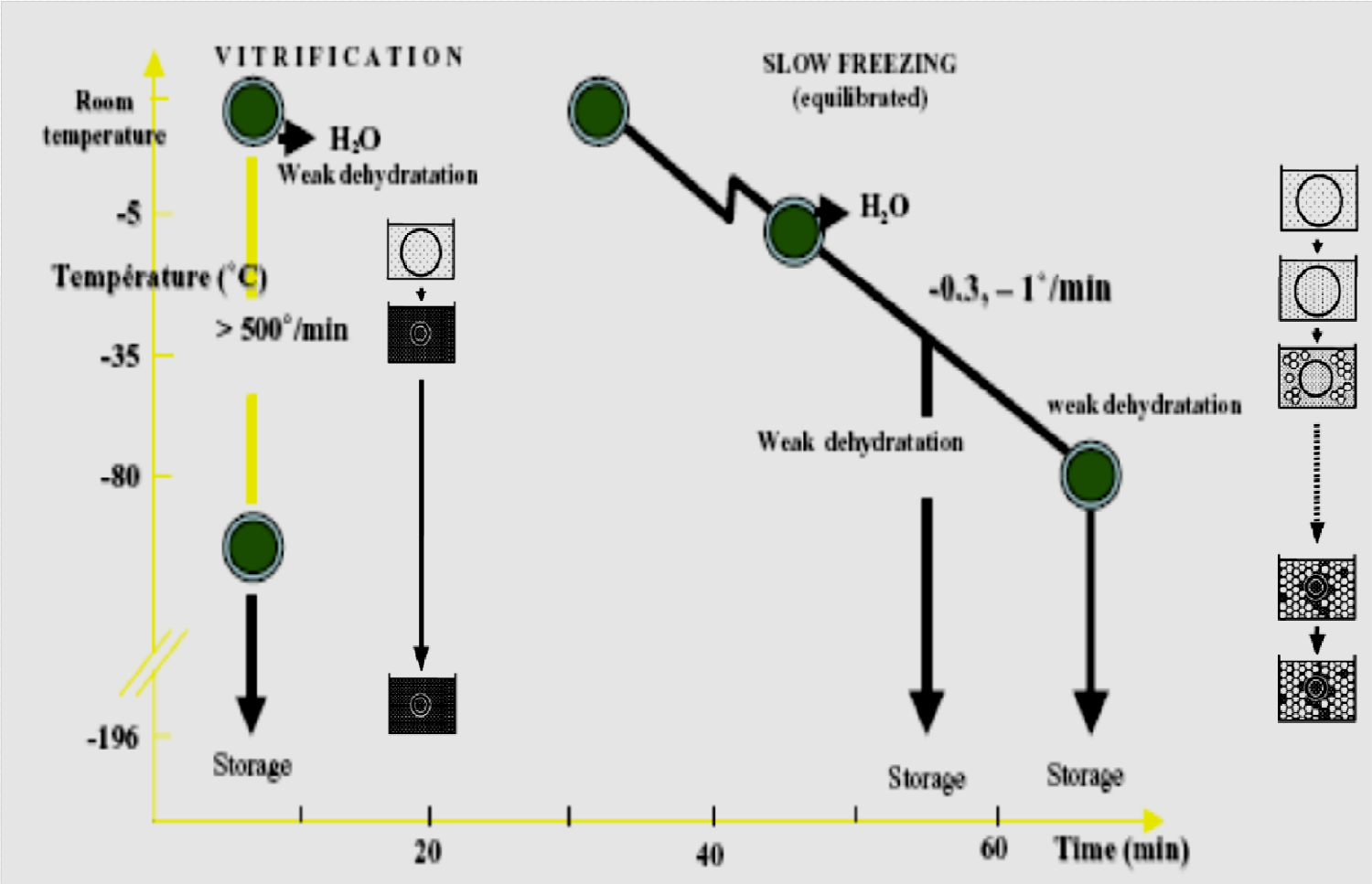
  Vitirification *vs.* Slow freezing

- Stages of embryo

- Re-cryopreservation

Cryopreservation methods

Two cryopreservation methods



Slow freezing (Programmed freezing)

- Cryopreservation of mouse embryo (Whittingham, 1972)
- Traditional method
- Controlled cooling rate
- Low cryoprotectant concentration
- Disadvantages of Slow freezing
  - Requirement for an expensive freezing machine
  - Time consuming

Slow freezing - Procedure

- Exposed to relative low concentration cryoprotectants
- Loaded in small volumes in to straw
- Cooled to -5 ~ -7℃
- Several minutes to equilibration
- Seeding – initiate extracellular freezing
- Cooled slowly -0.3 ~ -0.5℃/min
- To anywhere from -30 and -65℃
- Straws plunged into LN_2 for storage

Vitrification

- Glass like solidification
- Advantage
  - ice crystal ↓ (survival ↑)
  - simple method (freezing machine, time)
- Disadvantage
  - high cryoprotectant concentration
    : toxic & osmotic damage
  - direct contact to LN2

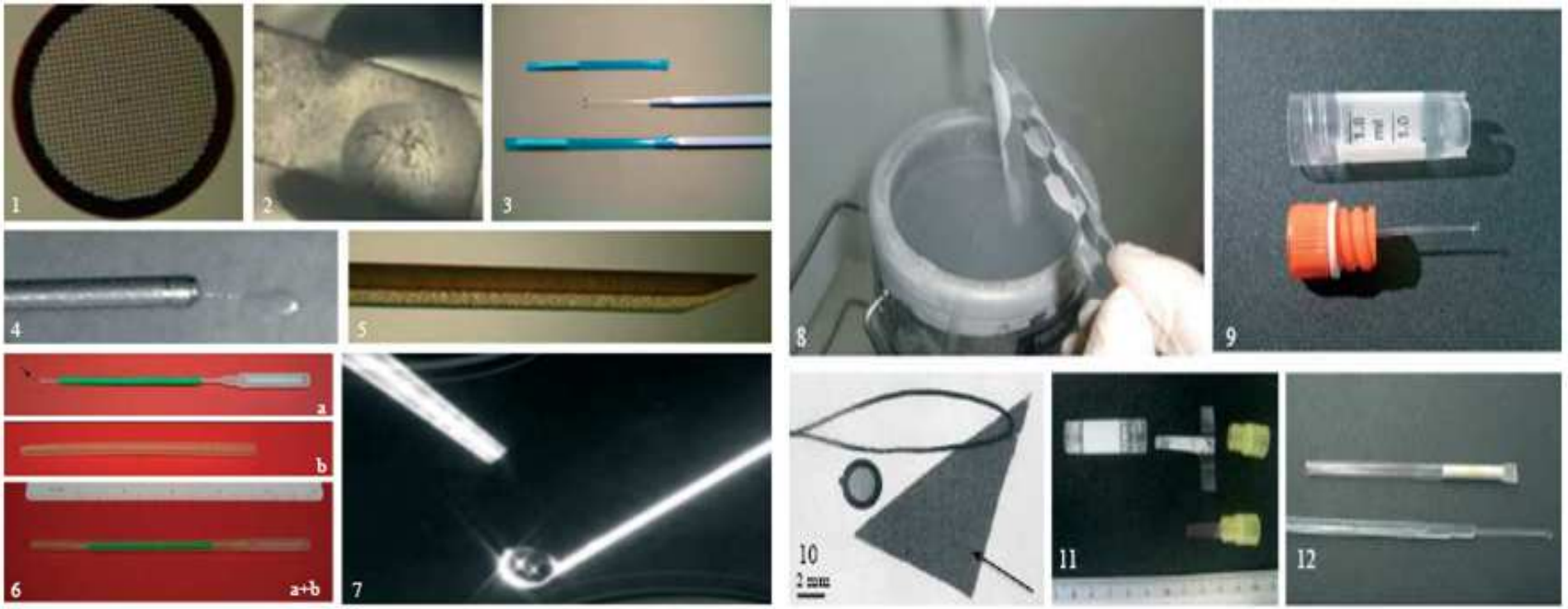
→ Closed vitrification system

Vitrification - Three important factors

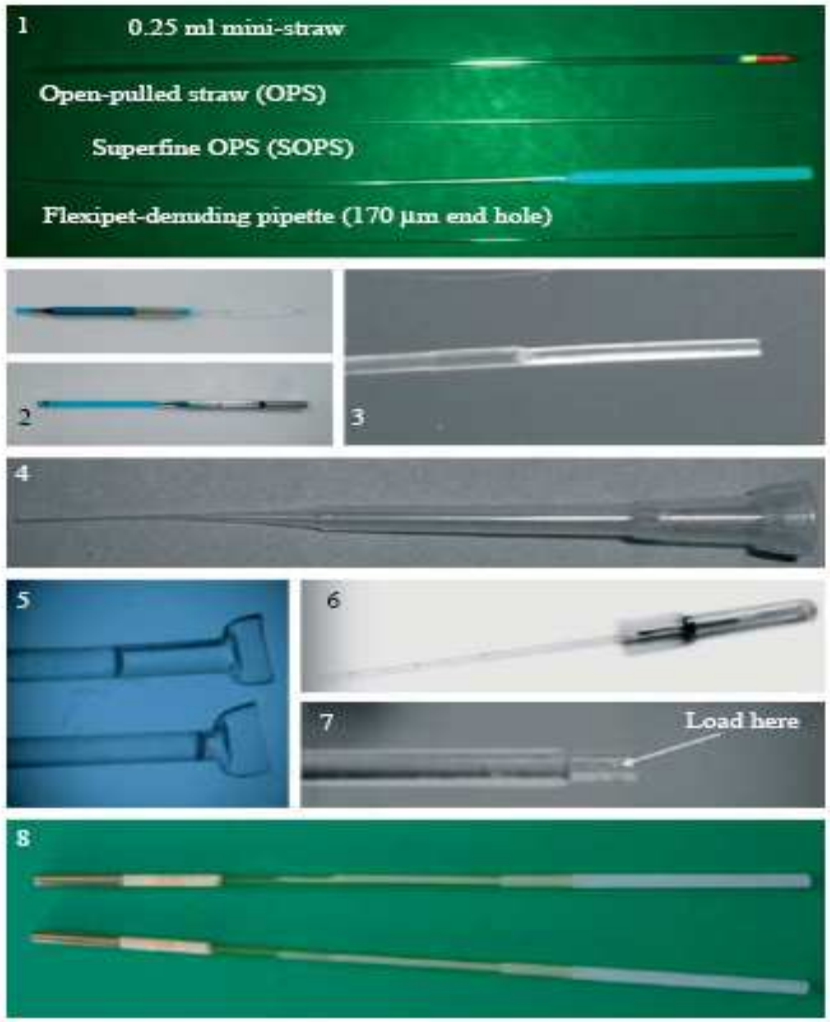
- Cooling rate ↑
- Viscosity ↑ : increase the concentration of cryoprotectant
- Loading volume ↓ : <1$\mu\ell$

$$\text{Probability of Vitrification} = \frac{\text{Viscosity} \times \text{Cooling rate}}{\text{Volume}}$$

Various types of carrier



1. Electron microscope grid
2. Minimum drop
3. Cryotop
4. Cryoloop
5. Hemi-straw
6. Cryoleaf
7. Fiber plug
8. Direct cover vitrification
9. Vitrifcation spatula
10. Nylon mesh
11. Plastic blade
12. Vitri-Inga



1, Plastic straw, open-pulled straw
2. CryoTip
3. High-security virification device
4. Pipette tip
5. Sealed pulled straw
6. Cryopette
7. Rapid-I
8. JY straw

Saragusty. J and Arav. A. Reproduction (2011)

Clinical outcomes: slow freezing *vs.* vitrification

Reprod Biomed Online. 2007 Mar;14(3):288-93.

Three years of routine vitrification of human zygotes: is it still fair to advocate slow-rate freezing?

Al-Hasani S, Ozmen B, Koutlaki N, Schoepper B, Diedrich K, Schultze-Mosgau A.

Hum Reprod. 2008 Sep;23(9):1976-82. Epub 2008 Jun 10.

A randomized controlled study of human Day 3 embryo cryopreservation by slow freezing or vitrification: vitrification is associated with higher survival, metabolism and blastocyst formation.

Balaban B, Urman B, Ata B, Isiklar A, Larman MG, Hamilton R, Gardner DK.

Reprod Biomed Online. 2010 Feb;20(2):209-22. Epub 2009 Nov 27.

Slow freezing, vitrification and ultra-rapid freezing of human embryos: a systematic review and meta-analysis.

AbdelHafez FF, Desai N, Abou-Setta AM, Falcone T, Goldfarb J.

Reprod Biomed Online. 2005 Nov;11(5):608-14.

Comparison of open and closed methods for vitrification of human embryos and the elimination of potential contamination.

Kuwayama M, Vajta G, Ieda S, Kato O.

Table 1. Survival and development rates of human pronuclear (PN) embryos cryopreserved by either slow cooling or vitrification using the Cryotop method.

	Slow cooling	*Vitrification*
Survived/cryopreserved rate (%)	1730/1944 (89)[a]	5881/5881 (100)[b]
Cleaved/surviving rate (%)	1557/1730 (90)[a]	5469/5881 (93)[b]
Blastocyst/cleaved rate (%)	796/1557 (51)[a]	3058/5469 (56)[b]
Blastocyst/cryopreserved rate (%)	796/1944 (41)[a]	3058/5881 (52)[b]

[a,b]Values within rows with different superscripts are significantly different ($P < 0.01$).

Table 2. Survival and pregnancy rates with human 4-cell embryos cryopreserved by either slow cooling or vitrification using the Cryotop method.

	Slow cooling	*Vitrification*
Survived/cryopreserved rate (%)	857/942 (91)[a]	879/897 (98)[b]
Pregnancy/transfer rate (%)	172/536 (32)[a]	136/504 (27)[a]

[a,b]Values within rows with different superscripts are significantly different ($P < 0.01$).

Kuwayama M. RBMOnline (2005)

Table 3. Survival and pregnancy rates with human blastocysts cryopreserved by either slow cooling as compared with vitrification using the Cryotop method.

	Slow cooling	*Vitrification*
Survived/vitrified rate (%)	131/156 (84)[a]	5695/6328 (90)[b]
Number of blastocysts transferred	127	5659
Pregnancy/transfer rate (%)	50/98 (51)[a]	2516/4745 (53)[a]
Live birth/transfer rate (%)	40/98 (41)[a]	2138/4745 (45)[a]

[a,b]Values within rows with different superscripts are significantly different ($P < 0.05$).

Table 4. Survival, pregnancy and delivery rates after single embryo transfer of human blastocysts vitrified with either the Cryotop or the CryoTip method.

	Cryotop	*CryoTip*
Survived/vitrified rate (%)	221/227 (97)	82/88 (93)
Pregnancy/transfer rate (%)	131/221 (59)	42/82 (51)
Delivery/transfer rate (%)	113/221 (51)	39/82 (48)

No significant differences between corresponding values were found.

Gene expression patterns between slow freezing and vitrification

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Clin Exp Reprod Med 2011;38(4):203-209

CERM

In vitro development and gene expression of frozen-thawed 8-cell stage mouse embryos following slow freezing or vitrification

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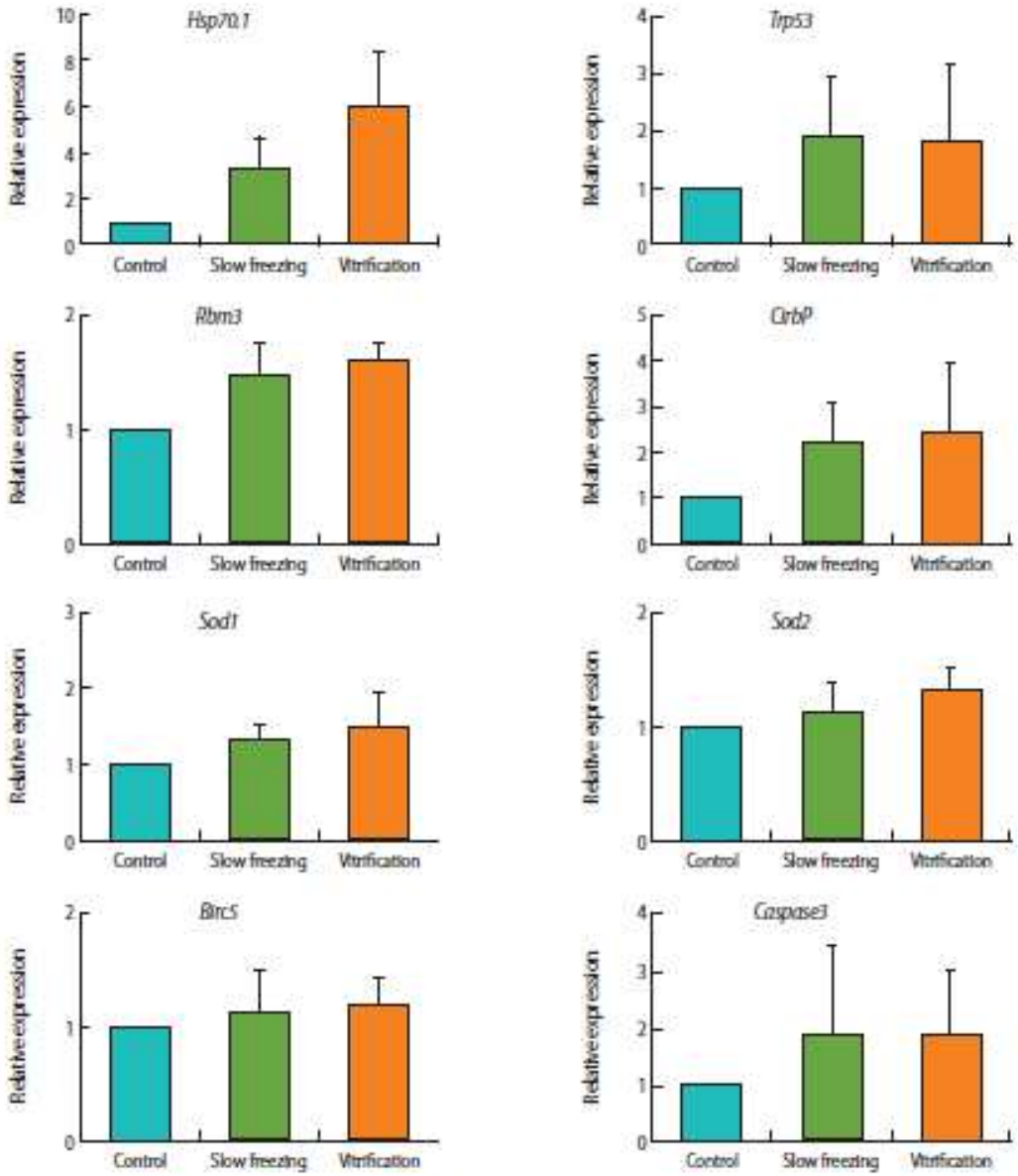
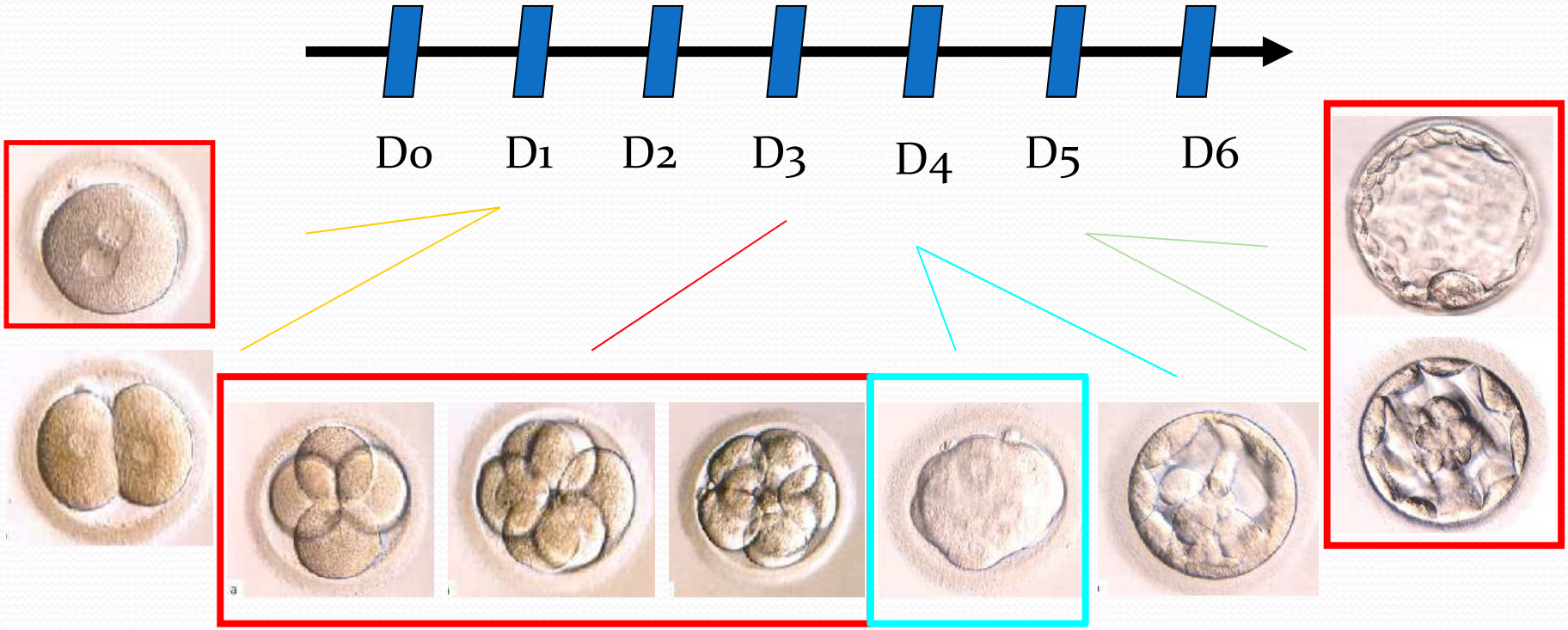


Figure 1. Relative expressions of the eight genes in thawed 8-cell mouse embryos frozen by slow freezing or vitrification. There was no significant difference in gene expressions among the groups. Relative expression levels are expressed as mean ± SE.

Developmental stage of embryos

Developmental stages of embryo



Clinical outcomes – developmental stage

J Assist Reprod Genet. 2005 Jan;22(1):33-5.

Successful pregnancy after the vitrification of zygotes using commercial vitrification solutions and conventional straws to protect against infections in liquid nitrogen.

Kumasako Y, Kumon M, Utsunomiya T, Araki Y.

J Assist Reprod Genet. 2009 Jun;26(6):347-54. Epub 2009 Jun 10.

Vitrification versus slow freezing gives excellent survival, post warming embryo morphology and pregnancy outcomes for human cleaved embryos.

Rezazadeh Valojerdi M, Eftekhari-Yazdi P, Karimian L, Hassani F, Movaghar B.

Fertil Steril. 2011 Mar 1;95(3):948-52. Epub 2010 Aug 1.

Prediction of pregnancy rate by blastocyst morphological score and age, based on 1,488 single frozen-thawed blastocyst transfer cycles.

Goto S, Kadowaki T, Tanaka S, Hashimoto H, Kokeguchi S, Shiotani M.

Fertil Steril. 2010 Mar 1;93(4):1353-5. Epub 2009 Oct 7.

Outcomes of day-1, day-3, and blastocyst cryopreserved embryo transfers.

Moragianni VA, Cohen JD, Smith SE, Schinfeld JS, Somkuti SG, Lee A, Barmat LI.

Day 4 – compaction/morula

Fertil Steril. 2001 Mar;75(3):629-31.

Pregnancies achieved after transferring frozen morula/compact stage embryos.

Tao J, Tamis R, Fink K.

Fertil Steril. 2004 Jul;82(1):108-18.

Cryopreservation of human embryos at the morula stage and outcomes after transfer.

Tao J, Craig RH, Johnson M, Williams B, Lewis W, White J, Buehler N.

TABLE 3

Relationship between embryo quality and post-thaw survival rate and transferable rate.

Embryo quality	Grade 1	Grade 2	Grade 3	Average
Post-thaw survival rate	84.9 (90/106)	84.4 (108/128)		
No. of transfers per thaw	69.8 (74/106)	68.8 (88/128)		

Survival rate – 89.2%

[a] Significant difference compared with grade 1 embryo ($P < .05$).
[b] Significant difference compared with grade 2 embryo ($P < .01$).
[c] Significant difference compared with grade 1 embryo ($P < .01$).

TABLE 4

Correlations between transferred post-thaw embryo qualities and outcomes.

Variables	Group A	Group B	Group C	Average
No. of cases	13	31	93	
No. of embryos thawed	3.5 ± 1.3	3.5 ± 1.4	3.1 ± 1.1	3.2 ± 1.3
No. of embryos transferred	2.5 ± 0.9	2.6 ± 0.8	2.4 ± 0.7	2.5 ± 0.7
Positive pregnancy test	46.2 (6/13)			
Clinical pregnancy	15.4 (2/13)			
Implantation rate	9.4 (3/32)			
Ongoing/live birth	15.4 (2/13)	29.0 (9/31)	61.3 (57/93)[b,d]	49.6 (68/137)

Clinical pregnancy rate – 57.7%

[a] Significant difference compared with group A ($P < .05$).
[b] Significant difference compared with group A ($P < .01$).
[c] Significant difference compared with group B ($P < .05$).
[d] Significant difference compared with group B ($P < .01$).

Tao J. Fertility and sterility (2004)

PGD (preimplantation genetic diagnosis) - blastocyst

Vitrification of preimplantation genetically diagnosed human blastocysts and its contribution to the cumulative ongoing pregnancy rate per cycle by using a closed device

María-José Escribá, Ph.D.,[a] *Jesús-Félix Zulategui, Ph.D.,*[a] *Aranzazu Galán, Ph.D.,*[a] *Amparo Mercader, Ph.D.,*[a] *José Remohí, M.D.,*[a,b] *and María-José de los Santos, Ph.D.*[a]

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Blastocyst vitrification significantly increased the cumulative ongoing pregnancy rate in PGD.

Fertility and Sterility, 2008

Re-cryopreservation

Re-cryopreservation

Fertil Steril. 2009 Feb;91(2):383-6. Epub 2008 Mar 4.

The efficacy of the transfer of twice frozen-thawed embryos with the vitrification method.

Kumasako Y, Otsu E, Utsunomiya T, Araki Y.

TABLE 2

Clinical results of once-frozen and twice-frozen groups.

	Once frozen	Twice frozen	*P*
Cancellation rate (%)	35/201 (17.4)	14/50 (28.0)	NS
Survival rate (%)	383/431 (88.9)	53/63 (84.1)	NS
Pregnancy per treatment cycle (%)	43/201 (21.4)	10/50 (20.0)	NS
Pregnancy rate per embryo transfer cycle (%)	43/166 (25.9)	10/36 (27.8)	NS
Spontaneous abortion rate (%)	14/43 (32.6)	2/10 (20.0)	NS
Implantation rate (%)	48/249 (19.3)	11/44 (25.0)	NS

Note: NS, not statistically significant.

Kumasako. Pregnancy using twice frozen embryos. Fertil Steril 2009.

Reprod Biomed Online. 2012 Mar;24(3):314-20. Epub 2011 Nov 30.

Vitrification of human embryos previously cryostored by either slow freezing or vitrification results in high pregnancy rates.

Stanger J, Wong J, Conceicao J, Yovich J.

Table 3 Survival rates of revitrified embryos compared with routine vitrification–warming.

Embryo age at revitrification	*Recryopreserved*	*Routine vitrification–warming*[a]
Day 3	16/16 (100)	129/173 (75)
Day 5/6	14/15 (93)	69/77 (90)
Total	30/31 (97)	198/250 (79)

Values are *n*/total (%).
[a]Warmed embryos vitrified between 2009 and 2010.

Summary & Conclusion

Summary I

- Vitrification resulted in significantly higher survival, and clinical pregnancy rates.
- In experienced groups, vitrification was not associated with a higher pregnancy than slow freezing.
- There is still no consensus as to the optimal development stage for embryo cryopreservation.

Summary II

- Recryopreservation of embryos is useful protocol. It provides the patient and the clinic with advantages by maximizing the chance of pregnancy while minimizing the number of transfers.

Conclusion

- A growing number of centers are incorporating vitrification as it is a simple, reproducible, robust, and inexpensive technique to cryopreservation embryos.

- Both techiniques (slow and vitrification) may offer good results in experienced hands, although vitrification results in higher survival rate, and most groups report better outcomes with vitrification than with slow freezing.

- In each centers, they can choice and establish the suitable cryopreservation method and embryonic stage.


THANK YOU!