Embryo vitrification and transfer in the natural cycle: redefining routine practice in IVF

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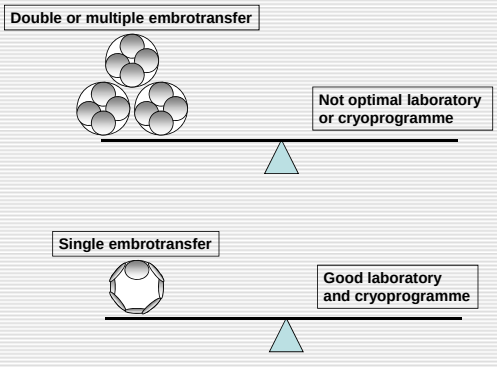
ESHRE guidelines for good practice in IVF laboratories:

Techniques and facilities for cryopreservation of gametes, zygotes and embryos should be available in each IVF centre.

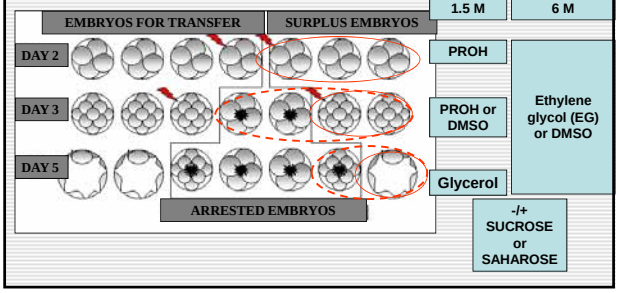
Aims (Why to freeze?):

- **Storing the spare embryos from IVF.**
- **Delaying ET in cases of OHSS w/o sacrificing the stimulated cycle.**
- **Cryo- quarantine enables a safe gamete donation programme.**
- **Storing gametes or embros for fertility preservation in patients with cancer or risk of early menopause.**

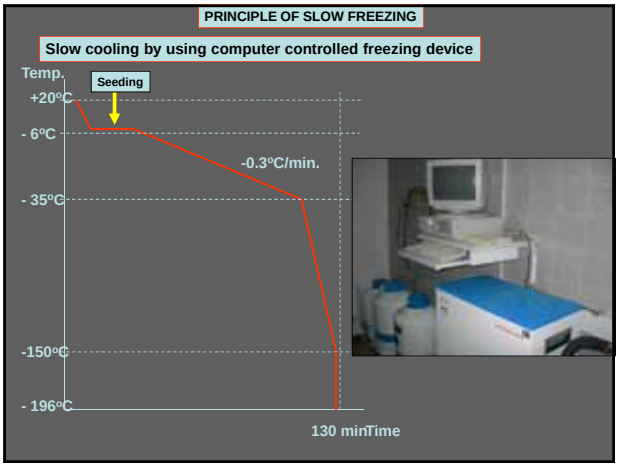


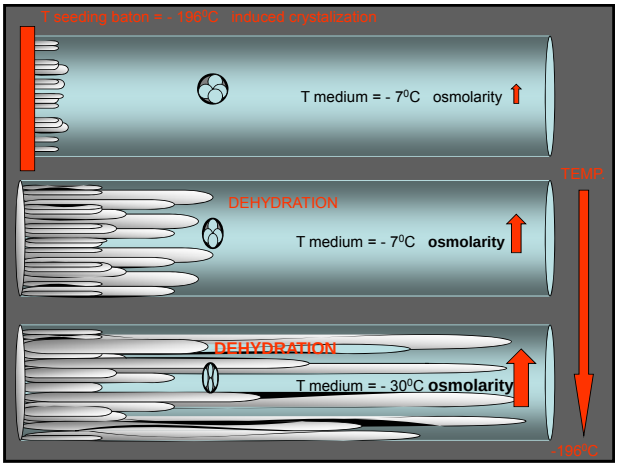
Different routine practices in IVF:

- How many to transfer, how many to freeze?
- Which to transfer, which to freeze? Selection !
- When to transfer, when to freeze? Selection !
- How to freeze?



CRYOPROTECTANTS	
SLOW FREEZING	VITRIFICATION
1.5 M	6 M
PROH	Ethylene glycol (EG) or DMSO
PROH or DMSO	
Glycerol	
-/+ SUCROSE or SAHAROSE	



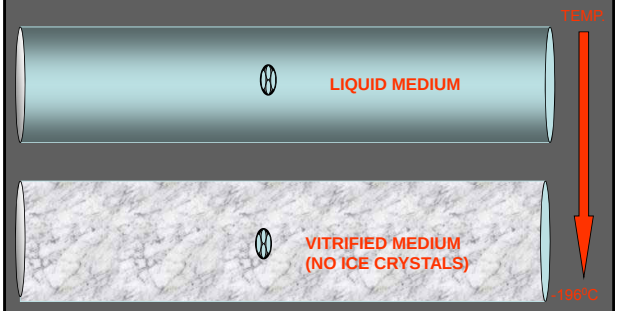


PRINCIPLE OF VITRIFICATION

Temp.
+20ºC
- 196ºC
Time

• High conc. of cryoprotectant
• Rapid cooling / warming

PRINCIPLE OF VITRIFICATION



Advantages of vitrification

- Avoids the growth of extra and intracellular ice cristals by solidification of surrounding medium.
- Does not require expensive equipment.
- Uses small amount of LN2.
- Reduces embryo exposuring out of incubator.
- Maintais the physiological temperature during equilibration.
- Allows the manipulation with the embryos of single patient.
- Is not time consuming. (?)
- Is more effective.

But

- Only few comparative studies on the effectiveness of different commercial vitrification solutions and straws (Guns et al., 2008; Hum Rep, O-134)

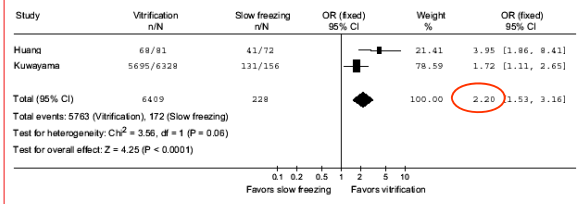
Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis

Kalliopi E. Loutradi, M.Med.Sc., Efstratios M. Kolibianakis, M.D., Ph.D., Christos A. Venetis, M.D., M.Sc., Evangelos G. Papanikolaou, M.D., Ph.D., George Pados, M.D., Ph.D., Ioannis Bontis, M.D., Ph.D., and Basil C. Tarlatzis, M.D., Ph.D.

FIGURE 2

FIGURE 3

Odds ratio of postthawing survival rate of blastocysts after vitrification and slow freezing.



Loutradi. Techniques and Instrumentation. Fertil Steril 2008.

Review

Current aspects of blastocyst cryopreservation

M Youssry[a], B Cornet[a], K Zohni, K Diedrich, S Al-Hasani[a]

Table 2. The outcome of several studies on slow freezing of human blastocysts.

Reference	Thawing blastocysts	Survival rate (%)	Pregnancy rate (%)	Implantation rate (%)
Liebermann and Tucker, 2006	254 cycles	Day 5: 91.4	42.8	29.6. Apparently higher implantation (not significant) with day 5 versus day 6 blastocysts
		Day 6: 94.8	43.1	28.2
Stehlik *et al.*, 2005	71	83.1 (59/71)	16.7 (4/24)	6.8 (4/59)
	76	89.5 (68/76)	18.5 (5/27)	7.4 (5/68)
Kuwayama *et al.*, 2005	156	84.0 (131/156)	51.0 (50/98)	NA
Veeck *et al.*, 2004	628	76.3 (479/628)	59.2	No differences between day 5 and day 6 embryos
Anderson *et al.*, 2004	202	81.2 (164/202)	69.0	43.0
Martin *et al.*, 2003	624	85.9	30.6	23.3
Nakayama *et al.*, 1995	69	Day 5: 78.3 (54/69)	1.7 (2/119)	Developmental rates were significantly lower for day 6 embryos 6.0 (3/50) than for day 5 embryos 18.8 (13/69)
	54	Day 6: 64.8 (35/54)		
Kaufman *et al.*, 1995	1239	83.4 (1033/1239)	21.7	13.4 high pregnancy rate in programmed cycles
Ménézo *et al.*, 1992	106	NA	21.0	NA
Troup *et al.*, 1990	34	38.2 (13/34)	No pregnancy	NA

NA = not available.

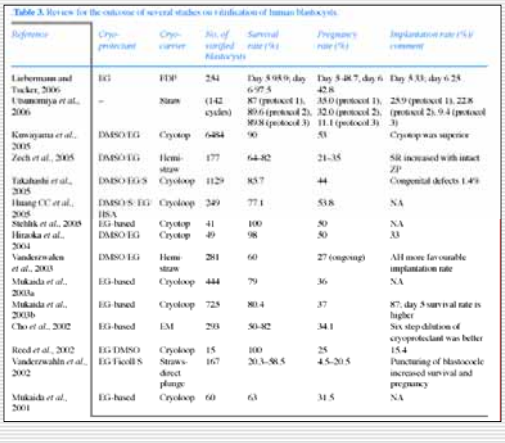


Table 3. Review for the outcome of several studies on vitrification of human blastocysts.

Reference	Cryo-protectant	Cryo-carrier	No. of vitrified blastocysts	Survival rate (%)	Pregnancy rate (%)	Implantation rate (%)/ comment
Liebermann and Tucker, 2006	EG	FDP	254	Day 5 98.9; day 6 97.5	Day 5 46.7, day 6 42.8	Day 5 33; day 6 28
Ubaramiya *et al.*, 2006	–	Straw	(142 cycles)	87 (protocol 1), 89.6 (protocol 2), 90.8 (protocol 3)	35.0 (protocol 1), 32.0 (protocol 2), 11.1 (protocol 3)	25.9 (protocol 1), 22.8 (protocol 2), 9.4 (protocol 3)
Kuwayama *et al.*, 2005	DMSO/EG	Cryotop	6484	90	53	Cryotop was superior
Zech *et al.*, 2005	DMSO/EG	Hemi-straw	177	64–82	21–35	SR increased with intact ZP
Takahashi *et al.*, 2005	DMSO/EG/S	Cryoloop	1129	85.7	44	Congenital defects 1.4%
Huang CC *et al.*, 2005	DMSO/S/ EG/ HSA	Cryoloop	249	77.1	53.8	NA
Stehlik *et al.*, 2005	EG-based	Cryotop	41	100	50	NA
Hiraka *et al.*, 2004	DMSO/EG	Cryotop	49	98	50	33
Vandervalden *et al.*, 2003	DMSO/EG	Hemi-straw	281	60	27 (ongoing)	AH more favourable implantation rate
Mukaida *et al.*, 2003a	EG-based	Cryoloop	444	79	36	NA
Mukaida *et al.*, 2003b	EG-based	Cryoloop	725	80.4	37	87; day 5 survival rate is higher
Cho *et al.*, 2002	EG-based	EM	293	50–82	34.1	Six step dilution of cryoprotectant was better
Reed *et al.*, 2002	EG/DMSO	Cryoloop	15	100	25	15.4
Vanderzwalmen *et al.*, 2002	EG/Ficoll/S	Straws-direct plunge	167	20.3–58.5	4.5–20.5	Puncturing of blastocoele increased survival and pregnancy
Mukaida *et al.*, 2001	EG-based	Cryoloop	60	63	31.5	NA

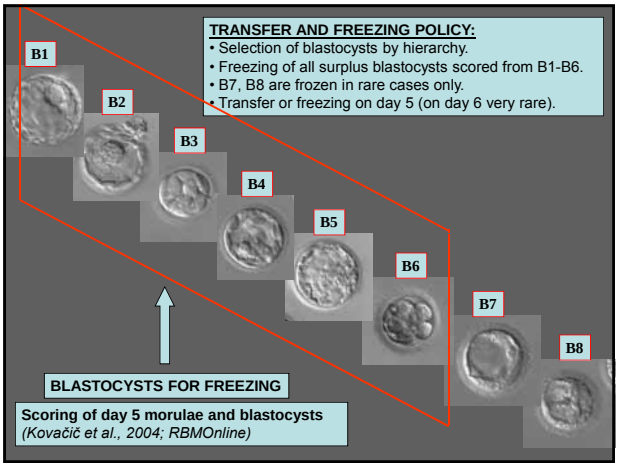
Our approaches:

- **From 2000 – 2003: all blastocyst cycles, also in natural cycles and poor responders** *(Vlaisavljević et al., 2001; Kovačič et al., 2002).*
  Transfer of mostly 2 blastocysts.
  Slow blastocyst freezing.

- **From 2004: individual approach:**
  Day 3 or day 5 transfer of 1 or 2 embryos or blastocysts.
  Slow blastocyst freezing.

- **From 2008: obligatory:**
  eSET of top quality embryo in patients aged <36 in 1.and 2. IVF cycle.
  Vitrification of blastocysts.
  Procedure was previously tested by mouse blastocysts.

  – Counsel on day 3 of embryo culture (gynecologist, embryologist, patients).
  – Consideration:
    - Insurrance conditions.
    - Risk of OHSS.
    - Infertility treatment history.
    - Embryo morphology and developmental stage.
    - Patients decision.



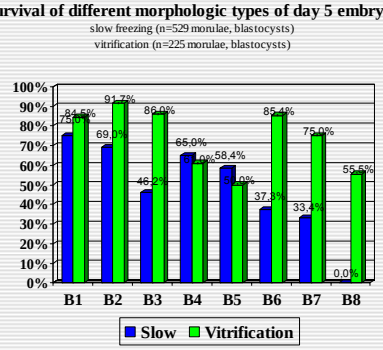
Methods:

- **Slow blastocyst freezing from 2000-2007:**
  – **Menezo's protocol** *(Menezo et al., 1992).*
  – **Medicult study media and Vitrolife Blast-Freeze /Thaw Kit.**
  – **Transfer 1.5 frozen/thawed blastocysts (> 50% of intact cells).**

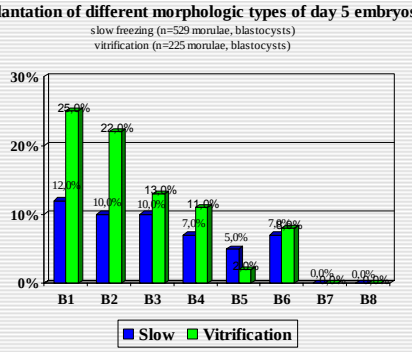
- **Vitrification from 2008:**
  – **Medicult and Irvine Scientific vitrification media** *(Kuwayama and Kato 2000).*
  – **Transfer 1.5 devitrified blastocysts (>50% of intact cells).**

- **Transfer in natural cycles in ovulatory patients** (urine LH test), **or in artificial cycles** (estradiol valerate (Estrofem) and progesterone (Utrogestan)) **in anovulatory patients.**
- **Ultrasound measurement of endometrial thickness from day 10.**

RESULTS: Survival rate of blastocysts after slow freezing or vitrification



RESULTS: Implantation rate of blastocysts after slow freezing or vitrification



RESULTS:

Retrospective analysis of nonselective blastocyst slow freezing (n=667 cycles)

	At least 1 optimal blastocyst (n=317)	Only nonoptimal blastocysts (n=350)
Survival rate (%)	77.3	65.6
Transfers (%)	100	91.3
Transferred blastocysts	1.6 ± 0.5	1.3 ± 0.7
Positive beta hCG (%)	33.3	22
Ongoing pregnancy (%)	**19.7***	**12.7**

Nonselective blastocyst vitrification (n=124 cycles)

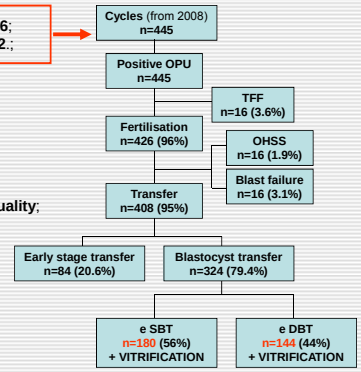
	At least 1 optimal blastocyst thawed (n=43)	Only nonoptimal blastocysts thawed (n=81)
Survival rate (%)	80	78.2
Transfers (%)	96	94.2
Transferred blastocysts	1.5 ± 0.6	1.4 ± 0.7
Positive beta hCG (%)	44.4	23.4
Ongoing pregnancy (%)	**33.3****	**18.8**

Statistical significance: * * *P*<0.01

RESULTS: Patients which should have eSET and vitrification:

Slovene Health Insurance requirement for **eSET** in:

- Women age: **<36**;
- Cycle number: **1. or 2.**;
- Embryo quality: **top quality**;



RESULTS:

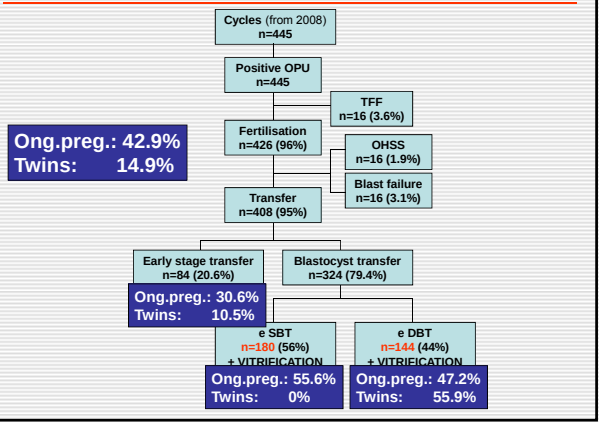
Pregnancy rates and cumulative pregnancy rates after elective single blastocyst transfers (eSBT) and double blastocyst transfers (eDBT).

Patients: age <36; 1. or 2. IVF attempt; day-5 transfer.

	eSBT	eDBT
No. of cycles	180	144
No. of cycles with vitrified blastocysts	147 (81.7)	79 (54.8)
Ongoing pregnancies (%)	100 **(55.6)**	68 **(47.2)**
Singletons (%)	100 (100)	30 (44.1)
Twins (%)	**0**	**38 (55.9)**
Blastocyst devitrification cycles*	40	31
Ongoing pregnancies (%)	13 **(32.5)**	4 **(12.9)**
Cumulative pregnancy rate (%)	113 **(62.8)**	72 **(50)**

* From the first year of vitrification programme.

RESULTS: Patients which should have eSET and vitrification:



ARTIFICIAL SHRINKAGE ?

Chen et al., 2005; Kader et al., in press;
Son et al., 2003; Hiraoka et al., 2004; Zech et al., 2005; Mukaida et al., 2006

ASSISTED HATCHING ?

Zhu et al., 1996; Cervera et al., 2003; Hershlag et al., 2005; Kung et al., 2003

Conclusions

- **Vitrification is a simple and successful method for cryopreservation of embryos and blastocysts.**
- **Vitrification of blastocysts results higher survival and implantation rates than slow freezing of blastocysts.**
- **Prolonged embryo culture and efficient cryopreservation (vitrification) of surplus blastocysts offer:**
  - **Good posibilities for elective single blastocyst transfer.**
  - **Easier decision for the cancellation of transfer in stimulated cycle because of side effects of gonadotrophins.**

RESULTS: Transfers of slowly frozen / thawed blastocysts in natural and artificial cycles

	Natural cycles	Artificial cycles
No. of thawing cycles	249	80
No. of survived / thawed blastocysts (%)	397/590 (67.3)	139/212 (65.6)
No. of transfers (ET)	226	73
Mean no. of transferred blastocysts	1.6 +/- 0.8	1.7 +/- 0.7
Positive beta hCG / thawing (%)	65/249 (26.1)	18/80 (22.5)
Clinical pregnancies / thawing (%)	52/249 (20.9)	10/80 (12.5)
Deliveries / thawing (%)	**43/249 (17.3) [b]**	**7/80 (8.8)**
Deliveries / transfer (%)	43/226 (19) [b]	7/73 (9.6)
Babies / thawed blastocyst (%)	49/590 (8.3) [a]	8/212 (3.8)
Babies / transferred blastocyst (%)	49/389 (12.6) [b]	8/134 (6)

Statistical significance: a $P<0.05$, b $P<0.01$