An overview of the vitrification of human embryos and blastocysts

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Introduction

ART success = Birth of a healthy singleton baby

- Multiple pregnancies

Treatment

SET: Day 2-3 or Day 5

sFRET: Day 2-3 or Day 5

- Ovarian stimulation

→ OHSS and suboptimal endometrium

Treatment

Freeze all - Transfer in natural cycles (sFRET)

Supernumerary embryos for cryopreservation

Increase efficiency of ART

Tool to reduce multiple pregnancies

→ **Efficient cryopreservation programmes**

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Introduction

Efficient cryopreservation programmes

→ *Data from national and international registers:*

- Not all embryos survive the cryopreservation with all cells intact
- The implantation potential of cryopreserved embryos is lower than of fresh embryos
- The implantation potential of fully intact embryos > embryos with blastomere loss after cryopreservation

The aim of a cryo programme should be to have fully intact embryos after thawing

Introduction

Cryopreservation of reproductive cells

↓

Stopping biological time

↓

-196°C

↓

Lethal intra-cellular ice formation

↓

Fate of cellular water

Equilibrium (quasi-equilibrium) cooling
(Freezing)

Non-equilibrium cooling
(Vitrification)

Dilemma

Which strategy is better for our patients: freezing or vitrification?

Recent developments: vitrification is the best strategy?

Rationale of vitrification

Vitrification:

- No extra and intra cellular ice crystal formation
- Dehydrate cell before cooling (no solution effects injury)
- Cool rapidly to “outrun” chilling injury
  - Low volumes to obtain very high cooling rates
  - Special devices available now
  - “Low toxicity” media formulations now available
- Flexibility

Overview of vitrification of embryos and blastocysts

Kuwayama et al (2005, 2007), Al - Hasani et al (2007), Mukaida et al (2007), Liebermann et al (2007), Desai et al (2007) ….

Vitrification is a simple, low cost, safe and efficient procedure for the cryopreservation of embryos and blastocysts

- Is vitrification a simple procedure?
- Is vitrification a low cost procedure?
- Is vitrification an efficient procedure?
- Is vitrification a safe procedure?

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Vitrification of embryos and blastocysts

Is vitrification a simple procedure (1) ?

→ Very quick procedure?

- Equilibration step(s) and Vitrification step
- Warming steps and several dilution steps
- One to one approach
- Artificial shrinking

Vitrification of embryos and blastocysts

Is vitrification a simple procedure (2) ?

→ Technical challenges

Probability of vitrification

$$\frac{\text{Cooling (}\textit{warming}\text{) rates x [CPA]}}{\text{Sample Volume}}$$

→ **Equilibrium "true" vitrification**: high [CPA], cooling rate independent, vol >100µl

→ **Non-equilibrium "apparent" vitrification**: low [CPA], high cooling rates, vol < 1µl

Vitrification of embryos and blastocysts

Is vitrification a simple procedure (3) ?

→ Technical challenges

- Succesfull vitrification depends on “sufficient” penetration of permeating CPA’s and “sufficient” dehydration by non-permeating CPA’s
  - Permeability characteristics of oocytes and embryos to water and CPA
  - Temperature and time dependency
  - Variability amongst embryos and blastocysts

Results highly dependent on the proficiency of staff

Vitrification of embryos and blastocysts

Is vitrification a low cost procedure?

No biological freezers required

Flexibility: manpower

Vitrification media and devices:

→ Commercial companies

- Expensive devices!
- Expensive media formulations!

Vitrification of embryos and blastocysts

Is vitrification an efficient procedure?

Evidence-based vitrification of embryos and blastocysts?

- → Efficiency (can it work)?
- → Effectiveness (does it work)?
- → Is it worth doing it?

Vitrification of embryos and blastocysts

Is vitrification an efficient procedure?

Data from literature:

Accepted papers in peer-reviewed journals (no abstracts, no case reports)

and chapters in books (Vitrification in Assisted Reproduction; Eds Tucker and Liebermann – 2007)

→ Immediate morphological surival

→ Developmental competence in-vitro

→ Metabolism

→ Pregnancies and implantation potential

Vitrification of embryos and blastocysts

Results from literature: some caution however!

→ Different devices and different media formulations used (>30 different vitrification protocols detected)

→ Oocyte collection cycle characteristics

- Patient selection
- Cryopreservation policy (selection of embryos before freezing)

→ Warming and transfer policy (selection of embryos for warming and for transfer)

→ Artificial shrinkage of blastocysts

→ No uniform reporting of data and (or) study endpoints

→ Commercial bias?

Vitrification of embryos: freezing versus vitrification (survival)

Loutradis et al (Fertil Steril 90, 186-193, 2008)

Systematic review and meta analysis on vitrification versus slow freezing of human embryos

→ *Comparative data on survival rates at the same developmental stage*

→ *Study should be published in a peer reviewed journal*

→ *Main outcome measures: post-thawing survival rate*

Potentially relevant studies evaluated: n = 873

Studies that were potentially able to answer the research aims: n = 90

Studies included in the meta analyses: n = 4

Properly designed RCT's n = 0!!

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Vitrification of embryos: freezing versus vitrification (survival)

Cleavage stage embryos morphological survival

	Vitrification	Slow freezing
Rama Raju	121/127	72/120
Zheng	46/49	8/52
Kuwayama	879/897	857/942

OR; 95% CI: 15.57 (3.68-65.82); p<0.001

Vitrification of embryos: freezing versus vitrification (survival)

Blastocysts morphological survival

	Vitrification	Slow freezing
Huang	68/81	42/71
Kuwayama	5695/6328	131/156

OR; 95% CI: 2.20 (1.53-3.16); p<0.0001

Vitrification of embryos: freezing versus vitrification (survival)

Conclusion

Vitrification ***appears*** to be associated with a significant higher post-warming survival rate as compared to slow cooling. Further prospective studies are necessary to confirm the above results and in addition, allow the evaluation of the two cryopreservation methods in terms of pregnancy achievement

Vitrification of embryos: freezing versus vitrification (RCT on survival, developmental potential in-vitro and metabolism)

Balaban et al (Hum Reprod 23, 1976-1982, 2008)

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

Women randomised 120 (60 vitrification and 60 freezing)

466 day 3 embryos (234 vitrification and 232 freezing)

Survival rate (fully intact embryos): Vitrification 173/234 (73.9%) and freezing 106/232 (45.7%) ($p < 0.01$)

Development to blastocyst (%): Vitrification (60.3%) and freezing (49.5%) ($p < 0.05$)

Pyruvate uptake: Vitrification > freezing(reflecting a higher metabolic rate)

Vitrification of embryos: freezing vs vitrification(meta-analysis on survival, developmental potential in-vitro implantation,clinical pregnancy and live birth rates)

Kolibianakis et al (Current opinion in OB/GYN 21, 270-274, 2009)

Cryopreservation of human embryos by vitrification or slow freezing: which one is better?

(Addition of 3 more published RCT's to the 2006 Loutradis paper)

Vitrification as compared with slow freezing, appears to be better in terms of post-thawing survival rates bi-oth for cleavage-stage embryos (odds ratio (OR): 6.35,95% CI: 1.14-35.26) and for blastocysts (OR:4.09, 95% CI:2.45-6.84)

Postthawing blastocyst development of embryos cryopreserved in the cleavage stage is significantly higher with vitrification as compared with slow freezing (OR: 1.56, 95% CI: 1.07-2.27)

No significant difference in clinical pregnancy rates per transfer could be detected between the two cryo methods (OR: 1.66, 95% CI: 0.98-2.79).

Vitrification of embryos: freezing vs vitrification(meta-analysis on survival, developmental potential in-vitro implantation,clinical pregnancy and live birth rates)

Kolibianakis et al (Current opinion in OB/GYN 21, 270-274, 2009)

Cryopreservation of human embryos by vitrification or slow freezing: which one is better?

Conclusion

Currently vitrification does not appear to be associated with an increased probability of pregnancy. However, a significant advantage of vitrification over slow freezing in terms of postthawing survival rates is present for embryos cryopreserved both at the cleavage stage and at the blastocyst stages. The above conclusions are based on limited data, and thus further properly designed controlled trials are needed

Vitrification of embryos and blastocysts

Pregnancies and implantation potential

Definitions:

Clinical pregnancy (CP): identification of a fetal sac

Evolutive clinical pregnancy (ECP): identification of a fetal sac with FHB

Implantation (I): fetal sac

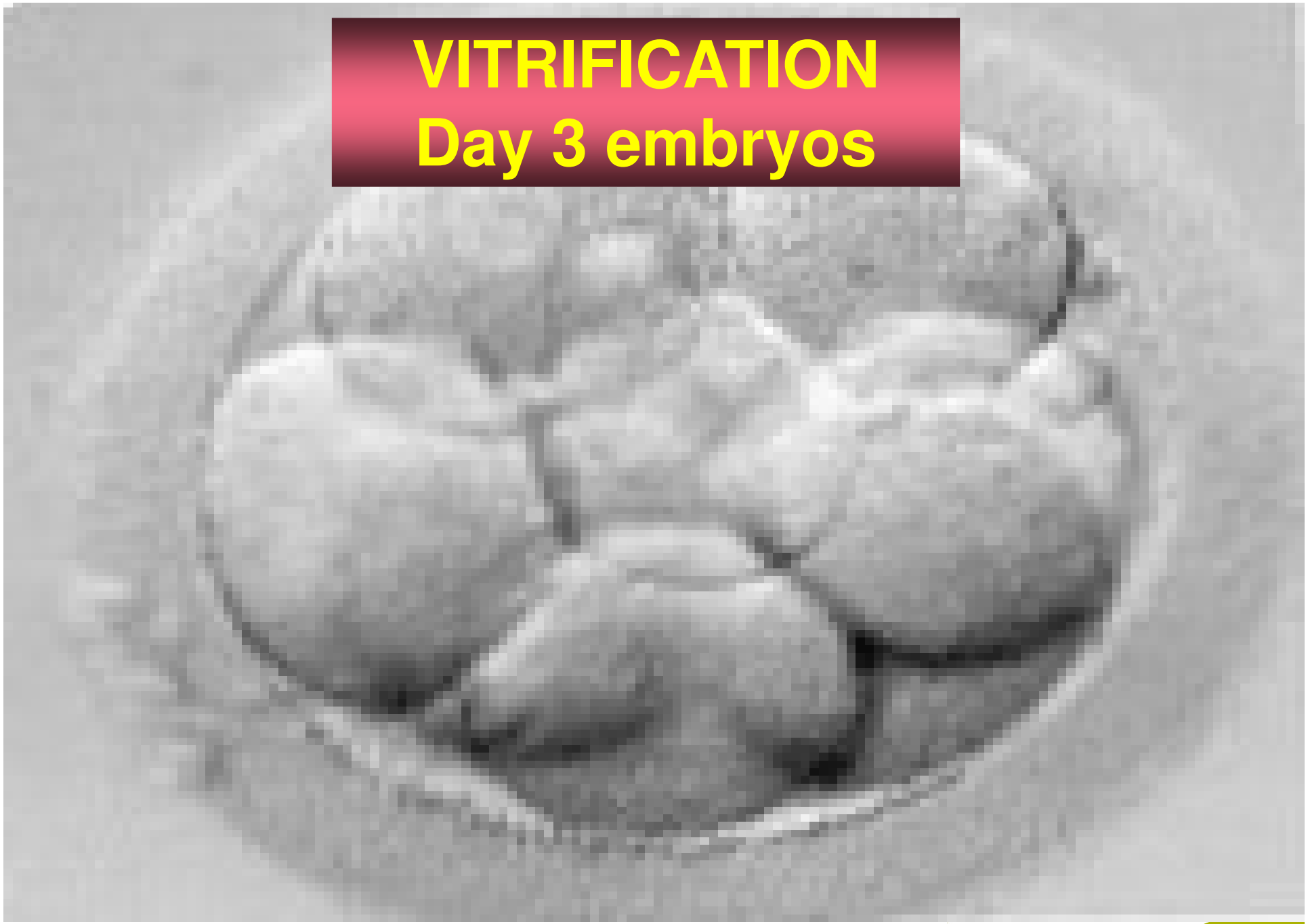
Evolutive implantation (EI): fetal sac with FHB


VITRIFICATION
zygotes

Vitrification of zygotes: outcome

	CP/ET(%)	I/E transferred (%)	I/E warmed (%)
Selman (2001)	2/4 (50.0)	2/11 (18.2)	2/27 (7.4)
Isachenko (2003)	4/10 (40.0)	4/26 (15.4)	4/59 (6.8)
Total (sacs)	*6/14 (42.9)*	*6/37 (16.2)*	*6/86 (7.0)*
	ECP/ET(%)	**EI/E transferred (%)**	**EI/E warmed (%)**
Al-Hasani (2007)	29/106 (27.4)	38/243 (15.6)	38/339 (11.2)
Total (FHB)	*29/106 (27.4)*	*38/243 (15.6)*	*38/339 (11.2)*

VITRIFICATION
Day 3 embryos



Vitrification of embryos: outcome

	CP/ET(%)	I/E transferred (%)	I/E warmed (%)
Kuwayama (2005)	136/504(27.0)	NA	NA
Mukaida (2007)	212/721 (29.4)	250/1764 (14.2)	250/2137 (11.7)
Total (sacs)	*348/1225 (28.4)*	*250/1764 (14.2)*	*250/2137 (11.7)*
	ECP/ET(%)	**EI/E transferred (%)**	**EI/E warmed (%)**
El Danasouri (2001)	11/36 (30.6)	NA	NA
Desai (2007)	34/77 (44.2)	40/201(19.9)	40/236 (16.9)
Balaban (2008)	36/73 (49.3)	50/168 (29.7)	50/241 (20.7)
Raju et al (2009)	105/285 (36.8)	148/817 (18.1)	148/904 (16.3)
Valojerdi et al (2009)	62/153 (40.5)	87/525 (16.6)	87/721 (12.1)
Total (FHB)	*248/624 (39.7)*	*325/1711 (19.0)*	*325/2105 (15.4)*

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VITRIFICATION BLASTOCYST

Vitrification of blastocysts: outcome

	CP/ET (%)	I/E transferred (%)	I/E warmed (%)
Huang (2004)	7/13 (53.8)	14/60 (23.3)	14/96 (14.6)
Kuwayama (2005)	2515/4745 (53.0)	NA	NA
Mukaida (2007)	750/1496 (50.1)	945/2722 (34.7)	945/3496 (27.0
Liebermann (2007)	228/541 (42.1)	310/1073 (28.9)	310/1140 (27.2)
Total (sacs)	*3500/6795 (51.5)*	*1269/ 3855 (32.9)*	*1269/4732 (26.8)*

Vitrification of blastocysts: outcome

	ECP/ET (%)	EI/E transferred (%)	EI/E warmed (%)
Choi (2000)	5/20 (25)	8/38 (21)	8/93 (8.6)
Yokota (2001)	6/18 (33.3)	7/32 (21.9)	7/45 (15.6)
Cho (2002)	14/41 (34)	19/92 (20.7)	19/120 (15.8)
Reed (2002)	1/4 (25)	1/13 (7.7)	1/15 (6.7)
Hiraoka (2004)	14/28 (50.0)	16/48 (33.3)	16/49 (32.7)
Hu et al (2004)	25/81 (30.9)	38/166 (22.9)	38/213 (17.8)
Stehlik (2005)	15/35 (42.8)	21/77 (27.3)	21/77 (27.3)
Takahashi (2005)	182/413 (44.1)	NA	327/1129 (29)
Son (2007)	498/1040 (47.9)	694/2698 (25.7)	694/3214 (21.6)
VD Zwalmen (2007)	124/414 (30.0)	146/848 (17.2)	146/1379 (10.6)
Hong (2009)	79/112 (70.5)	109/271 (40.2)	109/475 (22.9)
Ebner (2009)	49/113 (43.4)	61/158 (38.6)	61/273 (22.3)
Total (FHB)	*1012/2319 (43.6)*	*1120/4441 (25.2)*	*1447/7082 (20.4)*

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Vitrification of embryos and blastocysts

Is vitrification a safe procedure?

→ Open devices

- direct contact between samples and LN2

→ Long term LN2 storage (vapour storage) of apparently vitrified, minimal-volume (<1µl) samples

- Spontaneous devitrification possible

→ Cryoprotectants are NOT neutral

- Biological (long term) effects of vitrification?

→ Children follow-up? >2000 deliveries

- Perinatal outcome (~ 900 children)
  - Mukaida et al, 2009; Rama Raju et al, 2009; Wennerholm et al, 2009

General conclusions

Vitrification as clear as a glass?

→ Safety issues unanswered (long term safety issues)

- CPA's are NOT neutral

→ Technical challenges

- Development of more robust "true" vitrification procedures

→ Costs

General conclusions

Vitrification will it replace conventional freezing techniques?

→ Recent published data of the vitrification of human embryos and blastocysts indicate that vitrification works and produces even better results than conventional freezing. Prospective Randomised Controlled Trials?

- Effectiveness of vitrification:

The overall sucess rate (FHB/embryo warmed) is 11.2% for human zygotes, 15.4% for D3 embryos and 20.4% for blastocysts and these results are still lower than for their unfrozen counterparts (Blake et al, 2007)

→ Vitrification is worth doing it! It will replace conventional freezing techniques

→ Vitrification the next breakthrough in ART?