Assessment of frozen-thawed oocytes/embryos

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Introduction

Cryopreservation programme

↓

Freeze supernumerary (all or some) oocytes/embryos

↓

Thaw all frozen oocytes/embryos

↓

Transfer all "surviving" oocytes/embryos

Introduction

MULTIPLE pregnancy!!!
Successful ART treatment - Birth of a healthy child – SET

Ovarian (hyper) stimulation or not:

- Natural cycle: one oocyte – one embryo
- Mild stimulation: SET - no supernumerary embryos
- Ovarian hyper stimulation – SET - supernumerary embryos – cryopreservation - SFRET

→ Cryopreservation to increase the chances of success of ART

→ Cryopreservation as a tool to reduce multiple pregnancy

Efficient cryopreservation programmes

Introduction

Efficient cryopreservation programmes

Cryopreservation procedure

- Vitrification >>> slow controlled-rate freezing ??

Strategies to assess/select oocytes/embryos before freezing and after thawing

- Freezing policy
- Assessment of survival/transfer

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Human oocyte cryopreservation

Debra A. Gook[1,2,3] and David H. Edgar[1,2]

Table 5: Summary of clinical outcomes from oocyte cryopreservation using various protocols

	1.5 M PROH + 0.1 M sucrose	1.5 M PROH + 0.2 M sucrose	1.5 M PROH + 0.3 M sucrose	1.5 M PROH + 0.1 M sucrose (Na depleted)	1.5 M PROH + 0.2 M sucrose (Na depleted)	1.5 M PROH + 0.3 M sucrose (Na depleted)	Vitrification 2.7 M EG + 2.1 M DMSO + 0.5 M sucrose
Survival, % (no. of thawed oocytes)	50 (3537)	72 (926)	74 (4902)	52 (127)	62 (329)	59 (190)	91 (628)
Fertilization (ICSI), %	54	80	73	56	58	68	91
Cleavage, %	85	93	90	100	86	83	92
Embryos per 100 thawed oocytes	23	53	49	29	31	33	76
Implantation rate, %	10	17	5	21	11	16	14
Implantations per 100 thawed oocytes	2.3	9.1	2.4	6.1	3.4	5.3	11

Cryopreservation of embryos and blastocysts: freezing versus vitrification

Loutradis et al (Fertil Steril, in press) :

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 = 89

Studies included in the meta analyses: n = 4

Properly designed RCT's n = 0!!

Cryopreservation of embryos: freezing versus vitrification

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

Cryopreservation of blastocysts: freezing versus vitrification

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

Conclusion

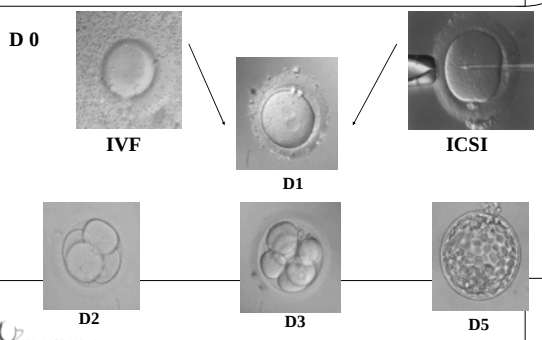
Vitrification ***appears*** to be associated with a significant higher post-thawing survival rate as compared to slow cooling. Further prospective studies are necessary to confirm the above results and in addition, allow the evaluation of the two cryopreservation methods in terms of pregnancy achievement

Introduction

Strategies to select oocyte/embryos before freezing and after thawing

Freezing policy
Assessment of survival/transfer

Freezing policy



Freezing policy

Freezing policy human oocytes

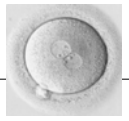
Donor – acceptor cycles
Cancer patients
Ethical/religious reasons

Freezing policy

Freezing policy human embryos and blastocysts

Freezing strategies

- Two strategies
  - S1: freezing embryos before morphology becomes a substantial factor: one-cell two pronucleate stage freezing



Freezing policy

Freezing policy human embryos and blastocysts

Freezing strategies

- S2: optimizing fresh transfer allowing the morphologically best embryos to be transferred: two- to 16-cell stage freezing or blastocyst stage freezing

Which embryos to freeze?

Freezing policy

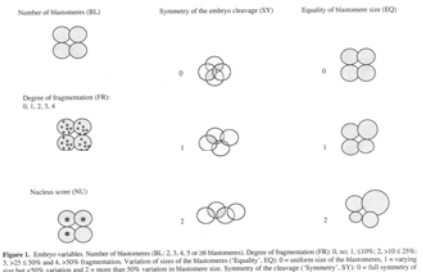
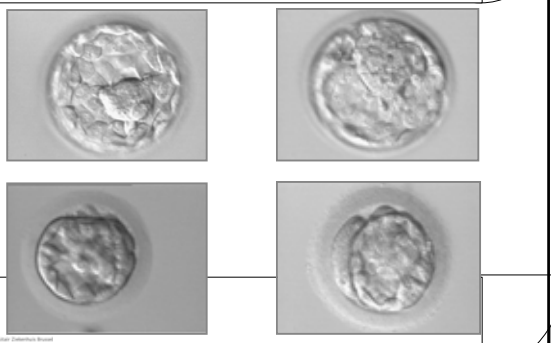


Figure 1. Embryo variables. Number of blastomeres (BL: 2, 3, 4, 5 or ≥6 blastomeres). Degree of fragmentation (FR): 0, no; 1, ≤10%; 2, >10 ≤ 25%; 3, >25 ≤ 50% and 4, >50% fragmentation. Variation of sizes of the blastomeres ('Equality', EQ): 0 = uniform size of the blastomeres, 1 = varying size but <50% variation and 2 = more than 50% variation in blastomere size. Symmetry of the cleavage ('Symmetry', SY): 0 = full symmetry of the cleaved embryo, 1 = slightly asymmetric cleavage and 2 = pronounced asymmetry. The parameter 'Nucleus score' (NU) was defined as the number of visible mononucleated blastomeres divided by the total number of blastomeres in the embryo (to correct for cleavage rate). Nucleus score 0 = a ratio of 0–0.25; Nucleus score 1 = ratio >0.25–0.50; Nucleus score 2 = ratio >0.50–0.75 and Nucleus score 3 = ratio >0.75. Nucleus score -1 denotes that the embryo contains at least one multinucleated blastomere.

Freezing policy



Freezing policy

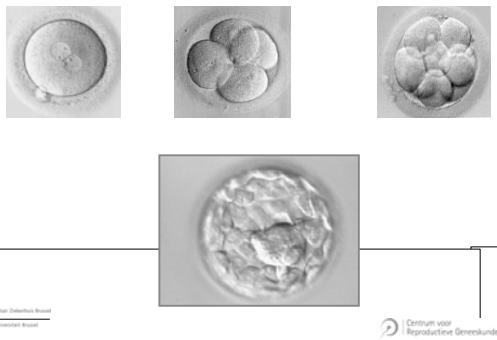
Freezing strategy

- Which stategy to use for each clinic is based on laboratory workload, general experience, success rates in fresh and cryopreserved cycles and legal/ethical considerations

Uniform reporting of the cryo data (Jones et al, 1995) (Hum Reprod 10, 2136-2138)

- Cycle cryopreservation rate (cycles with cryo/cycles with fresh transfer)
- Fresh embryo transfer rate (embryos transferred fresh/2PN embryos)
- Embryo cryopreservation rate (embryos frozen/2PN embryos)

Freezing policy



Freezing policy

Conclusion

Risk to throw out the child with the bathwater before freezing!!

Assessment of survival/transfer

Strategy to select oocyte/embryos before freezing and after thawing

Freezing policy
Assessment of survival/transfer

Assessment of survival/transfer

→ Developmental potential of an embryo:
- Birth of a healthy child

→ Intermediate embryo assessments:
- Morphological survival
- Developmental potential in-vitro

Assessment of survival/transfer

1. Morphological survival

1.1. Oocytes

Fully intact (ET)
- Polscope analysis of meiotic spindle (Rienzi et al 2004, Hum Reprod 19, 655-659)

Degenerated (no ET)

Assessment of survival/transfer

1. Morphological survival

1.2. Pronucleate stage

Fully intact (ET)
Degenerated (no ET)

Assessment of survival/transfer

1. Morphological survival

1.3. Intermediate stage embryos (2- to 16-cell stage)
Fully intact (ET)
≥50% intact (ET)
<50% intact (some ET)
0% intact (no ET)

Assessment of survival/transfer

Morphological survival and transfer

- Consequences of blastomere survival
  - No influence of blastomere loss:
    Hartshorne et al (1990) (Hum Reprod 5, 857-861)
    Testart et al (1990) (Adv Ass Reprod Technol, 573)
    Mandelbaum et al (1998) (Hum Reprod 1, (suppl 3) 161-177)

Assessment of survival/transfer

Van den Abbeel et al (1997) (Hum Reprod 12, 2006-2010)

Implantation potential (children born/ embryo transferred) of fully intact embryos : 10.4% (n = 431)

Implantation potential (children born/ embryo transferred) of damaged embryos only: 2.9% (n = 488)

Blastomere loss more important in 4-cell embryos as compared to 8-cell embryos?

Assessment of survival/transfer

Morphological survival and transfer

- Consequences of blastomere survival
  - Influence of blastomere loss:
    Speirs et al (1996) (Hum Reprod 11 (suppl 1) 107-192)
    Burns et al (1999) (Fertil Steril 72, 527-532)
    Edgar et al (2000) (Hum Reprod 15, 175-179)
    Guérif et al (2002) (Hum Reprod 17, 1321-1326)
    Pal et al (2004) (Fertil Steril
    Gabrielsen et al (2005) (RBM online 12, 70-76)
    Tang et al (2006) (Hum Reprod 21, 1179-1183)
    Edgar (2007) (RBM Online 14, 718-723)

Assessment of survival/transfer

To what extent intermediate-stage embryos may loose cells after thawing without subsequent viability loss?

- Day 2 embryos: Edgar (2007) (RBM Online 14, 718-723)
- Day 3 embryos: Van Landuyt and Van den Abbeel (unpublished observations)

Assessment of survival/transfer

Table 3. Outcome from single cryopreserved embryo transfer (SCET) in women under 36 years in relation to number of surviving blastomeres (day-2 embryos cryopreserved at the 4-cell stage).

Pre-freeze blastomeres	*Post-thaw blastomeres*	*SCET*	*FH*	*Implantation rate %*
4	4	615	160	26.0
4	3	131	36	27.5
4	2	85	8	9.4

FH = fetal heart beat.

Assessment of survival/transfer

Cell loss in human day 3 embryos and implantation in single frozen embryo transfers (Van Landuyt and Van den Abbeel 2004-2007: 547 single FRET cycles, cryo day 3, ET day 4)

Table I. Clinical pregnancy rates (%) in relation to cell loss after freezing

	Cell stage before freezing				
N° cells damaged	6	7	8	>8	Total
0	12/64 (18.8)	20/82 (24.4)	30/175 (17.1)	15/61 (24.6)	77/382 (20.2)
1	1/5 (20.0)	3/19 (15.8)	9/43 (20.9)	7/19 (36.8)	20/86 (23.3)
2	0/2 (0.0)	4/10 (40.0)	2/17 (11.8)	0/5 (0.0)	6/34 (17.6)
>2	1/3 (33.3)	0/3 (0.0)	2/10* (20.0)	0/8 (0.0)	3/24 (12.5)

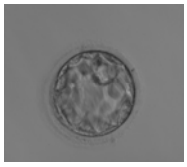
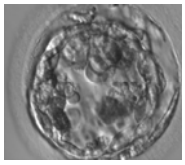
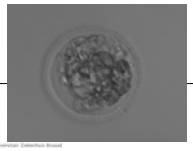
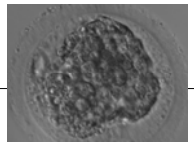
*2 pregnancies after transfer of embryos with 50% cell loss (4/8)

Assessment of survival/transfer

1. Morphological survival

1.4. Blastocyst stage
- Fully intact (ET)
- Moderately damaged (ET)
- Severely damaged (some ET)
- Degenerated (no ET)

Assessment of survival/transfer

Assessment of survival/transfer

1. Morphological survival

Conclusion:

Consequences of cellular loss:
- → Toxicity?
- → Hindering?
- → Totipotency?

It should be the aim of a cryopreservation programme to have fully intact oocytes/embryos after thawing

Assessment of survival/transfer

2. Developmental potential in-vitro

Fertilisation after IVF/ICSI

Further cleavage after 24 hours (resumption of mitosis)

Further cleavage up to the blastocyst stage

Reexpansion and expansion of thwad blastocysts

Oocytes, zygotes, intermediate-stage embryos, blastocysts

Assessment of survival/transfer

2.1. Developmental potential in-vitro of intermediate-stage embryos

- 2.1.1. Capability of resumption of mitosis after 24h

  - Van Der Elst et al (1997) Hum Reprod 12, 1513-1521)
  - Ziebe et al (1998) (Hum Reprod 13, 178-181)
  - Van den Abbeel et al (2000) (Hum Reprod 15, 373-378)
  - Tiitinen et al (2001) (Hum Reprod 16, 1140-1144)
  - Guérif et al (2002) (Hum Reprod 17, 1321-1326)
  - Edgar (2007) (RBM Online 14, 718-723)

Assessment of survival/transfer

Which embryos are likely to implant after a post-thaw 24 h culture period in-vitro

- Edgar (2007) (RBM Online 14, 718-723)
- Van Landuyt and Van den Abbeel (unpublished observations)

Assessment of survival/transfer

Table 4. Outcome from single cryopreserved embryo transfer (SCET) in women under 36 years in relation to post-thaw resumption of mitosis (day-2 embryos cryopreserved at the 4-cell stage).

Blastomere survival	*Overnight cleavage*	*SCET*	*FH*	*Implantation rate (%)*
4/4	Yes	540	148	27.4
4/4	No	75	12	16.0
3/4	Yes	99	31	31.3
3/4	No	32	5	15.6
2/4	Yes	64	7	10.9
2/4	No	21	1	4.8

FH = fetal heart beat.

Assessment of survival/transfer

Cell loss in human day 3 embryos, resumption of mitosis and implantation in single frozen embryo transfers
(Van Landuyt and Van den Abbeel
2004-2007: 547 single FRET cycles, cryo day 3, ET day 4)

	Fully intact embryos	Damaged embryos
% Compact (24h)	72.4	72.1
% Pregnant	29.9	28.8
% Not compact (24h)	27.6	27.9
% Pregnant	11.1	11.6

Assessment of survival/transfer

2.2. Developmental potential in-vitro of thawed blastocysts

→ Early blastocysts
- Capability of expansion
  Van den Abbeel et al 2005) (Hum Reprod 20, 2939-2945)
  Guerif et al (2003) (Theriogenology 60, 1457-1466)

→ Expanded blastocysts
- Capability of re-expansion
  Van den Abbeel et al 2005) (Hum Reprod 20, 2939-2945)
  Guerif et al (2003) (Theriogenology 60, 1457-1466)
  Shu et al 2008 (Fertil Steril, in press)

Assessment of survival/transfer

Is it necessar to culture thawed blastocysts overnight?

- Yes: 4 h culture versus 24 h culture
  Guerif et al (2003) (Theriogenology 60, 1457-1466)

- No: 4 h culture - degree of re-expansion
  Shu et al 2008 (Fertil Steril, in press)

Assessment of survival/transfer

Develomental potential in-vitro
Conclusion:

Developmental potential in-vitro of frozen/thawed oocytes/embryos ascertains their viability

Assessment of survival/transfer

Assessment of survival/transfer

Developmental potential in-vitro Morphological survival

Dilemma: thawing/warming policy?

→ How many oocytes/embryos/blastocysts to thaw?
- Developmental stage
- Expected survival rates
- Transfer policy

Risk to throw out the child with the bathwater after thawing!!

Assessment of survival/transfer

3. -Omics and cryopreservation

Cryopreservation affects physiology of oocytes and embryos
- Secretome, transcriptome?

- **Stokes et al (2007) Metabolism of human embryos following cryopreservation: implications for the safety and selection of embryos for transfer in clinical IVF(Hum Reprod 22, 829-836)**

Assessment of survival/transfer

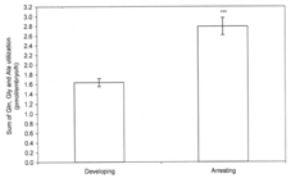


Figure 3. Sum of glutamine, glycine and alanine utilization by frozen–thawed human embryos from day 2 to day 3 of development. ***$P < 0.001$, significantly different from embryos that develop.

General conclusions

- The aim of a cryopreservation programme should be to have fully intact embryos after thawing. However, also damaged embryos can give rise to live births
- Resumption of mitosis or further development in-vitro of frozen-thawed surviving oocytes/embryos is capable of selecting the viable embryos for transfer. However, also not further cleaving embryos (intact ones and non-intact ones) can give rise to live births
- Freezing and thawing policy: risk to throw away the child with the bathwater before freezing and after thawing
- Metabolic assessment of frozen-thawed oocytes/embryos is still a research procedure
- Only one frozen-thawed embryo should be transferred/FRET