Morphology of oocytes and embryos after cryopreservation

Kersti Lundin

Sahlgrenska University Hospital

Gothenburg, Sweden

Lecture overview

- Aim of cryopreservation
- Methods
- The oocyte
- The embryo (early cleavage stage)
- Summary, conclusion

Aim of cryopreservation

- To preserve fertility
- To increase success rates

- To decrease multiple birth rates

- Oocytes; oocyte donation, ”social” egg freezing, legal issues

Damages of low temperature

- Low temperature per se – e.g. phase transitions in membranes, denaturation of proteins *(usually not harmful, due to almost no metabolism at very low temp)* ?
- Direct effects of freezing – intracellular ice formation, membrane damages
- Indirect effects of freezing – changes in ionic interactions (high salt concentrations), cellular ultrastructure changes (dehydration)

Slow freezing vs. vitrification

Slow freezing

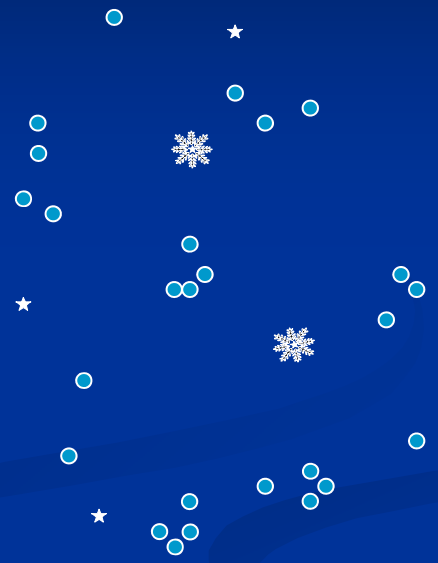
- Cooling rates: 0.3 °C / min
- Controlled ice crystal formation (nucleation / ”seeding”) at specified temperature

Vitrification

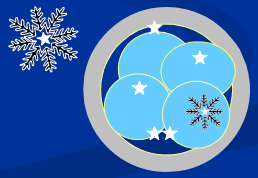
- Cooling rates: 2.000 - 20.000 °C / min
- No (less) ice crystal formation
- Very high CPA concentrations

Slow freezing

- At a certain temperature the kinetic energy of the molecules will become lower than the binding energy



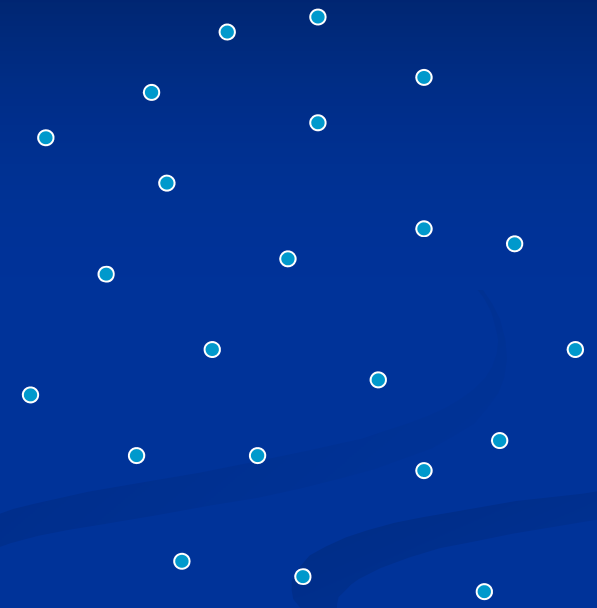
- Molecules will start to organise into clusters that may grow into structures (crystals)

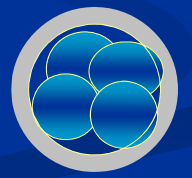


- They will try to organise into the energetically most favourable positions (equilibrium)

Glass transition

- If the cooling occurs fast enough, the molecules never reach their energetically preferred position
- They will form a glassy state: a non-equilibrium, amorphous, disordered state of extremely high viscosity.
- The transition to glass is a function of cooling rate and solute concentration





?

Does morphology of cryopreserved oocytes/embryos influence the implantation potential?

How should we select oocytes and embryos before vs. after cryopreservation

Oocyte morphology

- Oocyte diameter
- Zona pellucida abnormalities
- **Extra**cytoplasmic dysmorphims;
  - Polar body morphology
  - Perivitelline space
- Cytoplasmic dysmorphisms;
  - Vacuoles
  - Inclusions; refractile bodies, central granulation
  - sER aggregation

Oocyte morphology – predictive value for IVF?

- Rienzi et al 2011, Hum Rep Udate, systematic literature search, 50 papers included
- Variable and conflicting results.
- ”None of these features could be unanimously correlated with normal or compromised development, when evaluated by 15 outcome variables”

  (does not exclude that there is an impact)

The meiotic spindle
– influence on ICSI

- Meta-analysis, 10 papers included
- Presence of spindles resulted in:
  - Higher fertilisation rate ($p<0.0001$)
  - Increased cleavage rate ($p<0.0001$)
  - Increased no. of TQ embryos on day 3 ($p=0.003$)
  - Increased blastocyst rate ($p<0.0001$)

- However, no effect could be seen on implantation rates or clinical pregnancy rates per transfer

Petersen et al 2009

The ”normal” (= fertilisable) oocyte?

- Appropriate size
- Appropriate perivitelline space
- Single (intact?) polar body
- Appropriate zona thickness
- Healthy looking cytoplasm

Poor prediction for fertilisation and development

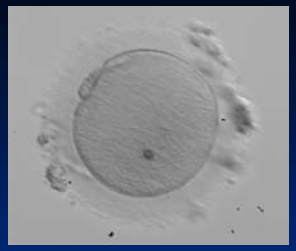
From Swain and Pool 2008

Oocyte cryopreservation

– possible consequences

- Metabolic changes
- Ultrastructural damage; microtubule depolymerisation, (MII spindle), (age dependent),
- Cortical granule loss, zona ”hardening”; fertilisation problems

- New protocols, increased implantation rates (differentiated sucrose concentrations, vitrification, optimised times prefreeze and post thaw)



Cryopreservation of oocytes

Implantation rate per aspirated oocyte

Slow cooling: 1.2 %

Vitrification: 3.4 %

(cleaved embryo ~ 7-9%)

Meta-analysis Oktay, 2006

Slow cooling: ⇨ 9 %

Vitrification: ⇨ 9-12 %

(cleaved embryo ⇨ 10 %)

Borini et al, 2009, Cobo et al 2008, Rienzi et al 2010

Frozen-thawed oocytes –

- dead or alive……?

	Slow freezing (n=53)	Vitrification (n=50)
Survival rate -	50%	87%
Mean zona thickness -	no change	no change
Cytoplasmic volume recovery -	86%	96%
Cytoplasmic appearance -	no change	no change
Meiotic spindle presence -	72%	94%
Spindle – PB angle -	no change	increased
DNA fragmentation -	no change	no change

Martinez-Burgos et al 2011

Summary oocyte morphology and cryopreservation

- Using routine light microscopic **volume** was the only detectable change after cryopreservation
- Using polarised microscope system, differences in presence and localisation of the **meiotic spindle** were detectable
- Other damages such as ZP hardening, CG loss, chromosome misalignement, not possible to ”see”
- Vitrification probably less detrimental than slow freeze, resulted in higher spindle repolymerisation rates

Embryo cryopreservation

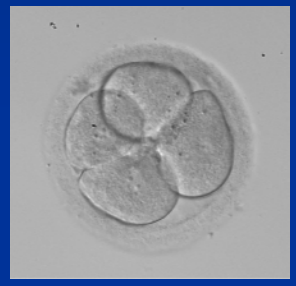
Embryo selection criteria

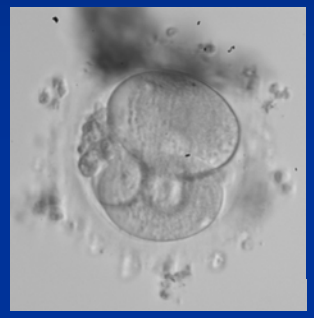
- PN morphology
- no MNB
- Cleavage rate: 4 cells (– 8)
- Even sized cells
- < 20 (-30?)% fragmentation
- First cleavage before 25-27-hours
- Presence of nuclei

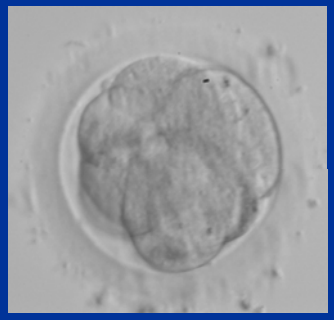
Possible consequences of cryopreservation of embryos

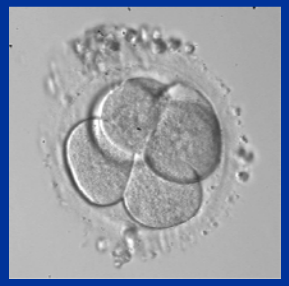
- Implantation potential is considered decreased for cryopreserved cells (metabolic changes?)
- Loss of cells not unusual (slow freezing), decreases the development potential /implantation rate further
- Initial slowing down of development (lag phase)?

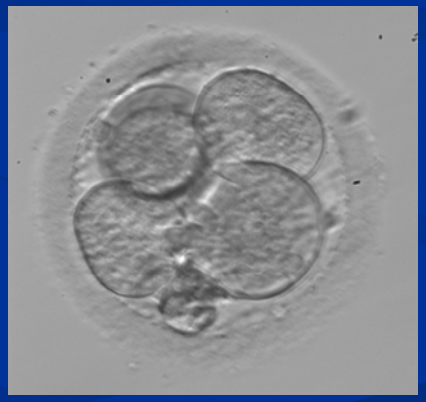
Embryo selection in FER cycles – what should we freeze??











Prefreeze embryo scoring variables vs. embryo survival and implantation rates:

- Development rate (No. of cells, early cleavage)
- Morphology

Implantation vs. number of cells

5572 embryon

2 cells frozen day 2	7.2%
4 cells frozen day 2	16.9%
4 cells frozen day 3	5.5%
Non-intact 4 cells day 2	<11%
Fresh 4 cells day 2	16.6%

Edgar et al 2000

Number of cells (prefreeze) -

Single/separate/similar embryo cryo

Cell survival	100%	60-80%	< 50%
4 cells (n=320)* #	55%	18%	27%
5 cells (n=94)*	37%	24%	39%
6 cells (n=44) #	34%	32%	32%

* p= 0.002, # p= 0.009

Sahlgrenska University Hospital

Blastocyst development after cryopreservation

	blastocyst rate	mean cell number
Intact embryos	40.9% (92/225)*	58.4**
Loss of blast.	24.6% (41/167)*	45.0**

* p<0.01 **p<0.05

Archer et al, 2003

2003-2006 (n= 1393 SET) Sahlgrenska University Hospital

Survival, %	Implantation (%)
100 *	232/967 (24)
70-90 *	56/325 (17)
60	9/63 (11)
40-50	7/65 (14)
Fresh 4-cells	28% (Thurin et al 2005)

* p= 0.011

Vitrification of cleavage stage embryos

- Survival rates of up to more than 90%
- Less blastomere loss

However,

- Pregnancy and inplantation rates remain the same
- Potential for increased cumulative rates

?

Embryo morphology prefreeze

=

Embryo morphology postthaw

Embryo morphology and survival rates

(640 4-cell embryos frozen separately on day 2)

Cell survival	100%	75%	< 50%
Grade 4:1+4:2A (n=435)	46% *	15%	39%
Grade 4:2B (n= 160)	36% *	15%	49%
Grade 4:2c (n= 45)	53%	10%	37%

A= <20% fragm
B= irregular cell size
c = slightly granular

*p= 0.034 for
100% 4:1+4:2A vs. 4:B

Early cleavage and survival rates
(297 embryos frozen separately on day 2)

Cell survival	100%	> 50%
Early cleavage	52%	14%
Late cleavage	59%	11%

NS

Summary – factors influencing success rates of oocytes and embryos after cryopreservation

- Oocyte:
  - Prefreeze morphology/meiotic spindle (survival)
  - Decreased cytoplasmic volume (?)
  - Loss of meiotic spindle (fertilisation rates)
- Embryo:
  - Prefreeze morphology
  - Prefreeze development speed
  - Loss of blastomeres

Conclusion

- Oocyte and embryo morphology **after** thawing = oocyte / embryo morphology **at** cooling
- Oocyte / embryo characteristics prefreeze influence survival rates after cryopreservation
- Survival rates after cryopreservation affects implantation rates….

Σ

….Morphology at cryopreservation affects implantation rates

Practical aspects….