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REGION

Criteria to select oocytes and embryos for cryopreservation

Josephine Lemmen, PhD, Clinical Embryologist,
Fertility Clinic, Rigshospitalet, Copenhagen, Denmark

Outline

- short introduction
- oocytes
- zygotes
- cleavage stage embryos
- blastocysts

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Main goals of cryopreservation

- increase pregnancy chances
- reduce multiple pregnancy rates
- preserve fertility (e.g. cancer)

As transfer becomes more restricted, cryopreservation of embryos and their transfer need accurate evaluation

Freezing embryos is a result of transferring less embryos

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FER-cycles worldwide

IVF-worldwide.com Survey by Dr. Ariel Weissman, results from 179 IVF centers representing 56 countries

Cycles per continents	Total IVF cycles	FET cycles
USA and Canada	25240	6730
Europe	47350	15832
South America	6400	1260
Australia & New Zealand	21300	8240
Asia	30100	6920
Africa	2900	170
Total	133290	39152

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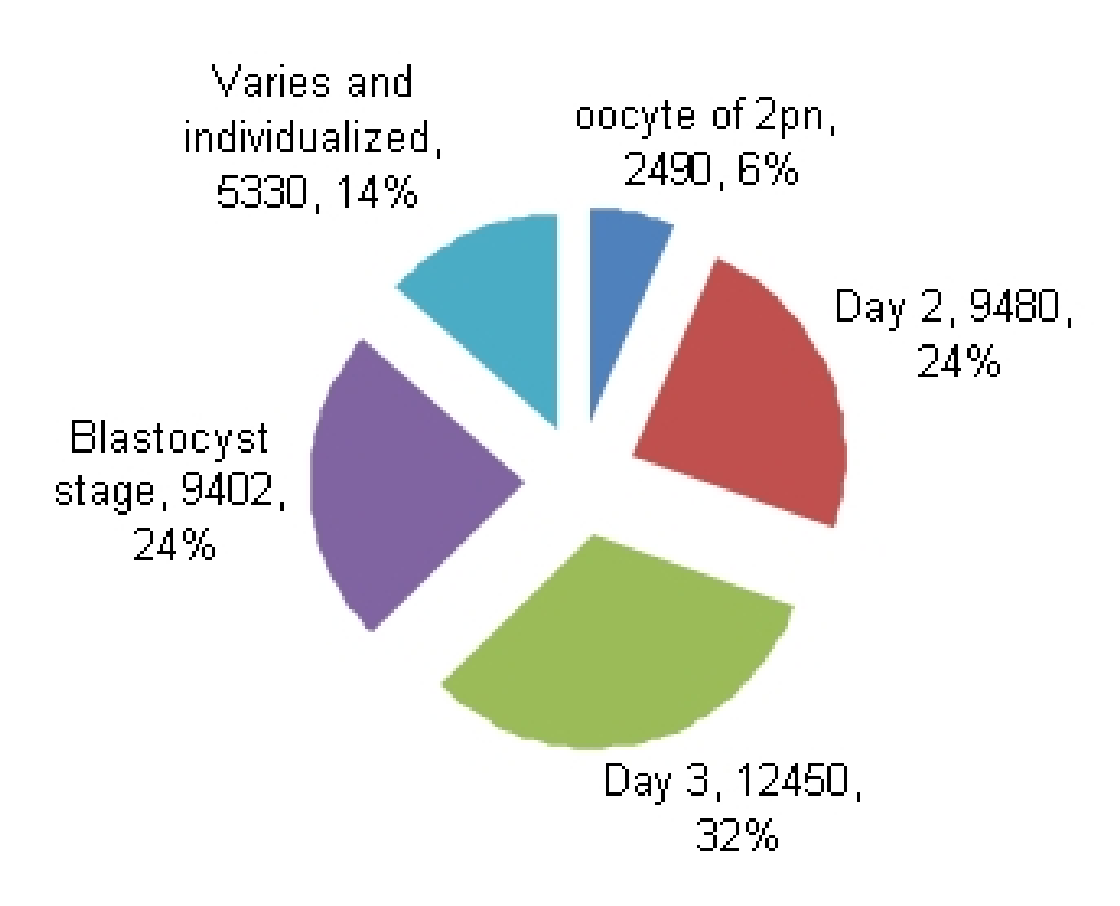
Preferred timing for cryopreservation



IVF wordwide.com Weissman

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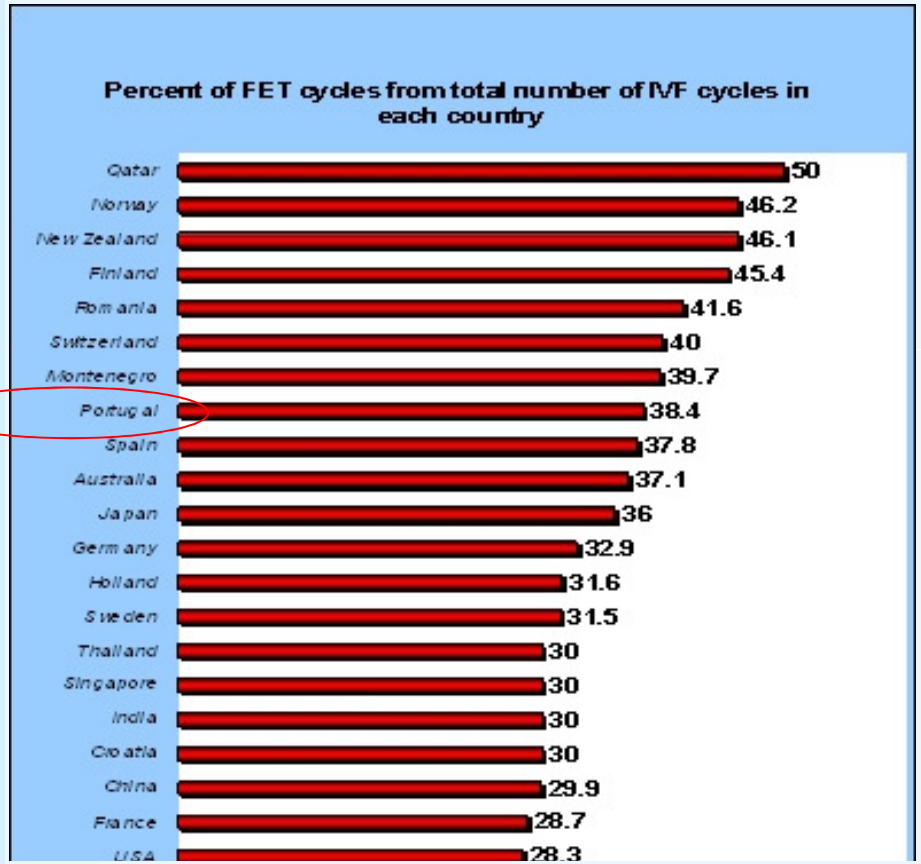
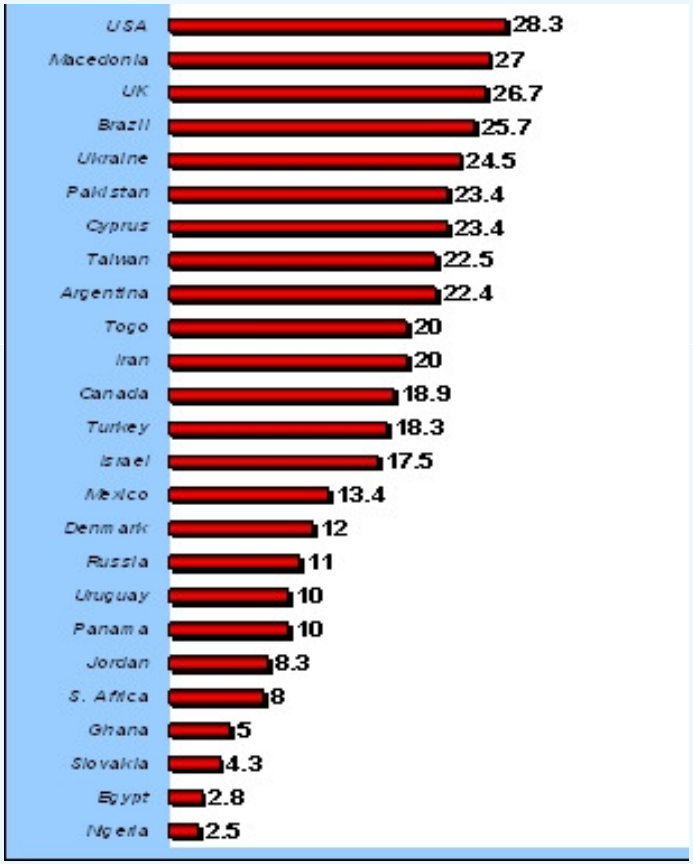
FER cycles worldwide





IVF wordwide.com Weissman

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% of pregnancies after FER in DK

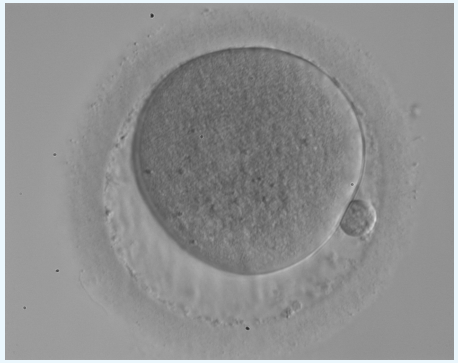
Children expected to be born in Denmark after treatment in 2007

Treatment	Cycles	Newborn	% of all newborn
IVF/ICSI	11.035	2.957	4.6 %
FER	2.668	457	0.7 %

- At RH ~25% of pregnancies are after FER

Why oocyte cryopreservation?

- preserving fertility in cancer patients
- interrupted IVF-cycles OHSS/no sperm
- oocyte banking for later own use/donor
- ethical concerns regarding embryo cryopreservation
- ..



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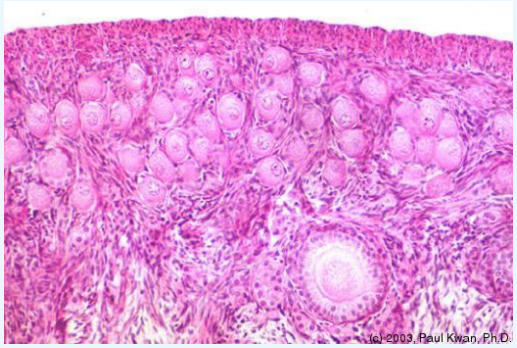
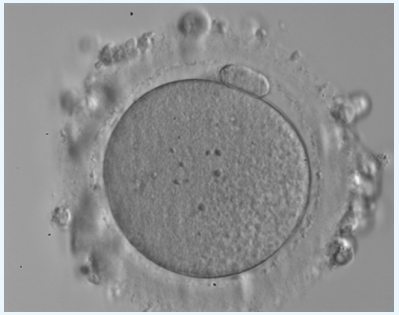
Stages for oocyte cryopreservation

- primordial follicles – ovarian cortex
- immature oocytes followed by IVM
- mature MII oocytes





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Challenges in oocyte cryopreservation

- large cell
- spindle
- cortical granule discharge –by DMSO PROH – reduced ability of sperm to bind ZP

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Oocyte cryopreservation slow freeze

- 50-76% survival
- 13-38% pregnancy/transfer

(Porcu 2000, Boldt 2006, Borini 2007)

- Freezing within 2 hrs from OR increases the efficiency of cryopreservation when using high sucrose

(Parmegiani 2008)

- Cumulative pregnancy rates embryo transfer and oocyte freezing up to 50% (Borini 2007)

- 91% survival with slow freeze (La et al 2010)

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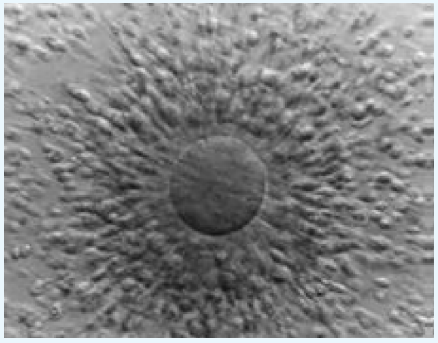
Oocyte cryopreservation vitrification

- 63-100 % survival
- 6.4-61 % implantionrate
- 21-83 % pregnancy/transfer

- Different devices and protocols



Review Cobo 2009

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Oocyte cryopreservation - outcomes

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

Table from D. Gook & D. Edgar 2007 HR

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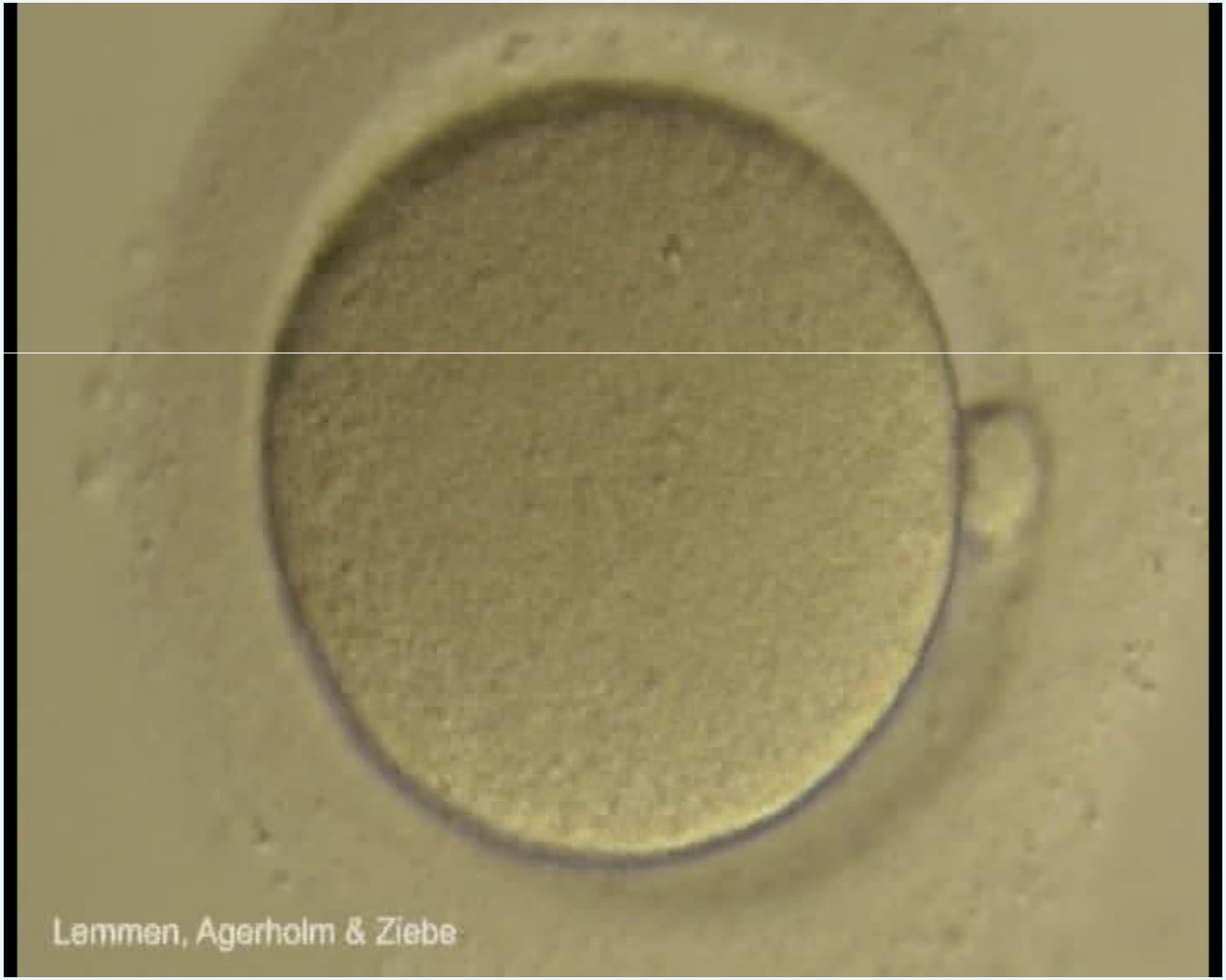
Oocyte cryopreservation in routine IVF

- only the required number of embryos will be created
- donor programs
- cancer/ other ovarian loss
- routine use?

- MII oocytes within 4hr of OR (Borini 2007)

From oocyte to zygote



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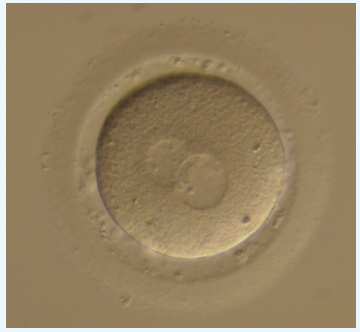
Why zygote stage cryopreservation?

- Legal situation, max result from 1 cycle
- Ethical ’not an embryo’
- Lack of spindle
- One cell
- Survival can be easily scored - syngamy



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Disadvantages zygote stage cryopreservation

- Many pre-embryos stored with unknown potential
- Leads to many FER cycles
- Time consuming FER cycles + freezing

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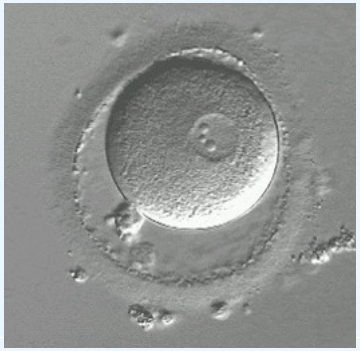
Which zygotes to cryopreserve?

- 2 PN
- size and alignment of pronuclei
- the number and distribution of nucleoli
- halo effect
- appearance of vacuoles
- appearance of the ooplasm



Optimal time is 16-20 h after fertilisation

Here are most at G2-phase

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Results of zygote cryopreservation

- zygote cryo gives the best survival rates and an increased cumulative pregnancy rate
(Veeck 1993; Nikolettos 2000; Damario 2000; Senn 2000; Salumets 2003)

- Better survival rate for zygotes than for day2 embryos
  Senn 2000: 80.4% vs. 71.8%;
  Salumets 2003: 86.5% vs.61.7%)

- similar results (Horne et al, 1997: 74.4% vs.77.4%)

- lower results (Kattera et al, 1999: 64.4% vs. 73.9%)

Results of zygote cryopreservation

Pregnancy and implantation rates – controversial

- better results after zygote than day2 FER
Senn 2000: 19.5% and 10.5% vs. 10.9% and 5.9%)

- lower results (Kattera 1999: 14.8% vs. 22.8%)

- similar results (Salumets 2003: 20.1% and 14% vs. 21.1% and 14.3%

Summary zygote cryopreservation

- Zygote-implantation rates are lower than cleavage stage embryos after cryo ??
- Cryopreserved zygotes have similar implantation rates as fresh zygotes

- Used mainly as an option when cleavage stage cryopreservation is unwanted

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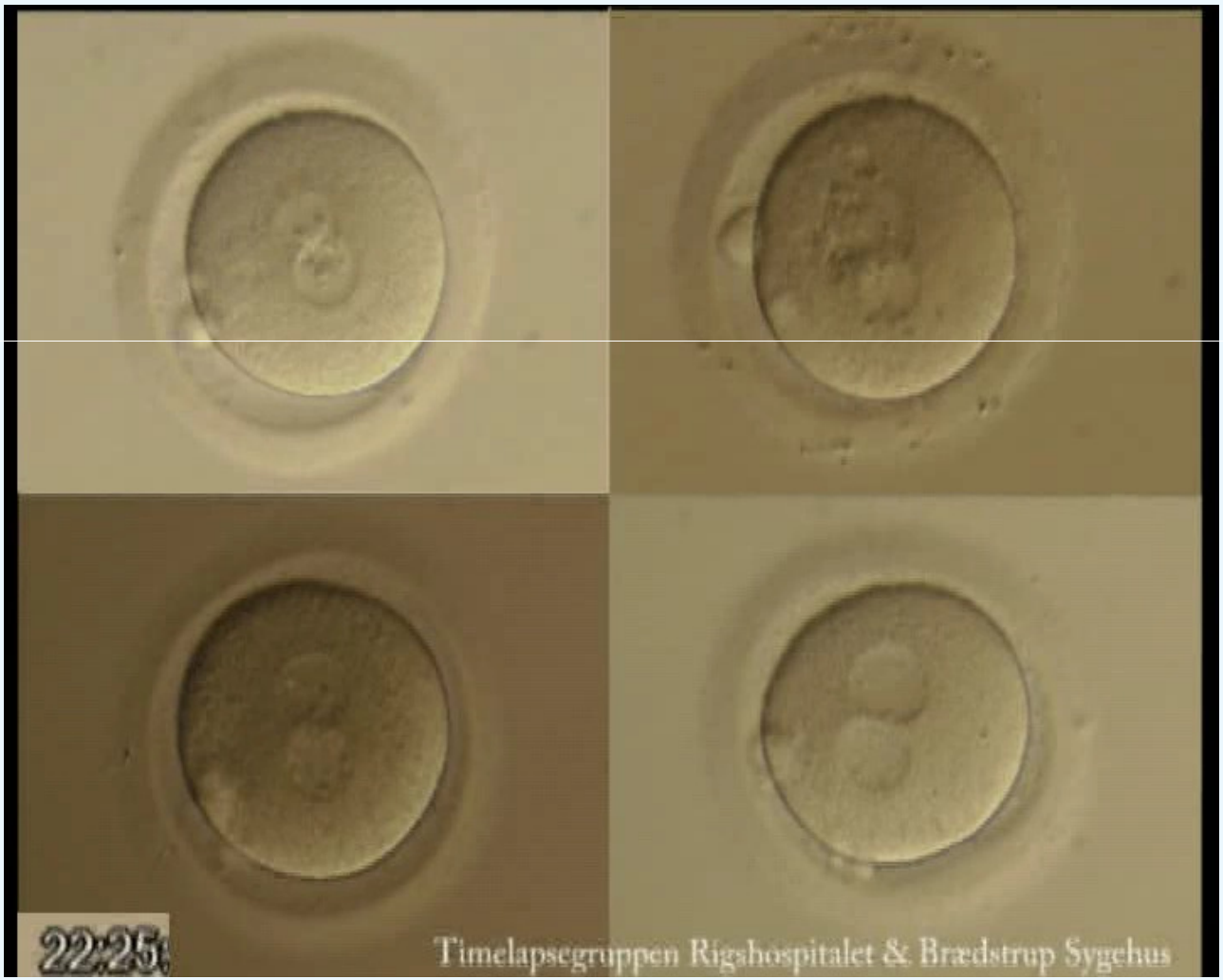
From zygote to cleavage stage



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Why cleavage stage cryopreservation

- increase pregnany chance pr. cycle
- prevention of OHSS
- better endometrium?
- massive data supporting safety

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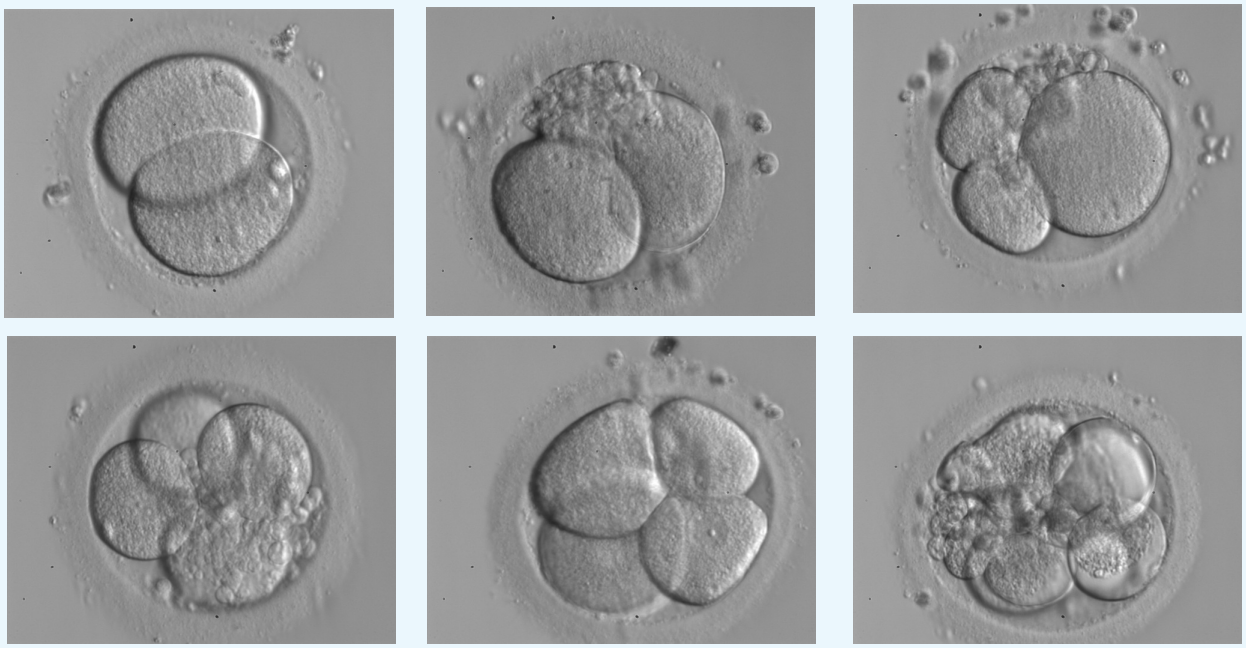
Criteria for cleavage stage cryopreservation

- the same as for transfer?
- more stringent or just everything not multinucleated?



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Criteria for cleavage stage cryopreservation

- number of blastomeres
- morphology - fragmentation, evenness of blastomeres

Influence outcome of FERcycle:

- outcome of the fresh cycle
- number of previous fresh cycles
- day of freezing
- fertilisation type

Cell no. prefreeze and implantationrate

Superiority of 4-cell embryos after freezing-thawing was clearly stated by many reports (day 2 cryo)

(Testart 1986/1987; Cohen 1988; Hartshorne 1990; Mandelbaum 1998;).

Salumets 2006: also moderate embryos implant...

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Cell no. prefreeze and implantationrate

2 blastomeres on day 2	7.2%
4 blastomeres on day 2	16.9%
4 blastomeres on day 3	5.5%

And fast 4 cells > slow 4 cells
"It therefore appears that the developmental potential of a thawed embryo is determined by its pre-freeze growth rate rather than the number of blastomeres at the time of freezing."

Edgar 2000

Blastomere evenness

- Equality of blastomeres and percentage of cytoplasmic exudates were reported as relevant criteria to predict the embryo survival

(Lassalle 1985; Van den Abbeel 1988; Camus 1989)

Fertilisation method

IVF as a fertilisation method was a predictive factor for live birth after FER

Only good quality embryos (GQE) were cryopreserved.

Olivius et al. 2008

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Prognostic value of fresh embryo transfer

- fresh embryo transfer outcome was reported to clearly influence the outcome of FET
- birth rate per transfer 17% vs. 5% (Lin 1995)
- pregnancy rate per transfer 29% vs. 21% (Karlström 1997)
- pregnancy rate 14.2% vs. 8.6% (Wang, 2001)
- ong. pregnancy rate per cycle 27.3% vs. 13.1% (El-Toukhy 2003)
- however not known at the time of freezing

So not usable as criterium for freezing

Cleavage stage cryopreservation - Day 2 or 3?

- higher survival rate as well as overall efficacy of cryopreservation are reported for day2 embryos compared to day3 embryos

survival rate: 61.7% vs. 43.1%

birth rate per thawed embryo: 7.6% vs. 4.2%

despite similar results in fresh embryo transfer.

Salumets 2003

See also: Testart 1986, cohen 1988

Summary cryopreservation of cleavage stage

- Prefreeze morphology influences survival, which in turn affects the implantation rate
- intact thawed embryos can have similar impl. potential as fresh embryos

- even number of blastomeres > uneven
- ≥4 cells on day2
- < 20% fragmentation
- day 2 > day3

From cleavage embryo to blastocyst

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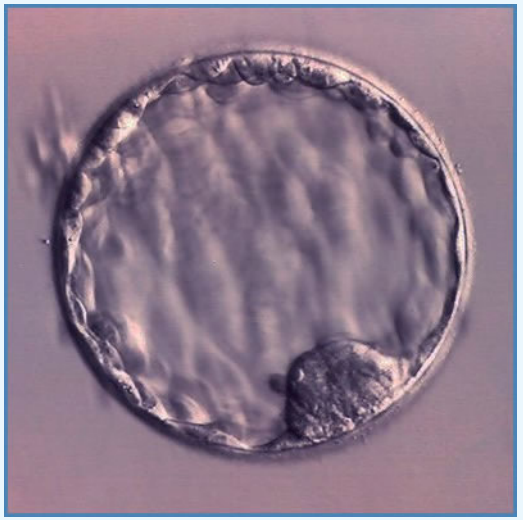
Why blastocyst cryopreservation

- higher cell no. of blastocysts – less vulnerable for cryodamage
- improved selection

- which blastocysts?
- day 5 or day 6?



Criteria for blastocyst cryopreservation

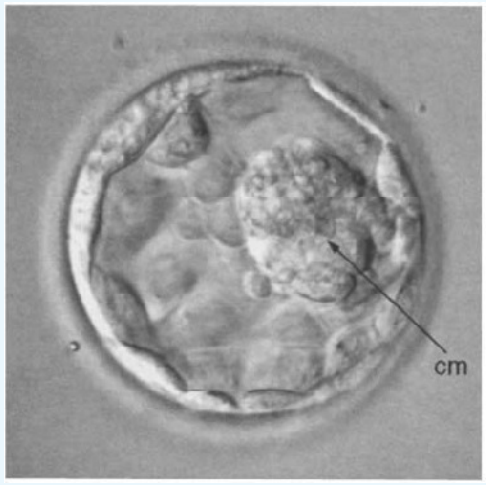
- which blastocysts?

At least 3BB or better on day 6

Preferrably not hatched??

Early cavitating blastocyst should be cultured further?

Vitrification allows for individual timing of cryopreservation



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Criteria for blastocyst cryopreservation

- which day?

Table 4. A comparison of retrospective data from the blastocyst cryopreservation programme at Fertility Centers of vitrified day 5 and day 6 from January 2004 until February 2009.

Day of development	*Day 5*	*Day 6*
Patient's age (years)	34.5 ± 5.2	34.6 ± 4.9
Warmed cycles	680	731
Transfers	678	720
Blastocysts warmed	1426	1409
Blastocysts survived (%)	1373 (96.3)	1357 (96.3)
Blastocysts transferred	1357	1351
Blastocysts transferred (mean)	2.0	1.8
Implantations (%)	460 (33.9)[a]	336 (24.9)[a]
Positive pregnancies from warming (%)	396 (58.2)[b]	299 (40.9)[b]
Positive pregnancies from VET (%)	396 (58.4)[b]	299 (41.5)[b]
Clinical pregnancies from warming (%)	338 (49.7)[b]	261 (35.7)[b]
Clinical pregnancies from VET (%)	338 (49.9)[b]	261 (36.3)[b]
Ongoing/delivered pregnancies from VET (%)	288 (42.5)	220 (30.6)
Live births	255	176

Values are numbers unless otherwise described.
VET = vitrified embryo transfer.
[a,b]Values within a row with the same superscript are significantly different ($P < 0.05$ and $P < 0.01$ respectively).

Liebermann 2009

Criteria for blastocyst cryopreservation

- First studies reported lower implantation rate for frozen-thawed blastocysts compared to fresh blastocysts (Hartshorne 1991; Kaufmann 1995).

- More recent studies have reported better results using frozen blastocysts transfer, especially vitrified (Langley 2001; Behr 2002; Gardner 2003; Mukaida 2003; Liebermann 2003, Kuwayama 2005).

Day 3 vs day 5

Day 5 (n = 72) versus day 3 (n = 119)

Implantation rate thawed: 21.9% vs. 10.1%

(fresh 54 vs 40%)

(transferred 2.35 vs 3.07)

Survival rate: 82 % vs 68.5% (Langley 2001).

- Results look promising for day5, one should notice that the frequency of embryo freezing is far lower at day 5 as compared to day 3, which may alter the final efficiency of such a procedure

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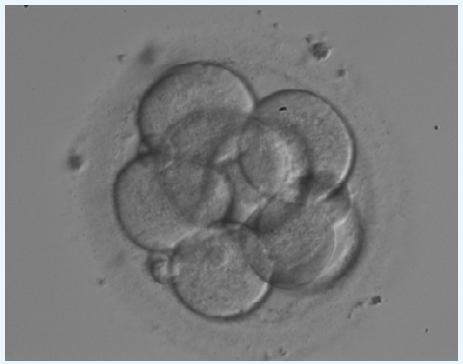
Day 2/3 vs day 5/6 Embryo freezing rate

- Rates of embryo freezing per couple showed a significant increase for the Day 2 to 3 transfers compared to Day 5 to 6
- . ....a significant difference in favour of more embryos frozen with early cleavage stage transfers.



Blake et al.,Cochrane review 2007 and 2009

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Wang study 150,376 transfers in AUS

Table II Pregnancy, live delivery and 'healthy baby' rates of embryo transfers cycles, Australia 2002–2006.

	Transfer cycles (No.)	Clinical pregnancy rate (%)	Live delivery rate (%)	'Healthy baby'* rate (%)
Fresh cleavage	69 308	28.0	21.7	14.8
Fresh blastocyst	19 483	35.9	27.9	21.0
Thawed cleavage	49 656	20.1	15.2	11.7
Blastocyst from thawed cleavage	2665	28.8	22.0	16.2
Thawed blastocyst	9264	22.5	16.3	13.2

*A 'healthy baby' was defined as a single baby born live at term (≥37 weeks gestation), weighing ≥2500 g, surviving for at least 28 days post birth and not having congenital anomalies.

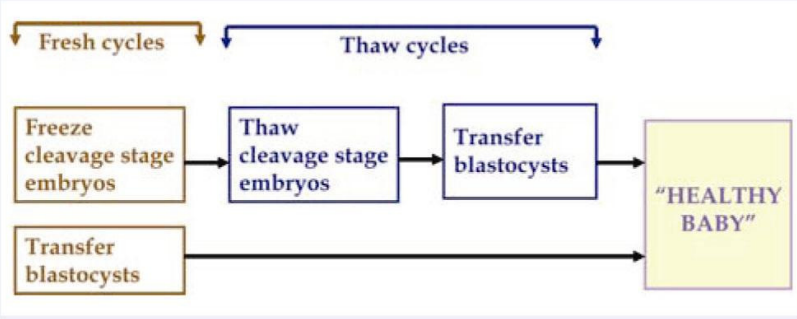

Figure 1 Suggested clinical practice model.

Wang et al., HR 2010

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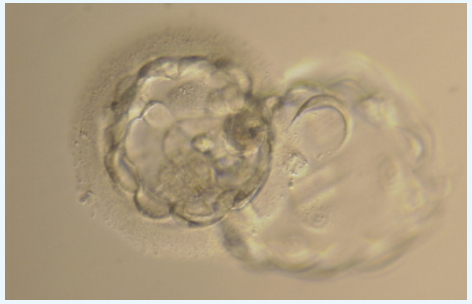
How to express succes rate of cryopreservation

- per thawed embryo
- per transferred/thawed embryo
- or the total of fresh and frozen-thawed cycles?

- comparing a new protocol to old protocol covering different periods
- have other things changed... eSET??



- better embryos for cryo

Slow freezing vs vitrification

Vitrification appears more attractive but most studies are retrospective, non-comparative

Recent meta-analysis of 4 studies (3 RCTs)(Loutradi 2008)

- Postthawing survival of cleavage stage/blastocyst favours vitrification
- Pregnancy data were not sufficient for analysis

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Summary

- Criteria for cryopreservation in general the same as for transfer of fresh zygotes/embryos/blastocysts
- On-going debate about the best stage and method for cryopreservation
- Vitrification may be an alternative for slowfreezing especially for blastocysts/oocytes
- Need for more RCT to prove this
- Outcome children
- More effort in further development of slowfreezing protocol
- Tendency of clinics to employ only one method

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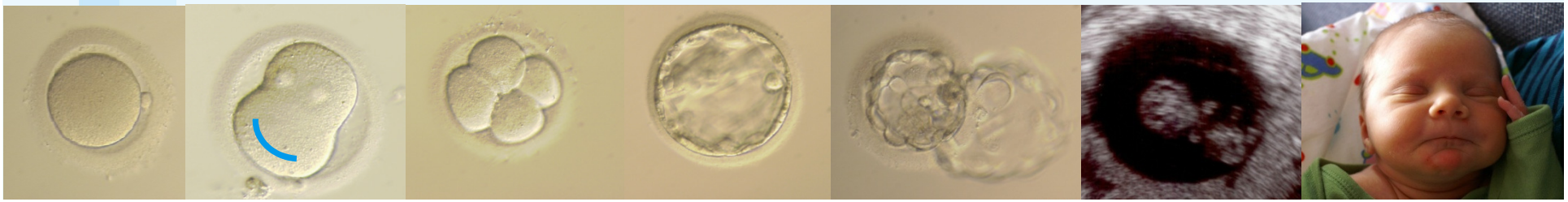
Take home message

Cryopreservation is a valuable tool to combine a low risk for multiple pregnancies by single embryo transfer with higher cumulative chances to achieve a pregnancy. Whether this strategy will benefit from cryopreservation at zygote, early cleavage-stage or blastocyst-stage needs to be further assessed by studies on cumulative birth rate per oocyte collection



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Discussion

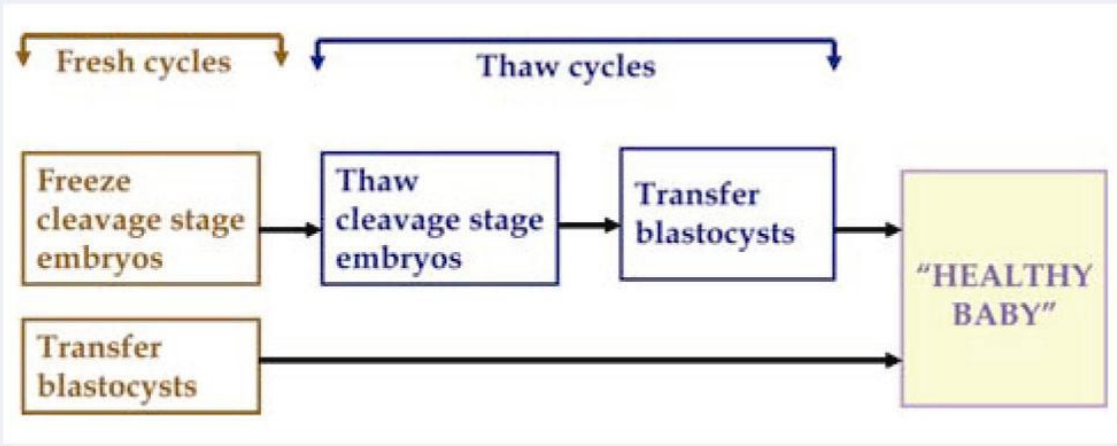
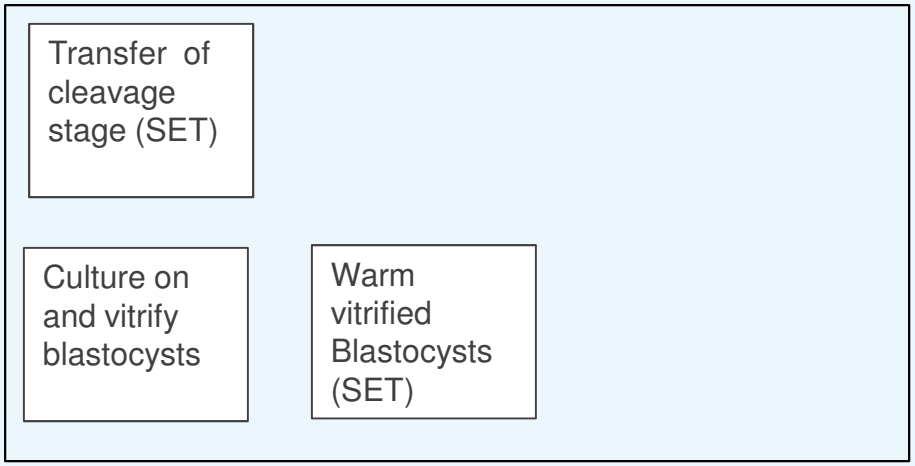


Figure 1 Suggested clinical practice model.

Wang et al., HR 2010



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