Is It Possible for Frozen Embryo Transfer Rates to Exceed those of Fresh Transfers?

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Please use the response keypad.
Is It Possible for Frozen Embryo Transfer Rates to Exceed those of Fresh Transfers?

A. Yes

B. No
SART - Percentage of ET’s Resulting in Live Birth (<35yo)

- 2003: 43.4%
- 2004: 43.4%
- 2005: 45.1%
- 2006: 46.9%
- 2007: 47.1%
- 2008: 47.1%
- 2009: 47.1%
- 2010: 45.1%
- 2011: 43.4%
- 2012: 43.4%

Fresh
SART - Percentage of ET’s Resulting in Live Birth (<35yo)

- Fresh: 29.5% to 47.1%
- Frozen: 29.5% to 42.4%

Year: 2003 to 2012
SART - Percentage of ET’s Resulting in Live Birth (<35yo)

Year


% Live Birth/Transfer

25 30 35 40 45 50

Fresh

Frozen

2003: 29.5%
2004: 42.4%
2005: 43.4%
2006: 45.0%
2007: 46.7%
2008: 47.1%
2009: 46.8%
2010: 45.7%
2011: 44.6%
2012: 43.4%

47.1%
42.4%
SART 2012 Percentage of ET’s resulting in live birth

![Graph showing the percentage of live births (LB) and the number of ET’s (ET’d) as a function of maternal age. The graphs compare fresh and frozen ETs.]

- **Percentage of Live Births (LB):**
  - The percentage decreases as maternal age increases. Fresh ETs show a higher percentage compared to frozen ETs across all age groups.
  - Below 35 years, fresh ETs have a higher percentage than frozen ETs. This gap narrows as age increases, with frozen ETs eventually surpassing fresh ETs in the >42 age group.

- **Number of ET’s (ET’d):**
  - The number of ETs increases with maternal age for both fresh and frozen ETs.
  - Fresh ETs consistently have a higher number of ETs across all age groups compared to frozen ETs.
  - The difference is most pronounced in the <35 age group, with fresh ETs having significantly more ETs.

- **Age Categories:**
  - <35, 35-37, 38-40, 41-42, >42

- **Lines:**
  - Red line: Fresh ETs
  - Blue line: Frozen ETs
What stage are most of your embryos currently being cryopreserved?

A. 2PN
B. Cleavage
C. Blastocyst
D. Combination
How do you cryopreserve your embryos?

A. All embryos/blastocysts are vitrified

B. All embryos/blastocysts are slow frozen

C. Some of each, depending on embryo stage

D. Some of each, as we explore best protocol
Background

- Historically, fresh ET had considerably better pregnancy rates than frozen transfers
- Slow freeze methods have not matched fresh
- Vitrification now yields increased pregnancy and implantations rates for most programs
Advantages of Vitrification and FET

- Allows for better embryo/uterine synchrony
- Can be scheduled & planned
- Less stressful for the patient
- Equivalent or better pregnancy rates to fresh
Advantages of Vitrification and FET

- Higher birth weights

- Possibly
  - Healthier uterine environment???
  - Healthier babies???
Factors Affecting Vitrification Success

- Pre-vitrification blastocyst/embryo selection
- Assisted Hatching
- Blastocoel collapse ???
- Vitrification vessel
- Vitrification freeze solutions & volumes
- Cryo storage and handling
- Vitrification thaw solutions & volumes
- Post-vitrification blastocyst/embryo selection
- Progesterone timing
Challenges of Vitrification

Slow  ➔  Vit

* J Saragusty & A Arav Reproduction 2011; 141:1–19
Vitrification tubing carrier systems:
(1a) plastic straw,
(1b) open-pulled straw,
(1c) superfine open-pulled straw,
(1d) flexipet-denuding pipette,
(2) CryoTip,
(3) HSV,
(4) pipette tip,
(5) sealed pulled straw,
(6) Cryopette,
(7) Rapid-i,
(8) JY Straw.

• J Saragusty & A Arav
Reproduction 2011; 141:1–19
If you vitrify embryos, which device do you use?

A. Kitazato Cryotop

B. Irvine HSV straws

C. Vitrolife Rapid-i

D. Regular 0.25cc straws

E. Other
Vitrification Method

- Irvine Scientific Vit Kit - vitrification solutions
- HSV straws
  - Similar design to Kitazato Cryotop
  - Closed or sealed system
Collapsing Blastocysts???

- Blastocysts are **NOT** routinely collapsed
- Zona breach on Day 3
- Blastocysts are hatching or completely hatched on Day 5/6 at time of vitrification
Blastocysts Collapsed with Laser:

- If highly expanded
- Vitrified before fully re-expanded after biopsy
Irvine Vit Kit & HSV straws
Vitrification Set up

150μl ES
10 min
HSV Straw with Brady Label
Vitrification Set up
Loading the blastocyst on HSV straw
Tips for loading HSV straws

● Drop of VS containing blastocyst must be very small
● Position the blastocyst just inside of stripper tip
● Touch & expell into the trough of the HSV straw near the tip (need to submerge for thaw)
● If too big, drop can touch the inside of the outer straw and embryo is difficult to recover
● To correct too big of a drop, spread the media and aspirate excess VS
Plunge into LN2 bath
Seal the outer HSV straw
Video of Loading an HSV straw
Storage of Vitrified Embryos
Options for Cryo Storage
Timing of Vitrification Post-Biopsy

- Most blastocysts seal & begin to re-expand in ~20 min
- Vitrify 30-90 minutes post-biopsy
- Before complete re-expansion
Vitrification Thaw

Center-well dish @ 37°C. Move straw quickly from LN2 to TS solution. Plunge the tip of the straw in TS and locate the blastocyst.
Embryo starts in TS ~1min
Merge DS1, DS2, DS3
Move to DS4
Merge WS1, WS2, WS3
Rinse through WS4
Move to culture plate

Lid of 60mm dish at room temp

~3min
~3min
~3min
Tips for vit warming

- Cut the outer straw while in LN2
- Move quickly from LN2 into TS (be aware of condensation & osmolarity changes of small volume)
- In event of straw malfunction... DO NOT PANIC
- Keep the straw submerged in LN2 until resolved
- Keep forceps, scissors and hemostats handy
- It is possible to recover an embryo from the inside of the outer straw and have it survive
<table>
<thead>
<tr>
<th>Pre-Biopsy</th>
<th>Post-Biopsy</th>
<th>At Vit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy, Vit, Thaw &amp; FET of single CCS normal</td>
<td>Singleton Delivered</td>
<td>FET</td>
</tr>
</tbody>
</table>
2012 Vitrification Results

- 137 thaw cycles
- 241 embryos thawed
- 96% survival
- 1.62 embryos FET’d
- 37% of vit FETs are single ET
# Fresh vs Vitrified Non-CCS Blastocyst Transfers

<table>
<thead>
<tr>
<th></th>
<th>Fresh</th>
<th>Vitrified</th>
</tr>
</thead>
<tbody>
<tr>
<td># of Transfers</td>
<td>399</td>
<td>115</td>
</tr>
<tr>
<td>Mean Maternal Age</td>
<td>33.1</td>
<td>32.1</td>
</tr>
<tr>
<td>Avg # Embryos Transferred</td>
<td>1.85</td>
<td>1.91</td>
</tr>
<tr>
<td>Positive βhCG</td>
<td>72.4%</td>
<td>76.5%</td>
</tr>
<tr>
<td>Clinical Pregnancy Rate</td>
<td>60.2%</td>
<td>67.0%</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>50.1%</td>
<td>50.5%</td>
</tr>
</tbody>
</table>

*Kelk et al. ASRM 2012*
2012 CCS Vitrification Results

• 44 CCS patient thaws in 2012
• 97.1% thaw survival
• 1.51 embryos transferred
• 48.8% of CCS FET’s are single ET
# Fresh vs Vitrified CCS Blastocyst Transfers

<table>
<thead>
<tr>
<th></th>
<th>Fresh Day 6</th>
<th>Vitrified</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>44</td>
<td>44</td>
<td>---</td>
</tr>
<tr>
<td>Mean Maternal Age</td>
<td>37.4</td>
<td>38.6</td>
<td>0.06</td>
</tr>
<tr>
<td>Positive βhCG</td>
<td>70.5%</td>
<td>75.0%</td>
<td>0.63</td>
</tr>
<tr>
<td>Clinical Pregnancy Rate</td>
<td>56.8%</td>
<td>63.6%</td>
<td>0.51</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>46.3%</td>
<td>53.7%</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Kelk et al. ASRM 2012
Day 3 Biopsy & Fresh BT vs TE Biopsy & FBT

Transfer of euploid blastocysts

Maternal Age

Implantation Rate

Day 3 Biopsy + Fresh BT
Day 5/6 Biopsy + FBT

Harton et al. ASRM 2011; (Euploid blastocysts by aCGH)
2013 CCS with Vitrification Outcomes – All Ages

- 2 Euploid ET’d → 80% clinical preg rate
  → 40% twin rate

- 1 Euploid ET’d → 59% clinical preg rate
Controlled ovarian hyperstimulation

- Multiple oocytes
- Multiple follicles
- Abnormally elevated estradiol levels
- Premature elevation of progesterone
- May shift the uterine window of implantation
- Creates asynchrony between embryo & endometrium
Natural Cycle Uterine Synchrony

-48 -24 0 24 48 72 96 120 144

Day 1
Day 2
Day 3
Day 4
Day 5

In Vivo Embryo

2PN  4-cell  8-cell  Morula  Blast

Natural Uterus

Ovulation  Day 3  Day 5

Day 1
Day 2
Day 3
Day 4
Day 5
Slow Developing Embryo Synchrony

-48 -24 0 24 48 72 96 120 144
Day 1 Day 2 Day 3 Day 4 Day 5

VOR Day 3 Day 5

In Vitro/Slow Embryo

In Vivo Embryo 2PN 4-cell 8-cell Morula Blast

Natural Uterus

Day 1 Day 2 Day 3 Day 4 Day 5

Legend:
- Day 1
- Day 2
- Day 3
- Day 4
- Day 5
Stimulated Cycle Synchrony

- Stimulated
- Stimulated Uterus
- In Vitro/Slow Embryo
- In Vivo Embryo:
  - 2PN
  - 4-cell
  - 8-cell
  - Morula
  - Blast
- Natural Uterus

Day 1
Day 2
Day 3
Day 4
Day 5

Day 1
Day 2
Day 3
Day 4
Day 5

VOR
Day 3
Day 5
Uterine Express has left the Station...

Uterine Express

P4 >5.0ng/ml

Uterine Express

Departure Times
P4 >5ng/ml: DEPARTED
P4 3.5-5.0ng/ml: 5 mins
P4 <3.5ng/ml: 10 mins
Window of Endometrial Receptivity

The degree of synchrony between the embryo & endometrium influences the probability of implantation.
Window of Endometrial Receptivity

Degree of synchrony can be controlled by:

1) initiating progesterone relative to the stage of embryo to be thawed

2) thawing & culturing embryos relative to progesterone stimulation
Patients in the high progesterone group had significantly lower clinical preg rates than those in the low prog group ($p=0.032^{a,c}$)

**Kelk et al. ASRM 2012**
Premature Progesterone Rise

- 2,566 GnRH agonist cycles
- P4 cutoff of >1.2ng/ml for long protocol
- P4 cutoff of >2.0ng/ml for short protocol
- PPR on day of hCG negatively correlated with LBR
- No adverse impact on FET cycles

Subtle Progesterone Rise

• 2,555 fresh embryo transfers
• P4 cutoff of >2.0ng/ml on day of hCG
• Lower β-hCG & lower implantation rates
• Reduced live birth rate
• Impaired early implantation

Thawed Embryo Synchrony

<table>
<thead>
<tr>
<th>Condition</th>
<th>Stages</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulated Uterus</td>
<td>VOR</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>In Vitro/Slow Embryo</td>
<td>2PN</td>
<td></td>
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<tr>
<td></td>
<td>4-cell</td>
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<td>Blast</td>
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<tr>
<td>Natural Uterus</td>
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</tbody>
</table>

VOR: Vaginal Ovulation Response
Hormonal induction of endometrial receptivity

- Oral E2 constant or increasing dose for 10-14 days
- Adequate endometrial thickness (>7 or 8mm)
- Start progesterone 50mg IM or 100mg vaginally
  - no advantage or disadvantage of higher doses
  - no difference in vaginal or IM P administration
- Optimal timing:

  → P Day 3 or 4
  → P Day 5 or 6


- Perinatal & neonatal outcomes of 494 babies delivered from 972 vitrified Day 3 embryos
- Vitrification using Cryotop
- Compared Day 3 vitrified with fresh ET
- Mean birth weight higher with vitrified group for both singleton and multiple gestations
- No significant difference in obstetrical and neonatal outcomes

- Neonatal outcome & birth defects in 6623 singletons
- Minimal ovarian stimulation
- Compared vitrified (Cryotop) versus fresh single ET
- Gestational age same (38.6 ± 2 vs 38.7 ± 1.9 weeks)
- Preterm delivery rate same (6.9% vs 6.9%)
- Vitrified embryos had:
  - Higher birth weight (3028 ± 465 vs 2943 ± 470 g, p<0.0001)
  - Lower rate of LBW (8.5% vs 11.9%)
  - Lower rate of SGA (3.6% vs 7.6%)
  - Total birth defect rate same (2.4% vs 1.9%)
  - Perinatal mortality rate same (0.6% versus 0.5%)
Obstetric outcomes of vitrified blastocysts (n=106), fresh blastocysts (n=207) & slow frozen cleavage (n=206)

Vitrification using Cryoloop

For singletons compared with fresh transfer:
- no significant differences in gestational age, mortality or birth defects
- birth weight higher
- lower rate of SGA (3.0% vs 12.1%, P = 0.0085)

- Large Baby Syndrome with FET vs fresh vs NC
- Singleton after FET have an increased risk of macrosomia & LGA (large for gestational age).
- Not solely explained by intrinsic maternal factors
- Observed in sibling pairs, where the sibling conceived after FET had an increased risk of LGA compared with the sibling born after fresh ET.
- Data from 1994-2008
Perinatal outcomes of singleton FET’s (n=6647), fresh (n=42,242) & natural conceptions (n=288,542)

FET singletons vs fresh singletons:
- Lower rates of LBW, SGA, PTB
- But higher rates of LGA & macrosomia

FET singletons had worse perinatal outcome compared with spontaneous conceptions:
- Higher rates of LBW, PTB, SGA, LGA & macrosomia
Advantages of Vitrification and FET

- Higher birth weights
- Possibly
  - Healthier uterine environment
  - Healthier babies
- Warning of large baby syndrome
Conclusions

- Data indicates little, if any compromise to embryo viability with vitrification.

- Vitrification & FET may allow for better synchrony between embryo & endometrium.

- Vitrification can match or exceed fresh implantation & pregnancy rates.
Conclusions

- Birth outcomes are promising; possibly better than with fresh ET.

- Programs should be comfortable in vitrifying blastocysts in freeze all cycles, OHSS patients, PGD/CCS cases, donor egg recipients & GC cycles.
Special Thank you to Embryology Team:

Erica Paganetti*
Jonathan Lo
Karen Reyes
EJ Testa
Questions???

Comments???