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

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Audience Response Question

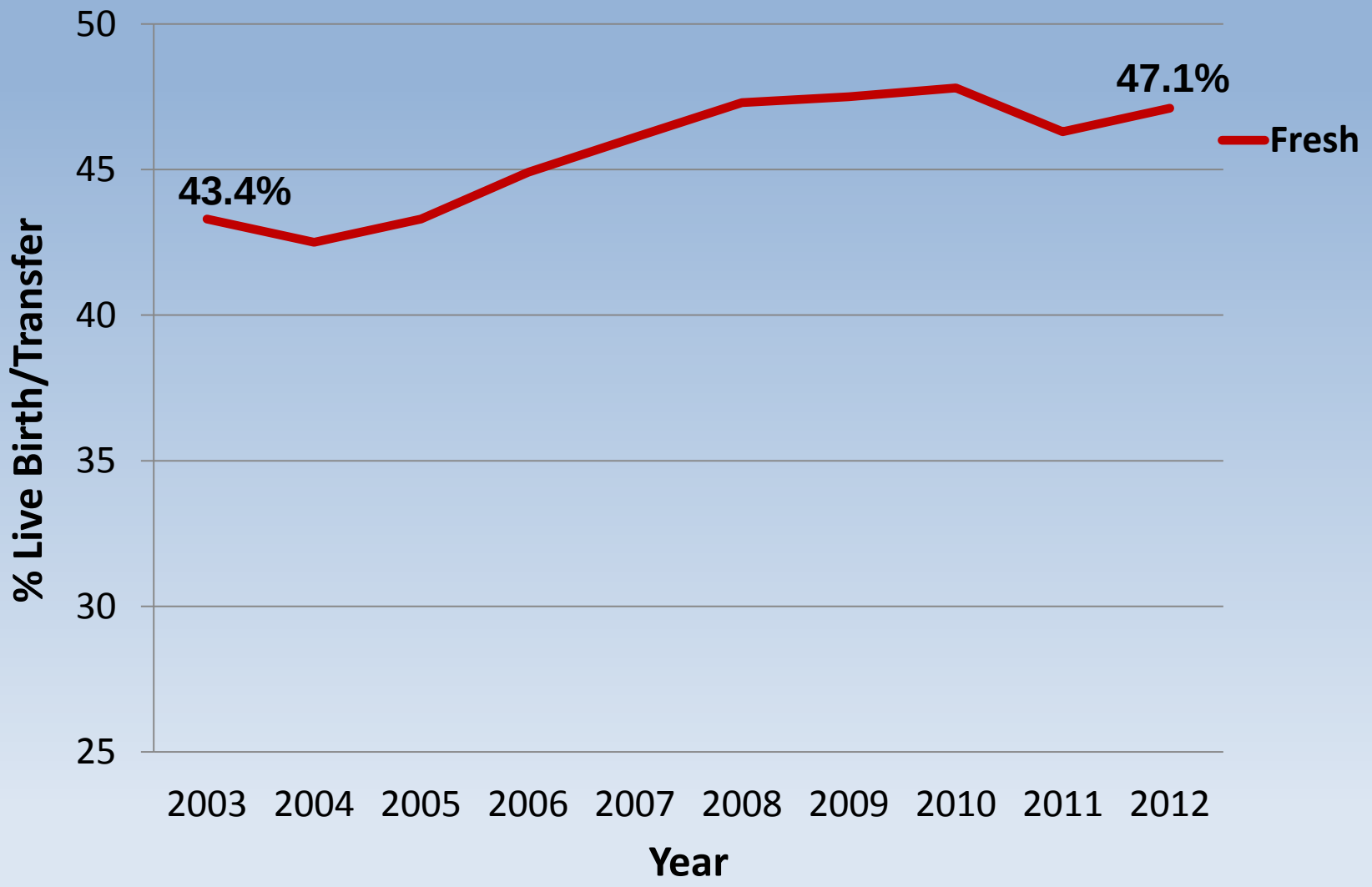
Please use the response keypad.

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)



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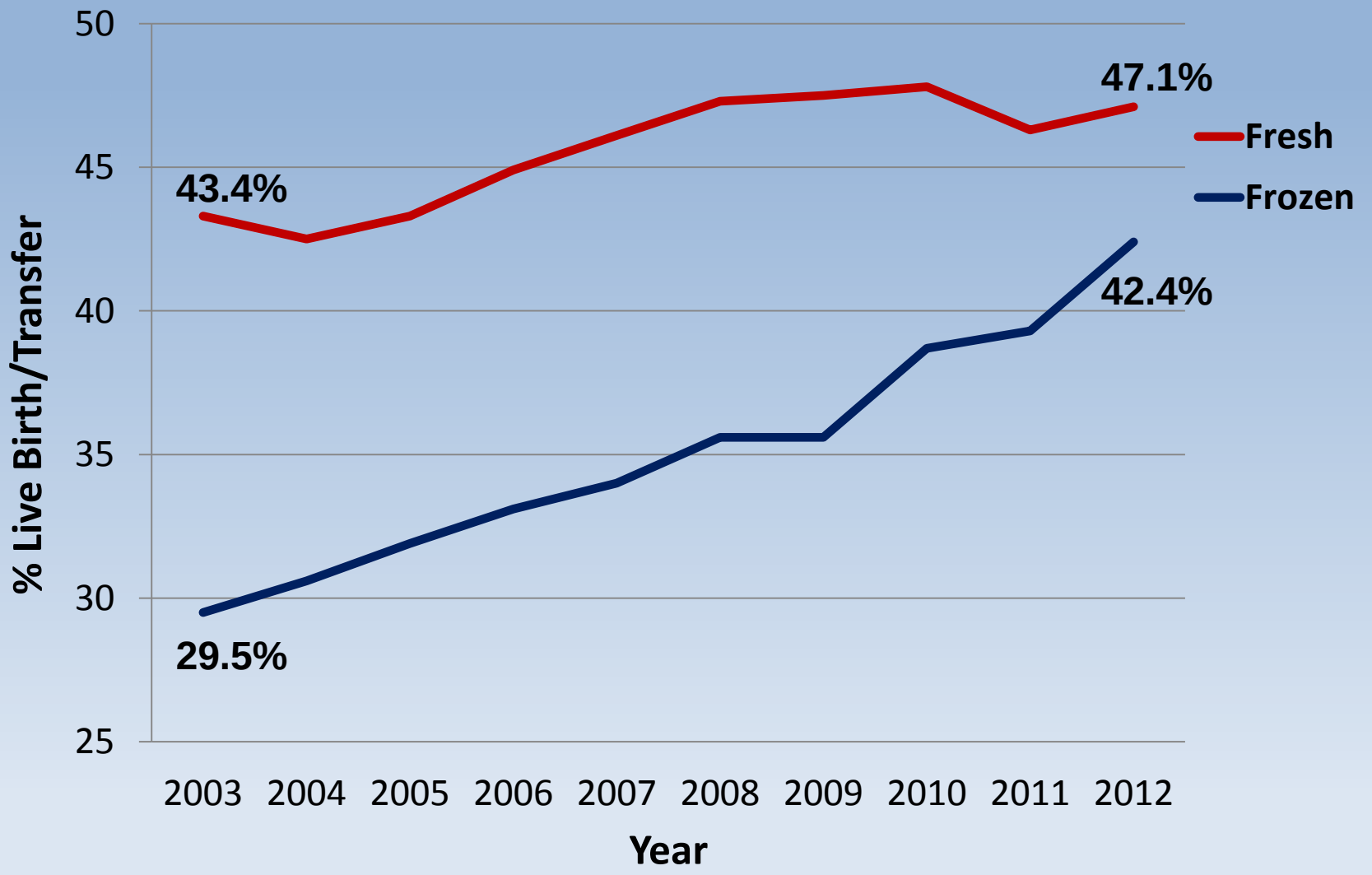
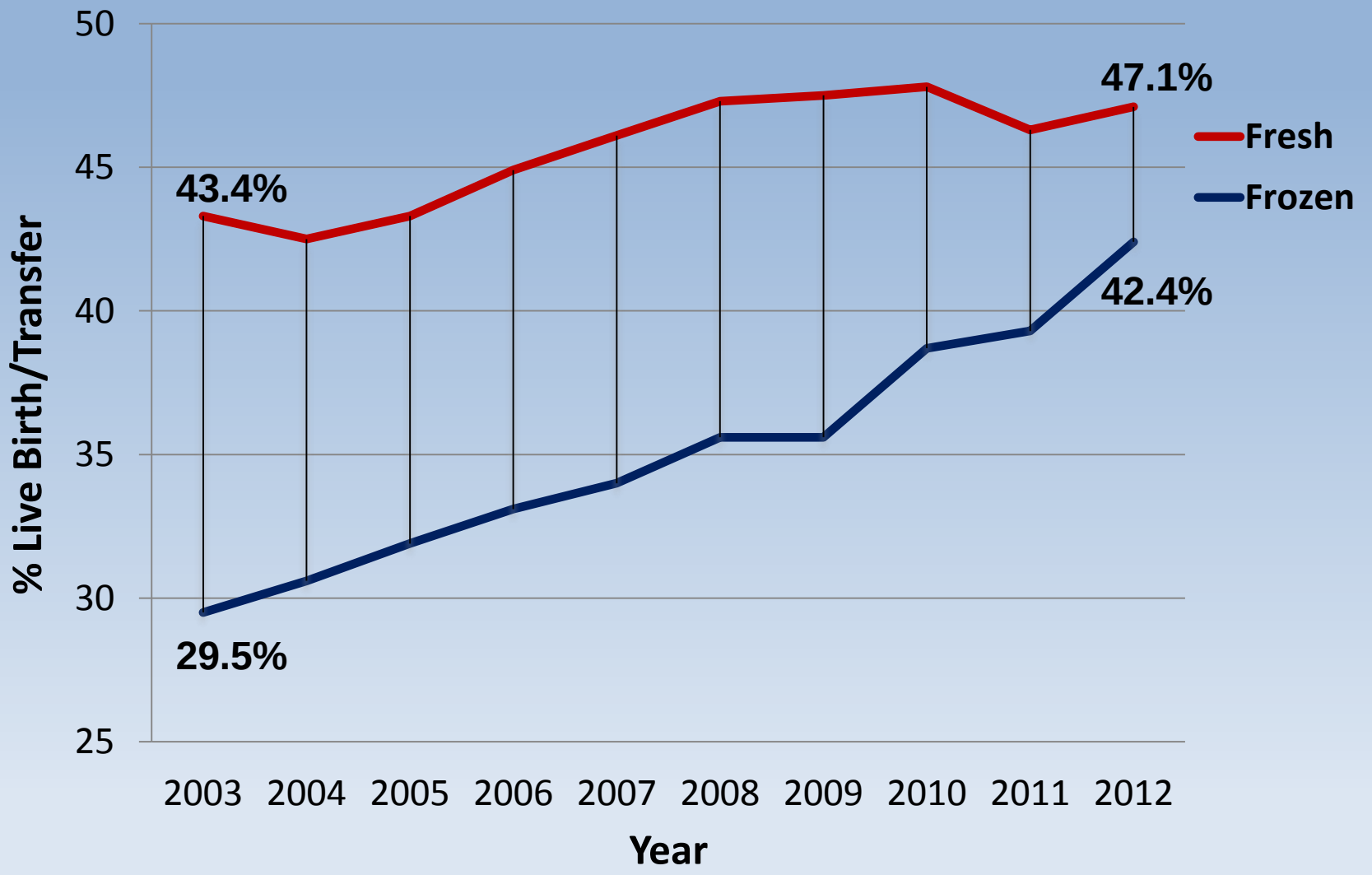
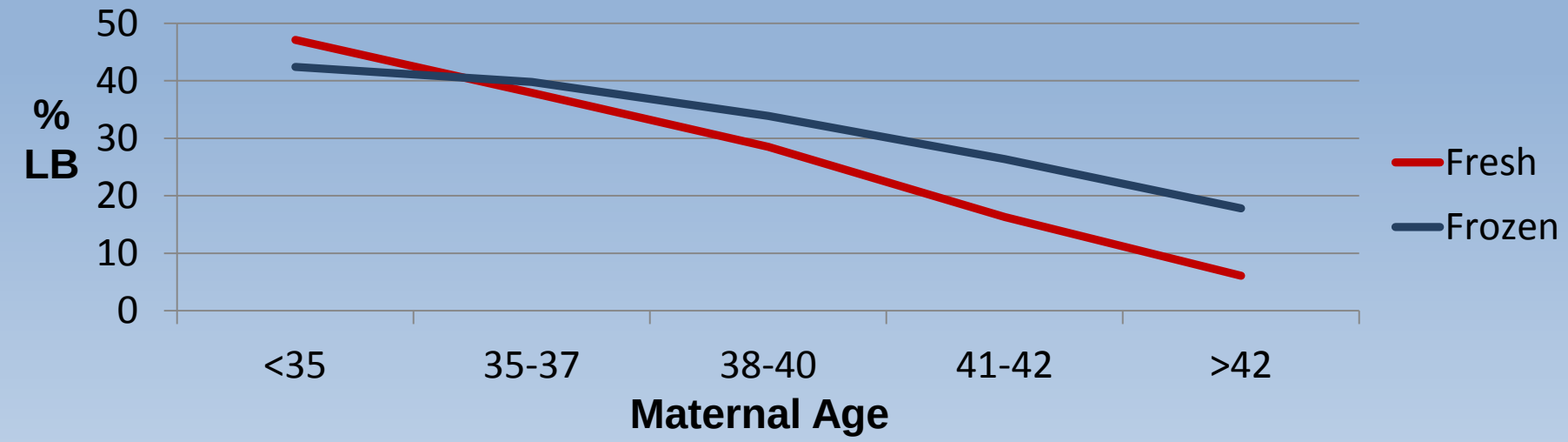
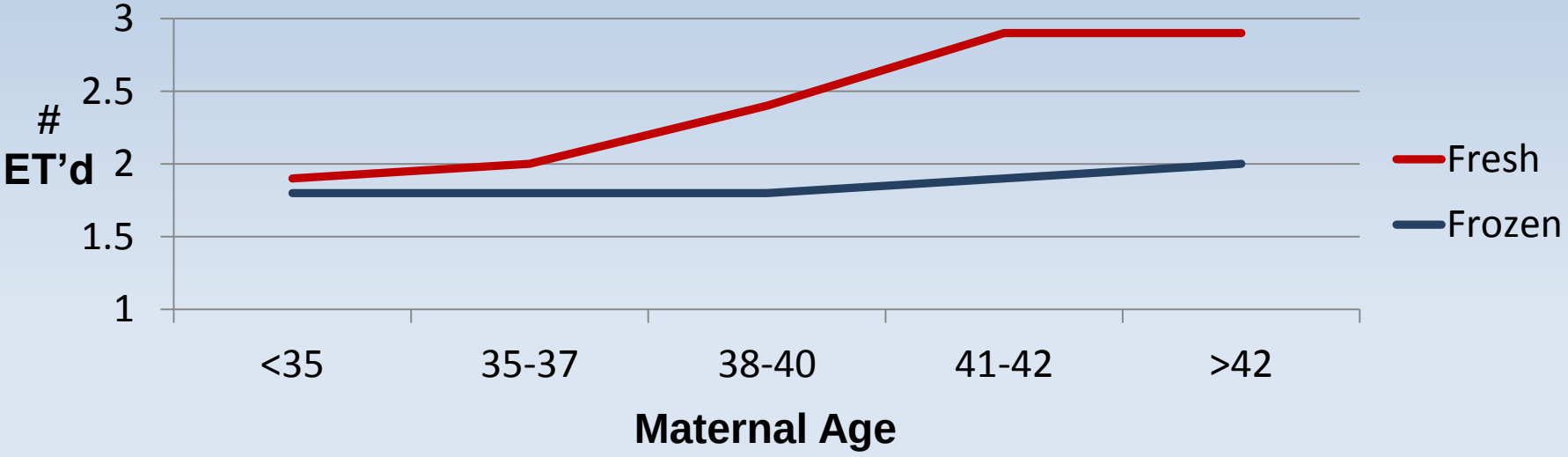


SART - Percentage of ET's Resulting in Live Birth (<35yo)



SART 2012 Percentage of ET's resulting in live birth





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

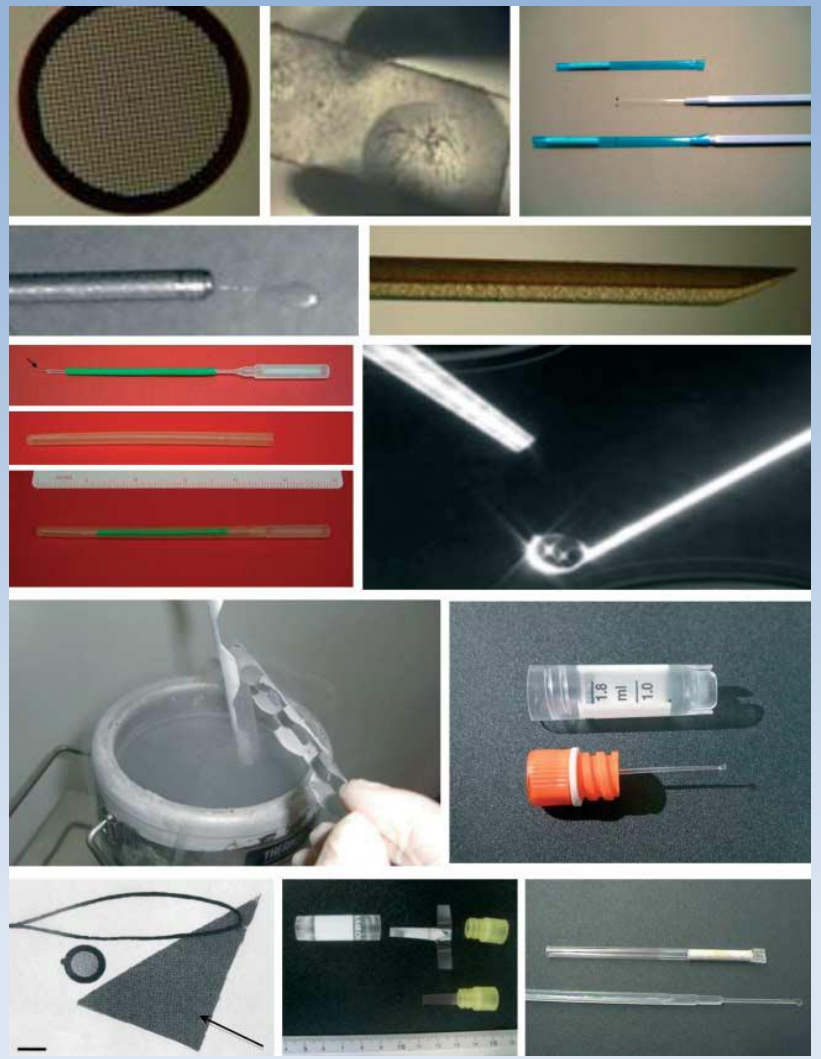
- Higher birth weights
- Possibly
  - Healthier uterine environment???
  - Healthier babies???

Factors Affecting Vitrification Success

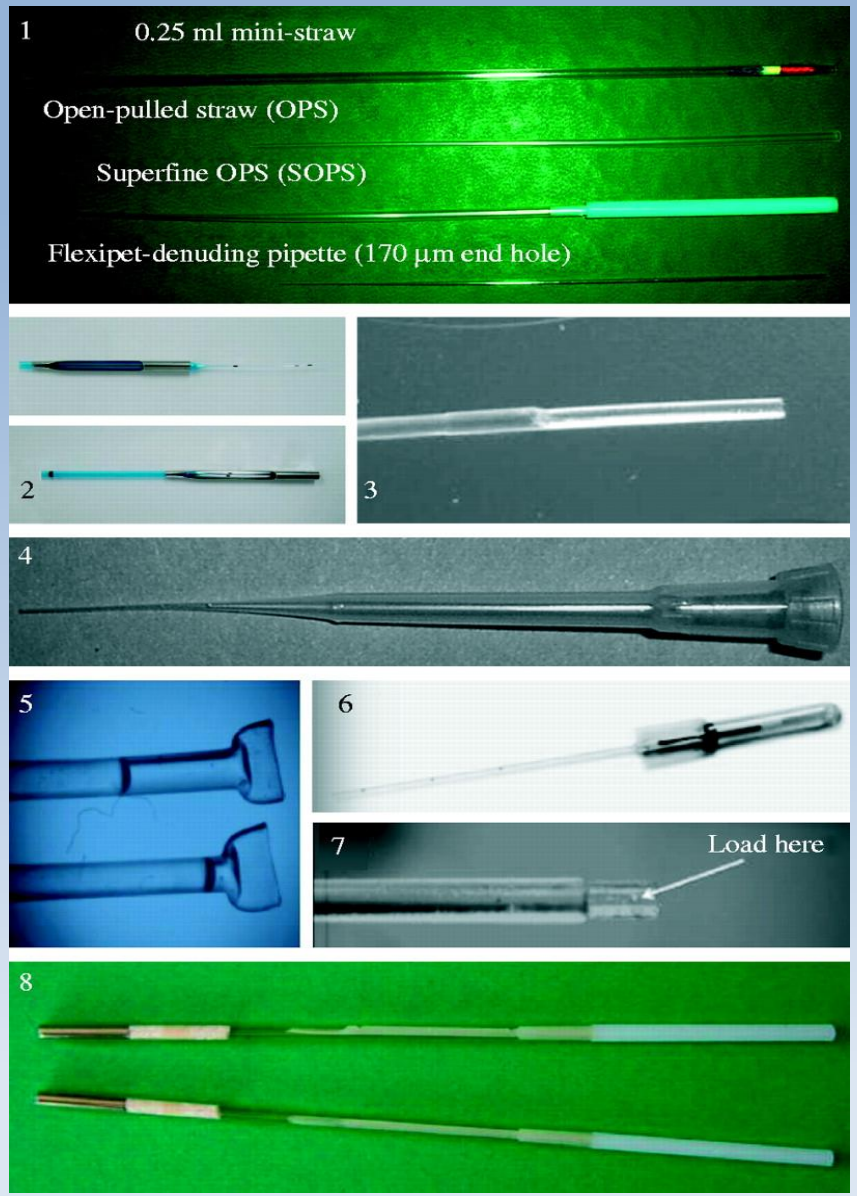
- Pre-vitrification blastocyst/embryo selection
- Assisted Hatching
- Blastocoel collapse ???
- Vitrification vessel
- Vitrification freeze solutions & volumes
- Cryo storage and handling
- Vitrification thaw solutions & volumes
- Post-vitrification blastocyst/embryo selection
- Progesterone timing

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

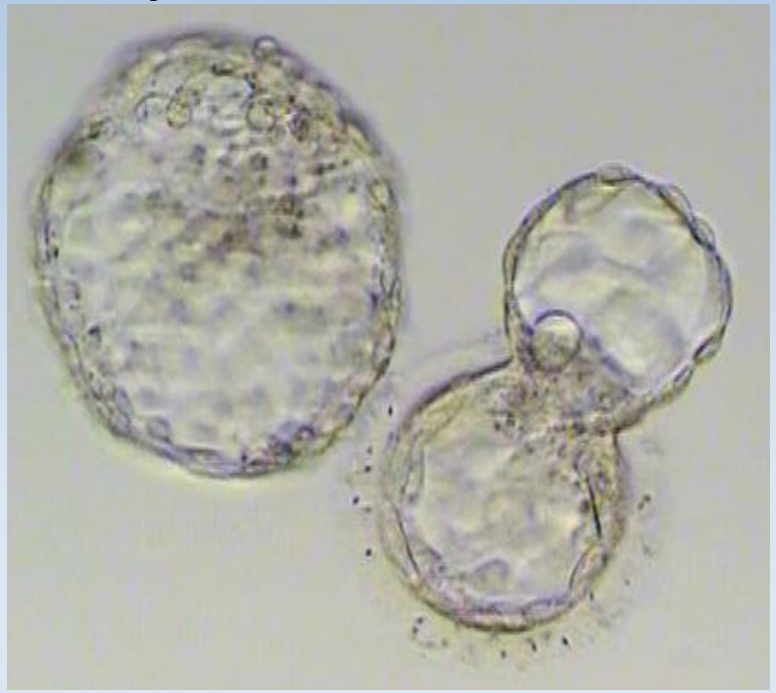
E. Other

Vitrification Method

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

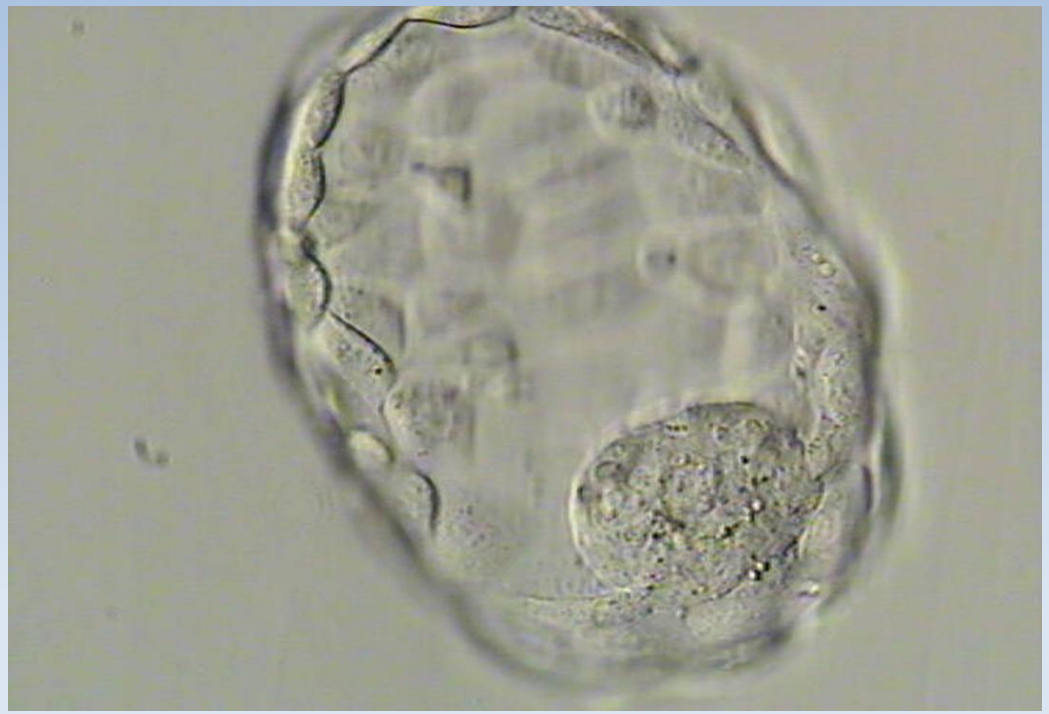
Collapsing Blastocysts???

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

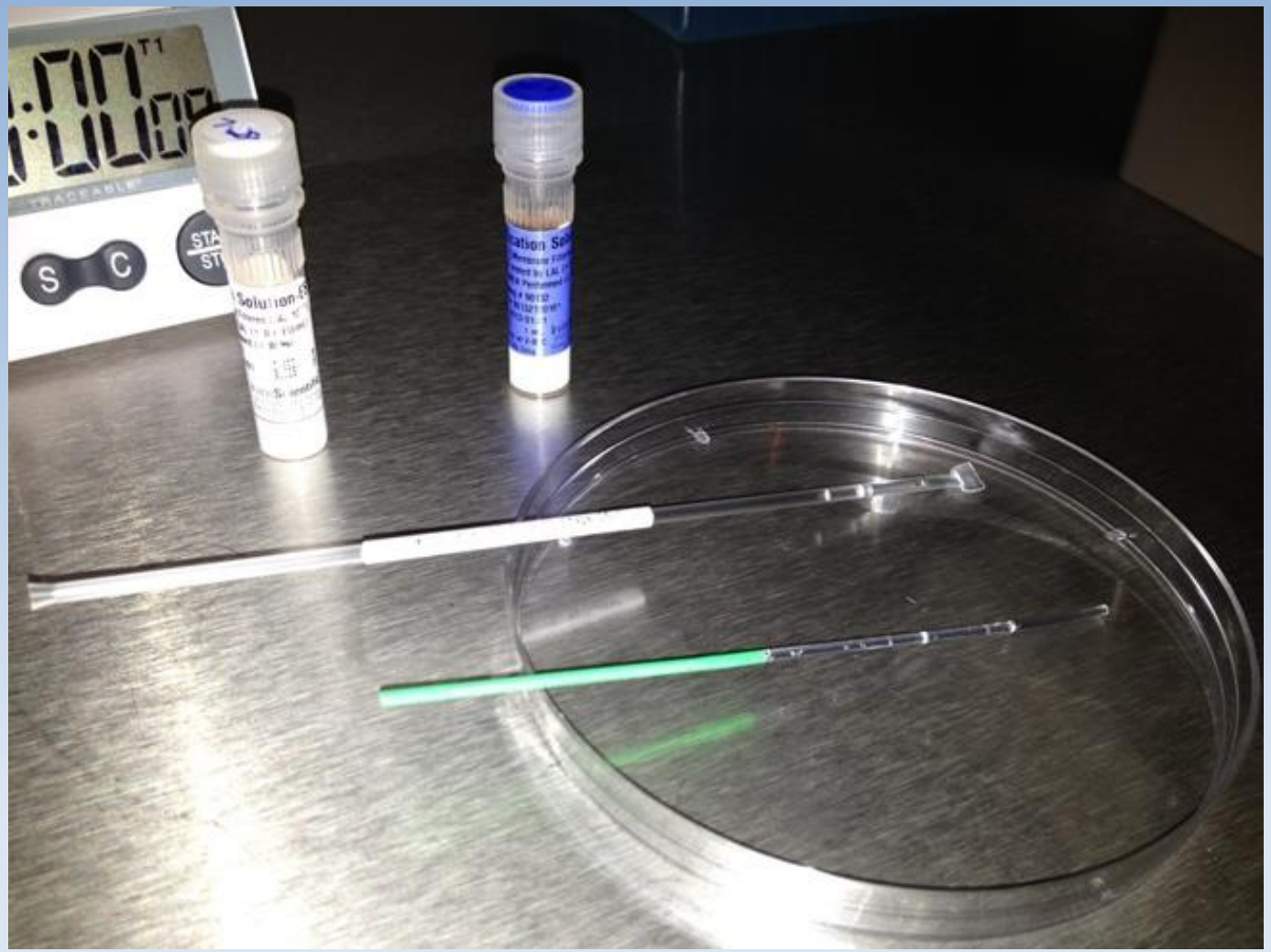


Blastocysts Collapsed with Laser:

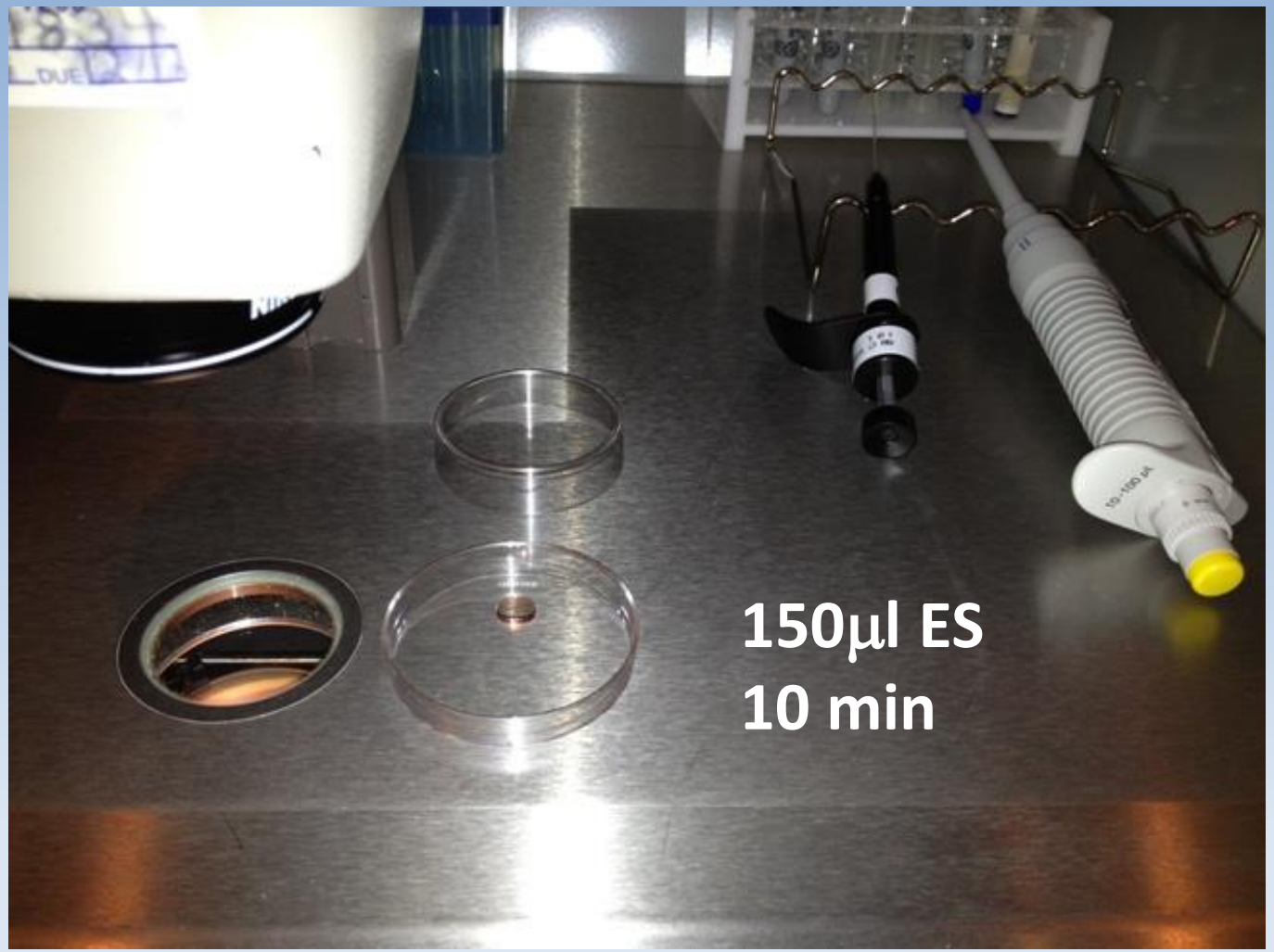
- If highly expanded
- Vitrified before fully re-expanded after biopsy



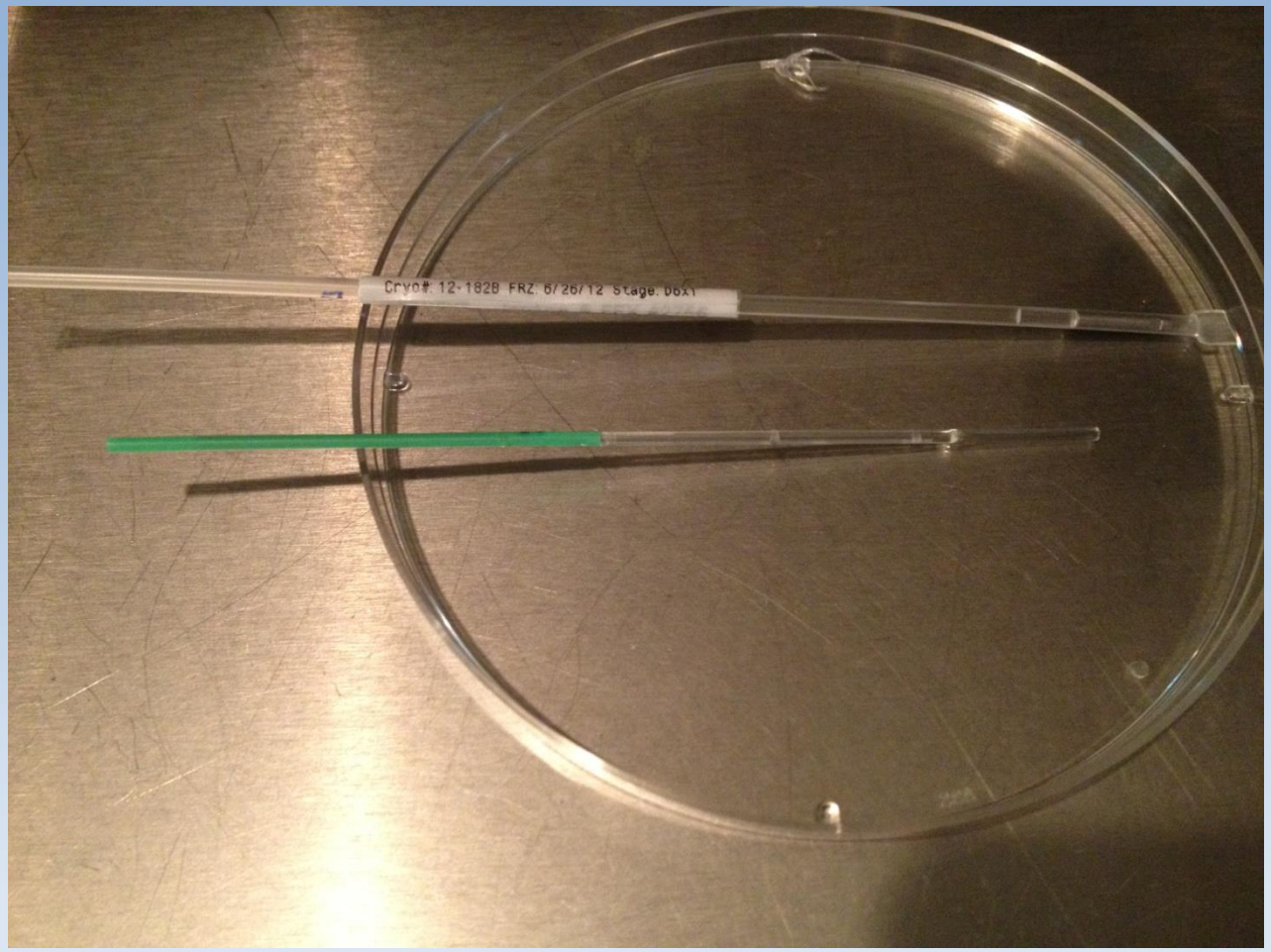
Irvine Vit Kit & HSV straws



Vitrification Set up

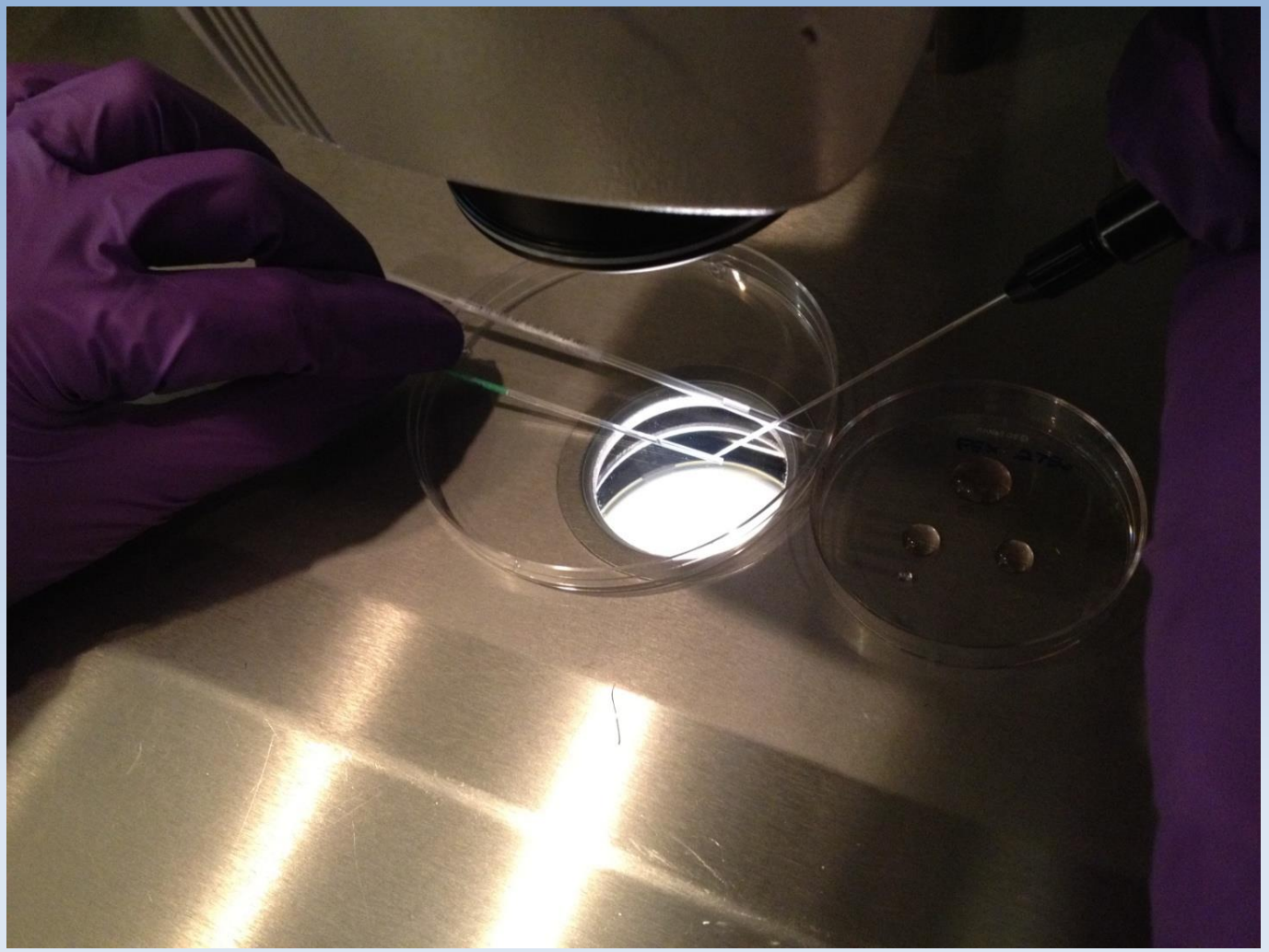


HSV Straw with Brady Label



Vitrification Set up

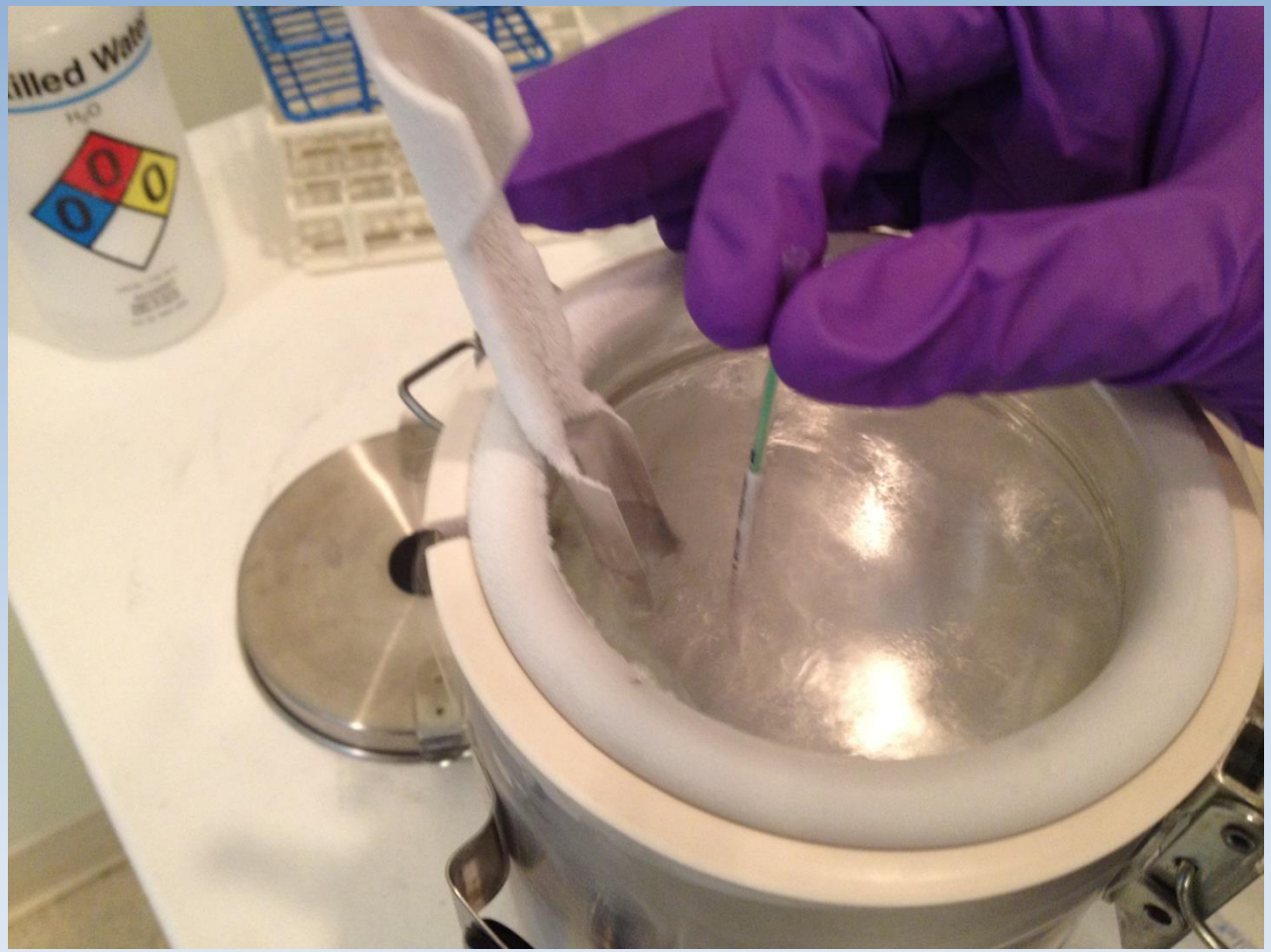
Loading the blastocyst on HSV straw



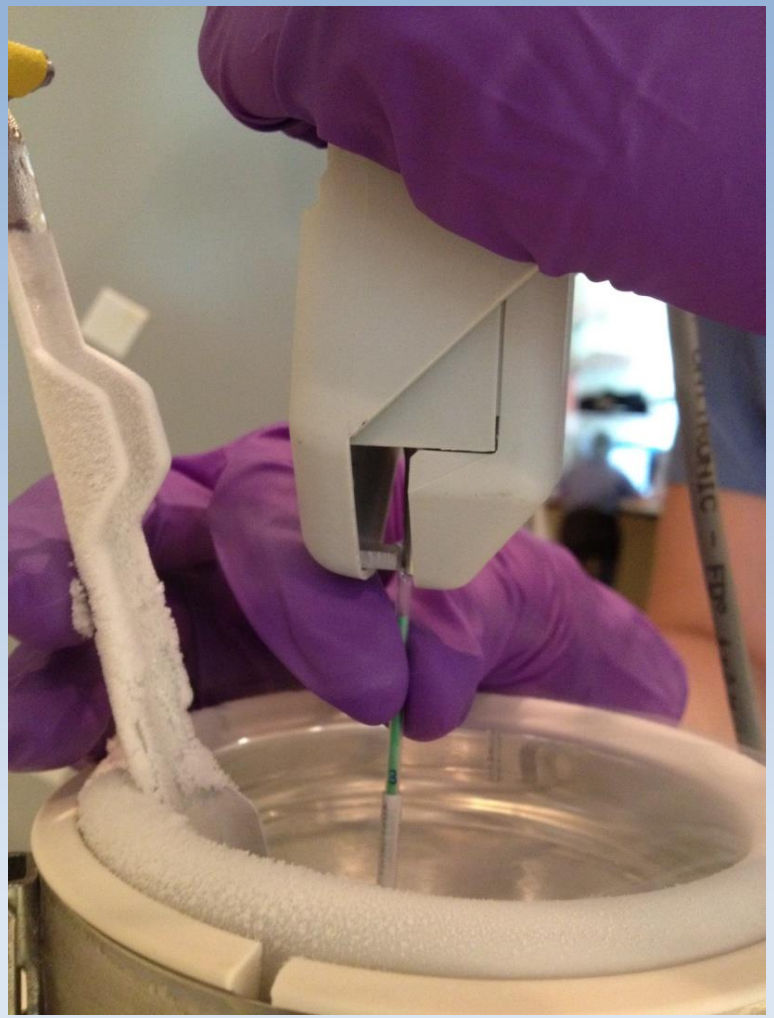
Tips for loading HSV straws

- 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

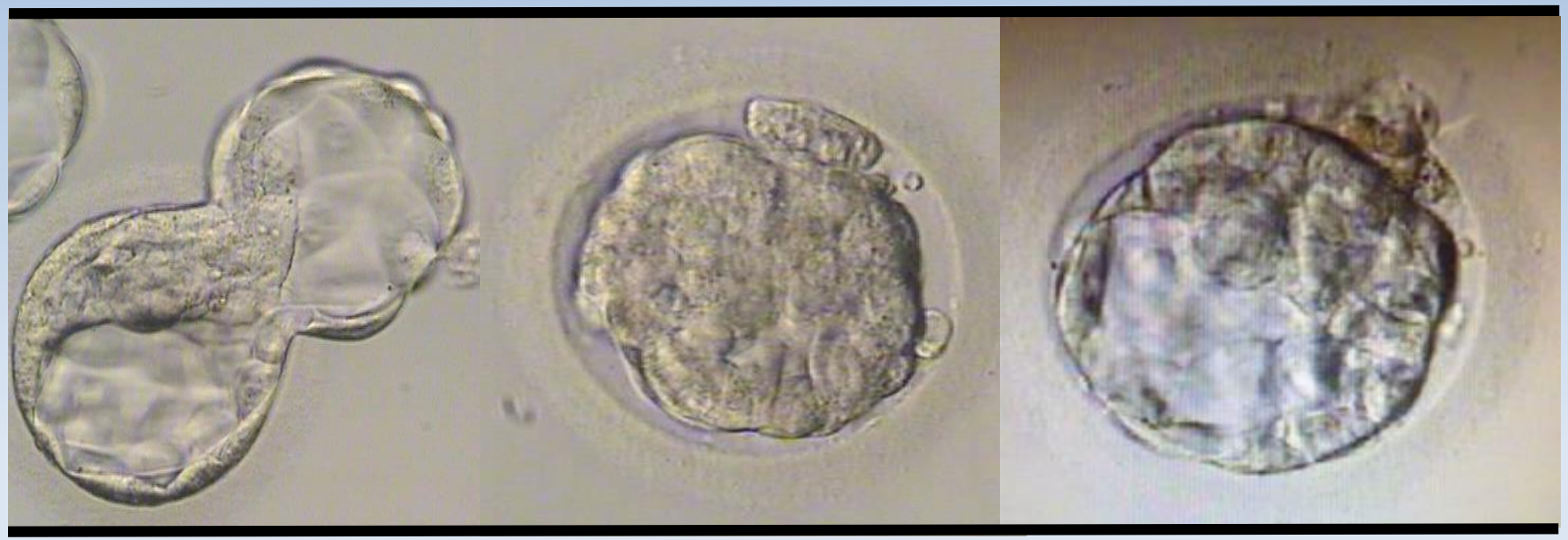
Storage of Vitrified Embryos

Options for Cryo Storage

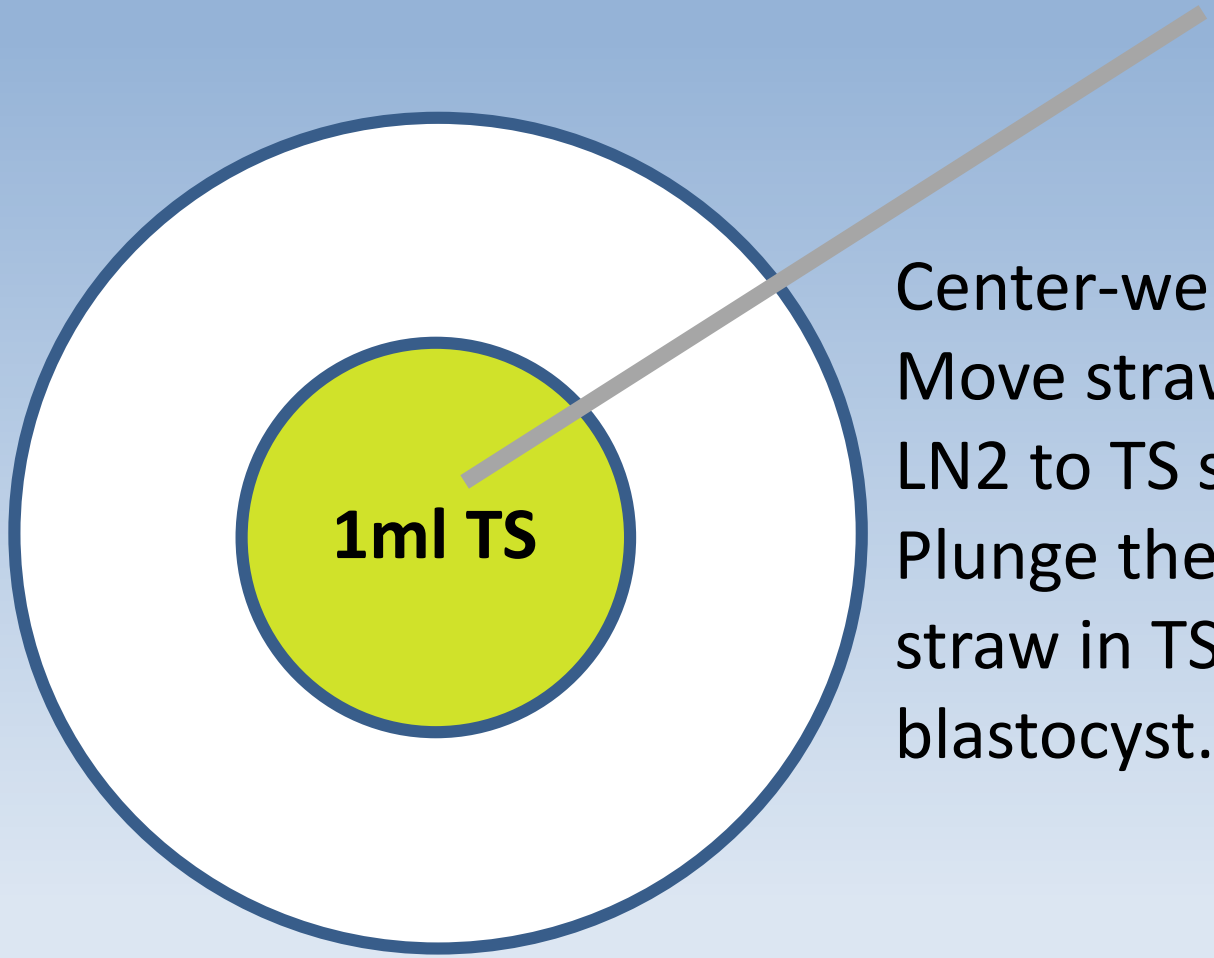
Timing of Vitrification Post-Biopsy

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

Pre-Biops y **Post-Biopsy** **At Vit**

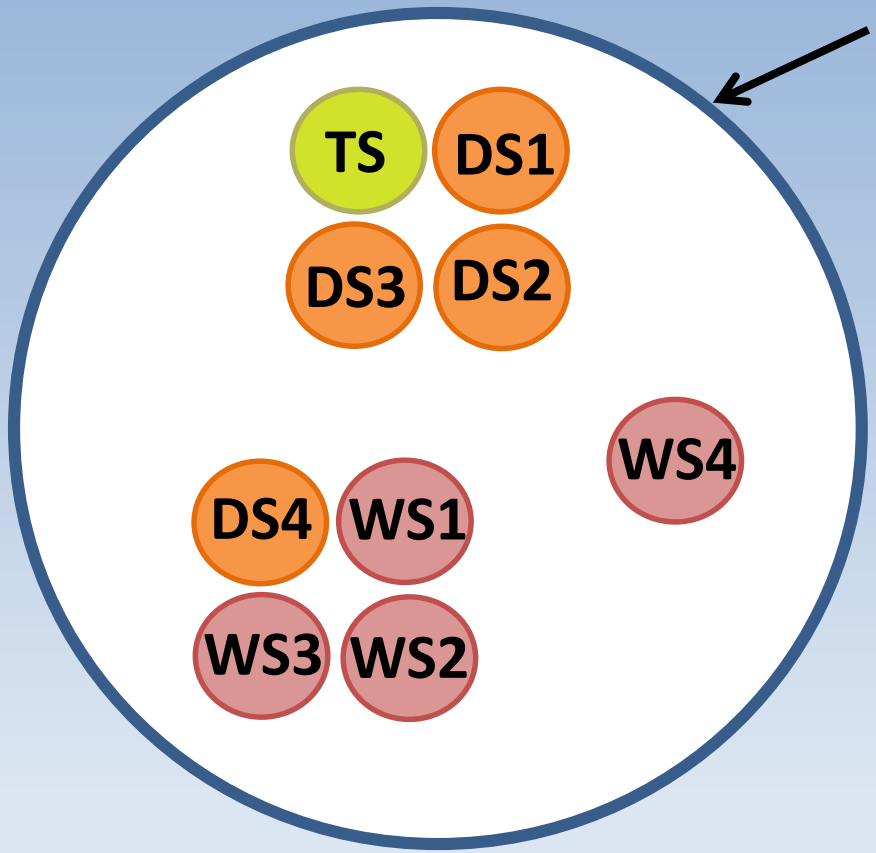


Vitrification Thaw



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.

Vitrification Thaw



Lid of 60mm dish at room temp

Embryo starts in TS ~1min

Merge DS1, DS2, DS3

Move to DS4

~3min

Merge WS1, WS2, WS3

Rinse through WS4

~3min

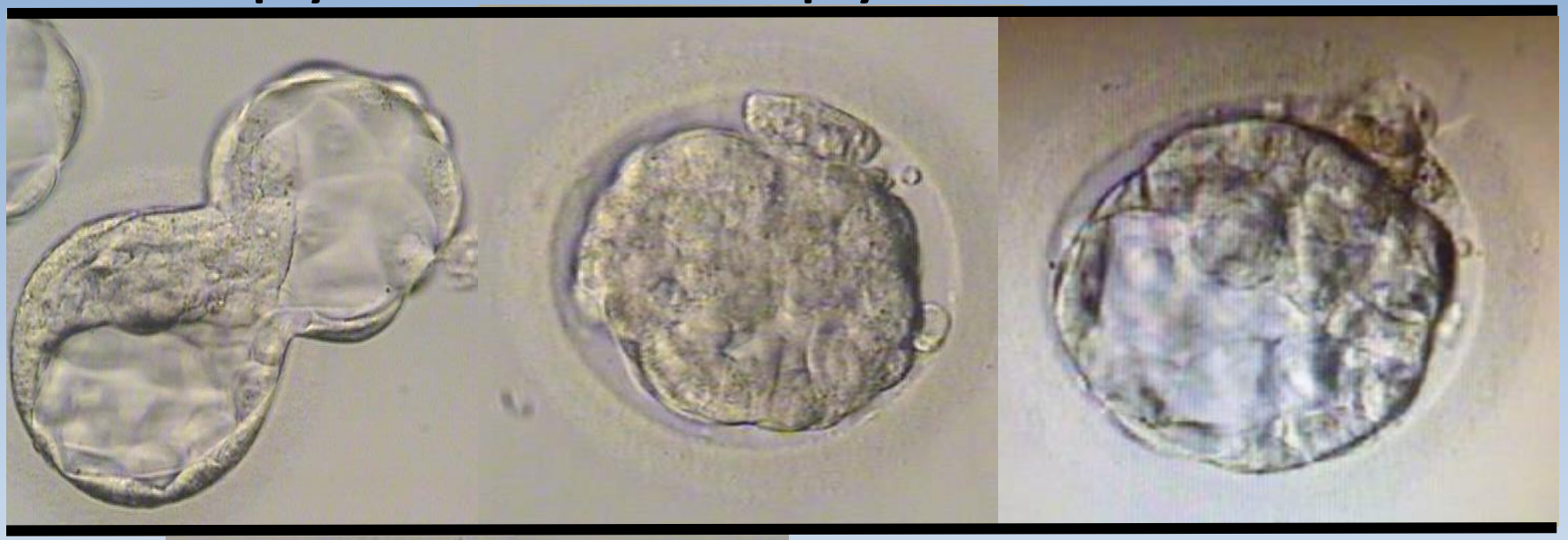
Move to culture plate

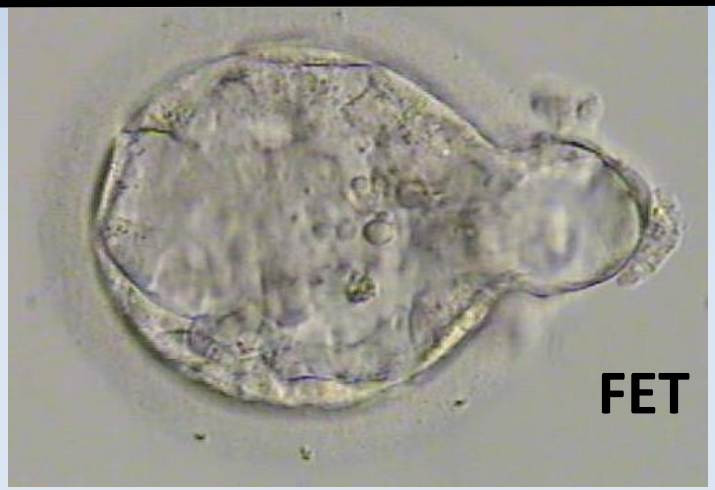
Tips for vit warming

- 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

Biopsy, Vit, Thaw & FET of single CCS normal

Pre-Biopsy | Post-Biopsy | At Vit





→ Singleton Delivered

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

	Fresh	Vitrified
# of Transfers	399	115
Mean Maternal Age	33.1	32.1
Avg # Embryos Transferred	1.85	1.91
Positive βhCG	72.4%	76.5%
Clinical Pregnancy Rate	60.2%	67.0%
Implantation rate	50.1%	50.5%

Kelk *et al.* ASRM 2012

2012 CCS Vitrification Results

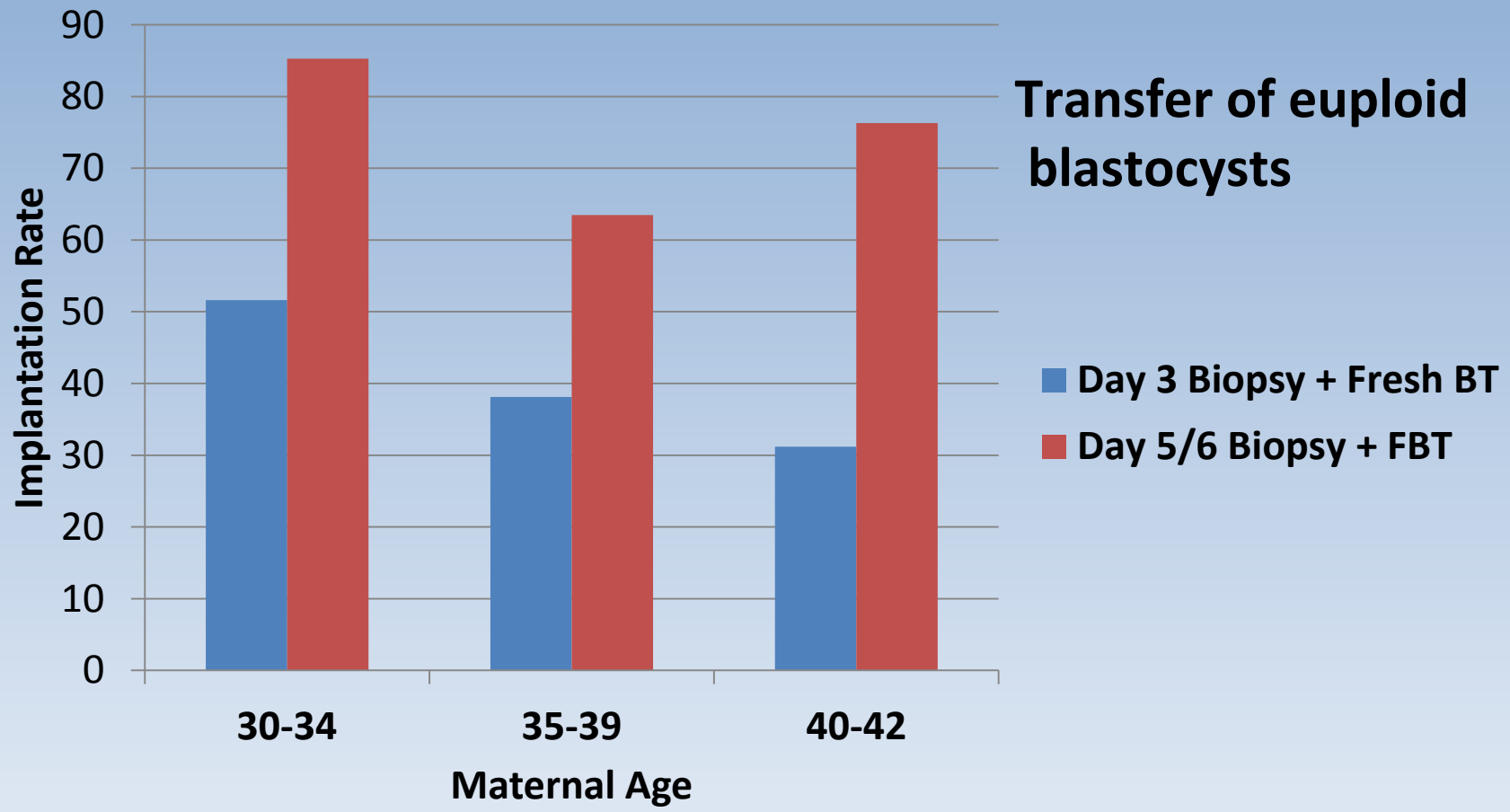
- 44 CCS patient thaws in 2012
- 97.1% thaw survival
- 1.51 embryos transferred
- 48.8% of CCS FET's are single ET

Fresh vs Vitrified CCS Blastocyst Transfers

	Fresh Day 6	Vitrified	p-value
N	44	44	---
Mean Maternal Age	37.4	38.6	0.06
Positive βhCG	70.5%	75.0%	0.63
Clinical Pregnancy Rate	56.8%	63.6%	0.51
Implantation rate	46.3%	53.7%	0.43

Kelk *et al.* ASRM 2012

Day 3 Biopsy & Fresh BT vs TE 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

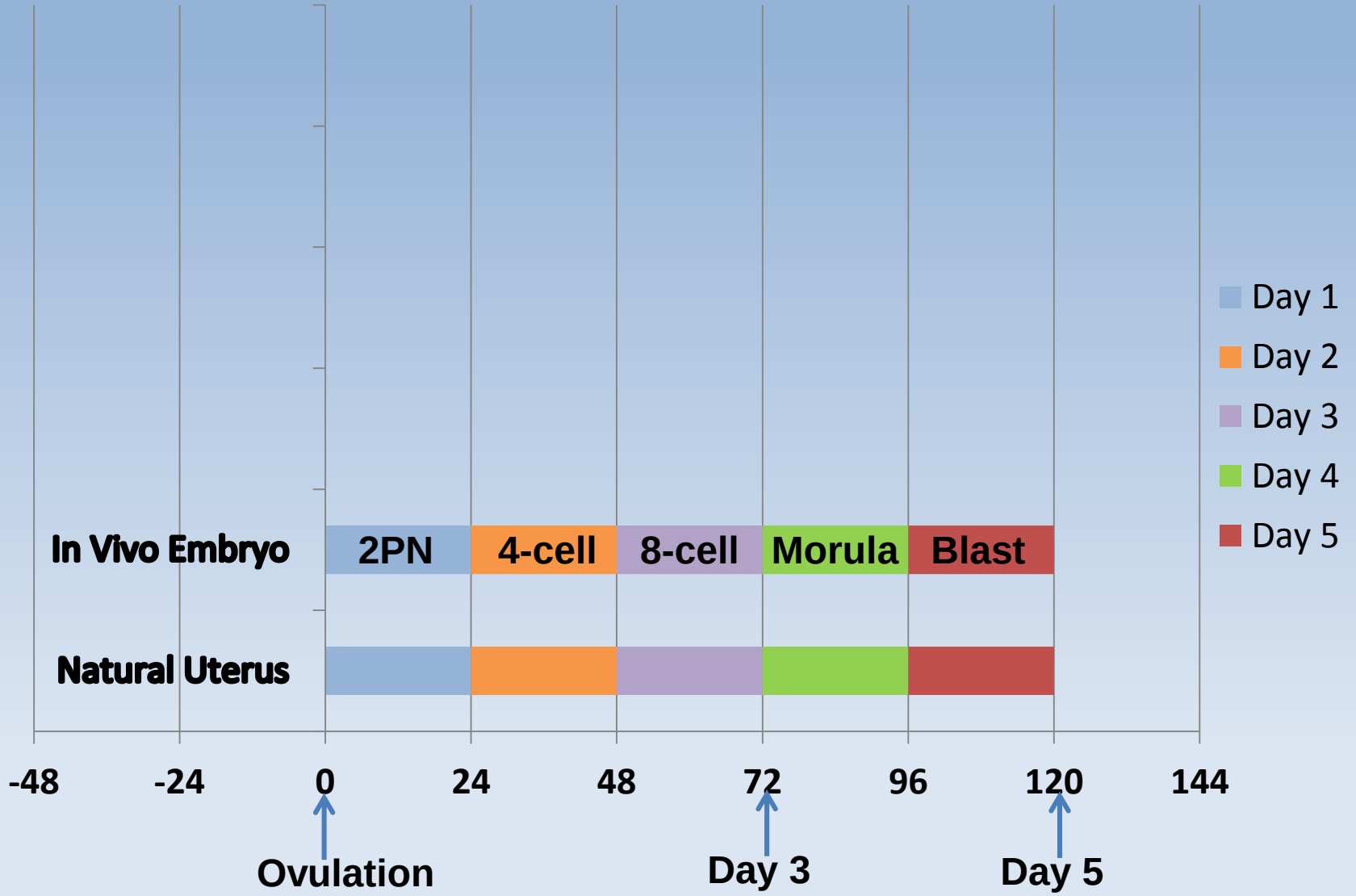
  → 40% twin rate

- 1 Euploid ET'd → 59% clinical preg rate

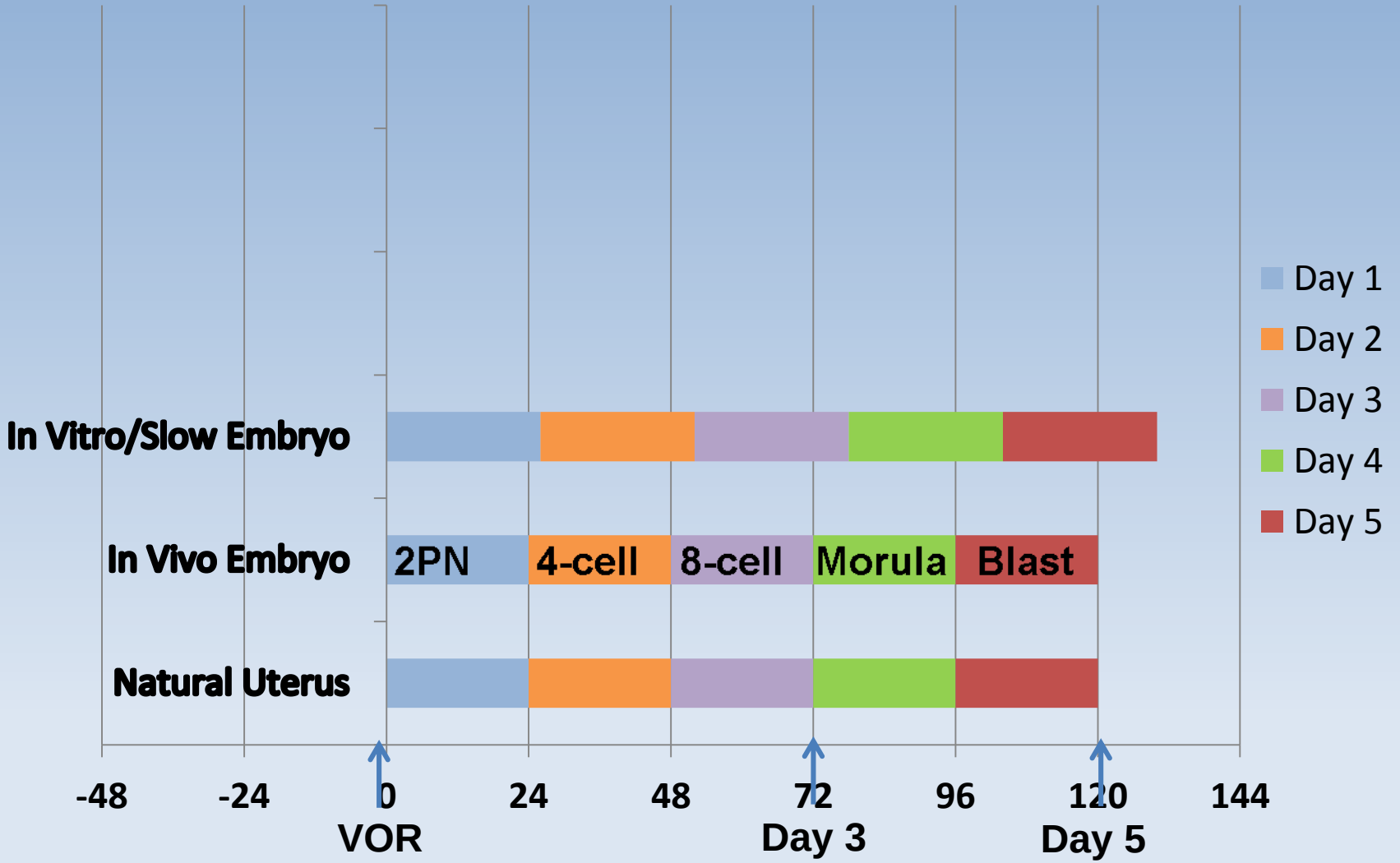
Controlled ovarian hyperstimulation

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

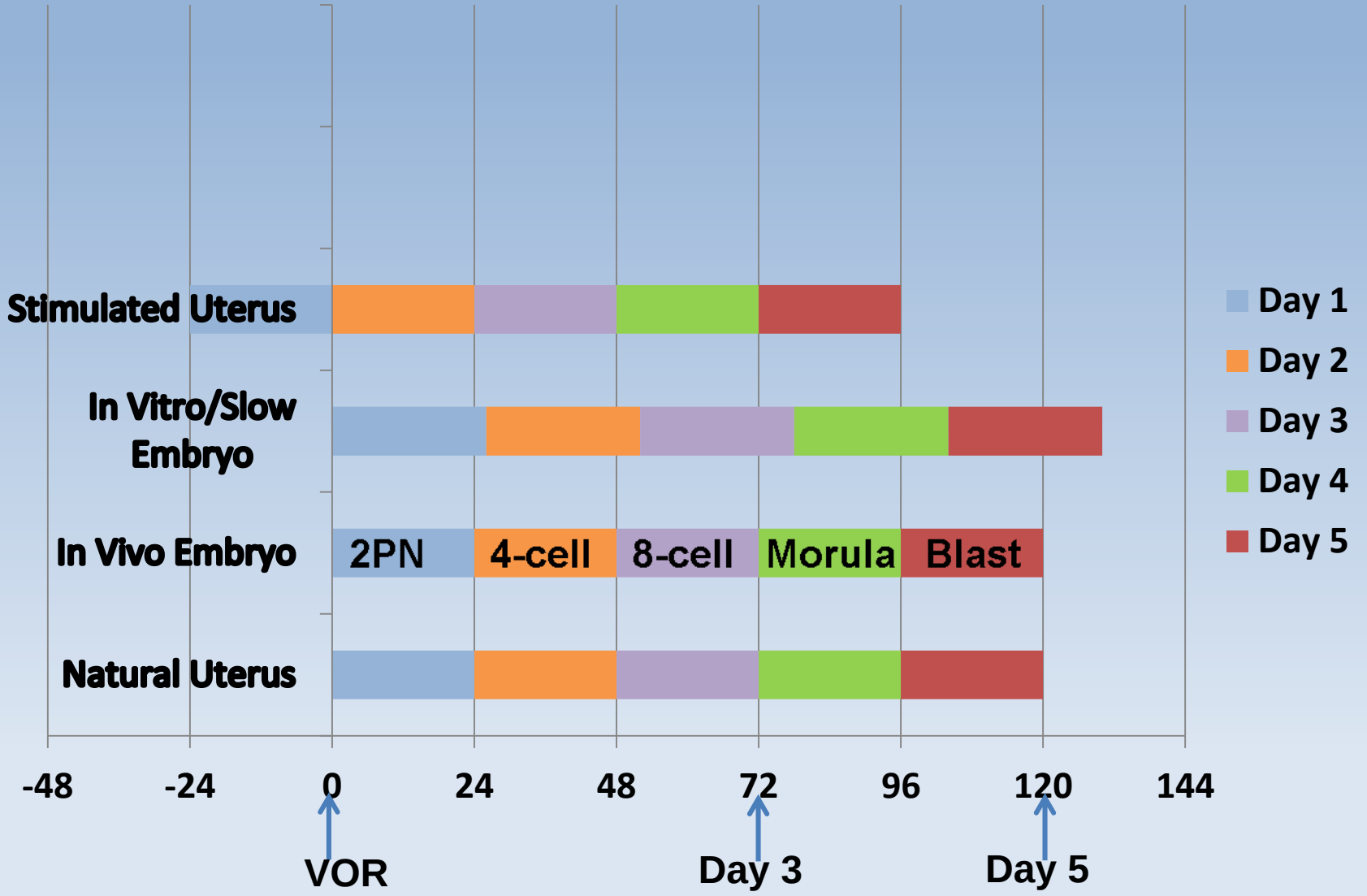
Natural Cycle Uterine Synchrony



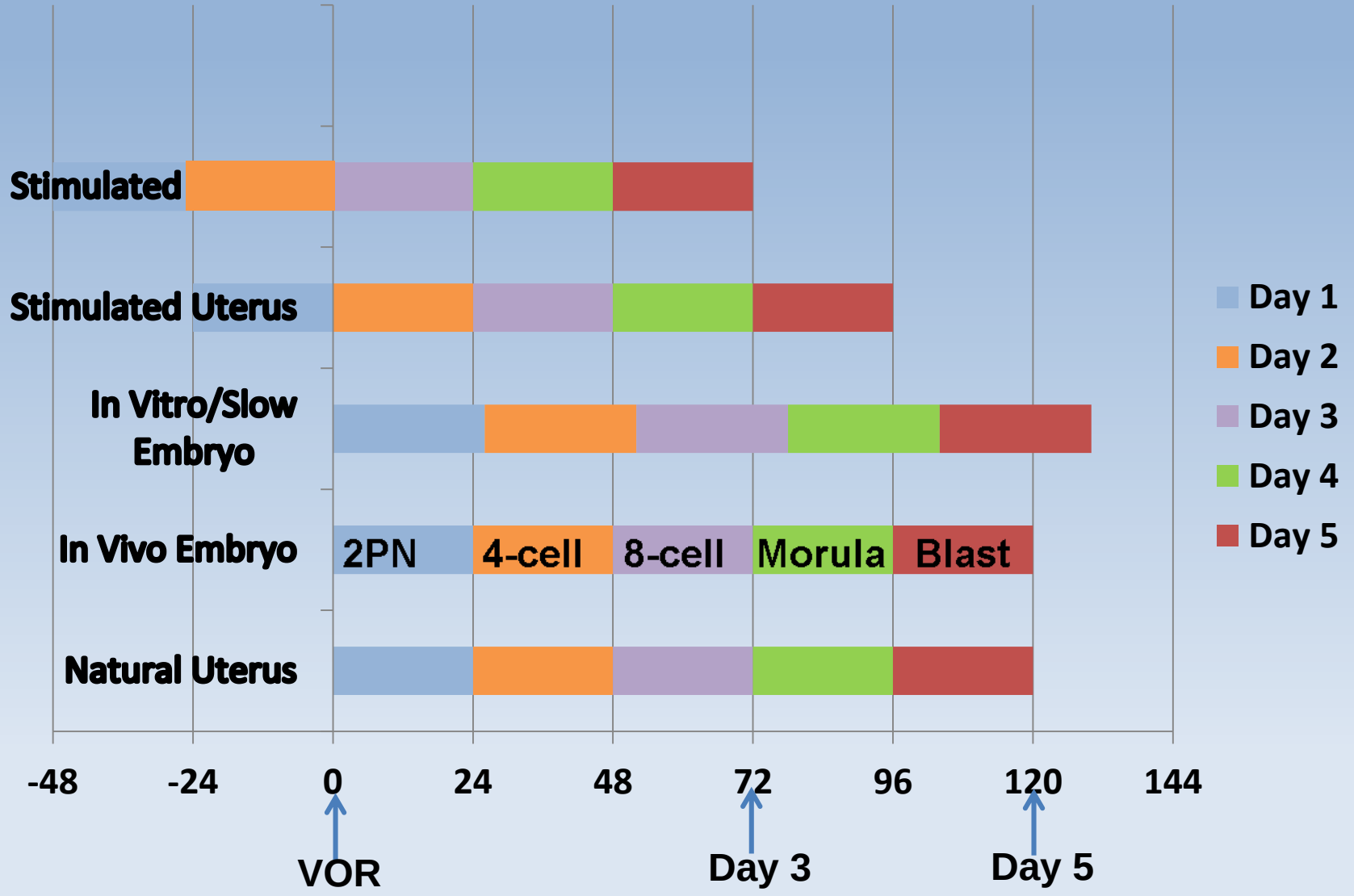
Slow Developing Embryo Synchrony



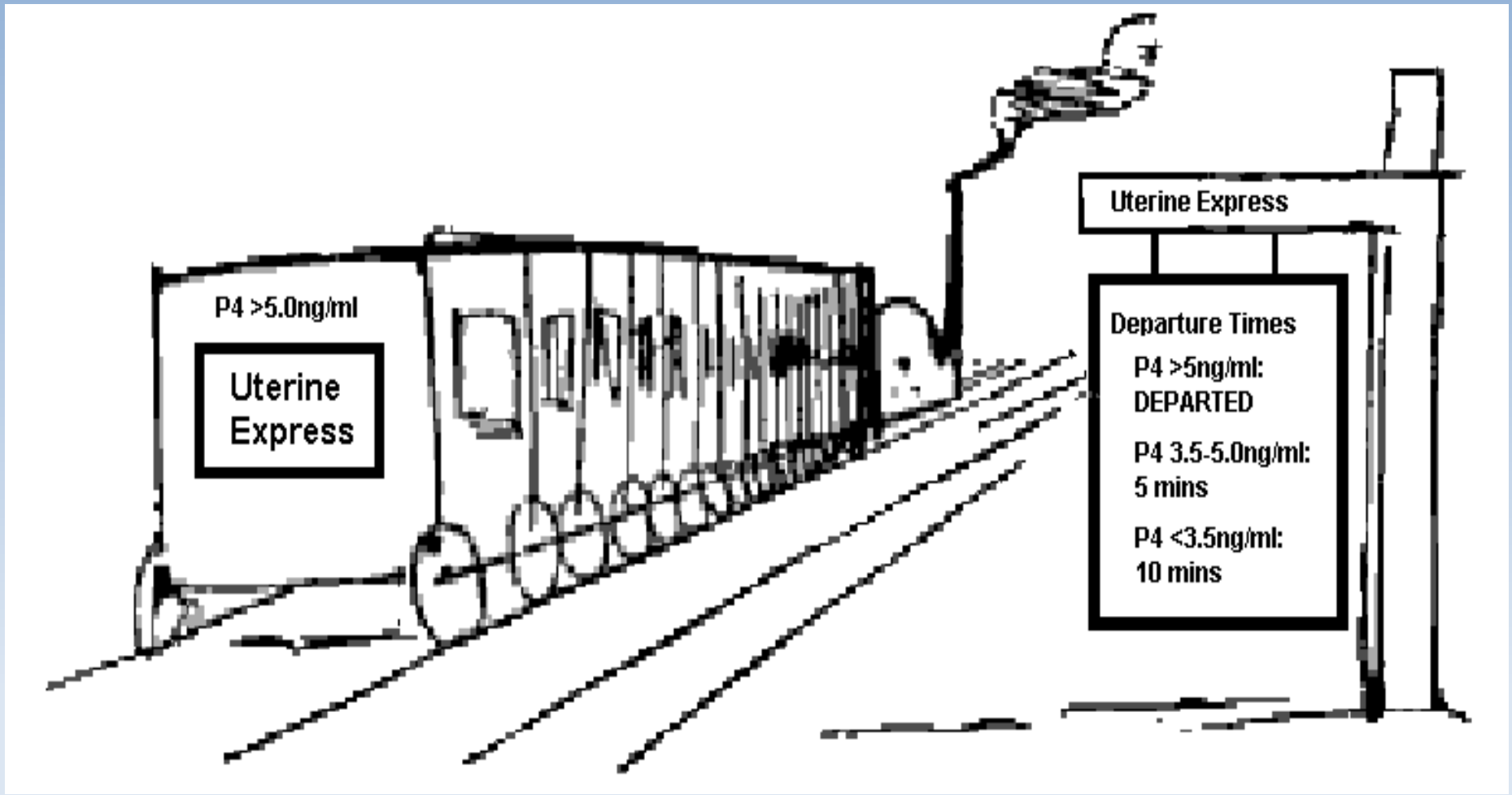
Stimulated Cycle Synchrony



Stimulated Cycle Synchrony

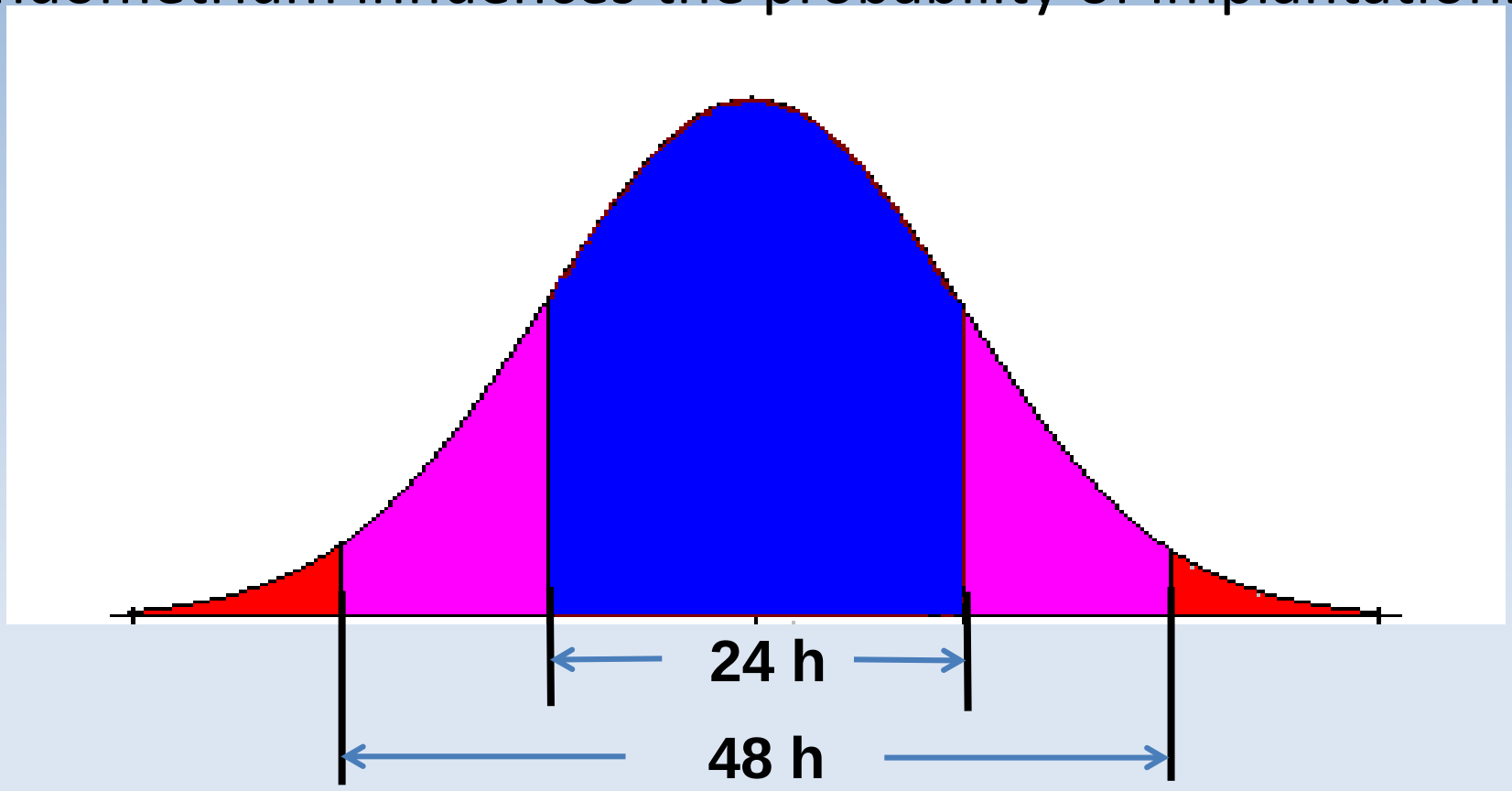


Uterine Express has left the Station…



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

Progesterone Level Before VOR & Preg Rates

Prog Level Day Prior to VOR	# Patients	Chem PR	Clin PR	Endomet. Thickness
<3.5 ng/ml	419	67.8%	59.6%[a]	9.9mm
3.5-5.0 ng/ml	212	61.5%	55.2%[b]	10.1mm
>5.0 ng/ml	140	63.7%	49.3%[c]	9.9mm

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

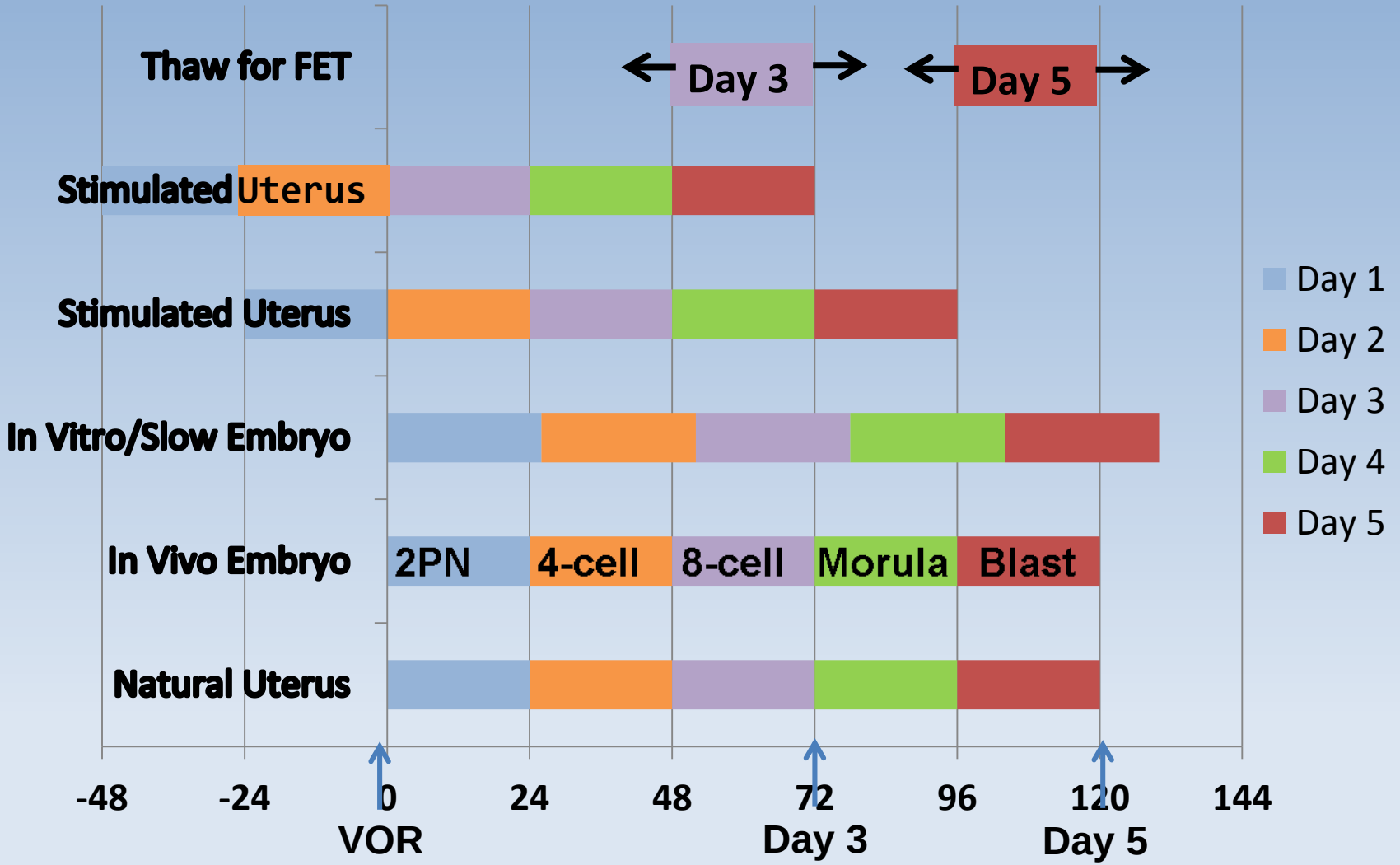
Huang *et al*. Fertil Steril. 2012; 98:664-70.

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

Ochsenkuhn *et al*. Fertil Steril. 2012; 98:347-54.

Thawed Embryo Synchrony

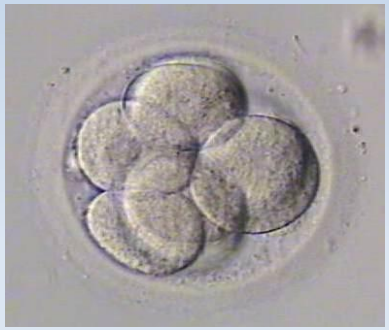


Hormonal induction of endometrial receptivity

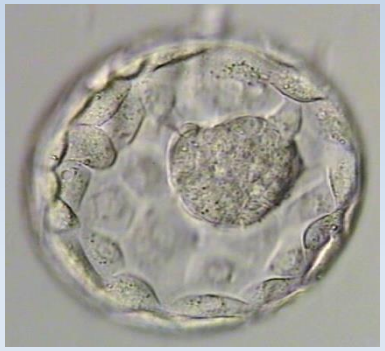
- Oral E2 constant or increasing dose for 10-14 days
- Adequate endometrial thickness (>7 or 8mm)
- Start progesterone 50mg IM or 100mg vaginally

  -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

Paulson, RJ. Fertil Steril. 2011; 96(3):530-5.

Shi *et al.* Fertil. Steril. 2012 Jun;97(6):1338-42.

- 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

Kato *et al.* Eur J Obstet Gynecol Reprod Biol 2012 Mar;161(1):46-50.

- 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%)

Wikland *et al.* Hum Reprod. 2010 Jul;25(7):1699-707.

- 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)

Pinborg *et al.* Hum Reprod. 2014 Mar;29(3):618-27.

- Large Baby Syndrome with FET vs fresh vs NC
- Singletons 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

Wennerholm *et al.* Hum Reprod. 2013 Sep;28(9):2545-53.

- 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???