Vitrification: Choosing which blastocysts to vitrify

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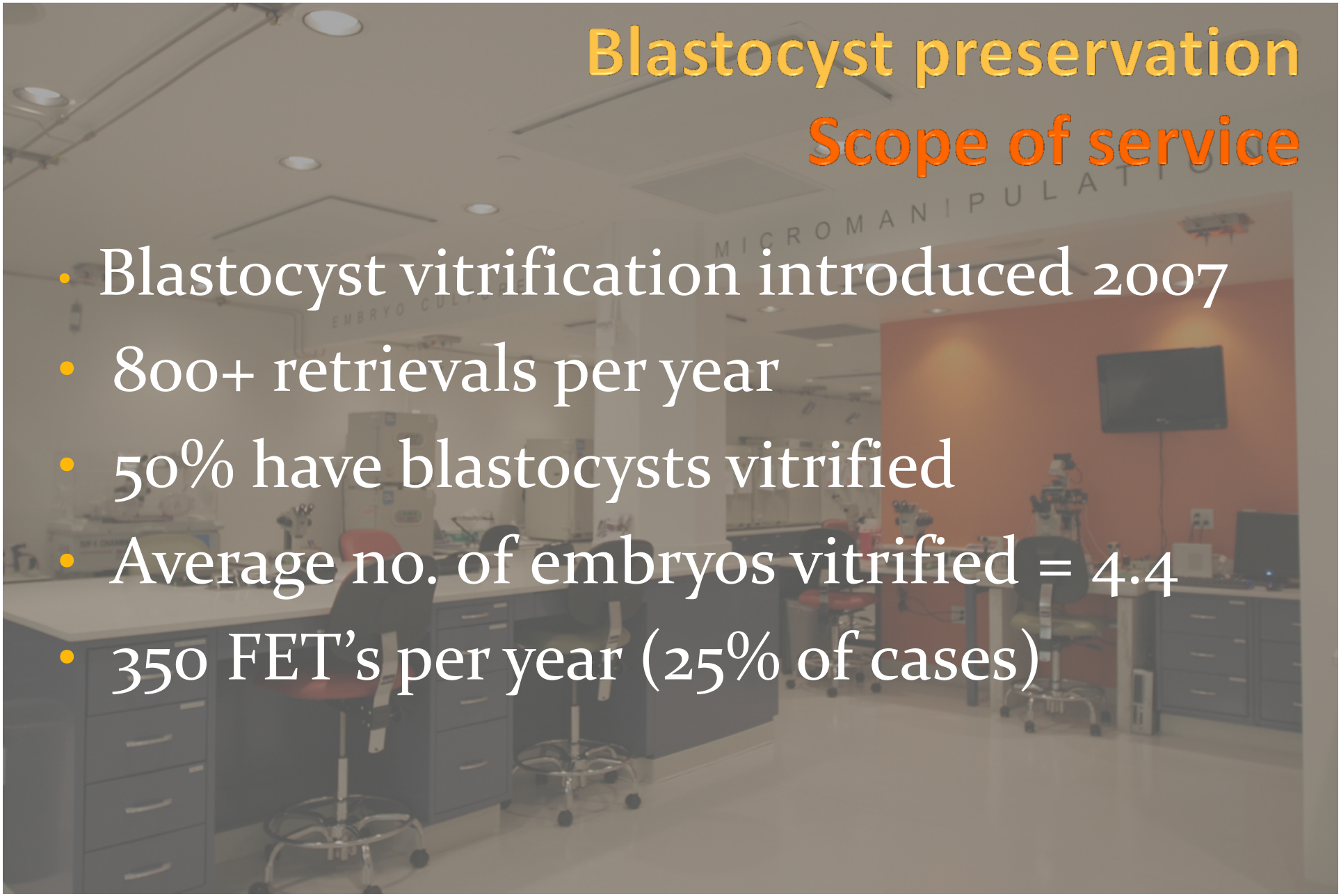
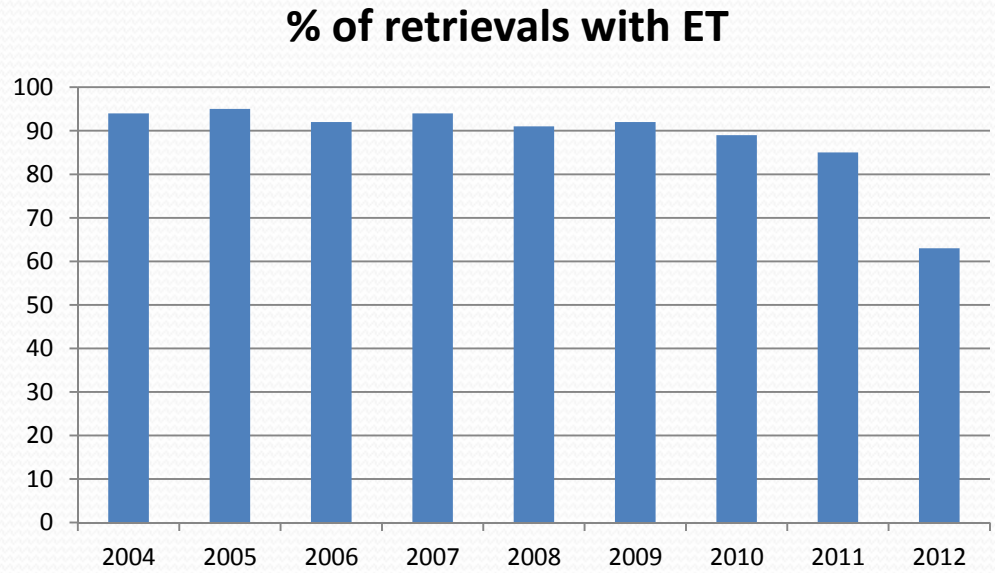
Las Vegas, ABB Mtg, May 17th 2013

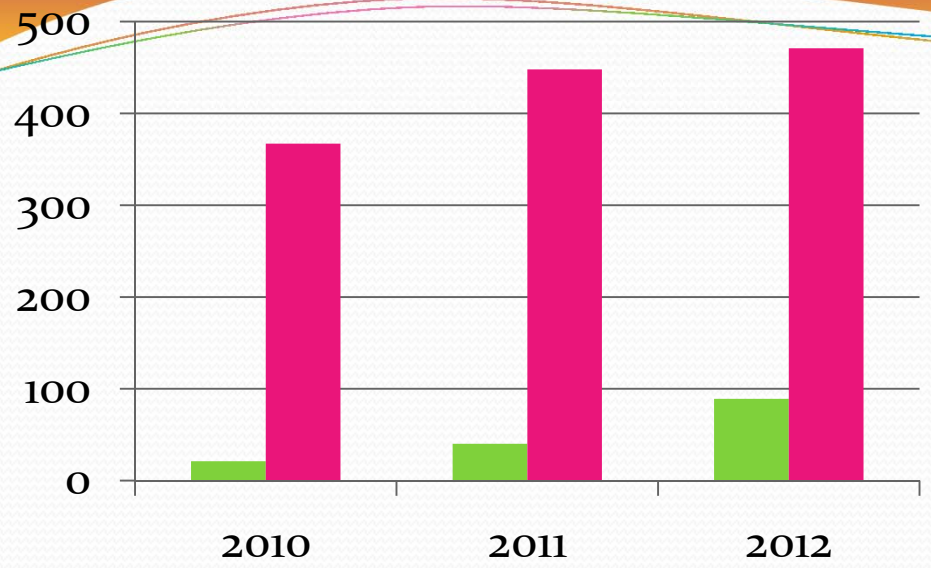


Blastocyst preservation
Scope of service

- Blastocyst vitrification introduced 2007
- 800+ retrievals per year
- 50% have blastocysts vitrified
- Average no. of embryos vitrified = 4.4
- 350 FET's per year (25% of cases)

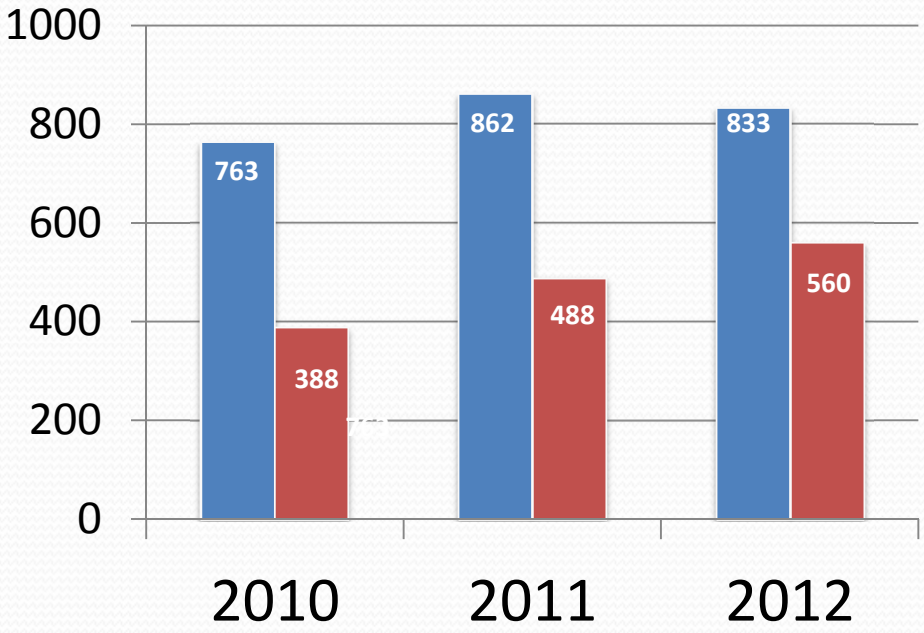
The changing face of the IVF lab





Oocyte Cryo

Embryo Cryo



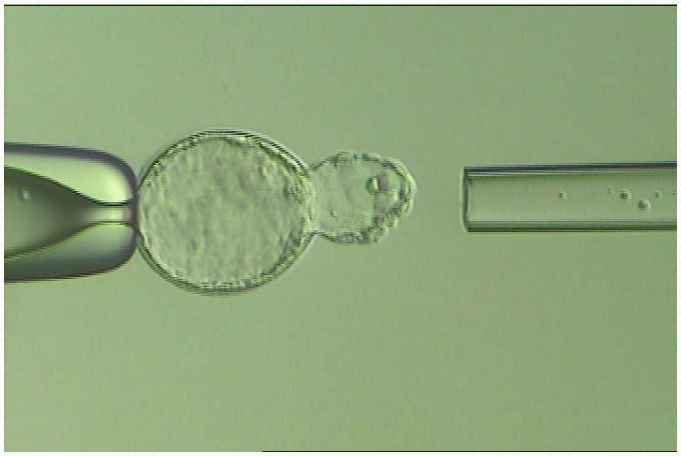
Retrievals

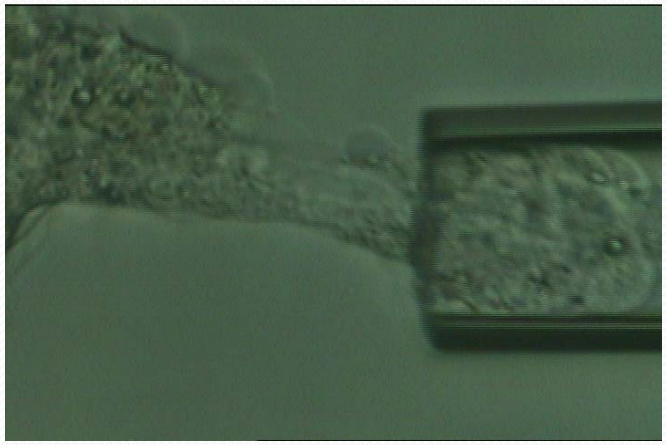
Oocyte + Embryo Cryo

Vitrification

Going forward

1. PGS with Troph biopsy gaining in popularity
2. More FET cycles
3. Embryologists will spend more time vitrifying and warming
4. Fertility preservation



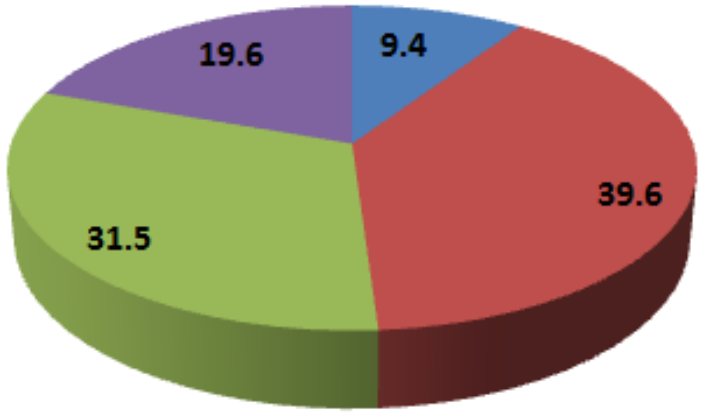


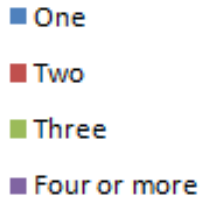
Elective single embryo transfer (eSET)

1. Ability to culture embryos
2. Choice of embryos for transfer and cryopreservation
3. Reliable freezing

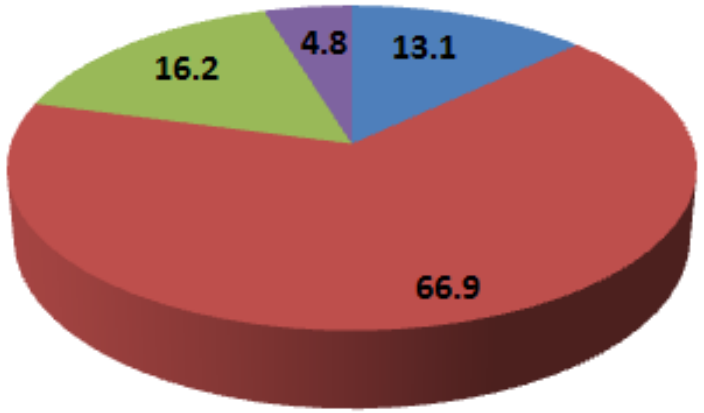
Fresh IVF Cycles: Number of embryos transferred

Day 3 ET





Day 5 ET



CDC Data: Own oocytes only

FET Results 2012

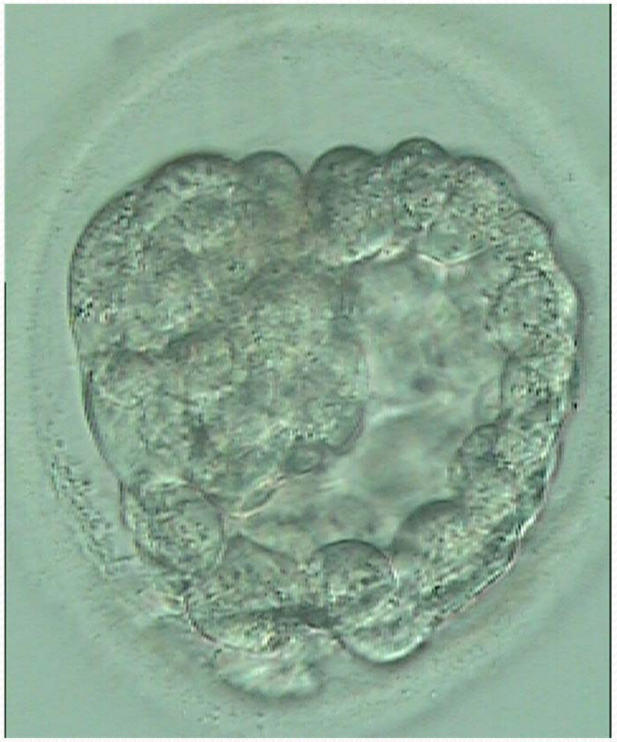
	<35	35-37	38-40	41-42	>42
Transfers	112	58	60	23	3
Clinical Pregnancies	66	41	40	11	1
Clinical Pregnancy Rate	0.59	0.71	0.67	0.48	0.33
Embryos Transferred	144	81	95	32	3
Embryos Implanted	79	52	54	11	1
Implantation Rate	0.55	0.64	0.57	0.34	0.33
Av. Embryos transferred	1.3	1.4	1.6	1.4	1.0

PGS Results 2012

	<35	35-37	38-40	41-42	>42
Transfers	18	13	21	11	2
Clinical Pregnancies	12	11	15	6	1
Clinical Pregnancy Rate	0.67	0.85	0.71	0.55	0.50
Embryos Transferred	22	14	24	11	2
Embryos Implanted	15	12	17	6	1
Implantation Rate	0.68	0.86	0.71	0.55	0.50
Av. Embryos transferred	1.2	1.1	1.1	1	1

Vitrification

Steps to success



1. Quality of embryo is not a factor
2. Collapse the big blastocysts
3. Use a simple protocol
4. Warming rate must be faster than cooling rate.
5. Make sure device is sealed

Blastocyst grading (SART)

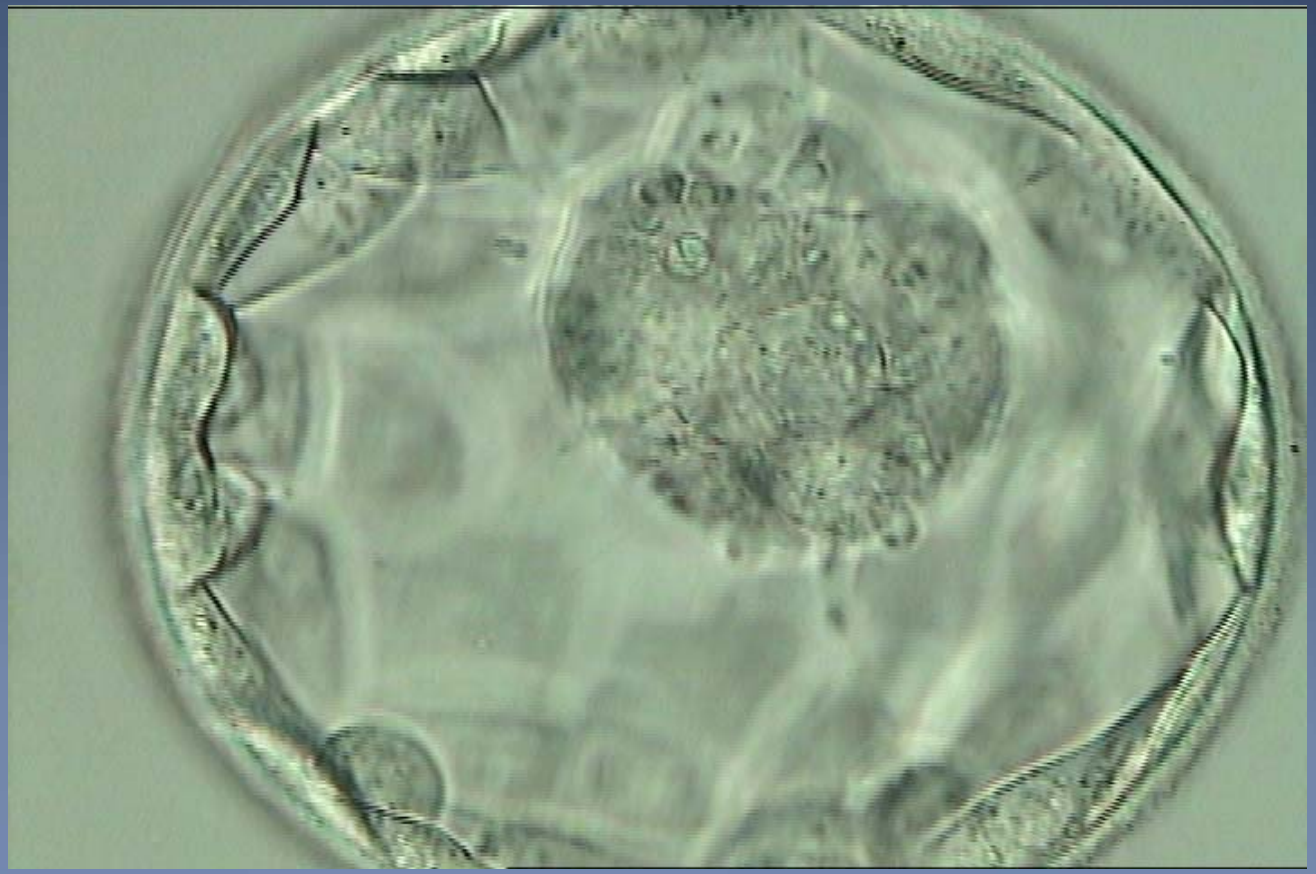
Stage	ICM	Trophectoderm
Early (cavity <50% vol.)	Good	Good
Blastocyst (Cavity 50% or more)	Average	Average
Expanded (Zona stretching)	Poor	Poor
Hatching		

Can we agree on grading?

Stage:

ICM:

TE:

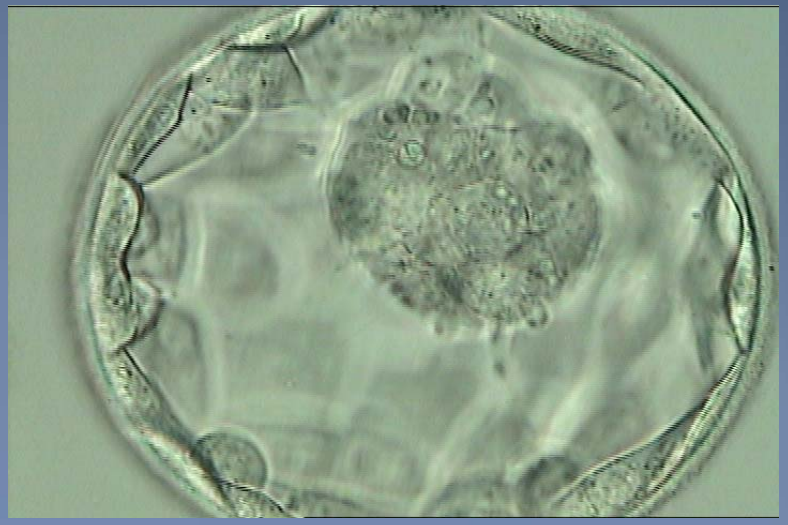


Can we agree on grading?

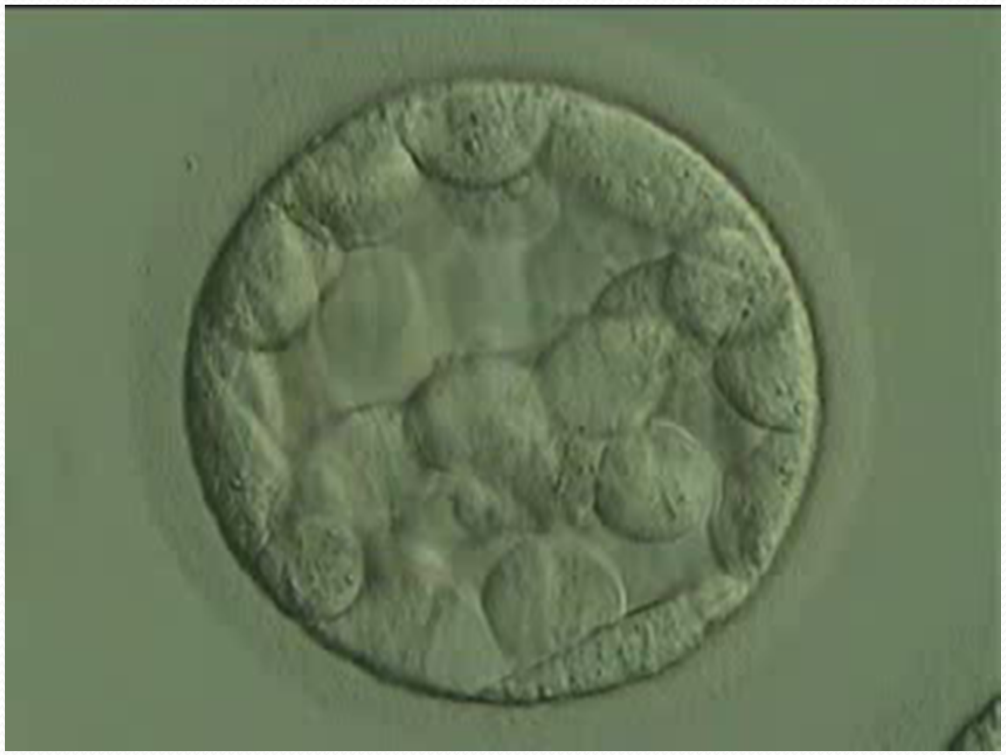
Stage: Expanded

ICM: Good

TE: Good



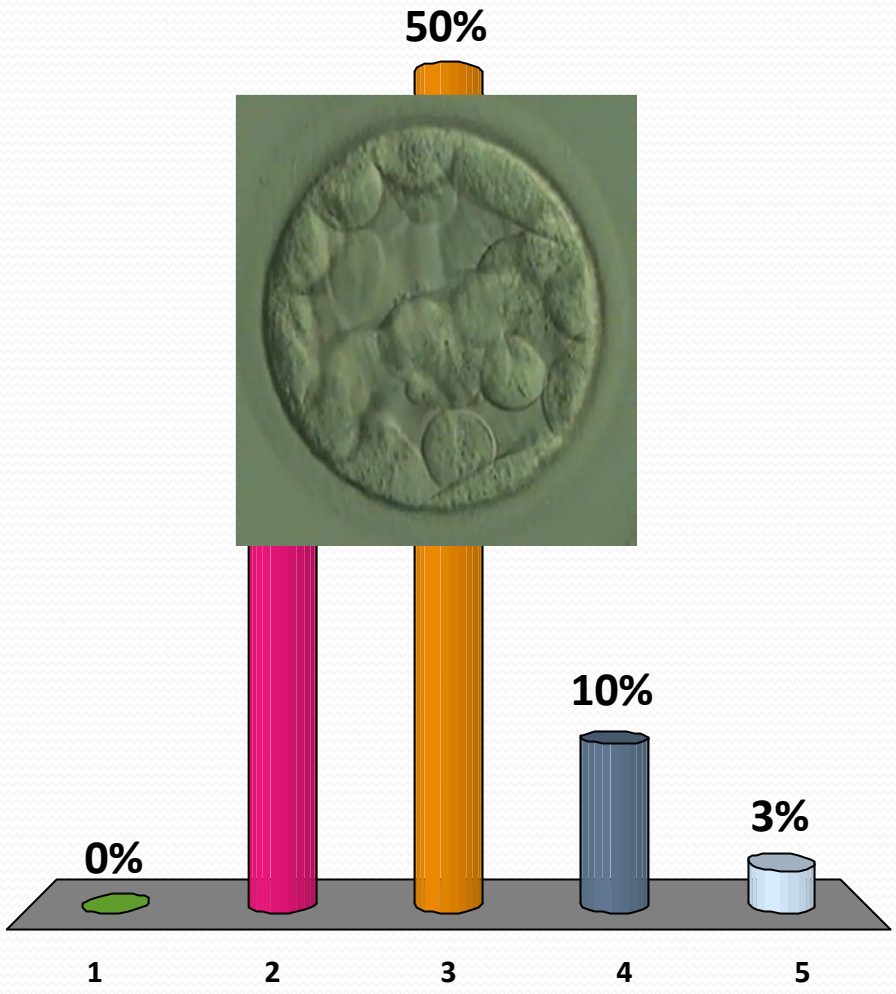
Can embryologists agree on embryo grading?



Stage	ICM	Troph
Morula		
Early Blast	Good	Good
Blastocyst	Average	Average
Expanded	Poor	Poor
Hatching		

What stage is this embryo at?

1. Morula
2. Early Blastocyst
3. Blastocyst
4. Expanded Blast
5. Hatching Blast



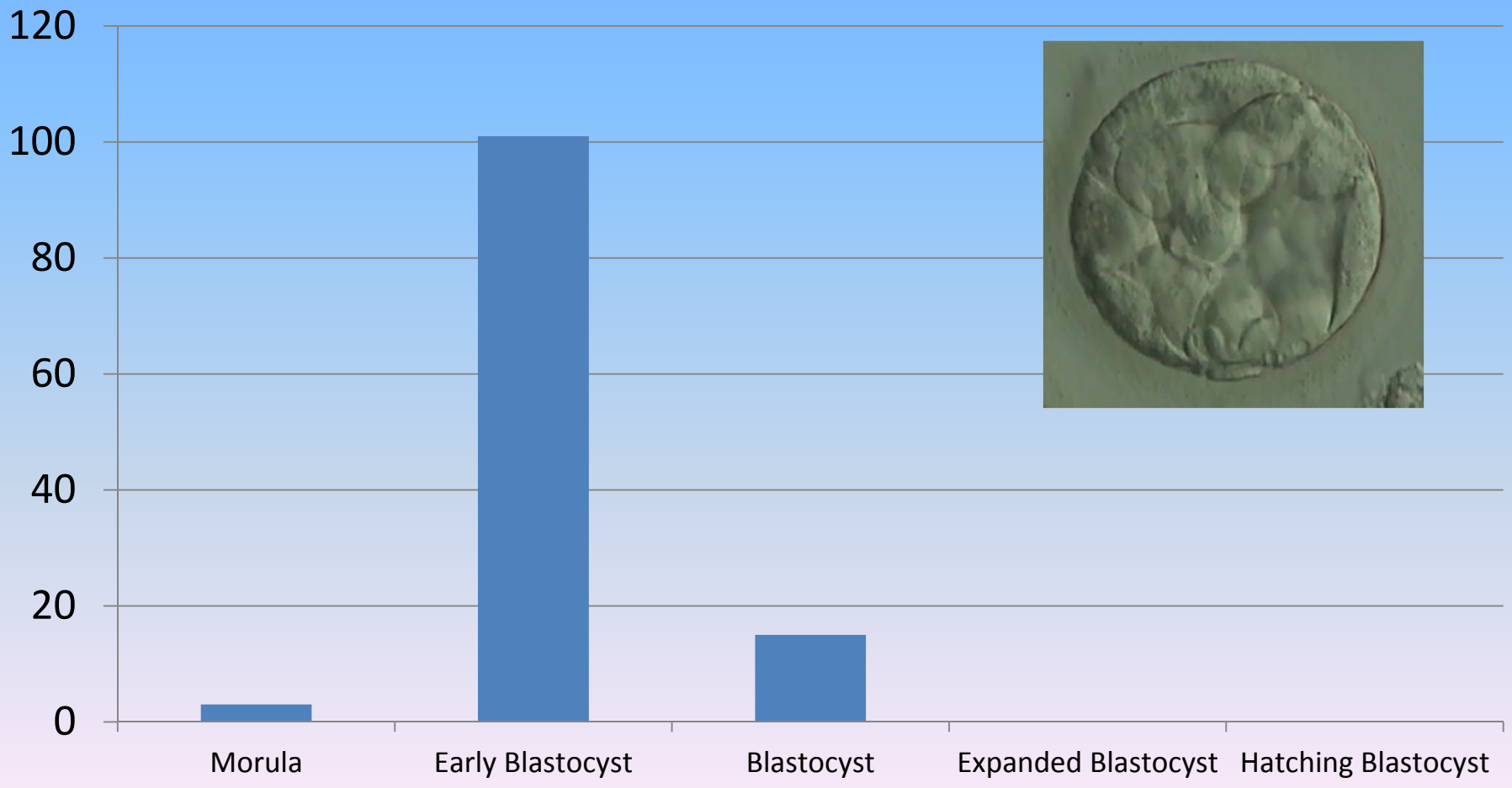
Blastocyst grading proficiency

Blastocyst grading results (n=119)

Let's try again

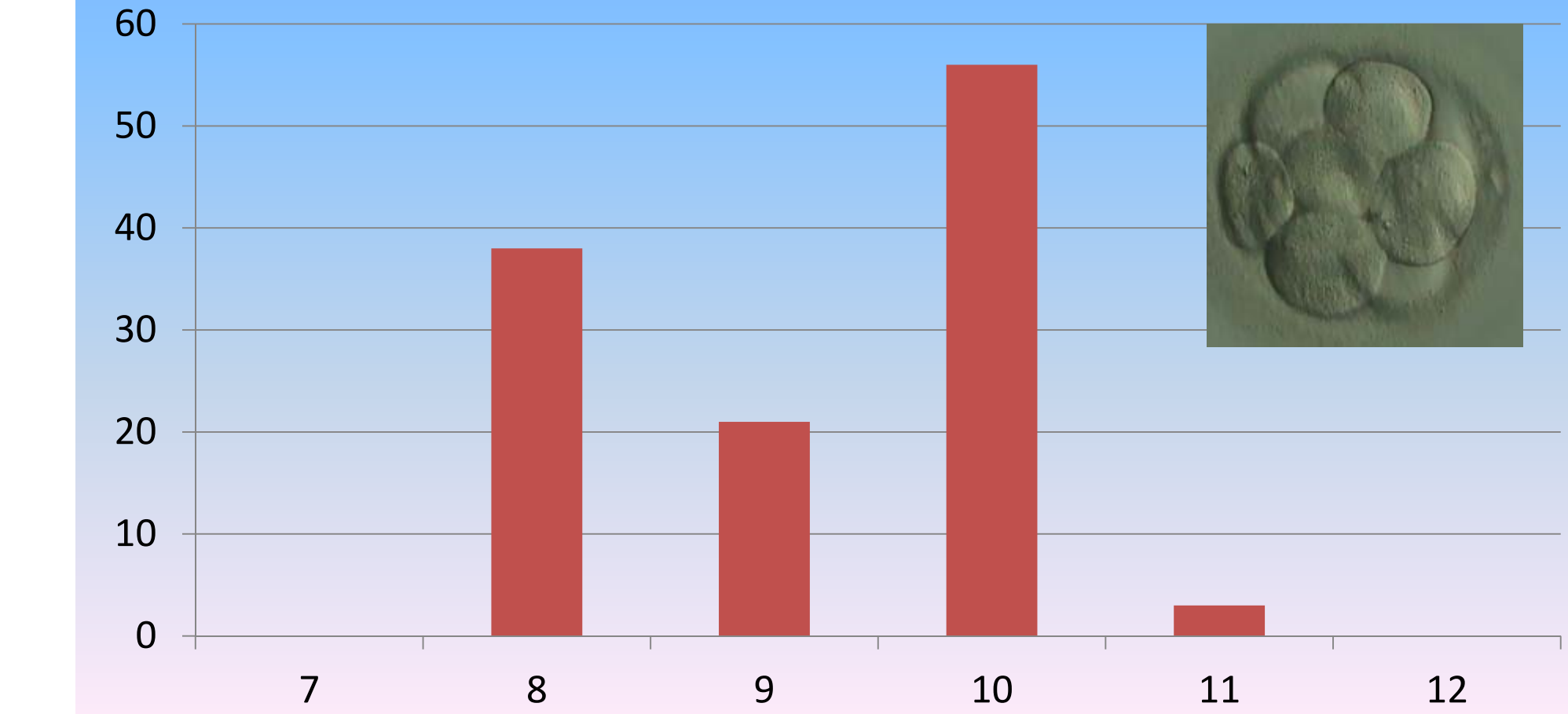
Stage	ICM	Troph
Morula		
Early Blast	Good	Good
Blastocyst	Average	Average
Expanded	Poor	Poor
Hatching		

Blastocyst grading results (n=119)

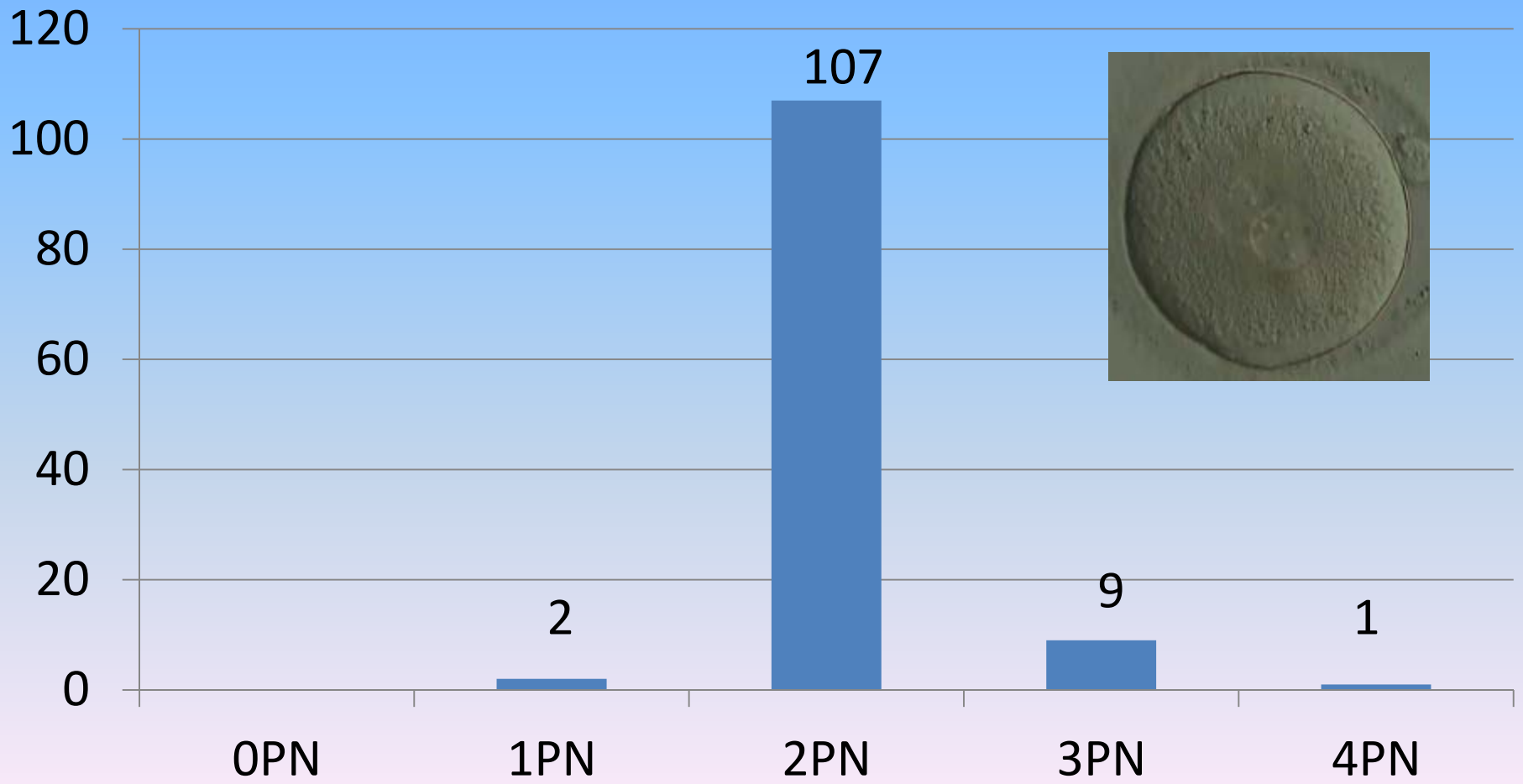


Blastocyst grading results (n=119)

Embryo grading results (n=119)



Zygote grading results (n=119)



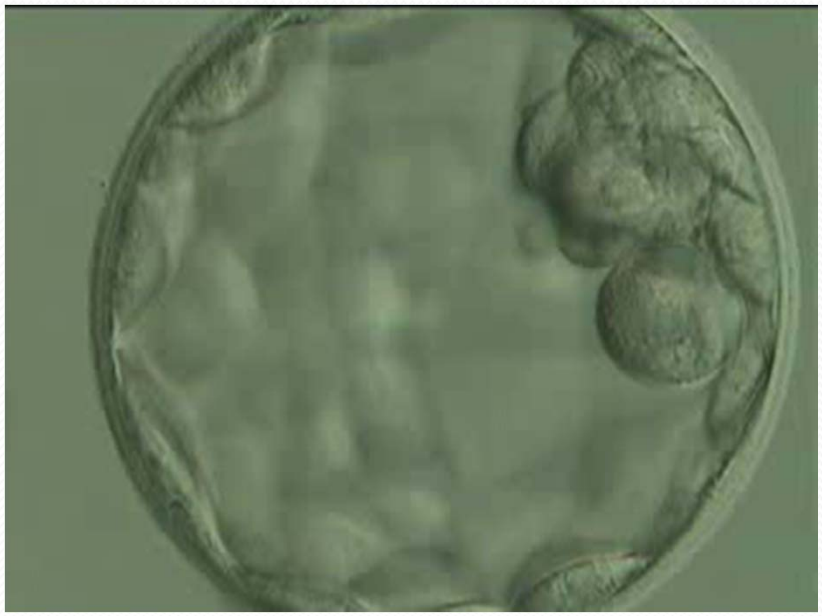
Sperm Count, Sperm Present/Absent

	Method	Absent No.	Absent %	Present No.	Present %
SEM-11	Conception Technologies Micro Cell	-	-	61	100.0
	Hemocytometer-bright field	4	0.6	634	99.4
	Hemocytometer-phase	-	-	66	100.0
	Humagen Counting Chamber	-	-	87	100.0
	Leja Standard Count	-	-	24	100.0
	Millennium Sciences Cell-VU	1	1.3	74	98.7
	Sefi Medical Makler	-	-	134	100.0
	Wet Mount	1	0.3	343	99.7
	Other, Specify	1	4.3	22	95.7
	All Methods	7	0.5	1447	99.5
	*Intended Response = **Present***				
SEM-12	Conception Technologies Micro Cell	61	100.0	-	-
	Hemocytometer-bright field	629	98.6	9	1.4
	Hemocytometer-phase	63	95.5	3	4.5
	Humagen Counting Chamber	86	98.8	1	1.1
	Leja Standard Count	24	100.0	-	-
	Millennium Sciences Cell-VU	73	98.7	1	1.4
	Sefi Medical Makler	133	98.5	2	1.5
	Wet Mount	341	99.1	3	0.9
	Other, Specify	22	95.7	1	4.3
	All Methods	1434	98.6	20	1.4
	*Intended Response = **Absent***				

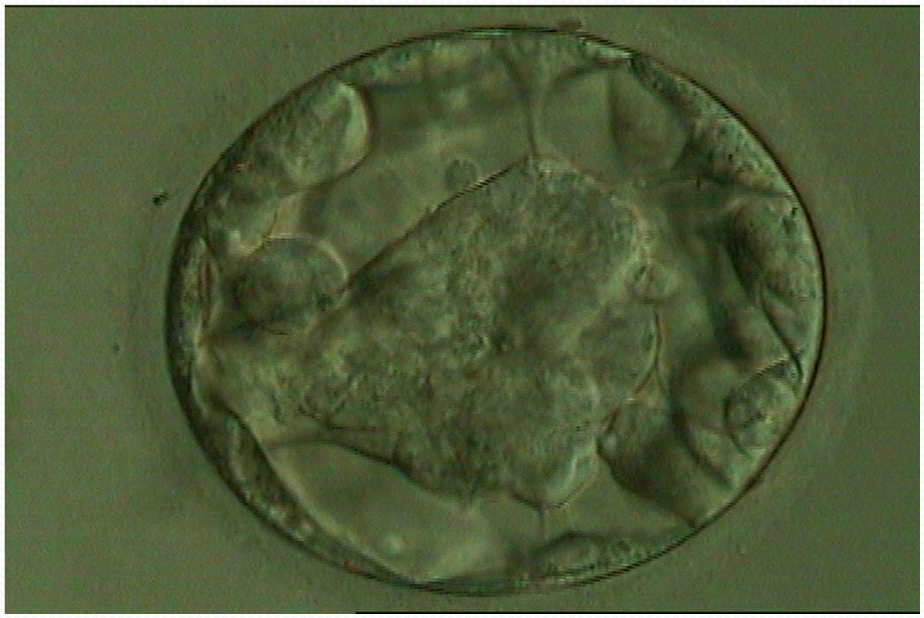
Blastocyst Quality

- Prefer to freeze embryos that have a distinct ICM and TE
- Early blastocysts may not have clear differentiated cell populations
- Pity freezes

Which embryo should we transfer? 30 year old patient / eSET. Embryos pictured on morning of D5.



A



B

This 30 year old woman only has these 2 embryos. Which one would you choose to transfer?

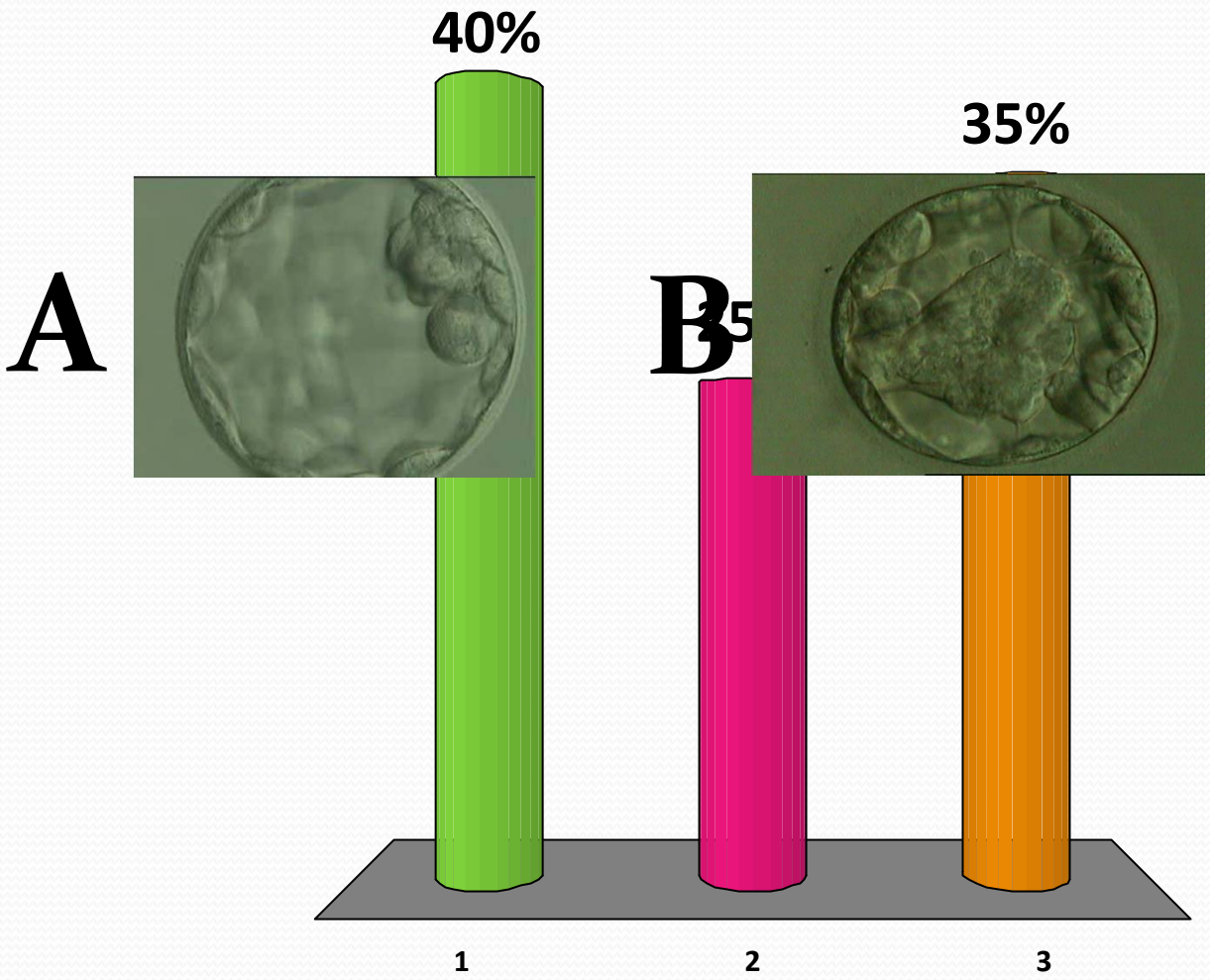
A 

B 

A. A
B. B
C. I'd encourage her to transfer both embryos

Which embryo would you transfer?

1. A
2. B
3. Both



Formation of the ICM

8-cell	Cell	Cell	Cell	Cell	Cell	Cell	Cell	Cell	
16-cell	O O	O I	O O	O I	O O	O I	O O	O I	12xO, 4xI

16-cell	O O	O O	O O	O O	O O	O O	I I	I I
32-cell	O O O O	O O O O	O O O O	O O O O	O O O I	O O O I	I I I I	I I I I

Average embryo should have 20-22 TE cells and 10-12 ICM cells

Moving from 32-cell to 64-cell, embryo can no longer make ICM cells if they don't already exist

Is trophectoderm more important?

- Embryo puts more energy into making TE
- If ICM is poor, embryo likely doomed
- But TE relatively more important

Transfer embryos with:

good TE/average ICM or good ICM/average TE

Criteria for vitrifying?

1. Loose criteria for D5 blastocysts
2. Tight control over D6 vitrification
3. No ICM = no vit
4. Not keeping embryos until D7
5. Assisted collapse used liberally

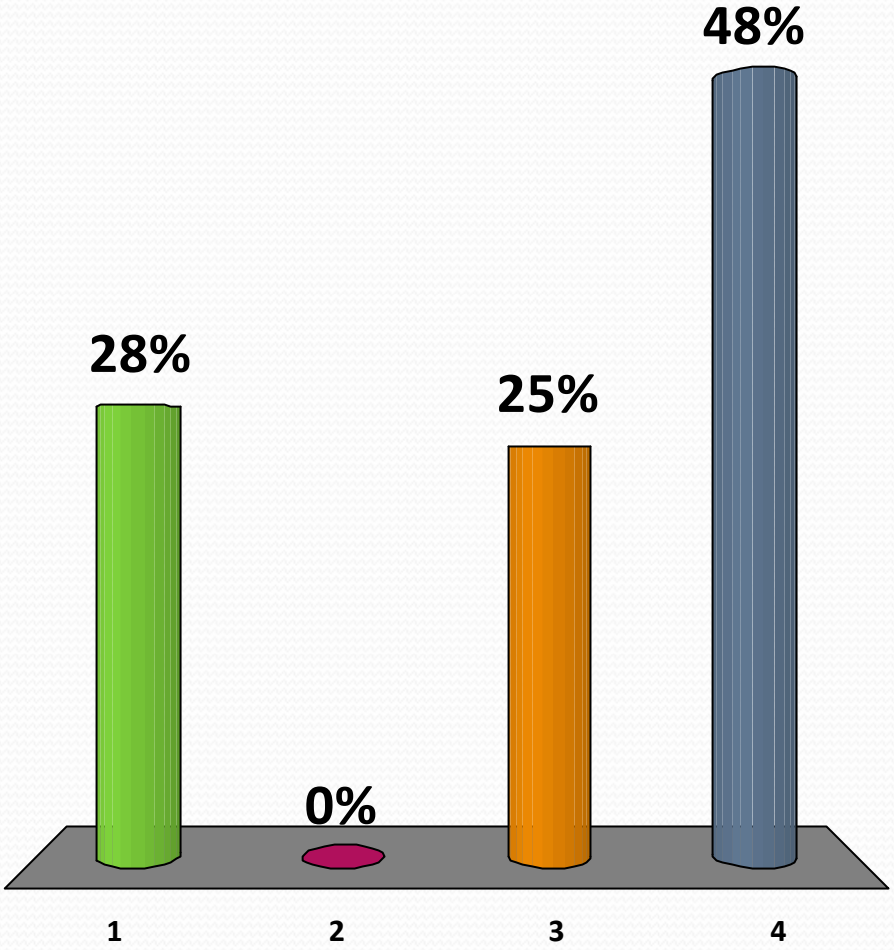
After fresh transfer, many embryos assessed by 2 embryologists to decide on freezing

This embryo belongs to a 41 year old woman and she has no other embryos to freeze. The picture was taken on the morning of Day 5. Would you vitrify the embryo?

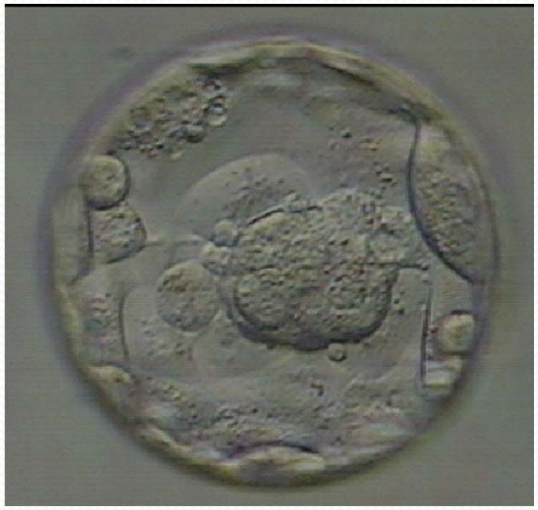
A. Yes
B. No
C. I'd look at it later
D. I'd culture it to D6

Would you vitrify?

1. Yes
2. No
3. I'd look at it later
4. Culture to day 6



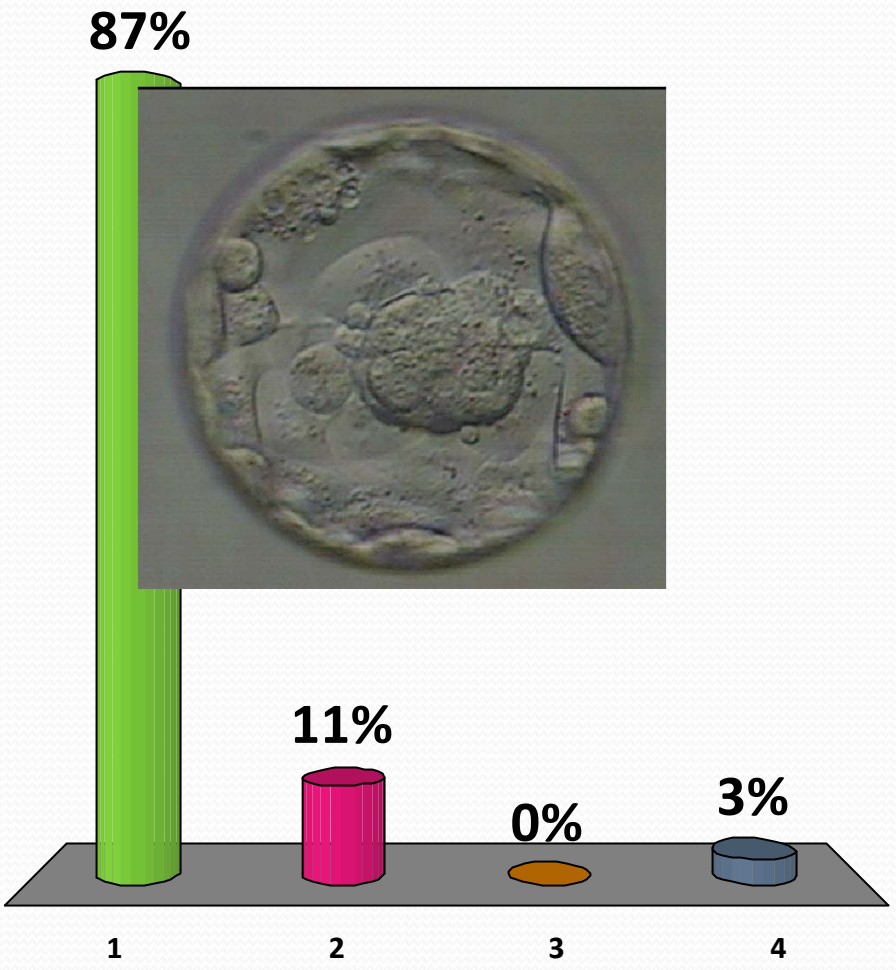
This embryo belongs to a 39 year old woman and she has 4 other embryos to freeze. The picture was taken on the morning of Day 6. Would you vitrify the embryo?



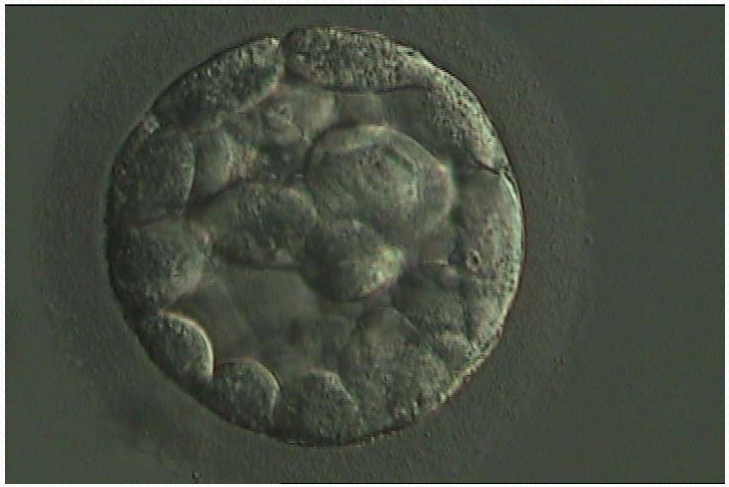
A. Yes
B. No
C. I'd look at it later
D. I'd culture it to D7

Would you vitrify?

1. Yes
2. No
3. I'd look at it later
4. Culture to day 7



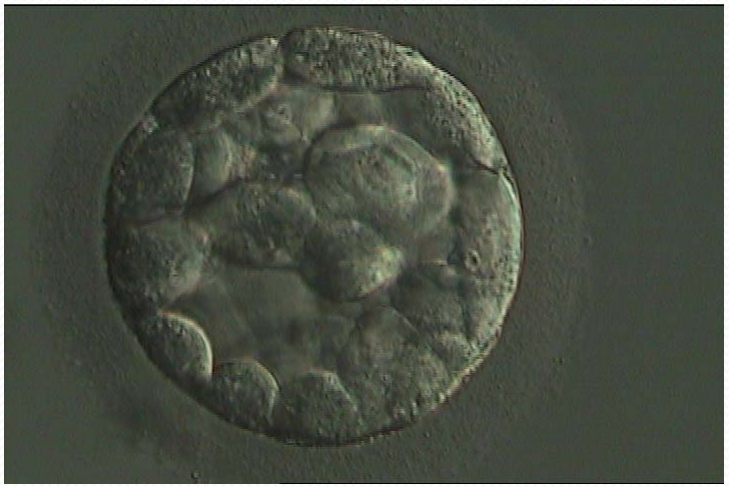
This embryo belongs to a 32 year old woman and she has 1 other embryo to freeze. The picture was taken on the morning of Day 5. Would you vitrify the embryo?

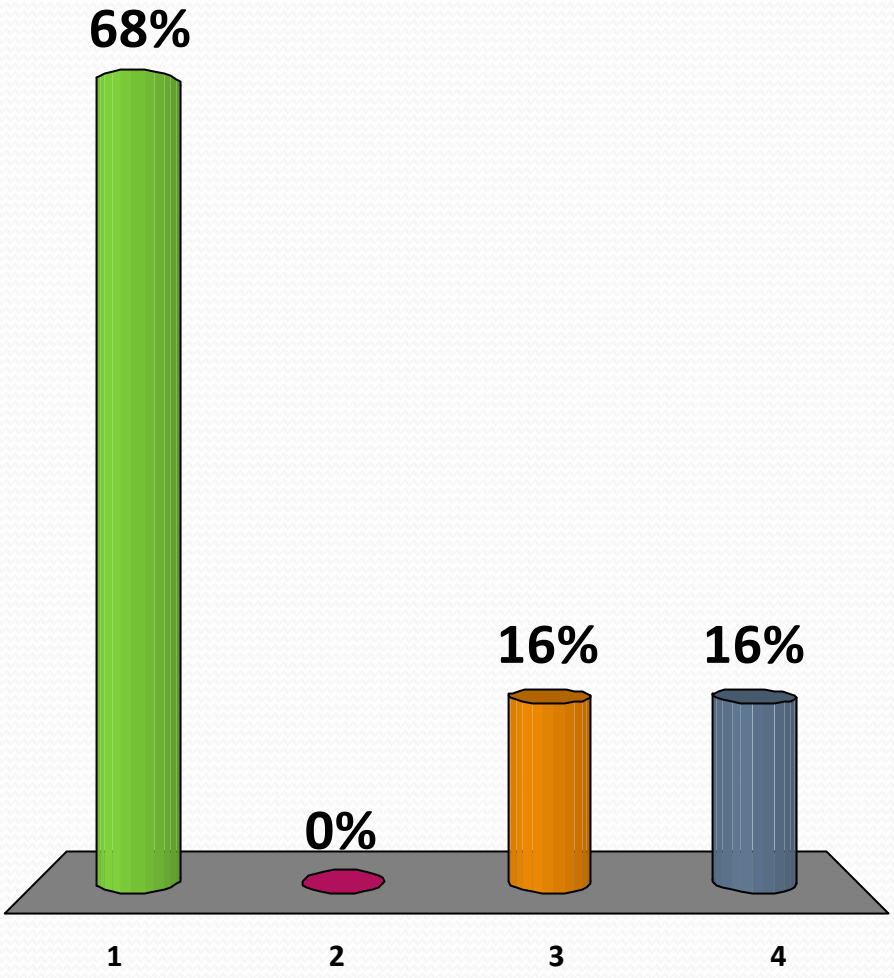


A. Yes, even if it was the only embryo
B. Yes, but only because she has another embryo
C. No
D. I'd look at it later
E. I'd culture it to D6

Would you vitrify?

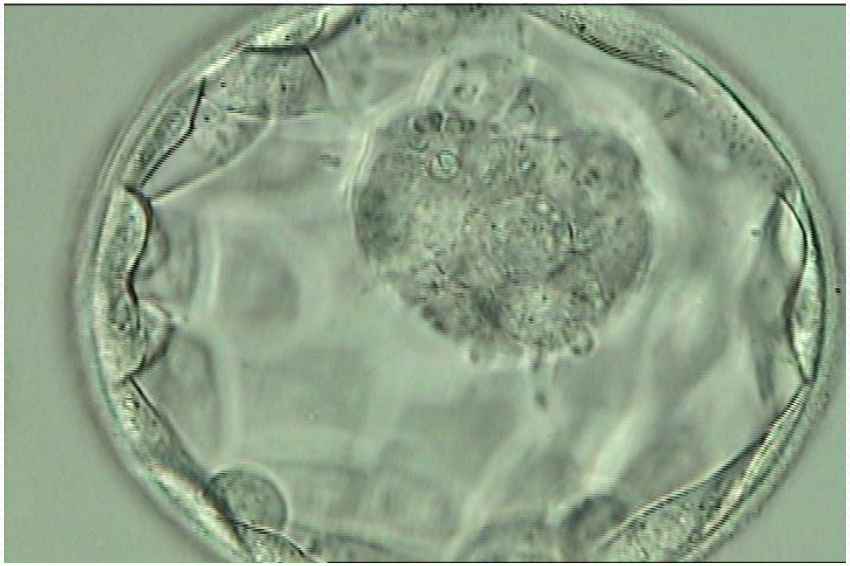
1. Yes
2. No
3. I'd look at it later
4. Culture to day 6

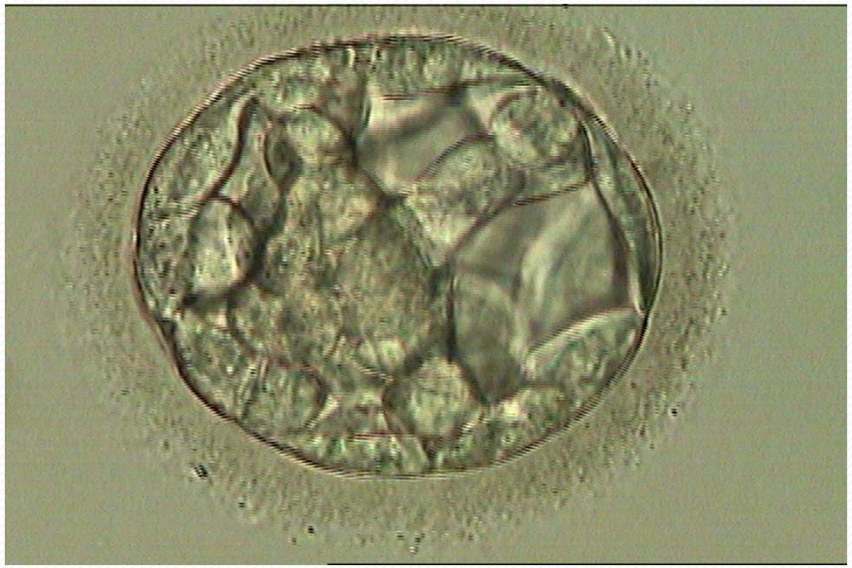




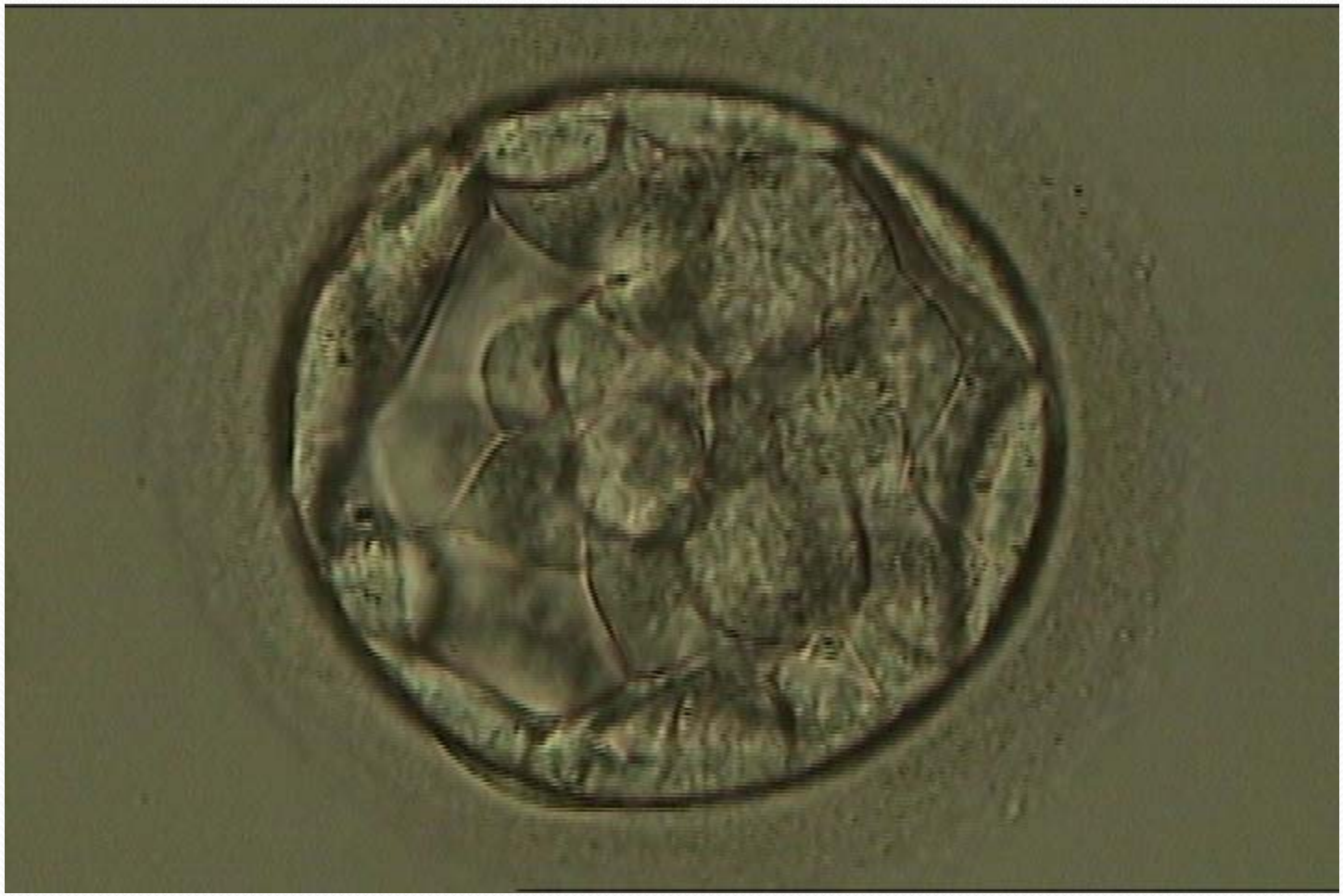
Choosing embryos to Vitrify

- Vitrification does not appear to reduce an embryo's potential for implantation
- Expect pregnancy rates post warming that are similar to fresh rates





We vitrify any embryo that we think has a chance



Borderline embryos

- Vitrifying poor embryos will hurt results
- If you can't decide, you should freeze
- If your results are too good, you are being too selective
- Recognize the importance of failure

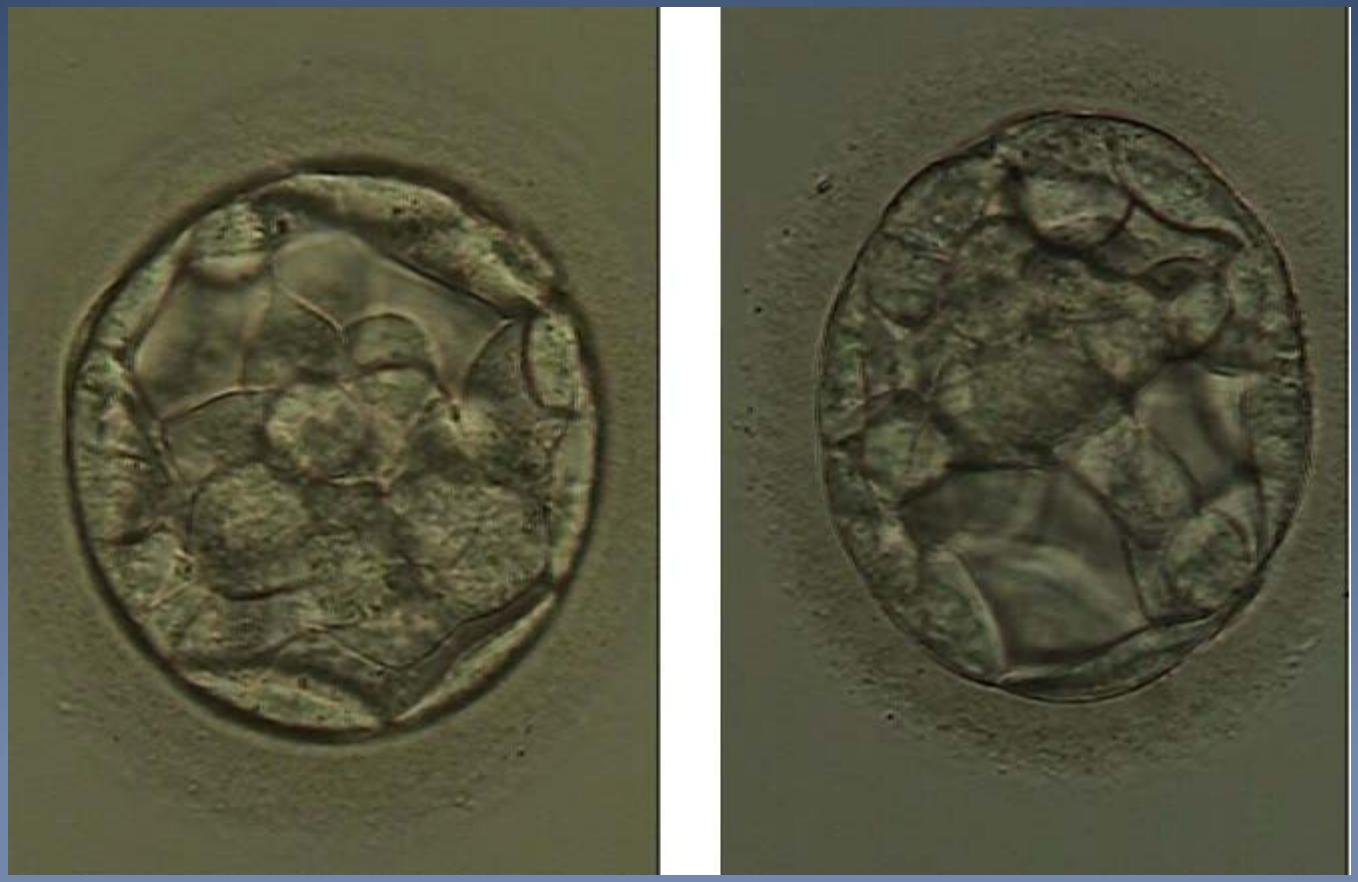
Given the choice, patients likely choose freezing

When to warm and transfer?

Natural cycle	hCG	Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Progesterone			Ov	P4 Day 1	P4 Day 2	P4 Day 3	P4 Day 4	P4 Day 5
D3's						Warm/ ET		
D5 or D6								Warm/ ET

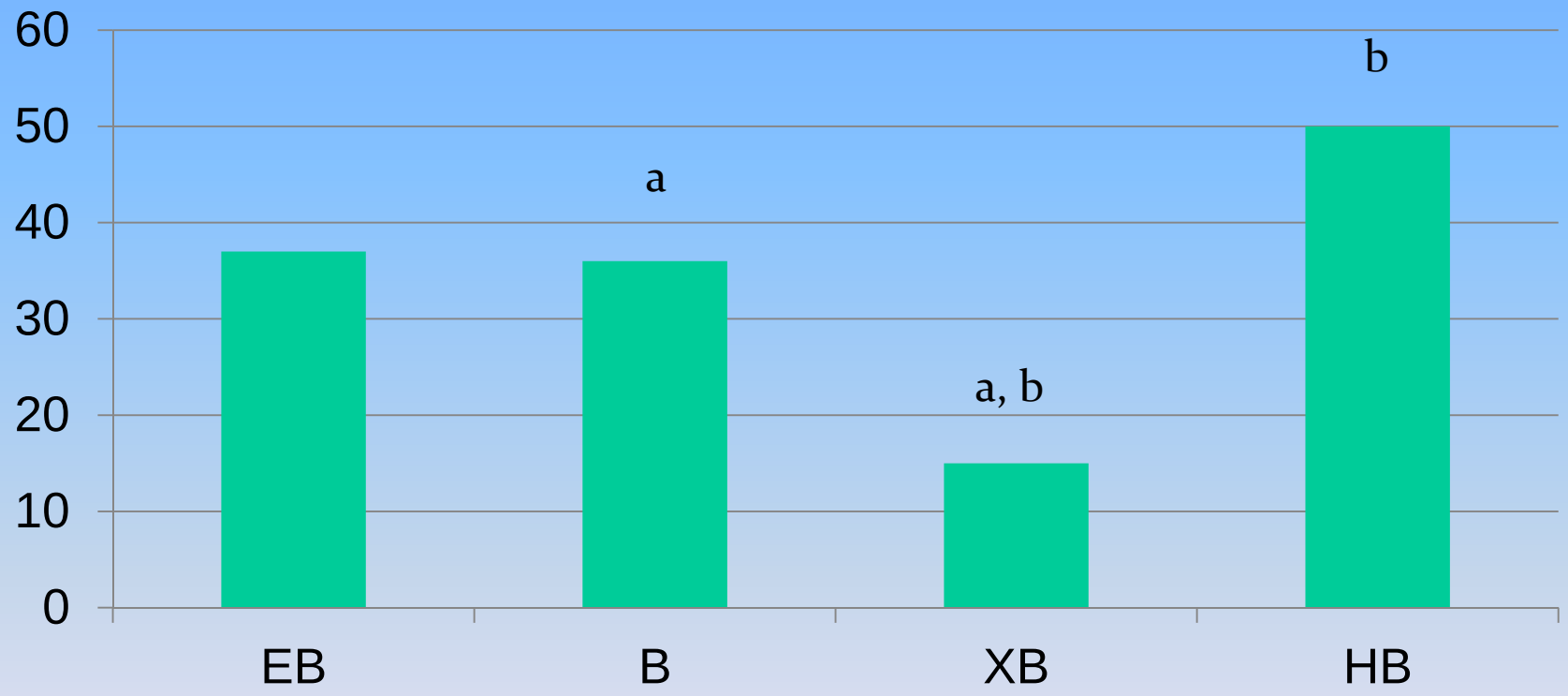
Common question

Do you freeze early blastocysts?



Implantation by stage

SET only, n = 182

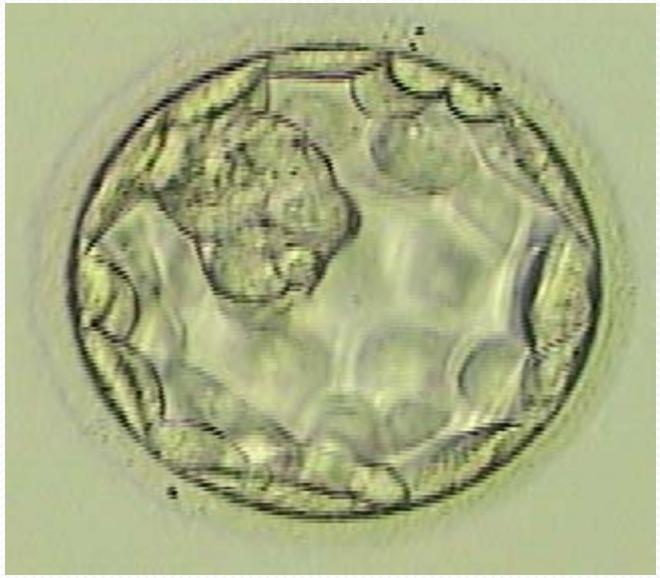


Early Blastocyst	Blastocyst	Expanded	Hatching
7/19	43/120	4/27	8/16

a, p = 0.04 and b, p = 0.03

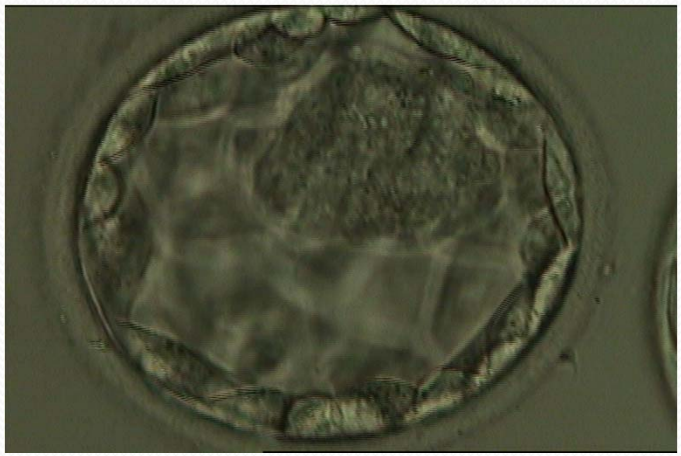
On the day of FET

- We do not wait for embryos to re-expand before transfer
- Assisted hatching after warming
- Embryologist discretion on when to warm a 2nd embryo

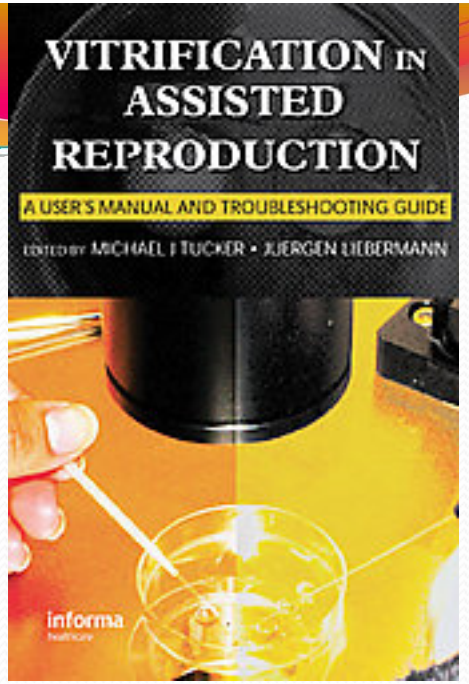


Is eSET working?

- 60% of OD recipients doing eSET
- 246 cycles (2008-10)
- 63% pregnancy rate
- Take home baby/retrieval 2010 cumulative
  - 80% (57/71) in OD recipients
  - 61% (39/64) for < age 35
- 3% twins



Conclusions



- Vitrification has driven eSET
- Physician sets expectations
- Embryologists make good embryos
- Collapsing widely used
- Very loose in choosing embryos for Vit.
- Good implantation rates post warming
- Aim is one embryo in fresh and FET cycle

Acknowledgements

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