Trophectoderm Biopsy & Comprehensive Chromosome Screening

Dawn A. Kelk, PhD, HCLD

Audience Response Question

Please use the response keypad.

Does your clinic biopsy for 24-chromosome PGS testing?

A. Yes

B. No

What do you currently biopsy in your lab?

A. Primarily polar body

B. Primarily blastomere

C. Primarily trophectoderm (TE)

D. Blastomere or TE depending on case

E. We are currently transitioning to TE

F. We don’t perform biopsies

Biopsy Background

- Biopsying >20 years, initially blastomere or polar body
- Improvements in blastocyst culture
- Improvements in blastocyst cryopreservation
- Limited evaluation of impact of biopsy
- Until relatively recently, no well controlled studies

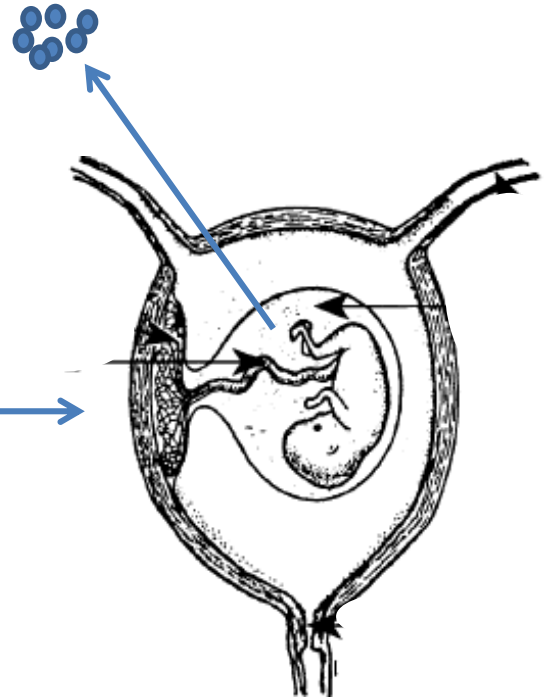
Limitations to a well controlled IVF study:

- Variability in stimulation dynamics
- Cycle to cycle variabililty in oocyte quality
- Batch to batch variablity in media
- Day to day variation in laboratory environment
- Embryo transfer techniques
- Endometrial receptivity
- Implantation window
- Hormonal mileu in luteal phase & early gestation

Impact of Biopsy Study

Fingerprint to determine if "biopsy" or "no biopsy" implanted

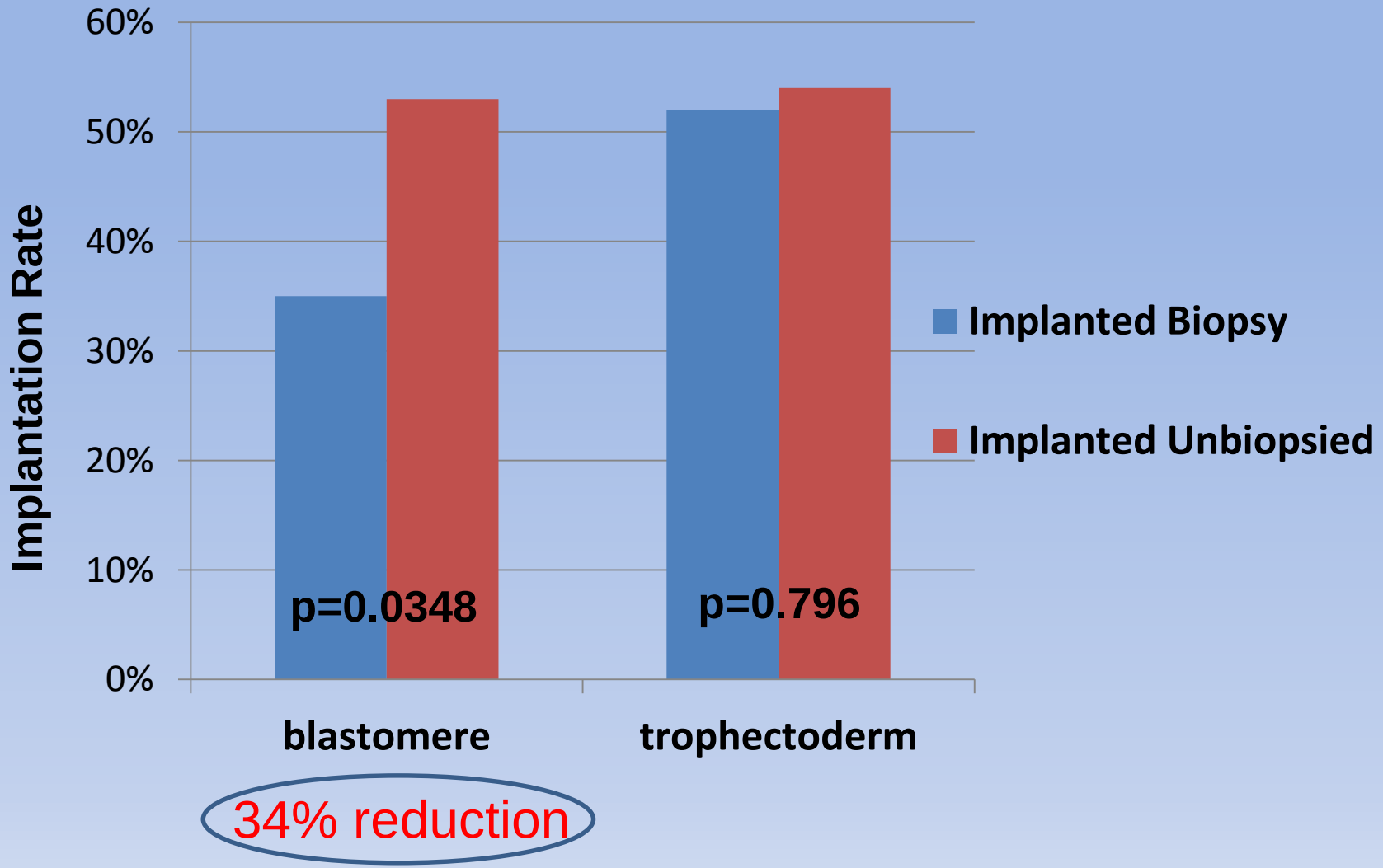
ET



Treff *et al*. Fertil Steril. 2010; 94:477-484. (blastomere or trophectoderm)

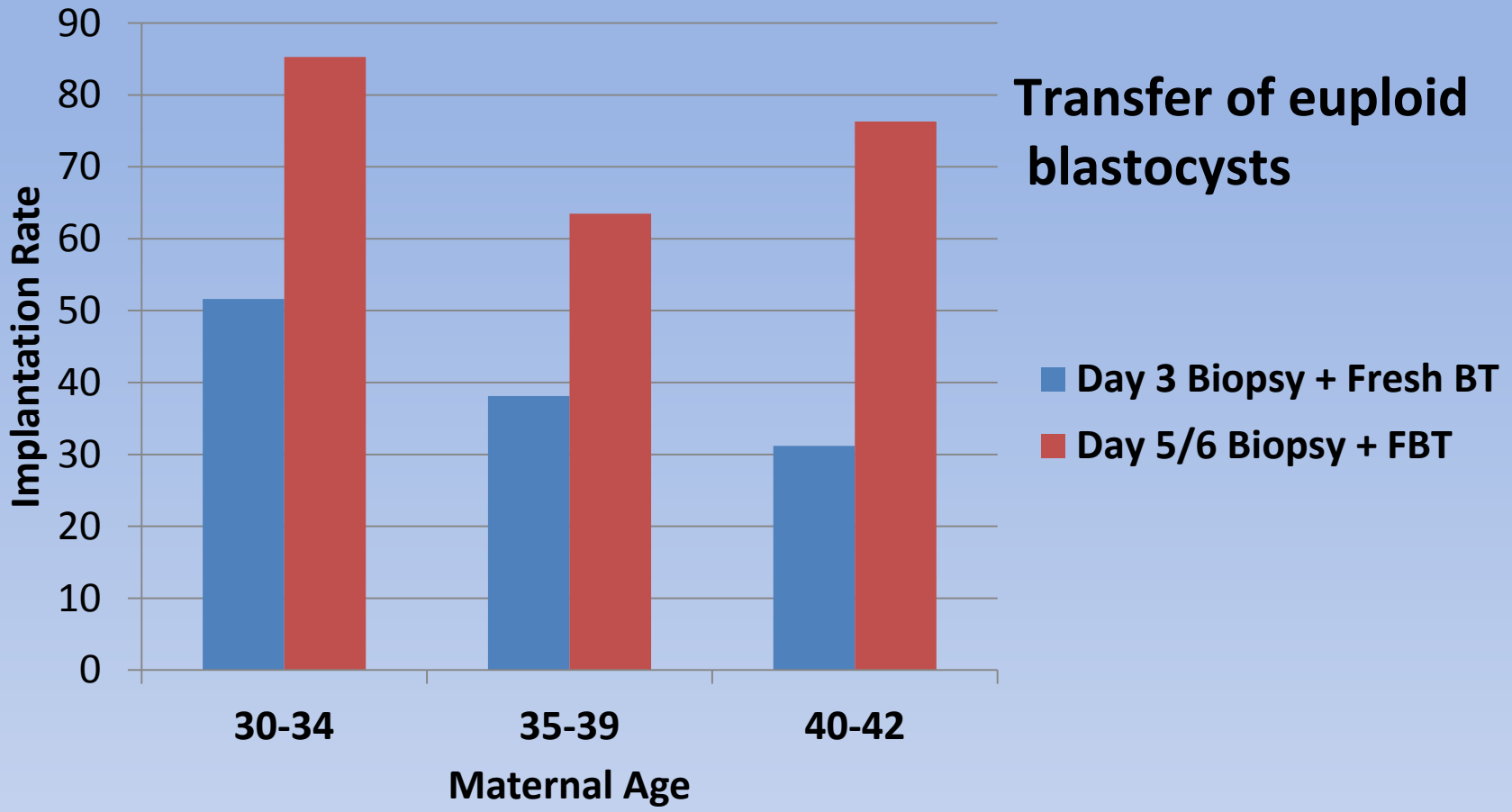
Treff *et al*. Fertil Steril. 2010; 93:2453-5. (polar body)

Impact of Biopsy Study



Treff *et al*, ASRM 2011

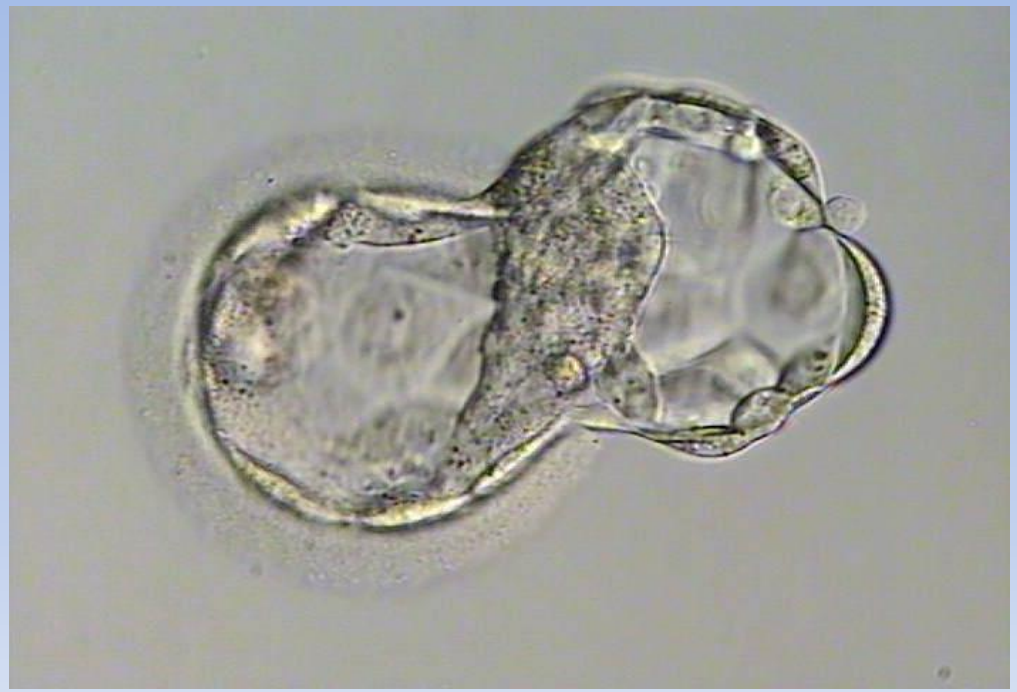
Day 3 Biopsy & Fresh BT vs TE Biopsy & FBT



Harton *et al.* ASRM 2011; (Euploid blastocysts by aCGH)

Blastocyst Biopsy

- Biopsy TE cells
- More cells available (3-10 cells)
- 3-5% vs 10-25% of embryo removed on Day 3



Reflections…

2008 Abstracts

D3 vs D5 eSET

- No 24-chrom
- Single gene PGD bx D3
- Blasts were slow frozen
- 2PN & Day 3 Cryo
- >50% of ET's were D3

2012 Abstracts

TE Biopsy & CCS

Vitrification

- All D3's are hatched
- 100% Day 5 ET
- Vit everything at blast
- ~33% of cases are CCS

Reflections…

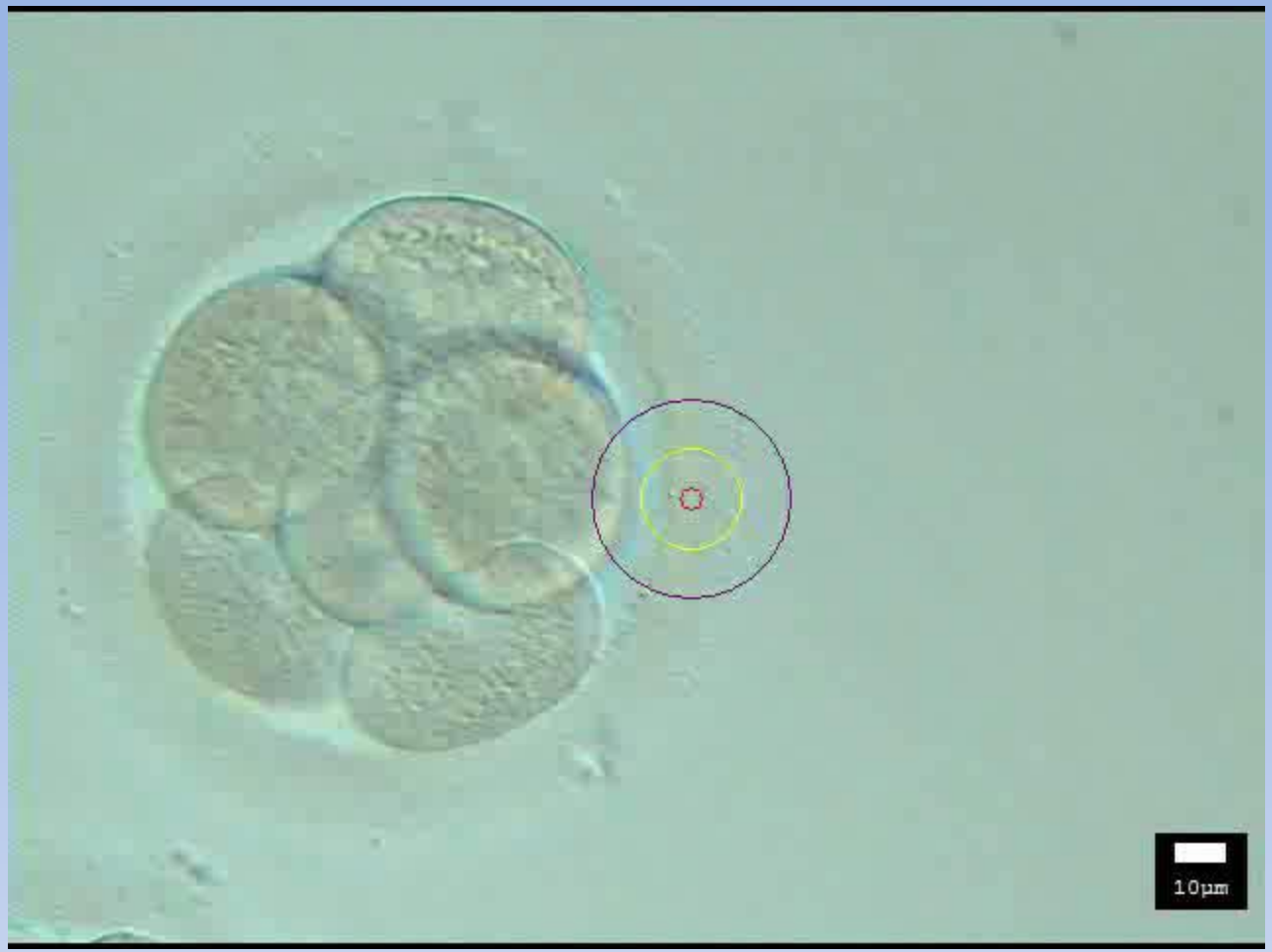
What hasn't changed:

- Global culture media
- MINC incubators
- Group culture
- Sort into like cell numbers on Day 2

Trophectoderm Biopsy

- Laser zona breach on Day 3
  - Allows embryo to herniate out of zona on Day 5/6
  - If hole is too small, embryo pinches, cells bleb off, biopsy is difficult
  - If hole is too large, the blastocyst pulls out of the zona, biopsy is more difficult
  - 400 μsec pulse

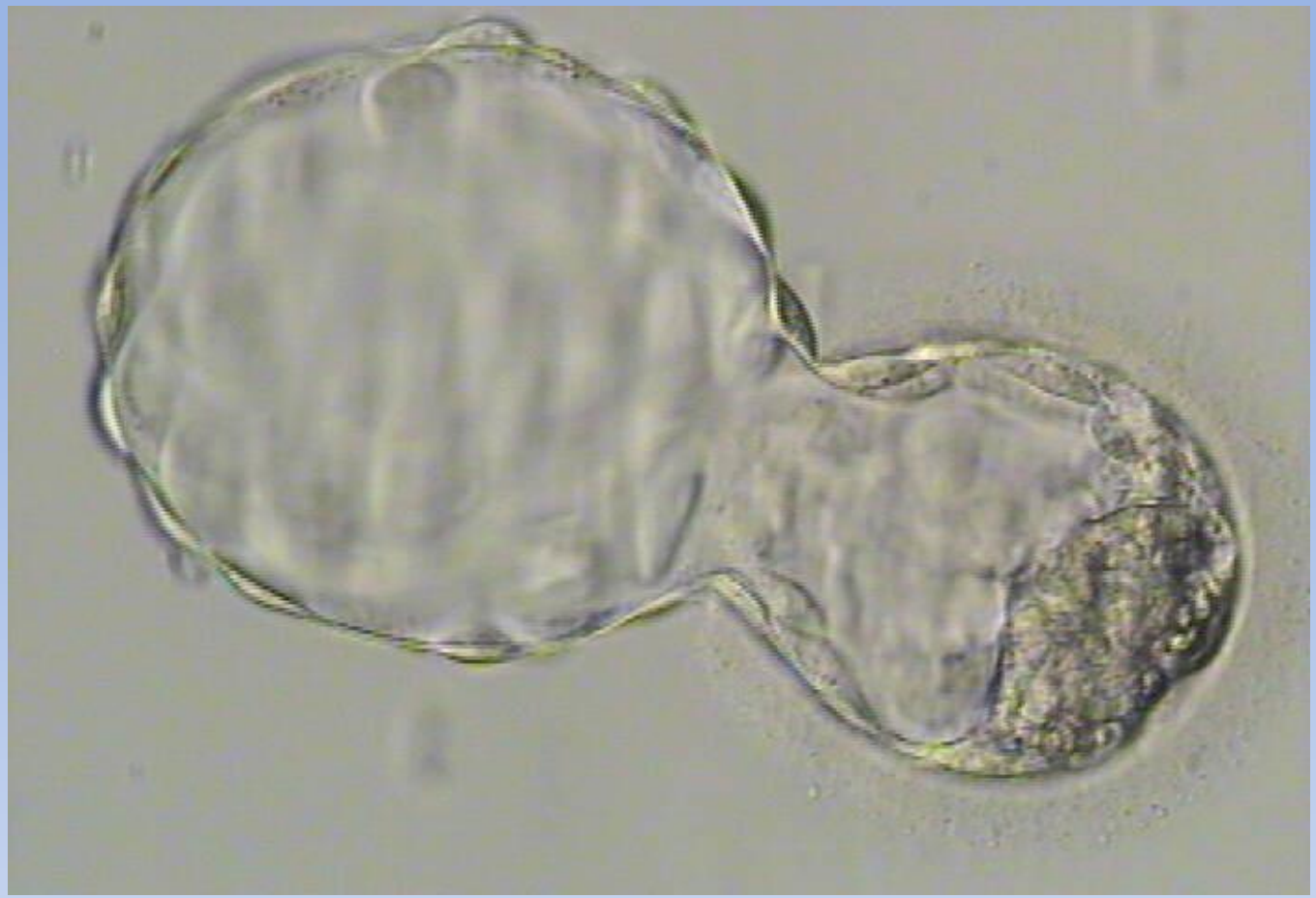
Zona Breaching on Day 3:



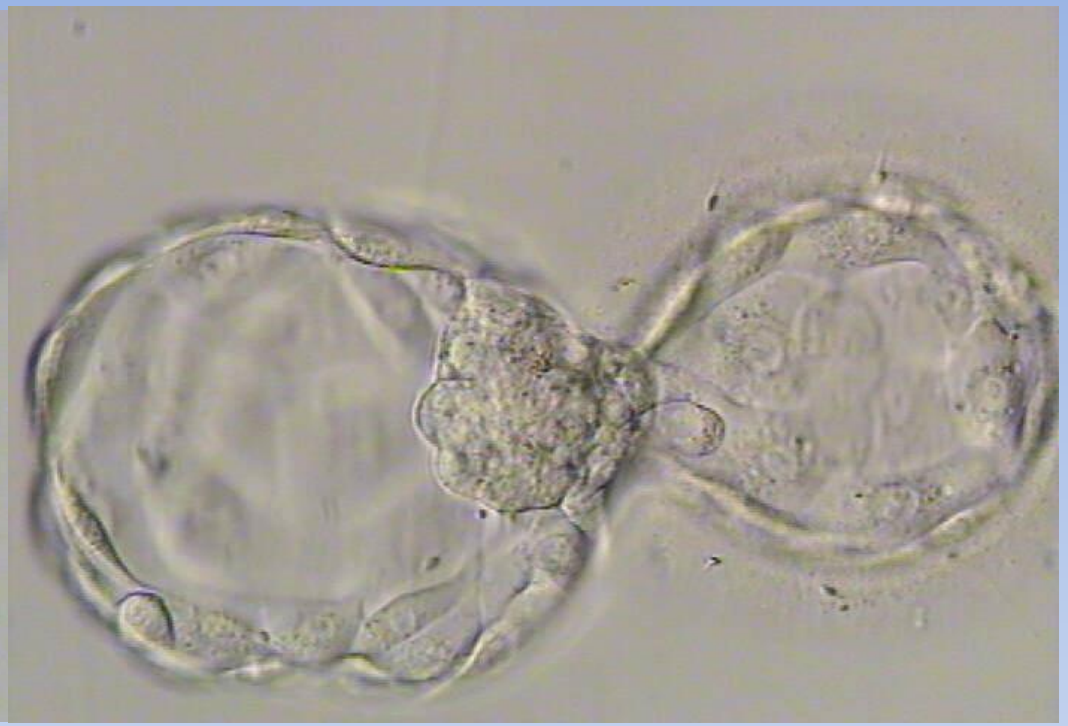
Trophectoderm Biopsy – Embryo Selection

- The more cellular the better
- If too early/too few cells, the embryo pulls apart
- Hatching/herniating blastocysts ideal
- Hatched blastocysts can also be biopsied

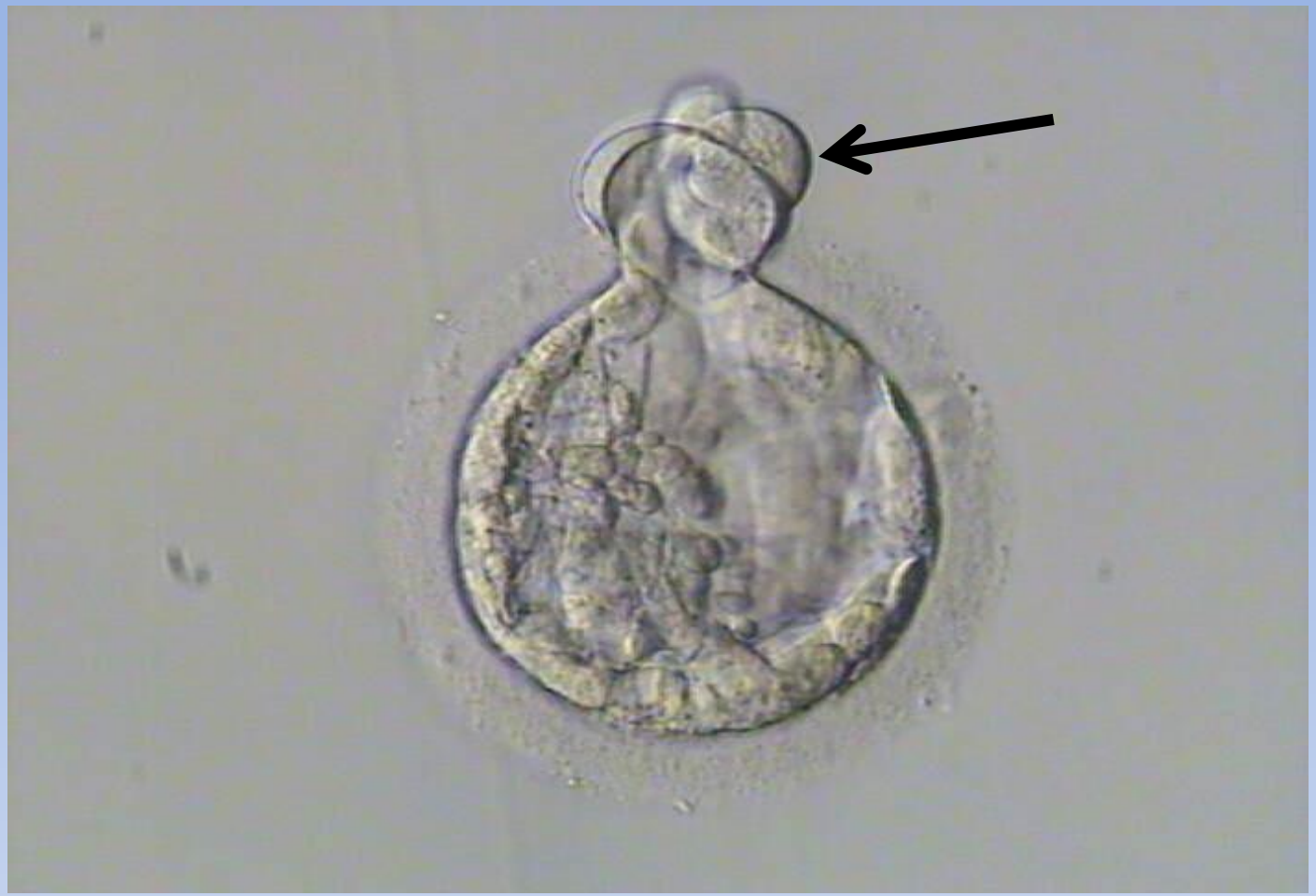
Ideal for biopsy:



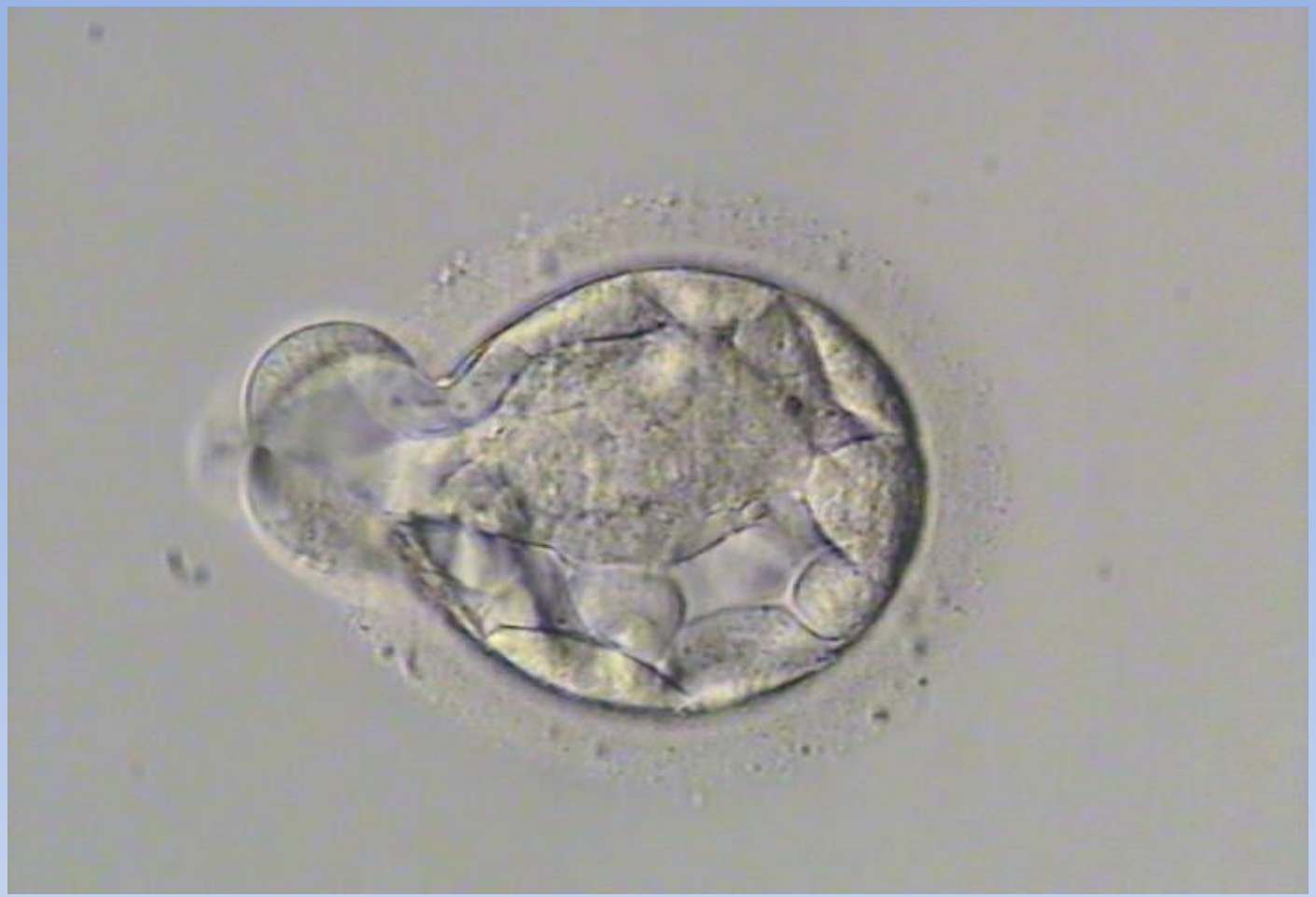
Embryo selection for TE biopsy:



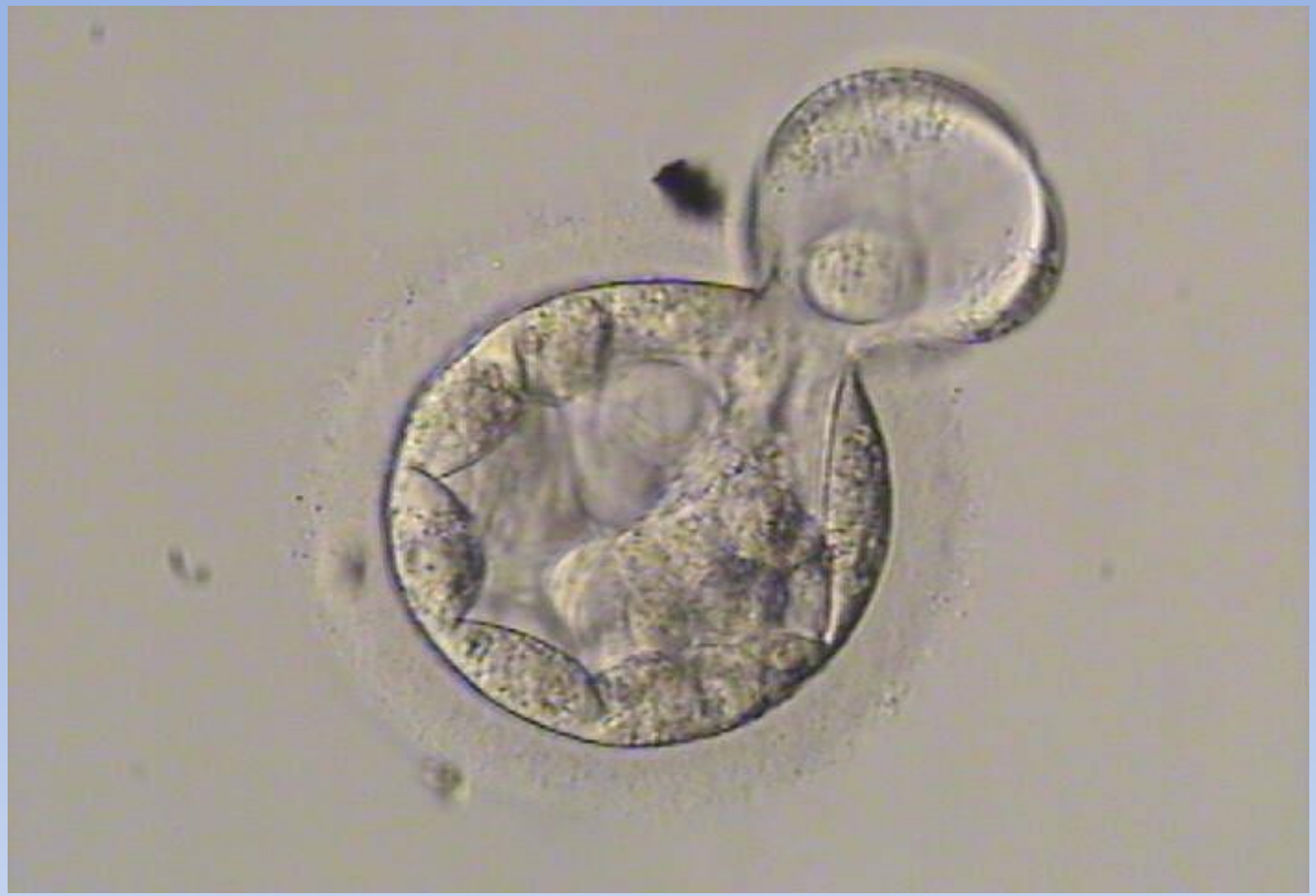
Loose cells/fragments extruding:



Too early to biopsy:

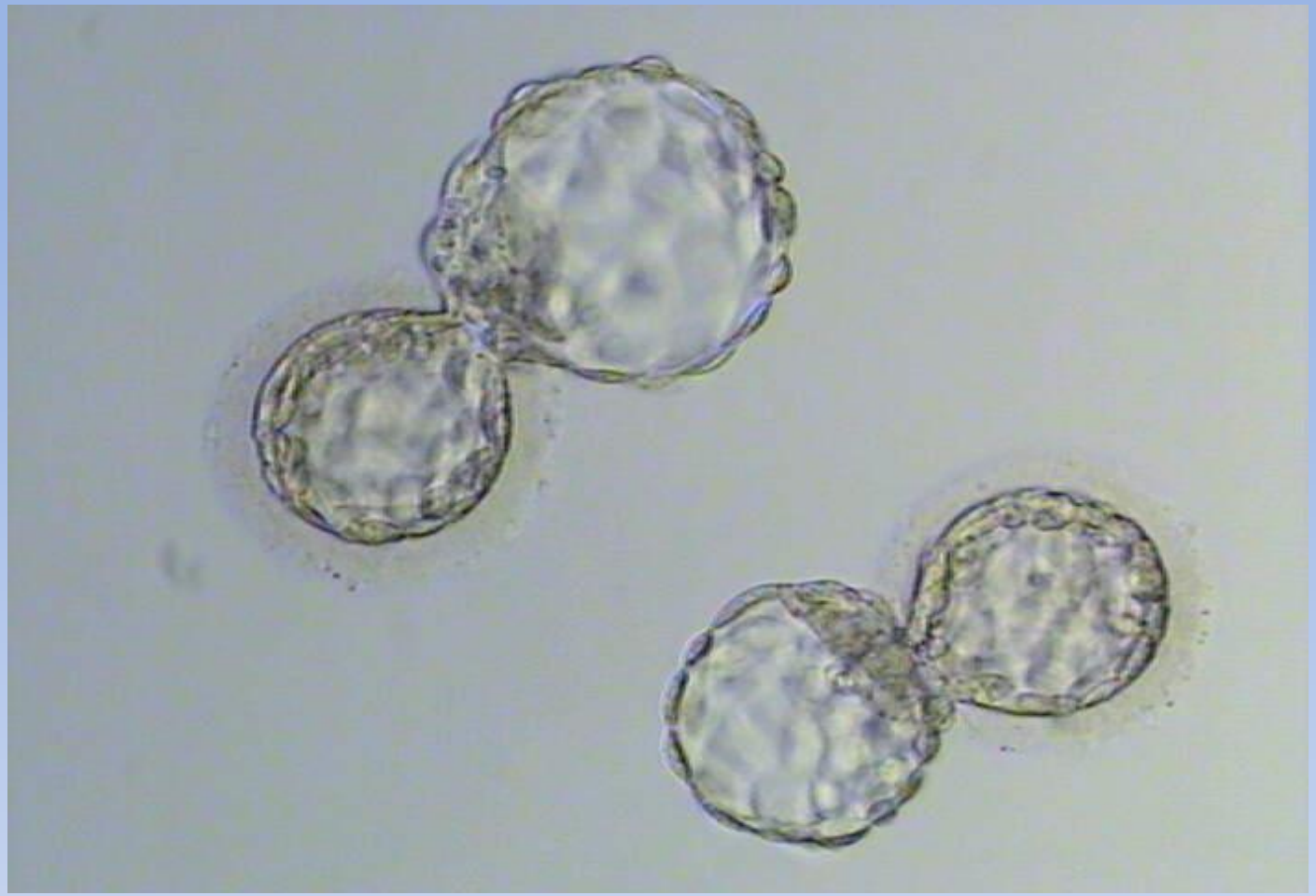


Early for biopsy:

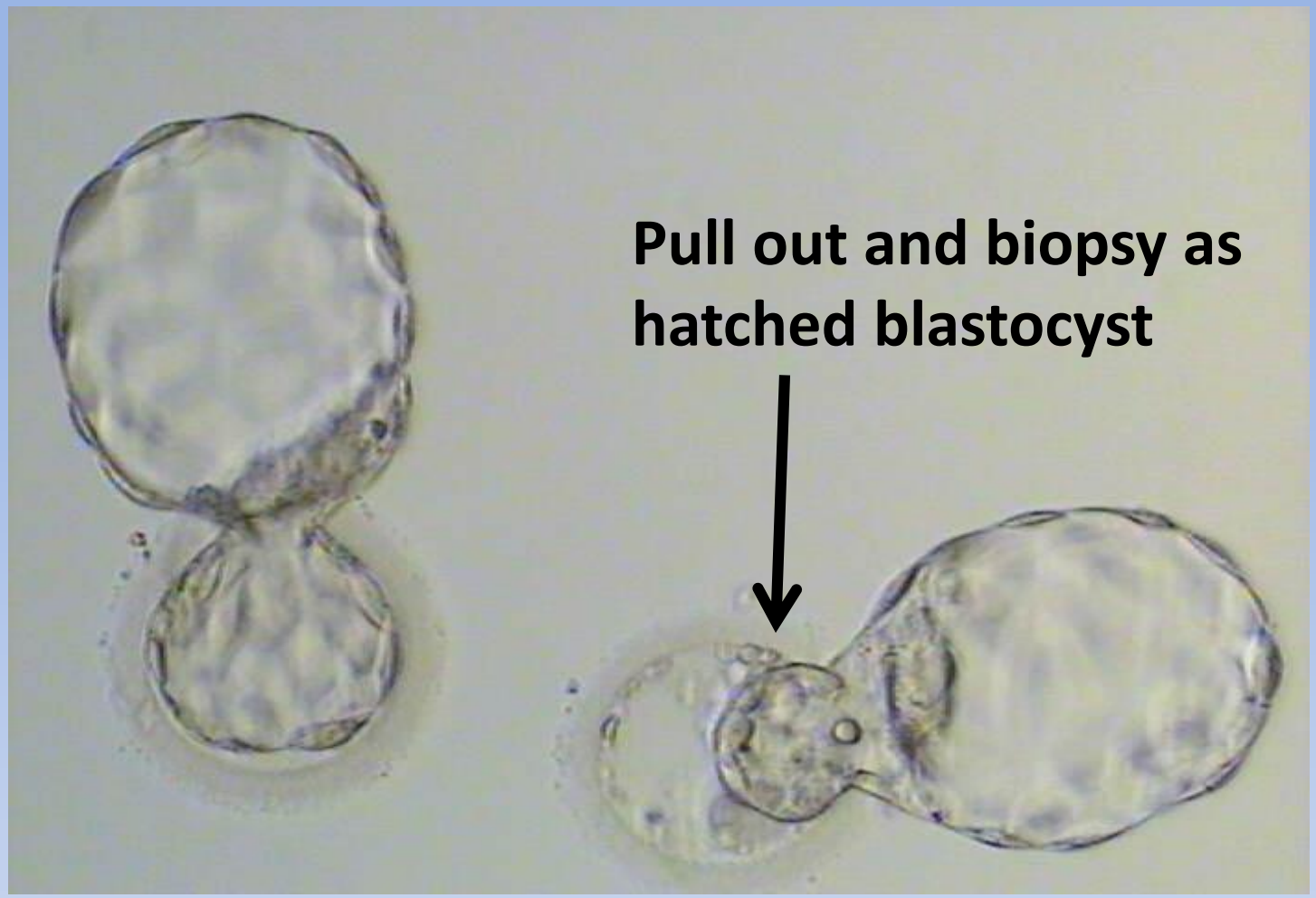


Borderline for biopsy:

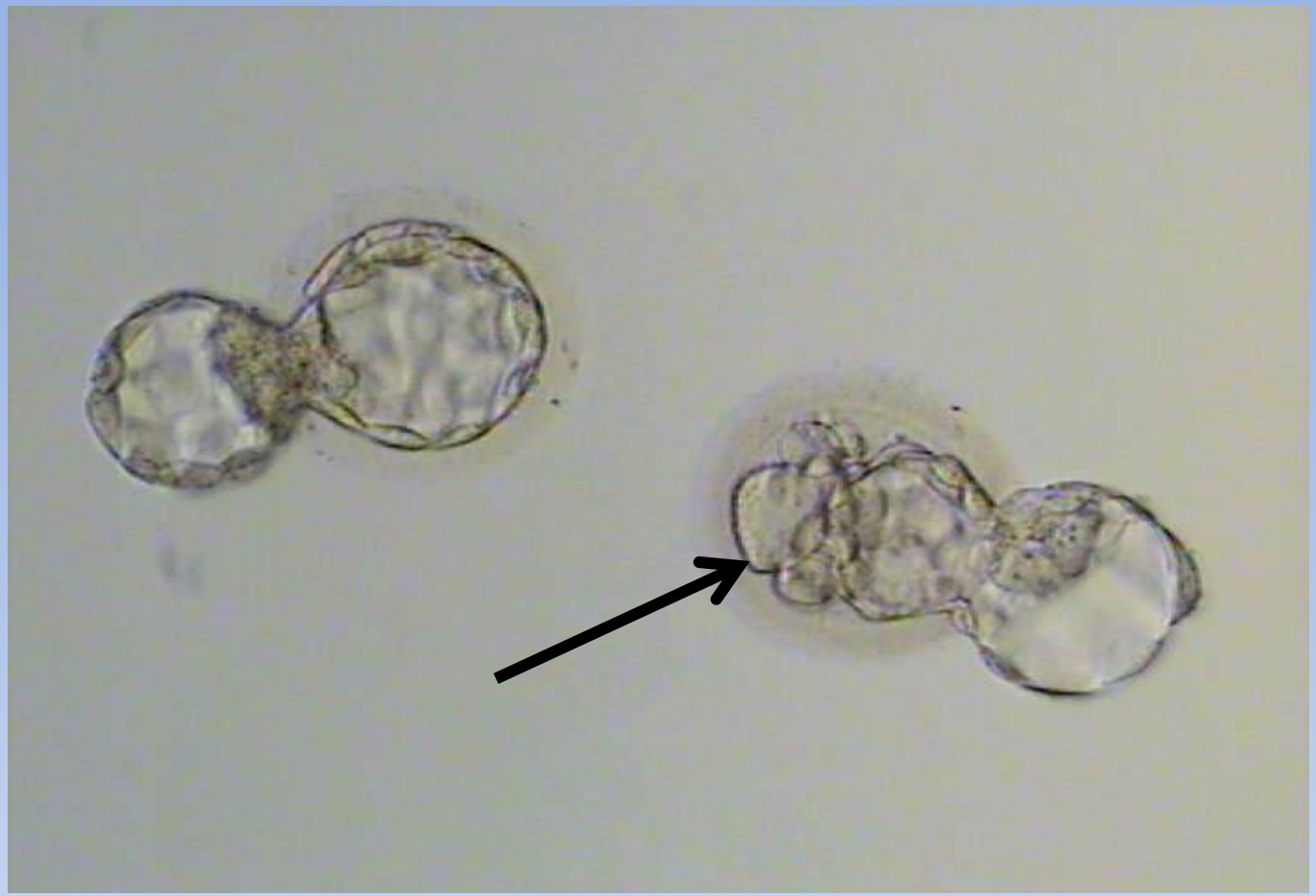
Hatching blastocysts for biopsy:



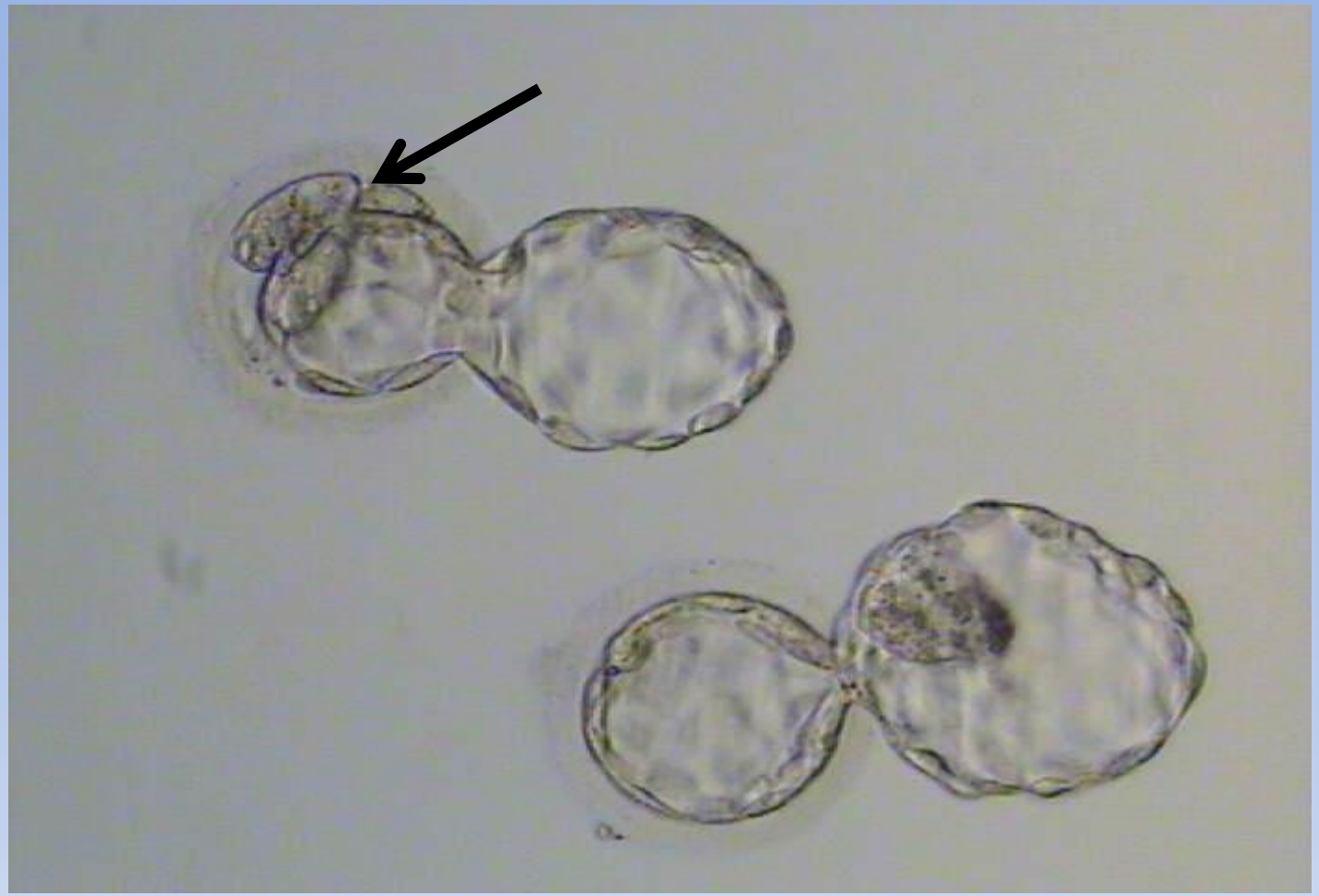
Hatching blastocysts for biopsy:



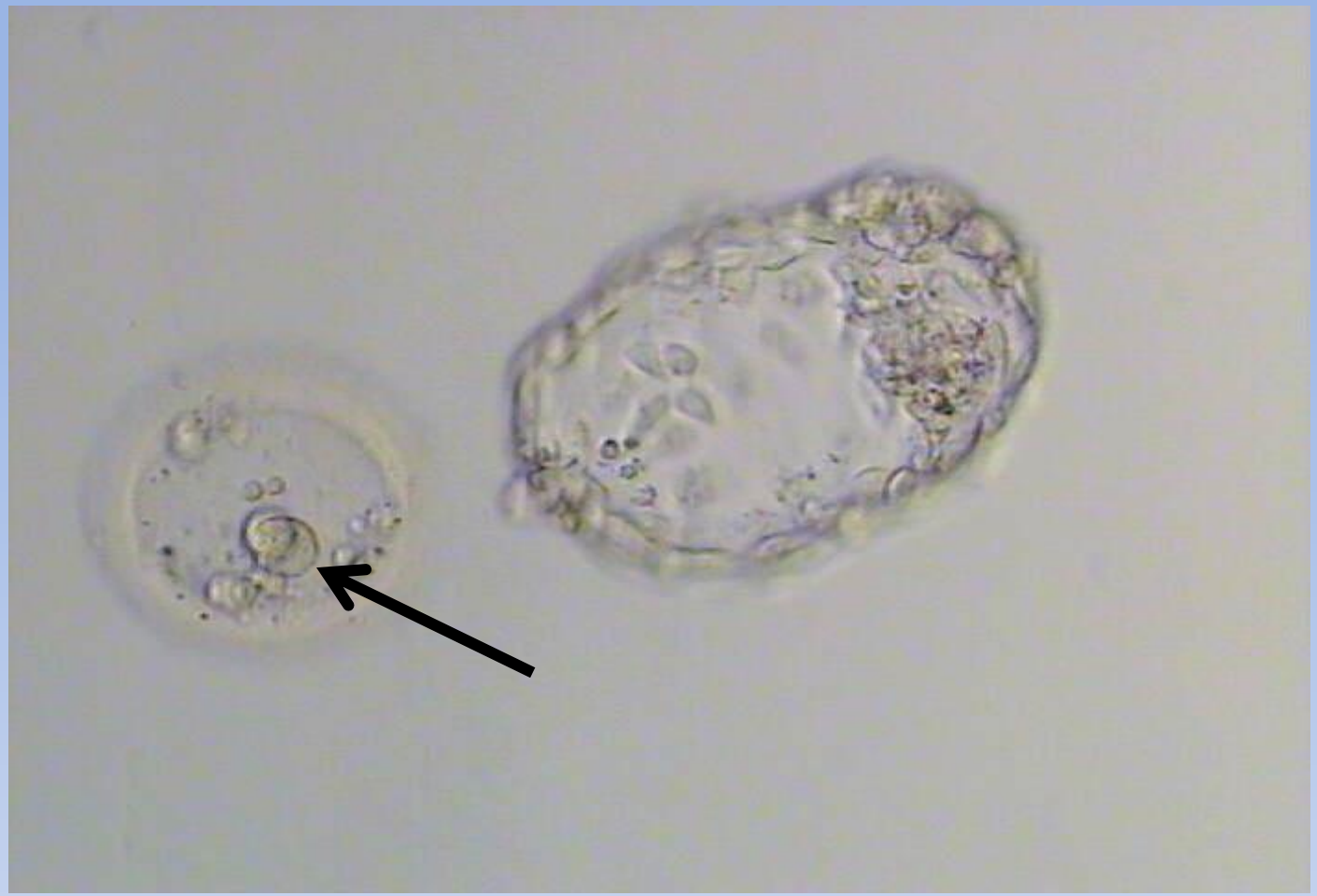
Cells/fragments frequently remain in zona:



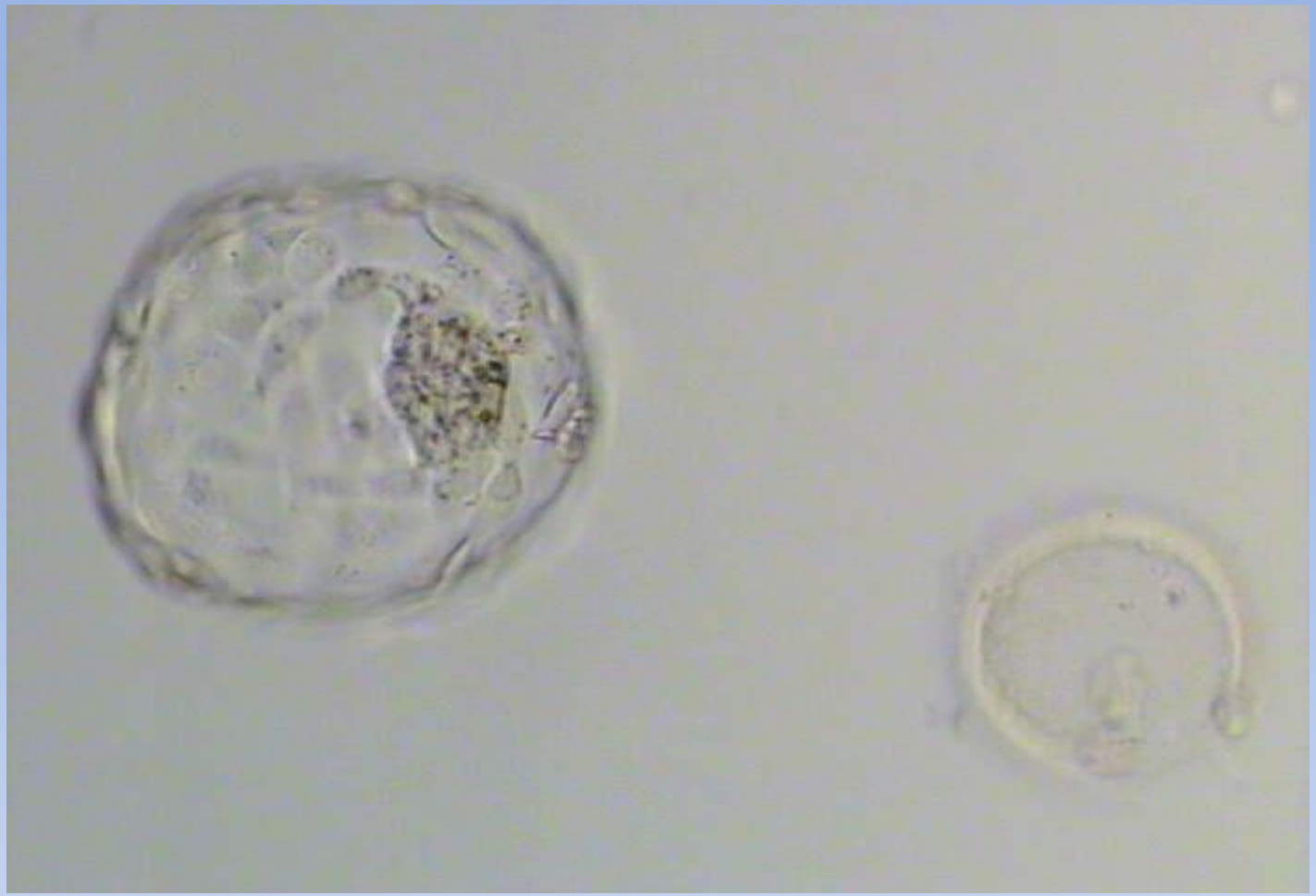
Cells/fragments frequently remain in zona:



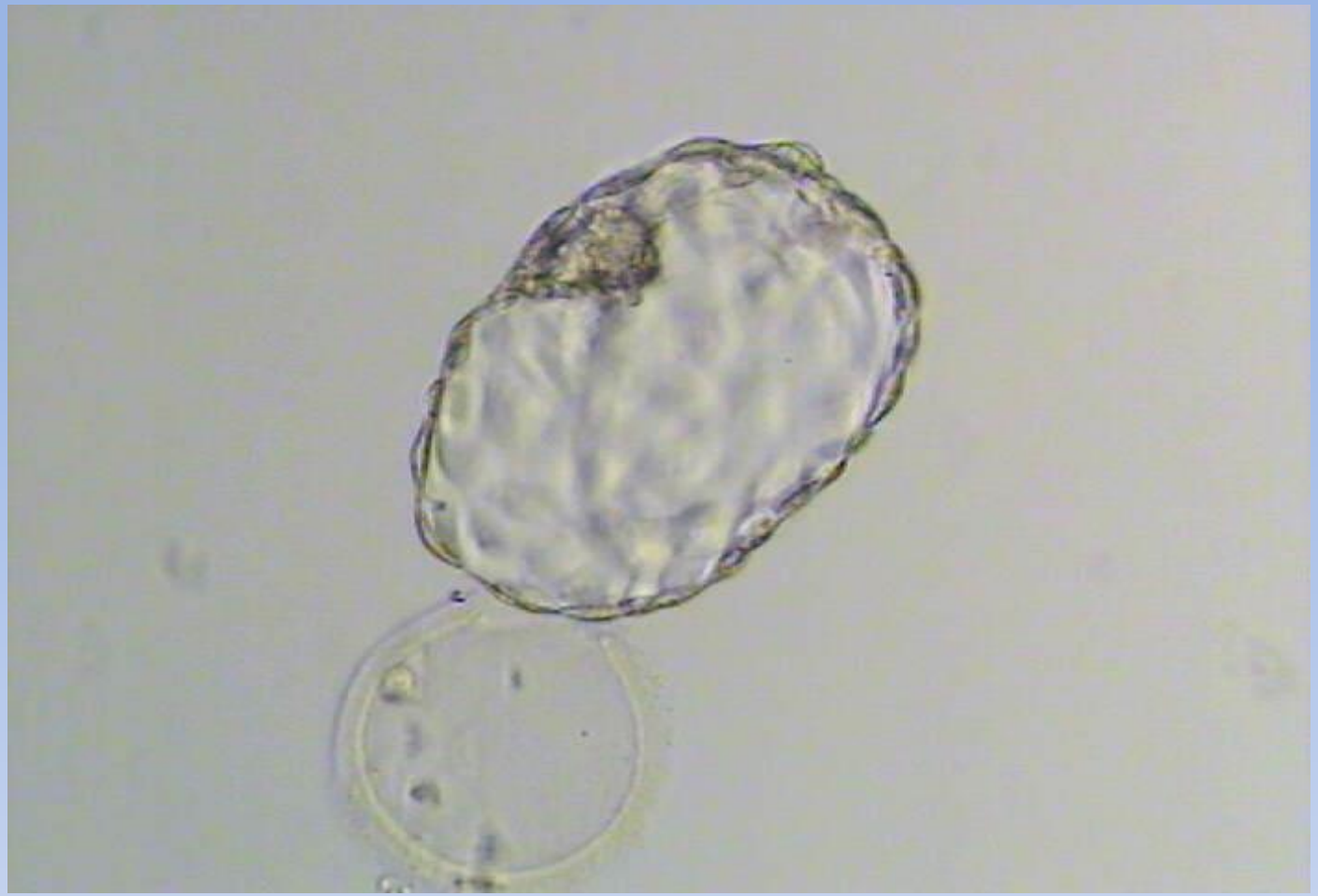
Cells/fragments frequently remain in zona:



Hatched Blastocyst (Day 6):

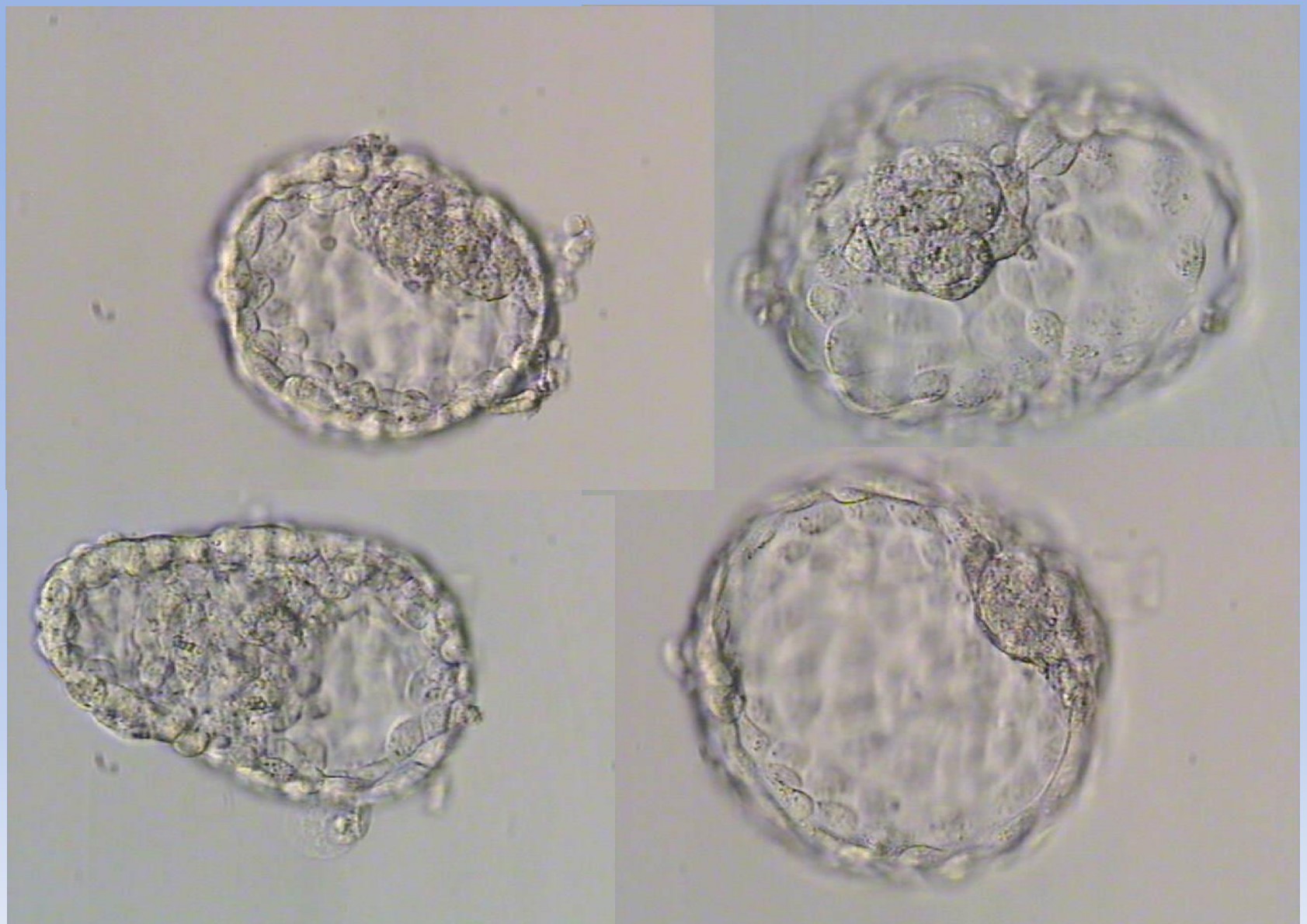


Hatched Blastocyst (Day 6):

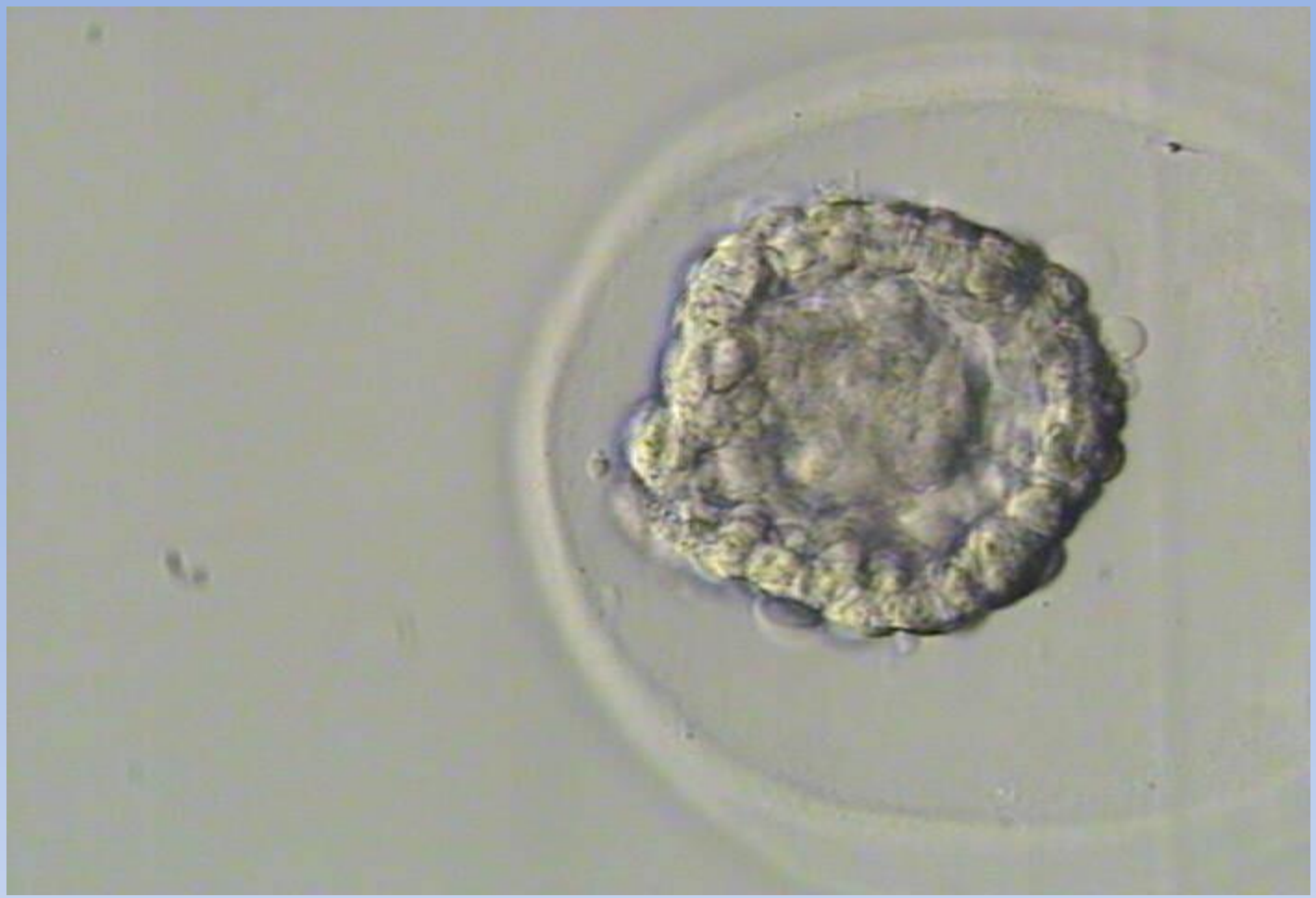


Hatched Blastocyst (Day 6):

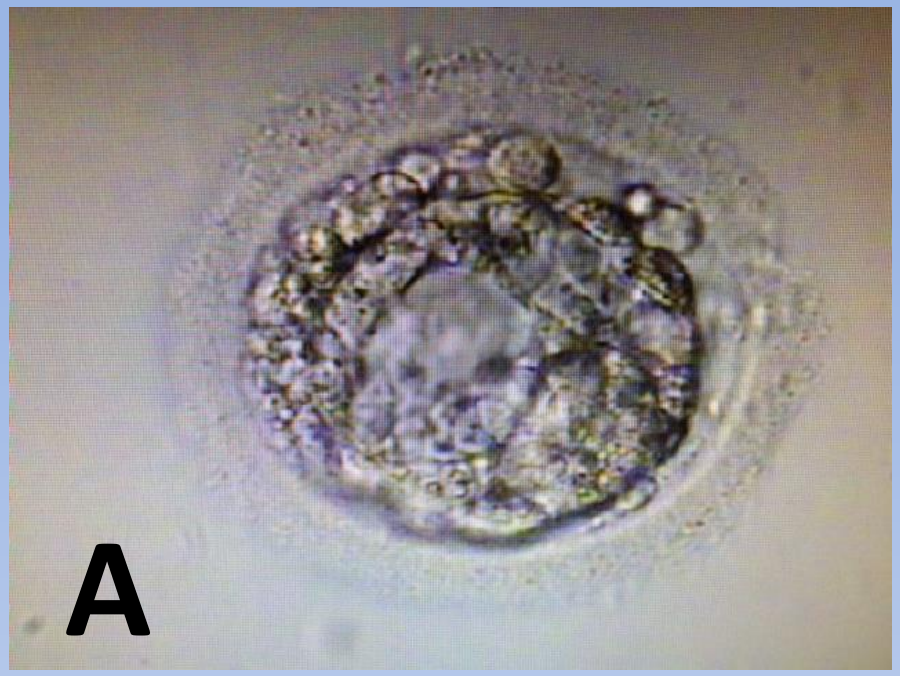
Hatched Blastocysts:

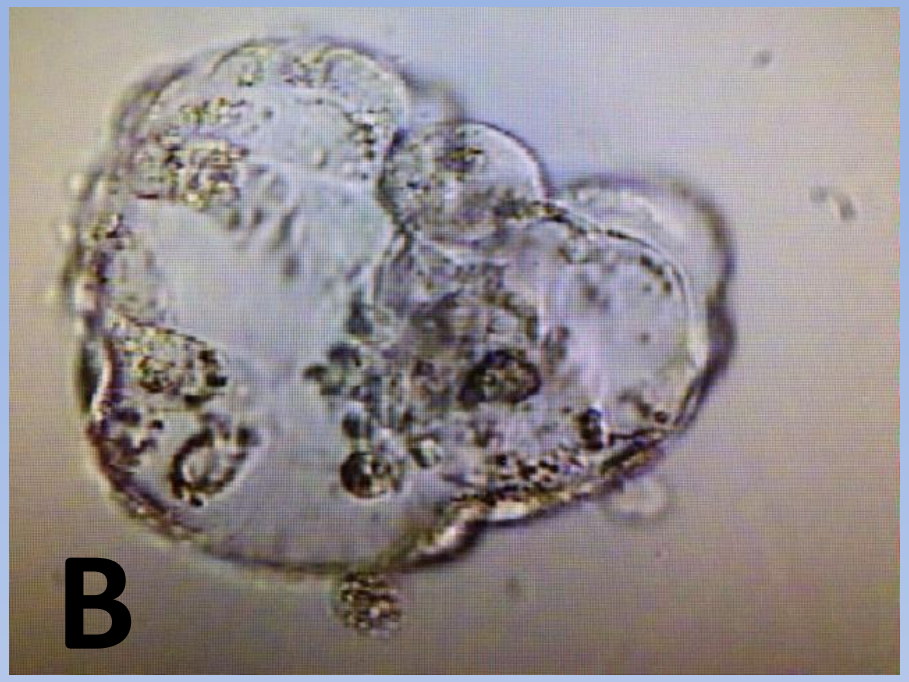


Collapsed Blastocyst:



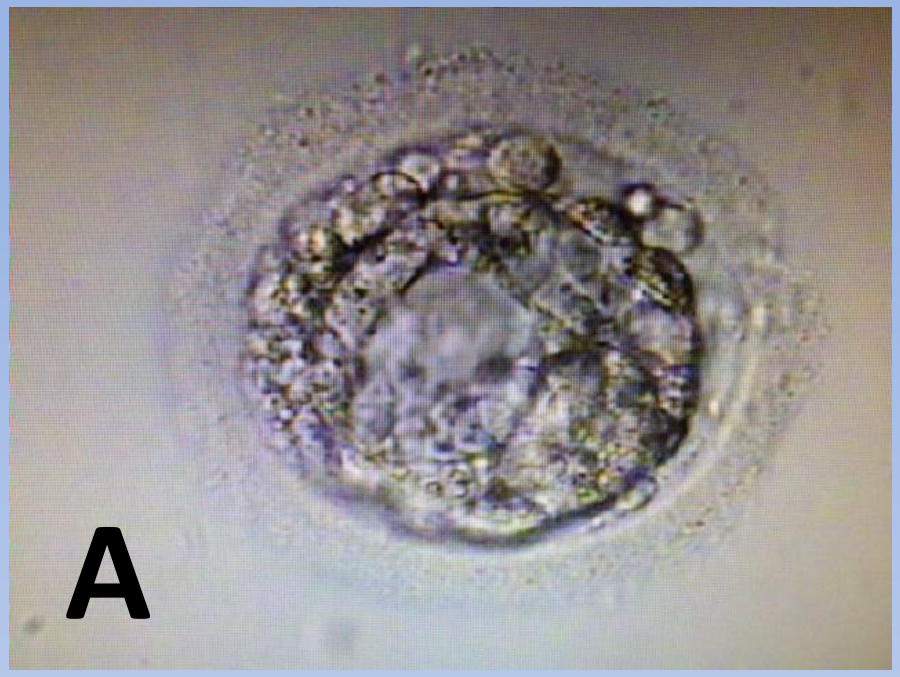
Which of these embryos made a baby?

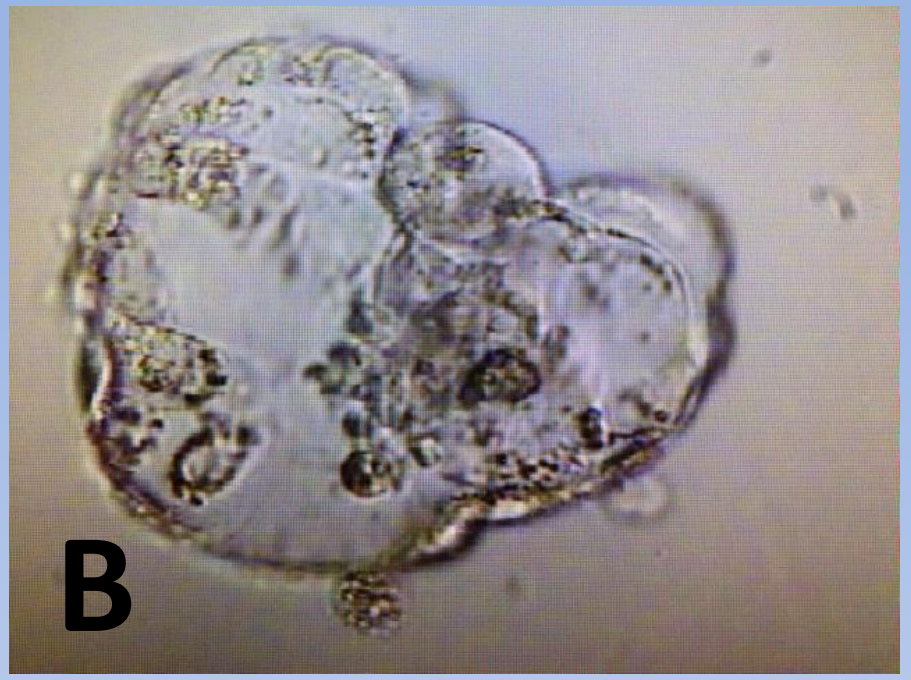




- Neither
- Embryo A
- Embryo B
- Both A & B

Which of these embryos made a baby?

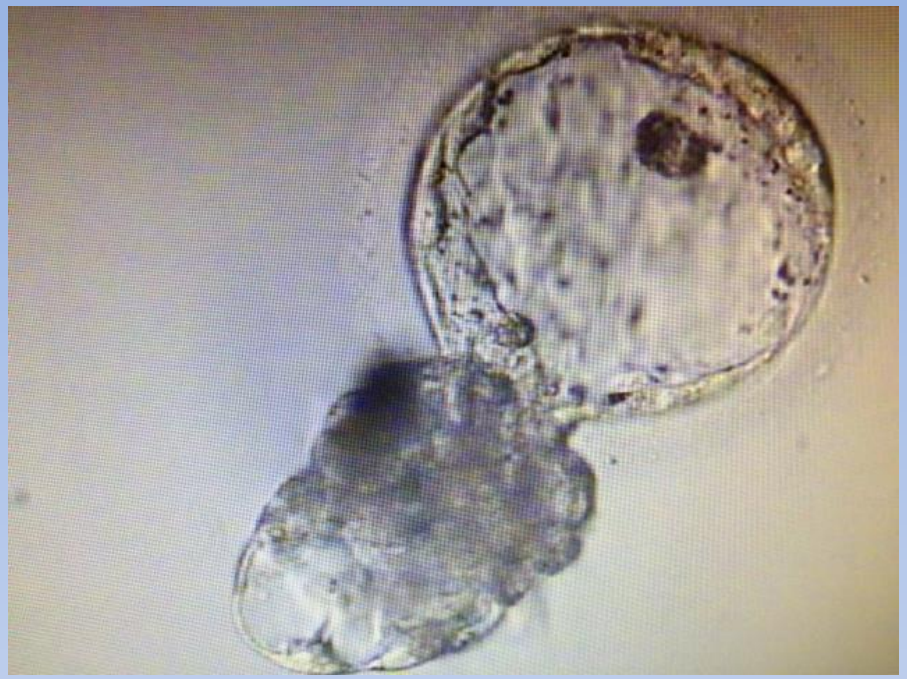


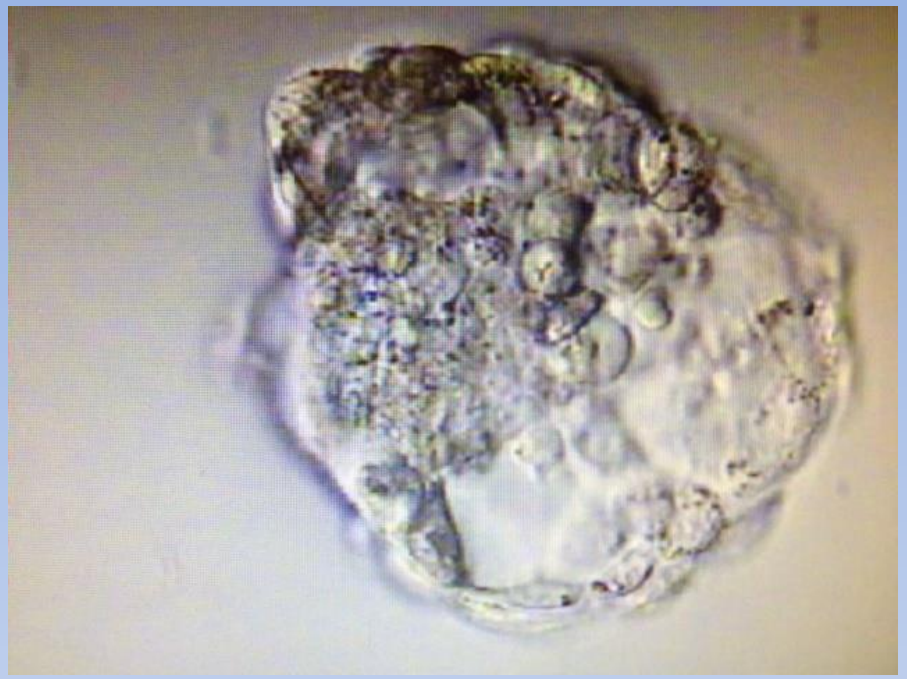


BOTH!

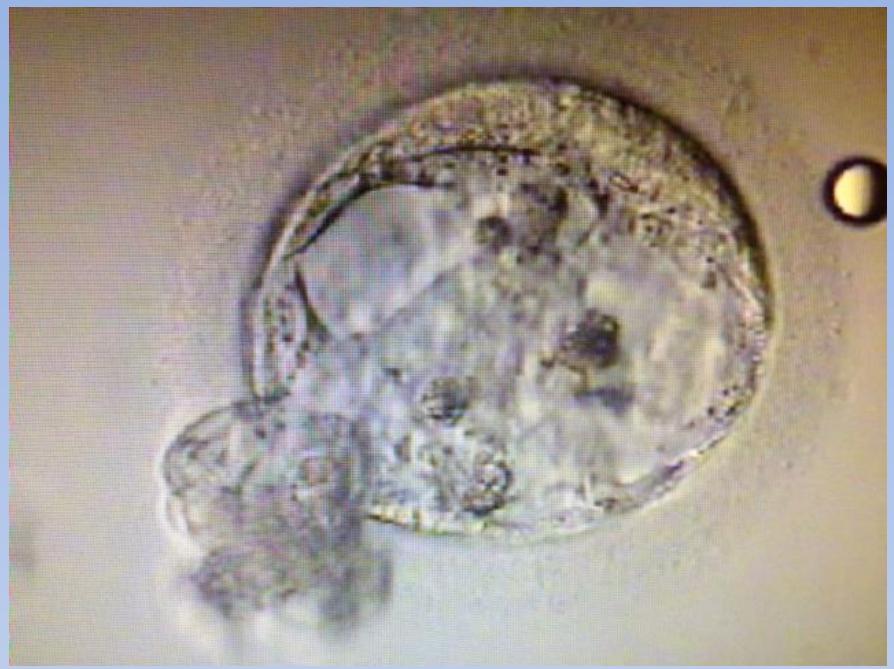
Twin Girls Delivered

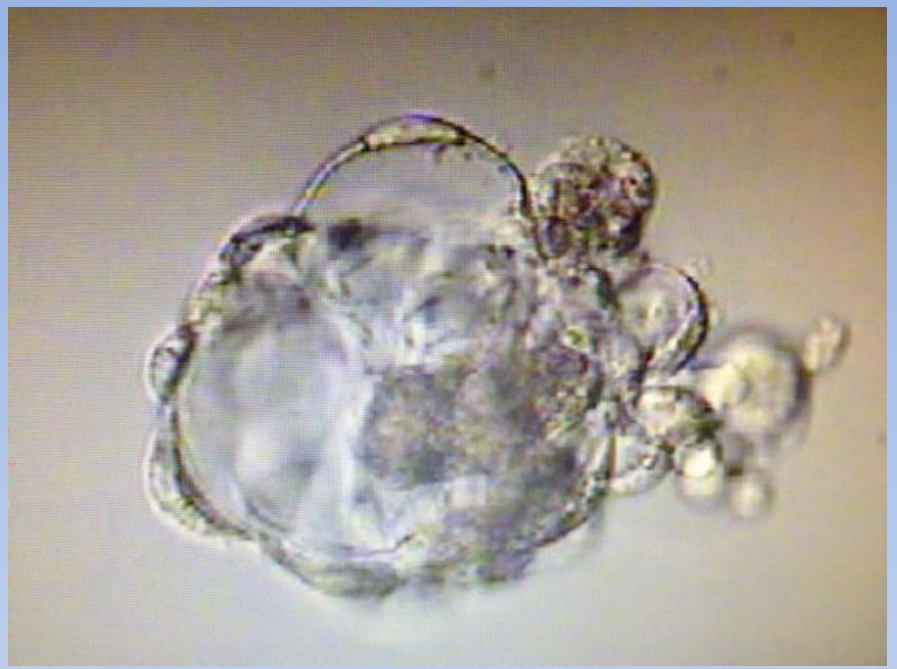
Twins delivered (boy & girl):



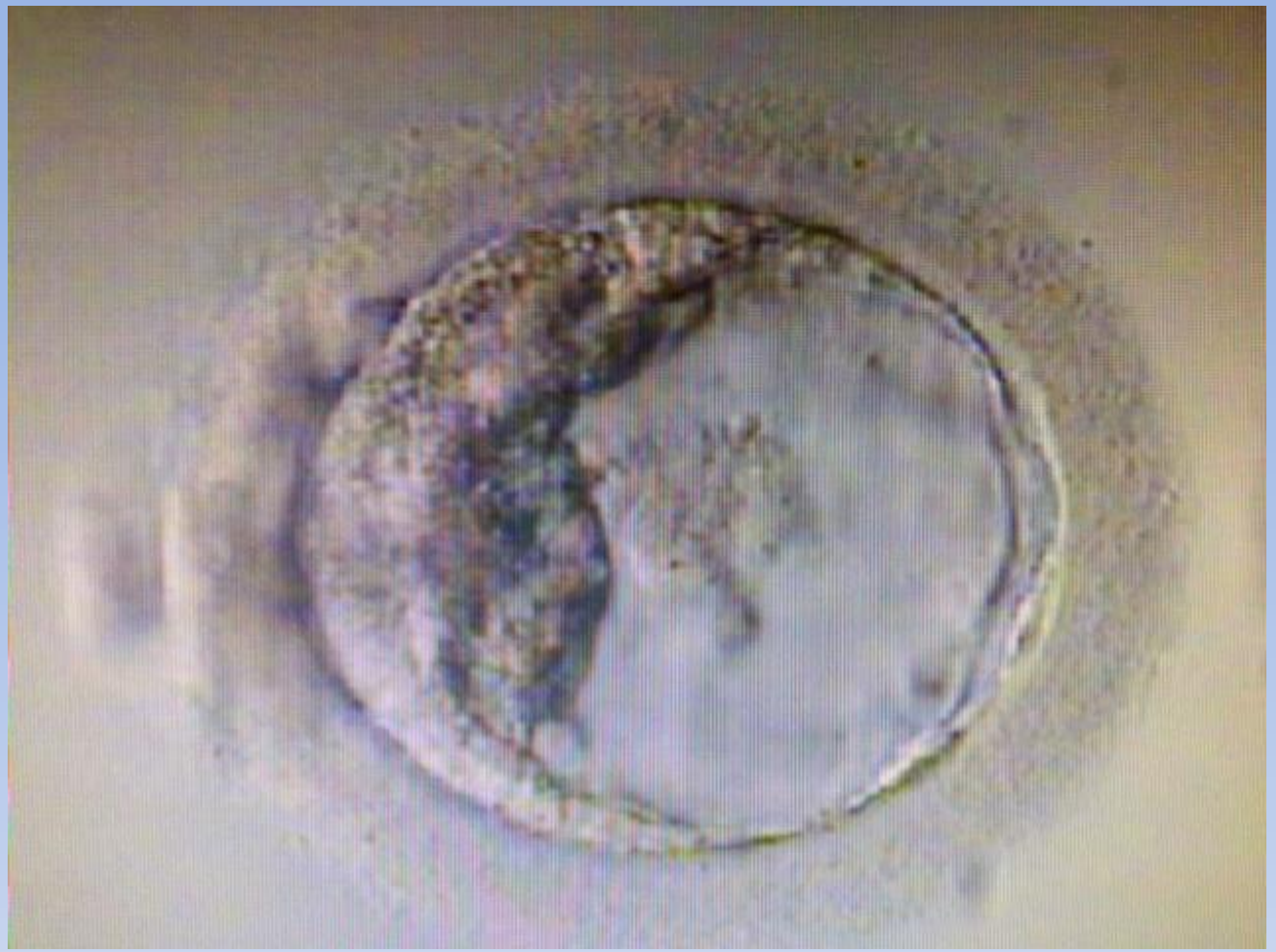


Twin girls delivered:





Delivered CCS normal singleton gestation:



Micromanipulator Set up

- Many labs still mouth pipetting
- Not necessary, Eppendorf Cell Tram Vario
- Prime with heavy silicone oil right through

Biopsy Dish Setup

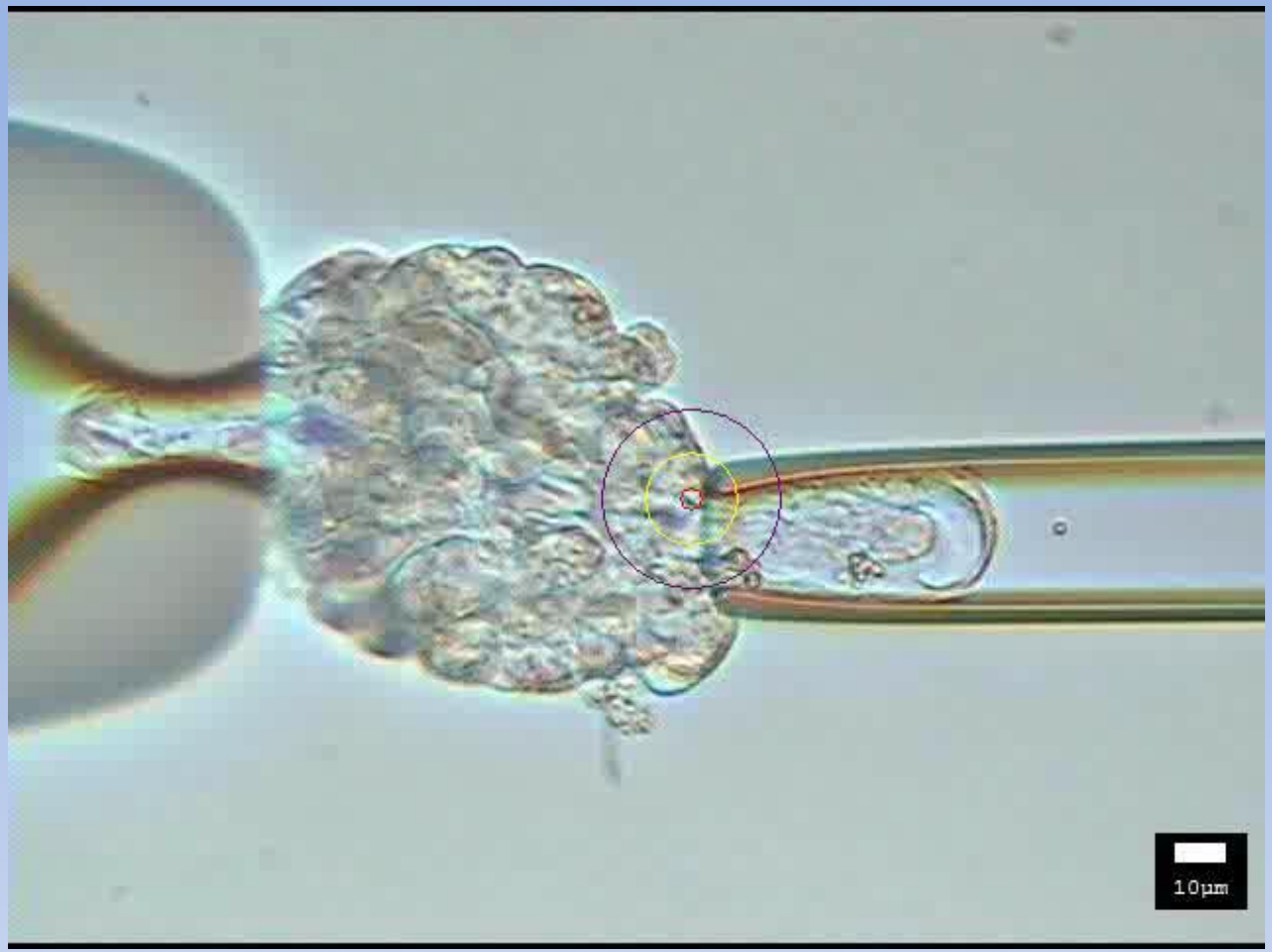
- Lid of 35mm dish
- 10 μl drops
- 2.5ml oil overlay
- Inverted 60mm dish

TE Biopsy Technique

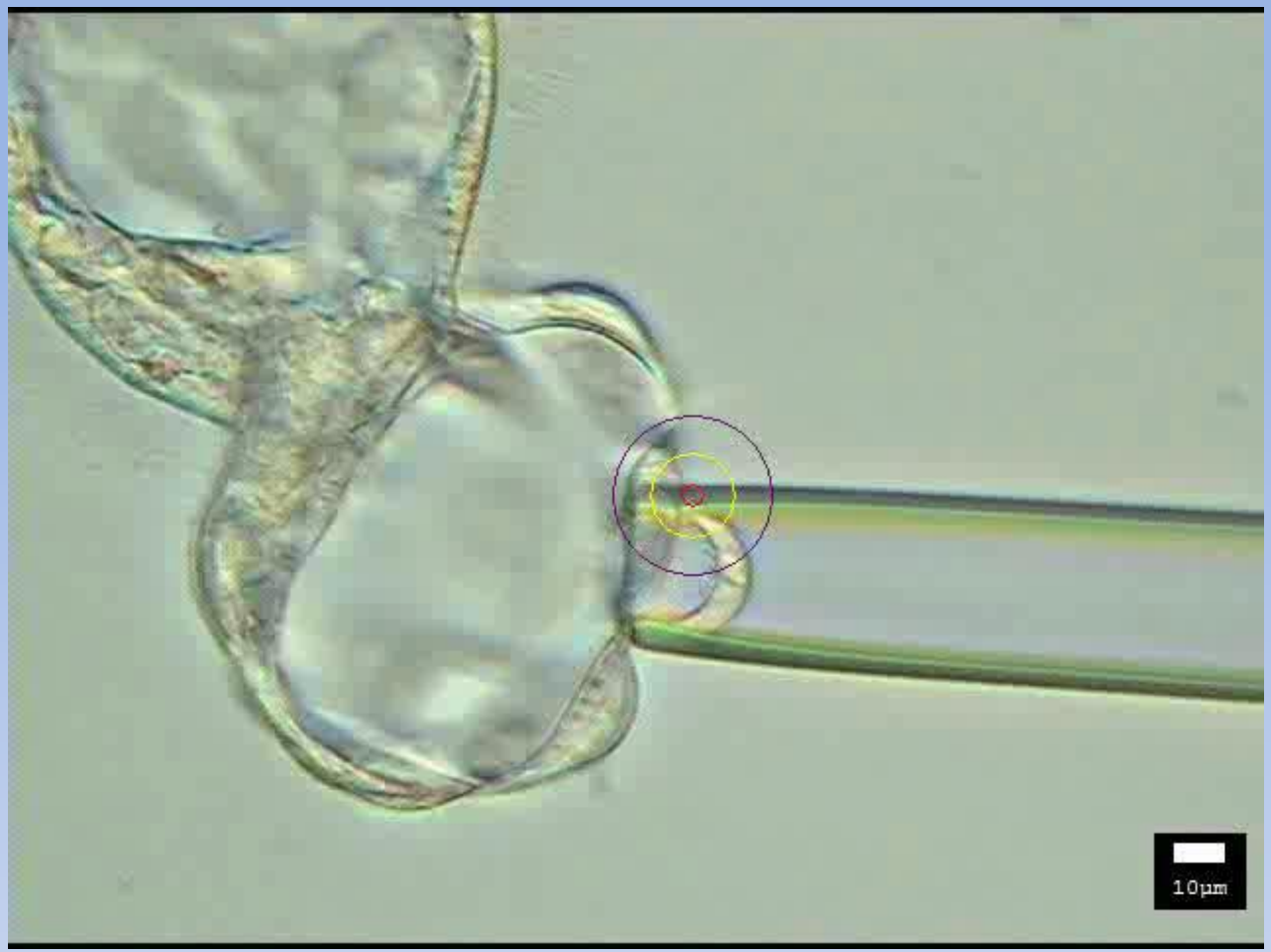
- Rotate the embryo so ICM is on holding pipet side
- Strong suction on zona or TE away from ICM
- PULL & s-t-r-e-t-c-h (more than you think you should)
- Cut with laser hits on cell junctions
- Do not cut through thick cytoplasmic areas
- When close to finishing cutting, as cells pull apart, lighten suction so biopsy doesn't shoot up biopsy pipet
- Release the biopsy away from the embryo

Trophectoderm Biopsy:

Trophectoderm Biopsy (Hatched blastocyst):



Trophectoderm Biopsy (ICM extruded):



Trophectoderm biopsies:

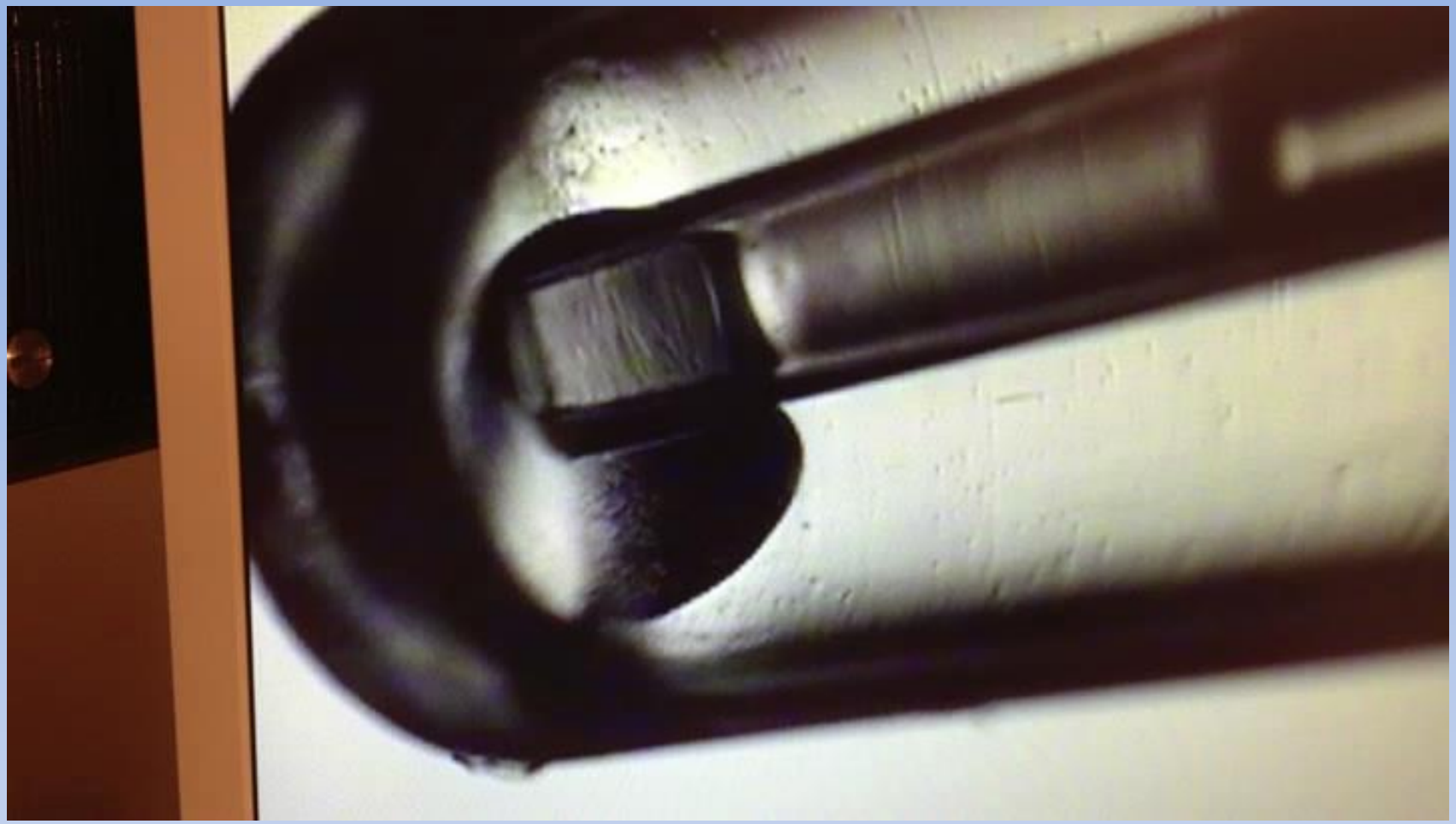
If the biopsy sticks to the biopsy pipet…

- Move the pipet down to firmly touch the bottom of dish
- Move the stage north & south (quick & firm)
- Stay near the center of the drop
- Do not contact the oil interface

Loading the biopsy in to the tube

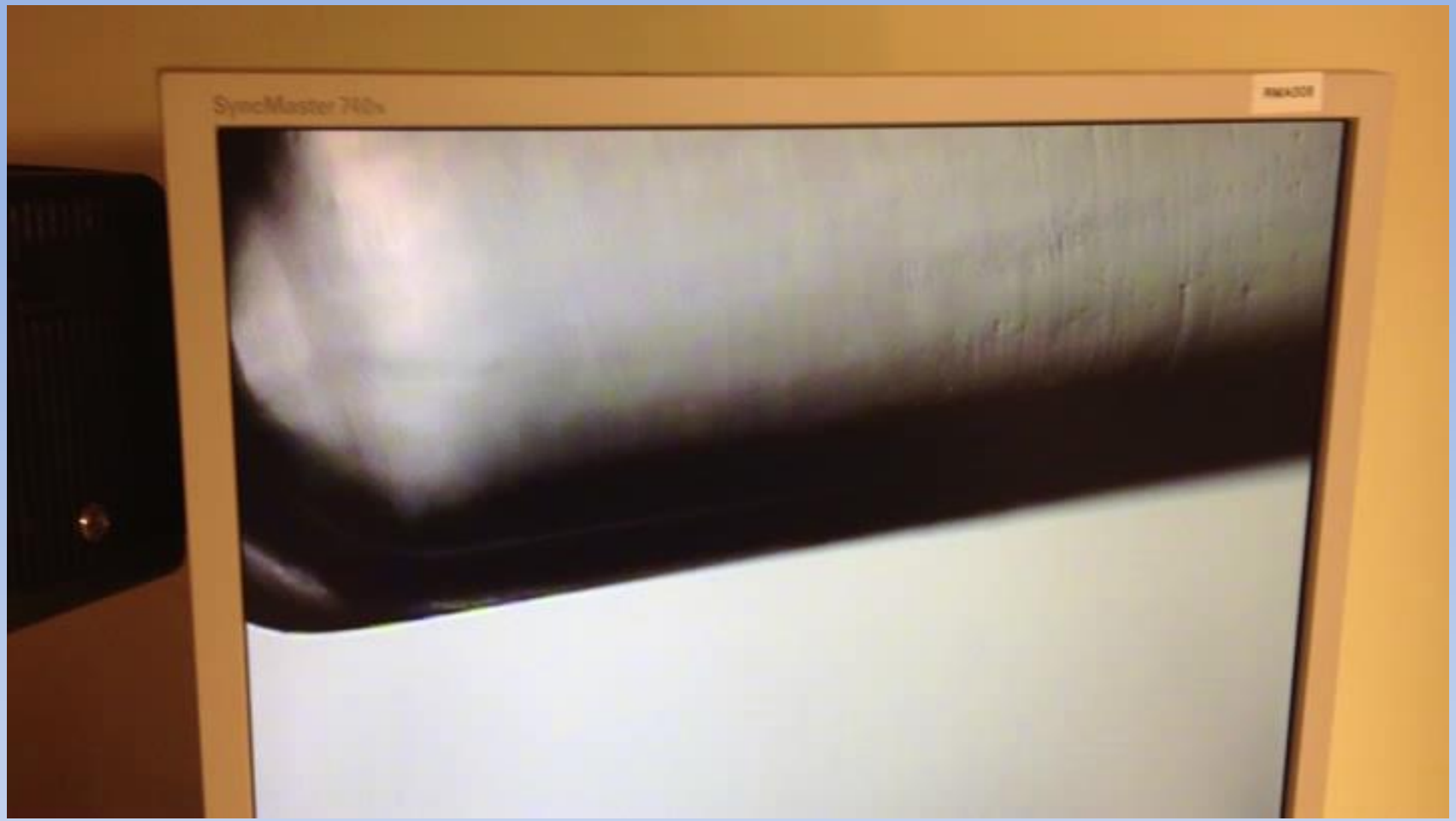
- Drag drop of buffer slightly up the side of the tube
- 1-2µl of buffer depending on the testing lab
- Rinse the biopsy in buffer using 130µm stripper tip
- Angle the tube under the stereoscope to focus on the drop
- View the biopsy going into the drop

Loading 1µl Buffer in PCR Tube:

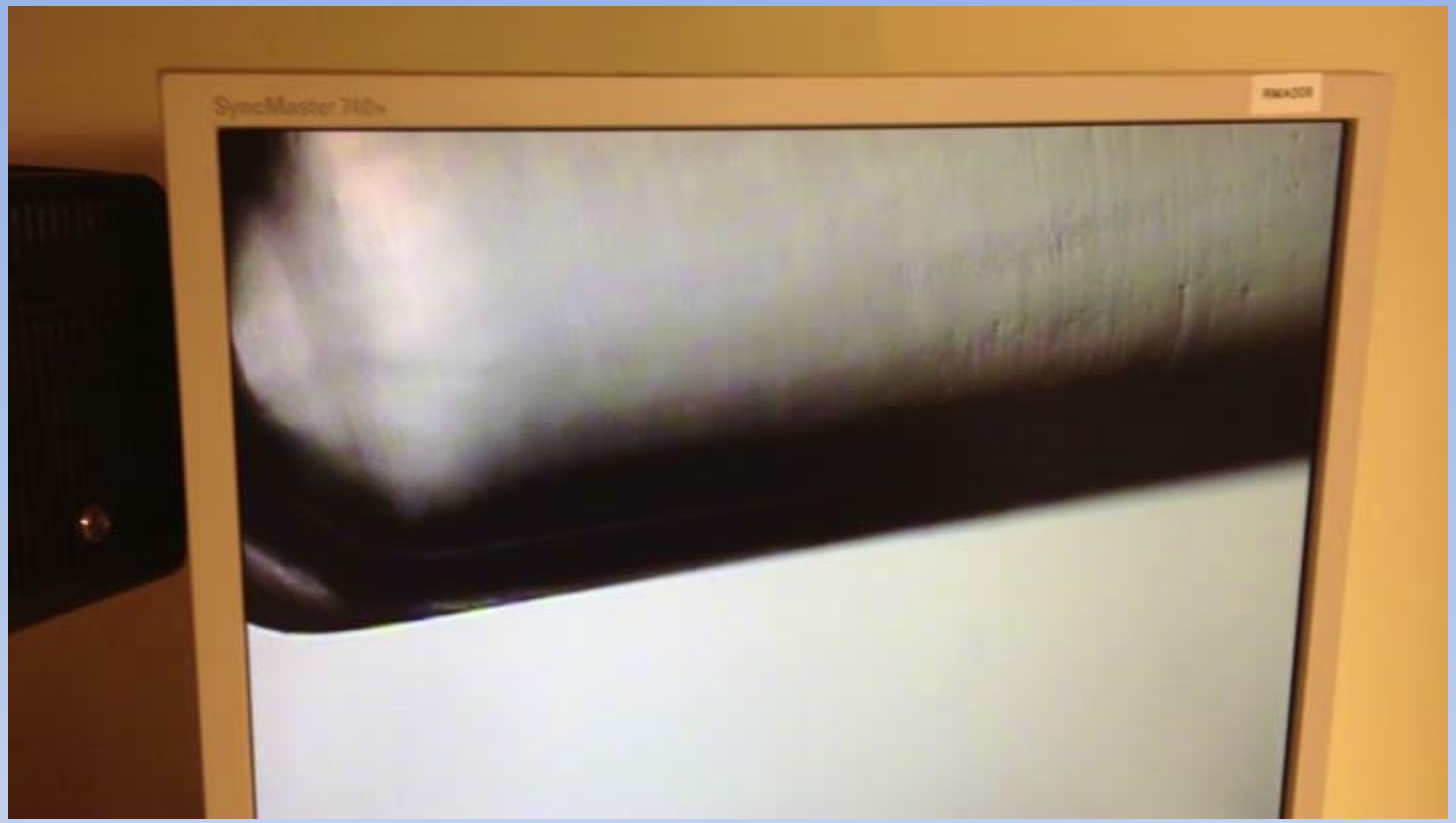


Note: This technique does not work for all PCR tubes.

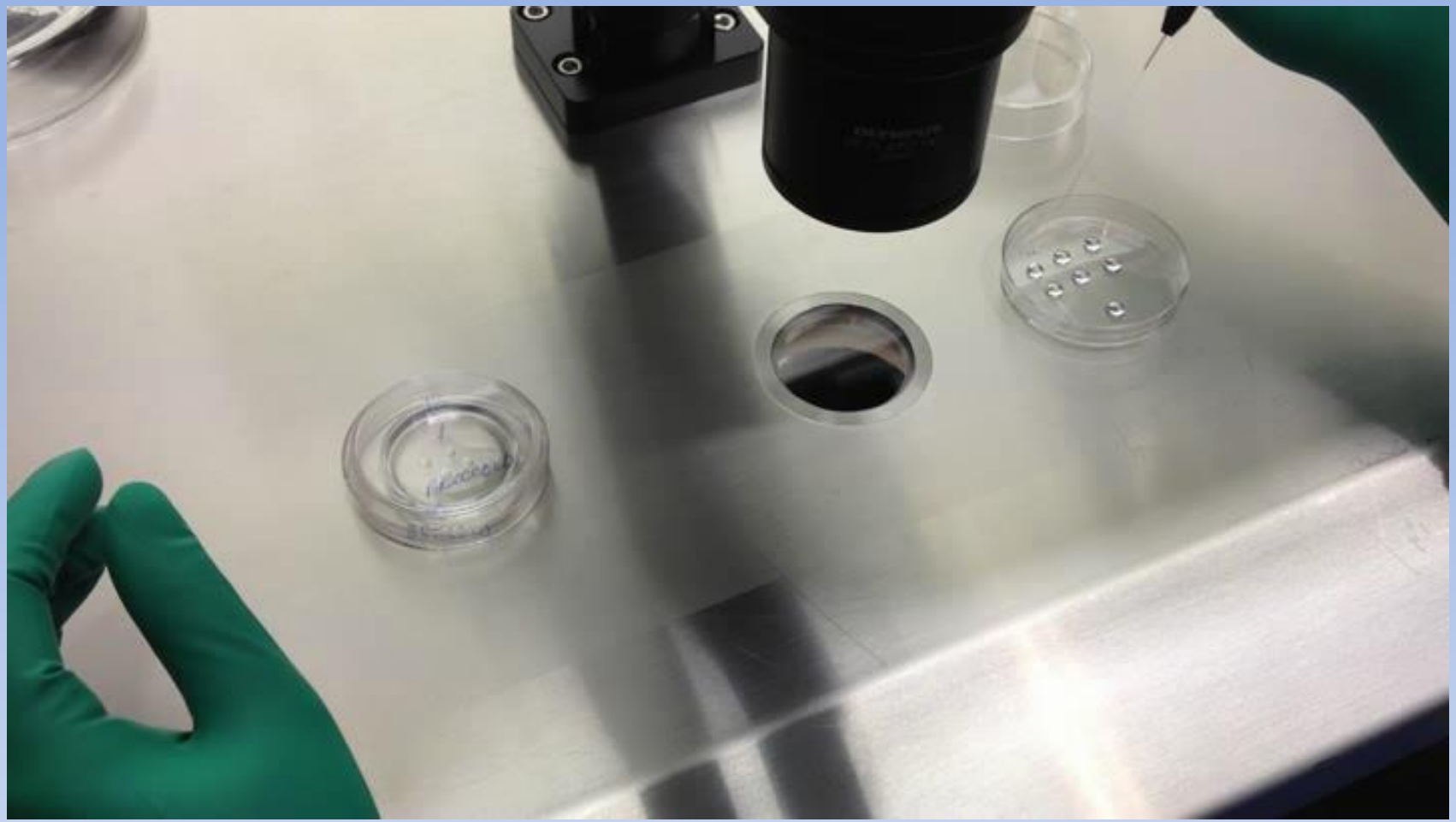
Loading 1µl Buffer in PCR Tube:



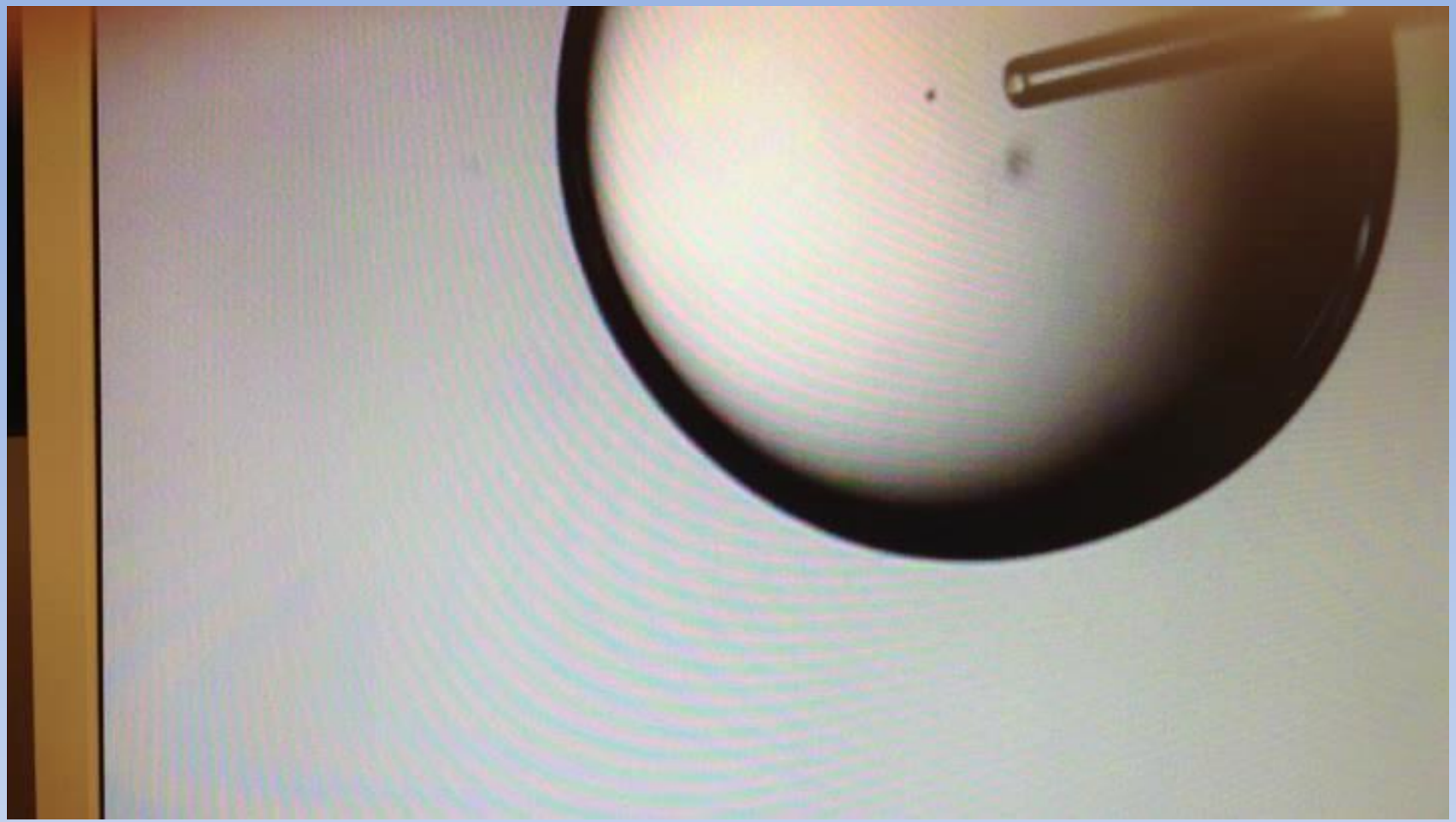
Loading 1µl Buffer in PCR Tube:



Loading Biopsy in PCR Tube:



Loading Biopsy in PCR Tube:



Handling Biopsied Hatched Blastocysts

- When moving embryos, they occasionally come into contact with air or oil bubbles.
- For zona intact embryos, generally a nuisance, requiring the embryo be knocked off the bubble.
- If a hatched blastocyst contacts an air or oil bubble, the surface tension causes the embryo to closely adhere to the bubble and the embryo is easily lost.

Handling Biopsied Hatched Blastocysts

- Biopsied, zona-free blastocysts are sticky and may be more likely to stick inside a handling pipet.
- When handling hatched blastocysts, minimize the possibility of the zona-free blastocyst touching an air/oil bubble or sticking inside a handling pipet.

Handling Biopsied Hatched Blastocysts

- Rinse & prime the handling pipet cleanly with culture medium prior to aspirating the hatched blastocyst (no air/oil bubbles).
- Visualize embryo at all times as it moves in & out of the pipet.
- Work quickly. Do not allow the embryo to settle in the pipet.
- Special attention to not touch the hatched blastocyst to the tip of the handling pipet. Draw the embryo from a short distance with fluid/media aspiration.

Nonconcurrent results (a warning):

We learned the hard way…

Problem:		Solution:
• Garbage in = Garbage out	→	• Practice on junk, but don't test junk
• Biopsy too early, cells pull apart	→	• Be patient, more cellular is better
• Biopsy sticks to holding	→	• Drag on bottom or flick
• Handling hatched blasts	→	• Avoid air & oil bubbles

Key Hints to Success:

- Pull and stretch when cutting biopsy
- Hit the cell junctions/membranes (not cytoplasm)
- Draw buffer up side of PCR tube
- Visualize biopsy expelled into buffer
- Good vitrification is key to success of CCS

Audience Response Question

Please use the response keypad.

If you do 24-chromosome testing, do you transfer any fresh tested embryos?

A. Yes

B. No, but we wish we could logistically

C. No, we do fine or better with FET

What percentage of your caseload do you estimate you do 24-chromosome testing?

A. 0%

B. 1-2%

C. ~5%

D. ~10%

E. ~20%

F. >25%

Where are your PGS biopsies currently tested?

A. Primarily by Reprogenetics

B. Primarily by Genesis Genetics

C. Primarily by RMA Genetics

D. Other or in-house testing lab

E. We send to multiple testing labs

F. We don’t perform biopsies for PGS

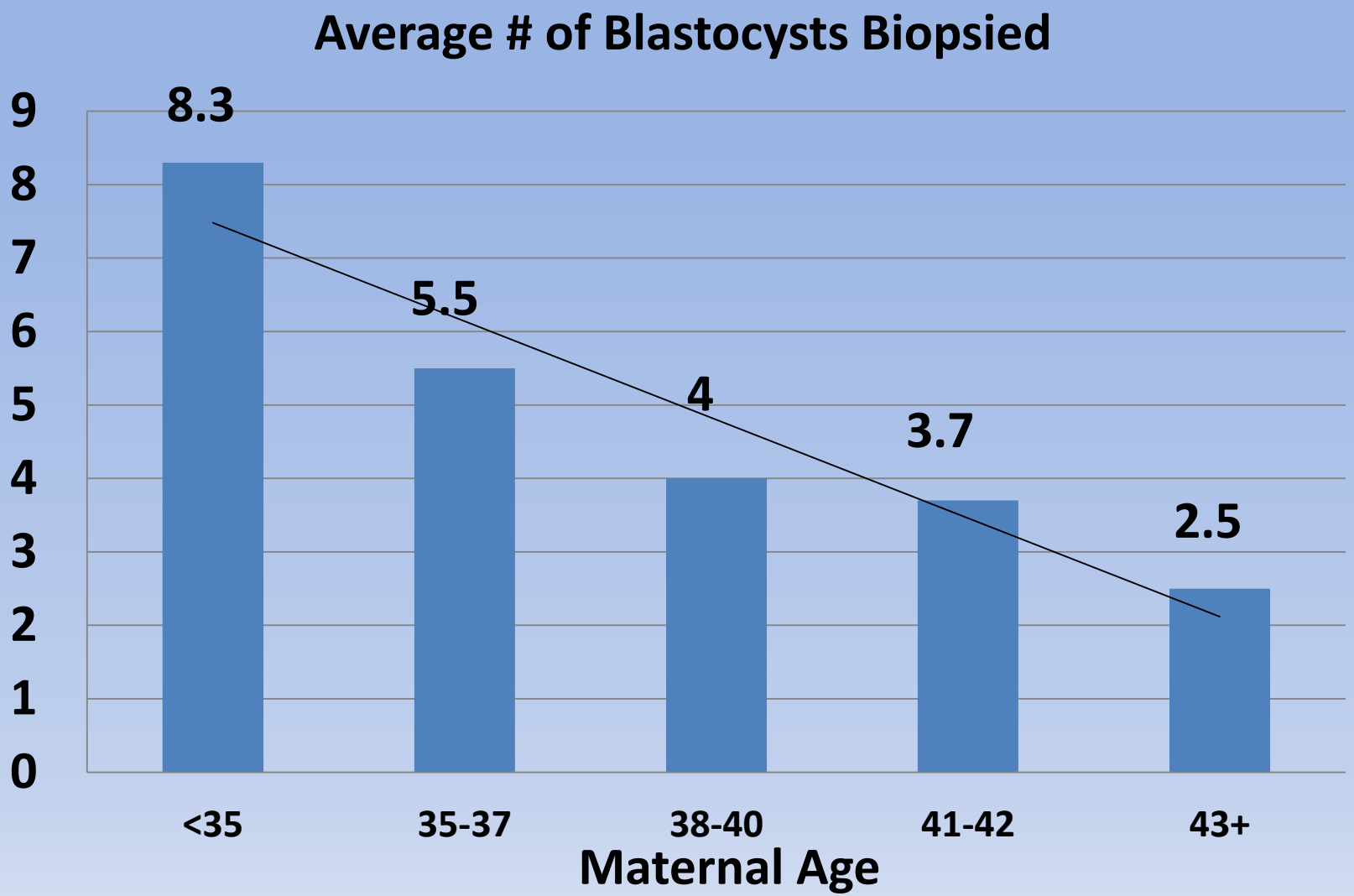
Advantages of 24-chromosome testing

- Transfer normal embryos
- Increase pregnancy rates
- Decrease miscarriage rates
- Decrease births of trisomies vs elective termination
  - Safer
  - Psychological benefit
  - More socially acceptable

Considerations for 24-chromosome testing

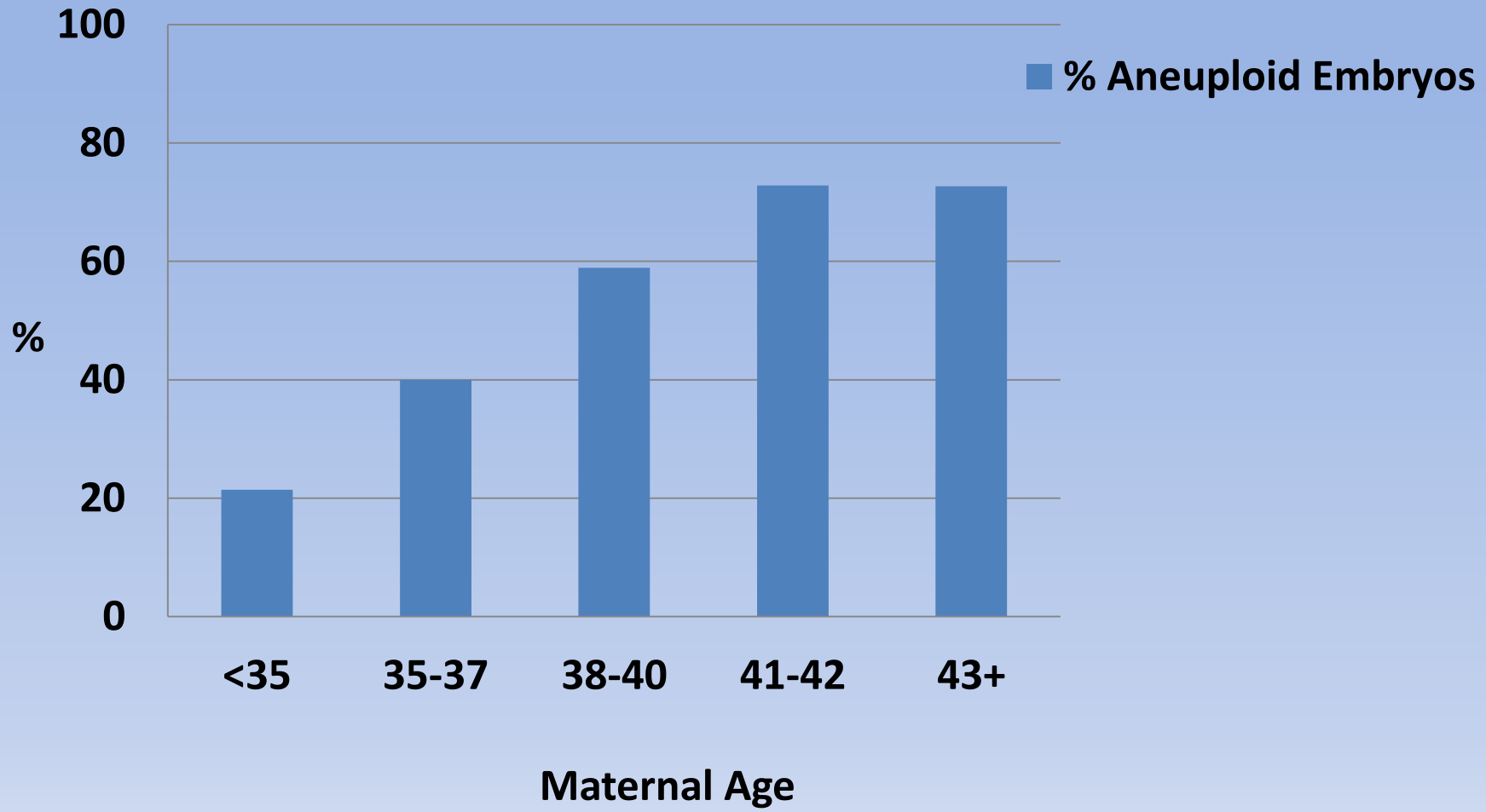
- More work, frequently need to biopsy Day5 & Day 6
- Possible damage to embryo during biopsy
- No normal embryos for transfer
- Expense (~$5000 for biopsy & testing)
- In most cases, requires cryopreservation of embryos

Blastocysts Available for Biopsy by Age



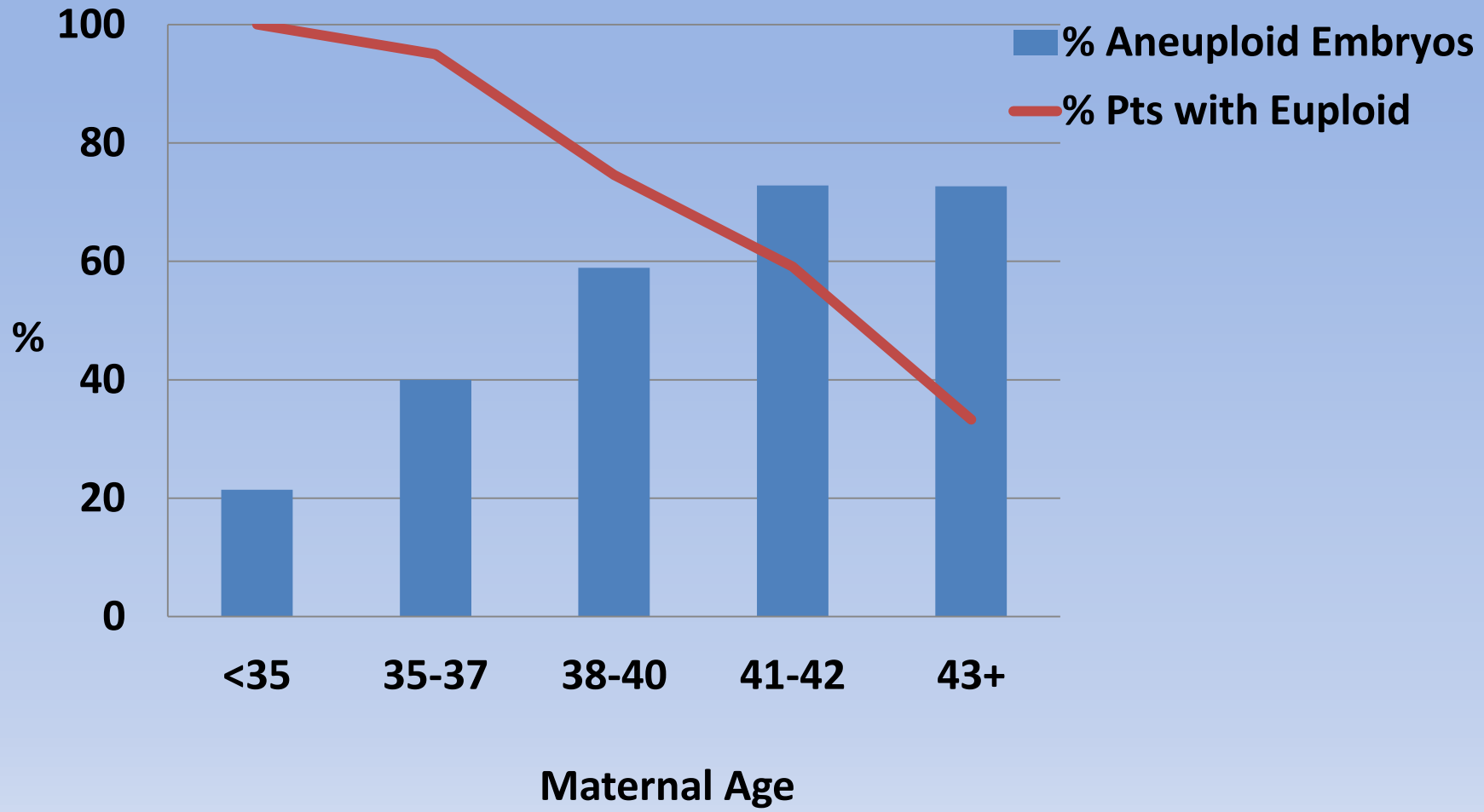
Kelk *et al*. ASRM 2012; O-198

Comprehensive Chromosome Screening Data



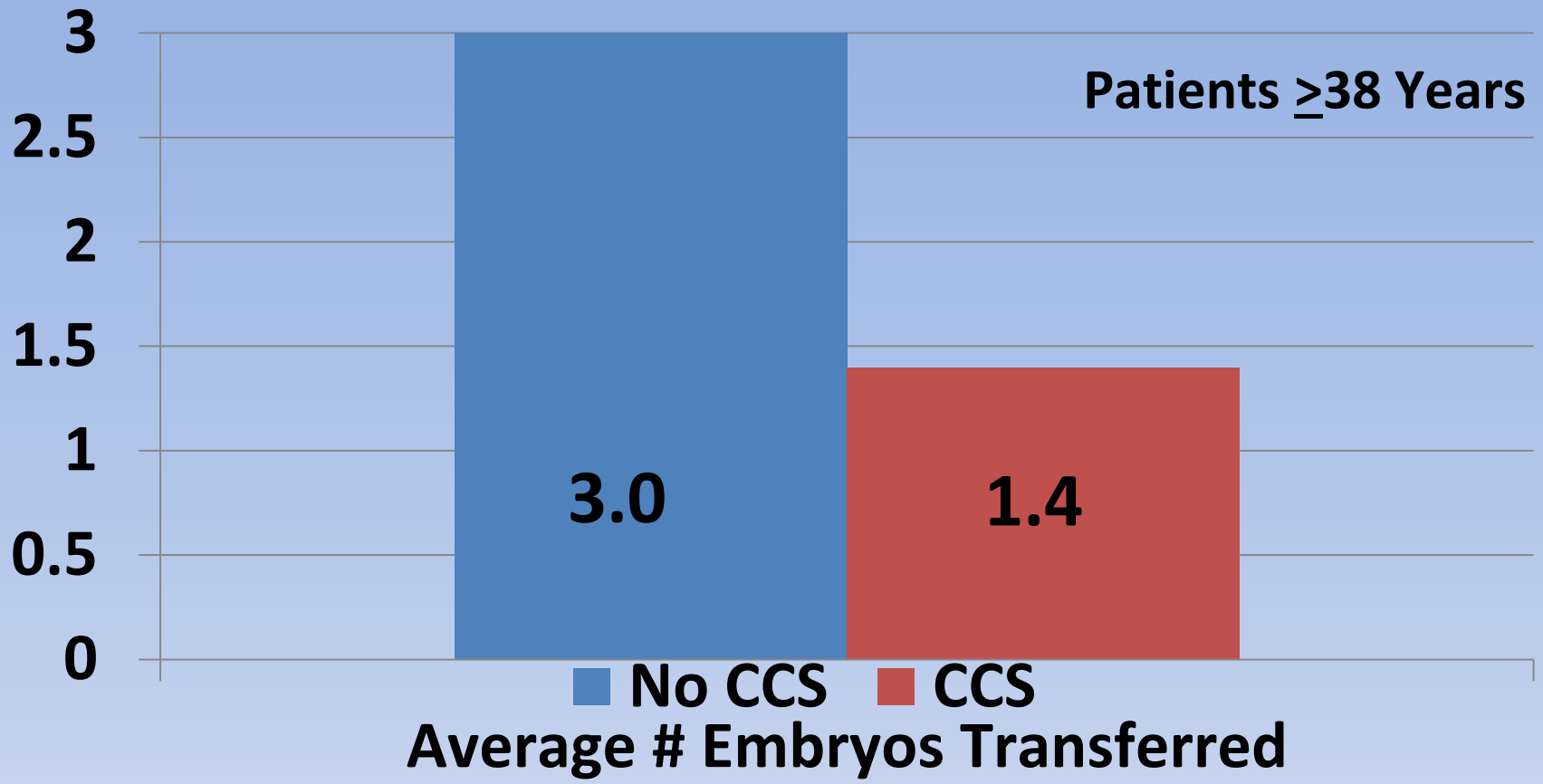
Kelk *et al.* ASRM 2012; O-198

Comprehensive Chromosome Screening Data

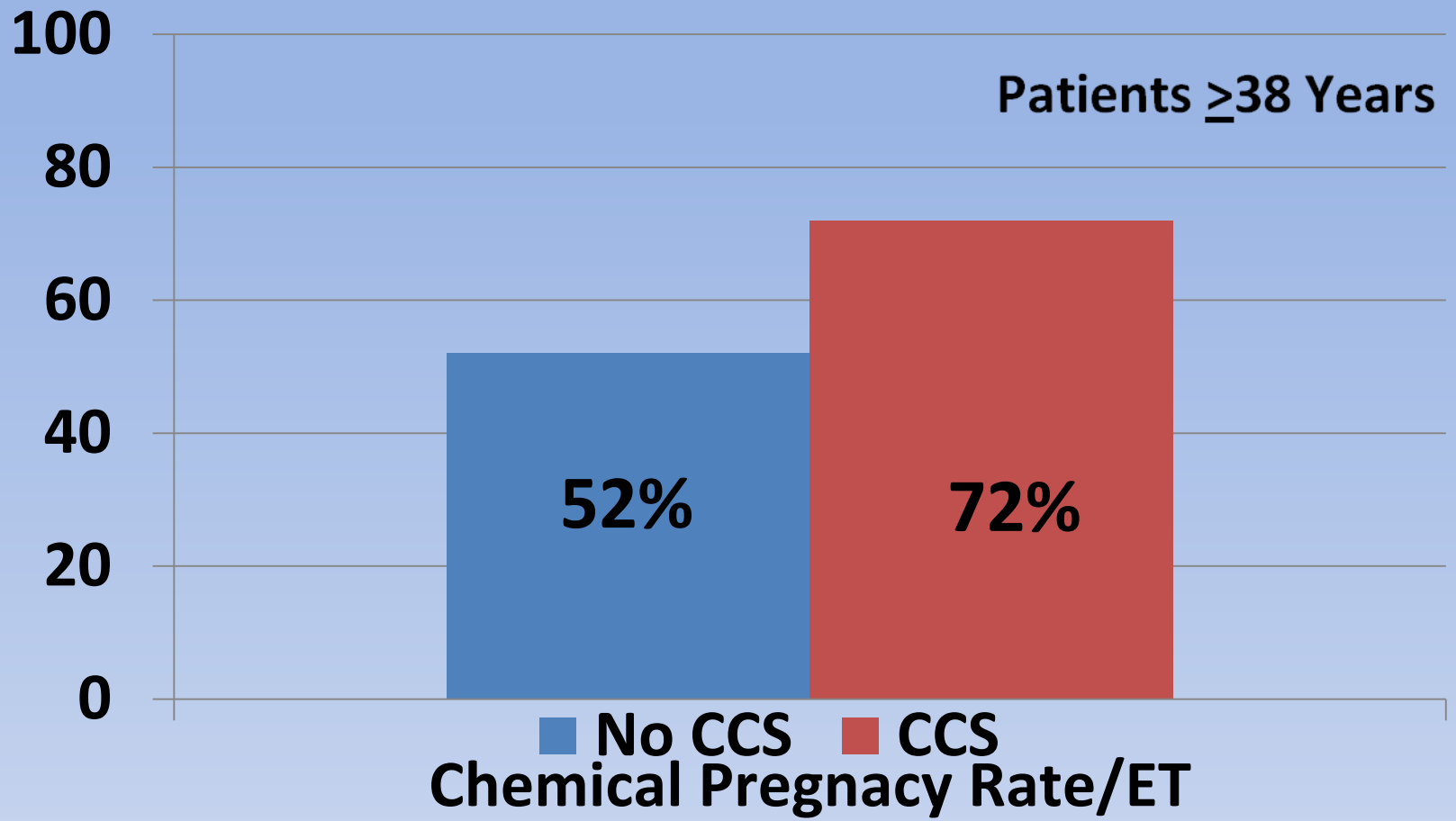


Kelk *et al*. ASRM 2012; O-198

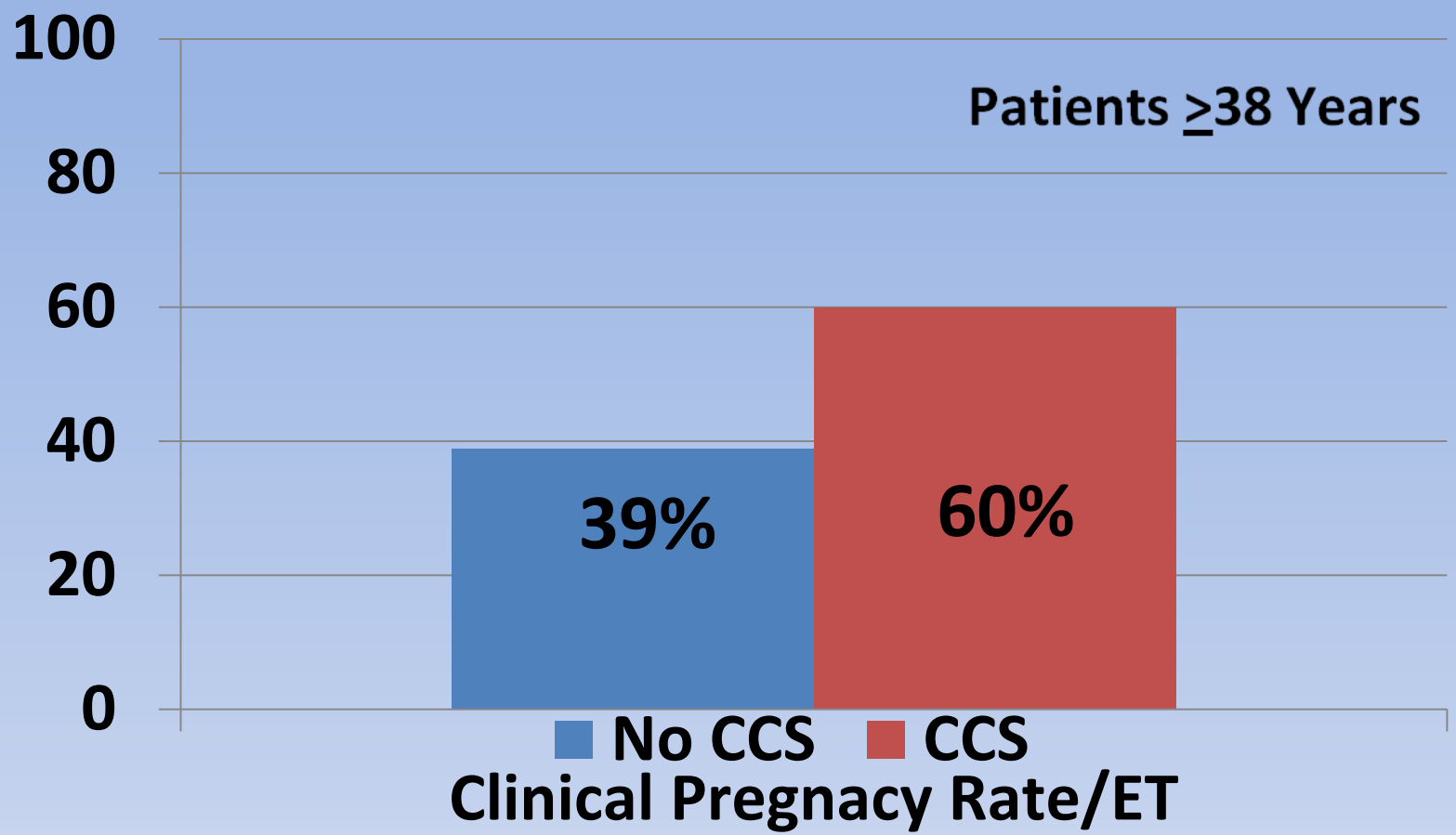
How CCS Affects # of Embryos Transferred:



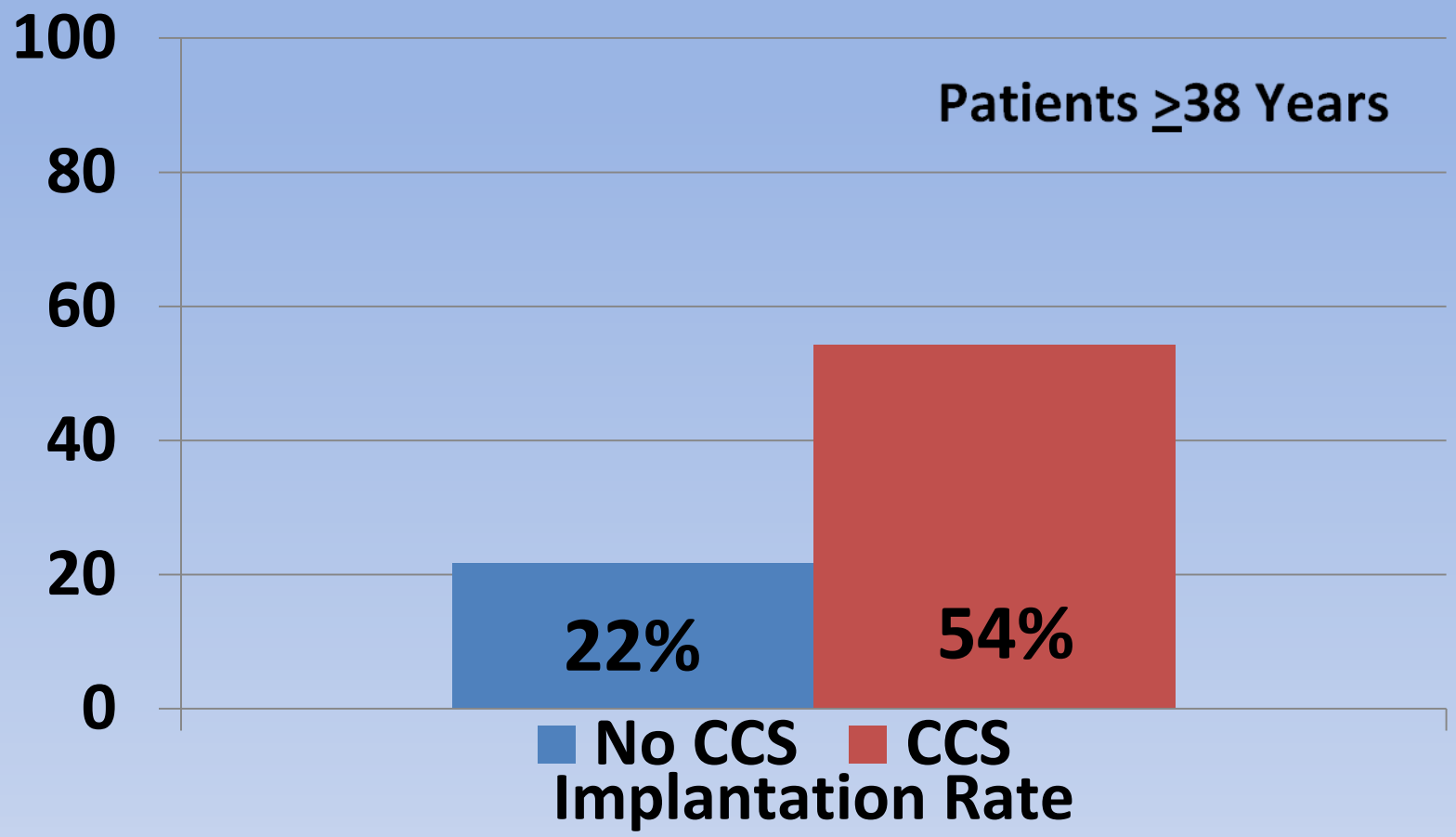
Chemical Pregnancy Rate With & Without CCS



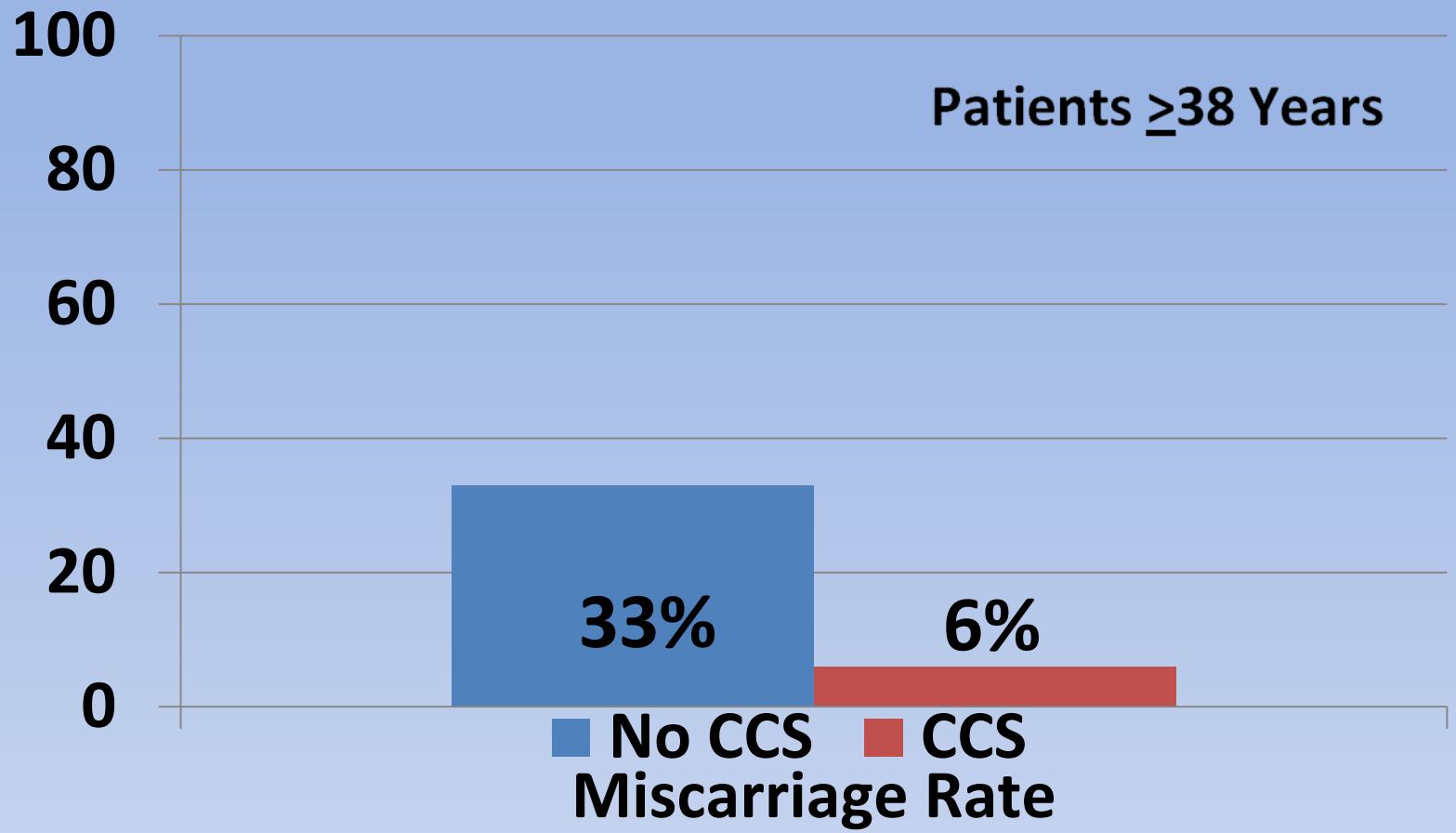
Clinical Pregnancy Rate With & Without CCS



Implantation Rate With & Without CCS



Miscarriage Rate With & Without CCS



Patient #1: (3/13/12 – 2/27/13; 40.7-42 yrs)

Cycle	# Eggs	# Mature	# 2PN	Bx'd	# Abnormal	# Normal
1	11	4	3	1	1	0
2	7	4	3	1	1	0
3	14	6	6	1	1	0
4	10	6	3	0	0	0
5	8	6	6	4	4	0
6	10	6	6	0	0	0
7	16	9	5	0	0	0
8	16	11	8	3	3	0
9	15	10	9	4	3	1
10	22	18	13	3	3	0
11	20	10	7	0	0	0
Total	**149**	**90 (60%)**	**69 (77%)**	**17 (25%)**	**13**	**1***

Patient #2: (10/8/12 – 4/30/13; 40.6-41.2 yrs)

Cycle	# Eggs	# Mature	# 2PN	Bx'd	# Abnormal	# Normal
1	13	11	6	5	5	0
2	8	8	5	3	3	0
3	19	14	12	9	7	2
4	19	13	6	5	5	0
5	15	12	9	7	4	2 (1)
6	17	13	7	4	4	0
7	27	22	18	9	6	3
Total	**118**	**93**	**63 (68%)**	**42 (67%)**	**34**	**7**

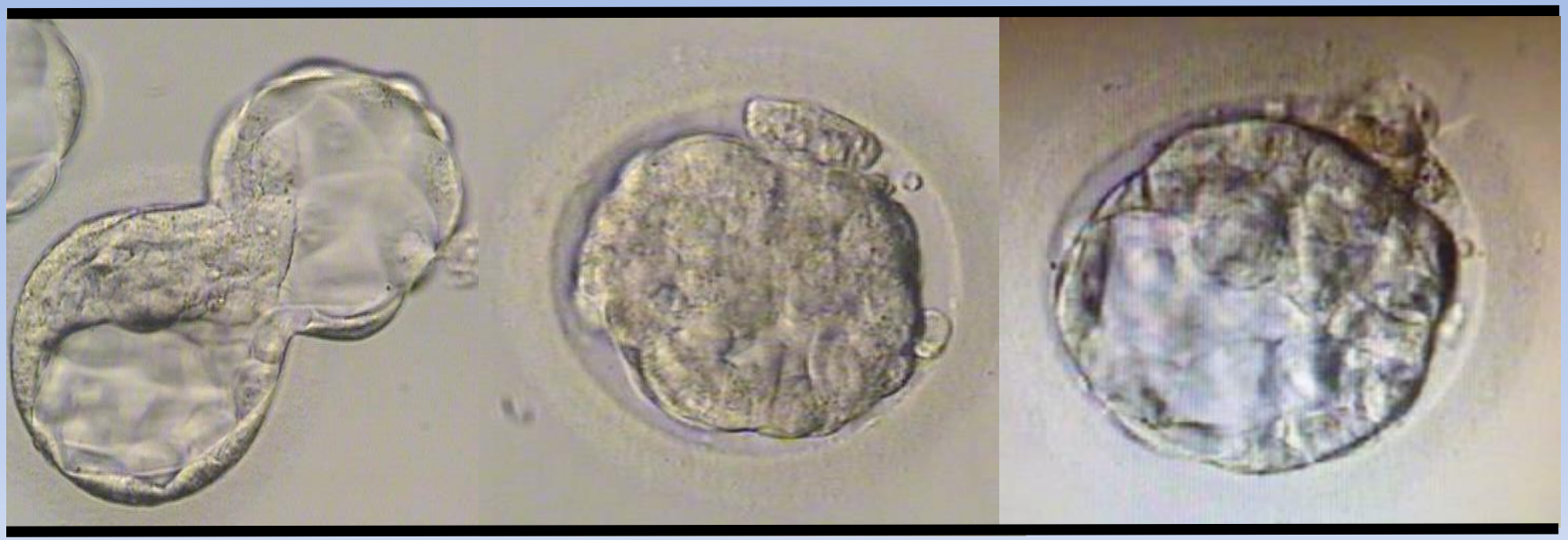
+1 nonconcurrent

2 CCS NORMAL FET'd → Ongoing Twins

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-Biopsy **Post-Biopsy** **At Vit**



Results – Fresh vs Frozen CCS Cycles

	Fresh Day 6	Frozen	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

CCS Vitrification Results

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

2013 CCS with Vitrification Outcomes – All Ages

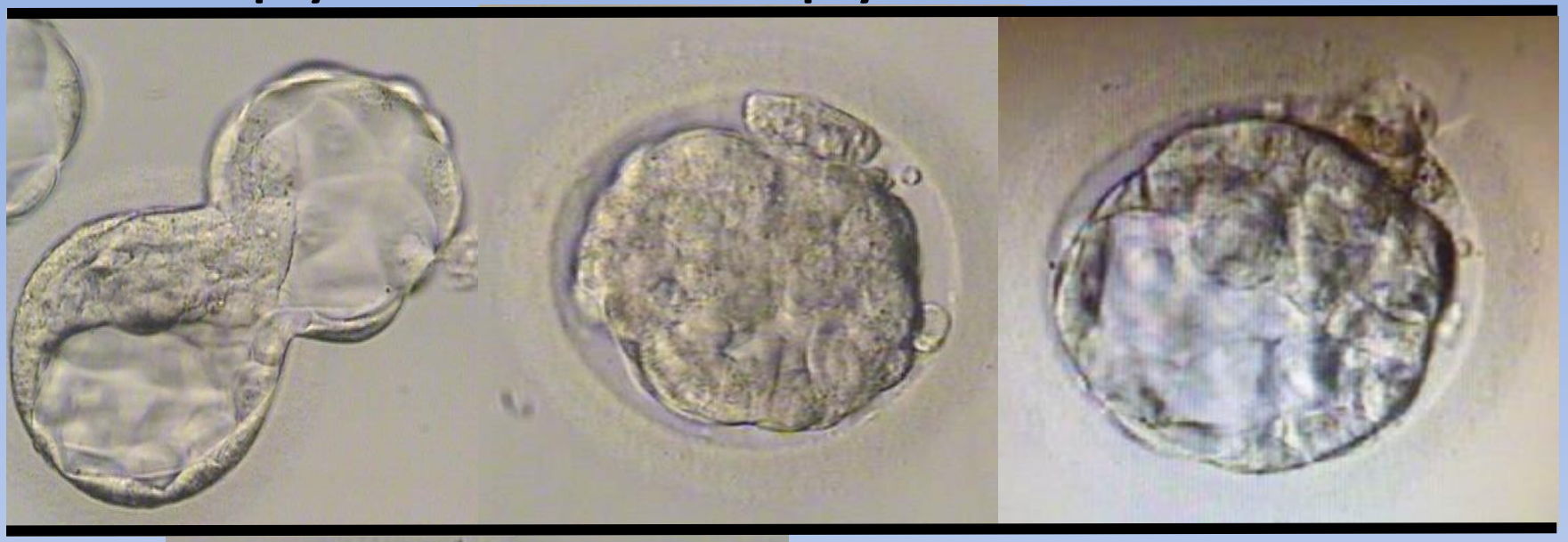
- 2 Euploid ET'd → 80% clinical preg rate

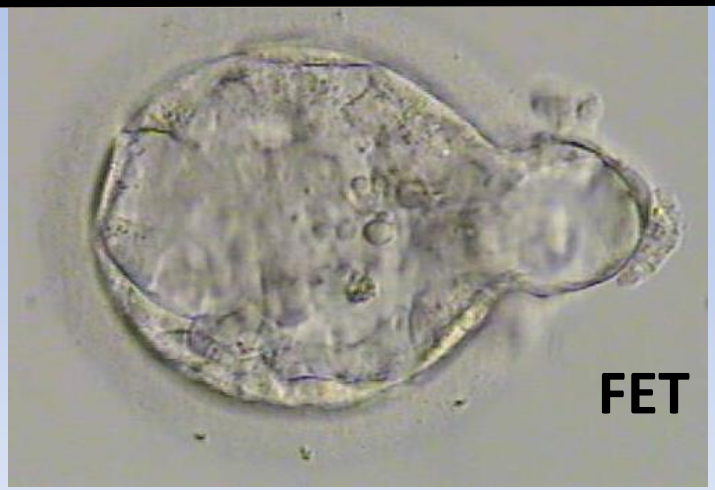
  → 40% twin rate

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

Biopsy, Vit, Thaw & FET of single CCS normal

Pre-Biopsy | Post-Biopsy | At Vit





→ Singleton Delivered

Special Thank you to

Embryology Team:

Jonathan Lo*

Karen Reyes*

Erica Paganetti

EJ Testa

Questions???

Comments???