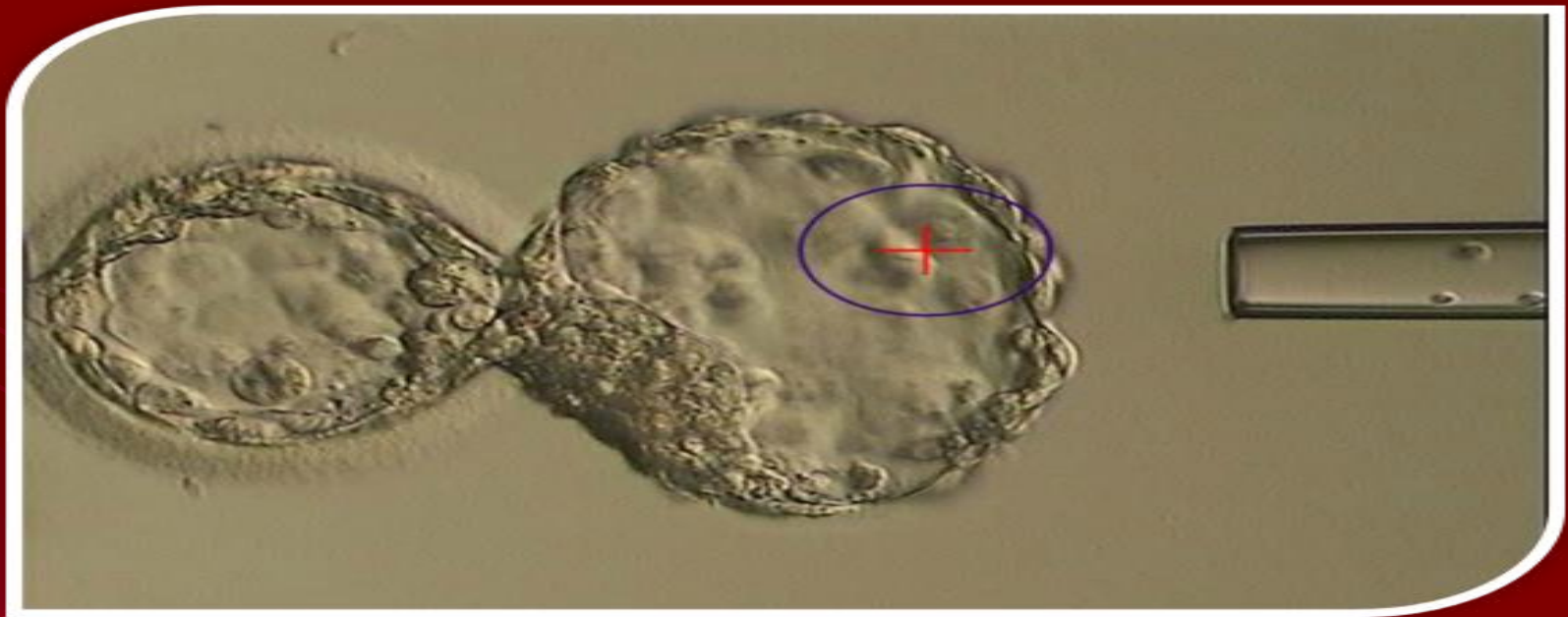


2014 CRB Trophectoderm Biopsy Workshop



Jean M. Popwell, PhD, HCLD

TE Biopsy Discussion

- Biopsy equipment and supplies
- Dish workflow
- Which blastocyst to biopsy?
- Biopsy Videos – The approach is everything!

- Tubing equipment and supplies
- Tubing setup

- Final Tips

TE Biopsy Workshop

- **Hands – On Experience !**
- **Work in Groups with Trainers – your choice:**
 - **Beginners – Never performed any type of embryo biopsy**
 - **Intermediate – Performed Day 3 embryo biopsy**
 - **Advanced – Currently training on Day 5 embryo biopsy**
- **Tubing Station – Wet and “Dry” tubing**

BIOPSY

EQUIPMENT AND SUPPLIES



PGS Setup



PGS Setup



PGS Setup



Embryo Biopsy Personnel

- Biopsy Tech x 1
- Biopsy Sample Tuber x 1
- Cryopreservation Tech x 1

If you are super lucky 😊

SUPPLY LIST

- **Inverted Scope: Joysticks and Mushrooms or Oil syringe controllers**
- **Foot Pedal Controller: Laser activation**
- **Laser setup: Saturn 5, Zilos / Lykos Power setting: 400 – 500**
- **Micropipettes:**
 - 1) **Holding: Humagen (Origio) MPH-LG-30**
 - 2) **Biopsy: Vitrolife: 35um ID, angle 30, REF: 15123**

•Dishes:

- 1) Embryo Culture: IVF Online, Embryo-GPS, REF: EGPS-010**
- 2) Biopsy: Falcon, Petri Dish, REF: 351006**
- 3) Tissue Prep for Tubing: Petri Dish, REF: 351008**

•Media:

- 1) PVP: In Vitro Care, Cat # 2210**
- 2) GMOPS Plus: Vitrolife**
- 3) Oil: Ovoil: Vitrolife**

•Drummonds/Strippers:

- 1) 300 um ID tips**
- 2) 170 um ID tips**
- 3) 130 um ID tip**

Sharpie Markers

BIOPSY SUPPLIES

MICROPIPETTES Vitrolife

TYPE: BIOPSY
SIZE: 35µm ID
ANGLE: 30°

REF 15123 (88-35-30)
LOT 120101
2016-01-31

CE
STERILE R

24
ECTREP

Vitrolife, Inc.
3800 Riverdale Drive, Suite 100
San Diego, CA 92121 USA
800-957-4887 / 619-454-2888
www.vitrolife.com

vitrolife Sweden AB
Sundsvall, Sweden 812 2
30-427 32 9
www.vitrolife.com

HOLDING
For use in in vitro fertilization

REF MPH-SM-30-NTP
LOT 101211174
Quantity: 10
Percent Blastocysts 100%
LAL < 8.83
2014-07
MADE IN THE USA
CE
STERILE R

100 **origio**
HUMAGEN PIPETS

100 origio, Inc.
1000 Main St., Shelton, CT 06484
www.origio.com

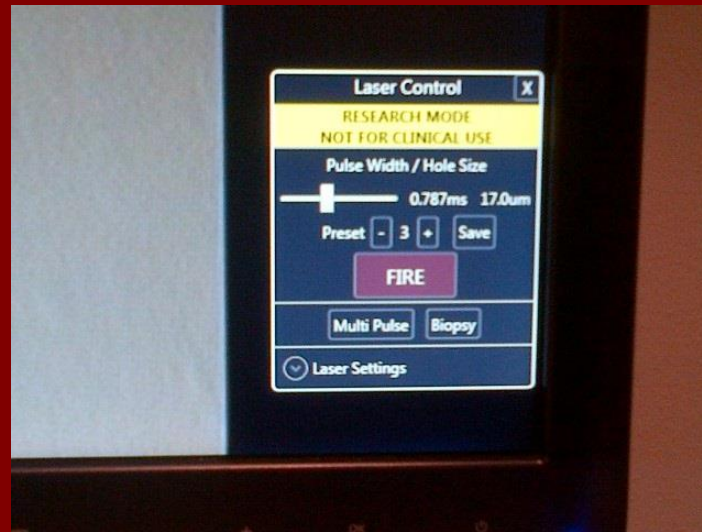


BIOPSY SUPPLIES

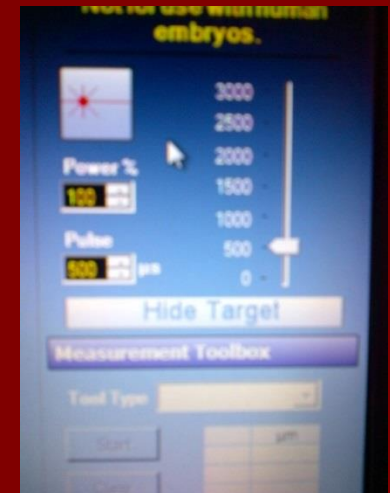


Power and Hole Sizes

- Saturn 5 :
0.787ms or 17.0 um



- Zilos : 450 to 500 power setting



Day 3 Breaching (AH)



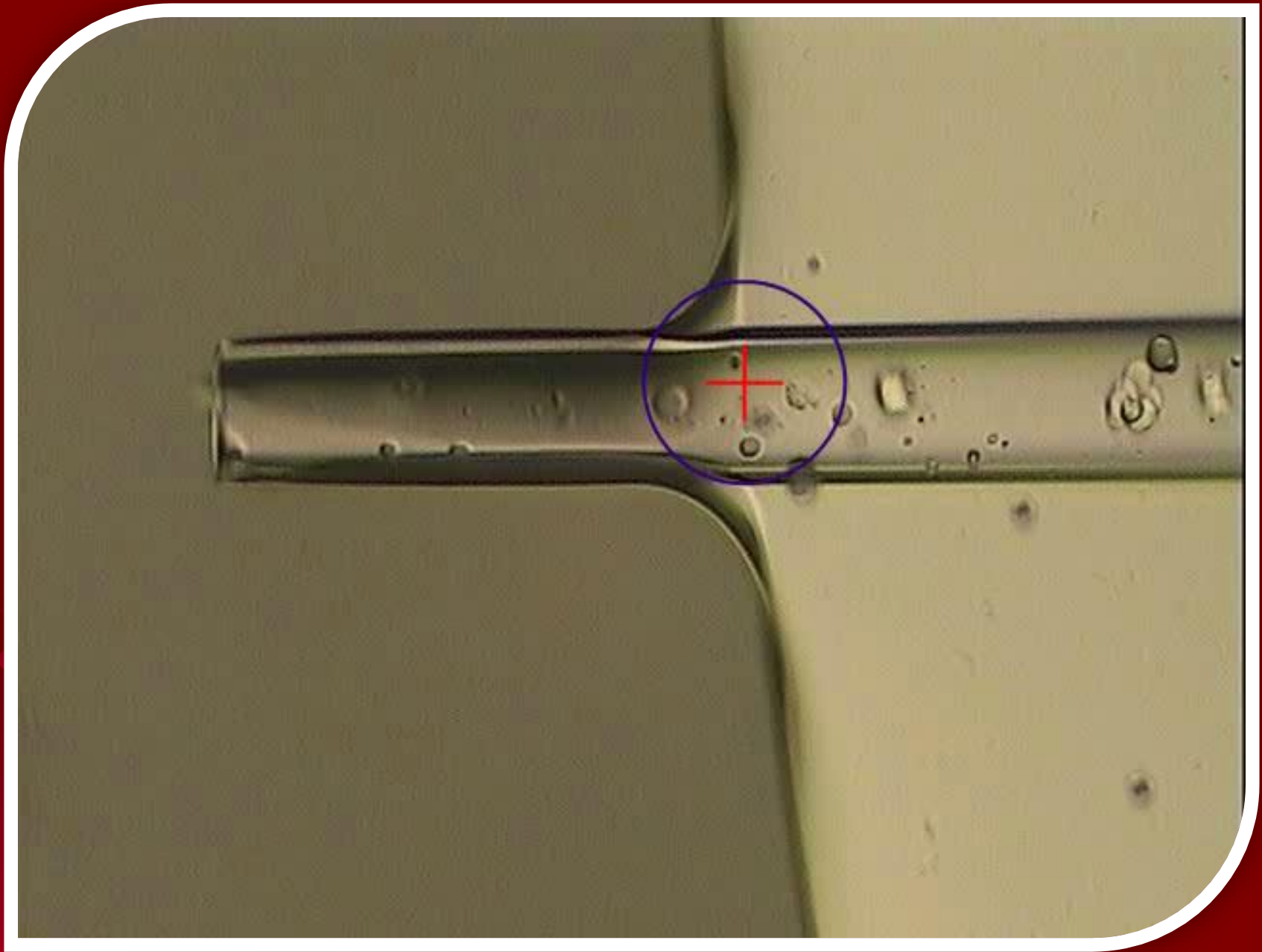
TE BIOPSY

VIDEOS

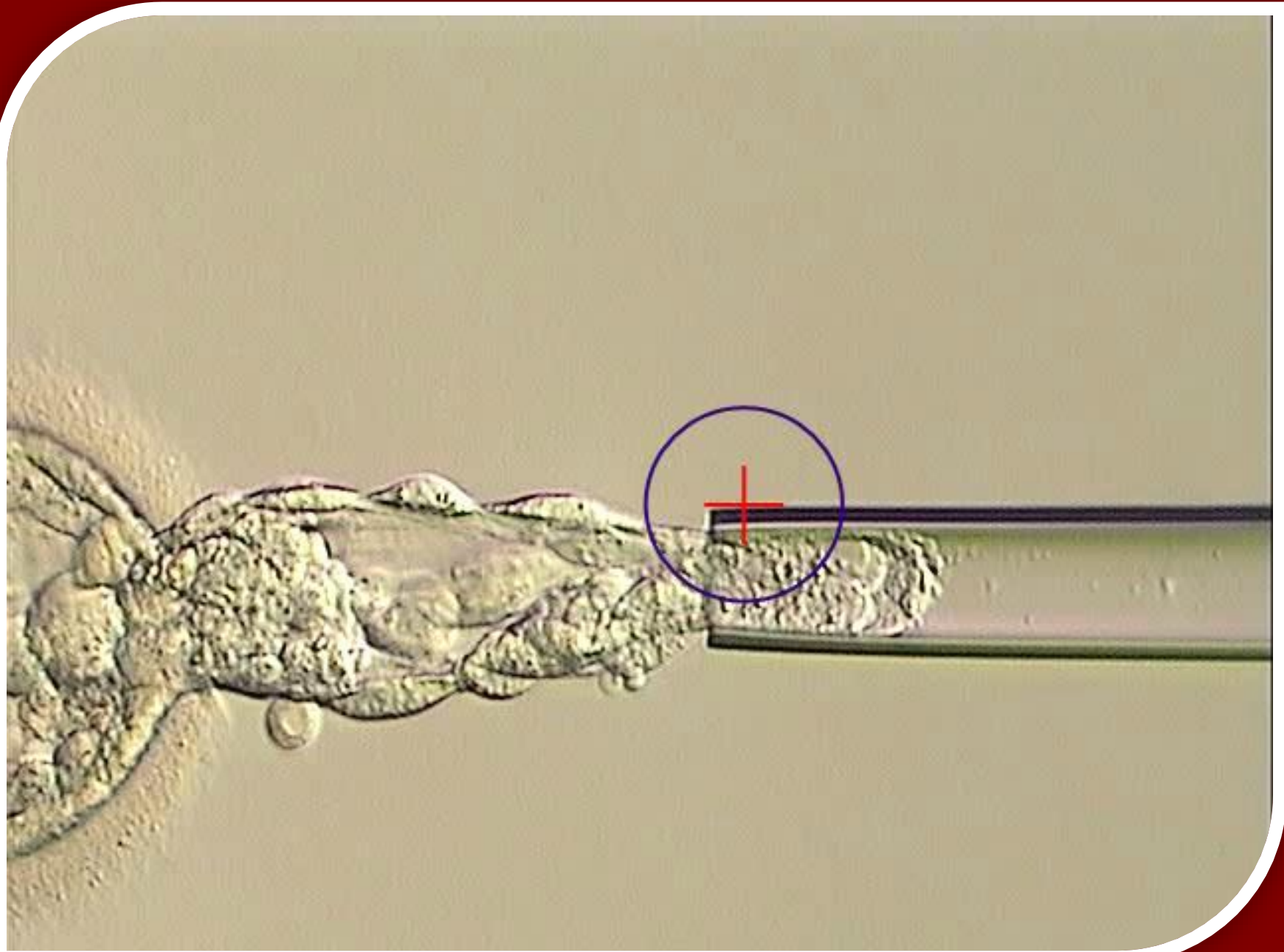


SCOPE SETUP – Crucial Step

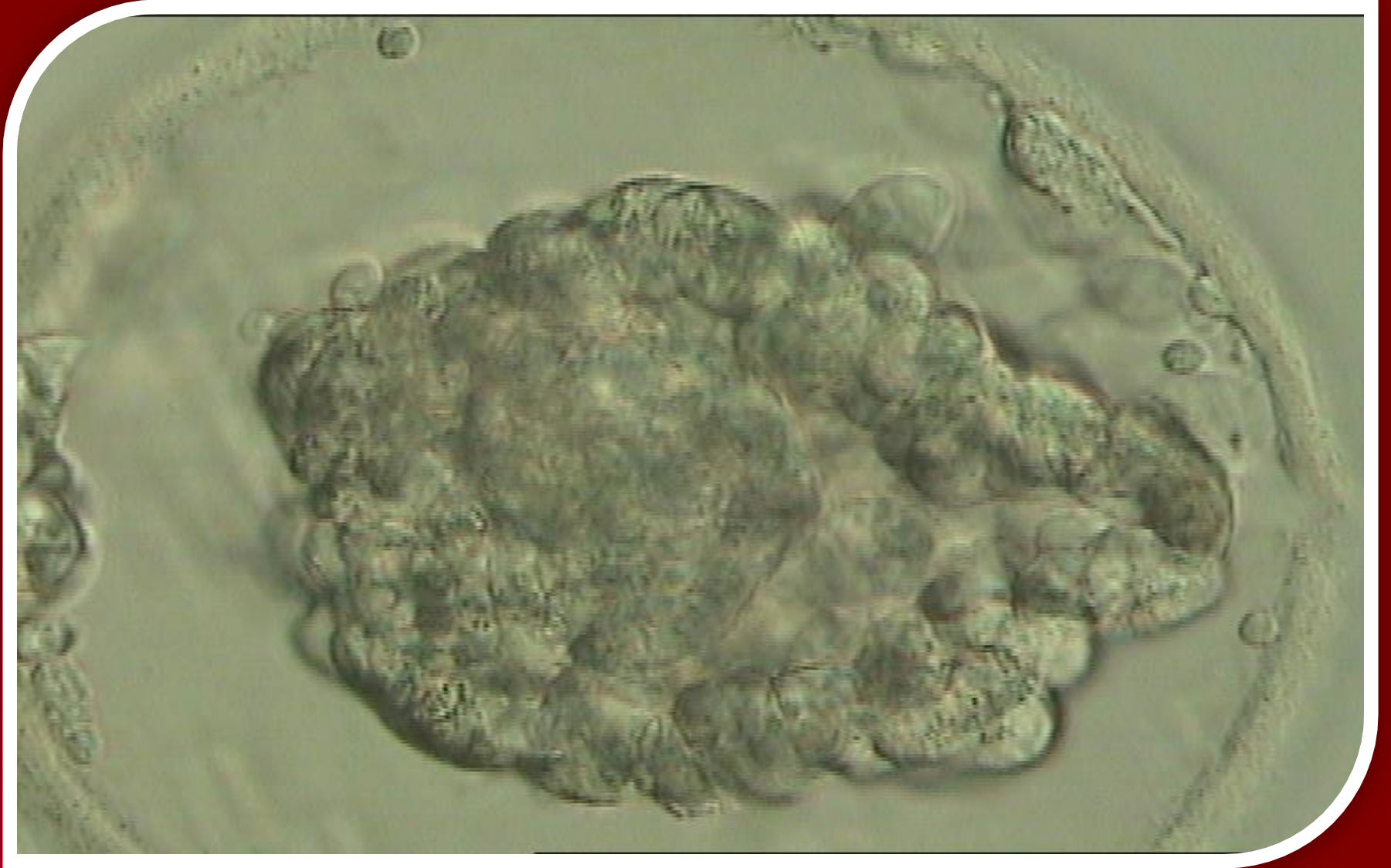




Biopsy Pipette Prep before each embryo: Blow bubbles first, suck in oil followed by PVP



POST BIOPSY COLLAPSE = READY FOR VITRIFICATION



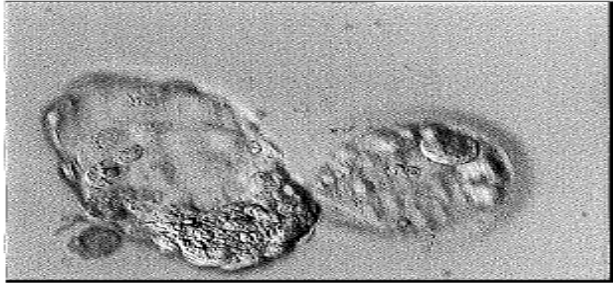
TE BIOPSY

**WHAT SHOULD I
BIOPSY?**

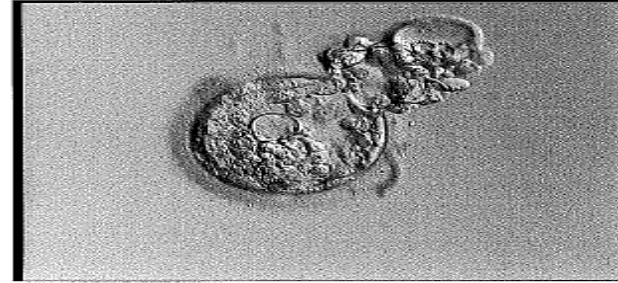


To Biopsy or Not to Biopsy?

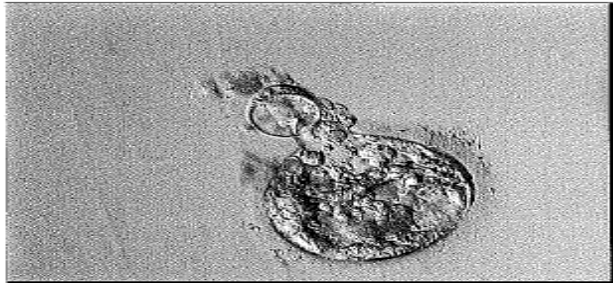
A



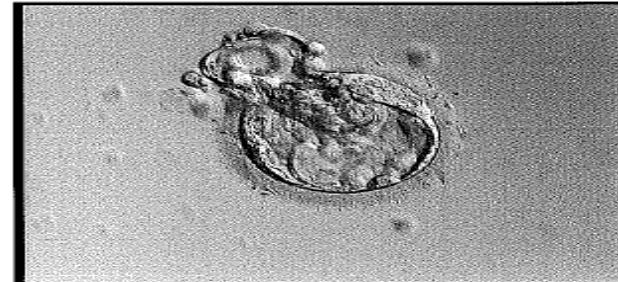
D



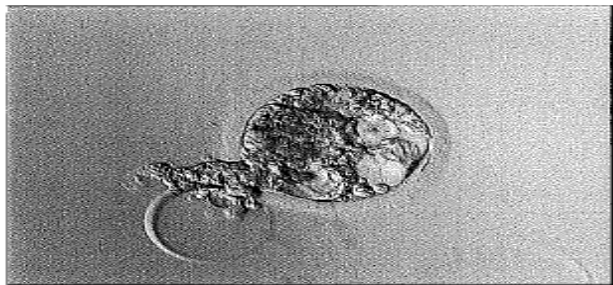
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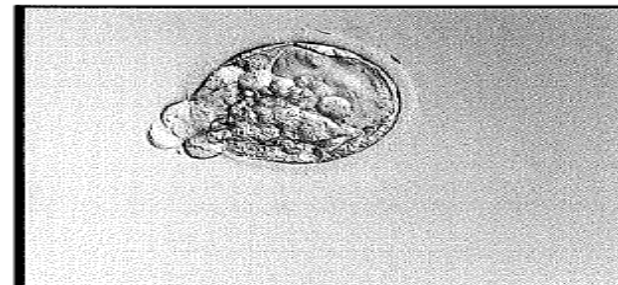
E



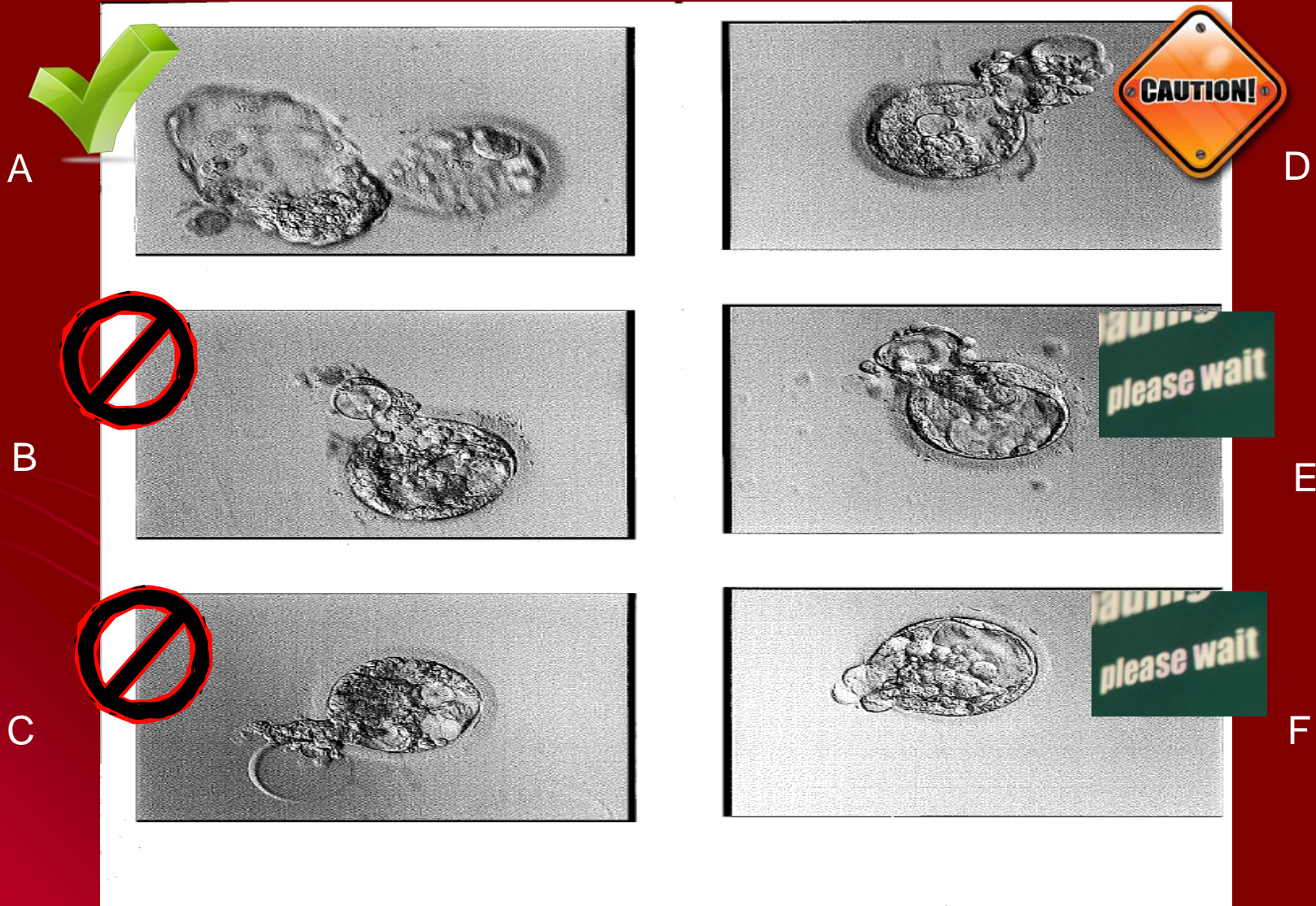
C



F



To Biopsy or Not to Biopsy?



TUBING

EQUIPMENT AND SUPPLIES



Tubing Attire



- Hat
- Gloves
- Mask
- Lab Coat

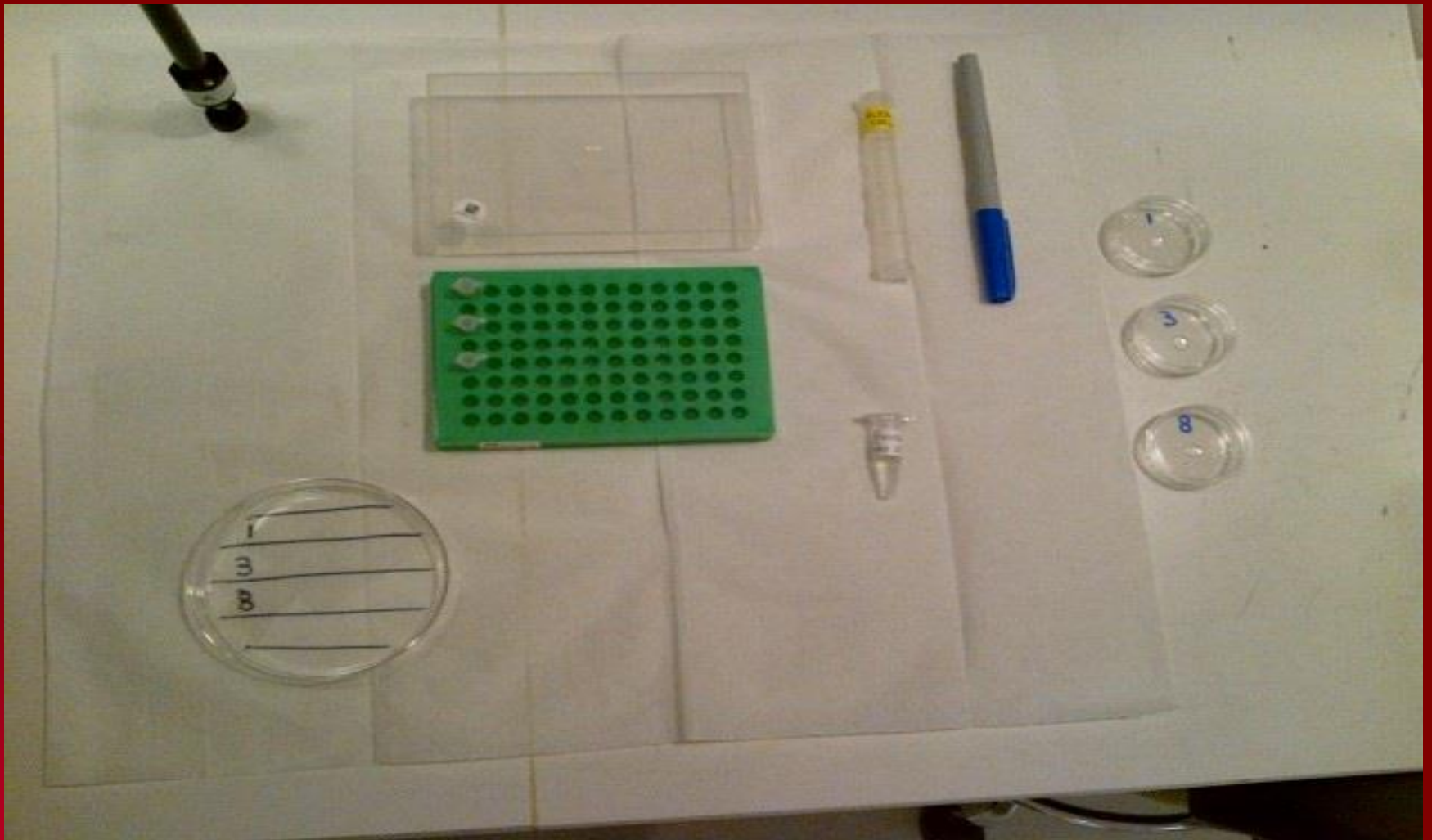
Tube Prep



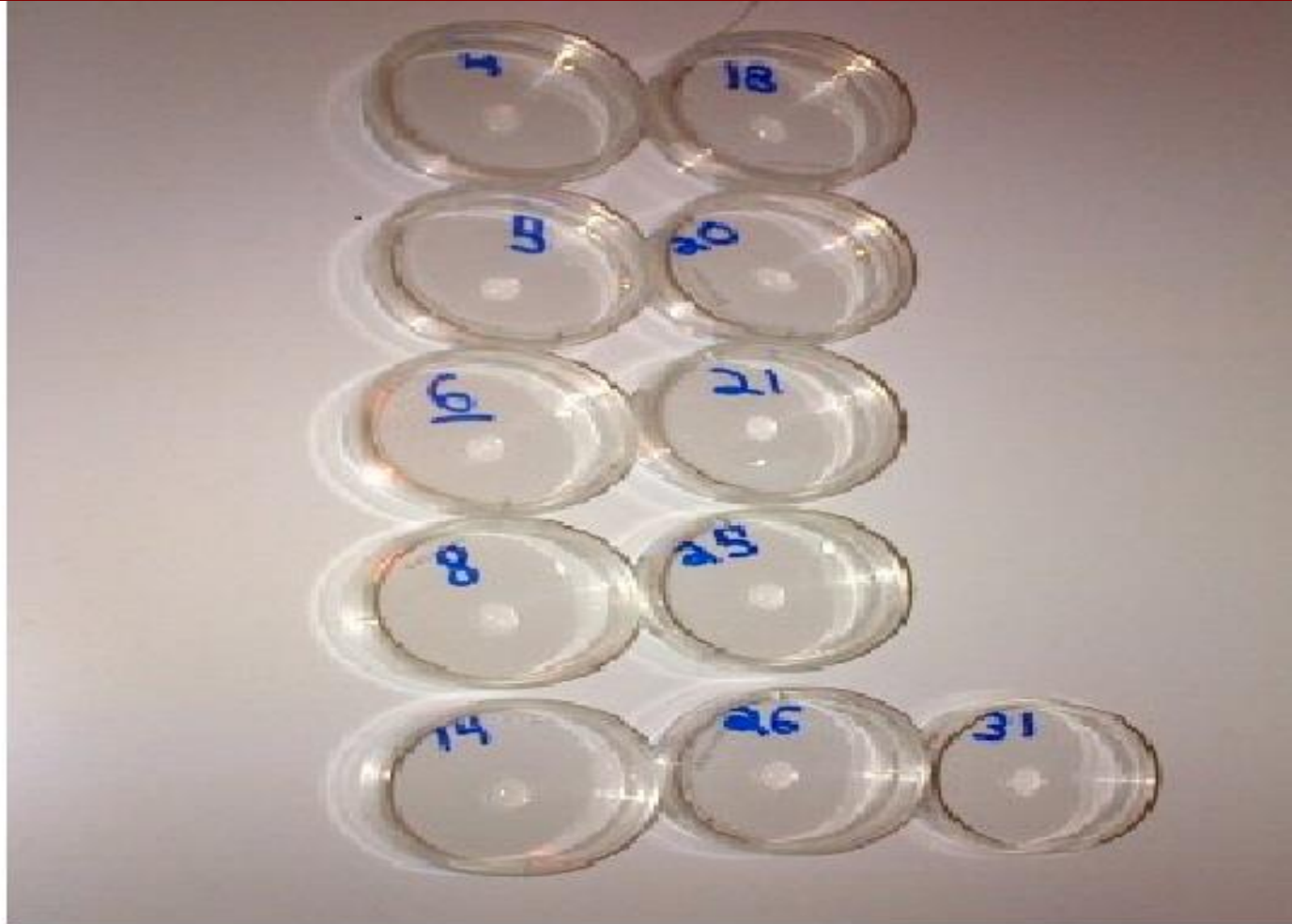
Tube Prep



Tubing Setup



Samples ready for Tubing





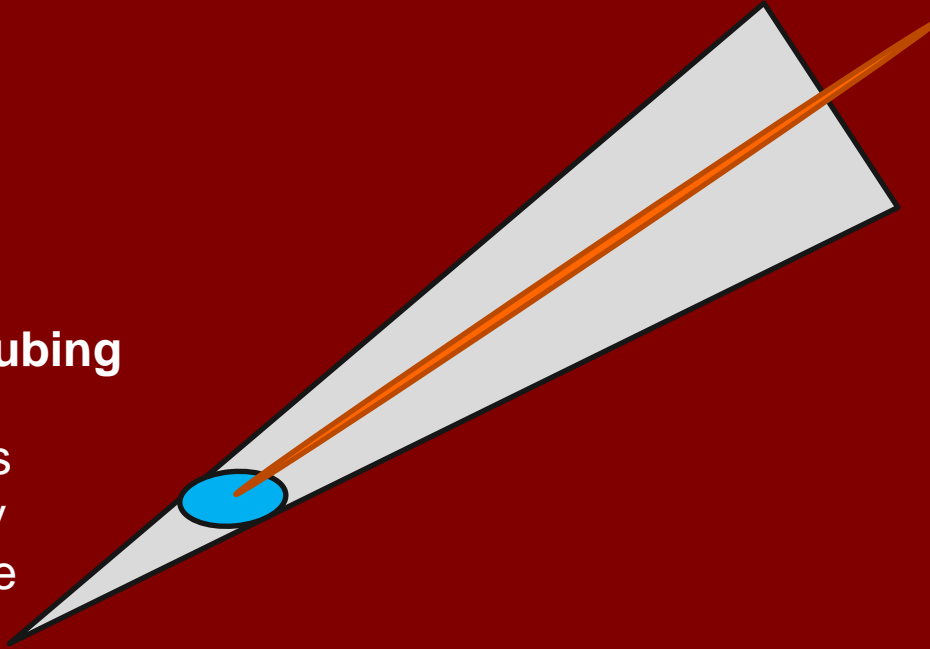




Tubing

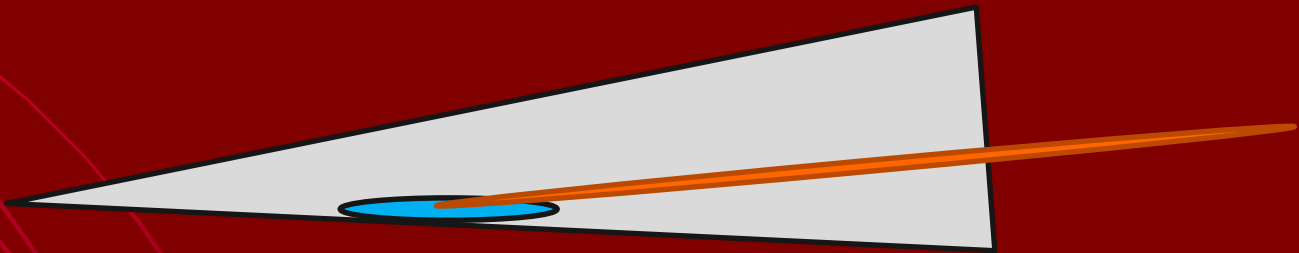
2 uL and 5 uL Tubing

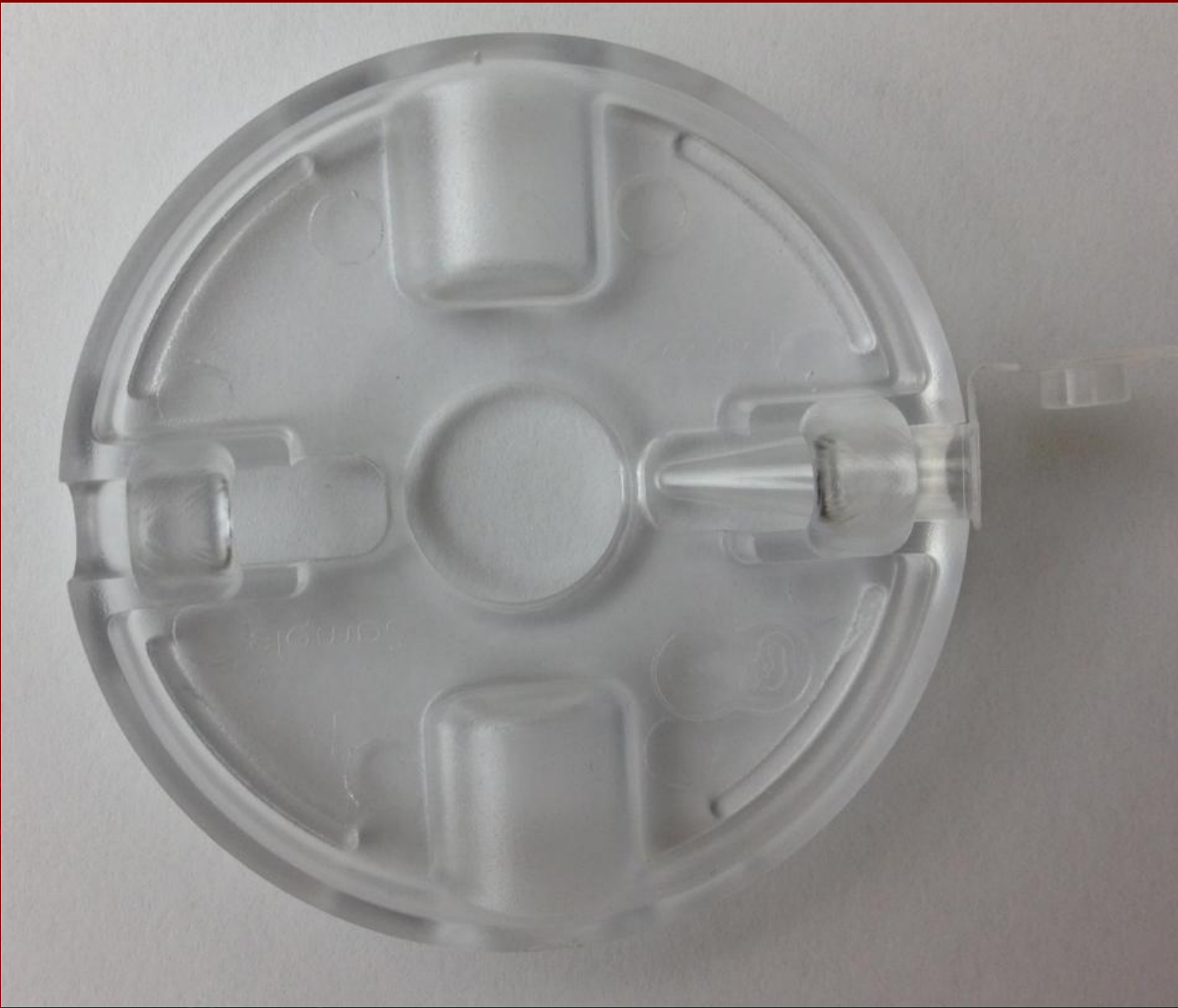
Aim for meniscus of liquid to visibly see sample leave the end of the stripper tip



Aim for flat side of tube, expel drop and smear to see sample leave the end of the stripper tip

“Dry” Tubing, 1 uL



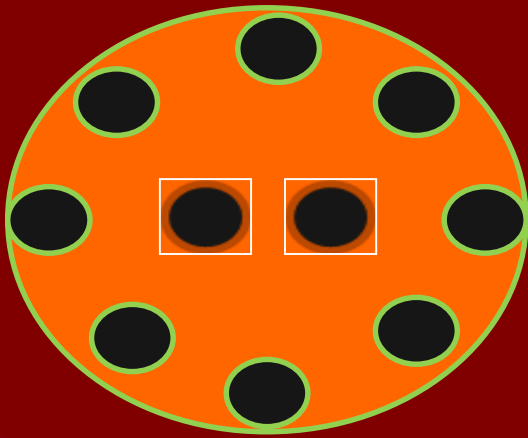


BIOPSY

DISH WORKFLOW



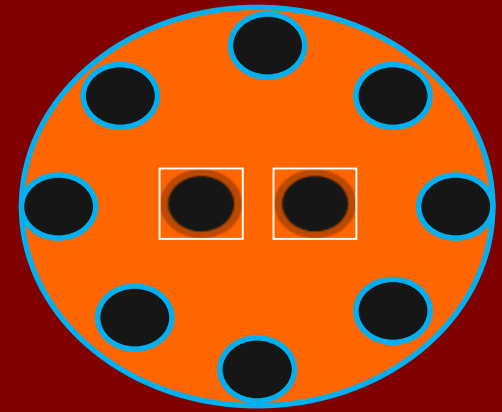
Day 3 to Day 5 Dish Workflow



Day 3 – G1 Plate



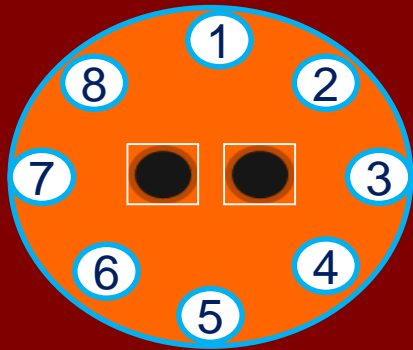
Assisted Hatching
(Breaching) of all
embryos



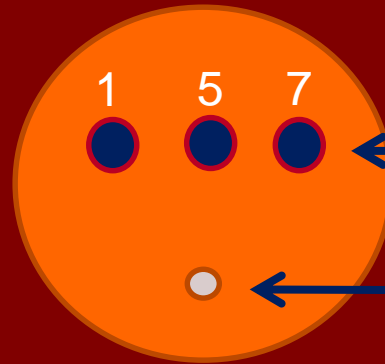
Day 5 – G2 Plate



Day 5 Dish Workflow



Day 5 Culture plate - G2



Biopsy Dish

to Biopsy = # biopsy drops

30 uL of G-MOPS

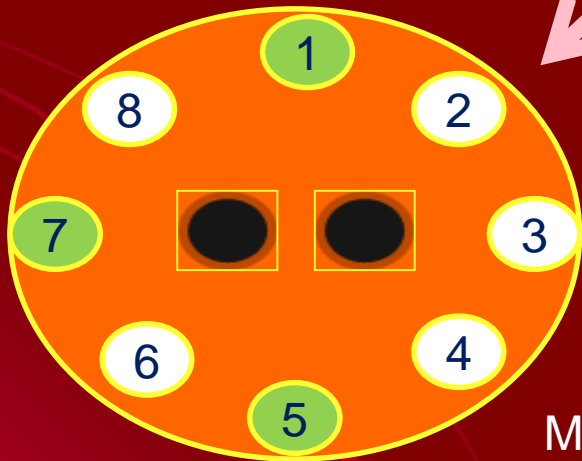
10 uL of PVP

Rinse post biopsy
in a 4-well of G-2



40 uL of
G-MOPS

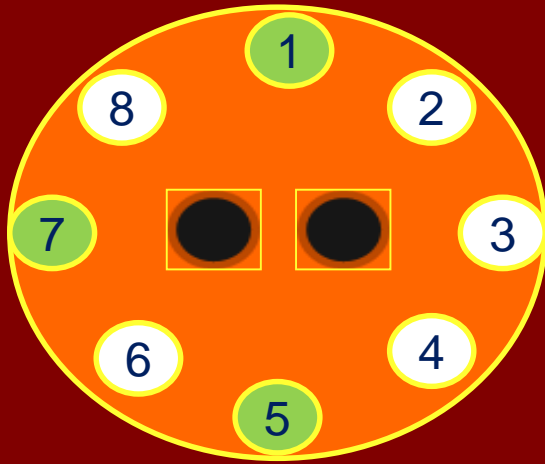
Sample waits to be Tubed



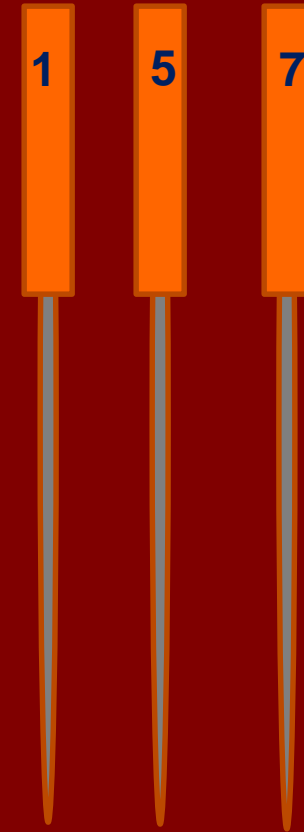
Move all embryos to GPS dish

GPS Culture Dish – 40 uL wells of G2

Day 5 Dish Workflow



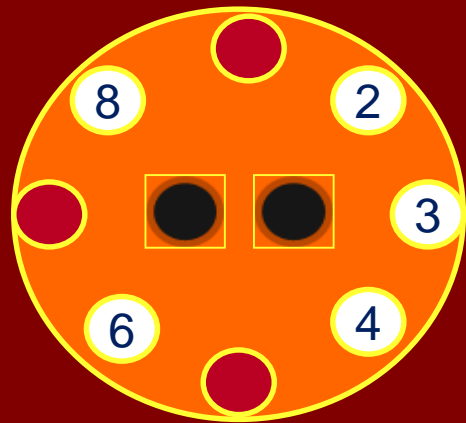
Artificial Collapse of
biopsied blastocyst



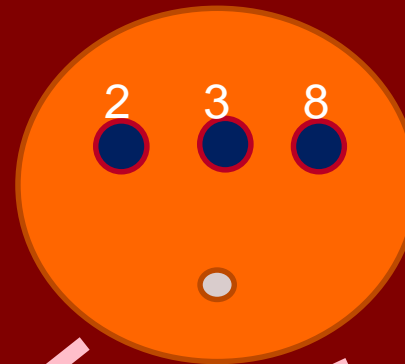
Vitrification

GPS Culture Dish –
40 uL wells of G2

Day 6 Dish Workflow



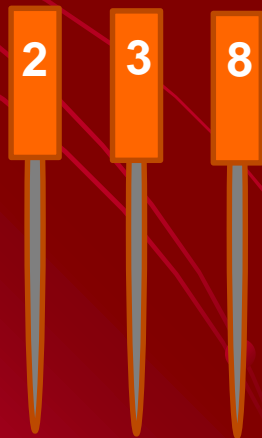
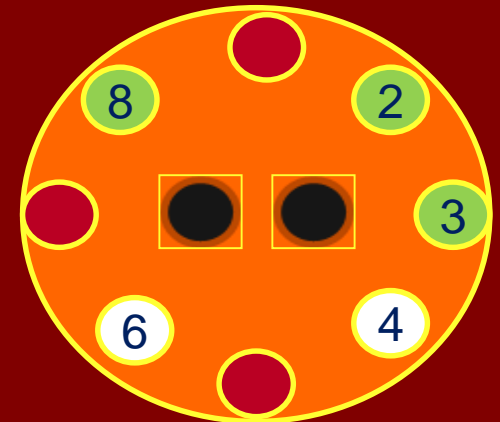
GPS Culture Dish –
40 uL wells of G2



Biopsy Dish



Sample waits to be Tubed



Vitrification

Why is Day 5 Biopsy Better ?

The blastocyst embryo is the preferred embryonic stage for embryo biopsy prior to PGS/PGD based on:

- decreased number of embryos to biopsy compared to Day 3, biopsy only the best quality embryos
- the ability to safely remove multiple cells for analysis
- a decreased mosaicism rate

Pros and Cons of Day 5 Biopsy

PROS of Day 5 Biopsy

- Ability to biopsy fewer but better quality advanced embryos
- Re-Biopsy is a rare event
- No Ca/Mg free media involved during embryo biopsy – take your time!

CONS of Day 5 Biopsy

- Some patients do not grow good Day 5 embryos in culture = no biopsy (Patient education)
- MUST have a superior Blastocyst Culture and freezing system, with great recovery and survival upon thawing

Aneuploidy Screening (PGS/CCS)

- Primary type of screening requested among IVF patients, as the average female patient age has increased towards age 40



2013

CCS Cases Only

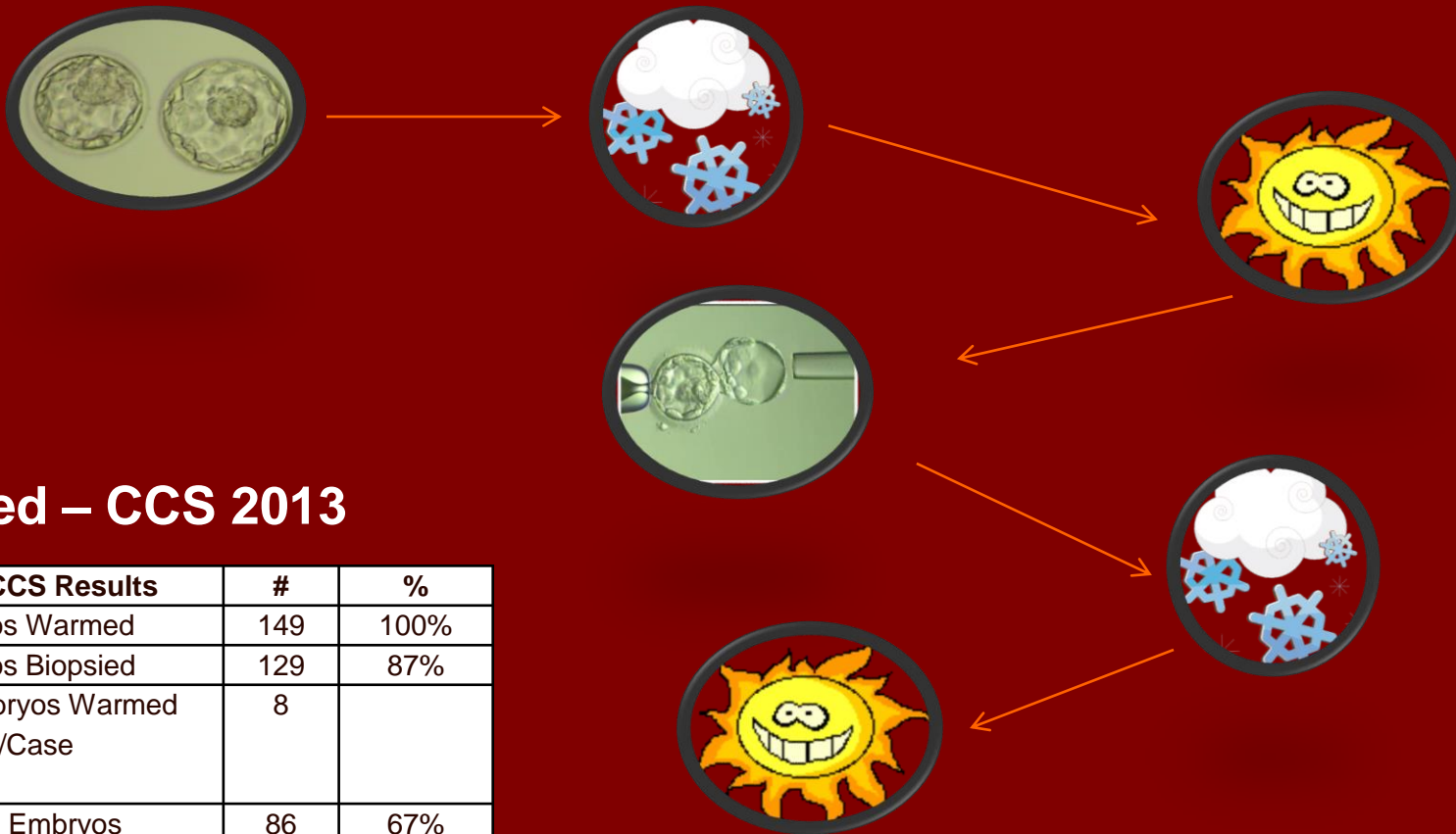
	<35	35-37	38-40	41-42	>42	OD	Total
Transfers	71	56	56	12	8	47	250
Clinical Pregnancies	34	37	42	9	4	35	161
Clinical Pregnancy Rate	0.48	0.66	0.75	0.75	0.50	0.74	0.64
Embryos Transferred	78	58	61	12	8	54	271
Embryos Implanted	38	41	45	9	4	40	177
Implantation Rate	0.49	0.71	0.74	0.75	0.50	0.74	0.65

FETs w/out CCS

	<35	35-37	38-40	41-42	>42	OD	Total
Transfers	100	36	35	5	1	131	308
Clinical Pregnancies	53	19	19	2	0	67	160
Clinical Pregnancy Rate	0.53	0.53	0.54	0.40	0.00	0.51	0.52
Embryos Transferred	122	42	51	7	5	167	394
Embryos Implanted	60	21	28	2	0	74	185
Implantation Rate	0.49	0.50	0.55	0.29	0.00	0.44	0.47

All FETs

	<35	35-37	38-40	41-42	>42	OD	Total
Transfers	171	92	91	17	9	178	558
Clinical Pregnancies	87	56	61	11	4	102	321
Clinical Pregnancy Rate	0.51	0.61	0.67	0.65	0.44	0.57	0.58
Embryos Transferred	200	100	112	19	13	221	665
Embryos Implanted	98	62	73	11	4	114	362
Implantation Rate	0.49	0.62	0.65	0.58	0.31	0.52	0.54



Warmed – CCS 2013

Table 1: W-CCS Results	#	%
Total Embryos Warmed	149	100%
Total Embryos Biopsied	129	87%
Average Embryos Warmed and Biopsied/Case	8	
Total Euploid Embryos	86	67%
Total Aneuploid Embryos	43	33%
Average Euploid Embryos/Case	5	67%

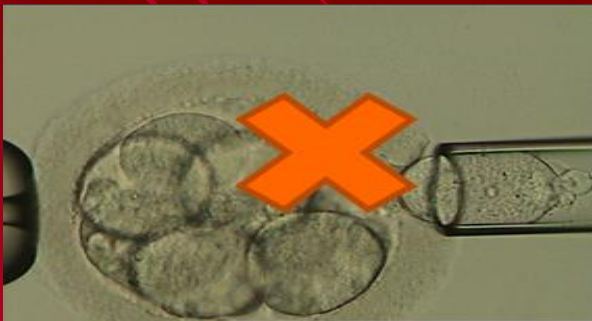
Table 2: Pregnancy/Implantation Rates	IVF/FET/ W-CCS 2013		IVF/FET/ CCS 2012
	#	%	
W-CCS Survival Rate	15	100%	
Total Transfers Completed	13*	100%	90
Clinical Pregnancy Rate	8	62%	62%
Implantation Rate	9	60%	61%

* 11 single embryo transfer (eSET) , 2 double embryo transfer (DET)

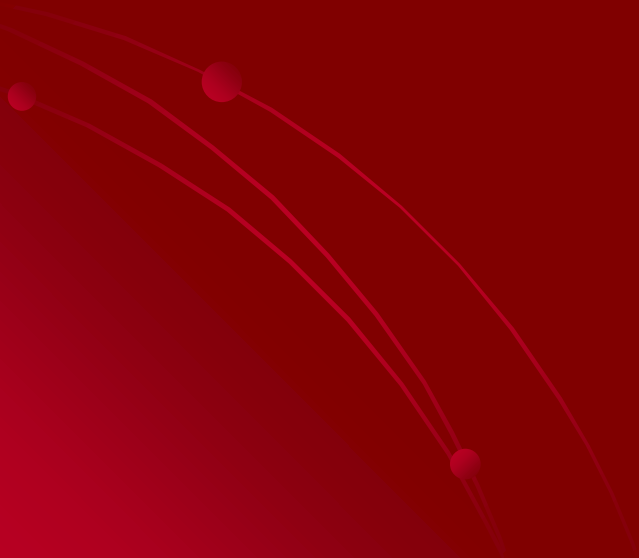
Day 5 vs Day 3

The replacement of a single unaffected euploid embryo in IVF cycles:

- decreases the multiple rate
- improves clinical pregnancy rates
- decreases SABs/RPLs



TE BIOPSY - FINAL TIPS



Quiz:

Good or Bad for TE Biopsy ?



Warmer



PVP



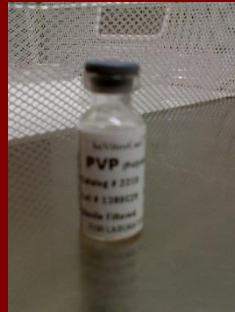
Ca/Mg Free Media

Quiz:

Good or Bad for TE Biopsy ?



Warmer



PVP



Ca/Mg Free Media

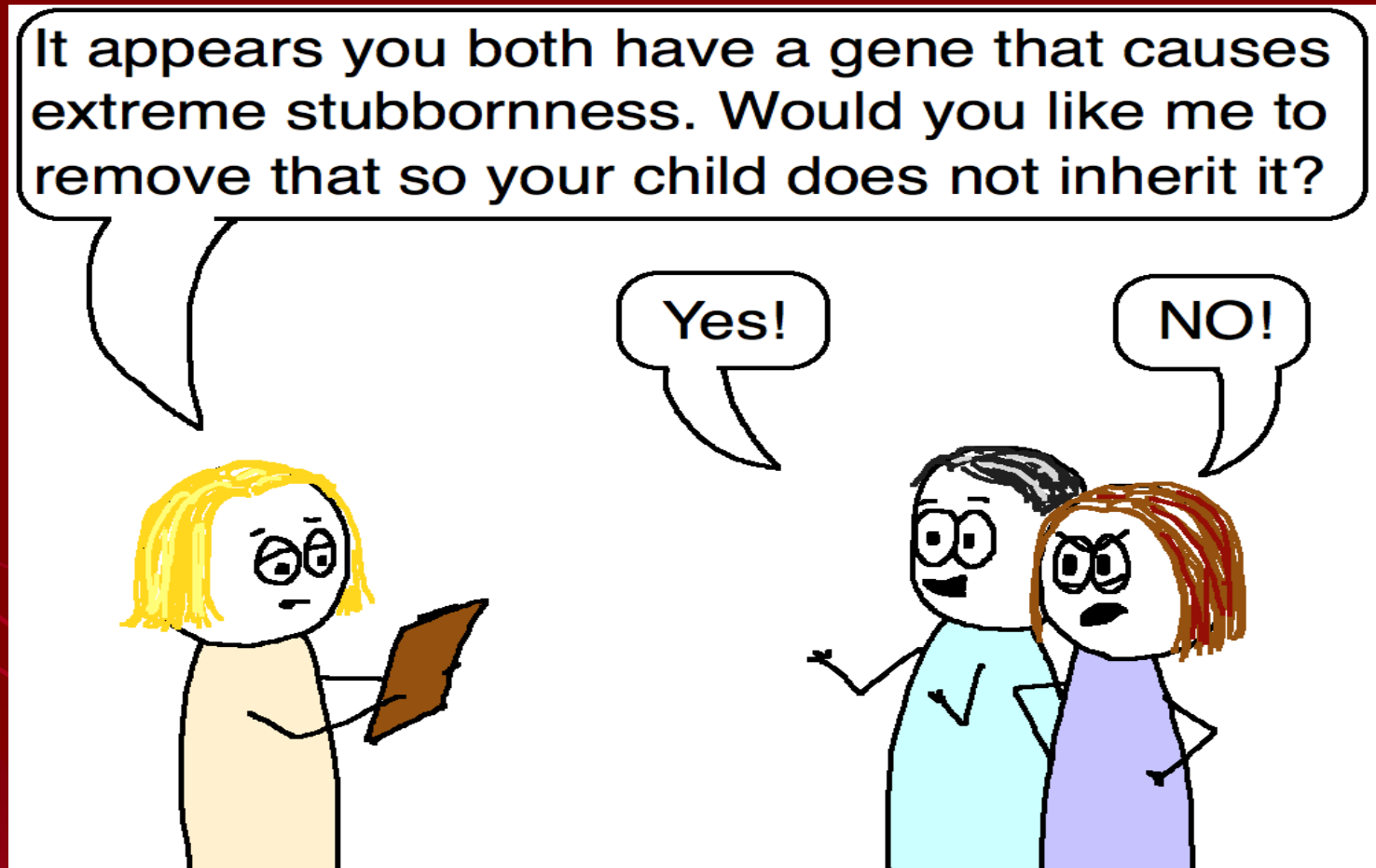
Final Tips: DANGER: DO NOT !

- Never use Ca^2/Mg^2 free media for Day 5 Biopsy : Blasts do not re-expand post biopsy and may die
- Never leave excess oil on samples or layer oil in tubes for analysis : may interfere with sample analysis
- Never leave biopsied samples on a warmer : leads to DNA degradation

Final Tips: For Good Results

- Perform QC on your laser BEFORE biopsy
- Take 4-6 cells from each blastocyst
- Read the reference genetics lab instructions BEFORE tubing: (5ul or 2ul or “dry” 1ul or less) , and tube/kit labeling if required
- Use a new stripper tip for each sample during tubing
- Insert tip first, then visualize the sample leaving the tip through the microscope
- Freeze samples in freezer (-20C) before shipping
- Ship samples on dry ice pellets

Any Questions ??



HANDS ON - WORKSHOP

- **Work in Groups with Trainers – your choice:**

GROUP A and B

Beginners – Never performed any type of embryo biopsy

GROUP C

Intermediate – Performed Day 3 embryo biopsy

GROUP D

Advanced – Currently training on Day 5 embryo biopsy

- **Tubing Station – Wet and “Dry” tubing**