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
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
Power and Hole Sizes

- **Saturn 5**: 0.787 ms or 17.0 μm

- **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?
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
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
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

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

# to Biopsy = # biopsy drops

30 uL of G-MOPS

10 uL of PVP

GPS Culture Dish – 40 uL wells of G2

Sample waits to be Tubed

Move all embryos to GPS dish
Day 5 Dish Workflow

Artificial Collapse of biopsied blastocyst

GPS Culture Dish – 40 uL wells of G2

Vitrification
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

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#### FETs w/out CCS

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#### All FETs

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<td>0.58</td>
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Table 1: W-CCS Results

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<tr>
<td>Total Embryos Warmed</td>
<td>149</td>
<td>100%</td>
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<tr>
<td>Total Embryos Biopsied</td>
<td>129</td>
<td>87%</td>
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<tr>
<td>Average Embryos Warmed and Biopsied/Case</td>
<td>8</td>
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<tr>
<td>Total Euploid Embryos</td>
<td>86</td>
<td>67%</td>
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<tr>
<td>Total Aneuploid Embryos</td>
<td>43</td>
<td>33%</td>
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<tr>
<td>Average Euploid Embryos/Case</td>
<td>5</td>
<td>67%</td>
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Table 2: Pregnancy/Implantation Rates

<table>
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<th>IVF/FET/W-CCS 2013</th>
<th>IVF/FET/CCS 2012</th>
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<tr>
<td>W-CCS Survival Rate</td>
<td>15 100%</td>
<td></td>
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<tr>
<td>Total Transfers Completed</td>
<td>13* 100%</td>
<td>90</td>
</tr>
<tr>
<td>Clinical Pregnancy Rate</td>
<td>8   62%</td>
<td>62%</td>
</tr>
<tr>
<td>Implantation Rate</td>
<td>9    60%</td>
<td>61%</td>
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* 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 Ca²/Mg² 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
It appears you both have a gene that causes extreme stubbornness. Would you like me to remove that so your child does not inherit it?

Yes!

NO!
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