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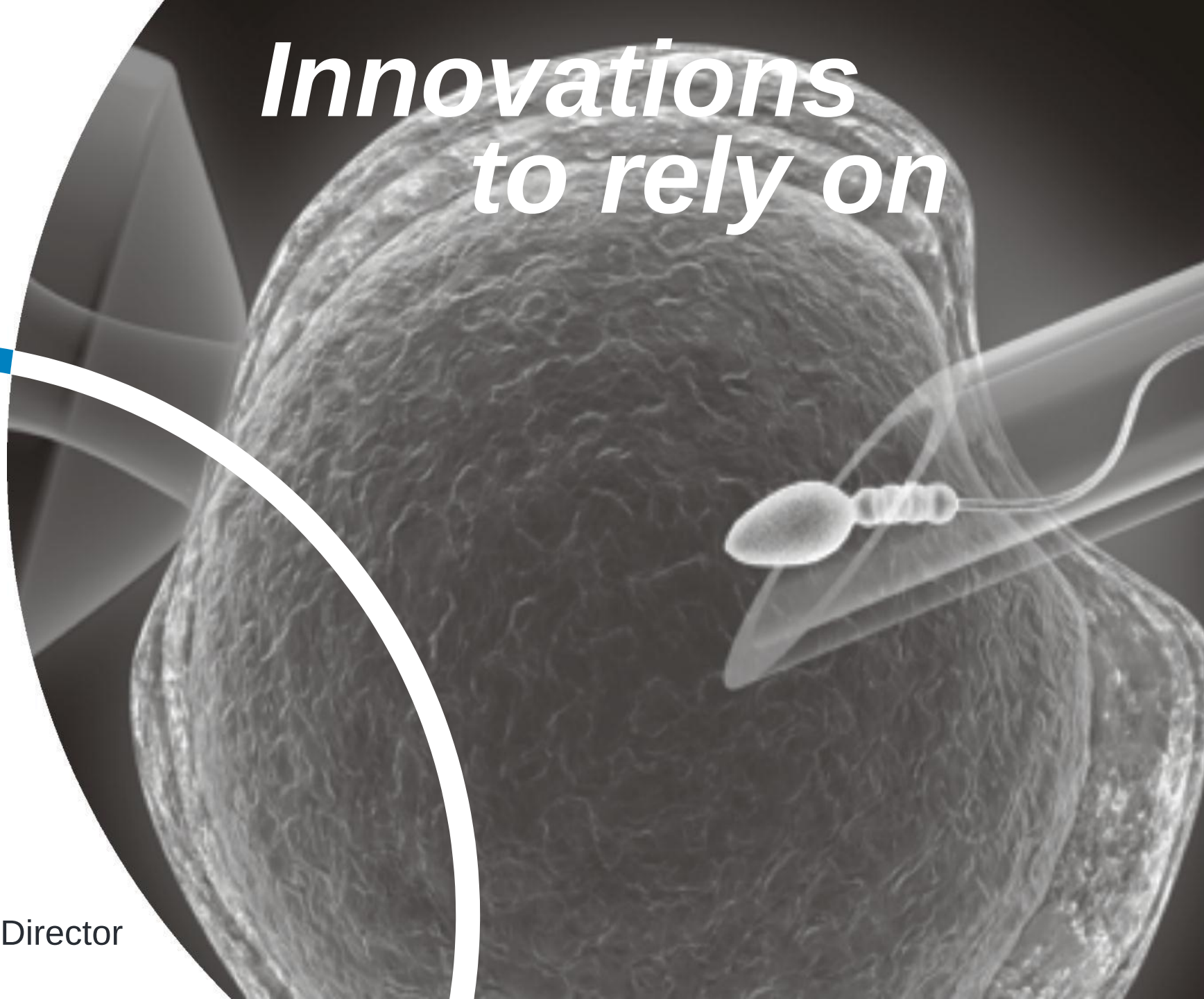
Innovations to rely on



Setting Up for Success – Tips and Tricks

October 2019

Catherine Welch, Regional Sales Director

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OVERVIEW

- Why TE Biopsy
- Laser Preparation
- Manipulation Preparation
- Assisted Hatching
- Biopsy
- Troubleshooting

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WHY TE BX?

- More material/more nuclei/more DNA
- Possible to reduce learning curve of technique
- Reduce error rate of PGT-A
- Less embryos to process
- Facilitate single embryo transfer

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WHY NOW?

- Improvements in IVF
- Higher yield of blastocysts
- ‘Better’ blastocysts
- ‘Better’ cryopreservation/vitrification

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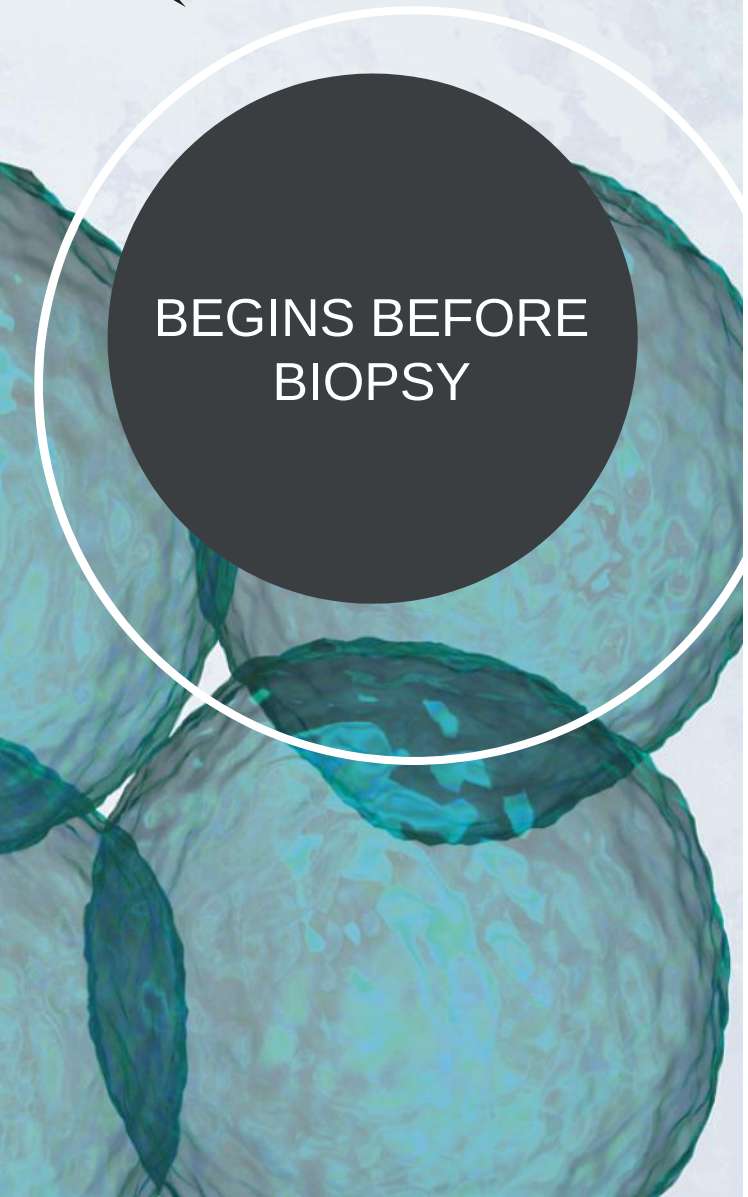
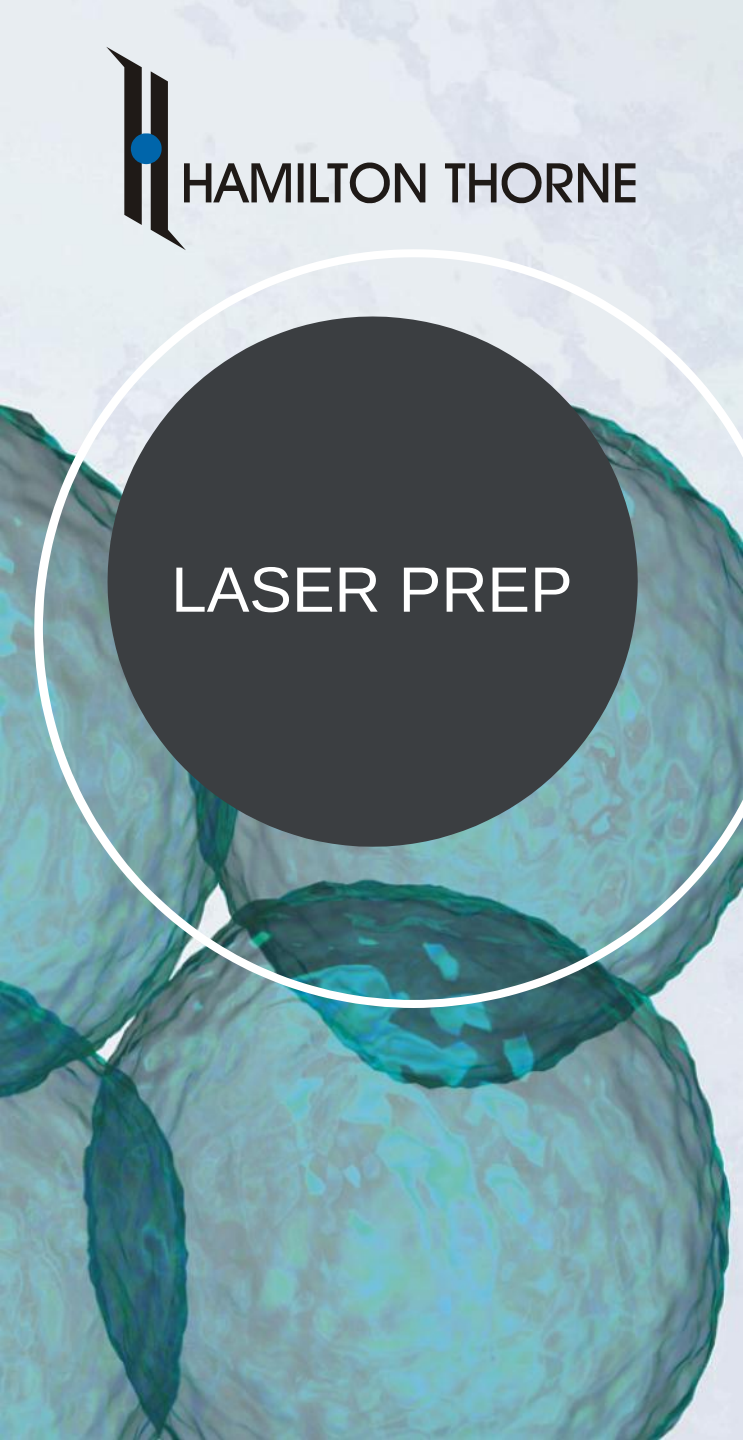


BEGINS BEFORE BIOPSY

- Know your equipment
- Set-up, set-up, set-up
- Confidence
- Adjust, as needed



- Checking laser alignment
  - Dangers of misdirected alignment
  - Use dry erase marker
  - Use dish that will be used for manipulation
  - Adjusting red eye
  - Ensure parfocal image
  - Changing magnification

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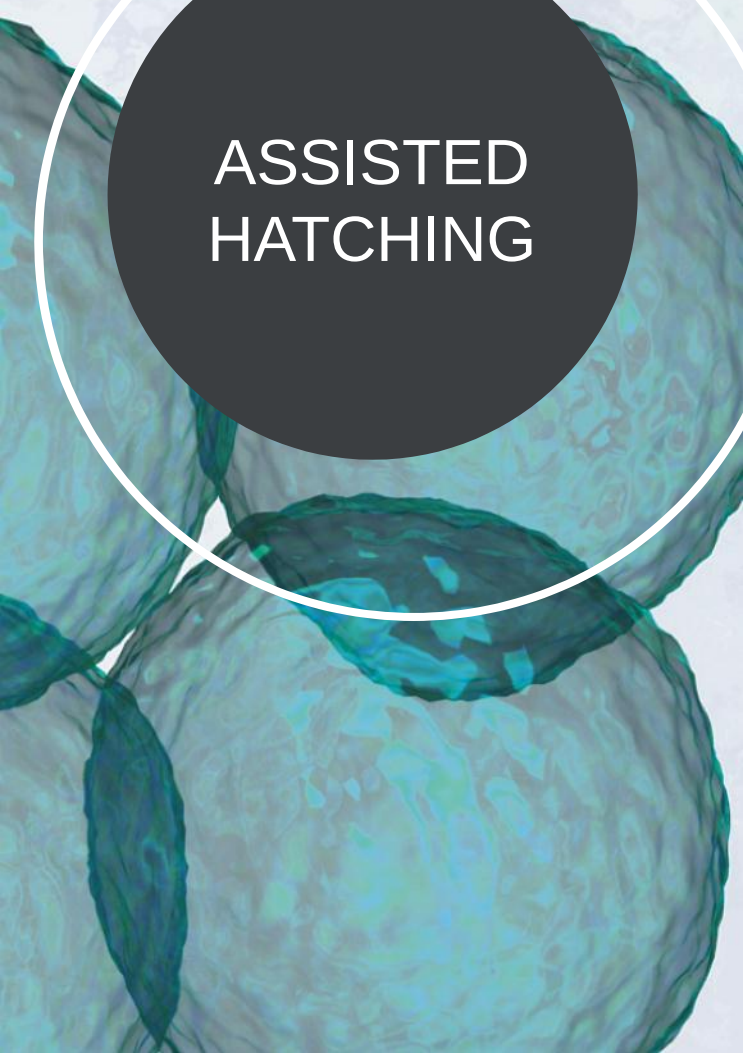
ASSISTED HATCHING



- Day 3 vs. Day 4 vs. Day 5/6
- Laser Opening
  - Multiple small pulses vs. one large pulse
  - Outside – In
  - Power: 100%
  - Pulse: 250µs
- Know your culture system and workflow

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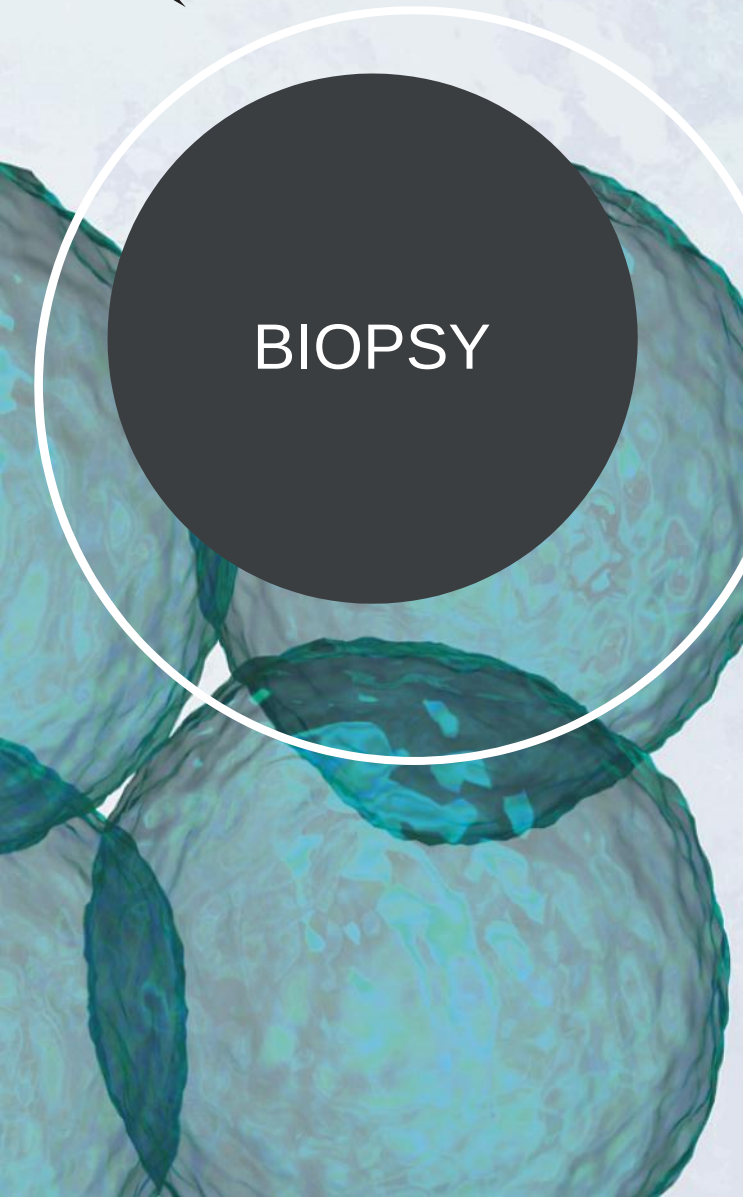

BIOPSY

- ICSI dish
- 10-20µl drops
- HEPES buffered media
- Keep tools wet
- PVP
- Time outside of incubator
- Change tools, as needed

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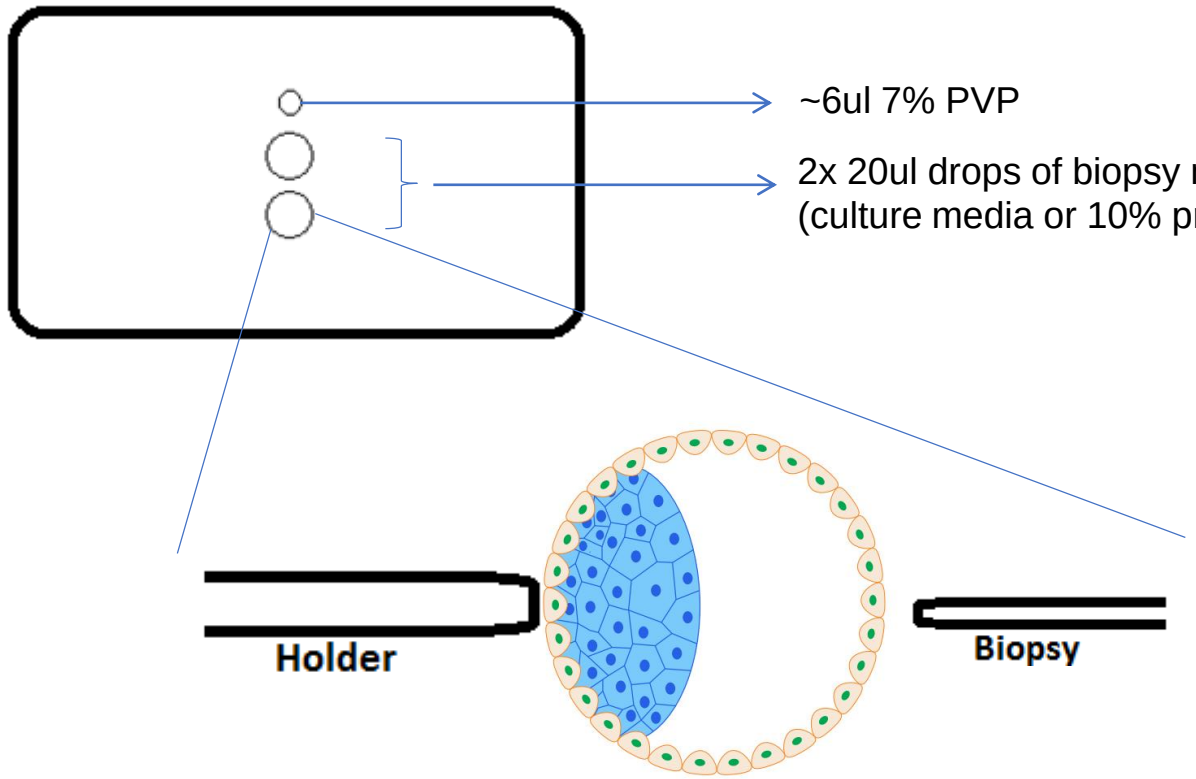
BIOPSY



- Larger diameter holding pipet
- Smaller diameter biopsy pipet
- Set-Up
  - Slightly tipped down
  - Ensures material kept close to bottom
  - Laser’s most effective focal point

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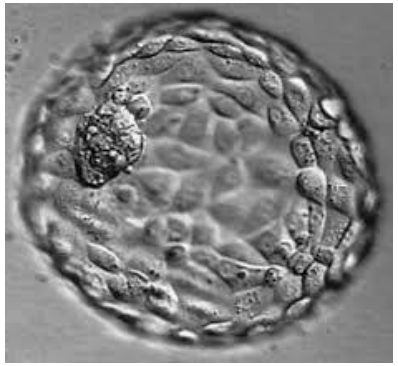
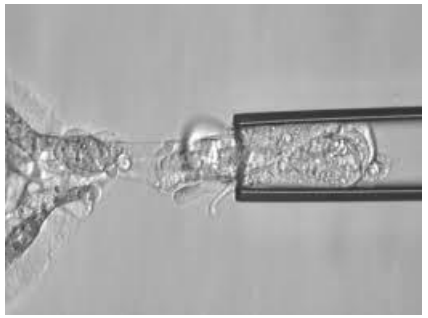
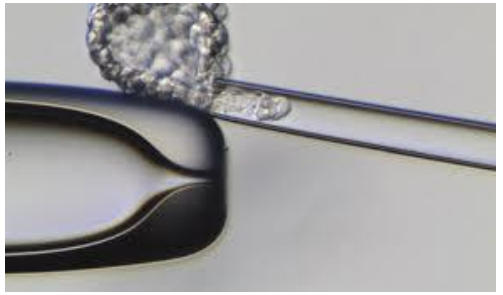
BIOPSY

- Position ICM between 7 and 11 o’clock distant from ZP opening
- Aspirate TE cells into biopsy pipette with gentle suction
- Laser pulses directed at junctions between cells
- If fully hatched excision is advisable using combination laser and flicking
- Minimize use and strength of laser – poor starting material *may* lead to poor amplification







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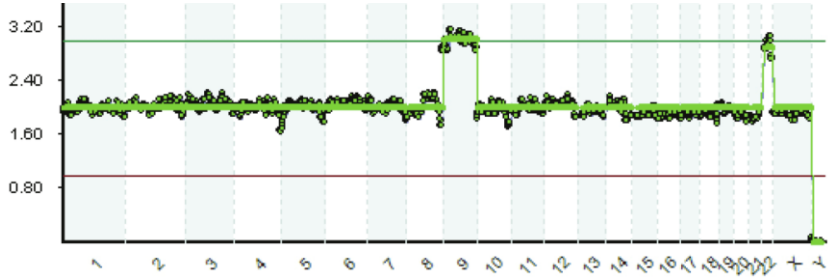
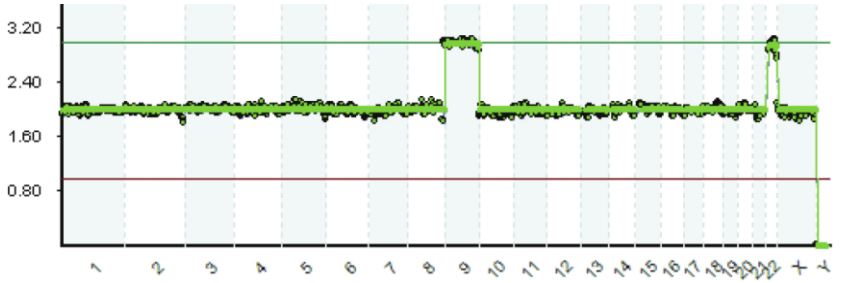
HOW MUCH IS TOO MUCH LASER?

Example profiles of biopsies derived from one embryo by varying laser exposure

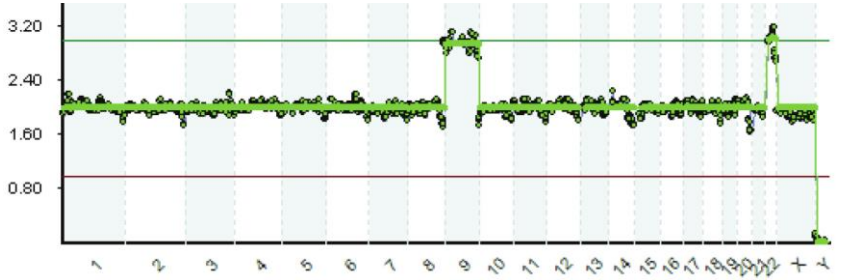
Original Interpretation
1-4 Pulses of 320µs



Laser Test Biopsy 1
9 Pulses of 700µs



Laser Test Biopsy 2
7 Pulses of 1600µs



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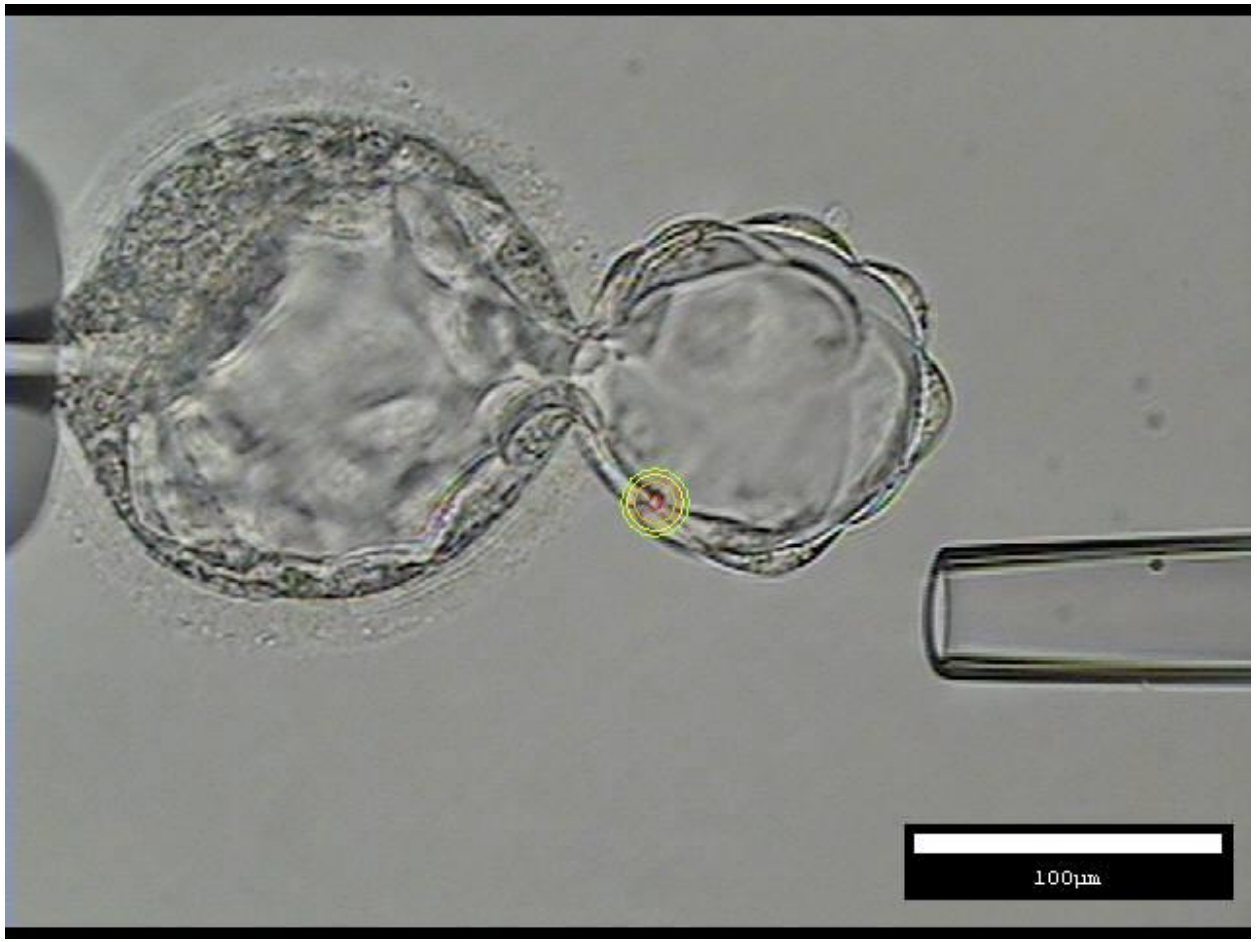
BIOPSY VIDEOS

- Day 3 Hatching



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BIOPSY VIDEOS

- Day 3 Hatching

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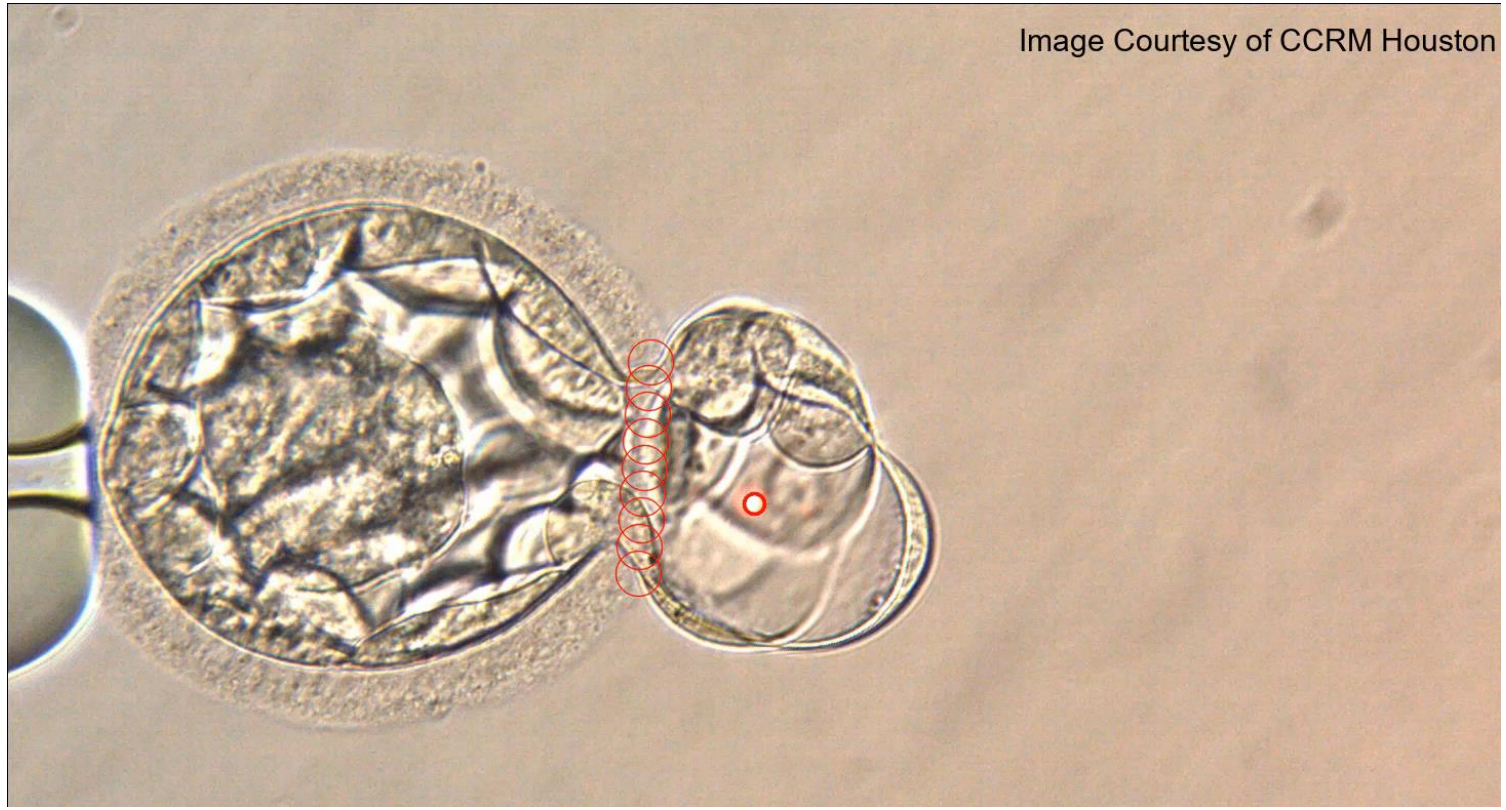
BIOPSY VIDEOS

- DTS



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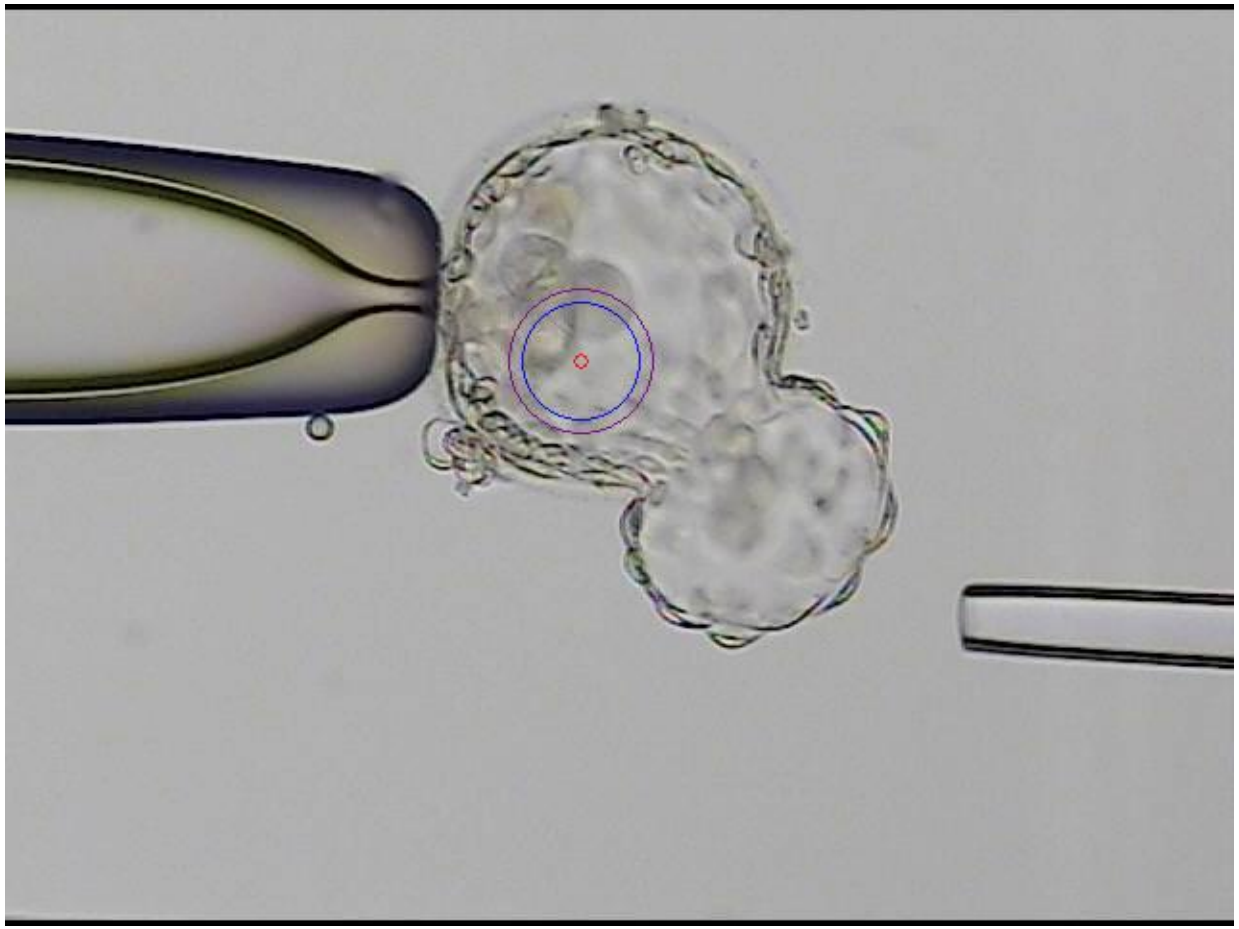
BIOPSY VIDEOS

- Flick technique



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TROUBLE-SHOOTING

- “Laser not working”
- Make sure you are on the laser objective
- Make sure the laser cable is plugged in all the way
- Make sure you are working on the bottom of your dish
- The laser is most effective in a very specific range. If you have inadvertently changed planes and risen above the bottom of the dish, it will not work
- Make sure the microscope magnification deck is in the correct position

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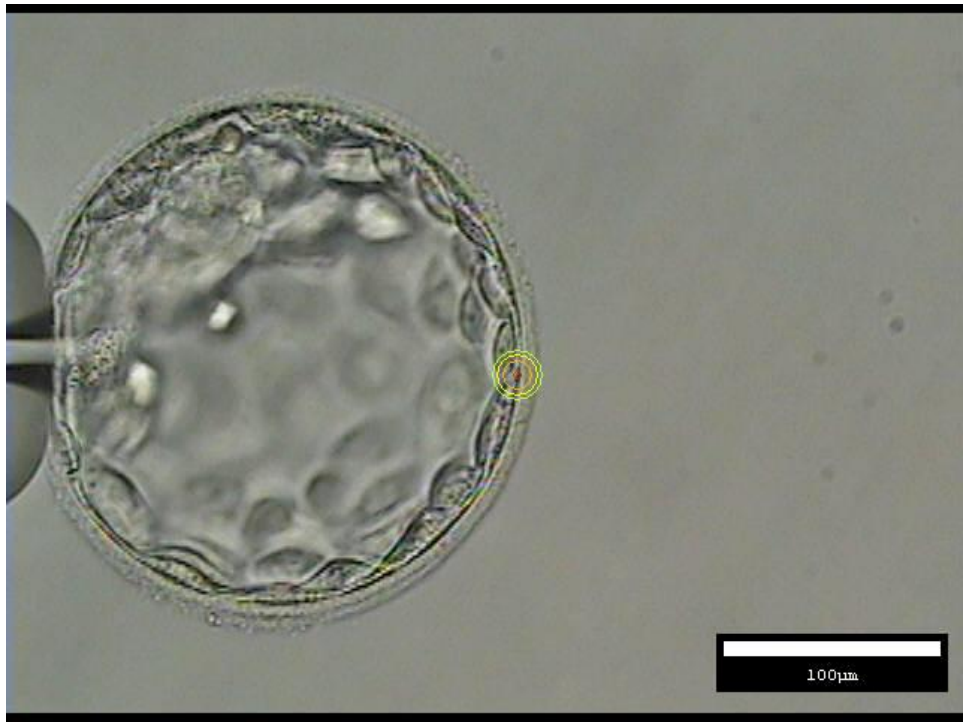
TROUBLE-SHOOTING

- For a non-hatching blastocyst, I cannot get the cells to pull out easily
- Insert whole pipet into the embryo by blowing
- Pull back through the zona to pinch it and create resistance
- Gently aspirate the cells by pulling slightly, then sucking…back and forth



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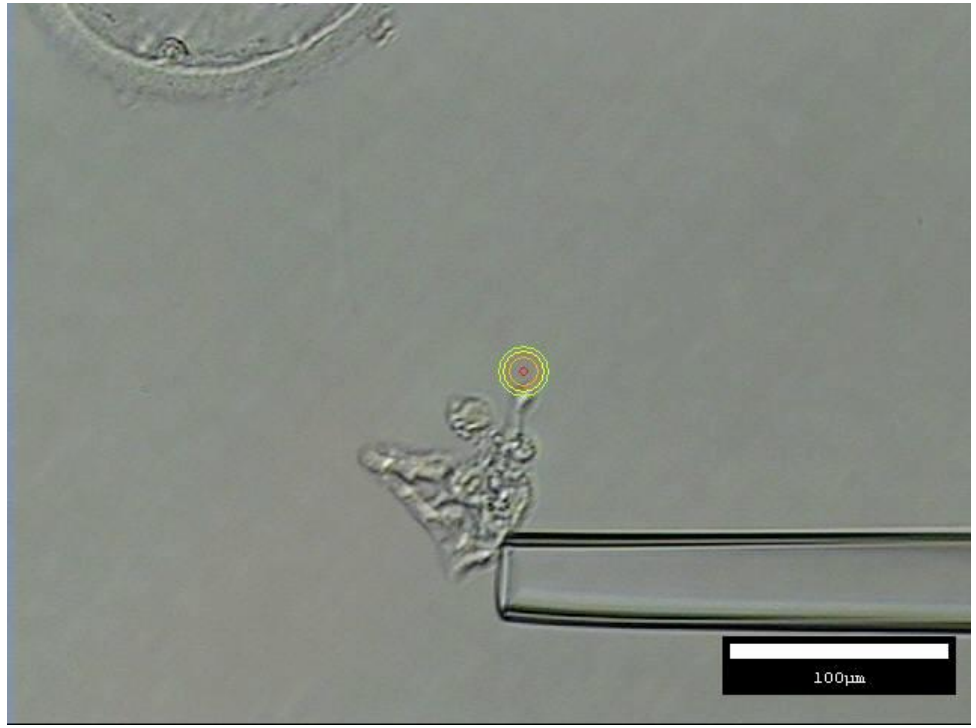
TROUBLE-SHOOTING

- Biopsy gets stuck on the tip of the pipet
- Flick the pipet holder with your finger
- Biopsy will come right off



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