Cleavage vs Blastocyst Transfer in the Time Lapse Era.

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Disclosure

- I am a founder of Auxogyn
- I am a founder of IviGen/Blastogen

Outline

- Lets make this interactive

Human Embryo Development

Why go to Blastocyst?

- Embryo Selection
- HAVING to go to blast removes some of the benefit of the TLM technology

What does a cleavage stage embryo need to do?

- Be euploid
- Cleave
- Compact
- Form a viable blastocyst

What does a Blastocyst need to do?

- Be euploid
- Differentiate into ICM ad TE
- Hatch
- Appose
- Attach
- Invade

$64,000 questions

- Are you doing fresh vs frozen ET?
- Are you doing PGD/S?
- When is the endometrium most receptive?

Cleavage vs Blastocyst TLM

- Simpler to measure early events
- Faster (ie can ET earlier)
- May be safer

What are we measuring with TLC?

- May determine which day we ET
  - Genetic (ie when is best to do a bx)
  - Metabolic
  - (both?)

Parameters measure cleavage stage events

Human Embryo Time-lapse Studies

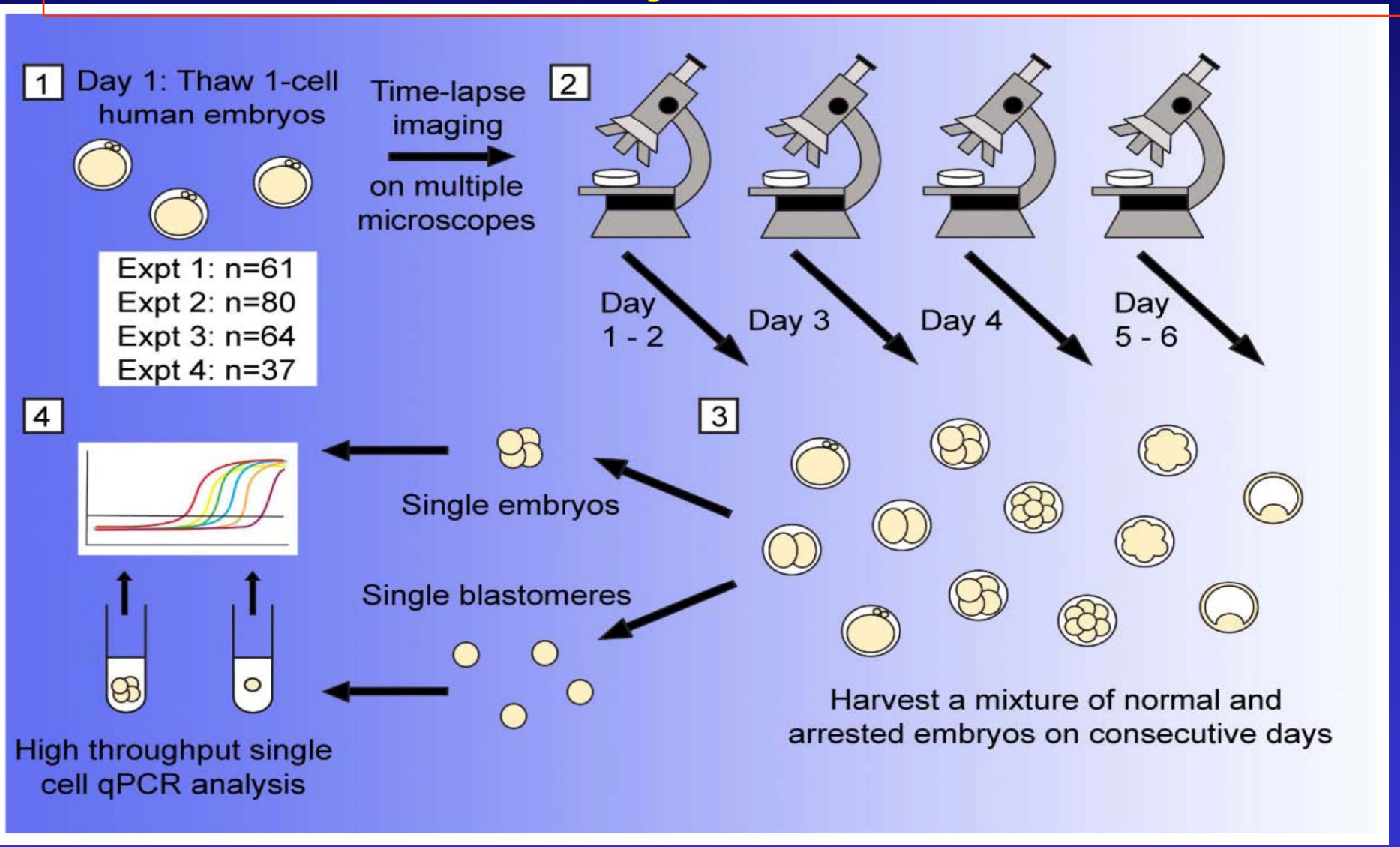
Paper	Published	Samples	Conclusions
Payne et al. Hum Reproduction	**1997**	50 2PN	• Observed details of the fertilization process to 20 hrs
Lemmen et al. RBM Online	**2008**	102 2PN oocytes	• Reported PN appearance & disappearance • Correlated synchrony in nuclei appearance after 1st cleavage with pregnancy success
Mio et al. Am J Obstet Gyn	**2008**	286 oocytes	• Observed details of the fertilization process • Reported two ICMs - monozygotic twins
Wong et al. Nature Biotechnology	**2010**	**242 2PN**	• Identified **cell cycle parameters** that predict blastocyst formation by Day 2 • Demonstrated that parameters correlate to **embryo gene expression data** • Developed **cell tracking software**
Pribenszky et al. RBM Online	**2010**	5 2PN	• Reported a live birth
Meseguer et al. Hum Reprod	**2011**	247 2PN	• Evaluated cell cycle parameters to implantation
Hashimoto et al. Fert Steril	**2012**	80 2PN	• Evaluated cell cycle parameters for blastocyst quality
Swann et. al. Fertil steril	**2012**	10 oocytes or zygotes	• Correlated cytoplasmic movements with Ca^{2+} oscillations.

nature biotechnology

Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage

Oct 2010

Imaging and Molecular Analysis of Embryonic Cells



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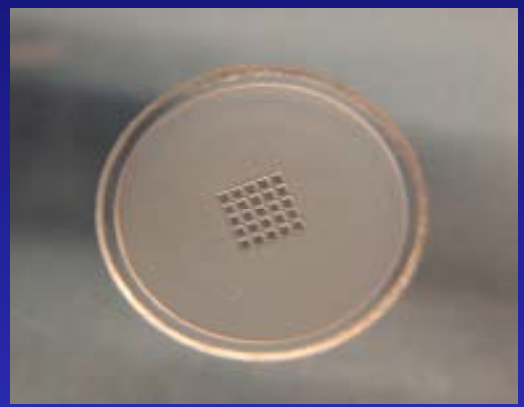
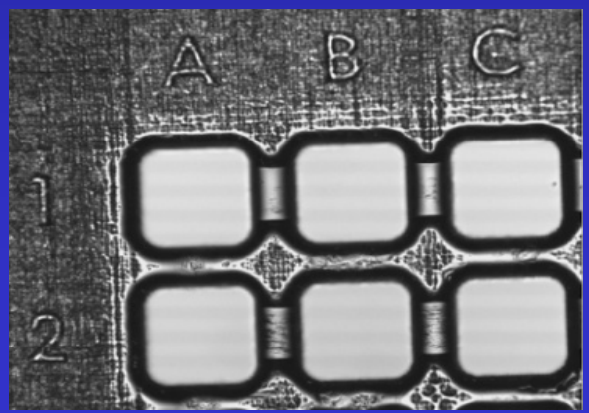
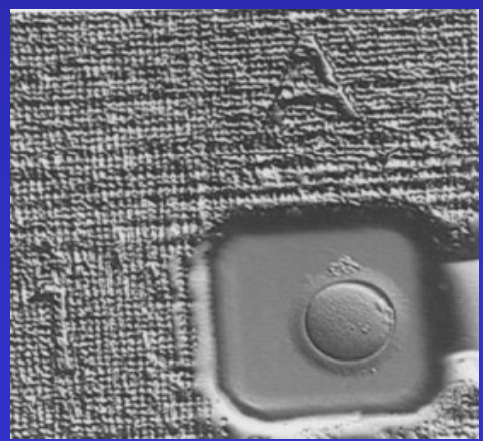
Custom Micro-Well Dishes

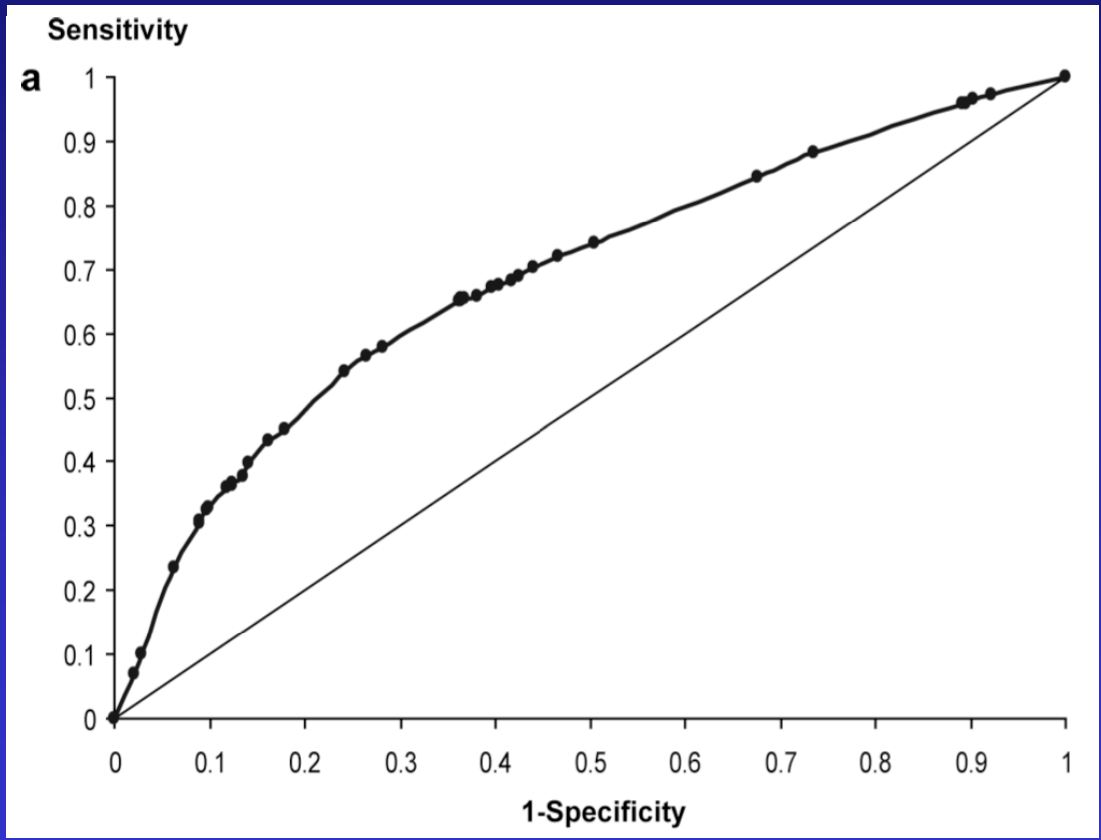
- Individual culture within the same media drop







Comparison to a Studyof Common Morphological Predictors[1]

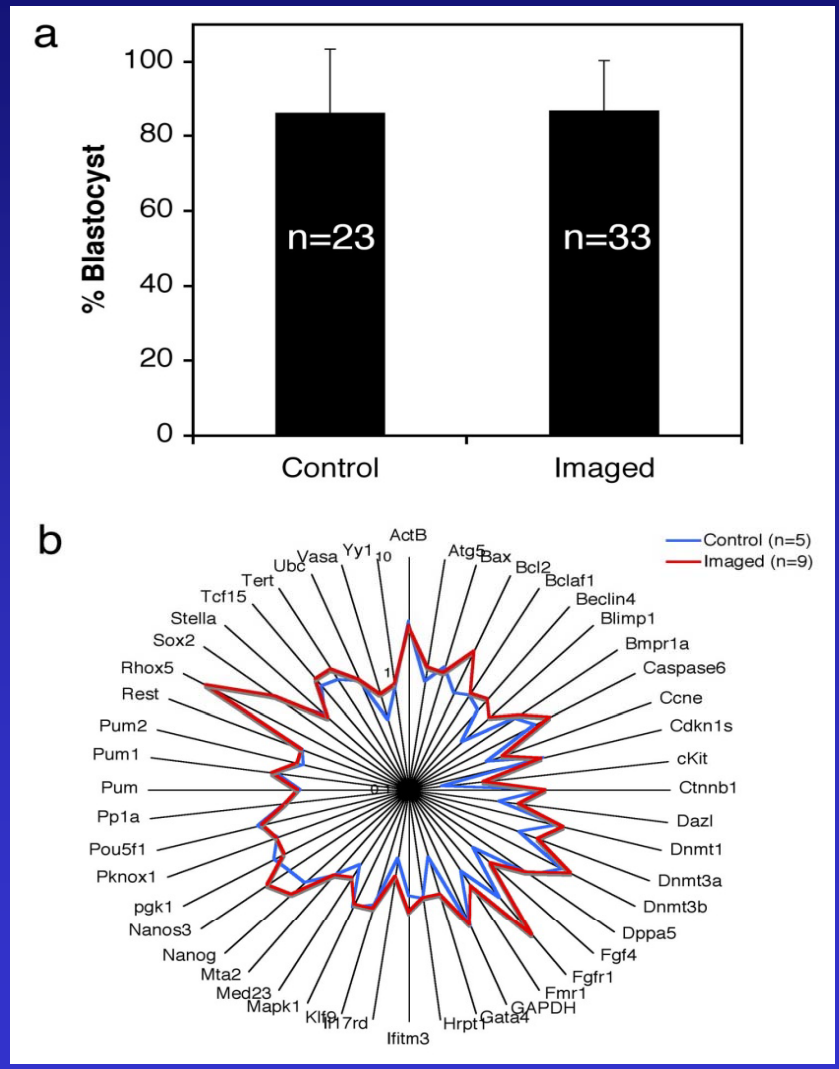


4 morphological criteria:

Pronuclear morphology on day 1; early cleavage, # cells, and fragmentation on day 2

1. F. Guerif et al., Limited value of morphological assessment at days 1 and 2 to predict blastocyst development potential: A prospective study based on 4042 embryos. Hum Reprod; 2007

Imaging Does Not Alter Fundamental Parameters



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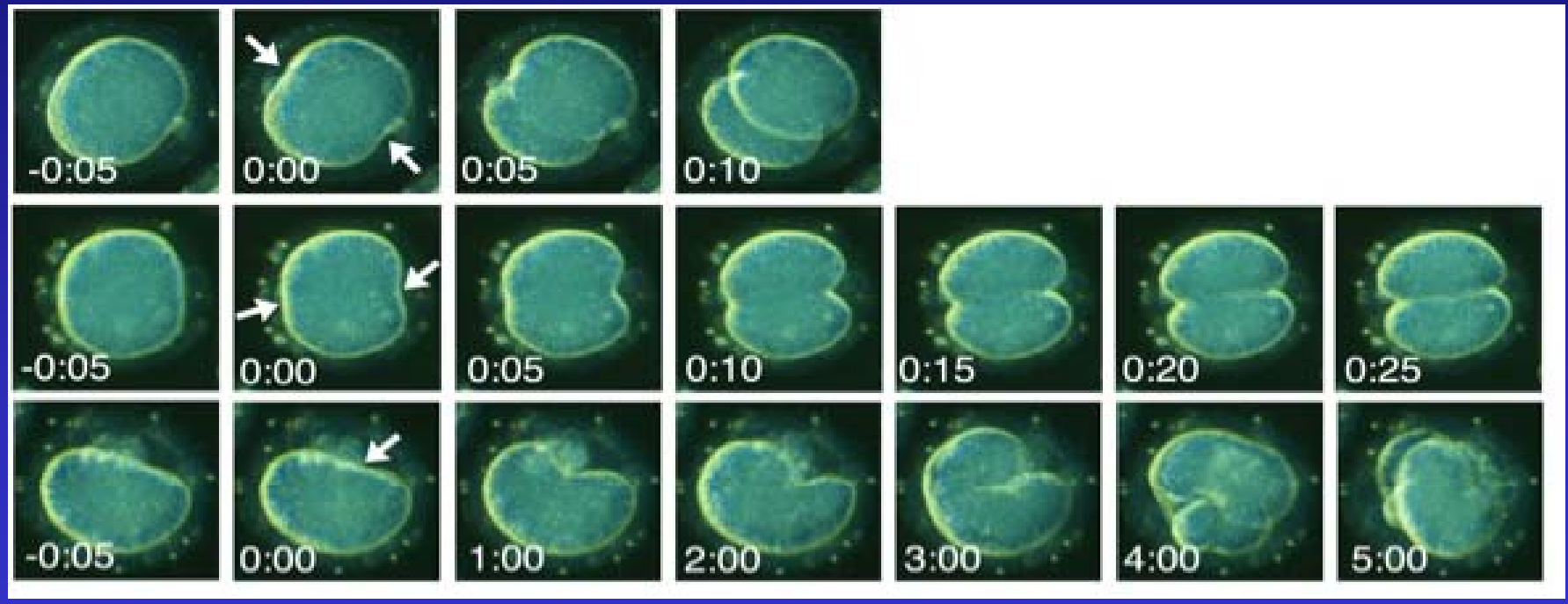
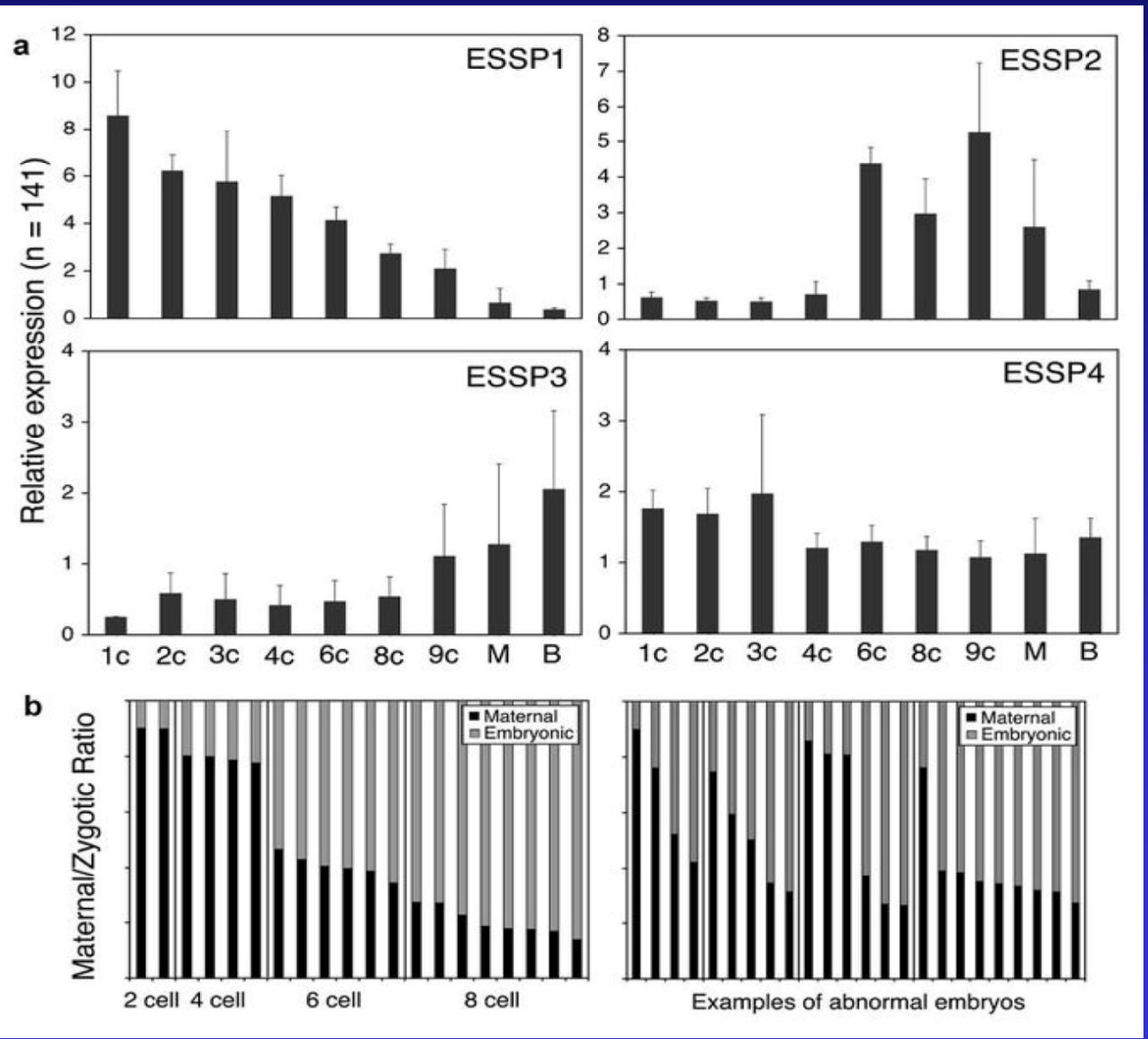
Arrested Embryos Exhibit Abnormal Cytokinesis During 1st Cleavage Division



4 ESSPs of Early Germ Cell Development



Maternal inherited (1)
EGA (2)
Blastocyst (3)
Ubiquitous (4)

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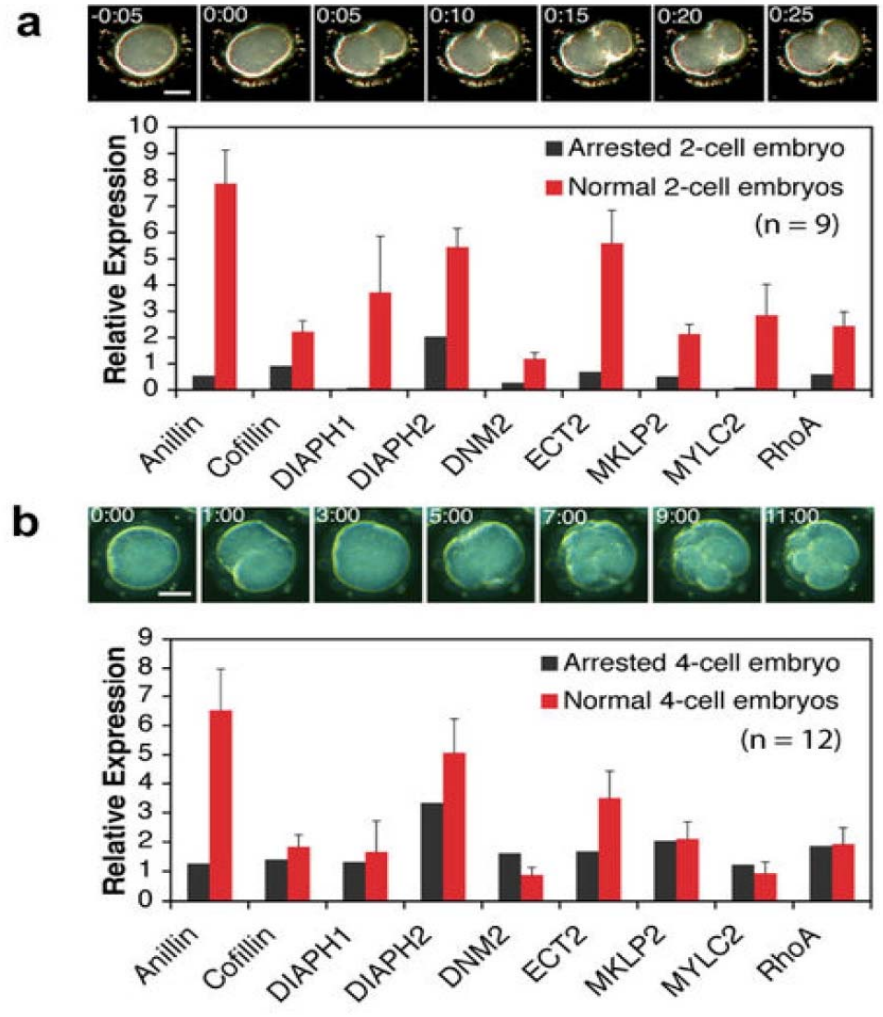
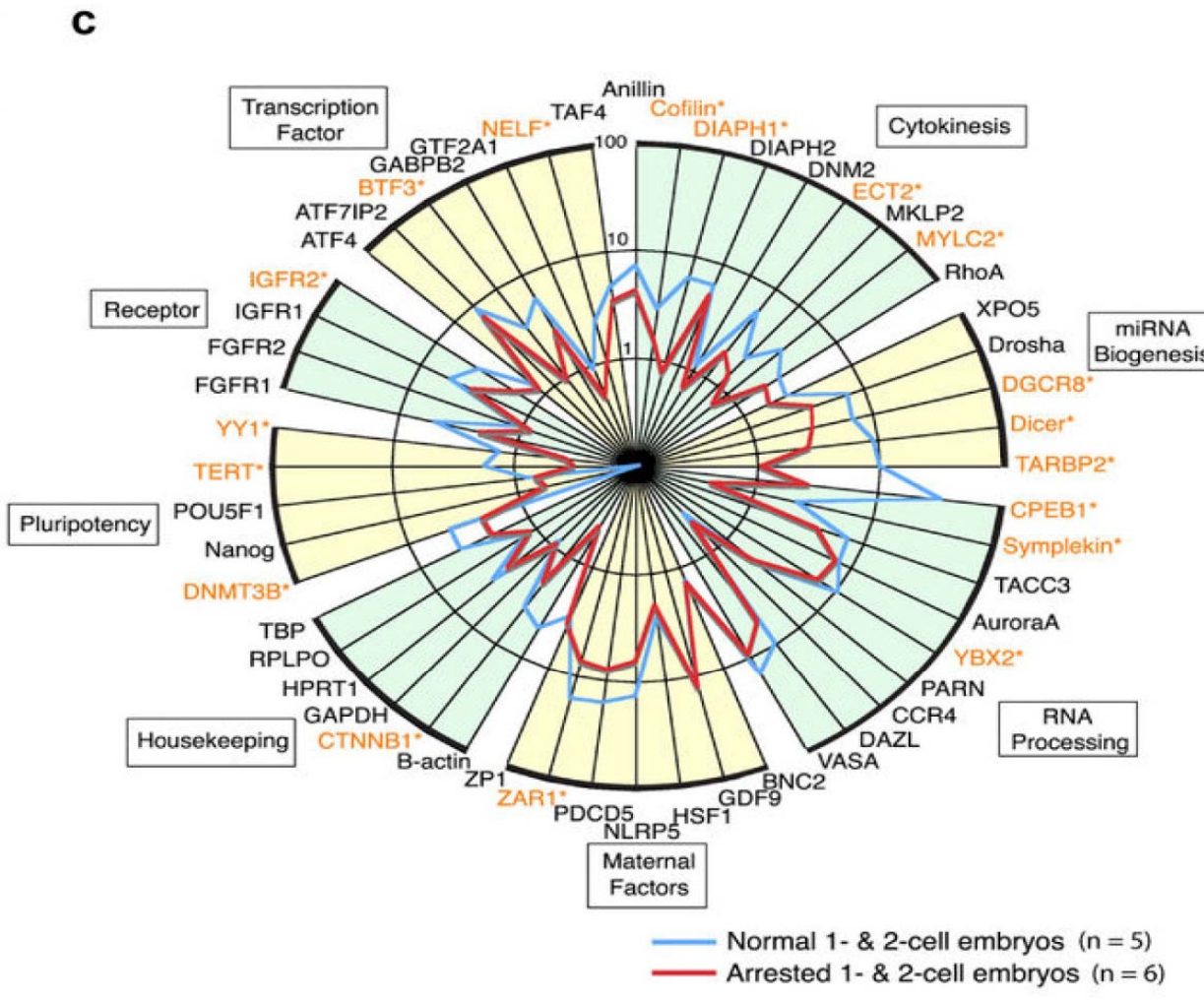
Embryo Arrest and Gene Expression in Individual Blastomeres





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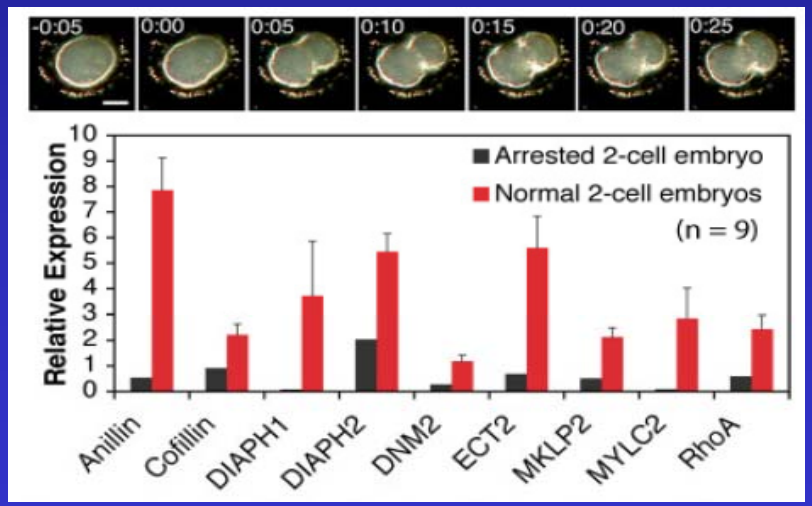
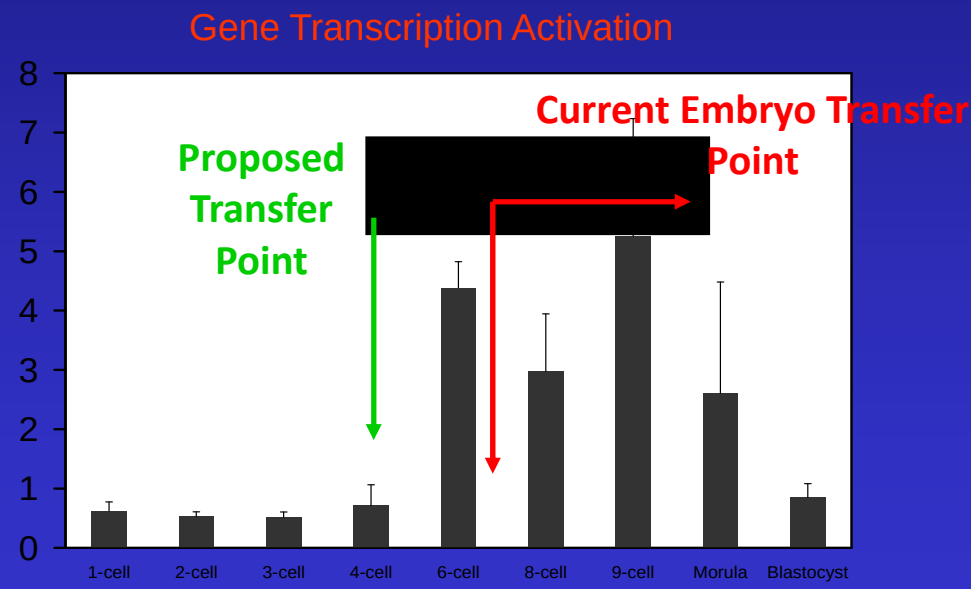
Molecular Analysis

Correlated gene expression profiling revealed:

1. Embryos with aberrant cytokinesis demonstrate underlying defects in molecular programs:

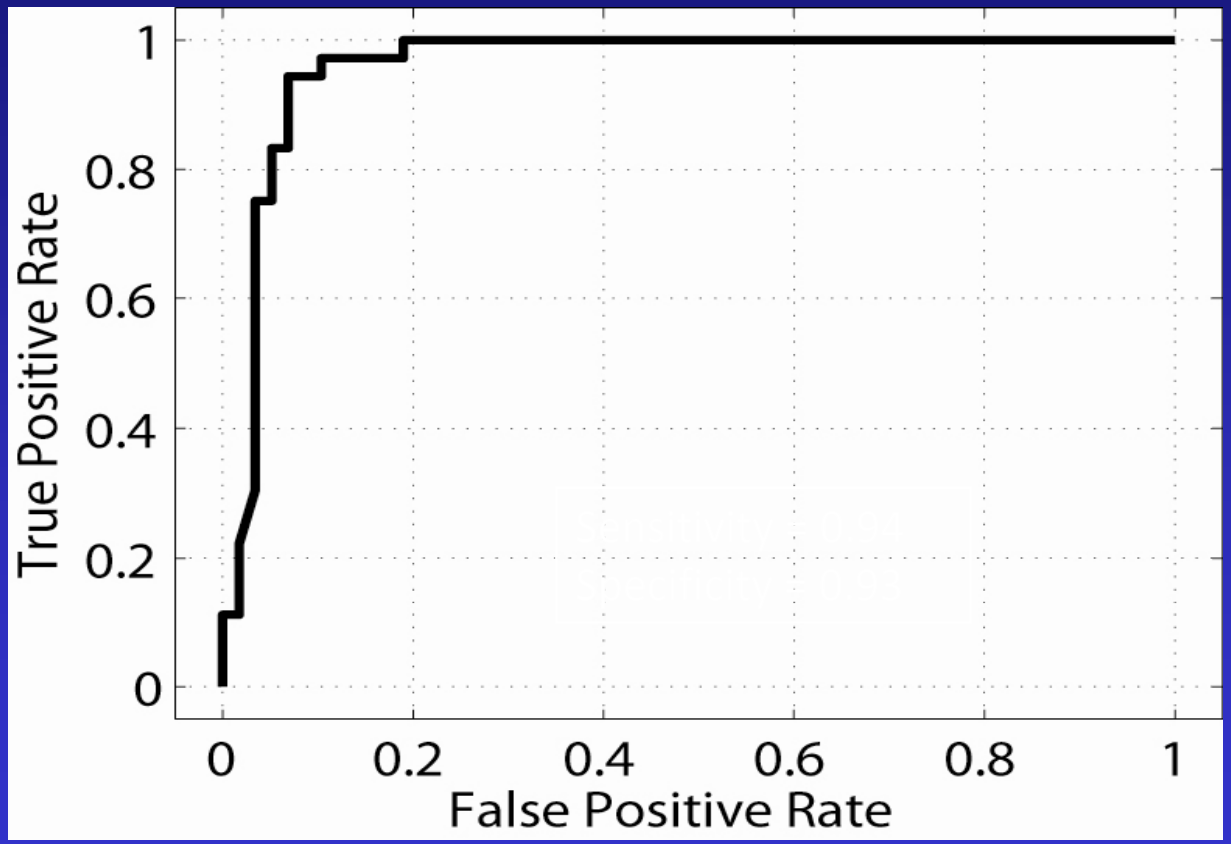


2. Using our predictors, embryo transfer can be performed prior to embryonic gene activation, minimizing risk of adverse outcomes:

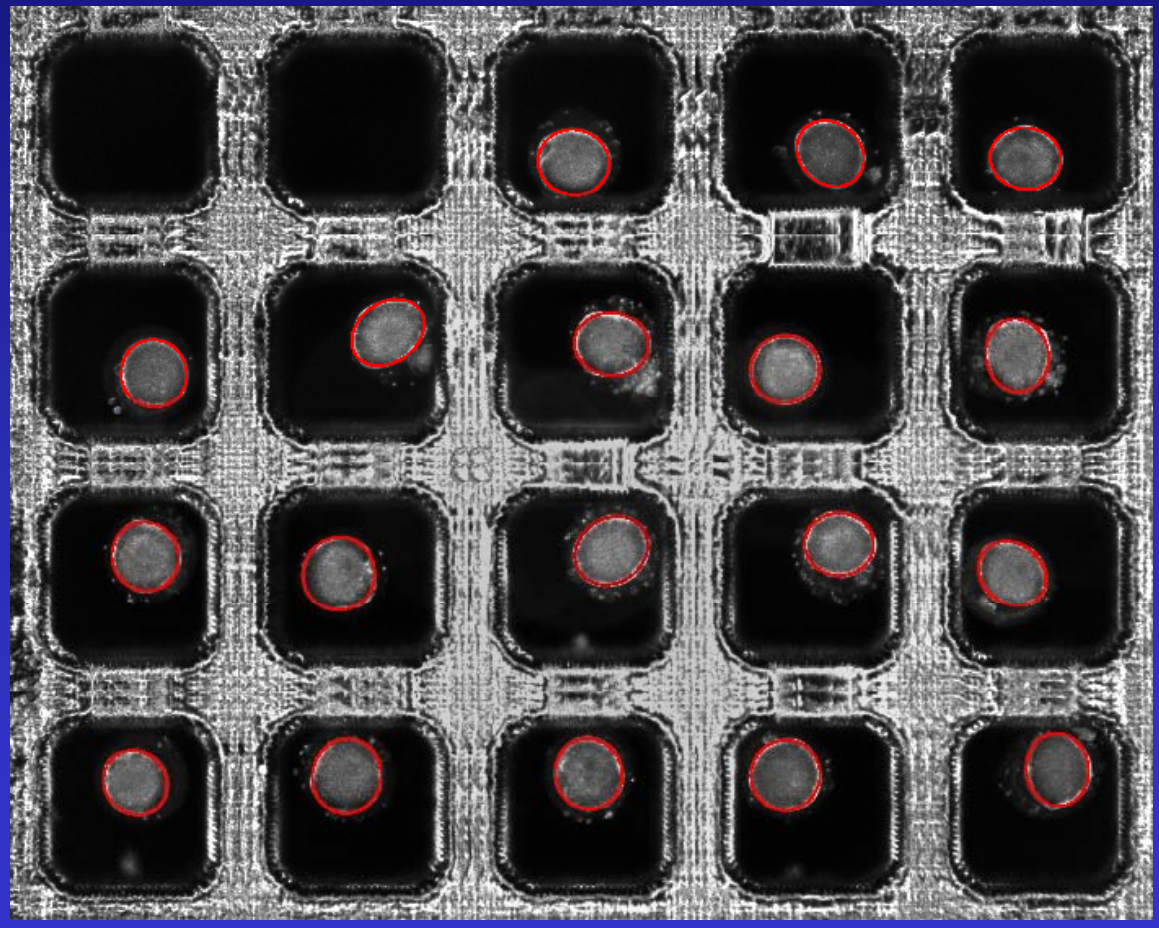


Discrimination Potential of Blastocyst Predictors

Receiver Operating Characteristic (ROC)

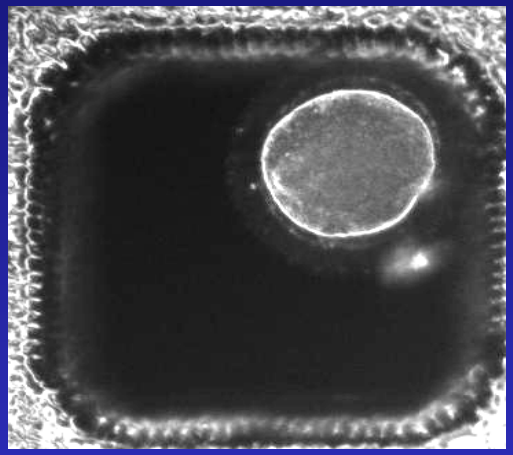


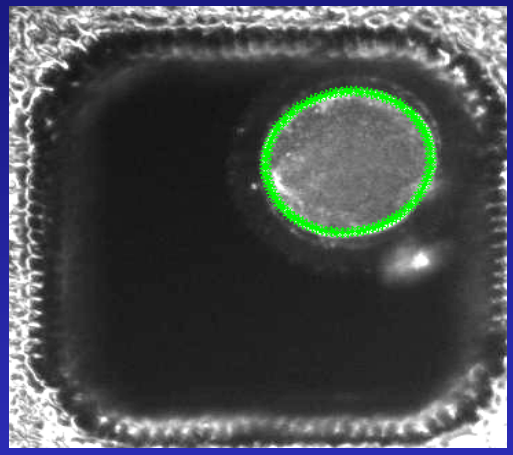
Example Tracking on Whole Dish

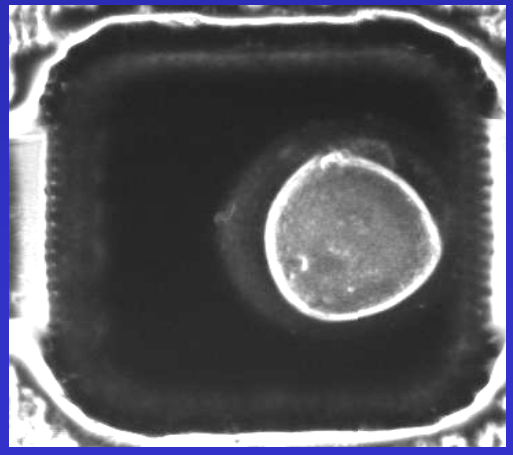


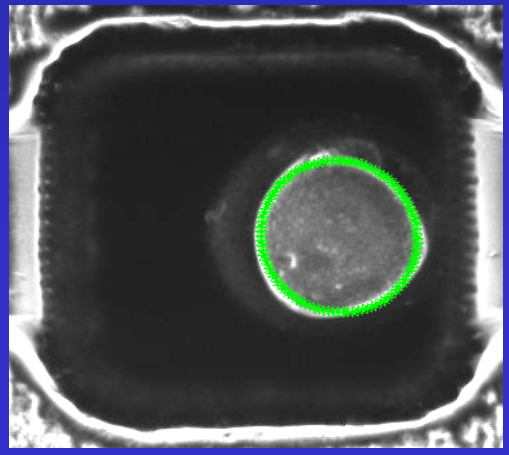
Eeva Analysis Software

Fully automated, state-of-the-art computer vision software predicts blastocyst formation by the end of Day 2







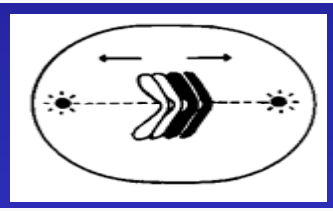


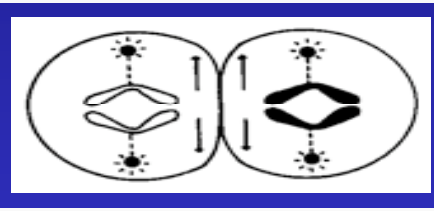
Eeva Parameter	Eeva Prediction	Timing
Duration of 1st cytokinesis	PASS	0min 30min, 20 min
Time from 1st to 2nd mitosis	PASS	8hr 14hr, 12 hrs
Time from 2nd to 3rd mitosis	PASS	0hr 5hr, 1 hrs

Duration of 1st cytokinesis	PASS	0min 30min, 20 min
Time from 1st to 2nd mitosis	FAIL	8hr 14hr, 15 hrs
Time from 2nd to 3rd mitosis	PASS	0hr 5hr, 2 hrs

Refinement of cell cycle imaging parameters

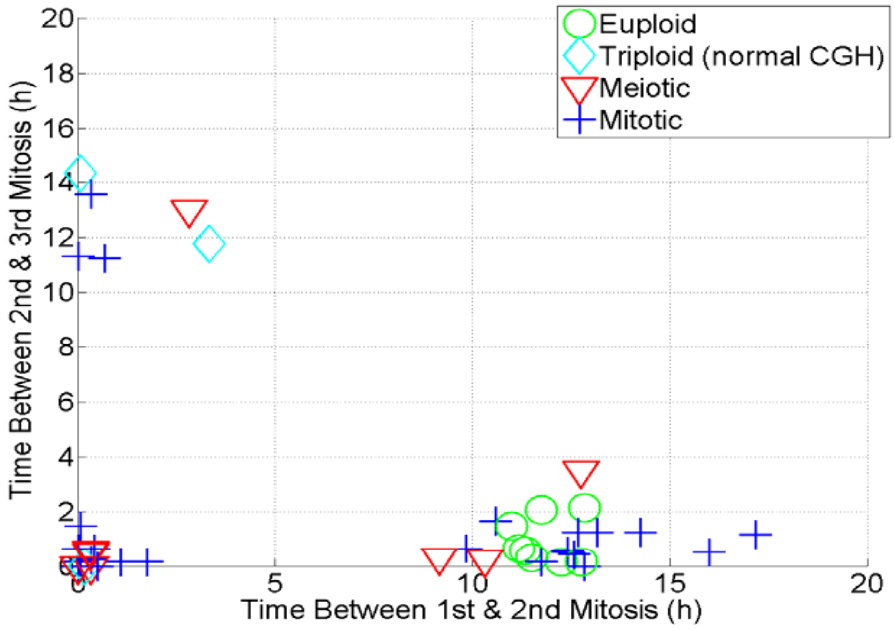
Parameter Measurements	Wong/Loewke et al.	Present Study
Duration of first cytokinesis	14.4±6 Min.	14.4±4.2 Min.
Time between first and second mitosis	11.1±2.2 Hours	11.8±1.2 Hours
Time between second and third mitosis	1±1.6 Hours	0.85±0.79 Hours

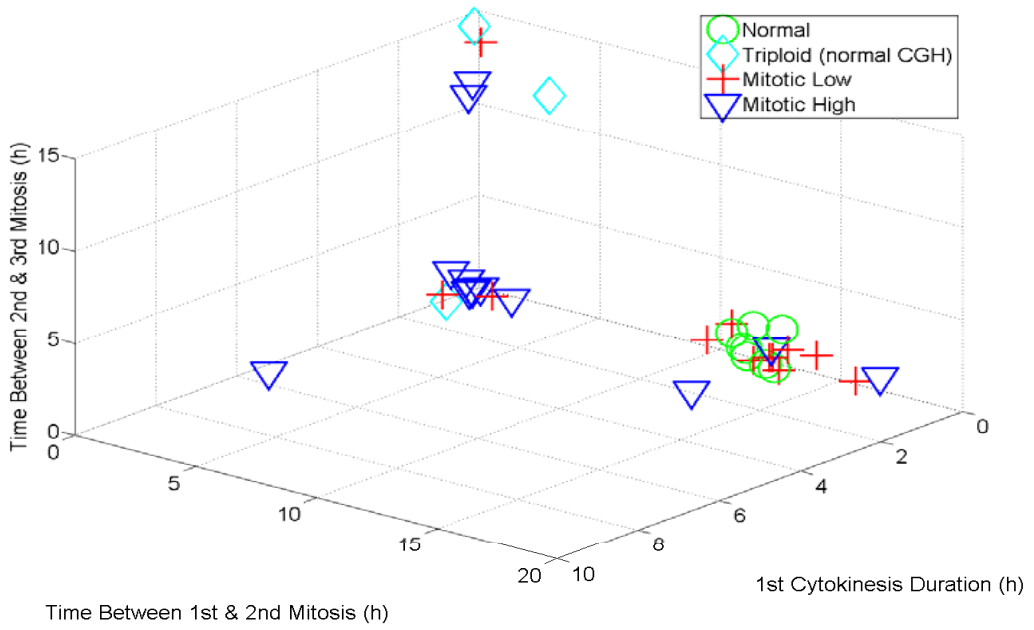




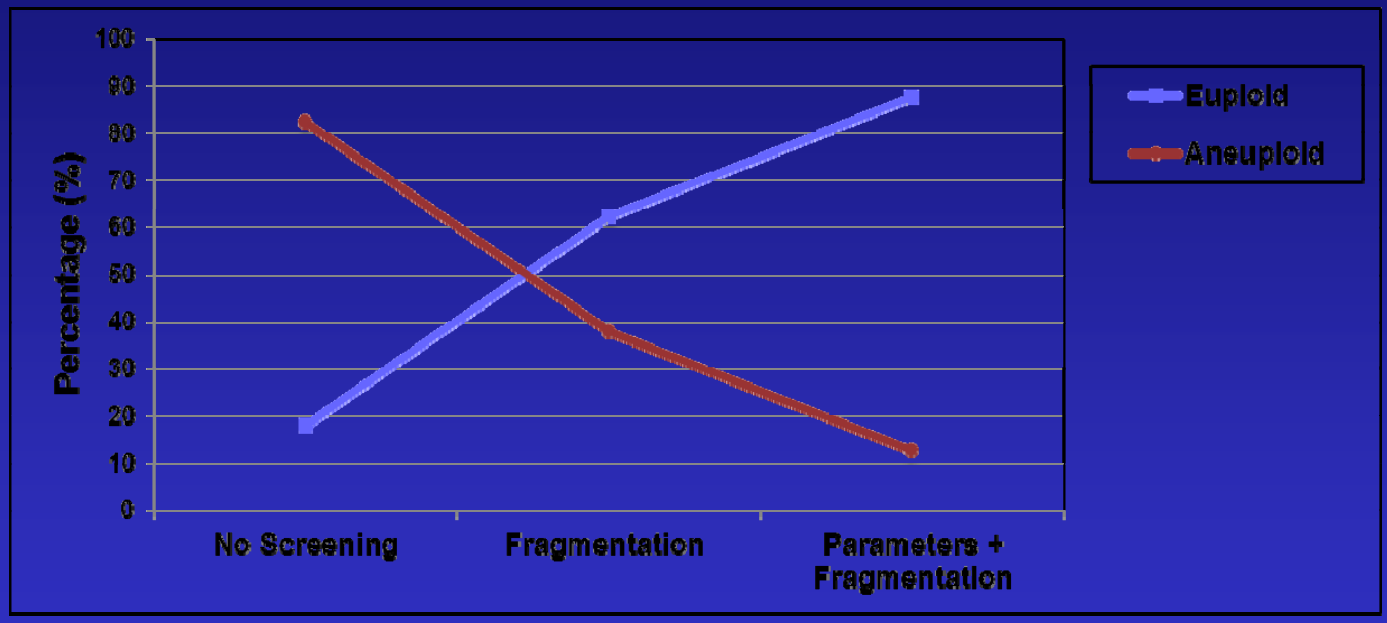
Parameter Measurements	Normal CGH	Meiotic Error	Mitotic Error
Duration of first cytokinesis	14.4±4.2 Min.	117.2±166.5 Min.	36.0±66.9Min.
Time between first and second mitosis	11.8±0.71 Hours	4.0±5.2 Hours	6.4±6.6 Hours
Time between second and third mitosis	0.96±0.84 Hours	2.0±4.3 Hours	2.0±3.9 Hours

Detection of chromosomal duplications/deletions and both simple and complex mosaicism





Calculation of Embryonic Euploidy Versus Aneuploidy Risk Using Morphological and/or Parameter Assessment



A table showing the calculation (number of euploid embryos/total number of embryos) and the resulting probability of embryonic euploidy expressed as a percentage for each morphological and/or parameter assessment. (B) Graphic representation of the table demonstrating that the highest percentage of embryonic euploidy over aneuploidy was obtained with the combination of cell cycle parameters that predict normal A-CGH and cellular fragmentation analysis.

Wong et al., 2010, Meseguer et al., 2011 and Hashimoto et al., 2012

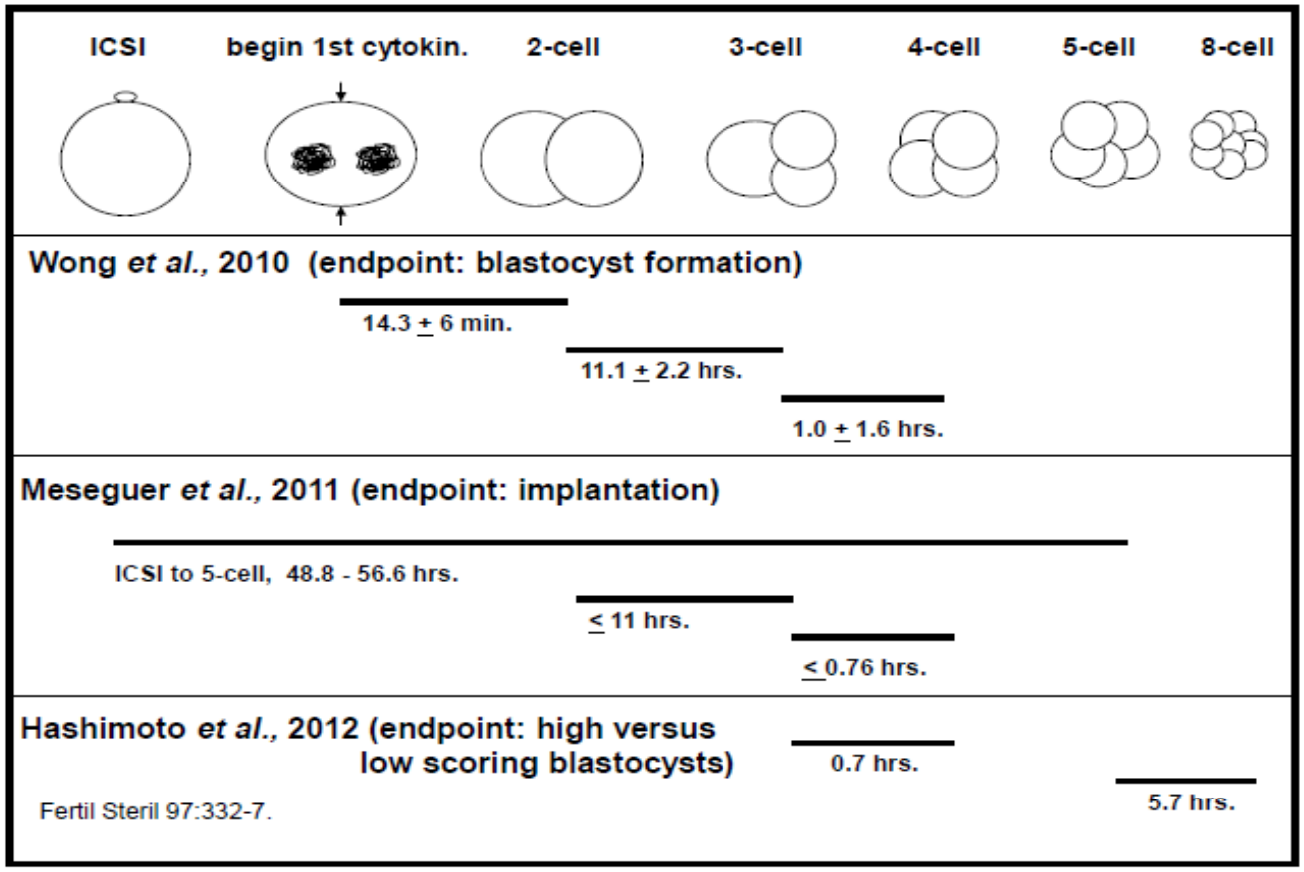
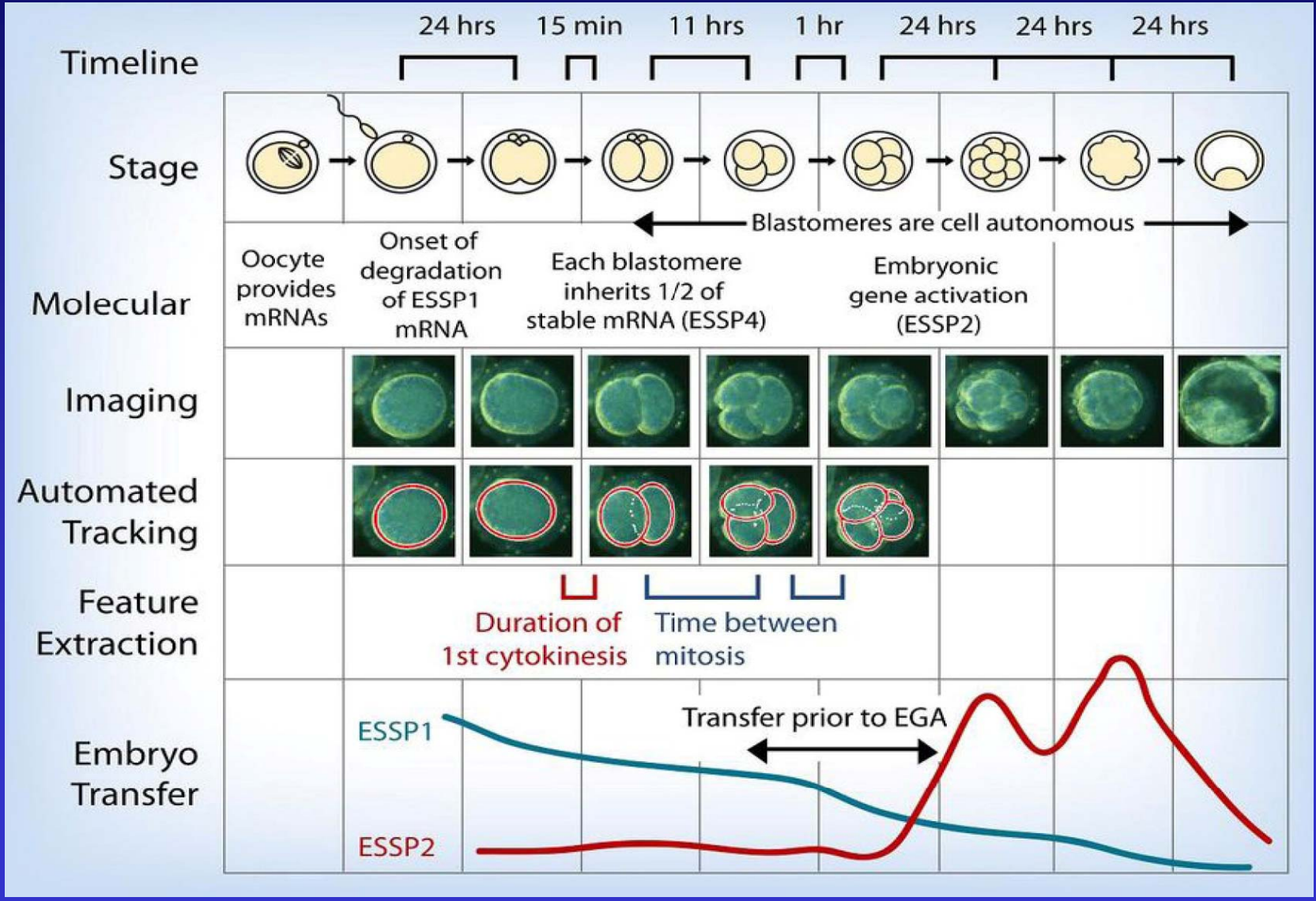


Table courtesy of Michael Tucker

Putting it all together



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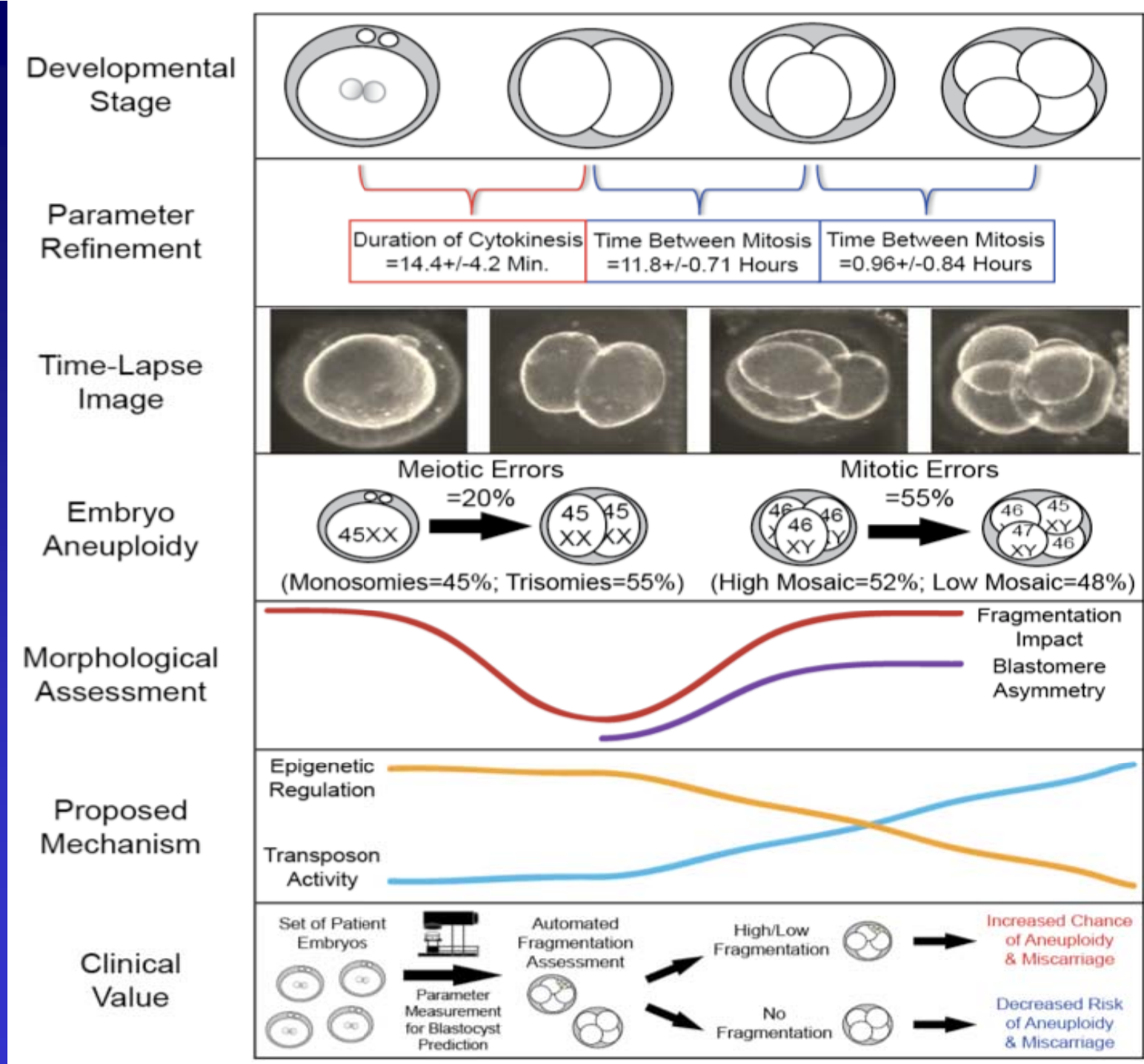
Summary


Developmental Stage
Parameter Refinement
Duration of Cytokinesis =14.4+/-4.2 Min.
Time Between Mitosis =11.8+/-0.71 Hours
Time Between Mitosis =0.96+/-0.84 Hours
Time-Lapse Image
Embryo Aneuploidy
Meiotic Errors
=20%
45XX
45 XX 45 XX
(Monosomies=45%; Trisomies=55%)
Mitotic Errors
=55%
46 XY
46 45 XY 47 XY 46
(High Mosaic=52%; Low Mosaic=48%)
Morphological Assessment
Fragmentation Impact
Blastomere Asymmetry
Proposed Mechanism
Epigenetic Regulation
Transposon Activity
Clinical Value
Set of Patient Embryos
Parameter Measurement for Blastocyst Prediction
Automated Fragmentation Assessment
High/Low Fragmentation
No Fragmentation
Increased Chance of Aneuploidy & Miscarriage
Decreased Risk of Aneuploidy & Miscarriage

Summary

- Any TLM approach is better than a static system
- Uterine receptivity will play a role in when we ET
  - Which will influence which system will dominate
- Practical matters like best time to bx or vit may also influence the decision

The End