Non-invasive embryo Evaluation

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Disclosure

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

Outline

- Background
- Historic perspective
- Current approaches
- Future technologies

What's Important ?

The Patient

stimulation

What culture system

What media

Conditions/Expertise

Which stage

Which embryo

IVF outcome

IVF Field Awaiting a Breakthrough

Dr. Howard Georgeanna Jones,
First IVF Baby and First IVF Clinic in the US

"Young fertility investigators today should figure out *which one embryo is likely to make a baby rather than transfer several.* That will reduce costs, the number of multiples births and significantly increase success rates of in vitro fertilization, which currently hover around 30%--surprisingly close to the 28% success rate his team was seeing in the 1980s."

The New York Times Epstein, Randi Hutter. "Pioneer Reflects on Future of Reproductive Medicine.", 22,March,2010

Risks of Multiple Embryos Transferred

- Short- and long-term risks to offspring
  - Increased chance of miscarriage
  - Low birth weight (LBW) and pre-term birth occur 7x and 5x, resp. (Barker Hypothesis)
  - 80% of infant mortalities result from 8% LBW, 13% pre-term births
- Risks to the mother
  - Pre-eclampsia / hypertensive disorder
  - Pregnancy-induced diabetes
  - Miscarriage and other prenatal complications often requiring hospitalization
- Selective fetal reduction

Which one?


Stanford Fertility and Reproductive Medicine Center
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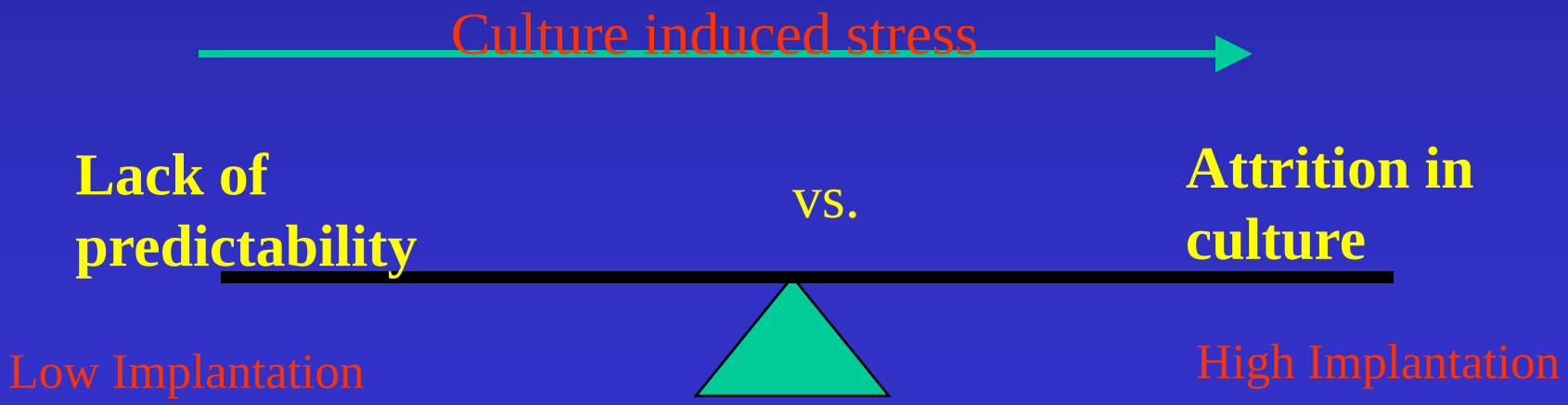
HAMILTON THORNE

Traditional Approach

- The Balancing Act !

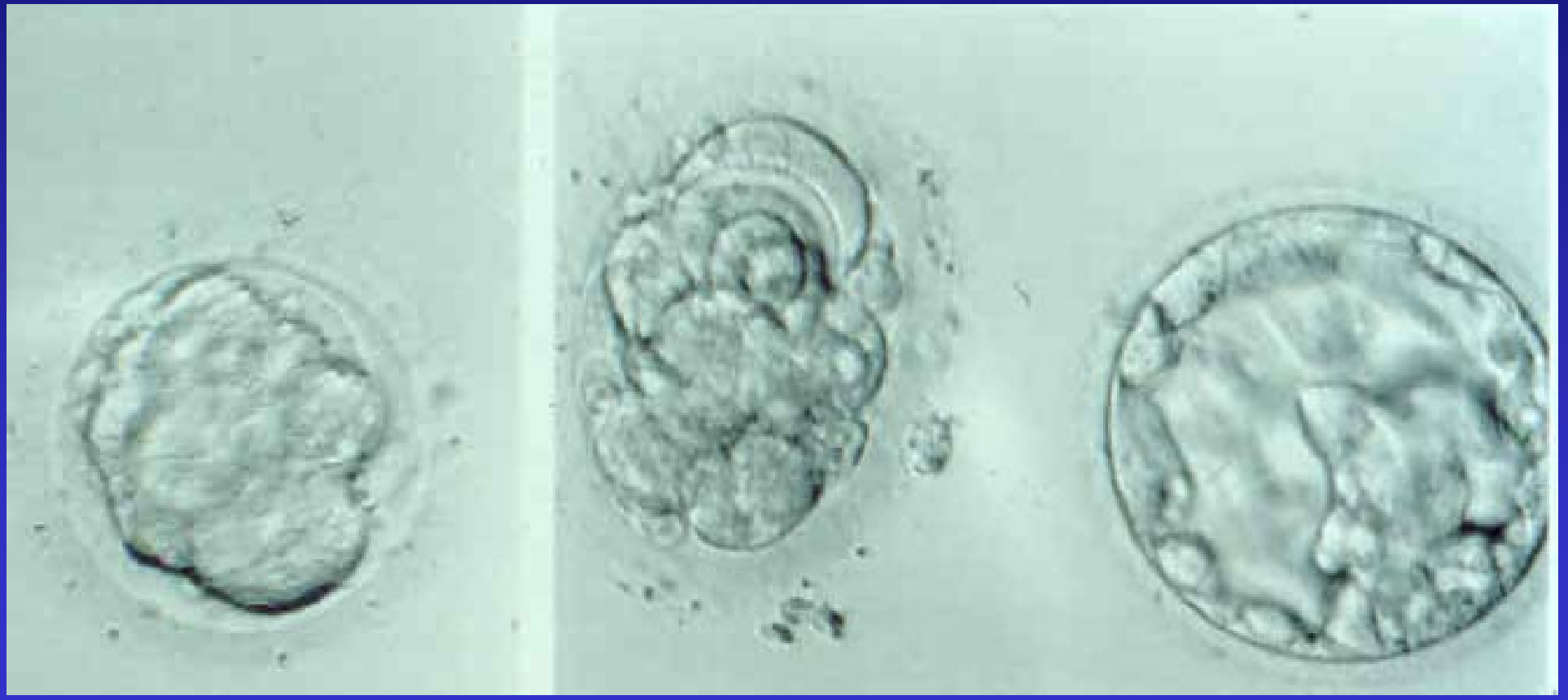


Problems with Embryo Assessment

- Subjective
- Poor standardization
- Timing dependent
- Can compromise development
- Pre vs Post genomic activation
- Paternal contribution assessment

What you see is not always what you get!

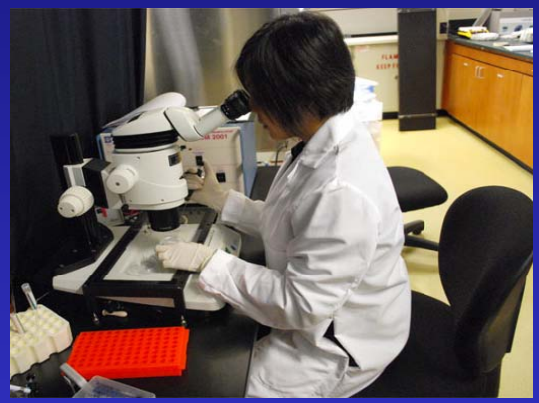
But…morphology isn't everything!



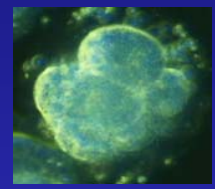
Current Embryo Assessment

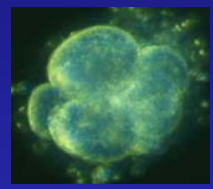
Embryos are evaluated based on simple morphological assessment on days 1 and 3

Step 1:
Embryos develop in incubator



Step 2:
Occasional monitoring and Day 3 selection


Viable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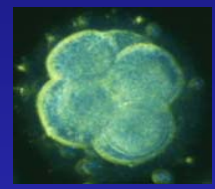
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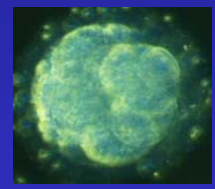
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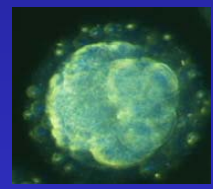
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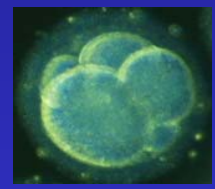
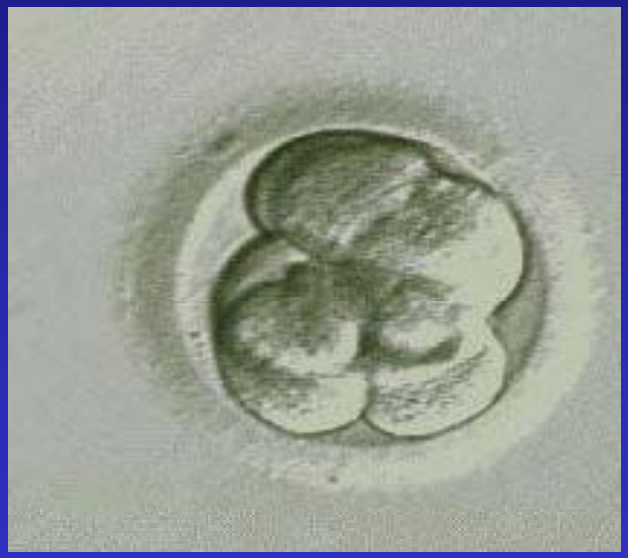
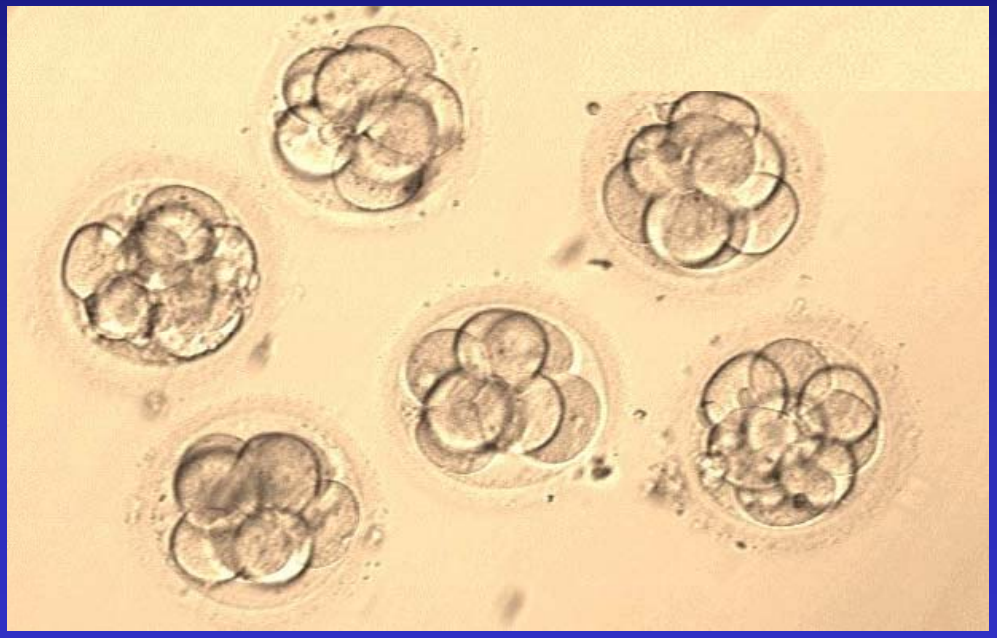

Non-Viable

The dilemma…….





BOTH RESULTED IN A SINGLETON

P.Nagy

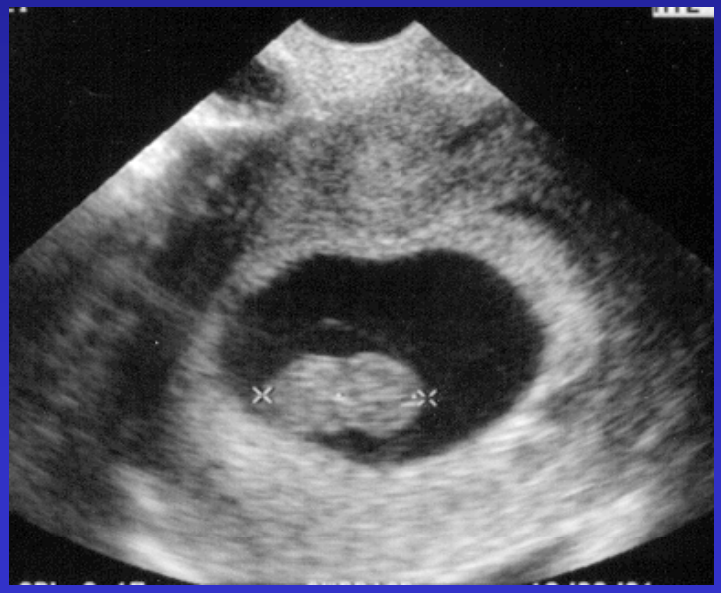
Human Embryo Development

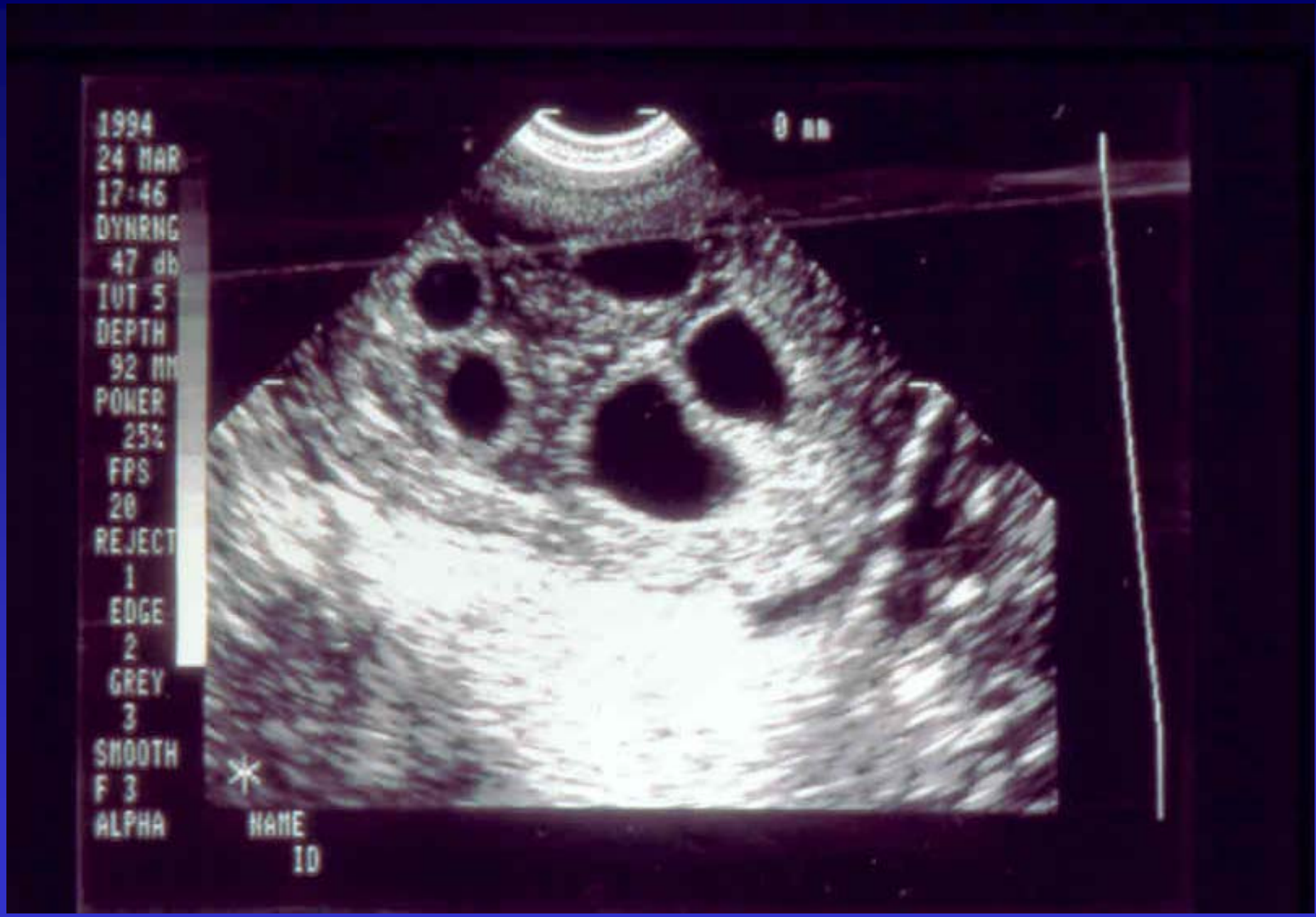
Important factors to consider

- Incubation chamber
- pH levels
- O_2 levels
- Oil vs. open culture
- Volume of medium
- Oocyte/Embryo density
- Mode of fertilization
- The time of gamete co-incubation
- Assisted hatching
- PGD
- Embryo transfer technique
- Ovarian stimulation protocols

Ultimate Goal

- Achieve a healthy singleton pregnancy
- Transfer fewer embryos (1 or 2)




1994
24 MAR
17:46
DYNRNG
47 db
IVT 5
DEPTH
92 MM
POWER
25%
FPS
20
REJECT
1
EDGE
2
GREY
3
SMOOTH
F 3
ALPHA
0 mm
NAME
ID

New Technologies

What can we do?

- What do you need to be healthy?
  - Good genes
  - Good metabolism

Embryo Selection Landscape

Most technologies lack clinical trials. No results exist to demonstrate improved blastocyst prediction or pregnancy rates.

Approach

Chemistry
- Metabolomic profiling
- *Amino Acid uptake*
- *Cummulus Cell analysis*

Genetics
- *Full Karyotype* - Pre-implantation Genetic Screening (PGS)

Imaging
- *Unisense/*Primo Vision – Time lapse imaging of embryos
- Olympus/Sanyo/*Nikon/Astec* – Instrumentation

Limitation

- Technology adoption challenge
- Doesn't fit in current workflow

- Mosaicism?
- Experts disagree on effectiveness?
- Invasive procedure

- No predictive parameters (yet)
- No prospective studies to show efficacy
- Lack of human data , emerging

Amino Acid Uptake: Novocellus

- **Immediate goal:** 30% increase in IVF success rates.
  Ultimate goal - 50% increase in Single Embryo Transfer rates to match those with two or more embryos replaced

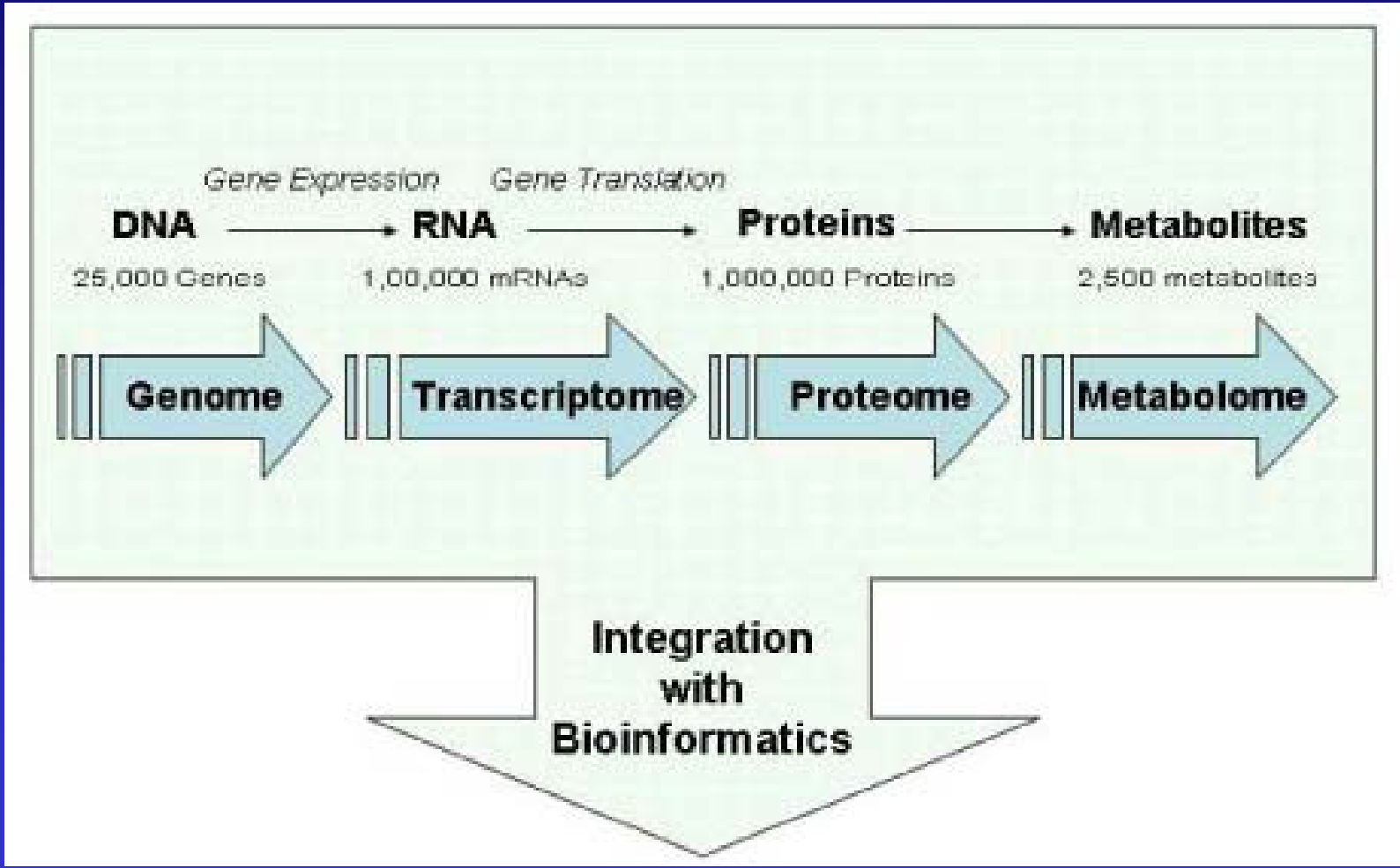
Novocellus

- Need to use proprietary media
- Culture in micro volumes

“Omics” era……

- Economics 
- Genomics
- Proteomics

The “omics”



Functional Phenotype

Proteomic analysis of individual human embryos to identify novel biomarkers of development and viability

Mandy G. Katz-Jaffe, Ph.D., David K. Gardner, Ph.D., and William B. Schoolcraft, M.D.

Colorado Center for Reproductive Medicine, Englewood, Colorado

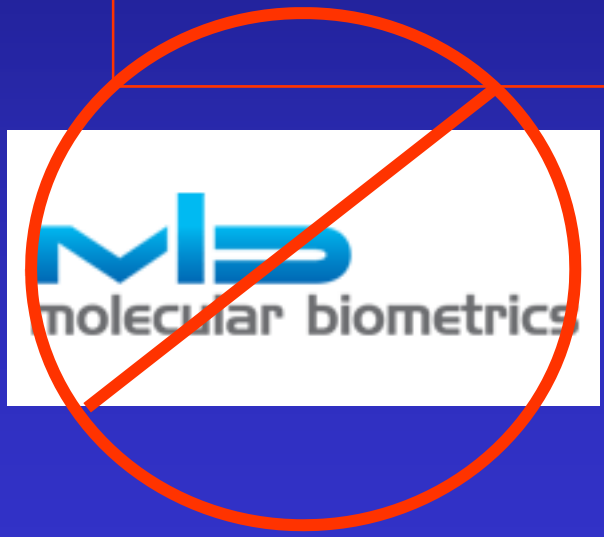
Proteomics

- Current approaches:
  - Not user friendly
  - Not rapid
  - Not high through put

Proteomics

- Most proteins are NOT secreted
- Proteins used for internal processes

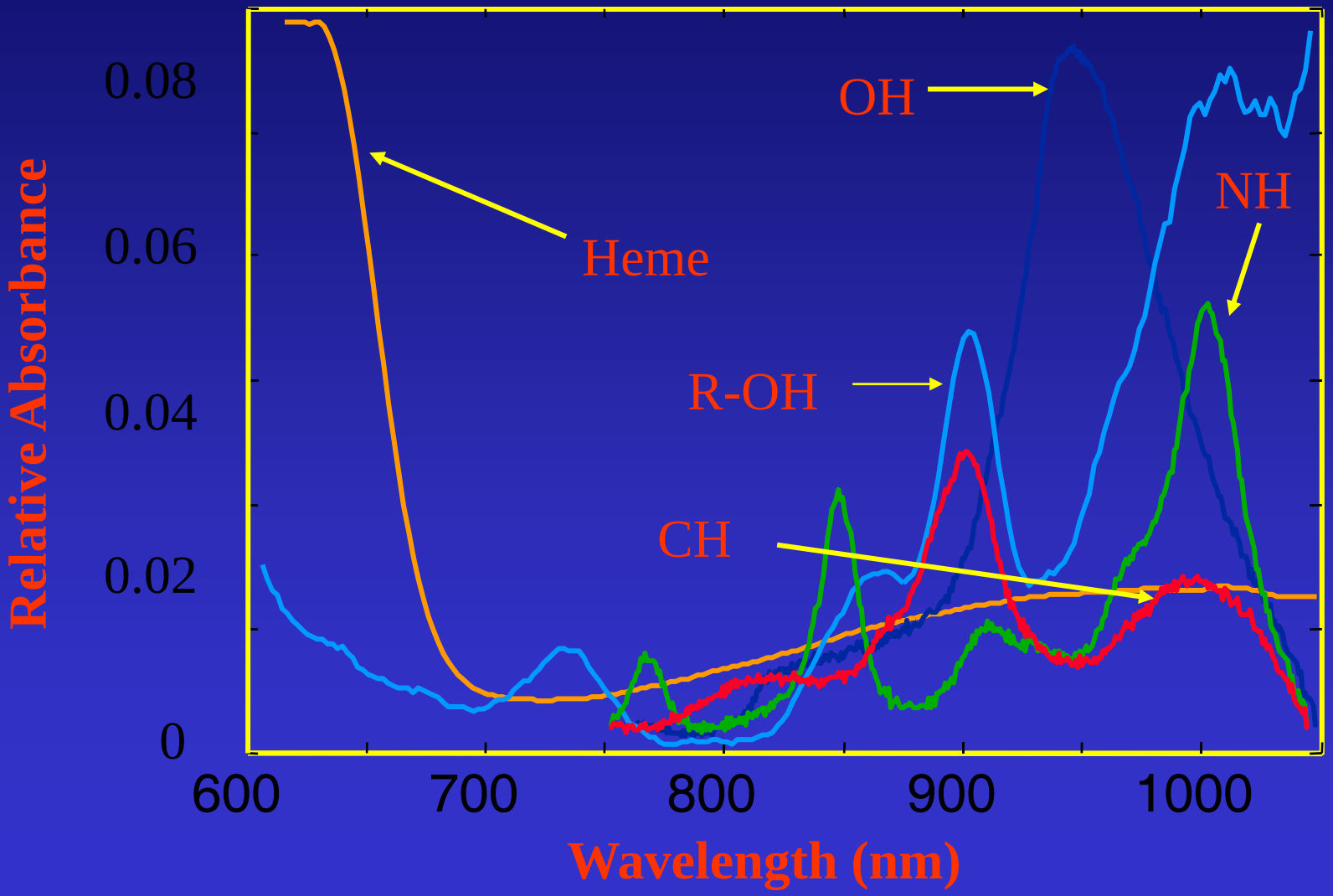
Metabolomics



Molecular Biometrics

Metabolon

Biomarker Spectral Signatures
(by NIR)



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.

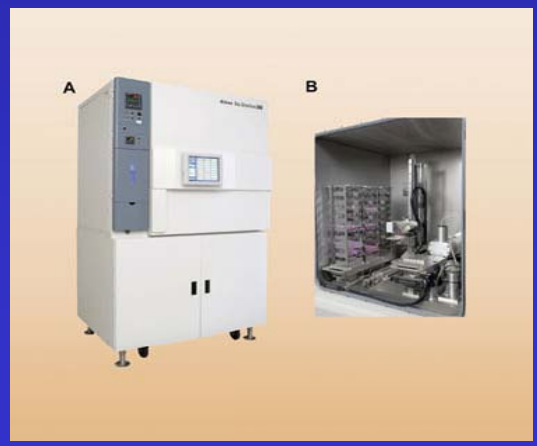
Imaging Systems (currently available in the USA)

Unisense EmbryoScope

Primo Vision

Olympus

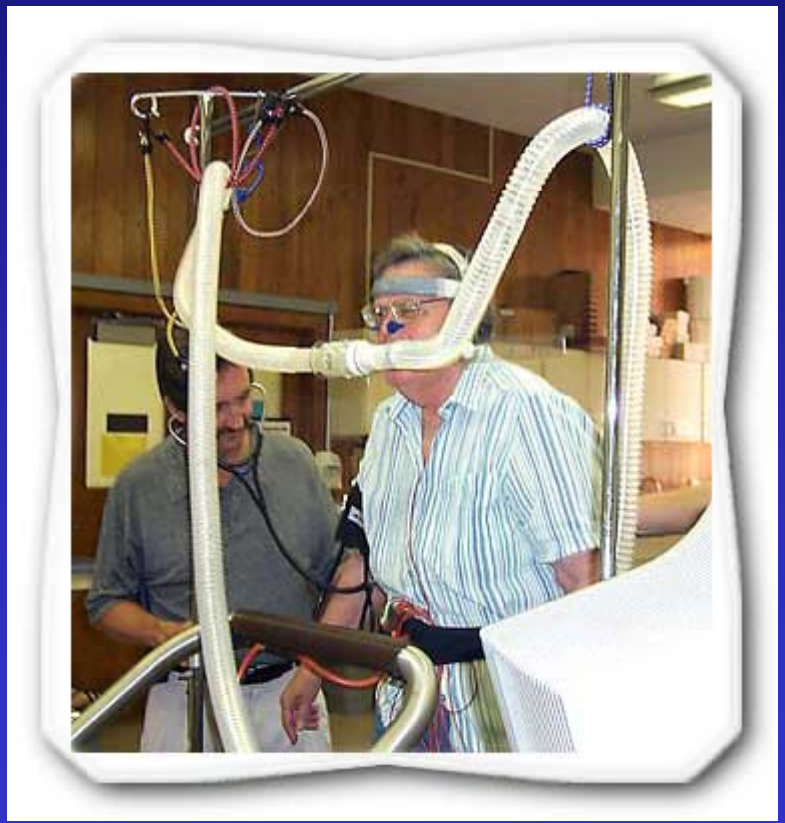
Nikon Biostation



Astec real time monitoring system

what is the embryo equivalent?

i.e. non–invasive, quantitative assessment



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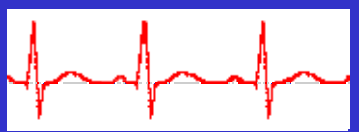

Summary

- Many opportunities to interrogate embryos.
- Time laps imaging offers a lot of promise. Prospective trials emerging.
- Parameters of the first three mitotic divisions prior to embryonic activation indicate success to blastocyst (>93% specificity and sensitivity); suggesting success/failure inherited (maternal).
- Defects in underlying molecular programs underlie aberrant blastomere behavior.
- Should be able to be measured other ways?
- Improved diagnostics = (Early transfer), fewer embryos, reduced adverse outcomes and increased success.