Metabolomics: approaches to assessing oocyte and embryo quality

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DISCLOSURE

Denny Sakkas, Ph.D.

Chief Scientific Officer of Molecular Biometrics LLC which are involved in developing instruments assessing embryo culture media

THE ULTIMATE AIM: TO IDENTIFY THE BEST EMBRYO FOR TRANSFER

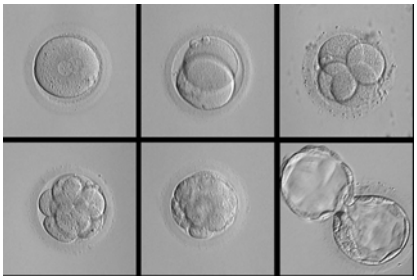
- We need to establish a more rigorous selection process for defining the quality of individual embryos so that the one we choose for transfer is more likely to be viable
- A limiting factor is that these measurements ideally need to be non-invasive and not time consuming

THE PARADIGM OF ASSISTED REPRODUCTION

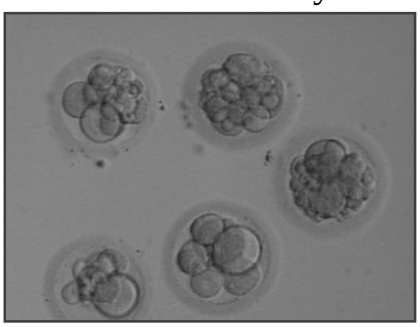
- **FAILURE (not good)**
- **MULTIPLE PREGNANCIES (too good)**
- **In relation to multiple pregnancy the current indications are that in the future we will be compelled via either a legal, financial or moral obligation to restrict the number of embryos transferred**

ASSESSMENT: NOW AND THE FUTURE

NOW: MORPHOLOGICAL CRITERIA



What would you do?



Case History

4 failed cycles

35 years of age

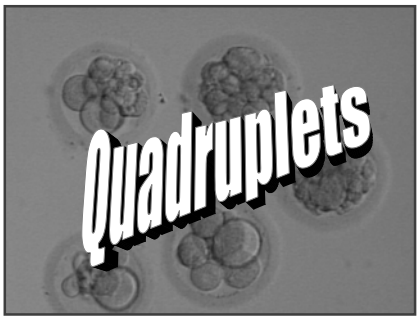
Cell number

Symmetry

Fragmentation rate

Courtesy of R. Scott, RMANJ

What would you do?



Case History

4 failed cycles

35 years of age

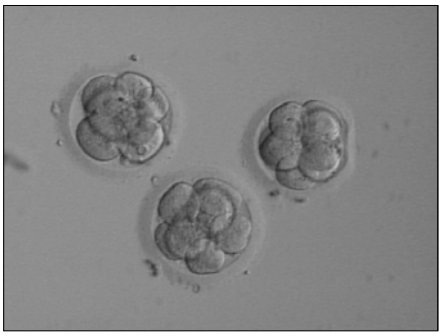
Cell number

Symmetry

Fragmentation rate

Courtesy of R. Scott, RMANJ

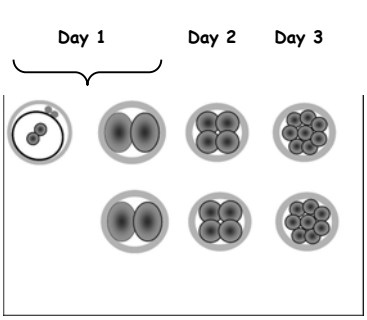
Are these Embryos Reproductively Competent?



No pregnancy occurred.

Courtesy of R. Scott, RMANJ

The accuracy of a day 3 morphological assessment in predicting blastocyst development on day 5



	% of embryos forming good blastocyst
	26/48 (54.2 %)
	35/70 (50.0 %)

[Sakkas et al., 2004]

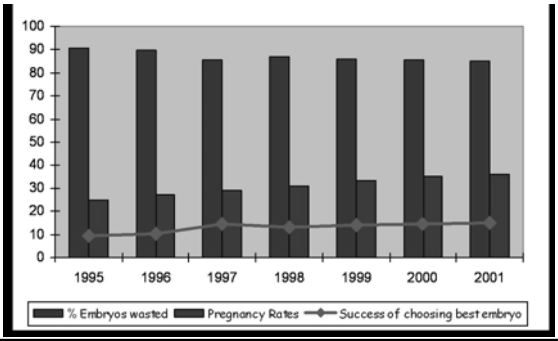
The accuracy of a day 3 morphological assessment in predicting blastocyst development on day 5

- Rijnders and Jansen (1998) found that only 51% of the embryos that were transferred on day 5 had been pre-selected for transfer on day 3.
- Milki et al. (2002) found that if on day 3, the embryologist selected two embryos to transfer, their accuracy on day 5 that:
  - both selections were transferred was only 23%
  - neither selection was transferred was 39%
  - only one selection was transferred was 38%

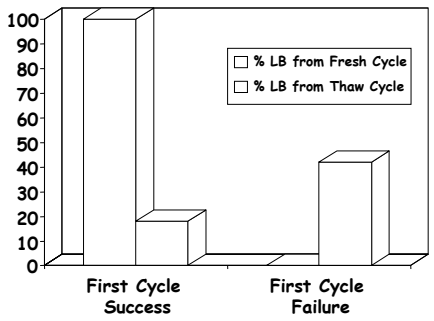
WHO TRANSFERS THE BEST EMBRYO IN TERMS OF MORPHOLOGY AND CLEAVAGE RATES?

WASTAGE OF EMBRYOS AFTER TRANSFER

(Kovalevsky And Patrizio, Fert Stert 2005)



SUCCESS OF EMBRYO TRANSFER AFTER SUCCESS OR FAILURE IN FIRST CYCLE

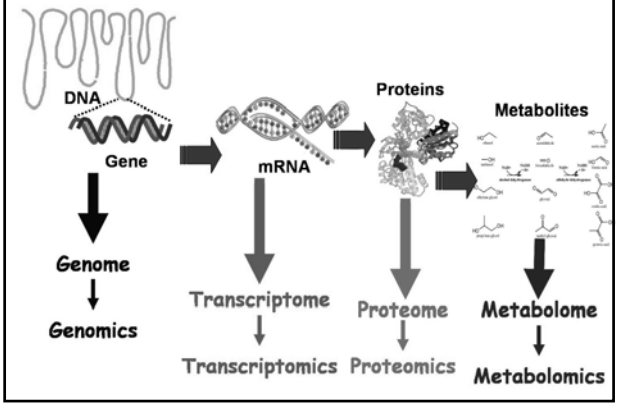


MORPHOLOGY DOES NOT ALWAYS REVEAL THE TRUTH

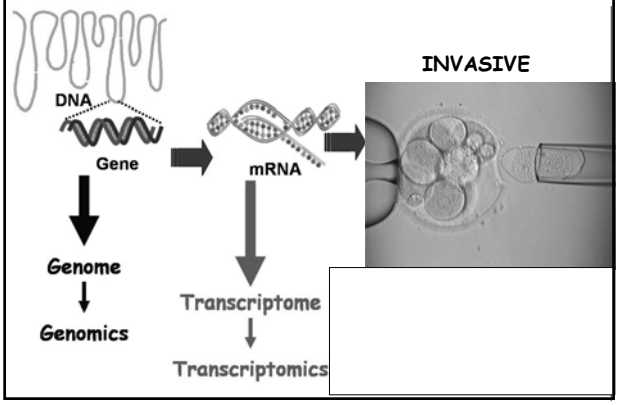
HOW WE WILL LOOK AT EMBRYOS IN THE NEAR FUTURE ?

- DNA FINGERPRINTING
- GENE EXPRESSION
- SECRETION OF FACTORS INDICATIVE OF VIABILITY
  - PROTEINS
  - METABOLIC

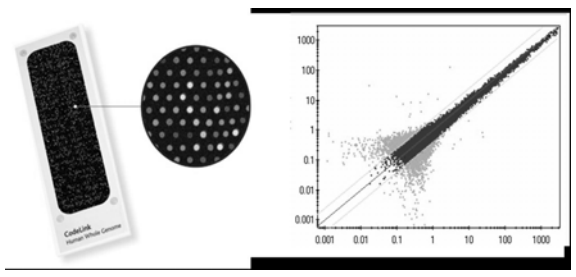
The “Omes and Omics”



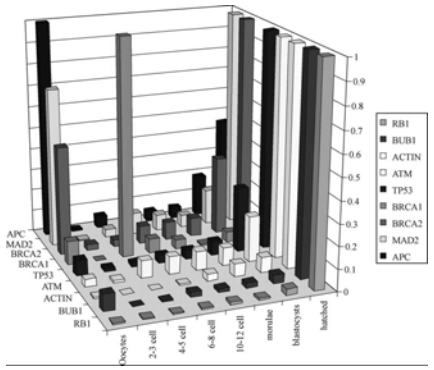
The “Omes” Part 1



Global Gene Expression

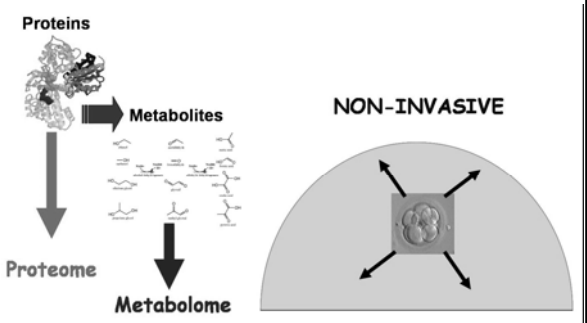


Fluctuations in gene expression during preimplantation development in the human

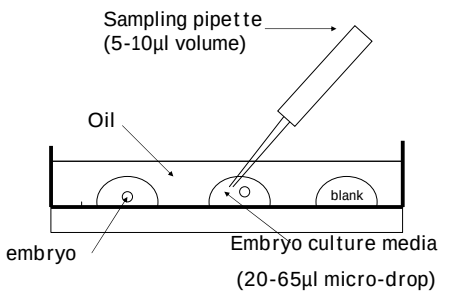


Wells. et al. Hum. Reprod. 2005 20:1339-1348

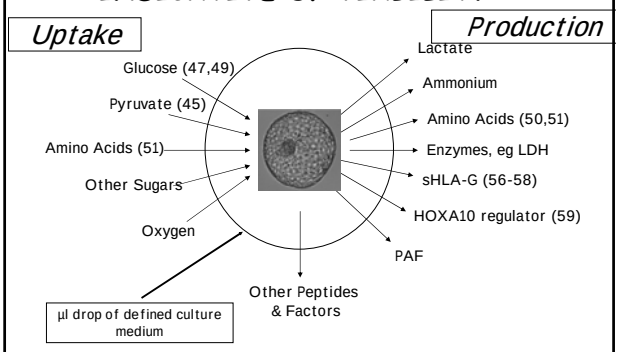
The "Omes" Part 2



Non-invasive Embryo Selection

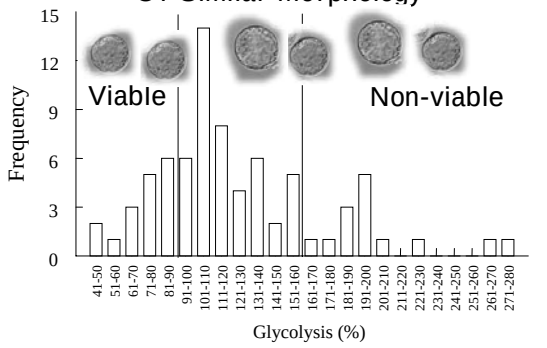


CHANGES IN THE CULTURE MEDIA INDICATIVE OF VIABILITY



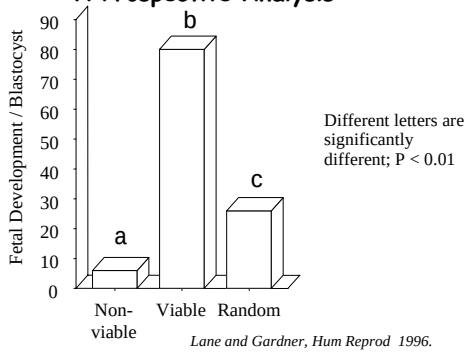
[Sakkas and Gardner, Curr. Opin. Obstet. Gynecol. 2005]

Prospective Selection Of Mouse Blastocysts Of Similar Morphology



[Lane and Gardner, Hum Reprod 1996]

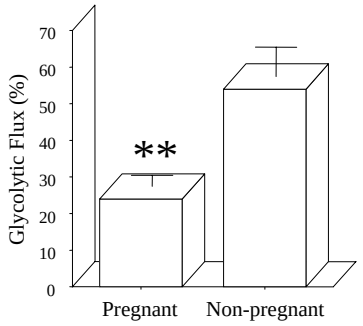
Glycolytic Activity and Mouse Blastocyst Viability: A Prospective Analysis



Different letters are significantly different; $P < 0.01$

Lane and Gardner, Hum Reprod 1996.

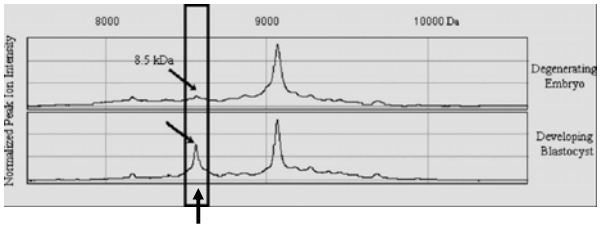
Relationship Between Human Blastocyst Glycolytic Activity and Pregnancy



Van den Bergh, RBMonline; 2001: 3, Suppl 1, O1

PROTEOMICS

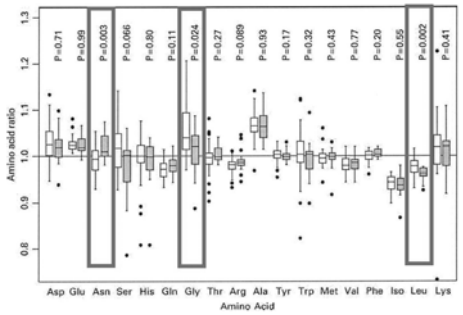
The expression of an 8.5-kDa protein biomarker appears to be directly associated with ongoing human blastocyst development.



Significant difference in expression

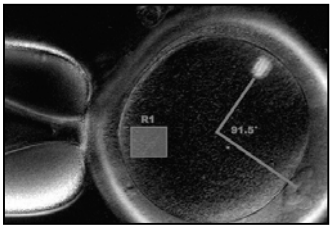
[Katz-Jaffe et al. Fertil Steril 2006]

Identifying viable embryos by non-invasive measurement of amino acid turnover using high performance liquid chromatography



[Brison et al. Hum Reprod. 2004]

Oosight Imaging System©



- Looks at presence, orientation, and size of meiotic spindle.
- Several minutes of additional exposure to ambient environment required to make assessment
- Not evaluated clinically to date

EmbryoScope© by Unisense

- Non invasive measurement of respiration rates of embryos during development
- Less than 1 minute per embryo
- Pregnancies reported of bovine embryos characterized by nano-respirometry prior to transfer
- Lopes et al Reprod Fertil Dev 2005
- Scott et al. ESHRE 2007

Metabolomics

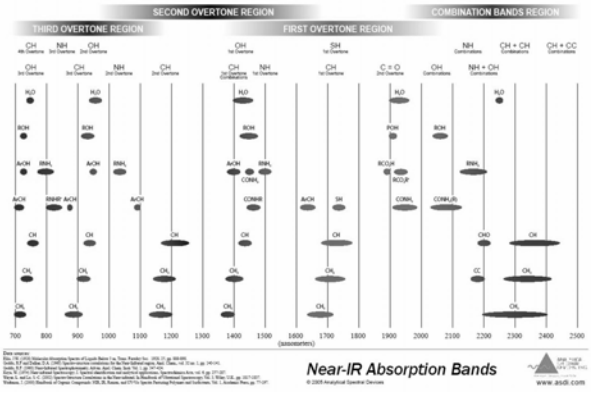
- **The complete array of small-molecule metabolites that are found within a biological system constitutes the metabolome**
- **Metabolomics provides a biochemical "snapshot" of the small molecule inventory produced during cellular metabolism, reflecting the physiological status of an organism**

Biospectroscopy in Industry

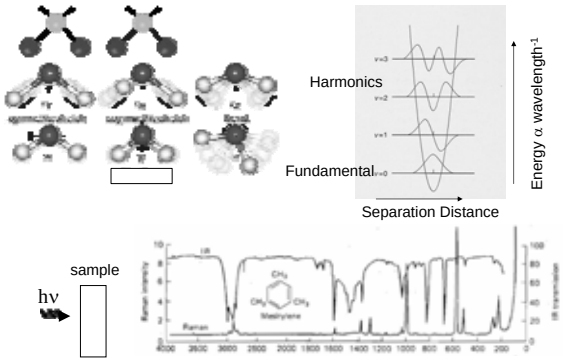
For Example in Milk:

Fat:	CO-O 5.723μm
	CH 3.48μm
Protein:	CO-N 6.465μm
Lactose:	OH 9.610μm

Foods: Snacks, starch, cereals, milk powders, infant food, coffee, chocolate, biscuits, ingredients, potato flakes, tea, breadcrumbs, flour, refined sugar, nuts, pasta dough.
Animal Feed: Sugar-beet pulp, dark grains, alfalfa, grass, fish meal, pet foods, soya meal, gluten meals, fibers, oilseed.
Pharmaceuticals: Batch drying and blending.
Oil Extraction: Residues from olives, palm and soy.
Fibers: Cotton, filter tow, acetate, textiles, rayon, tire cord.
Others: Sewage sludge, composted waste, cork.



Basis of Vibrational Biospectroscopy



What is Measured?

- Clinically
  - How the embryo modifies its environment
- Biologically
  - Changes in concentrations of:

Functional Groups	Constituents
•CH	•Albumin
•NH	•Lactate
•OH	•Pyruvate
•SH	•Glutamate
•C=C	•Glucose

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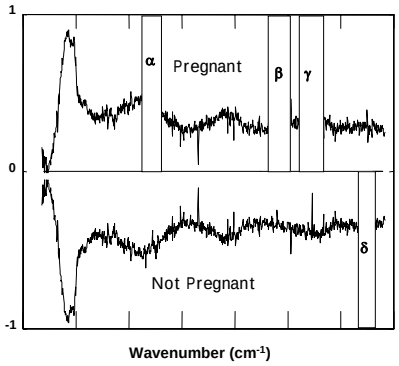
What is measured?



What is measured?

- Combinations of wavelength regions that reflect implantation outcomes are determined by inverse least-squares regression and a genetic algorithm optimization

VIABILITY SCORE =

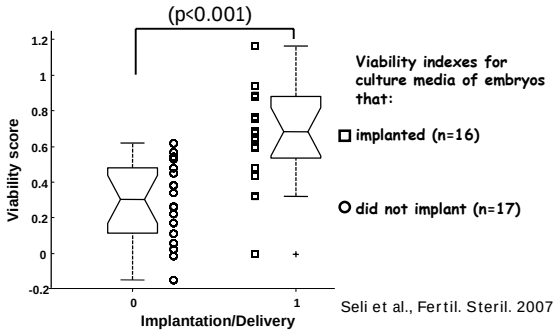
$\alpha(W\alpha) + \beta(W\beta) + \gamma(W\gamma) + \delta(W\delta)$

$\alpha,\beta,\gamma,\delta$ are the area measures of the spectrum and W is the weighting of their importance

The Dow Jones index of embryology

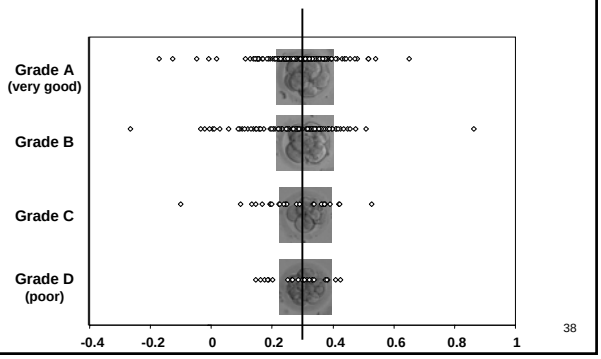
Viability scores calculated using NIR spectra of culture media

Notch box plots represent quartiles with green horizontal lines corresponding to the median of the data groups



Seli et al., Fertil. Steril. 2007

Distribution of the Viability Score of Individual Embryos of the same Morphology



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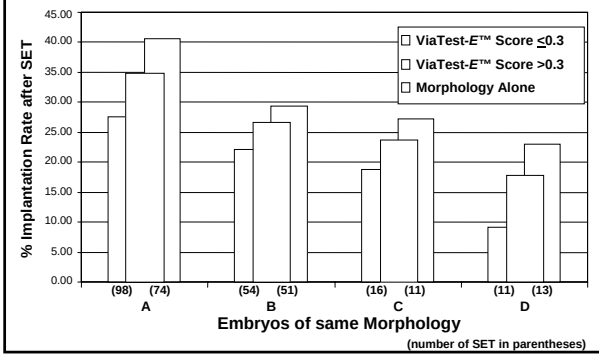
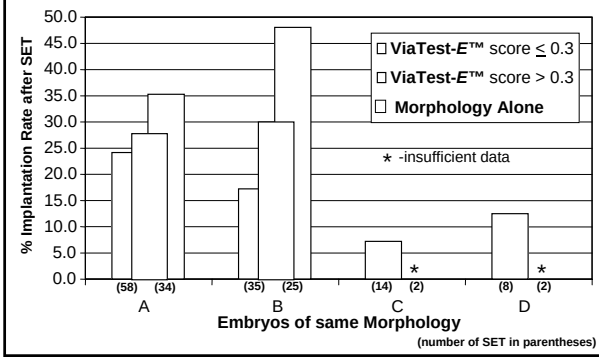
NIR analysis of samples from SET

1. SET was performed on the basis of morphology
2. Media was taken from the transferred embryo and stored at -80 degrees Celsius
3. Media samples were tested and algorithms generated according to clinical pregnancy outcome
4. Random media samples were then tested

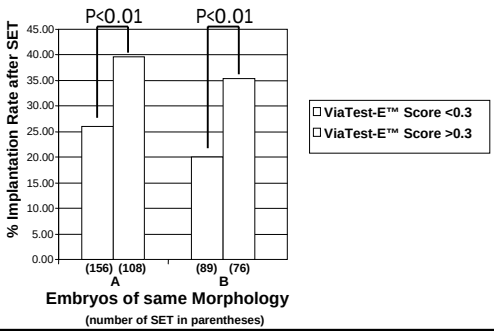
Amsterdam SET implantation rates of Day 3 embryos comparing the same morphology grade and a ViaTest-E™ score of ≤ or > than 0.3



KLC SET Implantation Rates of Day 2 Embryos Comparing the Same Morphology Grade and a ViaTest-E™ Score of ≤ or > than 0.3



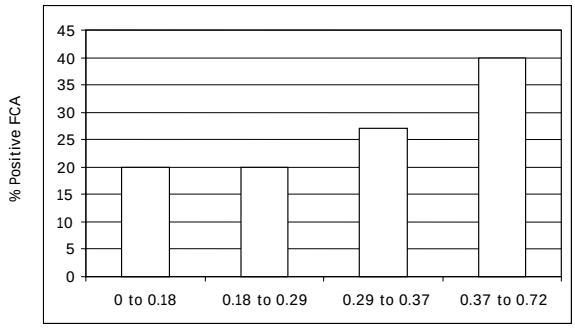
SET implantation rates of day 2 and 3 embryos comparing the same Morphology grade and a ViaTest-E™ Score of > 0.3



Kato Clinic Trial 2

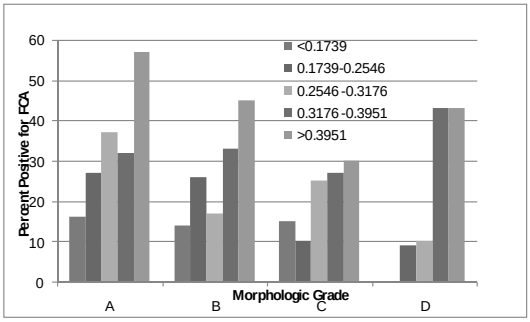
1. 181 samples run to generate spectra
2. 121 samples used to generate "genetic algorithm"
3. 60 samples tested blindly
4. ViaTest-E score generated for the 60 samples
5. ViaTest-E score correlated to pregnancy outcome

Kato Clinic Trial 2 separated into Quartiles From the 60 validation samples



Kato Clinic Trial 2 ViaTest-E Score

Proportion of FCA+ Patients by Morphology and ViaTest-E™ (N=577)



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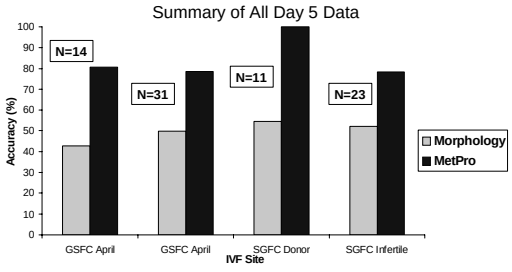
Accuracy

A measure of a test's ability to correctly identify positive and negative FCA pregnancy from a complete IVF patient population.
The accuracy of a test can be determined by calculating:

$$\text{Accuracy} = \frac{TP + TN}{TP + FN + TN + FP}$$

where TP = true positive [a good blastocyst or high score = pregnancy]
TN = true negative [a poor embryo or low score = no pregnancy]
FP = false positive
FN = false negative

Comparison of Accuracy: Morphology vs MetPro Based on Known Pregnancy Outcome

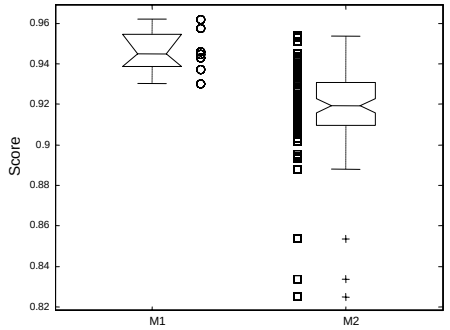


Accuracy of ViaTest-*E*™ vs. Morphology

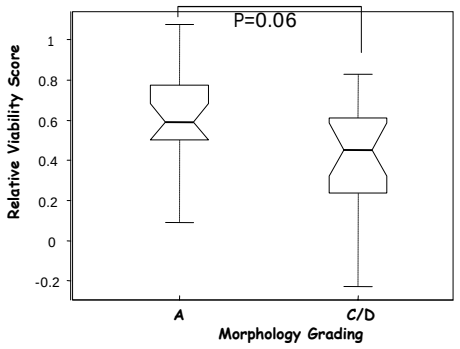
Day of Transfer	% Accuracy	
	Morphology	**ViaTest-*E*™**
Day 2	31.9	71.3
Day 3	55.0	74.0
Day 5	48.3	79.2
Global Average	45.1	74.8

Oocyte Maturation

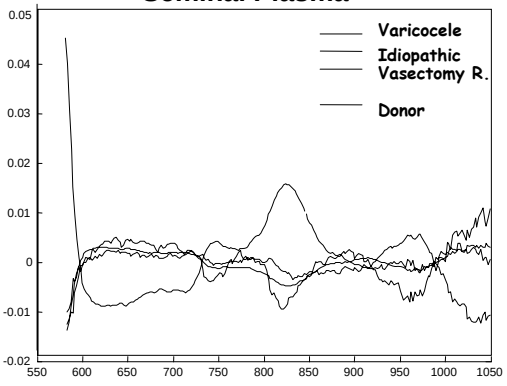
3 Hours Post Retrieval



NIR spectroscopic analysis of culture medium of oocytes that developed to grade-A and C/D embryos on day-3



Spectral analysis of Seminal Plasma

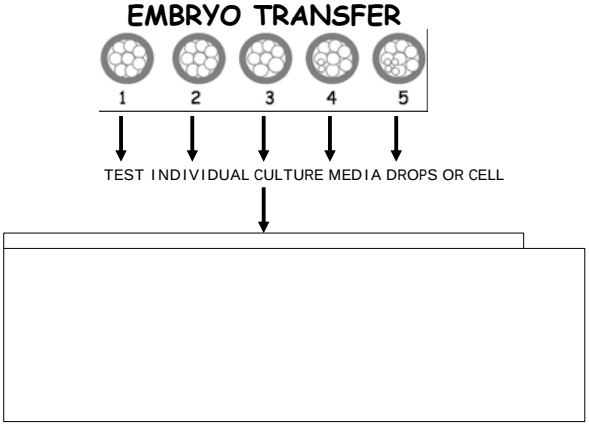


TECHNICAL CRITERIA FOR SELECTING THE BEST EMBRYO FOR TRANSFER:

The technique must:

- not damage the embryo
- measure the change rapidly
- measure the change consistently and accurately
- not be technically overwhelming

EMBRYO TRANSFER



IN THE NEAR FUTURE IN ADDITION TO MORPHOLOGY IDEAL DNA, RNA, PROTEIN OR METABOLITE PROFILES WILL BE PERFORMED ON:

1. Biopsied Blastomeres
2. Embryo culture medium
3. Follicular Fluid
4. Cumulus cells

ACKNOWLEDGEMENTS

Molecular Biometrics reproductive technology research group
-Dr. Emre Seli
-Dr. Peter Nagy
-Dr. Barry Behr
-Prof. A. Agarwal

-Lucy Botros
-Dr. Pieter Roos

Collaborating IVF Clinics

- *Kato Ladies Clinic, Tokyo, Japan*
- *VU University Medical Center, Reproductive Medicine, Amsterdam, the Netherlands*
- *Fertilitetscentrum, Göteborg, Sweden*
- *RMA-NJ, New Jersey, USA*
- *Shady Grove, Washington DC, USA*
- *Yale Fertility Center, New Haven, CT, USA*

Emerging Technologies for the Assessment of Gametes and Embryos: The "OMICS"

October 2-3, 2008
California, USA
SERONO BioSymposia

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- Metabolomics is the methodological analysis of target biological samples for changes in metabolite and small molecule composition. While genomics and proteomics have received considerable attention in recent years, metabolomics has assumed an increasingly prominent role with the realization that small molecules play a critical role in the chemistry of biological systems.
- In practice, metabolomics provides a biochemical “snapshot” of the small molecule inventory produced during cellular metabolism, reflecting the physiological status of an organism. Measuring and identifying individual analytes often results in incomplete, biased diagnostic “pictures.” This is because cellular metabolism involves multiple small-molecule metabolites. Thus, a more accurate diagnostic picture can be obtained by measuring multiple small molecule metabolites simultaneously as biomarkers. From these metabolomic profiles, biomarker interrelationships and changes can be extrapolated, resulting in a complete, objective analysis of the metabolic process and creation of an accurate, objective diagnostic picture.