Exogen markers

in human embryo culture

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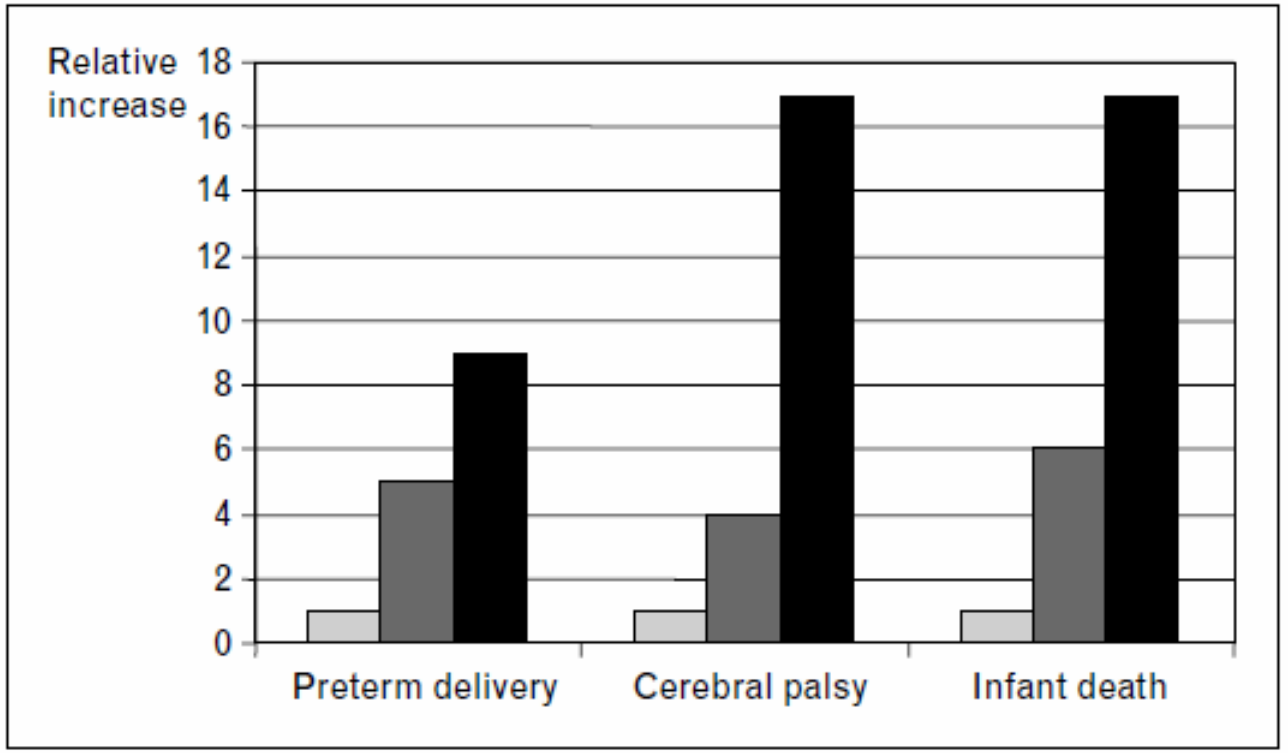
EMBRYO ASSESSMENT FOR TRANSFER

Reducing the risk of multiple gestation

Maximizing the probability of pregnancy

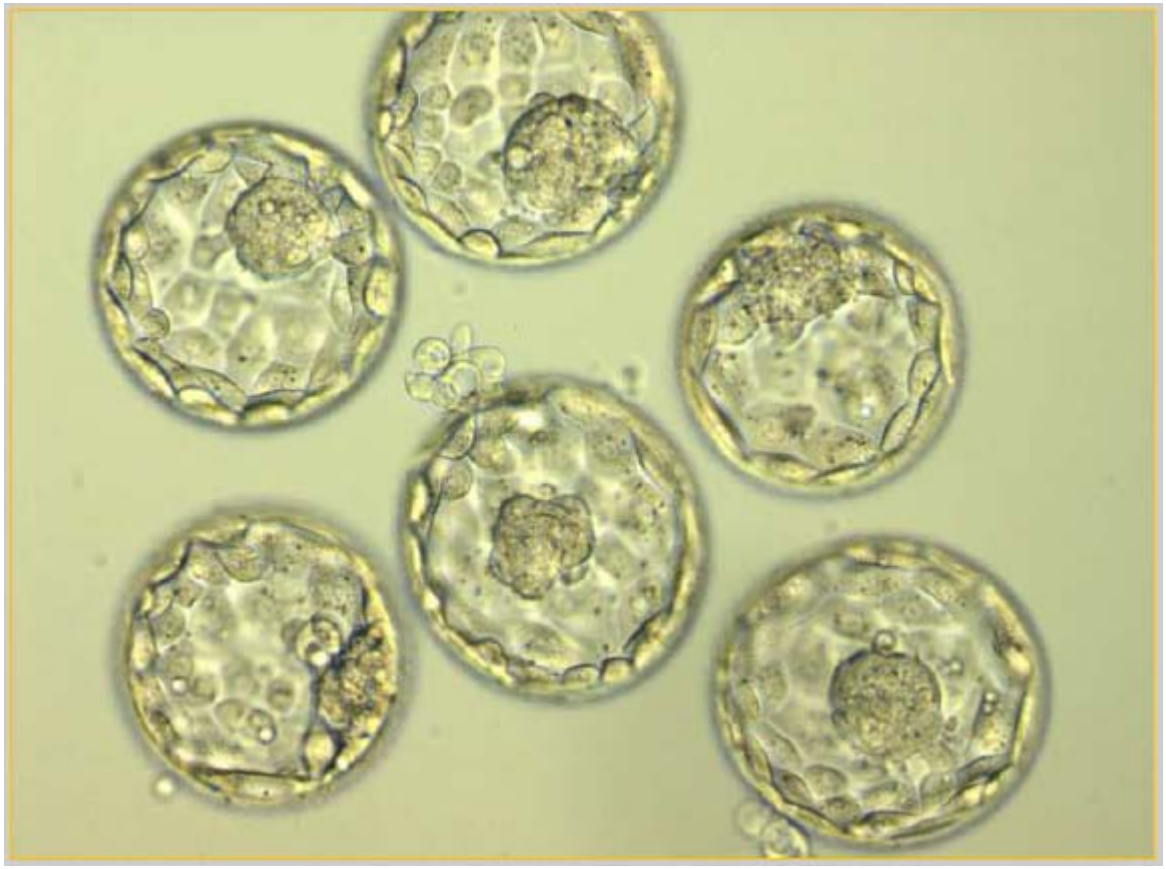
The selection of embryos with higher implantation potential is one of the major challenges in assisted reproduction technology

FETAL COMPLICATIONS OF MULTIPLE PREGNANCIES



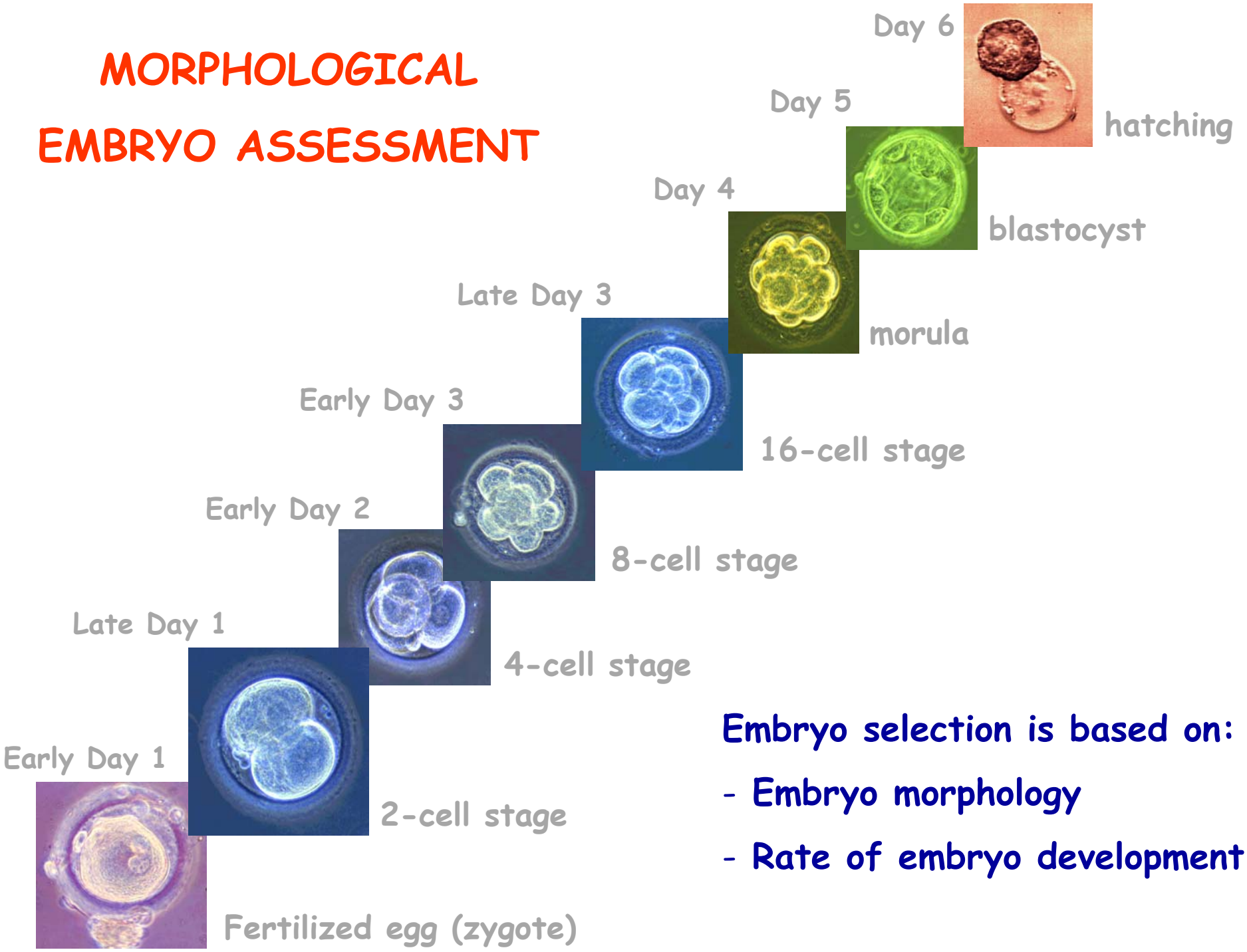
Twin and higher order multiple gestations are associated with significantly increased risks of infant morbidity and mortality compared to singletons. □, Singleton; ■, Twin; ■, Higher order.

Bromer & Seli. Curr Opin Obstet and Gynecol, 2008



WHICH EMBRYO TO SELECT FOR SET?

MORPHOLOGICAL EMBRYO ASSESSMENT



Embryo selection is based on:

- **Embryo morphology**
- **Rate of embryo development**

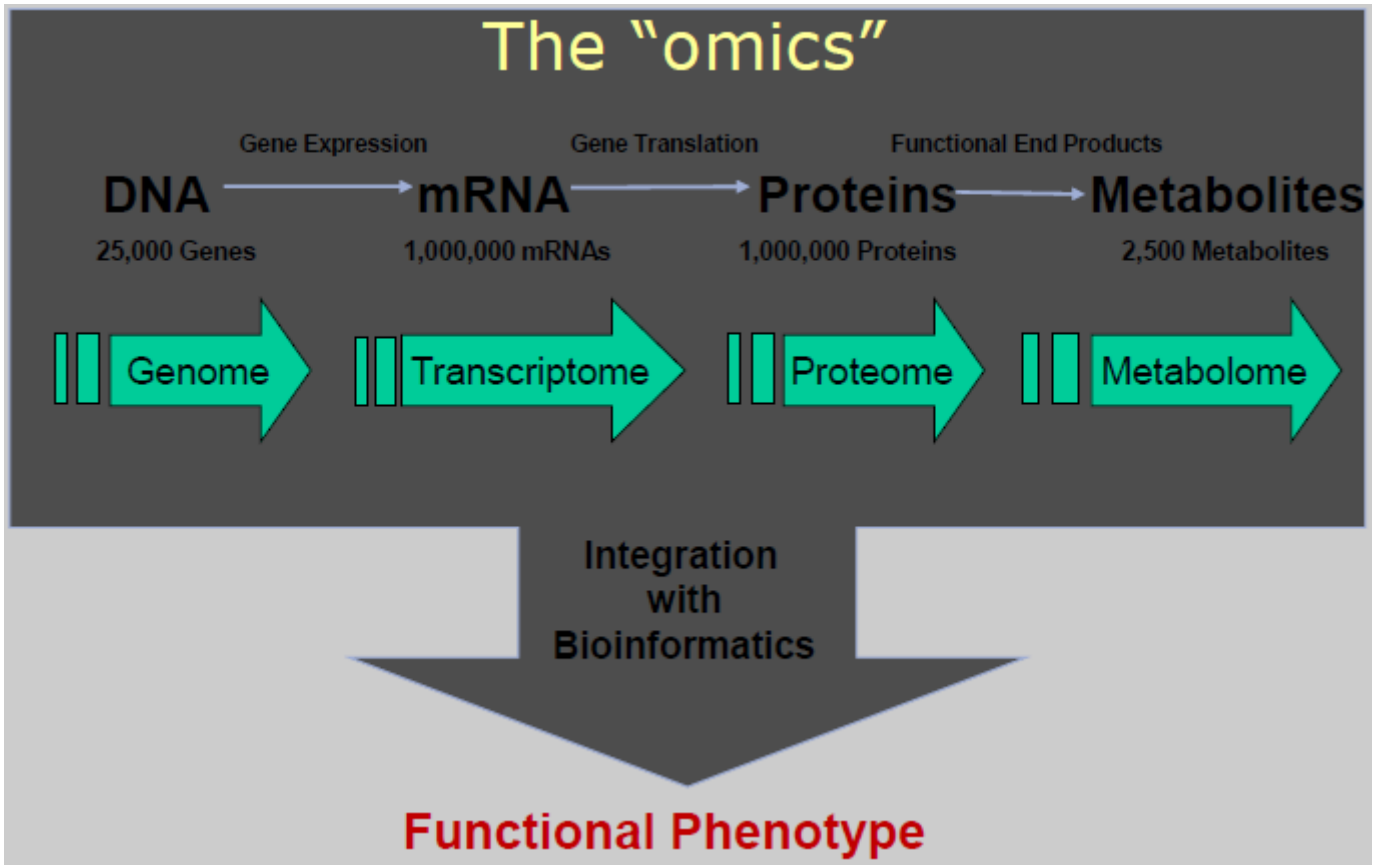
❖ An improvement over current embryo assessment strategies is needed

❖ Novel invasive (genomics: biopsy of blastomeres, transcriptomic analysis of blastomeres) and non-invasive (genomics: transcriptomic analysis of cumulus & granulosa cells; proteomics & metabolomics) approaches are investigated

❖ For clinical application, a technology should:

- Not damage the embryo
- Be easy
- Be rapid
- Be inexpensive
- Require a small sample

THE FUTURE FOR EMBRYO SELECTION

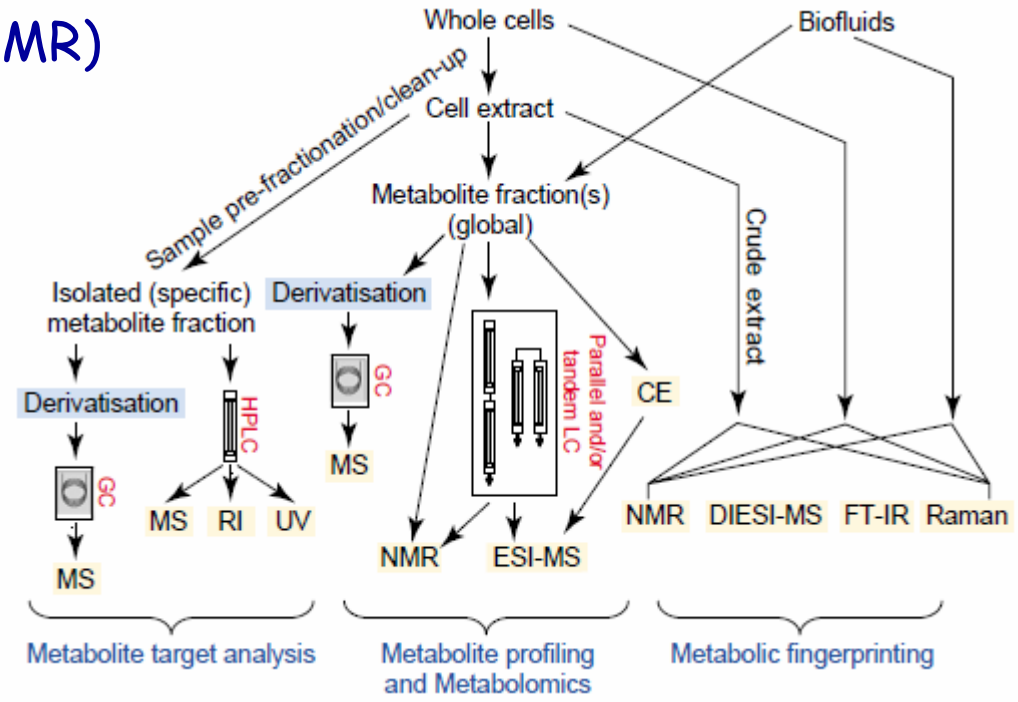


METABOLOME: the complete array of small molecule metabolites that are found in a biological system and reflects the functional phenotype (**SECRETOME**)

METABOLOMICS: studies the dynamic inventory of metabolites

TECHNOLOGY PLATFORMS FOR METABOLOMICS

- Capillary electrophoresis (CE)
- ELISA
- Mass spectrometry (MS)
- Nuclear magnetic resonance (NMR)
- Gas chromatography (GC)
- Liquid chromatography (LC)
- Raman spectroscopy
- Near-infrared spectroscopy
- Proton NMR
- Protein microarrays



Goodacre et al. Trends in Biotechnology, 2004

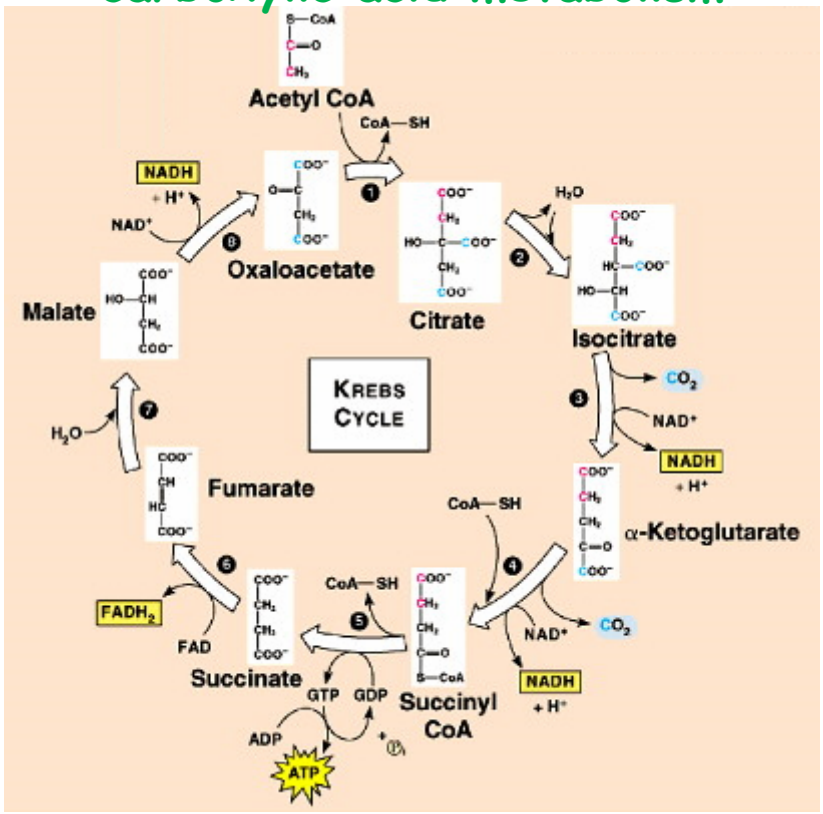
SECRETOME ANALYSIS TO ASSESS HUMAN OOCYTE AND EMBRYO QUALITY

Target Molecule	Method of analysis	Embryonic stage tested	Clinical practicality	Outcome
Single or specific molecule targeting				
Pyruvate	Ultramicrofluorescence	Day 1–5	High technicality, Less practical.	Contrasting results
Glucose	Ultramicrofluorescence	Oocytes, Day 1–5 embryos	High technicality, Less practical.	Contrasting results
Oxygen	Microspectrophotometry	Oocytes, blastocysts	High technicality, Less practical.	Acquired oxygen consumption rates for oocytes and blastocysts.
	Respirometry	Oocytes	Expensive equipment.	Respiration rates correlated to maturation and viability of oocytes
Amino acids	Reverse-phase high performance liquid chromatography	Day 2–5 embryos	High technicality, Practicality still to be demonstrated.	Predict blastocyst formation, pregnancy and live birth.
	Proton nuclear magnetic resonance spectroscopy	Day 3 embryos	High technicality, impractical.	Viability index correlated with pregnancy outcome.
HLA-G	Enzyme-linked immunoabsorbent assay	Follicular fluid, Day 0–5	High technicality, Less practical	Contrasting findings.
Leptin	Enzyme-linked immunoabsorbent assay	Day 5 embryos	High technicality, Less practical	Positive correlation between leptin secretion and blastocyst development
Groups of molecules targeted				
Protein compliment	Surface-enhanced laser desorption ionization time-of-flight mass spectrometry	Day 5 embryos	High technicality, Less practical. Expensive equipment.	Protein profiles are related to blastocyst morphology.
	Protein microarray	Day 5 embryos	High technicality, Less practical. Expensive equipment.	Implantation potential corresponds to specific protein secretion levels.
Metabolomic compliment	Non-optical spectroscopy	Day 3 embryos	High technicality, impractical. Expensive equipment.	Metabolomic profile correlates with reproductive potential of embryos.
	(Proton nuclear magnetic resonance) Vibrational spectroscopy (Near infrared; Raman)	Oocytes, Day 3–5 embryos	Simple, rapid procedure, inexpensive, practicality still to be demonstrated.	Oocyte viability score correlates to developmental potential. Embryo viability score predicts pregnancy independent of morphology.

METABOLIC PARAMETERS OF EMBRYOS (1)

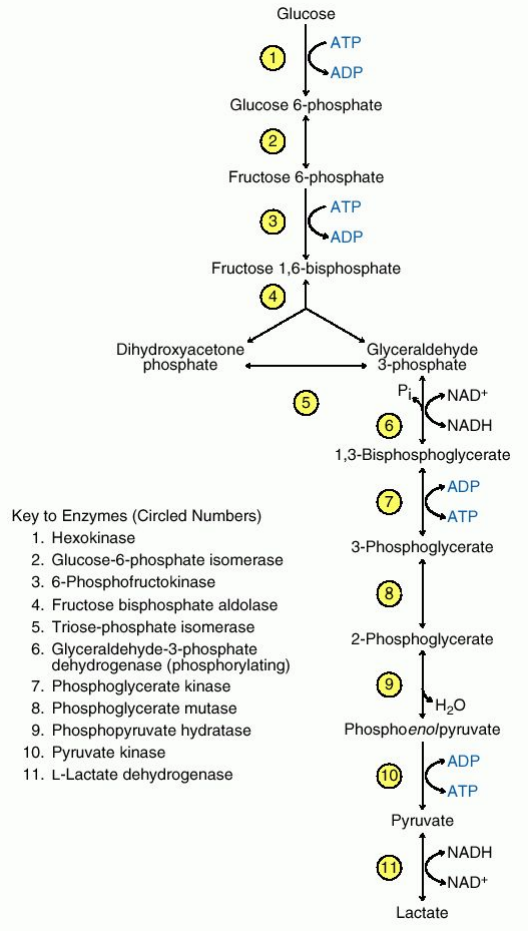
GLYCOLITIC ACTIVITY

Pre-compaction:
carboxylic acid metabolism



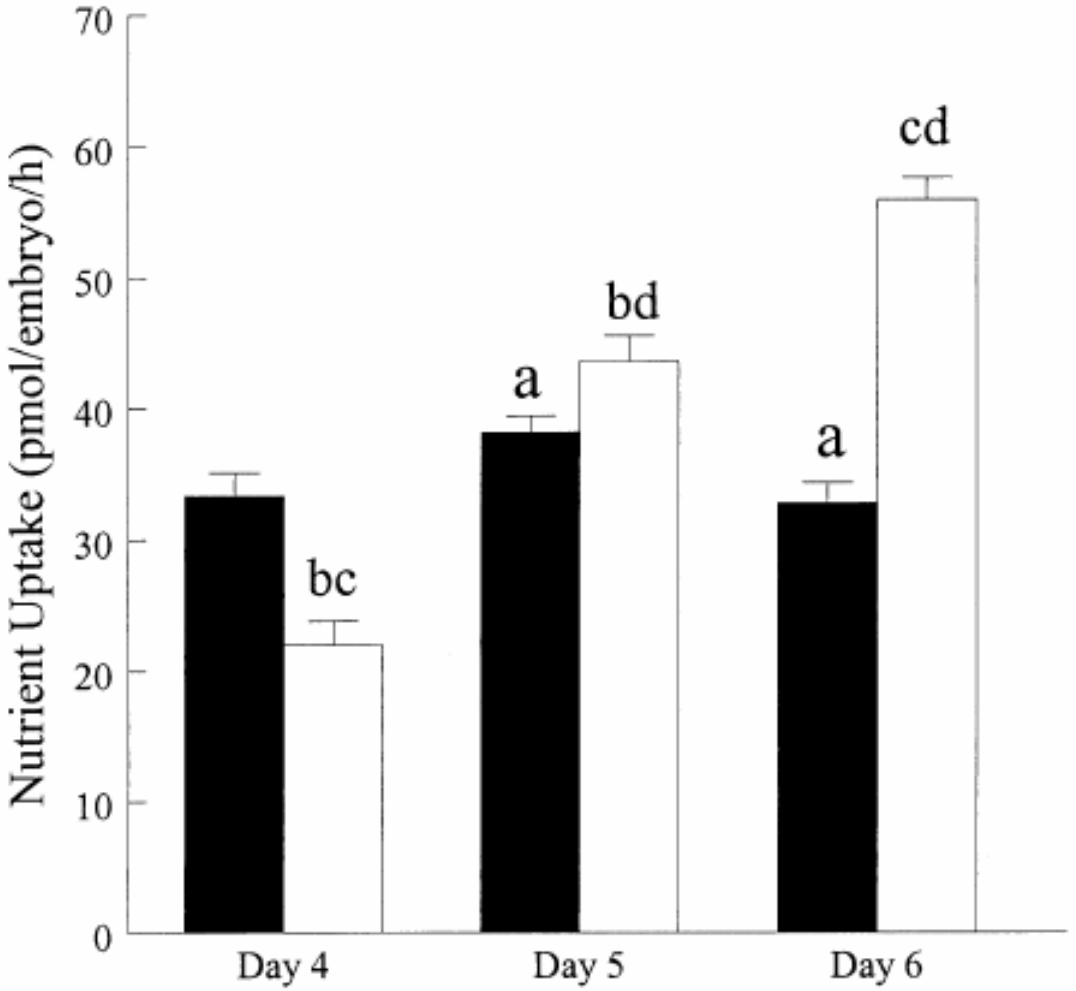
Pyruvate and lactate are the embryo's main sources of energy

Transition from morula to blastocyst



Post-compaction:
glucose metabolism

PYRUVATE AND GLUCOSE UPTAKE BY HUMAN EMBRYOS



Pyruvate uptake, closed bars; glucose uptake, open bars.
Statistically significant differences: a = P<0.05; b, c, d = P<0.01

Gardner et al. Fert & Ster, 2001

PYRUVATE AND GLUCOSE METABOLISM

Pyruvate, lactate and glucose metabolism as a predictor of embryo development and viability—human studies.

Study	Embryo stage examined	Altered metabolite associated with improved outcome	Technology used	Outcome
Hardy et al. 1989	Day 2-4	↑ pyruvate uptake No association with glucose uptake	Ultramicrofluorescence assay	Blastocyst development
	Day 5	↑ pyruvate uptake ↑ glucose uptake	Ultramicrofluorescence assay	Blastocyst development
Gott et al. 1990	Day 2-4	↑ pyruvate uptake ↑ lactate production No association with glucose uptake	Ultramicrofluorescence assay	Blastocyst development
	Day 5	↑ pyruvate uptake ↑ glucose uptake ↑ lactate production	Ultramicrofluorescence assay	Blastocyst development
Conaghan et al., 1993	Day 2 – 3	↓ pyruvate uptake	Ultramicrofluorescence assay	Clinical pregnancy
Turner et al., 1994	Day 2	Intermediate pyruvate uptake	Ultramicrofluorescence assay	Clinical pregnancy
Gardner et al., 2001	Day 4	↑ pyruvate uptake ↑ glucose uptake	Ultramicrofluorescence assay	Blastocyst development
Seli et al.	Day 2-3	A trend toward ↑ pyruvate uptake ↑ glucose uptake	Proton NMR	Pregnancy and delivery

Botros et al. Mol Human Repr, 2008

Inconclusive whether pyruvate and glucose uptake is predictive for embryo development and viability

? **Limiting factor**: stress on embryos due to a lack of essential nutrients in the culture media

METABOLIC PARAMETERS OF EMBRYOS (2)

AMINO ACID TURNOVER

Amino acid uptake and secretion by the embryo as a predictor of embryo development viability—human studies.

Study	Embryo stage examined		Altered metabolite associated with outcome	Technology used	Outcome
Houghton et al., 2002	Day 2 – 3	↓	amino acid turnover (sum of depletion and appearance)	HPLC	Blastocyst development
		↓	glutamine, arginine, methionine uptake		
		↓	alanine and asparagine release		
	8 cell-Morula	↓	amino acid turnover (sum of depletion and appearance)	HPLC	Blastocyst development
		↓	serine uptake		
		↓	alanine and glycine release		
Brison et al., 2004	Day 2	↓	glycine and leucine in culture media	HPLC	Clinical pregnancy and live birth
		↑	asparagine levels in culture media		
Seli et al. 2008	Day 3	↑	glutamate levels in culture media	Proton NMR	Clinical pregnancy and live birth

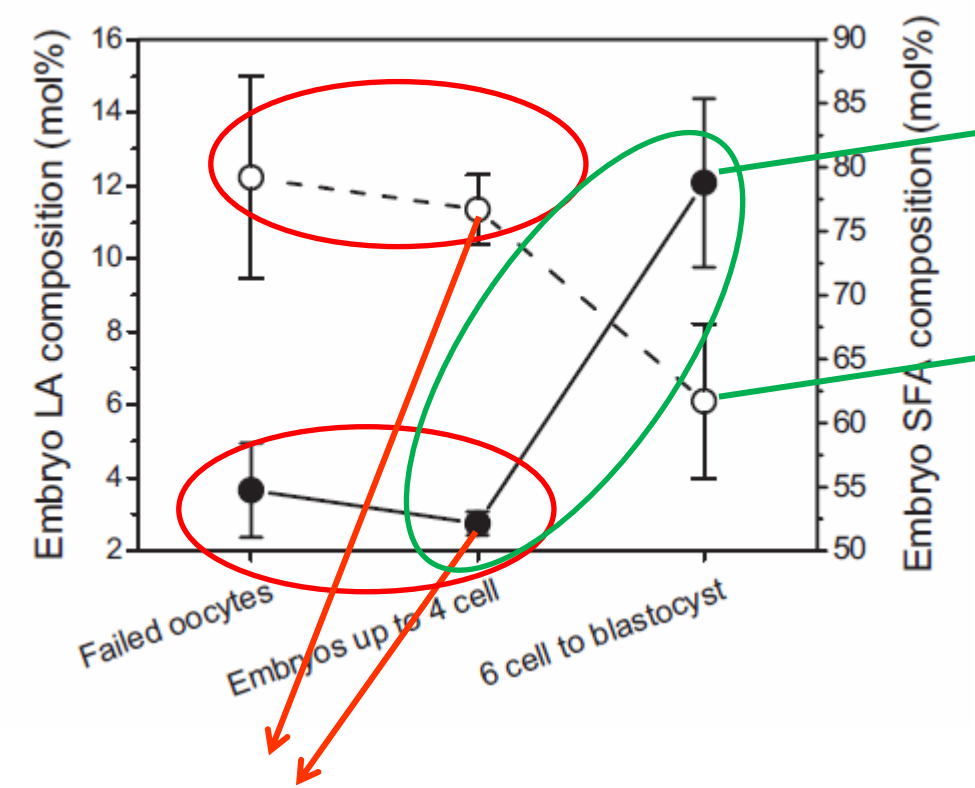
Botros et al. Mol Human Repr, 2008

Embryos with greater viability have a lower or quieter amino acid metabolism than those that arrest

METABOLIC PARAMETERS OF EMBRYOS (3)

FATTY ACID METABOLISM

Compositional changes as function of development:



Embryos which develop beyond the 4-cell stage have significantly higher concentrations of the **unsaturated acids**, particularly linoleic acid. Viceversa for saturated acids.

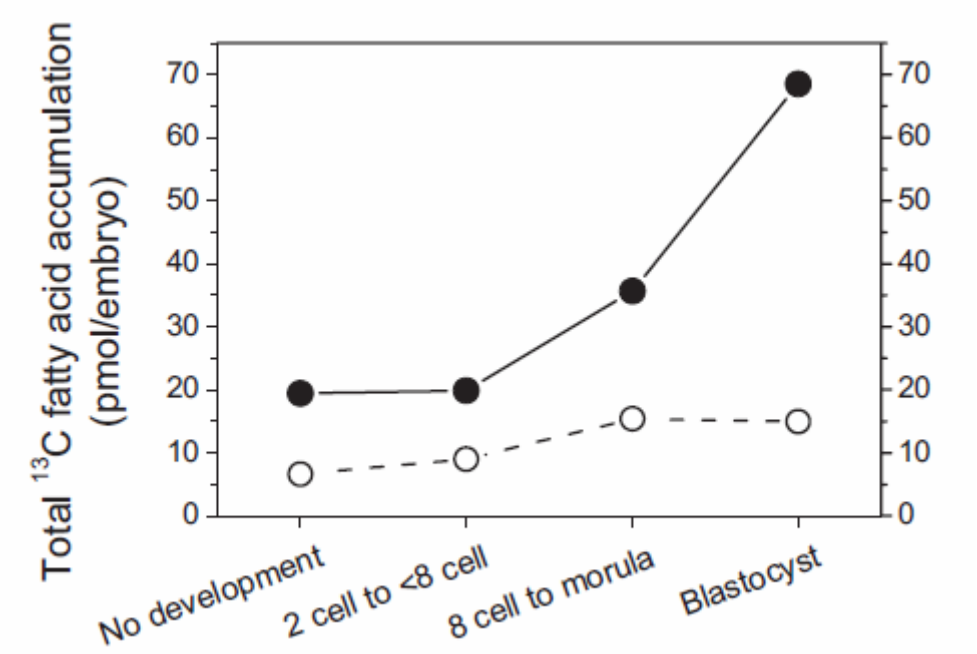
They **support developmental processes** in the embryos:
-Regulate gene expression
- Cell growth and differentiation by protein kinase C
- Essential components of membrane lipids which increase with each cell division

The fatty acid composition of embryos which fail to develop is similar to that of oocytes which fail to fertilize

Haggarty et al., Hum Reprod, 2006

FATTY ACID METABOLISM

Fatty acid uptake in relation to the stage of development:



Increasing uptake of linoleic acid and decreasing uptake of the saturated fatty acid with stage of development

The uptake of both fatty acid was very low prior to the 8-cell stage and increased with the stage of development for linoleic in particular

Haggarty et al., Hum Reprod, 2006

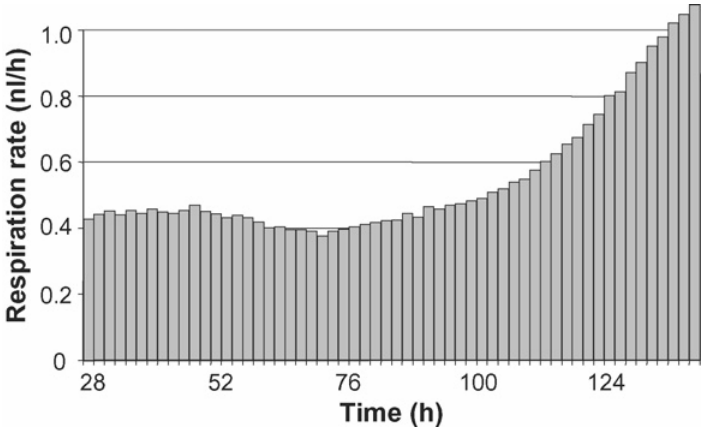
METABOLIC PARAMETERS OF EMBRYOS (4)

OXYGEN

Invasive techniques → **Non-invasive techniques**

- Microspectrophotometry
- Scanning electrochemical microscopy
- Loop-mediated isothermal amplification

- NanoRespirometer (individual measurement of embryonic respiration rates at a fixed time)
- Embryo Respirometer (individual, continous measurement of respiration rates + images of each embryo)



Respiratory profile of one embryo, measured continuously from the zygote to the expanding blastocyst stage

Lopes et al. Theriogenology, 2007

- Oxygen consumption is higher among embryos of superior morphological quality
- Positive correlation between oxygen consumption pregnancy rate
- Reduced respiration rates in oocyte cohorts with low or no fertilization

but not used for clinical applications (most on bovine embryos), not significant test

SINGLE PROTEIN ANALYSIS IN SECRETOME

PAF

-Autocrine trophic/survival factor
- Influences maternal physiology alterations (immune function and platelet activation)

ApoA1

Increased levels of ApoA1 are associated with blastocyst of higher morphological grade

Leptin

- During the window of implantation, leptin establishes a molecular dialogue with leptin receptors on the maternal side
- Competent human blastocysts secrete higher leptin concentrations than arrested embryos

Ubiquitin

It is increased in the secretome of developing blastocysts

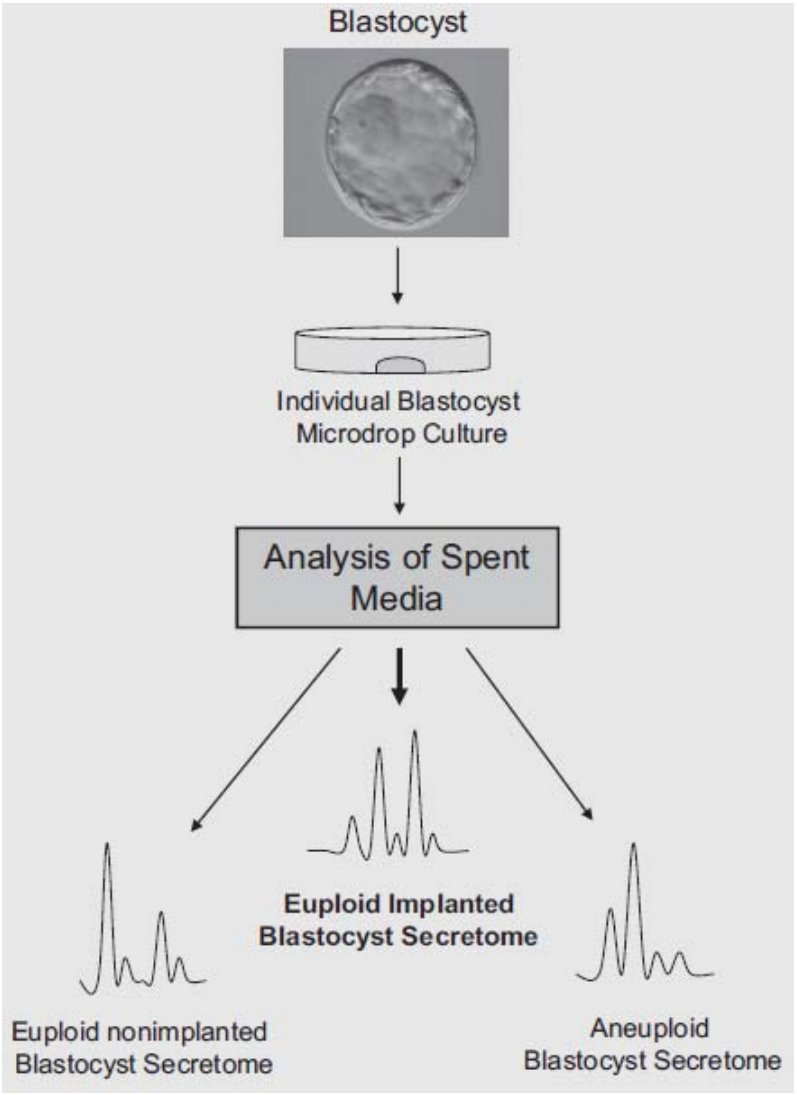
sHLA-G

- It is believed to play a role in immuno tolerance during pregnancy
- Its presence correlates with the improved embryo cleavage rate and pregnancy potential

HOXA10 regulator

Another protein involved in the reciprocal embryo-endometrial interaction.
HOXA10 expression by epithelial endometrial cells is regulated by an unknown soluble molecule secreted by human blastocysts.

PROTEIN SECRETOME AS POTENTIAL MARKERS OF EMBRYO ANEUPLOIDY



Katz-Jaffe & McReynolds. Fertil Steril, 2013

i.e., **Lipocalin-1**

- Overproduced under conditions of stress, inflammation, infection

- Increased secretion of lipocalin-1 from aneuploid blastocysts

- LCN-1 inhibits cysteine proteinases

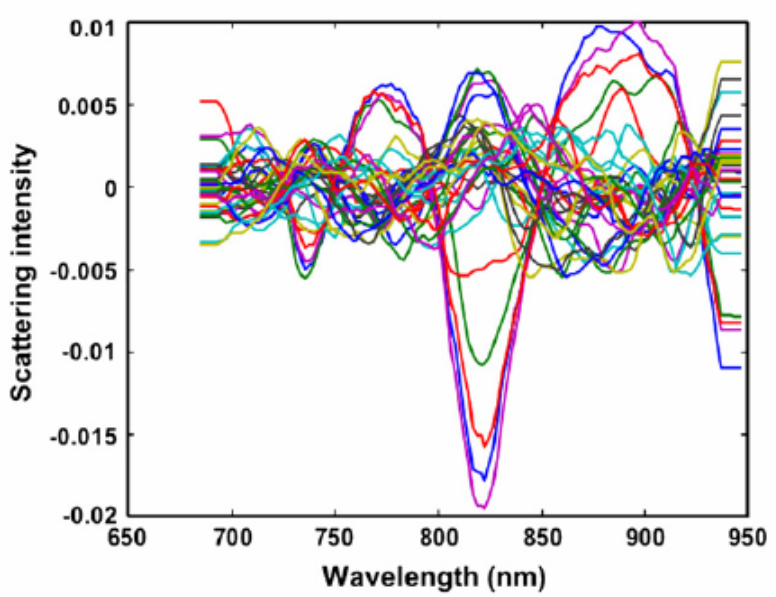
- Cysteine proteinases are important in embryo hatching and implantation

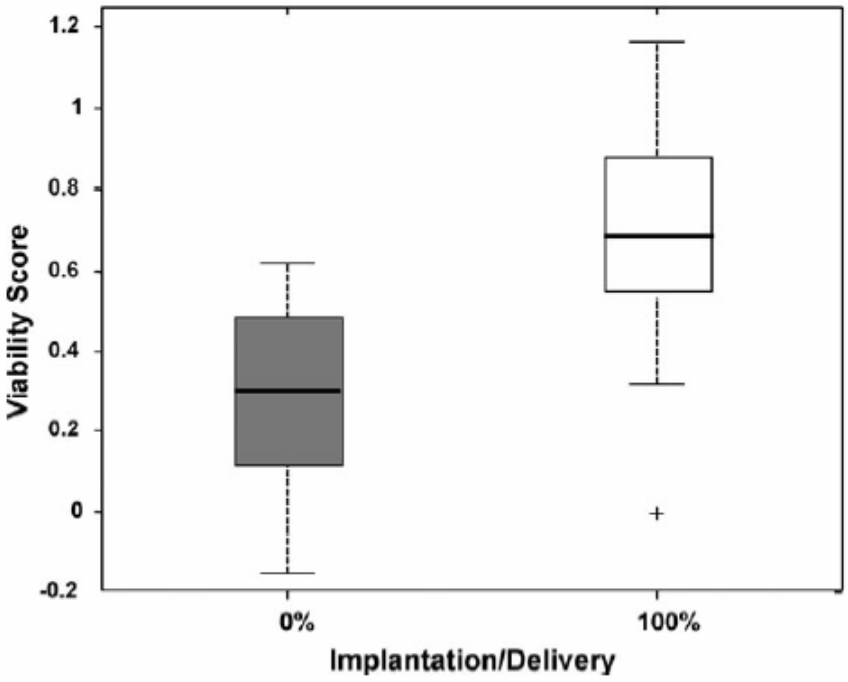
METABOLOMIC PROFILING

Raman and NIR spectroscopic analysis of functional groups :

-CH
-NH
-OH

Oxidative stress



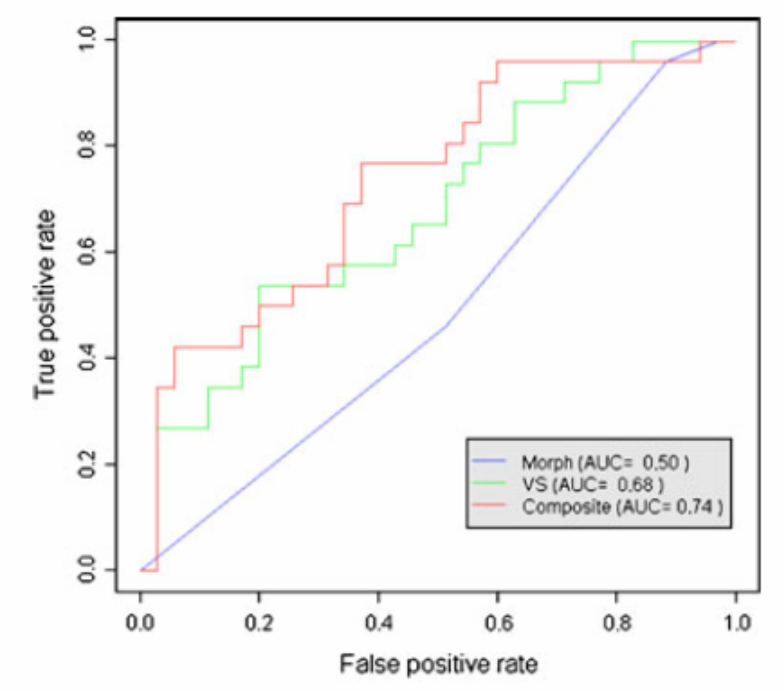


Viability score

Seli et al. Fertility and Sterility, 2007

Morphology grading and metabolomic Viability Score on predicting implantation outcome

- retrospective studies -



Seli et al. J Assist Reprod Genet, 2011

Prospective studies: *(Hardarson et al. Hum Reprod 2012; Vergouw et al. Hum Reprod, 2012)*

They **failed** to support the concept that NIR techniques can improve implantation rate in SET (day 2, 3 and 5)

CHALLENGES OF USING METABOLOMICS IN A CLINICAL SETTING

Limited template, low protein expression and lack of sensitivity of current proteomics plstform

The overwhelming presence of albumin and other serum proteins in the culture media makes it difficult to identify the low expressed secreted embryonic proteins

Proteomic technologies require installation and implementation of a system into a lab that does not specialize in the technologies

Sources of variability, *i.e.*, experimental design, data interpretation, lack of standardized sample collection and storage

To date there is no non-invasive platform that has been proven to be of true clinical predictive value or been examined in prospective randomized control trials to be better than current morphology-based selections methods


Thank you!