Embryo development and viability

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What is embryo viability

- An embryo that is ”alive”? (Blastocyst development?)
- An embryo giving rise to an implantation? To a (healthy) baby?
- An embryo that is chromosomally ”normal”?
- An embryo that is metabolically ”normal”?
- All or nothing? – or degrees?

Argument 1:

Viability = blastocyst development / (implantation)?

Embryo morphology/development that correlates to blastocyst development and implantation:

- Number of cells
- Cell size / fragmentation
- Number of nuclei…
- …..

Embryo morphology

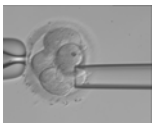
- Subjective
- Very much relying on experience
- Rather low predictive value
  - but ”validated” with development and implantation

Viability assessments *complementary* to morphology

- Invasive = using cell(s)
- Non-invasive = indirect

Argument 2:

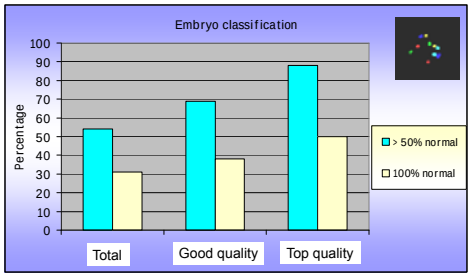
Viability = chromosomally normal?



Genetic status and success rates

- FISH
- CGH (comparative genomic hybridisation
- CGH – Microarray
- Non-invasive?

Chromosomal normality and embryo selection (n=144 embryos)



Ziebe et al 2003,

Genetic status and viability
(= blastocyst development)

- 1254 normal karyotype women, 6936 GQE embryos biopsied on day 3
- Analysed for chr. 13,16,18,21,22,X, Y

Blastocyst development:

- Euploid embryos = 68.2%
- Abnormal = 42.8%
- Mosaic = 53.7%
- Higher blastocyst rates for trisomies than for monosomies (autosomes)

Rubio et al 2007

Chromosomal status and viability
(= blastocyst development)

- I.e. low correlation
- Cut – offs?

PGS - FISH

- 11 randomised control trials (embryos) so far (age, poor/good prognosis patients)
- Show no improvement in delivery rates

  ?
- Limited number of analysed chromosomes
- High rates of embryo mosaicism
- Poor correlation between results and implantation? (*M. Hughes*)

- Polar body analysis? (ESHRE RCT ongoing)

CGH

- Allows analysis of all chromosomes
- Complex technique
- Needs DNA amplification
- Longer time for preparation/analysis (combined with cryopreservation?)

- Prospective trial showing increased live birth rates for CGH cycles
- No RCTs performed, needs to be validated
- Same problems with mosaicism

Wells et al 2008, Fragouli et al 2008

CGH - Microarrays

- Needs DNA amplification
- Faster analysis (<48h), more automated

- Invasive
- Not validated

Argument 3:

Viability = metabolically normal?

”Metabolic” assessments of the embryo or the surrounding, e.g.:

- Amino acid turnover (non-invasive)
- The ”omics” (invasive / non-invasive)
- sHLA-G (invasive / non-invasive)

Amino acid profiling of early embryos

- Depletion and/or appearance of AA:s (turnover)
- Analysed with high-performance liquid chromatography (HPLC)
- The pattern varies with development stage
- Varies with environmental conditions (eg. culture medium, cryopreservation)
- Different profiles from in vitro vs. in-vivo derived embryos (bovine)
- Different profiles ICM vs. TM

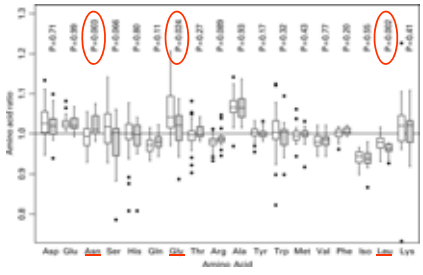
Houghton 2002, Brison et al 2004, Sturmey 2008

Amino acid profiling of early embryos - Results

- Developmental competent embryos have a lower AA turnover (”quiet embryo hypothesis”)
- AA profile independent of morphology
- Concentrations of **asparigine**, **glycine** and **leucin** in the medium at 24 h significantly associated with live birth
- The *overall* pattern of AA turnover significantly related to live birth
- Large prospective randomised study ongoing

Houghton 2002, Brison et al 2004, Sturmey 2008

Amino acid turnover



Brison et al Hum Rep 2004

”The Omics” – *looking at the*:

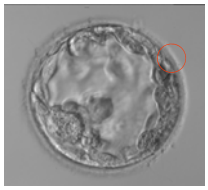
- *genome – genes, chromosomes*
- *transcriptome –mRNA*
- *proteome – proteins*
- *metabolome – metabolites*
- *secretome – secreted proteins*

Transcriptomics

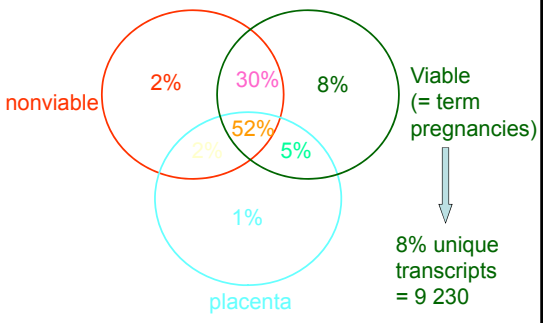
- Analysis of gene expression patterns
- mRNA amplification
- Slow, labour intensive
- Microarray techniques enables analysis of thousands of genes
- Invasive
- So far few studies

Trophectoderm analysis

- Blastocyst biopsy of 10-20 trophectoderm cells
- 48 patients
- 154 blastocysts
- Biopsy – microarray
- >40.000 gene transcripts
- 37 babies born
- Non-implanting vs. implanting embryos analysed (fingerprinting)



Cram et al ASRM 2005, Jones et al 2008



Jones et al 2008

Proteome / secretome

- Analysis of the proteins expressed and translated from the genome (proteomics)
- Analysis of the proteins secreted from the embryo into the medium (secretome)
- Mass spectroscopy methods

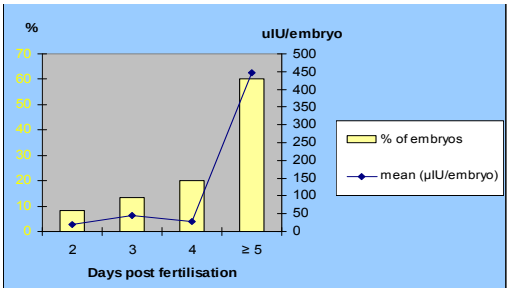
- Two ways to go:
  - Global approach (pattern")
  - Identification of individual proteins

Human leukocyte antigen-G (HLA-G) in embryos

- Detected in oocytes and preimplantation embryos
- HLA-G positive (mRNA expression) blastocysts show higher cleavage rate
- Correlation between HLA-G expression in blastocysts and implantation
- Embryotrophic (signal to cleave?)
- Immune response modulating?

Jurisicova 1996, Yao et al 2005, Warner et al 2008

hCG production in early embryos
(sandwich immunoassay, n=122)



Hansis et al ASRM 2005

Soluble Human leukocyte antigen-G (sHLA-G) in culture media

- Some studies show correlation between sHLA-G in culture media and implantation
- Not fully correlated to morphology (but to cleavage rate)
- Method not yet validated (optimal ELISA analysis protocol, single embryo culture, single embryo transfer)

Hansis et al ASRM 2005, Warner et al 2008

Secretome

- Culture media analysed every 24 hours
- Distinctive protein profiles
- Day 5 secretome from ongoing blastocyst development showed significantly upregulated protein (ubiquitin?)
- Different profiles from similar morphology blastocysts

Katz-Jaffe 2006

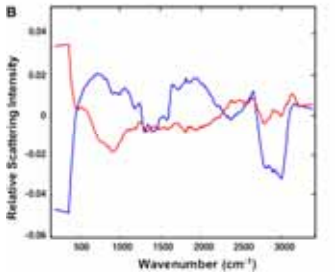
Metabolic fingerprints

- Metabolomic changes in the follicular fluid and/or culture medium (all small-molecule non-protein biomarkers, including metabolic intermediates, glucose, signalling molecules, ATP, etc.)
- Spectrophotometric techniques
- Provides a snap-shot of the current status
- Correlate with development and morphology assessment

The ”viability score”

- Differences in –CH, -NH, -SH, C=C and –OH functional groups
- Distinct different patterns day 3 between embryos that implanted or did not
- Independent of moprhology
- This pattern was used for validation in prospective study, producing a ”viability score” (fixed cut-off)
- Large RCT ongoing

Seli et al 2007, 2009



Seli et al 2007, 2009

Other non-invasive viabilityy assessments

- Respiration measurement
- Imaging

Respiration measurements

- May reflect mitochondrial capacity
- May reflect the amount of available ATP
- Reduced respiration rates in oocytes correlate with increasing age and FSH
- Not validated to implantation rates
- Correlates to development (i.e. decreased add-on value) - bovine

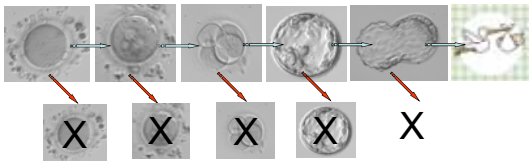
Scott et al 2008

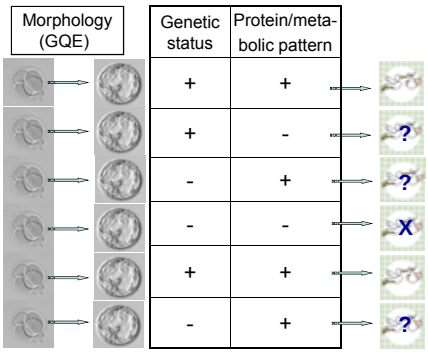
Back to microscopy…. : Continuous documentation, time-lapse

- Closed system
- Timing of cleavage
- Timing of nuclear appereance/disappereance
- Correlations with implantation and birth
- Will more accurate timing increase correlations?

Summary

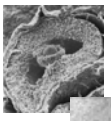
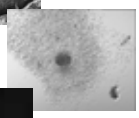
Assessment:		Correlation to "viability":
Morphology	→	Moderate correlation, well characterised
Genetic status	→	Low-moderate corr., mosaicism, cut-off?
Metabolic status	→	Higher corr.?, not yet validated





Can we influence embryo viability?

- Maternal factors
- Paternal factors
- Hormone stimulation
- Culture conditions
  - Media
  - Oxygen
  - Temperature


Thank you for listening!