Embryo development and viability

Kersti Lundin
Reproductive Medicine
Sahlgrenska University Hospital
Göteborg, Sweden

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 – off?

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

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 (e.g. culture medium, cryopreservation)
- Different profiles from in vitro vs. in-vivo derived embryos (bovine)
- Different profiles ICM vs. TM


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 asparagine, 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

"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 trophoderm 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

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?


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

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
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 morphology
- This pattern was used for validation in prospective study, producing a "viability score" (fixed cut-off)
- Large RCT ongoing

Sali et al 2007, 2009
Other non-invasive viability 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
Back to microscopy….
Continuous documentation, time-lapse

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

Summary

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Correlation to &quot;viability&quot;:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Moderate correlation, well characterised</td>
</tr>
<tr>
<td>Genetic status</td>
<td>Low-moderate corr., mosaicism, cut-off?</td>
</tr>
<tr>
<td>Metabolic status</td>
<td>Higher corr.?, not yet validated</td>
</tr>
</tbody>
</table>

Morphology (GQE)

<table>
<thead>
<tr>
<th>Genetic status</th>
<th>Protein/metabolic pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

X
Can we influence embryo viability?

• Maternal factors
• Paternal factors
• Hormone stimulation
• Culture conditions
  – Media
  – Oxygen
  – Temperature

Thank you for listening!