Seeing things from the embryo perspective

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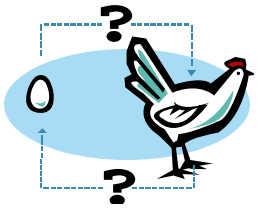
The embryo or the endometrium ?



Determining embryo viability / quality

- Being chromosomally ”normal”?
- Being metabolically ”normal”?
  - All or nothing? – or degrees?
- Blastocyst development?
- Implantation? To become a (healthy) baby?

Embryo ”quality”

Single embryo culture
Single embryo transfer

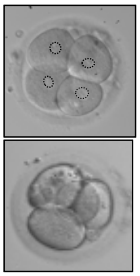
Embryo scoring

Things to look at….

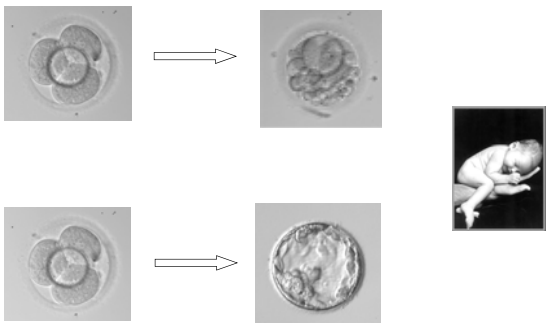
- Day 1
  - (PN score)
- Early cleavage
- Day 2/3
  - Number of cells
  - Fragmentation
  - Cell size
  - Number of nuclei
- Day 5/6
  - ICM
  - Trophectoderm
  - Expansion

Embryo morphology/development variables that independently predicts implantation:

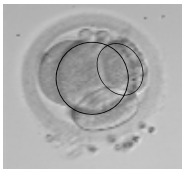
- Number of cells day 2 and 3
- Cell size
- Number of nuclei per cell
- Timing of cleavage



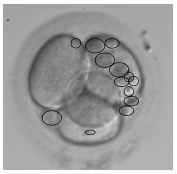
Lundin et al 2001, Saldeen and Sundström 2005, Thurin et al 2004, Ziebe et al 2007, Holte et al 2007



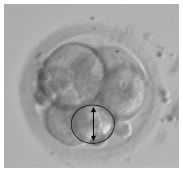
Morphology;
Are we scoring the same things?



Uneven size
(”Grade B”)



Grade of fragmentation
(”Grade A”)



Blastomere/fragment
(Grade A or B?)

Embryo morphology

- Subjective
- Very much relying on experience
- Correlates partly to chromosomal status
- Independent predictors, but rather low predictive value
  - ”validated” by experience
- What other variables could we use?

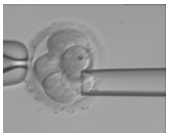
Viability assessments
complementary to morphology

- Invasive = using oocyte/embryo material
- Non-invasive = indirect

?

* Cytoplasmic maturity
* Genetic normality
* Metabolism
* Development (time-lapse)

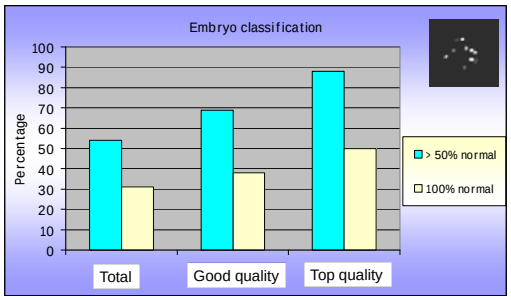
Chromosomally normal?



Measuring chromosomal / genetic status

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

Chromosomal normality and embryo selection (n=144 embryos)



Ziebe et al 2003, Munne 2008

Genetic status and 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 embryo/ blastocyst development

- Low correlation
- Cut – offs?

And what about performing genetic analysis of the embryo??

- PG Screening
- PG Diagnosis
- PG Testing

?

Munné et al 2010: ”Differences in opinion have arisen because the methods employed by the original studies reporting positive results were not used consistently by later RCTs. Given the serious deficiencies in the diagnostic strategies that these studies employed, it is unsurprising that they studies failed to find any improvement in IVF results. ”

N.B.: Observation studies using historical controls vs. RCT:s…

PGS - FISH

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

Why ? e.g. Harper et al 2010

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

- Polar body analysis? (ESHRE RCT ongoing)

PGS - FISH

Munné et al, performing the optimal study:

”Preimplantation Genetic Diagnosis for the Indication of Advanced Reproductive Age” –
- suspended….

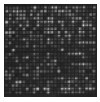
CGH

- 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 possible (<48h)

- Invasive
- Not validated

Metabolically normal?

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

- Amino acid turnover (non-invasive)
- The ”omics” (invasive / non-invasive)
- Respiration (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
  - environmental conditions (eg. culture medium, cryopreservation)
  - 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
- Prospective randomised trial ongoing

Houghton 2002, Brison et al 2004, Sturmey 2008

”The Omics” – *looking at the*:

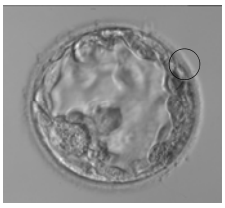
- *genome – genes, chromosomes*
- *transcriptome –mRNA*
- *proteome – proteins*
- *metabolome – metabolites*
- *secretome – secreted proteins*

Transcriptomics

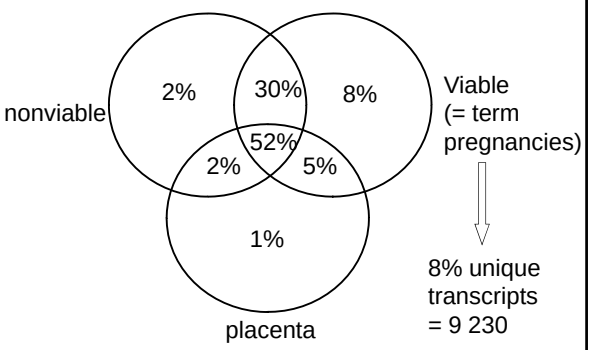
- Analysis of gene expression patterns
- mRNA amplification
- Slow, labour intensive
- Microarray techniques enables analysis of thousands of genes
- Invasive
- So far few studies

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

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

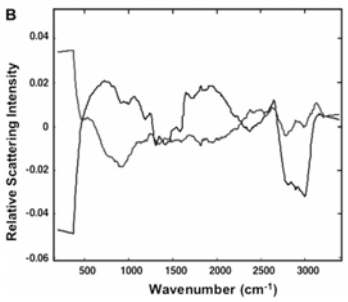
Metabolic fingerprints

- 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

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

Seli et al 2007, 2009



Seli 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

Scott et al 2008

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?

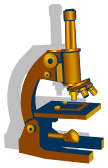
Jurisicova 1996, Yao et al 2005, Warner et al 2008

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

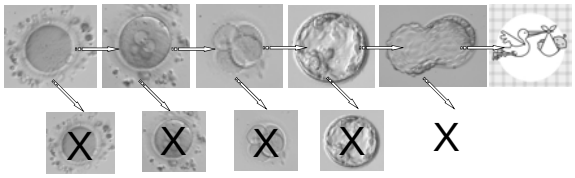
Back to microscopy…. - - Continuous documentation, time-lapse

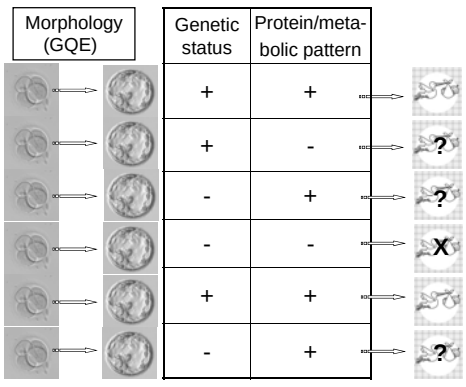


- Closed system
- Timing of cleavage
- Timing of nuclear appereance/disappereance
- Correlations with implantation and birth
- Will more accurate timing increase correlations?

Summary – around the corner?

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