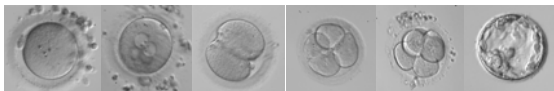


Morphological markers of embryo quality

Kersti Lundin
Sahlgrenska University Hospital
Göteborg, Sweden



What is "embryo quality"?
How do we define "the best embryo"?

"Embryo quality" (IVF)

An embryo that has the potential to implant in the uterus and give rise to the birth of a healthy baby

What are we measuring?

Embryo development variables

Implantation

Live birth



Nuclear status / cytoplasmic status /
metabolic status / environment /
chromosomal status

"Success rate" for early human embryos

- Morphology: ~ 40-60% GQE
- Chromosomal normality ~ 25-30%
- Implantation rates: ...→ 40%
- Births (SET): ...→ 35 %

Embryo "quality"



Embryo scoring

Embryo morphology/development

- MNB
 - Number of cells
 - Fragmentation
 - Cell size
 - First cleavage
 - Second cleavage
 - Visible nuclei
 - Cytoplasmic appearance
 - Compaction
- None
 - 4 / 8
 - < 20 (-30?)%
 - Even sized
 - < 25-27 hours
 - synchronised
 - 1 visible nucleus / cell
 - No vacuoles, no granulation ?
 - - day 2, + day 3

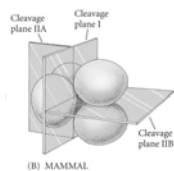
Why are these things important?

- Polarity / (a)symmetry
- Timing / Synchronisation

The first cleavages

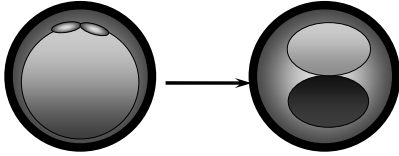
Mammals

- Rotational cleavage
- Holoblastic cleavage

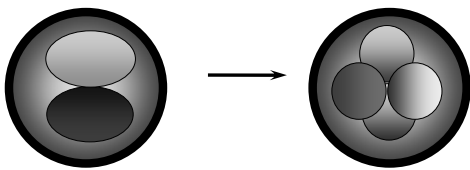


Embryo (a)symmetry

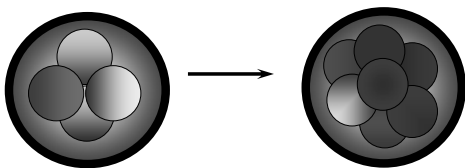
- First cleavage results in daughter cells with uneven content of transcription factors

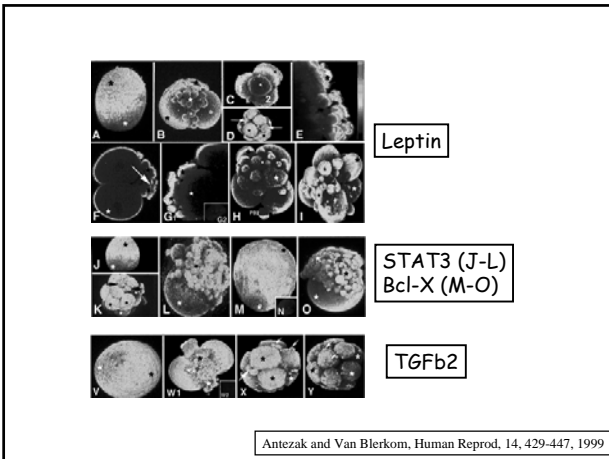


- The next cleavages increase the differences



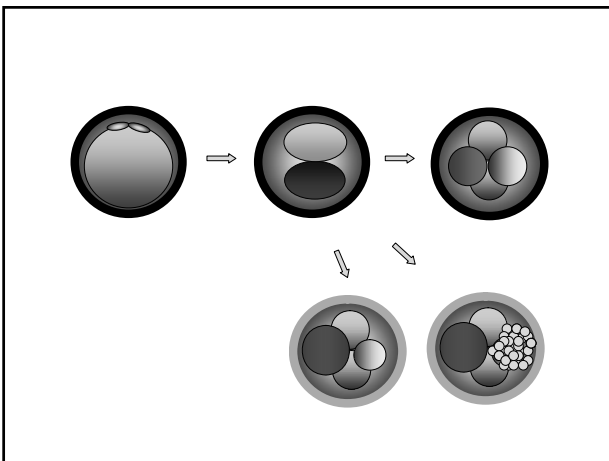
- From the 8-cell stage will some cells have positional identity





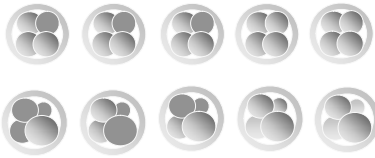
Embryo asymmetry

- Human embryos shows asymmetric distribution of factors believed to be important for establishing embryonic axes / positional identity
- Loss of blastomeres or part of blastomeres (fragmentation) or incorrect distribution of material (uneven sized) might impair the correct establishment of axis



And the chromosomal status...?

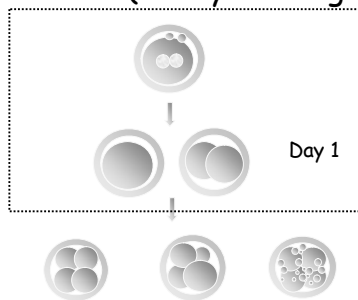
Chromosomes 13, 18, 21, X and Y	Even cleavage	Uneven cleavage	<i>P</i>
Embryo aneuploidy	4/13 (31%)	6/11 (55%)	0.24
Blastomere aneuploidy	4/47 (9%)	10/34 (29%)	0.014



Only GQE

Hardarson et al 2001

First mitotic cleavage ("early cleavage")



Scoring time
Medium
Temperature

Day 2

Early cleavage and embryo "quality"

	Early	Late
Numbers (%)	3 046 (26.9)	6 749 (73.1)
GQE (%)	1 903 (62.5)	2 593 (33.4)
Numbers (%)	85 (14.7)	494 (85.3)
Blastocysts (%)	19/59 (32.2)	61/367 (16.6)

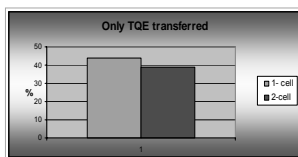
Lundin et al 2001; Fenwick et al 2002

Early first cleavage and ICSI (25 - 27 h, n =450 cycles)

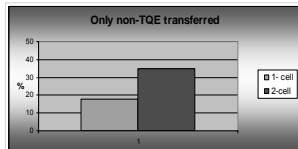
	<u>Pregnancy</u>	<u>Birth</u>
No. GQE	p <0.0001	p <0.0001
Early cleavage	NS	p =0.015

Lundin et al 2001

Early cleavage and embryo quality vs. pregnancy rates (cIVF)



N= 111
NS



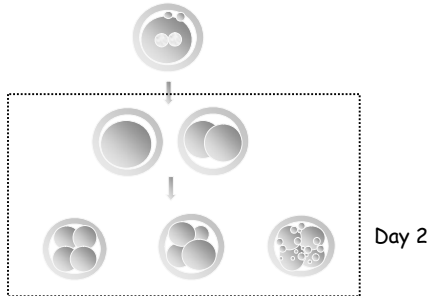
N= 266
p<<0.05

Lundin et al, ASRM 2005

More about early cleavage....

- Logistic regression analysis showed that EC is an independent predictor for both pregnancy and blastocyst development in addition to cell morphology and cell number
Van Montfoort et al 2004
- EEC (even early cleavage) strongly correlated to GQE
Terriou et al 2007
- EC a putative marker of embryo quality but weak input on clinical pregnancy rate
Rehman et al 2007

Second mitotic cleavage (time-lapse)



Cleavage rate - number of cells

- van Royen et al, 2002 - day 3

4 - 8/9 cells: 42% IR

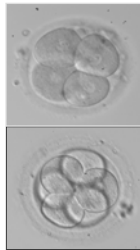
≠ 4 - 8/9 cells: <33% IR

- Thurin et al 2005, (SET) - day 2, multicenter study (661 cycles)

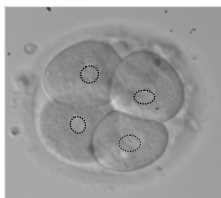
4 cells: 28% IR

≠ 4 cells: 16% IR

($p=0.013$)



Visible nuclei



744 embryos

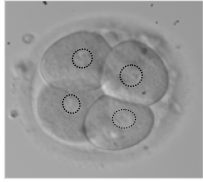
Overall IR 15.5%

Nuclei IR 26.1%

Top embryos with no visible nuclei IR 6.3%

Moriwaki et al 2004

Visible nuclei - 4 cells embryo



Nuclei	ET	IR	IR
0	28	14%	(20)*
1	74	22%	(21)
2	183	25%	(23)
3	237	27%	(22)
4	305	27%	(41)

- Sahlgrenska data non-selective, prospective
- Saldeen and Sundström 2005 selective, retrospective study

Multinucleation

- Associated with lowered pregnancy and implantation rates
- Occurs in ~ 25-50% of embryos on day 2/3
- Decreased incidence in good quality embryos ~ 15%

Palmstierna et al 1997, Kligman et al 1996, Jackson et al 1998, van Royen et al 2001, 2003, Hardarson, 2001, Hnida et al 2004

Cell size and multinucleation

Cleavage	Even	Uneven
Embryo multinuclearity (%)	1/13 (2.1)	5/11 (45.5)
		p=0.005

Cell size ($\mu\text{m}^3 \times 10^6$)	2 cell	4 cell
Mononucleated	0.210	0.118
Multinucleated	0.314	0.203
		p=<0.001

Hardarson et al 2001, Hnida et al 2004

Fragmentation vs. unequal size

	Top quality	< 20% frag	Uneven sized cells
No. cycles *	151	142	85
Pregnancy/ET (%) ^a	53.6	52.1	37.6
Implantation (%) ^b	39.3	33.3	23.9

^a p= 0.013 ^b p= 0.003

* All embryos transferred in a single cycle are of the same status

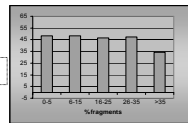
Hardarson et al 2001

Embryo fragmentation

Many different studies (univariate studies):

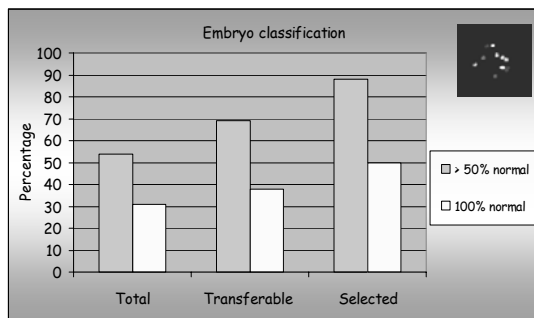
- Correlates to implantation and pregnancy...
or does not correlate...

Ziebe et al 2003



- No studies (multivariate) supporting an independent predictive influence of fragmentation (up to 20 (-30)%)

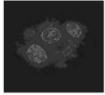
Chromosomal normality and embryo selection (n=144 embryos)



Ziebe et al 2003.

Chromosomal status vs. embryo development / morphology

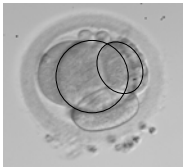
- Pronuclear scoring +
- Uneven cleavage +
- MNB +



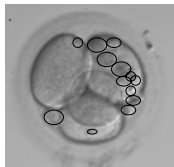
- Early cleavage -?
- Number of cells day 2 -?
- Number of cells day 3 -?

Gianaroli et al 2003, Ziebe et al 2003, Hardarson et al 2001

Are we scoring the same things? (in the same way...)



Uneven size



Grade of fragmentation



Blastomere/fragment



Can we predict PR/IR/BR from early embryo morphology?

Multivariate analyses

Independent embryo predictors

- Randomized multicenter trial (n=661)
- "Good prognosis women (<37 years, ≥ 2 GQE)
- Identification of specific maternal and/or embryo variables independently correlated with ongoing implantation:
- Number of cells independent predictors of ongoing implantation
- (Also fertilisation method and ovarian sensitivity)

Thurin et al 2005

Independent embryo predictors

- Retrospective analysis
- 861 SET transfers with a four-cell embryo
- Analysis of MONOnucleated blastomeres, equal-sized blastomeres and fragmentation:
- Number of MONOnucleate cells independent predictors of ongoing implantation
- Blastomere size and fragmentation not predictive

Saldeen and Sundström 2005

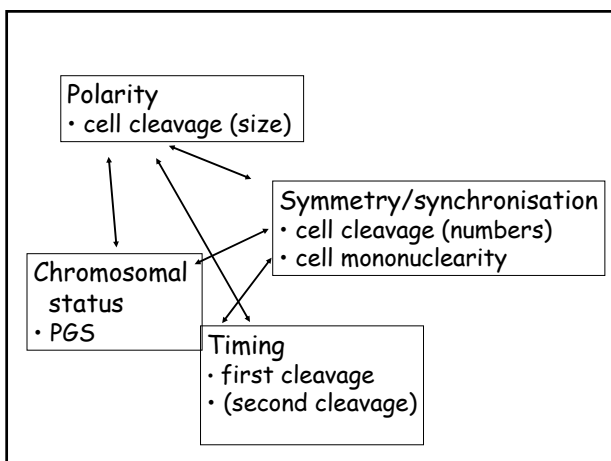
An integrated evidence-based model for prediction of implantation

- 2266 double embryo transfers
- Only 100% or 0% implantations assessed:
- Numbers of blastomeres, blastomere size and mononuclearity in the blastomeres independent predictors
- Fragmentation no predictor
- Early cleavage not analysed

Holte et al 2007

Summary

- Embryo scoring can be performed - to a large extent - according to a number of well defined morphological variables, correlating to implantation rates
- Morphologically "good" embryos are on average more genetically normal than surplus embryos
- What about metabolic "normality"??



Thank you for your attention!