Cleavage stage : morphology and implantation

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Disclosure of commercial and/or financial relationships

- I have no commercial interest with any pharmaceutical industry and other commercial industry
- I have no financial relationship with any pharmaceutical industry and other commercial industry
- Our project on cumulus cell biomarkers is supported by a Grant for Fertility Innovation from Merck-Serono company

Learning objectives

○ Defining embryo quality should not be based only on morphological parameters at day 1 or 2

○ Extended culture bring additional information on embryo quality

Evaluation of embryo viability in IVF

Selection of the best embryo (SET)

Identification of viable embryos (only cryopreserved)

Various methods (non-invasive)

○ **Common clinical practice (reliable / non-time consuming)**

In Vitro development   *Implantation rate*

Individual embryo culture   *Single embryo transfer*

Individual outcomes have more strength

Morphological and kinetics criteria available to select viable embryos

- **Oocyte morphology (day 0)**
- Pronuclear morphology (day 1)
- Early cleavage (day 1)
- Embryo morphology (day 2 and/or day 3)
- Blastocyst development (day 5/6)

Oocyte morphology (D0)

Cytoplasmic abnormalities
- Granularity, clustering of the cytoplasm
- Uneven cytoplasmic appearance
- ~~Vacuolization~~

Extracytoplasmic abnormalities
- Oocyte shape irregularity
- Oolemma consistency
- Perivitelline space : inclusions / enlargement
- Zona pellucida thickness, colour
- PB morphology

Available data remains controversial

Morphological and kinetics criteria available to select viable embryos

- Oocyte morphology (day 0)
- **Pronuclear morphology (day 1)**
- Early cleavage (day 1)
- Embryo morphology (day 2 and/or day 3)
- Blastocyst development (day 5/6)

Scott's classification (1998, 2000, 2002, 2003)
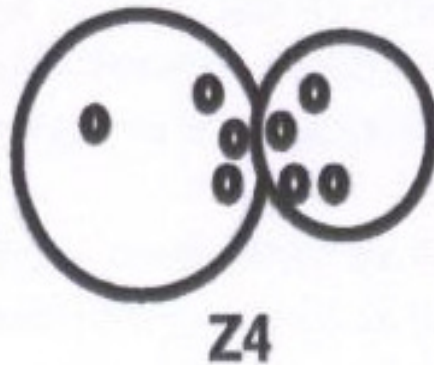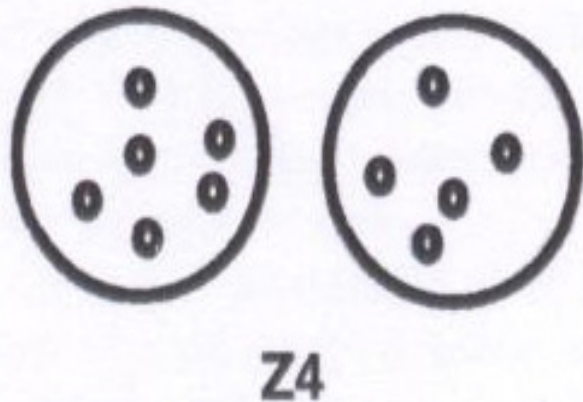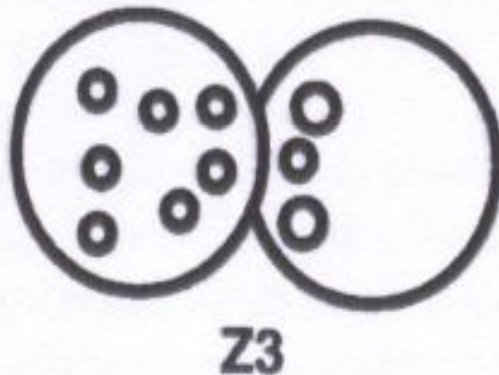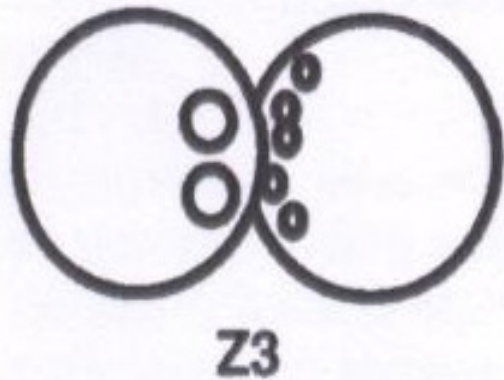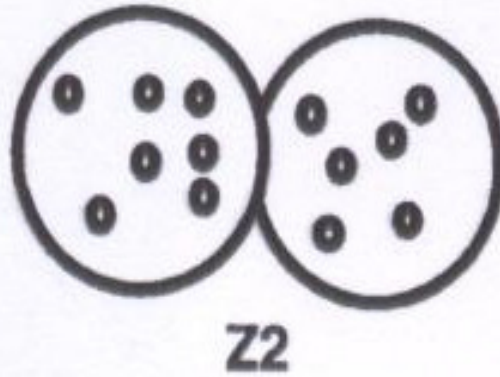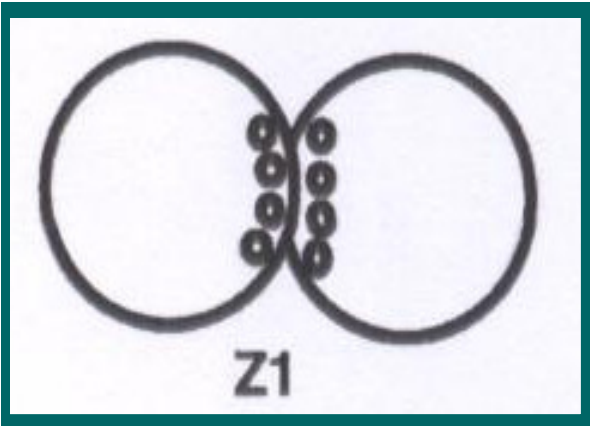
Pronuclei:

Position
Size

Nucleoli:

Number
Size
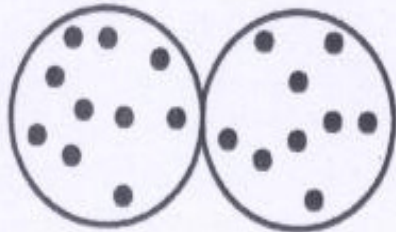**Alignment**
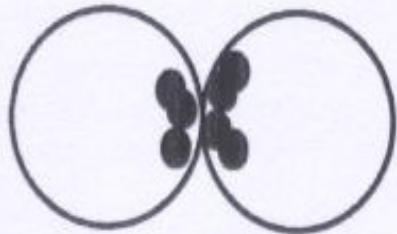
Nuclear membrane breakdown

Cytoplasmic appearance

Tesarik et Greco classification(1999)

Pattern 0A

Non Polarised NPB ≥7

Pattern 0B

Polarised NPB (3-6)

Pattern 1
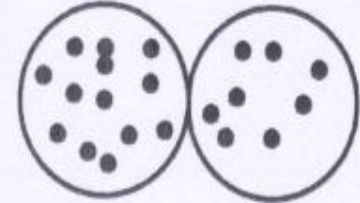
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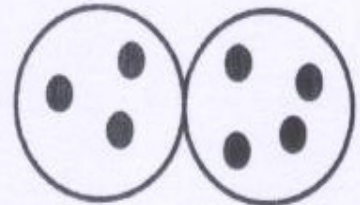
Pattern 3
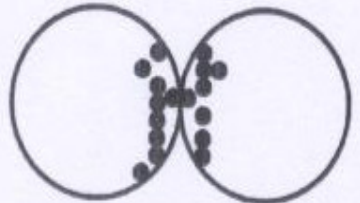
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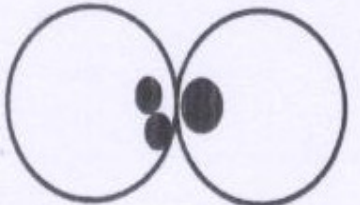
Pattern 5

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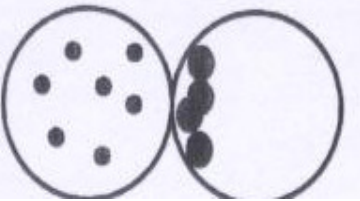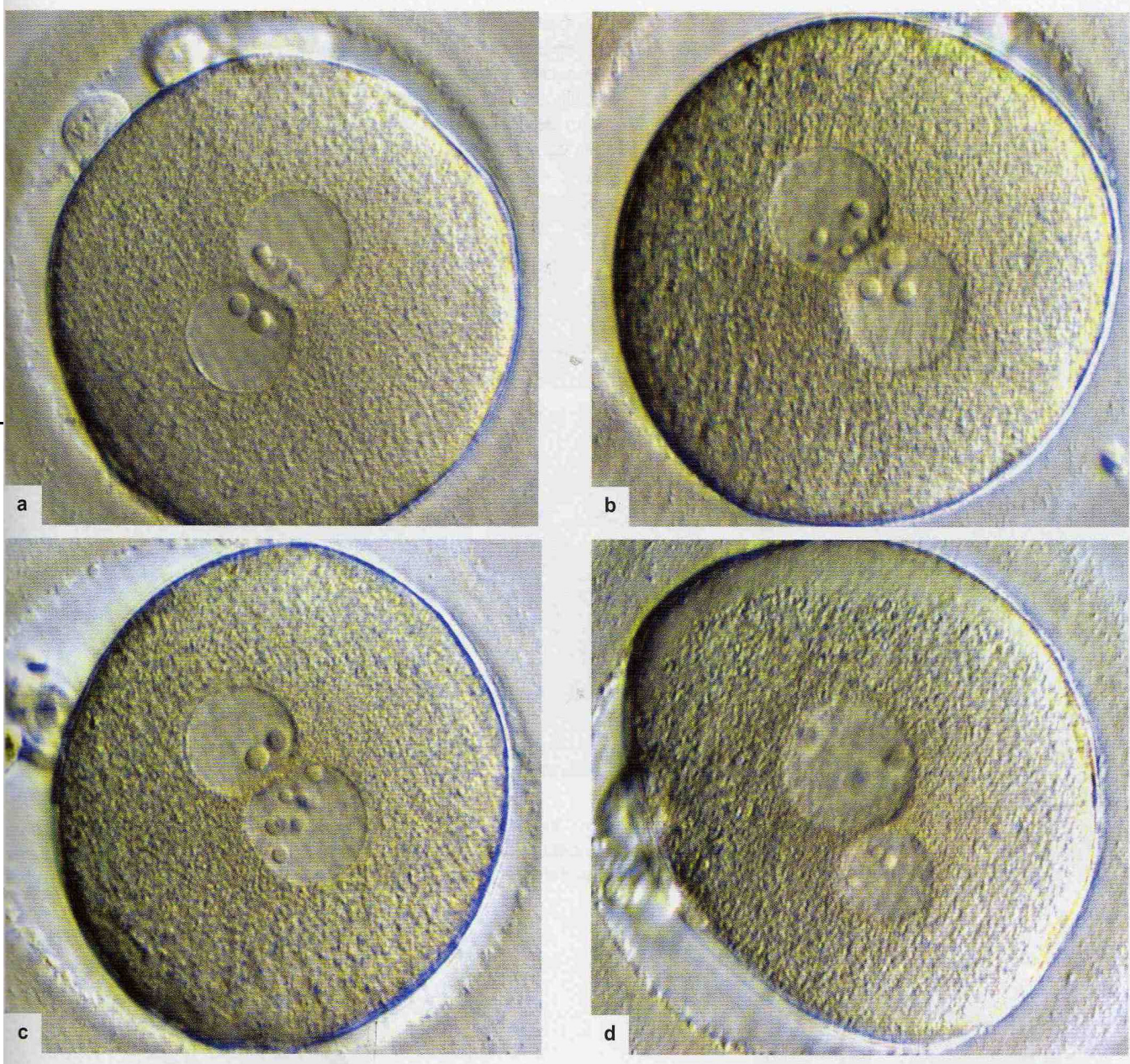
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Zygote scoring system (D1)

Observation 16-20 hours post-insemination

Zygote scoring and blastocyst development

	Zygote number	Classif.	Blastocyst development
Scott *et al.*, 2000	**654**	**Scott**	**56.5% Blasto D5 (Z1) vs 20% (Z2-Z4)**
Balaban *et al.*, 2001	**676**	**Tesarik**	**72% Blasto D5 (P0) vs 35.7% (P1-P5)**
Ebner *et al.*, 2003	**713**	**Tesarik**	**65.2% Blasto D5 (P0) vs 41.9% (P1-P5)**
Guerif *et al.*, 2007	**4042**	**Tesarik**	**56% Blasto D6 (P0) vs 41% (P1-P5)**

Zygote scoring and implantation

Scott's classification

- **Scott *et al.,* 1998, 2000, 2002, 2003**
- **Ludwig *et al.,* 2000**
- Salumets *et al.,* 2001
- Payne *et al.,* 2005
- James *et al.,* 2006
- Nicoli *et al.,* 2007

Tesarik and Greco's classification

- **Tesarik *et al.,* 1999, 2000**
- **Wittemer *et al.,* 2000**
- **Montag *et al.,* 2001**
- Salumets *et al.,* 2001
- Jaroudi *et al.,* 2004

- **Others classifications**
  - **Chen and Kattera, 2006**
  - **Senn *et al.,* 2006**

● **Correlation**

● No correlation

Zygote scoring and implantation / SET

	SET number	Classif.	Implantation Rate per cycle
Salumets *et al.,* 2001	**144**	**Tesarik**	**33.9% (P0)** **vs 31.8% (P1-P5)**
Salumets *et al.,* 2001	**105**	**Scott**	**29.2% (Z1)** **vs 31.7% (Z3) vs. 18.8% (Z2)**

Conflicting data

No standard zygote-grading system used throughout ART laboratories

Pronuclear morphology is a very dynamic process

timing is of extreme importance

Highly observer dependent

Morphological and kinetics criteria available to select viable embryos

- Oocyte morphology (day 0)

- Pronuclear morphology (day 1)

- **Early cleavage (day 1)**

- Embryo morphology (day 2 and/or day 3)

- Blastocyst development (day 5/6)
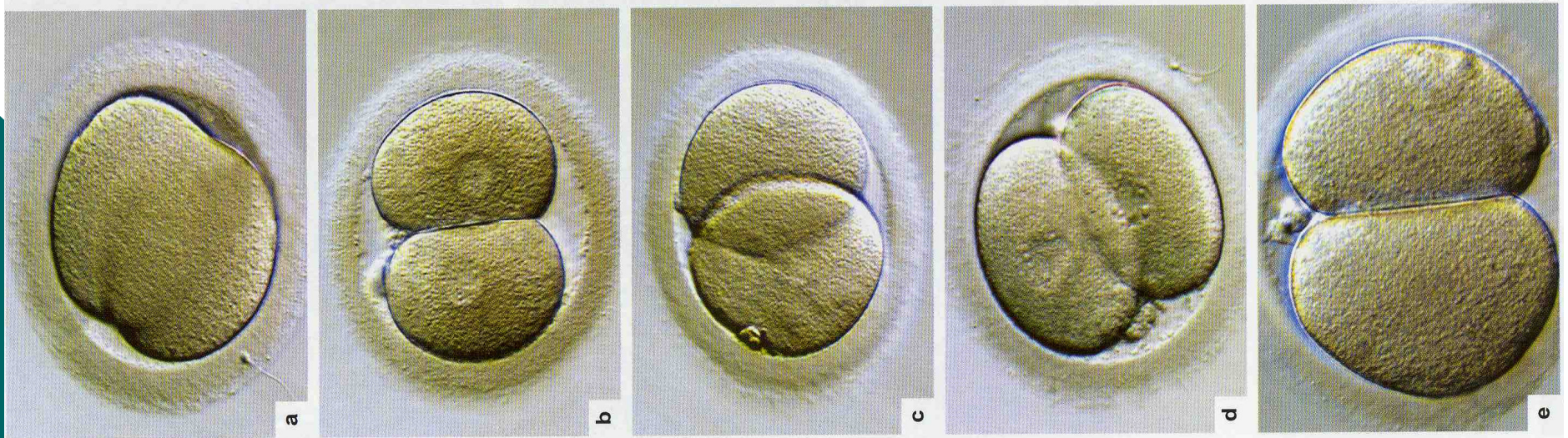
o **Observation of early cleavage**

- **26 ± 1 hrs post-ICSI**
- **28 ± 1 hrs post-IVF**

- **23 ± 1 hrs syngamy**

Early cleavage and blastocyst development

	Embryo Number	Blastocyst development
Fenwick *et al.,* 2002	**426**	**32.2% Blasto D7 (EC+) vs 16.6% (EC-)**
Van Montfoort *et al.,* 2004	**1160**	**66.1% Blasto D6 (EC+) vs 39.7% (EC-)**
Windt *et al.,* 2004	**NA**	**52% Blasto D5 (EC+) vs 22% (EC-)**
Guerif *et al.,* 2007	**4042**	**62% Blasto D5 (EC+) vs 35% (EC-)**

Early cleavage and implantation

Shoukir *et al.,* 1997 (n=143)   IR= 23.6% vs 7.5%   Het

Sakkas *et al.,* 1998 (n=88)   IR= 14.0% vs 3.2%   Het

Bos-Mikich *et al.,* 2001 (n=74) IR= 18.0% vs 8%   Het

Lundin *et al.,* 2001 (n=827)   IR= 28.0% vs 19.5%   Hom

Petersen *et al.,* 2001 (n=200)   IR= 17.5% vs 5.9%   Hom

Sakkas *et al.,* 2001 (n=230)   IR= 25.5% vs 14.8%   Het

Fenwick *et al.,* 2002 (n=70)   IR= 21.4% vs 6%   Het

Tsai *et al.,* 2002 (n=258)   IR= 18.6% vs 11.6%   Het

Early cleavage and implantation / SET

	SET number	Clinical IR per cycle
Salumets *et al.*, 2003	**178**	**50% (EC+)** **vs 26.4% (EC-)**
Van Montfoort *et al.*, 2004	**165**	**46.4% (EC+)** **vs 17.6% (EC-)**
Giorgetti *et al.*, 2006	**193**	**49.4% (EC+)** **vs 33.3% (EC-)**

Morphological and kinetics criteria available to select viable embryos

○ **Oocyte morphology (day 0)**

○ **Pronuclear morphology (day 1)**

○ **Early cleavage (day 1)**

○ **Embryo morphology (day 2 and/or day 3)**

○ **Blastocyst development (day 5/6)**

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Embryo morphology

- ○ **Observation at D2/D3**
  - **D2: 44 ± 1hrs (39-48)**
  - **D3: 68 ± 1hrs (64-69)**
- ○ **Evenness of blastomeres**
- ○ **Fragmentation**
- ○ **Observation of interphase nuclei**
- ○ **Assessment of multinucleation**
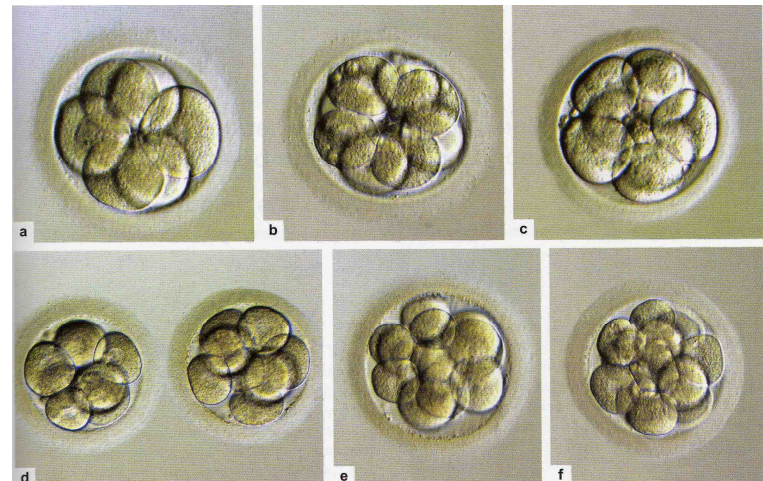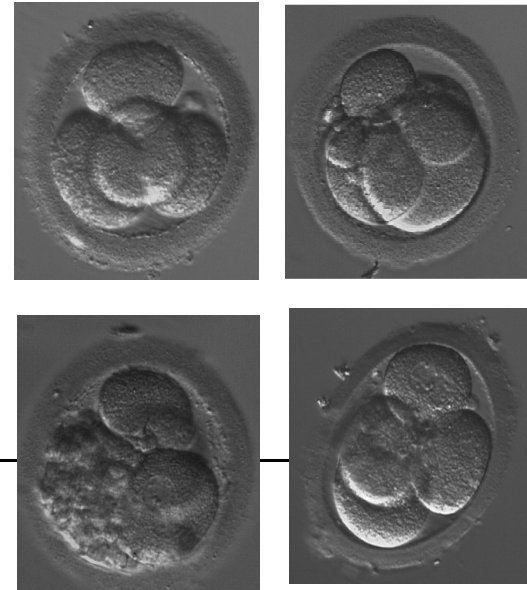
Number of blastomeres and blastocyst development

Day 2

- Shapiro *et al.,* 2000/Sjöblom *et al.,* 2006/Guerif *et al.,* 2007

4 cell-embryo> 5-6 cell-embryo> 2-3 cell-embryo

Day 3

- Shoukir *et al.,* 1998 / Racowsky *et al.,* 2000

Mean number of blastomeres <.....> Blastocyst rate

- Langley *et al.,* 2001 / Nomura *et al.,* 2006

8 cell-embryo> 9-10 cell-embryo> 2-7 cell-embryo

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Evenness of blastomeres and blastocyst development

○ **Day 2**

  ○ **Tours, IVF center**

  52.5% D6 (even) vs **38.5% D6 (uneven)**

○ **Day 3**

  ○ **Nomura *et al.,* 2006**

  48.7% D6 (even) vs **30.1% D6 (uneven)**

Degree of fragmentation and blastocyst development

- ○ **Day 2**
  - ○ **Alikani *et al.,* 2000**
  - **33.3% D5** (<15%) **vs 16.5% D5** (>15%)
  - ○ **Guerif *et al.,* 2007**
  - **50% D6** (<20%) **vs 38%** (>20%)
- ○ **Day 3**
  - ○ **Rijnders *et al.,* 1998**
  - **47% D5** (0-20%) **vs 21% D5** (>20%)
  - ○ **Nomura *et al.,* 2006**
  - **51.9% D6** (<50%) **vs 25.7% D6** (>50%)

Blastomere multinucleation and blastocyst development

- ## Day 2
    - ### Alikani *et al.,* 2000
    31.9% D5 (MNB-) vs **15.9% D5 (MNB+)**
- ## Day 3
    - ### Yakin *et al.,* 2005
    51.0% D5 (MNB-) vs **11.4% D5 (MNB+)**

Top quality embryos

Day 2

- 4-cell embryo
- Even-sized blastomeres
- No / minor fragmentation
- No multinucleation

Day 3

- 8-cell embryo
- Even-sized blastomeres
- No / minor fragmentation
- No multinucleation

D2/D3 morphology and implantation

	SET number	Clinical IR per cycle
Gerris *et al.,* 1999	26	38.5%
Vilska *et al.,* 1999	74	29.7%
De Sutter *et al.,* 2003	579	28.2%
Tiitinen *et al.,* 2003	470	34.4%
Martikainen *et al.,* 2004	308	34.7%
Thurin *et al.,* 2004	330	28.5%
Van Montfoort *et al.,* 2004	100	39.7%

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Selected patients

- **Prospective study**
- **Individual embryo culture (3226 embryos)**
- <u>**Investigation of the weight of five D2**</u> **parameters for implantation potential including no or twin implantation**
  - **Univariate analysis**
  - **Multivariate analysis**

Multivariate analysis is necessary:
- To find out which variable have independent power
- To find the correct power balance between such independent variables

Construction of an evidence-based integrated morphology cleavage embryo score for implantation potential of embryos scored and transferred on day 2 after oocyte retrieval

J.Holte[1,4], L.Berglund[2], K.Milton[1], C.Garello[3], G.Gennarelli[3], A.Revelli[3] and T.Bergh[1]

BACKGROUND: Evidence-based morphological embryo scoring models for ranking of implantation potential are still scarce, and the need for a precise model increases when aiming for singleton pregnancies. METHODS: 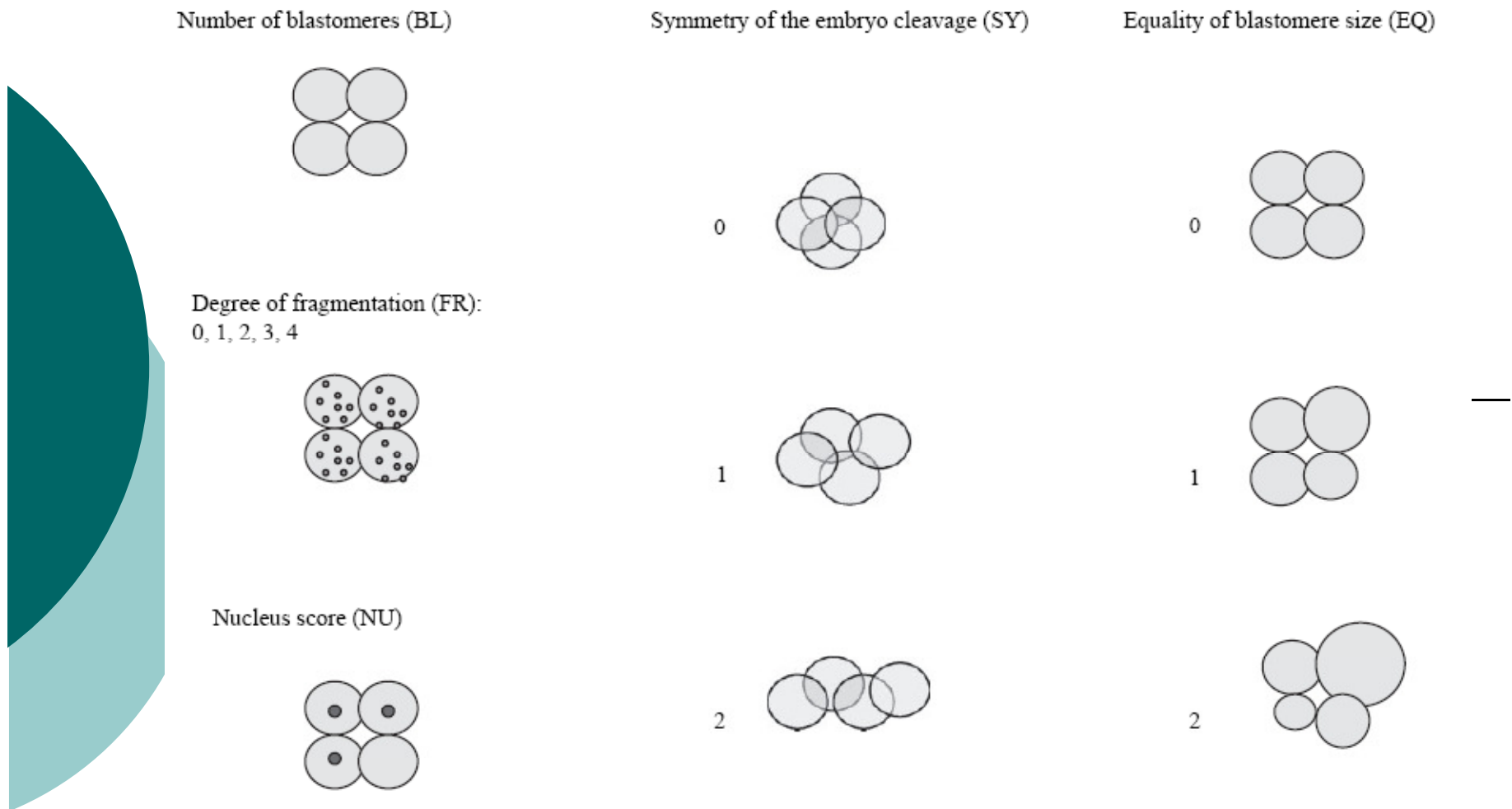Prospectively, 2266 IVF/ICSI double-embryo, day 2 transfers were studied. The five variables scored in 3- to 5-step scales for the embryos transferred are blastomere number (BL), fragmentation, blastomere size variation ('equality', EQ), symmetry of the cleavage and mononuclearity in the blastomeres (NU). The scoring results of embryos with an individual traceability from scoring to implantation, i.e. treatments resulting in either no implantation ($n = 1385$) or twin implantation ($n = 228$), were studied for prognostic potential. RESULTS: Although all five variables correlated highly with implantation potential, only BL, NU and EQ remained independently significant after regression analysis. The equation thus derived formed the basis for a 10-point integrated morphology cleavage (IMC) embryo score. A table with the scoring point for each possible combination of the embryo variables is presented. The scoring model was statistically validated on the singleton pregnancy group ($n = 653$). CONCLUSIONS: We suggest that this IMC embryo scoring, incorporating cleavage stage and information on the variation in blastomere size and the number of mononucleated blastomeres, may optimize embryo ranking and selection for day 2 transfers.

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Number of blastomeres (BL)

Symmetry of the embryo cleavage (SY)

Equality of blastomere size (EQ)

Degree of fragmentation (FR):
0, 1, 2, 3, 4

Nucleus score (NU)

0

1

2

0

1

2

Figure 1. Embryo variables. Number of blastomeres (BL: 2, 3, 4, 5 or ≥6 blastomeres). Degree of fragmentation (FR): 0, no; 1, ≤10%; 2, >10 ≤ 25%; 3, >25 ≤ 50% and 4, >50% fragmentation. Variation of sizes of the blastomeres ('Equality', EQ): 0 = uniform size of the blastomeres, 1 = varying size but <50% variation and 2 = more than 50% variation in blastomere size. Symmetry of the cleavage ('Symmetry', SY): 0 = full symmetry of the cleaved embryo, 1 = slightly asymmetric cleavage and 2 = pronounced asymmetry. The parameter 'Nucleus score' (NU) was defined as the number of visible mononucleated blastomeres divided by the total number of blastomeres in the embryo (to correct for cleavage rate); Nucleus score 0 = a ratio of 0–0.25; Nucleus score 1 = ratio >0.25–0.50; Nucleus score 2 = ratio >0.50–0.75 and Nucleus score 3 = ratio >0.75. Nucleus score –1 denotes that the embryo contains at least one multinucleated blastomere.
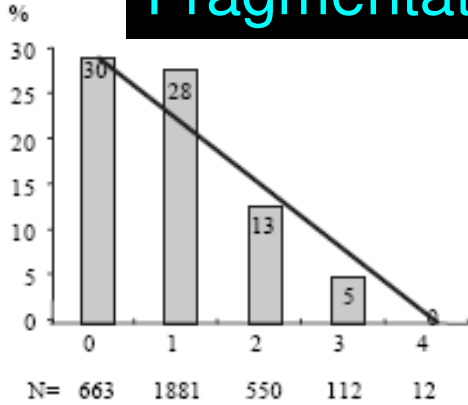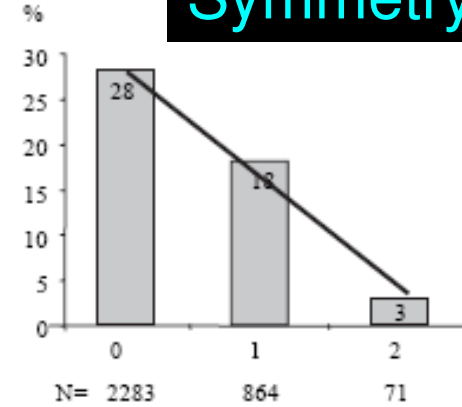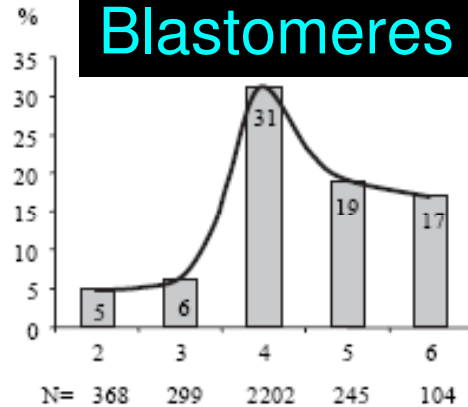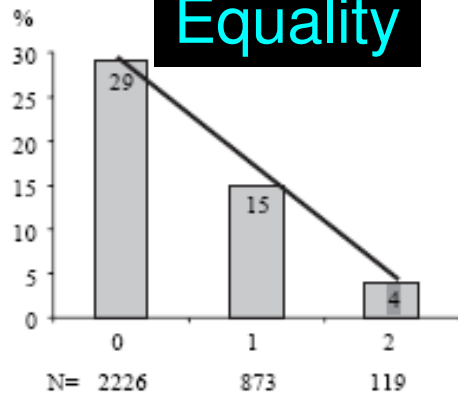
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Univariate analysis

Human Reproduction Vol.22, No.2 pp. 548–557, 2007
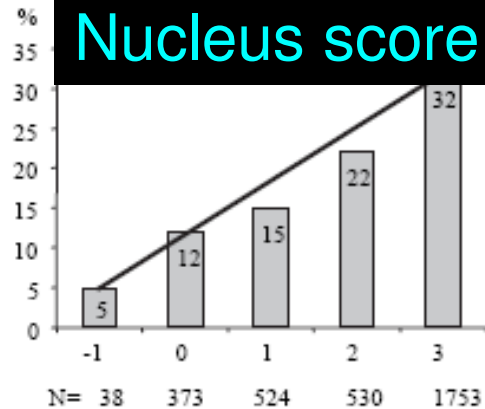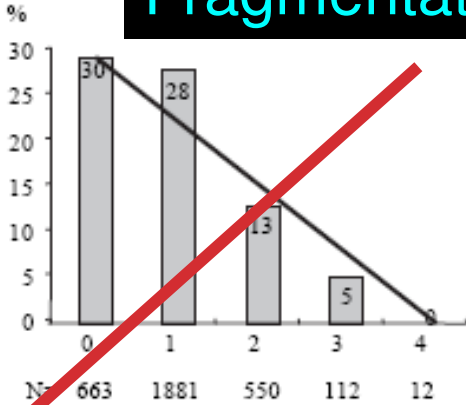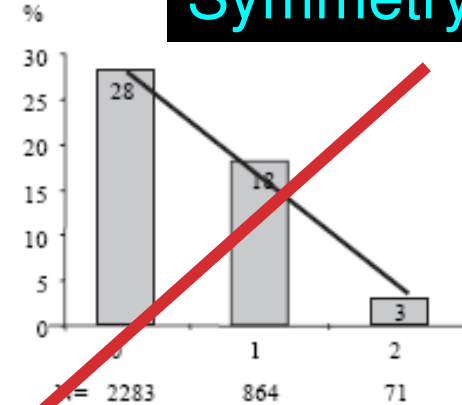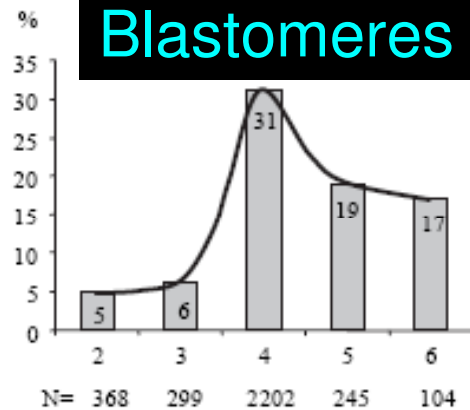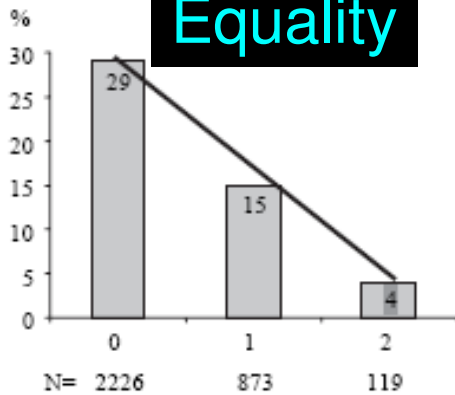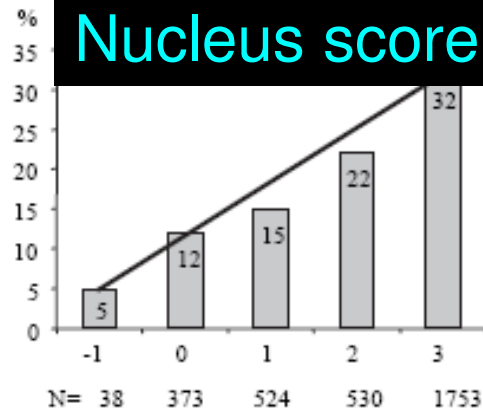
Table II. Results of a logistic regression analysis for prediction of implantation

Analysis of maximum likelihood estimates

Parameter	Estimate	SE	Wald Chi-square	$P >$ Chi-square
Intercept	−4.5386	0.8137	31.1113	<0.0001
Blastomere	0.6041	0.1908	10.0221	0.0015
A blast	−1.0395	0.2100	24.4931	<0.0001
Equality	−0.5929	0.2457	5.8219	0.0158
Nucleus	0.3507	0.1009	12.0772	0.0005

Odds ratio estimates

Effect	Point estimate	95% Wald confidence limits	
Blastomere	1.830	1.259	2.660
A blast	0.354	0.234	0.534
Equality	0.553	0.341	0.895
Nucleus	1.420	1.165	1.731

Blastomere, Equality and Nucleus were significant independent predictors, whereas Fragmentation and Symmetry were lost because of non-significance. Owing to its non-linear relationship with implantation rate, Blastomere is represented by two variables: Blastomere, the total number of blastomeres; and A blast, the absolute figure for the deviation from the ideal four blastomeres.

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Blastocyst development (D5-D6)
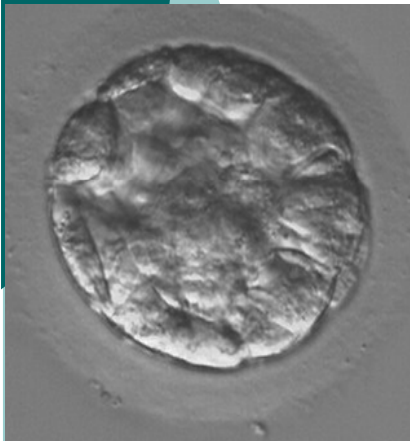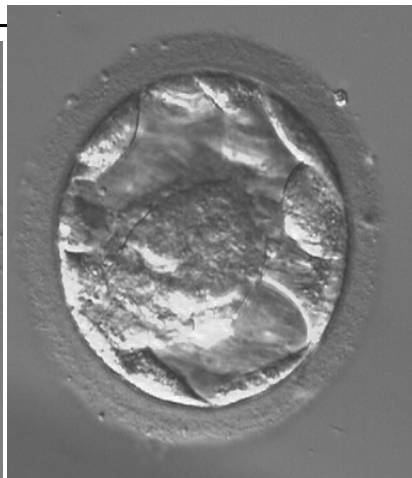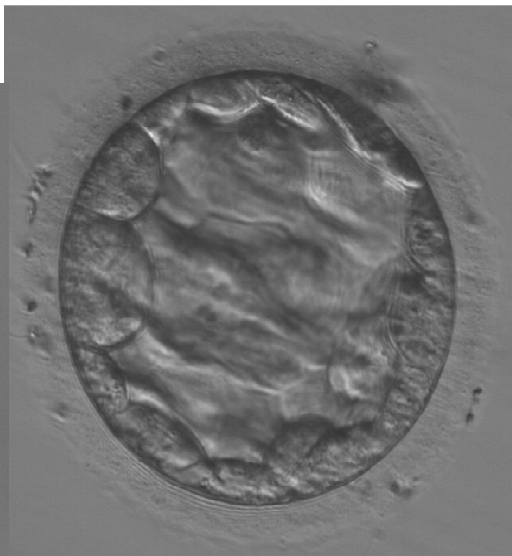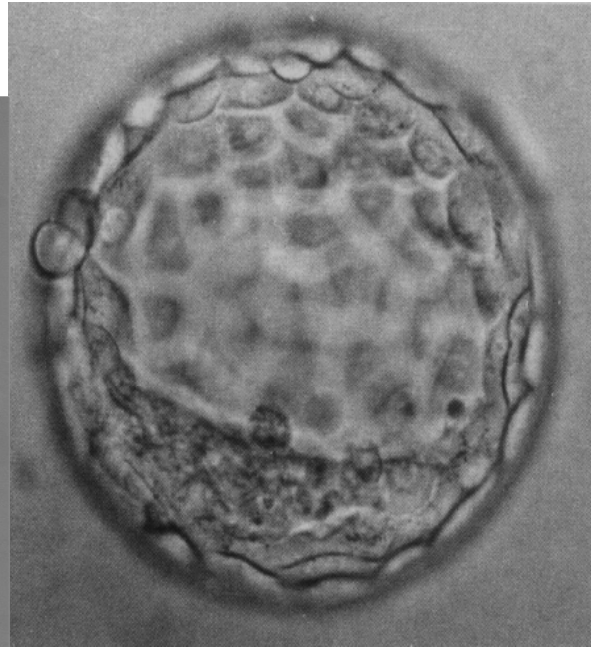
B1   B2   B3   B4-B5

Gardner and Schoolcraft 1999

Blastocyst and implantation

	SBT number	Clinical IR per cycle
Gardner *et al.,* 2004	23	60.9%
Criniti *et al.,* 2005	41	76%
Henman *et al.,* 2005	193	47.9%
Nilsson *et al.,* 2005	306	44.4%
Papanikolaou *et al.,* 2006	175	33.1%
Ryan *et al.,* 2007	83	62.7%
Davis *et al.,* 2007	45	51.1%
Guerif *et al.,* 2009	218	43.6%

Selected patients

Various factors are involved with embryo development.

=> Consequently a single early (D0 or D1 or D2 or D3) static observation is probably inadequate

to predict further development for an embryo

that appears normal at a first glance.

End point: blastocyst development or implantation

	D0	D1(PN)	D1(EC)	D2	D3
De Placido *et al.,* 2002		X		X	X
Nagy *et al.,* 2003		X			X
Fisch *et al.,* 2001, 2003		X	X	X	X
Lan *et al.,* 2003		X			X
Neuber *et al.,* 2003		X	X	X	X
Rienzi *et al.,* 2005		X	X	X	X
Sjöblom *et al.,* 2006	X	X	X	X	
Guerif *et al.,* 2007		X	X	X	
Scott *et al.,* 2007	X	X		X	X
Rehman *et al.,* 2007		X		X	X

Prediction of embryo developmental potential and pregnancy based on early stage morphological characteristics

Peter Sjöblom, Ph.D.,[a,c] Judith Menezes, Ph.D.,[b] Lisa Cummins, B.Sc.,[b]
Bagyalakshmi Mathiyalagan, Ph.D.,[b] and
Michael F. Costello, M.B.B.S., M.Med., FRANZCOG, CREI[a,b]

Objective: To analyze the association between morphological details at different stages of culture with blastocyst development, with an aim to improve selection for transfer.

Design: Retrospective audit of data.

Setting: Tertiary referral center and university hospital.

Patient(s): Two hundred sixty-eight couples underwent 357 treatment cycles.

Intervention(s): Oocyte pickups for IVF or intracytoplasmic sperm injection (ICSI) after ovarian stimulation. Embryos were individually cultured and examined on days 0–2 for morphological details and developmental characteristics, and selected for transfer, freezing, or further culture.

Main Outcome Measures: The association of blastocyst development and pregnancy with morphological characteristics.

Result(s): Five morphological characteristics (appearance of the cytoplasm, pronuclei and nucleoli, cytoplasmic deficit, and developmental rate) showed the strongest association with blastocyst development. By combining information from all days of culture into a cumulative score, prediction was greatly improved, compared to only using day 2 morphology. Cytoplasmic dysmorphisms of the oocyte, including accumulation of smooth endoplasmic reticulum, were associated with poor developmental performance. Differential weighting of these characteristics was included in a new embryo scoring system, which showed a strong correlation with implantation.

Conclusion(s): Weighting individual morphological characteristics of zygotes and embryos and combining them into a cumulative embryo score can improve selection of embryos for transfer. (Fertil Steril® 2006;86:848–61. ©2006 by American Society for Reproductive Medicine.)

Key Words: Embryo score, blastocyst, oocyte, zygote, morphology

TABLE 3

Description of the weighted scoring system.

Characteristic	Description	Score
A D1 (zygote) characteristics (day after oocyte pick-up)		
Polar body orientation	<45° with axis of pronuclei	3
	Other	0
Cytoplasmic halo	Present	3
	Absent	0
Cytoplasmic texture	Normal	6
	Slightly granular	3
	Vacuoles, dark patch, very granular	0
Membrane	Smooth	3
	Jagged	0
Nucleoli	Equal number (<8) and polarized	18
	Equal number and scattered	6
	Other	0
Pronuclei, size	Equal	3
	Unequal	0
Pronuclei, position	Central	3
	Eccentric	0
Pronuclei, apposition	Apposed	3
	Apart	0
Syngamy at 25 hpi	Cleavage to the 2-cell stage	8
	Breakdown of pronuclear membranes	4
	Intact PNs	0
	Maximum score	50
Aggregation of smooth endoplasmic reticulum on D0	No freezing or transfer, total score 0	
B D2 (embryo) characteristics at 42 hpi (2 days after oocyte pick-up)		
Zona pellucida thickness	Variable	3
	Uniform	0
Cytoplasm	Clear	3
	Granular, vacuoles	0
Membrane	Smooth	3
	Jagged	0
Blastomere size	Equal if 2^n blastomeres, otherwise in accord with cleavage stage	3
	Other	0
Cell shape	Spherical, regular	3
	Other	0
Perivitelline space	Blastomeres fill the space under the zona	5
	Large space between cells and zona	0
Fragmentation	<10% fragmented	10
	10%–30% fragmented	5
	>30% fragmented	0
Developmental rate	4 cells	20
	2, 3 or >4 cells	10
	Other	0
	Maximum score	50

Sjöblom. Embryo score and blastocyst development. Fertil Steril 2006.

TABLE 3

Continued.

Characteristic	Description	Score
Multinucleated blastomeres (MNBs)	Multiple (equal sized) or fragmented (varying sizes) nuclei	The score is decreased by the percentage of blastomeres with MNBs

Note: hpi = hours post insemination.

Sjöblom. Embryo score and blastocyst development. Fertil Steril 2006.

FIGURE 3

The association between implantation rate and the D2 weighted score (based on differential weighting of morphological characteristics) of transferred embryos. Numbers in parentheses above the bars are the number of embryos transferred in each category.

Sjöblom. Embryo score and blastocyst development. Fertil Steril 2006.

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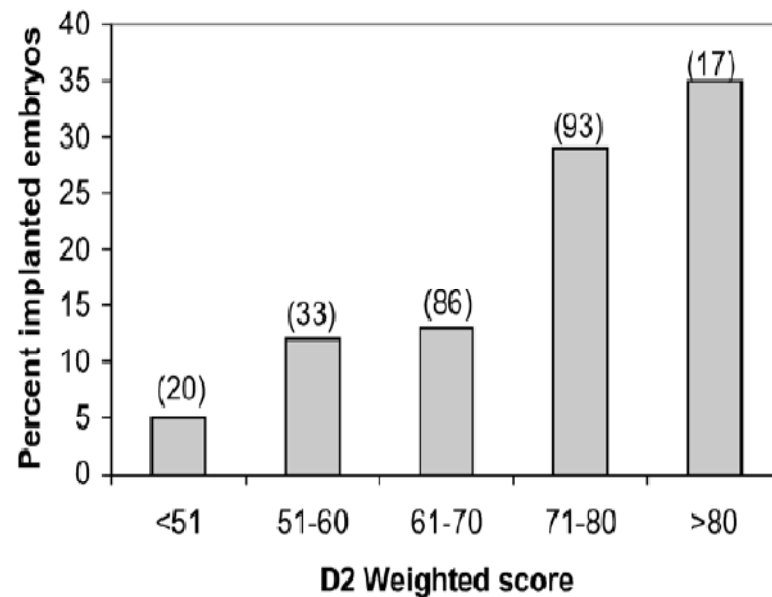
Morphologic parameters of early cleavage-stage embryos that correlate with fetal development and delivery: prospective and applied data for increased pregnancy rates

L.Scott[1], A.Finn, T.O'Leary, S.McLellan and J.Hill

group, and the number of embryos used decreased. CONCLUSION: In conclusion, early parameters that include PN morphology, number and ratio of NPBs per nucleus and the day 2 morphology of cleaving embryos are stronger positive predictors of implantation than day 3 morphology or the ability to achieve the blastocyst stage of development. Parameters that were most consistently correlated with no delivery were lack of PN symmetry, day 2 multinucleation and uneven cell size. Day 3 and day 5 parameters were not significant compared with the combination of early parameters.

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- Prospective study
- The outcome measure was to evaluate the usefulness of morphological characteristics (D0, D1, D2, D3, D5) in predicting implantation

---

- D1-D2 parameters are stronger predictive of implantation than D3 morphology or ability to achieve the blastocyst stage of development.

- Most significant D1/D2 factors:
  - PN morphology and NPB ratio
  - Day 2 cell number, blastomere symmetry, nucleation
  - Ability to cleave from D2 to D3

Limited value of morphological assessment at days 1 and 2 to predict blastocyst development potential: A prospective study based on 4042 embryos

F. Guerif[1], A. Le Gouge[2], B. Giraudeau[2], J. Poindron[1], R. Bidault[1], O. Gasnier[1] and D. Royere[1,3]

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	Embryos	Blastocysts at day 5/6		P value
	n	n	%	
Pronuclear morphology (day 1)				<0.0001
Pattern 0 zygotes	717	402	56	
Non-pattern 0 zygotes	3325	1360	41	
Early cleavage (day 2)				<0.0001
Early cleavage embryos	1298	803	62	
Non-early cleavage embryos	2744	959	35	
Number of cells (day 2)				<0.0001
2-3 cells	1637	511	31	
4 cells	1641	961	59	
5-8 cells	764	290	38	
Fragmentation rate (day 2)				<0.0001
< 20% fragmentation	1916	956	50	
20-50% fragmentation	1583	654	41	
>50% fragmentation	543	152	28	

Associations between pronuclear morphology, early cleavage, embryo morphology and blastocyst development at day 5/6 – Multivariate analysis (n=4042 embryos).

	OR*	95% CI**	P value	Overall P
Pronuclear morphology (day 1)				
Pattern 0 zygotes	1.00	-	-	
Non-pattern 0 zygotes	0.74	[0.62-0.88]	0.0007	
Early cleavage (day 2)				
Early cleavage embryos	1.00	-	-	
Non-early cleavage embryos	0.44	[0.38-0.51]	<0.0001	
Number of cells (day 2)				<0.0001
4 cells	1.00	-	-	
2-3 cells	0.46	[0.40-0.54]	<0.0001	
5-8 cells	0.46	[0.39-0.56]	<0.0001	
Fragmentation rate (day 2)				<0.0001
< 20% fragmentation	1.00	-	-	
20-50% fragmentation	0.81	[0.70-0.93]	0.0029	
>50% fragmentation	0.50	[0.40-0.62]	<0.0001	

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Guerif et al, 2007

Guerif *et al.,* 2007

Sensitivity

Combination of four D1/D2 parameters

$AUC_{ROC}=0.688$

0.9–1 = perfect
0.8-0.9 = excellent
0.7-0.8 = acceptable
0.6-0.7 = low
0.5-0.6 = no discr.
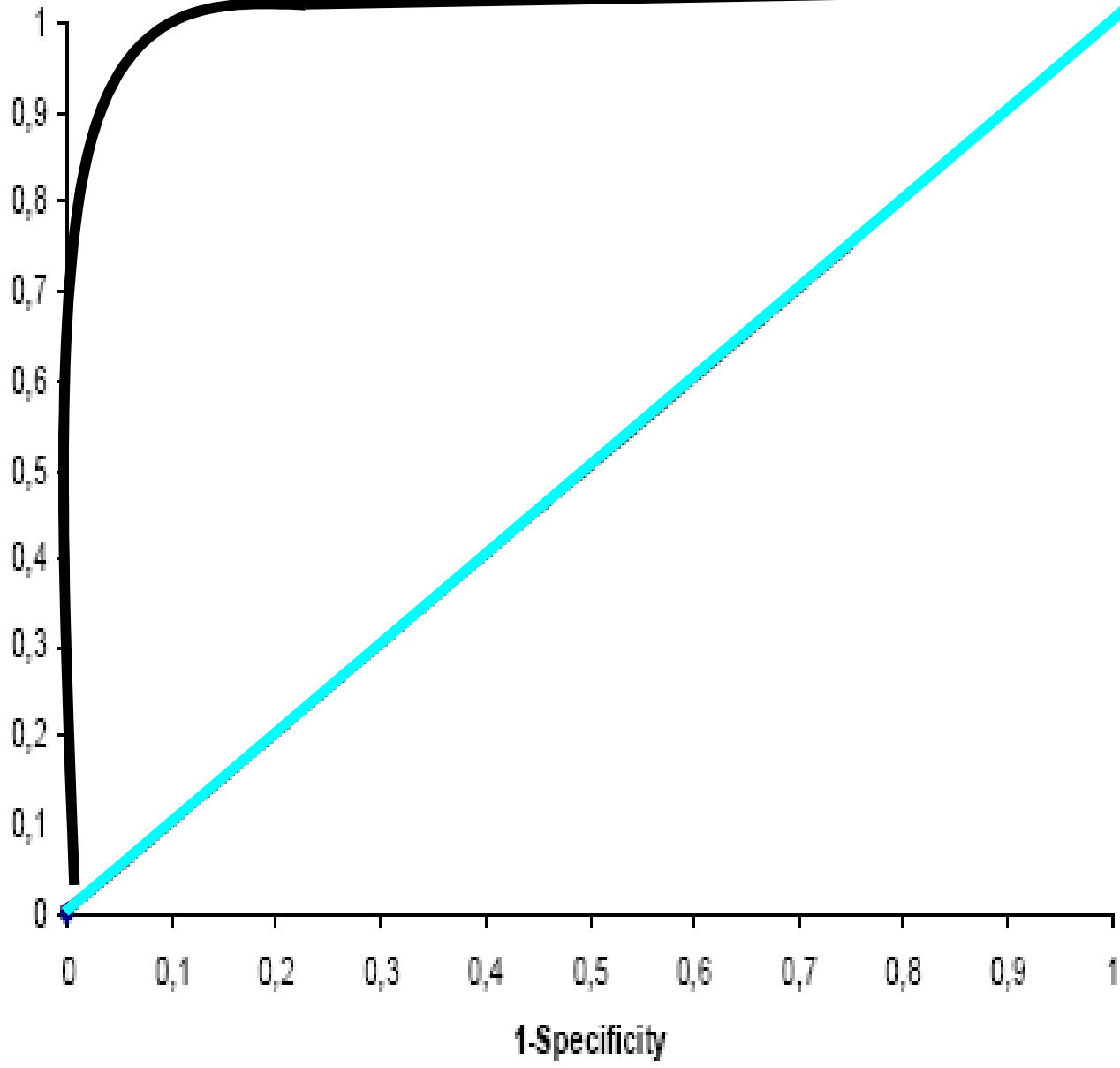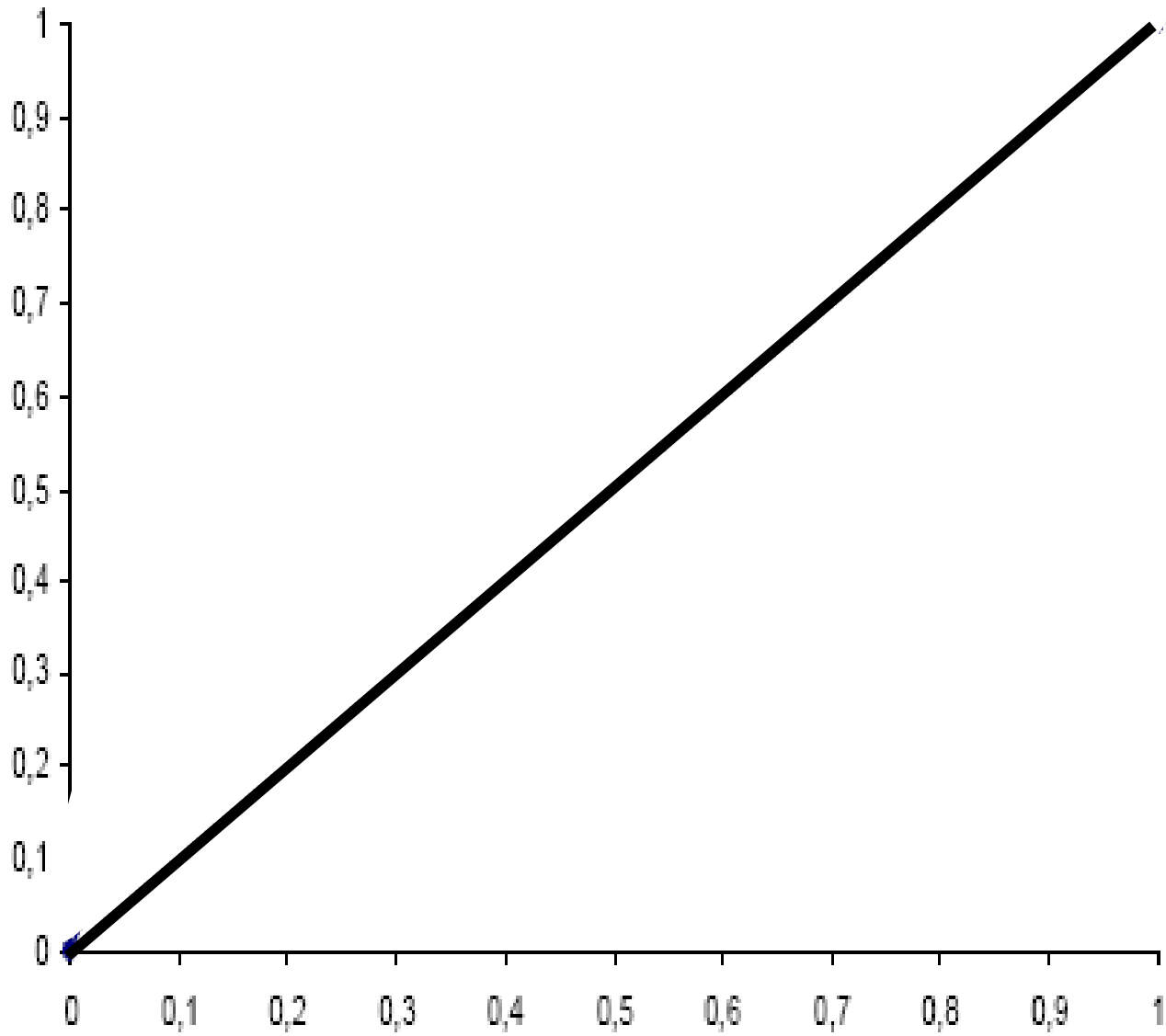
1-Specificity

Guerif *et al.*, 2007

Associations between pronuclear morphology, early cleavage, embryo morphology and good morphology blastocysts – Univariate analysis (n=1762 blastocysts).

	Total blastocyst at day 5/6	B3-B6 A/B A/B blastocysts at day 5		P value
	n	n	%	
Pronuclear morphology (day 1)				0.0002
Pattern 0 zygotes (2 PN)	402	162	40	
Non-pattern 0 zygotes (2 PN)	1360	414	30	
Early cleavage (day 2)				<0.0001
Early cleavage embryos	803	377	47	
Non-early cleavage embryos	959	199	21	
Number of cells (day 2)				<0.0001
2-3 cells	511	71	14	
4 cells	961	420	44	
5-8 cells	290	85	29	
Fragmentation rate (day 2)				0.0019
< 20% fragmentation	956	345	36	
20-50% fragmentation	654	194	30	
>50% fragmentation	152	37	24	

ESHRE CAMPUS SYMPOSIUM
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Guerif et al, 2007

Associations between pronuclear morphology, early cleavage, embryo morphology and good morphology blastocysts – Multivariate analysis (n=1762 blastocysts).

	OR*	95% CI**	P-value	Overall p
Early cleavage (day 2)				—
Early cleavage embryos	1.00			
Non-early cleavage embryos	0.40	[0.32-0.50]	<0.0001	
Number of cells (day 2)				<0.0001
4 cells	1.00			
2-3 cells	0.30	[0.22-0.40]	<0.0001	
5-8 cells	0.54	[0.41-0.72]	<0.0001	

Guerif et al, 2007

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Clinical outcome of transferred blastocysts as a function of their morphology.

	Morphology of blastocyst(s) transferred		
	At least one B3-B6 A/B A/B blastocyst at day 5	All other blastocysts at day 5 or 6	*p*
Nb of transfers	204	138	
Nb of transferred blastocysts	305	202	
Mean of transferred blastocysts	1.5 ± 0.5	1.5 ± 0.5	NS
Clinical PR per transfer (%)	65 / 204 (31.9%)	26 / 138 (18.8%)	0.0108
Live birth rate per transfer (%)	53 / 204 (26.0%)	20 / 138 (14.5%)	0.045
Clinical IR per transfer (%)	75 / 305 (24.6%)	31 / 202 (15.3%)	0.015
Ongoing IR per transfer (%)	63 / 305 (20.7%)	25 / 202 (12.4%)	0.0244

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Guerif et al, 2007

Late stages of embryo progression are a much better predictor of clinical pregnancy than early cleavage in intracytoplasmic sperm injection and in vitro fertilization cycles with blastocyst-stage transfer

Khurram S. Rehman, M.D.,[a,b] Orhan Bukulmez, M.D.,[a,b] Martin Langley, B.Sc.,[a]
Bruce R. Carr, M.D.,[b] Anna C. Nackley, M.D.,[a,b] Kathleen M. Doody, M.D.,[a,b]
and Kevin J. Doody, M.D.[a,b]

Objective: To define and validate metrics of embryo progression and morphology during extended embryo culture and to compare the effects of early cleavage (EC) vs. blastulation stages on clinical pregnancy.

Design: Retrospective observational study.

Setting: University-affiliated assisted reproduction center.

Patient(s): One thousand two hundred ninety-two intracytoplasmic sperm injection and 842 IVF blastocyst-transfer cycles.

Intervention(s): The embryo progression index (EPI) was calculated as the area under the curve of total cell number (TCN) over time, by using observed TCN for cleavage-stage embryos and estimated blastocyst TCN according to morphology. The EPI from days 1–3 measured early cleavage, and blastulation was assessed by EPI over extended embryo culture. Blastocyst morphology was converted into numerical blastocyst quality scores (BQSs). Receiver operating characteristic curve analysis was used to evaluate predictors for clinical pregnancy.

Main Outcome Measure(s): Clinical pregnancy.

Result(s): Per-cycle mean EPI and mean BQS for all embryos developing into blastocysts, as well as mean BQS of the transferred embryos, were significant predictors of clinical pregnancy in intracytoplasmic sperm injection and IVF cycles. Mean EPI for days 1–3 did not predict outcome.

Conclusion(s): Early cleavage is a putative marker of embryo quality. Late-stage embryo development is more sensitive and specific in predicting clinical pregnancy than is early cleavage, supporting the use of extended embryo culture for embryo selection. The embryo progression index and BQS may also be used for this purpose. (Fertil Steril® 2007;87:1041–52. ©2007 by American Society for Reproductive Medicine.)

Key Words: Blastocyst, morphology, embryo progression, early cleavage, in vitro fertilization, clinical pregnancy

FIGURE 1

The method of conversion of blastocyst morphology grading into the numerical BQS. Blastocyst quality score is the product of degree of expansion (numbered 1 to 6) and ICM and TE grades, where grade A = 3, grade B = 2, and grade C = 1. The embryo illustrated represents an expanded blastocyst with excellent morphology, with a BQS of 36.

Inner cell mass - ICM

Trophectoderm - TE

Gardner and Schoolcraft Morphology grading
(Gardner and Schoolcraft, 1999)

Expansion	ICM grade	TE grade
4	A	A

$$4 \times 3 \times 3 = 36 \quad \text{BQS}$$

Blastocyst Quality Score

Rehman. Blastulation vs. early cleavage. Fertil Steril 2007.

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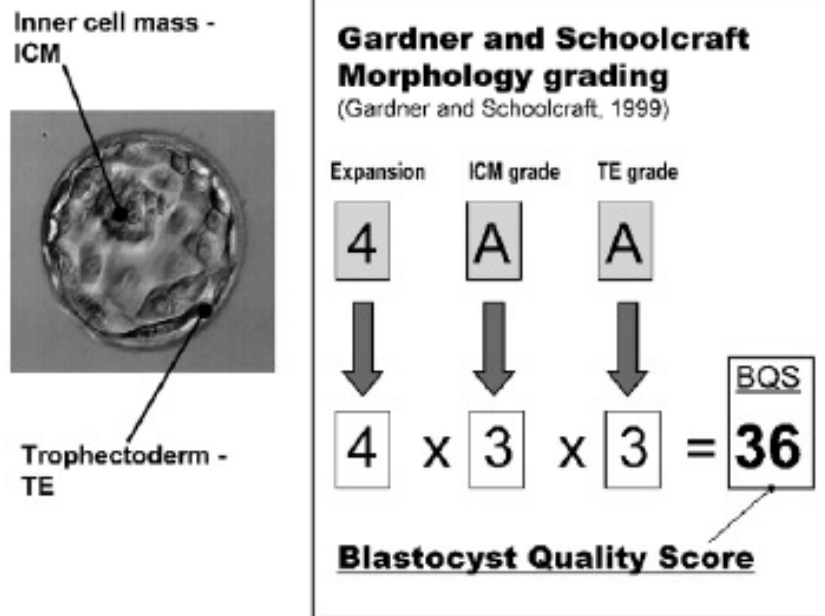
TABLE 2

Gardner and Schoolcraft blastocyst morphology grades, with corresponding BQS.

Morphology	BQS	Morphology	BQS	Morphology	BQS
1AA	9	3AA	27	5AA	45
1AB/1BA	6	3AB/3BA	18	5AB/5BA	30
1BB	4	3BB	12	5BB	20
1AC/1CA	3	3AC/3CA	9	5AC/5CA	15
1BC/1CB	2	3BC/3CB	6	5BC/5CB	10
1CC	1	3CC	3	5CC	5
2AA	18	4AA	36	6AA	54
2AB/2BA	12	4AB/4BA	24	6AB/6BA	36
2BB	8	4BB	16	6BB	24
2AC/2CA	6	4AC/4CA	12	6AC/6CA	18
2BC/2CB	4	4BC/4CB	8	6BC/6CB	12
2CC	2	4CC	4	6CC	6

Rehman. Blastulation vs. early cleavage. Fertil Steril 2007.

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Examples of TCN values for cleavage-stage embryos and compacting morulae.

Stage	TCN
Zygote (2PN, etc.)	1
2 cell	2
4 cell	4
5 cell	5
6 cell	6
7 cell	7
8 cell	8
10 cell	10
12 cell	12
Morula	24[a]

[a] Estimated TCN.

Rehman. Blastulation vs. early cleavage. Fertil Steril 2007.

Calculation of EPI. The embryo progression curve shown represents an early blastocyst reaching an estimated TCN of 48 cells on day 5 after fertilization. Embryo progression index is the AUC of observed or estimated TCN plotted against time in days after fertilization; EPI for days 1–5 is the sum of the areas of the four shaded trapezoids: a, b, c, and d.

Rehman. Blastulation vs. early cleavage. Fertil Steril 2007.

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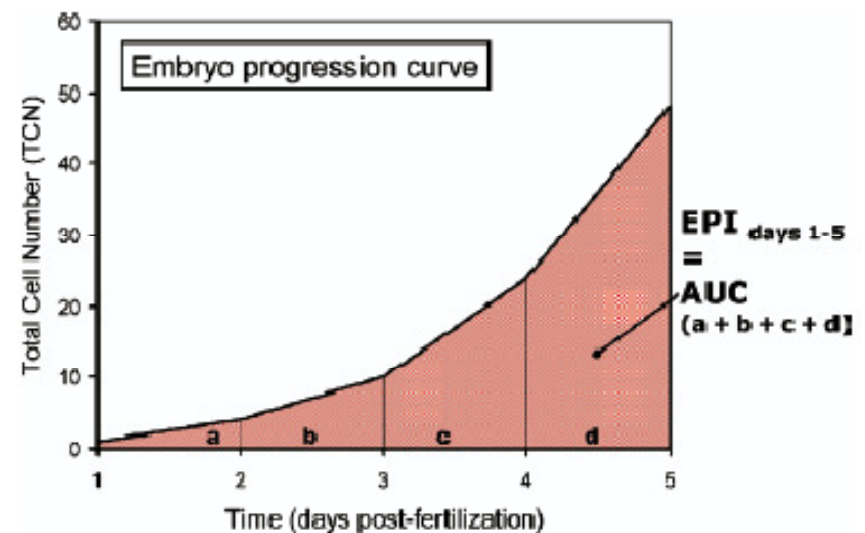
FIGURE 3

Use of the EPI to quantify early cleavage vs. blastulation stages of embryo development. The embryo progression curves shown represent TCN plotted on a linear scale against time in days after fertilization. On the left, EPI from days 1 to 3 is shown as the shaded AUC, measuring early cleavage. On the right, EPI from days 1 to 6 is shown as the shaded AUC, measuring blastulation.

Rehman. Blastulation vs. early cleavage. Fertil Steril 2007.

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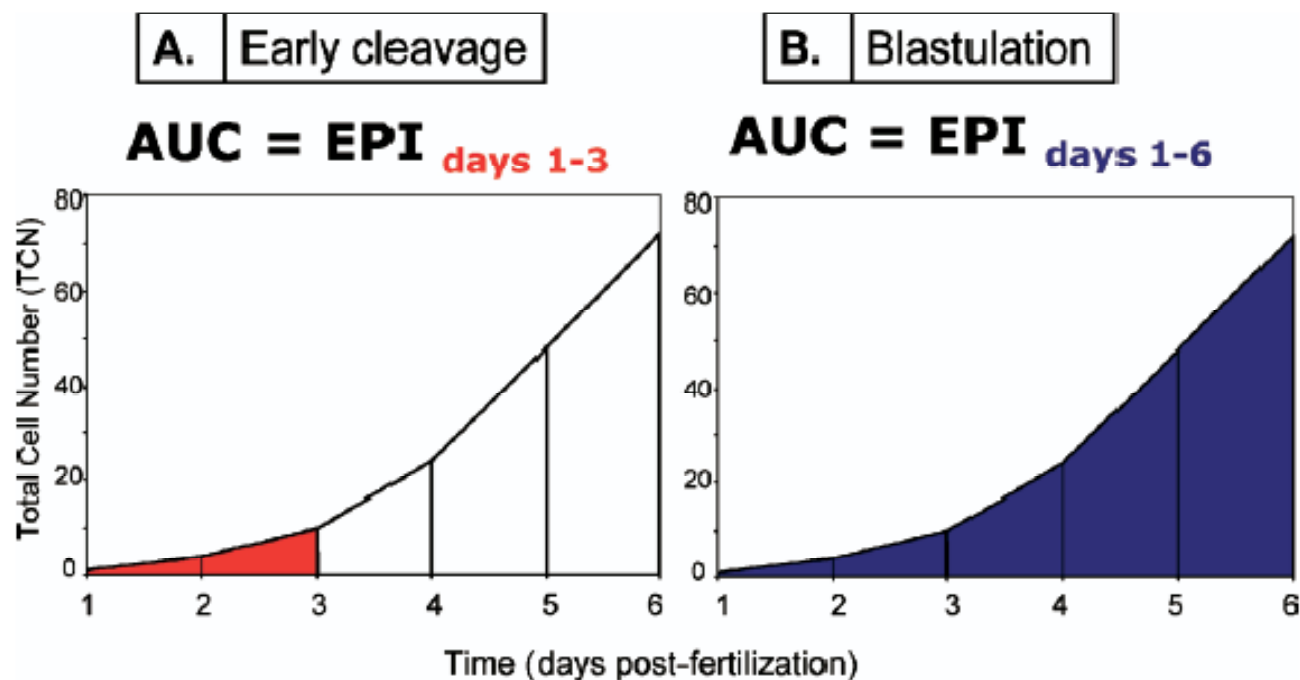
ROC Curves in the prediction of clinical PR

Rehman. Blastulation vs. early cleavage. Fertil Steril 2007.

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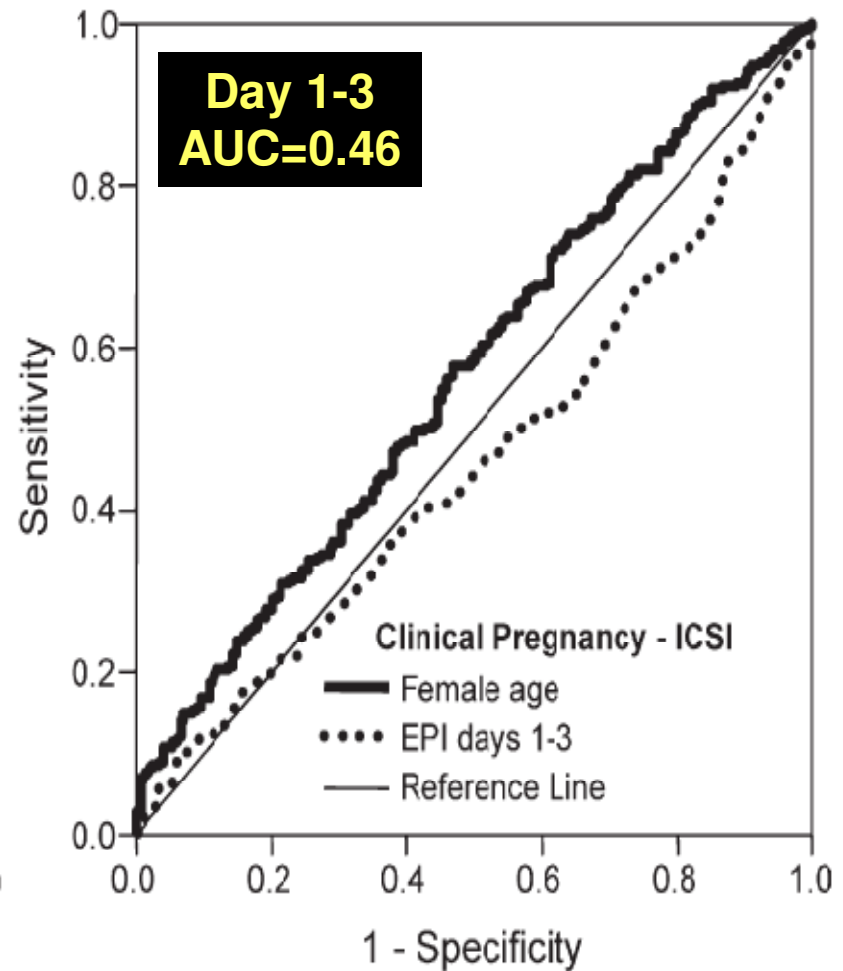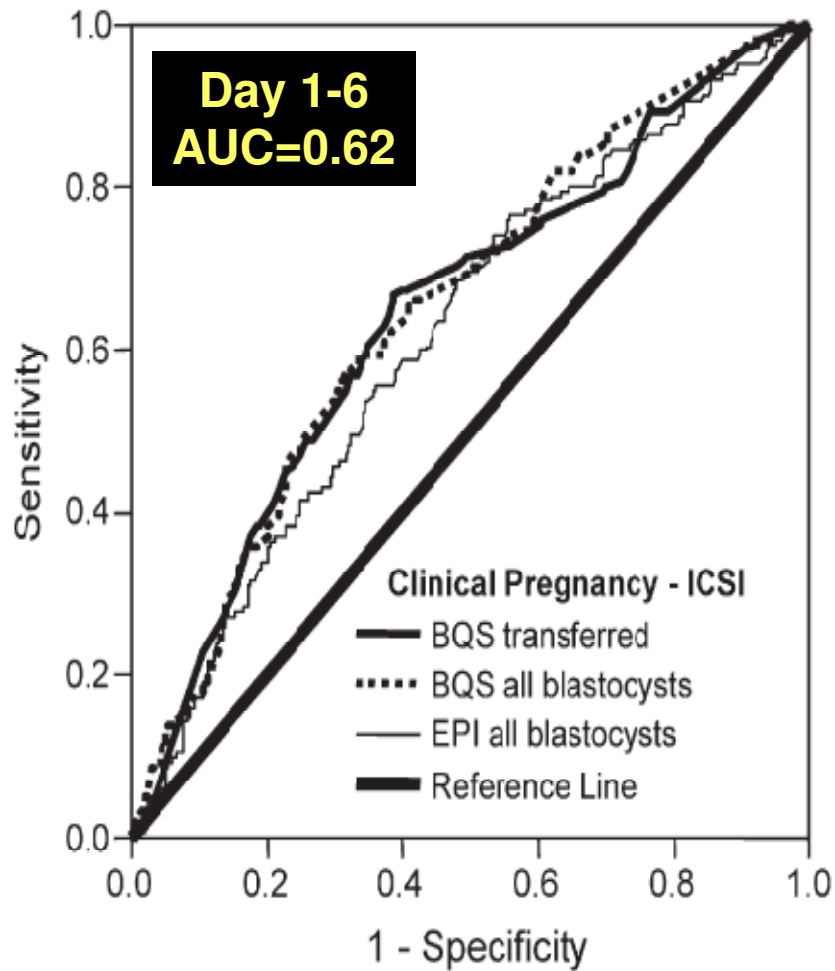
Early parameters and blastocyst implantation ?

	Della Ragione et al., 2007 SBT Day 5 (B3-B5 A/B)		IVF Unit, Tours, France SBT Day 5 (B3-B5 A/B)	
	100% Implant.	Non Implant.	100% Implant.	Non Implant.
n	93	110	83	97
% Pattern 0 zygotes	NA	NA	20.5%	20.6%
% Early cleavage	NA	NA	43.5%	58.6%
% 4-cell embryos	86.0%	86.4%	78.3%	78.4%
% <10% fragment.	88.2%*	76.4%*	75.9%	69%

IR=45.8%   IR=46.1%

At the blastocyst stage, the embryo has already reached a high developmental status and the morphological characteristics of blastocysts seem more important than the previous cleavage patterns.

=> Reaching the blastocyst stage on D5 with a good morphology seems to brings us additional information compared to early parameters of development.

Does early morphology provide additional selection power to blastocyst selection for transfer?

F.GUERIF[1,2,3,4], M.LEMSEFFER[1,2,3], J.LEGER[5], R.BIDAULT[1], V. CADORET[1], C. CHAVEZ[1], O.GASNIER[1], MH SAUSSEREAU[1], D.ROYERE[1,2,3],

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Embryo morphology and blastocyst outcome

- **Prospective observational study oct2007-dec2008**

- **2617 embryos from 511 couples assigned to SBT for 1st or 2nd attempt**

- **Individual embryo follow up with embryo and blastocyst scoring**

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Morphological appearance	n	Blastocyst rate on day 5/6	Number of points
Day 1			
2 PN with 0 pattern	250/338	74.0%	5
2 PN with non 0 pattern	1354/2062	65.7%	4
0 pronucleus	59/124	47.6%	2
1 pronucleus	40/93	43.0%	2
Day 2			
Early cleavage	726/944	76.9%	5
Non-early cleavage without PN	533/841	63.4%	4
Non-early cleavage with 2 PN	444/832	53.4%	3
4-cell embryos	1046/1339	78.1%	5
5/6-cell embryos	215/376	57.2%	3
2/3-cell embryos	288/566	50.9%	2
Regular cells	886/1211	73.2%	5
Irregular cells	817/1406	58.1%	3
Fragments <20%	1195/1660	72.0%	5
Fragments 20-50%	414/715	57.9%	3
Fragments >50%	94/242	38.8%	1

○ **Embryo scoring**
- **Poor :11-14**
- **Medium : 15-18**
- **Good : 19-21**
- **Excellent : 22-25**

FIGURE 1

The method of conversion of blastocyst morphology grading into the numerical BQS. Blastocyst quality score is the product of degree of expansion (numbered 1 to 6) and ICM and TE grades, where grade A = 3, grade B = 2, and grade C = 1. The embryo illustrated represents an expanded blastocyst with excellent morphology, with a BQS of 36.

Inner cell mass - ICM

Trophectoderm - TE

Gardner and Schoolcraft Morphology grading
(Gardner and Schoolcraft, 1999)

Expansion | ICM grade | TE grade
4 | A | A

4 x 3 x 3 = 36 | BQS

Blastocyst Quality Score
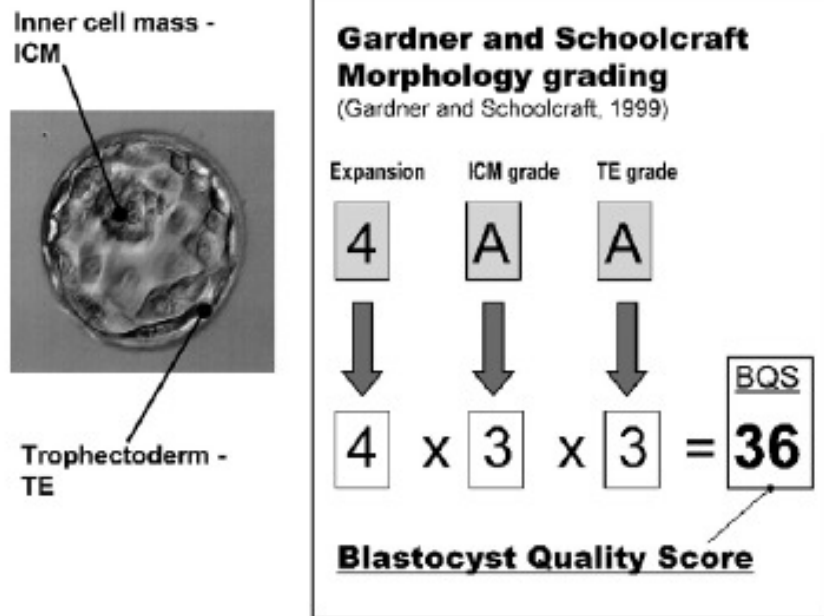
Rehman. Blastulation vs. early cleavage. Fertil Steril 2007.

○ **Blastocyst scoring**
- **Poor :** 1 - 5
- **Medium :** 6 – 18
- **Good :** 20 - 54
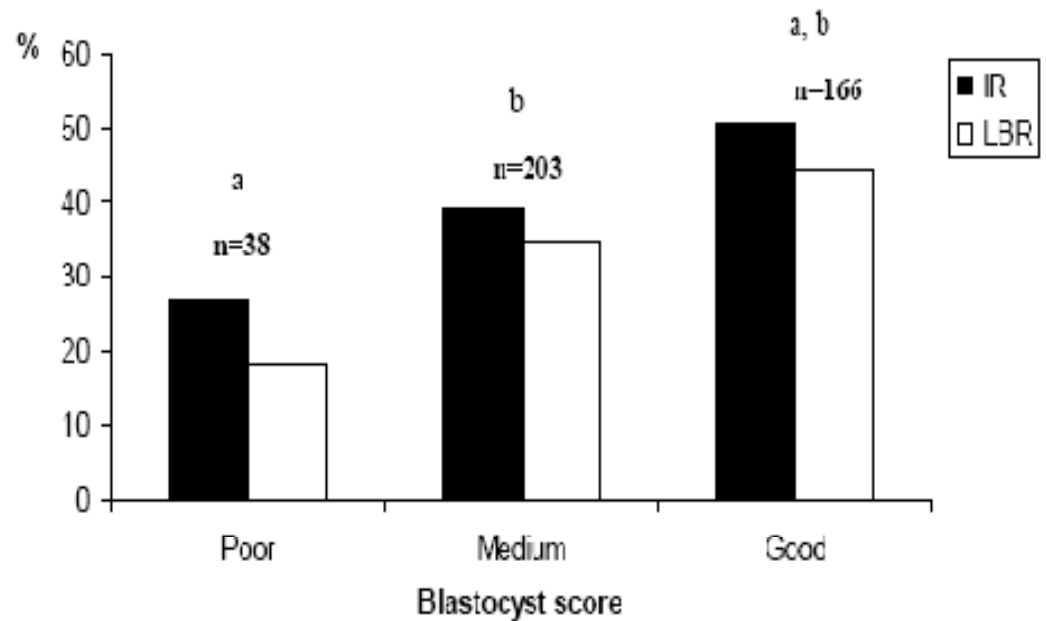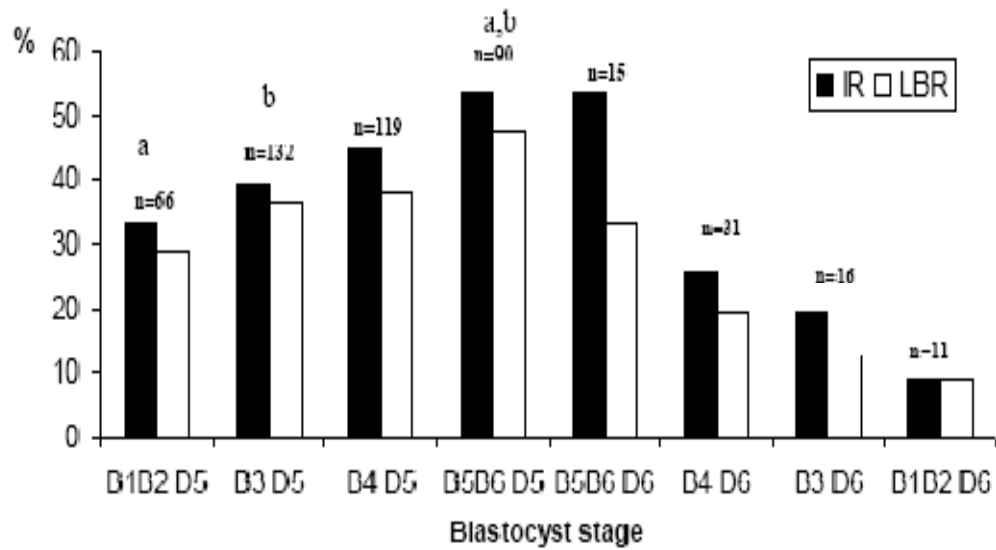
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LISBON 2010

RBM online, 2010, doi:10.1016/j.rbmo.2010.06.043

Associations between blastocyst score on day 5, blastocyst stage, trophectodermal cells and

inner cell mass and blastocyst outcome – multivariate analysis.

Blastocyst score	OR	95% confidence interval		p-value	AUC
Poor	-	-	-	-	
Medium	2.337	0.983	5.556	NS	0.579
Good	3.562	1.484	8.580	0.004	

|	OR	95% confidence interval		p-value
Blastocyst stage				NS
1	-	-	-	-
2	1.076	0.381	3.040	NS
3	1.257	0.512	3.086	NS
4	1.306	0.520	3.282	NS
5	2.084	0.809	5.368	NS
Trophectodermal cells				NS
Poor	-	-	-	
Medium	1.020	0.525	1.982	NS
Good	0.984	0.453	2.141	NS
Inner Cell Mass				NS
Poor	-	-	-	
Medium	1.788	0.790	4.051	NS
Good	1.956	0.787	4.861	NS

OR = Odd Ratio – NS = Non-significant

AUC – Areas under the receiver operating characteristics curve

RBM online, 2010, doi:10.1016/j.rbmo.2010.06.043
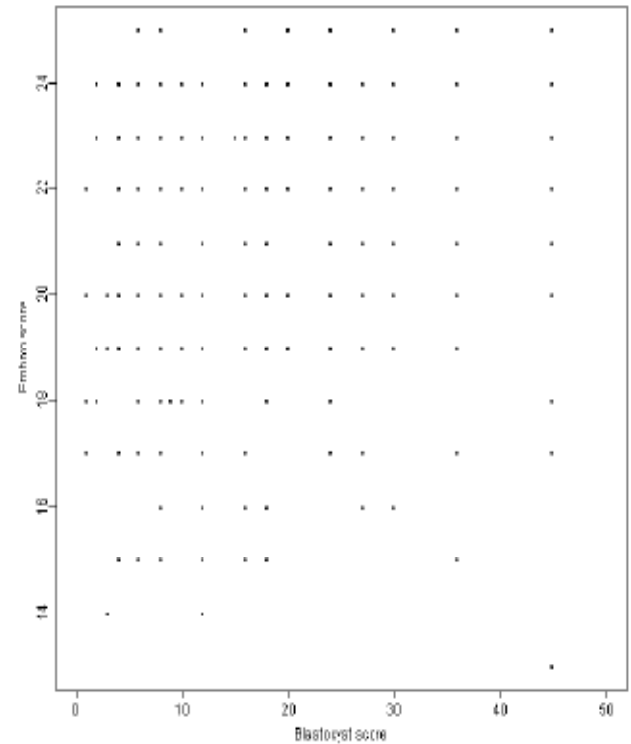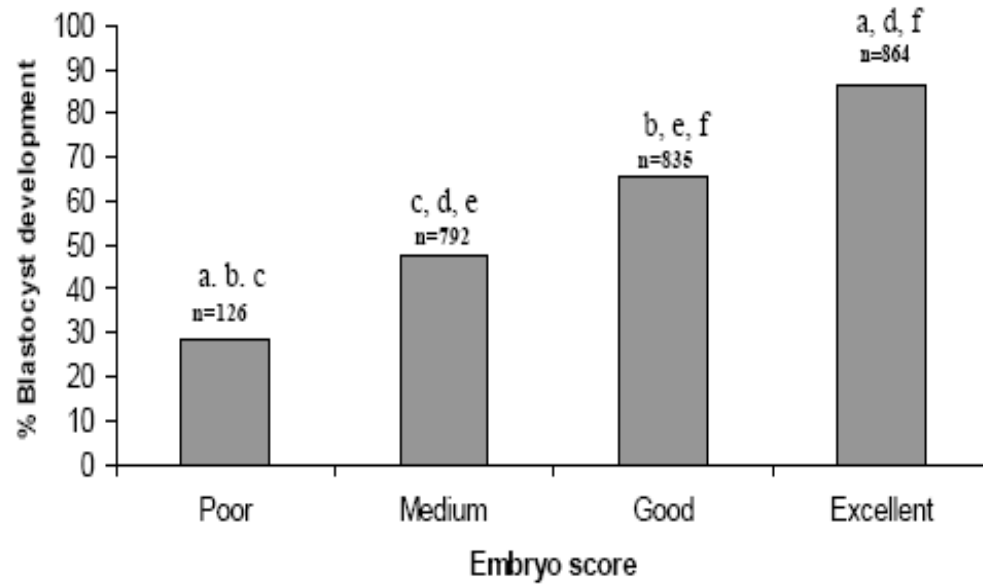
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RBM online, 2010,
doi:10.1016/j.rbmo.2010.06.043

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Clinical outcome of single blastocysts transferred on day 5 according to blastocyst and embryo scores and detailed morphology on day 1/2.

	Birth		p
	YES	NO	
n	155	252	
Mean blastocyst score	19.8 ± 12.4	17.2 ± 11.3	0.03
Good score rate	46.9%	34.5%	0.007
Medium score rate	48.7%	53.8%	
Poor score rate	4.4%	11.7%	
Mean embryo score	21.3 ± 2.4	21.0 ± 2.6	NS
Excellent score rate	51.8%	51.9%	NS
Good score rate	34.7%	31.4%	NS
Medium score rate	12.9%	15.5%	NS
Poor score rate	0.6%	1.2%	NS
0 pattern zygotes rate	15.3%	12.1%	NS
Early cleavage rate	49.4%	45.3%	NS
4-cell rate	74.7%	70.6%	NS
Regular cell rate	61.8%	57.8%	NS
Fragmentation rate <20%	72.9%	75.5%	NS

Associations between embryo score, day 1 and day 2 morphological characteristics and blastocyst outcome – multivariate analysis.

Embryo score	OR	95% confidence interval		p-value	AUC
Poor-Medium	-	-	-	-	
Good	1.311	0.692	2.485	NS	0.518
Excellent	1.244	0.683	2.266	NS	

	OR	95% confidence interval		p-value	AUC
Pronuclei				NS	
0 or 1 PN	-	-	-	-	
2 PN with non 0 patternr	1.066	0.427	2.663	NS	
2 PN with 0 pattern	1.431	0.511	4.008	NS	
Cleavage				NS	
Non-early cleavage with 2PN	-	-	-	-	
non-early cleavage without PN	1.085	0.542	2.174	NS	
Early cleavage	1.161	0.586	2.297	NS	
Number of blastomeres				NS	
2-3 cells	-	-	-	-	0.546
≥ 5 cells	1.069	0.410	2.785	NS	
4 cells	1.061	0.524	2.150	NS	
Shape of cells				NS	
Irregular	-	-	-	-	
Regular	1.059	0.685	1.637	NS	
Fragmentation rate				NS	
>50%	-	-	-	-	
20-50%	1.580	0.456	5.477	NS	
<20%	1.522	0.461	5.018	NS	

Deep phenotyping to predict live birth outcomes in in vitro fertilization

Prajna Banerjee[a,1], Bokyung Choi[b,1], Lora K. Shahine[a,c], Sunny H. Jun[a,d], Kathleen O'Leary[a], Ruth B. Lathi[a], Lynn M. Westphal[a], Wing H. Wong[e], and Mylene W. M. Yao[a,2]
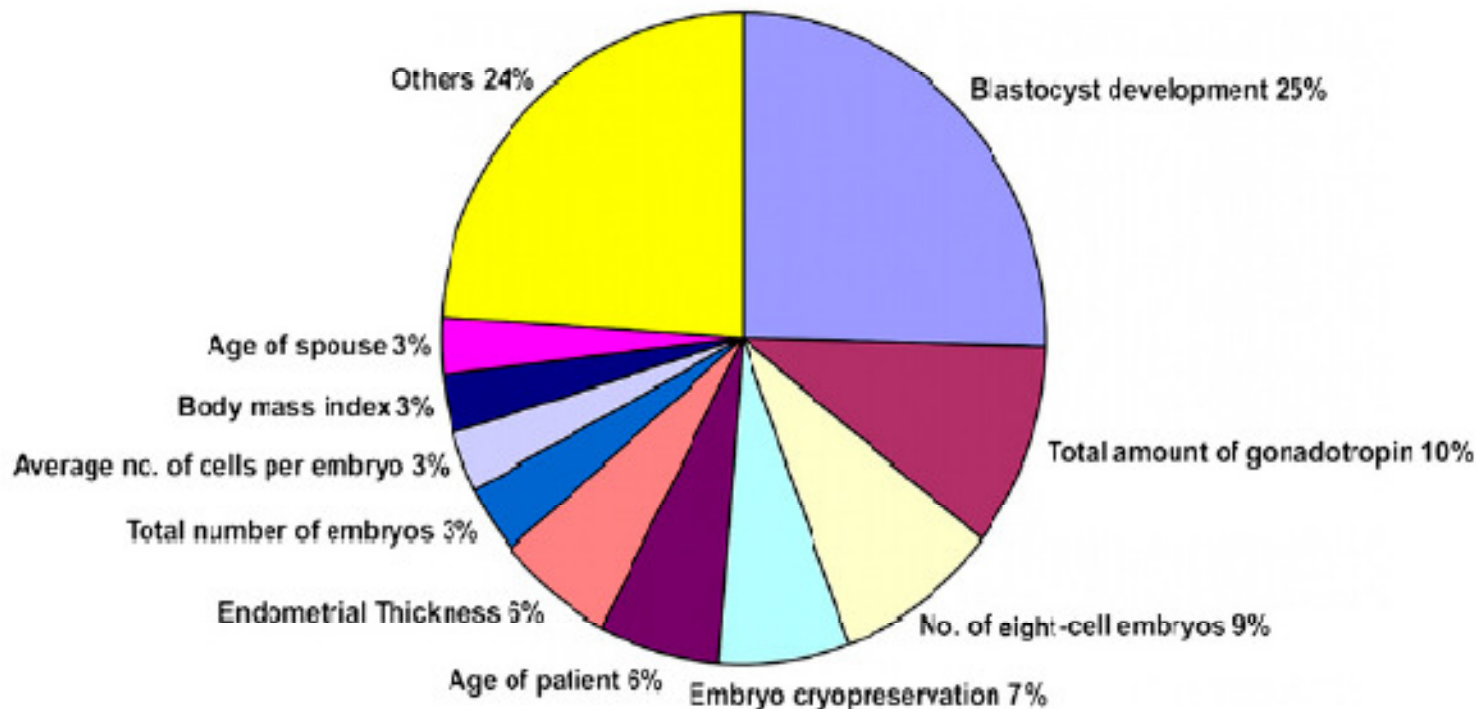
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CONCLUDING REMARKS
PERSPECTIVES

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Different ways to select embryos...

- **Using D1 (and D0) parameters** when approaches involving embryo selection cannot be implemented in countries with restrictive IVF legislation.

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Pro

- **Only realistic option is the use of early scoring systems**
  - **Improvement of the identification of viable oocytes ?**

However

- **Further publications of SET based on pronuclear morphology are necessary to validate the weight of zygote scoring systems.**

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Different ways to select embryos...

○ **Using D3/D2 (and D1, D0) parameters when you think that**

drawbacks > advantages with extended culture

    * Suboptimal conditions of culture

    * Increased rate of cancelled transfers

    * Time consuming or expensive cost in laboratory

<u>Pro</u>

○ **Promising results of early combined parameters.**

○ **Additional benefit of new parameters ?**
  ○ **Blastomere mononucleation**

<u>However…</u>

○ **Discriminating value of D3/D2 parameters remains to be confirmed.**

○ **Deleterious effects of multiple observations ?**

Different ways to select embryos...

○ **Using later stages parameters (D5/D6) when you think that**

advantages > drawbacks with extended culture

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TOP QUALITY EMBRYOS AND BLASTOCYST DEVELOPMENT

	No Emb	Characteristics	Blastocyst dev.D5 TQ Embryos
Fisch *et al.,*2001	1245	PN + EC + D3	64 % blastocysts
Lan *et al.,* 2003	1894	PN + D3	92 % blastocysts
Neuber *et al.,* 2003	1550	PN + EC + D2 + D3	54 % blastocysts
Rienzi *et al.,* 2005	993	PN + EC + D2 + D3	77 % blastocysts
Guerif *et al.,* 2007	4042	PN + EC + D2	59 % blastocysts

	Rijnders and Jansen, 1998	Milki *et al.,* 2002	IVF Unit, Tours-France
Number of cycles	48	100	140
Day of observ.	Day 3	Day 3	Day 2
No. Emb transfer.	2-3	2	1
Total agreement	**20%**	**23%**	**32%**
Partial agreement	56%	38%	34%
No Agreement	24%	39%	34%
Blastocyst IR	30%	NA	41.5%

Predictive value of embryo morphology on day 2/3 for subsequent blastocyst formation seems limited

What about SET vs SBT ?

	Papanikolaou et al., 2006		Zech et al., 2007		Guerif et al, 2009	
	SET (D3)	SBT (D5)	SET (D3)	SBT (D5)	SET + FET (D2)	SBT + FBT (D5/6)
Number of cycles	176	175	99	128	243	235
Ongoing IR per cycle	23.3%*	33.1%*	23.2%	32.8%	34.2%	37.9%

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Conclusions, perspectives

- Morphology and kinetics have a rather limited power to predict implantation
- Blastocyst development have some independent additive predictive value

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- Several criteria or parameters ("omics") have been proposed to better select the embyo for transfert
- Which among them will be the more robust and useful in clinical practice?
- They will have to satisfy a true prospective and blinded evaluation, neither "series" ou "experiment" dependent.
- While they should be at least in part unrelated to morphological criteria, they should have a reasonnable cost-effectiveness ratio.

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