Morphokinetic Analysis of Early Stage Human Embryos: Does It Have Any Clinical Significance?

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MAYBE

Time-Lapse Imaging Systems

Comparison of the technical parameters of TLS

	Embryoscope	Primo vision	EEVA
Illumination	Bright field, low intensity red LED	Bright field, low intensity green LED	Dark field
Microscope/incubator	Incubator with integrated time-lapse system	Microscope that can be placed in standard incubators	Microscope that can be placed in standard incubators
Culture dish	Embryoslide	9-16 well Primo vision embryo culture dish	EEVA dish
Embryo culture	Single culture	Group culture	Group culture
Planes of view	7 focal planes	11 focal planes	Single plain
Max.# of embryos monitored	72	96	Depends on the dish
Other	Comes with software	Comes with software	Automated, software scores blastocyst formation potential

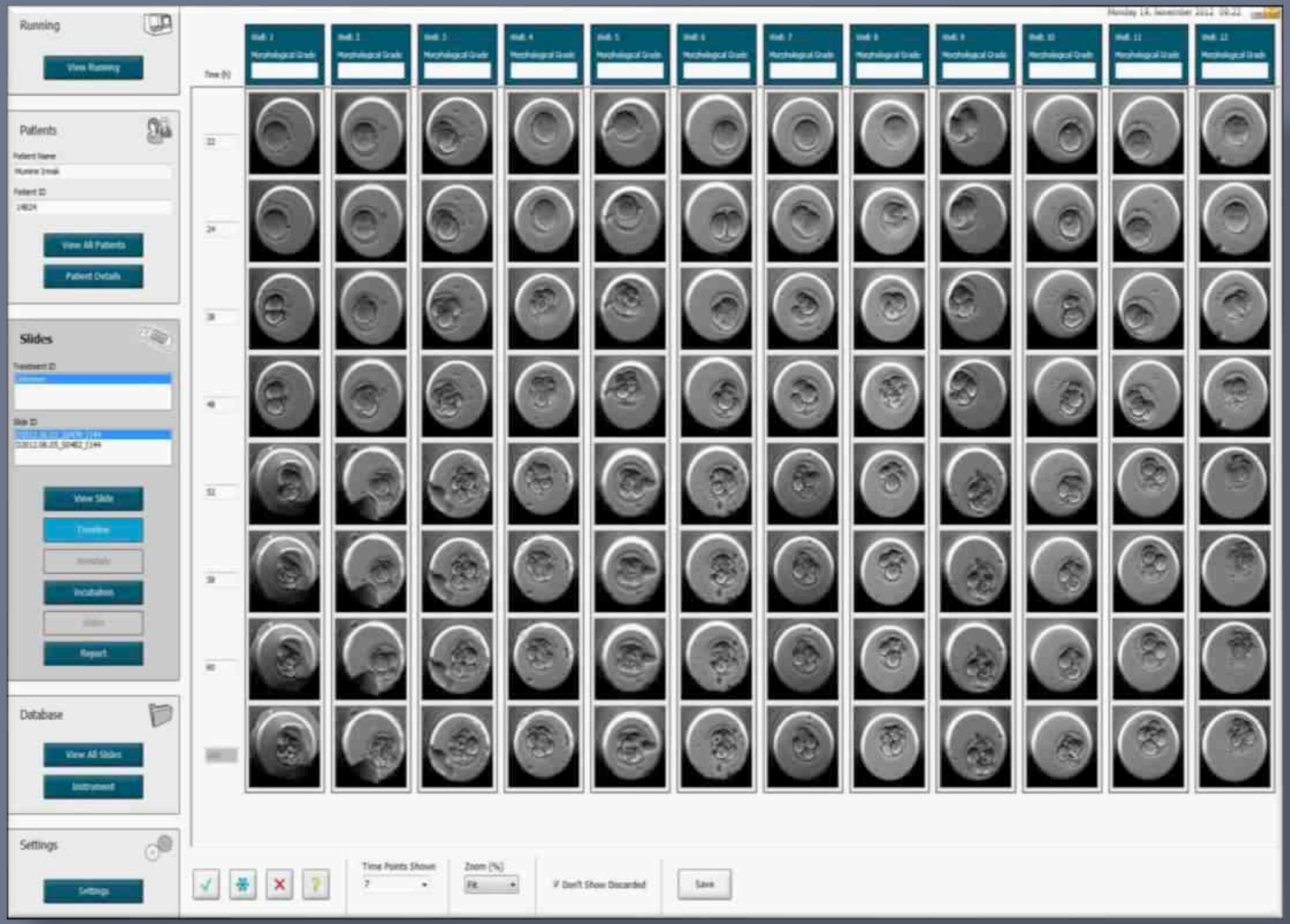
P Kovacs Reproductive Biology and Endocrinology 2014

Undisturbed contidions

Flexible evaluation

Teaching

More observations



Hierarchical Classification Model

Version 1

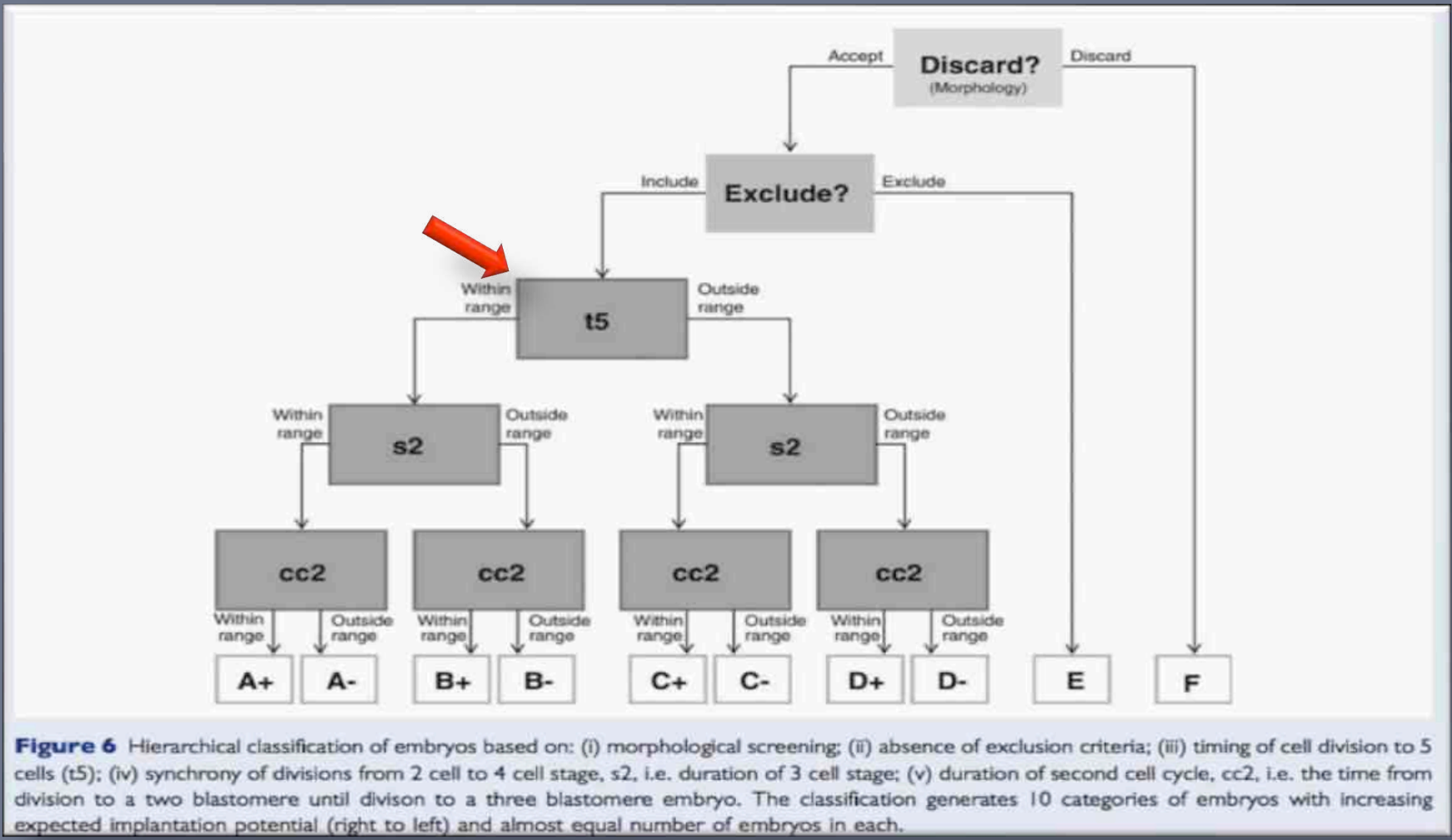


Figure 6 Hierarchical classification of embryos based on: (i) morphological screening; (ii) absence of exclusion criteria; (iii) timing of cell division to 5 cells (t5); (iv) synchrony of divisions from 2 cell to 4 cell stage, s2, i.e. duration of 3 cell stage; (v) duration of second cell cycle, cc2, i.e. the time from division to a two blastomere until divison to a three blastomere embryo. The classification generates 10 categories of embryos with increasing expected implantation potential (right to left) and almost equal number of embryos in each.

M Meseguer Human Reprod 2011

Hierarchical Classification Model

Version 2

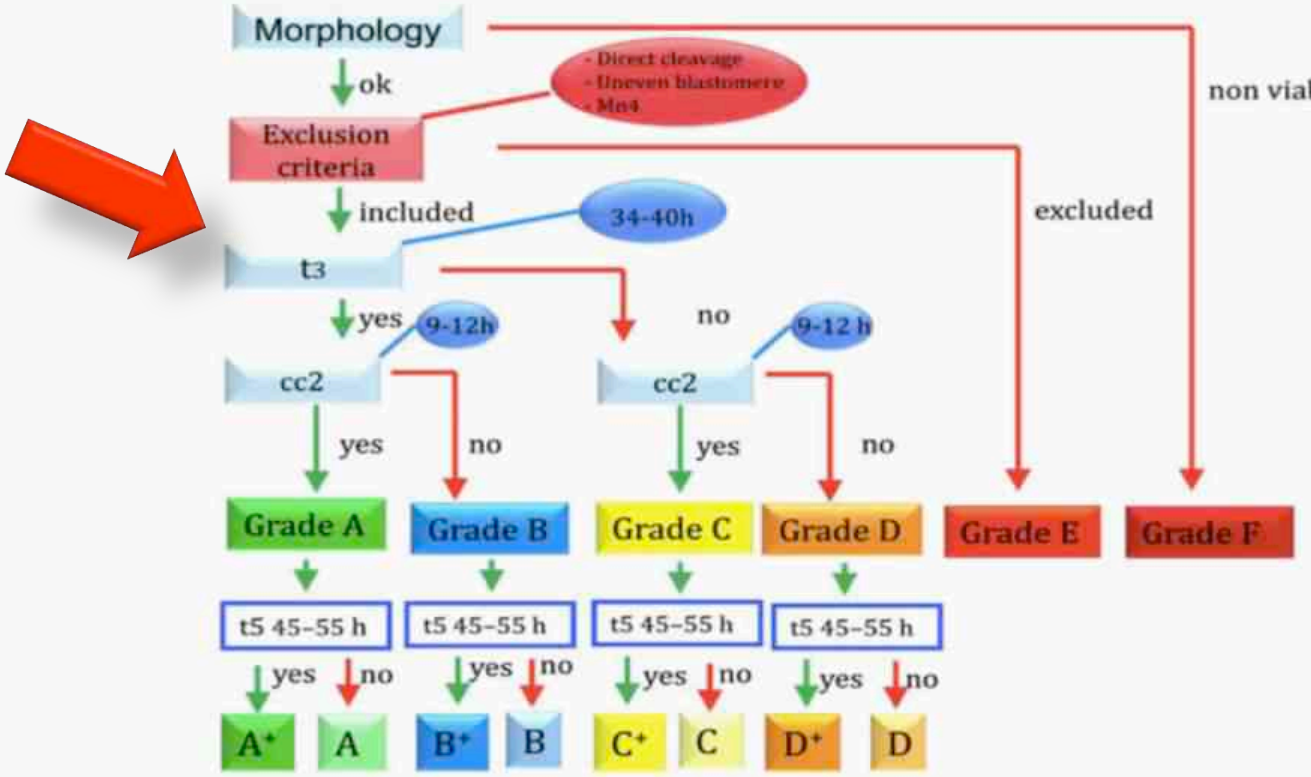
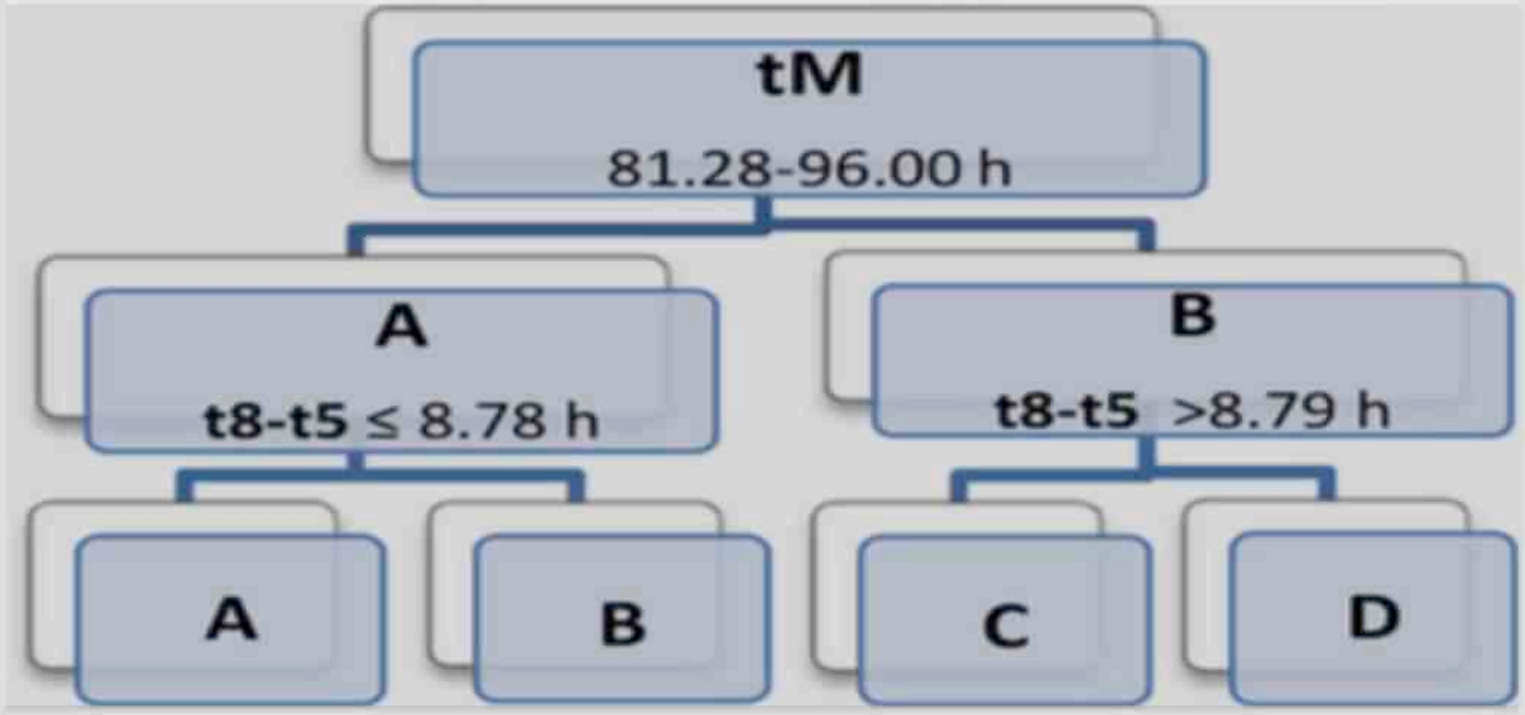


Figure 3 Hierarchical classification of embryos based on: (i) Morphological screening; (ii) the new morphological criteria; (iii) timing of cell division to three cells (t3); (iv) duration of second cell cycle, cc2, i.e. the time from division to a two blastomere until division to a three blastomere embryo; (v) timing of cell division to five cells . The classification generates 10 categories of embryos with increasing expected implantation potential (right to left) and almost equal number of embryos in each.

N Basile Human Reprod 2015

Hierarchical Classification Model

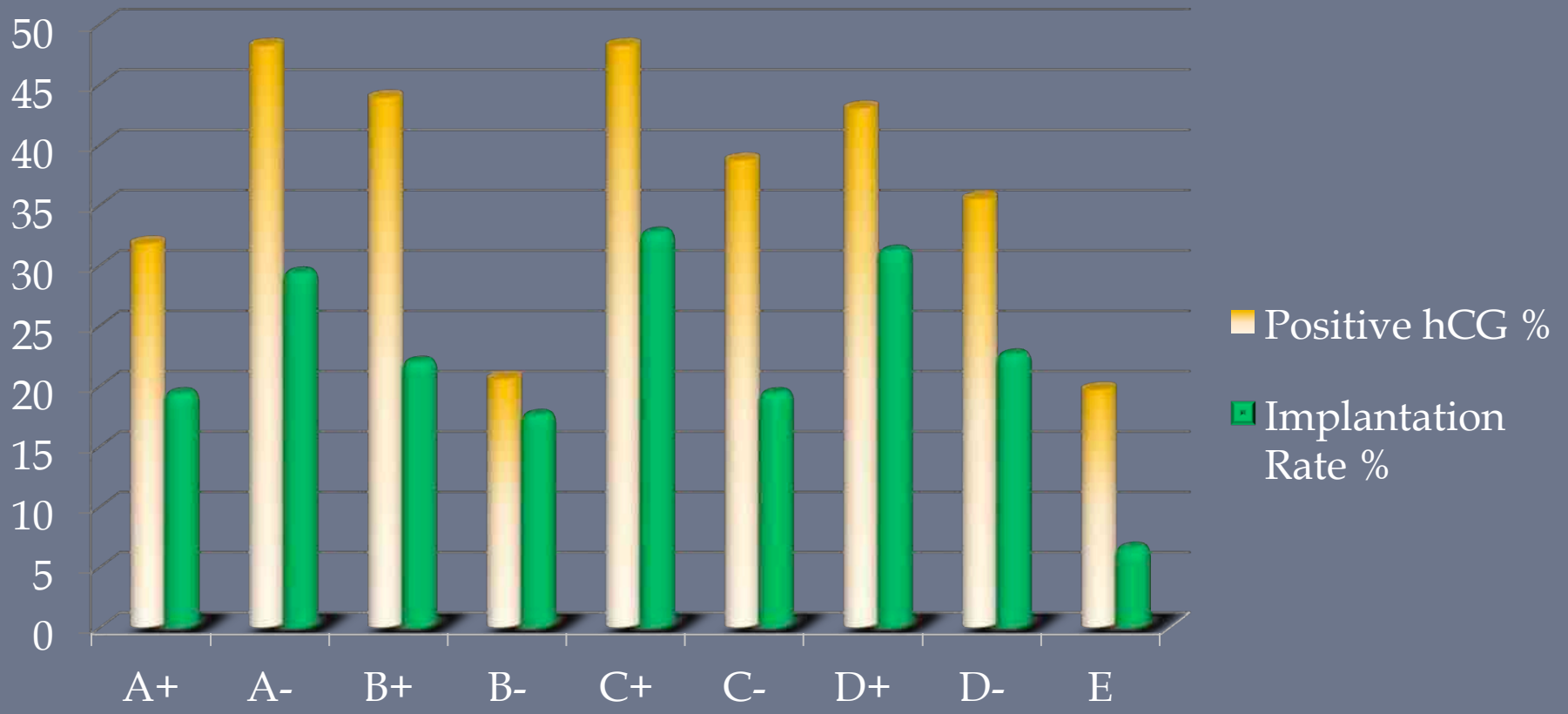
Version 3



Hierarchical classification of embryos based on [1] timing of morula, optimal range 81.28–96.00 hours; [2] timing of transition from 5-blastomere embryo to until 8-blastomere embryo (t8–t5), optimal range ≤8.78 hours. The classification generates four categories of embryos with increasing expected blastocyst formation (right to left).

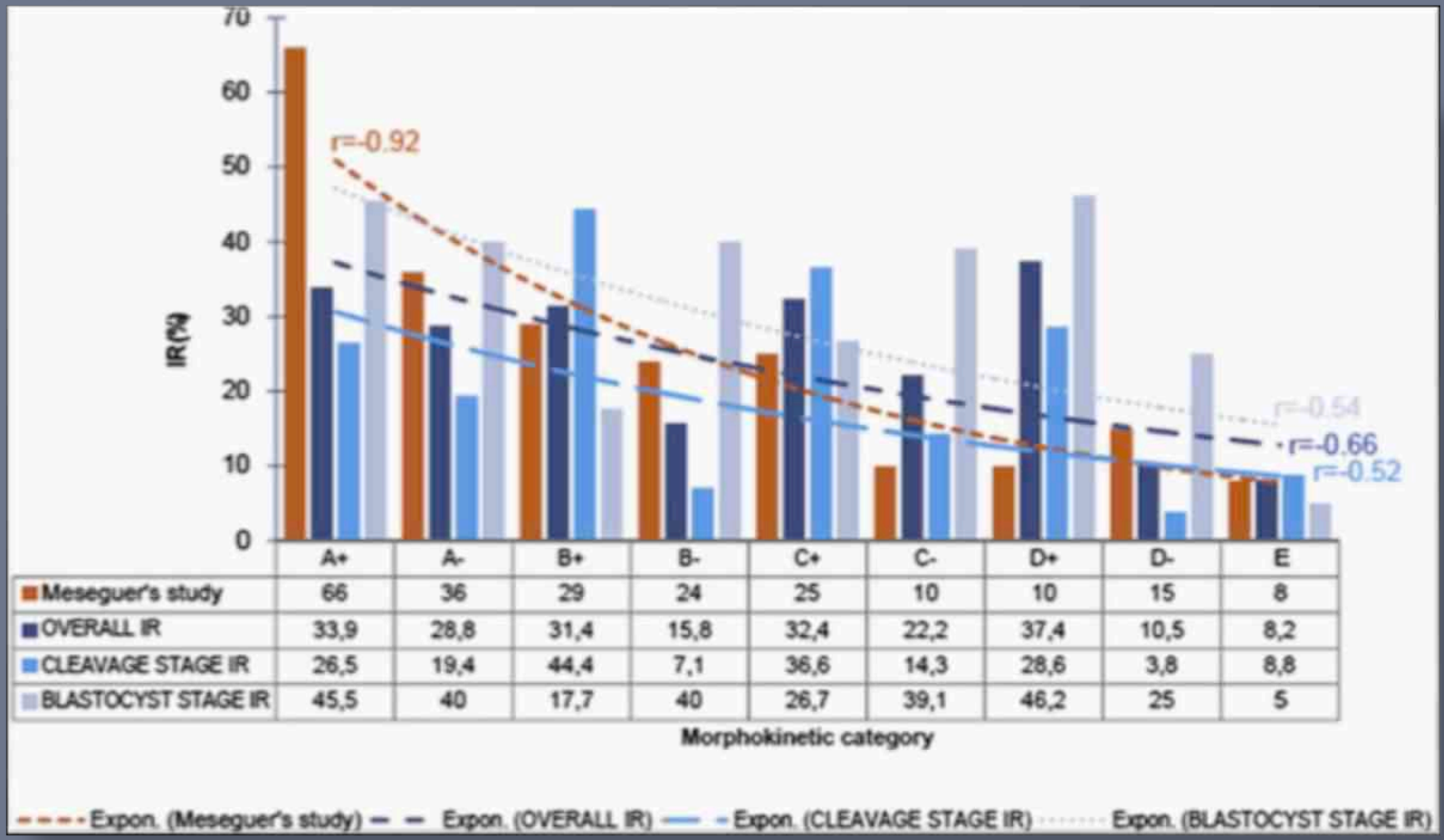
Y Motato Fertil Steril 2016

Reproducibility of a time-lapse embryo selection model based on morphokinetic data in a sequential culture media setting



E.Yalçınkaya JTGAA 2014

External validation of time-lapse prediction model



	A+	A-	B+	B-	C+	C-	D+	D-	E
Meseguer's study	66	36	29	24	25	10	10	15	8
OVERALL IR	33,9	28,8	31,4	15,8	32,4	22,2	37,4	10,5	8,2
CLEAVAGE STAGE IR	26,5	19,4	44,4	7,1	36,6	14,3	28,6	3,8	8,8
BLASTOCYST STAGE IR	45,5	40	17,7	40	26,7	39,1	46,2	25	5

T Freour Fertil Steril 2015

Limitations of a time-lapse blastocyst prediction model: a large multicentre outcome analysis

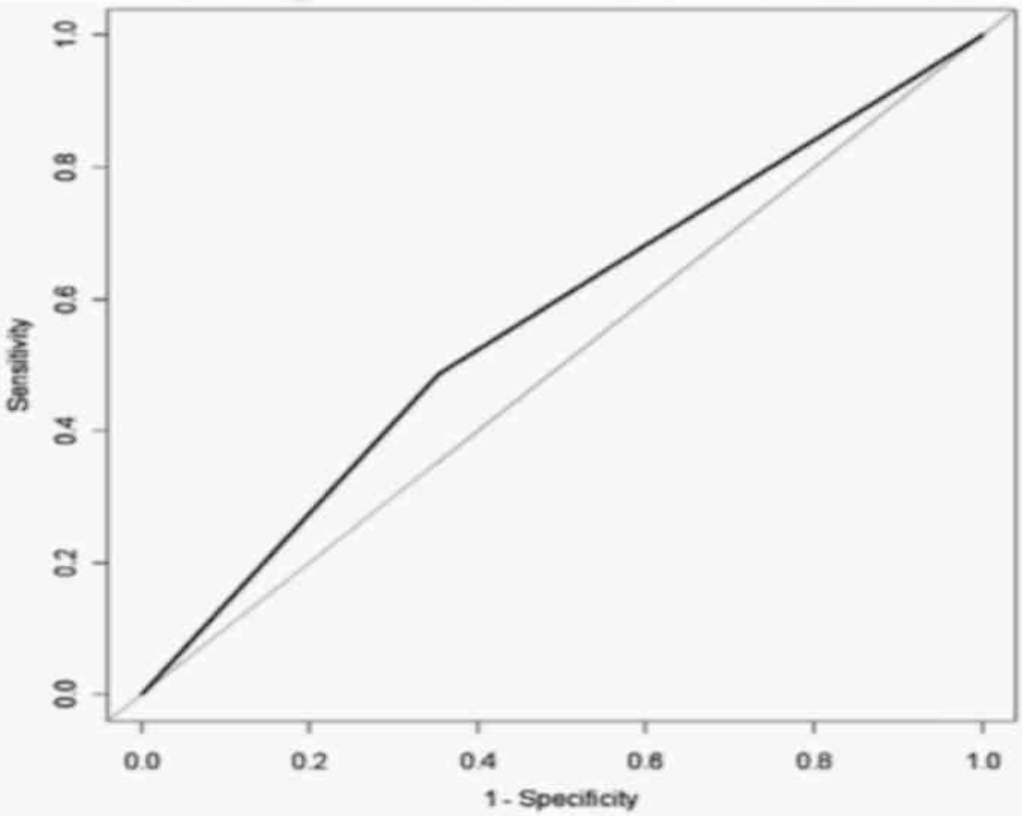
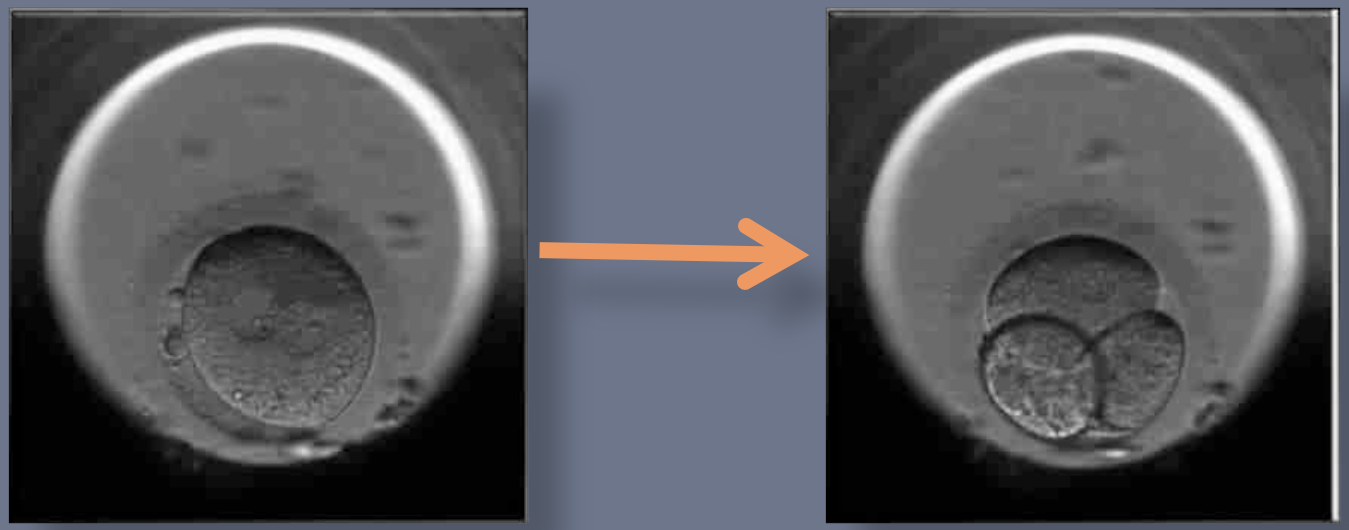


Figure 1 Receiver operating characteristic curve for prediction of pregnancy by the parameters $t_3 - t_2$ and $t_4 - t_3$ from the test model.

K Kirkegaard RBM Online 2014

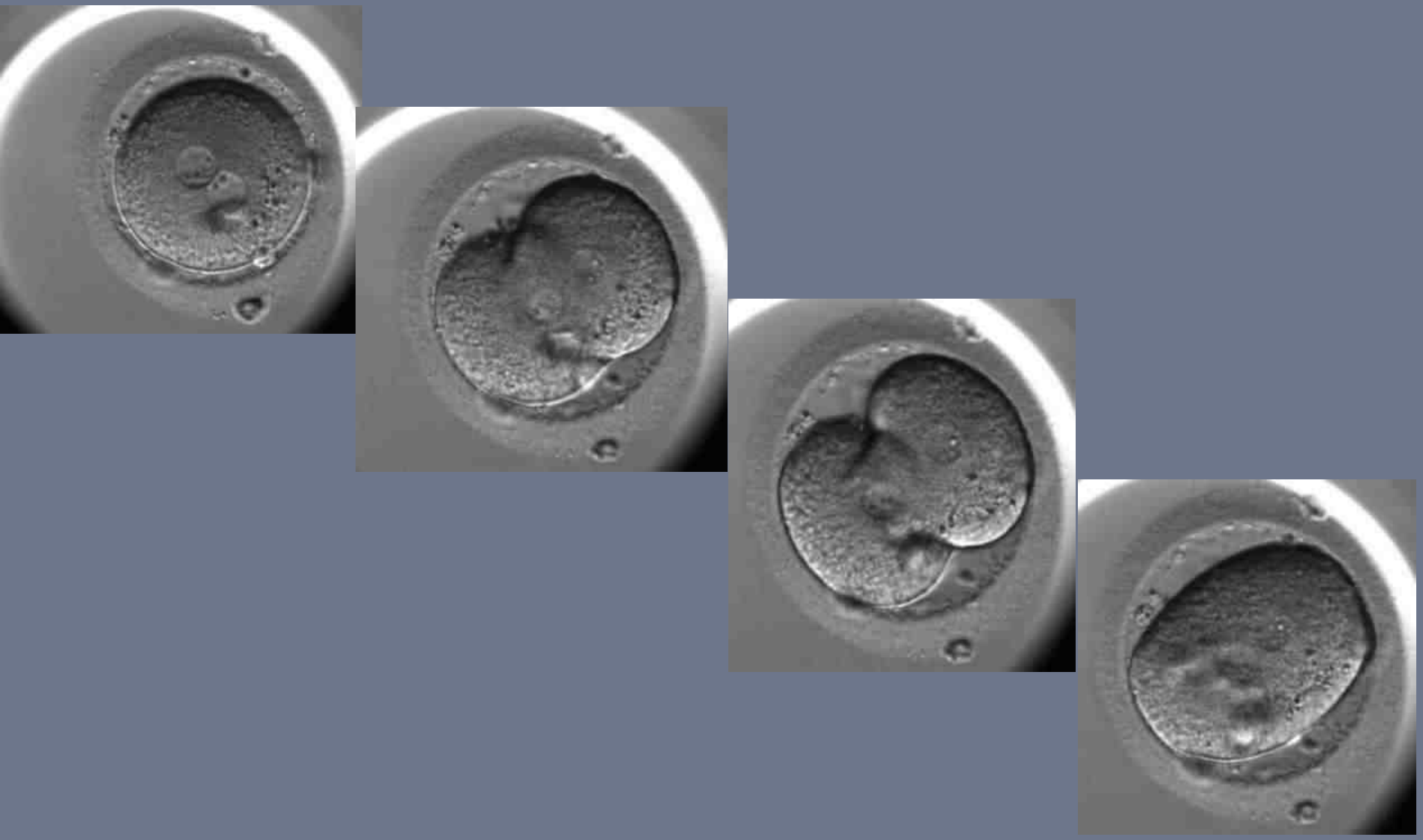
Qualitative Deselection Parameters

Direct Cleavage

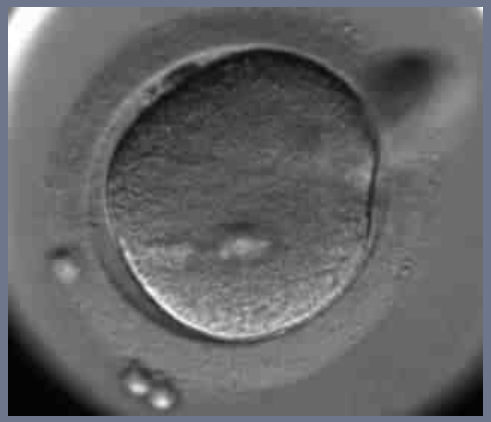
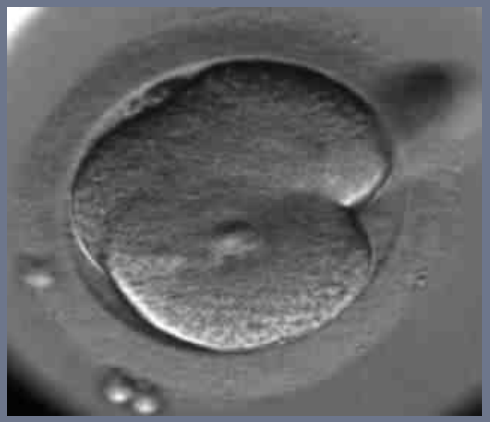


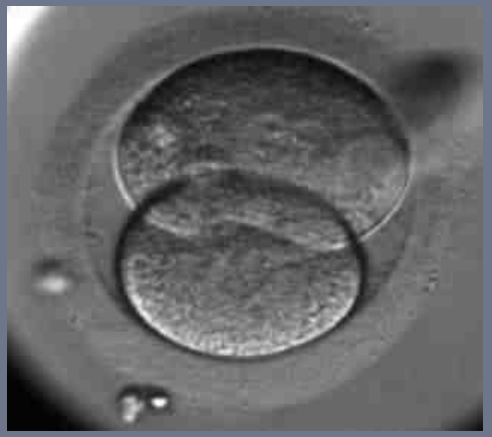
I Rubio Fertil Steril 2012

Reverse Cleavage



Reverse Cleavage



Reverse Cleavage

- It occurred 27,4% of embryos
- Higher incidance in antagonist cycles
- Higher incidance in ICSI cases
- More multinucleation
- Less embryo reached the 6 cell on day 3
- Poor embryo morphology and morphokinetic
- Less implantation rate

Y Liu Fertil Steril 2014

Abnormal division patterns

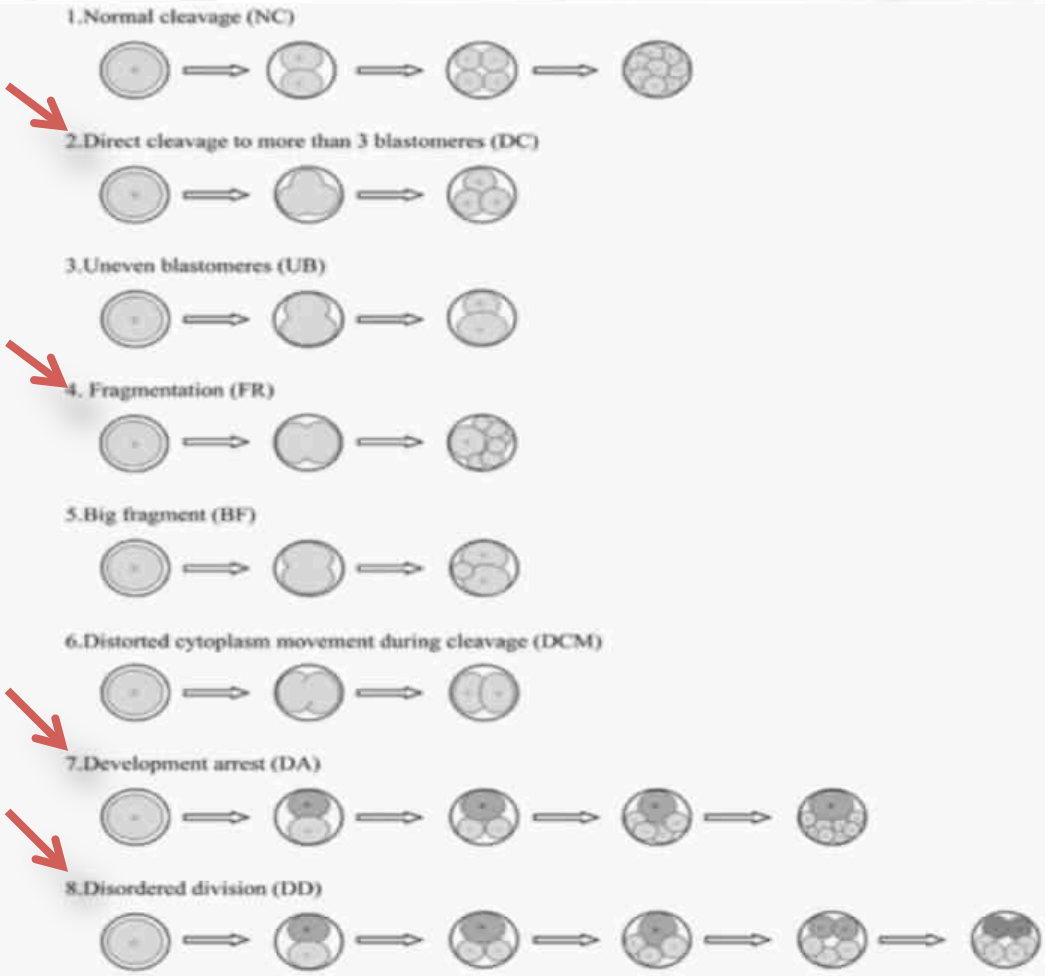
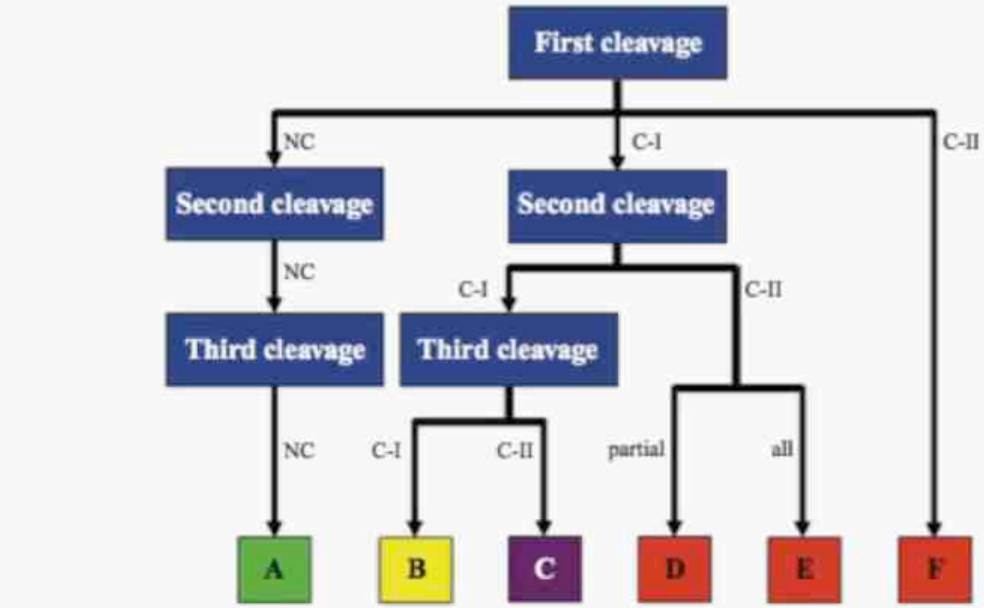


Figure 1 Abnormal cleavage during early embryonic development as assessed by time-lapse imaging. Classification of division behaviours occurring in early embryonic development is presented (see definitions in text): (1) normal cleavage; (2) direct cleavage (3); uneven blastomeres (4) fragmentation (5) big fragment (6) distorted cytoplasm movement during cleavage (7) development arrest and (8) disordered division.

ST Yang RBM Online 2015

Abnormal division patterns

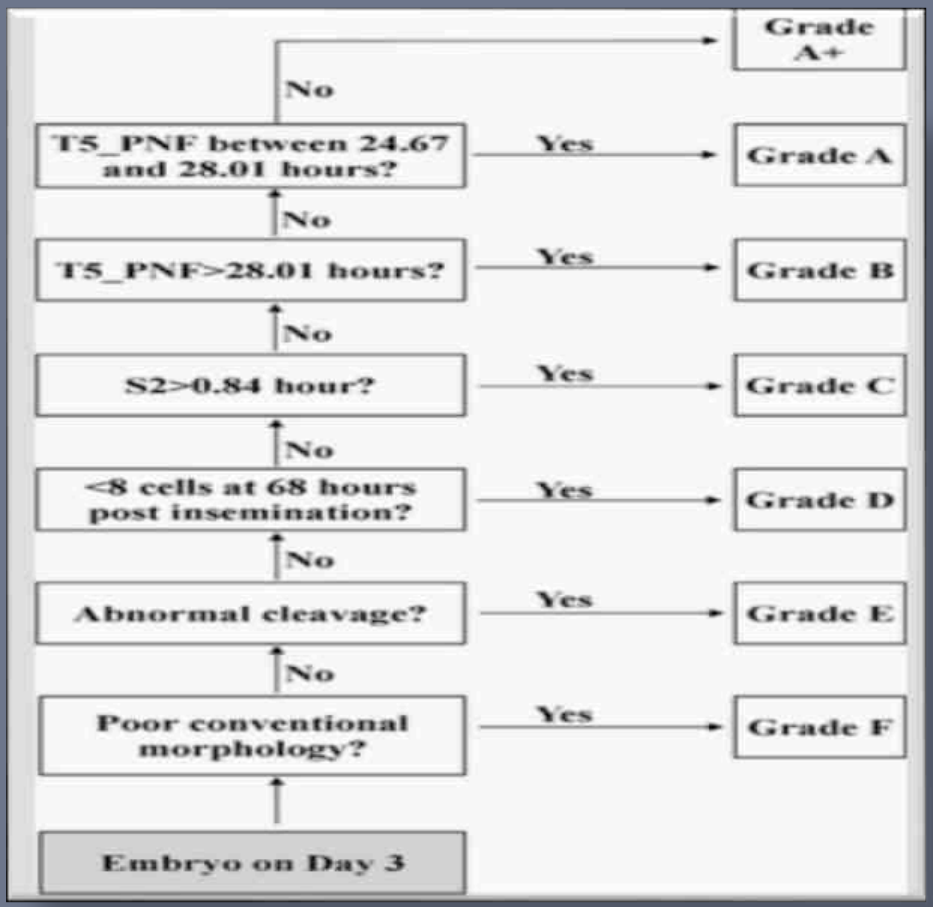


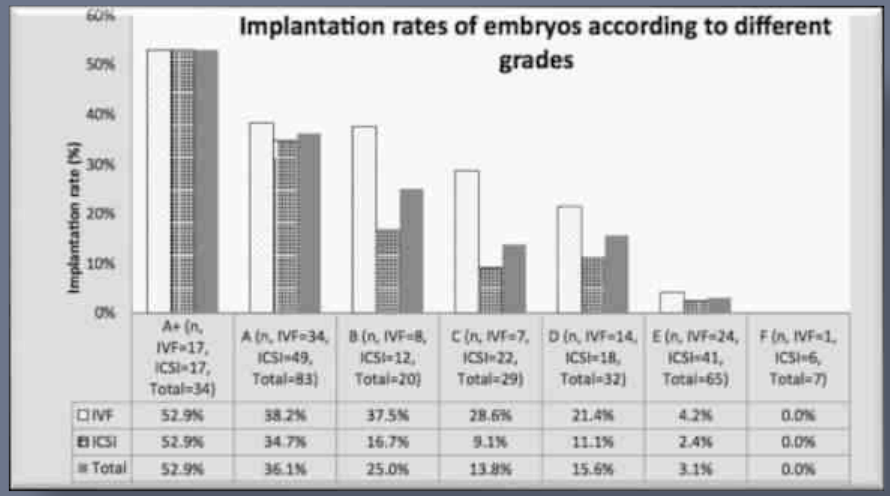
Blastocyst formation (%)	94.8 (91/96)	85.3 (87/102)	48.6 (18/37)	51.3 (20/39)	47.4 (9/19)	21.2 (11/52)
Good quality blastocyst formation (%)	70.8 (68/96)	54.9 (56/102)	13.5 (5/37)	10.3 (4/39)	0 (0/19)	3.8 (2/52)

Figure 4 A hierarchical predictive model for embryo assessment and selection showing the six categories of classification (A to F). The corresponding evaluation of the present study of normal fertilized embryos, showing their viability from the first cleavage to the third is shown below the model. C-I: category 1, C-II: category 2. NC, normal cleavage.

ST Yang RBM Online 2015

Time-lapse deselection model for human day 3 in vitro fertilization embryos: the combination of qualitative and quantitative measures of embryo growth





Y Liu Fertil Steril 2015

Why should we use time-lapse?

Which morphological scoring system is relevant in human embryo development?

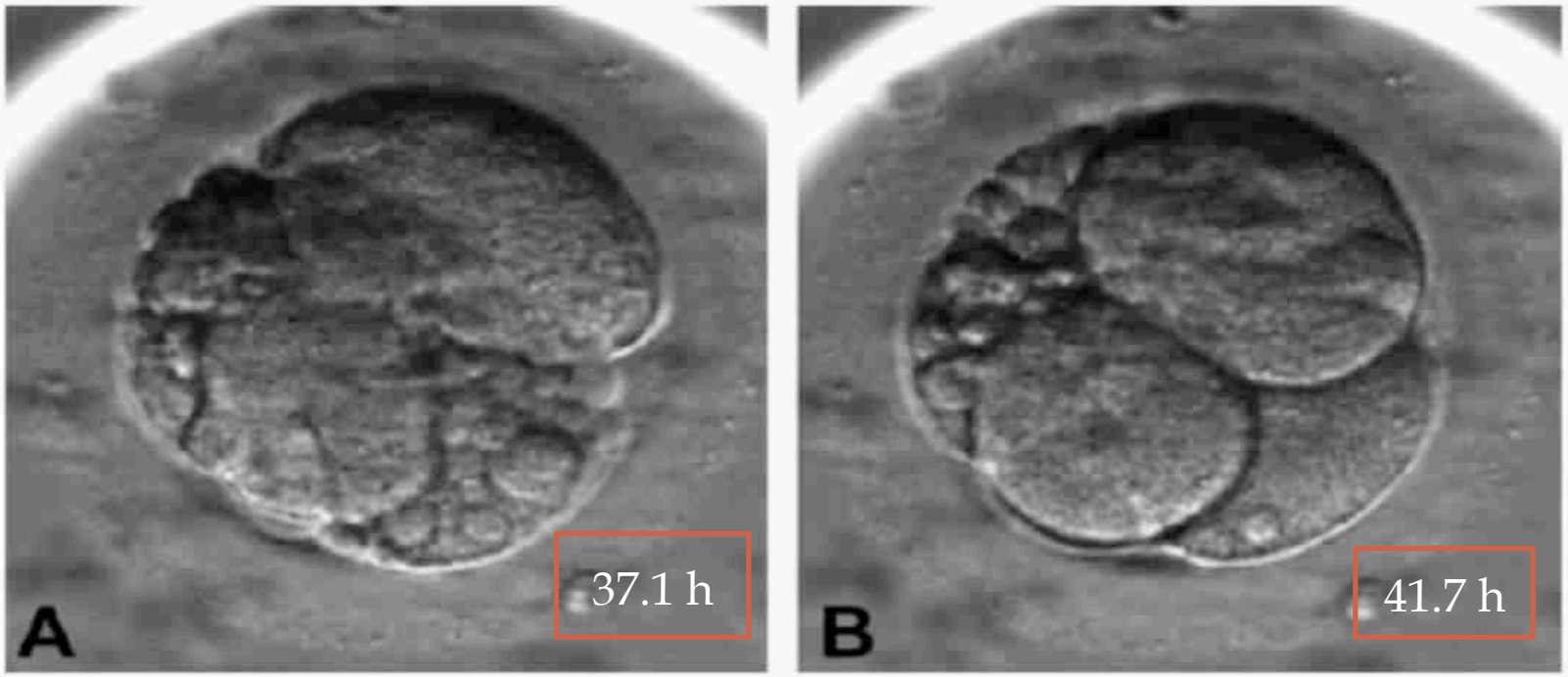


Fig. 2. Changes of embryo morphology after cleavage. An example of an embryo is shown with a highly irregular morphology at 37.1 h after ICSI (A) and regular blastomeres and less fragments at 41.7 h after ICSI (B).

M. Montag Placenta 2010

Which morphological scoring system is relevant in human embryo development?

Table 1
Scoring markers assessed at different time points.

Pronuclear morphology	Same pattern		Different pattern
16–18 h: 212 2PN oocytes compared to 14–15 h:	75% (159/212)		25% (53/212)
19–20 h: 65 2PN oocytes[a] compared to 16–18 h:	66.2% (43/65)		33.8% (22/65)
Time of first cleavage	<25 h	25–26 h	>26 h
$n = 59$	27.1% (16/59)	30.5% (18/59)	42.4% (25/59)
"Early" cleavage	57.6% (34/59)		
Embryo morphology	Same score		Different score
40 h versus 38 h	50.8% (29/57)		49.1% (28/57)
42 h versus 40 h[b]	67.3% (33/49)		32.6% (16/49)

[a] Numbers for 2PN oocytes are lower at 19–20 h as some have been frozen at the 2PN stage after the second assessment.

[b] Numbers of embryos analyzed are lower at 42 h versus 40 h as some were removed for transfer in-between 40 h and 42 h.

M. Montag Placenta 2010

Frequency of embryo multinucleation detected by time-lapse system and its impact on pregnancy outcome

Cycle characteristics and pregnancy outcome.

Variable	Group 1 (n = 145)	Group 2 (n = 366)	*P* value
Antagonist cycles	143 (98.6)	360 (98.4)	.83
Total gonadotropin dose (IU)	2,443 ± 963	2,509 ± 1,114	.50
MII oocytes aspirated	10.9 ± 6.1	11 ± 5.6	.85
Single ETs	112 (77.2)	205 (66.1)	<.05[a]
Double ETs	33 (22.7)	161 (43.9)	
Positive ß-hCG	54 (37.2)	193 (52.7)	.002[a]
Clinical pregnancy rate	34 (23.4)	161 (44.0)	<.001[a]
Spontaneous abortion rate	4 (2.8)	13 (3.6)	.65
Biochemical pregnancies	16 (11)	19 (5.1)	.01[a]
Implantation rate (N = 686)	37/159 (23.3)	230/527 (43.6)	<.001[a]

Note: The data are presented as n (%) or mean ± SD from the mean.
[a] Statistically significant, $P < .05$, χ^2-test.

Group 1: Multinucleated at 2 cell

Group 2: Non Multinucleated at 2 cell

E Ergin Fertil Steril 2014

Results:

Detection of multinucleation within embryo check times proposed by ESHRE/ALPHA consensus.

Embryo check times by ESHRE/ALPHA consensus	No. of embryos with multinucleated blastomeres (n/N = 44/159)
22, 23, 24 h (post-ICSI)	7/44
25, 26, 27 h (post-ICSI)	30/44
43, 44, 45 h (post-ICSI)	15/44

E Ergin Fertil Steril 2014

How sould time-lapse systems be tested?

Trial design 1:

TLS vs CI

Assess the culture conditions

Use morphologic parameters for embryo selection (for both)

- *M. Cruz J Assist Reprod Genet 2011*
- *K. Kirkegaard J Assist Reprod Genet 2012*
- *H. Park Human Reprod 2015*

S. Amstrong Human Reprod 2015

How sould time-lapse systems be tested?

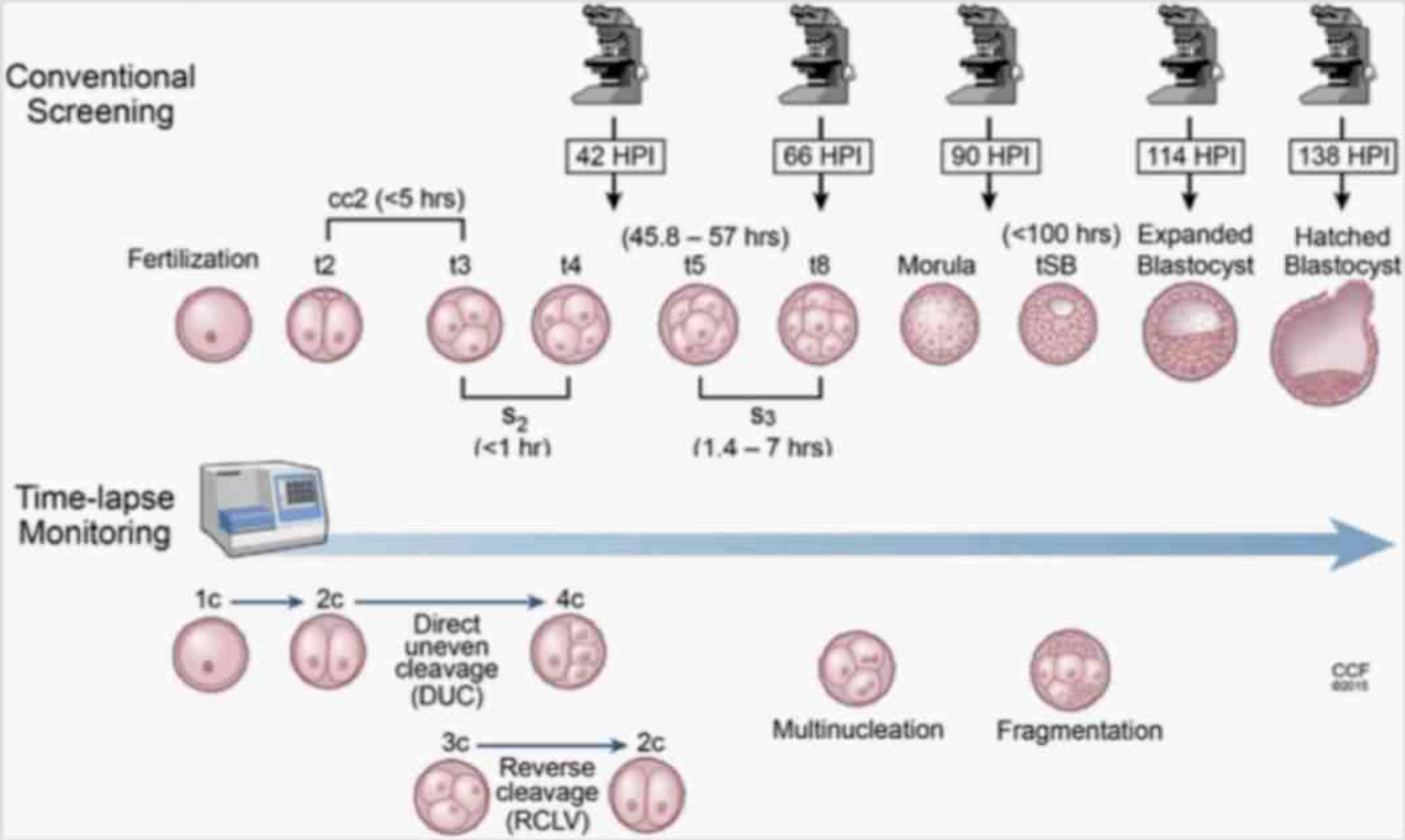
Trial design 2:

TLS cell-tracking algorithims vs routine morphology culturing in TLM systems

- *L. Goodman Fertil Steril 2016*

S. Amstrong Human Reprod 2015

Trial design 2:



Embryo growth and development over time. The top of the figure depicts the conventional screening standard viewing points ± 1 hour (HPI = hours after insemination). Time-lapse monitoring occurred continuously. Dysmorphisms that were evaluated are pictured at the bottom. Established time frames incorporated into the morphokinetic score (cc2, s2, t5, s3, and tSB) are shown.

Goodman. Time lapse–assisted embryo selection. Fertil Steril 2016.

L Goodman Fertil Steril 2016

Trial design 2:

Outcome results in cycles with selection, stratified by day of transfer and age.

Clinical outcome	TLM	CS	*P* value
All transfers (day 3 and 5)	n = 119	n = 116	
CPR	81/119 (68.1%)	73/116 (62.9%)	.41
IR	122/239 (51.0%)	100/221 (45.2%)	.21
All transfers, <40 y old	n = 110	n = 110	
CPR	79/110 (71.8%)	72/110 (65.5%)	.10
IR	119/211 (56.4%)	99/205 (48.3%)	.31
Blastocyst transfers	n = 91	n = 89	
CPR	67/91 (73.6%)	61/91 (67.0%)	.33
IR	96/173 (55.5%)	83/162 (51.2%)	.44
Pregnancy outcomes	n = 81	n = 73	.23
Viable singleton pregnancy	48 (59.3%)	48 (65.8%)	
Viable twin pregnancy	29 (35.8%)	21 (28.8%)	
Viable triplet pregnancy	2 (2.5%)	1 (1.4%)	
Spontaneous abortion	2 (2.5%)	3 (4.1%)	

Note: Patients with 1–3 embryos were excluded from analysis. CPR = clinical pregnancy rate; IR = implantation rate; other abbreviations as in Supplemental Table 1.

Goodman. Time lapse-assisted embryo selection. Fertil Steril 2016.

L Goodman Fertil Steril 2016

How sould time-lapse systems be tested?

Trial design 3:

TLS(cell-tracking) vs CI(morphologic assessment)

- *I. Rubio Fertil Steril 2014*

S. Amstrong Human Reprod 2015

Trial design 3:

Outcome results per intention to treat, per cycle, per transfers and per embryo transferred.

Outcome	TMS group	Control group	RR	*P* value
All cycles with oocyte retrieval	438	405		
Pregnancy (% of all treated cycles)	61.6 (56.9–66.0)	56.3 (51.4–61.0)	1.09 (0.98–1.23)	.12
Ongoing pregnancy (% of all treated cycles)	51.4 (46.7–56.0)	41.7 (37.0–46.6)	1.23 (1.06–1.43)	.005
All transfers	415	373		
Pregnancy (% of all transfers)	65.3 (60.6–69.7)	61.1 (56.1–65.9)	1.07 (0.95–1.19)	.22
Ongoing pregnancy (% of all transfers)	54.5 (49.6–59.2)	45.3 (40.3–50.4)	1.20 (1.04–1.39)	.01
All pregnant cycles	271	228		
Early pregnancy loss (% of all pregnancies)	16.6 (12.6–21.4)	25.8 (20.6–31.9)	0.64 (0.45–0.91)	.01
All transferred embryos	775	699		
Implantation rate (% of all transferred embryos)	44.9 (41.4–48.4)	37.1 (33.6–40.7)	1.43 (1.05–1.39)	.02

Note: Results shown as proportion with 95% confidence limits in brackets, relative risk (RR) with 95% confidence limits in brackets and the corresponding *P* value (Fisher's exact test). Total number of cycles are also presented in brackets.

Rubio. Clinical validation of EmbryoScope. Fertil Steril 2014.

I Rubio Fertil Steril 2014

Factors Affecting Embryo Morphokinetcs

- Culture medium
- Oxygen concentration in culture
- Patient population
- Ovarian stimulating protocols
- Hormon levels in the female partner
- Sperm DNA fragmentation

How can we choise the best embryos by using time-lapse ?

- Which morphokinetic criteria?
- Which cleavage timing?
- Which selection criteria?
- Which deselection criteria?
- Which embryo development day?
- Which time-lapse system?
- Which culture media?
- Which patient?

Conclusion

- More information about embryo development for qualitative morphological evatuation
- Using quantitative morphokinetic parameters to select best embryos
- Each lab that uses TLM should create their unique predictive model based on their own data.
- More prospective RCT's are needed to prove TLM's effectiveness

THANK YOU