PRE-CONGRESS COURSE 3

Time-lapse monitoring in the ART lab

Special Interest Group Embryology
Munich - Germany, 29 June 2014

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Time-lapse monitoring in the ART lab

Munich, Germany
29 June 2014

Organised by
The ESHRE Special Interest Group Embryology

Contents

Course coordinators

Maria José de los Santos (SIGE), Basak Balaban (Alpha) and Kersti Lundin (SIGE)

Course description

The aim of this basic course is to give an up-to-date, comprehensive and evidence-based overview of the use of time-lapse monitoring in the ART laboratory.

Target audience

Mainly embryologists, scientists and clinicians interested in embryo development and embryo selection.

Scientific programme

Chairmen: Maria Jose De Los Santos - Spain and Basak Balaban - Turkey

09:00 - 09:30 Time-lapse monitoring – the technique; possibilities and restrictions
Thorir Hardarson - Sweden
09:30 - 09:45 Discussion
09:45 - 10:15 Embryo morphokinetics: A predictive tool in the IVF laboratory?
Marcos Meseguer Escriva - Spain
10:15 - 10:30 Discussion

10:30 - 11:00 Coffee break

Chairmen: Kersti Lundin - Sweden and Carlos Plancha - Portugal

11:00 - 11:30 Effects of culture conditions on embryo morphokinetics
Kirstine Kirkegaard - Denmark
11:30 - 11:45 Discussion
11:45 - 12:15 What have we learned from morphokinetics about early human development: a comparison with classical scoring systems
Zsolt Peter Nagy - U.S.A.
12:15 - 12:30 Discussion

12:30 - 13:30 Lunch

Chairmen: Thorir Hardarson - Sweden and Zsolt Peter Nagy - U.S.A.

13:30 - 14:00 Expanding applications of live cell microscopy to the study of oogenesis and oocyte quality
Giovanni Coticchio - Italy
14:00 - 14:15 Discussion
14:15 - 14:45 The biology behind time-lapse monitoring of embryos: correlates of aneuploidy and gene expression
Shawn L Chavez - U.S.A.
14:45 - 15:00 Discussion

15:00 - 15:30 Coffee break

Chairmen: Giovanni Coticchio - Italy and Susanna Jamina Apter - Finland

15:30 - 16:00 An evidence-based evaluation of time-lapse monitoring in clinical embryology
Sjoerd Repping - The Netherlands
16:00 - 16:15 Discussion
16:15 - 16:45 Time-lapse monitoring of human embryos: A clinician's perspective
Charles Kingsland - United Kingdom
16:45 - 17:00 Discussion

17:00 - 17:30 Business meeting SIG Embryology

Time-lapse monitoring

The technique; possibilities and restrictions

- *Thorir Hardarson, PhD*
- *Fertility Centre Scandinavia, Gothenburg, Sweden*

Conflict of interest?

Our lab uses primo-vision time-lapse equipment

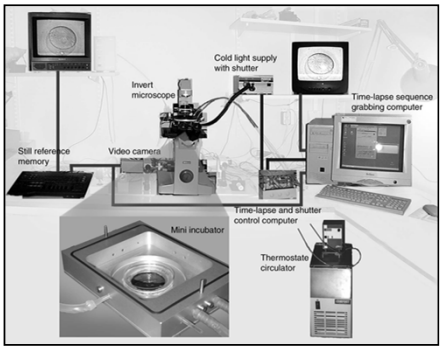
Learning objectives

1. History of time-lapse
2. Time-lapse – The possibilities ☺
3. Time-lapse – The restrictions ☹
4. Time-lapse – The future

History of time-lapse

- PubMed “Time-lapse” = 7900 publications
- Used in embryo research almost a century
- **First**: Lewis & Gregory, **1929** (*Science*, 69)
- **First in human**: Eriksson *et al*. 1981
- **Second**: Payne *et al*. 1997
- After 2000=> Many, ….too many?

Time-lapse setup in 1999



Epithelial injury of the eye[1365]

Recorded by Time-lapse

Thorir Hardarson
Charles Hanson
Ulf Stenevi

Time-lapse: the possibilities ☺

1. Detects and documents the dynamics of early embryo development
2. Educational – "A picture tells a 1000 words"
3. Freeing the IVF lab from time restrictions
4. Clinical – Use as a selection tool?

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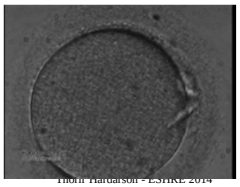
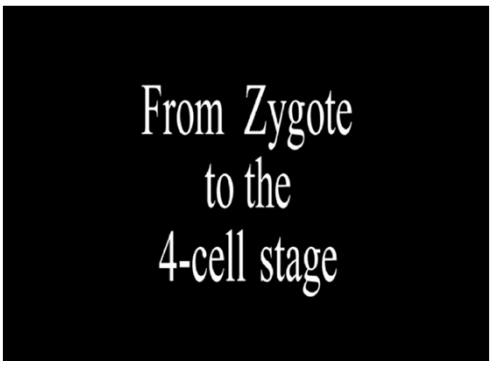
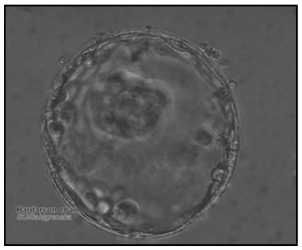
Discover



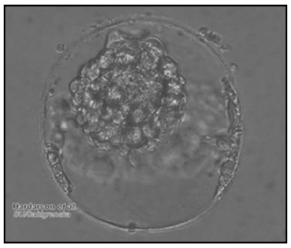
Educational



Blastocyst

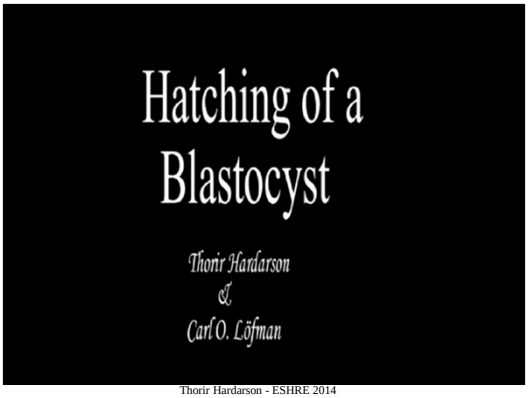


Blastocyst 3 min. later



Thorir Hardarson - ESHRE 2014

Educational



Thorir Hardarson - ESHRE 2014

Freeing the IVF lab from time restrictions

1. Able to perform ICSI anytime – that correlates with hCG and oocyte maturation
2. Short insemination, denudation in Std.IVF
3. Will not miss the fast and/or the late fertilizations

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Time-lapse: the restrictions ☹

1. Overbelieve! We have been there before!!
2. Embryo kinetics may be a blunt instrument to detect normal embryos (metabolically, chromosomally….)
3. Cost benefit – if it does not improve pregnancy rates then why invest?

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Proposed time-lapse measurements/definitions

Name of dev. event	Explanation
PN appearance	The first time-point where at least one PN is visible
PN abuttal	The time when no visible space is between the two PN
PN breakdown	The time-point where the PN´s are no longer visible
1st cytokinesis	Start of 1st cleavage marked by the appearance of the cleavage furrow
1-7 divisions (t2-t8)	When cell cleavage is finished. 2-cell stage (t2) etc…..
Duration 1-7 div	The duration of the first cleavages
1st and 2nd nuclei	The appearance and disappearance of the nuclei in the first 2 cells
Compaction	Timing of the initiation and completion of compaction
Blastulation	Timing of the initiation of blastulation (first blastocoel visible)
Full blastocyst	Timing when the blastocoel fills out most of the ZP
Hatching status	Timing of the initiation and completion of blastocyst hatching
Contractions	Frequency and duration of blastocyst contractions

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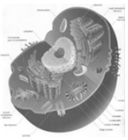
Cleavage rate



Cleavage rate = Rate of cell division

Cell division is the process by which a parent cell divides into two or more daughter cells*, i.e. mitosis.*

A human being's body experiences about 10,000 trillion cell divisions in a lifetime

Before division can occur, the chromosomes must be replicated, and the duplicated genome separated cleanly between cells.

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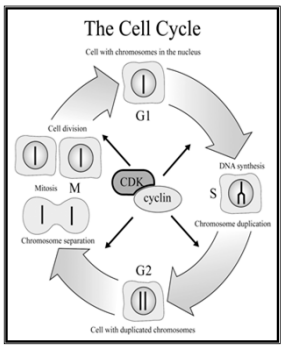


The Nobel Prize in Physiology or Medicine 2001

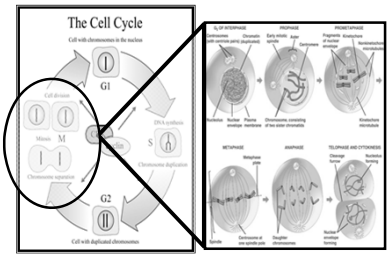
Leland H. Hartwell, Tim Hunt, Sir Paul Nurse

For their discoveries of "key regulators of the cell cycle"

Start-Genes
Checkpoints
CDK (Cyclin dependent kinase)
Cyclins, (proteins that regulate CDK)

Cell cycle control

Hundred of genes and macromolecules involved
Checkpoints of DNA damage and order of cell cycle events



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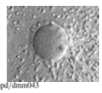
Checkpoints in oocytes



Human Reproduction Update, Vol.14, No.2 pp. 143–158, 2008
Advance Access publication December 14, 2007
doi:10.1093/humupd/dmm043

Meiosis in oocytes: predisposition to aneuploidy and its increased incidence with age

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High chromosomal aberration rates in the human oocytes

*Has evolution **favored** low fecundity in humans?*

Fertil. Steril. 2011

The relationship between blastocyst morphology, chromosomal abnormality, and embryo gender

Samer Alfarawati, M.S.[a,b] *Elpida Fragouli, Ph.D.,*[a,b] *Pere Colls, Ph.D.,*[c] *John Stevens, M.S.,*[d] *Cristina Gutiérrez-Mateo, Ph.D.,*[c] *William B. Schoolcraft, M.D.,*[d] *Mandy G. Katz-Jaffe, Ph.D.,*[d] *and Dagan Wells, Ph.D., F.R.C.Path.*[a,b]

Existing, but weak correlation between morphology and chromosomal abnormalities

Supports earlier findings at all developmental stages

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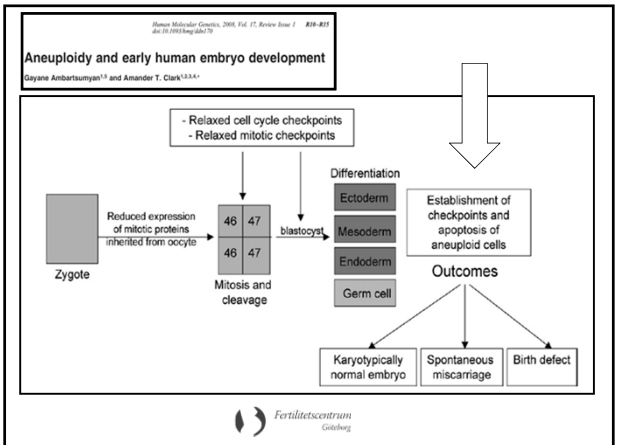

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Summarizing….

We have…

Checkpoints that in somatic cells keep the cells in check

These checkpoints work badly in humans gametes

Allows for oocytes and embryos that are aneuploid

A weak correlation between morphology and euploidy

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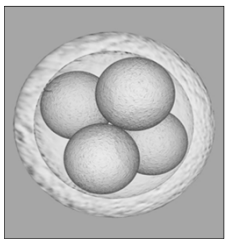
Study	n	Endpoint(s)	Predictive param.	Embryos source
Payne et al. 1997	30	Day 3 morphology	PB extrusion, PN appearance/abuttal	Fresh
Lemmen et al. 2008	19	Pregnancy	1st division and PN breakdown	IVF/ICSI
Lemmen et al. 2008	102	Day 2 blastomere number	1st division and PN breakdown	IVF/ICSI
Wong et al 2012	100	Blastocyst development & gene expr.	Dur. 1st cytokinesis & 2-3 cell stages.	Frozen/thawed surplus embryos.
Hashimoto et al. 2012	80	Blastocyst morphology/development	Timing 7/8 cell stage, duration of the 3-cell stage and 3rd cleavage	
Cruz et al. 2012	834	Blastocyst morphology/development	Tim 4-cell, dur. 3-cell, morula, dir to 3 cells	Donor ICSI
Dal Canto et al. 2012	459	Blastocyst development	All divisions and durations except 1st division	Surplus
Hlinka et al. 2012	180	Blastocyst development	Time intervals for cleavage and interphases	Fresh ICSI
Meseguer et al. 2011	247	Pregnancy, day 3 ET	Timing for div. up to 5-cells. Dur. 2/3 cell etc.	Don/fresh/ICSI
Azzarello et al. 2012	159	Live birth, day 2 ET		Fresh ICSI
Cruz et al. 2012	120	Pregnancy	None	Donor ICSI
Dal Canto et al. 2012	124	Pregnancy, day 2 & 5 ET	Time-point of the 8-cell stage	IVF/ICSI
Hlinka et al. 2012	114	Pregnancy, day 5 ET	Timings for cleavage and interphases	Fresh ICSI
Campbell et al. 2013	98	Blastocyst development & chr. status	Compaction/blastulation	Fresh

Why these different results?

Milieu:
- pH
- Temperature
- 0_2
- MEDIA
- Medication



Embryos:
- Fragmentation
- Multinucleation
- Metabolism
- Mosaicism!!

Patients:
- Maternal age
- Cause of infertility
- BMI?
- Previous cycles?

Time-lapse measurements:
10-20 minutes between frames
3 different systems

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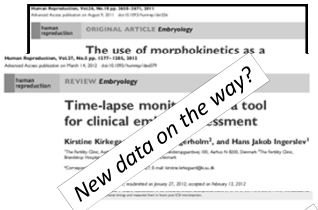
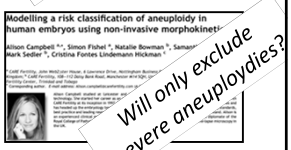
Time-lapse to select embryos?

Improving results?



Detect aneuploidy?



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CHALLANGE

To determine the optimal clevage timings

Cleavage patterns and strange divisions

Improve picture quality?

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Will time-lapse have a future in IVF?

ABSOLUTELY!

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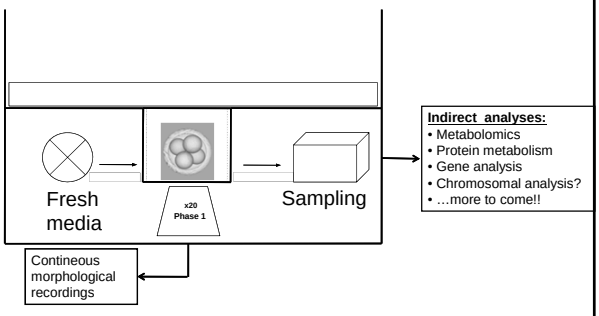
Time-lapse: The future?



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Thank you for your attention!

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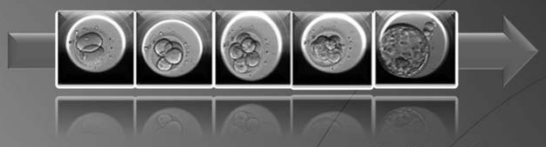
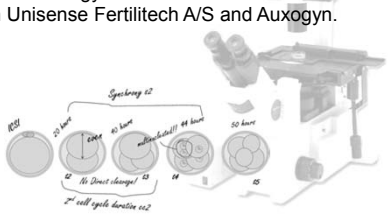
MORPHOKINETICS; A PREDICTIVE TOOL IN IVF?



Dr. Marcos Meseguer
Marcos.meseguer@ivi.es

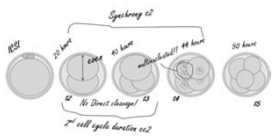
Disclosure

This work has not received any financial support from any commercial entity and the instrumentation, disposables and utensils belong to IVI. None of the authors have any economic affiliation with Unisense Fertilitech A/S nor Auxogyn but IVI is a minor shareholder in Unisense Fertilitech A/S and Auxogyn.



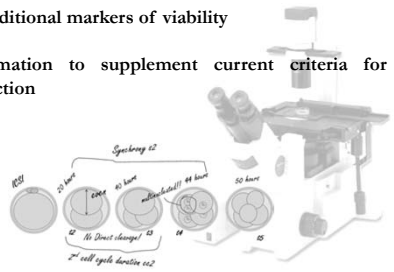
Learning Objectives

- ✓ Introduce the concepts of time-lapse and morphokinetics.
- ✓ Key findings of time-lapse research.
- ✓ Describe the existing algorithms of embryo selection.
- ✓ Introduction of a time-lapse system in a clinical setting; outcomes after combination of a new embryo culture and selection method.



Introduction

- ✓ Standard methods of embryo assessment:
  Subjective morphology evaluation.
- ✓ Search of additional markers of viability
- ✓ More information to supplement current criteria for embryo selection



PÁG.4

Time-Lapse

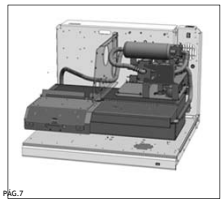
PÁG.5

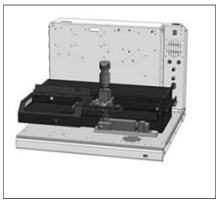
The main challenge of applying time-lapse microscopy in everyday IVF practice is:

- ✓ Ensuring embryo culture and light exposure **safety**
- ✓ Defining and validating **predictive** parameters using evidence-based data
- ✓ Processing abundant imaging data in **real-time**
- ✓ Fitting time-lapse instrumentation into current laboratory settings without significant **workflow** interruption and expense

PÁG.6

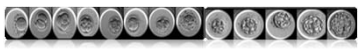
Time-lapse technology in a closed system

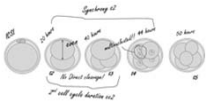




PAG.7

Key Findings of time-lapse research

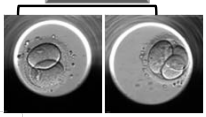


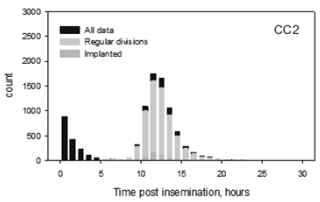


PAG.8

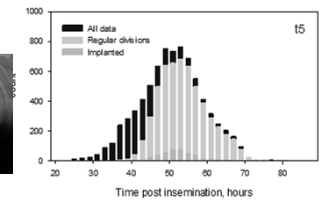
Time-Lapse Knowledge

cc2= t3-t2





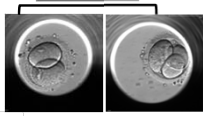
t5



PAG.9

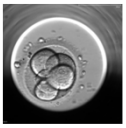
Time-Lapse Knowledge

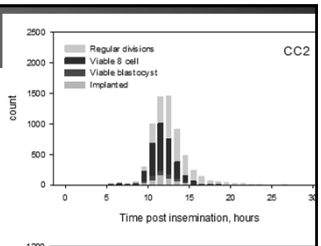
cc2= t3-t2

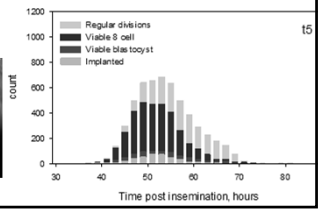


A time to look back: analysis of morphokinetic characteristics of human embryo development

t5







PÁG.10

Prediction of Implantation

The use of morphokinetics as a predictor of embryo implantation†

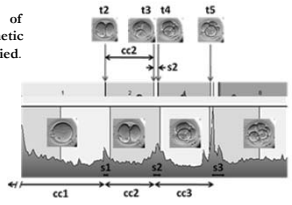
Marcos Meseguer[1,*], Javier Herrero[1], Alberto Tejera[1], Karen Marie Hilligsøe[2], Niels Birger Ramsing[2], and Jose Remohi[1]

[1]Instituto Valenciano de Infertilidad, Universidad de Valencia, Plaza de la Policía Local, 3, Valencia 46015, Spain [2]Unisense FertiliTech A/S, Aarhus, Denmark

*Correspondence address. Tel: +34-96-305-0988; Fax: +34-96-305-0999; E-mail: marcos.meseguer@ivi.es

Submitted on March 25, 2011; resubmitted on June 27, 2011; accepted on July 4, 2011

The precise timings of numerous morphokinetic parameters were identified.



PÁG.11

The use of morphokinetics as a predictor of embryo implantation

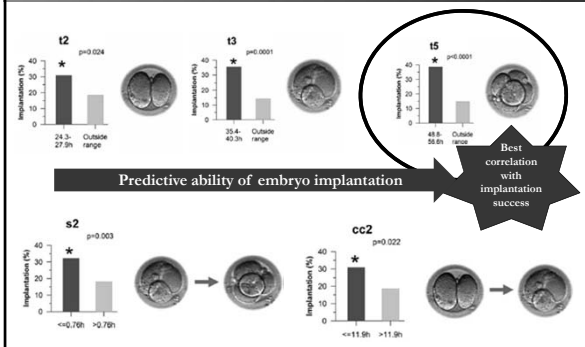
Table II Exact timing of the first deavages grouped in quartiles (Q1, Q2, Q3 and Q4) from 247 transferred embryos.

Parameter	Q1		Q2		Q3		Q4	
	Limit (h)	Implantation (%)	Limit (h)	Implantation (%)	Limit (h)	Implantation (%)	Limit (h)	Implantation (%)
t2	<24.3	23	**24.3–25.8**	**32**	**25.8–27.9**	**30**	>27.9	15
t3	<35.4	18	**35.4–37.8**	**39**	**37.8–40.3**	**32**	>40.3	11
t4	<36.4	23	**36.4–38.9**	**36**	**38.9–41.6**	**31**	>41.6	10
t5	<48.8	16	**48.8–52.3**	**37**	**52.3–56.6**	**40**	>56.6	14
cc2	**<11.0**	**23**	**11.0–11.9**	**39**	11.9–12.9	18	>12.9	19
s2	**<0.30**	**36**	**0.30–0.76**	**28**	0.76–1.50	20	>1.50	16

✓ Optimal ranges were established taking into account the two consecutive quartiles with the highest implantation probabilities in each category.

PÁG.12

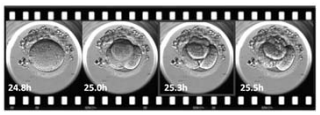
The use of morphokinetics as a predictor of embryo implantation



PÁG.13

Morphology dynamics; direct cleavage

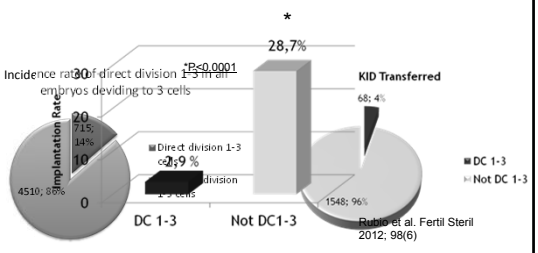
1 to 3 cells in less than 5 hours



cc2 < 5 h

PÁG.14

Clinical Multicenter Study

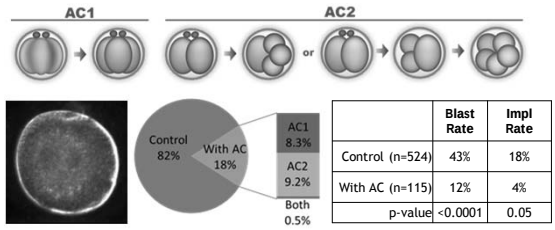


Rubio et al. Fertil Steril 2012; 98(6)

Exclusion criteria

PÁG.15

Abnormal Cleavage = Direct Cleavage confirmation by automatic time-lapse



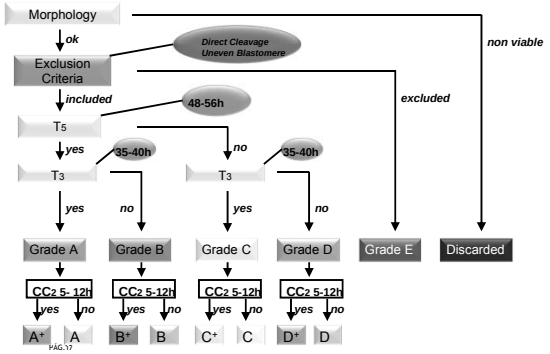
	Blast Rate	Impl Rate
Control (n=524)	43%	18%
With AC (n=115)	12%	4%
p-value	<0.0001	0.05

- **AC1 and AC2 embryos are often selected for Day 3 transfer** (28.6%)
- **AC embryos are often good quality** (46.9% 6-10 cells, ≤10% frag)
- **Morphology is unable to detect AC embryos**
- **Implantation Rate: 3.7%**

PAG.16

Athayde Wirka et al. *Fertil & Steril*, In Press

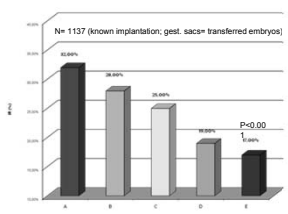
Resulting model



PAG.17

Algorithm: implantation

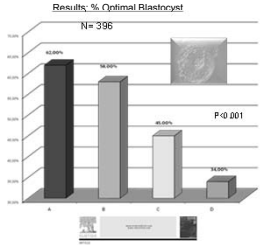
Results: 885 ICSI cycles



Embryo incubation and selection in a time-lapse monitoring system improves pregnancy outcome compared with a standard incubator: a retrospective cohort study

Algorithm: blastocyst

Results: % Optimal Blastocyst



Timing of cell division in human cleavage-stage embryos is linked with blastocyst formation and quality

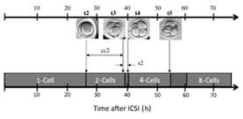
PAG.

Clinical validation through retrospective studies

Embryo Development: Implantation

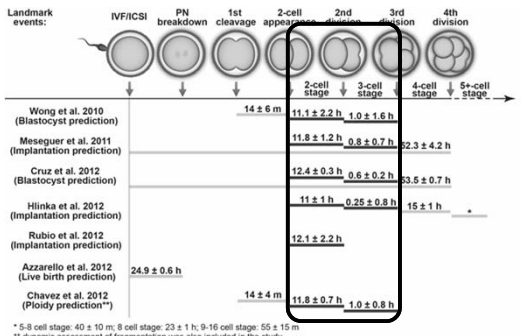
- **2011, Meseguer et al.** Hum Reprod: *Added ICSI to 5-cell*
- **2012, Hlinka et al.** Physiol Res: *Added cell cycles timings to 16-cell*
- **2012, Dal Canto et al.** Reprod Biomed Online: *Added cell cycles timings to 8-cell*
- **2012, Rubio et al.** Fertility & Sterility: *Suggested DC2-3 (P2) <5h should be excluded*
- **2012, Azarello et al.** Hum Reprod: *Suggested ICSI to PN breakdown <20.75h be excluded*

- **Embryo Development: Blast Quality**
- **2012, Hashimoto et al.** Fertility & Sterility
- **2012, Cruz et al.** Reprod Biomed Online



PÁG.19

Summary of early predictive time-lapse markers

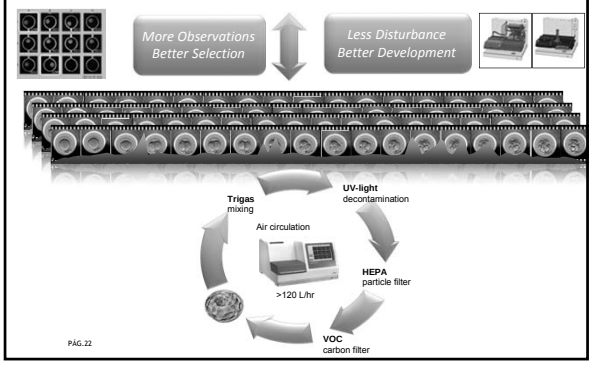


Chen et al. *Fertility & Sterility,* (2013)

Embryo incubation and selection

PÁG.21

Hypothesis



PÁG.22

New technology **VS** Conventional Incubator

Prospective Study:
Controlled study where patients are randomly distributed between both groups.

Retrospective Study:
Where patients were [illegible] between both groups before the study was designed.

FIRST APPROXIMATION

PÁG.23

Retrospective Study

Limitation:

✓Patients may not be evenly distributed between the 2 different incubators which can affect final conclusions.

Analyse for possible confounding factors:

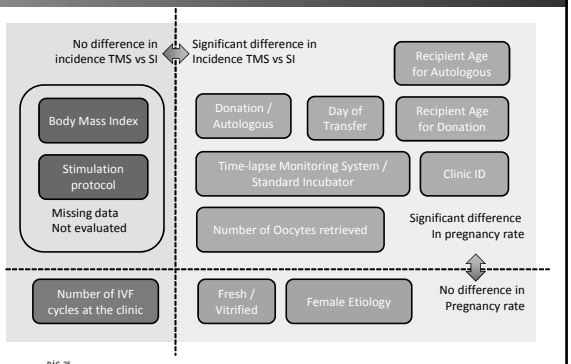
✓ Significantly influence pregnancy rates

✓Uneven distribution between incubators.

✓Type of incubator ✓Autologous ICSI/oocyte donation ✓ Tansference day ✓ Oocyte origin ✓ Nº of MII	✓Patient age ✓ Nº of prvious treatments ✓ Nº of embryos transferred ✓ IMCI ✓ Clinic

PÁG.24

Bias or Confounding factors analysis



PÁG.25

Odds Ratio for clinical pregnancy based on 7305 cycles

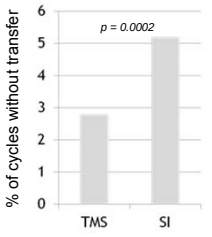
Factor	Comparison	Crude Estimate	Logistic regression model Estimate	Lower CL	Upper CL	P-value
Incubation	**TMS versus SI**	1.190	**1.201**	1.059	1.363	**0.0043**
Day of transfer	Day 5 versus Day 3	1.272	1.169	1.039	1.312	0.0092
Donation cycle	Donation versus autologous	1.786	1.921	1.674	2.205	0.0000
Per year of age	Per year less in autologous	1.057	1.100	1.080	1.121	0.0000
	Per year less in donation cycles	0.971	1.019	1.003	1.035	0.0194
Number of oocytes	Per oocyte less in autologous cycles	0.951	0.974	0.959	0.989	0.0005
	Per oocyte less in donation cycles	0.914	0.946	0.925	0.966	0.0000

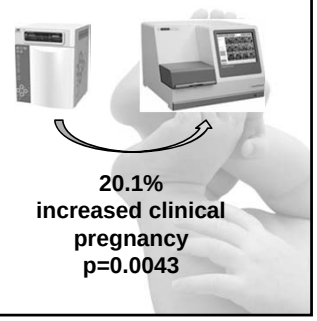
Odds Ratio for clinical pregnancy based on 6691 cycles with ET

Factor	Comparison	Crude Estimate	Logistic regression model Estimate	Lower CL	Upper CL	P-value
Incubation	**TMS versus SI**	1.141	**1.157**	1.018	1.315	**0.0254**
Day of transfer	Day 5 versus Day 3	1.598	1.612	1.419	1.832	0.0000
Donation cycle	Donation versus autologous	1.653	1.709	1.483	1.969	0.0000
Per year of age	Per year less in autologous	1.035	1.078	1.057	1.100	0.0000
	Per year less in donation cycles	0.979	1.013	0.997	1.03	*0.1227*
Number of oocytes	Per oocyte less in autologous cycles	0.958	0.992	0.976	1.007	*0.283*
	Per oocyte less in donation cycles	0.923	0.967	0.946	0.989	0.0033
Number of transferred	3 Embryos transferred versus 1	1.448	1.714	1.199	2.451	0.0031
	3 Embryos transferred versus 2	0.768	0.899	0.640	1.262	*0.5376*

Logistic Regression Analysis ; Clinical Impact

Significantly reduced cancellation rate





20.1% increased clinical pregnancy p=0.0043

PÁG.28

Randomized Control Trial

ClinicalTrials.gov
Protocol Registration System

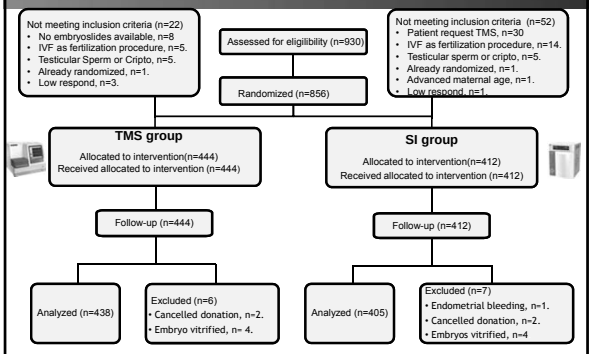
Sponsor:	Instituto Valenciano de Infertilidad, Spain
Collaborators:	
Information provided by (Responsible Party):	Marcos Meseguer, Instituto Valenciano de Infertilidad, Spain
ClinicalTrials.gov Identifier:	NCT01549262

PÁG.:

Eligibility Criteria

Inclusion Criteria	ICSI
MII	≥6
Age	20-38
Previous Cycles	≤2
BMI	18-25
Basal FSH	<12
AMH	>7 pmol/L
Exclusion	Uterine Pathologies
	Hidrosalpinx
	Recurrent Miscarriage
Male factor	< 1 mill progressive sperm (A+B)
	Endometriosis

Patient Flow Chart



PÁG.31

Characteristics of patients and IVF lab practise

	TMS GROUP(n=438)	CONTROL GROUP(n=404)	p
Age (Years)	34.7 (34.4-34.9)	34-6 (34.4-34.9)	NS
BMI (kg/m²)	23.2 (22.6-23.7)	23.04 (22.5-23.5)	NS
Long GnRH agonist (%)	14.8	16.4	NS
Short GnRH antagonist (%)	85.2	83.6	NS
Total dose of FSH	1781 (1709-1853)	1832 (1763-1900)	NS
Total dose of hMG	1127 (1035-1219)	890 (813-966)	<0.0001
E_2 on hCG day (pg/ml)	1981 (1882-2079)	1964 (1860-2067)	NS
P_4 on hCG day (ng/ml)	0.76 (0.72-0.80)	0.74 (0.70-0.78)	NS
Days of stimulation	13.0 (12.5-13.6)	13.2 (12.6-13.8)	NS
Oocyte donation (%)	47.1	49.1	NS
Metaphase II oocytes (n)	8.0 (7.76-8.26)	8.1 (7.8-8.3)	NS
Fertilization rate (%)	75.3 (73.8-76.9)	74.0 (72.3-75.7)	NS
Day3 ET (% of total)	72.5 %	75.5 %	NS

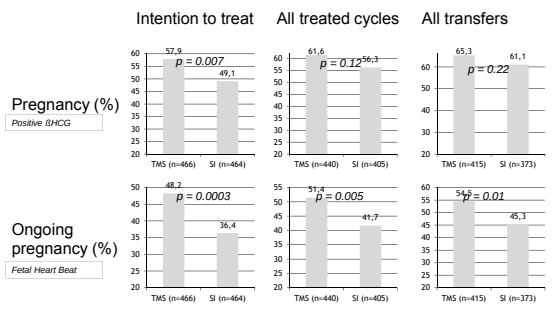
PÁG.32

Difference in cancellations

	TMS GROUP(n=438)	CONTROL GROUP(n=404)	*p*
Blastocyst rate (%)	27.5	24.5	NS
Embryo Fragmentation (%)	7.5 (7.2-7.9)	6.9 (6.5-7.1)	0.006
Number of Blastomeres	6.9 (6.8-6.9)	6.9 (6.8-7.0)	NS
Optimal Embryos (D3) (%)	46.2	43.1	0.010
Blastocyst rate (%)	52.3	50.5	NS
Optimal Blastocyst (D5) (%)	20.9	16.6	0.001
Transferred embryos (per treatment)	1.86 (1.8-1.9)	1.86 (1.8-1.9)	NS
Cryopreserved embryos (per treatment)	3.9 (3.6-4.1)	3.6 (3.4-3.9)	NS

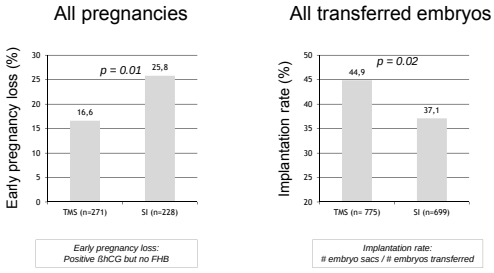
PÁG.33

Results per Outcome meassure



PÁG. 34

Results per Outcome meassure



PÁG. 35

Logistic Regression Analysis

Model effect	values	OR	p value
Incubation	TMS versus SI	1.41 (1.06-1.871)	0.017
Day of Transfer	Day 5 versus Day 3	1.76 (1.22-2.52)	0.002
Oocyte source	Autologous versus Donation	0.83 (0.60-1.14)	ns
Age	years	per year 0.99 (0.94-1.05)	ns

PÁG. 36

How morphology worked out?

Embryo Morphology category	Implantation [%]
I (n=72)	38.8
II (n=224)	35.7
III (n=146)	24.7
IV (n=27)	11.1
V (n=0)	-

PÁG. 37

How morphokinetics worked out?

Embryo category	N total [#]513	N implanted [#]	Implantation [%]	Embryo category	Implantation [%]
A+	122	74	60.6	A	52.9
A-	80	33	41.2		
B+	46	24	52.2	B	43.9
B-	36	12	33.3		
C+	67	30	44.8	C	39.5
C-	52	17	32.7		
D+	28	13	46.5	D	29.3
D-	30	4	13.3		
E	51	7	13.7	E	13.7

PÁG. 38

Conclusions of prospective study

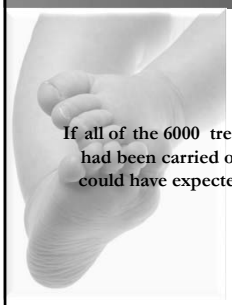
Incubation in the time-lapse incubator

- ✓Increases the number of optimal embryos on day 3 and day 5

..and in combination with a set of morphokinetic selection and deselection criteria

- ✓Increases implantation rate
- ✓ Increases ongoing pregnancy rate
- ✓ Decreases Early pregnancy loss

PÁG. 39



If all of the 6000 treatments in the conventional incubator had been carried out using Time-Lapse Incubator, we could have expected about 545 additional pregnancies.

References

- Basile N, Nogales MC, Bronet F, Florensa M, Riqueiros M, Rodrigo L, García-Velasco J, Meseguer M. Increasing the probability of selecting chromosomally normal embryos by time-lapse morphokinetics analysis. *Fertil Steril* 2014 In Press.
- Chen AA, Tan L, Suraj V, Reijo Pera R, Shen S. Biomarkers identified with time-lapse imaging: discovery, validation, and practical application. *Fertil Steril* 2013;**99**: 1035-1043.
- Cruz, M, Gadea B, Garrido N, Pedersen KS, Martinez M, Perez-Cano I, Munoz M, Meseguer M. Embryo quality, blastocyst and ongoing pregnancy rates in oocyte donation patients whose embryos were monitored by time-lapse imaging. *J Assist Reprod Genet* 2011;**28**: 569-573. Scott L. The biological basis of non-invasive strategies for selection of human oocytes and embryos. Human Reproduction Update. 2003; 9:237-49
- Aparicio B, Cruz M, Meseguer M. Is morphokinetic analysis the answer? Reprod Biomed Online. 2013 Dec;27(6):654-63.
- Herrero J and Meseguer M. Selection of high potential embryos using time-lapse imaging: the era of morphokinetics. Fertil Steril. 2013 Mar 15;99(4):1030-4
- Sundvall, L., Ingerslev, H.J., Knudsen, U.B., Kirkegaard, K.; Inter- and intra-observer variability of time-lapse annotations *Hum. Reprod.* 2013 28 (12): 3215-3221.
- Meseguer M, Herrero J, Tejera A, Hilligsøe KM, Ramsing NB, Remohí J. The use of morphokinetics as a predictor of embryo implantation. Hum Reprod. 2011 26:2658-71
- Meseguer M, Rubio I, Cruz M, Basile N, Marcos J, Requena A. Embryo incubation and selection in a time-lapse monitoring system improves pregnancy outcme compared with a standard incubator: a retrospective cohort study. Fertil Steril. 2012 Dec;98(6):1481-9
- Kirkegaard K, Hindkjaer JJ, Grøndahl ML, Kesmodel US, Ingerslev HJ. A randomized clinical trial comparing embryo culture in a conventional incubator with a time-lapse incubator. J Assist Reprod Genet. 2012;29:565-572.
- Nakahara T, Iwase A, Goto M, Harata T, Suzuki M, Ienaga M, et al. Evaluation of the safety of time-lapse observations for human embryos. J Assist Reprod Genet.

References

- Wong C, Loewke K, Bosser NL, Behr B, De Jonge CJ, Baer TM, Pera RAR. Non-invasive imaging of human embryos before embryonic genome activation predicts development to blastocyst stage. Nature Biotechnol. 2010;28:1115-1121
- Dal Canto M, Coticcio G, Renzini M, De Ponti E, Novara PV, Brambillasca F, Comi R, Fadini R. Cleavage kinetics analysis of human embryos predicts development to blastocyst and implantation. Reprod Biomed Online. 2012 Nov;25(5):474-80
- Kirkegaard K, Kesmodel US, Hindkjaer JJ, Ingerslev HJ. Time-lapse parameters as predictor of blastocyst development and pregnancy outcome in embryos from good prognosis patients: a prospective cohort study. Hum Reprod. 2013;28:2643-2651
- Kirkegaard, K., J.J. Hindkjaer, and H.J. Ingerslev, Effect of oxygen concentration on human embryo development evaluated by time-lapse monitoring. Fertil Steril, 2013. 99(3): 738-744
- Ciray HN, Aksoy T, Goktas C, Ozturk B, Bahceci M. Time-lapse evaluation of human embryo development in single versus sequential culture media-a sibling oocyte study. J Assist Reprod Genet. 2012 29(9): 891-900
- Fréour T, Dessolle L, Lammers J, Lattes S, Barrière P. Comparison of embryo morphokinetics after in vitro fertilization-intracytoplasmic sperm injection in smoking and nonsmoking women. Fertil Steril. 2013. 99(7): 1944-50

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PÁG.43

Thank you for your attention!

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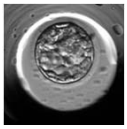
PÁG.44

Effect of culture conditions on embryo morphokinetics



Kirstine Kirkegaard MD, PhD
Aarhus University Hospital

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”I have no commercial relationsships or professional activities that pose a potential conflict of interest”

Aarhus University Hospital

Aims:

To understand how culture conditions and patient/treatment related factors influence timing of development and the implications for time-lapse as a diagnostic test

Objectives:

State the most import influencing factors

Explain the consequences for model building and interpretation of data

”…Up-to-date, comprehensive and evidence-based overview…”

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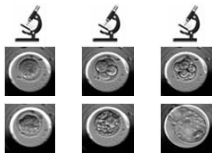
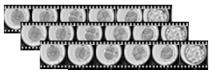
Morphology	**Time-lapse**
Limited information	Detailed information
Dependent on timing	Flexible
Subjective	Precise*)
Validated	Not validated





*Sundvall et al 2013

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Refined assessment

↓

Quality control?

↓

Improved selection?

↓

Improved treatment?

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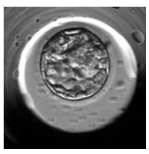
Embryo selection – strategies for building time-lapse models



While we wait for the RCTs…

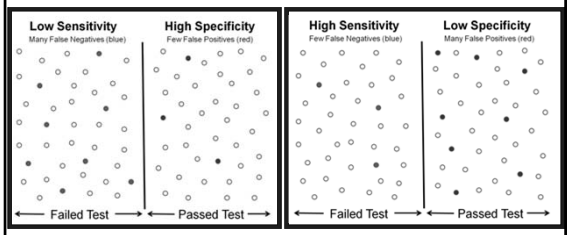
Diagnostic tests:

- Accuracy, precision, sensitivity and specificity

Interpretation of results

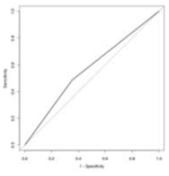
- What is normal?
- Analytical and biological variation

Sensitivity and specificity



	Implanted	Not implanted	Implantationrate
Usable*	n=131	n=445	22,7 %
Non-usable#	n=134	n=809	14.2%
Entire cohort	n=265	n=1254	17.4%

*P2 : 9.33-11.45 hours and P3: 0-1.73 hours.
#P2 outside 9.33-11.45 hours and P3> 0-1.73 hours.



Kirkegaard et al, 2014

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Precision

human reproduction ORIGINAL ARTICLE *Embryology*

Inter- and intra-observer variability of time-lapse annotations

Linda Sundvall[1,2,*], Hans Jakob Ingerslev[1,2], Ulla Breth Knudsen[1,2], and Kirstine Kirkegaard[1,2]

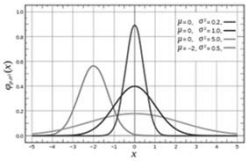
Aarhus University Hospital

Does one model fit all? - Establishing reference intervals

Laboratory/culture conditions
- IVF/ICSI
- Oxygen
- Culture media

Treatment ?

Patients (Age, diagnosis, BMI, hormones) ?



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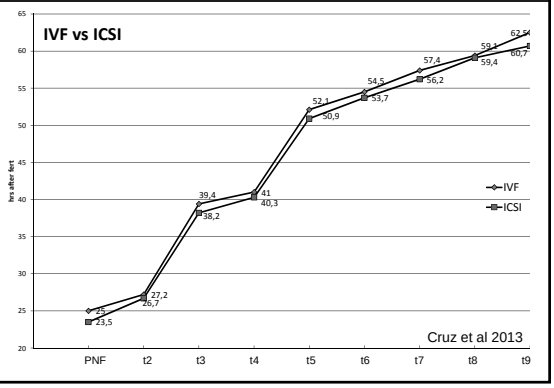
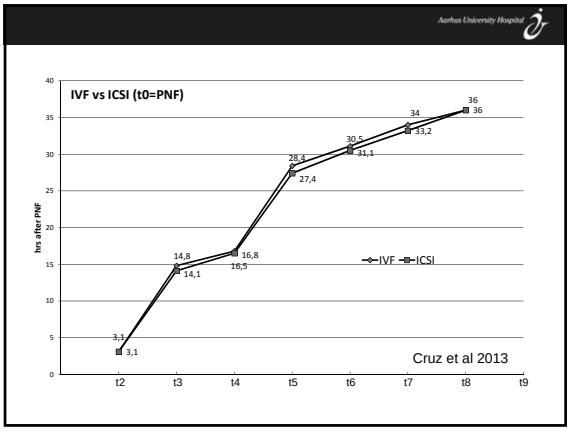
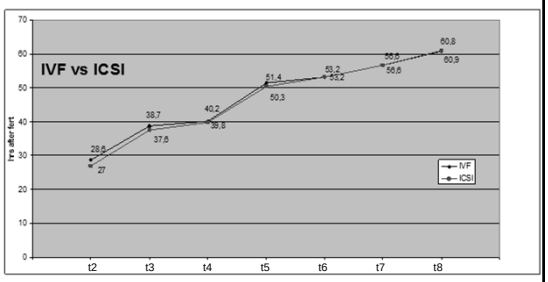
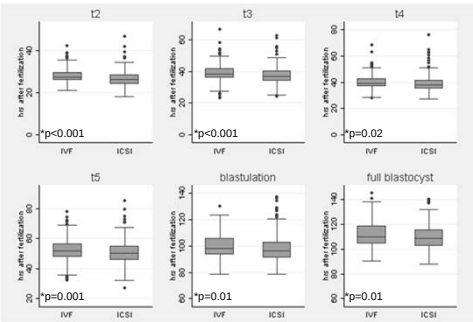






Dal Canto 2012

IVF vs ICSI



*Students t-test. Blastocysts only n_{IVF}=262 n_{ICSI}=375, unpublished data

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Hatching of in vitro fertilized human embryos is influenced by fertilization method

Kirstine Kirkegaard, M.D., Johnny Juhl Hindkjaer, M.Sc., and Hans Jakob Ingerslev, D.M.Sc.
Fertility Clinic, Aarhus University Hospital, Aarhus, Denmark

TABLE 2

Fertilization method according to type of hatching.

	IVF (n = 165)	ICSI (n = 215)
Type 1	92 (55.8%)	210 (97.7%)
Type 2	45 (27.3%)	3 (1.4%)
Type 1 + 2	28 (17.0%)	2 (1.0%)

Note: P value testing the hypothesis of no relation between fertilization method and type of hatching (Fisher exact test): < .001. Percentages are reported for columns. Abbreviations as in Table 1.

Kirkegaard. In vitro hatching of human embryos. Fertil Steril 2013.

TABLE 3

Completed hatching versus fertilization method and type of hatching.

	Total	Completed	Not completed
Type 1	302	3 (1.0%)	299 (99.0%)
Type 2	48	31 (64.6%)	17 (35.4%)
Type 1 + 2	30	19 (63.3%)	11 (36.6%)
IVF	165	50 (30.3%)	115 (69.6%)
ICSI	215	3 (1.4%)	212 (98.6%)

Note: P value testing the hypothesis of no relation between type of hatching: < .001; and fertilization method: < .001. Percentages are reported for rows. Abbreviations as in Table 1.

Kirkegaard. In vitro hatching of human embryos. Fertil Steril 2013.

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Wale and Gardner 2010

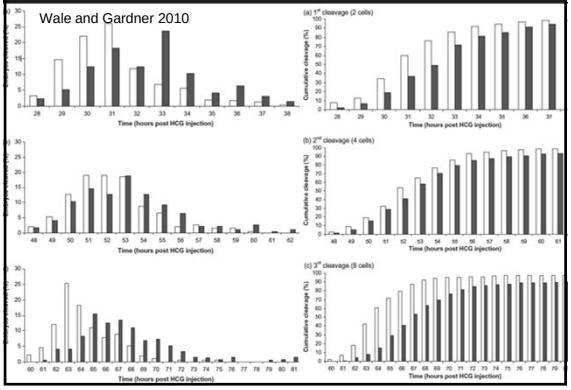

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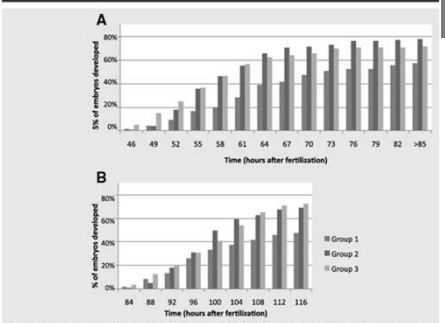
FIGURE 1

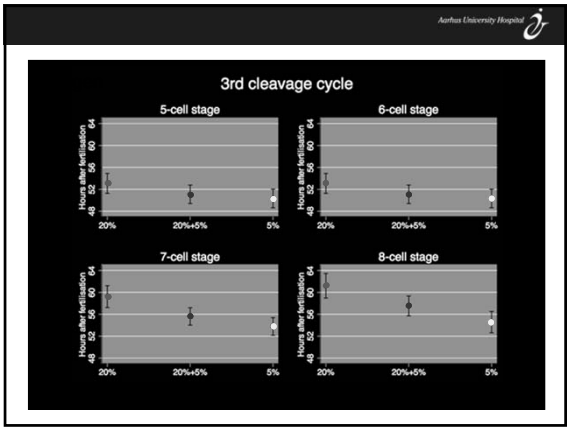


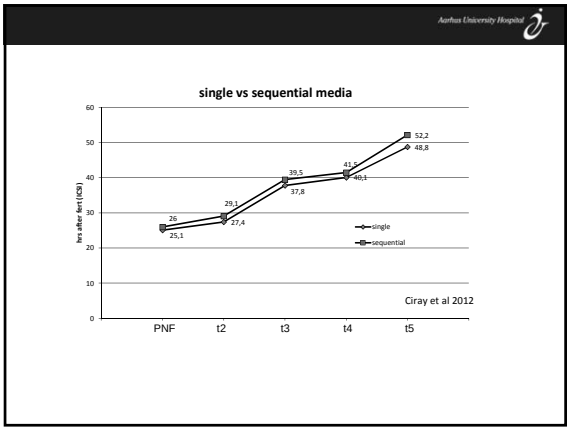
The cumulative development of embryos progressing through (A) third cleavage cycle and (B) early blastocyst stage.

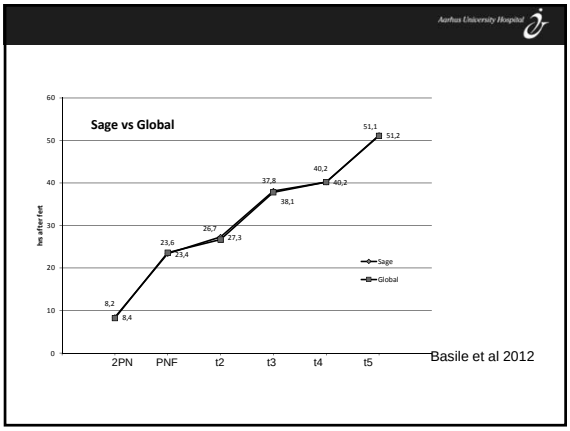
Kirkegaard. Temporal effect of O_2 on human embryos. Fertil Steril 2013.

Oxygen-Human embryos

- 20% O_2
- 5%+20% O_2
- 5% O_2


3rd cleavage cycle
5-cell stage
6-cell stage
7-cell stage
8-cell stage
Hours after fertilisation
48
52
56
60
64
20%
20%+5%
5%


single vs sequential media
hrs after fert (ICSI)
0
10
20
30
40
50
60
26
25,1
29,1
27,4
39,5
37,8
41,5
40,1
52,2
48,8
single
sequential
PNF
t2
t3
t4
t5
Ciray et al 2012


Sage vs Global
hrs after fert
0
10
20
30
40
50
60
8,2
8,4
23,6
23,4
26,7
27,3
37,8
38,1
40,2
40,2
51,1
51,2
Sage
Global
2PN
PNF
t2
t3
t4
t5
Basile et al 2012

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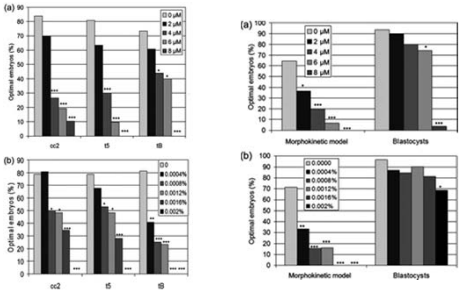
Response to toxicity



Percentage of embryos meeting optimal criteria (<95% CI of controls) in response to (**a**) CH or (**b**) Triton X-100. *P < 0.05, **P < 0.01, ***P < 0.001 versus the control ($\chi 2$). Wolff et al 2013

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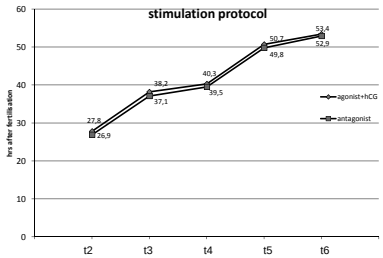

Munoz et al 2012 + 2013

+FSH dose

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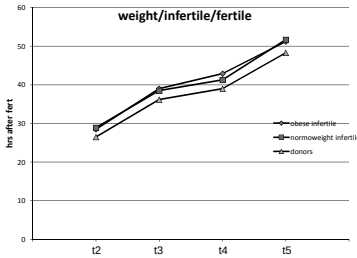
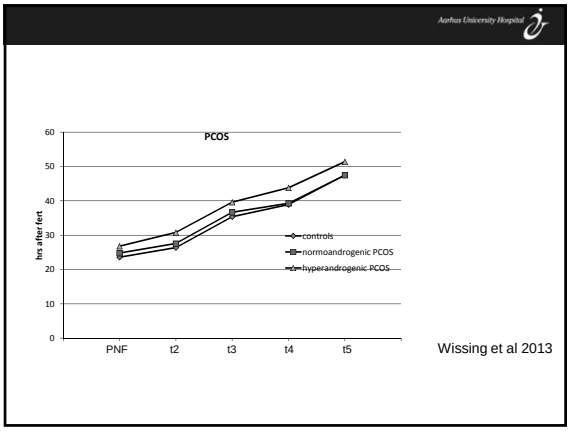
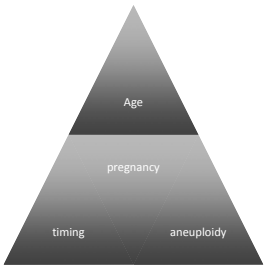


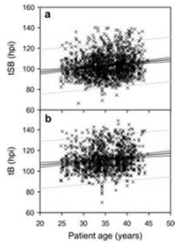
group	age
Obese infertile	34.7 (SD 3.4)
Normo weight infertile	33.3 (SD 2.9)
Donors	27.1 (SD 4.2)

Bellver et al 2013



Wissing et al 2013





Age, aneuploidy and tSB

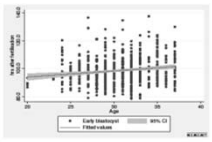
Campbell et al 2014

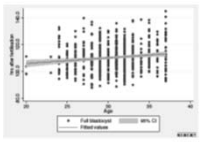
Table 1 Logistic regression analysis of the combined effect of t_{SB} and age on aneuploidy.

Model effect	Estimate (logOR)	Significance of additional effect	AIC* if removing single effect (full model = 238)
Intercept	−11 (3)	<0.0001	
Age (continuous)	0.16 (0.04)	<0.0001	254
t_{SB} (continuous)	0.05 (0.02)	0.016	242

*AIC = Akaike information criterion.

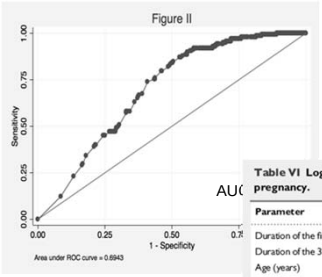
Age and blastocyst timing





	Regression coefficient (95% CI)	P-value	Coefficient of determination (R^2)
Early blast	0.44 (0.16; 0.71)	0.002	0.02
Full blast	0,42 (0.10;0.74)	0.01	0.02

Clustered linear regression, n=653, npt=149



Prediction of blastocyst formation vs. pregnancy

AUC

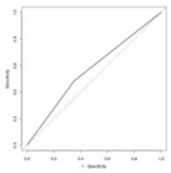
Table VI Logistic regression analysis of predictors of pregnancy.

Parameter	OR (95% CI)	P-value
Duration of the first cytokinesis (h)	0.84 (0.45; 1.57)	0.59
Duration of the 3-cell stage (h)	0.84 (0.59; 1.22)	0.36
Age (years)	0.84 (0.73; 0.98)	0.03
Number of previous cycles	1.2 (0.62; 2.4)	0.56
Number of GQE on Day 2	1.0 (0.78; 1.3)	0.98
Number of GQE on Day 3	1.1 (0.83; 1.4)	0.57
Total FSH dose(100 IU)	0.99 (0.93; 1.1)	0.82
Cause of infertility (categorical)	0.34 (0.05; 2.2)	0.25

Kirkegaard et al, 2013

Implications

Transferability
Interpretation of studies
Modelbuilding
Predictive power



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Thoughts on modelbuilding

Sensitivity/specificity

Population

Confounders

- Oxygen
- Fertilisation method
- Age
- ??

Type of model

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References

BASILE, N., MORBECK, D., GARCIA-VELASCO, J., BRONET, F. and MESEGUER, M., 2013. Type of culture media does not affect embryo kinetics: a time-lapse analysis of sibling oocytes. *Human reproduction* **28**(3), pp. 634-641

BELLVER, J., MIFSUD, A., GRAU, N., PRIVITERA, L. and MESEGUER, M., 2013. Similar morphokinetic patterns in embryos derived from obese and normoweight infertile women: a time-lapse study. *Human reproduction* **28**, pp. 794-800.

CAMPBELL, A., FISHEL, S. and LAEGDSMAND, M., 2013. Aneuploidy is a key causal factor of delays in blastulation: author response to 'A cautionary note against aneuploidy risk assessment using time-lapse imaging'. *Reproductive biomedicine online*.

CIRAY, H.N., AKSOY, T., GOKTAS, C., OZTURK, B. and BAHCECI, M., 2012. Time-lapse evaluation of human embryo development in single versus sequential culture media-a sibling oocyte study. J assist reprod Genet **29**, pp. 891-900.

CRUZ, M., GARRIDO, N., GADEA, B., MUNOZ, M., PEREZ-CANO, I. and MESEGUER, M., 2013. Oocyte insemination techniques are related to alterations of embryo developmental timing in an oocyte donation model. *Reproductive biomedicine online*, **27**(4), pp. 367-375.

DAL CANTO, M., COTICCHIO, G., MIGNINI RENZINI, M., DE PONTI, E., NOVARA, P.V., BRAMBILLASCA, F., COMI, R. and FADINI, R., 2012. Cleavage kinetics analysis of human embryos predicts development to blastocyst and implantation. *Reproductive biomedicine online* **25**, pp. 474-80.

KIRKEGAARD, K., HINDKJAER, J.J. and INGERSLEV, H.J., 2013. Hatching of in vitro fertilized human embryos is influenced by fertilization method. *Fertil Steril*, **99**(3), pp. 738-744

KIRKEGAARD, K., HINDKJAER, J.J. and INGERSLEV, H.J., 2012. Effect of oxygen concentration on human embryo development evaluated by time-lapse monitoring. *Fertil Steril*, **100**(5), 1277-1282

KIRKEGAARD, K., KESMODEL, U.S., HINDKJAER, J.J. and INGERSLEV, H.J., 2013. Time-lapse parameters as predictors of blastocyst development and pregnancy outcome in embryos from good prognosis patients: a prospective cohort study. *Human reproduction* **28**, pp. 2643-51.

KIRKEGAARD, K., CAMPBELL, A., AGERHOLM, A., BENTIN-LEY, U., GABRIELSEN, A., KIRK, J., SAYED, S. and INGERSLEV H.J.2014: Limitations of a time-lapse blastocyst prediction model - demonstrated by a large multicentre outcome analysis. *Reproductive biomedicine online*, accepted

MUNOZ, M., CRUZ, M., HUMAIDAN, P., GARRIDO, N., PEREZ-CANO, I. and MESEGUER, M., 2013. The type of GnRH analogue used during controlled ovarian stimulation influences early embryo developmental kinetics: a time-lapse study. *European journal of obstetrics, gynecology, and reproductive biology* **168**, pp. 167-72.

MUNOZ, M., CRUZ, M., HUMAIDAN, P., GARRIDO, N., PEREZ-CANO, I. and MESEGUER, M., 2012. Dose of recombinant FSH and oestradiol concentration on day of HCG affect embryo development kinetics. *Reproductive biomedicine online* **25**, pp. 382-9.

SUNDVALL, L., INGERSLEV, H.J., BRETH KNUDSEN, U. and KIRKEGAARD, K., 2013. Inter- and intra-observer variability of time-lapse annotations. *Human reproduction*, **28**(12), pp. 3215-3221.

WALE, P.L. and GARDNER, D.K., 2010. Time-lapse analysis of mouse embryo development in oxygen gradients. *Reproductive biomedicine online* **21**, pp. 402-10.

WISSING, M.L., BJERGE, M.R., OLESEN, A.I.G., HOEST, T. and MIKKELSEN, A.L., 2013. *Impact of PCOS on early embryo cleavage kinetics. Reproductive biomedicine online, (ePub ahead of print)*

WOLFF, H.S., FREDRICKSON, J.R., WALKER, D.L. and MORBECK, D.E., 2013. Advances in quality control: mouse embryo morphokinetics are sensitive markers of in vitro stress. *Human Reproduction* **28**, pp. 1776-82.

What have we learned from morphokinetics about early human development: a comparison with classical scoring systems

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Disclosure

MERCK MSD	- Speaker's Bureau
Origio	- Advisory Board
Fertilitech	- Advisory Board
MEB	- Stock holder

Learning Objectives

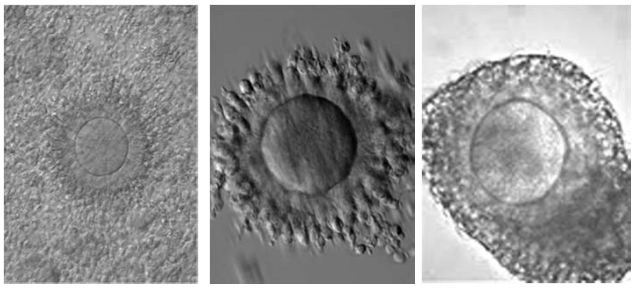
- To review traditional embryo assessment and principles of scoring systems
- To appraise outcomes achieved with current tools
- To describe potential benefits of morphokinetics
- To summarize findings / Conclusions

Traditional assessment

Day 0		Aspect of cumulus-corona complex and oocyte maturity and morphology
Day 1		Oocyte quality: zona, cytoplasm, PB's 2PN Assessments - Z scoring
Day 2	 	Early cleavage/ Multinucleation
Day 3		Genetic screen (PGD); metabolic evaluation, morphologic evaluation; extended culture decision
Day 4		?
Day 5		Selection blastocyst for transfer, embryo cyopreservation, PGD

Cumulus–corona-oocyte complex

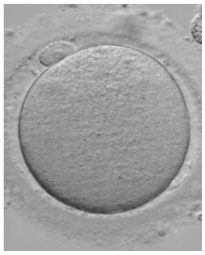
Assessment of human oocyte developmental competence by cumulus cell morphology…(Sato et al., Reproductive BioMedicine Online, Volume 14, Number 1, January 2007 , pp. 49-56(8))



An Atlas of Human Oocyte and Embryo Morphology by L. Veeck – Parthenon Publishing 1986

Oocyte's Morphological Characteristics

A. Cumulus-enclosed oocyte (cumulus score)
B. Oocyte maturation stage
C. Oocyte size and shape
D. Cytoplasmic features
  D.1 Ooplasm
  D.2 Metaphase plate
E. Extracytoplasmic features
  E.1 Zona pellucida
  E.2 Perivitelline space
  E.3 Polar body



Early Stage (day-1) Morphological Characteristics

Evaluation of the pronuclear-stage oocyte

1. Number of pronuclei

0 Not fertilized
1 Parthenogenetic activation (or asynchronous development)
2 Normal fertilization
3+ Abnormal fertilization

2. Size of pronuclei (in case of 2 PN fertilization)

Optimal Normal Size and Equal Size
Suboptimal Larger/Smaller Size and/or Unequal Size

3. Nucleoli (in case of 2 PN fertilization)

Number of nucleoli – in each pronuclei
Size of nucleoli (small or medium or large – in each of the two pronuclei)
Polarization of nucleoli
1, POLARIZED
2, non-POLARIZED

Nagy, RBA

Early Stage (day-1) Morphological Characteristics

Evaluation of the pronuclear-stage oocyte

4. Aspect of Polar Bodies

1, Single non-fragmented Polar Body
2, Single fragmented Polar Body
3, Two Polar Bodies (fragmented or non-fragmented)

5. Distance between the Polar Bodies (in case of the presence of 2 PB)

Optimal Close
Suboptimal Distant

6. Concentration of cytoplasmic organelles

For each oocyte the % of cytoplasmic organelles concentration/oocyte diameter (%C) is estimated
(usually this is between 10% and 20%)

7. Aspect of the zygote cytoplasm

Suboptimal presence of vacuoles
Suboptimal presence of the refractal body

Nagy, RBA

Human Pronuclei as a Mode of Predicting Viability

Table III. Implantation and pregnancy rates in patients grouped according to corrected embryo score (CS ⩾15; CS ⩽14)

	⩾15	⩽14
Mean CS (SD)	19.7 (3.4)	11.2 (4.1)
Mean age (SD)	32.4 (3.7)	35.3 (3.8)
No. retrievals	48	49
No. of embryo transfers	48	44
No. mature oocytes	480	439
Fertilization rate (SD %)	78 (3.2)	71 (3.8)
No. embryos transferred	175	178
Mean (SD)	3.7 (1.3)	4.6 (2.5)
No. clinical pregnancies (%)	34 (71)	4 (8)
No. ongoing/born (%)	31 (65)	1 (2)
No. implantations (%)	49 (28)	4 (2)
No. ongoing/born (%)	41 (23)	1 (1)
Multiple pregnancies (%)	10/34 (29)	0
Delivery rate/OR (%)	65	0

The successful use of pronuclear embryo transfers the day following oocyte retrieval. LA Scott and S Smith *Human Reproduction, 1998, 1003-1013*

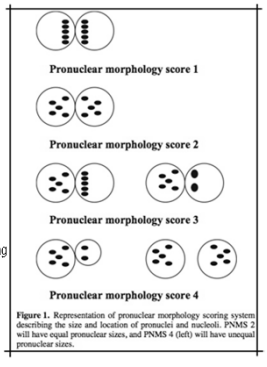


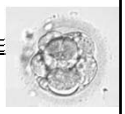
Figure 1. Representation of pronuclear morphology scoring system describing the size and location of pronuclei and nucleoli. PNMS 2 will have equal pronuclear sizes, and PNMS 4 (left) will have unequal pronuclear sizes.

The limited importance of pronuclear scoring of human zygotes James et al., *Human Reproduction 2006 21(6):1599-1604*

Nagy et al. Fertil Steril. 2003 Jul;80(1):67-74.

Early Stage (day-2/3) Morphological Characteristics

EMBRYO EVALUATION CRITERIA — RELATIVE IMPORTANCE



1. Number of blastomeres — **STRONG**

	Day 2	Day 3
Optimal	4-(6) cells	7-8 cells
Medium	3 cells	5-6 cells
Poor	2 cells	2-4 cells

2. Dimension of blastomeres – Day 2 / Day 3 — **MODERATE/STRONG**

Optimal similar or equal size blastomeres
Suboptimal different size blastomeres

3. Proportion of anucleate fragments – Day 2 / Day 3 — **STRONG**

Optimal between 0-10%
Good between 10-30%
Medium between 30-50%
Poor more than 50%

Nagy, RBA

Early Stage (day-2/3) Morphological Characteristics

EMBRYO EVALUATION CRITERIA — RELATIVE IMPORTANCE

4. Quality of the cytoplasm — **MODERATELY**

Optimal: normal appearance
Suboptimal: presence of cytoplasm abnormalities (granulated, vacuoles, refractile bodies)

5. Multinucleation of blastomeres — **MODERATELY**

Easier to evaluate at the 2-4 cells stage.
Optimal Number of cells with a single nucleus
Suboptimal Number of cells with multinucleation
Poor Number of cells with multinucleation

6. Early cell compaction — **MODERATELY**

Cells compaction start after 8-10 cell stage embryo (end of day 3 or day 4)
Optimal Cell compaction is not observed (or not strong) until the end of day 3
Suboptimal Cell compaction is strongly present late day 2 or early day 3

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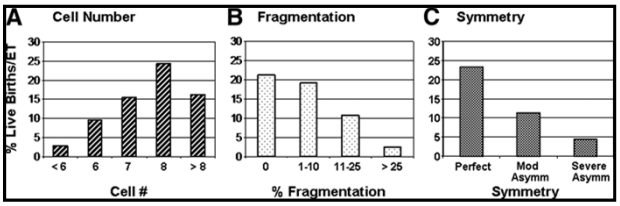
Associations among day 3 cell number, fragmentation and blastomere asymmetry, and live birth rate



Relationship between live birth rate (per embryo transferred) and (**A**) cell number, *P*<.0001; (**B**) fragmentation, *P*<.0001; and (**C**) blastomere symmetry, *P*<.001 – 7528 transfer cycles

Racowsky et al., FertilSteril. 2011 May;95(6):1985-9.

Consensus scoring system for cleavage stage embryos

Grade	*Rating*	*Description*
1	**Good**	<10% Fragmentation Stage-specific cell size No multinucleation
2	**Fair**	10–25% Fragmentation Stage-specific cell size for majority of cells No evidence of multinucleation
3	**Poor**	Severe fragmentation (>25%) Cell-size not stage-specific Evidence of multinucleation

ALPHA Scientists In Reproductive Medicine; ESHRE Special Interest Group Embryology. Reprod Biomed Online. 2011 Jun;22(6):632-46.

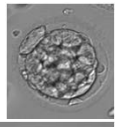
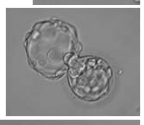
Blastocyst Stage (day-5/6) Morphological Characteristics

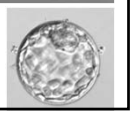
Classification of Blastocyst Development

Stage A - Hatched blastocyst
Stage B - Hatching blastocyst
Stage C - Fully Expanded
- Thin zona p.
- Distinct ICM
- Distinct Trophectoderm

Stage D - A single cavity occupying >50% of the volume of the embryo
- Regular zona p.
- The ICM and trophectoderm may not be clear

Stage E - A distinct single cavity 25- 50% of the volume of the embryo.
- The diameter of the embryo is unchanged.
- Zona is unchanged



Classification of Blastocyst Quality

ICM
Grade 1 - Tightly packed , many cells
Grade 2 - Loosely grouped, several cells
Grade 3 - Very few cells

Trophoblast
Grade 1 - Many cells forming a cohesive epithelium
Grade 2 - Few cells forming a loose epithelium
Grade 3 - Very few cells

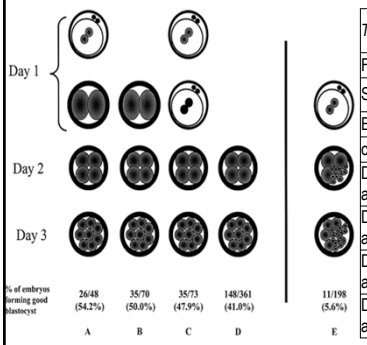
Nagy, RBA

Consensus scoring system for blastocysts

	Grade	*Rating*	*Description*
Stage of development	**1**		Early
	2		Blastocyst
	3		Expanded
	4		Hatched/hatching
Inner cell mass	**1**	Good	Prominent, easily discernible, with many cells that are compacted and tightly adhered together
	2	Fair	Easily discernible, with many cells that are loosely grouped together
	3	Poor	Difficult to discern, with few cells
Trophectoderm	**1**	Good	Many cells forming a cohesive epithelium
	2	Fair	Few cells forming a loose epithelium
	3	Poor	Very few cells

ALPHA Scientists In Reproductive Medicine; ESHRE Special Interest Group Embryology. Reprod Biomed Online. 2011 Jun;22(6):632-46.

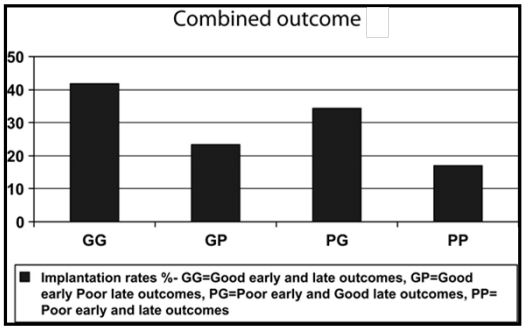
Cumulative Scoring to Maximize Prediction
and recommended time-line of observations



Type of observation	*Timing (hours post insemination)*
Fertilization check	17±1
Syngamy check	23±1
Early cleavage	26±1 post ICSI
check	28±1 post IVF
Day-2 embryo assessment	44±1
Day-3 embryo assessment	68±1
Day-4 embryo assessment	92±2
Day-5 embryo assessment	116±2

Neuber E et al. Hum. Reprod. 2003;18:1307-1312
ALPHA Scientists In Reproductive Medicine; ESHRE Special Interest Group Embryology. Reprod Biomed Online. 2011 Jun;22(6):632-46.

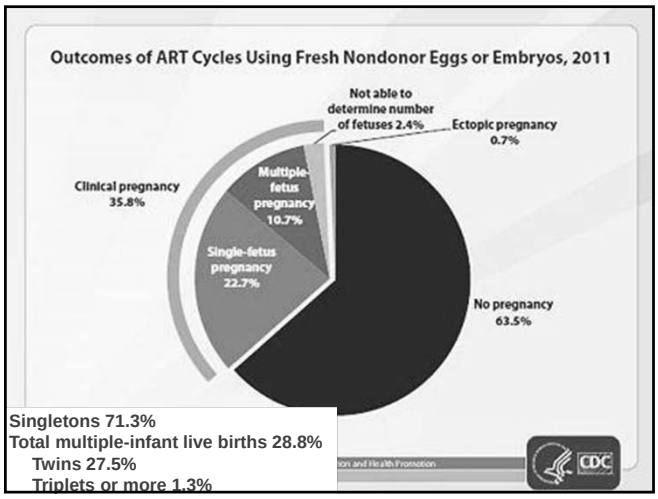
Implantation outcomes in 426 fresh IVF day 3 transfers of 852 embryos in women <36 years



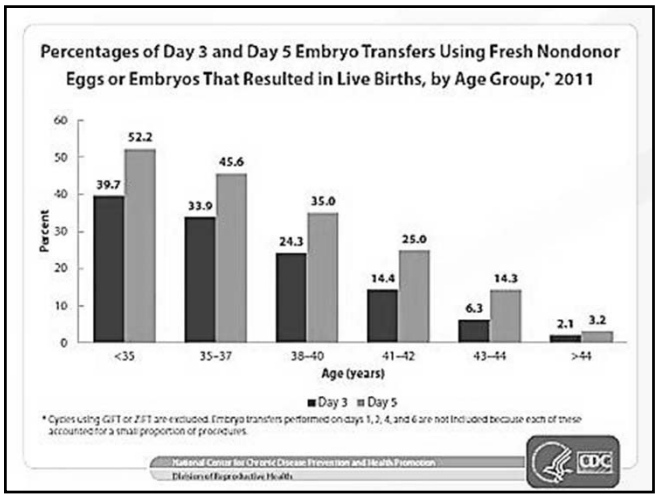
Weitzman et al., Fertil Steril. 2010 Feb;93(2):658-62

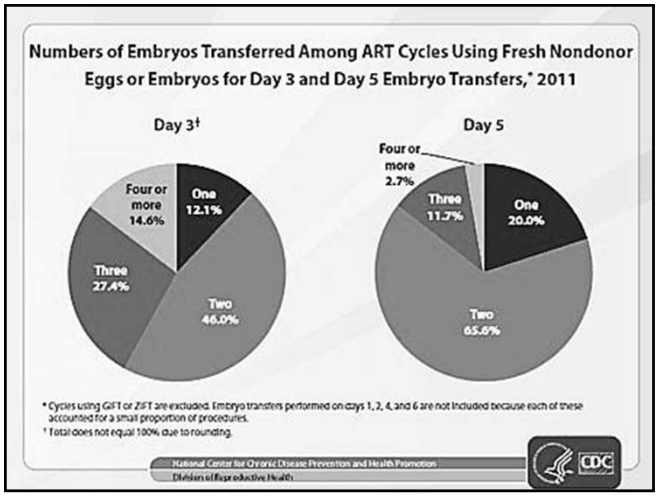
Outcomes achieved using traditional assessment
Brief review of latest CDC (USA) statistics - 2011

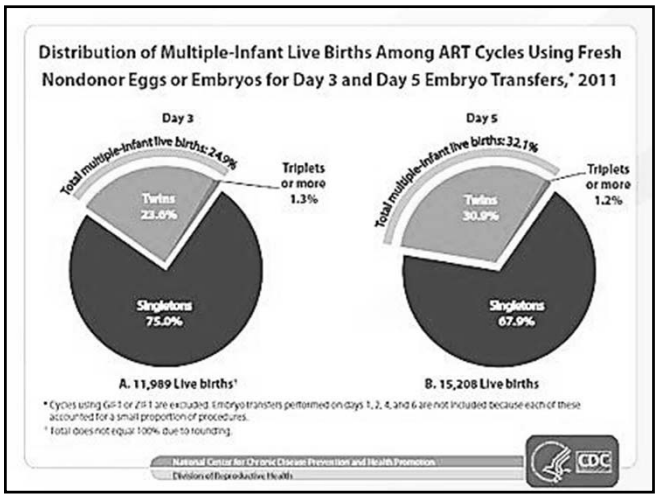
Number of ART clinics in the United States in 2011	**481**
Number of ART clinics that submitted data in 2011	**451**
Number of ART cycles reported in 2011	**151,923**
Number of live-birth	**47,818**
Number of infants born	**61,610**

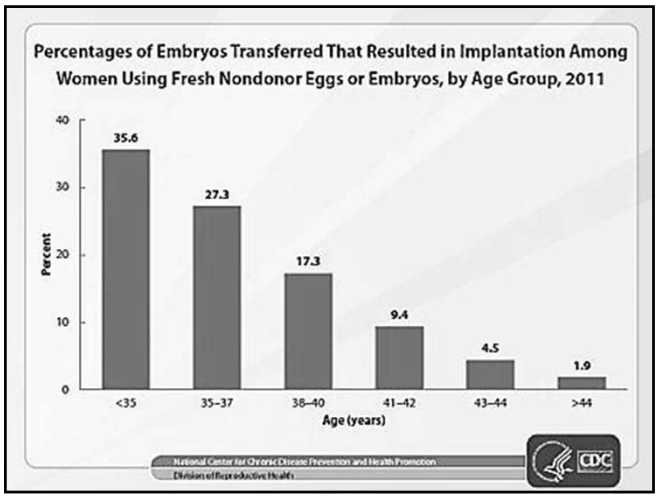


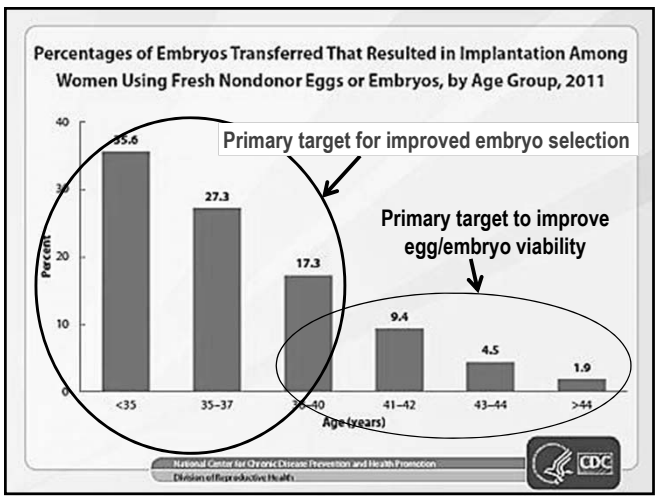
Singletons 71.3%
Total multiple-infant live births 28.8%
Twins 27.5%
Triplets or more 1.3%

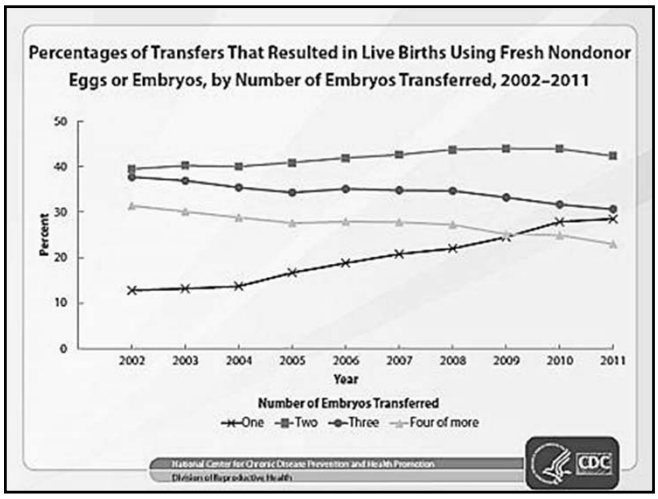




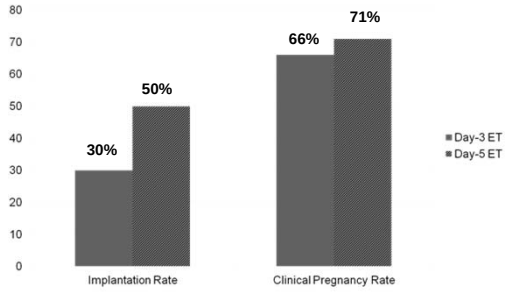

Distribution of Multiple-Infant Live Births Among ART Cycles Using Fresh Nondonor Eggs or Embryos for Day 3 and Day 5 Embryo Transfers,* 2011
Day 3
Total multiple-infant live births: 24.9%
Triplets or more 1.3%
Twins 23.6%
Singletons 75.0%
A. 11,989 Live births†
Day 5
Total multiple-infant live births: 32.1%
Triplets or more 1.2%
Twins 30.9%
Singletons 67.9%
B. 15,208 Live births
* Cycles using GIFT or ZIFT are excluded. Embryo transfers performed on days 1, 2, 4, and 6 are not included because each of these accounted for a small proportion of procedures.
† Total does not equal 100% due to rounding.
National Center for Chronic Disease Prevention and Health Promotion
Division of Reproductive Health
CDC


Percentages of Embryos Transferred That Resulted in Implantation Among Women Using Fresh Nondonor Eggs or Embryos, by Age Group, 2011
Percent
40
30
20
10
0
35.6
27.3
17.3
9.4
4.5
1.9
<35
35–37
38–40
41–42
43–44
>44
Age (years)
National Center for Chronic Disease Prevention and Health Promotion
Division of Reproductive Health
CDC


Percentages of Embryos Transferred That Resulted in Implantation Among Women Using Fresh Nondonor Eggs or Embryos, by Age Group, 2011
Primary target for improved embryo selection
Primary target to improve egg/embryo viability
Percent
40
30
20
10
0
35.6
27.3
17.3
9.4
4.5
1.9
<35
35–37
38–40
41–42
43–44
>44
Age (years)
National Center for Chronic Disease Prevention and Health Promotion
Division of Reproductive Health
CDC



What pregnancy rates can be achieved using traditional embryo assessment in "ideal" situation?

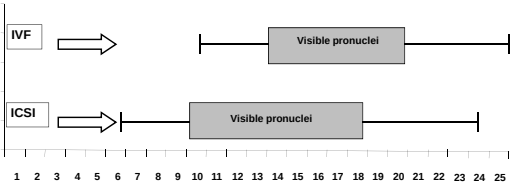


- Average maternal age was 34.5 and 33.6 in the day-3 and day-5 ET groups respectively.
- 3.7 and 2.2 Es were transferred, and high multiple pregnancy rate was obtained.

A prospective randomized trial of blastocyst culture and transfer in in-vitro fertilization. Gardner et al. Hum Reprod. 1998 Dec;13(12):3434-40.

The promise of morphokinetics for improved embryo assessment

"Pre-historic" morphokinetics: Visible pronuclei after insemination (IVF) or microinjection (ICSI) of human oocytes – *observations every 2 hours -1994*



Nagy et al., Hum Reprod. 1994 Sep;9(9):1743-8. Time-course of oocyte activation, pronucleus formation and cleavage in human oocytes fertilized by intracytoplasmic sperm injection.

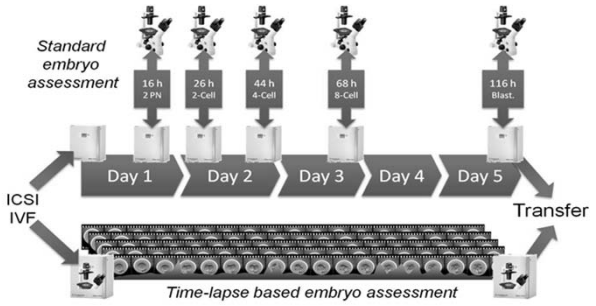
Nagy et al., Hum Reprod. 1998 Jun;13(6):1606-12. Timing of oocyte activation, pronucleus formation and cleavage in humans after intracytoplasmic sperm injection (ICSI) with testicular spermatozoa and after ICSI or in-vitro fertilization on sibling oocytes with ejaculated spermatozoa.

The promise of morphokinetics for improved embryo assessment

The real start of morphokinetics: Preliminary observations on polar body extrusion and pronuclear formation in human oocytes using time-lapse video cinematography. **Payne** et al., Hum Reprod. 1997 Mar;12(3):532-41.
2008, Lemmen et al. Reprod BioMed Online PN appearance, disappearance, synchrony (n=102 to pregnancy)
2008, Mio et al. Am J Obstet Gynecol Fertilization to first cleavage (n=286)
2010, Pribenszky et al. Reprod Biomed Online Reported a live birth (n=5)
2010, Wong et al. Nature Biotech Early cell division timings to 4-cell Successful development and molecular health (n=242 to blast)
2011, Meseguer et al. Hum Reproduction Early cell division timings to 5-cell (n=247 tx implantation)
Embryo Development: Blast Quality
2012, Hashimoto et al. Fertility & Sterility
2012, Cruz et al. Reprod Biomed Online
Embryo Development: Implantation
2011, Meseguer et al. Hum Reprod: Added ICSI to 5-cell
2012, Hlinka et al. Physiol Res: Added cell cycles timings to 16-cell
2012, Dal Canto et al. Reprod Biomed Online: Added cell cycles timings to 8-cell
2012, Rubio et al. Fertility & Sterility: Suggested DC2-3 (P2) <5h should be excluded
2012, Azarello et al. Hum Reprod: Suggested ICSI to PN breakdown <20.75h be excluded
>300 papers and abstracts on morpokinetics (rapidly increasing)
>50,000 embryos included in the studies (over 80% retrospective) Montag et al., 2011 Change of scores over time; Kirkegaard et al., 2012 Blastomere biopsy and late morphokinetics; Rubio et al., 2012 Direct cleavage as deselection criterion; Kirkegaard et al., 2013 Reduced oxygen and development; Campbell et al., 2013 Morphokinetics to assess aneuploidy risks; Wolf et al., 2013 Morphokinetics and quality control

Superior amount of information with time-lapse

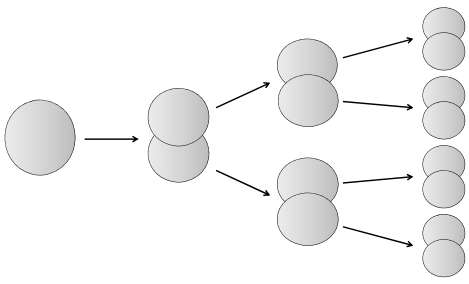
- The difference is not only "quantity"



Over 5 days per embryo: approx. 5000 images (700 time values / 7 focal planes)

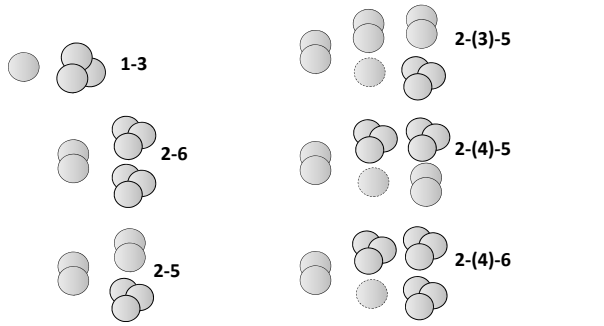
Cleavage patterns

"Regular" cleavage pattern ➡ Establish "positive selection" criteria

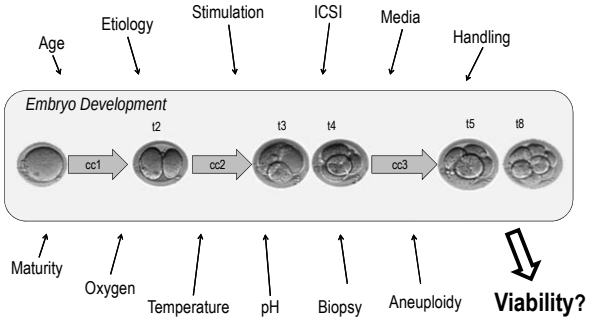


Cleavage patterns

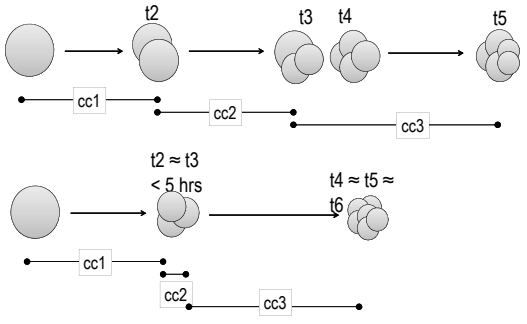
Unusual cleavage patterns → Establish "negative selection" criteria



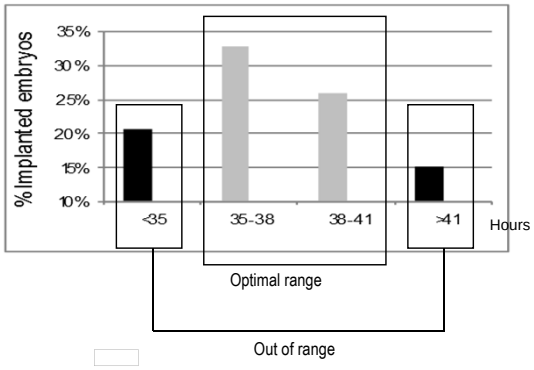
Factors affecting cleavage patterns



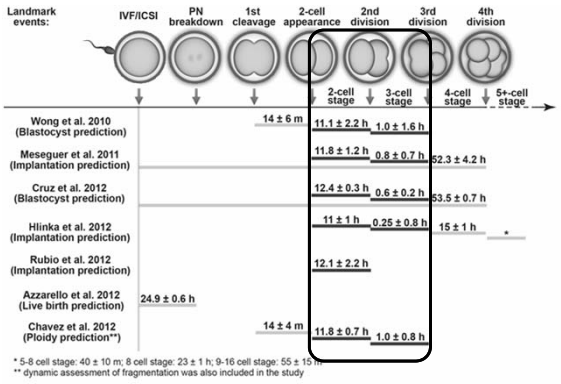
Cleavage patterns – varying nomenclature



Establishing optimal ranges



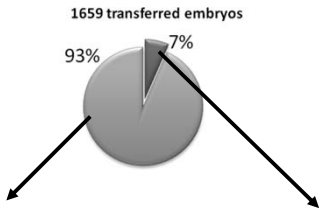
Time-lapse markers described by different studies



Chen et al. *Fertility & Sterility*. (2013)

Direct cleavage from 1-3 cells

Static observation will miss 63%



Regular cleavage from 1 to 2

Implantation rate < 2%
(n=109)

Direct cleavage from 1-3 cells

Implantation rate >13%
(n=1550)

Rubio I et al. 2012 Fertility and Sterility

Morphokinetic markers correlate with implantation

Type of abnormal development	Day 3, 6-10 cells		Blastocyst Formation Rate	Blastocyst Overall Grade Good/Fair	Rate of Transferred or Frozen Embryos	Known Implantation Data
Abnormal Syngamy (AS)	**Overall Grade Good/fair**	**≤10% frag**				
Control: Without AS (n=443)	90.7% (313/345)	61.7% (213/345)	44.9% (131/292)	60.0% (79/131)	50.1%(222/443)	17.9% (19/106)
With AS (n=163)	78.6% (70/89)	41.6% (37/89)	21.5% (23/107)	30.0% (7/23)	26.4% (43/163)	0.0% (0/14)
p-value	0.002	<0.001	<0.0001	0.008	<0.0001	0.08
Abnormal First Cytokinesis ($A1^{cyt}$)						
Control: Without $A1^{cyt}$ (n=443)	90.7% (313/345)	62.9% (217/345)	44.6% (131/294)	52.3% (69/131)	48.5% (215/443)	16.5% (15/91)
With $A1^{cyt}$ (n=196)	79.7% (94/119)	40.7% (48/119)	21.7% (26/120)	69.2% (18/26)	34.2% (67/196)	6.2% (2/32)
p-value	0.001	<0.0001	<0.0001	0.1	0.0005	0.1

Slide is courtesy of: Auxygen

Wirka et al. *Fertil & Steril* 2014 (*in press*)

Morphokinetic markers correlate with implantation

Type of abnormal development	Day 3, 6-10 cells		Blastocyst Formation Rate	Blastocyst Overall Grade Good/Fair	Rate of Transferred or Frozen Embryos	Known Implantation Data
Abnormal Cleavage (AC)	**Overall Grade Good/fair**	**≤10% frag**				
Control: Without AC (n=524)	88% (337/383)	59.3% (227/383)	43.1% (149/346)	55.0% (82/149)	44.8% (239/524)	18.0% (19/105)
With AC (n=115)	86.4% (70/81)	46.9% (38/81)	11.7% (8/68)	62.5% (5/8)	37.4% (43/115)	3.7% (1/27)
p-value	0.4	0.04	<0.0001	0.7	0.1	0.05
Chaotic Cleavage						
Control: Without chaotic cleavage (n=543)	94.6% (388/410)	64.1% (263/410)	42.3% (148/350)	55.4% (82/148)	49.5% (269/543)	18.2% (24/132)
With chaotic cleavage (n=96)	35.2% (19/54)	3.7% (2/54)	14.0% (9/64)	55.6% (5/9)	13.5% (13/96)	0.0% (0/7)
p-value	<0.0001	<0.0001	<0.0001	0.99	<0.0001	0.3

Slide is courtesy of: Auxygen

Wirka et al. *Fertil & Steril* 2014 (*in press*)

Morphokinetic markers correlate with implantation

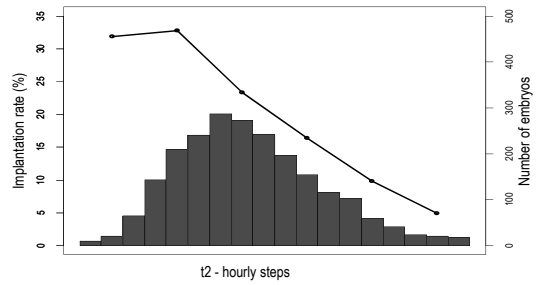
Retrospective analyzes

Patient Population	# Pts	# Embs	Avg Age (years)	Implantation Rate	Clinical Pregnancy Rate	Ongoing Pregnancy Rate
At least 1 Eeva High transferred	47	89	32.1 ± 5.2	**49%** (44/89) 	**60%** (28/47) 	**55%** (26/47) 
Only Eeva Lows transferred	30	52	32.2 ± 5.1	**21%** (11/52) 	**40%** (12/30) 	**37%** (11/30) 
p-value			*p=0.9*	*p<0.001*	*p=0.09*	*p=0.11*

Chen et al. *Fertility & Sterility* (2013)

Slide is courtesy of: Auxygen

t2 and implantation rates

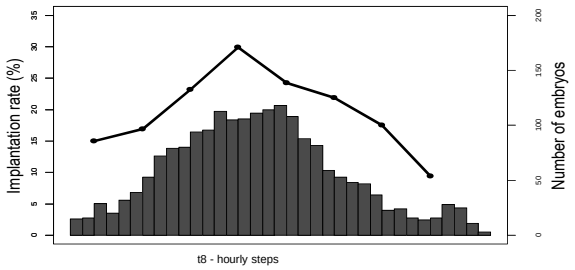


Early cleavage is an important parameter

But the exact definition of „early" depends on the individual laboratory and the conditions

Based on n > 2000 treatment cycles from different clinics
Courtesy of: FertiliTech

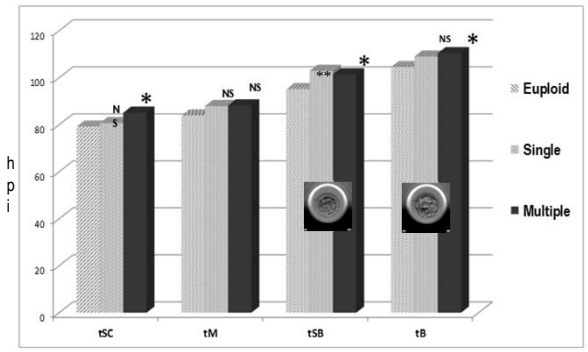
t8 and implantation rates



- Embryos being 8-cell too early or too late have a much lower implantation potential
- Chosing day 3 embryos with cell numbers that are much higher than 8 as standard is not beneficial

Based on n > 2000 treatment cycles from different clinics
Courtesy of: FertiliTech

tSB / tB and euploidy/aneuploidy

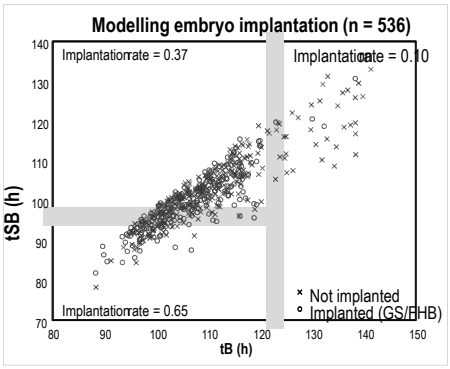


* P<0.05 ** P<0.01 (MWW test)

Courtesy of: FertiliTech

tSB time from insemination to start of blastulation (h)
tB time from insemination to reach 'full' blastocyst (h)

Implantation validation of the aneuploidy model in normal cycles



Based on n=536 embryos from different clinics; Source: FertiliTech database

Embryos from 27 clinics as they distribute into the three risk classification groups from Campbell et al. (2013a). Implanted embryos are shown with green circles and non-implanted with red crosses.

The grey area indicates that though the criteria seem universal, the actual timing intervals may be different between clinics.

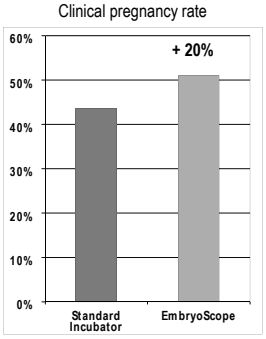
Summary

The benefits of morphokinetics

Less Disturbance = Better Development

More Observations = Better Selection

P value 0.0043
Odds ratio **1.201** (CL 95% 1.059-1.362)



Meseguer et al 2012, Fertil & Steril

Conclusions

- The current "traditional" evaluation methods have limited capability to accurately and reliably assess gamete / embryo viability, and developmental potential.

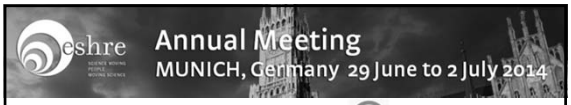
- Time-lapse systems may improve embryo development potential and efficacy of assessing embryo viability – increasing IVF efficiency.

- Time lapse systems may improve predictability of blastocyst formation and implantation – facilitating eSET

- Further studies are required to fully assess benefit / cost / logistics of time-lapse relative to "traditional" embryo evaluation and alternative assessment techniques.

References

- Sato et al., Reproductive BioMedicine Online, Volume 14, Number 1, January 2007 , pp. 49-56(8)
- An Atlas of Human Oocyte and Embryo Morphology by L. Veeck – Parthenon Publishing 1986
- James et al., Human Reproduction 2006 21(6):1599-1604
- LA Scott and S Smith Human Reproduction, 1998, 13, 1003-1013
- Nagy et al. Fertil Steril. 2003 Jul;80(1):67-74.
- ALPHA Scientists In Reproductive Medicine; ESHRE Special Interest Group Embryology. Reprod Biomed Online. 2011 Jun;22(6):632-46.
- Neuber, E. et al. Hum. Reprod. 2003 18:1307-1312;
- Racowsky et al., FertilSteril. 2011 May;95(6):1985-9.
- Weitzman et al., Fertil Steril. 2010 Feb;93(2):658-62
- Gardner et al. Hum Reprod. 1998 Dec;13(12):3434-40.
- Nagy et al., Hum Reprod. 1994 Sep;9(9):1743-8. Time-course of oocyte activation, pronucleus formation and cleavage in human oocytes fertilized by intracytoplasmic sperm injection.
- Nagy et al., Hum Reprod. 1998 Jun;13(6):1606-12. Timing of oocyte activation, pronucleus formation and cleavage in humans after intracytoplasmic sperm injection (ICSI) with testicular spermatozoa and after ICSI or in-vitro fertilization on sibling oocytes with ejaculated spermatozoa.
- Payne et al., Hum Reprod. 1997 Mar;12(3):532-41. Preliminary observations on polar body extrusion and pronuclear formation in human oocytes using time-lapse video cinematography.
- Chen et al., Fertil Steril. 2013 Mar 15;99(4):1035-43. Biomarkers identified with time-lapse imaging: discovery, validation, and practical application.



Pre-congress course 3
"Time-lapse monitoring in the ART lab"

Organized by SIG Embryology

"Expanding applications of live cell microscopy to the study of oogenesis and oocyte quality"

Giovanni Coticchio, PhD
Biogenesi
Monza - Italy

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Embryologists
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Declaration of potential conflict of interest

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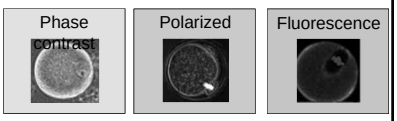
Learning objectives

Appreciate	that live cell imaging can be used to uncover dynamic activities in oocytes
Observe	fundamental behaviors/properties of oocyte maturation unrecognized until recently
Envisage	novel methods and criteria of oocyte quality?

Live cell microscopy approaches and their objects of study

Game field: Time lapse microscopy / oocyte maturation

Imaging:



Object of Analysis:

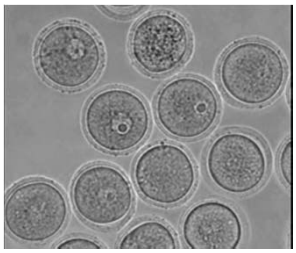
- Morphokinetics (e.g. polar body emission)
- Organelle movement (e.g. germinal vesicle)
- Cytoplasmic dynamics

Newly observed phenomena

Oocyte maturation	- Germinal vesicle (GV) breakdown in relation to GV position - Contractions of the oocyte cortex
Cytoskeletal dynamics	- Spindle migration and localization - Cytoplasmic streaming

Ordinary transmitted light time-lapse microscopy has limited detection power

Oocyte maturation (mouse)



Long acquisition intervals (>20 min) only scratch the surface of oocyte maturation

We can only assess spatio-temporal aspects of

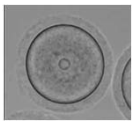
- **GV breakdown**
- **polar body I (PBI) emission**

… but there is more that meets the eye

An amazing story revealed by live cell microscopy

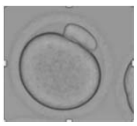
Generation of asymmetry and polarity in mouse oocytes during maturation

Oocyte meiosis demands an asymmetric geometry



At meiotic resumption chromosomes condense centrally while the GV breaks down

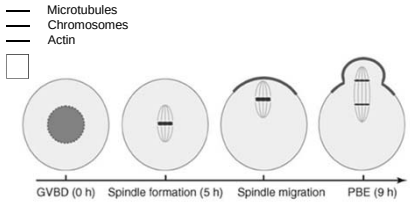
… and yet



Segregation of a set of chromosomes in the PBI is a highly asymmetric phenomenon

How is symmetry breaking achieved?

A current model of generation of asymmetry during maturation

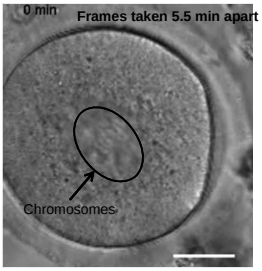


The MI spindle first forms in the centre and only afterwords moves to the cortex

Yi, Li et al., 2013

Examples of contributions of live cell microscopy to the model

Phase contrast and fluorescence microscopy detect chromosome movement during maturation



Hoechst-stained oocyte

Chromosomes move from the centre to the cortex while remaining aligned

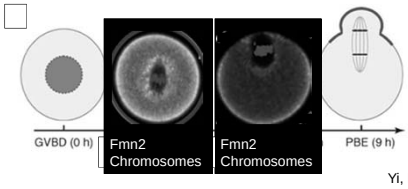
This suggests that also the MI spindle moves together with chromosomes

Yi, Li et al., 2013

Live cell fluorescence microscopy has revealed the role of key elements controlling spindle migration

Actin fibers, but not microtubules, are responsible for spindle migration

Formin 2 (Fmn2, actin nucleating protein) clusters around the condensing chromosomes and forming spindle

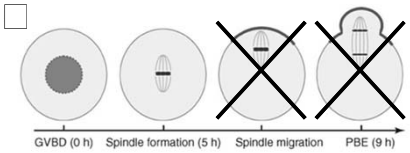


Yi, Li et al., 2013

Actin fibers, but not microtubules, are responsible for spindle migration

Formin 2 (Fmn2, actin nucleating protein)
clusters around the condensing chromosomes
and forming spindle
and generate pushing forces

Fmn2-knockout oocytes

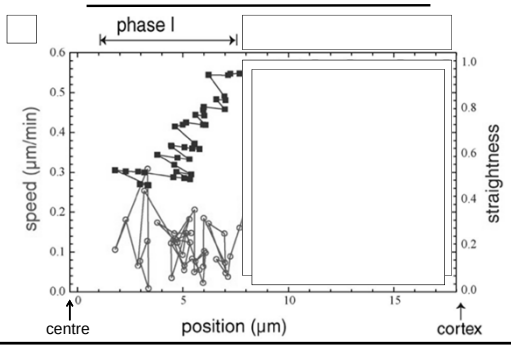


Yi, Li et al., 2013

Time lapse microscopy can precisely track spindle movement

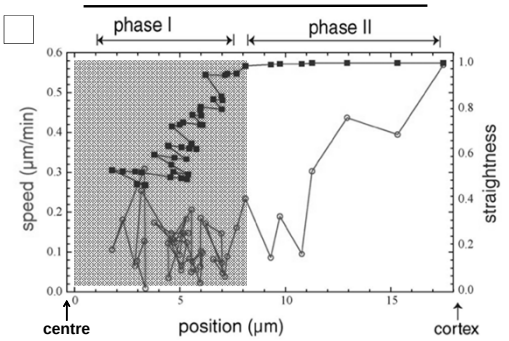
Biphasic spindle migration from the centre to the cortex

Yi, Li et al., 2013

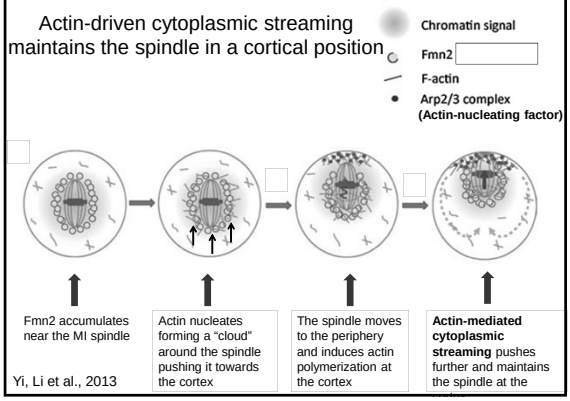


Biphasic spindle migration from the centre to the cortex

Yi, Li et al., 2013



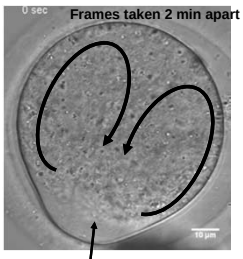
Actin-driven cytoplasmic streaming maintains the spindle in a cortical position



Yi, Li et al., 2013

How was cytoplasmic streaming detected?

Cytoplasmic streaming emerges from transmitted light TLM observation



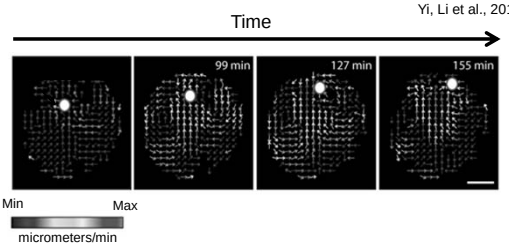
Chromosomes
(and MI spindle)

Can cytoplasmic streaming be assessed objectively?

Yi, Li et al., 2013

Cytoplasmic streaming can be quantified by image analysis

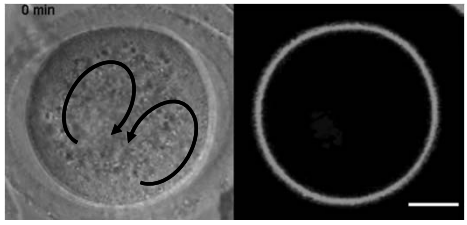
Yi, Li et al., 2013



Orientation and color of vectors describe movement of cytoplasmic domains

Vicinity of the spindle to the cortex is associated to increased speed of cytoplasmic streaming

Cytoplasmic streaming also maintains the MII spindle in position after PBI emission

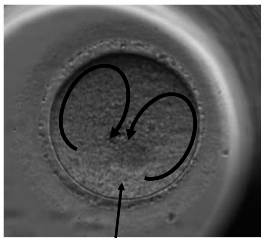


Frames taken 11 min apart over 840 min

Yi, Li et al., 2013

Does cytoplasmic streaming occur in human oocytes?

Mature oocyte

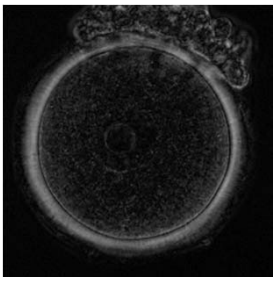


Polar body I

Biogenesi video

What can we learn through other live cell microscopy approaches?

Polarized light TLM offers a unique view of cytoskeletal dynamics



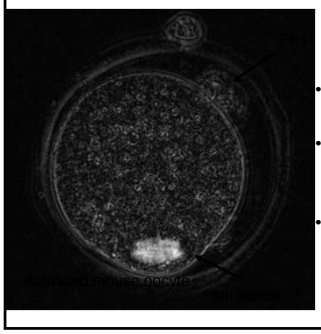
Presence of birefringent elements around the GV and nucleolus

Cortical tension

MI spindle
- formation
- vectorial movement (pole first)
- rotation

Liu, Keefe and Albertini, unpublished

Polarized light TLM shows new features of PBII extrusion

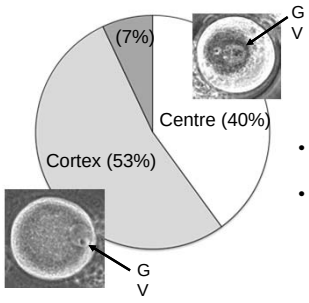


- Spindle elongates
- The spindle equator establishes contact with actin
- Waves of cortical contractions accompany PBII emission

Liu, Keefe and Albertini, unpublished

What do we know about GV breakdown and other meiotic events in the human oocyte?

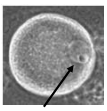
In immature human oocytes the GV is positioned in the oocyte centre or cortex



- Different from the mouse
- Do these categories have different meiotic competence?

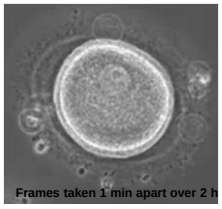
Coticchio et al., 2011

A cortical position is often retained by the GV and is followed by GV breakdown



Frequent maintenance of cortical position → GVBD approaching 100%

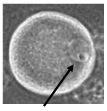
The oocyte cortex as the preferential domain for GV localization and GV breakdown



GV positioned cortically can travel a long way across the cortex before undergoing GVBD

Biogenesi video

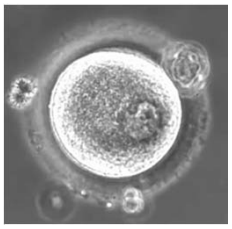
A cortical position is often retained by the GV and is followed by GV breakdown



Frequent maintenance of cortical position → Nearly 100% GVBD

Occasional disanchoring and drifting to the centre → GVBD strongly suppressed

Disanchoring from the cortex leads to GV breakdown failure

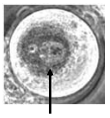


Frames taken 1 min apart for over 24 h

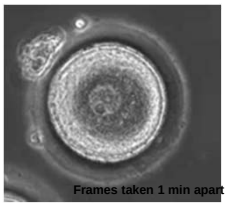
Play speed X8

Biogenesi video

If a central GV does not re-localize to the cortex, meiotic resumption is unlikely to occur



Inability to migrate to the cortex very often coincides with GVBD failure

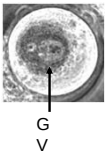


GV arrest observed for over 30 hours

GVBD failure occurs despite the GV and chromatin are dynamically active

Biogenesi video

If a central GV does not re-localize to the cortex, meiotic resumption is unlikely to occur

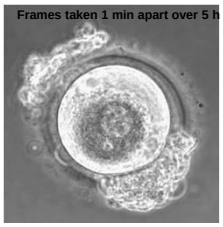


G
V

Maintenance of central position → GV arrest

Displacement to the cortex → GVBD

Nuclear motility preceeds GV relocation to the cortex

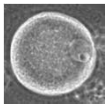


- GV relocation to the cortex is accompanied by
  - rotational and translational motility
  - dynamic chromatin reorganization
- Cytoskeletal forces (probably actin-driven) act within the GV

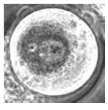
Biogenesi video

Overall, GV breakdown is a process associated with the cortex

Chances of GV breakdown

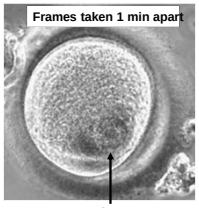


High



Low

GV breakdown is followed by massive contractions of the cytoplasm



Biogenesi video

- Contractions occur rhythmically with a period of 1-2 hours
- Overall phenomenon not observed in the mouse
- Significance currently obscure

Final considerations

Time lapse microscopy is contributing to the understanding of oocyte maturation

- **Mouse oocyte maturation**
  - Symmetry breaking and polarization
  - Spindle positioning
  - Quantitation of cytoskeletal and cytoplasmic dynamics
- **Human oocyte maturation**
  - GV rotational and translational motility
  - Intranuclear contractility
  - The cortex as the preferential domain for GVBD
  - Ripetitive cortical contractions as a cellular manifestation of oocyte meiosis

References

- Almonacid M, Terret ME, Verlhac MH. Actin-based spindle positioning: new insights from female gametes. Journal of Cell Science. 2014 Jan 30;127(3):477–83.
- Azoury J, Verlhac M-H, Dumont J. Actin filaments: key players in the control of asymmetric divisions in mouse oocytes. Biology of the Cell. 2012 Jan 3;101(2):69–76.
- Brunet S, Verlhac MH. Positioning to get out of meiosis: the asymmetry of division. Human Reproduction Update. 2010 Dec 13;17(1):68–75.
- Coticchio G, Guglielmo M, Dal Canto M, Brambillasca F, Albertini DF, Mignini Renzini M, et al. Time-lapse imaging of immature human oocytes reveals a role for the oocyte cortex in germinal vesicle anchoring and meiotic resumption. Human Reproduction. 2011;26 (suppl.1):i191–2.
- Coticchio G, Guglielmo MC, Albertini DF, Dal Canto M, Mignini Renzini M, De Ponti E, et al. Contributions of the actin cytoskeleton to the emergence of polarity during maturation in human oocytes. Molecular Human Reproduction. 2014 Mar;20(3):200–7.
- Holubcová Z, Howard G, Schuh M. Vesicles modulate an actin network for asymmetric spindle positioning. Nature Cell Biology. Nature Publishing Group; 2013 Jul 21;15(8):937–47.
- Li R, Albertini DF. The road to maturation: somatic cell interaction and self-organization of the mammalian oocyte. Nat Rev Mol Cell Biol. Nature Publishing Group; 2013 Mar 1;14(3):141–52.
- Yi K, Rubinstein B, Unruh JR, Guo F, Slaughter BD, Li R. Sequential actin-based pushing forces drive meiosis I chromosome migration and symmetry breaking in oocytes. J Cell Biol. 2013 Feb 25;200(5):567–76.
- Yi K, Li R. Actin cytoskeleton in cell polarity and asymmetric division during mouse oocyte maturation. Robinson DN, Bement WM, Balasubramanian MK, Sanger JW, editors. Cytoskeleton. 2012 Aug 13;69(10):727–37.
- Yi K, Unruh JR, Deng M, Slaughter BD, Rubinstein B, Li R. Dynamic maintenance of asymmetric meiotic spindle position through Arp2/3-complex-driven cytoplasmic streaming in

References

- Almonacid M, Terret ME, Verlhac MH. Actin-based spindle positioning: new insights from female gametes. Journal of Cell Science. 2014 Jan 30;127(3):477–83.
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The biology behind time-lapse monitoring of embryos: correlates of aneuploidy and gene expression

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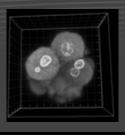
ESHRE Pre-Congress
Course #3
06-29-14



Disclosures

Neither I, nor any member of my immediate family, have current financial relationships to disclose relevant to the content of this presentation.

Learning objectives

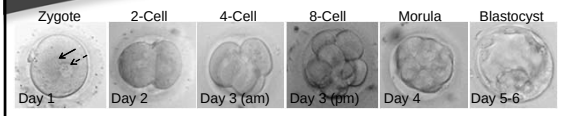
I. Review the fundamental aspects of pre-implantation development with focus on timing in the human.

II. Examine chromosomal instability in human embryos with methods for aneuploidy detection.

III. Assess embryonic chromosomal status via time-lapse imaging and automated image analysis of human embryo behavior.

IV. Elucidate the causative mechanisms underlying increased aneuploidy rates in human embryos with current and future research aims.

Fundamental aspects of human pre-implantation development



Maternal programs

Embryonic genome activation

Epigenetic reprogramming

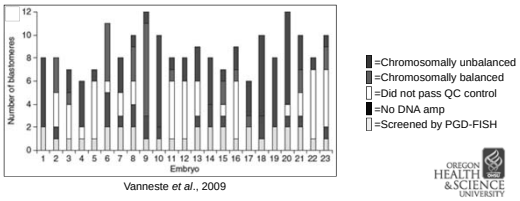
Cell lineage specification

Chromosomal stability?

Increased chromosomal instability in cleavage-stage human embryos

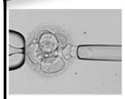
Previous studies using array-based methods have demonstrated that chromosomal abnormalities (aneuploidy) are remarkably common, occurring in as many as **50-80%** of cleavage-stage human embryos, **including those from fertile couples and regardless of whether fresh or cryopreserved**.

(Vanneste *et al*., 2009; Johnson *et al*., 2010; Chavez *et al*., 2012)



Vanneste *et al*., 2009

Methods to detect aneuploidy in embryos following assisted reproduction



- Previously, the most frequently used method for diagnosing aneuploidy was pre-implantation genetic screening (PGS) of day 3 biopsied blastomeres, **which suffers from mosaicism**.

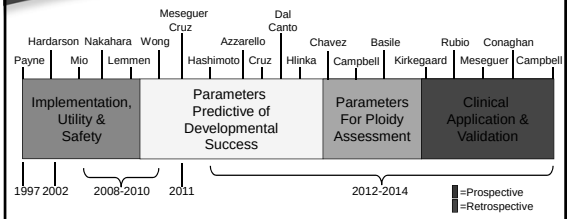
  (Kuo *et al*., 1998; Baart, *et al.*, 2006)

- Alternative approaches such as extended culture of embryos to the blastocyst stage and analysis of chromosomal status via trophectoderm biopsy have also been used to evaluate aneuploidy.

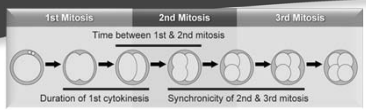
- However, there are additional potential risks associated with prolonged embryo culture such as the **introduction of epigenetic changes, embryo arrest and other factors that may disrupt embryo integrity.**

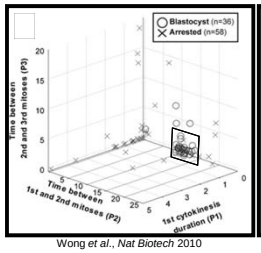
  (Peramo, *et al*., 1999; Khosla *et al*.; 2001; Katari *et al*., 2009; Kallen *et al*., 2010)

An evolutionary timeline of time-lapse technologies for human embryo assessment

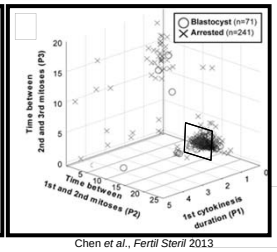


Dynamic imaging parameters predict success in human development



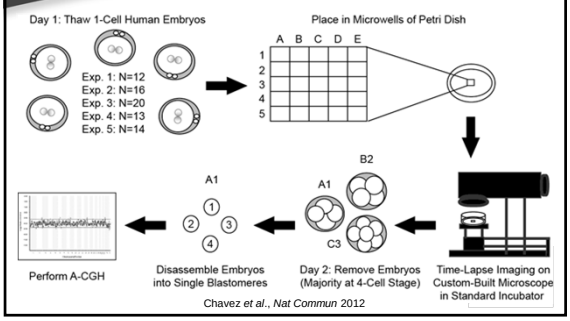


Wong *et al.*, *Nat Biotech* 2010



Chen *et al.*, *Fertil Steril* 2013

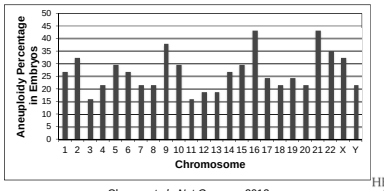
Experimental design of human aneuploidy analysis at the cleavage-stage



Chavez *et al.*, *Nat Commun* 2012

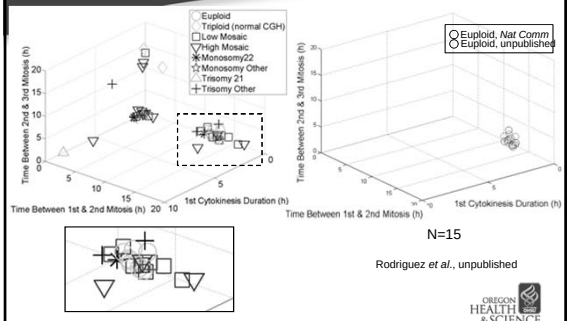
High frequency of aneuploidy is often mitotic and not preferential to a subset of chromosomes

	Progressed Beyond 1-Cell Stage	Conclusive A-CGH Data	Euploid (Normal A-CGH)	Aneuploid (Abnormal A-CGH)	Triploid (Normal A-CGH)	Meiotic Errors	Mitotic Errors
Number of Embryos	53	45	8	34	3	9	25
Total Number of Embryos	75	53	45	45	45	45	45
Percentage	70.7%	84.9%	17.8%	75.5%	6.7%	20%	55%



Chavez *et al.*, *Nat Commun* 2012

Distinction between euploid, aneuploid and triploid embryos by cell cycle parameter analysis



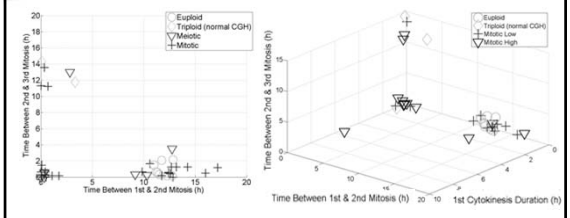
N=15

Rodriguez *et al.*, unpublished

Chavez *et al.*, *Nat Commun* 2012

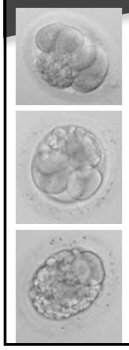
Distinction between euploid, aneuploid and triploid embryos by cell cycle parameter analysis

Mitotic Low = ≤ 4 chromosomes
Mitotic High = > 4 chromosomes



Chavez *et al.*, *Nat Commun* 2012

Characteristics of cellular fragmentation in human pre-implantation embryos



- Generally thought to be cytoplasmic fragments
- Frequently observed in human embryos
- Distinct from the DNA fragmentation that can occur following cell death late in pre-implantation development
- Some evidence to suggest that cellular fragmentation occurs in human embryos *in vivo*
- May negatively correlate with implantation potential

(Antczak and Van Blerkom, 1999; Hardy *et al.*, 2001; Pereda and Croxatto, 1978; Buster *et al.*, 1985; Alikani, M. *et al.*, 1999; Pelinck, *et al.*, 2010)

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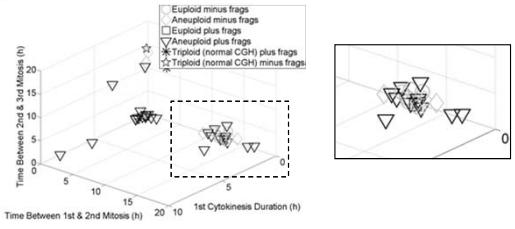
Cellular fragmentation and cell cycle parameter assessment predicts ploidy status



Chavez *et al.*, *Nat Commun* 2012

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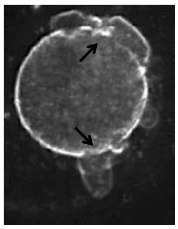
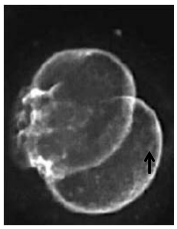
Correlation between the type of chromosomal error and fragmentation timing

Meiotic/Triploid = 1-cell stage



Mitotic = 2-cell stage



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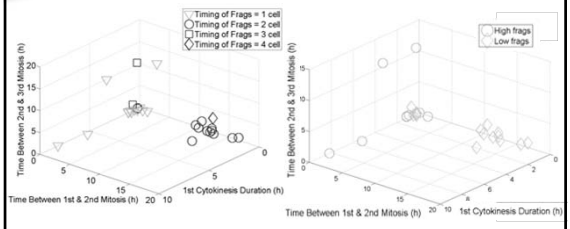
Fragmentation timing and degree have differential effects on cell cycle parameters



Chavez *et al*., *Nat Commun* 2012

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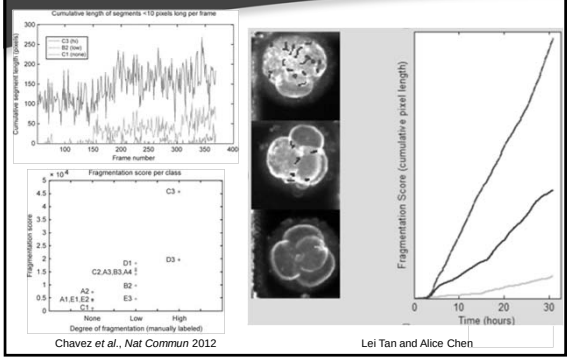
Automated real-time tracking of cellular fragmentation over time



Chavez *et al*., *Nat Commun* 2012

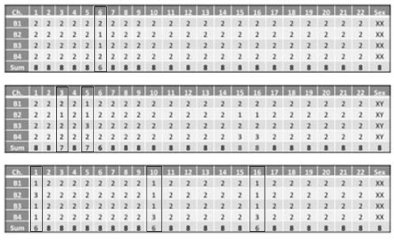
Lei Tan and Alice Chen

Human blastomeres often exhibit loss of one or more chromosomes

2 copies of Ch. 6

1 copy of Ch. 3 & 5

2 copies of Ch. 1, 10 & 16



Chavez *et al*., *Nat Commun* 2012

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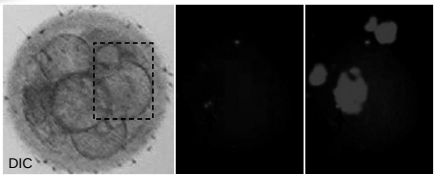
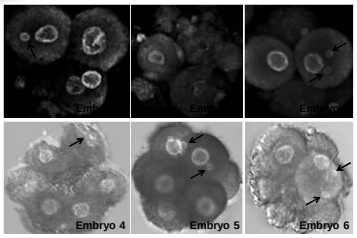
Detection of chromosomes distinct from the primary nuclei of blastomeres



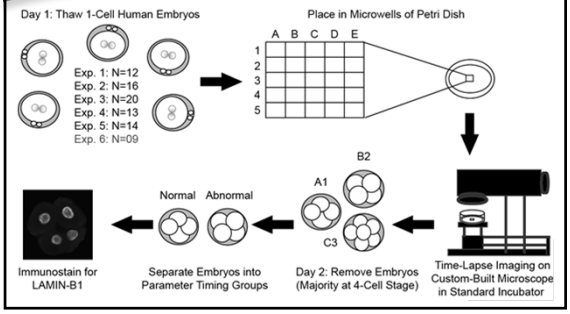
Chavez *et al.*, *Nat Commun* 2012

Identification of embryonic micronuclei in the blastomeres of human embryos

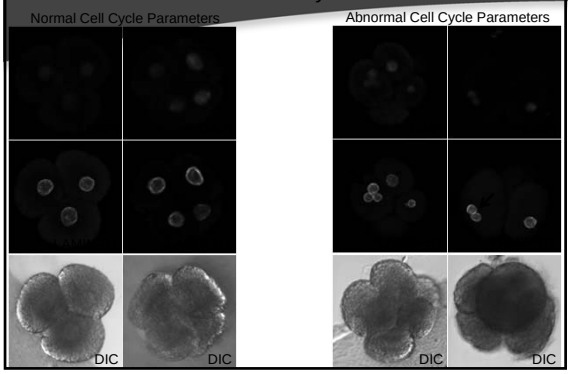


Chavez *et al.*, *Nat Commun* 2012

Experimental design for determining the effects of embryonic micronuclei on developmental potential



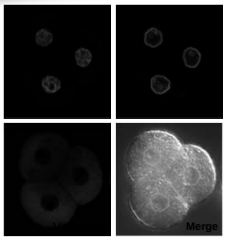
Correlation between parameter timing and the visualization of embryonic micronuclei



Mouse pre-implantation embryos exhibit a low incidence of aneuploidy

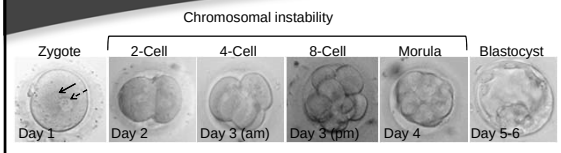
Mouse embryos are estimated to exhibit less than 1% aneuploidy rates during pre-implantation development and micronuclei formation is not frequently observed at the cleavage-stage.

(Bond and Chandley, *Oxford University Press* 1983; Lightfoot *et al*., *Dev Biol* 2006; Chavez *et al*., *Nat Commun* 2012)



Why are there such high rates of aneuploidy in cleavage-stage human embryos?

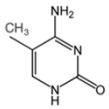
Chromosomal and epigenetic aspects of human embryonic development



The two main types of epigenetic mechanisms

- **DNA methylation**

Mediated by a family of DNA methyltransferases (DNMTs), which catalyze the transfer of a methyl group to the 5'-position of cytosine residues within CpG dinucleotides.



- **Histone modifications**

The tail of histones are unique in that certain amino acid residues have the capacity to undergo post-translational modifications.

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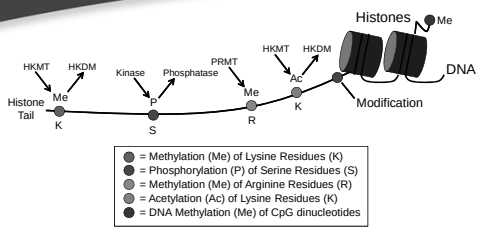
Association between DNMTs, histone modifications and histone modifying enzymes



DNA methylation and the regulation of histone modifications are intricately associated, working together to influence gene expression and chromatin structure.

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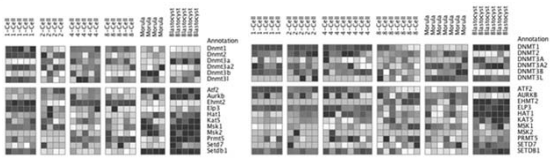
Differential epigenetic expression patterns between individual mouse and human embryos

Mouse embryos

Human embryos



Chavez *et al.*, *Hum Mol Genet* (submitted)

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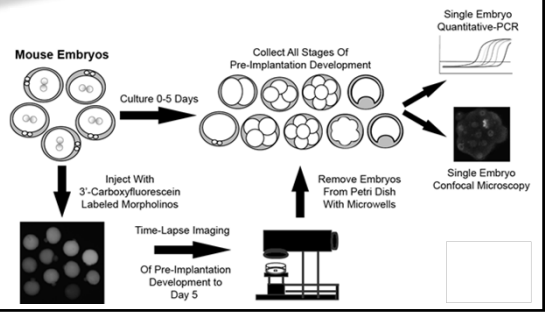
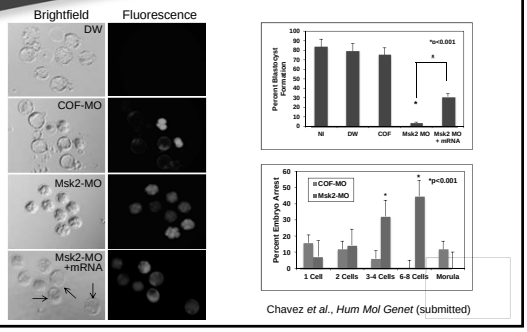
Experimental design to test the function of the histone modifying enzyme, Msk2

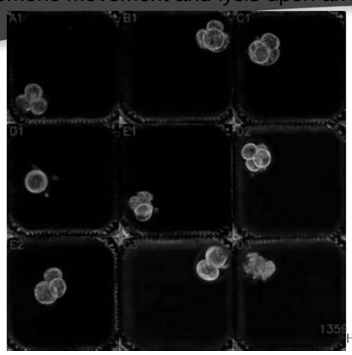


Msk2 knockdown induces embryo arrest at the 3-8 cell stage



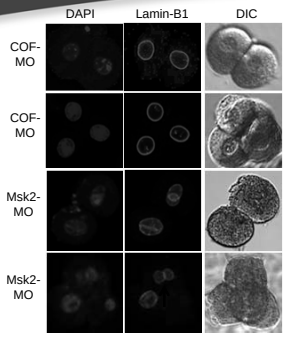
Chavez *et al.*, *Hum Mol Genet* (submitted)

Msk2-MO-injected embryos exhibit increased blastomeric movement and lysis upon arrest



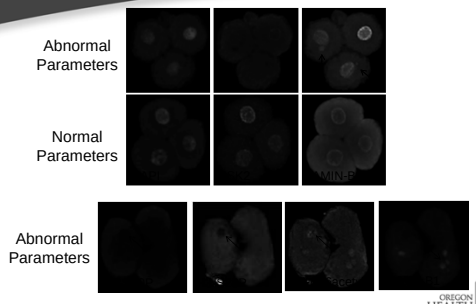
Chavez *et al.*, *Hum Mol Genet* (submitted)

Msk2 knockdown results in the formation of mouse embryonic micronuclei



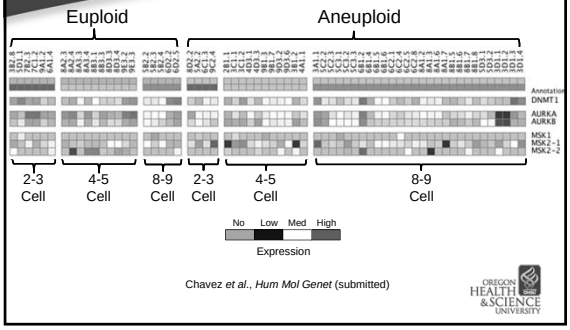
Chavez *et al.*, *Hum Mol Genet* (submitted)

Association between MSK2 expression, micronuclei and cell cycle parameters in human embryos



Chavez *et al.*, *Hum Mol Genet* (submitted)

Correlation between ploidy status and gene expression in single human blastomeres



Chavez *et al.*, *Hum Mol Genet* (submitted)

Summary and conclusions

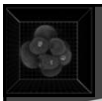
- Assessment of cell cycle parameters in conjunction with **dynamic fragmentation analysis** can assist in the differentiation between euploid and aneuploid embryos.
- The high frequency of human embryonic aneuploidy may have contributions from the encapsulation of chromosomes in **embryonic micronuclei/fragments**.
- Mouse and human embryos appear to deal with chromosomal instability differently, which may be due to differences in the **extent or timing of epigenetic reprogramming** during pre-implantation development.
- The **knockdown of a single epigenetic factor** can have profound effects on chromosomal stability in cleavage-stage embryos and developmental progression.

Current & future research aims

- To continue to investigate why there are such high rates of aneuploidy at the cleavage-stage of human pre-implantation development
  (precise underlying causes)

- To determine if chromosomal abnormalities can be detected earlier in development to avoid the unnecessary creation of embryos that are destined to fail
  (in oocytes and/or sperm)

- To examine alternative biomedical optics or other non-invasive imaging techniques with the potential for automated assessment
  (focus on nuclear structure)



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Santiago Munne
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- Alikani, M. *et al.* Human embryo fragmentation in vitro and its implications for pregnancy and implantation. *Fertil Steril* 71, 836–842 (1999).
- Antczak, M. & Van Blerkom, J. Temporal and spatial aspects of fragmentation in early human embryos: possible effects on developmental competence and association with the differential elimination of regulatory proteins from polarized domains. *Hum Reprod* 14, 429–447 (1999).
- Azzarello, A. *et al*. The impact of pronuclei morphology and dynamicity on live birth outcome after time-lapse culture. *Hum Reprod* 27, 2649-2657 (2012).
- Baart, E.B. *et al*. Preimplantation genetic screening reveals a high incidence of aneuploidy and mosaicism in embryos from young women undergoing IVF. *Hum Reprod* 21, 223–233 (2006).
- Basile, N. *et al*. Increasing the probability of selecting chromosomally normal embryos by time-lapse morphokinetics analysis. *Fertil Steril* 101, 699-704 (2014).
- Bond, D.J. and Chandley, A.C. Aneuploidy. *Oxford Monographs on Medical Genetics No. 11*, Oxford University Press (1983).
- Buster, J.E. *et al*. Biologic and morphologic development of donated human ova recovered by nonsurgical uterine lavage. *Am J Obstet Gyn* 153, 211–217 (1985).
- Campbell, A. *et al*. Modelling a risk classification of aneuploidy in human embryos using non-invasive morphokinetics. *Reprod Biomed Online* 26, 477-485 (2013).
- Campbell, A. *et al*. Retrospective analysis of outcomes after IVF using an aneuploidy risk model derived from time-lapse imaging without PGS. *Reprod Biomed Online* 27, 140-146 (2013).
- Chavez, S.L. *et al*. Dynamic blastomere behaviour reflects human embryo ploidy by the four-cell stage. *Nat Commun* 3:1251 (2012).
- Chavez, S.L. *et al*. Comparison of epigenetic mediator expression and function in mouse and human embryonic blastomeres. *Human Molecular Genetics* (submitted).
- Chen, A.A. *et al*., Biomarkers identified with time-lapse imaging: discovery, validation, and practical application. *Fertil Steril* 99, 1035-1043 (2013).
- Conaghan, J. *et al*. Improving embryo selection using a computer-automated time-lapse image analysis test plus day 3 morphology: results from a prospective multicenter trial. *Fertil Steril* 100, 412-419 (2013).
- Cruz, M. *et al*. Embryo quality, blastocyst and ongoing pregnancy rates in oocyte donation patients whose embryos were monitored by time-lapse imaging. *J Assist Reprod Genet* 28, 569-573 (2011).
- Cruz, M. *et al*. Timing of cell division in human cleavage-stage embryos is linked with blastocyst formation and quality. *Reprod Biomed Online* 25, 371-381 (2012).
- Dal Canto, M. *et al*. Cleavage kinetics analysis of human embryos predicts development to blastocyst and implantation. *Reprod Biomed Online* 25, 474-480 (2012).
- Hardarson, T., *et al*. Internalization of cellular fragments in a human embryo: time-lapse recordings. *Reprod Biomed Online* 5, 36-38 (2002).
- Hardy, K. *et al*. From cell death to embryo arrest: mathematical models of human preimplantation embryo development.

- Hashimoto, S. *et al*. Selection of high-potential embryos by culture in poly(dimethylsiloxane) microwells and time-lapse imaging. *Fertil Steril* 97, 332-337 (2012).
- Hlinka, D. *et al*. Time-lapse cleavage rating predicts human embryo viability. *Physiol Res* 61, 513-525 (2012).
- Johnson, D.S. *et al*. Preclinical validation of a microarray method for full molecular karyotyping of blastomeres in a 24-h protocol. *Hum Reprod* 25, 1066–1075 (2010).
- Kallen, B. *et al*., Blastocyst versus cleavage stage transfer in in vitro fertilization: differences in neonatal outcome? *Fertil Steril* 94, 1680-1683 (2010).
- Katari, S, *et al*., DNA methylation and gene expression differences in children conceived in vitro or in vivo. *Hum Mol Genet* 8, 3769-3778 (2009).
- Khosla, S. *et al*. Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. *Biol Reprod* 64, 918-926 (2001).
- Kirkegaard, K. *et al*. Time-lapse parameters as predictors of blastocyst development and pregnancy outcome in embryos from good prognosis patients: a prospective cohort study. *Hum Reprod* 28, 2643-2651 (2013).
- Kuo, H.C., Ogilvie, C.M. & Handyside, A.H. Chromosomal mosaicism in cleavage-stage human embryos and the accuracy of single-cell genetic analysis. *J Assist Reprod Genet* 15, 276–280 (1998).
- Lemmen, J.G. *et al*. Kinetic markers of human embryo quality using time-lapse recordings of IVF/ICSI-fertilized oocytes. *Reprod Biomed Online* 17, 385-91 (2008).
- Lightfoot, D.A. *et al*. The fate of mosaic aneuploid embryos during mouse development. *Dev Biol* 289, 384–394 (2006).
- Meseguer, M. *et al*. The use of morphokinetics as a predictor of embryo implantation. *Hum Reprod* 26, 2658–2671 (2011).
- Meseguer, M. *et al*. Embryo incubation and selection in a time-lapse monitoring system improves pregnancy outcome compared with a standard incubator: a retrospective cohort study. *Fertil Steril* 98, 1481-1489 (2012).
- Mio, Y. & Maeda, K. Time-lapse cinematography of dynamic changes occurring during in vitro development of human embryos. *Am J Obstet Gynecol* 199, 661-665 (2008).
- Nakahara, T., *et al*. Evaluation of the safety of time-lapse observations for human embryos. *J Assist Reprod Genet* 27, 93-96 (2010).
- Payne, D., *et al*. Preliminary observations on polar body extrusion and pronuclear formation in human oocytes using time-lapse video cinematography. *Hum Reprod* 12, 532-541 (1997).
- Pelinck, M.J. *et al*. Embryo quality and impact of specific embryo characteristics on ongoing implantation in unselected embryos derived from modified natural cycle in vitro fertilization. *Fertil. Steril.* 94, 527–534 (2010).
- Peramo, B. *et al*. Blastocyst transfer and monozygotic twinning. *Fertil Steril* 72, 1116-1117 (1999).
- Pereda, J. & Croxatto, H.B. Ultrastructure of a seven-cell human embryo. Biol.Reprod. 18, 481–489 (1978).
- Rubio, I. *et al*. Limited implantation success of direct-cleaved human zygotes: a time-lapse study. *Fertil. Steril* 98, 1458-1463 (2012).
- Vanneste, E. *et al*. Chromosome instability is common in human cleavage-stage embryos. *Nat Med* 15, 577–583 (2009).
- Wong, C.C. *et al*. Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage. *Nat Biotechnol* 28, 1115-1121 (2010).

An evidence-based evaluation of time-lapse monitoring in clinical embryology

- to select or not to select that's the question -

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S.Repping@amc.uva.nl

Universiteit van Amsterdam

am center for reproductive medicine

Conflict of interest

- I am a scientist
  - I aim to seek the truth
- I believe in the God of Spinoza
  - Everybody else: bring your data
- I head a University based Center for Reproductive Medicine
  - Our aim is to aid patients to achieve life birth safely and effectively
- I have no commercial interests in assisted reproduction
  - My salary is fixed
- I have been paid to lecture
  - ESHRE, ASRM, ECE, ACE, EAA, ASA, ESHG, PFCP, MSD, Merck

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Spinoza (1677) Ethica ordine geometrico demonstrata

Learning objectives

- To understand the value of morphological selection
- To understand the basis of and evidence for time-lapse
  - What is really different?
  - What does it really add?
- To understand the importance of any selection method
  - Revolution of cryopreservation
  - Freeze-all strategies

am center for reproductive medicine

Embryo selection rationale

- To achieve the best possible live birth rates after IVF, while minimizing the risk for multiple pregnancy, one or two embryos that are considered to have the best chance of implanting are selected for transfer

Alpha/ESHRE, Istanbul consensus workshop, HR (2011)

Morphology

- Most commonly used method for embryo selection is morphological evaluation
- Multiple morphological characteristics at one or several stages of preimplantation development

Alpha/ESHRE, Istanbul consensus workshop, HR (2011)

Morphology

- Timing of observation
- Oocyt scoring
  - COC, ZP, PB
- Fertilization check
  - PN scoring
- Cleavage stage embryos
  - Cell number, fragmentation, multinucleation, cell size
- Morula stage
  - Compaction, number of excluded cells
- Blastocyst stage
  - Stage of development, ICM, TE

Table VII Consensus scoring system for Day-4 embryos.

Grade	Rating	Description
1	Good	• Entered into a fourth round of cleavage. • Evidence of compaction that involves virtually all the embryo volume.
2	Fair	• Entered into a fourth round of cleavage. • Compaction involves the majority of the volume of the embryo
3	Poor	• Disproportionate compaction involving less than half of the embryo, with two or three cells remaining as discrete blastomeres

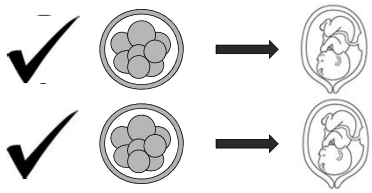
Alpha/ESHRE, Istanbul consensus workshop, HR (2011)

Prediction model

- All IVF/ICSI cycles
  - Cycles with TESE / MESA / HIV-1 positive patients excluded
- Center for Reproductive Medicine, Amsterdam, the Netherlands
- Development set: January 2004 and July 2009
  - 5,028 embryos with exact traceability
- Validation set: August 2009 and September 2011
  - 2,617 embryos with exact traceability

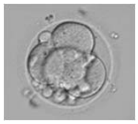
Methods

- Only cycles with exact traceability of embryos



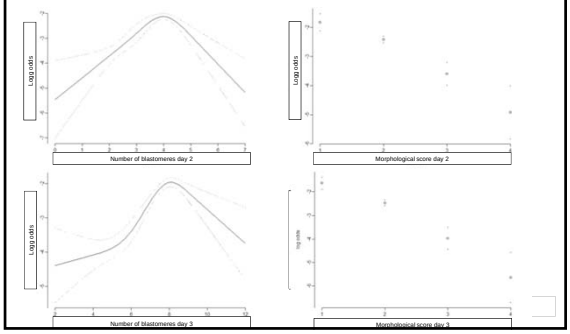
Scoring embryo predictors

- Pronuclear scoring was performed 17-22 hrs
- Early cleavage was scored 23-28 hrs
  - Number of blastomeres on day 2 and 3
- Morphological score on day 2 and 3
  - Score 1: no fragmentation
  - Score 2: <20% fragmentation
  - Score 3: <20-50% fragmentation
  - Score 4: > 50% fragmentation
  - Non-uniform blastomere size: score was augmented with 1
- On day 3 compaction: morula
  - Score 1: full compaction
  - Score 2: 50 -<100% compaction
  - Score 3: < 50% compaction



Puissant et al., HR (1987)

Results – Continuous variables



Results – Univariable analysis

	β	OR	95% CI	P-value
Pronuclear score				0.26
2 PN (reference)				
1 PN	-1.25	0.28	(0.07-1.17)	0.08
0 PN	-0.18	0.84	(0.55-1.28)	0.41
Unknown	-1.15	0.32	(0.04-2.32)	0.26
2-blastomeres	0.48	1.61	(0.37-7.07)	0.53
Early cleavage	0.65	1.92	(1.42-2.59)	0.00
Number of blastomeres day 2	0.24	1.28	(1.15-1.41)	0.00
Number of blastomeres day 3	0.32	1.37	(1.28-1.47)	0.00
Morulae	0.32	1.37	(0.84-2.23)	0.20
Morphological score day 2	-0.83	0.04	(0.35-0.54)	0.00
Morphological score day 3*	-1.05	0.35	(0.28-0.43)	0.00
Progression from 4 blastomeres on day 2 to 8 blastomeres on day 3	1.28	3.59	(2.89-4.45)	0.00

Embryo selection model

Predictors	Embryo Score
Intercept	103
Early cleavage	2
Number of blastomeres on day 2 calculated as: value of the deviation from 4	-3
Number of blastomeres on day 3 calculated as: value of the deviation from 8	-3
Morphological score day 3	-5
Morula on day 3	-11

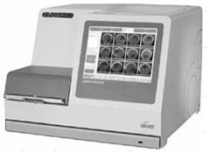
van Loendersloot, et al., RBM Online (2014) in press

Ranking embryos

	Early cleavage	Number of blastomeres day 2†	Number of blastomeres day 3‡	Morphological score day 3	Morula on day 3	Total score
Embryo 1	no	2	3	3	no	67
Embryo 2	no	3	5	3	no	76
Embryo 3	no	4	12	2	no	81
Embryo 4	no	5	7	2	no	87
Embryo 5	no	3	NA	NA	yes	89
Embryo 6	yes	4	9	2	no	92
Embryo 7	no	4	8	2	no	93
Embryo 8	yes	4	NA	NA	yes	94
Embryo 9	no	4	8	1	no	98
Embryo 10	yes	4	8	1	no	100

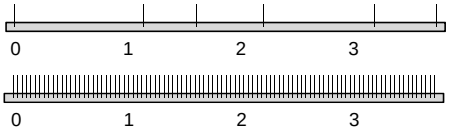
van Loendersloot, et al., RBM Online (2014) in press

Time-lapse



Time-lapse….

- What is different?
  - Continuous monitoring (video)

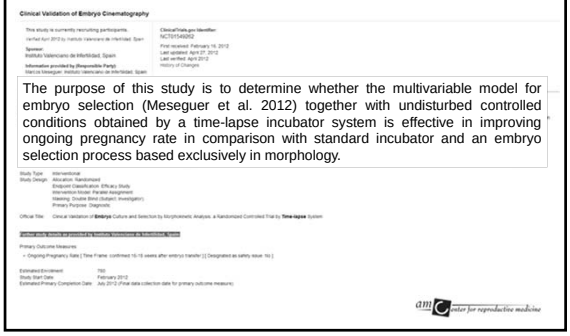


- But also
  - Different incubator
  - No removal from incubator
  - Different container / dish

Time-lapse

- Is it really the time-lapse versus the multiple time-point analysis?
  - RCT
    - Culture in time lapse machine with time-lapse selection
    - Culture in time lapse machine with multiple time-point selection
- No trial conducted
- Ongoing trials compare BOTH time-lapse and the overall culture procedure
  - Incubator, number of times removed from incubator, environment oustide the incubator, the culture dish

Embryoscope

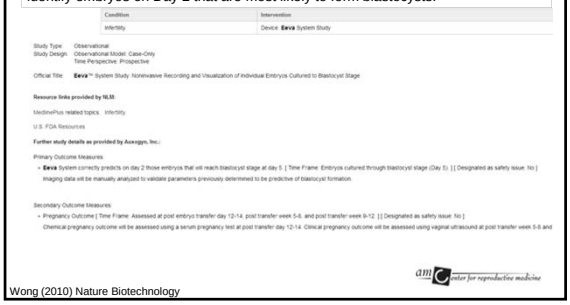


Clinical Validation of Embryo Cinematography

The purpose of this study is to determine whether the multivariable model for embryo selection (Meseguer et al. 2012) together with undisturbed controlled conditions obtained by a time-lapse incubator system is effective in improving ongoing pregnancy rate in comparison with standard incubator and an embryo selection process based exclusively in morphology.

EEVA

The purpose of this study is to demonstrate that the Eeva System may be used to identify embryos on Day 2 that are most likely to form blastocysts.



Condition	Intervention
Infertility	Device: **Eeva** System Study

Study Type: Observational
Study Design: Observational Model: Case-Only
Time Perspective: Prospective

Official Title: **Eeva**™ System Study: Noninvasive Recording and Visualization of Individual Embryos Cultured to Blastocyst Stage

Resource links provided by NLM:

MedlinePlus related topics: Infertility

U.S. FDA Resources

Further study details as provided by Auxogyn, Inc.:

Primary Outcome Measures:

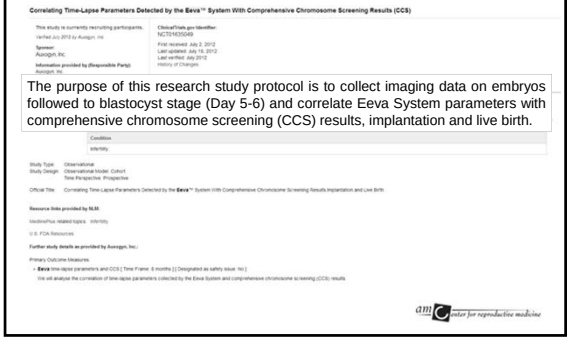
- **Eeva** System correctly predicts on day 2 those embryos that will reach blastocyst stage at day 5. [Time Frame: Embryos cultured through blastocyst stage (Day 5).] [Designated as safety issue: No]
  Imaging data will be manually analyzed to validate parameters previously determined to be predictive of blastocyst formation.

Secondary Outcome Measures:

- Pregnancy Outcome [Time Frame: Assessed at post embryo transfer day 12-14, post transfer week 5-8, and post transfer week 9-12.] [Designated as safety issue: No]
  Chemical pregnancy outcome will be assessed using a serum pregnancy test at post transfer day 12-14. Clinical pregnancy outcome will be assessed using vaginal ultrasound at post transfer week 5-8 and

Wong (2010) Nature Biotechnology

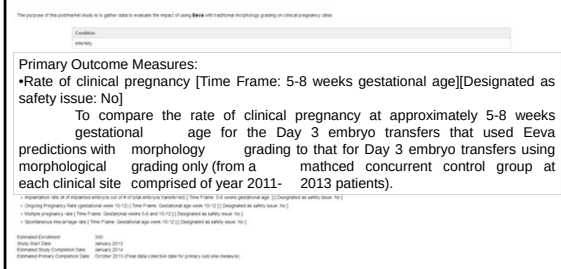
EEVA



Correlating Time-Lapse Parameters Detected by the Eeva™ System With Comprehensive Chromosome Screening Results (CCS)

The purpose of this research study protocol is to collect imaging data on embryos followed to blastocyst stage (Day 5-6) and correlate Eeva System parameters with comprehensive chromosome screening (CCS) results, implantation and live birth.

EEVA



Primary Outcome Measures:

•Rate of clinical pregnancy [Time Frame: 5-8 weeks gestational age][Designated as safety issue: No]

To compare the rate of clinical pregnancy at approximately 5-8 weeks gestational age for the Day 3 embryo transfers that used Eeva predictions with morphology grading to that for Day 3 embryo transfers using morphological grading only (from a mathced concurrent control group at each clinical site comprised of year 2011-2013 patients).

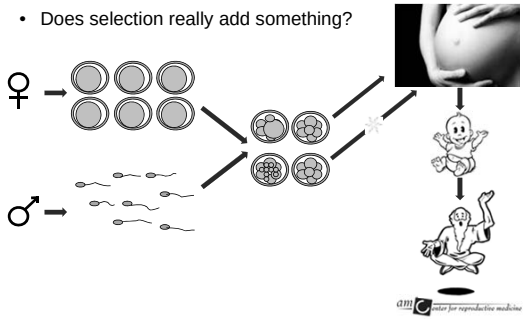
Who wants this?

- The professional
  - Improved results
  - Looks cool
- The patients
  - Improved results
  - Feels good
- The company
  - Improved results
  - More profit

Will selection ever work?

Selection

- Does selection really add something?



Selection

The embryological technician was asked to identify the morphologically best embryo in the treatment group to analyze the percentage of patients where another embryo would have been transferred when selection was based on morphology plus the viability score instead of morphology alone. The embryological technician was unaware of the viability scores of the embryos. In 75.4% (104 of 138) of the transfers, the embryo with the best morphology did not have the highest viability score. The live birth rate in this group of 104 embryos (30.8%) was not significantly different from the live birth rate in the control group (31.3%). This strongly suggests that within a group of good quality embryos, there is more than one embryo able to develop into an ongoing pregnancy. Therefore, to avoid multiple pregnancies,

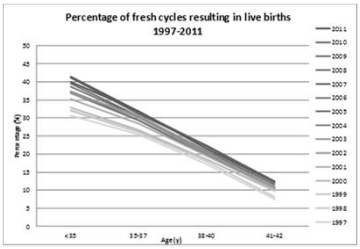
Vergouw, et al., HR (2012)

Is selection the way forward?

- History embryo selection
  - Low success / multiple embryos
  - Increased success / fewer embryos
- Selection necessary
  - reduced success after cryopreservation
- Recent developments challenge this concept
  - Improved cryopreservation
  - Endometrial receptivity affected by hyperstimulation
    - In natural cycles increased endometrial quality
    - Could counterbalance negative effect of cryopreservation

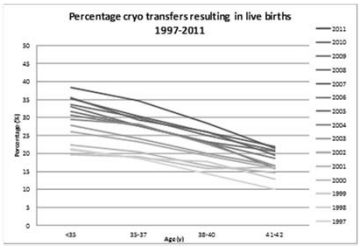
Paulson, et al., F&S (1990), Check, et al., JARG (1999), Shapiro, et al., F&S (2008)

Improved cryopreservation



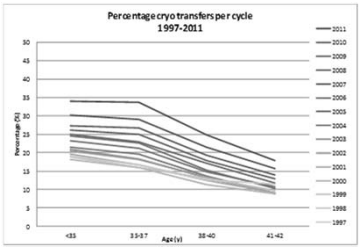
Data derived from SART (http://www.cdc.gov/art/)

Improved cryopreservation



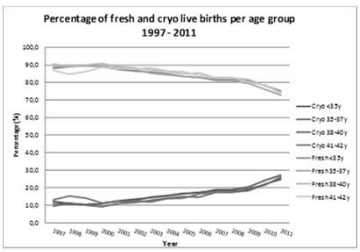
Data derived from SART (http://www.cdc.gov/art/)

Improved cryopreservation



Data derived from SART (http://www.cdc.gov/art/)

Improved cryopreservation



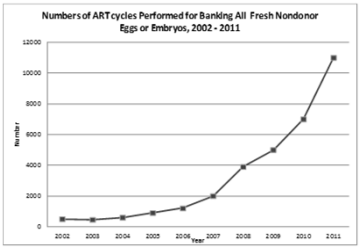
Data derived from SART (http://www.cdc.gov/art/)

Is selection the way forward?

- Cycles with disengagement of transfer (freeze-all)
  - egg donation / OHSS
  - comparable / increased pregnancy rates
  - using slow freezing protocols
- Even better with vitrification
  - Higher pregnancy rates after vitrification in comparison with slow freezing
- First RCTs promising
  - Higher pregnancy rates after IVF without fresh transfer compared to IVF with fresh transfer
    - High responders
    - Normal and high responders, different protocol

Abdelhafez ,et al., RBMO (2010), Aflatoonian, et al., JARG (2010)
Shapiro, et al., F&S (2011), Mastenbroek, et al., HR (2011)

Freeze-all strategies



Data derived from SART (http://www.cdc.gov/art/)

Is selection the way forward?

- Future…
  - Cryopreservation of all embryos
    - No fresh transfer
  - Transfer in subsequent natural cycles
- Selection methods
    - Will not improve overall pregnancy rates
      - If not 100% accurate → decrease pregnancy rates
        - » Holds for all current selection techniques
        - » See the PGS saga….
    - Might (at best) improve time to pregnancy
      - Earlier transfer of best embryos

Mastenbroek, et al., HR (2011)

Time Lapse Monitoring of Human Embryos: A Clinicians Perspective

Professor Charles Kingsland MD FRCOG
Liverpool Women's Hospital
ESHRE Munich 2014

Commercial Relationships

- Medical Advisor – Smiths Industries

The Hewitt Fertility Centre

- Largest of its kind in Europe (over 2000 cycles pa)
- State-of-the-art facility
- 3/4 state-funded; 1/4 private
- 14 Clinical Embryologists (& 7 Biomedical Andrologists)
- 10 Consultant Clinicians

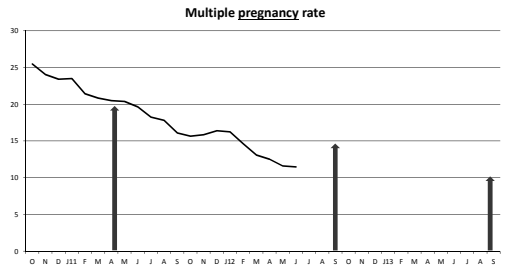
Our ethos ...

- Evidence-based medicine
- Less ‘commercial’
- Open to new ideas
- Approach by Auxogyn

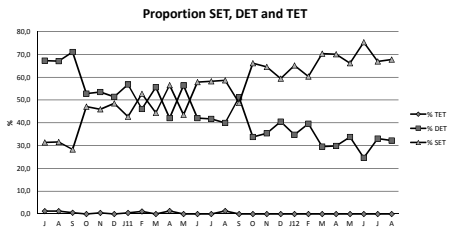
Regulation ...

- Human Fertilisation & Embryology Authority
- Multiple Birth Minimisation Strategy

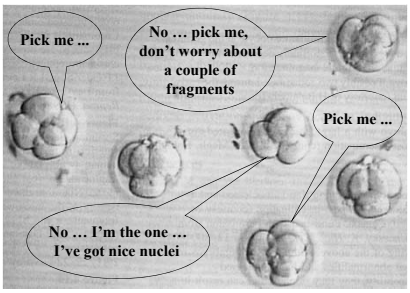
UK multiple birth rate targets



Increasing elective SET ...



↑ ↑ embryological responsibility ...

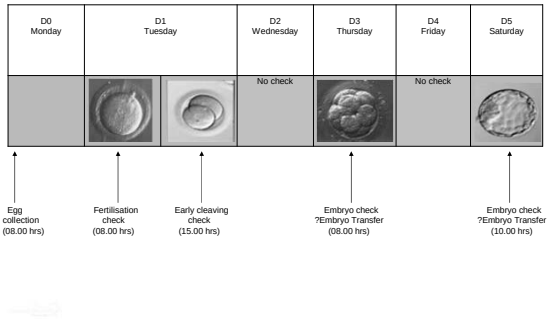


The (not too distant) future ...

- The 'omics
  - Transcriptomics / Genomics
  - Proteomics
  - Metabolomics
  - Secretomics
- Robotic ICSI & embryo biopsy

Conventional observations ...

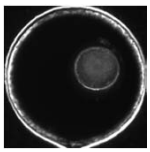
Conventional embryo observations ...



Comparison of time-lapse systems ...

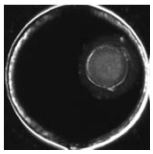
	EmbryoScope™	PRIMO Vision	Eeva™
Company	Unisense® Fertilitech	Vitrolife/ Cryo Management Ltd	Auxogyn Inc
Capacity (patients/embryos per patient)	6/12	User-defined/16	User-defined/20
Equipment	Incubator and image capture	Image capture	Image capture and real-time prediction
Image capture frequency	User-defined (10 or 20 mins)	User-defined (minimum 5 mins)	5 mins
Culture	Single	Group	Group
Image analysis	Manual	Manual	Automated
Prediction	NO	NO	YES
Reproducibility between clinics	?	?	YES

Eeva™ Imaging and Image Analysis



HIGH probability to form a blastocyst if:

P2, time from 2-cell to 3-cell: **9.3 to 11.5 hrs**
P3, time from 3-cell to 4-cell: **0 to 1.7 hrs**



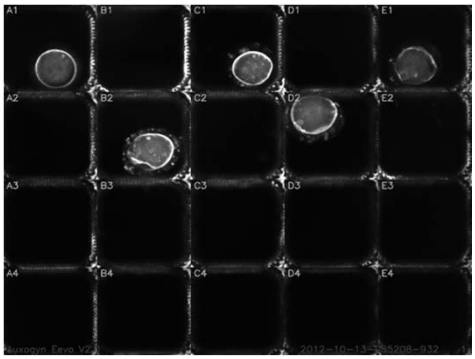
- **LOW** probability to form a blastocyst if:

  P2 or **P3** were out of range

City-born miracle baby is a world first

Tot is born with new IVF technology

ECHO



Case report 1

(IVF age 34)

Day 3 morphology	Conventional plan	Eeva prediction	Day 5 morphology	Conventional morphology alone	Adjunct use of Eeva
13c 4/4 (good) 7c 3/3 (good) 8c 3/3 (good) 7c 2/3 (borderline) 5c 2/2 (poor) 6c 2/2 (poor) 6c 3/2 (borderline) 4c 2/4 (poor) 5c 3/2 (poor)	Culture all embryos to day 5	High Borderline Borderline Borderline Borderline Borderline Low Borderline Borderline	4 C/b (borderline) 2 C/b (borderline) 2 E/b (poor) 2 B/b (good) CM CM 3 C/c (poor) CM Nec	Transfer	Transfer

How did Eeva change clinical practice?

1 x Eeva high embryo → Day 5 transfer of a Eeva high / slightly 'poorer' morphology blastocyst → 1 x FH

Case report 2

(IVF age 36)

Day 3 morphology	Conventional plan	Eeva prediction	Day 5 morphology	Conventional morphology alone	Adjunct use of Eeva
10c 3/3 (good) 8c 4/4 (good) 5c 2/2 (poor) 7c 2/3 (borderline) 9c 2/3 (borderline) 8c 3/3 (good) 8c 3/3 (good)	Culture all embryos to day 5	High Low Low Low Low Low Low	4 C/b (borderline) 5 C/c (borderline) CM (poor) 8c 2/3 (poor) 3 C/c (poor) 8c 2/3 (poor) CM(poor)	Select embryo for eSET	Transfer

How did Eeva change clinical practice?

1 x Eeva high embryo → Day 5 transfer of 1 x Eeva high/discounting 1 x similar morphology blastocyst on Day 5 → 1 x FH

Case report 3

(IVF age 33)

Day 3 morphology	Conventional plan	Eeva prediction	Adjunct use of Eeva
10c 3/3 (good) 8c 3/3 (good) 7c 2/2 (poor) 7c 2/3 (poor)	Select embryo for eSET day 3*	Borderline High Low Borderline	Transfer

How did Eeva change clinical practice?

Provided confidence to perform day 3 eSET

**** SOP requires ≥ 3 x good quality embryos on day 3 for extended culture***

Case report 4

(IVF age 31)

Day 3 morphology	Conventional plan	Eeva prediction	Day 5 morphology	Adjunct use of Eeva
8c 3/3 (good) 8c 2/2 (poor) 7c 2/2 (poor) 6c 3/2 (poor)	Transfer on day 3* Disc Disc Disc	Low Low Borderline Borderline	7c 3/3 (arrested) 3 A/b (good) 2 E/c (poor) M (poor)	Transfer

How did Eeva change clinical practice?

2 x Eeva borderline embryos → Day 5 transfer of a 'different' embryo → Preg

**** SOP requires ≥ 3 x good quality embryos on day 3 for extended culture***

Day Five Transfers

Elective single embryo transfers

D5 per eSET (<35)		%
No. patients	82	
Bio	57	**69.5**
Clin	47	**57.3**
FH	47	
IR	82	**57.3**

D5 per eSET (40yrs)		%
No. patients	3	
Bio	0	**0.0**
Clin	0	**0.0**
FH	0	
IR	0	**0.0**

D5 per eSET (35-39)		%
No. patients	46	
Bio	30	**65.2**
Clin	25	**54.3**
FH	25	
IR	46	**54.3**

D5 per eSET (all ages)		%
No. patients	131	
Bio	87	**66.4**
Clin	72	**55.0**
FH	72	
IR	131	**55.0**

Lessons learned ...

- Back-up systems
  - Already installed!
  - But worst case = conventional incubator
- Dish handling
  - Vibration
  - New dish design
- Patients assume ££ or $$ = deliverable

Informing patients ...

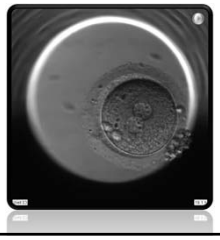
Patients like .....

- Usage = 15-20% of patients
  - Both private and NHS patients pay
  - £750/£800
- Now price inclusive
- Understand undisturbed culture
- Like technology
  - Sign of the times
- To talk to embryologists
- Like images and videos
  - Memory sticks
- Request for day 3 ET
  - Concerns about blastocyst transfer

Facilitating the Doctor- patient interaction

Patient consultation

- Introducing embryo culture and development
- View patient´s embryos prior to embryo transfer



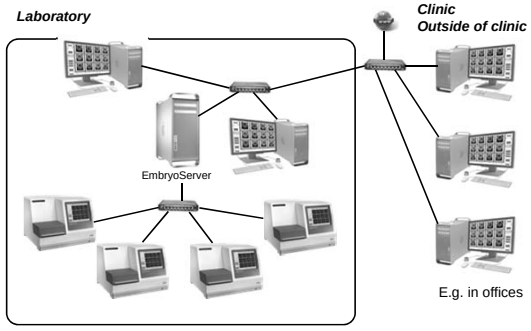
Patients don't like .....

- When the machines are full
- Making decisions (ES vs Eeva)
- Technology failing
- Computers making decisions

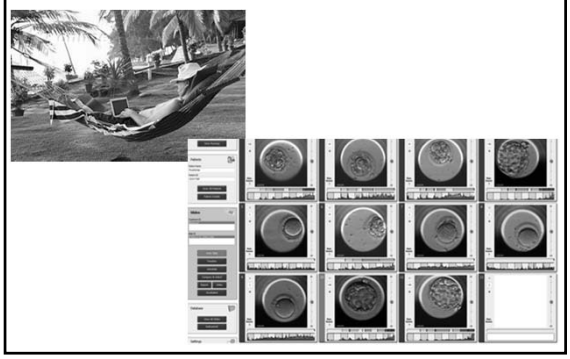
A Clinicians View Summary

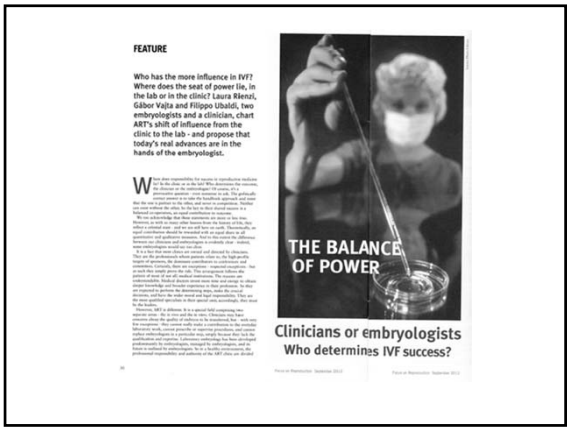
- Patients Get it
- Does no harm
- Brings Embryology into the clinic
- De mystifies embryology
- 100% Time Lapse

Remote office: Full access in clinic or even outside



Remote office: Expert opinion




FEATURE
Who has the more influence in IVF? Where does the seat of power lie, in the lab or in the clinic? Laura Rienzi, Gábor Vajta and Filippo Ubaldi, two embryologists and a clinician, chart ART's shift of influence from the clinic to the lab - and propose that today's real advances are in the hands of the embryologist.
THE BALANCE OF POWER
Clinicians or embryologists
Who determines IVF success?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