EARLY EMBRYO VIABILITY ASSESSMENT OF HUMAN EMBRYOS FOR TRANSFER

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Fertility academe
Personalized medical education

Disclosures

- NOTHING TO DISCLOSE

The views expressed in the presentation are those of the presenter and not necessarily that of Merck.

This presentation may contain unregistered indications or products for medical education purposes only.

Learning objective

- After this presentation participants should be able to:

  - discuss the importance of developing and validating novel technologies for embryo monitoring as to improve outcomes in ART.
  - discuss the current situation of embryo assessment in ART.
  - be updated on the clinical outcomes obtained by the use of embryo monitoring technologies.

Trying to find the best embryo

Assessment of embryo viability

- Improve implantation
- Decrease miscarriage rates
- Decrease risk of aneuploid offspring

Time-lapse: the remaining questions to be answered

In 2009, the first automatic time-lapse devices became commercially available for the in vitro fertilization (IVF) units, moving from very primitive cinematography to a massive analysis of growing embryos in a few hours. From that point, embryologists started to question its utility and two major issues were raised:

- Are culture conditions improved by avoiding embryo manipulation outside the incubator?
- Are the variables related to timing of cleavages and dynamic changes in morphology useful markers of embryo viability (morphokinetics and morphology dynamics)?

This study is the first randomized controlled trial in which all embryos are similarly cultured in the closed EmbryoScope system. Although they did not reach significance, there were increased clinical pregnancy and implantation rates with the use of the additional parameters. These facts are inspiring to us, since with a similar sample size as that used by Rubio et al. (4), results would be probably comparable. Unfortunately, resources and logistics needed for such a sample size are rarely available, and Cleveland Clinic performed a tremendous but insufficient effort to achieve that objective. In any case results are confident and I assume that Nina Desay and her team of embryologists will keep using time lapse technology as a routine clinical tool.

In addition, the authors confirmed that the time of the start of blastulation, the absence of multinucleation and the use of a score based on morphology were significant predictors of implantation. These are very relevant findings to our scientific community. Apart from these two main questions raised in this editorial, many other related as blastocyst, euploidy or implantation prediction have been extensively analyzed by retrospective designed studies.

The first question was elegantly answered by Wong et al. (1) and further detailed and clinically applied by our group (2). Both papers defined selection and deselection parameters based on optimal time-ranges of the embryo cell cycle and abnormal cleavage patterns. Wong's algorithm is commercially available as a diagnostic test and ours has been widely applied in IVI clinics, actually we have the largest number of cycles reported by time-lapse.

An attempt to answer the second question was initially made by Cruz et al. through a sibling oocytes study on recipients for oocyte donation. An additional investigation undertaken by Park et al. in a randomized controlled trial (RCT) attempted to calculate the effect of undisturbed culture conditions provided by the EmbryoScope in comparison with the standard incubator. Both studies were properly designed but failed to have adequate power (sample size) to demonstrate a non-inferiority or equivalent hypothesis and/or a subtle or moderate effect (that should be expected from an improved embryo culture environment) reviewed by Basile et al. (3).

The only properly powered RCT (focused on time-lapse) was achieved by Rubio et al. (4), in which more than 800 patients were enrolled to demonstrate that a time-lapse strategy (combined culture conditions and morphokinetic embryo selection) is better than box incubators combined with single point morphological observation. Although it was clearly reflected that time-lapse strategy is improving our clinical results, many questions remained unreturned, as the project was unable to demonstrate whether the improvement were from the un-

The remaining questions are: is blastocyst prediction clinically useful; is there any morphokinetic algorithm available for all time-lapse devices and clinics; can it be sold as a diagnostic test; regarding embryo euploidy, can it be forecast by a non-invasive morphokinetic test; and is time-lapse useful for selecting euploid embryos? For all these questions, no prospective evidence is published yet, and may never be, as the logistics and resources needed would make it impossible to undergo such kind of research. Embryology is becoming more exciting every year, time-lapse is significantly contributing to increase our knowledge in the field, and I firmly believe that this technology is here to stay.

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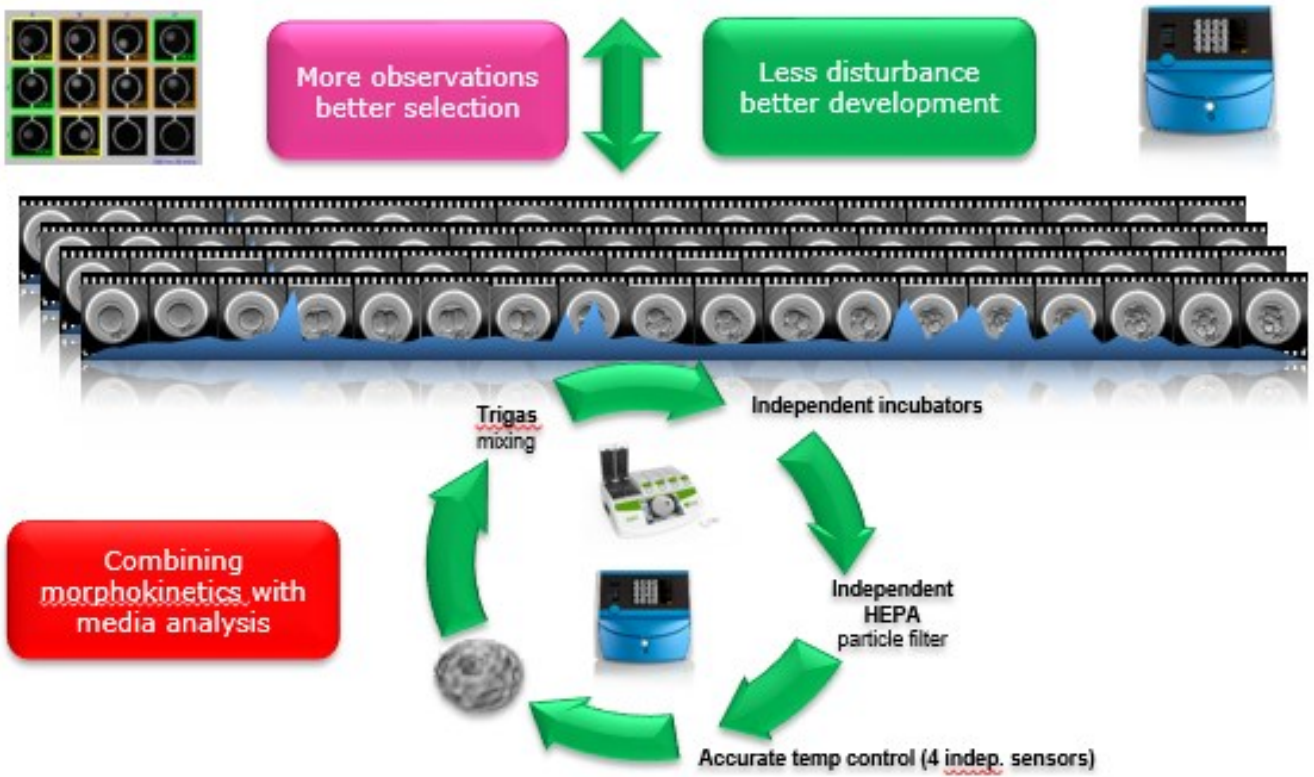
You can discuss this article with its authors and with other ASRM members at
http://fertstertforum.com/meseguerm-time-lapse-remaining-questions/

Why time-lapse?

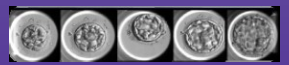


HEPA, high efficiency particulate air.

Zaninovic N, et al. Fertil Steril. 2017;108:722-9.

Quality of the data in embryology

RESULTS FROM QUALITY-CONTROL ASSESSMENT IN MORPHOLOGY
50 BLASTOCYST 20 EMBRYOLOGIST

Kappa index: grade of expansion  **0.56 (95% CI 0.50–0.61)**

Kappa index: ASEBIR ASEBIR Asociación para el Estudio de la Biología de la Reproducción **0.51 (95% CI 0.44–0.57)**

Kappa index: embryo viability **0.58 (95% CI 0.53–0.63)**

0.40–0.60 MODERATE AGREEMENT (NOT GOOD 0.6-0-0.8, NOT VERY GOOD)

ASEBIR, Asociación para el Estudio de la Biologia de la Reproducción; CI, confidence interval.

Quality of the data: results from QC assessment in morphokinetics

Objective variables

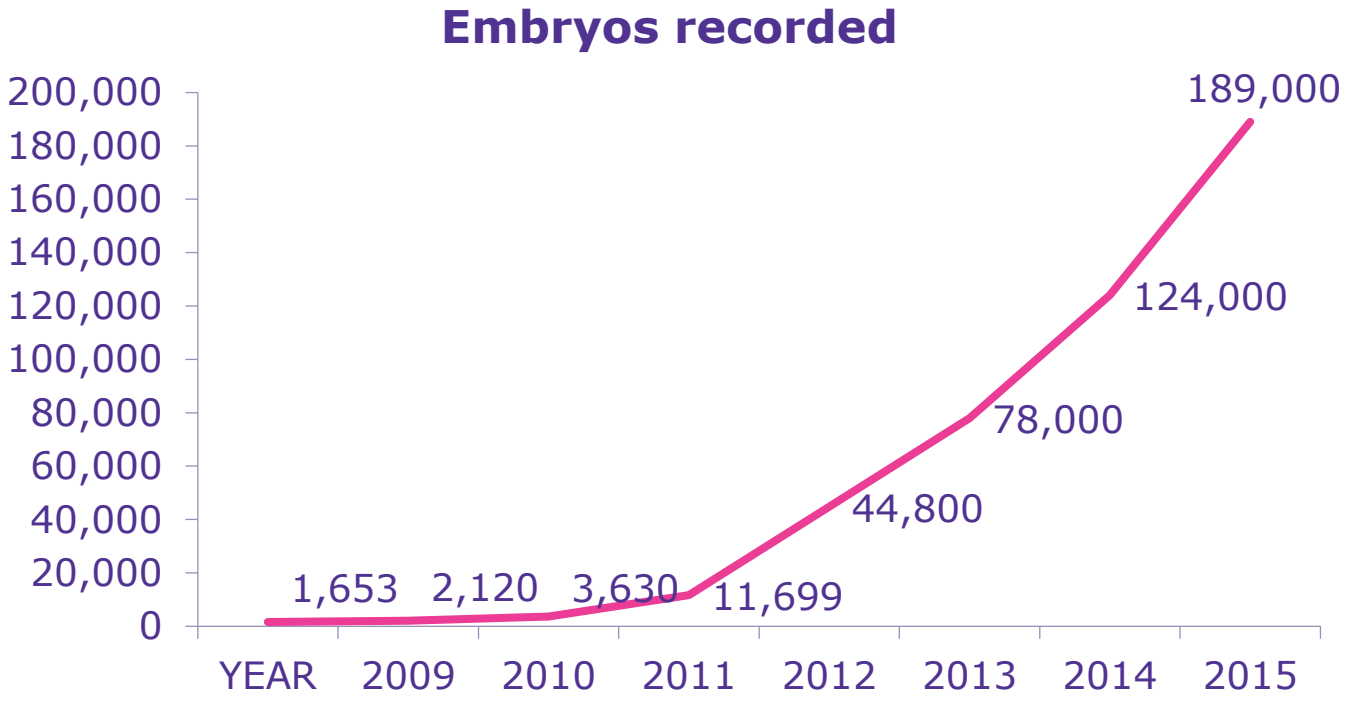
Interobserver and intraobserver agreement for assessed parameters

Parameter	Inter ICC	N	95% CI
PN appearance	0.678	113	0.568–0.764
PN abuttal	0.773	90	0.692–0.838
PN breakdown	0.999	130	0.998–0.999
First cytokines	0.92	138	0.895–0.94
1st nuclei appearance	0.984	90	0.977–0.989
2nd nuclei appearance	0.967	78	0.953–0.978
1st nuclei disappearance	0.989	86	0.985–0.993
2nd nuclei disappearance	0.75	75	0.66–0.824
1st cleavage division	0.958	134	0.945–0.969
2nd cleavage division	0.885	133	0.85–0.913
3rd cleavage division	0.809	141	0.756–0.853
4th cleavage division	0.863	125	0.821–0.897
5th cleavage division	0.868	123	0.826–0.901
6th cleavage division	0.906	99	0.872–0.933
7th cleavage division	0.9	68	0.852–0.935
Final division	0.599	82	0.336–0.757
Compaction	0.686	114	0.581–0.769
Morula	0.737	111	0.642–0.81
Early blastocyst	0.894	114	0.8–0.939
Full expanded blastocyst	0.913	116	0.869–0.941
Hatching blastocyst	0.872	75	0.816–0.914
Hatched blastocyst	0.99	11	0.972–0.997
Blastocoel contractions	0.653	75	0.536–0.752

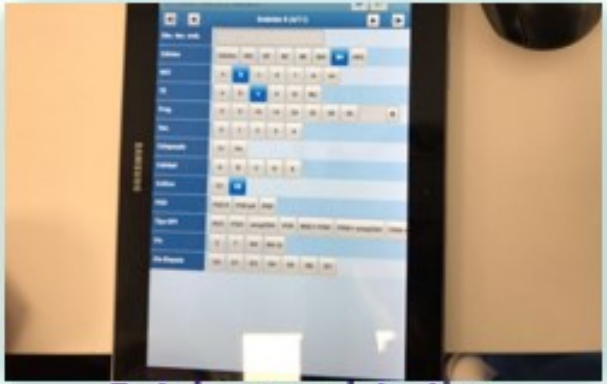
ICC, intra-class correlation coefficient; PN, pronuclei.

Sundvall L, et al. Hum Reprod. 2013;28:3215-21.

Evolution of data; exponential growth



Exponential growth of time required for data acquisition/annotation…



Technology solutions: automatic algorithm-based analysis – Eeva™

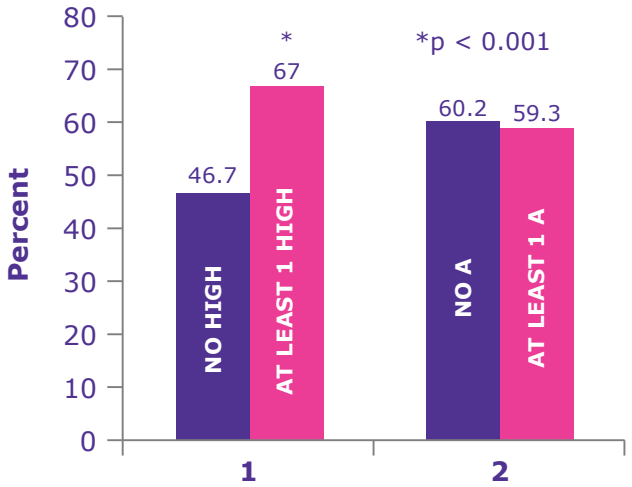
Logistic regression analysis for pregnancy rates according to Eeva™ and ASEBIR Day 3 categories of the transferred embryos

	OR	95% CI	p value
At least 1 Eeva™ high transferred embryo	2.567	1.302–5.062	0.05
At least 1 ASEBIR A transferred embryo	0.563	0.268–1.182	NS
Transfer D3/D5	0.795	0.244–2.597	NS

Automatic time-lapse instrument is superior to single-point morphology observation for selecting viable embryos: retrospective study in oocyte donation

Belén Aparicio-Ruiz, Ph.D.,[a] Natalia Basile, Ph.D.,[b] Sonia Pérez Albalá, Ph.D.,[a] Fernando Bronet, Ph.D.,[b] José Remohí, M.D.,[a] and Marcos Meseguer, Ph.D.[a]

[a] Instituto Valenciano de Infertilidad (IVI) Valencia, INCLIVA-Universidad de Valencia, Valencia; and [b] IVI Madrid, Madrid, Spain



(**1**) Ongoing pregnancy rate when at least 1 transferred embryo was high according to Eeva™ or none were high
(**2**) Ongoing pregnancy when at least 1 transferred embryo was rated A according to ASEBIR or none were rated A

Eeva™, early embryo viability assessment; NS, not significant; OR, odds ratio.

Aparicio-Ruiz B, et al. Fertil Steril. 2016;106:1379-85.

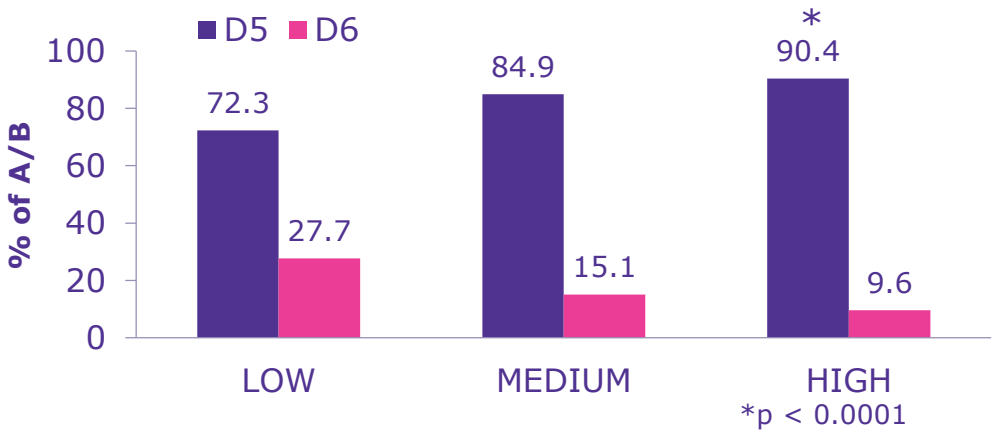
Regarding these viable blastocysts, we distinguished those with QUALITY A/B (ASEBIR)

We had a higher number of A/B blastocysts on Day 5 than on Day 6

Number of embryos (n) that were A/B blastocyst in each Eeva™ category on Day 5 or Day 6

Eeva™	D5	D6
LOW	219	84
MEDIUM	118	21
HIGH	122	13

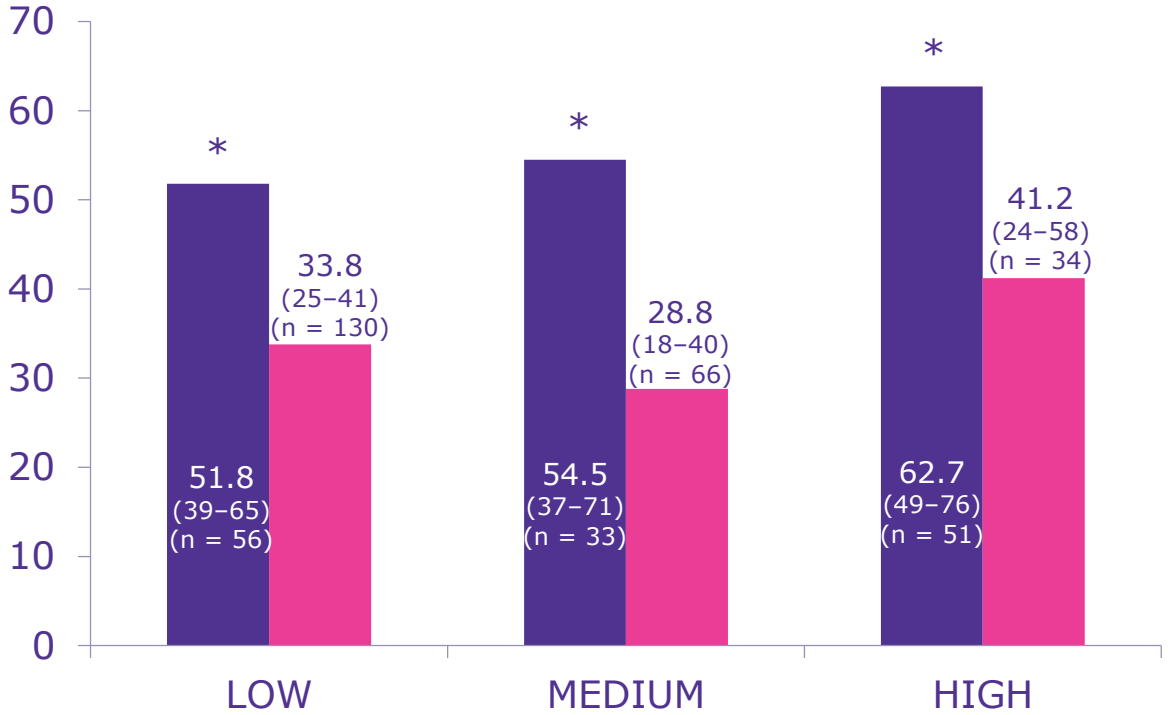
% of A/B on Days 5/6 according to each category



D, day.

Meseguer M, personal data.

KID + (%) according to Eeva™ category



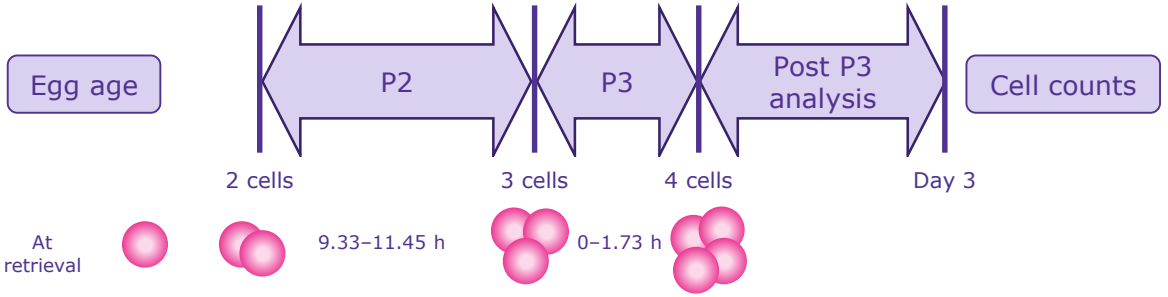
- Fresh embryos qualified as HIGH have higher implantation rates
- Implantation rates are correlated with Eeva™ category
- Inside each category, fresh transfers have higher implantation rates than frozen transfers
- Higher proportion of LOW and MEDIUM categories are present in frozen cases

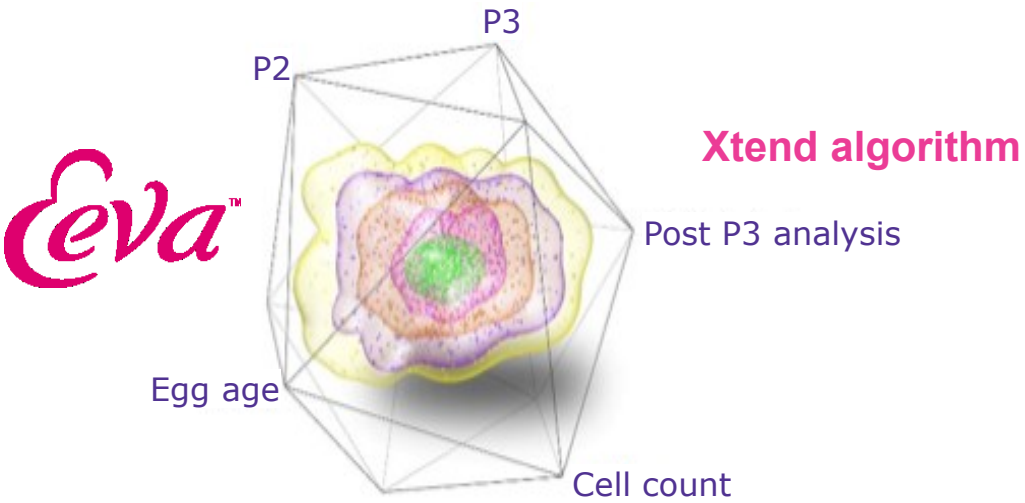
*$p < 0.05$

KID, known implantation data.

Meseguer M, personal data.

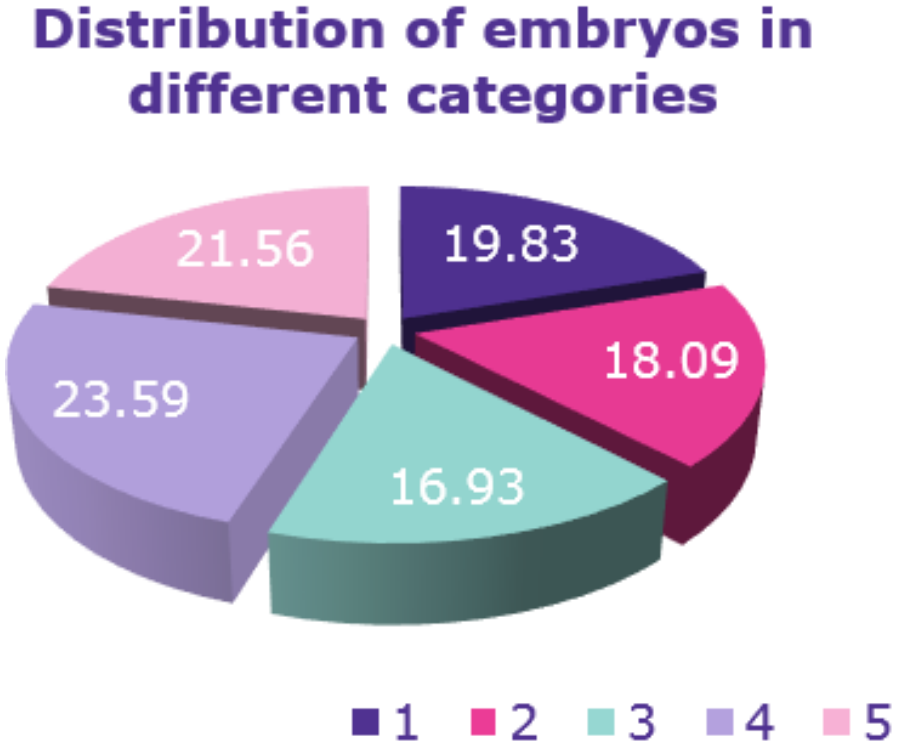
Technology solutions; automatic annotations computer analysis





Perez Albala S, et al. ESHRE. 2017;P-214.

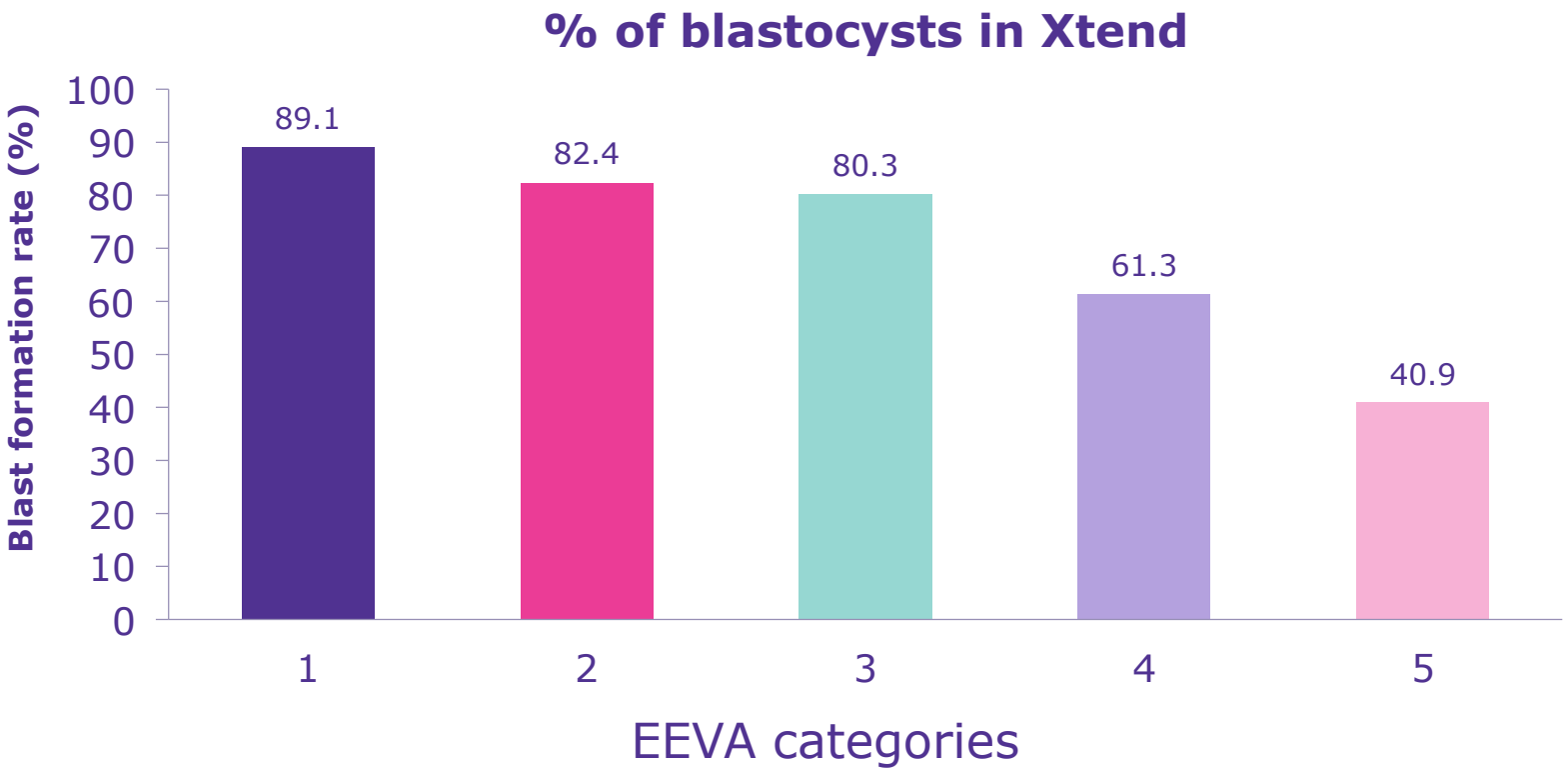
Xtend categories (1-5) embryo analysis



Perez Albala S, et al. ESHRE. 2017;P-214.

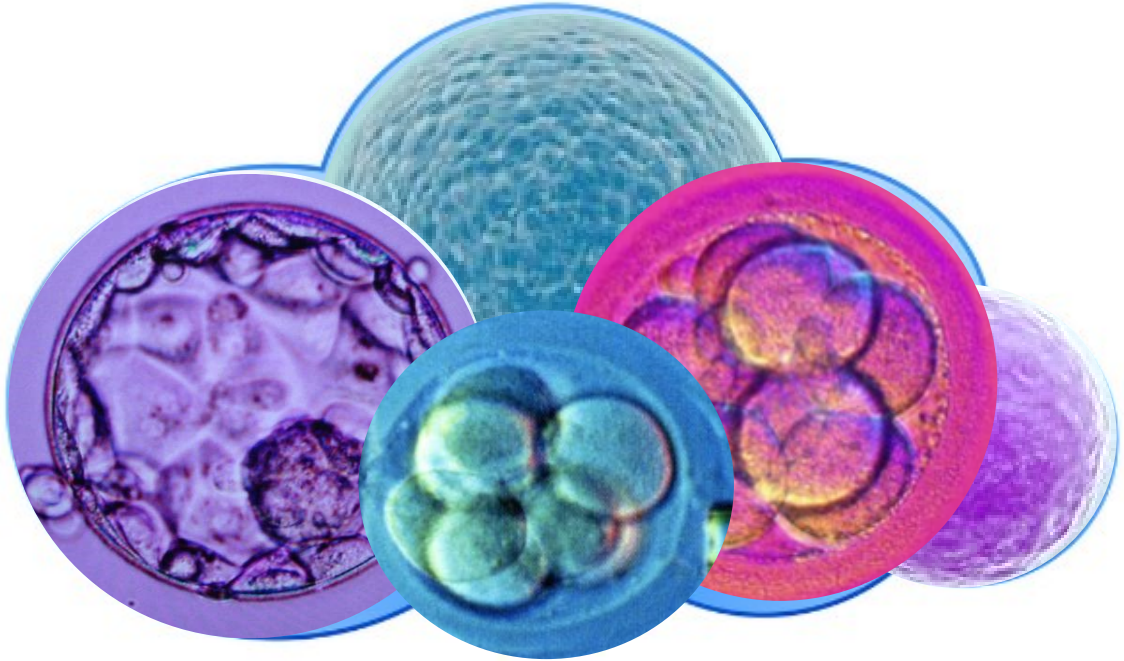
Xtend embryo analysis

More observations Better selection



Perez Albala S, et al. ESHRE. 2017;P-214.

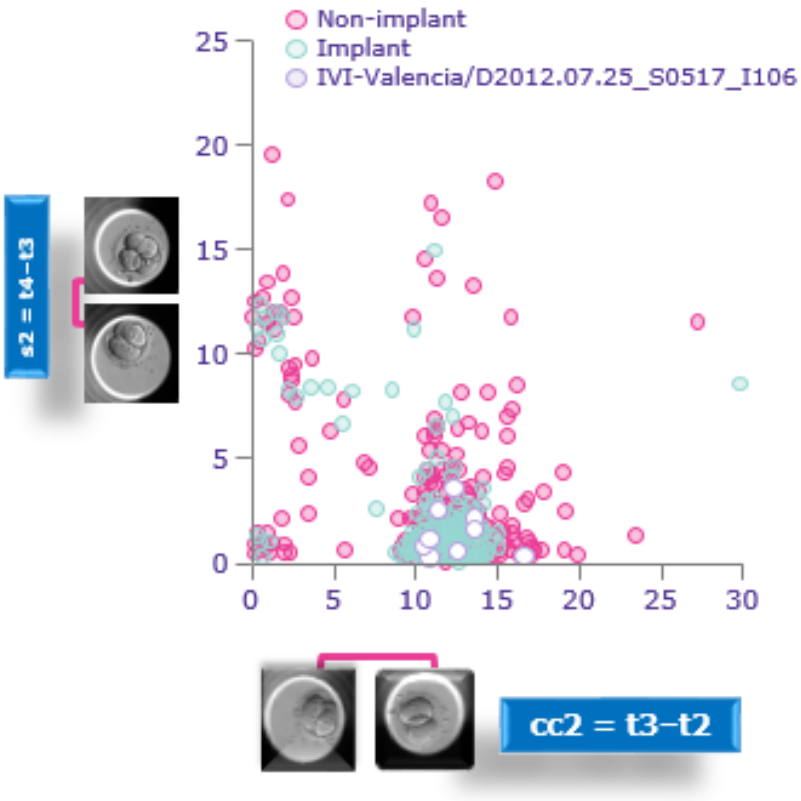
Technology solutions: the embryo cloud concept – DANA



Alegre L, et al. Fertil Steril. 2017;108:e239 (P-347).

The evolution of software analysis in TL:V1.0

More observations Better selection



Ranking

X-axis param: 2-cell→3-cell
Y-axis param: 3-cell→4-cell

Top rank	Avg dist (hr)
3	0.22
6	0.25
8	0.31
7	0.34
2	0.75
11	0.79
12	0.94
10	1.45
1	2.65
4 (no rank)	-
5 (no rank)	-
9 (no rank)	-
Bottom rank	

Note: selected parameters not measured for wells: 4, 5, and 9

Plot by
○ Clinic ◉ Outcome ○ Both

Outcome type
◉ Implant ○ Blastocyst ○ Transfer

X-axis
2-cell ▼ to 3-cell ▼

Y-axis
3-cell ▼ to 4-cell ▼

Filter by clinic Select ▼

Filter by outcome Select ▼

Filter by year Select ▼

Overlay my patient
Select clinic ▲

Marker size: < 6 >

Marker fill: < 20% >

DANA

Alegre L, et al. Fertil Steril. 2017;108:e239 (P-347).

The evolution of software analysis in TL:V1.0

More observations
Better selection

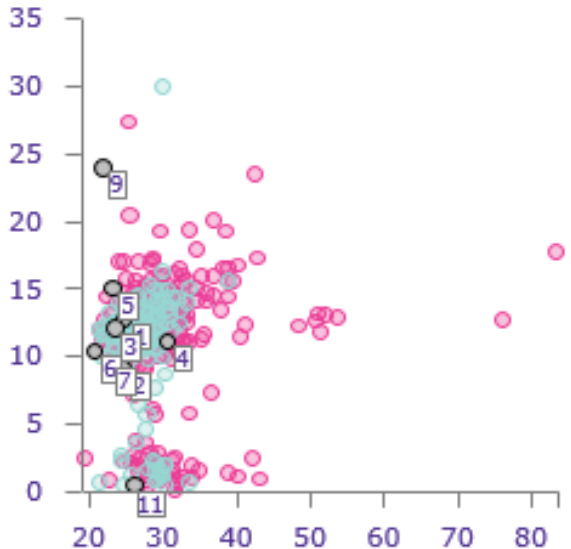
Phase 1 Retrospective	Phase 2 Prospective	Phase 3 Prospective Automatic
799 cycles	274 cycles	120 cycles
3,781 analysed embryos	> 409 analysed embryos	> 190 analysed embryos
1,676 transferred embryos	389 transferred embryos	147 transferred embryos
1,021 KID embryos	261 KID embryos	110 KID embryos

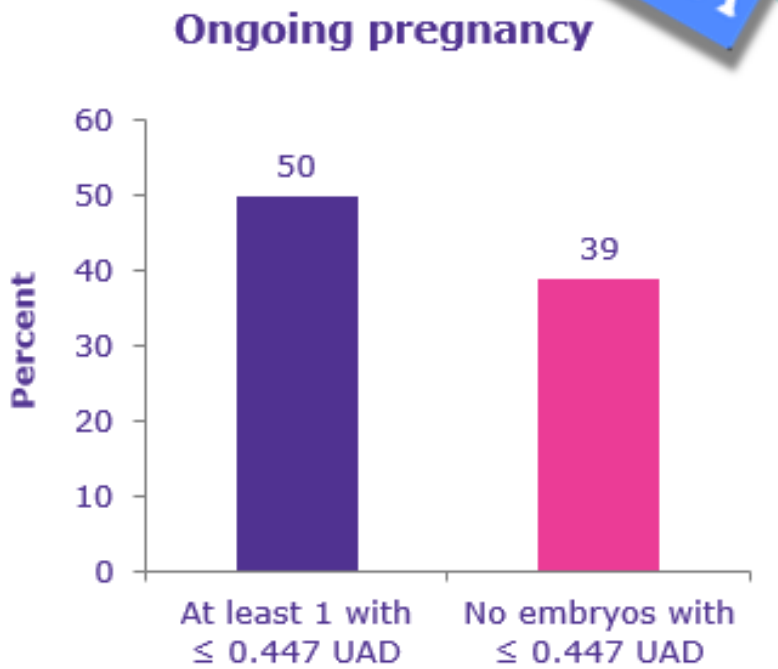
Alegre L, et al. Fertil Steril. 2017;108:e239 (P-347).

The evolution of software analysis in TL:V1.0

Phase 3
Prospective
Automatic

- 120 cycles
- > 190 analysed embryos
- 147 transferred embryos
- 110 KID embryos





UAD, unit average distance.

Alegre L, et al. Fertil Steril. 2017;108:e239 (P-347).

Es seguro | https://api.danaivf.com/demo

Candle 2.0 | DEMO | Marcos

Demo

UPLOAD A GENEA VIDEO TO CLASSIFY

Click here or drag file to upload videos (max 16)

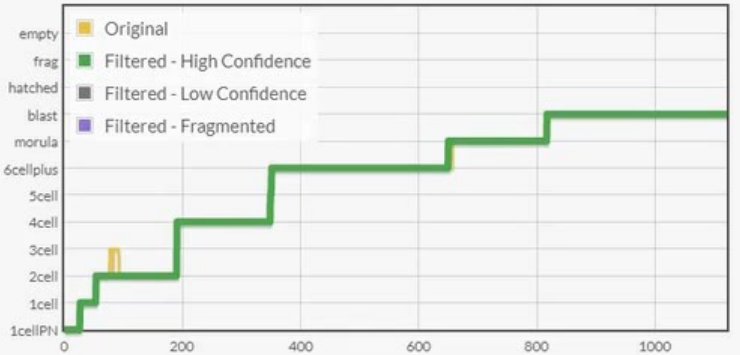
EXPERIMENT LIST

10

Date Uploaded	Video Name	Status	Save	Delete
2018-03-15 09:02:17	24603865_well11_zid05	queued		
2018-03-15 09:01:43	24603865_well10_zid05	queued		
2018-03-15 09:00:33	24603865_well09_zid05	queued		
2018-03-15 09:00:02	24603865_well07_zid05	queued		
2018-03-15 08:58:50	24603865_well06_zid05	queued		
2018-03-15 08:56:38	24603865_well03_zid05	57%		
2018-03-15 08:55:20	24603865_well02_zid05	analyzed		

ANALYSIS OUTPUT: 16001957_well06_zid07

Fragmented:	False
Reverse Cleavage or Failed Cytokinesis:	93
Direct Cleavage 1-3:	False
Direct Cleavage 2-5:	False
Number of Collapses:	1
Expanded Blast Frame:	994



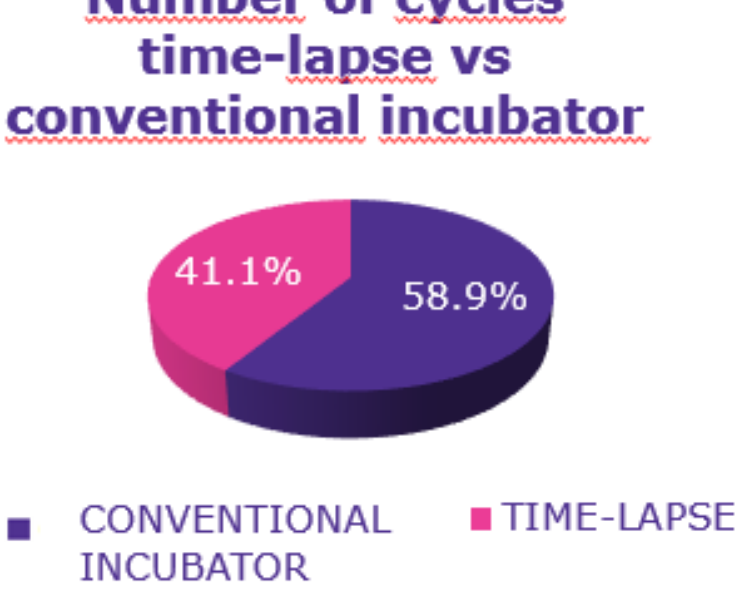
ezvid RECORDER PAUSE STOP DRAW

CLINICAL RESULTS JANUARY 2016–JUNE 2017

All blastocysts 90% SET

Standard incubator (no evaluation on cleavage stage) vs time-lapse

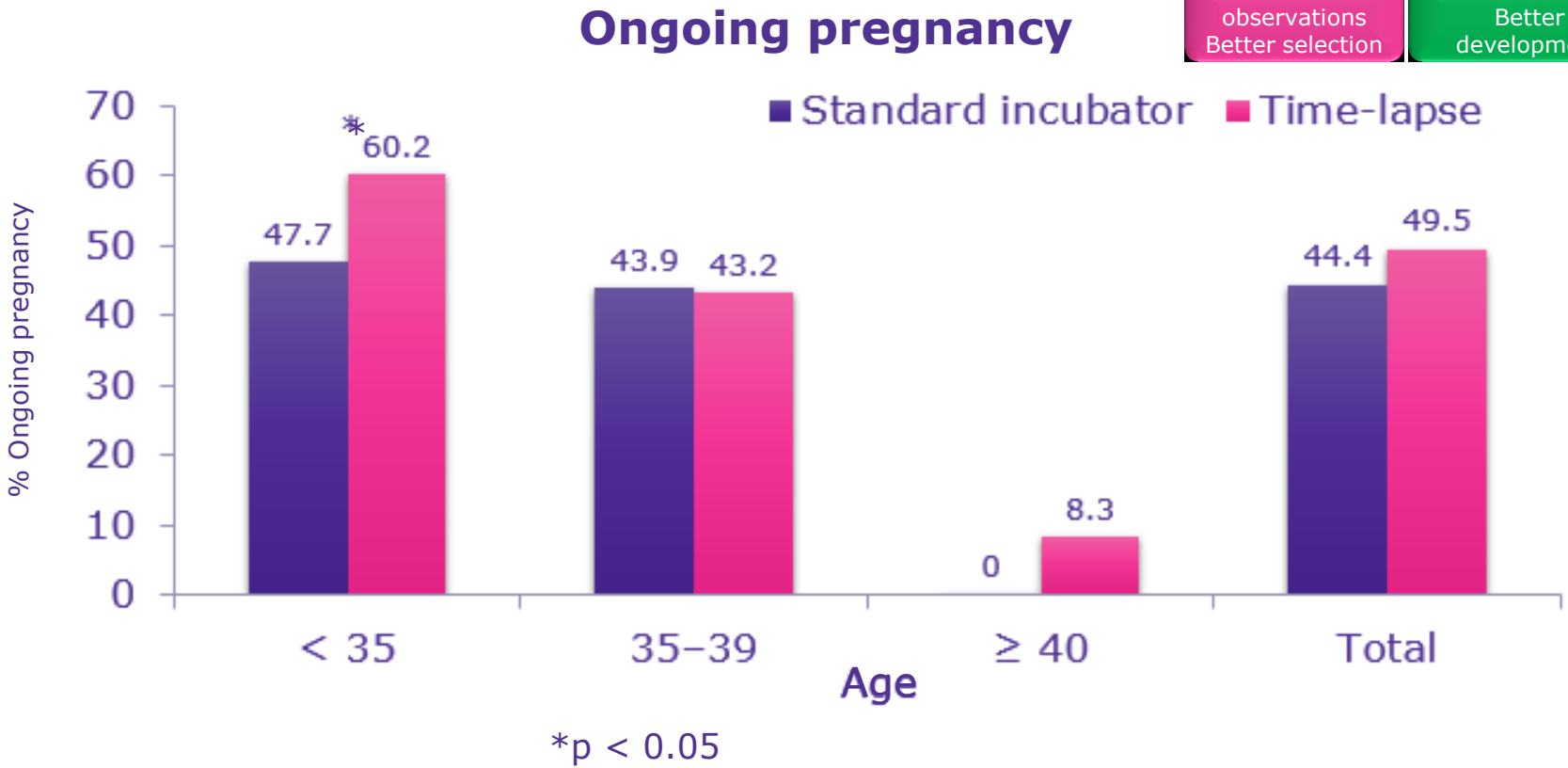
Conventional incubators vs time-lapse incubators
(n = 5,574)



Fresh or deferred first-transfer time-lapse - NO PGS

More observations Better selection

Less disturbance Better development



PGS, preimplantation genetic screening.

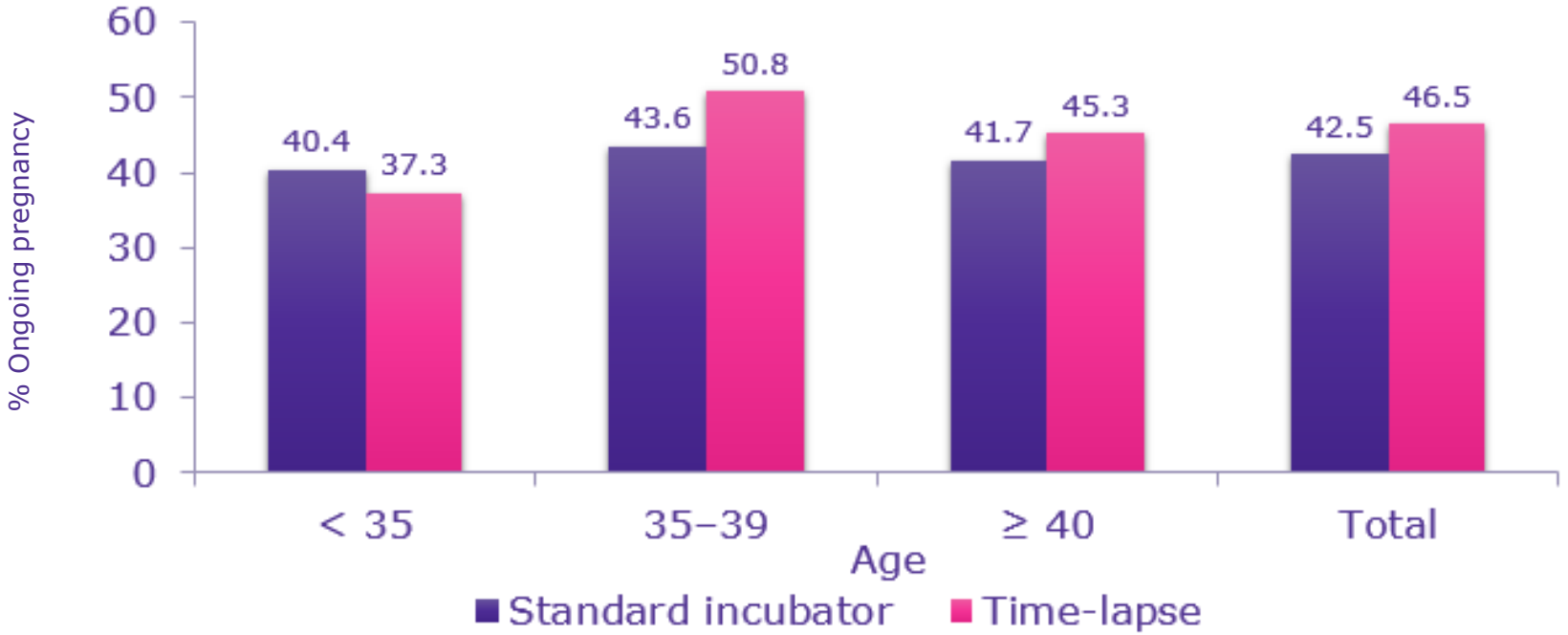
Meseguer M, personal data.

PGS first-transfer trophoectoderm biopsy time-lapse vs standard incubator

Ongoing pregnancy

More observations Better selection

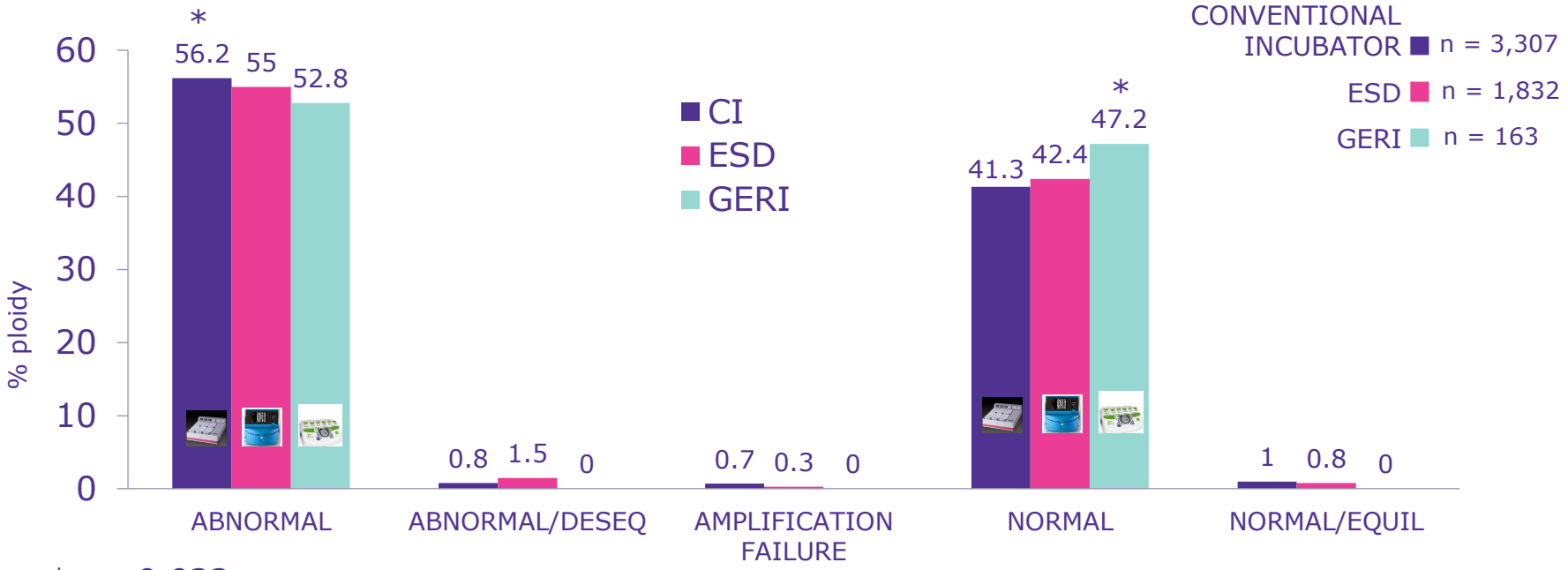
Less disturbance Better development



Meseguer M, personal data.

% ploidy of the results in each incubator
N = 5,311 blastocysts

Less disturbance
Better
development

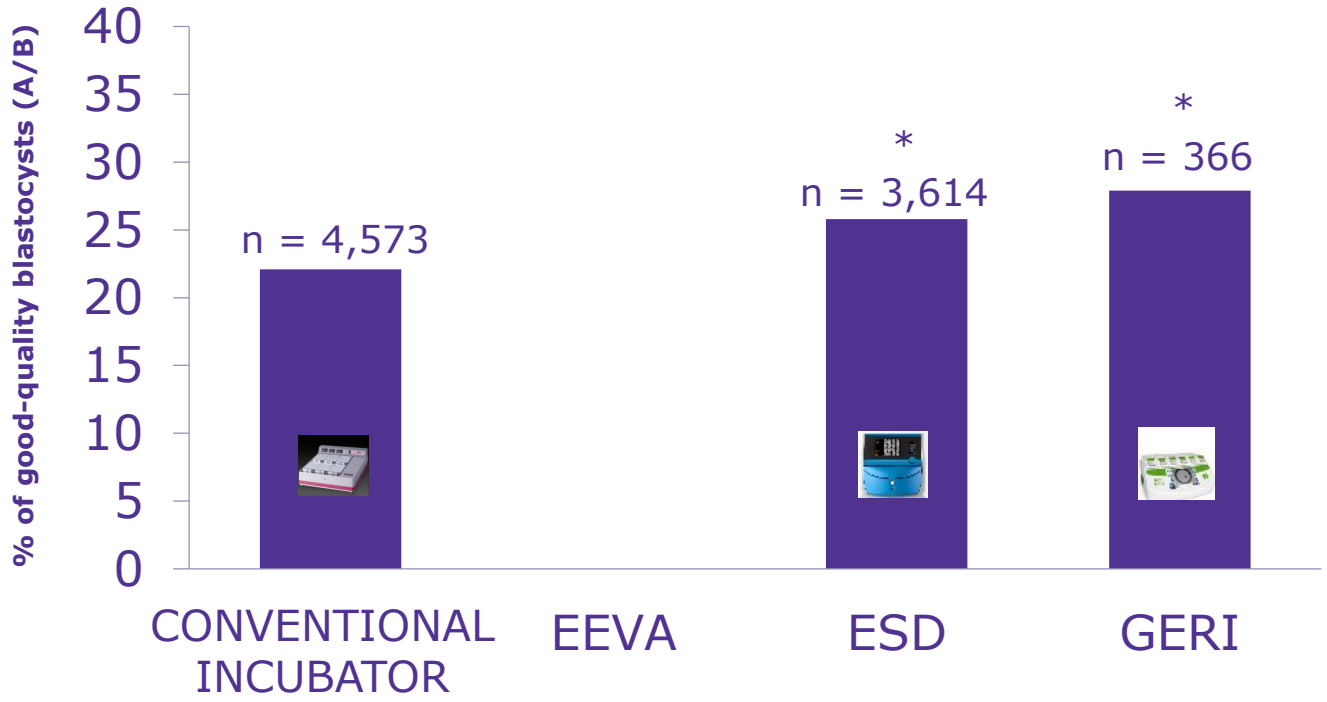


*p = 0.022

Meseguer M, personal data.

Less disturbance
Better
development

% of **good-quality** blastocysts (A/B) on Day 5 in each incubator



Meseguer M, personal data.

Recent report on live birth

Obstetric and perinatal outcomes of pregnancies conceived with embryos cultured in a time-lapse monitoring system

Maria Fernanda Insua, Ph.D.,[a] Ana Cristina Cobo, Ph.D.,[a] Zaloa Larreategui, Ph.D.,[b] Marcos Ferrando, M.D.,[b] Vicente Serra, Ph.D.,[a] and Marcos Meseguer, Ph.D.[a]

[a] IVI Valencia, Valencia; and [b] IVI Bilbao, Bilbao, Spain

More observations Better selection

Less disturbance Better development

Logistic regression analysis (for all cycles or only singleton pregnancy cycles) of newborn delivery, female neonates, very preterm births (< 34 weeks), low birth weight (< 2,500 g), and very low birth weight (< 1,500 g) as affected by incubation type (TLS vs SI)

Outcome variable	All cycles		Singleton pregnancy cycles	
	OR (95% CI)	p value	OR (95% CI)	p value
Delivery crude	1.44 (1.09–1.92)	0.011	1.32 (0.96–1.80)	NS
Delivery (adjusted)	1.41 (1.06–1.86)	0.015	0.33 (0.97–1.81)	NS
Female neonates	1.42 (0.88–2.28)	NS	2.04 (1.14–3.64)	0.016
Female neonates (adjusted)	1.23 (1.05–1.45)	0.012	1.28 (1.07–1.52)	0.008
Preterm births (< 37 week)	1.16(0.67–2.01)	NS	0.83 (0.36–1.89)	NS
Preterm births (< 37 week) (adjusted)	1.16 (0.68–1.99)	NS	0.78 (0.39–1.99)	NS
Preterm births (< 34 week)	1.38 (0.60–1.30)	NS	0.91 (0.19–4.29)	NS
Preterm births (< 34 week) (adjusted)	1.42 (0.59–3.38)	NS	0.96 (0.21–4.39)	NS
Low birth weight (< 2,500 g)	1.41 (0.74–2.70)	NS	0.97 (0.38–2.48)	NS
Low birth weight (< 2,500 g) (adjusted)	1.44 (0.85–2.43)	NS	1.25 (0.67–2.32)	NS
Low birth weight (< 1,500 g)	1.59 (0.39–6.39)	NS	0.72 (0.09–5.48)	NS
Low birth weight (< 1,500 g) (adjusted)	2.70 (0.87–8.35)	NS	2.479 (0.66–9.27)	NS

Note: in each multivariate model day of transfer, oocyte source, and age of the oocyte were included as a potential bias variables!

IVF, in vitro fertilization ; SI, standard IVF incubator; TLS, time-lapse system.

Insua MF, et al. Fertil Steril. 2017;108:498-504.

More observations Better selection

Less disturbance Better development

The last metanalysis reported

Outcomes	Assumed risk (conventional incubation, median-risk population)	Corresponding risk (time-lapse, Median-risk population)	Relative effect (95% CI)	No. of participants (studies)	Quality of evidence (GRADE)
Ongoing pregnancy	410/1,000	517/1,000 (457–577)	OR 1.542 (1.211–1.965)	1,637 (5 RCTs)	Moderate [1.5]
Early pregnancy loss	196/1,000	139/1,000 (103–186)	OR 0.662 (0.469–0.935)	904 (5 RCTs)	Moderate [2.5]
Live birth	321/1,000	441/1,000 (349–537)	OR 1.668 (1.134–2.455)	481 (3 RCTs)	Moderate [3.5]
Stillbirth	29/1,000	69/1,000 (23–188)	OR 2.483 (0.794–7.759)	481 (3 RCTs)	Low [4.5]

GRADE Working Group grades of evidence

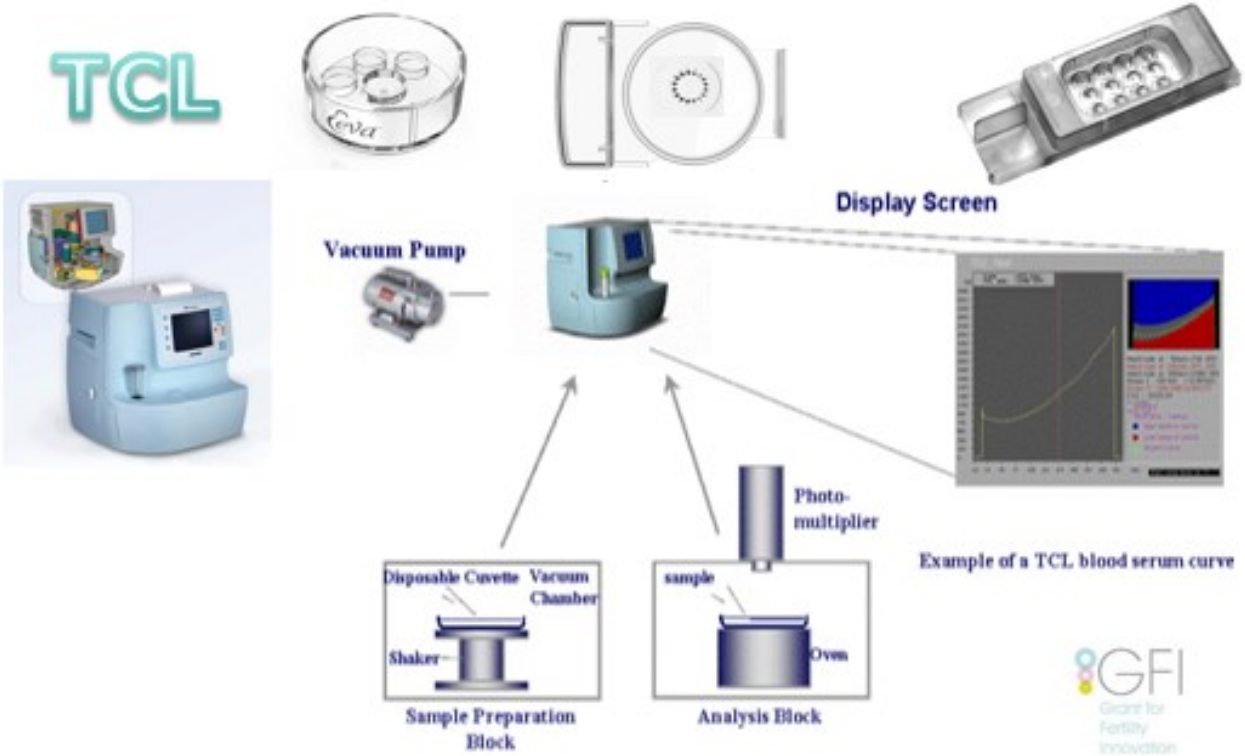
- High quality: further research is very unlikely to change our confidence in the estimate of effect
- Moderate quality: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate
- Low quality: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate
- Very low quality: we are very uncertain about the estimate

RCT, randomized controlled trial.

Pribenszky C, et al. Reprod Biomed Online. 2017;35:511-20.

Combining morphokinetics and TCL

Combining morphokinetics with media analysis



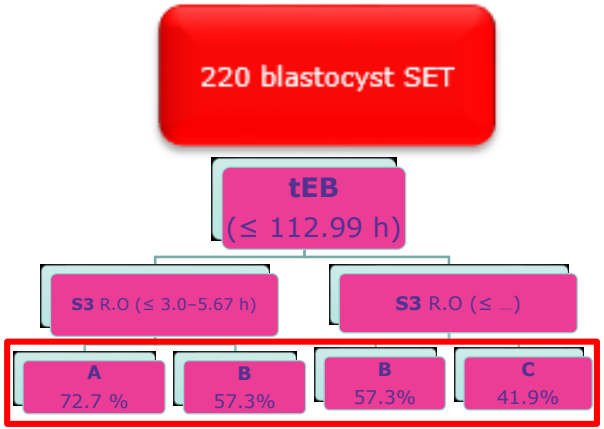
Alegre L, et al. ESHRE. 2017;P-179.

Combining morphokinetics and TCL

Combining morphokinetics with media analysis

Blastocyst morphology and morphokinetics

Combining morphokinetics with media analysis



ORIGINAL ARTICLE: ASSISTED REPRODUCTION

GFI Grant for Fertility Innovation

Morphokinetic analysis and embryonic prediction for blastocyst formation through an integrated time-lapse system

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Expansion grade	Inner cell mass (ICM) quality	Trophectoderm (TE) quality	ASEBIR category
Bei BE BH Bhi	A	A B C D	A B C D
	B	A B C D	B C D
	C	A B C D	C D
BC (zp thick)			C
Morula			D

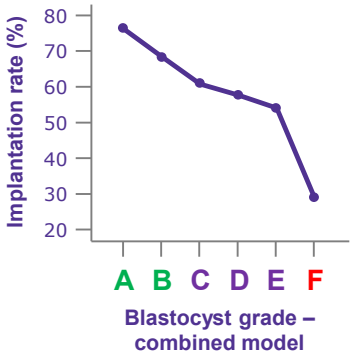
Category	Total (n = 220)	Blastocyst implantations (n = 117)	% (95% CI)
A	52	27	51.9 (53.99–98.95)
B	141	78	55.3 (43.24–94.26)
C	18	7	38.9 (44.38–77.84)
D	9	5	55.6 (41.45–74.34)

Bei, initiating blastocyst expansion; BE, expanded blastocyst; BH, hatching blastocyst; Bhi, initiating blastocyst hatching.

Motato Y, et al. Fertil Steril. 2016;105:376-84.

Combining morphokinetics and TCL

Combining morphokinetics with media analysis



Alegre L, et al. ESHRE. 2017;P-179.

Take-home message

1. Objective technologies will help improve outcomes in ART
2. We can apply different strategies to increase objectivity in our daily practice
   - Continuous embryo-monitoring incubators are useful tools in an IVF setting for 2 main reasons
     1. Provide better culture conditions
     2. Provide more observations; better selection
3. Embryo monitoring is in permanent evolution and future hardware (devices) and software (selection programs) are being developed for immediate clinical use

MR, miscarriage rates.