Key findings learned through ESD; IVI experience

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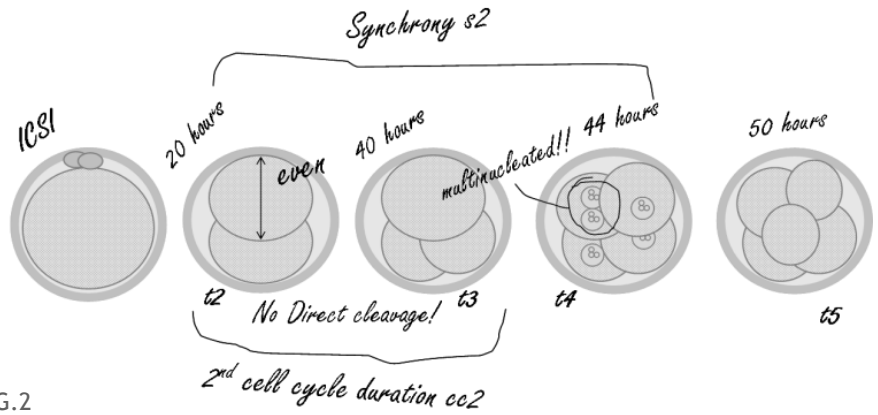
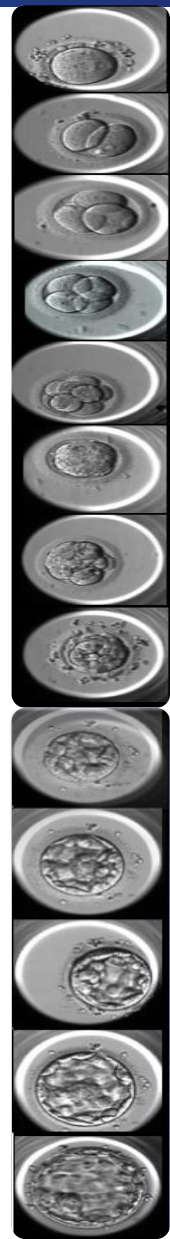
Introduction

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



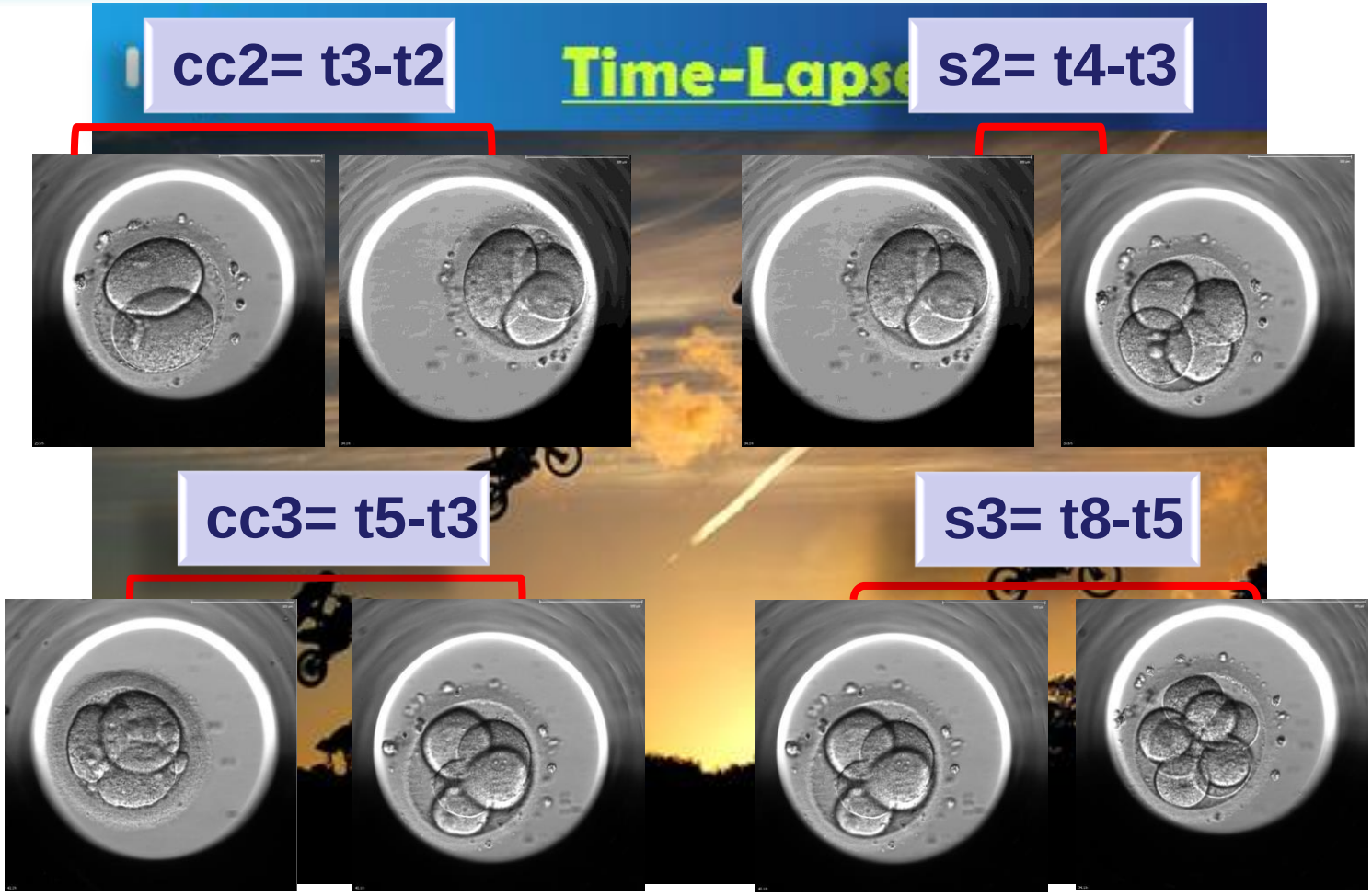
Key Finfings of time-Lapse research

- ✓ New embryo behavior
- ✓ New categories for embryo evaluation
- ✓ Checking the best timing to IVM
- ✓ New markers of implantation: exclusion and inclusion criteria
- ✓ New algorithm of embryo selection
- ✓ Validation of ESD with RCT



New variables analyzed

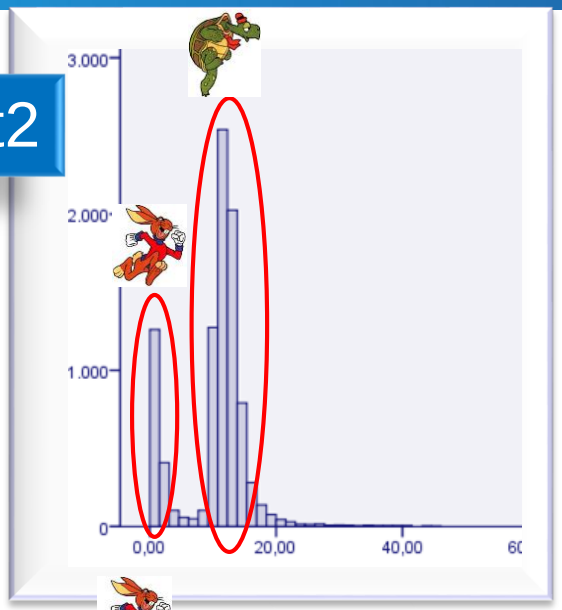
- ✓ New embryo behavior



2 types of embryo populations

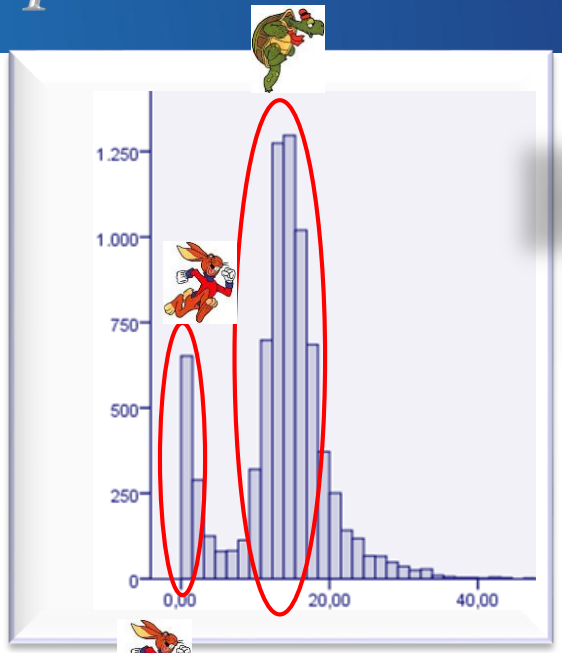
cc2= t3-t2

5-12 h



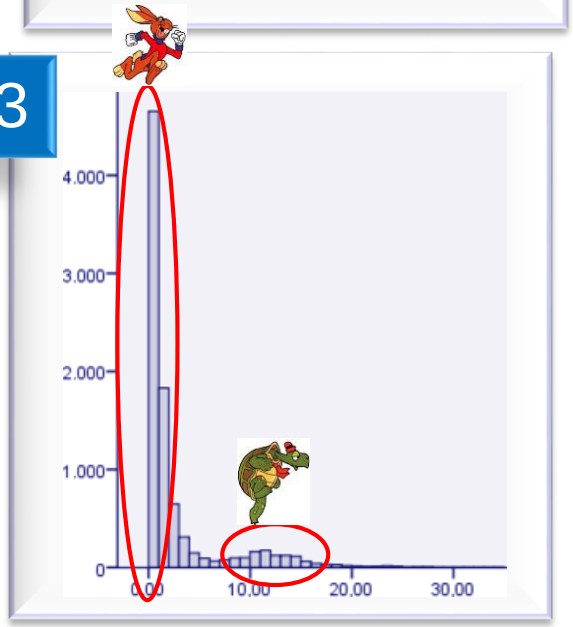
cc3= t5-t3

12-16 h



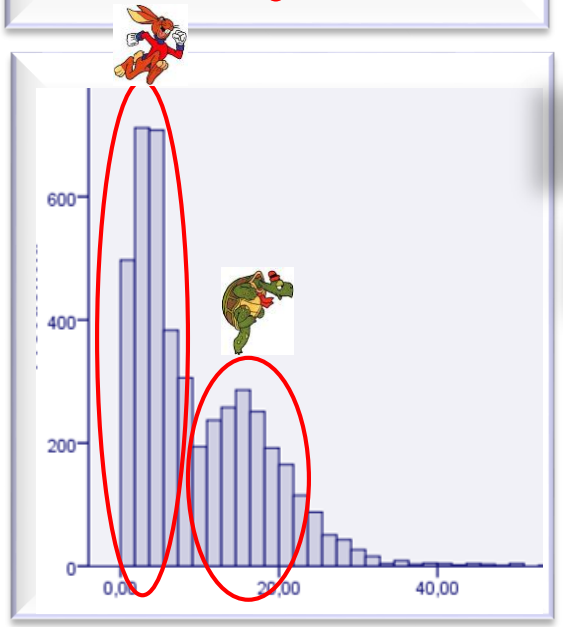
s2= t4-t3

<0,75



s3= t8-t5

0-3 h

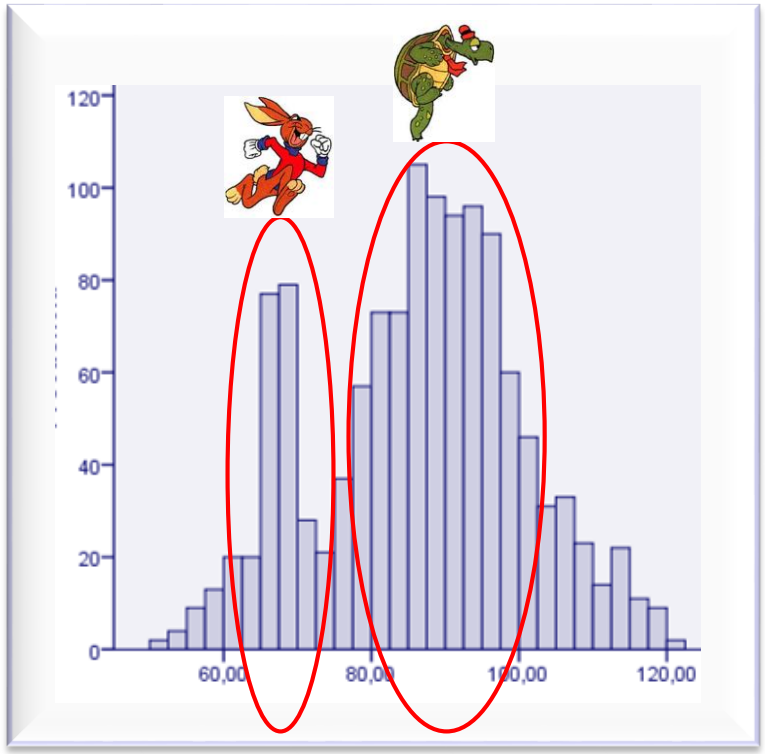


2 types of embryo populations

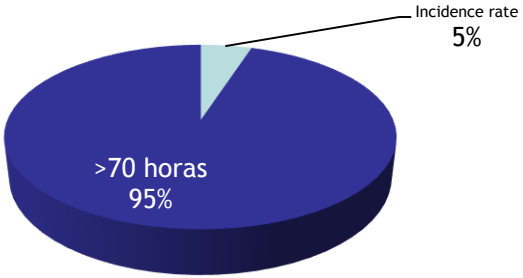
Time of compaction

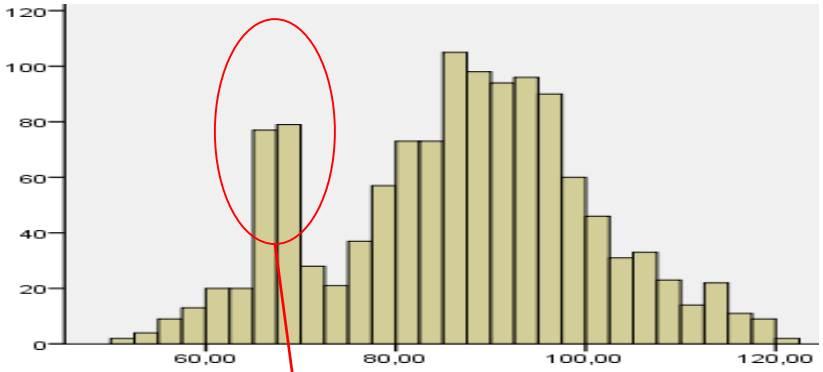
70 h

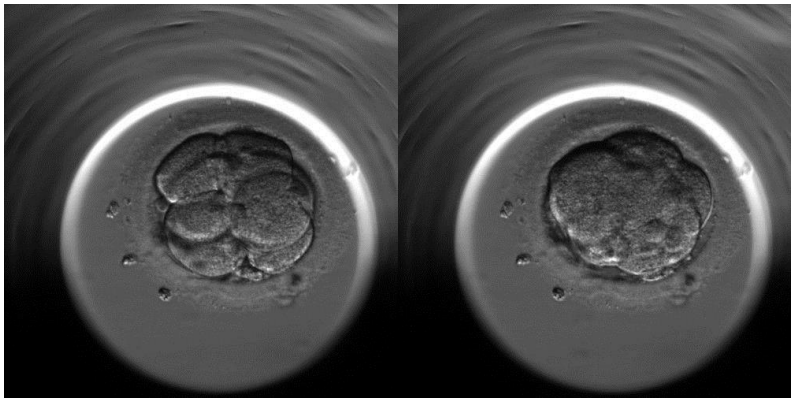
tM

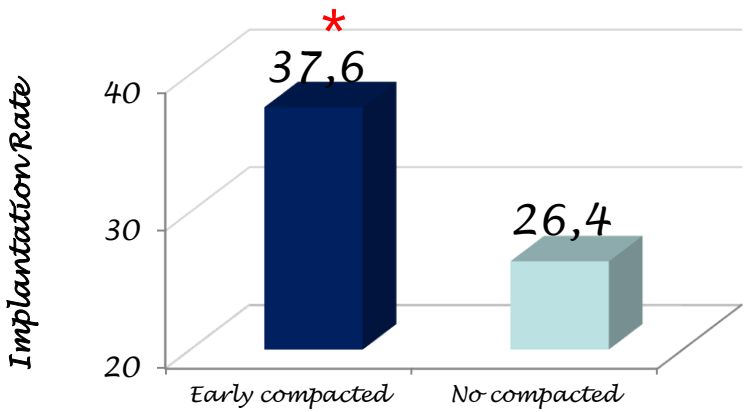


Discovering new parameters







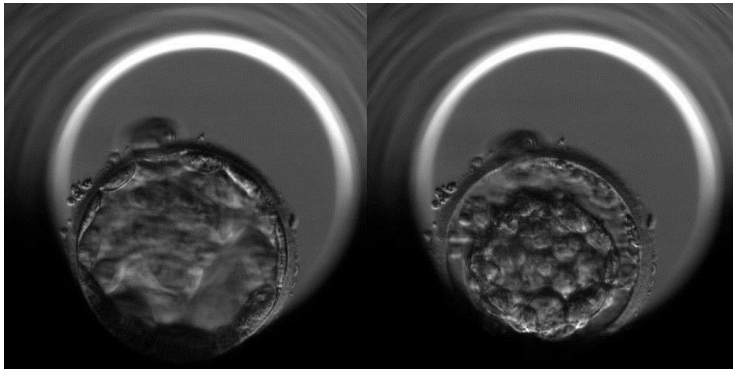


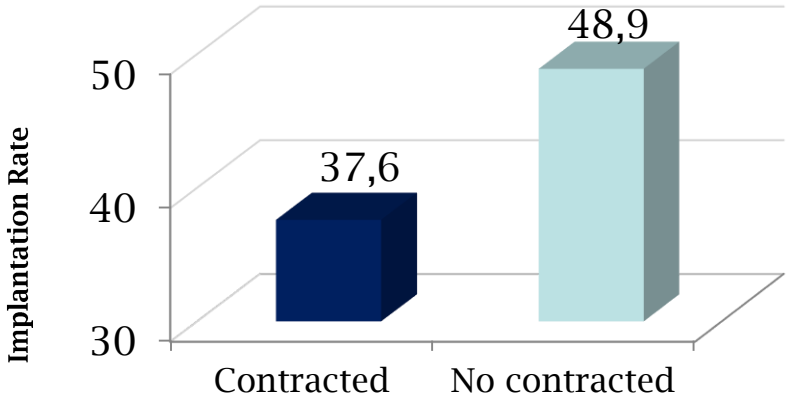
Early compaction

N= 3532 couples
N= 3782 embryos with KID(Known implantation data)

During Blastocyst expansion, embryo is partially or fully collapsed or contracted one or several times prior to transfer.

INCIDENCE RATE
19,1%



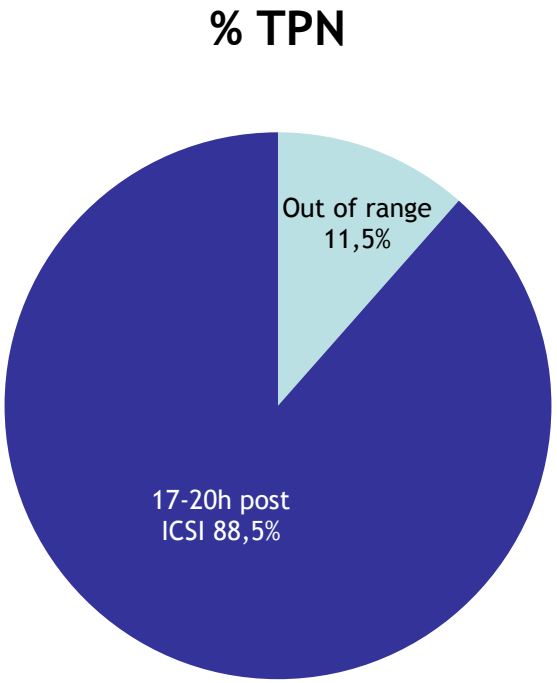


N= 507 cases
N= 715 blastocysts

Collapse or blastocyst contraction

Key Findings of time-lapse research

With the ESD we can detect triploid zygotes that we couldn´t detect with a conventional evaluation



Detection of Triploid zygotes

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- ✓ New markers of implantation: exclusion and inclusion criteria
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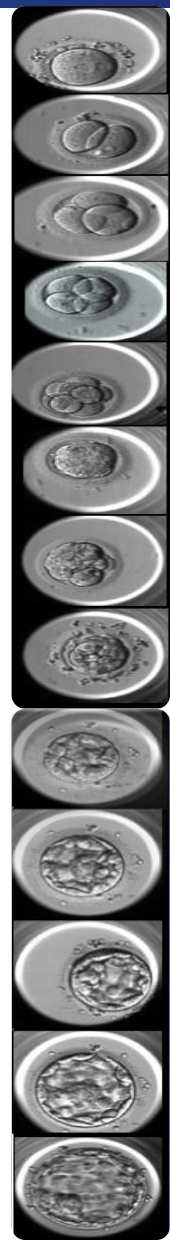
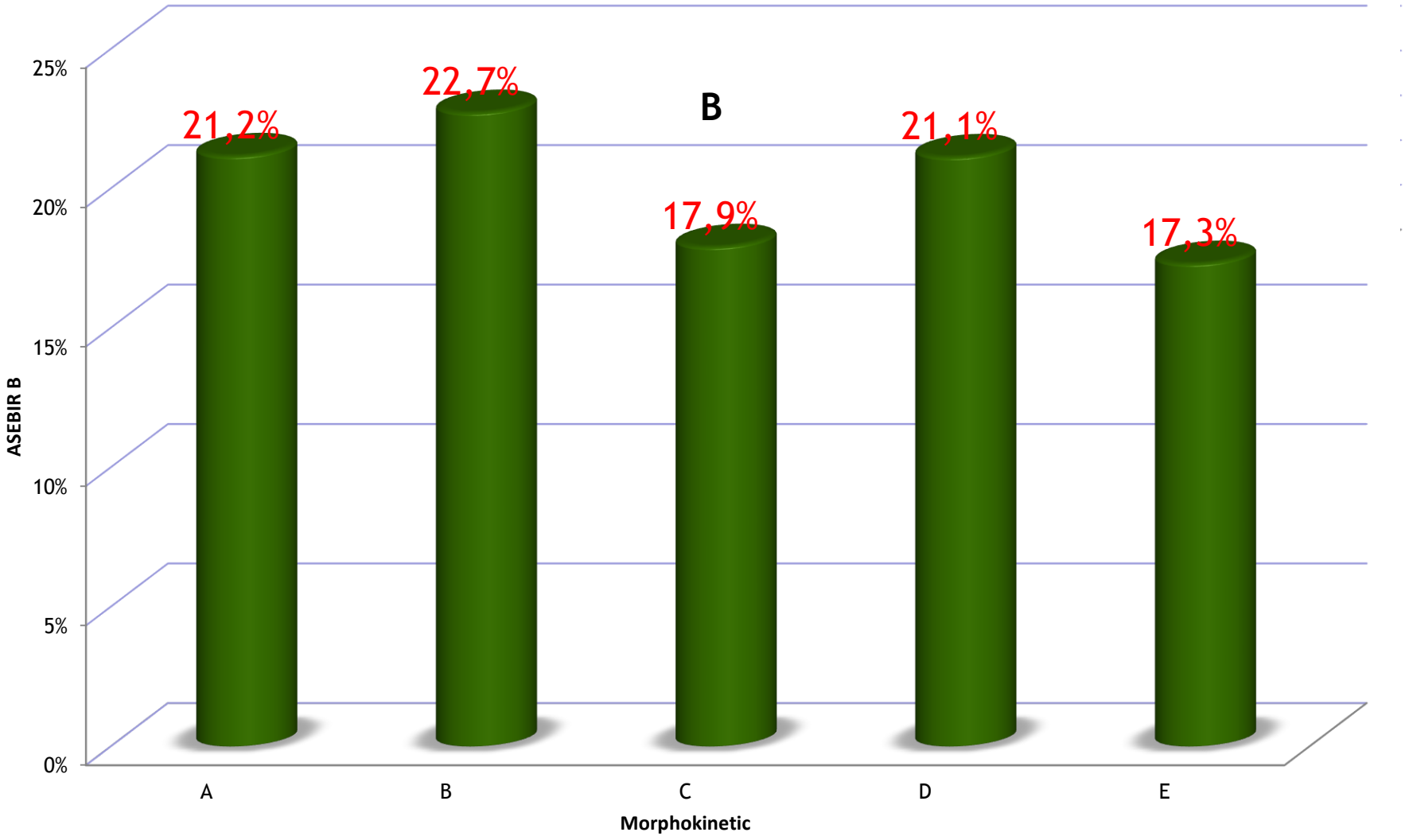


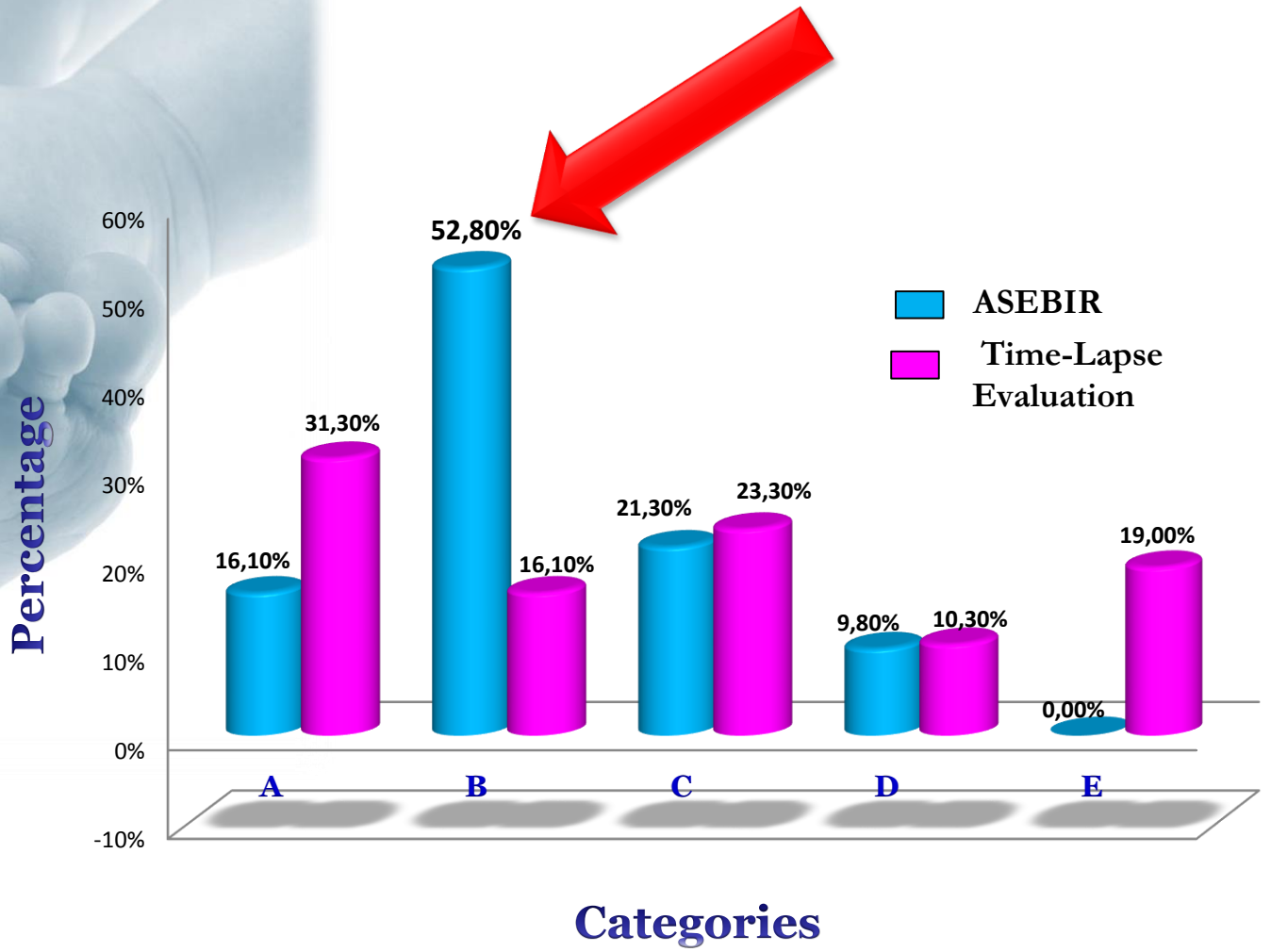
Morphokinetic vs Morphology

It seems that the morphological classiffication is not very precise.



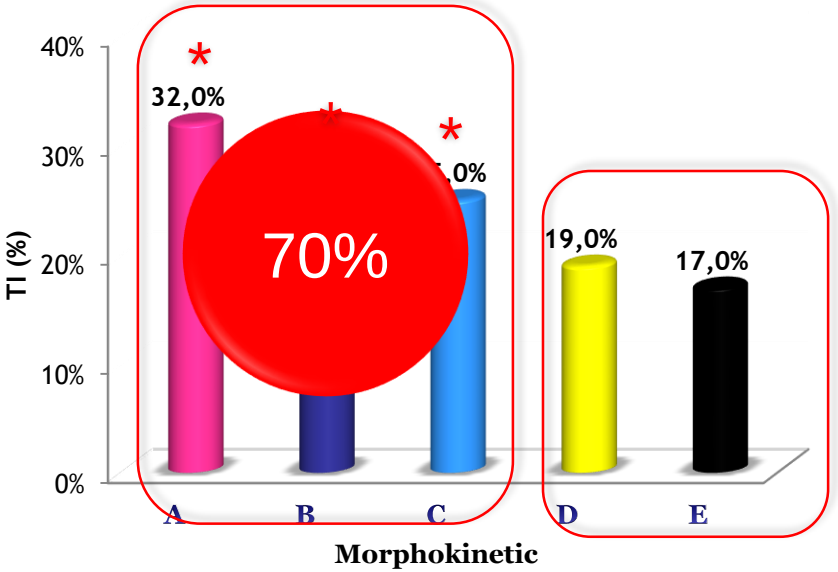
Morphokinetic vs Morphology

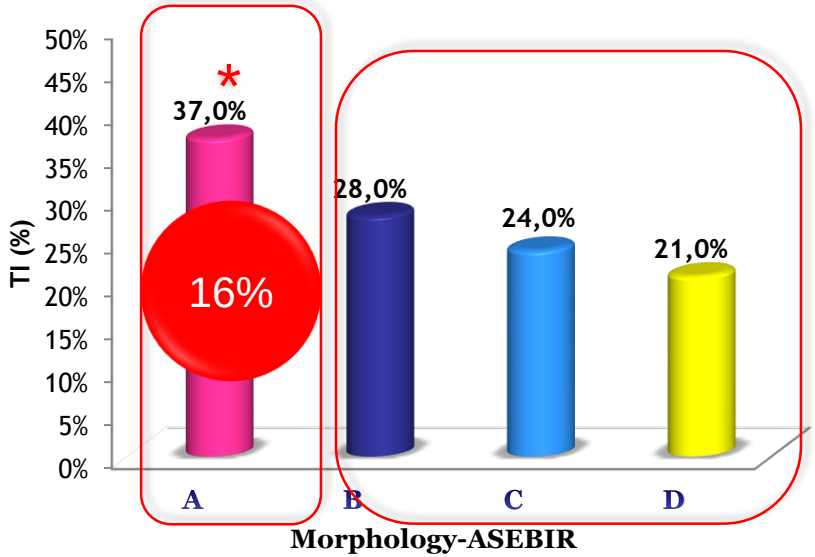
Different point of view…



Morphokinetics vs Morphology

Comparing implantation rates





✓Only morphology criteria, is not able to select the best embryo from a cohort.

✓ It is important to use a combination of both classifications

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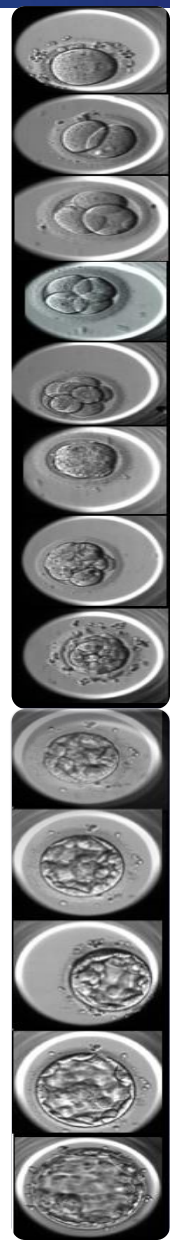


Checking the best IVM timing

Day 1 or 2

Checking maturation

n=88

A23187	Puromycin
8mM	10µg/mL
5 min	5 hrs

Partenogenetic activation

Response after activation

Day 2 o 3

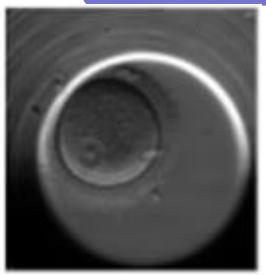
No activation (MII)	Incomplete Activation (IA)	Normal Activation (NA)	Abnormal Activation I (ANAI)	Abnormal Activation II (ANAII)
(1PB+0PN)	(1PB+2PB+0PN)	(1PB+2PB+1PN)	(1PB+2PB+>1PN)	(1PB+≥1PN)

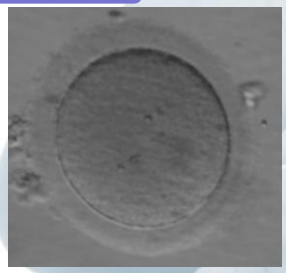
Checking the best IVM timing

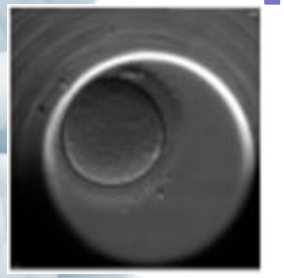
Early IVM (70.5%)

n=62
≤22 hours

18.4±2.7h*







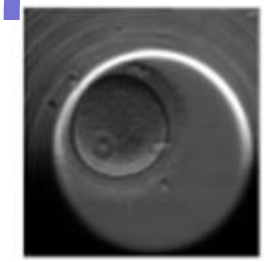
4.8±2.3hrs *

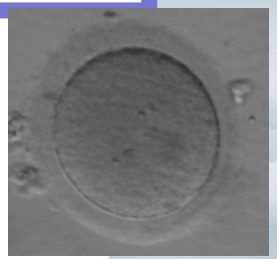
13.1±2.7hrs

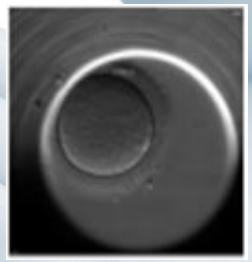
Late IVM (29.5%)

n=26
>22 hours

26.3±3.8hrs *



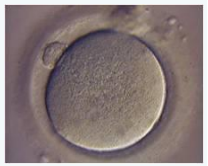
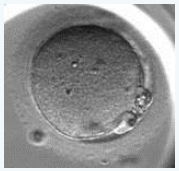
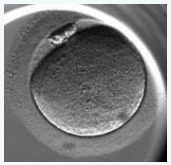




10.3±4.1hrs *

15.7±3.0hrs

The early IVM gives rise to higher % of normal activation compare to late IVM (61.3% vs. 34.6%)

	Activation	No activation (MII)	Incomplete Activation (IA)	Normal Activation(NA)	Abnormal Activation I (ANAI)	Abnormal Activation II (ANAII)
	(IA+NA+ANAI +ANAII)	(1PB+0PN)	(1PB+2PB+0PN)	(1PB+2PB+1PN)	(1PB+2PB+>1PN)	(1PB+≥1PN)
						
E-IVM (n=62)	57 (91.9%) a	5 (8.0%)	0	38 (61.3%) a	16 (25.8%) a	3 (4.8%)
L-IVM (n=26)	21 (80.8%) a	5 (19.2%)	2 (7.7%)	9 (34.6%) b	7 (26.9%) a	3 (11.5%)
Total (n=88)	78 (88.6%)	10 (11.4%)	2 (2.3%)	47 (53.4%)	23 (26.1%)	6 (6.8%)

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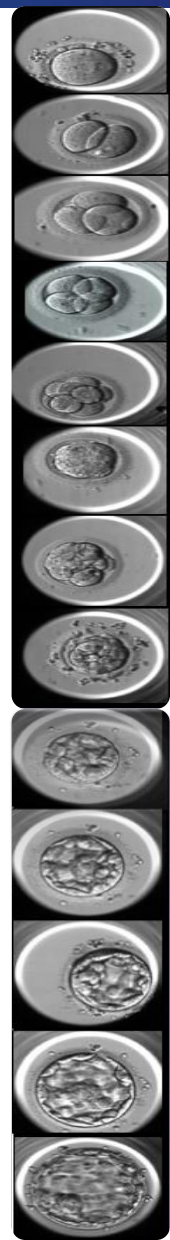
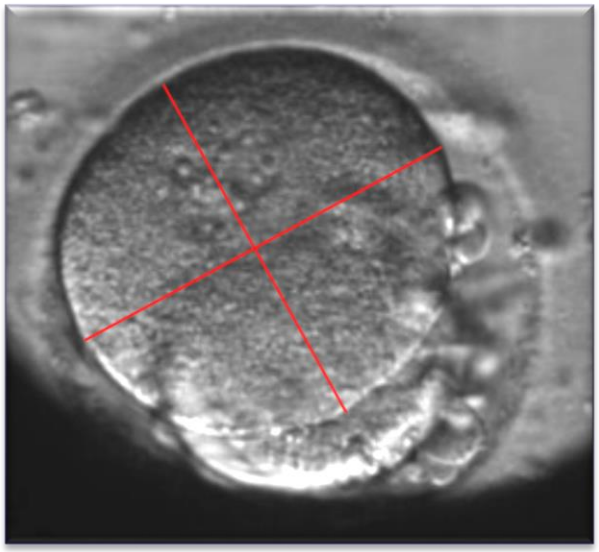
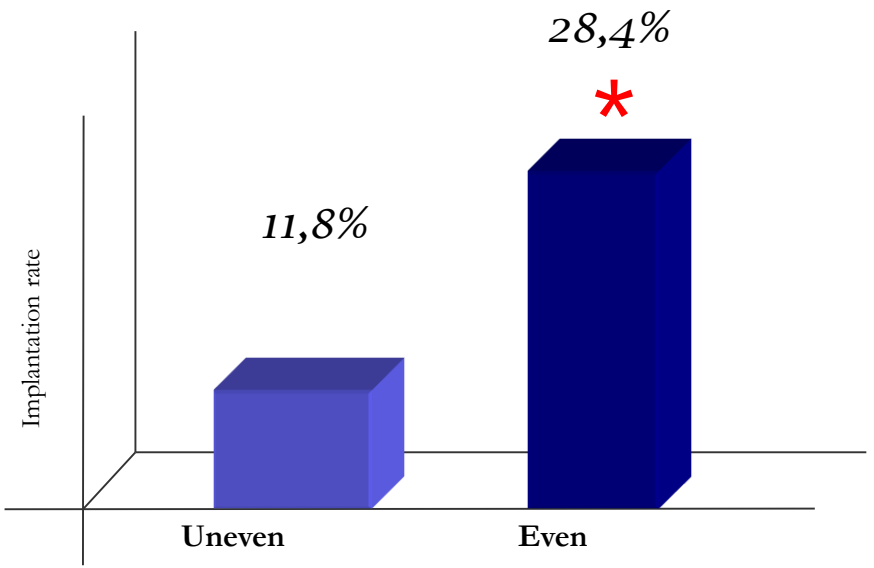


New markers of implantation: exclusion criteria

1st Exclusion criteria: Uneven blastomere size after the first cleavage

Definition: Blastomeres were considered uneven sized if the average diameter of the largest blastomere was more than 25% larger than the average diameter of the small blastomere.

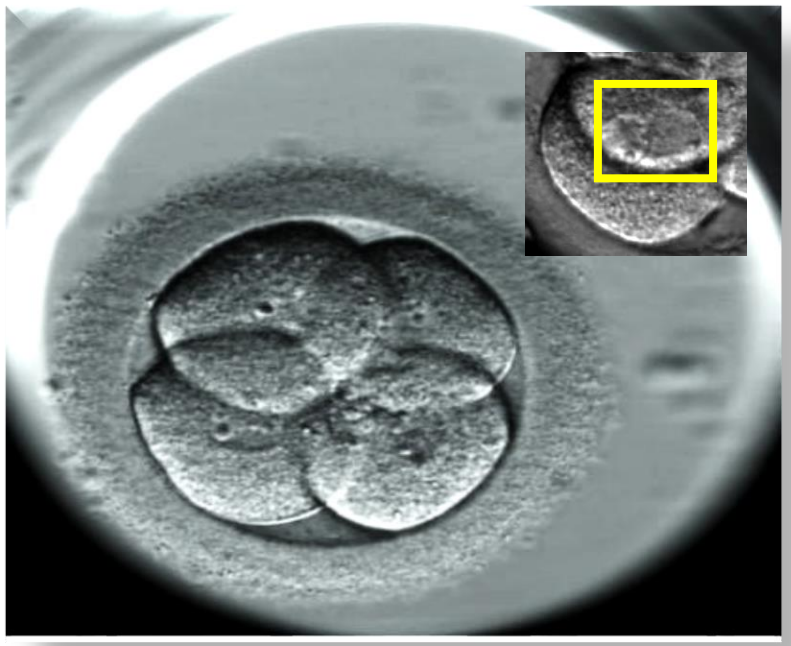


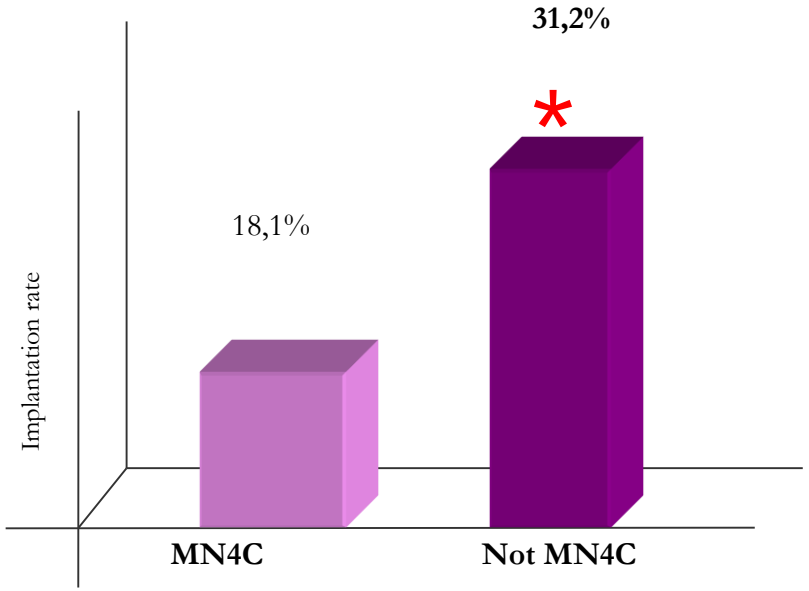


New markers of implantation: exclusion criteria

2nd *Exclusion criteria:* Multi-nucleation at the four-cell stage

Definition: Embryos were considered MN at the 4 cell stage when more than one distinct nucleus was observed in one (or more) blastomeres.

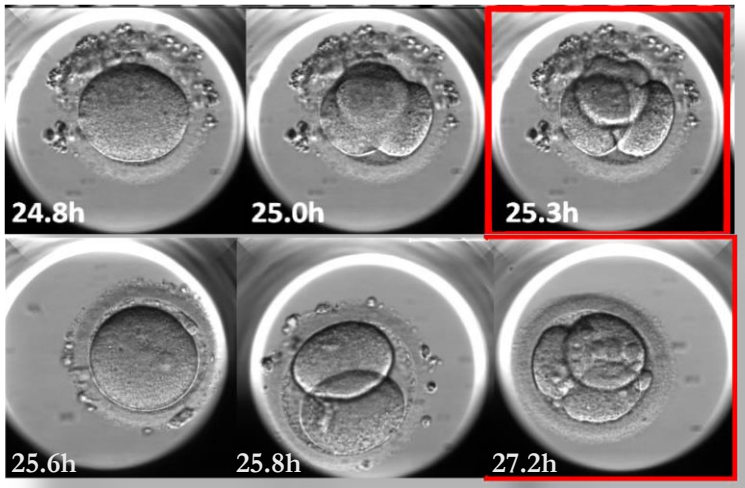




New markers of implantation: exclusion criteria

3rd *Exclusion criteria:* Abrupt division from one to three cells

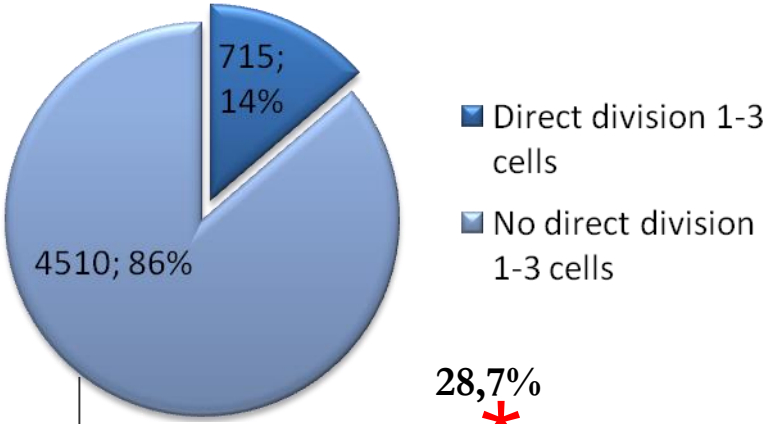
Definition: A cleavage from zygote to three blastomere embryo or a cleavage from zygote to two blastomeres in less than 5 hours.

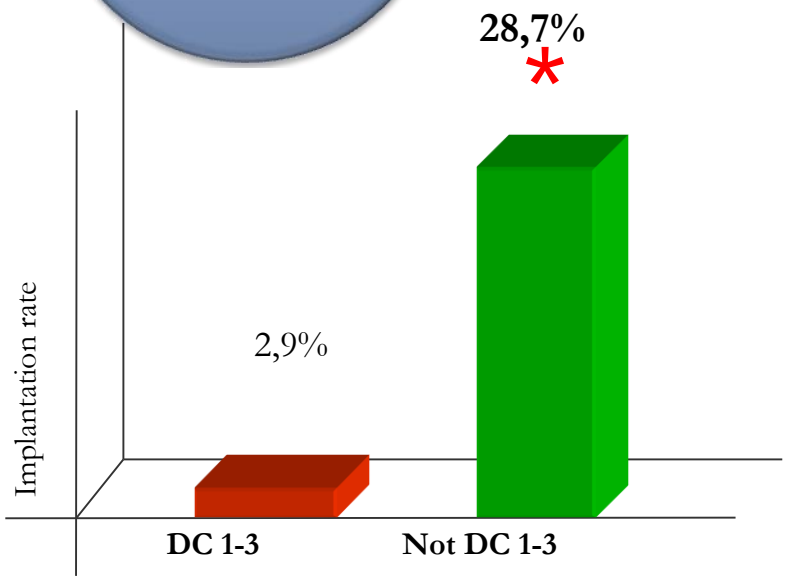


Limited implantation success of direct-cleaved human zygotes: a time-lapse study

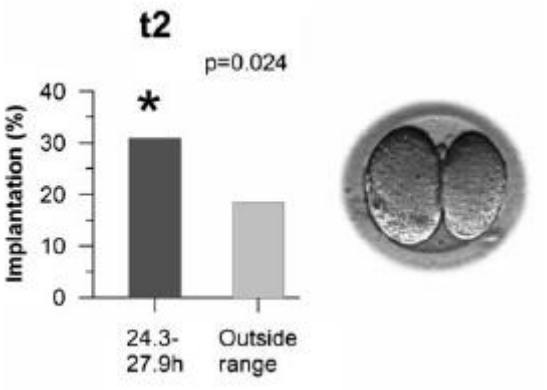
Irene Rubio, Ph.D.,[a] Reidun Kuhlmann,[b] Inge Agerholm, Ph.D.,[c] John Kirk, M.D.,[b] Javier Herrero, Ph.D.,[a] María-José Escribá, Ph.D.,[a] José Bellver, Ph.D.,[a] and Marcos Meseguer, Ph.D.[a]

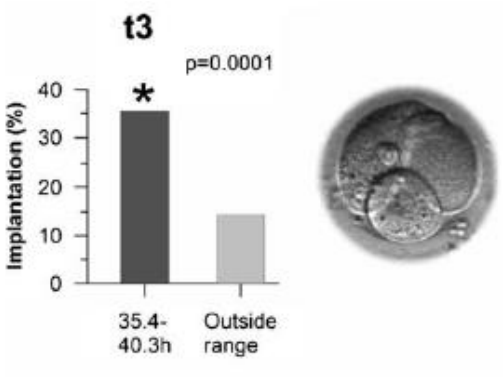
[a] Instituto Valenciano de Infertilidad Valencia, Universidad de Valencia, Valencia, Spain; [b] Malgaard Fertility Clinic, Aarhus, Denmark; and [c] Fertility Clinic Braedstrup, Braedstrup, Denmark

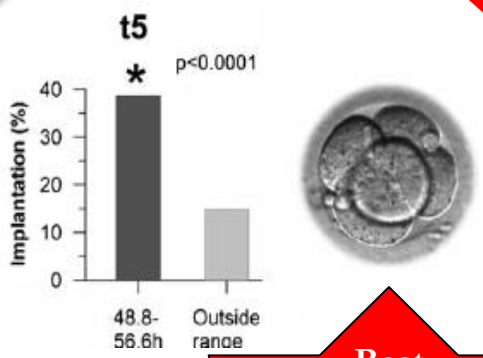




New markers of implantation: inclusion criteria

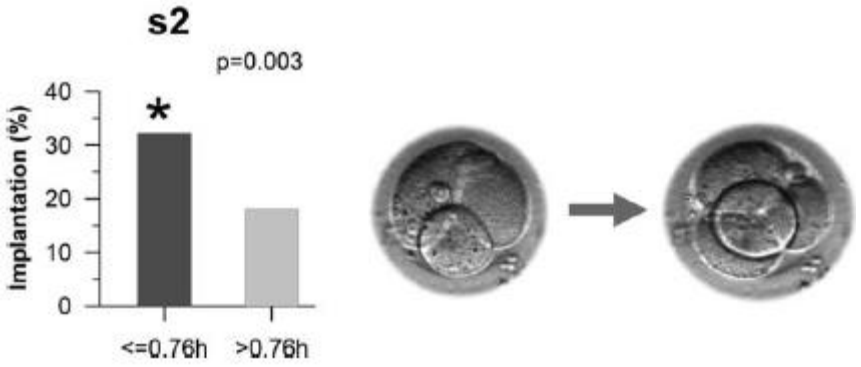


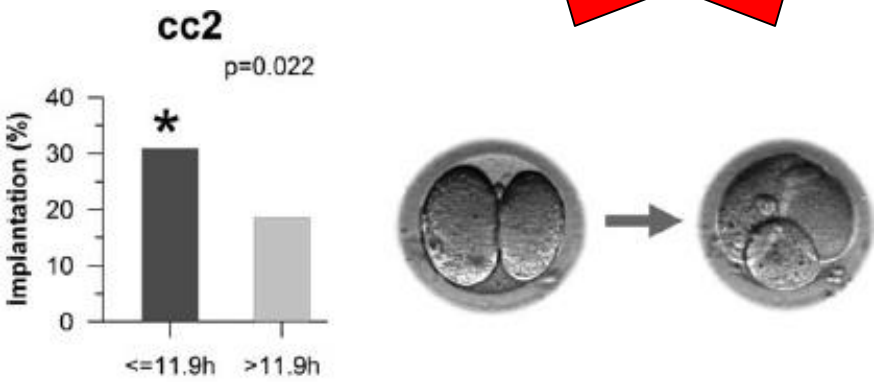




Predictive ability of embryo implantation

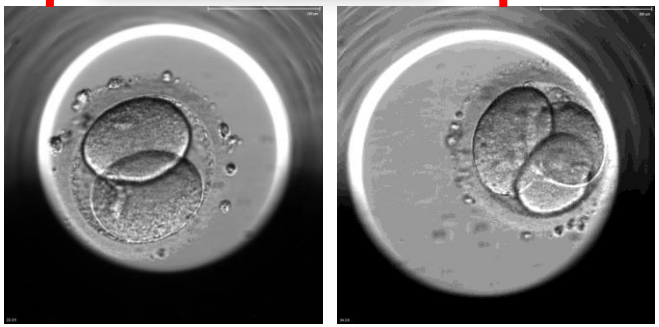
Best correlation with implantation success

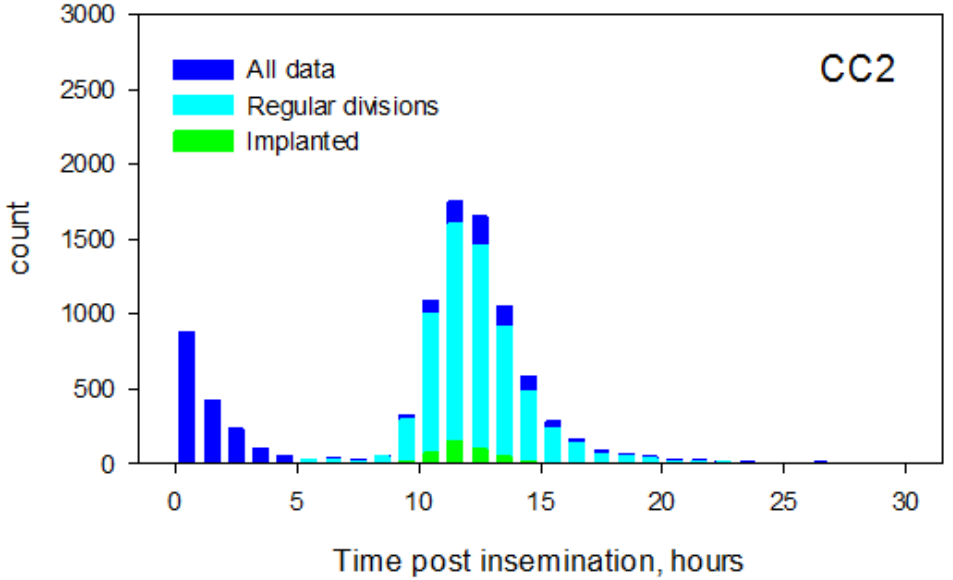




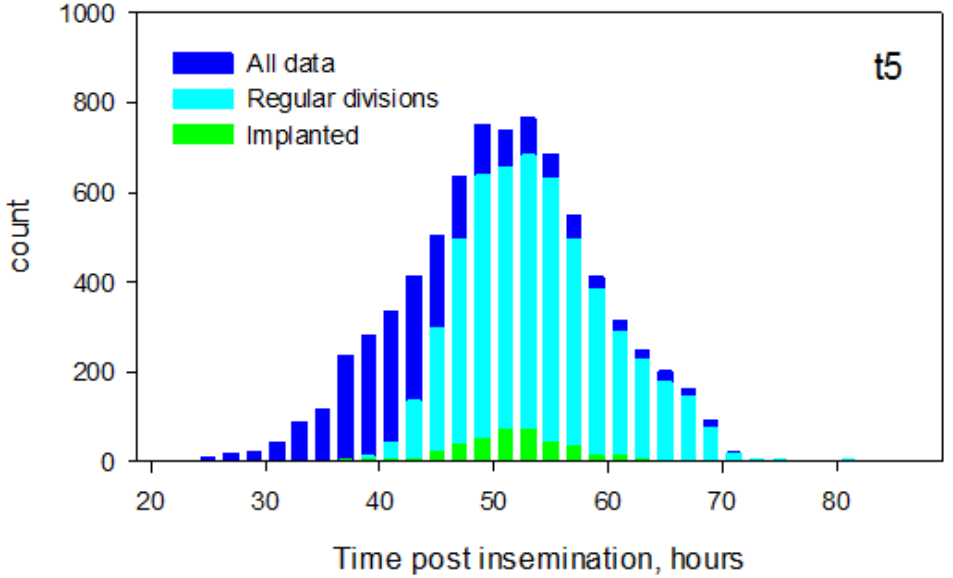
New markers of implantation: inclusion criteria

cc2= t3-t2





t5



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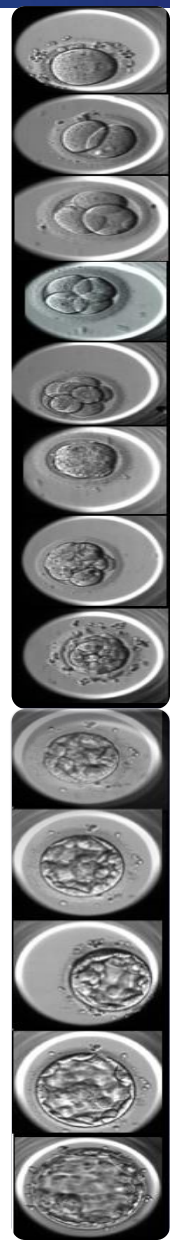
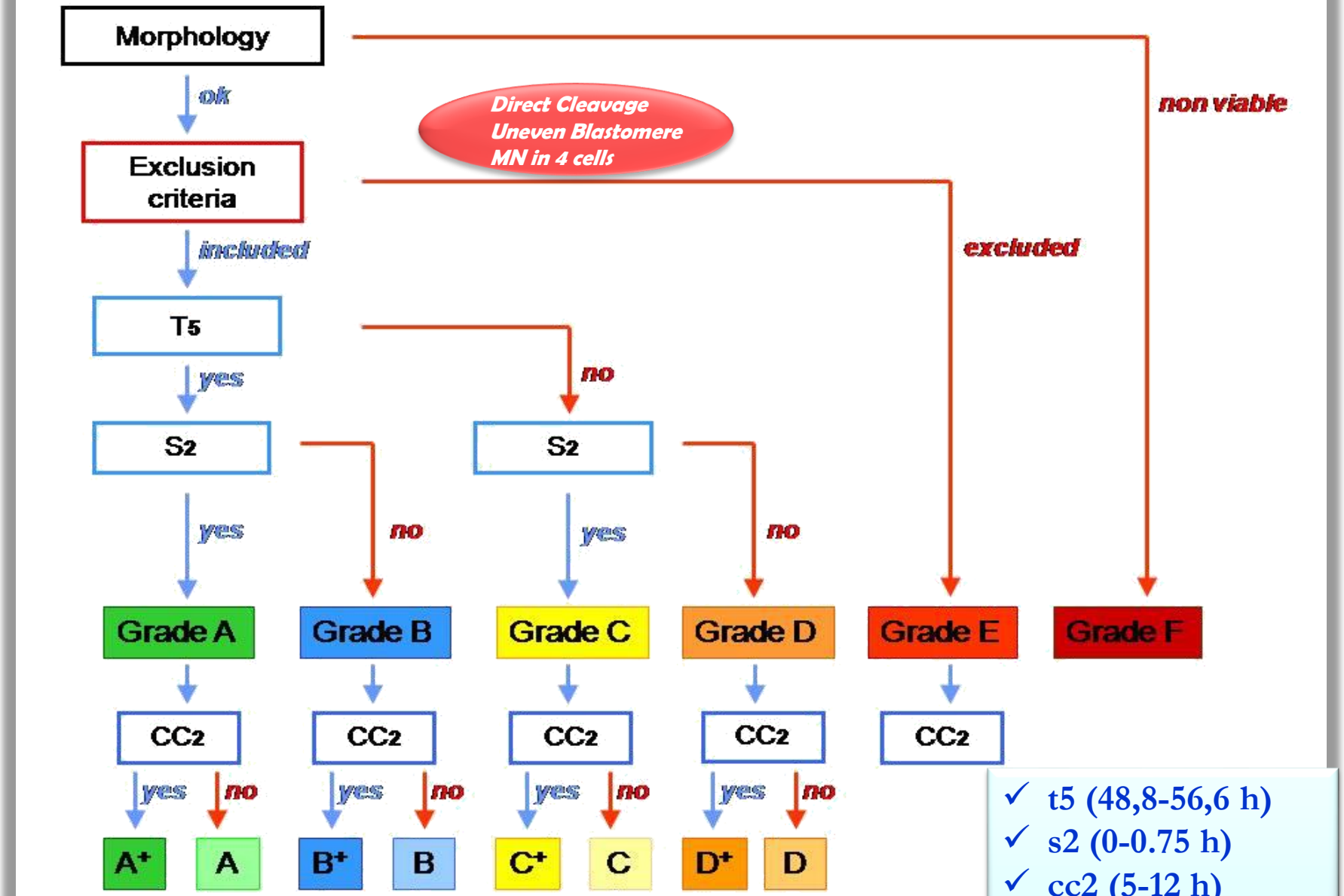
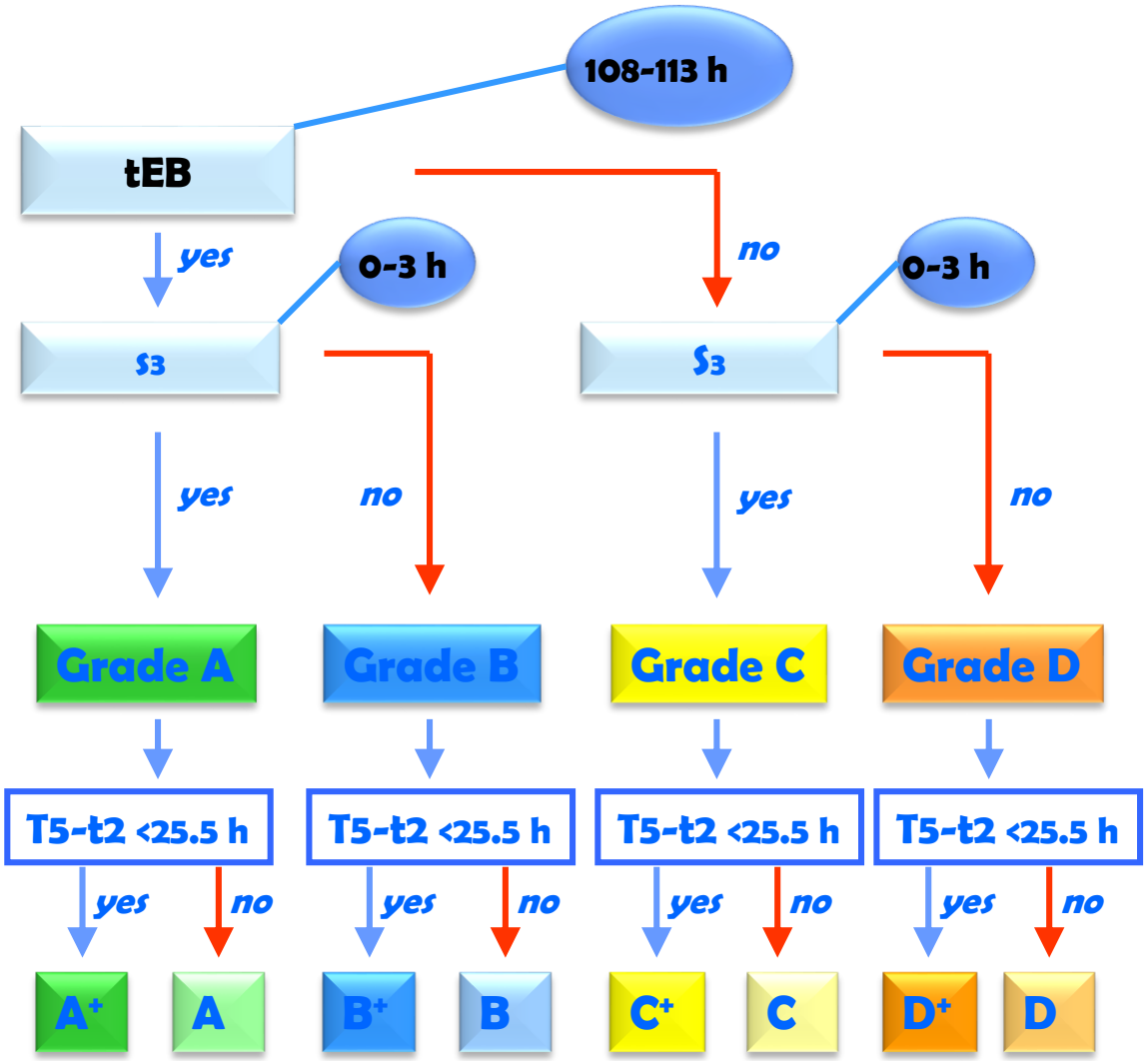


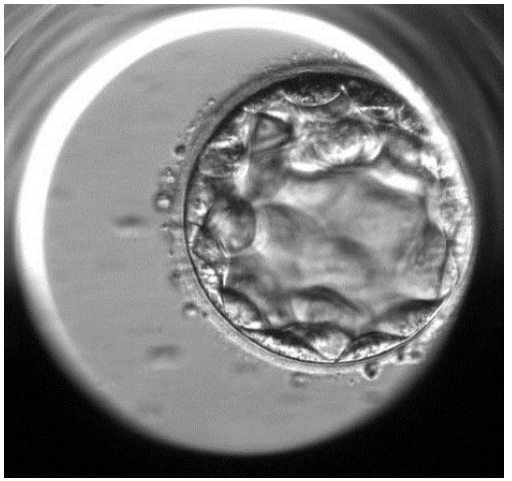
New algorithm of embryo selection: day 3



- ✓ t5 (48,8-56,6 h)
- ✓ s2 (0-0.75 h)
- ✓ cc2 (5-12 h)

Algorithm for Blastocyst selection: day 5-6

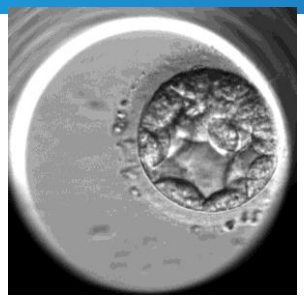


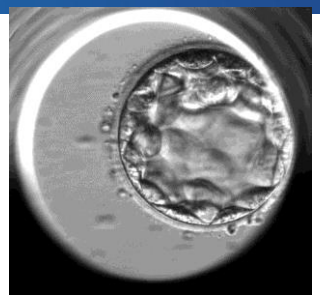


Tejera and Meseguer, ESHRE (2014)

Based on a Logistic Regression model

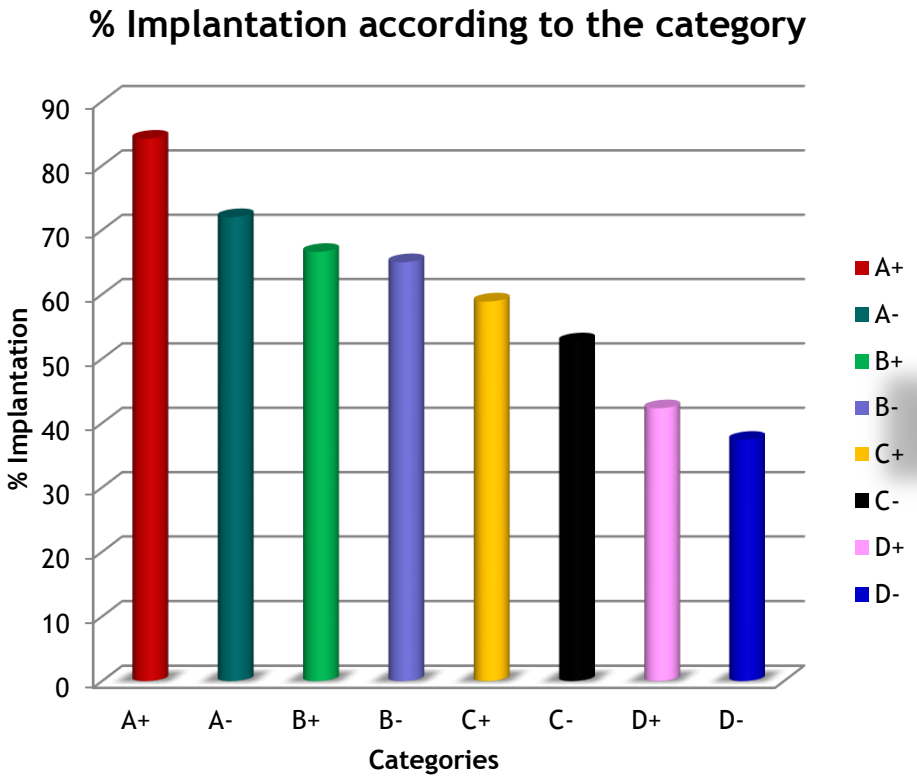
Algorithm for Blastocyst selection: day 5-6



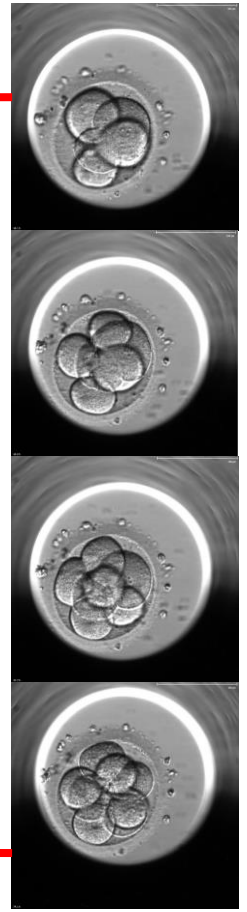


tEB

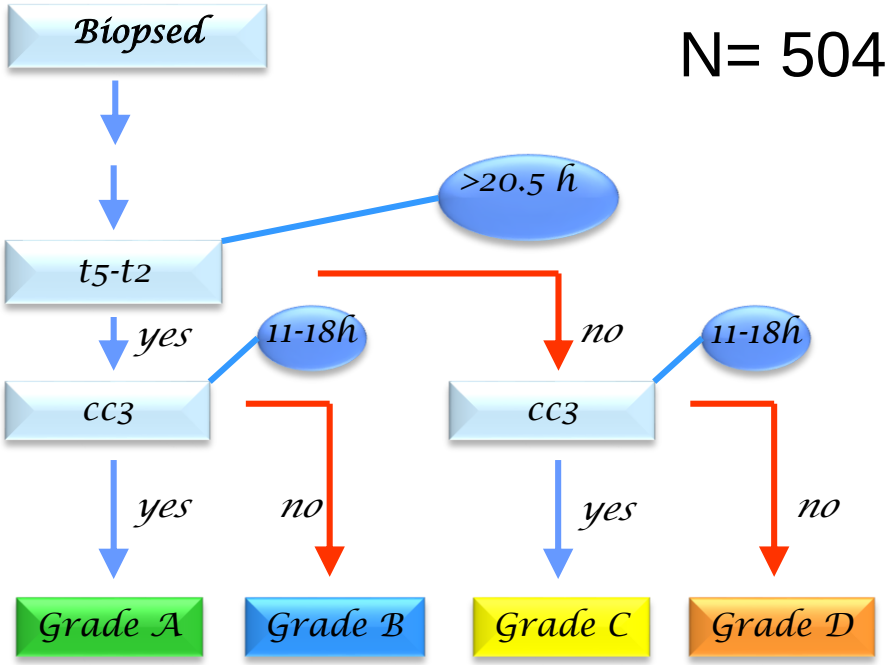
t5-t2



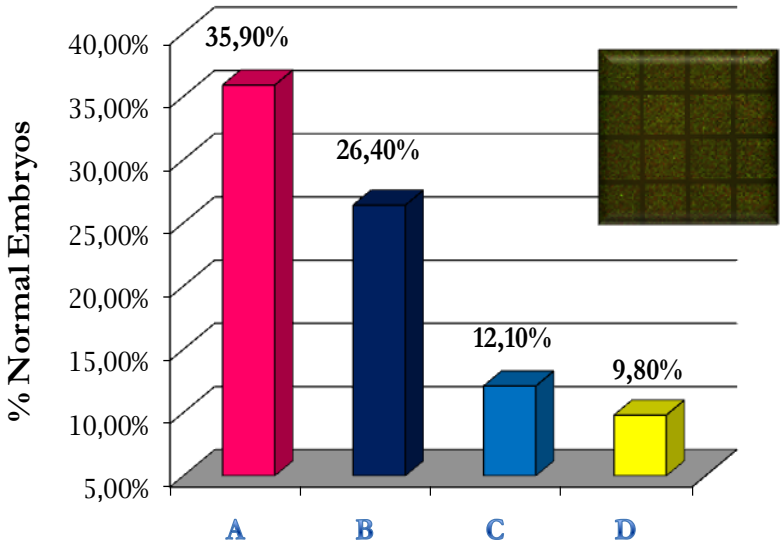
S3=t8-t5

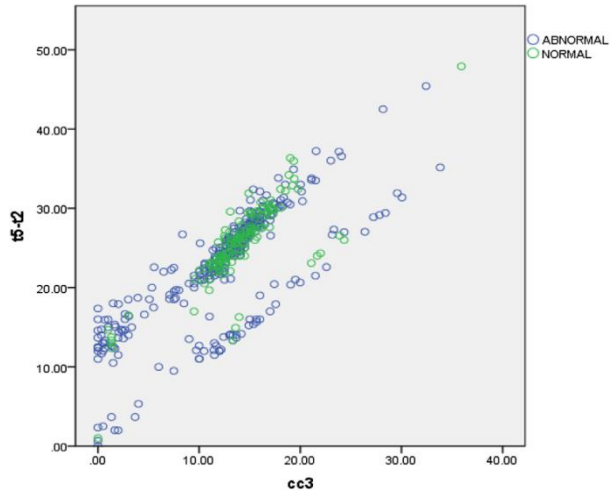


Morphokinetic algorithm based on parameters on day 3 and Genetic content



N= 504 embryos





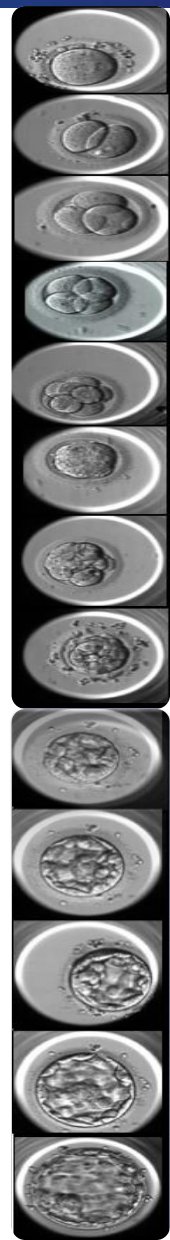
Increasing the probability of selecting chromosomally normal embryos by time-lapse morphokinetics analysis

Natalia Basile, Ph.D.,[a] Maria Del Carmen Nogales, Ph.D.,[a] Fernando Bronet, Ph.D.,[a] Mireia Florensa, Ph.D.,[b] Marissa Riqueiros, Ph.D.,[b] Lorena Rodrigo, Ph.D.,[c] Juan García-Velasco, M.D.,[a] and Marcos Meseguer, Ph.D.[d]

[a] IVI Madrid, Madrid; [b] IVI Barcelona, Barcelona; [c] IVIOMICS, Valencia; and [d] Instituto Valenciano de Infertilidad, Universidad de Valencia, Valencia, Spain

Key Finfings of time-Lapse research

- ✓ New embryo behaviors
- ✓ New categories for embryo evaluation
- ✓ Checking the best timing to IVM
- ✓ New markers of implantation: exclusion and inclusion criteria
- ✓ New algorithm of embryo selection
- ✓ Validation of ESD with RCT



Estimated Improvement of ESD

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

Marcos Meseguer, Ph.D.,[a] Irene Rubio, Ph.D.,[a] Maria Cruz, Ph.D.,[b] Natalia Basile, Ph.D.,[c] Julian Marcos, Ph.D.,[d] and Antonio Requena, M.D.[c]

[a] IVI, Universidad de Valencia, Valencia; [b] IVI Alicante, Alicante; [c] IVI Madrid, Madrid; and [d] IVI Murcia, Murcia, Spain

New technology

VS

Conventional Incubator

Prospective Study:

Controlled study where patients are randomly distributed between both groups.

Retrospective St

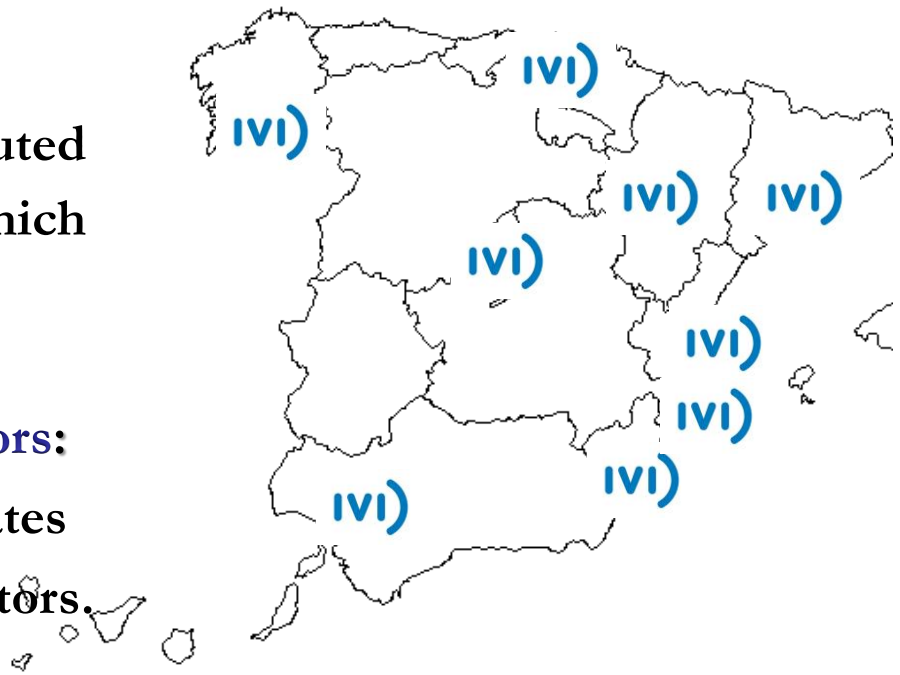
Where pati distributed betwee ups before the study h esigned.

FIRST APPROXIMATION

Estimated Improvement of ESD

Limitation:

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



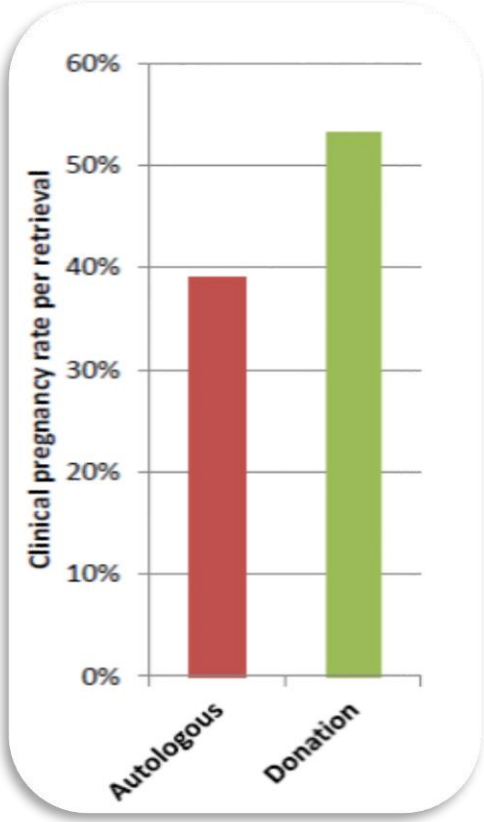
Analyse for possible confounding factors:

- ✓ Significantly influence pregnancy rates
- ✓Uneven distribution between incubators.

- ✓Type of incubator
- ✓Own treatment/oocyte donation
- ✓ Transfer day
- ✓ Oocyte origin
- ✓ Nº of MII
- ✓Patient age
- ✓ Nº of prvious treatments
- ✓ Nº of embryos transferred
- ✓ IMCI
- ✓ Clinic

Estimated Improvement of ESD

Type of treatment: autologous or donation



Conventional incubator

Disadvantage

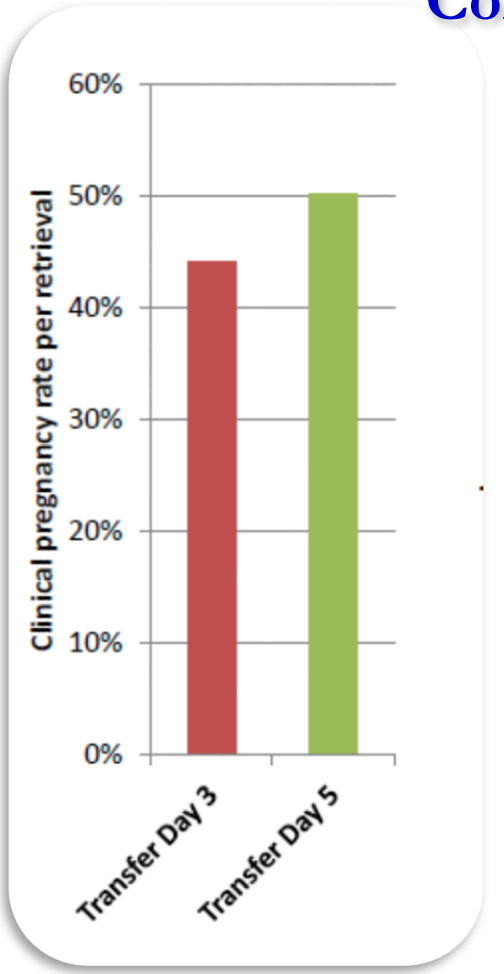
TMS Incubator

Advantage

- ✓Uneven distribution
- ✓This factor gives advantage to TMS (and higher pregnancy rates than CI

Estimated Improvement of ESD

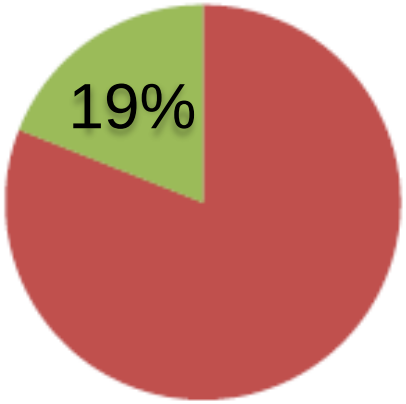
Transfer day:



Conventional incubator

Advantage

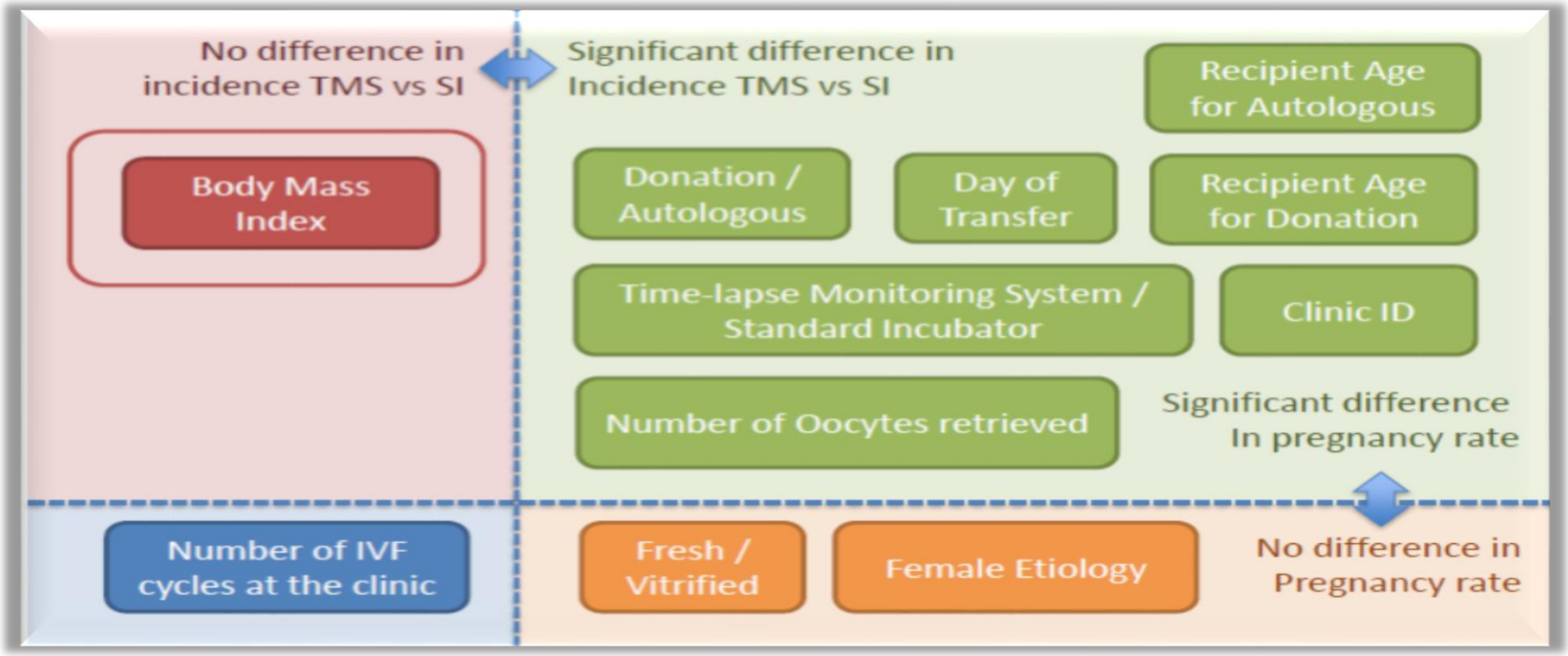
TMS Incubator



Disadvantage

Disadvantage of TMS vs conventional incubator

Estimated Improvement of ESD



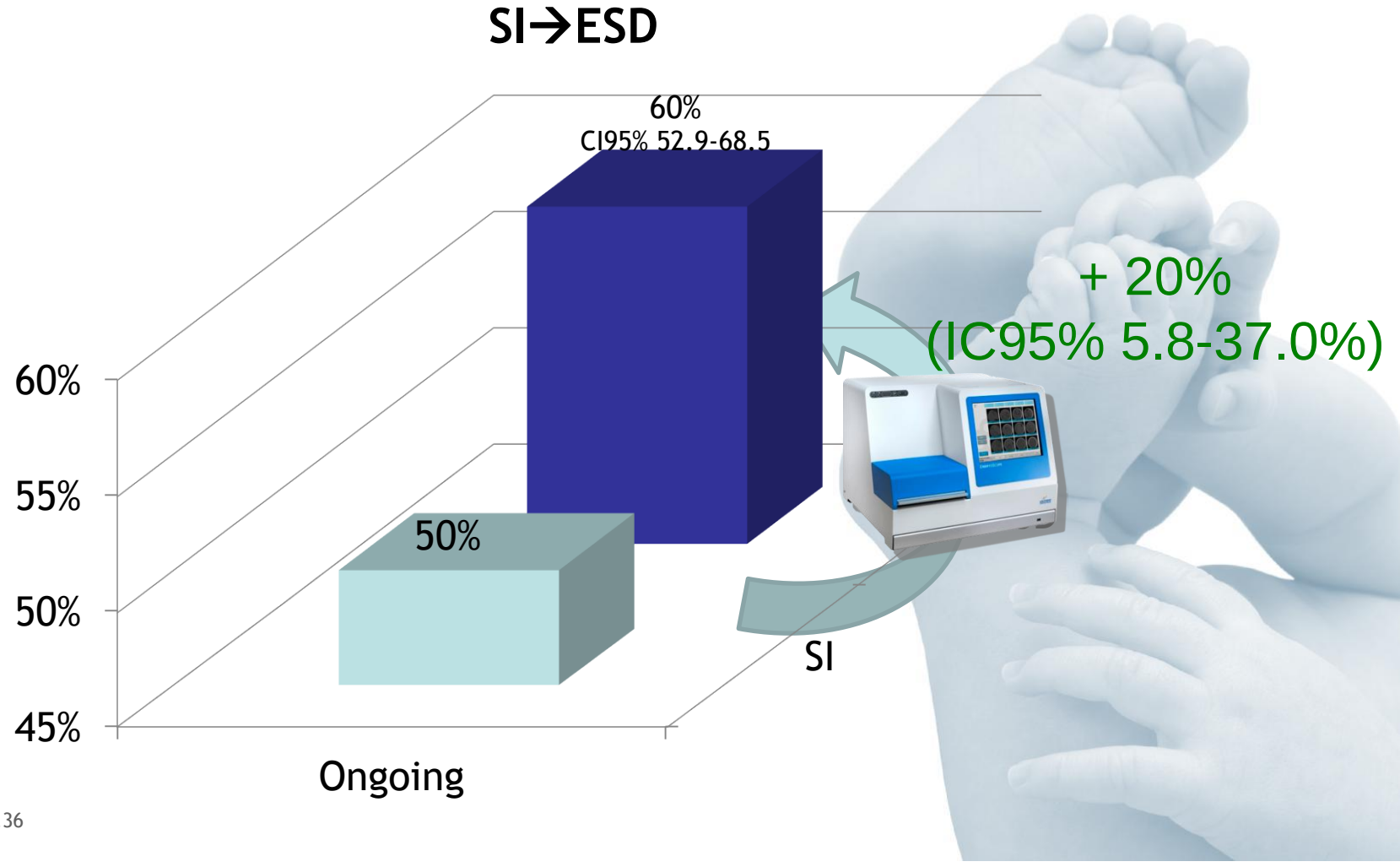
Odds Ratio for clinical pregnancy based on 7305 cycles with oocyte retrieval

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 Autocycles	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	Peer oocyte less in Autologues 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

Estimated Improvement of ESD

- Effect of ESD on clinical pregnancy;

OR= 1.201 (CI95% 1.059-1.363) p=0.0043



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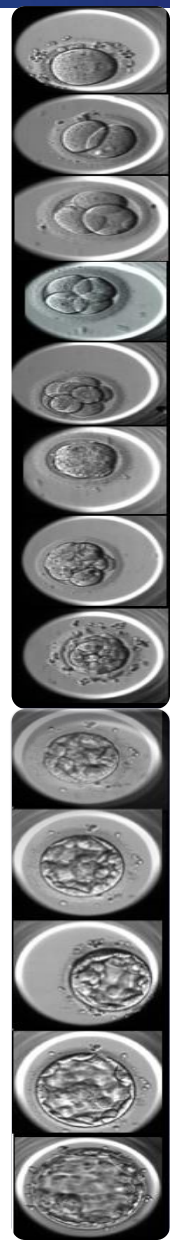

Randomized Control Trial

ARTICLE IN PRESS

ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Clinical validation of embryo culture and selection by morphokinetic analysis: a randomized, controlled trial of the EmbryoScope

Irene Rubio, Ph.D.,[a] Arancha Galán, Ph.D.,[a] Zaloa Larreategui, Ph.D.,[b] Fernando Ayerdi, Ph.D.,[b] Jose Bellver, M.D.,[a] Javier Herrero, Ph.D.,[a] and Marcos Meseguer, Ph.D.[a]

[a] Instituto Universitario IVI Valencia, University of Valencia, Valencia; and [b] IVI Bilbao, Bilbao, Spain

Real Improvement of ESD

Randomized Control Trial

TABLE 1

Descriptive characteristics of the patient and IVF laboratory practice in the time-lapse and control groups.

	TMS group (n = 438)	Control group (n = 405)	*P* value
Age (y), patients and recipients	34.7 ± 2.7 (34.4–34.9)	34.6 ± 2.7 (34.4–34.9)	NS
Age (y), patients and donors	30.4 ± 5.5 (29.9–31.0)	30.0 ± 5.5 (29.5–30.5)	NS
BMI (kg/m^2)	23.2 ± 3.7 (22.6–23.7)	23.04 ± 2.8 (22.5–23.5)	NS
COS protocol			
Long GnRH agonist (%)	14.8 (65)	16.4 (66)	NS
Short GnRH antagonist (%)	85.2 (373)	83.6 (339)	NS
Total dose of FSH	1,781 ± 631 (1,709–1,853)	1,832 ± 603 (1,763–1,900)	NS
Total dose of hMG	1,127 ± 664 (1,035–1,219)	990 ± 538 (913–1,066)	NS
E_2 on hCG day (pg/mL)	1,981 ± 943 (1,882–2,079)	1,964 ± 962 (1,860–2,067)	NS
P_4 on hCG day (ng/mL)	0.76 ± 0.38 (0.72–0.80)	0.74 ± 0.35 (0.70–0.78)	NS
Days of stimulation	13.0 ± 3.45 (12.5–13.6)	13.2 ± 3.42 (12.6–13.8)	NS
Donor recipients (%)	47.8 (208) (43.2–52.6)	49.1 (200) (44.2–53.9)	NS
Metaphase II oocytes (n)	8.0 ± 2.7 (7.76–8.26)	8.1 ± 3.0 (7.8–8.3)	NS
Fertilization rate (%)	75.3 ± 16.5 (73.8–76.9)	74.0 ± 17.4 (72.3–75.7)	NS
Day 3 ET (% of total)	72.5 % (318) (68.3–76.7)	75.5 % (306) (71.3–79.7)	NS
Blastocyst ET (%)	27.5 (120) (23.3–31.7)	24.5 (99) (20.3–28.7)	

Note: Values are mean, and values in brackets are 95% confidence intervals or the total number of patients. BMI = body mass index; COS = controlled ovarian stimulation; E_2 = estradiol; ET = embryo transfer; FSH = follicle-stimulating hormone; GnRH = gonadotropin-releasing hormone; hCG = human chorionic gonadotropin; hMG = human menopausal gonadotropin; NS = not statistically significant; P_4 = progesterone; TMS = time-lapse monitoring system.

Rubio. Clinical validation of EmbryoScope. Fertil Steril 2014.

Real Improvement of ESD

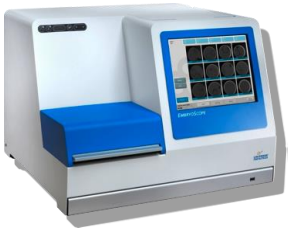

TABLE 2

Descriptive characteristics of the embryo development and fate in the time-lapse and control groups.

	TMS group (n = 2,638)	Control group (n = 2,427)	*P* value
Embryo fragmentation (%)	7.5 ± 0.1 (7.2–7.9)	6.9 ± 9.4 (6.5–7.1)	.006
No. of blastomeres	6.9 ± 2.3 (6.8–6.9)	6.9 ± 2.7 (6.8–7.0)	NS
Embryo symmetry	1.7 ± 0.5 (1.7–1.7)	1.7 ± 0.5 (1.7–1.7)	NS
Optimal embryos (day 3) (%)	46.2 (1,219) (44.3–48.1)	43.1 (1,046) (41.3–45.1)	.010
Blastocyst rate (%)	52.3 (576) (50.3–54.2)	50.5 (471) (48.5–52.5)	NS
Optimal blastocyst (day 5) (%)	20.9 (230) (19.4–22.4)	16.6 (155) (15.1–18.1)	.001
Transferred embryos	1.86 ± 0.37 (1.8–1.9)	1.86 ± 0.40 (1.8–1.9)	NS
Cryopreserved embryos	3.9 ± 2.2 (3.6–4.1)	3.6 ± 2.2 (3.4–3.9)	NS

Note: Values are mean, and values in brackets are 95% confidence interval or the total number of embryos.

Rubio. Clinical validation of EmbryoScope. Fertil Steril 2014.

Real Improvement of ESD

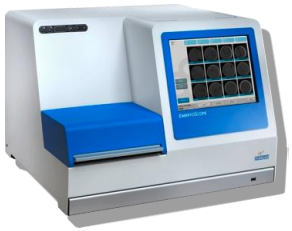

TABLE 3

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

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

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

Rubio. Clinical validation of EmbryoScope. Fertil Steril 2014.

Real Improvement of ESD

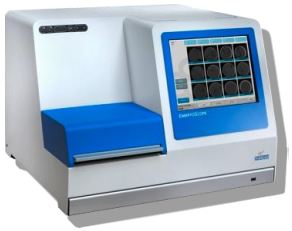

TABLE 4

Logistic regression analysis of ongoing pregnancy after week 12 as affected by incubation type, day of transfer, oocyte source, and age of the patient.

Model effect	Values	OR (95% CI)	*P* value
Incubation	TMS versus SI	1.41 (1.06–1.86)	.015
Day of transfer	Day 5 versus day 3	1.53 (1.08–2.16)	.016
Oocyte source	Autologous versus donation	0.89 (0.60–1.30)	NS
Age	Years	Per year, 1.01 (0.98–1.05)	NS

Note: CI = confidence interval; OR = odds ratio; NS = not statistically significant; SI = standard incubator; TMS = time-lapse monitoring system.

Rubio. Clinical validation of EmbryoScope. Fertil Steril 2014.

Real Improvement of ESD

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

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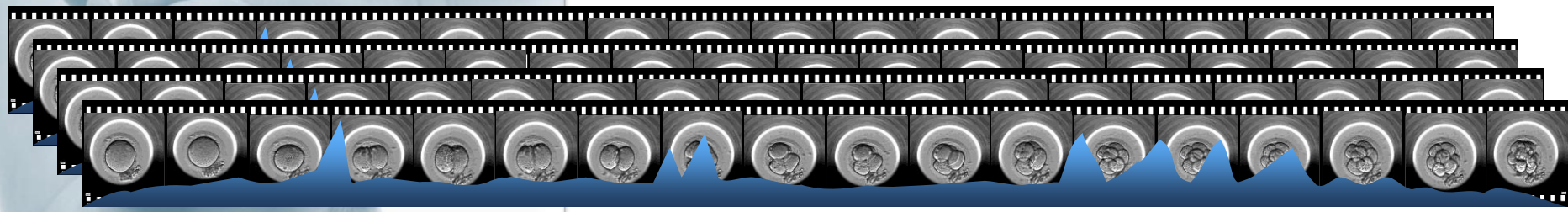
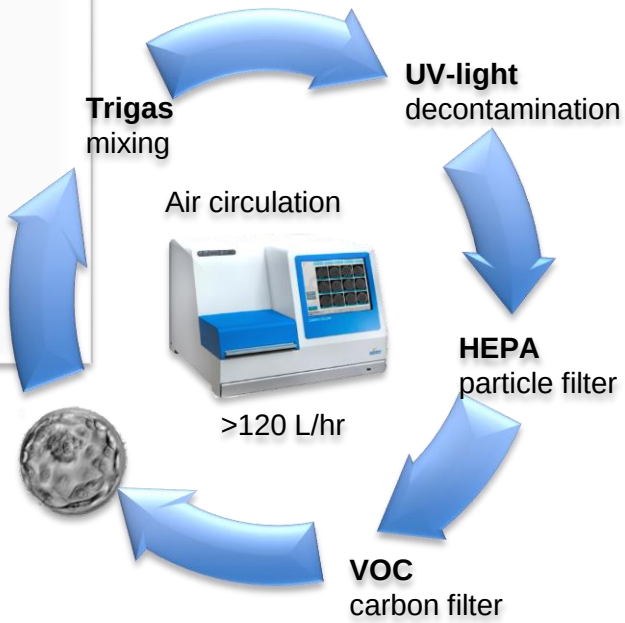
* Instituto Universitario IVI Valencia, University of Valencia, Valencia; and † IVI Bilbao, Bilbao, Spain

Hypothesis

More Observations
Better Selection

Less Disturbance
Better Development





Conclusions

✓Morphokinetics are related with morphology but it is necessary to combine both of them for embryo selection

✓ Embryo time-lapse analysis makes it possible to identify the best moment of IVM.

✓ Time-lapse provides new markers of implantation with rigor and objectivity: exclusion and inclusion criteria

✓ Algorithm for embryo selection has been established, but the variables could change, so, each lab should develop its own algorithm

✓ Kinetics are related with chromosome content

✓ The clinical use of time-lapse for embryo selection is able to improve reproductive outcome.