

Time-lapse technology in clinical setting: outcome results

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Time-lapse imaging systems

EmbryoScope®

Incubator + microscope, 20X

Users annotations and algorithms



EEVA: in incubator,
mathematical algorithms,
no annotations, dark field, 10x



Primovision: in incubator,
LED light, 10x



ESCO: incubator + microscope

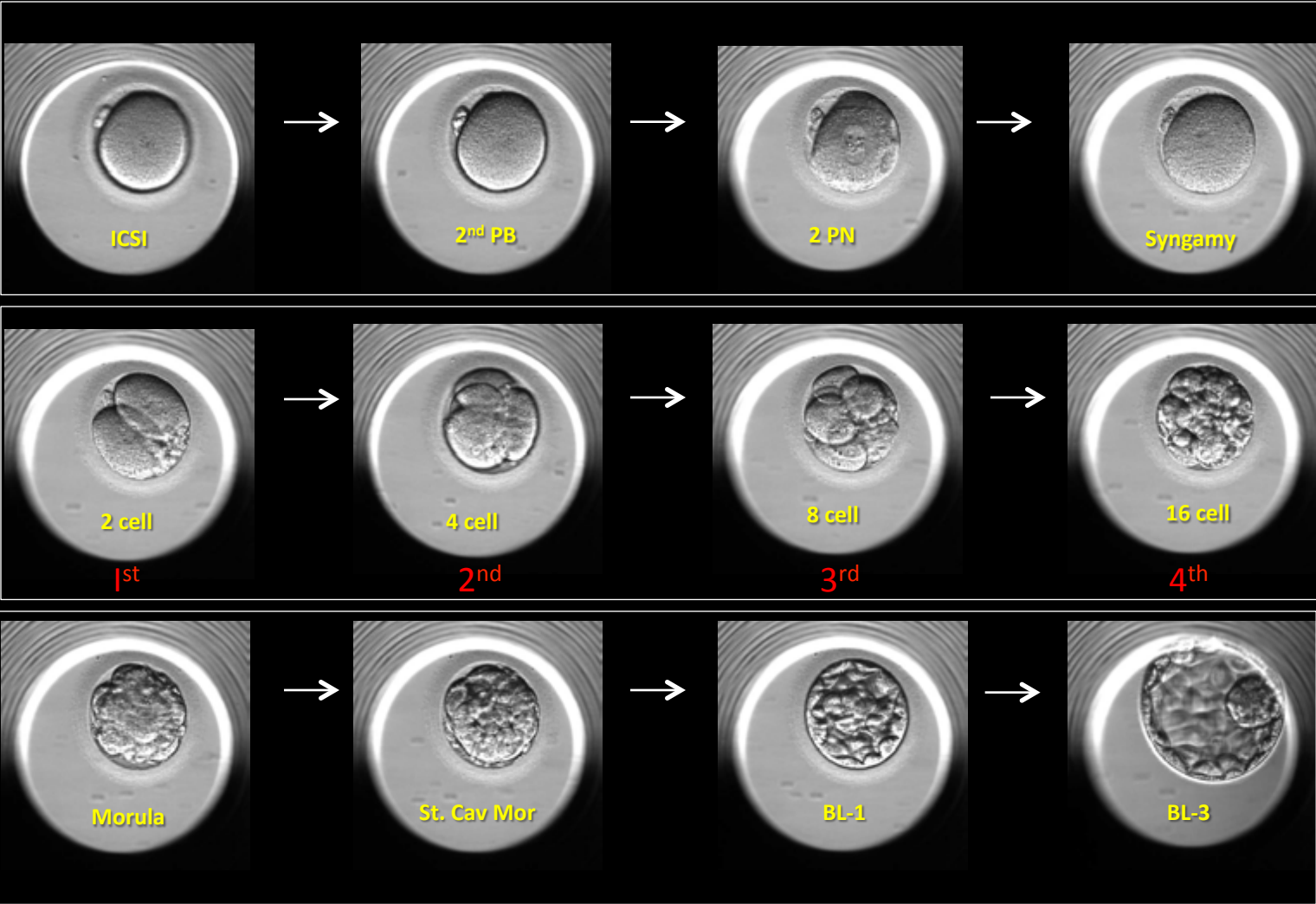


ASTEC: in incubator, red light, 10x

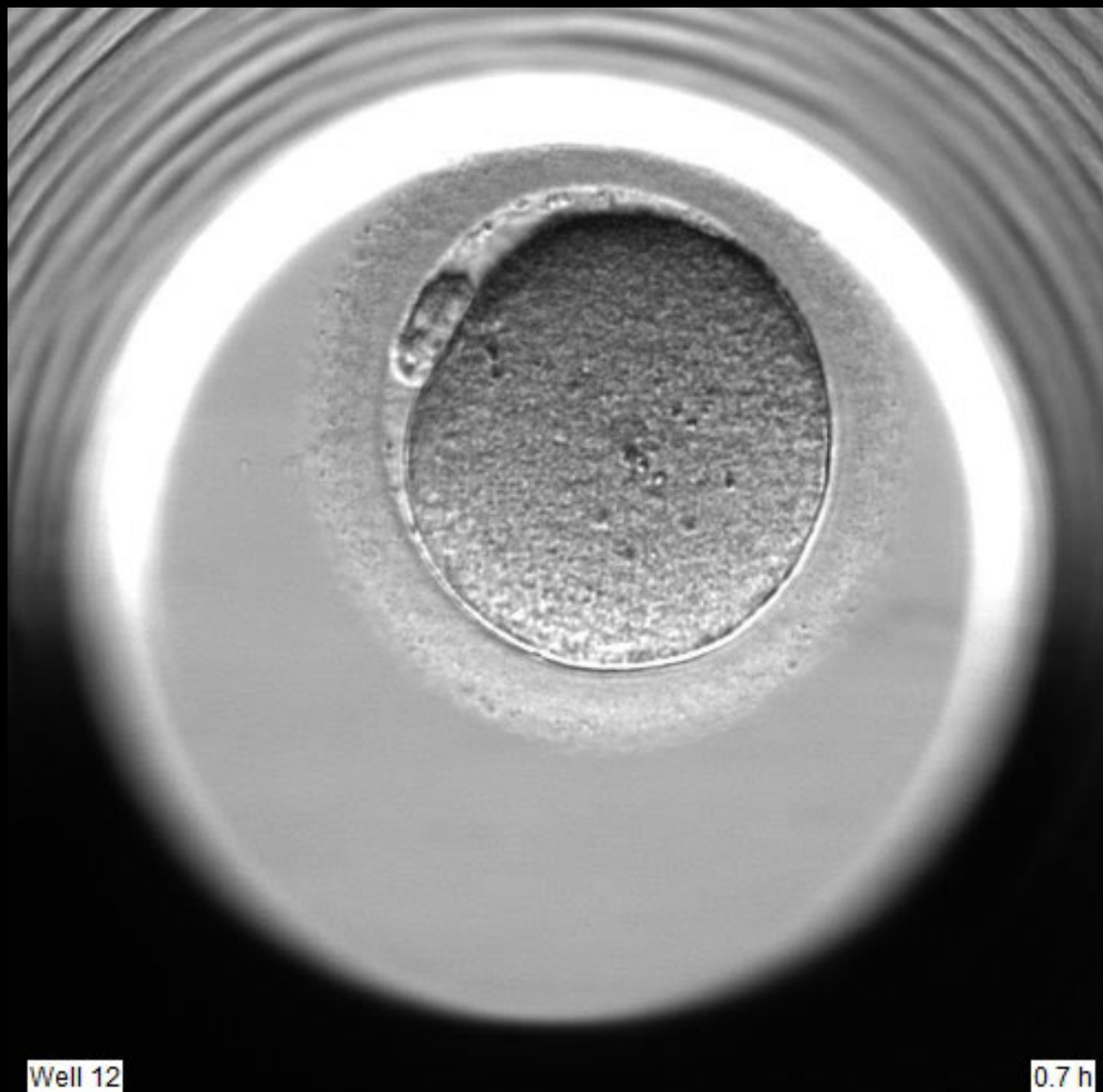
Implementing EmbryoScope® at CRM, Weill Cornell

- November 2011: 1st E instrument (20% O₂)
 - 2012 two E instruments (5% and 20% O₂)
 - 2013: 6 E instruments (all 5% O₂)
 - March 2013 EmbryoScope® incubation for all IVF patients
 - 2014: 8 E instruments
 - 2015: 11 E instruments (server incorporated)
 - Over 4,500 cycles in E
 - **Babies born** (March 2015): **1,053**
 - 801 IVF
 - 128 DER
 - 124FET
- >500 ongoing preg. in 2014/15 pending deliveries

Human Embryo Development



Optimal embryo development: Time-lapse



Selection of human embryos for ET using Time-lapse (Cornell)

Two - step process

1. Embryo de-selection

- Selection of embryos with abnormal karyokinesis, cytokinesis or other abnormalities for exclusion

Selection of human embryos for ET using Time-lapse (Cornell)

Two - step process

1. Embryo de-selection

Selection of embryos with abnormal karyokinesis, cytokinesis or other abnormalities for exclusion

2. Embryo selection

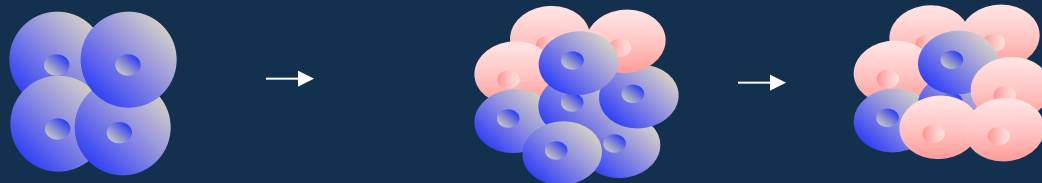
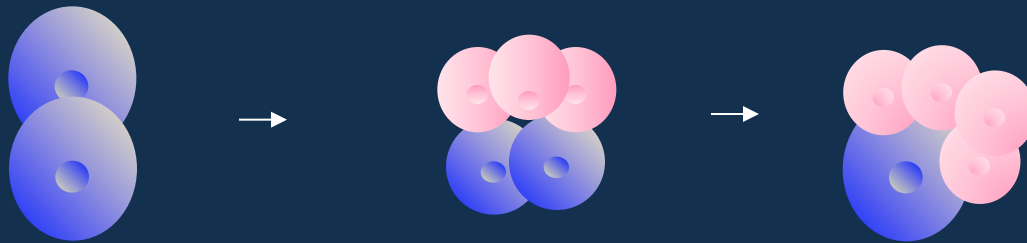
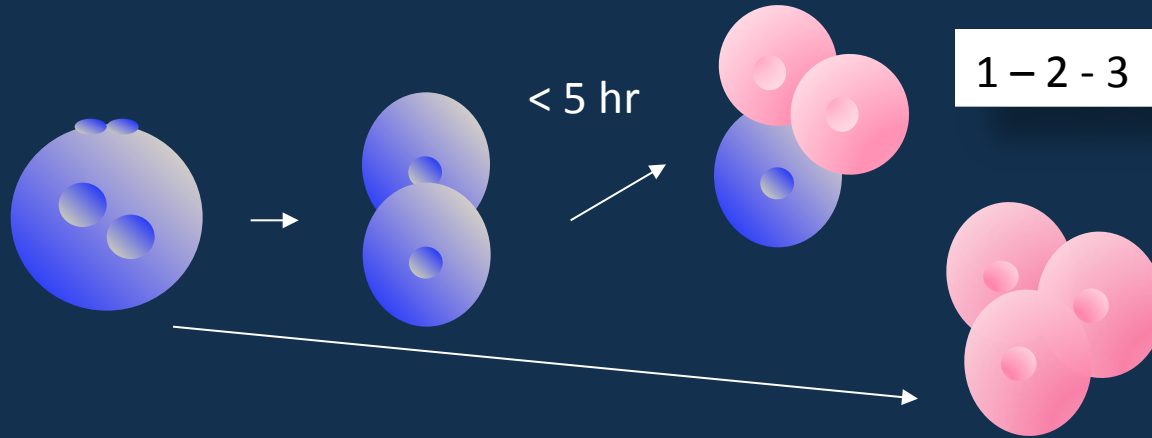
Selection of embryo(s) with “optimal” morphokinetics

Embryo De-Selection

Abnormalities of human embryo development identified by Time -lapse: Embryo de-selection

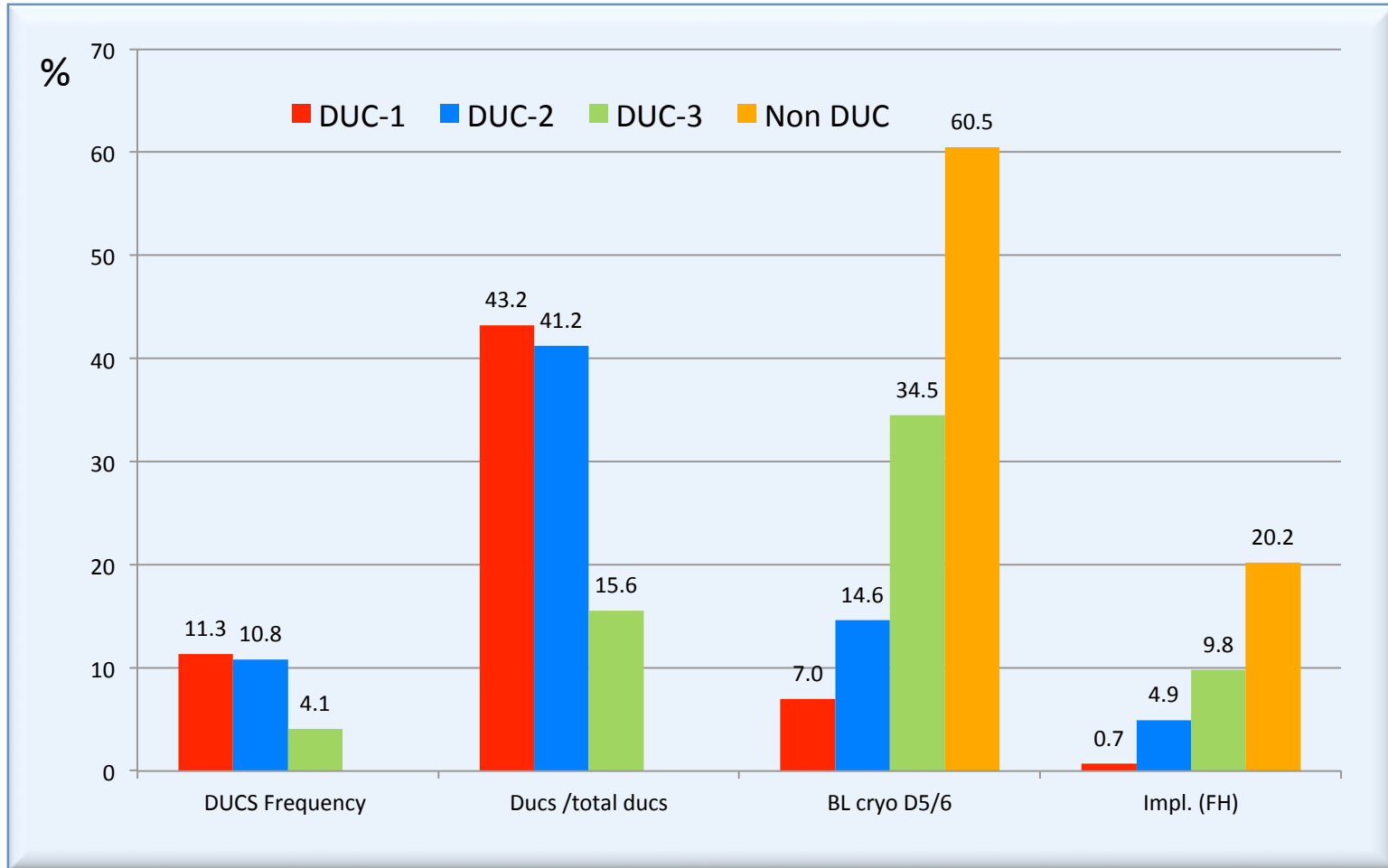
- DUCS: Direct Unequal Cleavages
- Cell Fusion
- NUK: Nuclear division without cytoplasmic division
- 1 cell MNU (Multinucleated 1-cell)
- Premature compaction
- Compaction of few cells, partial blastocyst
- Cell extrusion
- Blastocyst collapse

Direct Unequal Cleavages (DUCS)



DUCS: frequency and outcome

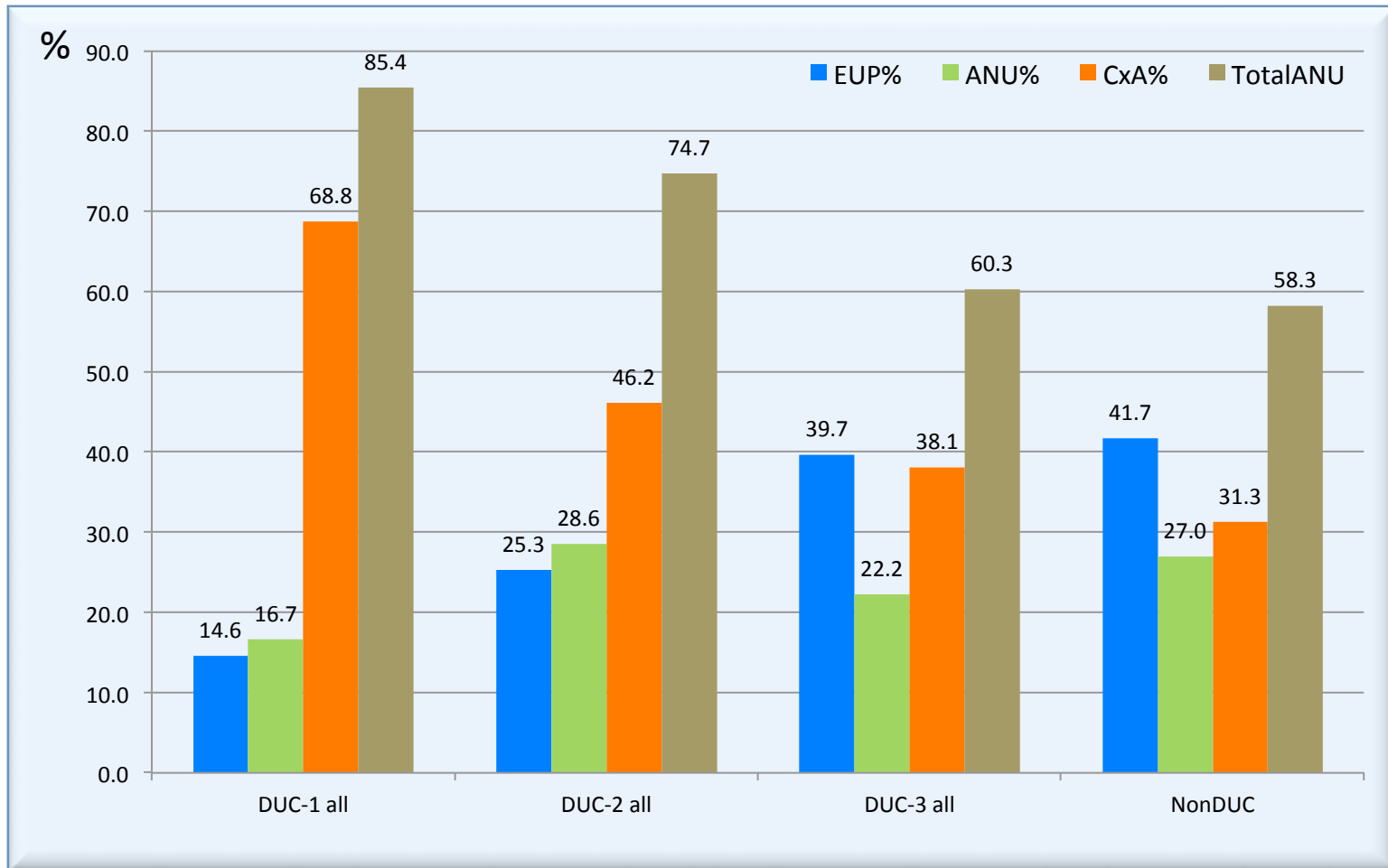
5,597/21,368 (26.2%) embryos



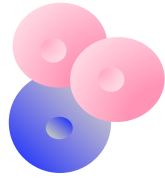
No LB with DUC-1

DUCS: Chromosomal Analysis

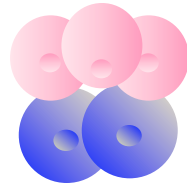
310 PGS cycles; 202/1,462 DUCS (13.8%) biopsied embryos (D3 or D5 bx)



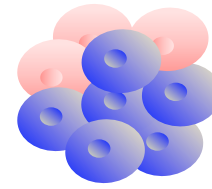
Chances of DUCS embryos to be euploid BL (PGS data)



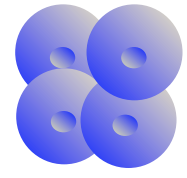
DUC-1 (1-3)



DUC-2 (2-4)

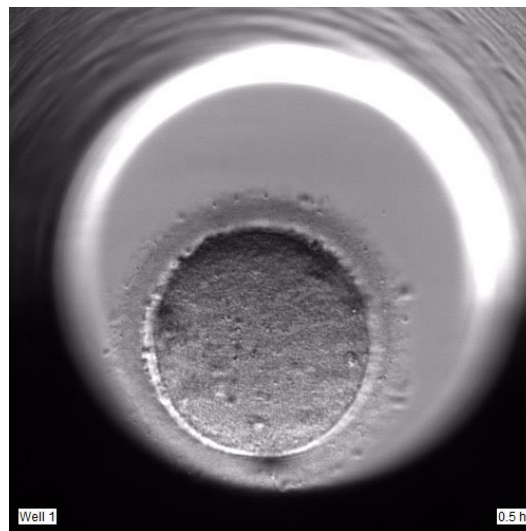


DUC-3 (4-6)



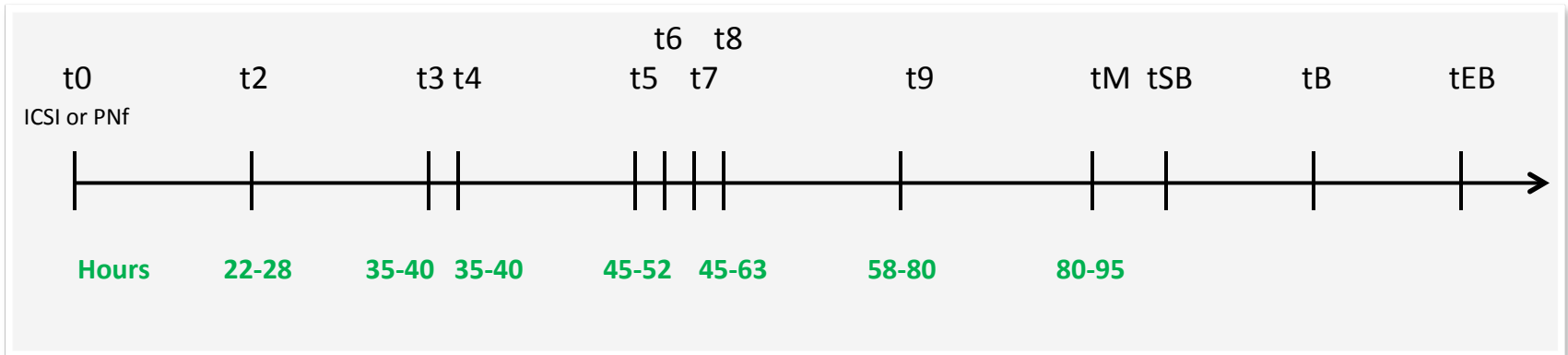
Non-DUC

	DUC-1 (1-3)	DUC-2 (2-4)	DUC-3 (4-6)	Non-DUC
Good embryos D3	27%	58%	82%	56%
BL D5/6	8.2%	19%	40%	61%
Euploid BL	25%	28%	40%	38%
Euploid %	1-3%	5%	16%	24%



Embryo selection using Time-Lapse kinetic parameters

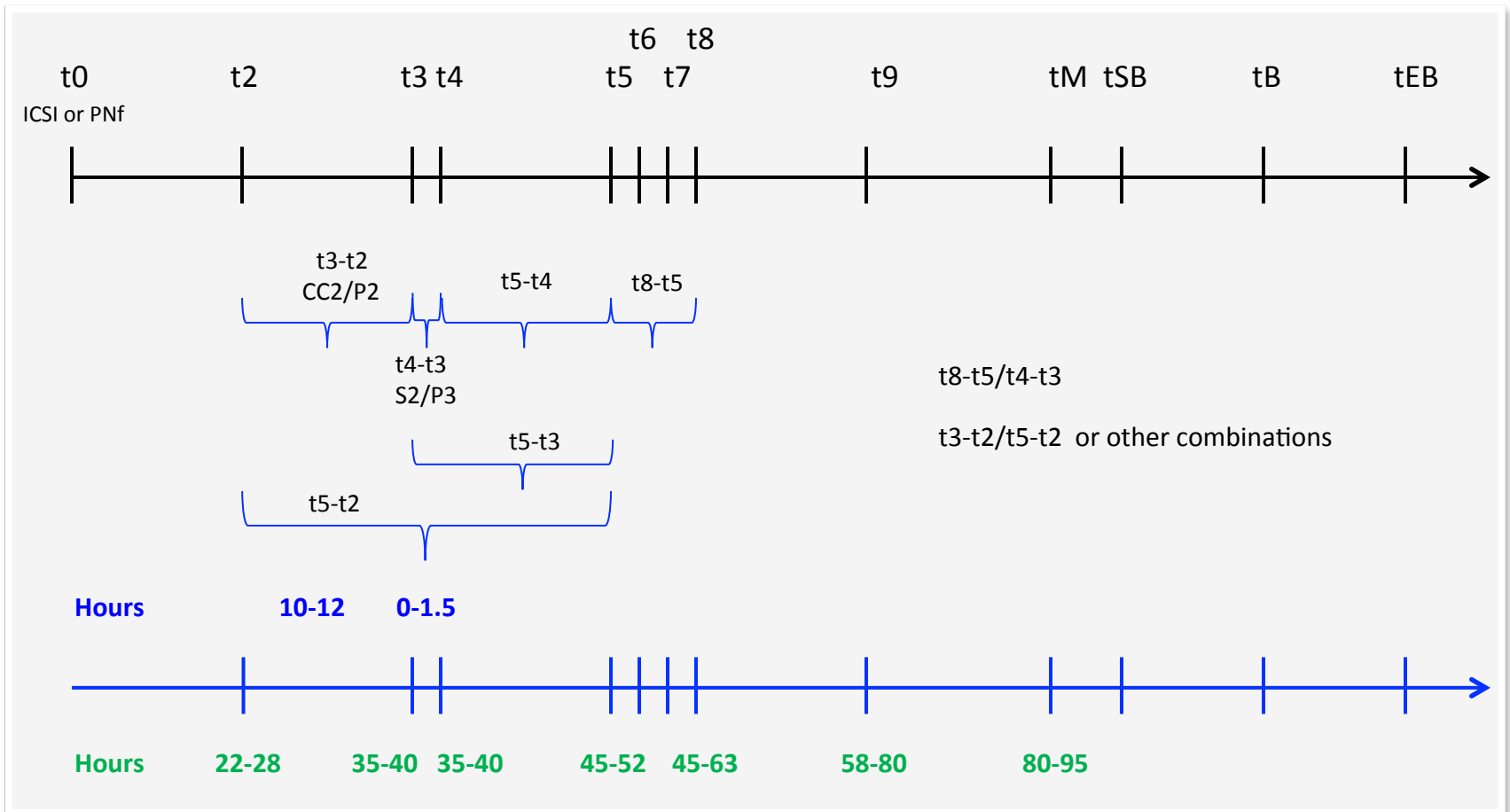
“Optimal” Embryo Kinetic?



Individual cleavage time points

Insufficient for embryo selection

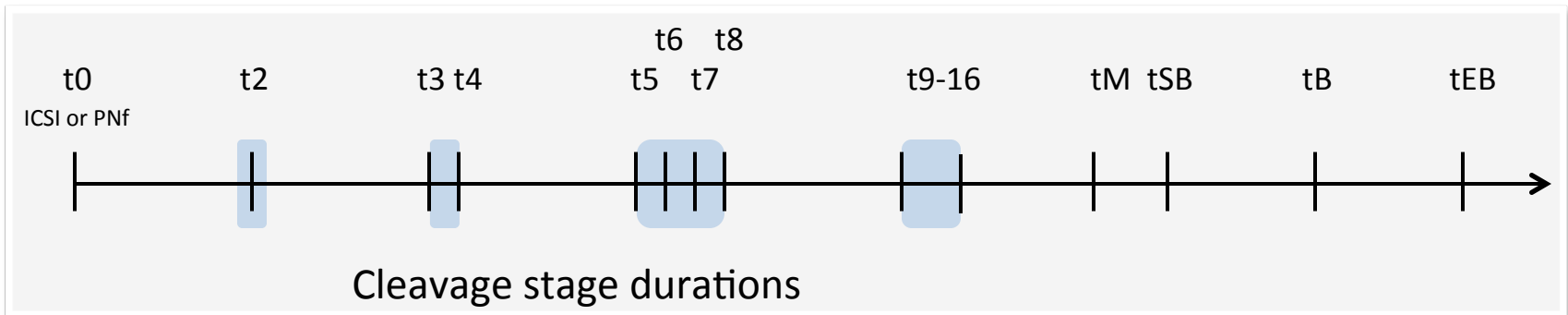
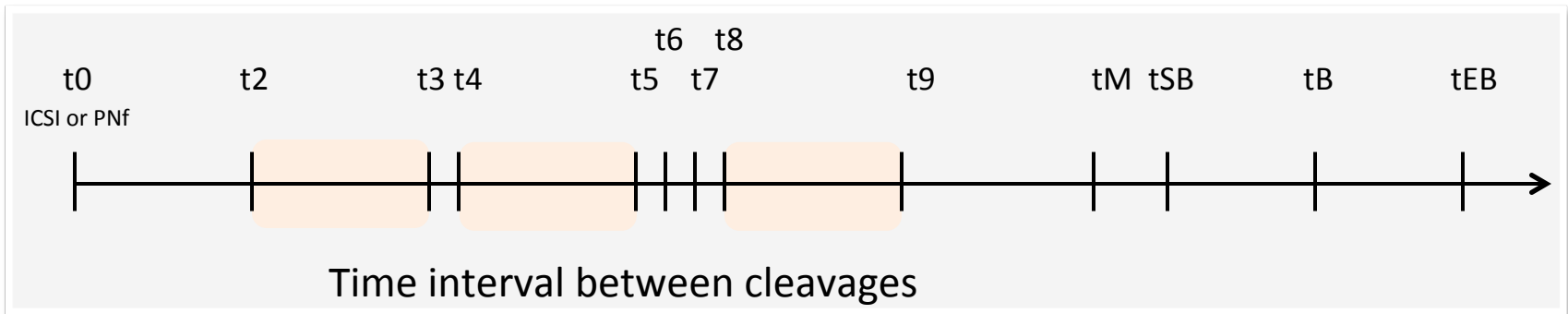
“Optimal” Embryo Kinetic?



Individual time points + Synchronized cleavages

Enhanced embryo selection

Embryo Selection



Embryo morphology: fragmentation, cell symmetry

Can blastocyst development be predicted by embryo kinetic by day 3?

Can embryo kinetic by day 3 predict embryo viability/implantation?

Can we develop universal embryo selection model(s) based on embryo kinetic?

EmbryoScope[®] vs. standard incubator

Retrospective analysis

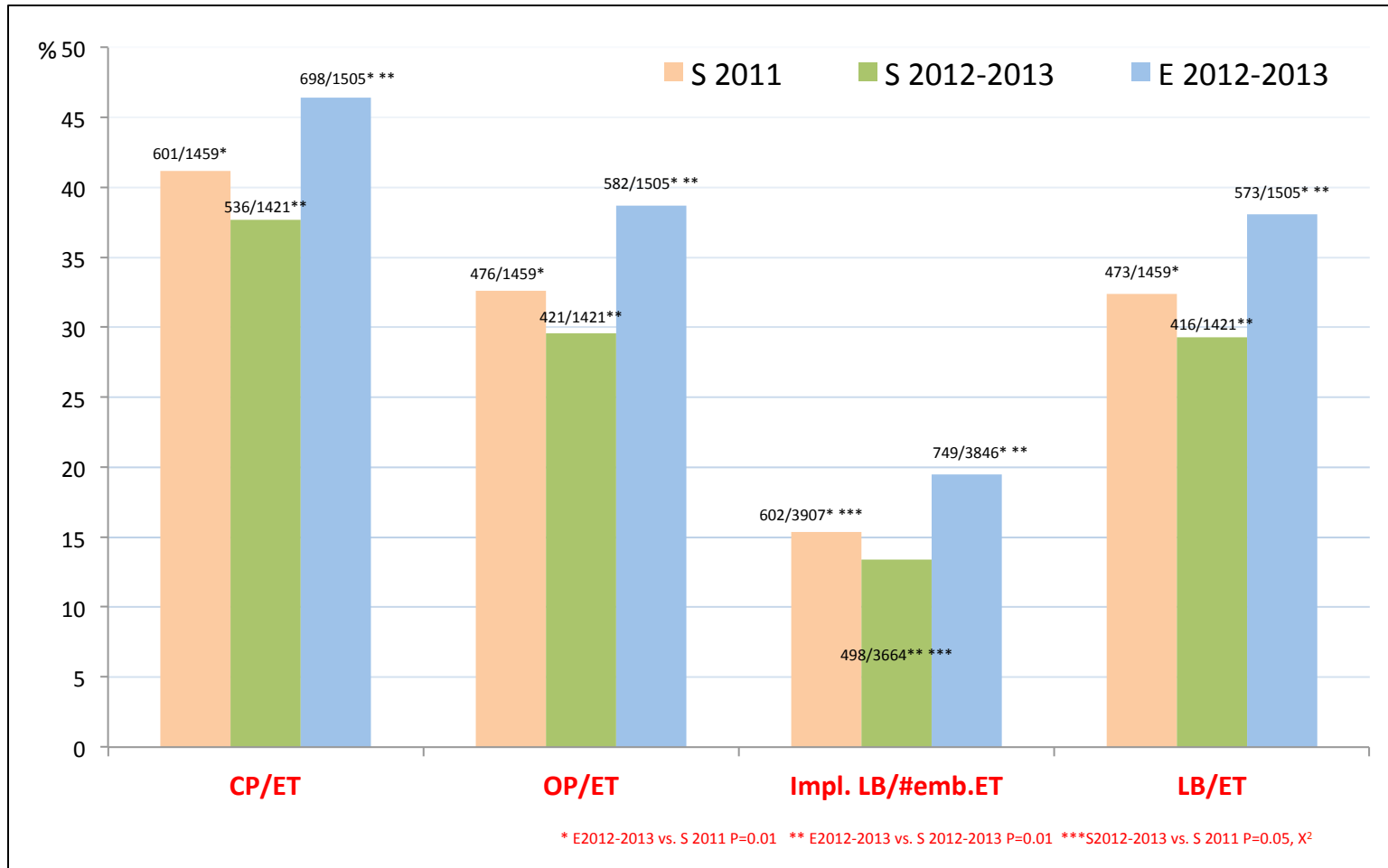
2011 (S) vs. 2012-2013 (S) vs. 2012-2013 (E)

Based on the live births

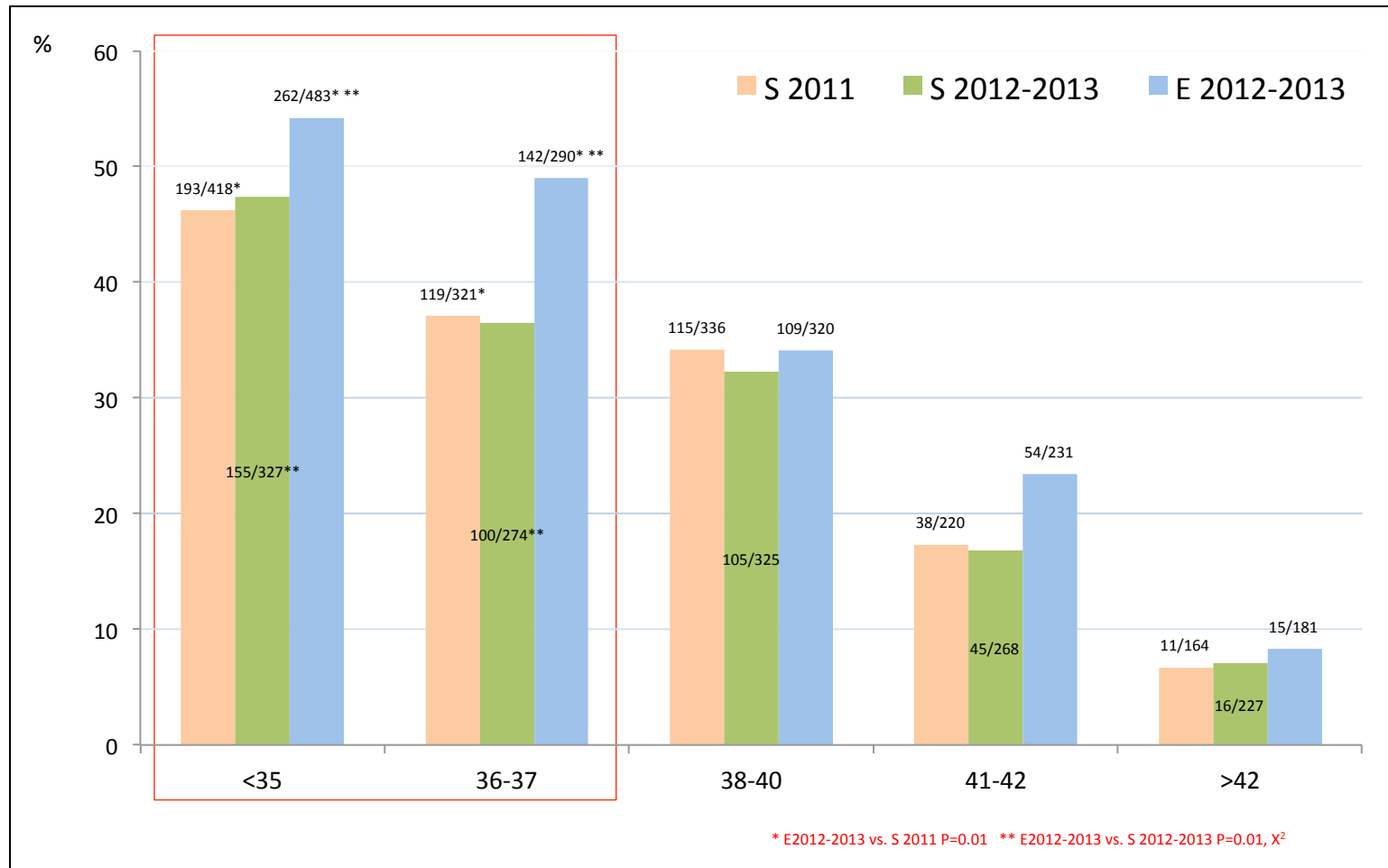
Excluded: PGS, cryo U/F, CC, split cycles

IVF cycles	2011 (S)	2012-2013 (S)	2012-2013 (E)
Number of patients	1231	1199	1318
Number of ET cycles	1459	1421	1505
Number of oocytes (avg./cycles)	15888 (avg. 12.9)	12961 (avg. 10.8)	17263 (avg. 13.1)
Number of MII (Avg.)	12965 (Avg. 10.5)	10465 (Avg. 8.7)	14308 (Avg. 10.9)
Number of 2PN (Avg.)	9646 (Avg. 7.8)	7401 (Avg. 6.2)	10918 (Avg. 8.3)
Number of embryos ET (Avg.)	3907 (Avg. 2.7)	3664 (Avg. 2.6)	3846 (Avg. 2.6)
Number of D3-ET	1321	1324	1214
Number of D5-ET (%) (few D2, D4 or D6 ET)	136 (9.3%)	91 (6.4%)	290 (19.3%)
Number of SET (D5)(%)	20 (14.7%)	22 (24.2%)	70 (24.1%)
Number of cycles with BL cryo (%/cycle)	401 (27.5%)	310 (21.2%)	633 (42.1%)
Number of BL frozen (avg./cryo cycle)	1011 (Avg. 2.5)	718 (Avg. 2.3)	2113 (Avg. 3.3)

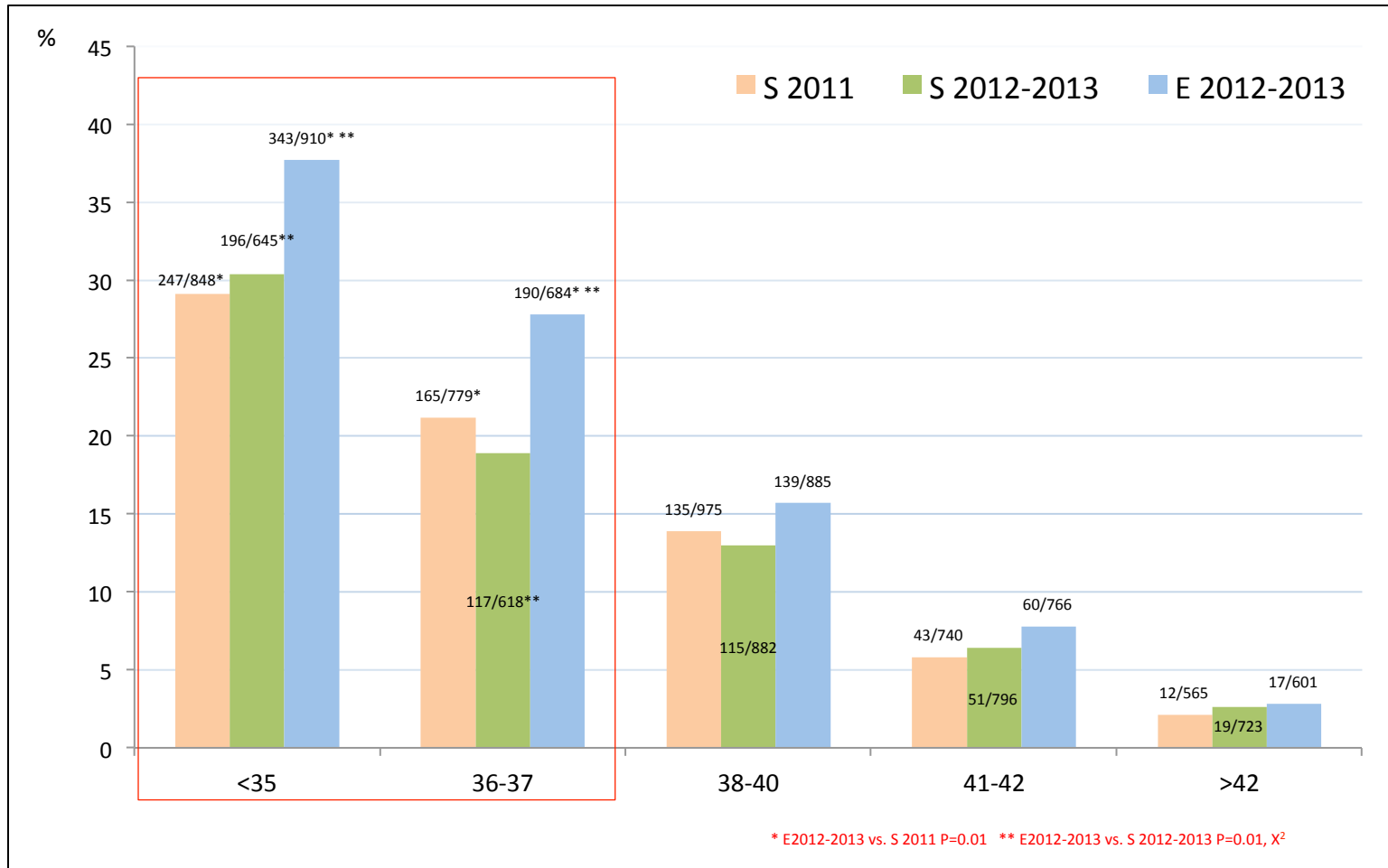
Overall clinical outcome data for **CP** (sac seen), **OP** (per LB), **IMPL** (LB per ET) and **LB cycles** (per ET) between 2011 S, 2012-13 S and 2012-13 E cycles.



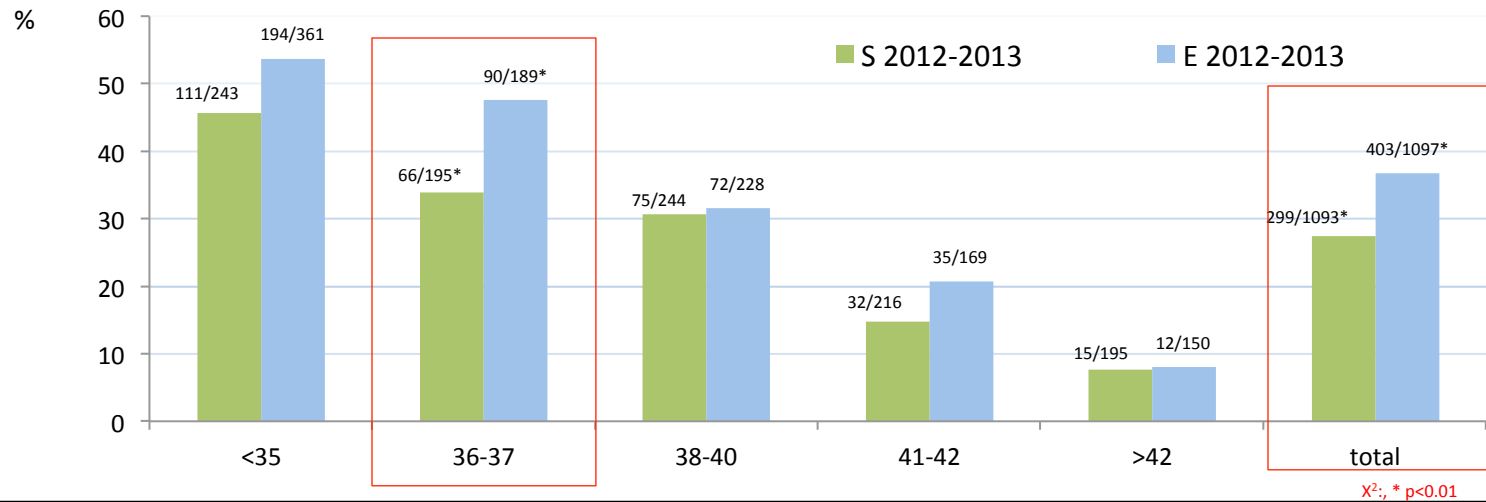
Ongoing pregnancies per ET (LB/ET) by maternal age in S 2011, S 2012-13 and E 2012-13 groups.



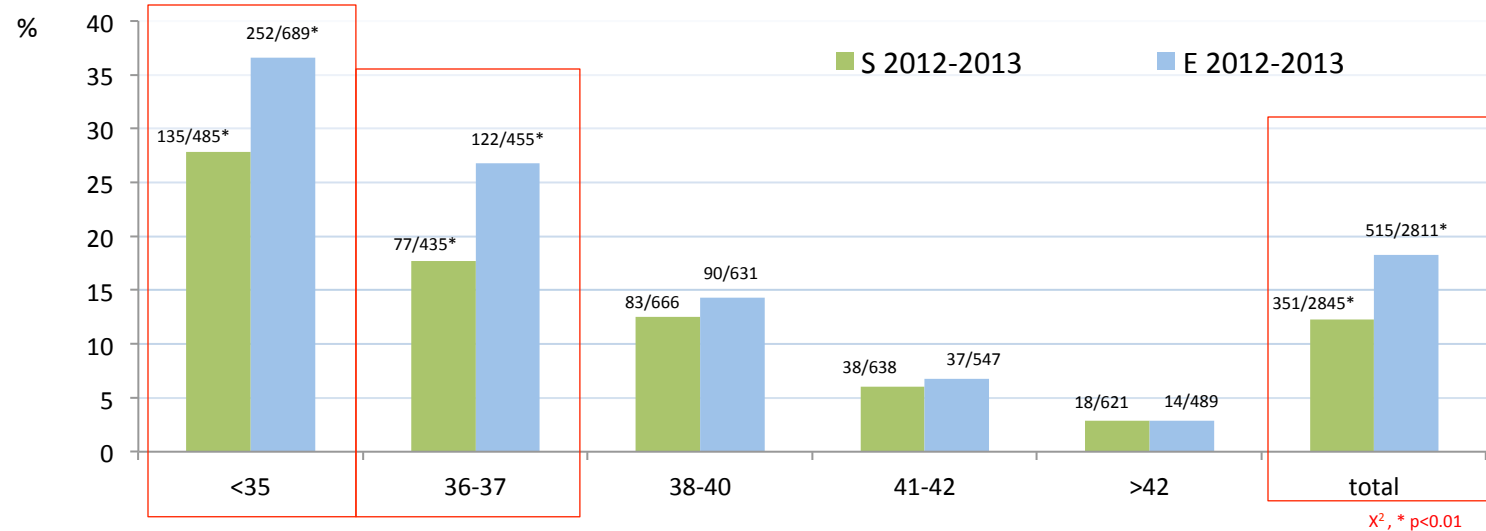
Implantation rate (number of LB/ embryos ET) by maternal age in S 2011, S 2012-13 and E 2012-13 groups



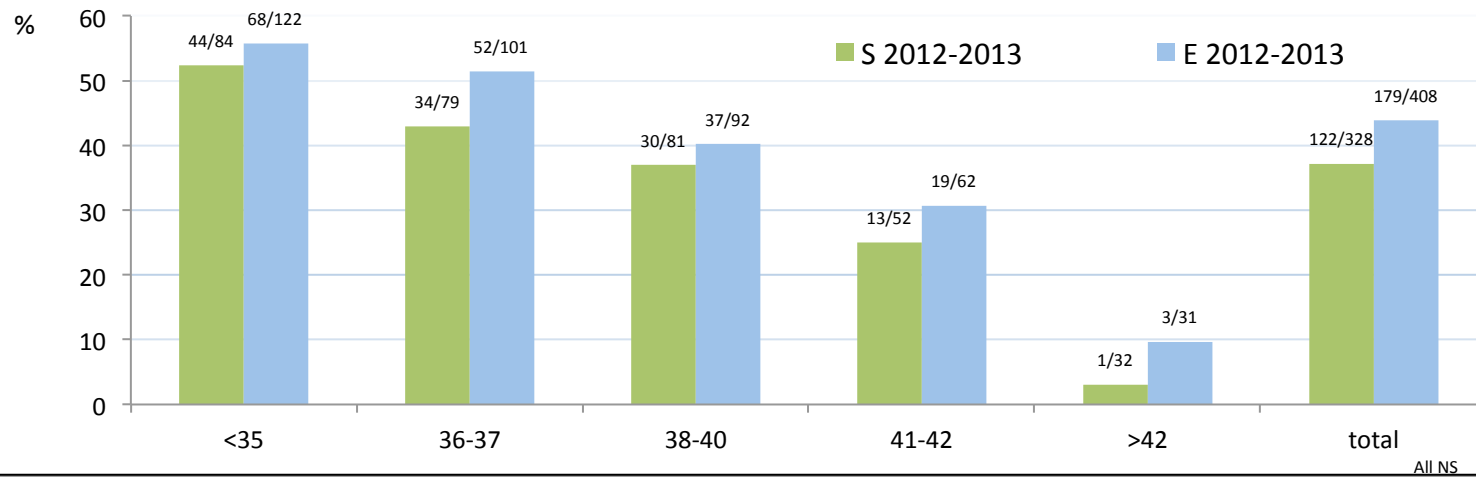
Ongoing pregnancies per ET (LB) in ICSI cycles by maternal age:
S 2012-13 vs. E 2012-13 groups



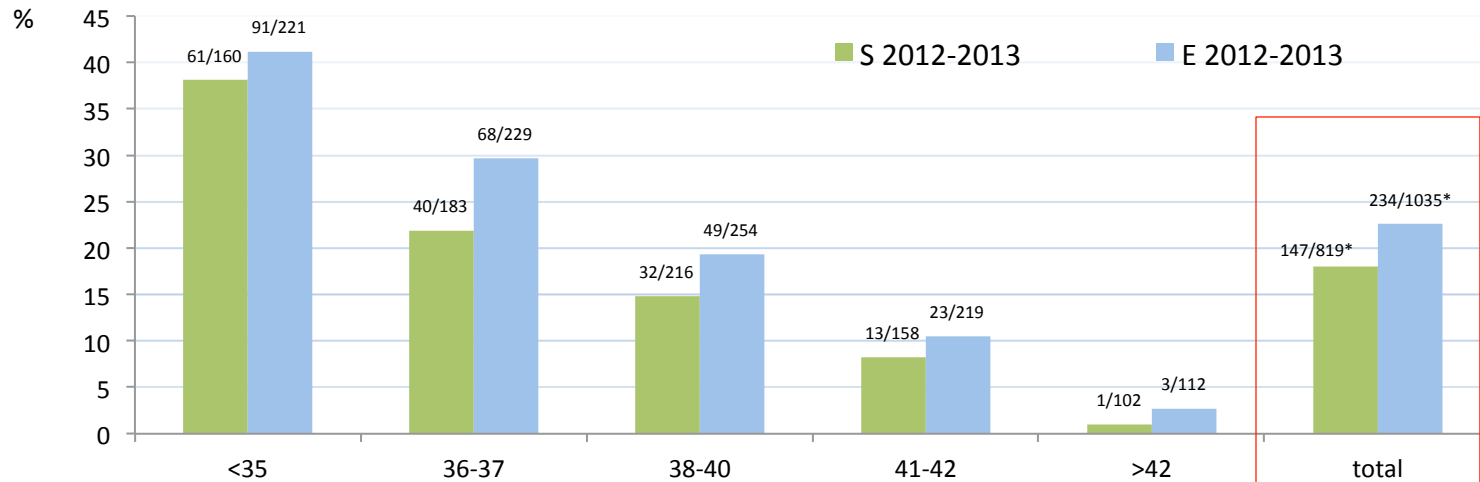
Implantation rate (LB/embryos ET) in ICSI cycles by maternal age:
S 2012-13 vs. E 2012-13 groups



Ongoing pregnancies per ET (LB) in **inseminated cycles** by maternal age:
S 2012-13 vs. E 2012-13 groups

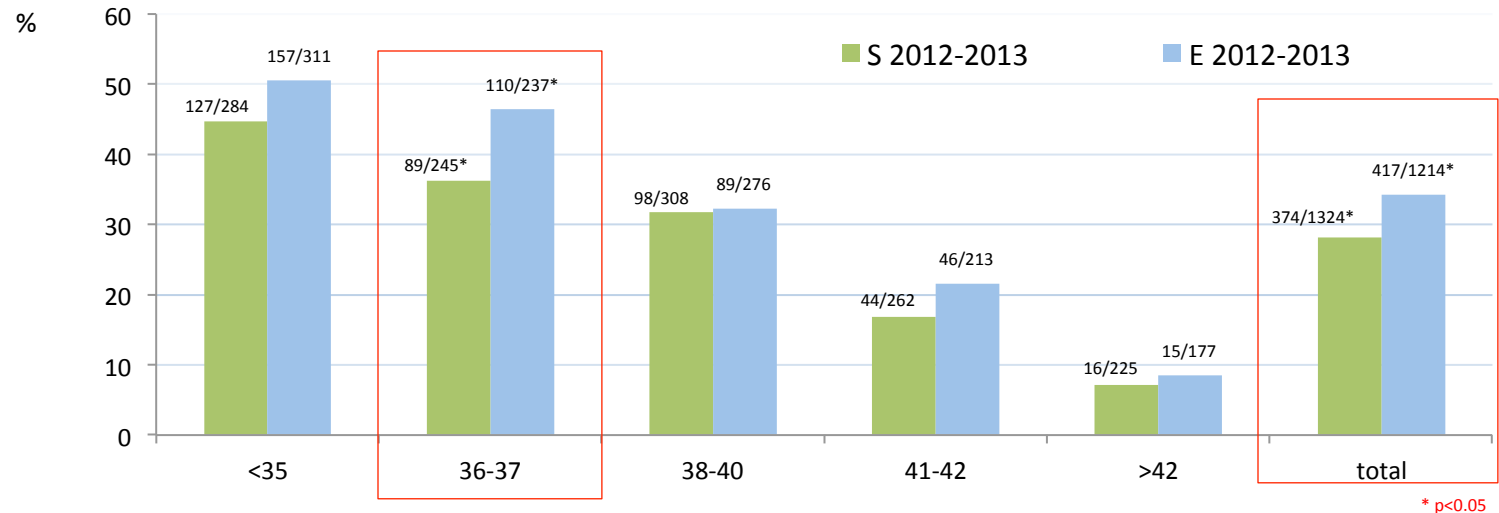


Implantation rate (LB/embryos ET) in **inseminated cycles** by maternal age:
S 2012-13 vs. E 2012-13 groups

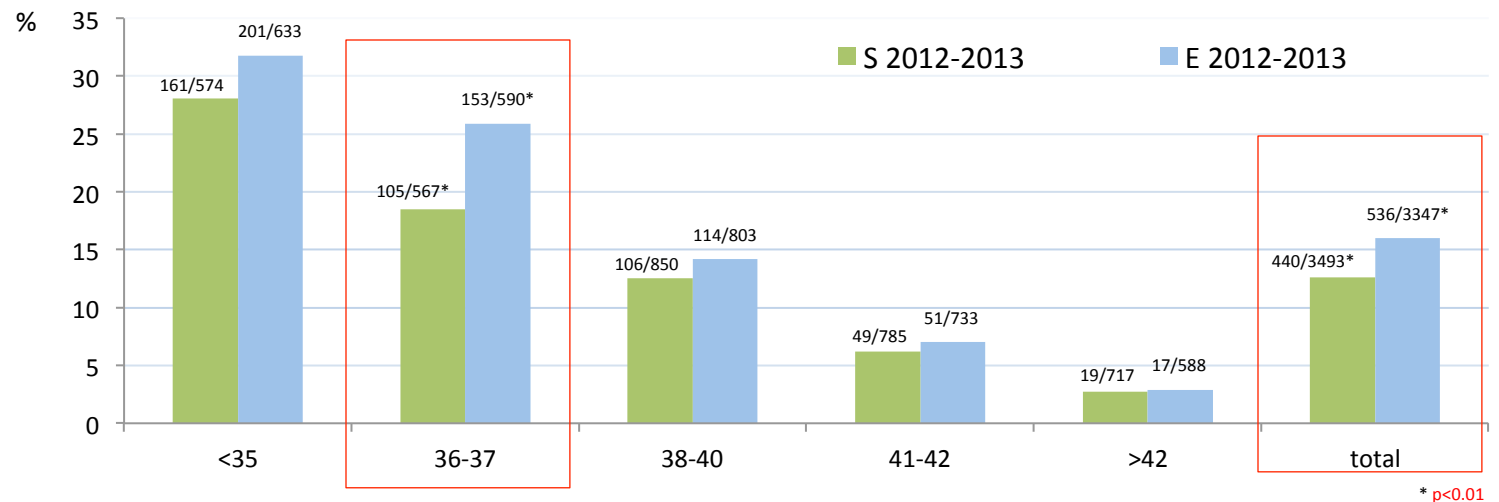


X², * p=0.02

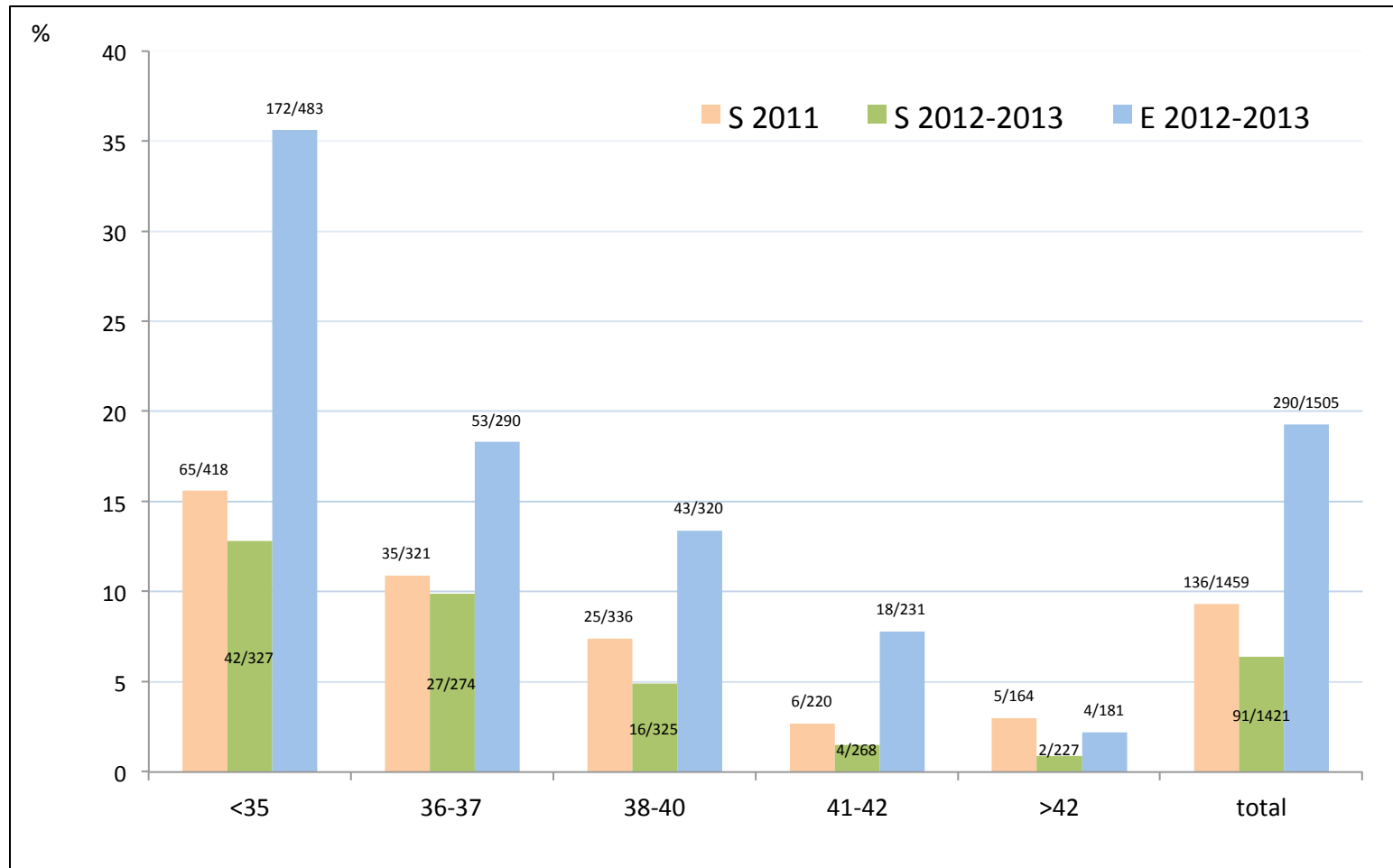
Ongoing pregnancies per ET (LB) in **day 3 ET** cycles by maternal age:
S 2012-13 vs. E 2012-13 groups



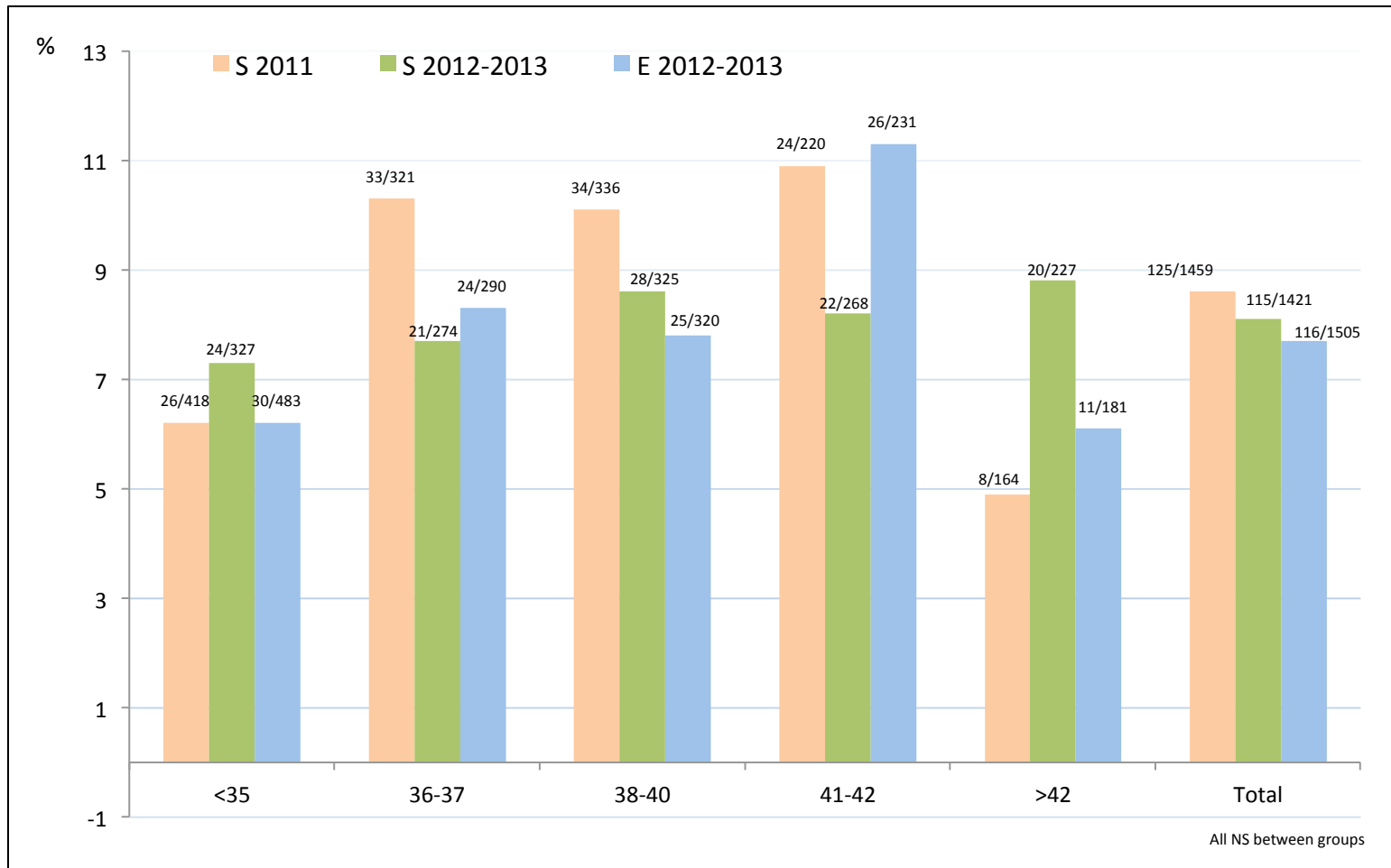
Implantation rate (LB/embryos ET) in **day 3 ET** cycles by maternal age:
S 2012-13 vs. E 2012-13 groups



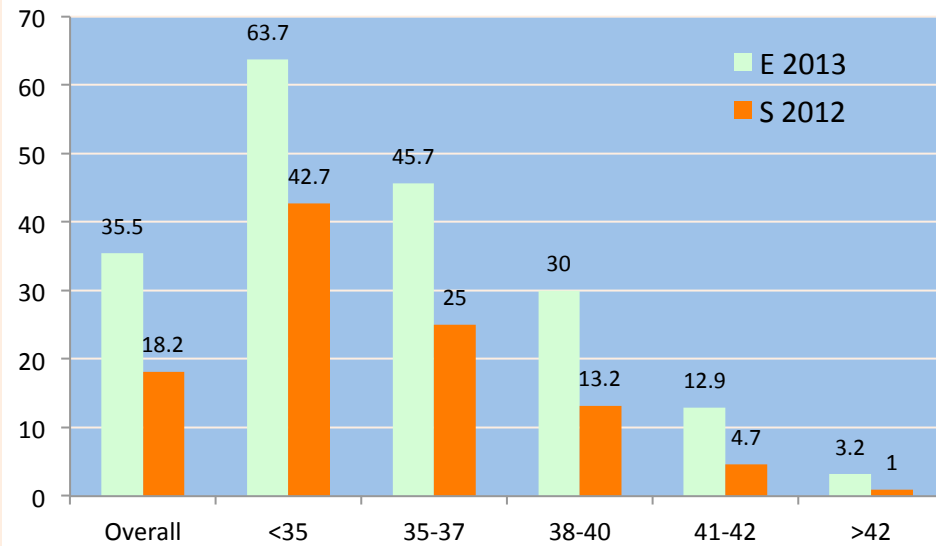
Percent of day 5 ET in correlation with maternal age



Miscarriage cycle rate by maternal age

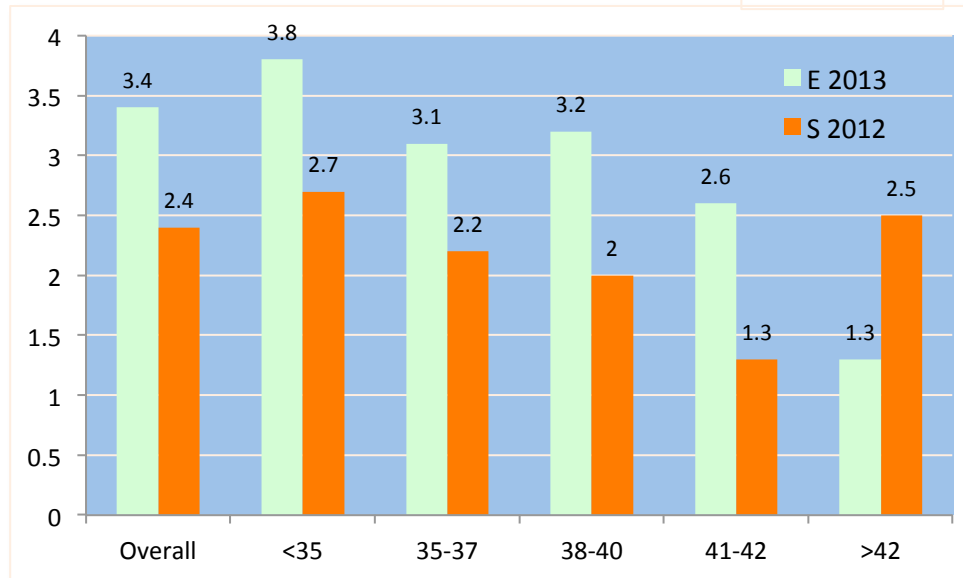


% BL cryo cycles



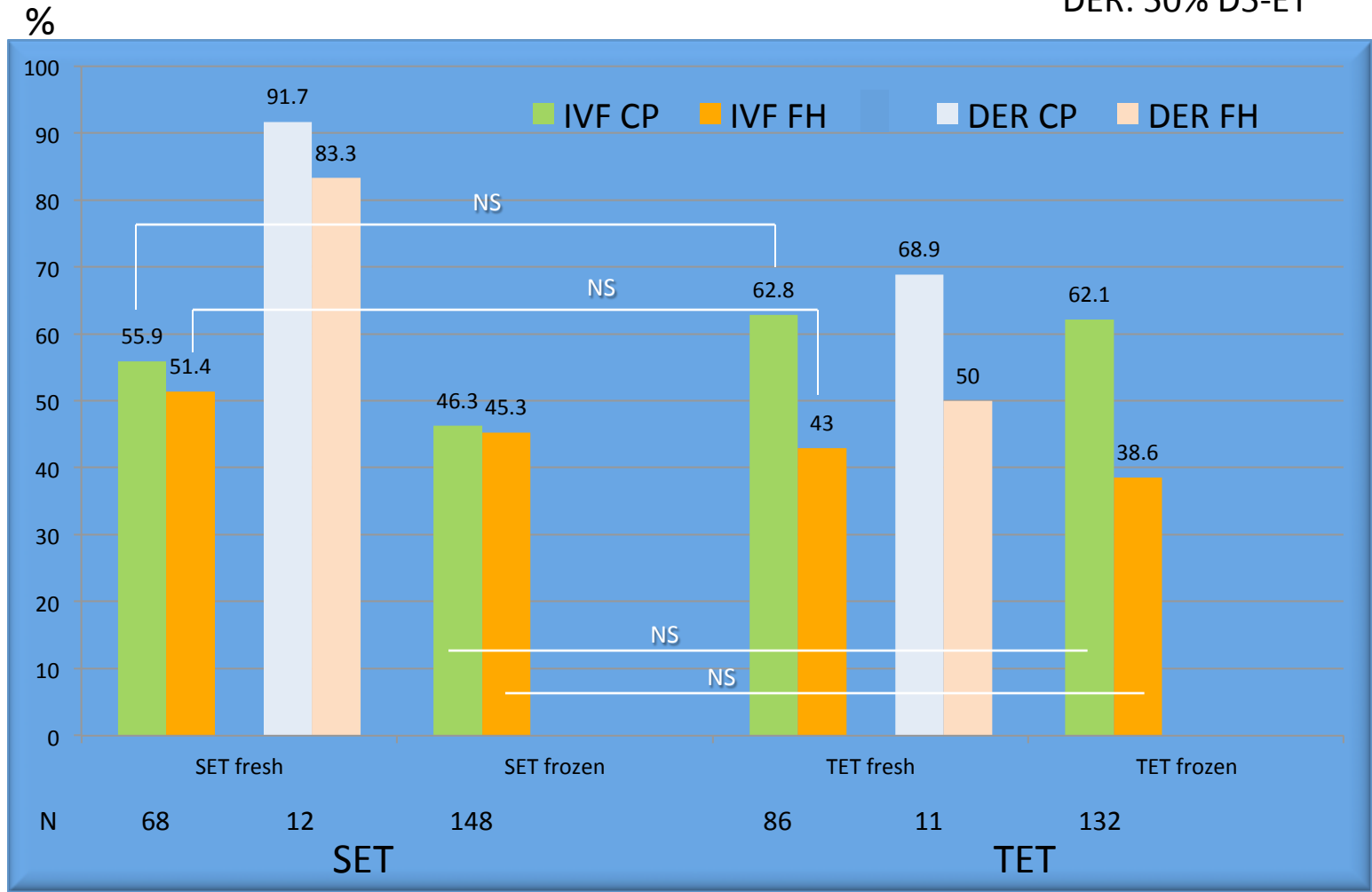
BL cryo D5/6 E vs. S
(excluded: CRYO U/F, CC, PGD/S, DER)

Avg. BL frozen

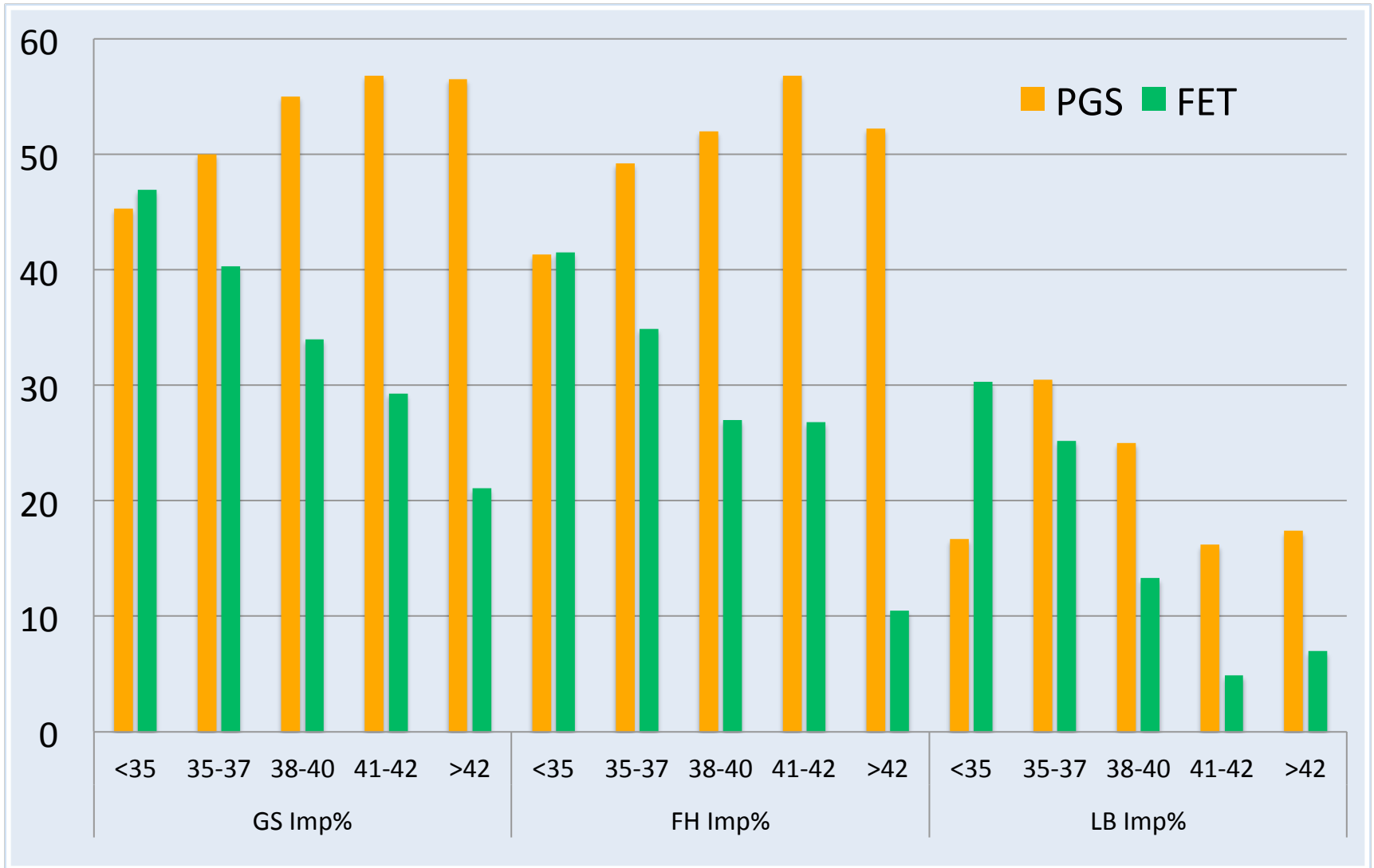


SET vs. TET (January to July 2014); fresh vs. frozen; IVF and DER

DER: 50% D5-ET



Implantation rate in PGS-FET (n=176) vs. FET (n= 902) cycles



PGS: day5/6 bx; 24 chr screening

Acknowledgements

Zev Rosenwaks, MD, Medical director

Embryology Lab:

Director:

Nikica Zaninovic, MS PhD, HCLD, ELD

Supervisors:

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Embryologists:

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Rosemary Berrios, MS

Richard Bodine, MS

Hang Yin, PhD

Jiying Hao, MD

Sam Jones, BS

Qiansheng Zhan, MD, PhD

Christopher Norberg, BS

Sandra Benedict, MS

Pavle Josipovic, PhD

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June Hariprashad, BA

Dina Weiss, MS

Ameeta Bahia, BS

Laboratory Assistants:

Katherine Larkins, BS

Rosa Robles, MA

Administration:

Naomi Lourie, BA (manager)

Abigail O'Connell, BA

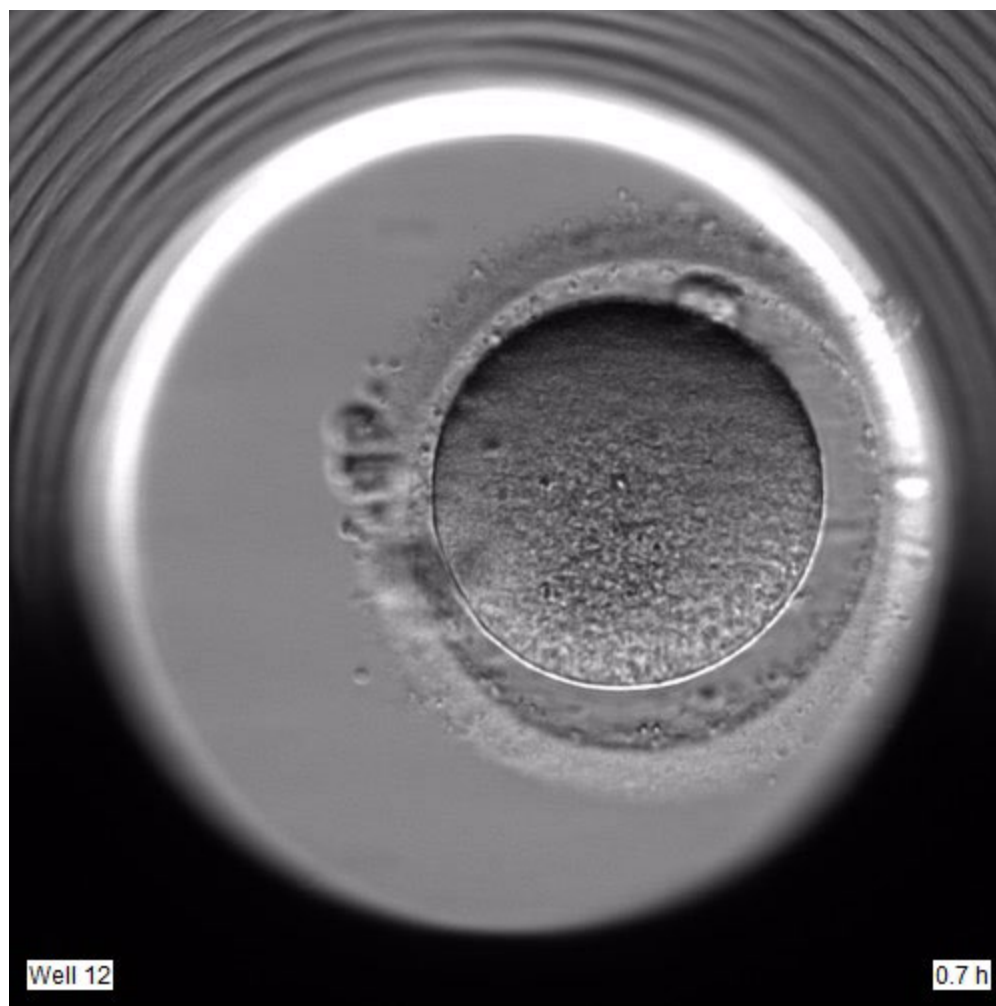
IVF physicians , nurses and administrative staff

Special thanks:

Joel Goldschlag

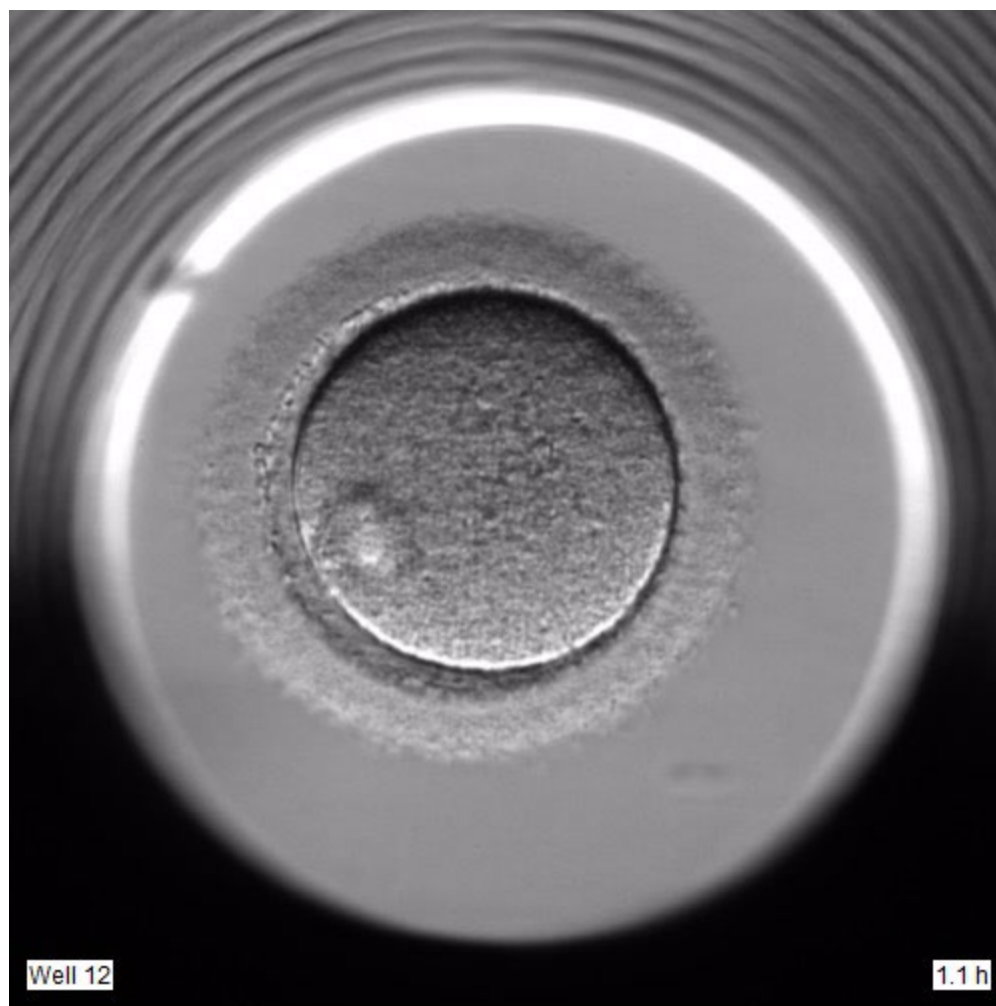
Jose Tabora-Sierra, PhD (statistic)

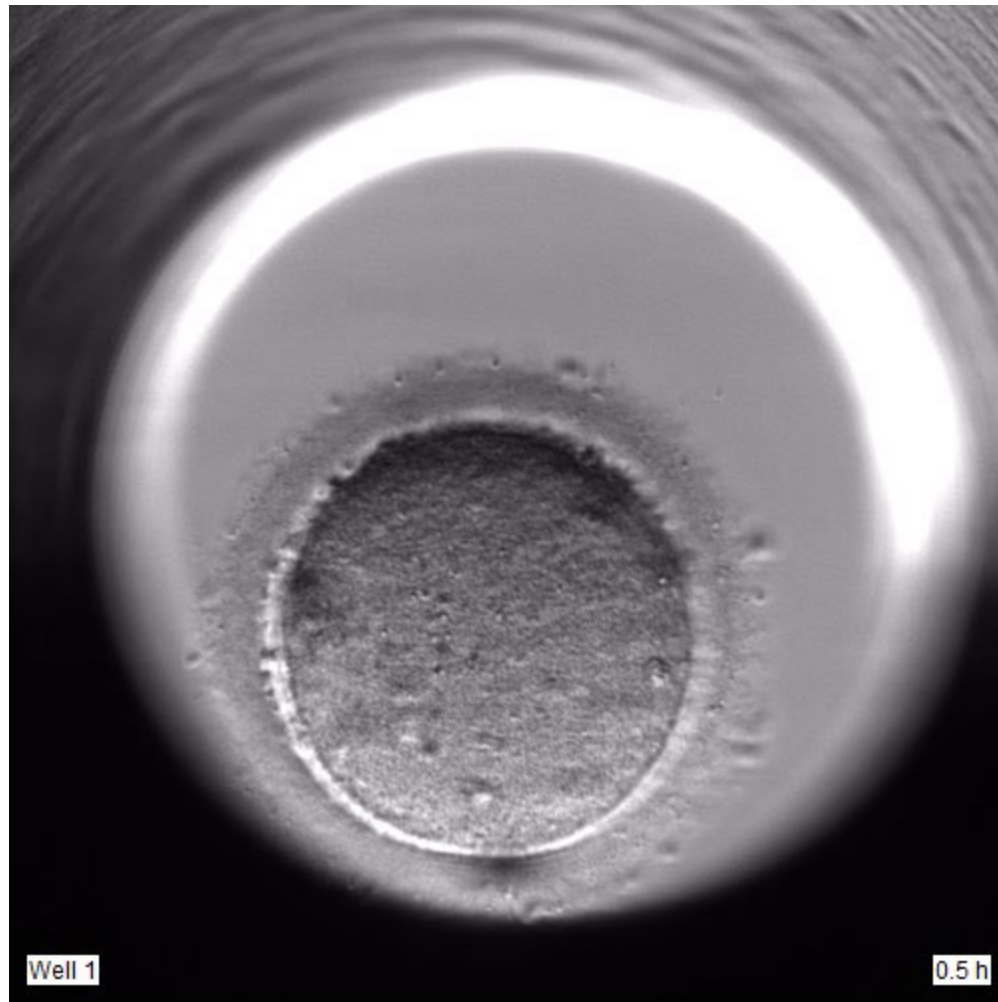
Jonas Malmsten



Well 12

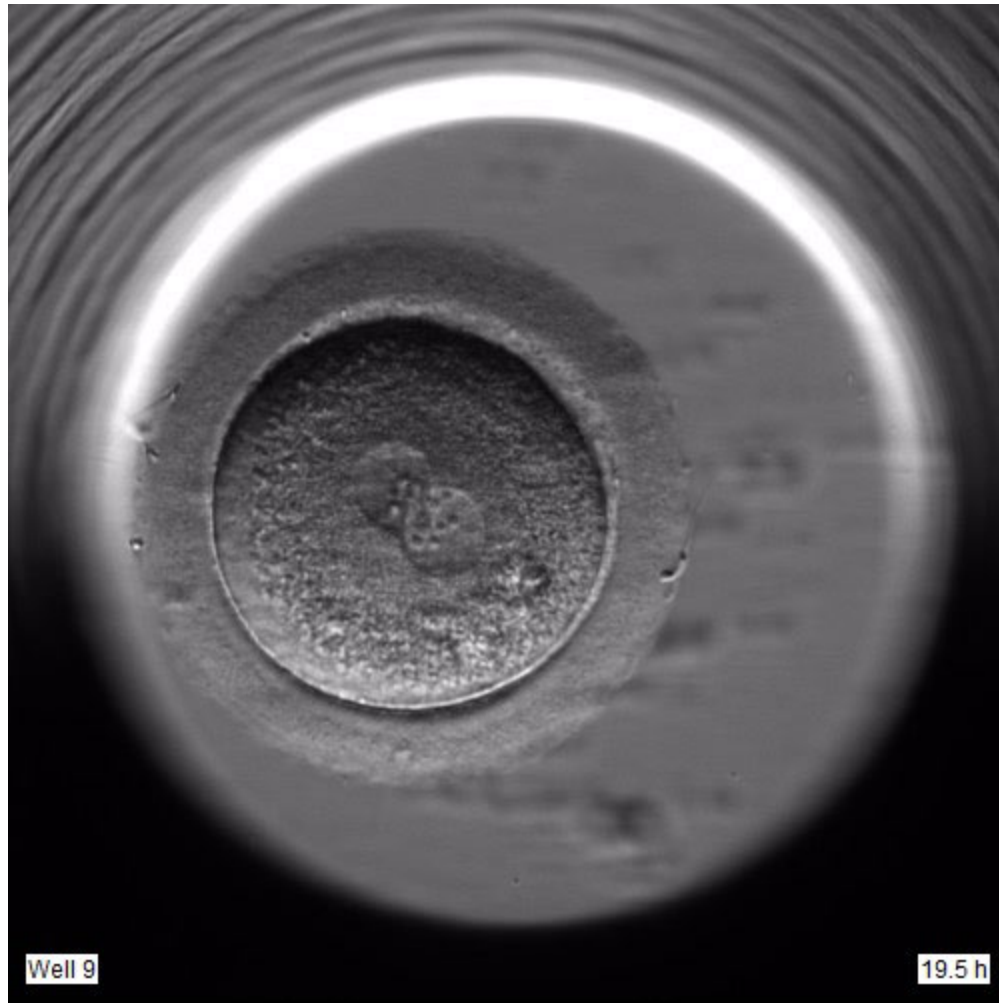
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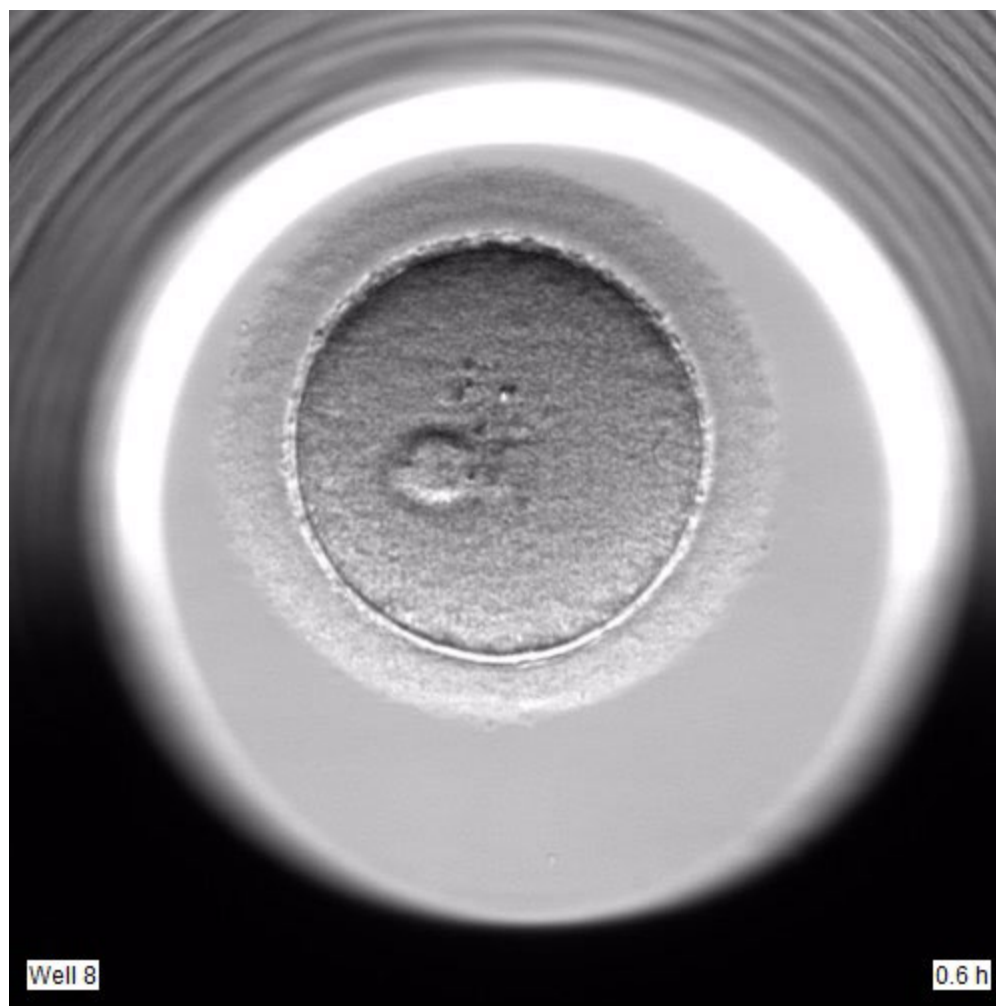
Well 1

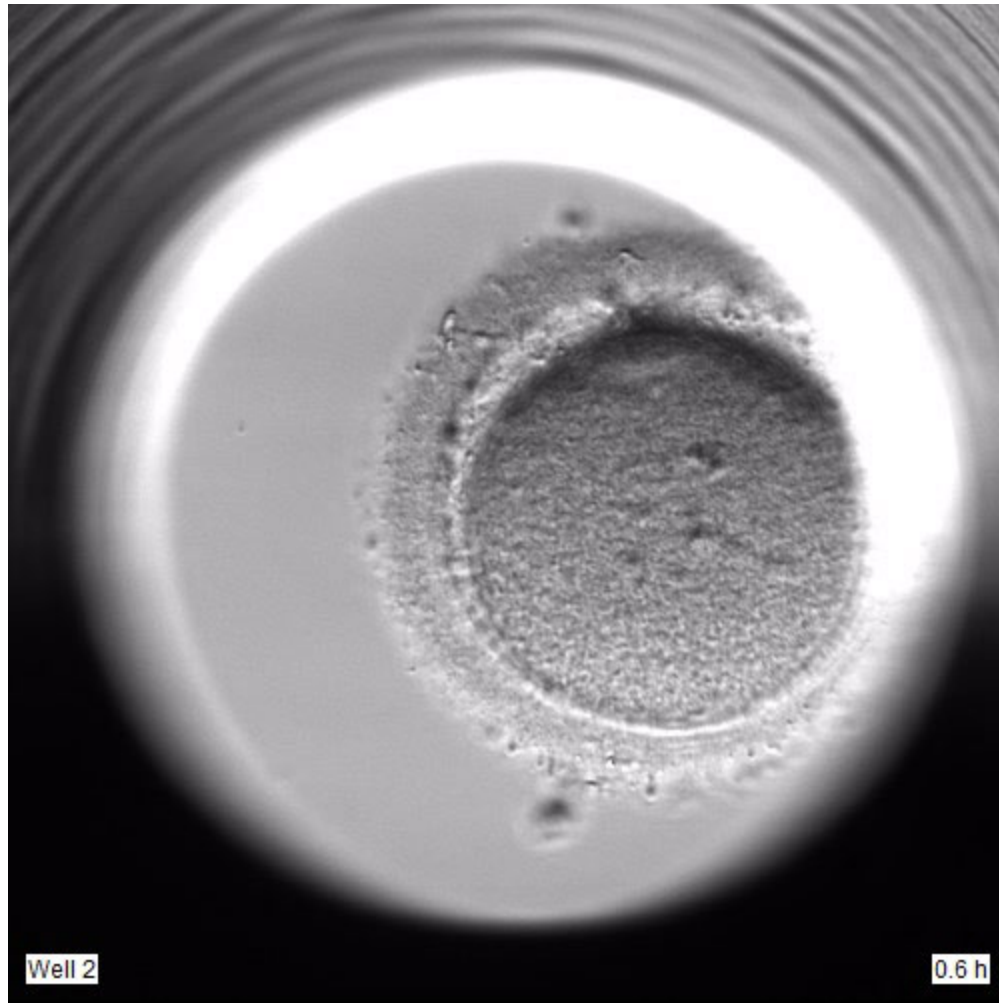
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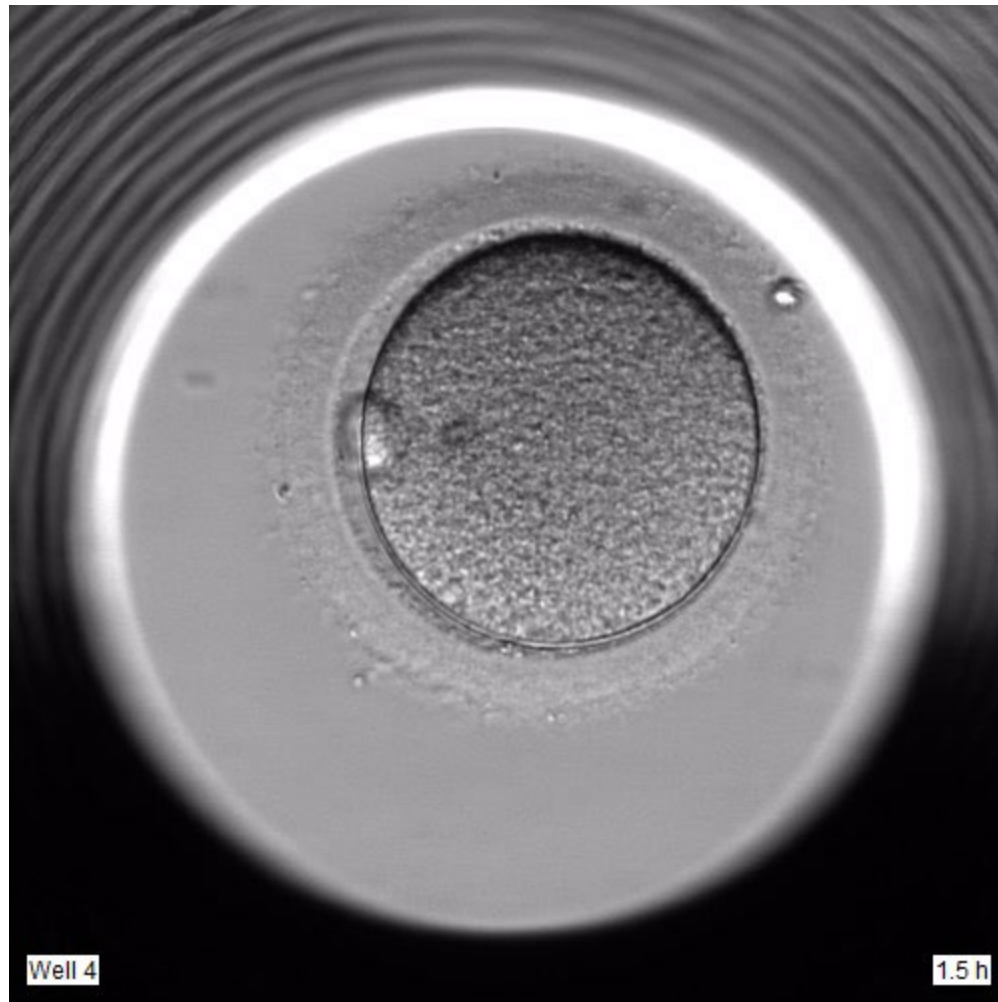


Well 9

19.5 h

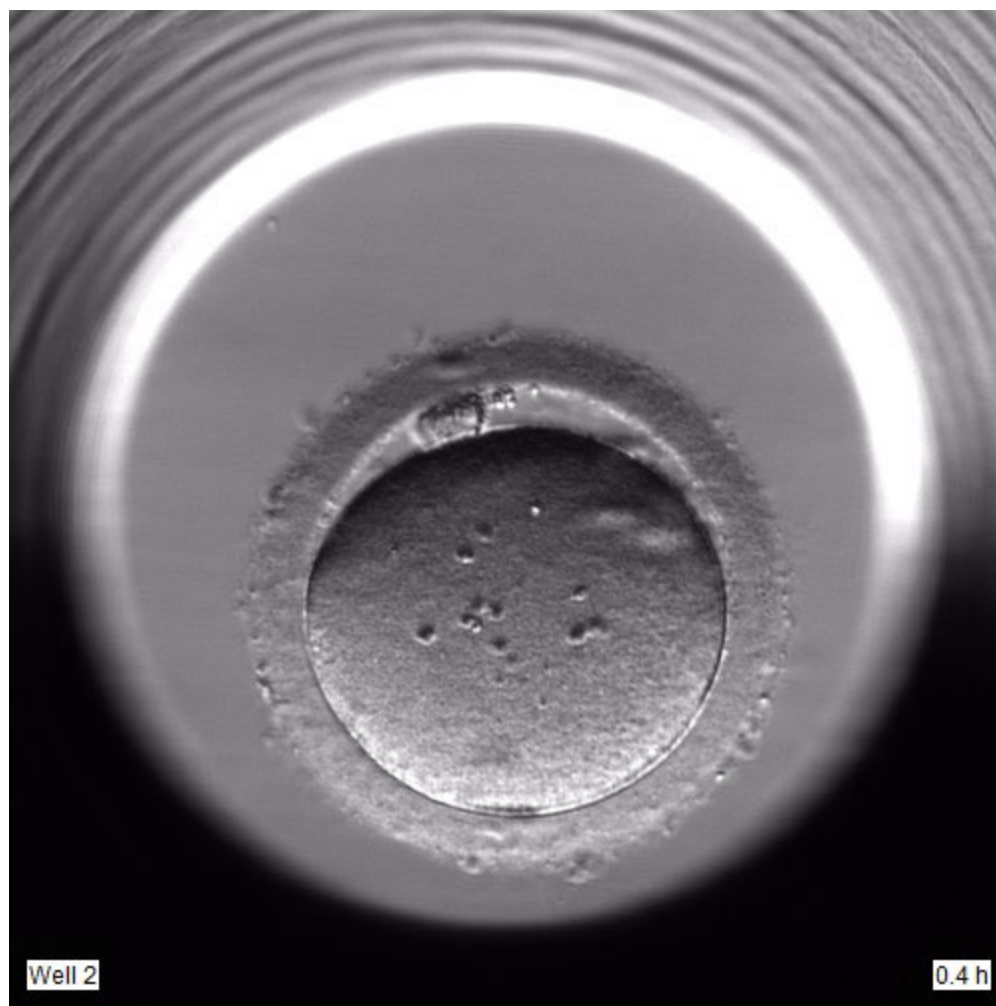


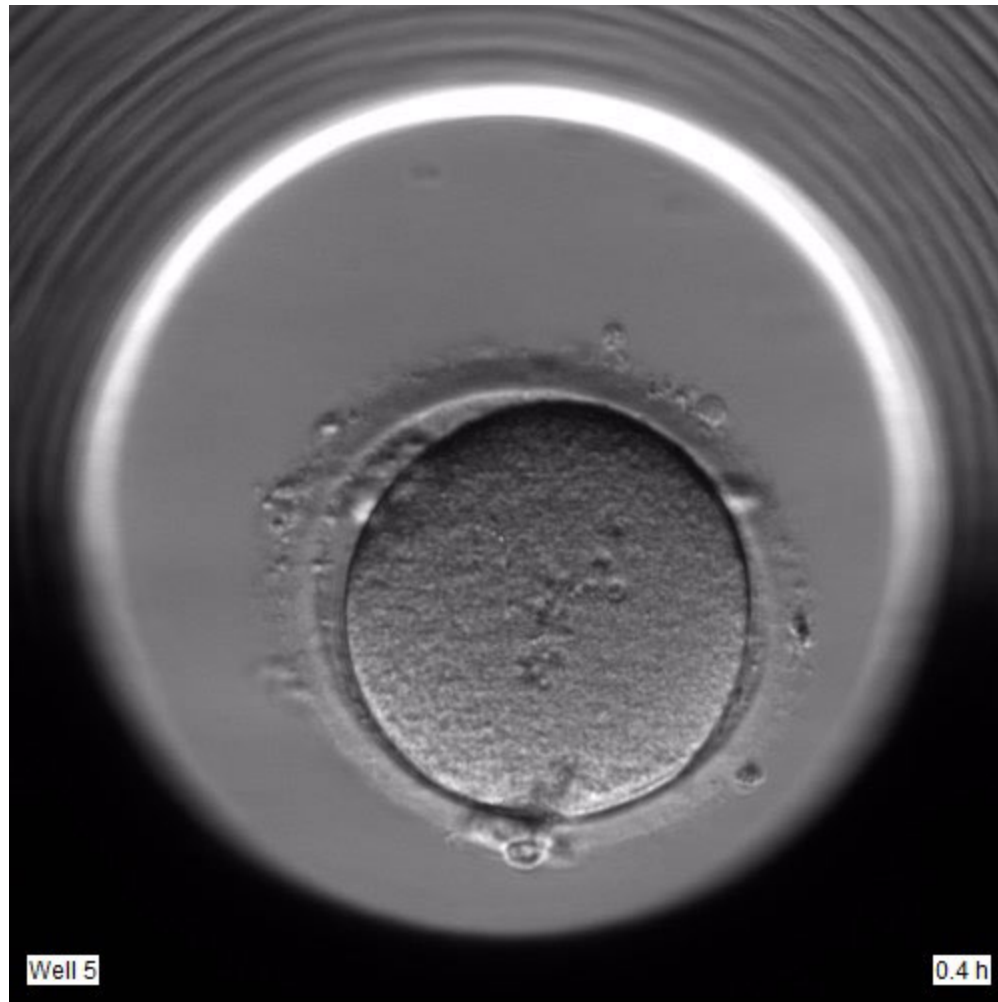




Well 4

1.5 h



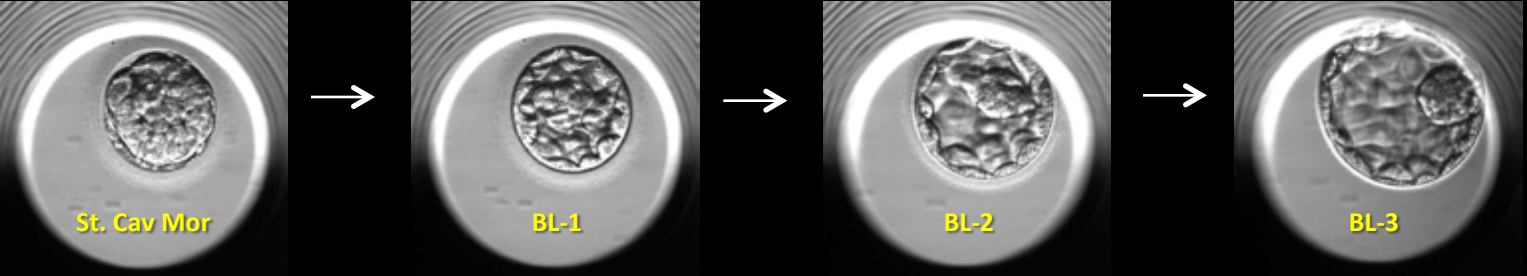
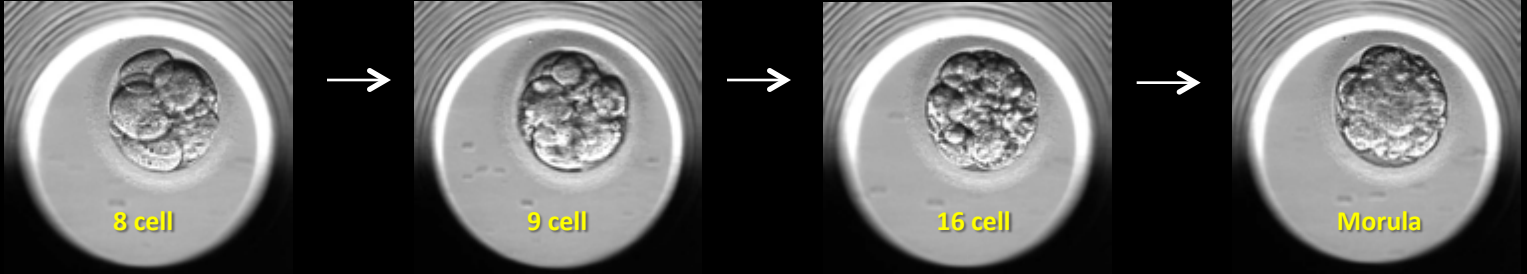
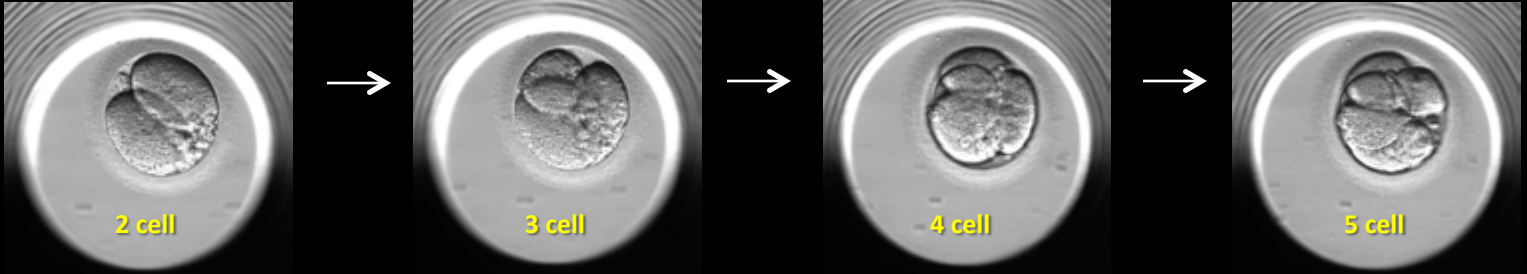
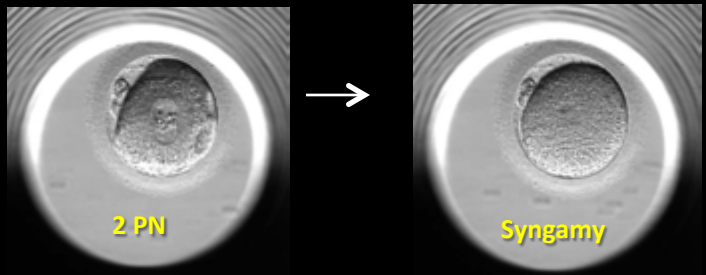
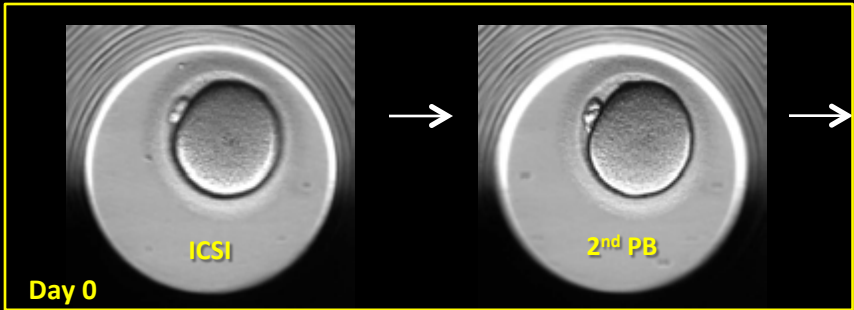


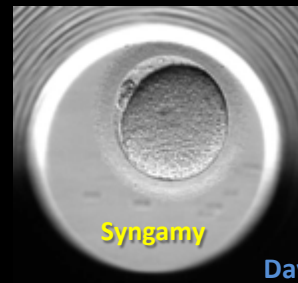
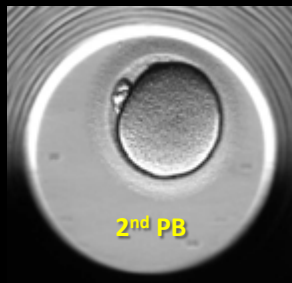
Well 5

0.4 h



Thank You

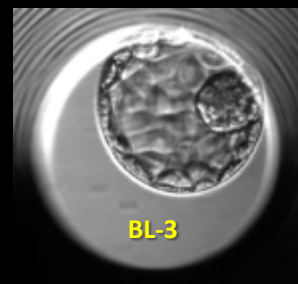
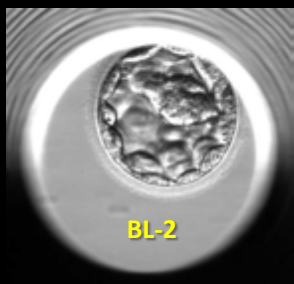
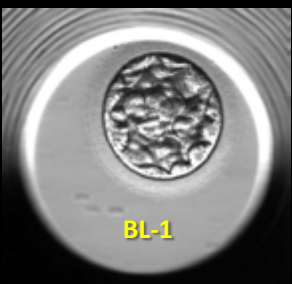
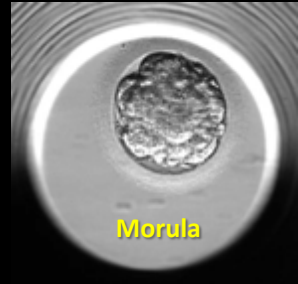
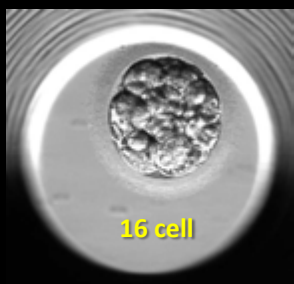
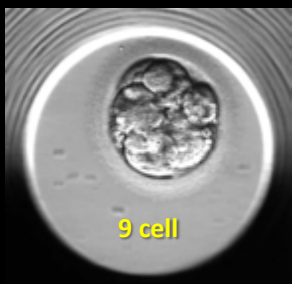
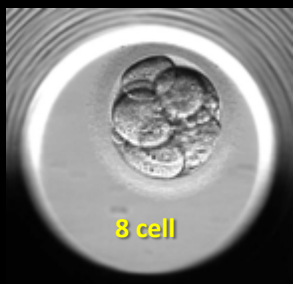
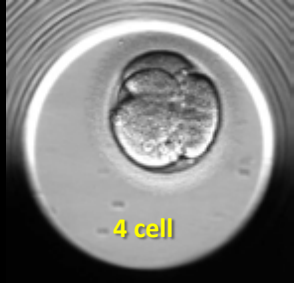
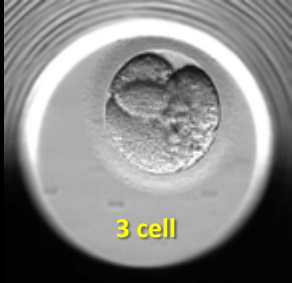


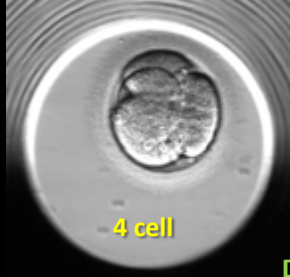
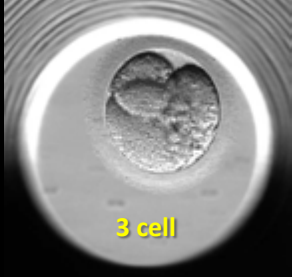
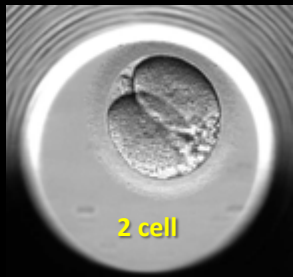
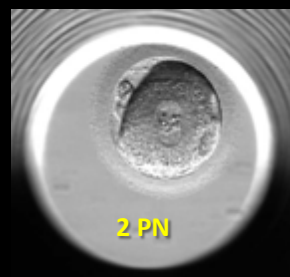
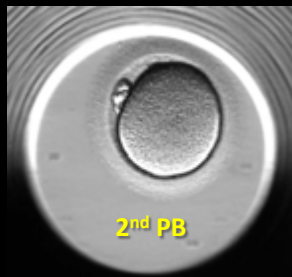
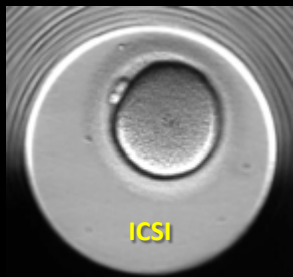


Day 1

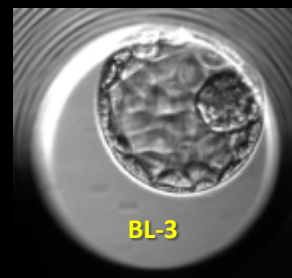
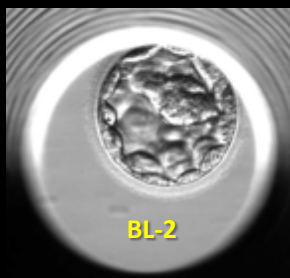
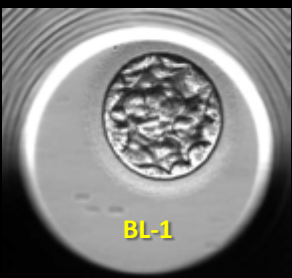
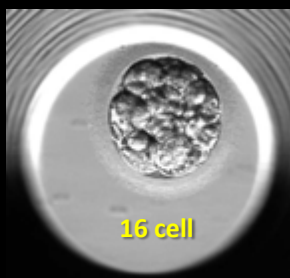
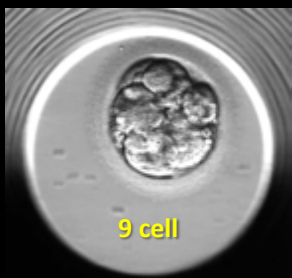
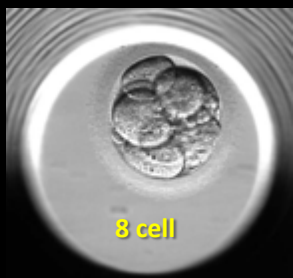


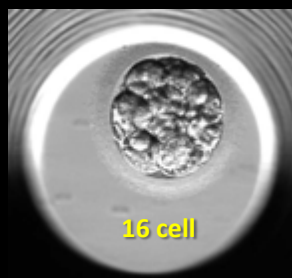
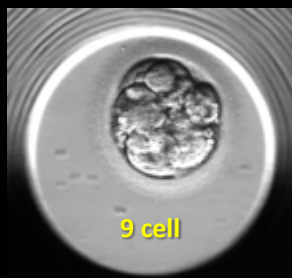
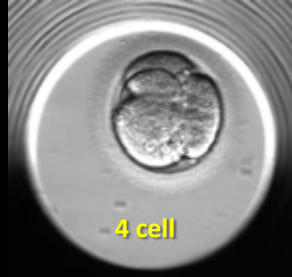
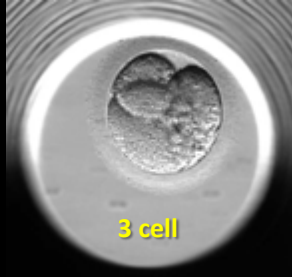
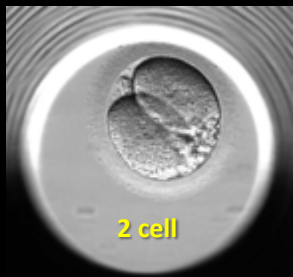
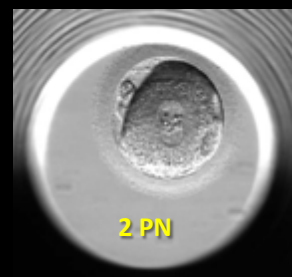
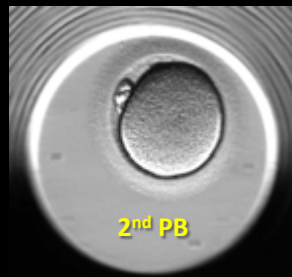
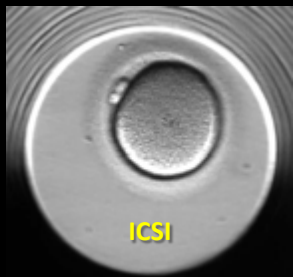
Day 1



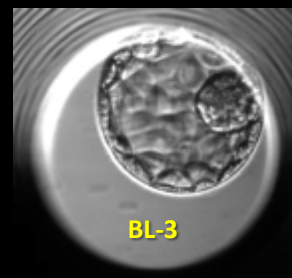
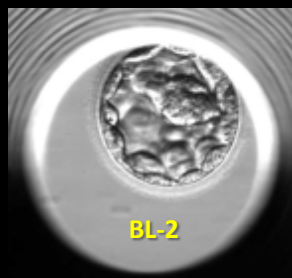
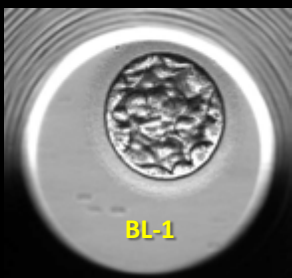


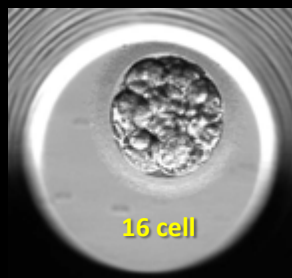
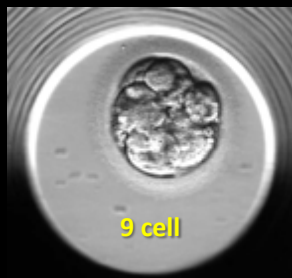
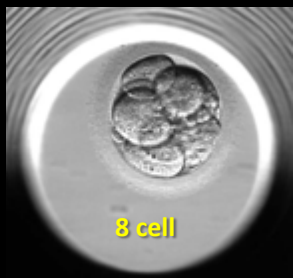
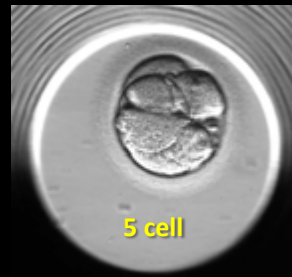
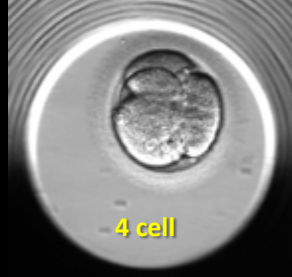
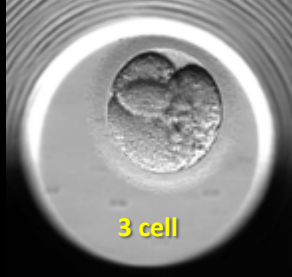
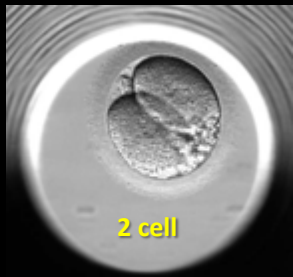
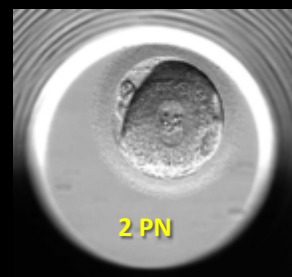
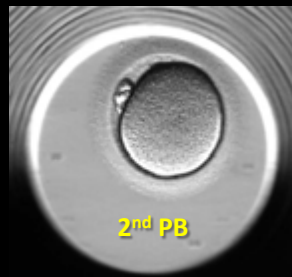
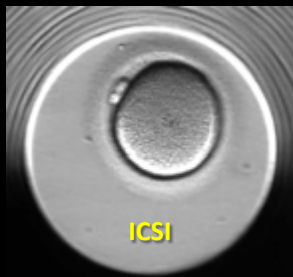
Day 2





Day 3

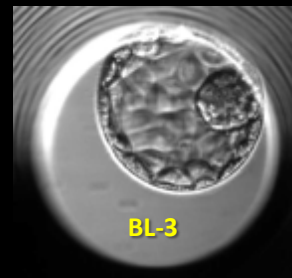
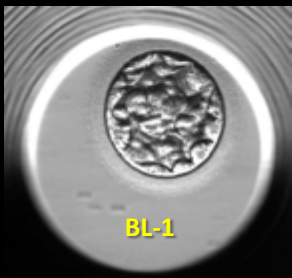


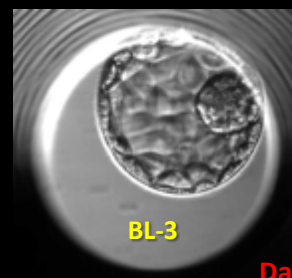
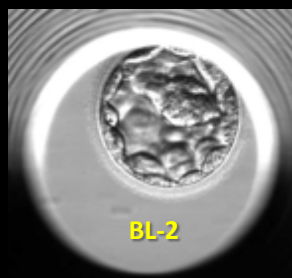
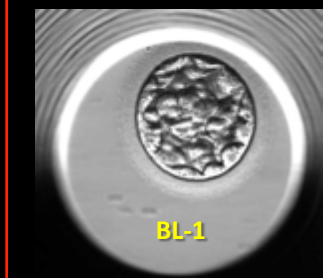
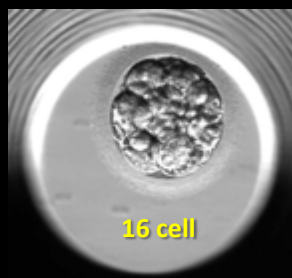
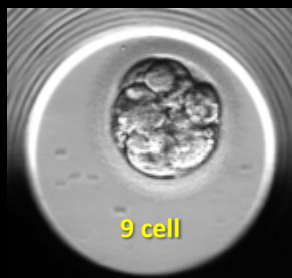
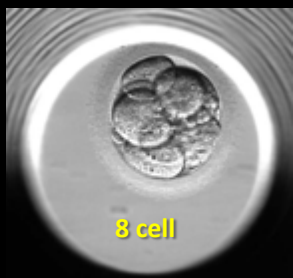
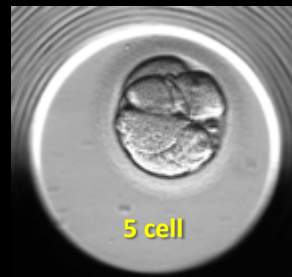
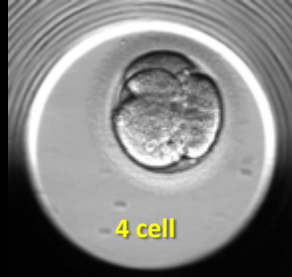
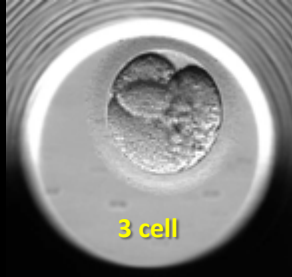
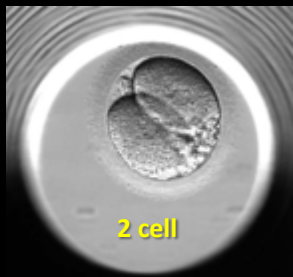
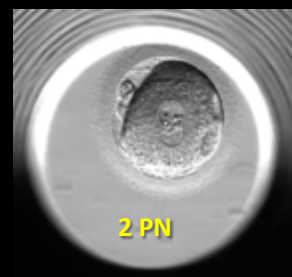
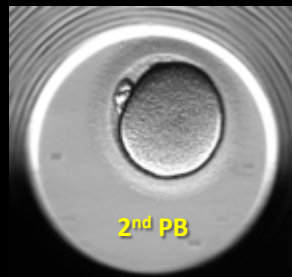
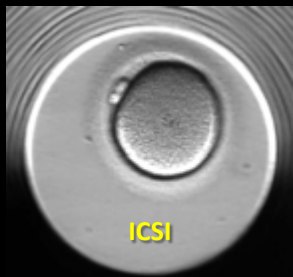


Day 4



Day 4

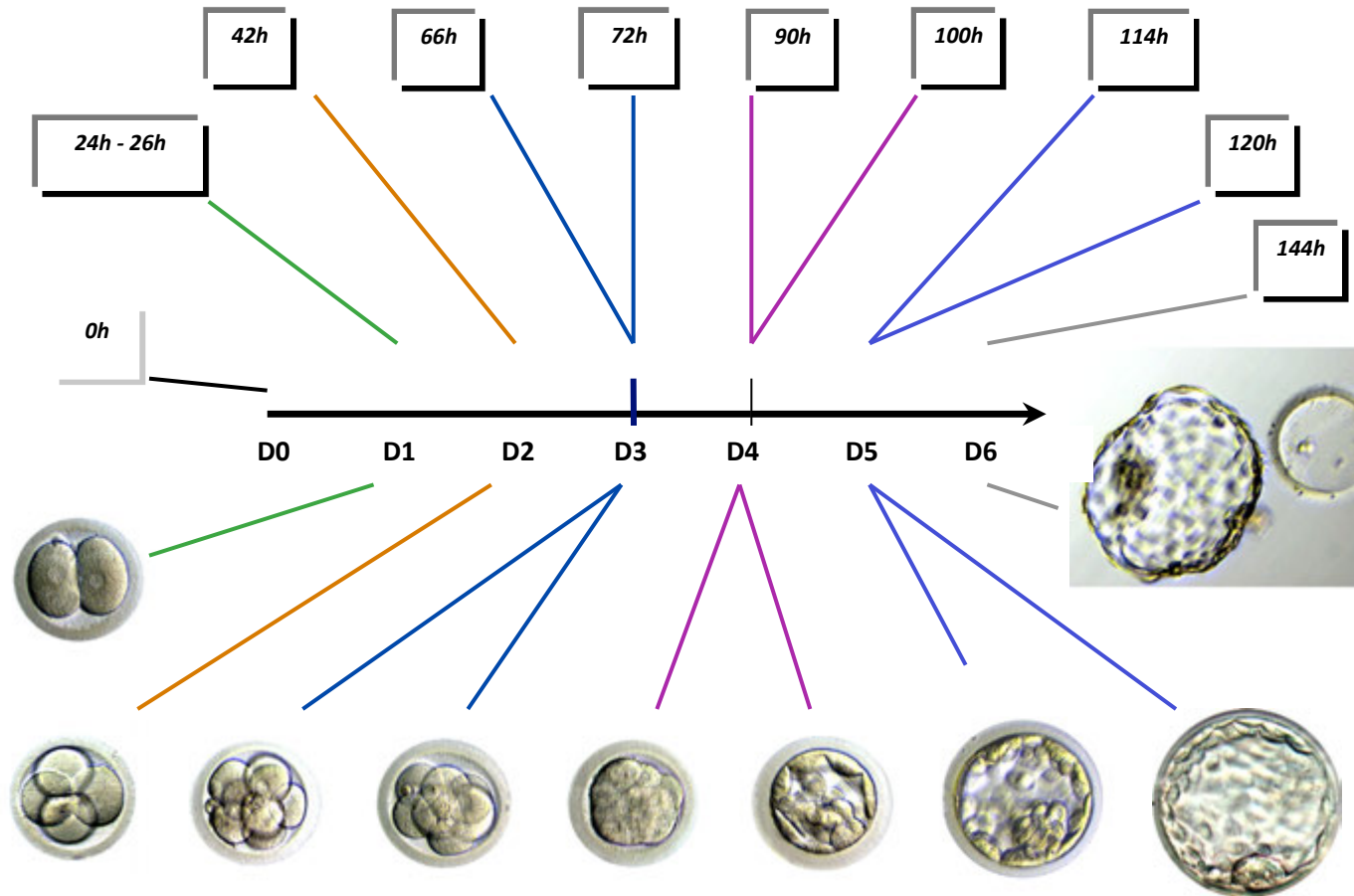




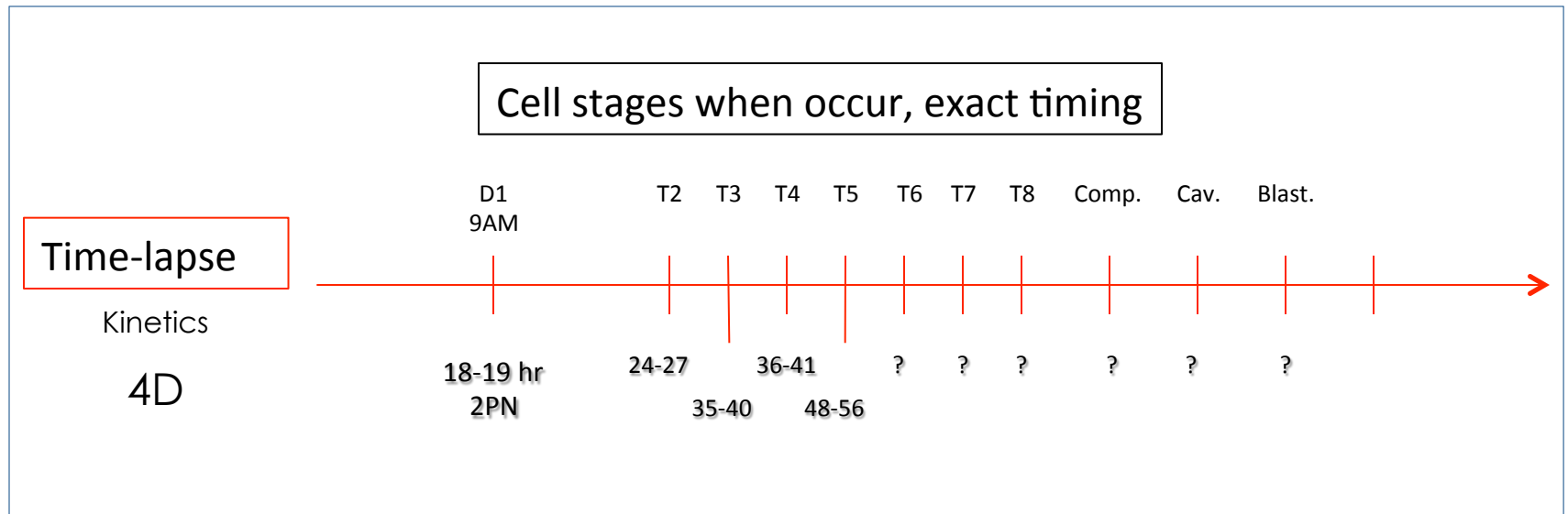
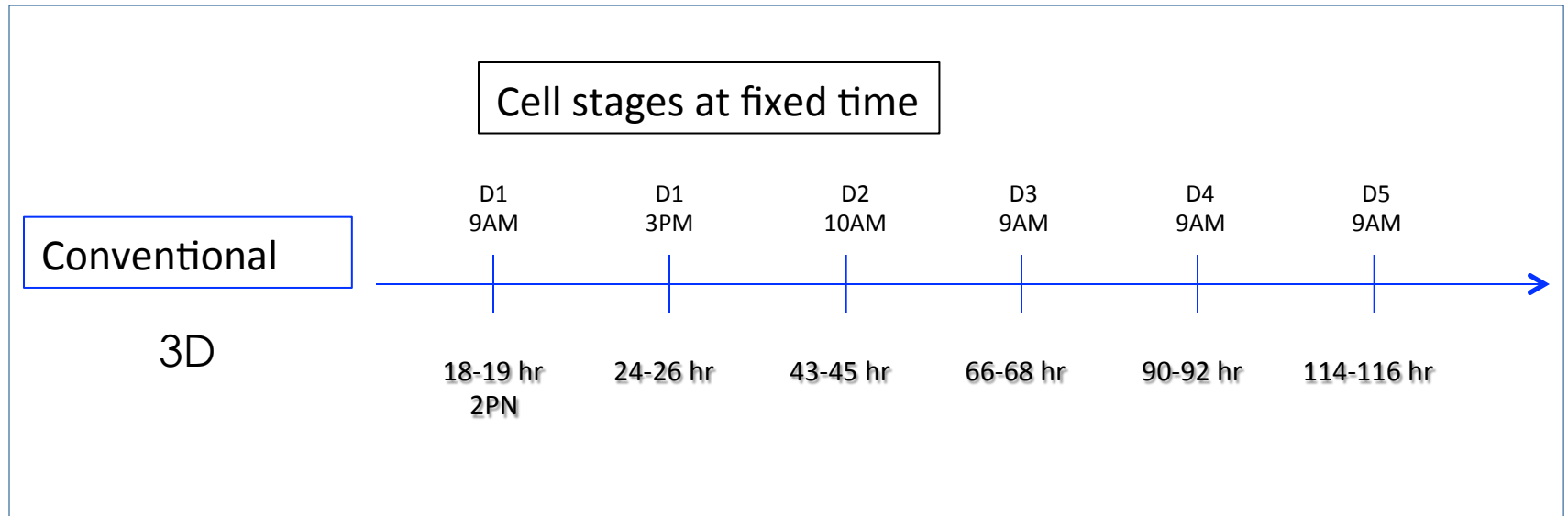
Day 5

Time-line of human embryo development in vitro

(from Veeck and Zaninovic, *An atlas of human blastocyst*, 2003)



Conventional annotation vs. time-lapse



EmbryoScope[®] vs. standard incubator

Cornell validation

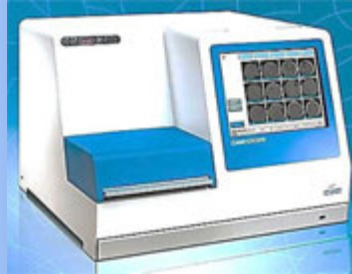
Study design

3E



5.8 % CO₂
5.0 % O₂
37 ° C

E



5.8 % CO₂
20.0 % O₂
37 ° C

S (standard)



5.8 % CO₂
20.0 % O₂
37 ° C

3E vs. E (Study 2)

E vs. S (Study 1)

3E vs. S (study 3)

3E vs. E vs. S

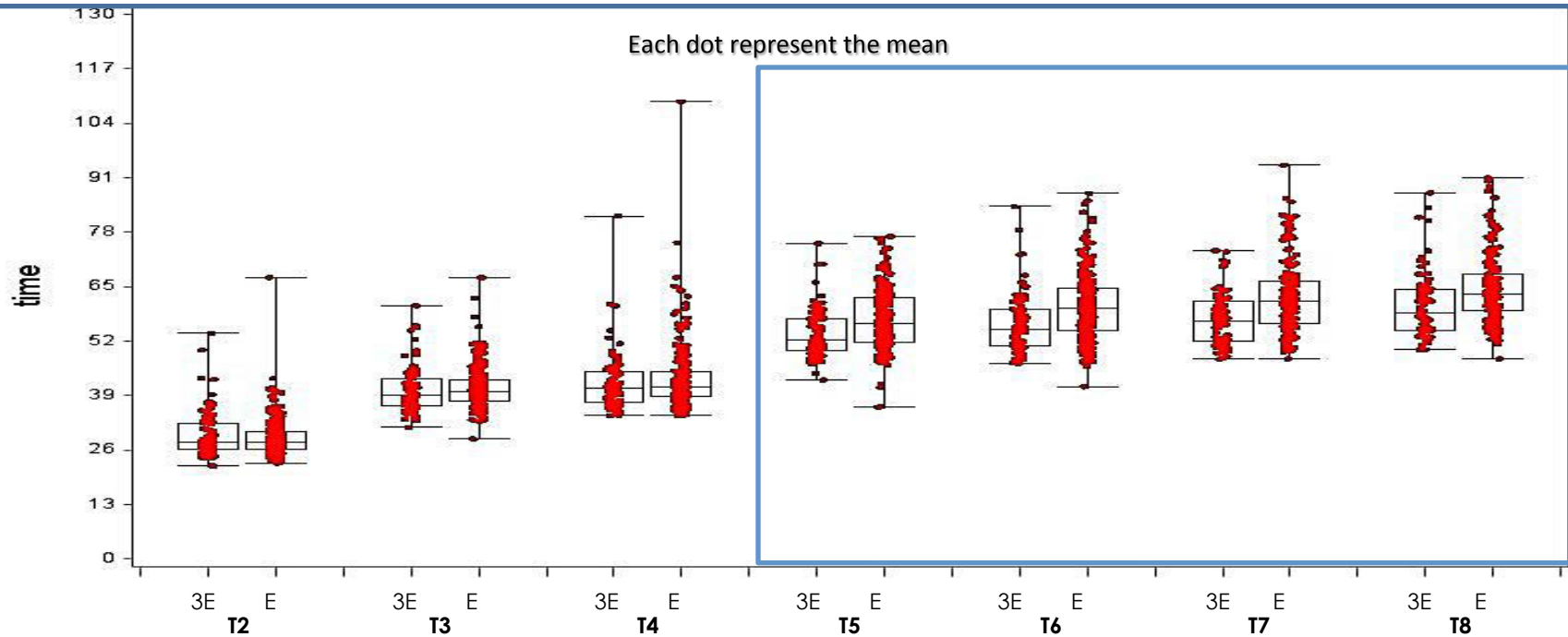
Culture medium: In house sequential medium; pH: 7.35-7.45, in incub. 7.2-7.3; Osm: 260-265 mOsm

Study 1. EmbryoScope (20% O₂) vs. S (20% O₂) on sibling oocytes; n=77 patients

	E	S	P
No. 2PN	491	466	
Day2 cell number (Avg. ± SD)	3.5 ± 1.1	3.4 ± 1.1	NS
Day 2 fragmentation (Avg. ± SD)	8.7 ± 11.9	8.9 ± 11.1	NS
Day 3 cell number (Avg. ± SD)	6.6 ± 2.0	6.3 ± 2.2	0.047
Day 3 fragmentation (Avg. ± SD)	10.7 ± 13.6	10.9 ± 12.1	NS
≥ 4-cells day 2 (48 hours)	307/491 (62.5%)	271/466 (58.2%)	NS
≥ 8-cells day 3 (66 hours)	206/491 (42.0%)	166/466 (35.6%)	0.052
No. embryos ET (%) n=163*	93/163 (57.1%)	70/163 (42.9%)	< 0.05
D3-ET	67/93 (72.0%)	60/70 (85.7%)	
D5-ET	26/93 (28.0%)	10/70 (14.3%)	
No. embryos frozen (D5/6) (%) n=186*	104/186 (56.0%)	82/186 (44.0%)	< 0.05
Embryos used (ET + frozen)*	197/491 (40.1%)	152/466 (32.6%)	< 0.05
* 74 pt. with ET, 3 pt. all cryo (not included)			
Paitred t-test for avg.			
X ² for proportions			

Study 2. EmbryoScope (5% O₂, 3E) vs. EmbryoScope (20% O₂, E) on sibling oocytes; n=28 patients

	3E	E	P
No. 2PN	154	142	
Day2 cell number (Avg. \pm SD)	3.4 \pm 1.1	3.4 \pm 1.1	NS
Day 2 fragmentation (Avg. \pm SD)	8.9 \pm 12.2	6.7 \pm 9.6	0.07
Day 3 cell number (Avg. \pm SD)	6.8 \pm 2.4	6.4 \pm 2.1	0.034
Day 3 fragmentation (Avg. \pm SD)	9.2 \pm 13.5	7.4 \pm 11.7	NS
\geq 4-cells day 2 (48 hours)*	75/154 (48.7%)	65/142 (45.8%)	NS
\geq 8-cells day 3 (66 hours)*	65/154 (42.2%)	54/142 (38.1%)	NS
No. embryos ET (%) n=78	44/78 (56.4%)	34/78 (43.6%)	< 0.05
D3-ET	34/44 (77.3%)	30/34 (88.2%)	
D5-ET	10/44 (22.7%)	4/34 (11.8%)	
No. embryos frozen (D5/6) (%) n=60	37/60 (61.7%)	23/60 (38.3%)	< 0.05
Embryos used (ET + frozen)	78/154 (50.6%)	57/142 (40.1%)	< 0.05
DUCS	28/154 (18.2%)	23/142 (16.2%)	NS
* DUCS excluded			
Paired t-test for avg.			
X ² for proportions			



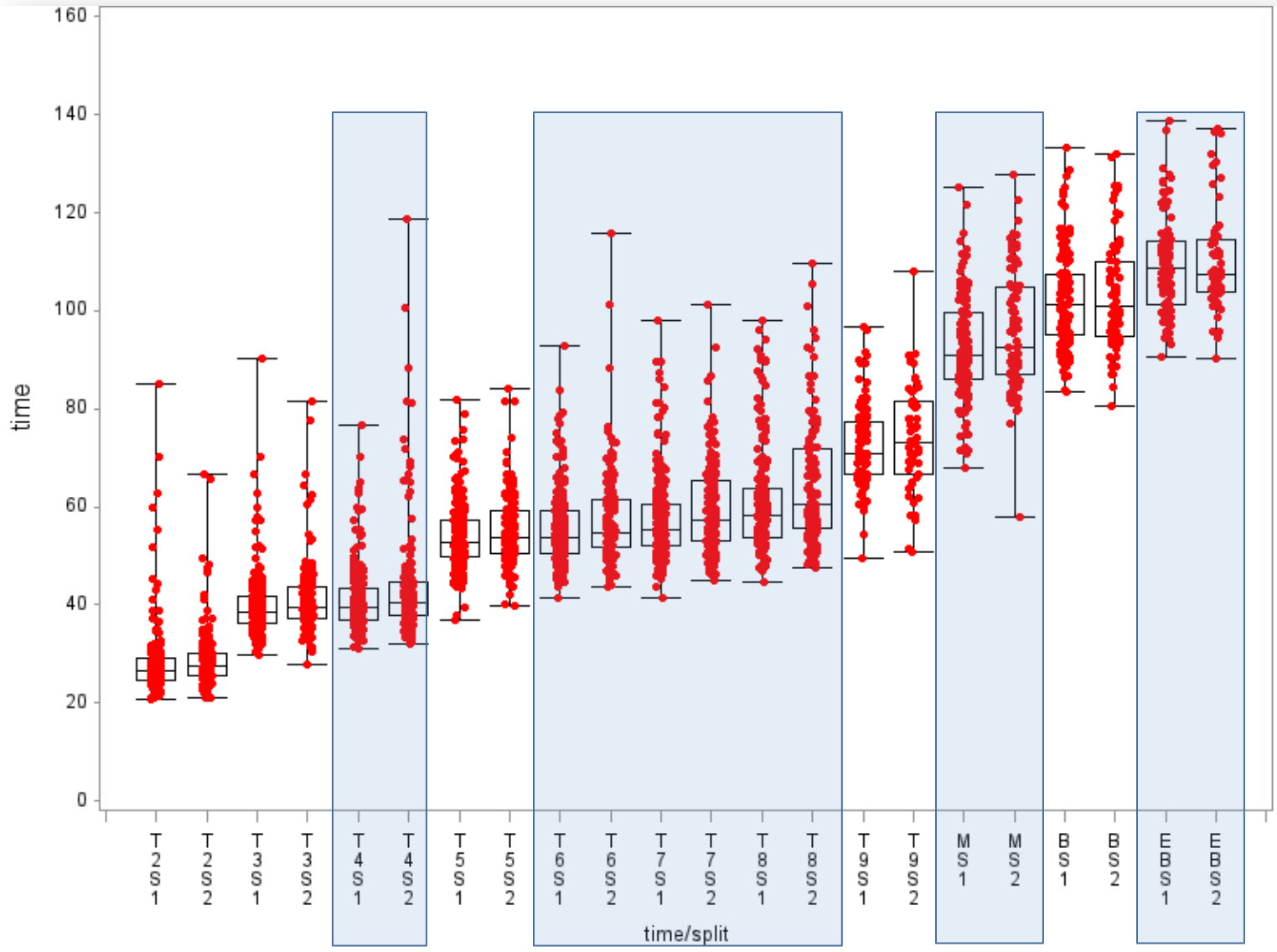
time	Incub	N	Minimum	Maximum	Mean	Std Dev	Lower Quartile	Median	Upper Quartile	p-value (from Wilcoxon to test Median)
2	3E	115	22.14	80.82	32.06	9.52	26.49	28.65	33.86	0.2389
	E	297	22.93	72.92	30.07	6.36	26.57	28.41	31.30	
3	3E	115	28.79	89.76	41.02	8.89	36.29	39.15	43.86	0.1157
	E	296	25.53	78.88	41.26	6.75	37.51	40.01	43.37	
4	3E	111	29.36	81.90	42.70	7.61	37.50	41.28	45.41	0.3022
	E	291	31.25	109.29	43.59	8.09	38.85	41.20	46.17	
5	3E	108	31.68	75.40	52.49	6.82	48.25	51.39	55.88	0.0002
	E	281	33.78	114.63	55.84	9.47	50.42	54.79	61.63	
6	3E	105	39.61	84.16	55.43	7.54	50.42	54.44	58.93	<.0001
	E	264	37.38	91.30	59.69	8.39	53.90	59.49	64.44	
7	3E	96	41.36	77.54	57.54	7.01	52.28	56.65	61.74	<.0001
	E	241	37.38	94.12	62.65	8.91	56.29	61.77	66.16	
8	3E	93	49.93	87.47	60.87	8.37	54.55	59.12	64.76	<.0001
	E	207	47.03	91.07	64.54	8.58	59.40	63.57	68.06	

Study 3. EmbryoScope (5% O₂, 3E) vs. S (20% O₂) on sibling oocytes; n=35 patients			
	3E	S	P
No. 2PN	242	208	
Day2 cell number (Avg. ± SD)	3.6 ± 1.1	3.5 ± 1.2	NS
Day 2 fragmentation (Avg. ± SD)	10.2 ± 11.8	9.8 ± 12.8	NS
Day 3 cell number (Avg. ± SD)	7.0 ± 2.3	6.3 ± 2.2	0.0004
Day 3 fragmentation (Avg. ± SD)	10.1 ± 13.2	11.1 ± 13.9	NS
≥ 4-cells day 2 (48 hours)	165/242 (68.2%)	130/208 (62.5%)	NS
≥ 8-cells day 3 (66 hours)	137/242 (56.6%)	83/208 (40.0%)	0.0006
No. embryos ET (%) n=94	64/94 (68.0%)	30/94 (31.9%)	< 0.05
D3-ET	47/64 (73.4%)	21/30 (70.0%)	
D5-ET	17/64 (26.6%)	9/30 (30.0%)	
No. embryos frozen (D5/6) (%) n=95	55/95 (57.9%)	40/95 (42.1%)	< 0.05
Embryos used (ET + frozen)	119/242 (49.2%)	70/208 (33.7%)	< 0.05
Paired t-test for avg.			
X ² for proportions			

ICSI day 0 (DO) vs. ICSI day 1 (D1) on sibling oocytes; n=77 patients

	DO	D1	P
No. 2PN	330	281	
Day2 cell number (Avg. \pm SD)	3.9 \pm 1.1	3.7 \pm 1.2	0.044
Day 2 fragmentation (Avg. \pm SD)	7.8 \pm 9.8	7.3 \pm 9.4	NS
Day 3 cell number (Avg. \pm SD)	7.5 \pm 2.1	7.0 \pm 2.2	0.013
Day 3 fragmentation (Avg. \pm SD)	8.1 \pm 11.3	9.5 \pm 14.3	NS
\geq 4-cells day 2 (48 hours)*	200/330 (60.6%)	145/281 (51.6)	0.031
\geq 8-cells day 3 (66 hours)*	171/330 (51.8%)	108/281 (38.4%)	< 0.05
No. embryos ET (%) n=161	100/161 (62.1%)	61/161 (37.9%)	< 0.05
D3-ET	70/100 (70%)	41/61 (67.2%)	
D5-ET	30/100 (30%)	20/61 (32.8%)	
No. embryos frozen (D5/6) (%) n=162	101/162 (62.3%)	61/162 (37.7%)	< 0.05
Embryos used (ET + frozen)	201/330 (60.1%)	122/281 (43.4%)	< 0.05
DUCS	74/330 (22.4%)	72/281 (25.6%)	NS
* DUCS excluded			
Paired t-test for avg.			
X ² for proportions			

ICSI D0 vs. D1: 77 pt. t-times



1=D0; 2=D1

Wilcoxon Signed Ranked test

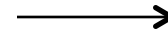
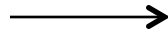
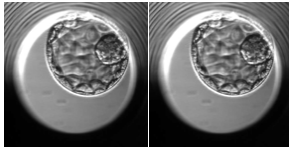
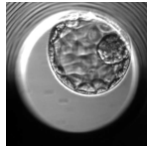
ICSI 2PN Fertilization rate: 898 emb. in E (2013) vs. 900 emb. in S (2012) cycles
Retrospective analysis, avg. MII=NS



$\chi^2 * p < 0.05$

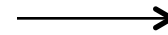
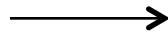
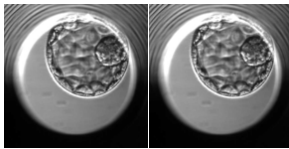
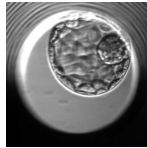
KID (Known Implantation Data)

1 emb ET

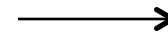
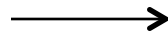
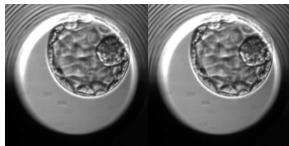


KID +

2 emb ET



KID -



KID ?