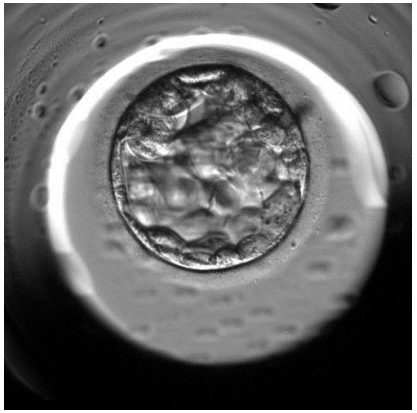
Building robust time-lapse models

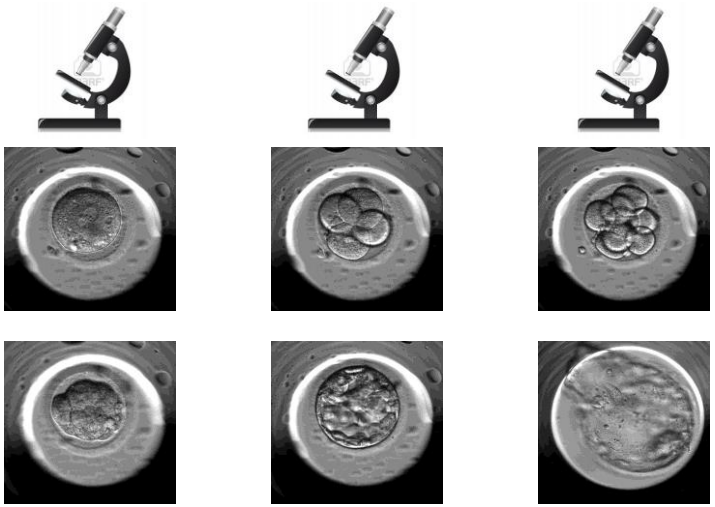


Kirstine Kirkegaard
The Fertility Clinic
Aarhus University Hospital

Time-lapse as a diagnostic test

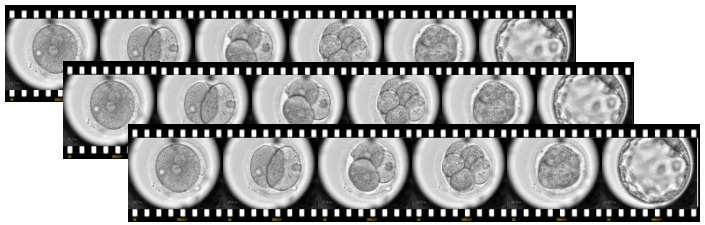
Morphology

- Limited information
- Dependent on timing
- Subjective
- Validated

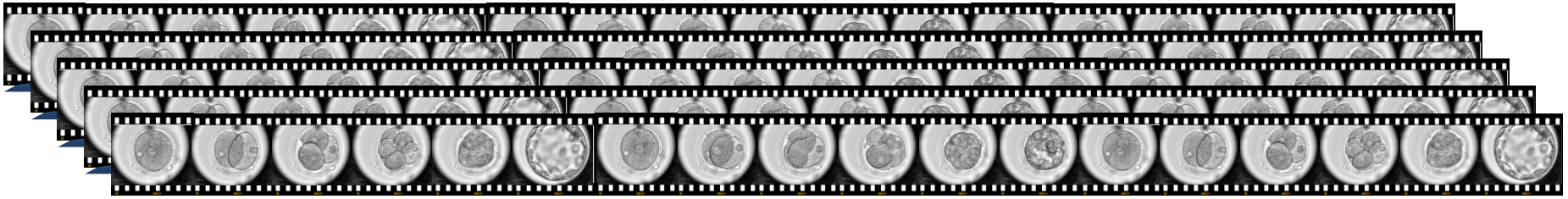


Time-lapse

- Detailed information
- Flexible
- Precise*)
- Validated?



*Sundvall et al 2013



Time-lapse incubation

Improved culture?

Improved selection?

Improved treatment?

Improved pregnancy rates

Assumptions:

1. Culture conditions
   - Un-interrupted culture
   - Incubator
2. Selection

Culture conditions

J Assist Reprod Genet (2012) 29:565–572
DOI 10.1007/s10815-012-9750-x

TECHNICAL INNOVATIONS

A randomized clinical trial comparing embryo culture in a conventional incubator with a time-lapse incubator

Kirstine Kirkega
Marie Louise Gre
Hans Jakob Inge

ASSISTED REPRODUCTION TECHNOLOGIES

Embryo quality, blastocyst and ongoing pregnancy rates in oocyte donation patients whose embryos were monitore

María Cruz · Blanca Ga
Kamilla Søe Pedersen · I
Inma Pérez-Cano · Man

human reproduction ORIGINAL ARTICLE *Embryology*

No benefit of culturing embryos in a closed system compared with a conventio number of from an R

H. Park, C. Bergh,

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

Embryo selection

Assupmtion: Timing of development can distinguish viable from non-viable embryos

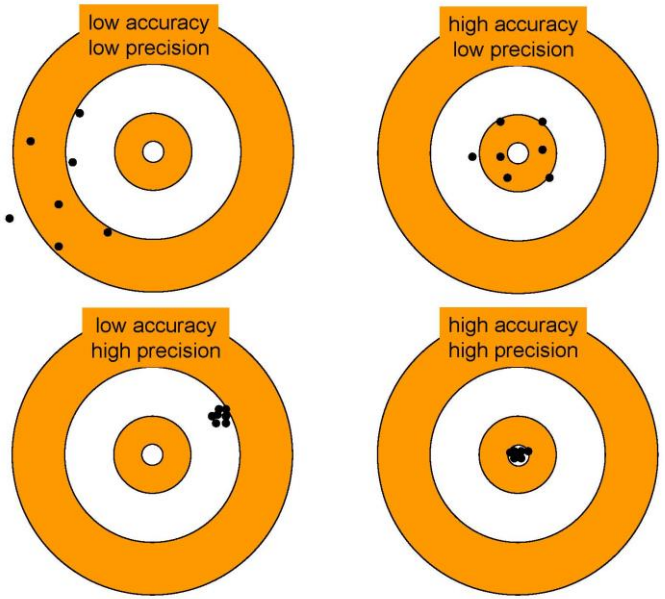
Accuracy and precision

Viable?

What is normal?

Sensitivity and specificity

Precision and accuracy



human reproduction

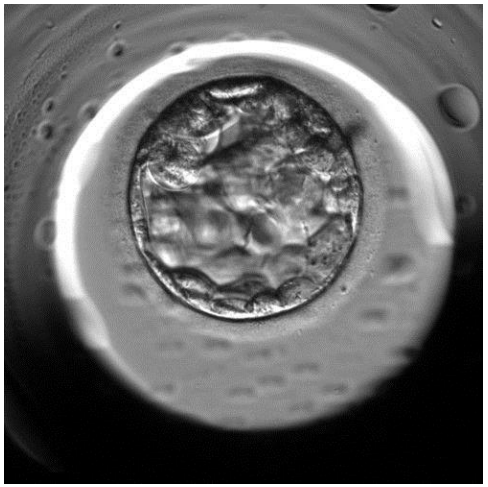
ORIGINAL ARTICLE *Embryology*

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

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

ACCURACY:
Dependent on time between image recordings

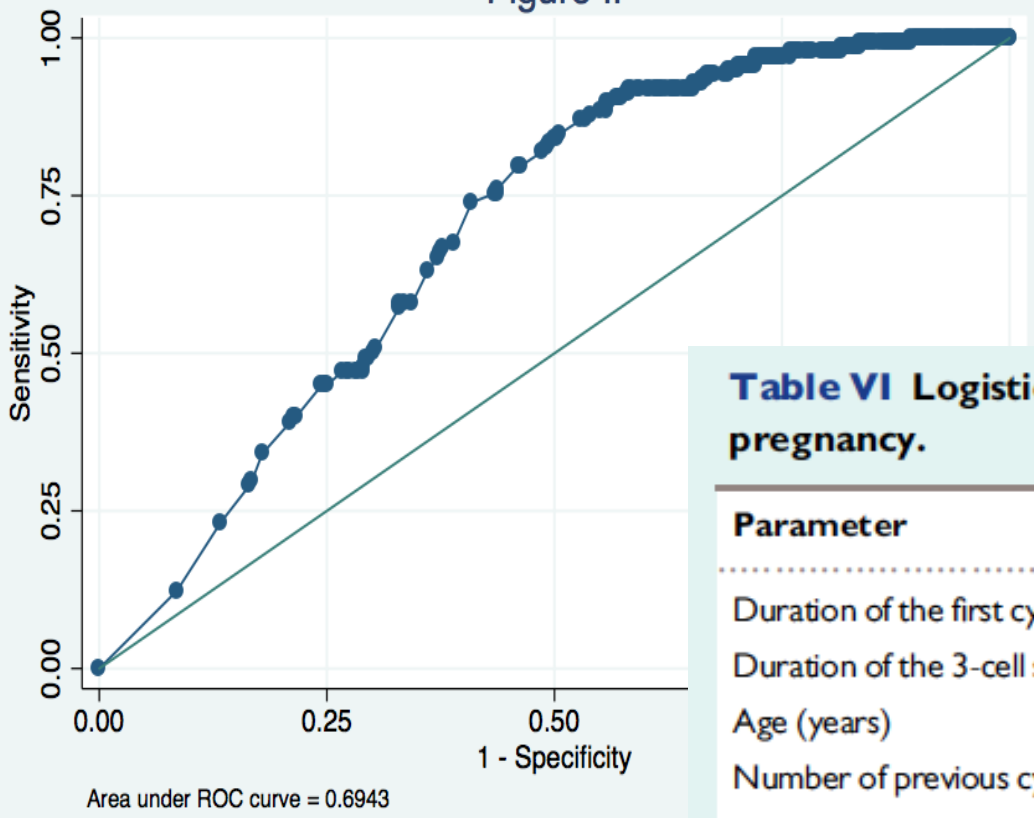
What is viable?



Blastocyst development

STUDY	ENDPOINT	PARAMETERS
Wong 2010	Blastocyst development (n=100)	1st cytokinesis, duration 2- and 3-cell stage
Hashimoto 2012	Blastocyst score (n=80)	T(7/8), 3rd cell cycle, duration 3-cell stage
Cruz 2012	Blastocyst score (n=834)	T(4), duration 3-cell stage, morula, direct cleavage 1-3, uneveness 2-cell stage
Dal Canto 2012	Expanded blastocyst (n=459)	T(3)-t(8), duration of all cleavage stages
Hlinka 2012	Blastocyst development (n=180)	Cleavage cycles and interphases.
Conaghan 2013	Blastocyst quality (n=1233)	Duration 2- and 3-cell stage
Kirkegaard 2013	High quality blastocysts (n=571)	1st cytokinesis, duration 3-cell stage, direct cleavage 1-3

Figure II



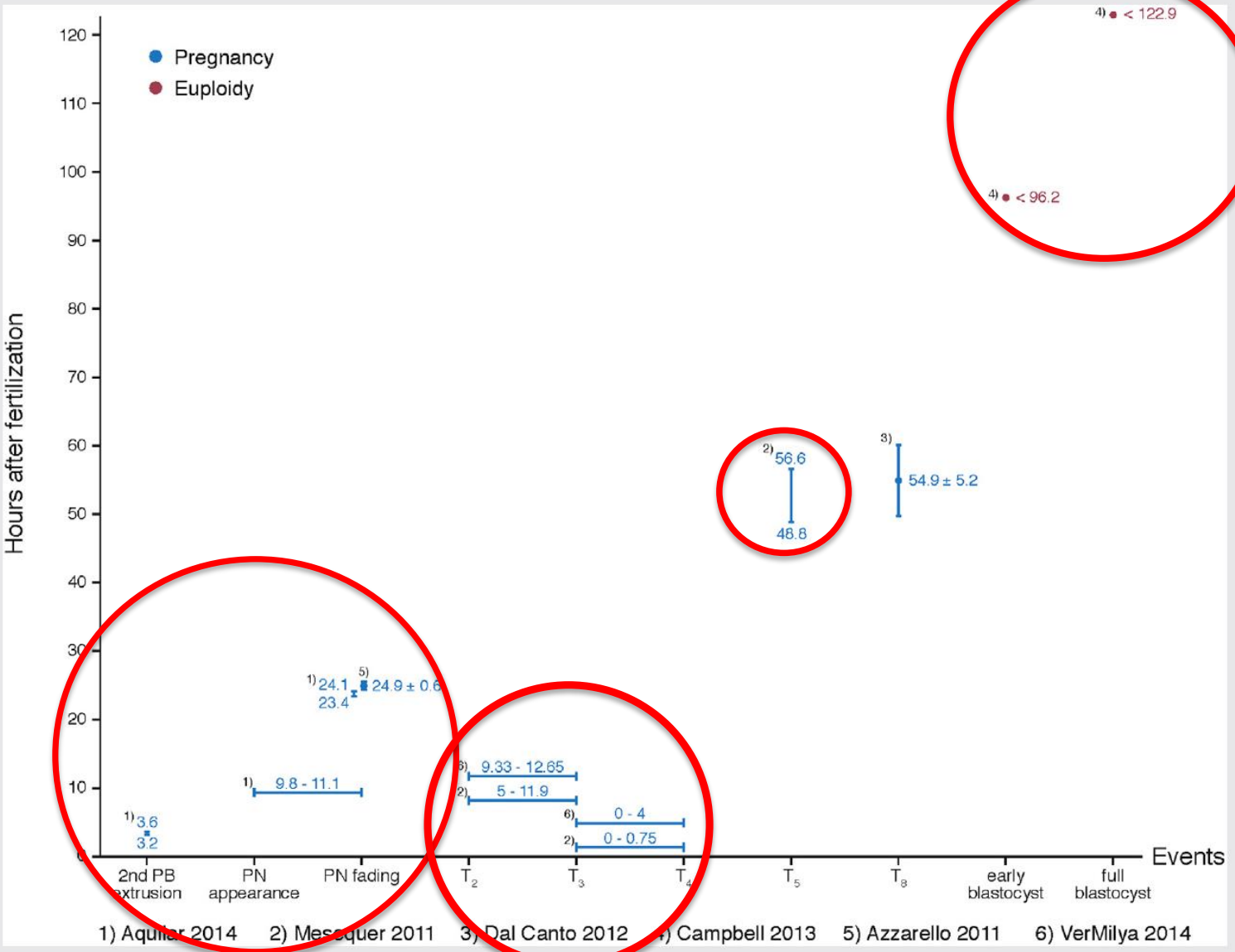
Prediction of blastocyst formation vs. pregnancy

Table VI **Logistic regression analysis of predictors of pregnancy.**

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

Kirkegaard et al, 2013

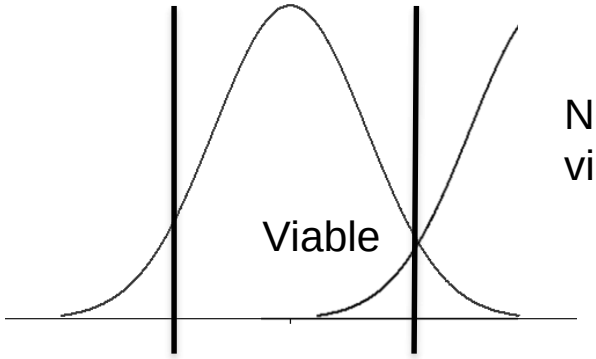
FIGURE 1



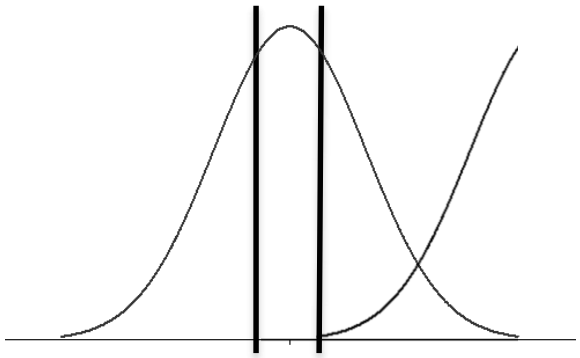
Time-points for embryonic events proposed in time-lapse selection models. PB = polar body; PN = pronuclei.

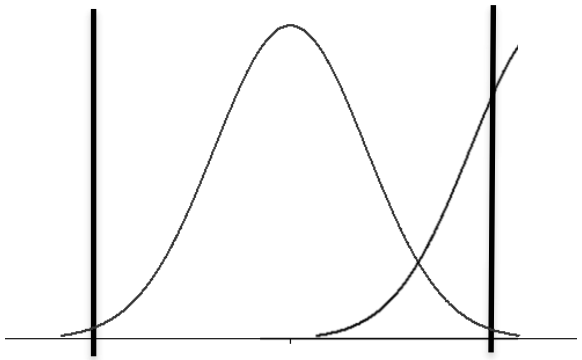
Kirkegaard. Embryo selection using time lapse. Fertil Steril 2014.

What is normal?

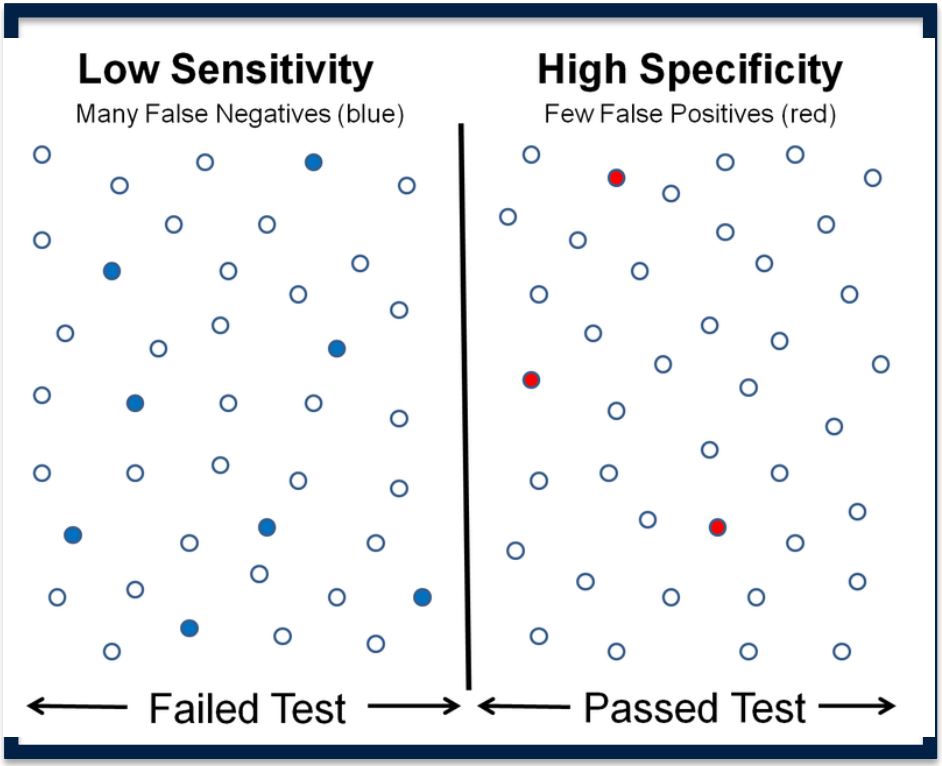


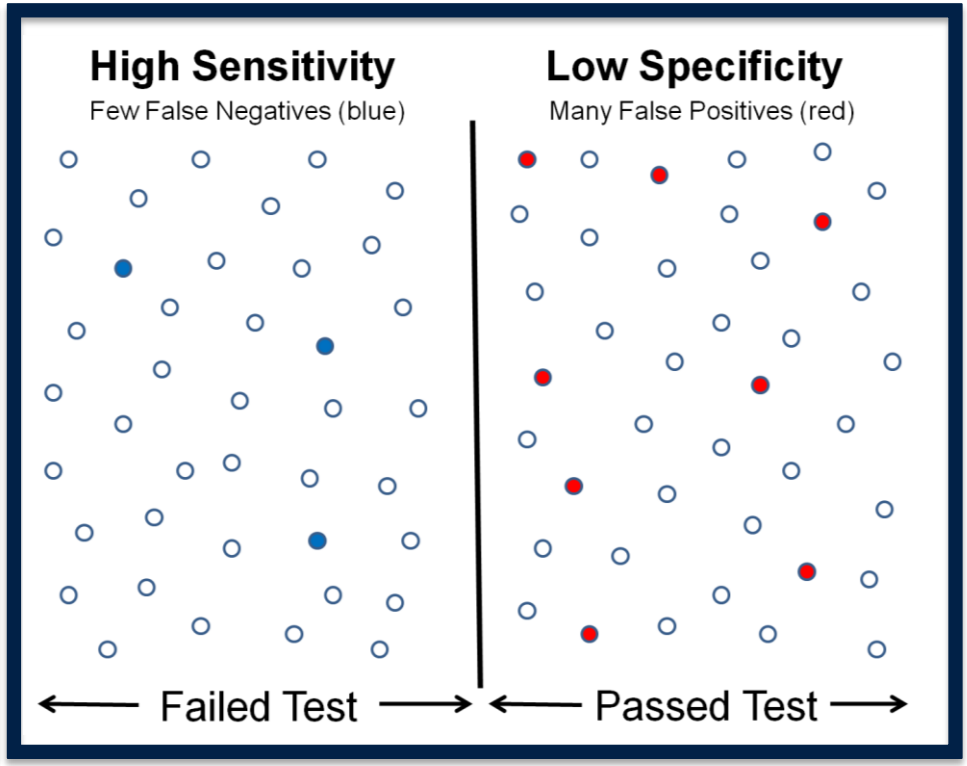
Non-viable





Diagnostic sensitivity and specificity



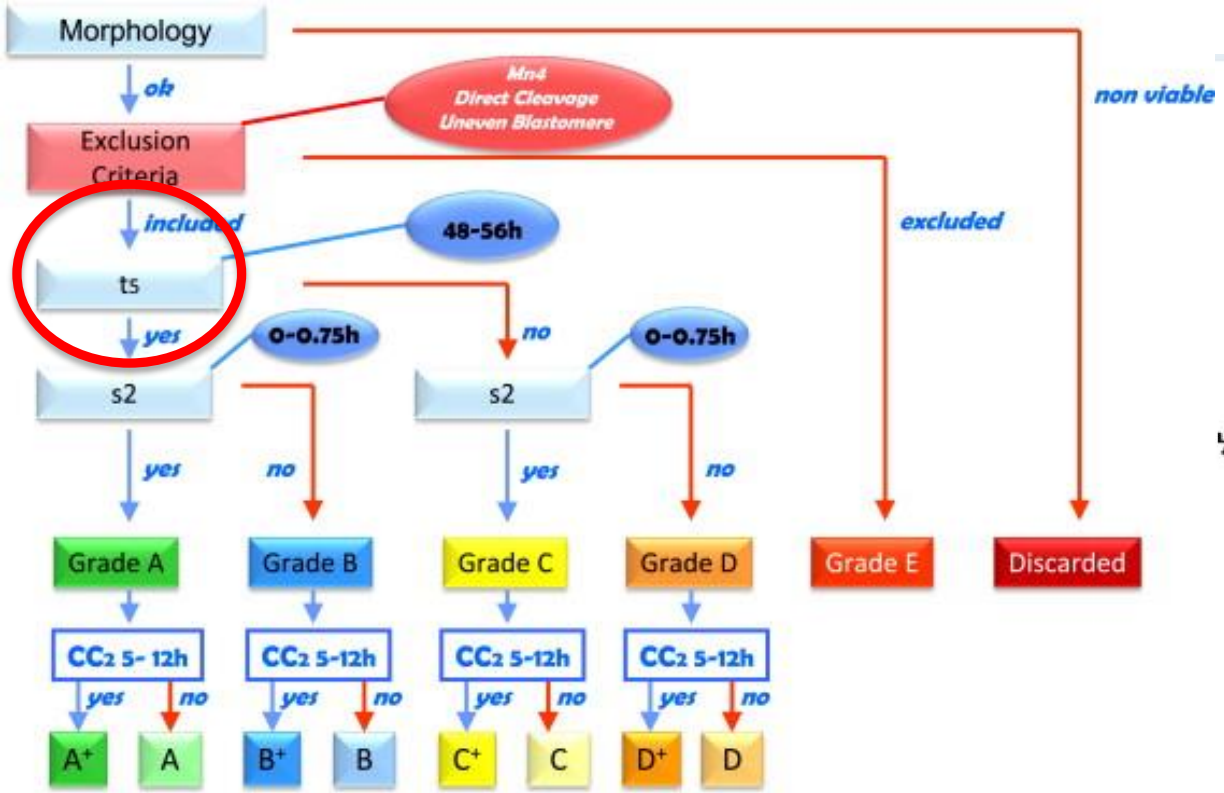


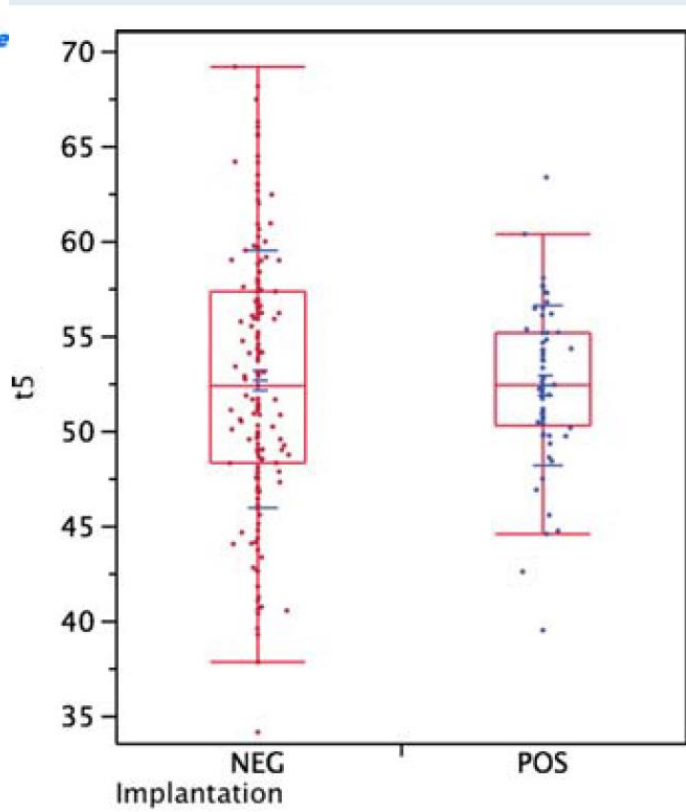
Depends on the reference intervals/cut off levels!
In practice there is rarely a sharp demarcation between normal/abnormal

human reproduction ORIGINAL ARTICLE *Embryology*

The use of morphokinetics as a predictor of embryo implantation†

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

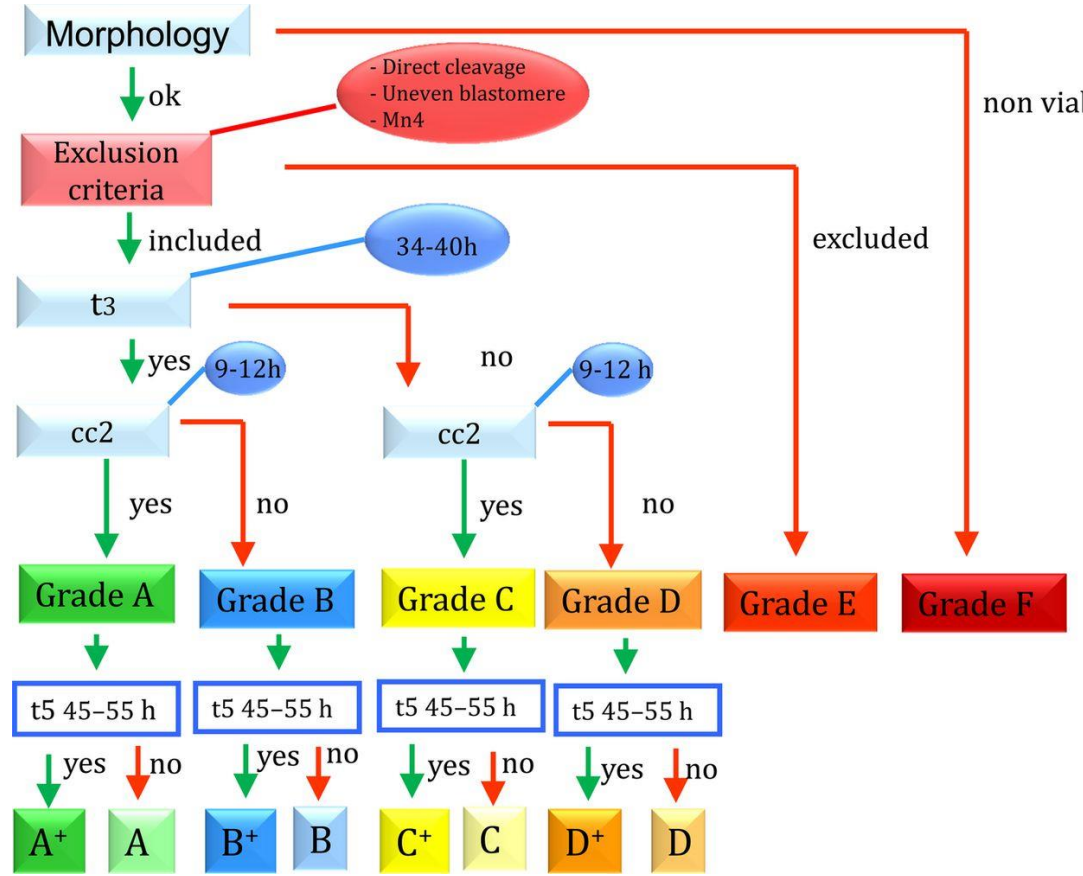


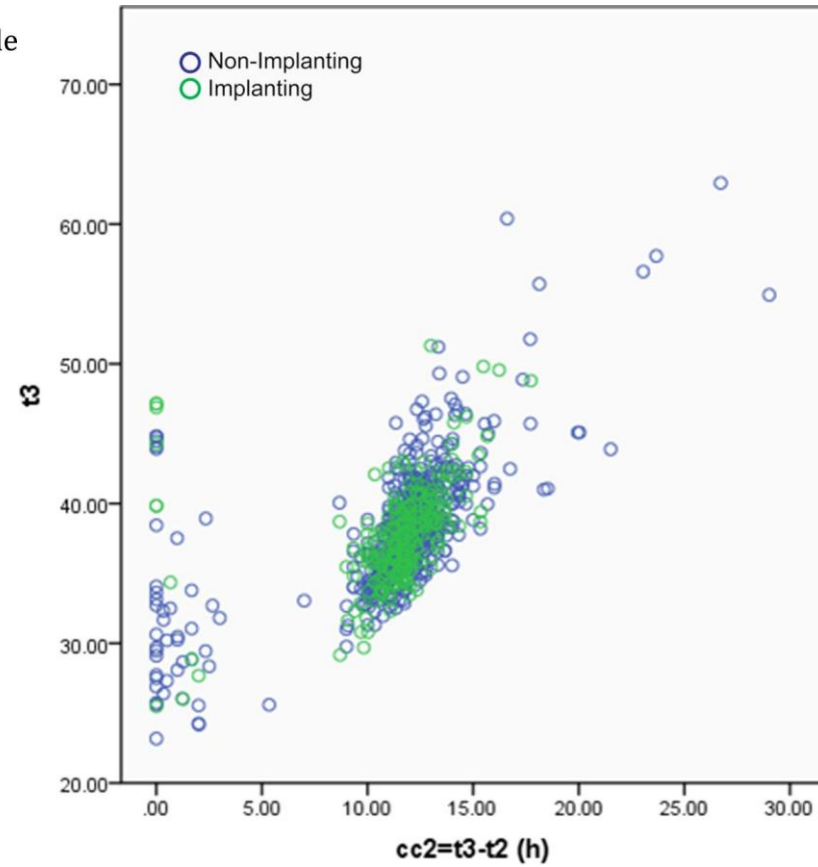


t5:48–56; s2(t4-t3): ≤0.75; cc2(t3-t2): 5-12

human reproduction ORIGINAL ARTICLE *Embryology*

The use of morphokinetics as a predictor of implantation: a multicentric study to define and validate an algorithm for embryo selection



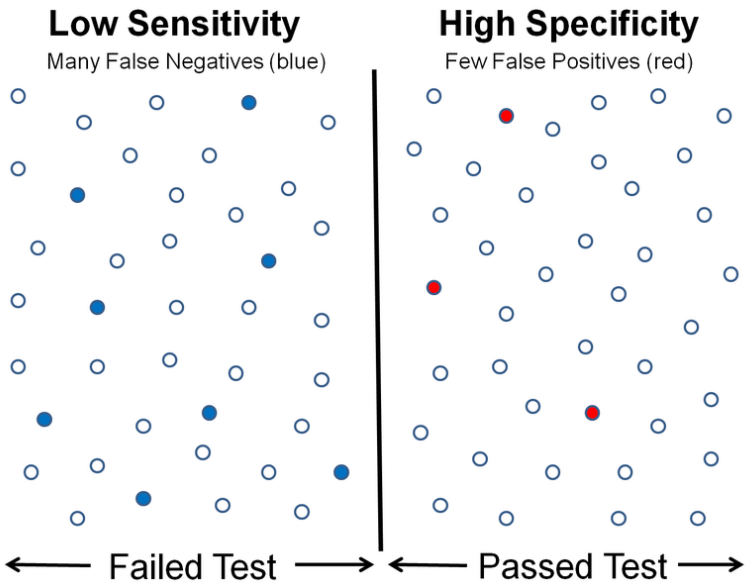


T3 : 31/34–40 h; cc2 9–12 h; t5: 45-55

Improving embryo selection using a computer-automated time-lapse image analysis test plus day 3 morphology: results from a prospective multicenter trial

Joe Conaghan, Ph.D.,[a] Alice A. Chen, Ph.D.,[b] Susan P. Willman, M.D.,[c] Kristen Ivani, Ph.D.,[c] Philip E. Chenette, M.D.,[a] Robert Boostanfar, M.D.,[d] Valerie L. Baker, M.D.,[e] G. David Adamson, M.D.,[f] Mary E. Abusief, M.D.,[f] Marina Gvakharia, M.D., Ph.D.,[f] Kevin E. Loewke, Ph.D.,[b] and Shehua Shen, M.D.[b]

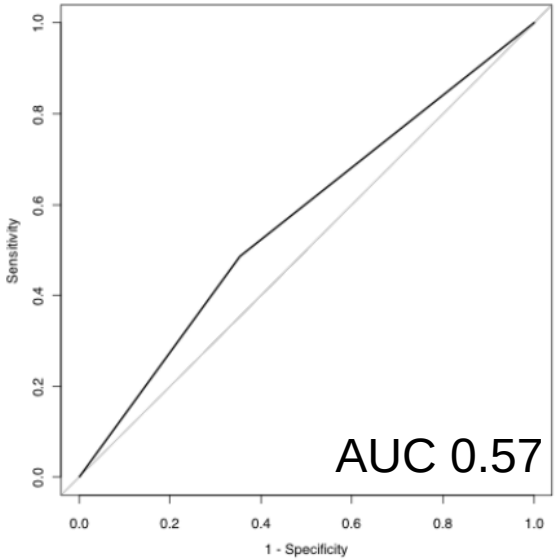
P2 (duration 2 cell stage) 9.33–11.45 hours
P3 (duration 3 cell stage) 0 – 1.73 hours
specificity of 84.7% sensitivity of 38.0%,
PPV of 54.7%, NPV of 73.7%



	usable	**non-usable**	
Test usable	TP	FP	PPV 54.7%
Test non-usable	FN	TN	NPV 73.7%
	Sensitivitet 38.0%	Specificitet 84.7%	

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

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



Kirkegaard et al, 2014

Computer-automated time-lapse analysis results correlate with embryo implantation and clinical pregnancy: A blinded, multi-centre study

Matthew D VerMilyea [a], Lei Tan [b], Joshua T Anthony [a], Joe Conaghan [c], Kristen Ivani [d], Marina Gvakharia [e], Robert Boostanfar [f], Valerie L Baker [g], Vaishali Suraj [b], Alice A Chen [b], Monica Mainigi [a], Christos Coutifaris [a], Shehua Shen [b,*]

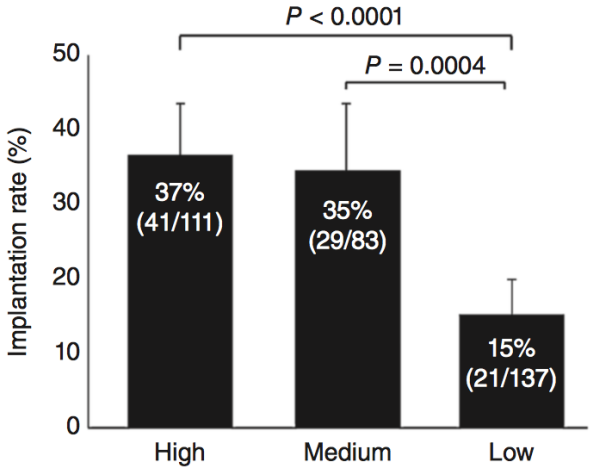
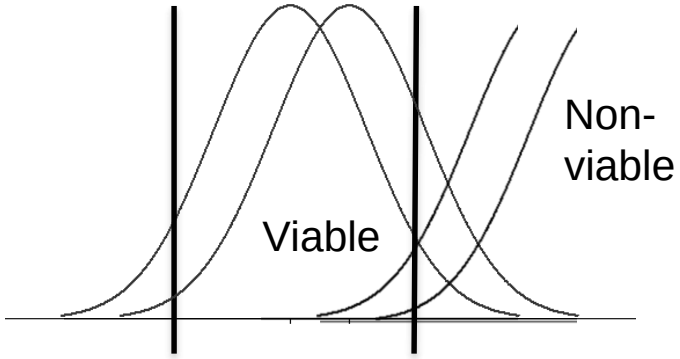
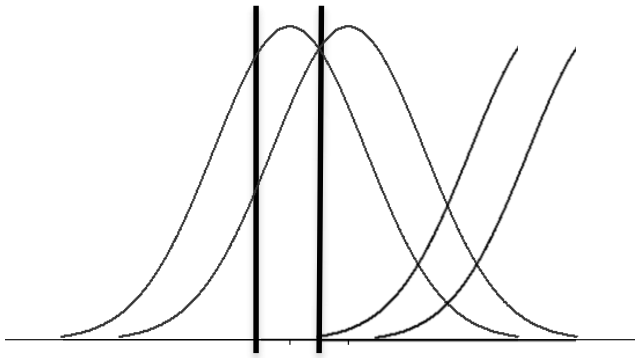
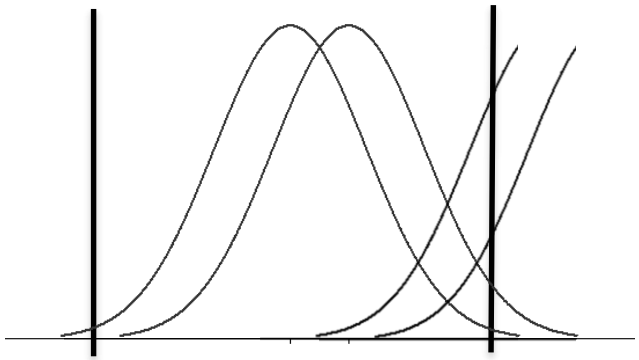


Figure 2 Implantation rates for Eeva three-category High, Medium and Low scored embryos. Error bars represent 95% upper confidence limit. Implantation rates between High versus Low and Medium versus Low were significantly different ($P < 0.0001$ and $P = 0.0004$, respectively).

What is normal?







What is normal?

Laboratory/culture conditions

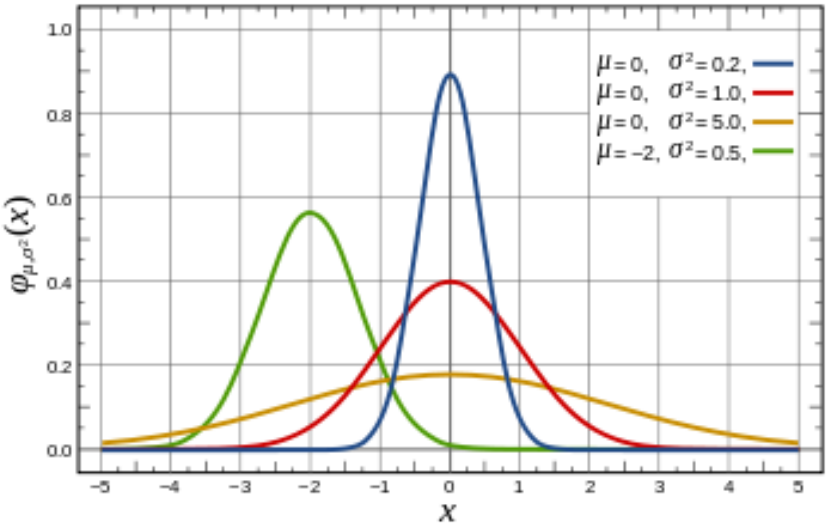
- IVF/ICSI
- Oxygen
- Culture media

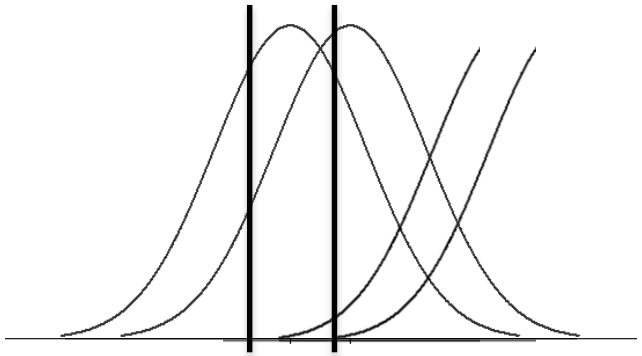
Treatment ?

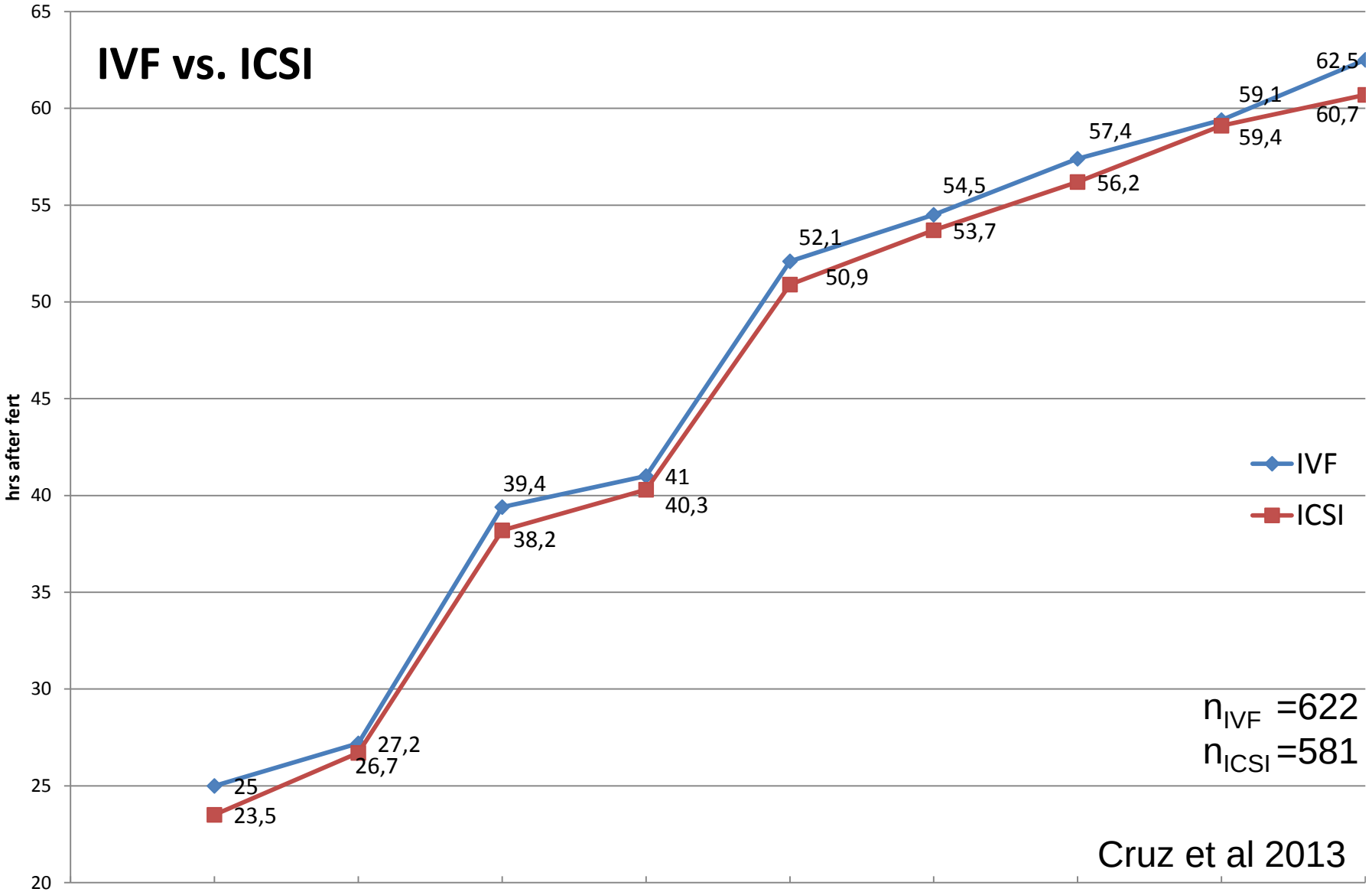
Patients (Age, diagnosis,

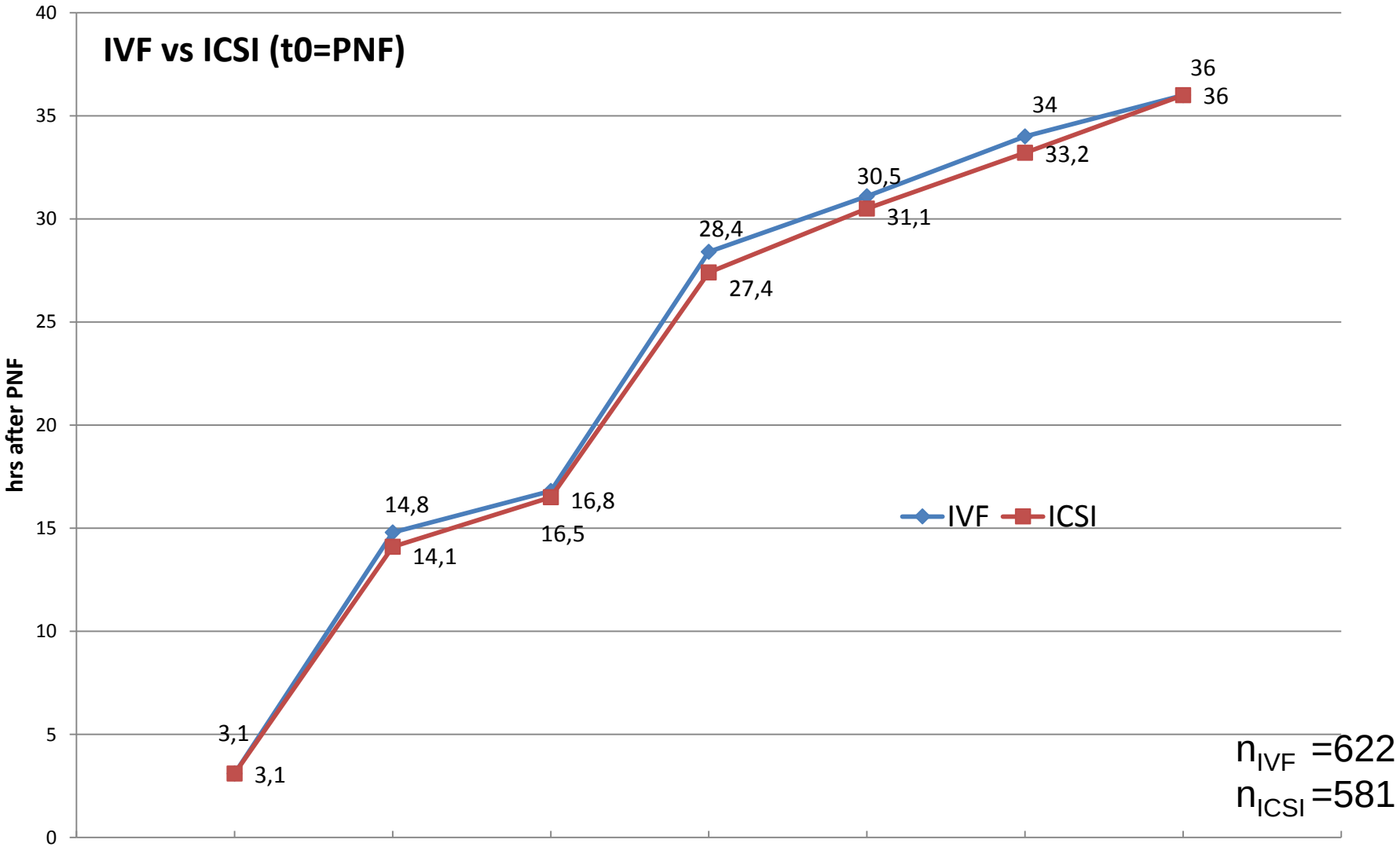
- BMI, hormones) ?

Cell cycle check points



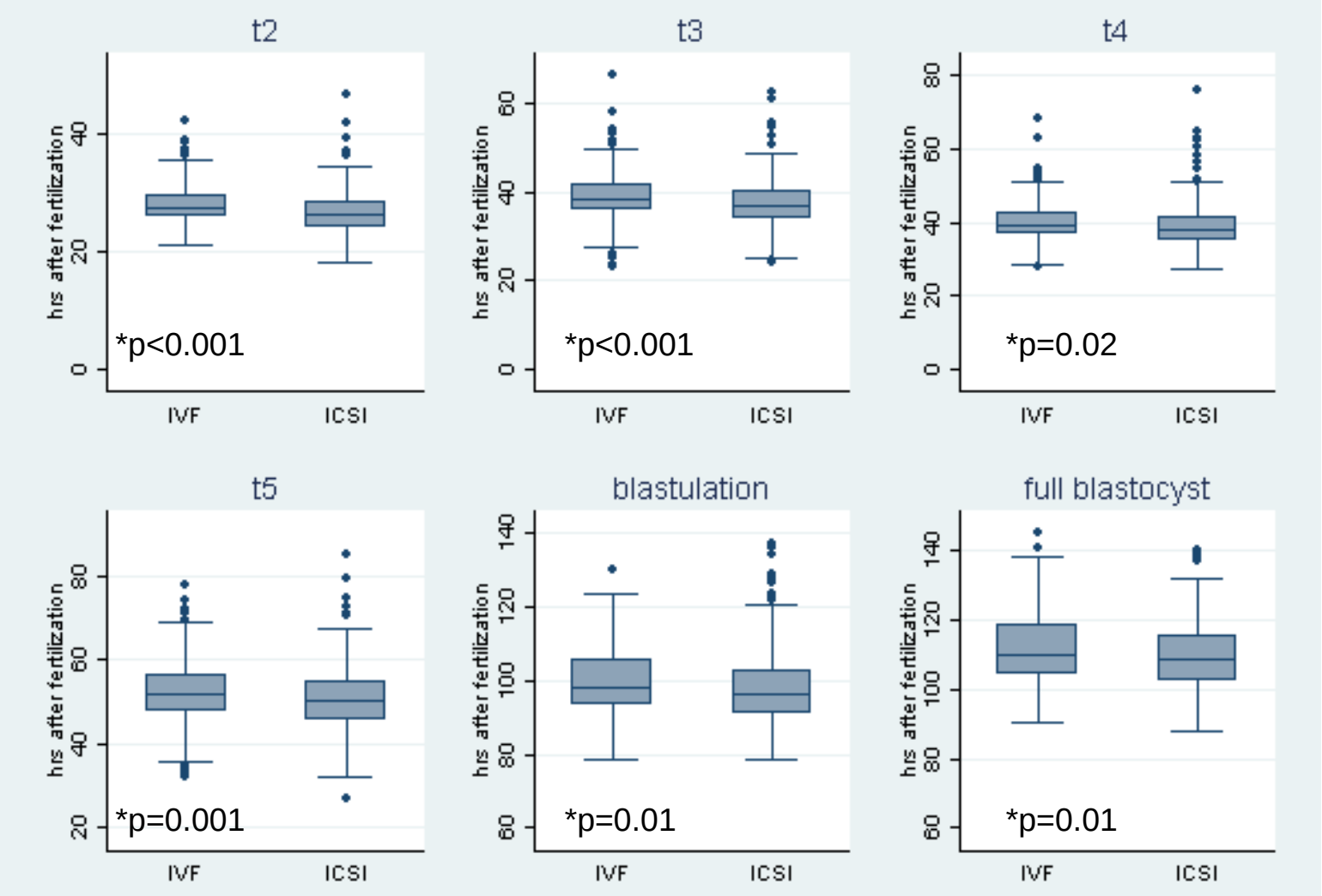



IVF vs. ICSI
hrs after fert
65
60
55
50
45
40
35
30
25
20
25
23,5
27,2
26,7
39,4
38,2
41
40,3
52,1
50,9
54,5
53,7
57,4
56,2
59,1
59,4
62,5
60,7
IVF
ICSI
n_{IVF} =622
n_{ICSI} =581
Cruz et al 2013



Cruz et al 2013

IVF vs. ICSI



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

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

Kirstine Kirkegaard, M.D., Johnny Juhl Hindkjaer, M.Sc., and Hans Jakob Ingerslev, D.M.Sc.

Fertility Clinic, Aarhus University Hospital, Aarhus, Denmark

TABLE 2

Fertilization method according to type of hatching.

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

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

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

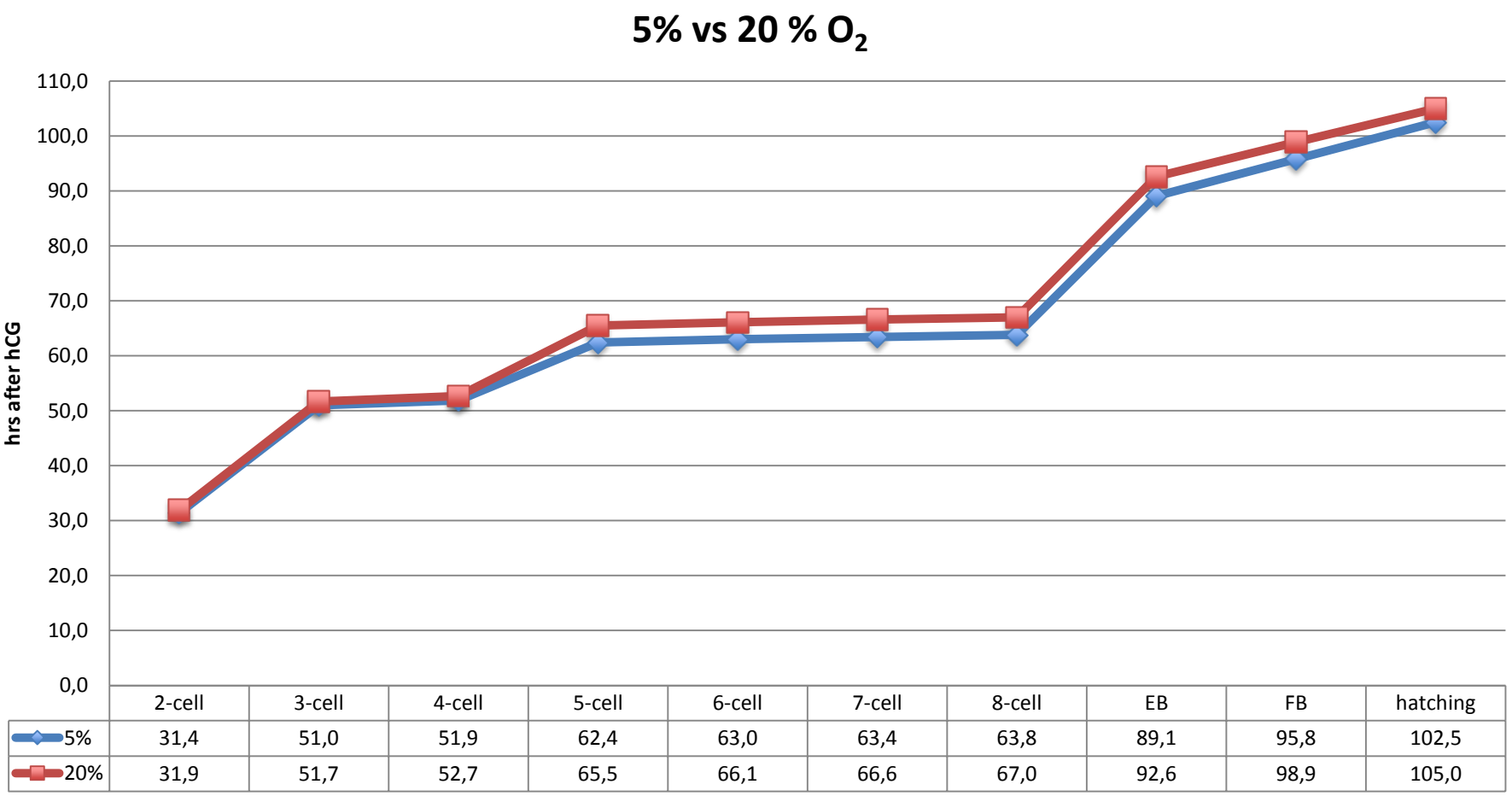
TABLE 3

Completed hatching versus fertilization method and type of hatching.

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

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

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



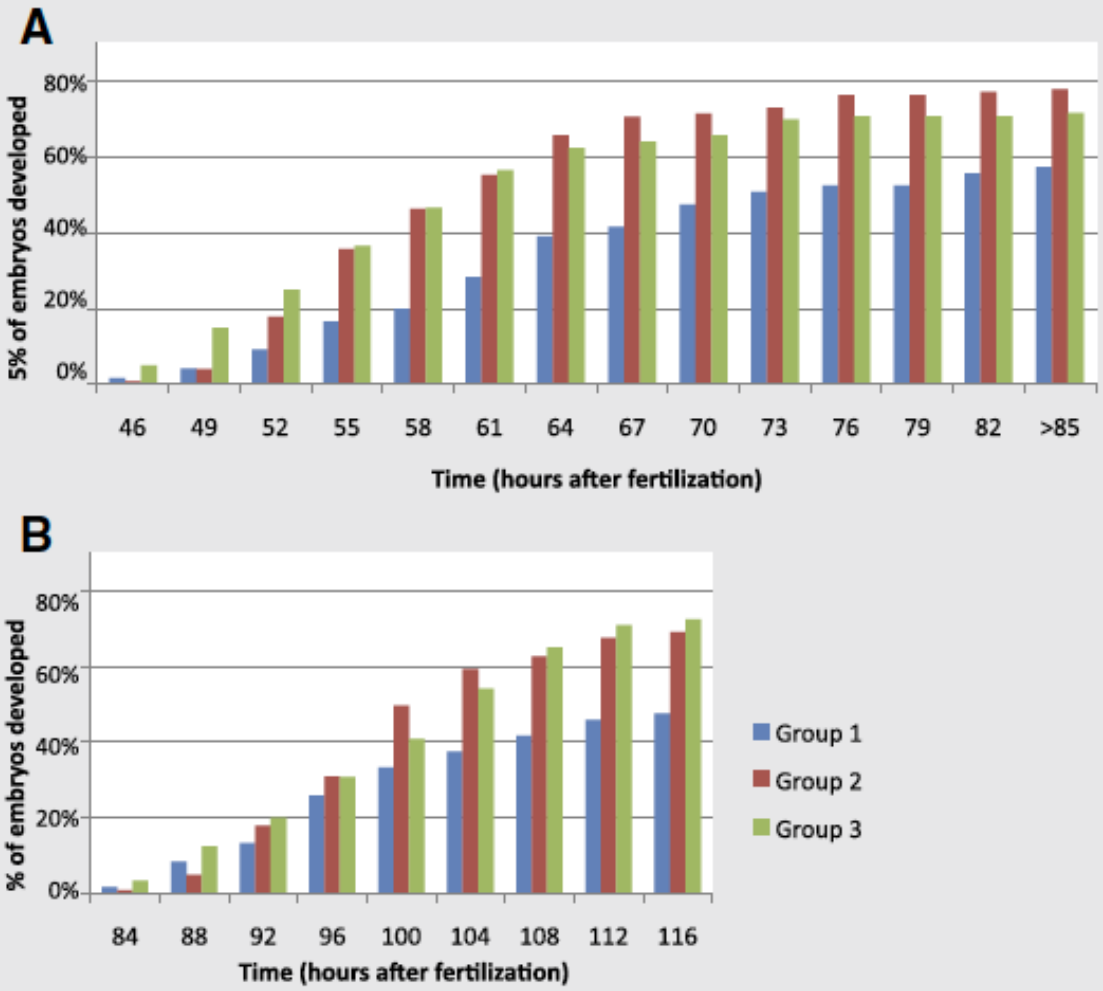
	2-cell	3-cell	4-cell	5-cell	6-cell	7-cell	8-cell	EB	FB	hatching
5%	31,4	51,0	51,9	62,4	63,0	63,4	63,8	89,1	95,8	102,5
20%	31,9	51,7	52,7	65,5	66,1	66,6	67,0	92,6	98,9	105,0

Culture under 20% oxygen results in cummulative delayed development and increased developmental arrest at all stages

Wale and Gardner 2010

Oxygen-Human embryos

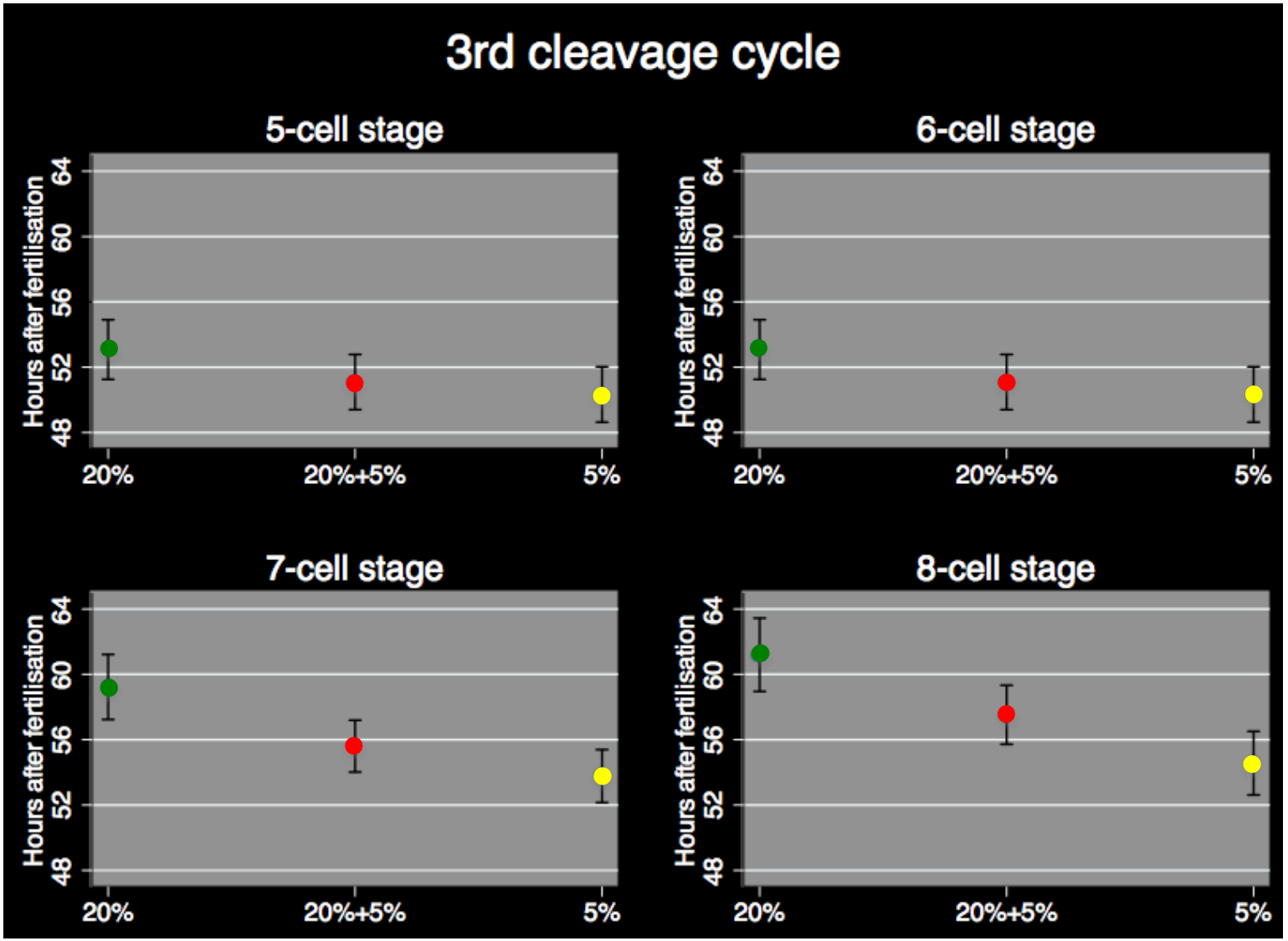
FIGURE 1



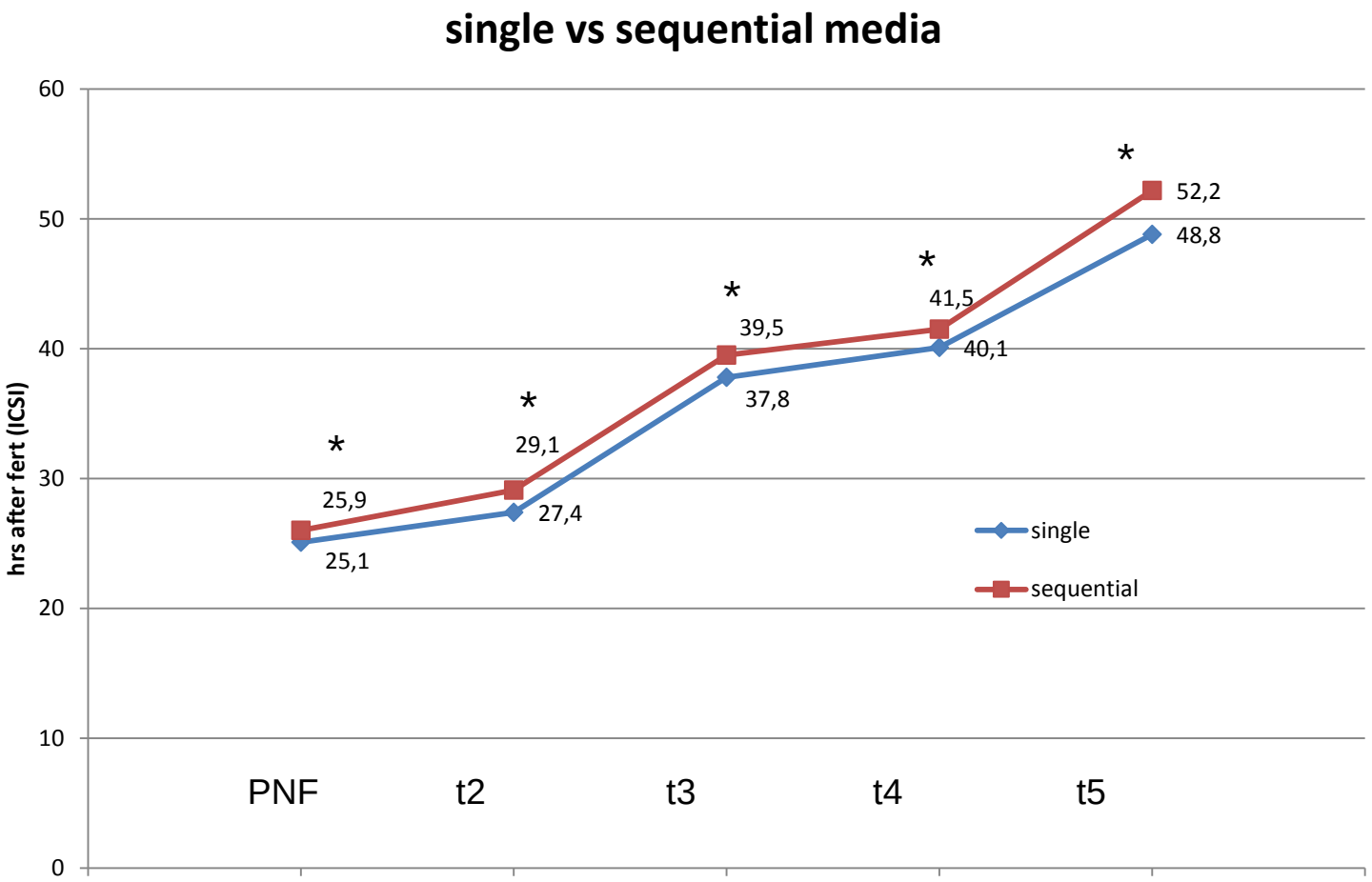
The cumulative development of embryos progressing through (A) third cleavage cycle and (B) early blastocyst stage.

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

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

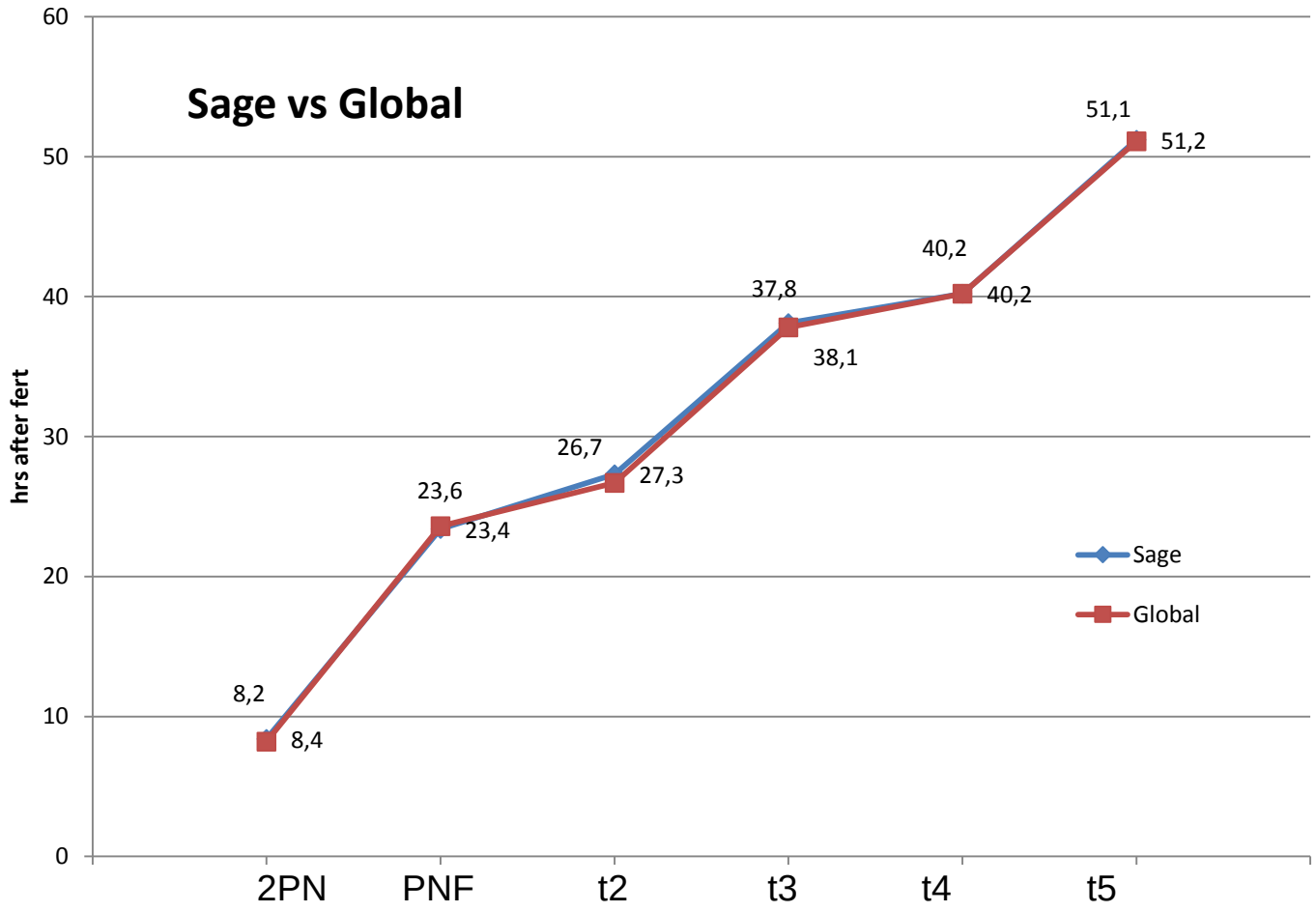


Kirkegaard et al 2013

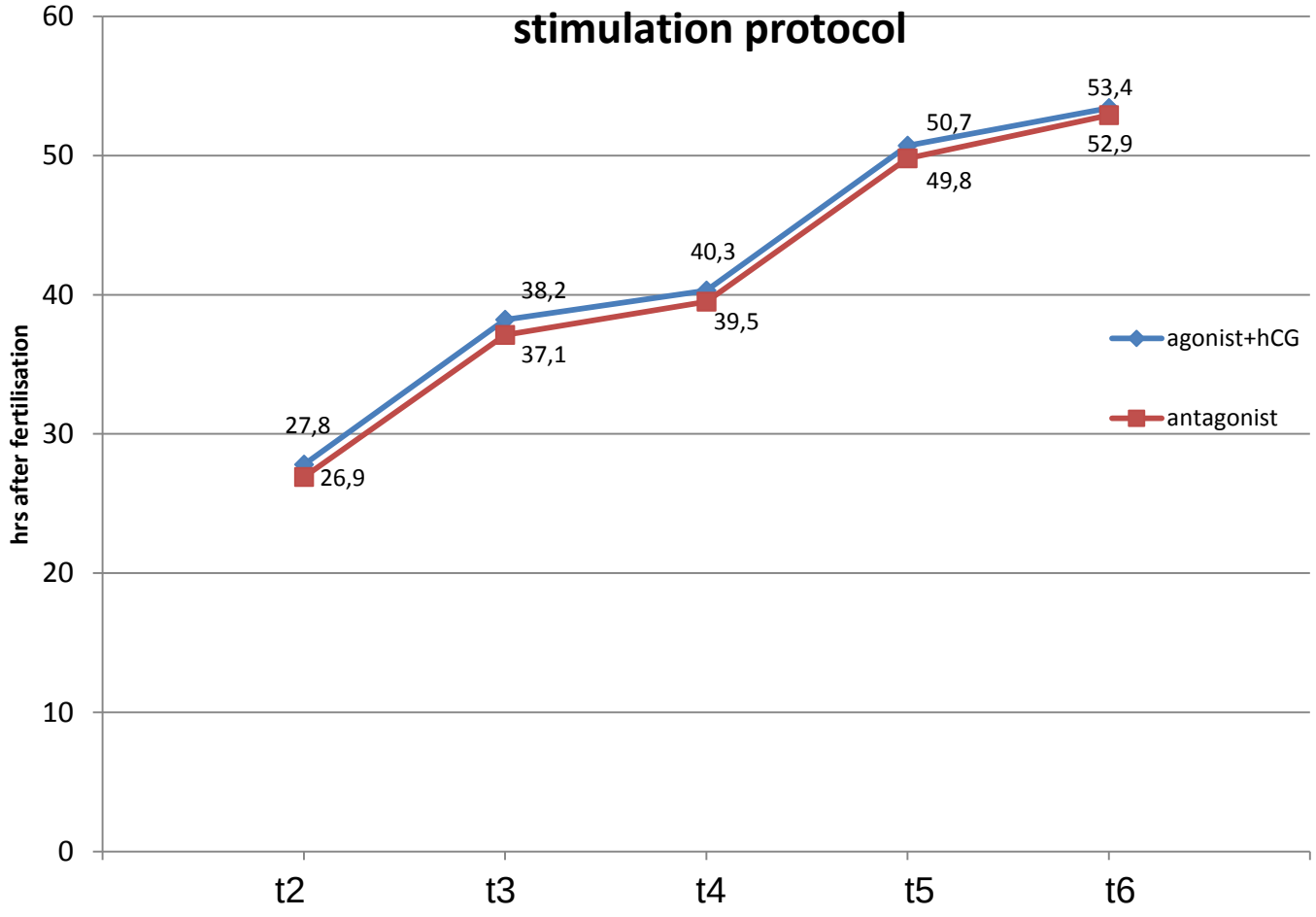


Ciray et al 2012

n_{single}=231 (2 PN=170), n_{seq}=215 (2 PN=149)
71 cycles



Basile et al 2012



n=2101

n=713

Munoz et al 2012 + 2013

+FSH dose

What is normal?

Laboratory/culture conditions

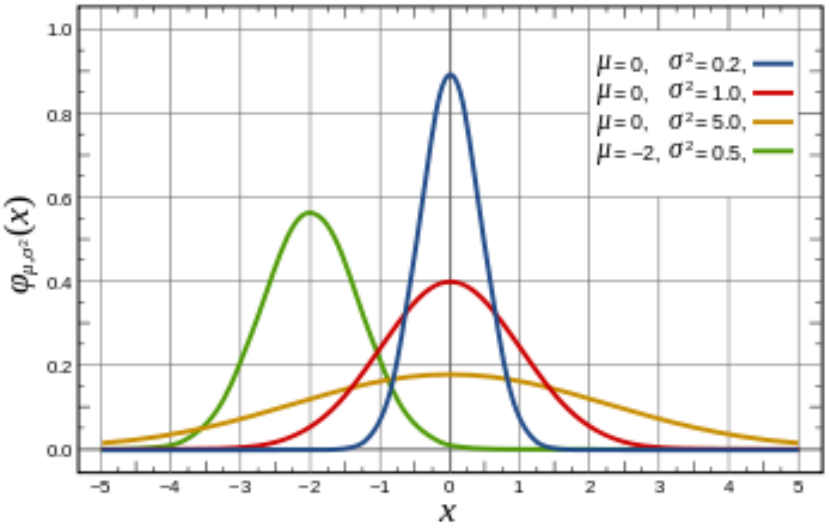
- IVF/ICSI
- Oxygen
- Culture media

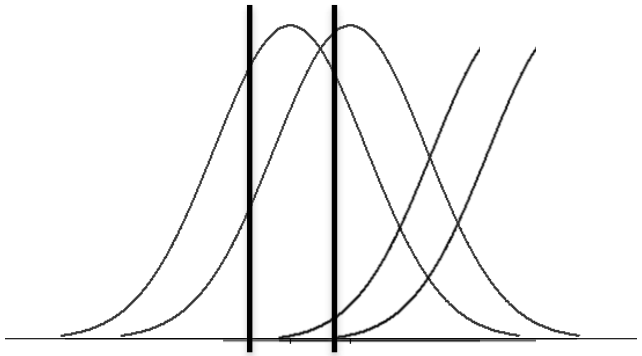
Treatment ?

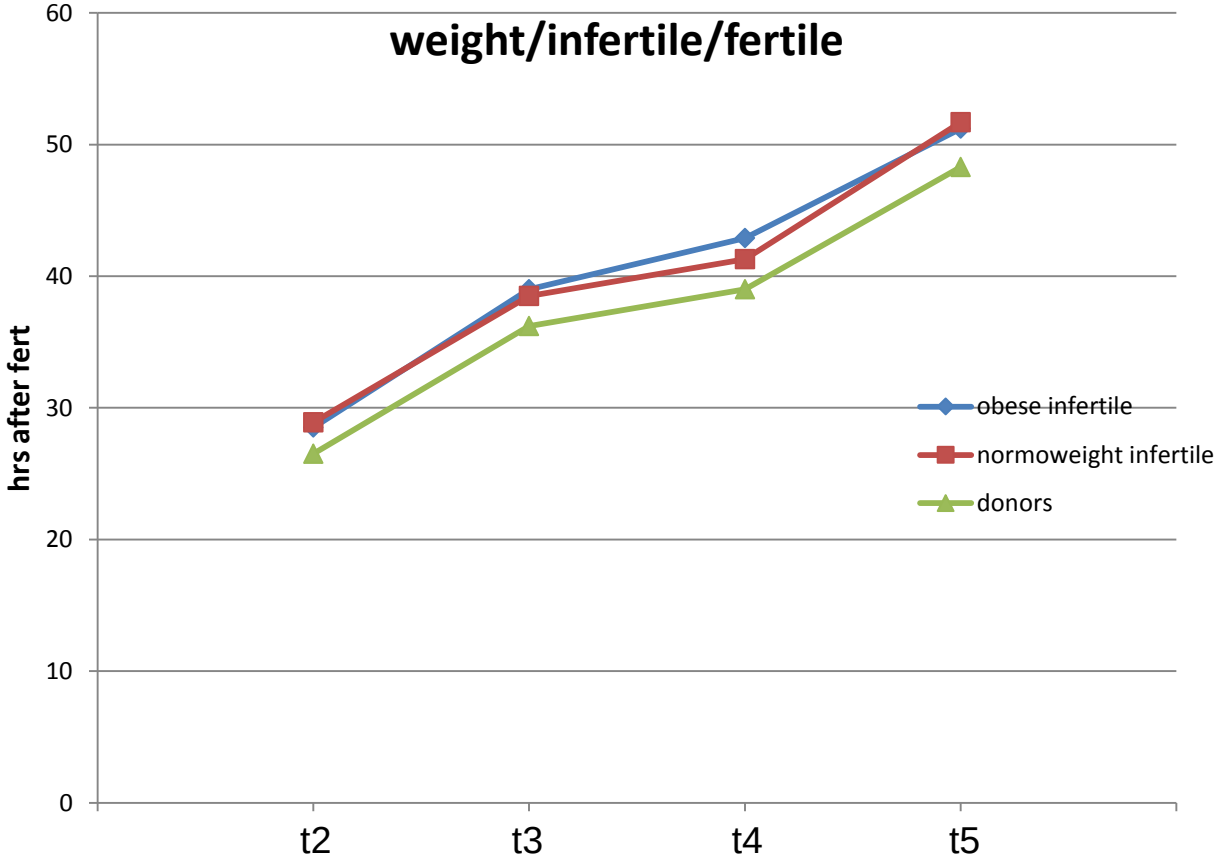
Patients (Age, diagnosis,

- BMI, hormones) ?

Cell cycle check points

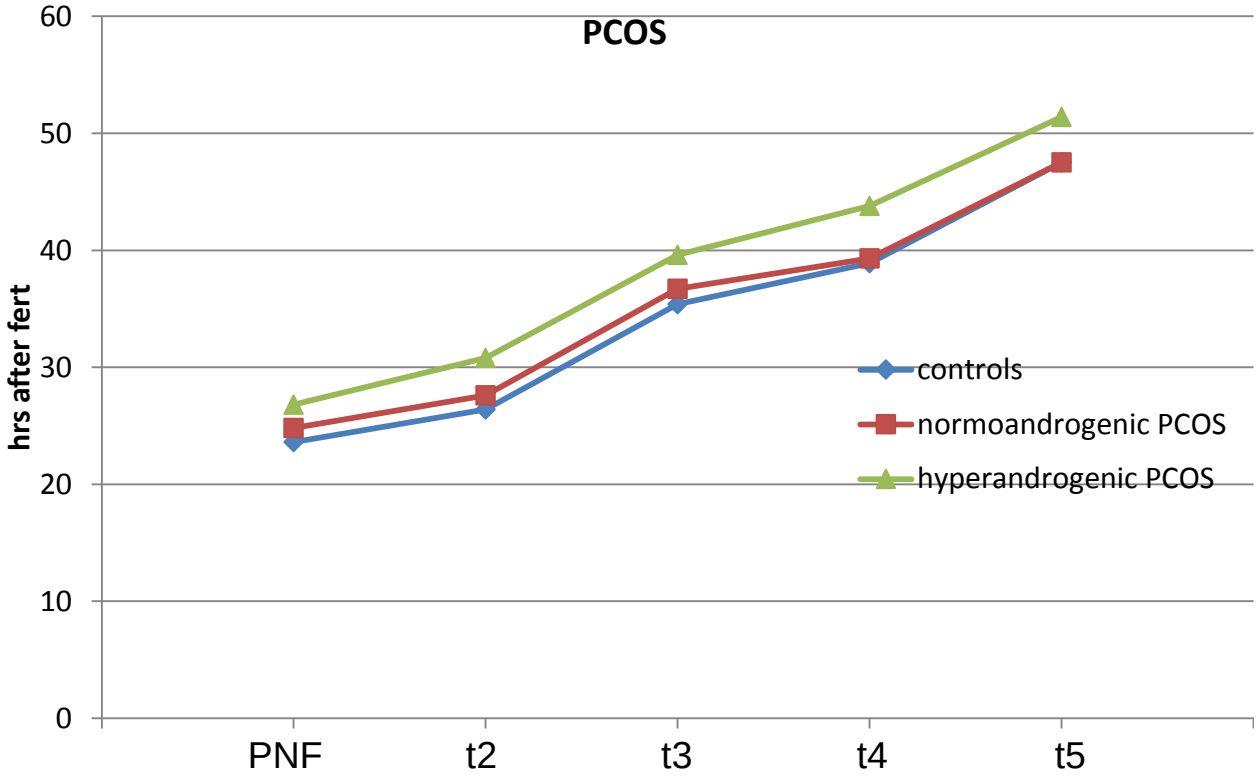






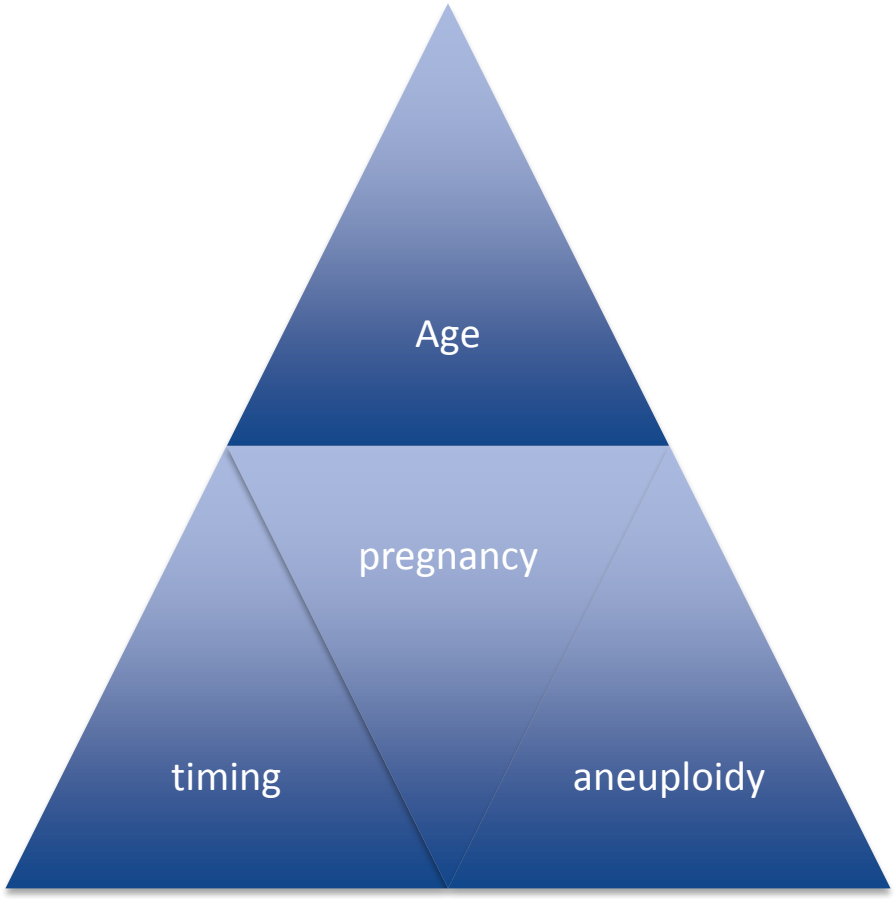
group	age
Obese infertile	34.7 (SD 3.4)
Normo weight infertile	33.3 (SD 2.9)
Donors	27.1 (SD 4.2)

Bellver et al 2013



Wissing et al 2013

Confounding

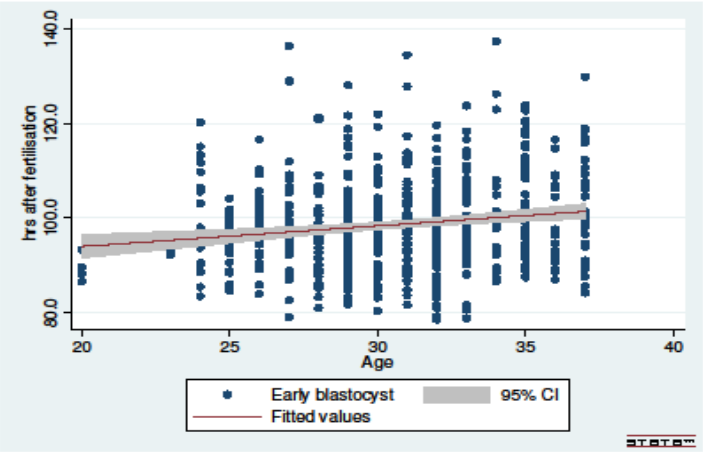


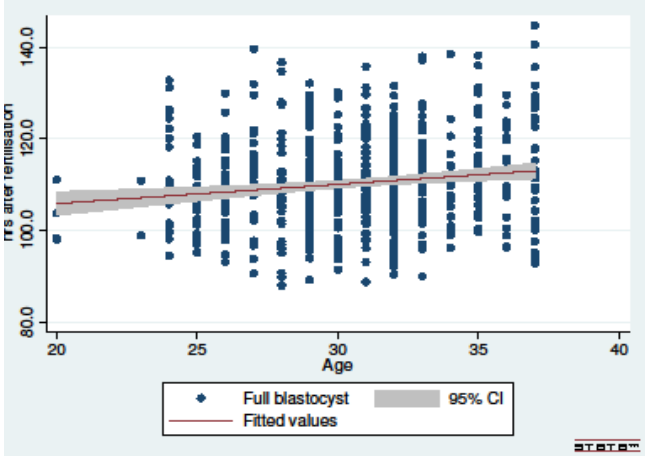
Age, aneuploidy and tSB

Model effect	Estimate	Significance of additional effect
Age	0.16 (0.04)	0.016
tSB	0.05 (0.02)	0.0001

Logistic regression analysis of the effect of age and tSB on aneuplodi.
Campbell et al 2014

AGE AND BLASTOCYST TIMING

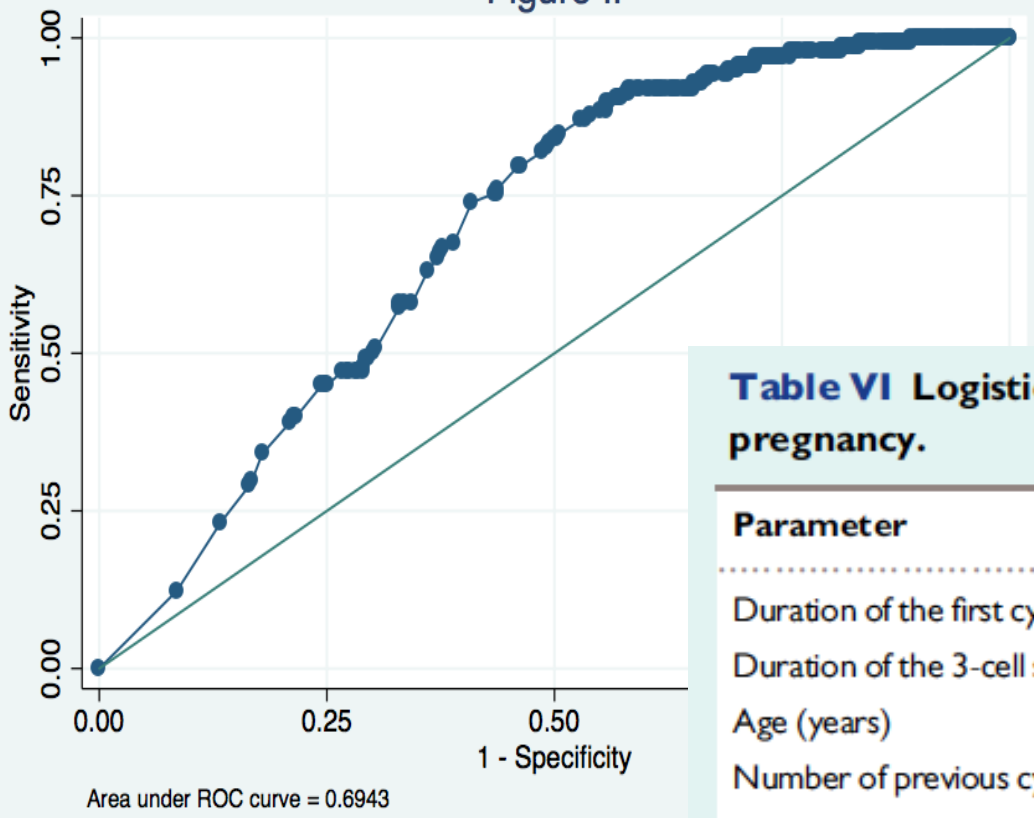




	Regression coefficient (95% CI)	P-value	Coefficient of determination (R^2)
Early blast	0.44 (0.16; 0.71)	0.002	0.02
Full blast	0,42 (0.10;0.74)	0.01	0.02

Clustered linear regression, n=653, npt=149

Figure II



Prediction of blastocyst formation vs. pregnancy

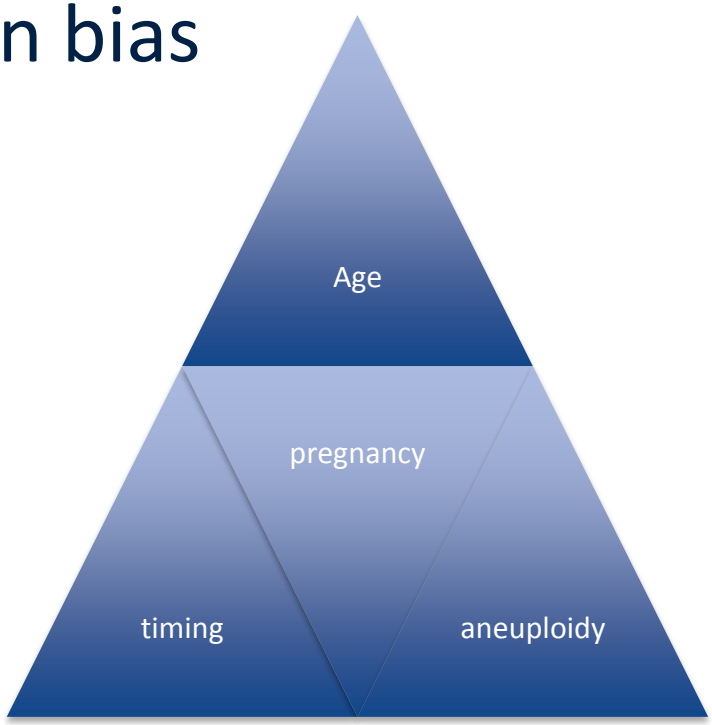
Table VI **Logistic regression analysis of predictors of pregnancy.**

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

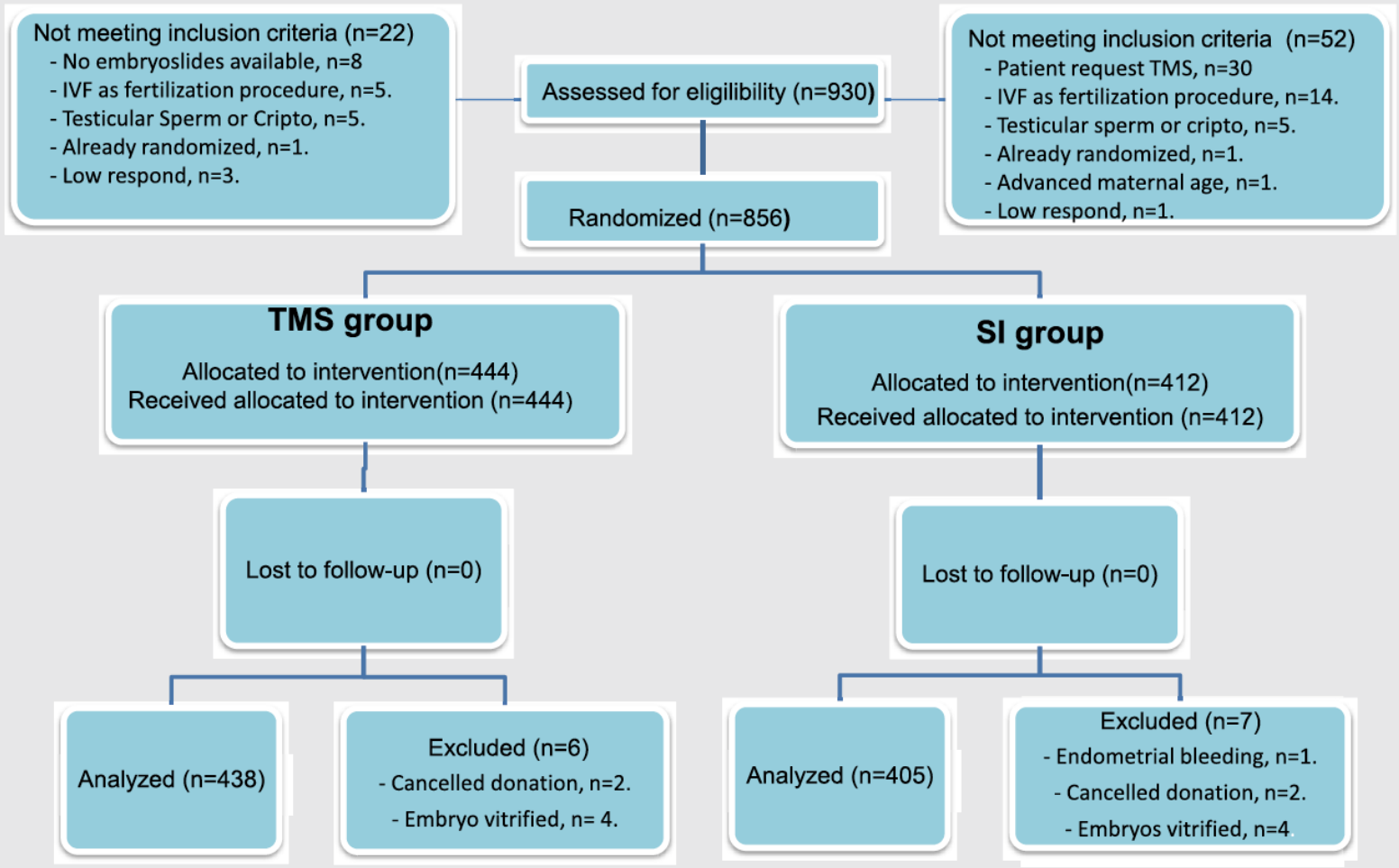
Kirkegaard et al, 2013

A few words about RCT´s

- Equal distribution of known and unknown confounders
- Elimination of selection bias



SUPPLEMENTAL FIGURE 2



Patient flow chart presentation. Number of patients and descriptions of patients are included in every box.

Rubio. Clinical validation of EmbryoScope. Fertil Steril 2014.

What is normal?

Laboratory/culture conditions

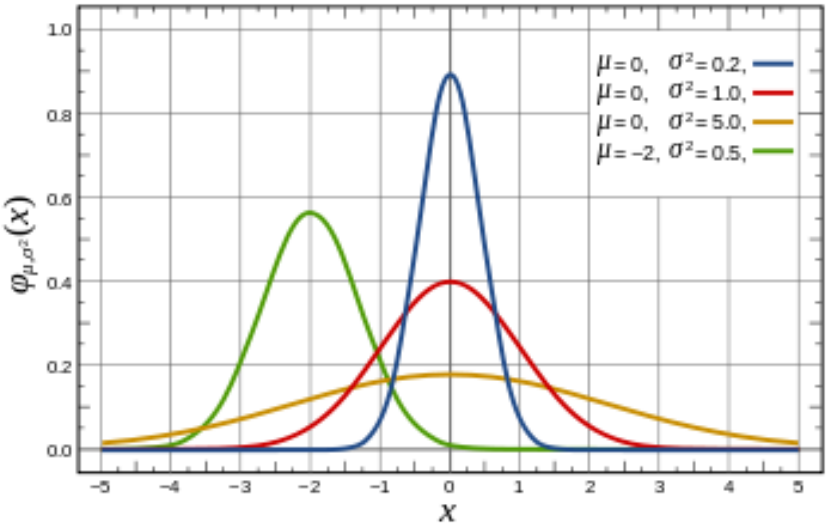
- IVF/ICSI
- Oxygen
- Culture media

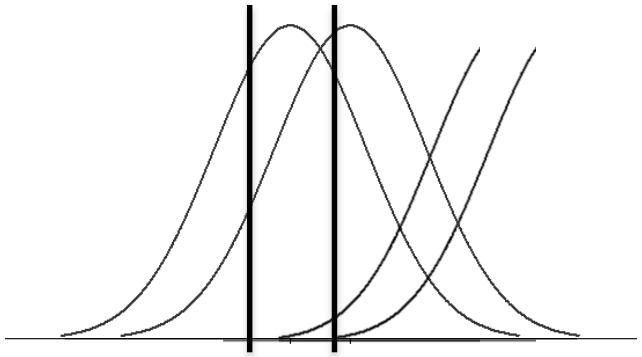
Treatment ?

Patients (Age, diagnosis,

- BMI, hormones) ?

Cell cycle check points





Choosing the best embryo by time lapse versus standard morphology

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TABLE 3

Studies evaluating aneuploidy.

Study	No. of embryos	End point(s)	Predictive parameters
Chavez et al., 2012	45	Aneuploidy at the 4-cell stage (a-CGH)	Duration of the first cytokinesis, duration of the 2- and 3-cell stage
Campbell et al., 2013	88	Aneuploidy at the blastocyst stage (a-CGH and SNP array)	Time to blastulation and full blastulation
Basile et al., 2014	504	Aneuploidy at day 3 ($\geq$6 cells) (a-CGH)	Time ranges for division to 5 cells (t5), duration of the 2-cell stage (t3-t2), t5-t3, and t5-t2
Rienzi et al., 2014	455	Aneuploidy at the blastocyst stage (q-PCR based comprehensive chromosome screening)	None

Sensitivity/specificity

Ranking

Confounders

Validation