Preimplantation Aneuploidy Testing: on day 3 embryos or on blastocyst?

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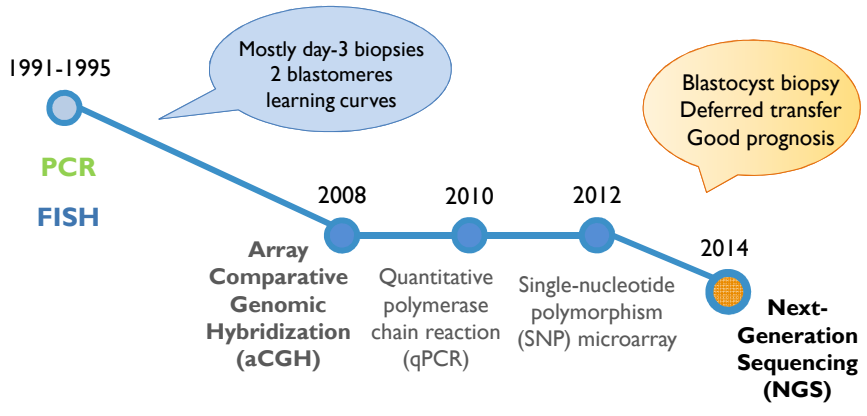
igenomix
Pioneers in Reproductive Medicine and Genetics

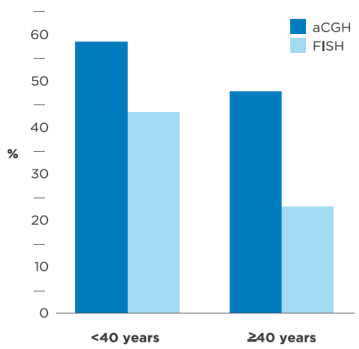
PGS: technology evolution



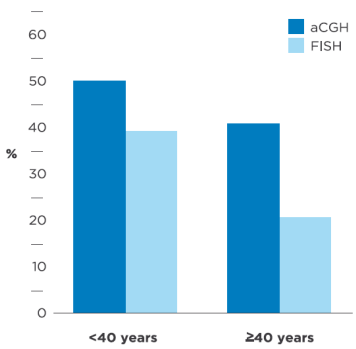
Day-3 biopsy: aCGH vs. FISH

PGS with aCGH achieves better reproductive outcome than FISH (24-chr. vs. 9 chr.) (2010-2011)

Pregnancy Rate/transfer



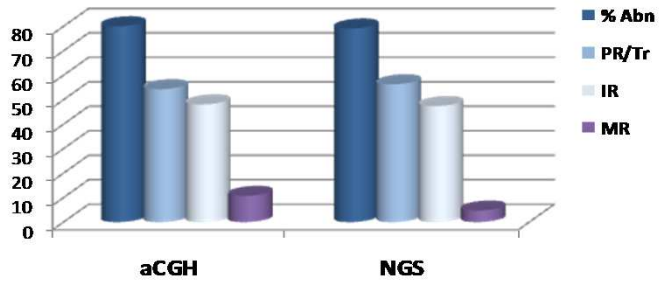
Implantation Rate



Nº cycles-AMA: 880, IF:187, RM:204, MF:116
Pregnancy of 12 weeks gestation or more

aCGH vs. NGS

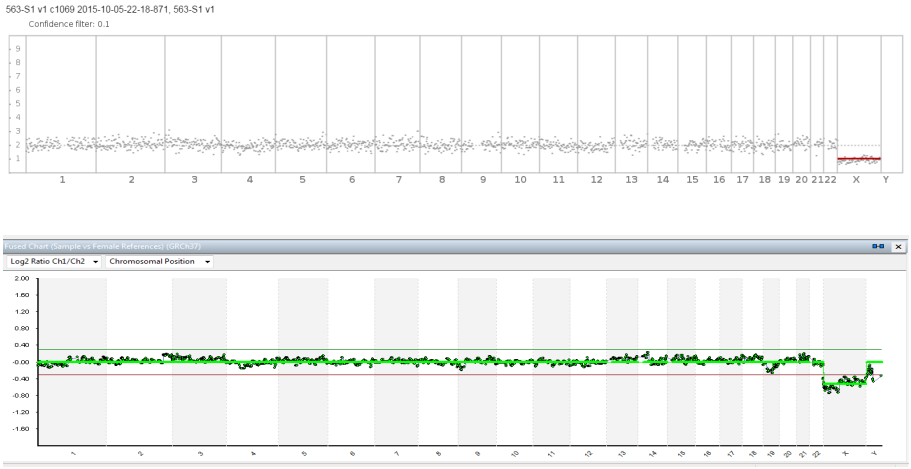
Day-3 biopsies: N= 6,934 aCGH vs. 284 NGS cycles



NGS and aCGH inform of similar % abnormal embryos, pregnancy and implantation rates, with a trend towards lower miscarriage rates in NGS

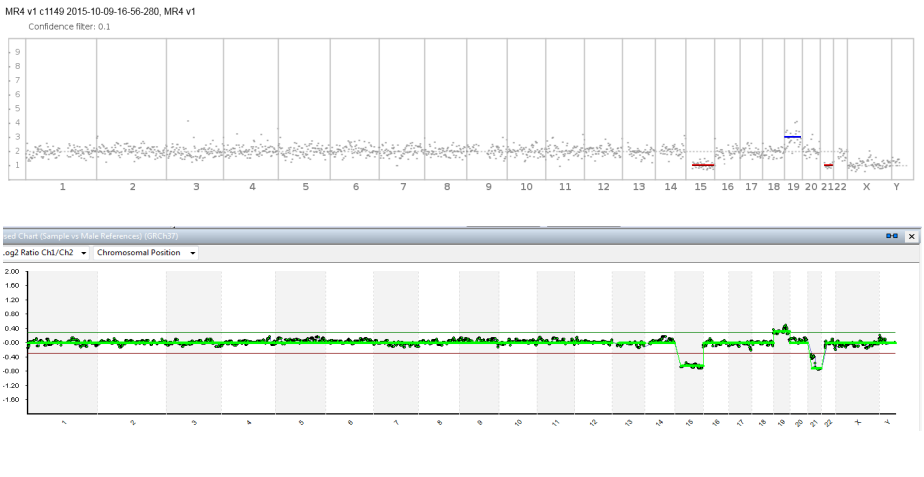
Validation of PGS-NGS

Concordance with aCGH: euploid embryo

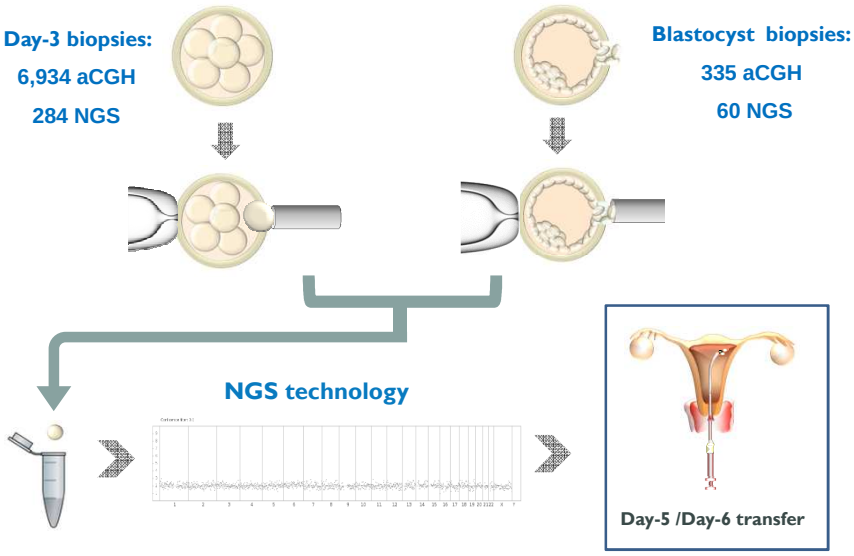


Validation of PGS-NGS

Concordance NGS/aCGH: aneuploid embryo



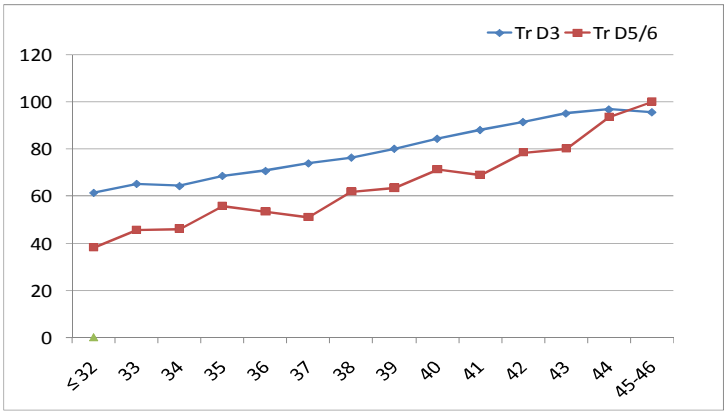
PGS with aCGH and NGS



aCGH: abnormal embryos

Percentage of abnormal embryos (38,031 embryos analyzed)

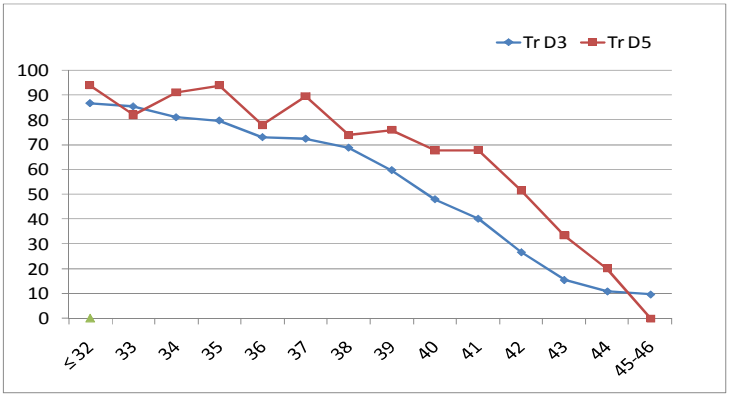
36,281 embryos with day-3 biopsies
1,750 embryos with day-5/6 biopsies



Day-3 vs. blastocyst biopsies

Percentage of cycles with euploid embryos (7,269 cycles)

6,934 cycles with day-3 biopsies
335 cycles with day-5/6 biopsies

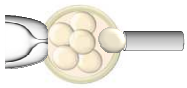


aCGH: abnormal embryos

	Day-3	Blastocyst
Mean age	39.5	37.6
Mean MII	9.6	13.1
Mean analyzed	5.2	5.4
% abnormal embryos	81.0	54.4*
% chaotic pattern	16.1	2.1*
% complex aneuploidy	18.6	6.2*
% partial aneuploidies	5.4	6.1

*(*p<0.05)*

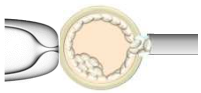
NGS: day-3 biopsies



	NORMAL	ABNORMAL	CHAOTIC
BLOQ	**4.1**	**18.3**	**56.4**
MC	**6.0**	20.0	**22.1**
BT	13.4	21.7	11.4
BC	17.7	18.0	6.9
BE	2.7	2.5	0.3
BH	3.6	1.3	2.8
BHi	52.0	21.7	0
Total Blast	**90.0**	**61.5**	**21.4**

*(*p<0.05)*

Blastocyst biopsies



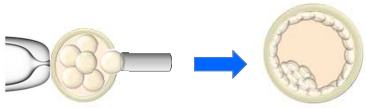
	% Cycles	Transfer type
Arrested before biopsy	**11%**	
All Day-5 biopsy	11%	Fresh
Day-5 and day-6 biopsy	50%	Defered
All Day-6 biopsy	28%	Defered

Mercader, IVI-Valencia

aCGH: false positive rates

Accuracy of aCGH on day-3 and day-5 biopsies

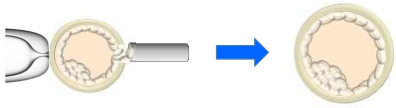
N= 76 reanalysed abnormal embryos



Day-3 aCGH reconfirmation with FISH on day-5

	Confirmed Diagnosis	NO confirmed
aCGH day-3	74/76 (97.4%)	2/76 (2.6%)

N= 37 reanalysed abnormal embryos



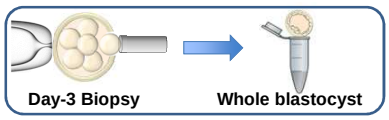
Day-5 aCGH reconfirmation with FISH on day-5

	Confirmed Diagnosis	NO confirmed
aCGH day-5	36/37 (97.3%)	1/37 (2.7%)

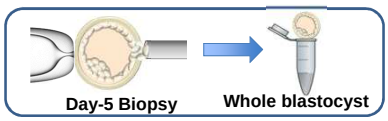
Mir et al., ASRM 2011

aCGH: false positive rates

Low false positive rate in both types of biopsies

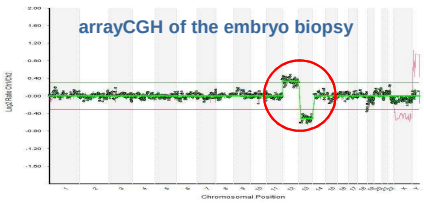


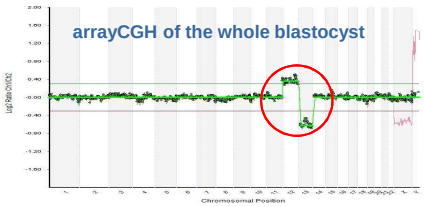
Vs.



	Confirmed	NO confirmed
aCGH Day-3	49/50 (98%)	1/50 (2%)

	Confirmed	NO confirmed
aCGH Day-5	57/59 (96.6%)	2/59 (3.4%)

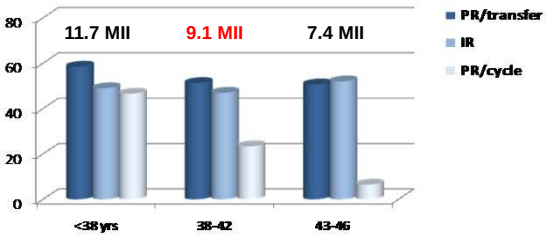




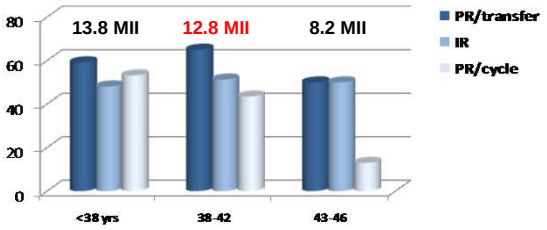
Mir et al., JARG 2015

aCGH: Day-3 vs. blastocyst biopsies

Day-3 biopsies:
N= 6,934 cycles
(36,281 embryos)



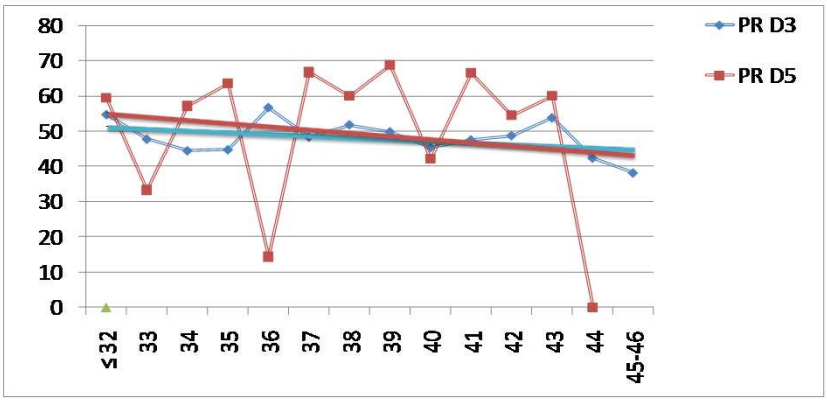
Day-5/6 biopsies:
N= 335 cycles
(1,750 embryos)



aCGH: Day-3 vs. blastocyst biopsies

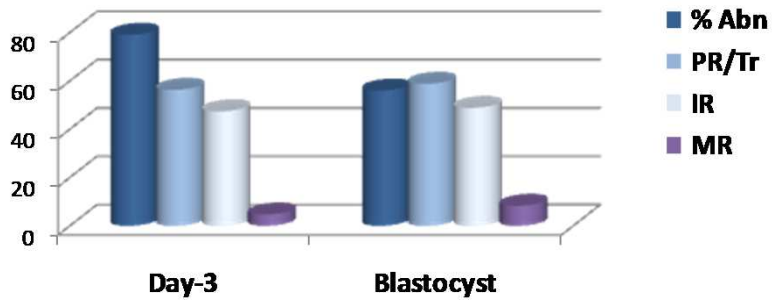
Cycles with <u>one</u> embryo transferred (2,328 cycles)

2,174 cycles with day-3 biopsies (31.3%)
154 cycles with day-5/6 biopsies (46%)



NGS: Day- 3 vs. Blastocyst

NGS: 284 day-3 vs. 60 blastocyst cycles

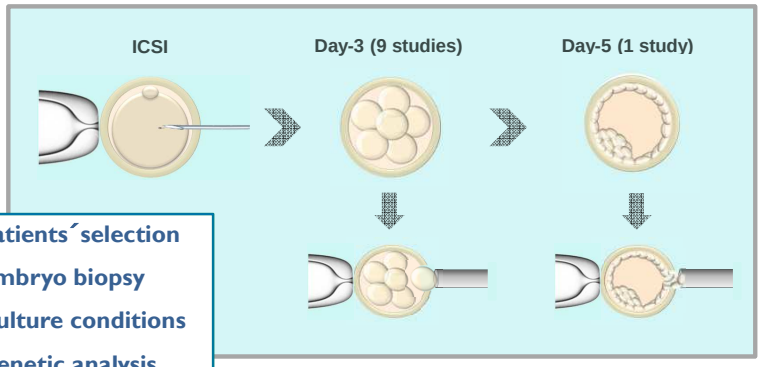


Similar results between day-3 and blastocyst biopsies by NGS aneuploidy screening

Published RCTs in PGS mostly day-3

Advanced Maternal Age (5 RCTs)
Recurrent implantation failure (1 RCT)
Young IVF couples w/o risk (4 RCTs)
} **FISH for 5-7 chromosomes**

No clinical improvement



- Patients´selection
- Embryo biopsy
- Culture conditions
- Genetic analysis

Published RCTs in PGS mostly day-3

IVI RCTs studies using 9-chromosomes FISH on day-3 biopsies

AMA (41-44 years; ≥5 MII)	Blastocyst	PGS	P-value
No. of cycles	90	93	----
Mean Age (SD)	41.7 (0.9)	41.8 (0.9)	NS
Ongoing implantation rate (%)	20/152 (13.1)	40/114 (35.1)*	p<0.0001
Live birth rate (%)	14/90 (15.5)	**30/93 (32.3)***	p=0.0099

RIF (≥ 3 IVF failures ; < 40 yrs)	Blastocyst	PGS	P-value
No. of cycles	43	48	----
Mean Age (SD)	35.3 (2.9)	35.2 (3.5)	NS
Ongoing implantation rate (%)	15/70 (21.4)	26/71 (36.6)*	P=0.0112
Live birth rates (%)	12/43 (27.9)	**23/48 (47.9)**	NS

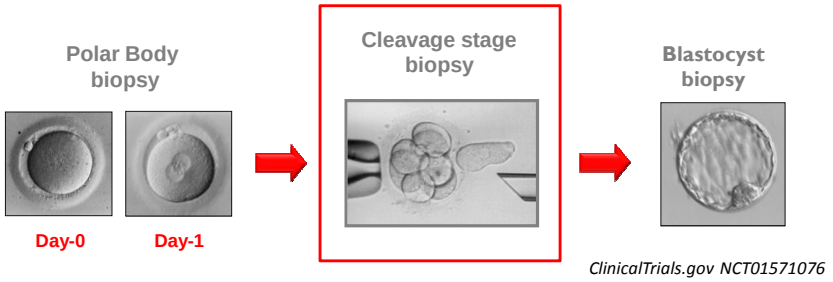
Two-side Fisher´s test

Rubio et al., FS 2013

aCGH: day-3 biopsy RCT

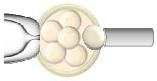
Study groups

- Advanced maternal age (38 - 41 years)
- Severe male factor (≤ 2 million/mL)



ClinicalTrials.gov NCT01571076

AMA - RCT with aCGH on day-3 biopsies

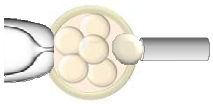
 VS. 

38-42 years	Blastocyst	PGS	P-value
No. of cycles performed	110	102	---
Mean age (SD)	39.4 (1.1)	39.5 (1.1)	NS
Percentage of transfers	**99.1**	**66.7**	***p<0.0001***
Mean embryos/transfer	1.8 (0.6)	1.3 (0.7)	*p<0.0001*
No. of pregnancies	48	38	---
No. of miscarriages (%)	**23 (47.9)**	**1 (2.6)**	***p<0.0001***
Delivery rate/transfer	22.9	54.4	*p<0.0001*
Delivery rate/cycle	**22.7**	**36.3**	***p=0.0349***
Ongoing IR	14.9	46.7	*p<0.0001*
Liveborns/cycle	**26.4**	**42.1**	***p=0.02***

Two-side Fishers´ test *ClinicalTrials.gov NCT01571076*

ASRM 2015

MF - RCT with aCGH on day-3 biopsies

 **VS.** 

	Blastocyst	PGS	P-value
No. of cycles performed	41	44	---
Mean age (SD)	32.6 (3.4)	33.0 (2.8)	NS
Percentage of transfers	97.6	88.6	NS
Mean embryos/transfer (SD)	**1.9 (0.6)**	**1.5 (0.7)**	*P=0.0079*
No. of pregnancies	23	29	---
No. of miscarriages (%)	**6 (26.1)**	**1 (3.4)**	*P=0.0237*
Ongoing PR/transfer*	42.5	71.8	*P=0.0078*
Ongoing PR/cycle*	**41.5**	**63.6**	*P=0.0334*
Ongoing IR	22.7	55.2	*P=0.0001*

**12 weeks ongoing pregnancies* ** *p<0.05 T-Student and one-side Fishers´ test*

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aCGH: blastocyst biopsy RCT

Single embryo transfer (SET)

Women < 35 years
First IVF attemp
No previous miscarriages
(Yang et al., 2012)

Table 1 Characteristics of patients whose embryos were randomized to assessment by morphology with aCGH (Group A) and blastocyst morphology only (Group B)

	Group A ($n = 55$)	Group B ($n = 48$)
Age (yrs)	31.2 ± 2.5	31.5 ± 2.7
Total oocytes retrieved	19.5 ± 8.2	19.3 ± 8.1
MII (mature) oocytes	16.6 ± 7.8	16.3 ± 7.6
Oocytes fertilized (2*pn*)	13.1 ± 6.7	12.8 ± 6.4
Day 3 embryos	12.9 ± 1.8	12.6 ± 1.9
Day 5 blastocysts	8.3 ± 2.1	8.1 ± 2.4

Table 3 Comparison of laboratory findings and clinical outcome among IVF patients undergoing SET with embryo assessment by aCGH + morphology (Group A) and blastocyst morphology alone (Group B)

	A		
Fresh blastocyst transfer *according to morphology assessment:*	5		
Grade 5/6	3		
Grade 4	21		7[a]
Grade 3	3 (5.4)		
Clinical pregnancy	39 (70.9)	22 (45.8)	0.017[a]
Ongoing pregnancy (≥20wks GA)	38 (69.1)	20 (41.7)	0.009[a]
Missed abortion	1 (2.6)	2 (9.1)	0.597[b]

Notes: All data reported as *n* (%). SET = single embryo transfer; aCGH = array comparative genomic hybridization; GA = gestational age [a] by Chi-squared test [b] by Fisher's exact test

IVI MF RCT
Ongoing PR
63.6 vs. 41.5

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