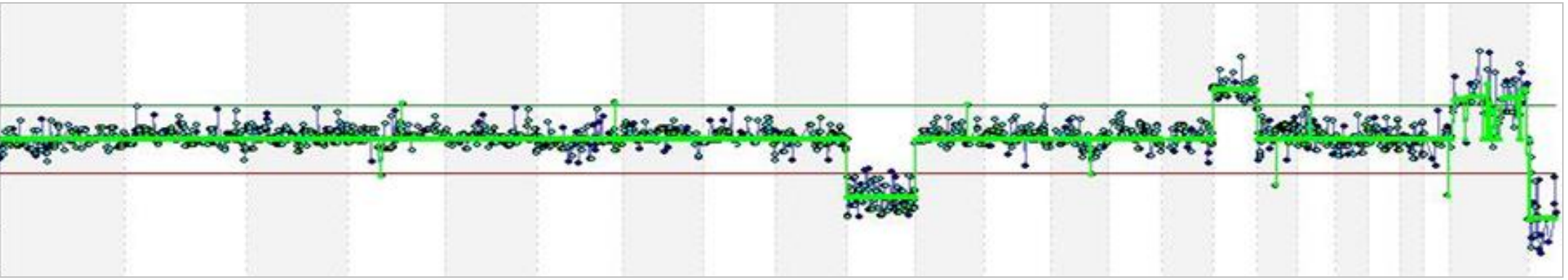
# Prevention of genetic disease before pregnancy

Reprogenetics

***Santiago Munné, PhD***



**Reprogenetics Laboratories**

**US:** Livingston (NJ), Los Angeles (CA), Chicago (IL), Portland (OR), Boca Raton (FL) / **Europe:** Barcelona (Spain), Oxford (UK), Hamburg (Germany) / **Asia:** Kobe (Japan), Macao, Abu Dhabi (UAE) / **Latin America**: Lima (Peru), Buenos Aires (Argentina), Sao Paulo (Brazil), DF (Mexico)

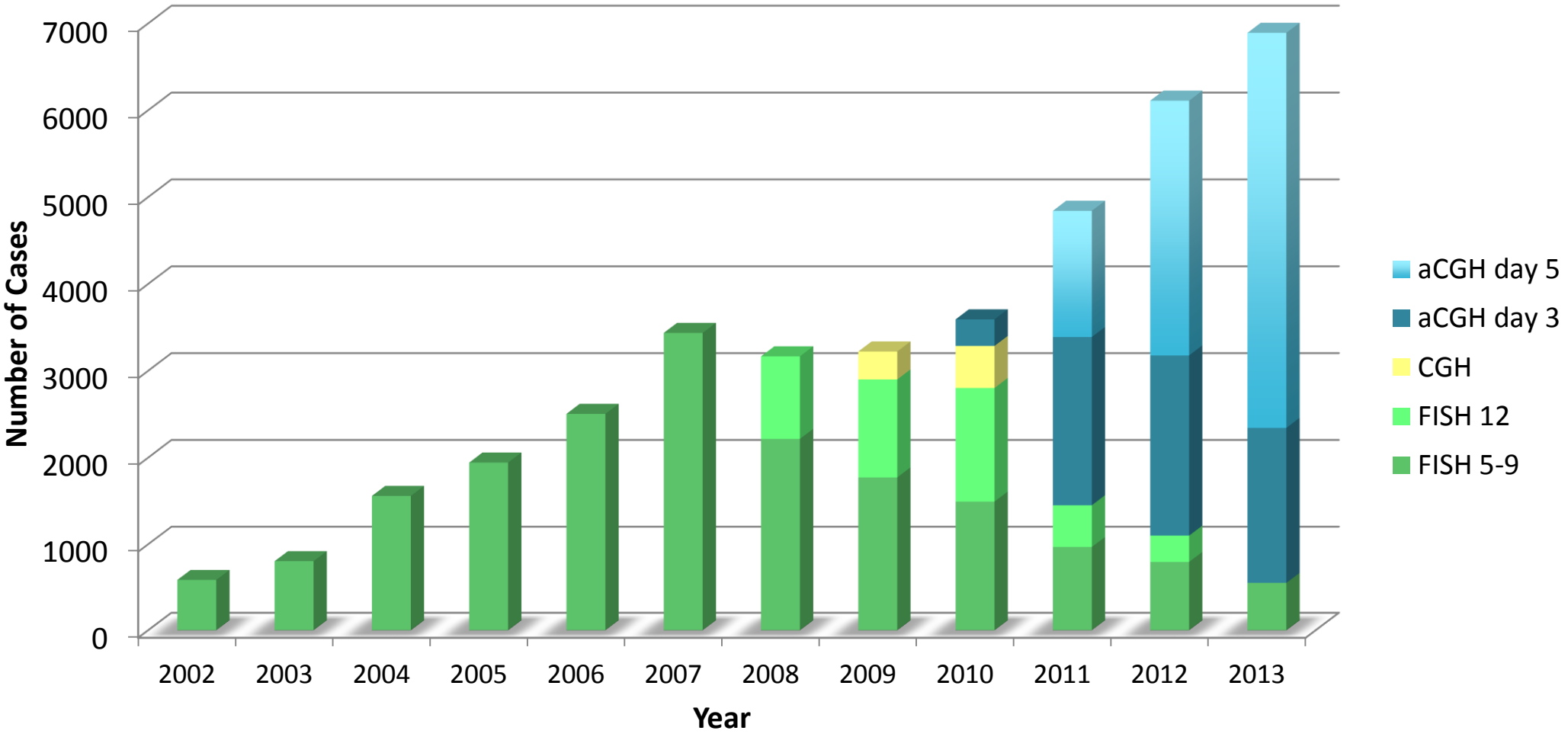
# Genetics and infertility

Reprogenetics

| Problem | frequency | Detection in couple | Solution |
|---|---|---|---|
| AMA | 50% of cycles | interview | PGS |
| RPL | 1% of fertile couples | interview | PGS |
| Translocations | 9% RPL, 2% MF | karyotype | PGD |
| Hereditary gene defects | 2% of couples | Carrier screen **(CarrierMap)** | PGD |
| Genetic susceptibility to infertility | Unk. | Carrier screen **(FertilityMap)** | Pharmaco-genetics |
| De novo gene defects | 1/100 autistic babies | N/A | PGD with whole gene sequencing |

# Evolution of PGS: Reprogenetics data

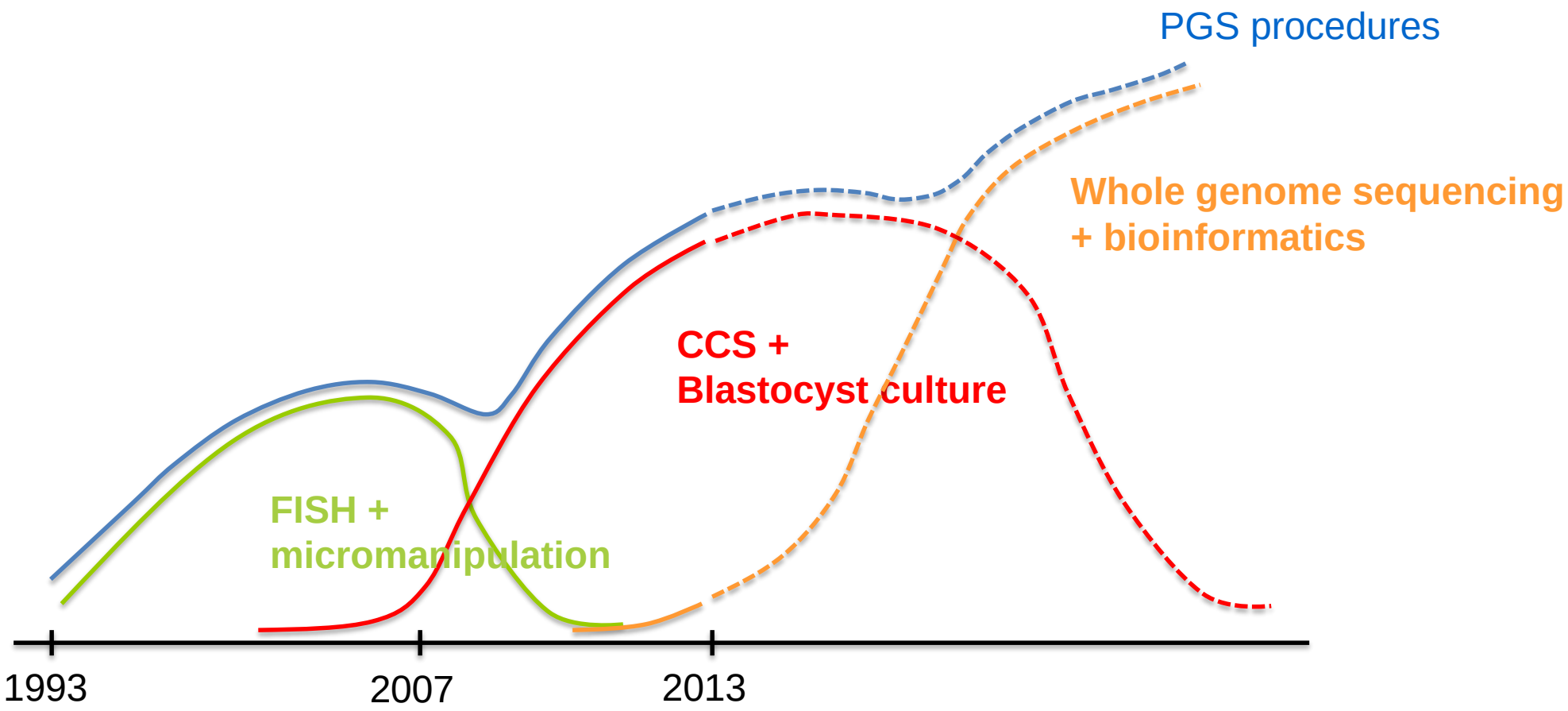
Reprogenetics




Reprogenetics Laboratories: 39,000 PGD procedures up to 12/2013

# Waves of technology

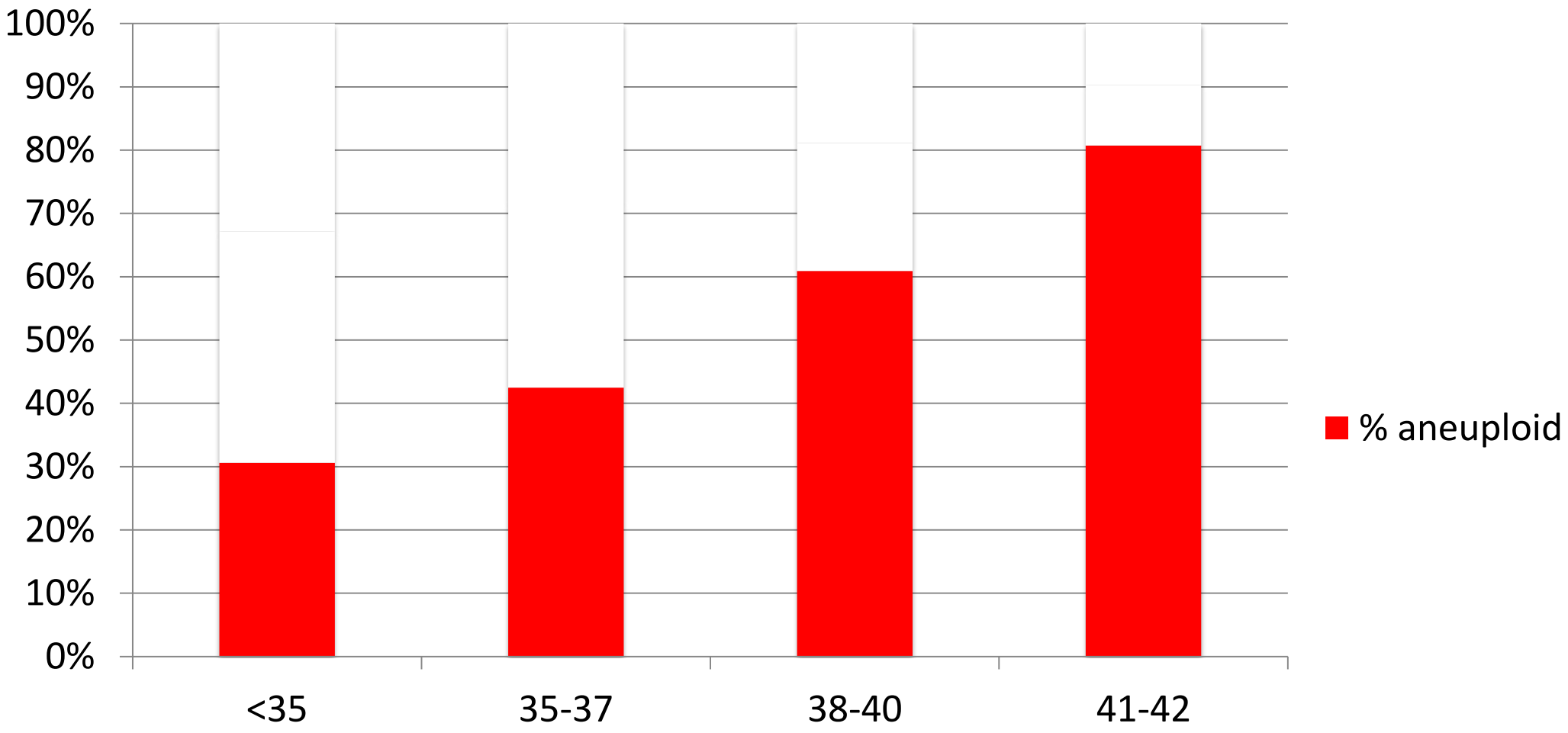
Reprogenetics



# Why PGS?

# Most loss of implantation is caused by chromosome abnormalities

Reprogenetics




*Reprogenetics data: 96 centers, >3500 cycles, >19,000 blastocysts analyzed by aCGH to 9/2013*

# The PGS hypothesis - proven

Reprogenetics

- **50%** of blastocysts are aneuploid,
- Aneuploidy increases with maternal age
- Maternal age is inversely proportional to implantation
- The **error rate** of PGS with CCS is low (**<2%**)
- And blastocyst biopsy is **non-detrimental**

Therefore PGS with CCS and blastocyst biopsy:

- Should double Implantation rates
- Should eliminate the Maternal age effect on implantation

**CCS**: comprehensive chromosome analysis, such as **array CGH**, qPCR, Karyomaping, NGS

QUESTION

Do you think that the PGS hypothesis has now been proved?

1. YES
2. NO

# PGD v.2
# (or CCS)

- 24 chromosome analysis by arrays
- Blastocyst biopsy

# Effect of day 3 biopsy: Mastenbroek et al. (2007)

Reprogenetics

- 20% of <u>cycles</u> undiagnosed and replaced (third arm)

| | implantation | |
|---|---|---|
| Control | 14.7% | 59% reduction |
| Biopsied, undiagnosed | **6.0%** | |
| Biopsied and PGD | 16.8% | |

- 59% implantation reduction due to biopsy
- PGD vs. Biopsied undiagnosed: 2.8x improvement

# Effect of day 3 biopsy and blastocyst biopsy

Reprogenetics

| | cleavage stage | | blastocyst | |
|---|---|---|---|---|
| | biopsy | not | biopsy | not |
| **Implantation rate** | **31%** | **53%** | **52%** | **54%** |
| | P<0.05 (42% reduction) | | N.S. | |

**…but biopsy is an operator-dependent procedure and its effect may vary**

*Scott et al. (2013) Fertil Steril, in press*

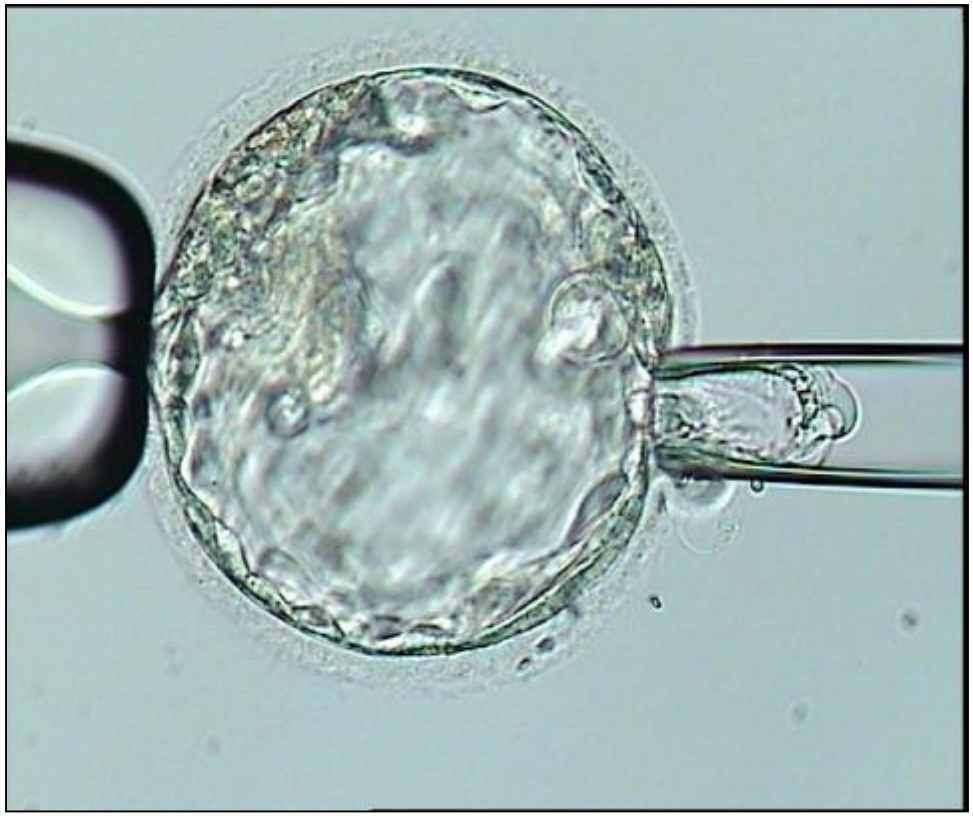
Patients randomized to cleavage of blastocyst biopsy. Two best embryos randomized 1 to biopsy and 1 to no biopsy, both replaced. Biopsied embryos fingerprinted and compared with the fetus.

# blastocyst biopsy: Advantages

Reprogenetics

**Advantages:**

- More DNA: less no results
- Less mosaicism = low error rate
- Reduced impact of embryo biopsy
- Less embryos to process
- Facilitates single embryo transfer
- Frozen cycle: Uterine environment optimized after thaw



**Disadvantages:**

- Not all embryos reach blastocyst the same day
- 4.5% monozygotic twins (Morin et al. 2013)

# No results:
# Day 3 vs. day 5

Reprogenetics

| Biopsy stage | Embryos undiagnosed | Centers* range |
|---|---|---|
| Cleavage | 3.2% | 1% - 5% |
| Blastocyst | 2.3% | **0%** - 18% |

Most experienced — Untrained

*Gutierrez-Mateo et al. (2011) Fertil Steril and Reprogenetics data on **9049** embryos*

** Centers with >20 cycles done for that biopsy stage*

# Is the trophectoderm representative of the ICM?

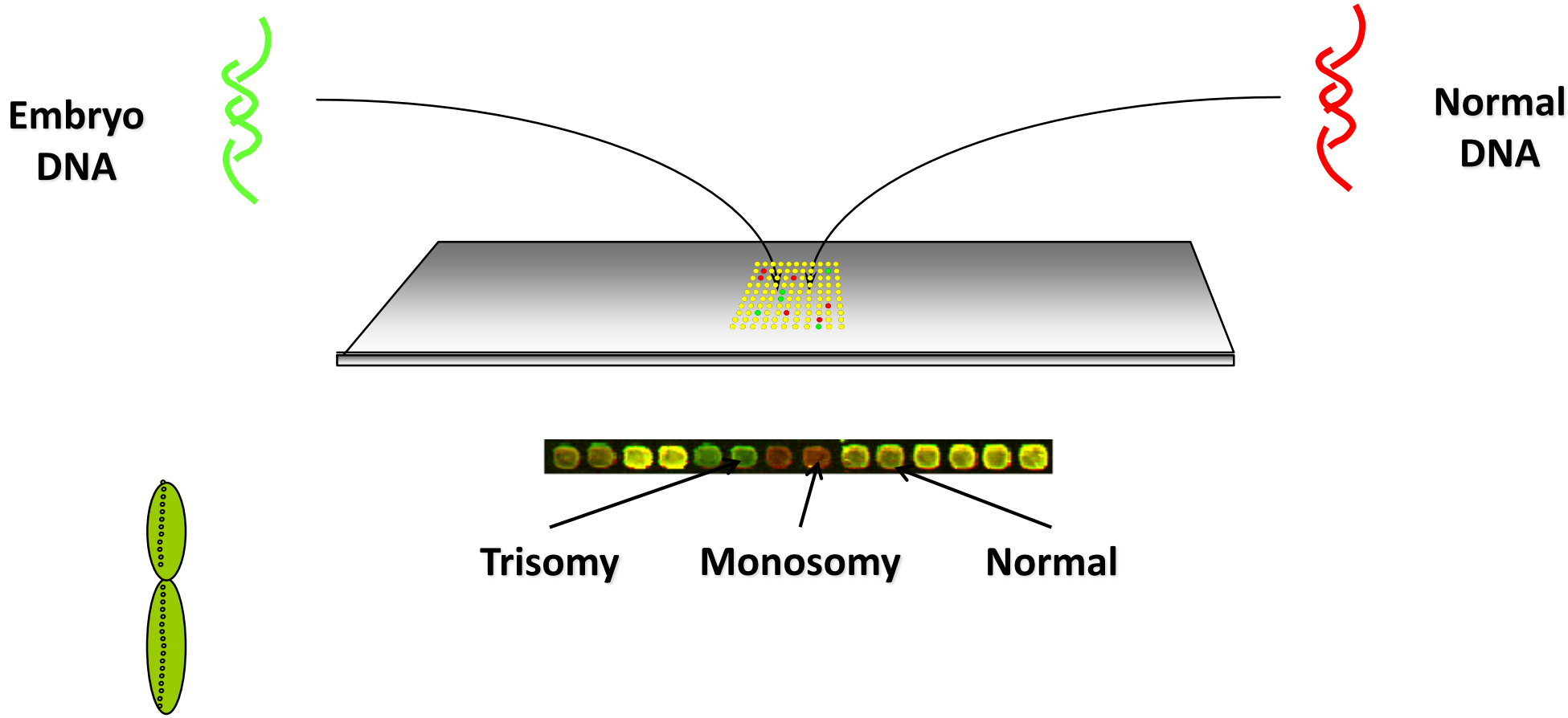
Reprogenetics

- ICM and TE were concordant in **97%** (31/32) embryos when analyzed by aCGH ([a]).
- Blastocysts analyzed by aCGH as abnormal were reanalyzed by FISH and were **97.5%** (39/40) abnormal ([b]).

*(a) Capalbo et al. (2013) in press, (b) Colls et al. (2013) ASRM*

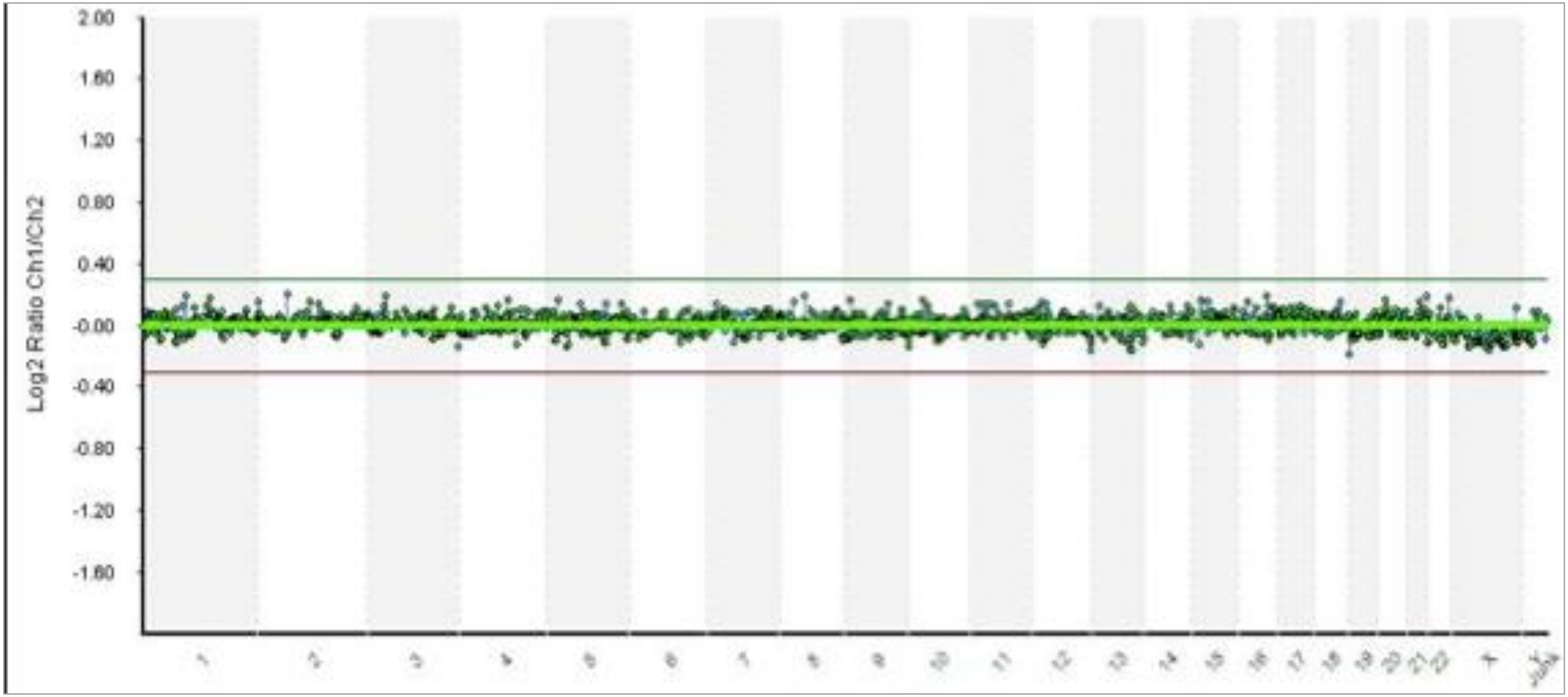
# Array Comparative Genome Hybridization

Reprogenetics



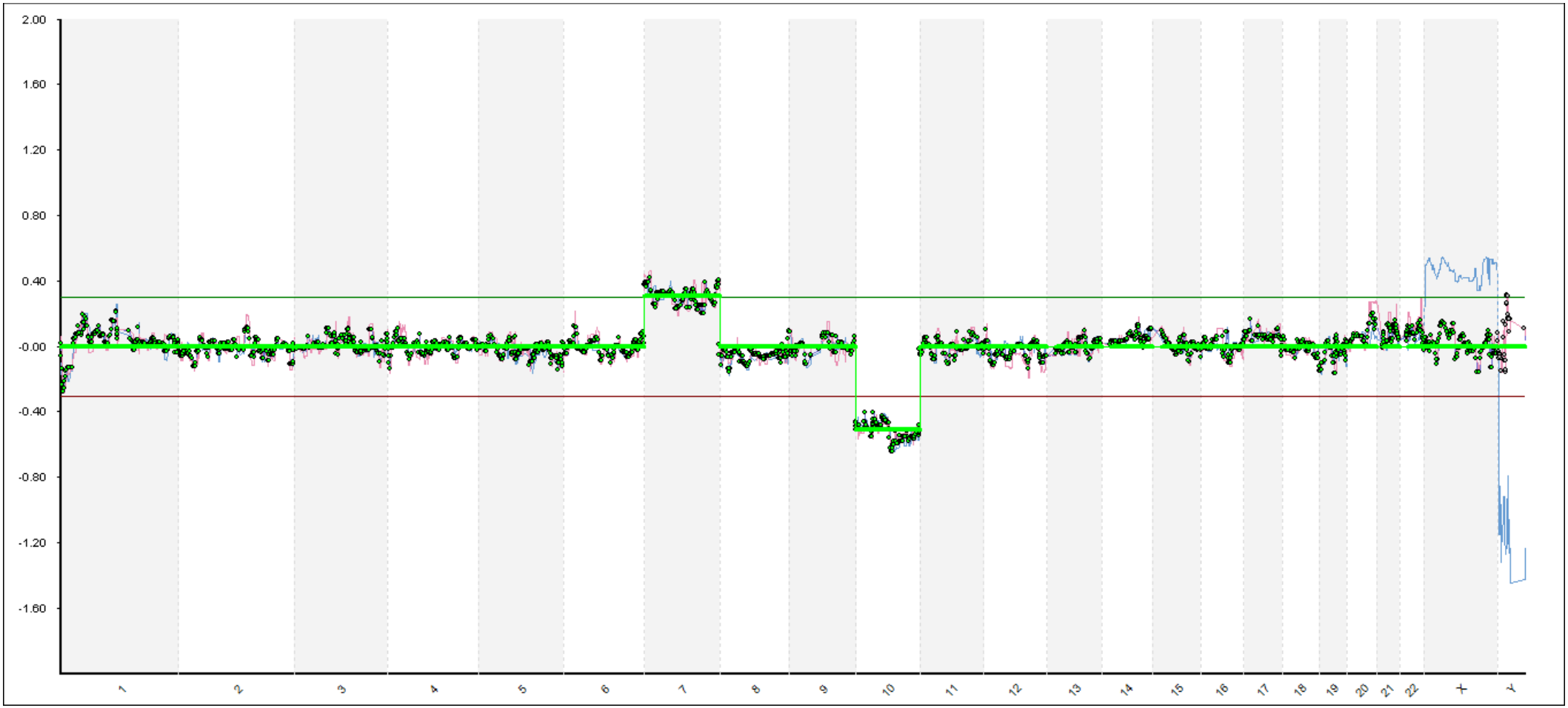
46,XY

Reprogenetics



# 46,XX+7-10

Reprogenetics



# aCGH advantages

Reprogenetics

- All **24 chromosome** aneuploidies and translocations detected.
- Results in <**16** hours:
  allows for day 5 biopsy and **10am day 6** transfer
- Parental DNA **not** required: ad hoc decisions possible.
- ICSI **not** required.

# aCGH validation: PBs, Day 3 embryos

Reprogenetics

| Approach | Errors | Reason |
|---|---|---|
| Cell lines: karyotype and aCGH same passage [a] | **2.0%** | unk |
| Day 3: FISH reanalysis of non-replaced embryos [b] | **1.8 - 3.0%** | mosaics |
| Day 3: aCGH reanalysis of non-replaced embryos [c] | **0.0 - 1.2%** | mosaics |
| PBs: aCGH comparison of PBs and eggs [d] | **6.0%** | unk |
| **Day 3: aCGH comparison to NGS** [e] | **0.0%** | |

*[a] BlueGnome unpublished data, [b] Gutierrez-Mateo et al (2011) Fertil Steril, 95:953 and Mir et al. (2011) ASRM, [c] Biricik et al. (2011) ASHG, Montreal, and Reprogenetics unpublished data, [d] Geraedts et al. (2011) Human Reprod, in press, [e] Wells et al. (2013) ASRM*

# aCGH validation: reanalysis of blastocysts

Reprogenetics

| | Reanalysis method | Confirmed Euploid | Confirmed abnormal | TOTAL |
|---|---|---|---|---|
| Fragouli et al 2011 | FISH, aCGH | 23/25 | 27/27 | 50/52 |
| Capalbo et al. 2013 | FISH | 19/20 | 50/50 | 69/70 |
| Colls et al. 2013 | FISH, aCGH | 7/7 | 39/40 | 46/47 |
| Wells et al. 2013 | **Next Gen. Sequencing** | 23/23 | 67/67 | 90/90 |
| **Total** | | **96% Sensitivity** | **99.5% Specificity** | **1.6% Error rate** |

Fragouli et al. (2011) Hum. Reprod. 26: 480-90, Colls et al. (2013) ASRM P-168, Capalbo et al. (2013) Hum Reprod, in press, Wells et al. (2013) ASRM O-435 and unpublished data from Reprogenetics

# Speed of different techniques

Reprogenetics

| | Biopsy | Reception | Results by |
|---|---|---|---|
| **qPCR:** | day 5 | day 5, **6pm** | day 6, **am** |
| **aCGH:** | day 5 | day 5, **6pm** | day 6, **noon** |
| **NGS:** | day 5 | day 5, **6pm** | day 6, **noon** |
| **SNPs:** | day 5 | day 5, **noon** | day 6, **6pm** |

# aCGH vs other techniques: Detection differences

Reprogenetics

| | aCGH | SNPs | qPCR | NGS | frequency |
|---|---|---|---|---|---|
| 69,XXX w/o aneuploidy | no | yes | yes | yes | 0.2% [a] |
| 69,XXX with aneuploidy | yes | yes | yes | yes | 7.8% [a] |
| UPD w/o other abnormalities | no | yes | no | yes | >0.01% [b] |
| Trisomy w/o recombination | yes | unk | yes | yes | **3%** |
| Duplications, deletions | yes | yes | no | yes | 5% |
| Translocations | all | some | no | yes | unk |
| Error rate (day 3-5 biopsy) | 2-3%[c] | 2-4%[d] | 1%[e] | 0%[f] | |

[a] *Bisignano, Wells, Harton and Munne (2011) RBO*

[b] *www.ncbi.nlm.nih.gov/omim,* [c] *Gutierrez-Mateo et al. (2011),* [d] *Scott et al. (2012),*

[e] *Treff et al. (2012) Fertil Steril 97:819–24.* [f] *Wells wt al. (2013) ASRM*

# Chromosome abnormalities detected with array CGH

# Euploidy decreases with age but not with cohort size

Reprogenetics

| # of blastocysts | % normal embryos | | | | | |
|---|---|---|---|---|---|---|
| | egg donors | <35 years | 35-37 years | 38-40 years | 41-42 years | >42 years |
| 1-3 | 58% | 61% | 51% | 39% | 22% | 13% |
| 4-6 | 62% | 60% | 52% | 38% | 23% | 17% |
| 7-10 | 65% | 62% | 51% | 36% | 21% | 14% |
| >10 | 68% | 63% | 55% | 37% | 25% | n/a |

*N = 4,747 cycles and 29,803 embryos, up to 12/2013.*

*Ata, Munne et al. (2012) Reprod Biomed Online and unpublished data.*

# Prognosis depending on age and ovarian response

Reprogenetics

| # of blastocysts | % of patients with normal embryos | | | | | |
|---|---|---|---|---|---|---|
| | egg donors | <35 years | 35-37 years | 38-40 years | 41-42 years | >42 years |
| 1-3 | 86% | 85% | 72% | 60% | 58% | 24% |
| 4-6 | 95% | 97% | 95% | 88% | 69% | 54% |
| 7-10 | 100% | 99% | 96% | 92% | 85% | 65% |
| >10 | 100% | 100% | 98% | 98% | 92% | 83% |

*N = 3,571 cycles and 19,356 embryos, up to 8/2013.*

*Ata, Munne et al. (2012) Reprod Biomed Online and unpublished data.*

# Overall clinical results

# 1st randomized clinical trial: CGH and frozen transfer

Reprogenetics

| | Cycles cycles | Mat. age | Prev. failed | embryos replaced | implant. (+ sac) |
|---|---|---|---|---|---|
| CGH : | 45 | 37.7 | 2.4 | 2.0 | **72%** |
| control : | 113 | 37.1 | 1.2 | 2.7 | **46%** |

p=0.0003

*Schoolcraft et al. (2010) Fertil. Steril. 94:1700*

# 2nd Randomized Clinical Trial: aCGH + fresh transfer, <35 years old

Reprogenetics

| | Control | PGS | |
|---|---|---|---|
| **patients** | 48 | 55 | |
| **age** | <35 | <35 | |
| **replacement** | Day 6 | Day 6 | |
| **replaced** | 48 (1) | 55 (1) | |
| **Pregnancy rate** | 45.8% | 70.9% | **P<0.05** |
| **Ongoing preg rate** | 41.7% | 69.1% | **P<0.05** |
| **multiples** | 0 | 0 | |

*Yang et al. (2012) Molec Reprod*

# 3rd randomized clinical trial: qPCR + fresh transfer

Reprogenetics

**Good prognosis patients (average 8 blastocysts)**
Control replaced on day 5, test biopsied on day 5 and replaced on day 6

| | PGD | Control | |
|---|---|---|---|
| **age** | 32.2 | 32.2 | |
| **N** | 72 | 83 | |
| **blastocysts** | 8 | 7.9 | |
| **Emb replaced** | 1.9 | 2.0 | |
| **implantation** | 79.8% | 63.2% | **P=0.002** |
| **Sustained implant** | 66.4% | 47.9% | **P=0.03** |
| **Delivery rate** | 84.7% | 67.5% | **P=0.01** |

*Scott et al., 2013 Fertil Steril.*

# 4th Randomized Clinical Trial: 1 tested vs. 2 untested

Reprogenetics

| | ongoing pregnancy rate | | |
|---|---|---|---|
| | 1 euploid blastocyst | 2 untested blastocyst | |
| **Fresh transfer** | 65% | 70% | **NS** |
| **Frozen transfer** | 55% | 52% | **NS** |

*Forman et al. (2013) Fertil Steril*
*Mean maternal age 35 (patients <43)*

# Metanalysis

Reprogenetics

## IMPLANTATION RATES IN RCT STUDIES USING PGS v2:

| | Control | PGS | |
|---|---|---|---|
| Yang et al. 2012 | 46% | 69% | |
| Scott et al. 2013 | 63% | 80% | |
| Forman et al. 2013 | 40% | 58% | |
| **TOTAL** | **53%** | **73%** | **P<0.001** |

# Array CGH with blastocyst biopsy: Unselected compiled results

Reprogenetics

| | total | range / center |
|---|---|---|
| Centers doing d5 biopsy: | 96 | |
| Cycles included: | 3571 | 11 - 522 |
| Maternal age: | 35.4 | 34.7 - 38.6 |
| Av. blasts biopsied: | 5.6 | 4.5 - 8.4 |
| Av. Embryos replaced | 1.1 | 0.8 - 1.4 |
| Implantation rate | 51% | 35 - 79% |
| Pregnancies / cycle | 49% | 28 - 72% |
| Pregnancies / transfer | 71% | 49 - 90% |
| Ong preg / cycle | 45% | 26 - 65% |
| Ong preg / transfer | 64% | 43 - 86% |

*Reprogenetics data to 8/2013*

# Is it worthy to biopsy day 6 blastocysts?

Reprogenetics

The differences between day 5 biopsy and fresh transfer vs. day 5-6 biopsy and vitrification is that the later includes day 6 biopsies:

- Day-5 morulas were cultured to day-6 and biopsied if reached blastocyst

- SET of blastocysts either biopsied on day 5 or on day 6, thawed transfer

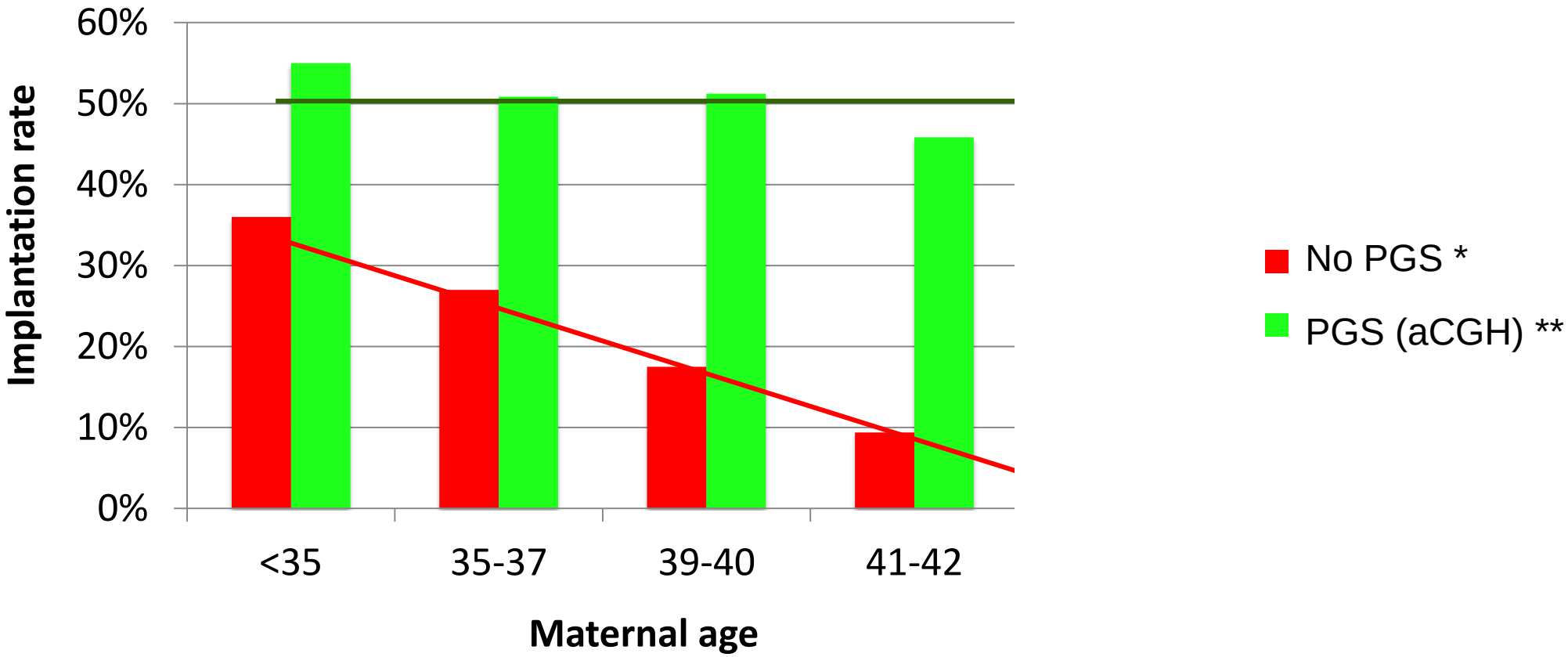
| | Day 5 biopsy | Day 6 biopsy | |
|---|---|---|---|
| Implantation | 61% | 60% | N.S. |
| Euploidy | 56% | 42% | P<0.025 |

*Reprogenetics, unpublished*

# maternal age effect disappears with full chromosomes analysis

# aCGH eliminates the negative effect of maternal age on implantation

Reprogenetics



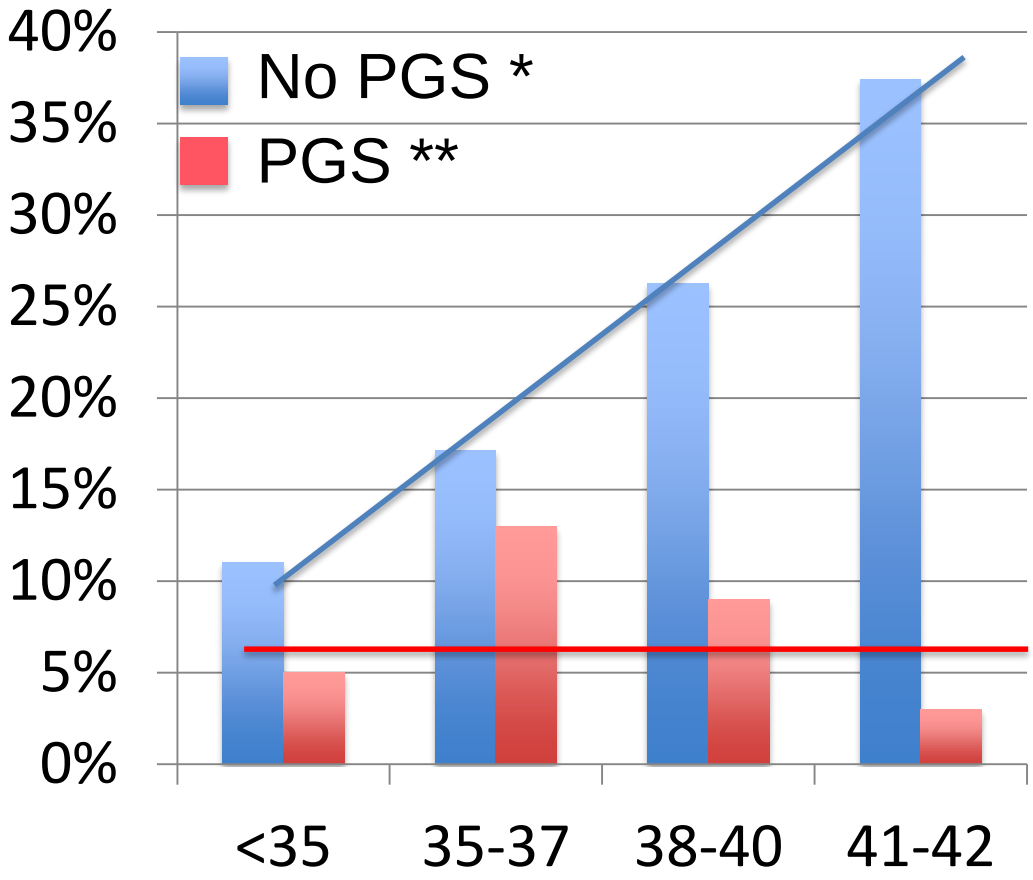

** SART 2011*

*** Harton, Munné et al. (2013) Fertil Steril. And unpublished data to 8/2013. N >800 blast biopsies*

# Miscarriage rate after blastocyst biopsy

Reprogenetics

## Compared to SART:



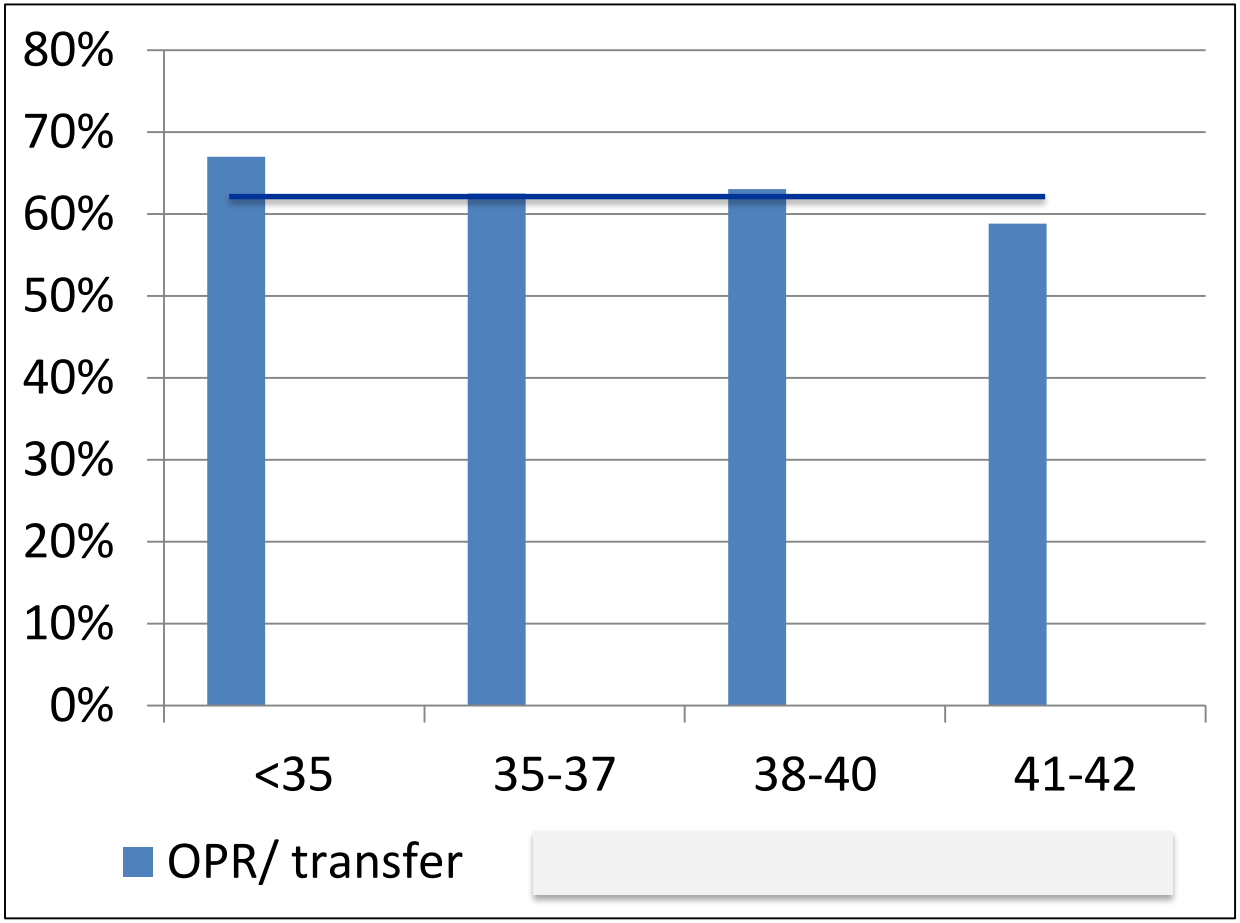

## Compared to other studies:

| | Pregnancies | age | SAB |
|---|---|---|---|
| This study | 307 | 34.9 | 7.5% |
| Scott et al. 2013 | 72 | 32.2 | 8.3% |

*SART, ** Harton et al. (2013) Fertil Steril, and unpublished data

# Ongoing pregnancy rate does not change with maternal age but …

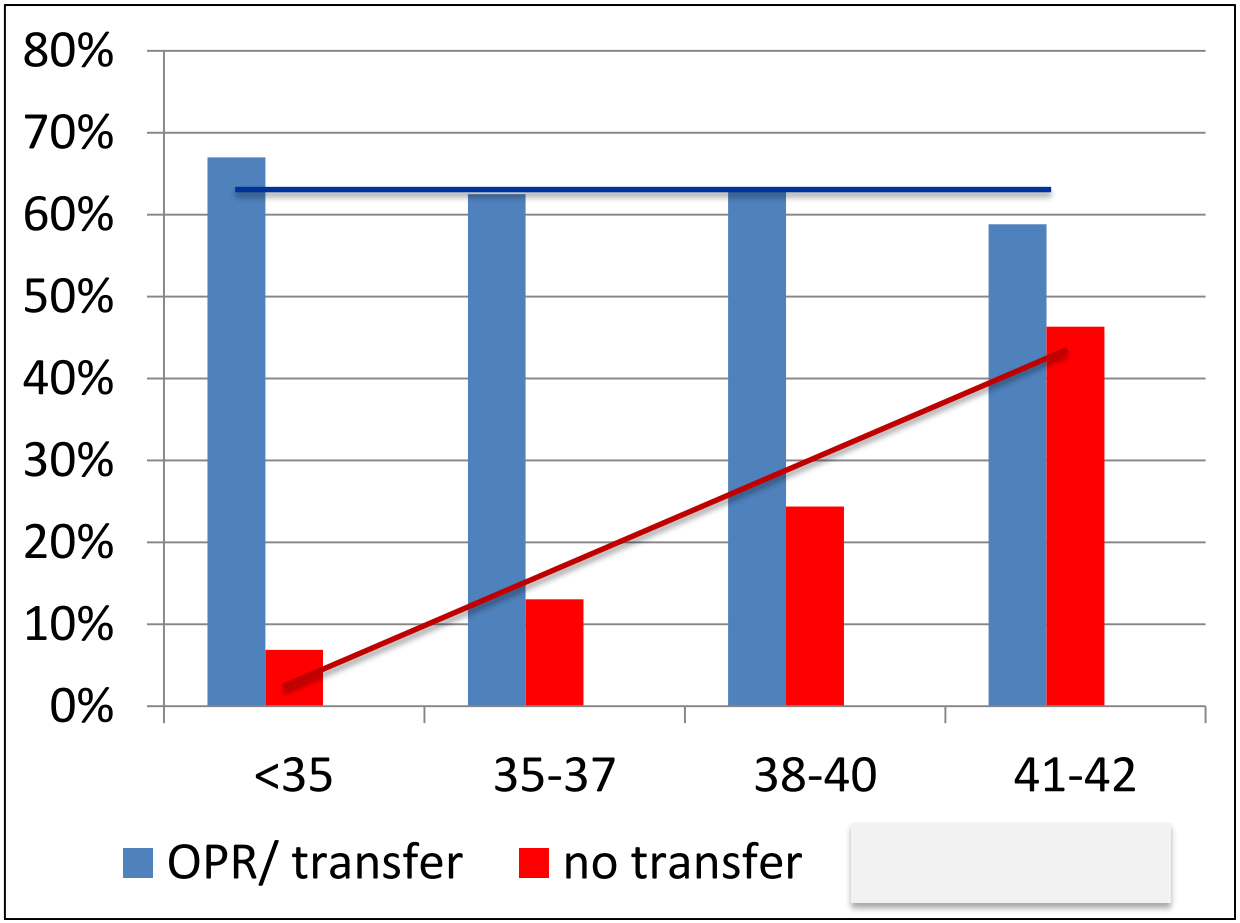
Reprogenetics




*Harton, Grifo, Munne, Wells et al. (2013) Fertil Steril, and unpublished data.*
*N >800 cycles of blast biopsy with follow up, up to 8/2013.*

# … Cycles with no euploid embryos do increase with maternal age …

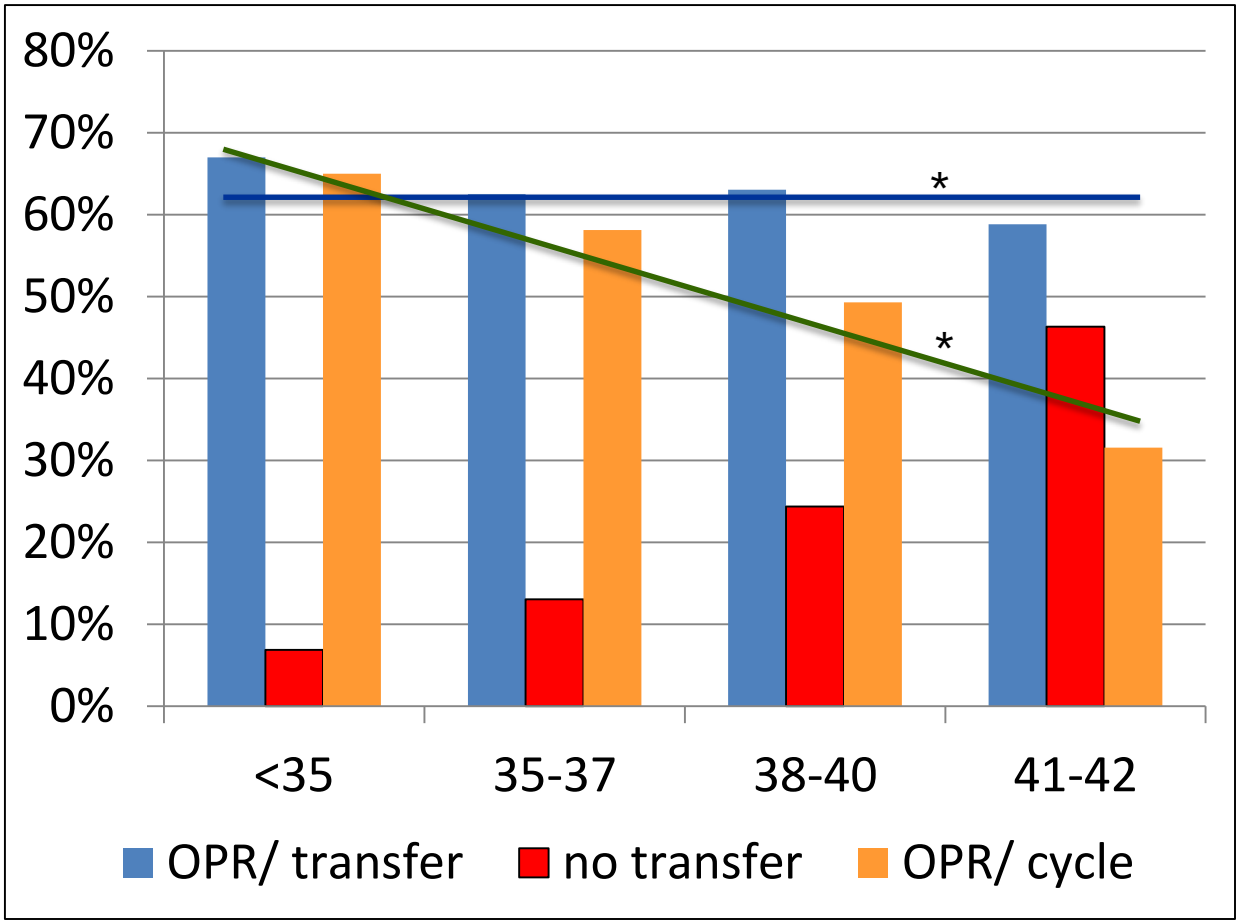
Reprogenetics




*Harton, Grifo, Munne, Wells et al. (2013) Fertil Steril, and unpublished data.*
*N >800 cycles of blast biopsy with follow up, up to 8/2013.*

# … resulting in a decrease in pregnancy rate per cycle

Reprogenetics




*Harton, Grifo, Munne, Wells et al. (2013) Fertil Steril, and unpublished data.*
*N >800 cycles of blast biopsy with follow up, up to 8/2013. *p<0.001*

# Maternal age effect and aCGH: conclusions

Reprogenetics

- Euploid embryos implant at the same high rate irrespective of maternal age
- However with maternal age there are more cycles without euploid embryos
- Therefore pregnancy rates / transfer are independent of maternal age but pregnancy rates per cycle still decrease with age

QUESTION

Do you think that the PGS hypothesis has now been proved?

1. YES
2. NO

# To replace 1 or 2 euploid blastocysts?

# Cohort size as a predictor of SET success

Reprogenetics

- Grade of transferred embryo has been correlated to embryo cohort size
- The presence of supernumerary embryos is a possible indirect marker for embryo quality
- ASRM acknowledges surplus embryos as being indicative of "good prognosis"

DEVREKER, *et al.* 1999. Selection of good embryos for transfer depends on embryo cohort size: implications for the 'mild ovarian stimulation debate'. *Hum Reprod,* 14, 3002-08.
STEINBERG, *et al.* 2013. Elective single embryo transfer trends and predictors of a good perinatal outcome – United States, 1999 to 2010. *Fertil Steril*; 99, 1937-43.
Practice Committee of Society for Assisted Reproductive Technologies. 2013. Criteria for number of embryos to transfer: a committee opinion. *Fertil Steril,* 99, 44-46.

# 1 vs. 2 euploid blastocysts replaced: Effect on pregnancy and multiple rates

Reprogenetics

| | ongoing pregnancy rate | | |
|---|---|---|---|
| | 1 euploid blastocyst | 2 untested blastocyst | |
| Fresh transfer | 65% | 70% | NS |
| Frozen transfer | 55% | 52% | NS |

**Average of 3.2 euploid balstocysts**

*Forman et al. (2013) Fertil Steril*

# Success of SET by Euploid Cohort Size

Reprogenetics

| No. Euploid Embryos | CPR |
|---|---|
| 1 | 23/55 (41.8%) |
| 2 | 13/27 (48.1%) |
| 3 | 9/19 (47.4%) |
| 4 | 16/21 (76.2%) |
| 5 | 8/11 (72.7%) |
| 6 | 6/8 (75.0%) |
| >7 | 11/15 (78.6%) |

$p < 0.01$

S. Morin, K. Melzer, J. Grifo, P. Colls, Z. Zheng, S. Munné (2014) JARG

# 1 vs. 2 euploid blastocysts replaced: Effect on pregnancy and multiple rates

Reprogenetics

| # euploid | # replaced | preg / transfer | | multiples | |
|---|---|---|---|---|---|
| 1-3 | 1 | **42%** (47/111) | P<0.01 | **0%** (0/111) | p<0.001 |
| 1-3 | 2 | **65%** (37/57) | | **38%** (14/37) | |
| 4 or more | 1 | **75%** (41/55) | N.S. | **10%** ( 4/41) | p<0.001 |
| 4 or more | 2 | **78%** (58/74) | | **52%** (30/58) | |

S. Morin, K. Melzer, J. Grifo, P. Colls, Z. Zheng, S. Munné (2014) JARG

# EMBRYO BANKING

# Embryo banking for low responders or bad prognosis patients

Reprogenetics

| # of blastocysts | % of patients with normal embryos | | | | | |
|---|---|---|---|---|---|---|
| | egg donors | <35 years | 35-37 years | 38-40 years | 41-42 years | >42 years |
| 1-3 | 86% | 85% | 72% | 60% | 58% | 24% |
| 4-6 | 95% | 97% | 95% | 88% | 69% | 54% |
| 7-10 | 100% | 99% | 96% | 92% | 85% | 65% |
| >7-10 | 100% | 100% | 98% | 98% | 92% | 83% |

*N = 3,571 cycles and 19,356 embryos, up to 8/2013.*

*Ata, Munne et al. (2012) Reprod Biomed Online and unpublished data.*

# Embryo banking aneuploidy rates Remain constant

Reprogenetics

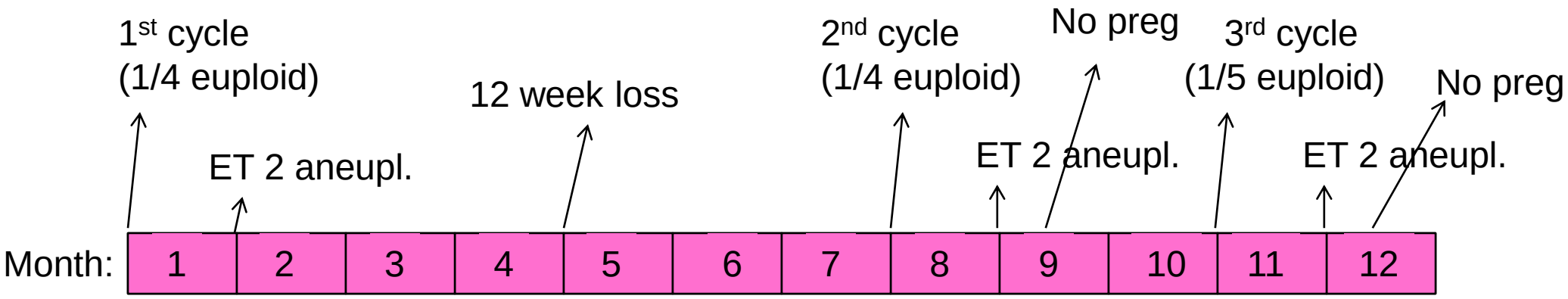
| | 1st cycle | 2nd cycle | 3rd cycle | Total |
|---|---|---|---|---|
| Euploidy rate | 29% | 29% | 27% | 28% |
| # euploid blastocysts | 0.7 | 0.9 | 0.7 | **2.2** |

*Reprogenetics data, unpublished*
*>300 cycles of embryo banking, average age 39.9*

# Example: 41 years old

Reprogenetics

**Without PGD:**

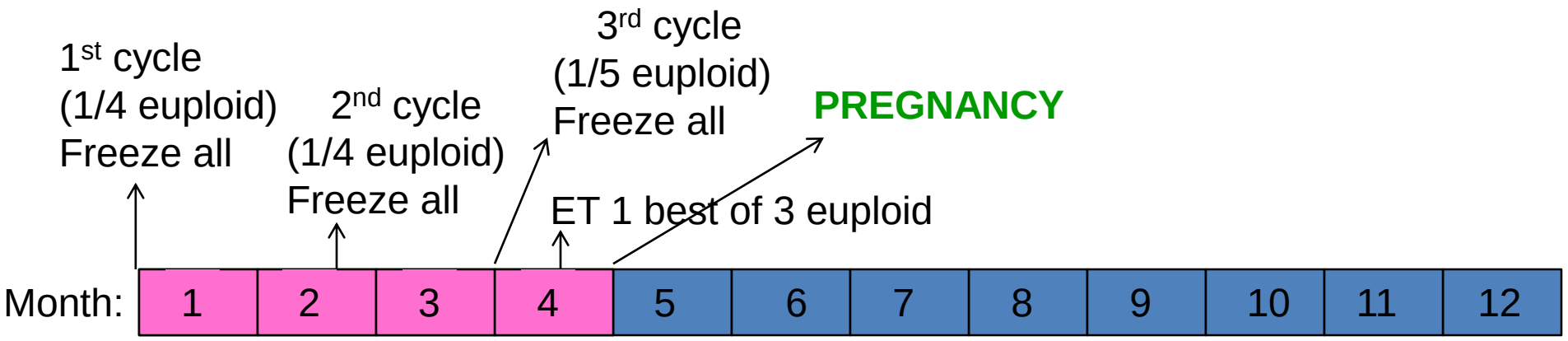



- **Risk of patient drop off**
- **Longer time to pregnancy**
- **Risk of miscarriage**

# Example: 41 years old

Reprogenetics

**Embryo banking, one PGD at the end:**




- **Less time to pregnancy**
- **No risk of patient dropping off**
- **Less cost of PGD**
- **More cost of freezing**

# Advantages

Reprogenetics

- Less patient “fatigue”: less drop out from cycle to cycle.
- Cheaper PGD: One fee per package of IVF cycles
- Facilitates “guaranteed baby” plans

# PGD FOR RECURRENT PREGNANCY LOSS (RPL)

# Background of RPL

Reprogenetics

- Defined as 3 or more lost pregnancies
- Occurs in 1% of fertile population
- Attributed to anatomic, endocrine, immunological or genetic problems but …
- …>50% of RPL cases are UNEXPLAINED

# All controlled PGD studies on idiopathic RPL show a decrease in miscarriages

Reprogenetics

**Idiopathic RPL :**


Werlin L, et al. (2003) Preimplantation genetic diagnosis (PGD) as both a therapeutic and diagnostic tool in assisted reproductive technology. Fertil Steril, 80:467

Munné et al. (2005) Preimplantation genetic diagnosis reduces pregnancy loss in women 35 and older with a history of recurrent miscarriages. Fertil Steril 84:331

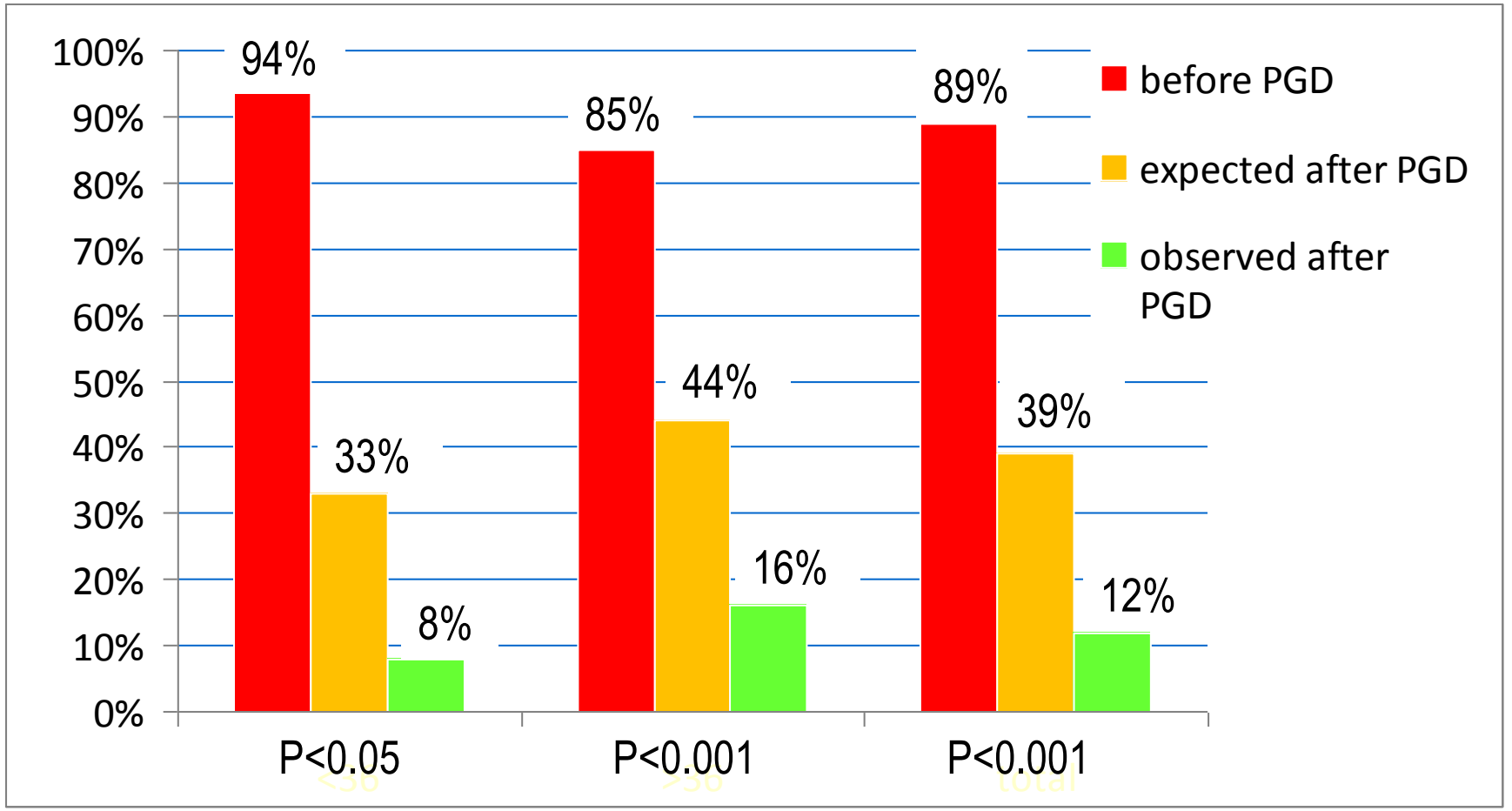
Garrisi et al. (2009) Effect of infertility, maternal age, and number of previous miscarriages on the outcome of preimplantation genetic diagnosis for idiopathic recurrent pregnancy loss. Fertil. Steril 92: 288

Rubio et al. (2009) Prognosis factors for Preimplantation Genetic Screening in repeated pregnancy loss. Reprod Biomed Online

Hodes-Wertz et al. (2012) Idiopathic recurrent miscarriage is caused mostly by aneuploid embryos. Fertil Steril. 98(3):675-80

# Reduction in miscarriages in RPL patients after PGD-FISH

Reprogenetics




*Munné et al. 2005*

*N=122 procedures of PGD of couples with >2 previous loses*

# Reduction in miscarriages in RPL patients after PGD-FISH

Reprogenetics

PGD results according to fertility:

| method conception | cycles | % loss expected | % loss after PGD | p | % to term |
|---|---|---|---|---|---|
| **IVF** | 115 | 35% | **14%** | **p<0.01** | 34% |
| **natural** | 124 | 41% | **15%** | **p<0.005** | 37% |

*Average maternal age: 37.5*
*Garrisi et al. (2009)*

# Results of PGD by aCGH for RPL: age effect

Reprogenetics

| maternal age | preg. cycles | % loss expected | % loss after PGD | |
|---|---|---|---|---|
| <35 | 27 | 26.3% | 3.7% | p<0.001 |
| ≥35 | 59 | 36.7% | 8.5% | p<0.001 |
| *Total* | *89* | *33.5%* | *7.0%* | *p<0.001* |

*Grifo et al. (ASRM 2011), and Grifo et al. (submitted)*

# Results of PGD by aCGH for RPL: biopsy stage effect

Reprogenetics

| day biopsy | preg. cycles | % loss expected | % loss after PGD | |
|---|---|---|---|---|
| Day 3 | 59 | 36% | 9% | p<0.001 |
| Day 5 | 40 | 42% | 3% | p<0.001 |
| *Total* | *99* | *38%* | *6%* | *p<0.001* |

Hodes-Wertz et al. (2012) Fert Ster

# Results of PGD by aCGH for RPL: 2 vs 3 or more loses

Reprogenetics

| Prev. loses | day biopsy | preg. cycles | % loss expected | % loss after PGD | |
|---|---|---|---|---|---|
| 2 | Day 5 | 34 | 32% | 9% | p<0.05 |
| >2 | Day 5 | 40 | 42% | 3% | p<0.001 |
| *Total* | | *74* | *37%* | *5%* | *p<0.001* |

Hodes-Wertz et al. (2012) Fert Ster

# Multiple pregnancies with euploid and aneuploid fetuses

Reprogenetics

Real case: 35 years old, triplet pregnancy miscarriage

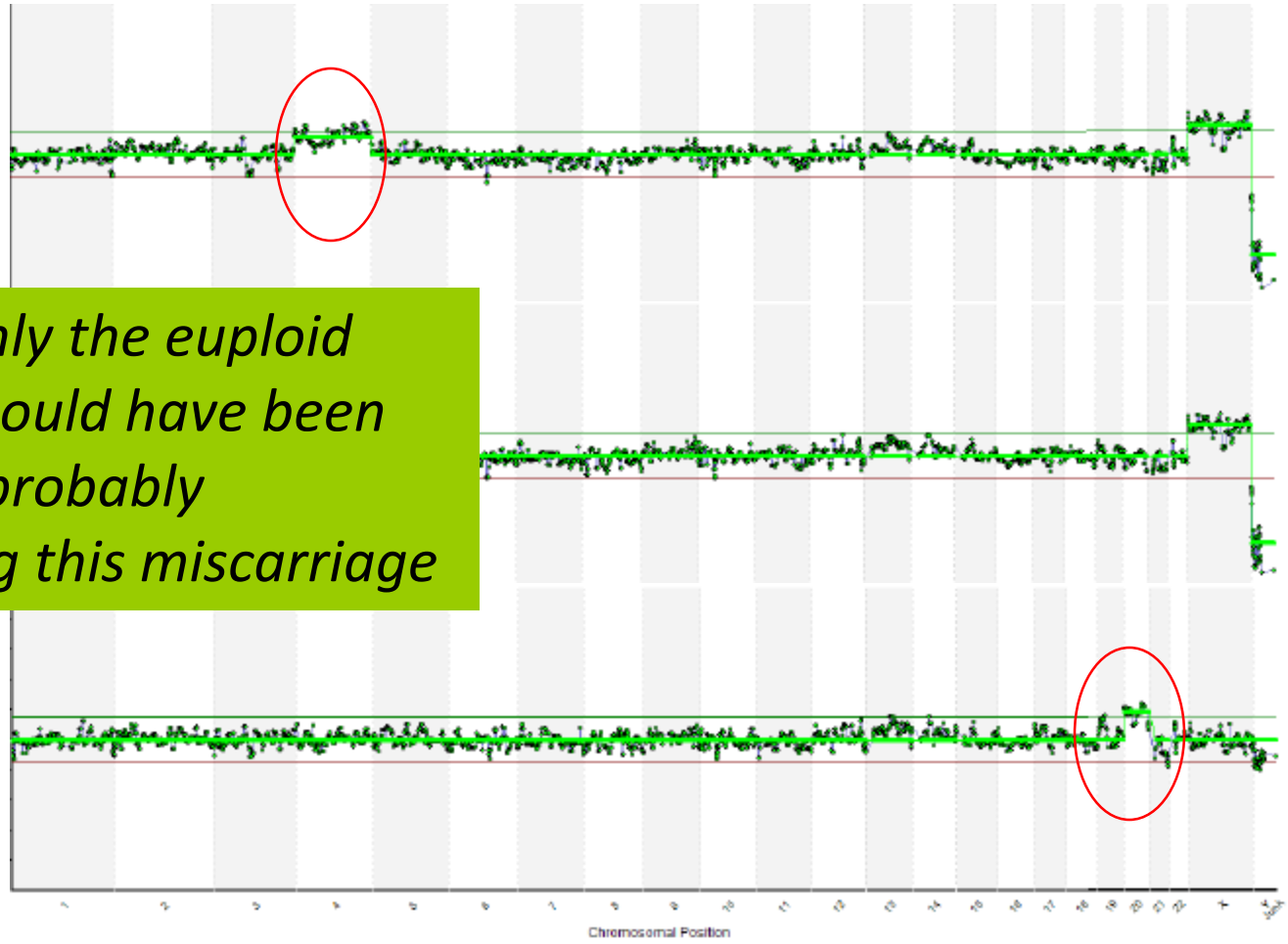
POC analysis:

47,XX + 4

46,XX

47,XY + 20

*By PGD only the euploid embryo would have been replaced probably preventing this miscarriage*



# PGD for translocations and 24 chromosome abnormalities

# All PGD studies on RPL for translocations show a decrease in miscarriages

Reprogenetics

**RPL due to translocations:**

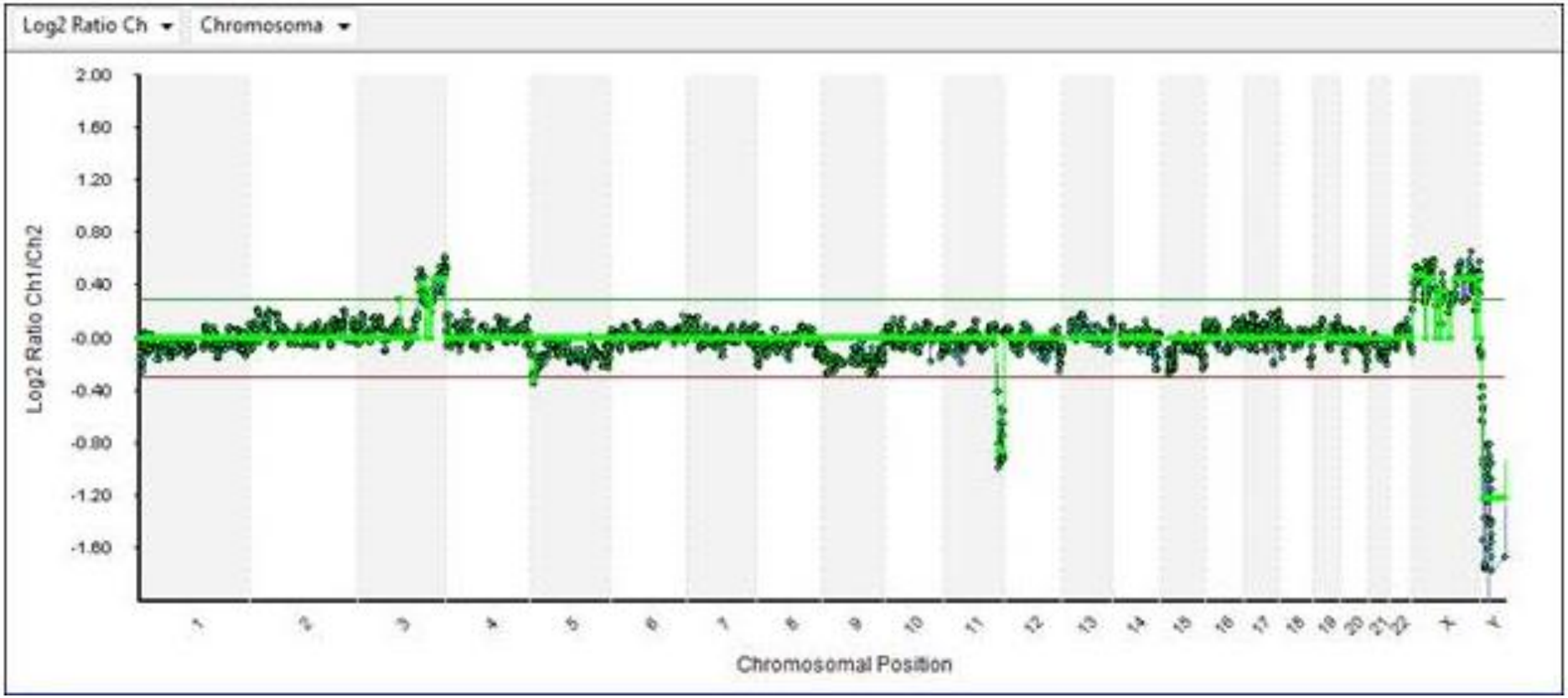
- Munné et al (1998). Spontaneous abortions are reduced after pre-conception diagnosis of translocations. J Assisted Reprod Genet 290:
- Munné S et al. (2000) Outcome of Preimplantation Genetic Diagnosis of translocations. Fertil Steril. 73:1209
- Verlinsky et al. (2005) Preimplantation testing for chromosomal disorders improves reproductive outcome of poor prognosis patients. Reprod Biomed Online 11:219

  Munné S (2006) Preimplantation genetic diagnosis for translocations. Hum Reprod 21: 839
- Otani et al.(2006) Preimplantation genetic diagnosis significantly improves the pregnancy outcome of translocation carriers with a history of recurrent miscarriage and failing to produce a live birth. Reprod Biomed Online 13: 879
- Fischer J, Colls P, Escudero T, Munné S (2010) Preimplantation Genetic Diagnosis (PGD) improves pregnancy outcome for translocation carriers with a history of recurrent losses. Fertil Steril, In press

# aCGH for translocations and 24 chromosome aneuploidy

Reprogenetics

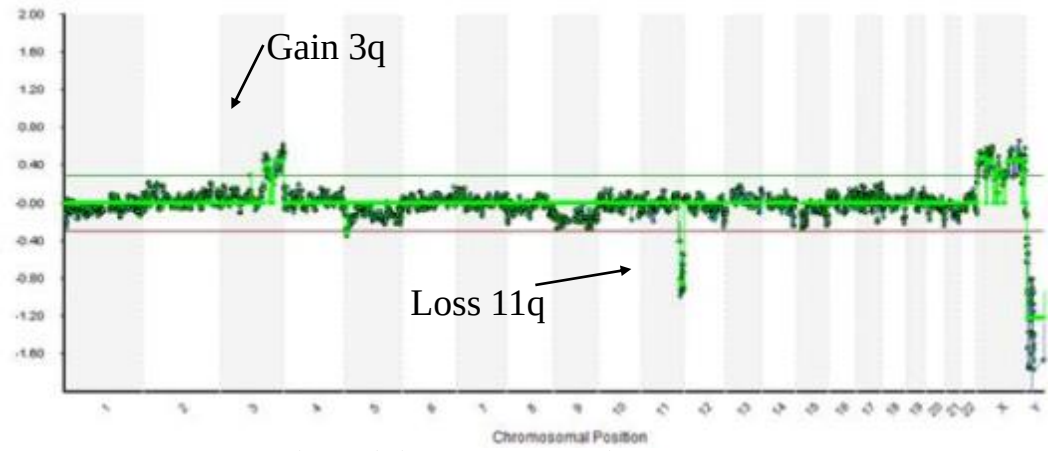
Patient: 46,XX,t(3;11)(q22.2;q23.3)



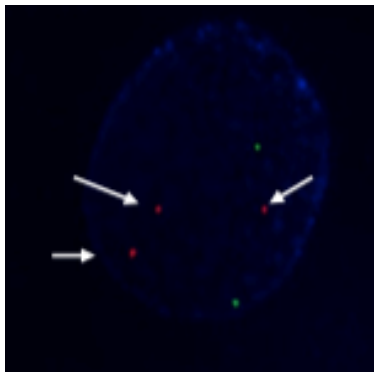
# Validation of aCGH for Translocations + Aneuploidy

Reprogenetics

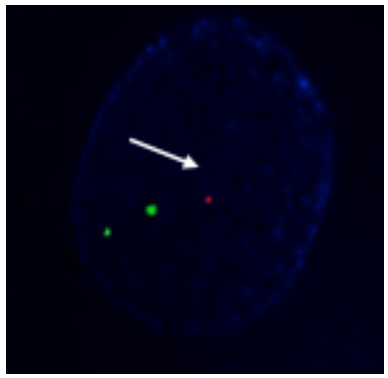
- 0%-2% error rate with aCGH [a,b]
- All 931 translocations previously studied at Reprogenetics by FISH can be identified by aCGH [a]




Patient: 46,XY,t(3;11)(q22.2;q23.3)



Tel 3p (green)
Tel 3q (orange)



Cent 11 (green)
Tel 11q (orange)

*a: Colls et al. (2012) RBO, b: Fiorentino et al. (2011) Human Reprod*

# PGD for gene defects

# PGD for gene disorders

Reprogenetics

## We can do PGD for any disease with known mutation

**Disease tested:** Acetil Co Oxidase type I defficiency, Adrenoleucodistrophy, Alpha-thalassemia, Alport syndrome, Autosomal Dominant Polycystic Kidney Disease (ADPKD), Autosomal Recesive Polycystic Kidney Disease (ARPKD), Beta-thalassemia, Branchio-Oto-Renal syndrome (BOR), BRCA1 breast cancer predisposition, BRCA2 breast cancer predisposition, CanavanCharcot-Marie-Tooth type IA (CMT1a), Choroideremia, Congenital adrenal hyperplasia (CAH), Congenital neutropenia, Connexin 26 hearing loss, Cystic fibrosis, Duchenne/Becker Muscular Dystrophy (DMD), Ectrodactyly, Ectodermal dysplasia, and Cleft lip/palate syndrome (EEC1), Fabry Disease, Familial adenomatous poliposis coli (FAP), Familial dysautonomia, Familial intrahepatic cholestasis 2, Fanconi anemia, Fragile site mental retardation , Gangliosidosis type 1 (GM1), Gaucher disease, Glomuvenous malformations (GVM), Glycogen-storage disease type I (GSD1), Glycosylation type 1C, Hemoglobin SC disease, Hemophilia A, Hemophilia B, Hereditary nonpolyposis colon cancer (HNPCC), Hereditary pancreatitis, HLA matching Huntington disease, Hurler syndrome, Hypophosphatasia, Incontinential pigmenti, Krabbe disease (Globoid cell leukodystrophy), Long QT syndrome, Marfan syndrome, Meckle gruber, Metachromatic leukodystrophy (MLD), Methylmalonic aciduria cblC type (MMACHC), Myotonic Dystrophy 1, Myotubular myopathy, Neurofibromatosis 1, Neurofibromatosis 2, Niemann-Pick Disease, Noonan syndrome, Oculocutaneous albinism 1 (OCA1), Ornithine carbamoyltransferase deficiency (OTC), Osteogenesis Imperfecta 1, Rapp Hodgkin ectodermal dysplasia, Retinitis pigmentosa, Retinoblastoma, Sickle Cell Anemia, Smith-Lemli-Opitz syndrome (SLOS), Spinal bulbar muscular atrophy (SBMA), Spinal Muscular Atrophy Type 1 (SMA1), Tay Sachs, Tuberous sclerosis 1 (TSC1), Tuberous sclerosis 2 (TSC2), Von Hippel-Lindau Syndrome (vHL), X-linked dominant Charcot–Marie–Tooth (CMTX), etc…… *(see review Gutierrez et al. (2008))*

# Day 3 vs. day 5 biopsy for PGD
# Of gene defects

Reprogenetics

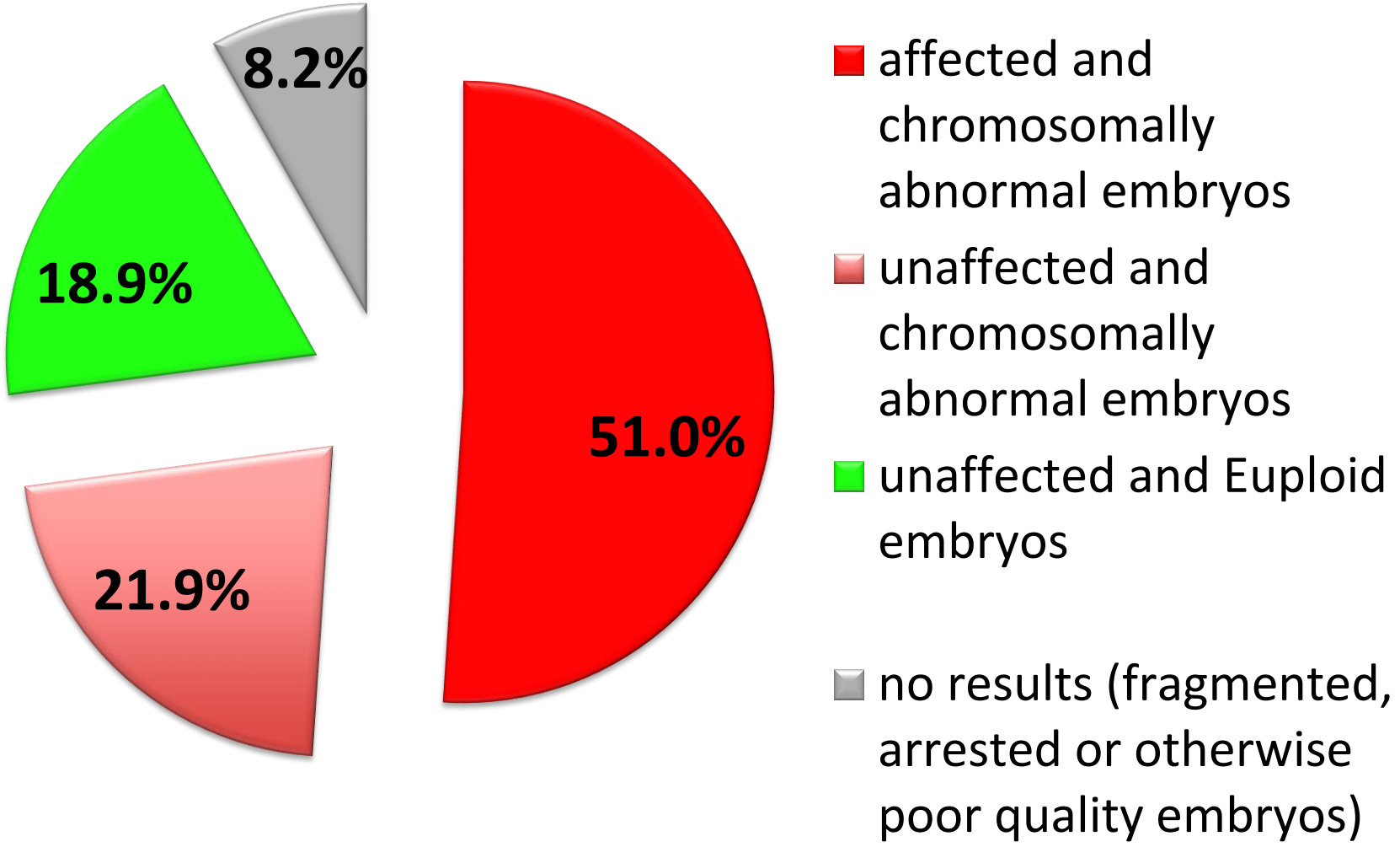
| | Day 3 | Day 5 | |
|---|---|---|---|
| Total embryos | 2634 | 797 | |
| No Results | 12.1% | 5.3% | <0.0001 |
| ADO rate | 9.8% | 1% | <0.0001 |

Prates et al. (2013) Fertil Steril, ASRM

# aCGH and Single Gene Disorders: Results

Reprogenetics

N= 329 embryos tested




*Prates et al. (2013) ASRM*

# Improved pregnancy results

Reprogenetics

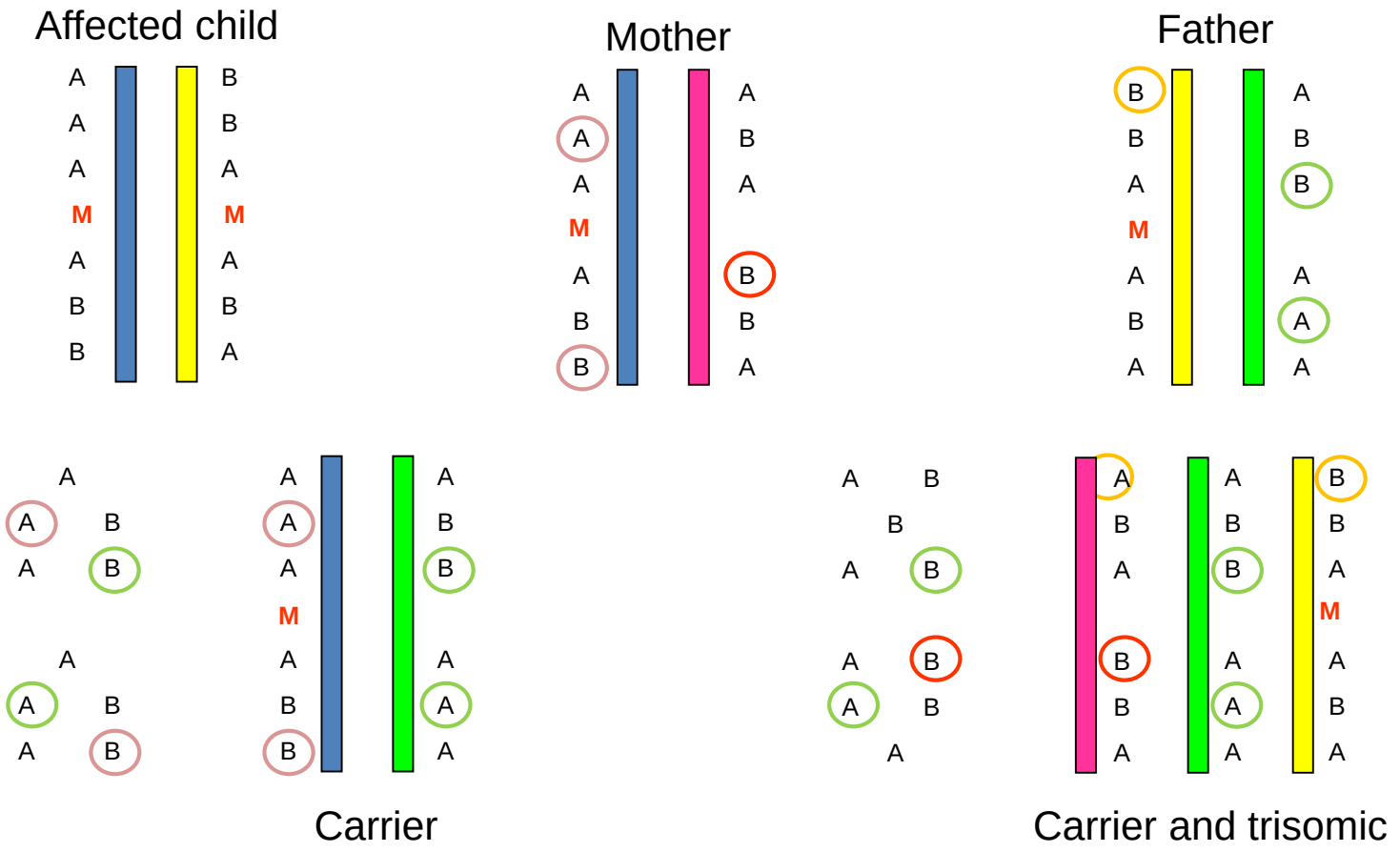
| Test type | Av age | Pregnancy rate |
|---|---|---|
| SGD | 31.6 | 54% (14/26) |
| SGD + CCS | 32.3 | 86% (12/14) |
| | | **p<0.05** |

# Reducing work-up time for PGD of single gene disorders - Karyomapping

Reprogenetics

Thousands of polymorphisms on each chromosomes

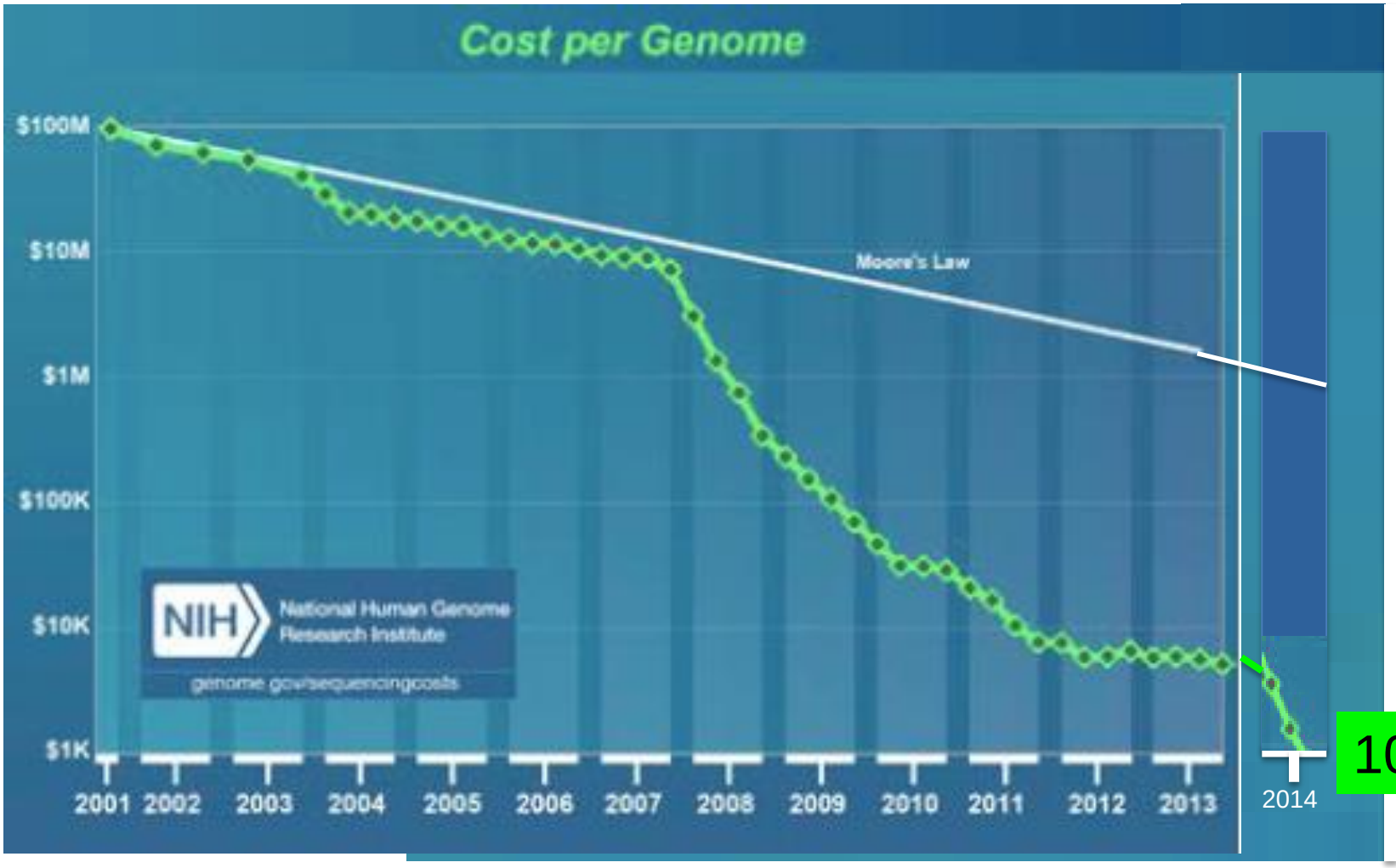
Each chromosome (region) has a unique DNA fingerprint



# PGD v3:
# Next Generation Sequencing

# The $1000 genome is here

Reprogenetics




* Not including equipment, labor, overhead, analysis, etc

# Differences between PGD methods

Reprogenetics

| | PCR | PCR + aCGH | SNP arrays* | Next Gen Sequencing |
|---|---|---|---|---|
| Detects aneuploidy | no | yes | yes | yes |
| Detects gene defects | yes | yes | yes | yes |
| Detects mitotic errors | no | yes | no | yes |
| >2 month of Preparation | yes | yes | no | no |
| Requires affected proband | no | no | yes | no |
| # genomes / run | 0 | 0 | 0 | 1 * |

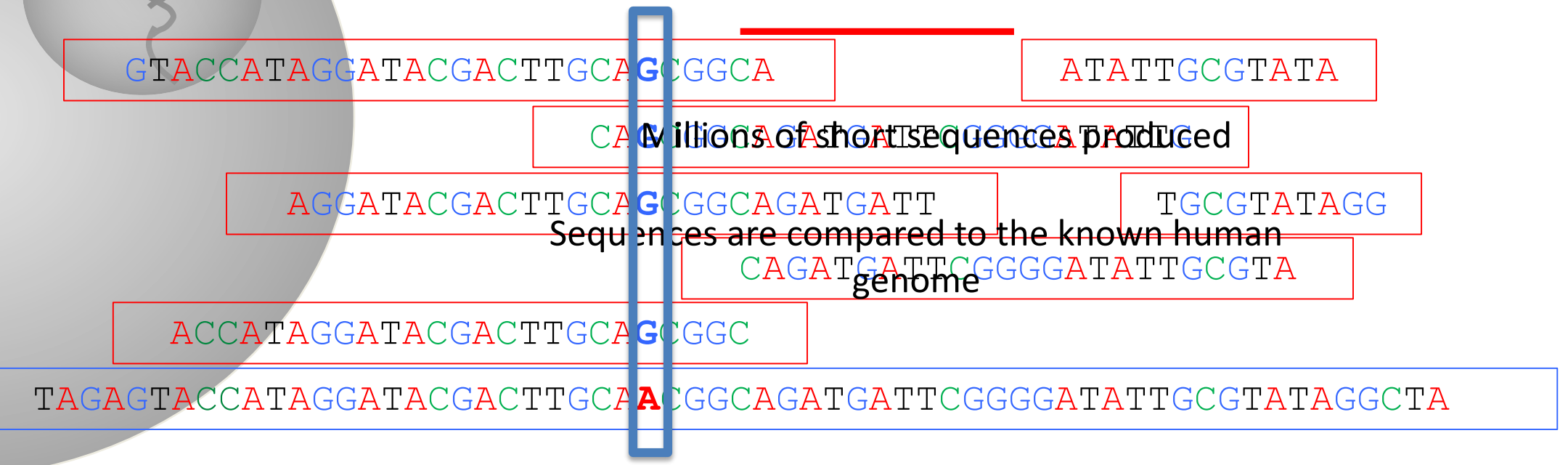
* Karyomapping using BlueGnome
** with NextSeq

# Next Generation Sequencing (NGS)

Reprogenetics

Fragmentation

Each region of the genome sequenced multiple times




Millions of short sequences produced

Sequences are compared to the known human genome

Known sequence (CFTR gene chromosome 7)

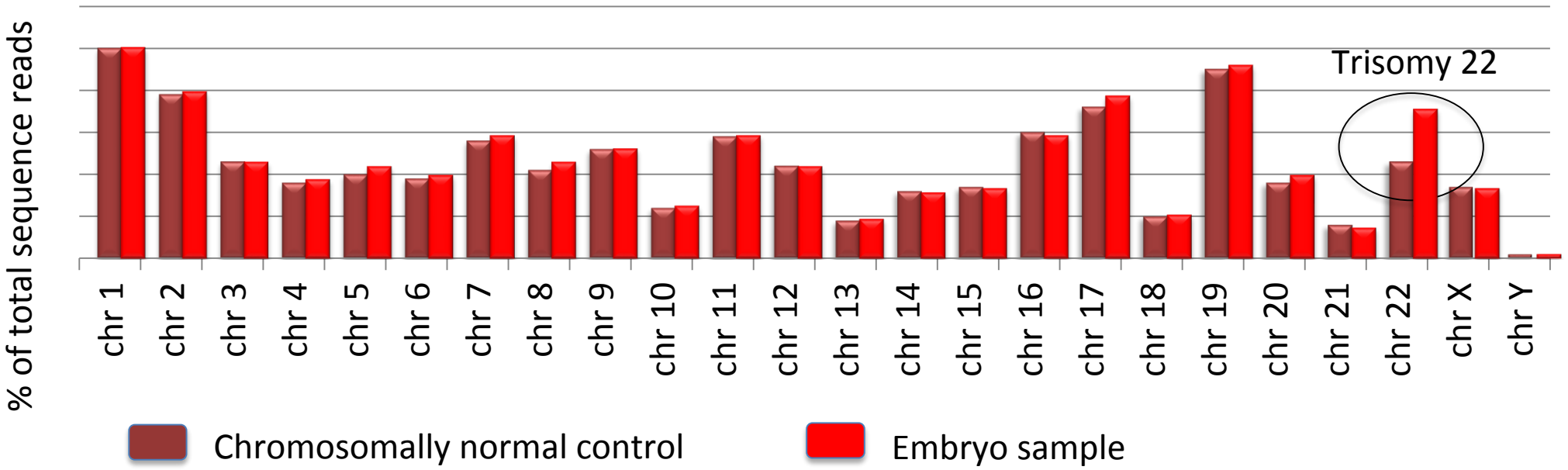
Mutations identified and amount of DNA (aneuploidy) revealed

*Slide adapted from D. Wells*

# PGS: Not all regions amplify equally

Reprogenetics

NGS analysis of amplified DNA from single cells:



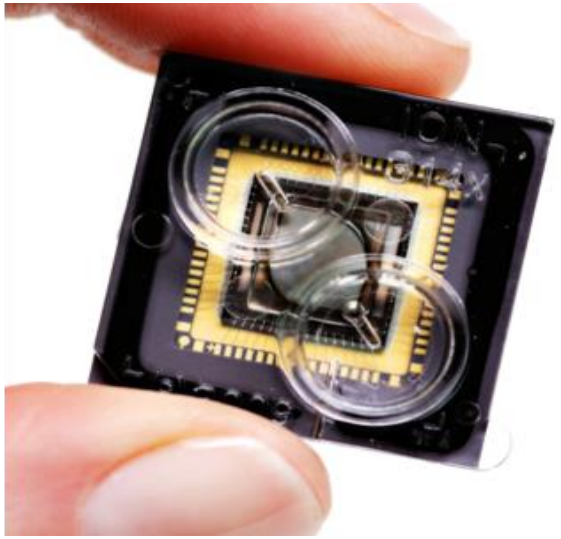

*Slide adapted from D. Wells*

# Platforms used for PGS

Reprogenetics

**Ion torrent** (ThermoFisher):

- PGM
- Proton



**Illumina:**

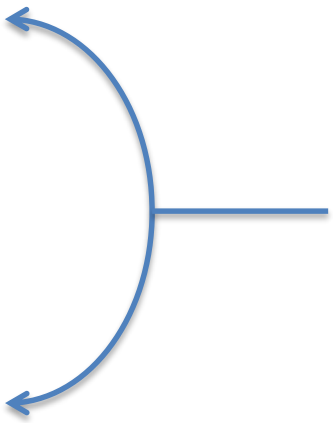
- MiSeq
- NextSeq
- HiSeq

**Complete genomics** (BGI)

# Different output for different needs

Reprogenetics

| | samples / run * |
|---|---|
| **Genome** | **1** |
| Exome | 6-12 |
| NIPT | 16-20 |
| Carrier screen | 24-96 |
| **PGS** | **96** |



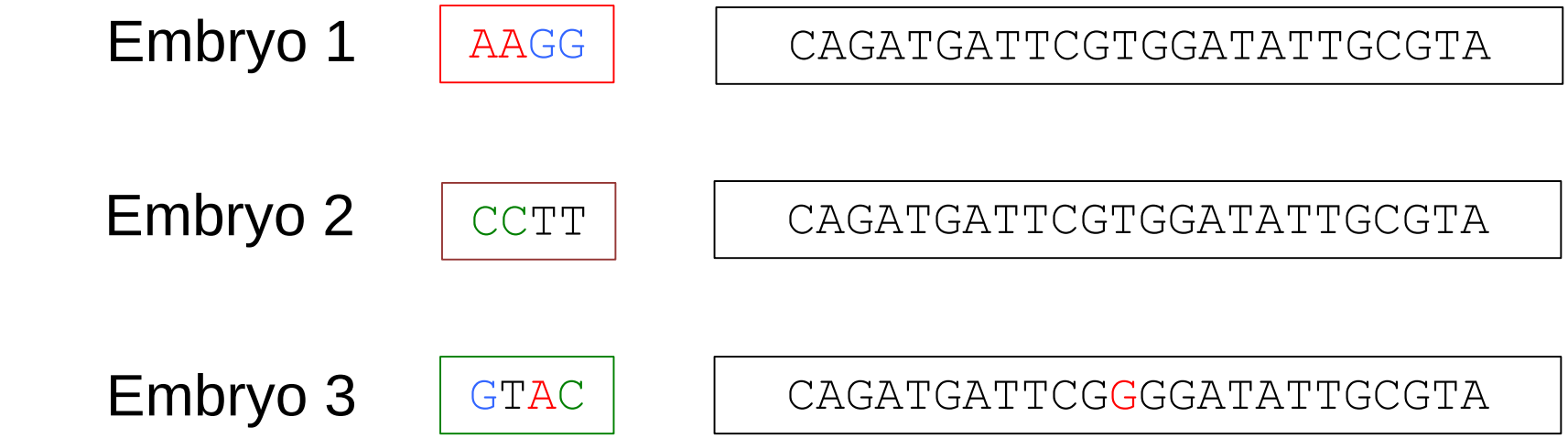
chromosome screening needs less output

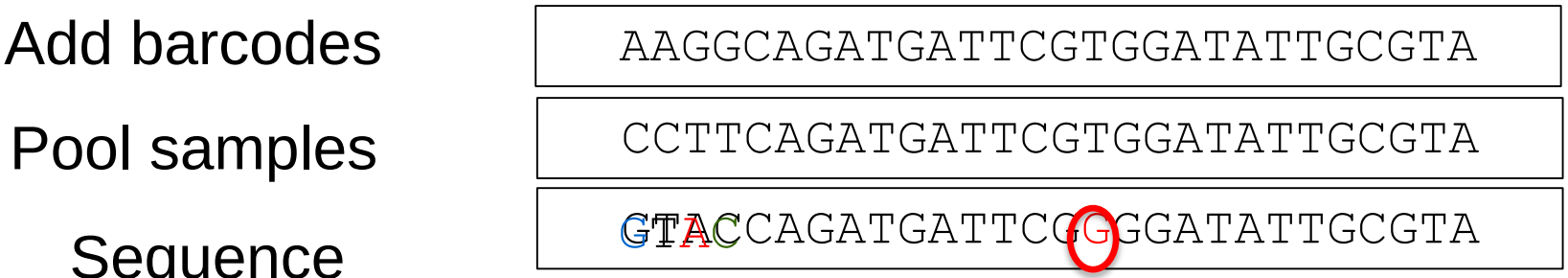
* using NextSeq, x30 coverage, 120 Gb

# Barcoding

Reprogenetics

However, price per sample can be competitive using barcodes




*Slide adapted from D. Wells*

# Barcoding:
# More samples, less sequence

Reprogenetics

| | samples / run | genome coverage | depth of coverage |
|---|---|---|---|
| **Genome** | 1 | 100% | x 30 |
| **PGS *** | 16- 96 | ≤ 10% | x1 to x3 |

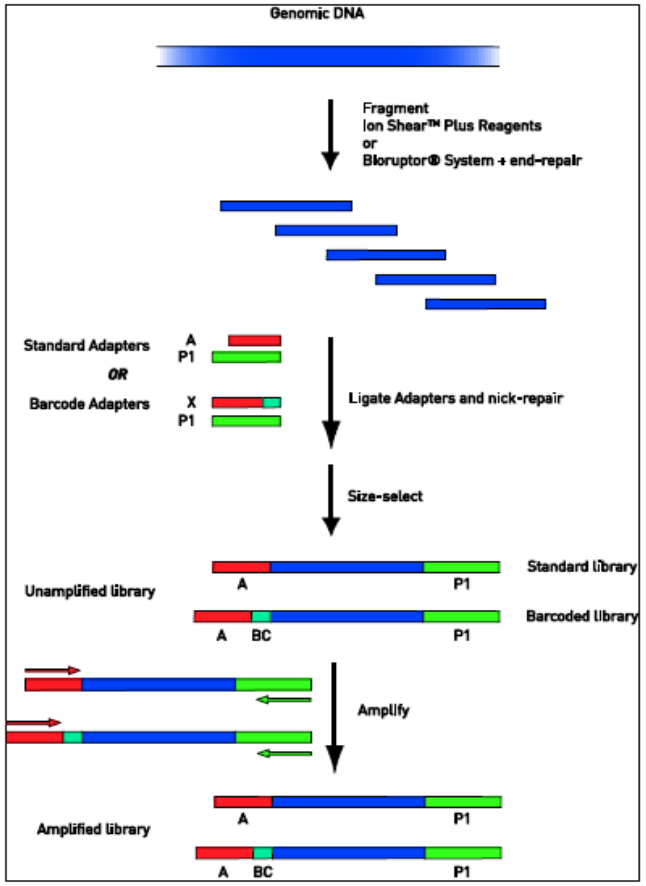
Wells, Kaur, Rico, Grifo, Anderson, Sherlock, Taylor, Munne (2013) ESHRE, Yin et al (2013) Biol Reprod 88, 69

* Output: MiSeq = PGM << NextSeq

# PGS with NGS: Method

Reprogenetics

- Whole Genome Amplification of Sample
- Library preparation:
  - Fragment DNA
  - Ligate adapters and barcodes (≥16)
- Sequence




D Wells, K Kaur, A Rico, J Grifo, S Anderson, J Sherlock, JC Taylor , S Munne (2013) ESHRE

# validation of Next Generation Sequencing (NGS)

Reprogenetics

78 blastocysts previously diagnosed by aCGH were reanalyzed by NGS in a blinded experiment.

21/21 euploid
55/56 aneuploid
1 polyploid

**1.3%** discordance with aCGH, polyploidy detected.

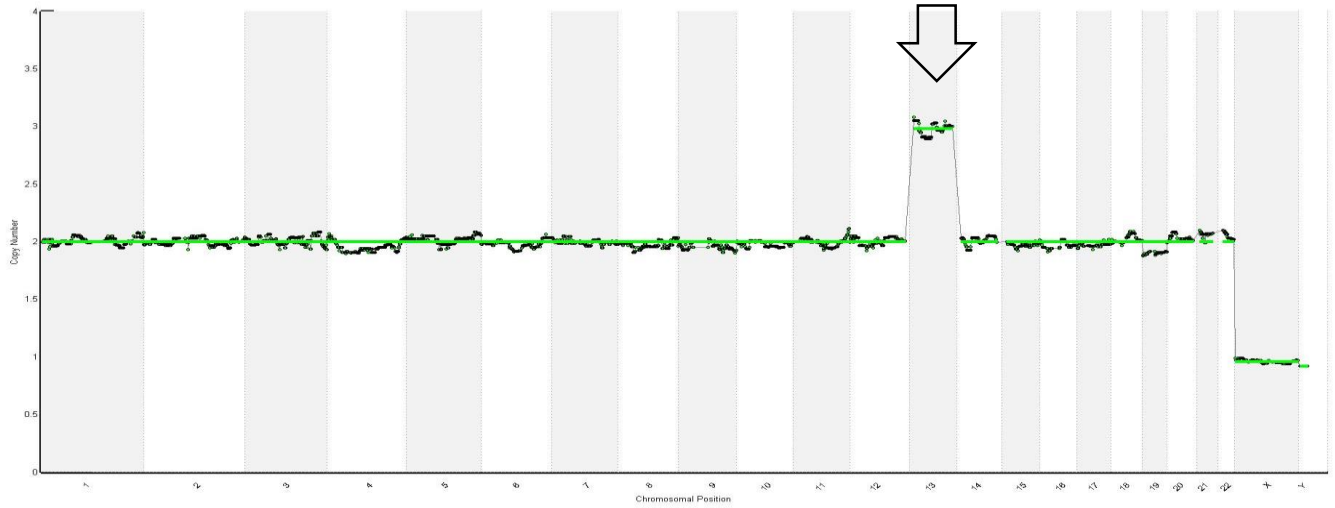
Allen Kung et al. (2014) ESHRE

# Example

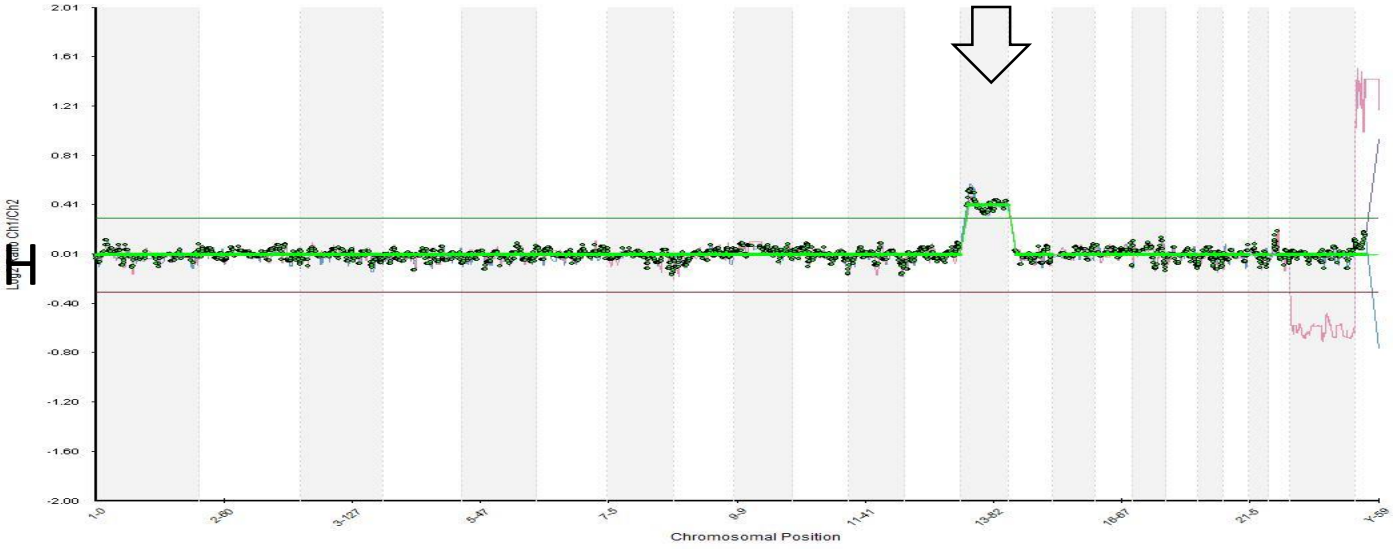
Reprogenetics

Trisomy 13 male
(47,XY,+13)

NGS




aCGH

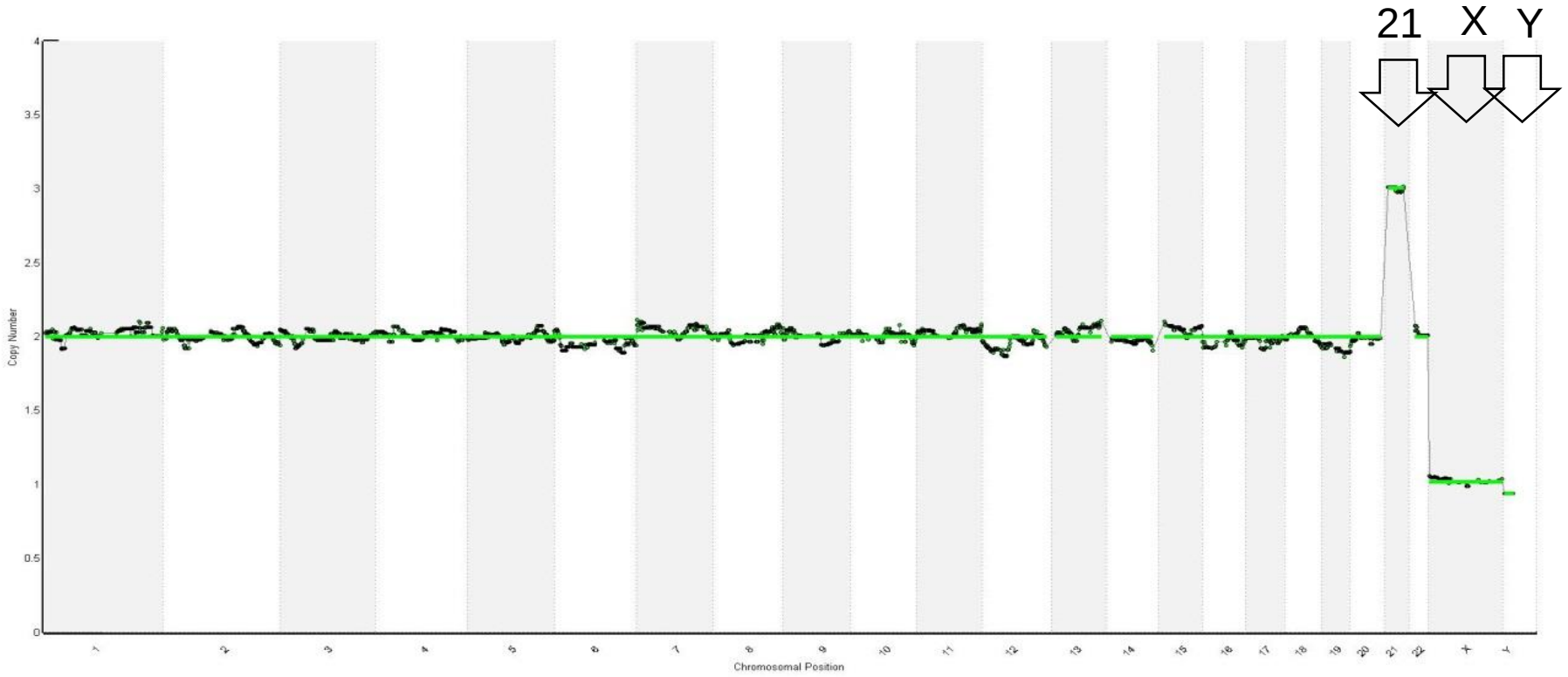



*Adapted from G.Harton, platform: MiSeq*

# Example

Reprogenetics

Trisomy 21, male (47,XY + 21)

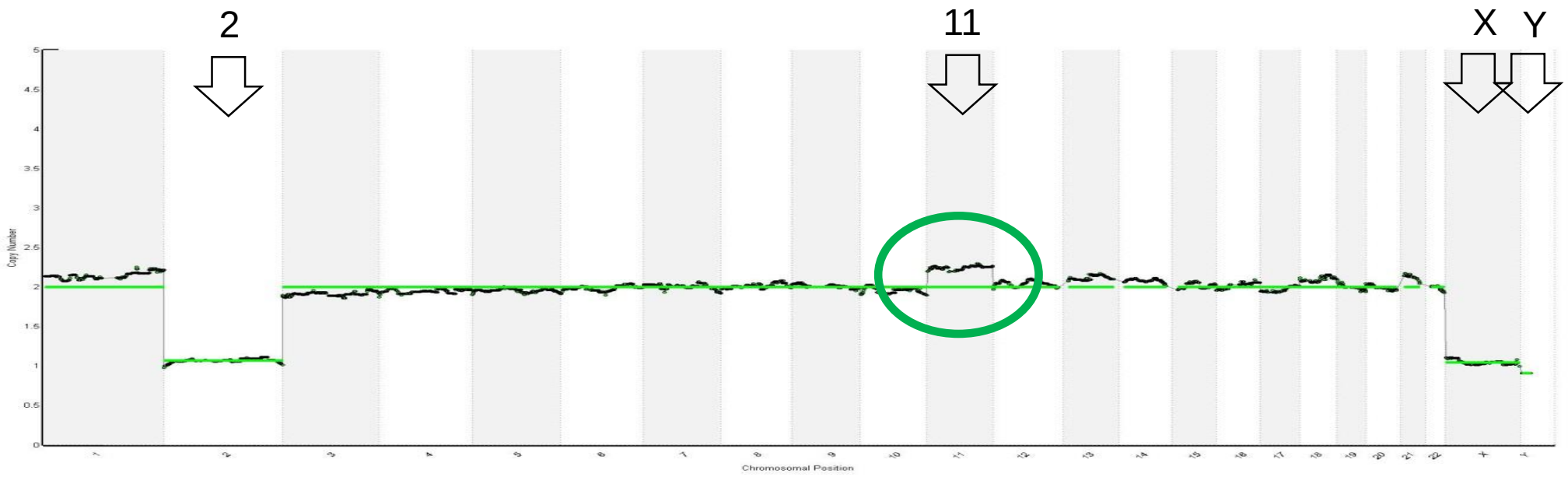



*Adapted from G.Harton, platform: MiSeq*

# Example

Reprogenetics

Monosomy 2, male (45,XY -2)
And mosaic for 11?

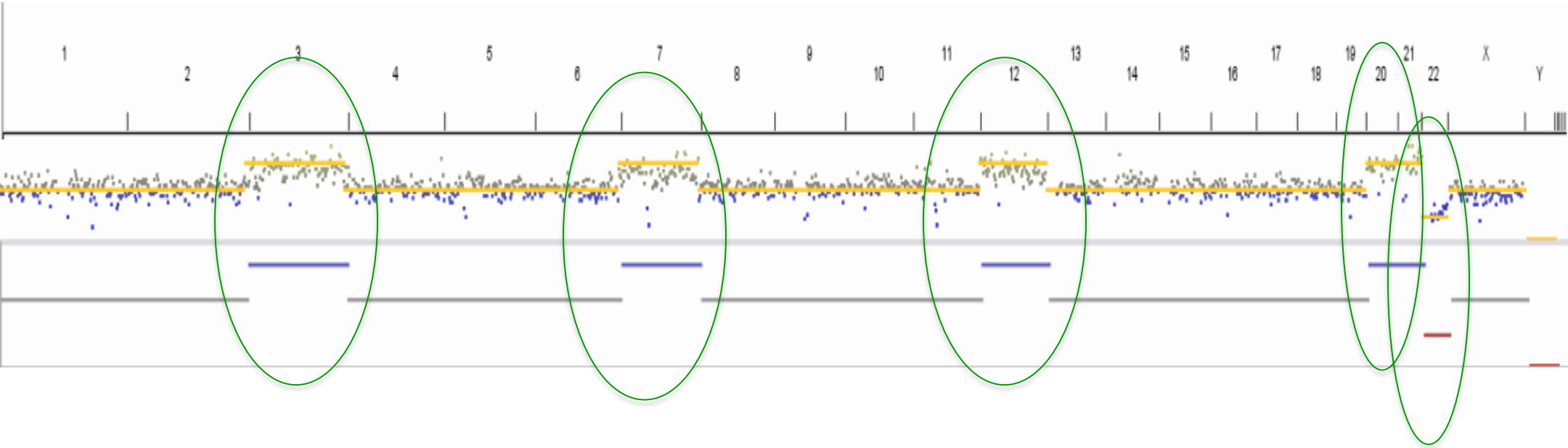



*Adapted from G.Harton, platform: MiSeq*

# Example

Reprogenetics

49,XY +3 +7 +12 +21 -22




By Reprogenetics, using ion torrent PGM

# First baby born from NGS

Reprogenetics


First NGS baby:
David Levy

A collaboration of Reprogenetics-US, Reprogenetics-UK (Dagan Wells) and Main Line Fertility (Dr. Glassner)

# Conclusions

# Conclusions

Reprogenetics

- Euploid embryos implant at the same high rate irrespective of maternal age
- However with maternal age there are more cycles without euploid embryos
- Therefore pregnancy rates per transfer are independent of maternal age but pregnancy rates per cycle still decrease with age

# Conclusion

Reprogenetics

- Arrays are fully validated and combined with Blastocyst biopsy provide a significant improvement in ongoing pregnancy rates.
- Arrays alone or in combination with karyomapping can screen for aneuploidy and gene defects simultaneously.
- Next generation sequencing will allow further information to be detected once prices decrease.

**Year:** 2013

**Issue:** Vol 100 | No. 3 | September 2013 | Pages 593-896


VIEWS AND REVIEWS

# Introduction: Preimplantation genetic screening is alive and very well

David R. Meldrum, M.D.

Reproductive Partners Medical Group, Redondo Beach, California

With 24-chromosome PGS, the rate of miscarriage is running only at <10%, a remarkable finding.

marked decrease in multiple pregnancies, without loss of pregnancy potential.

# Reprogenetics

Reprogenetics

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Samer Alfarawati (UK)
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