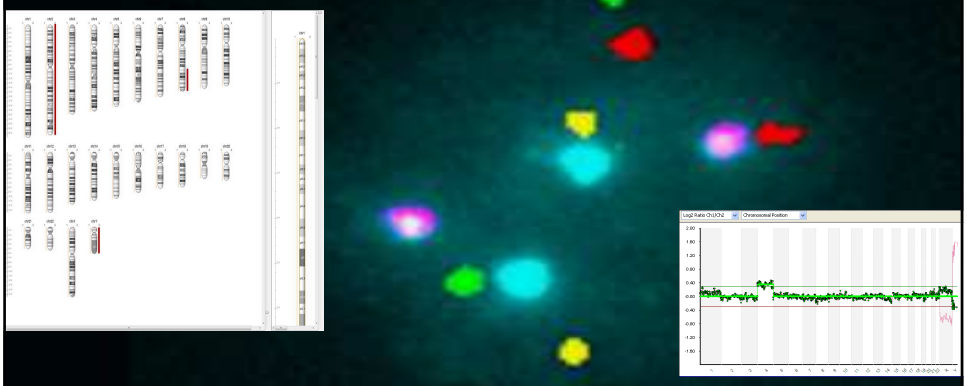
EVOLUTION OF PGD FOR TRANSLOCATIONS



Anver Kuliev, Zeev Zlatopolsky,Li Wang, Yua
Yao, David Cram, Svetlana Rechitsky

Evolution of PGD for Translocations 1996-2016

- PB1 FISH and WCP
- Sequential PB1 and PB2 FISH
- PB2 nuclear conversion
- Blastomere interphase FISH
- Blastomere nuclear conversion
- Chemical blastomere nuclear conversion
- Haplotyping
- SNP-Array/Array-CGH/NGS
- MPS

Present RGI Experience -

Over 12,500 PGD Cycles

<5,000 FOR MENDELIAN

Over 487 CONDITIONS

Over 500 H L A cases

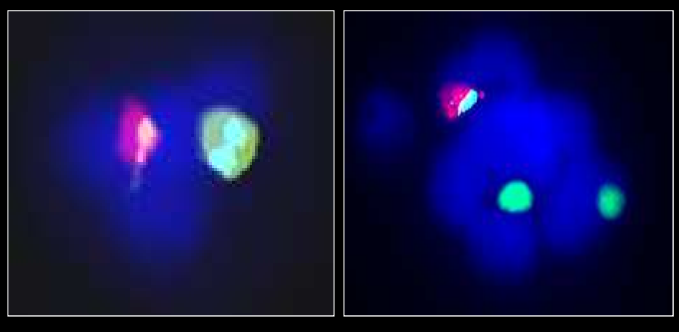
<900 For Translocations

<2,000 Mendelian +Aneuploidy Testing

PGD for Translocations was Introduced on PB1.

1st Polar Bodies, Oocyte Status Inferred

Unbalanced | Balanced

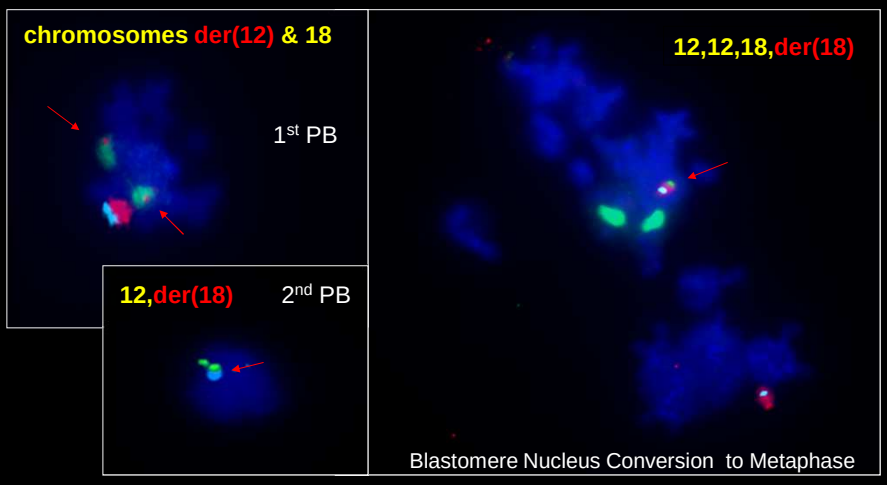


der(7),18 | 12,18

Reproductive Genetics Institute, 1999

Sequential PB1 and PB2 Analysis

46,XX,t(12;18)(p13.31;q21.32)

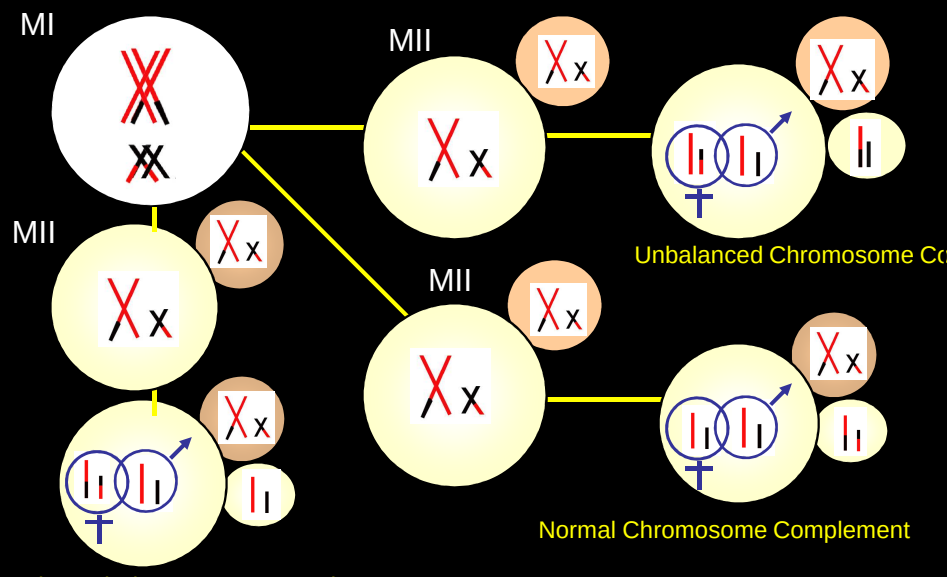


Probes: WCP 12, WCP 18, CEP 18, telomeric 12p, telomeric 18q

Reproductive Genetics Institute

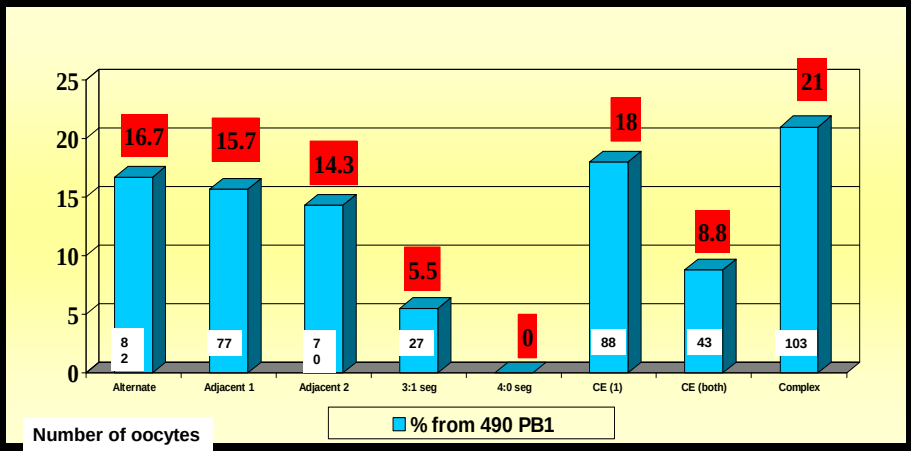
Reciprocal Translocation

Chromatid Exchange Involving Both Chromosomes



Segregation Patterns for Female Reciprocal Translocations

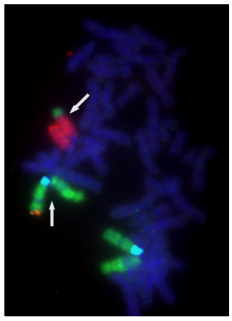
1st Polar Body Analysis



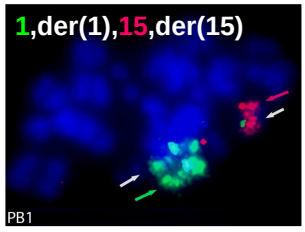
Reproductive Genetics Institute

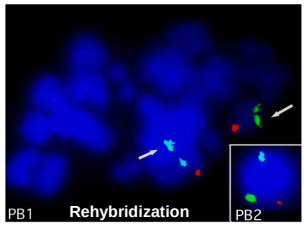
*Following alternate segregation in MI

Chromatid Exchange of both normal and derivative chromosomes – Accurate diagnosis by 2nd PB analysis

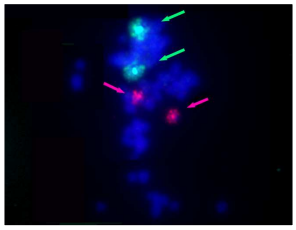


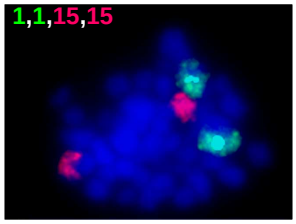
46,XX,t(1;15)(q32;q26)





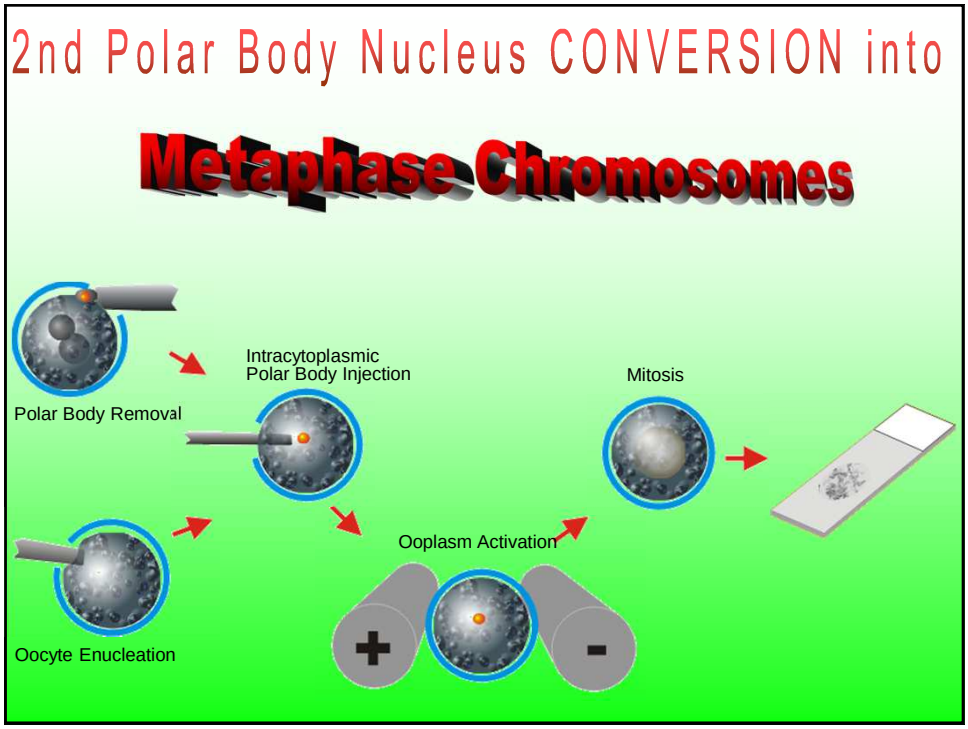
Polar Body Analysis

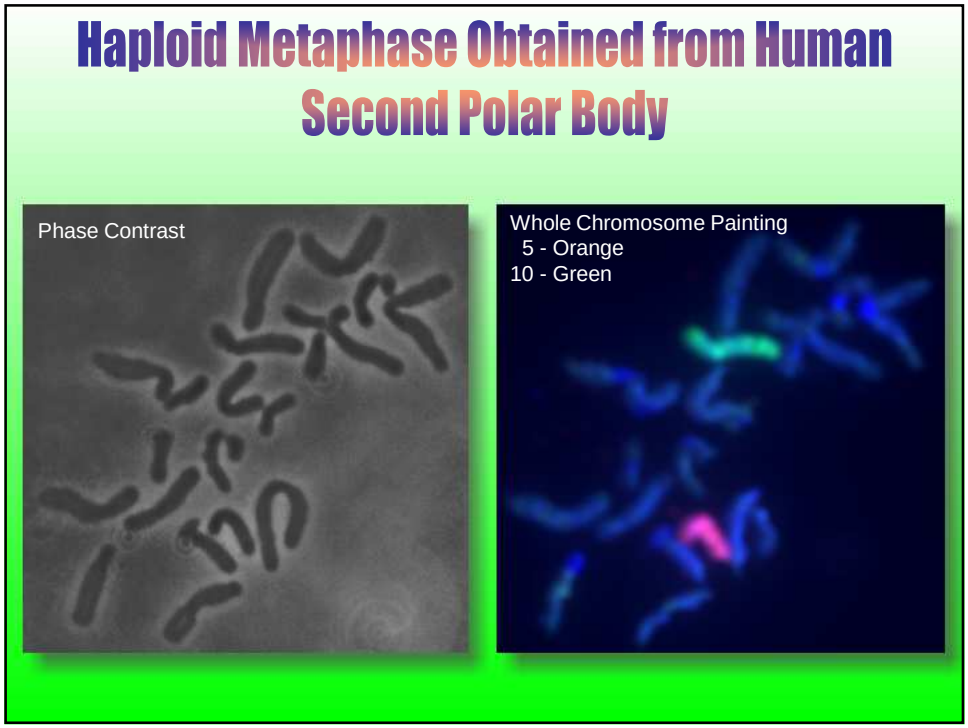




Blastomere Analysis

Reproductive Genetics Institute


2nd Polar Body Nucleus CONVERSION into
Metaphase Chromosomes
Polar Body Removal
Intracytoplasmic
Polar Body Injection
Mitosis
Oocyte Enucleation
Ooplasm Activation
+
-


Haploid Metaphase Obtained from Human
Second Polar Body
Phase Contrast
Whole Chromosome Painting
5 - Orange
10 - Green

PRENATAL DIAGNOSIS
Prenat. Diagn. **18:** 1437- 1449 (1998)

REVIEW

CHROMOSOME TRANSLOCATIONS: SEGREGATION MODES AND STRATEGIES FOR PREIMPLANTATION GENETIC DIAGNOSIS

P. N. SCRIVEN [1]*, A. H. HANDYSIDE [2] AND C. MACKIE OGILVIE [1]

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SUMMARY

Preimplantation genetic diagnosis (PGD) offers polymerase chain reaction tests for an increasing range of single gene defects, and fluorescence *in situ* hybridization tests for sex determination (for X-linked conditions) and for aneuploidy detection. Patients carrying chromosome translocations with a high reproductive risk are increasingly seeking to increase their chances of a normal pregnancy with the help of PG D, for which they present a special challenge. This paper describes the behaviour of reciprocal translocations at meiosis, discusses current methods of detecting meiotic outcomes at the preimplantation stage and outlines ways forward for preimplantation diagnosis of these common rearrangements. We also propose a more general strategy using recently developed chromosome specific sub-telomeric probes, combined , if possible, with proximal probes, to form a strong diagnostic tool.

KEY WORDS: preimplantation genetic diagnosis (PGD); reciprocal translocations; sub-telomeric probes; fluorescence *in situ* hybridization (FISH); meiotic segregation

INTRODUCTION

Preimplantation genetic diagnosis (PGD) is now established at around 35 centres world-wide, offering polymerase chain reaction (PCR) tests (Handyside *et al.*, 1990; Liu *et al.*, 1994; Daniels *et al.*, 1997) for an increasing range of single gene defects, or fluorescence *in situ* hybridization (FISH) tests for sex determination (for X-linked conditions) and for aneuploidy detection (Munne and Weier, 1996; Handyside and Delhanty, 1997). Around 600 cycles of PGD following cleav age stage biopsy (Handyside, 1991; Ao and Handyside, 1995; Lissens and Sermon, 1997) of preimplantation embryos had been carried out up to the end of 1996, resulting in over I 00 pregnancies and nearly I 00 babies born, with a pregnancy rate of 26 per cent per embryo transfer (Harper, personal comm unication). Polar body biopsy for aneuploidy detection in older women has also resulted in the bi rth of healthy children (Verlinsky *et al.*, 1996).

Reciprocal translocations (usually an exchange of two terminal segments from different chromo somes) are the commonest form of chromosome abnorma lity, occurring in approximately I in every 500 live births (Hook and Hamerton, 1977).

Carriers of these translocations are nearly always phenotypically normal, as no loss of genetic material is involved. (Abnormalities can arise, however, if breakpoints disrupt important genes). Translocations are therefore usually detected when the individual presents with recurrent pregnancy loss or phenotypically abnormal offspring due to

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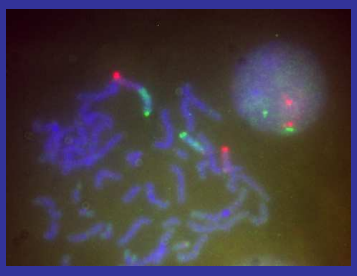
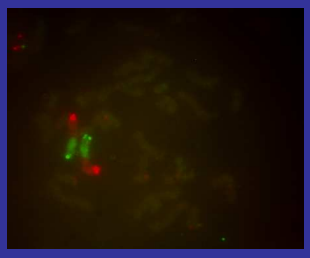
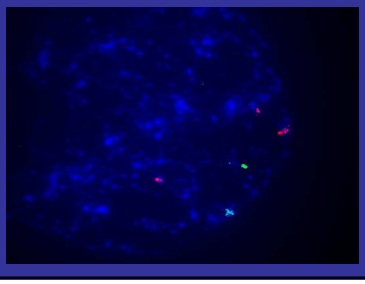
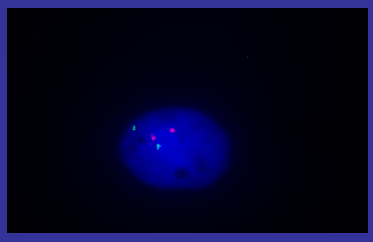
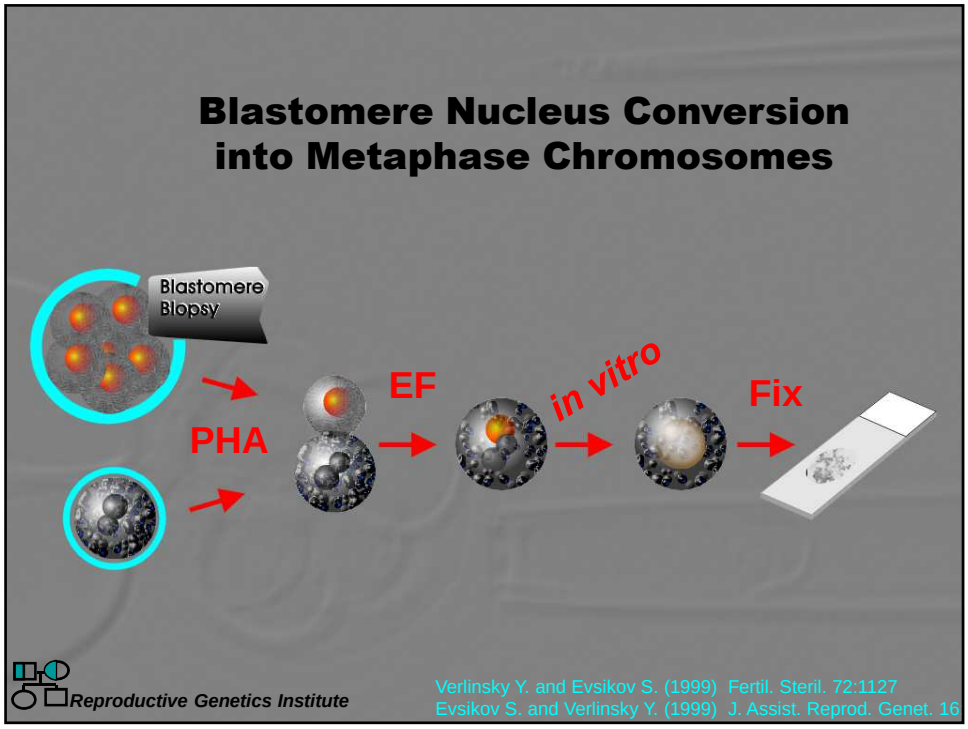
CCC 0197-3851/98/131437- 13$17.50

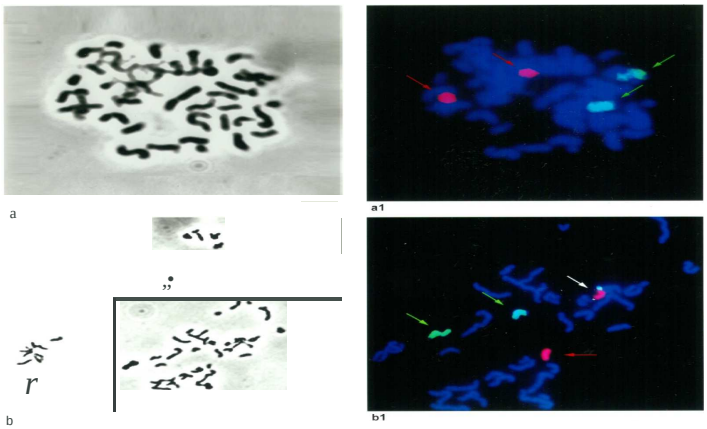
PGD in a family with both maternally and a paternally derived Robertsonian translocation 45,XX,der(13;14)(q10;q10) with involvement of both pairs of chromosomes 13 & 14

(44,XY,der(13;14)(q10;q10),der(13;14)(q10;q10)



PGD for translocation 46, XY, t(**13**;**20**)(q22;p11.2) by blastomere nuclear conversion



(Chemical) Conversion of interphase nu to metaphase chromosomes involves:

1) Morphological selection of the blastomere (i.e. largest blastomere with 1-2 large nucleoli)

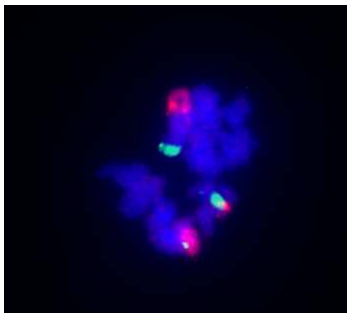
2) Exposure to medium containing caffeine (1mmol/l) to reduce the incubation time

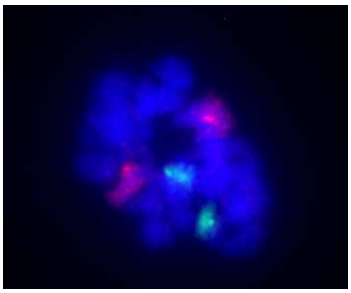
3) Low colcemid dose (<0.1 µg/ml)

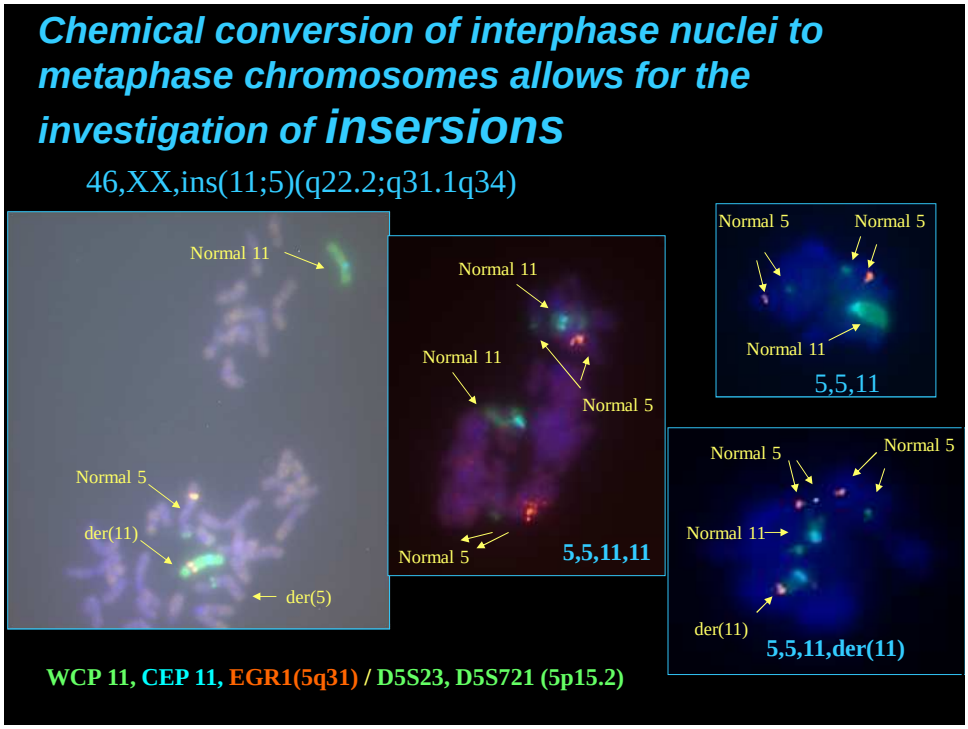
Results in 80% conversion to metaphase chromosc with an incubation time of 3.6 +/- 2.5 hours

Shkumatov, A et al. Reproductive BioMedicine Online 2007

PGD for translocation 46,XX,t(5;13)(p15.3;q34), using the chemical conversion







A molecular strategy using STRs for routine PGD in both reciprocal and Robertsonian translocation carriers

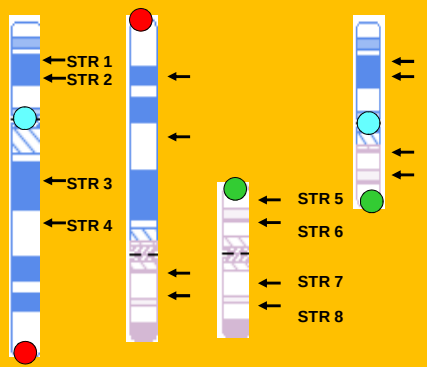
Traversa M, *and Leigh, D.*

PGD using PCR technology

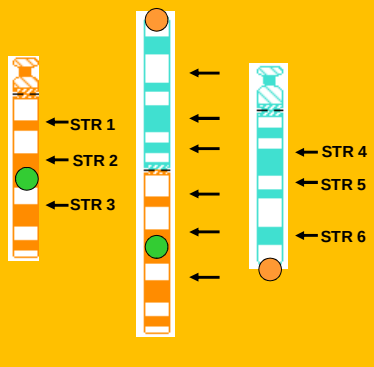
- Choose polymorphic markers at least two band locations away from either side of reported breakpoints
  - -cytogenetic limitations in defining breakpoints
  - -mapping of breakpoints using microarrays
- Individual carrying translocation must be fully informative against partner (i.e. have 2 distinguishable alleles)
- Number of peaks at each marker represents number of chromosome segments present

Placement of STR markers

Reciprocal Translocations
e.g. 46 XX t(9;19)(q12;p12)

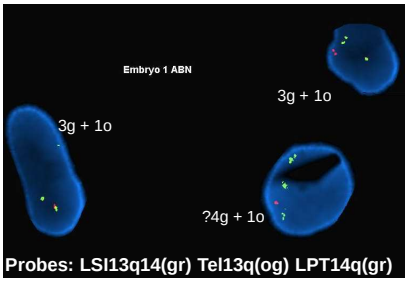


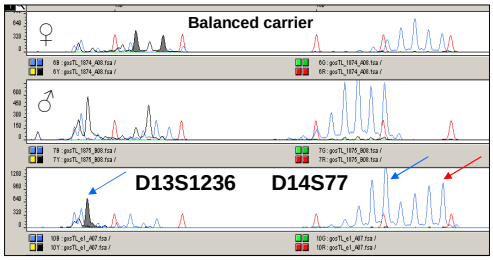
Robertsonian Translocations
e.g. 45 XY t(13;14)(q10;q10)

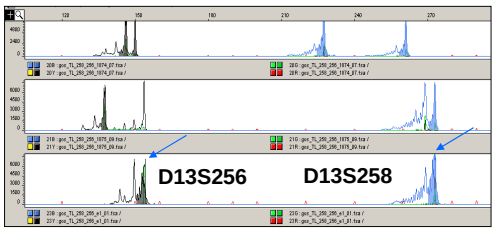


Embryo results: FISH & PCR

46, XX, (13;14)(q14.3;q11.2)



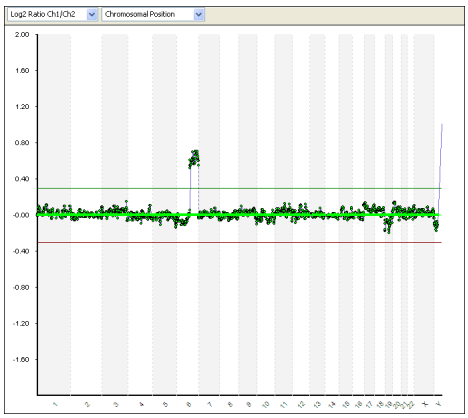


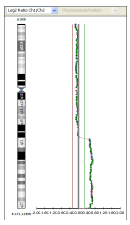


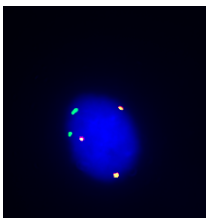
- 3 cells indicating abnormal signal pattern
- indicating 3:1 (likely interchange monosomy 13)

3:1 Interchange monosomy 13

Validation of aCGH PGD for duplication of q arm of chromosome 6 (dup(6)(q21q27)) by FISH

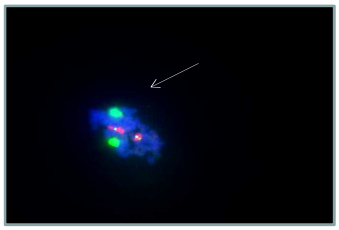




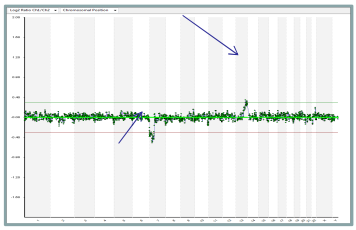


Incidental Translocation Finding in aCGH Aneuploidy Testing

Day 3 blastomere from same embryo : normal and derivative chromosomes 7 present (red) ; normal copies of chromosome 13 (green)

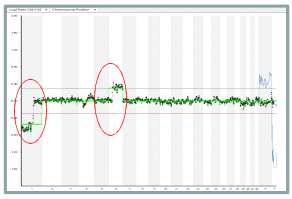


46, XY,der(7)(t(7;13)(p22;q22): missing genetic material at 7p22 and extra genetic material at 13q22

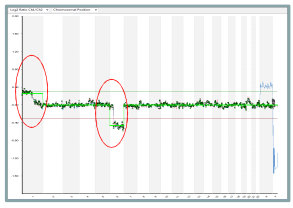


Pattern of structural abnormalities detected during routine aneuploidy study with abnormalities in chromosomes 1 & 6.
Paternal karyotype diagnosed as 46,XY,t(1;6)(p13.3;q14) after request.

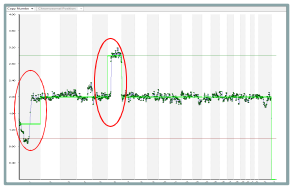
Deletion at 1p and duplication at 6q- aCGH



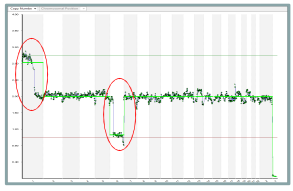
Duplication at 1p and deletion at 6q- aCGH



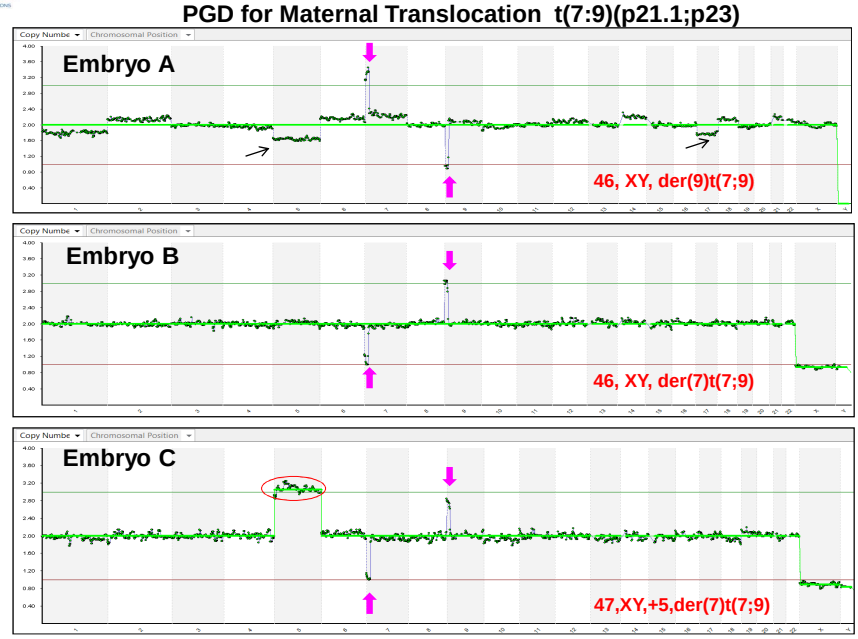
Deletion at 1p and duplication at 6q-NGS on same embryo as above



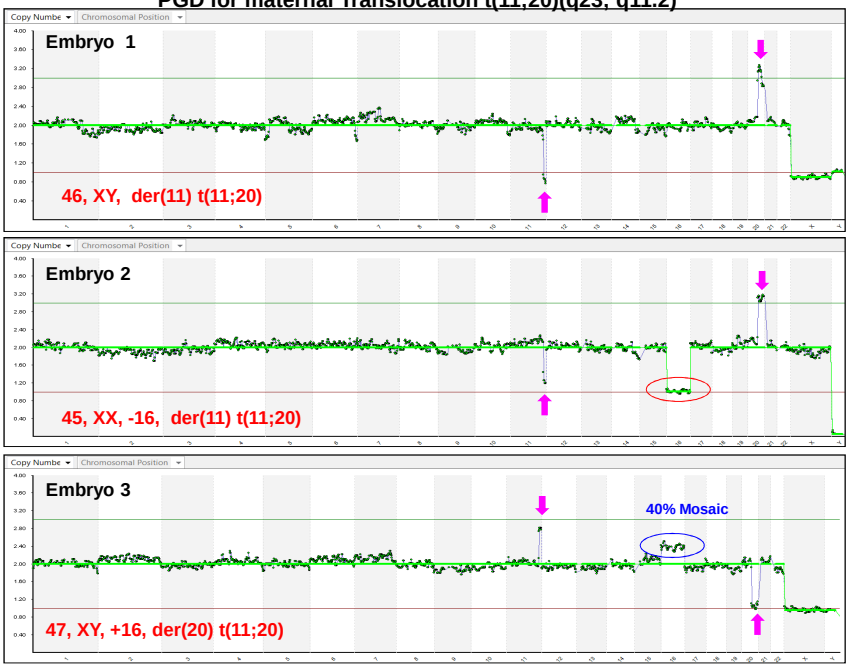
Duplication at 1p and deletion at 6q-NGS on same embryo as above



PGD for Maternal Translocation t(7:9)(p21.1;p23)

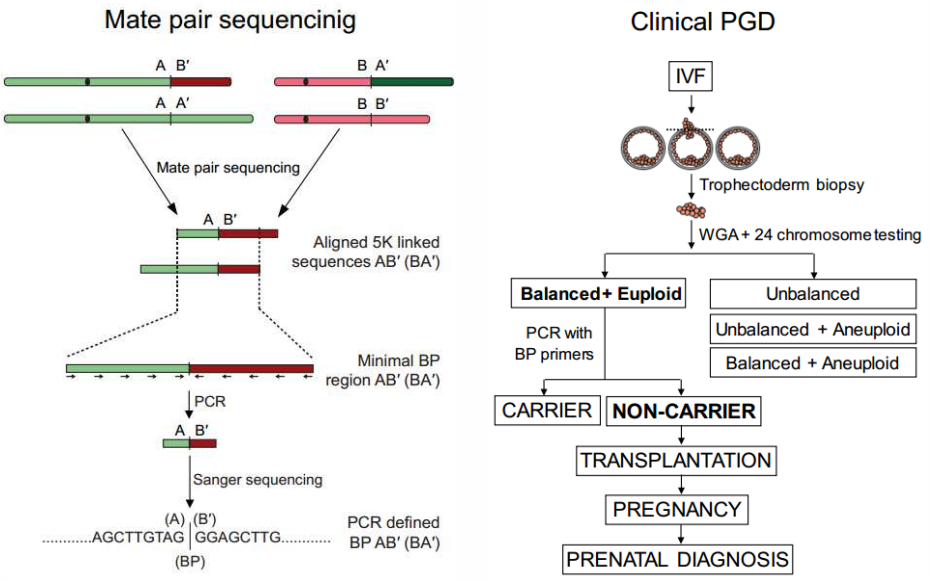


PGD for maternal Translocation t(11;20)(q23; q11.2)



Technology for Life
科技关爱生命

New strategy



Technology for Life
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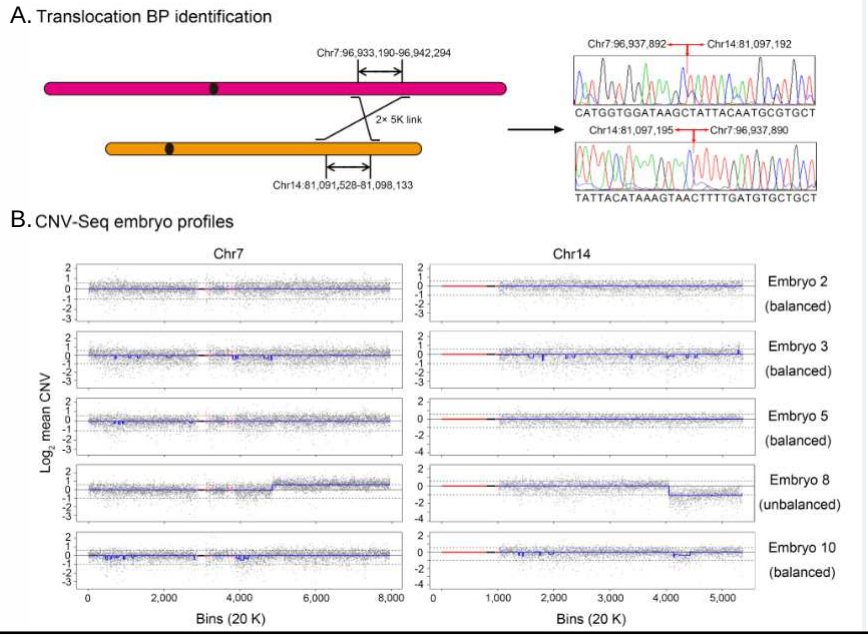
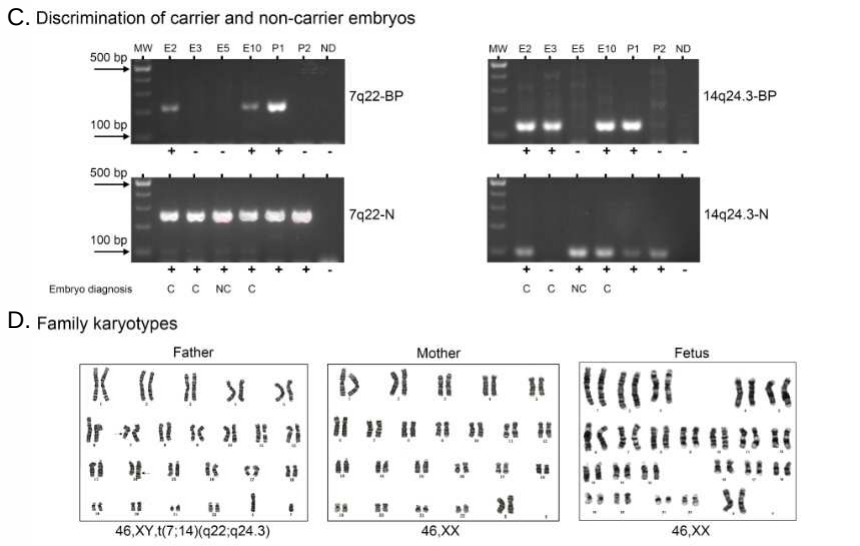
Clinical proof of concept – 46,XY,t(7;14)(q22;q24.4)



Technology for Life
科技关爱生命

Clinical proof of concept – 46,XY,t(7;14)(q22;q24.4)



Outcome of PGD for Translocations by FISH and NGS

Patients	Cycles	Embryo Transfers	Pregnancy	Delivery	Children born	SAB
FISH 433	685	486	193 (40%)	145*	176	29(15%)
NGS 93	217	117	76 (64.9%)	73	77	3 (3.9%)

Evolution of PGD for translocations

- **Anver Kuliev, Zev Zlatopolsky, Svetlana Rechitsky**
  Reproductive Genetics Institute
- **Li Wang, Yuanqing Yao**
  PLA General Hospital, Beijing, China
- **David Cram**
  Berry Genomics, Beijing, China