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ESTIU 2006

# S10. II Simposi sobre Fertilitat Femenina i Masculina: Genètica i Ambient

Sabadell, 7 de juliol

# RIESGO EN LA DESCENDENCIA DE PORTADORES DE ANOMALÍAS CROMOSÓMICAS ESTRUCTURALES. CONSEJO GENÉTICO

**Maria Oliver-Bonet,** Universitat Autònoma de Barcelona.

Organitzadors: Ajuntament de Sabadell · ESDi · Fundació Parc Taulí · Universitat Autònoma de Barcelona · Centre d'Innovació i Formació Tecnològica · UOC Universitat Oberta de Catalunya

Patrocinadors: Fundació BancSabadell · BS · Obra Social Caixa Sabadell

Il Simposio sobre infertilidad femenina y masculina: genética y ambiente

# Riesgo en la descendencia de portadores de anomalias cromosomicas estructurales
# Consejo genético

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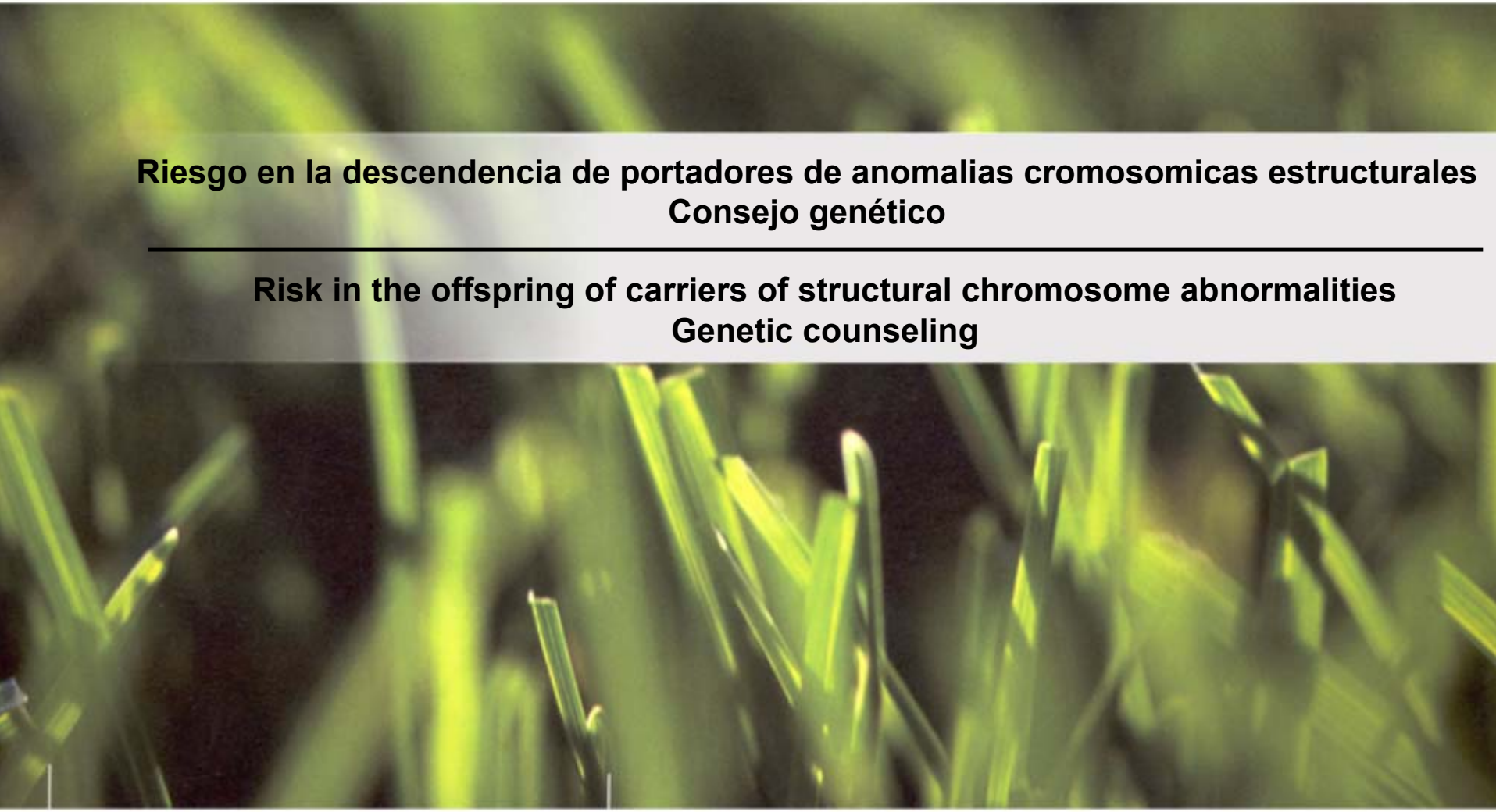
# Risk in the offspring of carriers of structural chromosome abnormalities
# Genetic counseling



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# Structural abnormalities

- Balanced Structural Abnormalities do not usually change the amount of chromosomal material, but the order of the genetic material

- They are known to cause infertility in humans (high incidence in infertile patients):

  Production of gametes with chromosome abnormalities (abortions, risk of affected offspring)
  Interruption of gametogenesis (reduction/no production of gametes)

  - Reciprocal translocations
  - Robertsonian translocations
  - Inversions (paracentric and pericentric)

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# Incidence of structural abnormalities at birth

**Table III.** Comparison between frequencies of chromosomal aberrations that had a normal phenotype at birth in six large surveys

| Authors | Newborn number | Robertsonian translocations | Reciprocal translocations | Autosomal inversions | 47,XYY | Marker chromosomes |
|---|---|---|---|---|---|---|
| Jacobs *et al.* (1974) | 11 680; *7849 males, 3831 females* | 10 *(0.85)* [6 t(13;14) *(0.5)*] | 10 *(0.85)* | 2 *(0.17)* | 12 *(1.5)* [2 mosaics *(0.25)*] | Not reported |
| Hamerton *et al.* (1975) | 13 939; *7176 males, 6763 females* | 13 *(0.93)* [10 t(13;14) *(0.7)*] | 11 *(0.78)* | 0 | 7 *(0.9)* [3 mosaics *(0.4)*] | Not reported |
| Buckton *et al.* (1980) | 3993; *2072 males, 1921 females* | 3 *(0.7)* [3 t(13;14)] | 5 *(1.2)* [1 t(Y;15)] | 2 *(0.5)* | 4 *(1.9)* | 2 *(0.5)* |
| Hansteen *et al.* (1982) | 1830; *955 males, 875 females* | 4 *(2.1)* [3 t(13;14) *(1.6)*] | 5 *(2.7)* | 0 | 1 *(1)* | 4 *(2.1)* |
| Maeda *et al.* (1991) | 14 835; *7608 males, 7227 females* | 11 *(0.74)* [9 t(13;14) *(0.6)*] | 11 *(0.74)* | 2 *(0.13)* | 5 *(0.66)* [3 mosaics *(0.4)*] | 3 *(0.2)* |
| Nielsen and Wohlert (1991) | 34 910; *17 860 males, 17 050 females* | 43 *(1.2)* [34 t(13;14) *(0.97)*] | 49 *(1.4)* | 12 *(0.34)* | 19 *(1)* [2 mosaics *(0.1)*] | 23 *(0.66)* |
| [redacted] | 81 187; *43 520 males, 37 667 females* | 84 *(1)* [65 t(13;14[redacted] | 9[redacted] | 1[redacted] | 48 *(1.1)* [10 mosaics *(0.22)*] | 3[redacted] |

Frequencies are given per 1000 in italics/brackets.

Adapted from Ravel et al, 2006

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# Incidence of structural abnormalities according to the number of spontaneous abortions

| Chromosomal abnormality | 1 Sp. Abortions | 2 Sp. Abortions | ≥ 3 Sp. Abortions |
|---|---|---|---|
| Reciprocal translocation | 0.50% | 1.38% | 1.51% |
| Robertsonian translocation | 0.36% | 0.62% | 0.66% |
| Inversions | 0.14% | 0.20% | 0.21% |

Adapted from De Braekeleer (2006) and Mau-Holzmann (2005)

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# Distribution of structural abnormalities according to the sex of the carrier

| Chromosomal abnormality | Males | Females |
|---|---|---|
| Reciprocal translocation | 36.3% | 63.7% |
| Robertsonian translocation | 31.3% | 68.7% |
| Inversions | 40.3% | 59.7% |

Adapted from De Braekeleer (2006)

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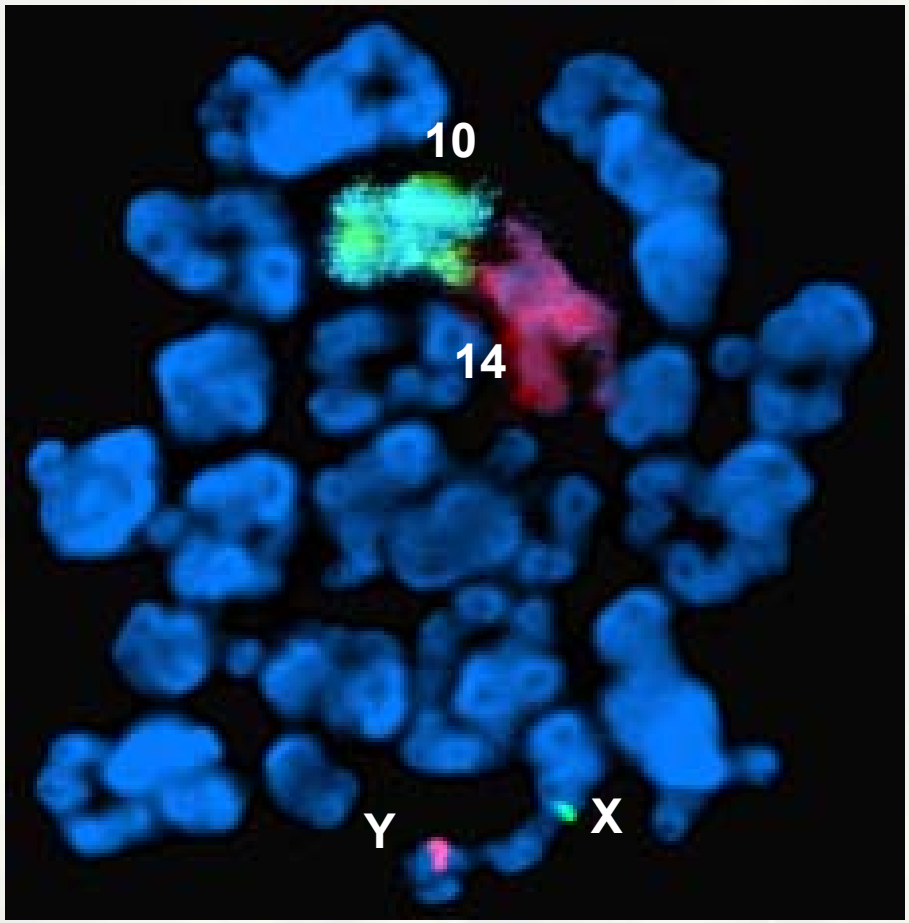
# Segregation analysis methodologies

## Males:

- FISH on testicular tissue

# FISH on testicular tissue: Example

**46,XY,t(10;14)(q24;q32)**

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# Segregation analysis methodologies

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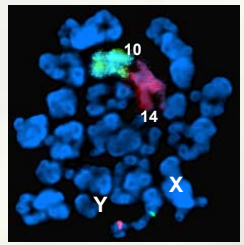


- Heterologous fertilization of hamster oocytes

(Sperm chromosome complements)

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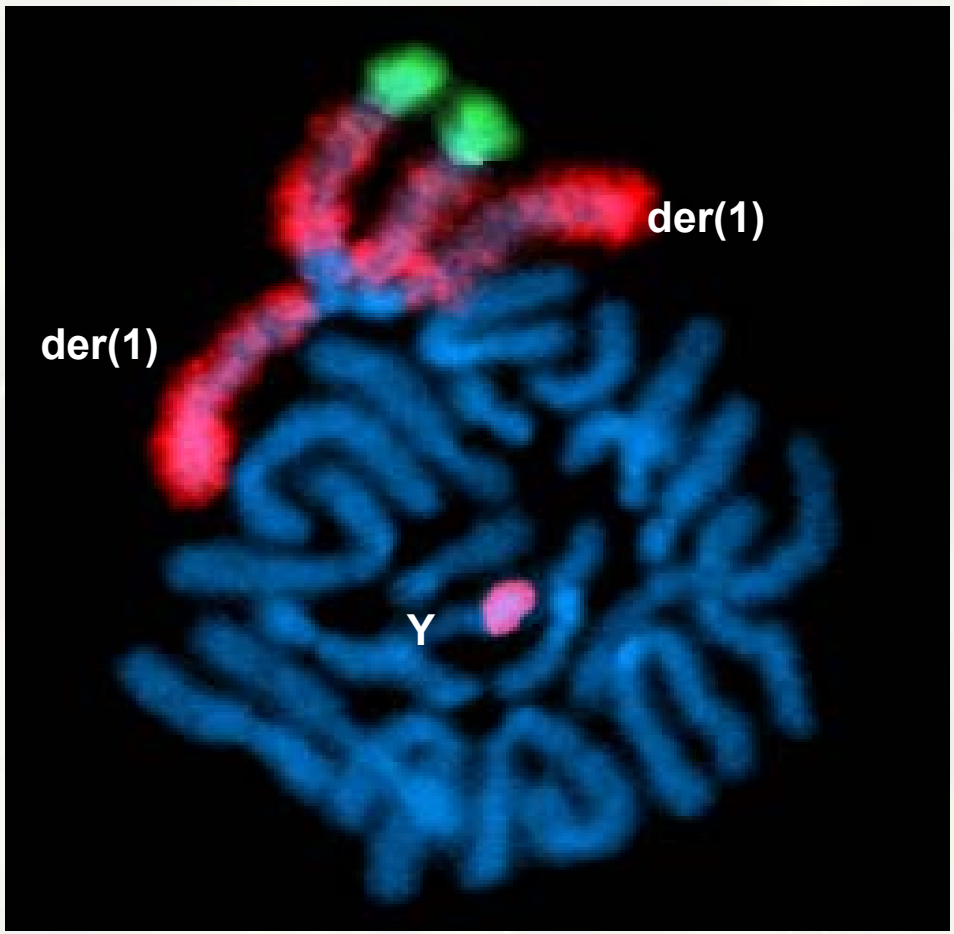
# Sperm chromosome complements: Example

**46,XY,t(1;13)(q41;q22)**

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# Segregation analysis methodologies

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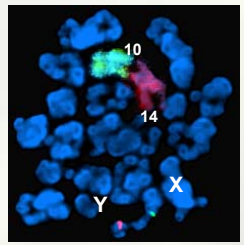


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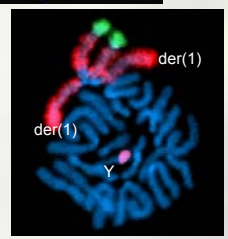


- FISH on decondensed sperm nuclei

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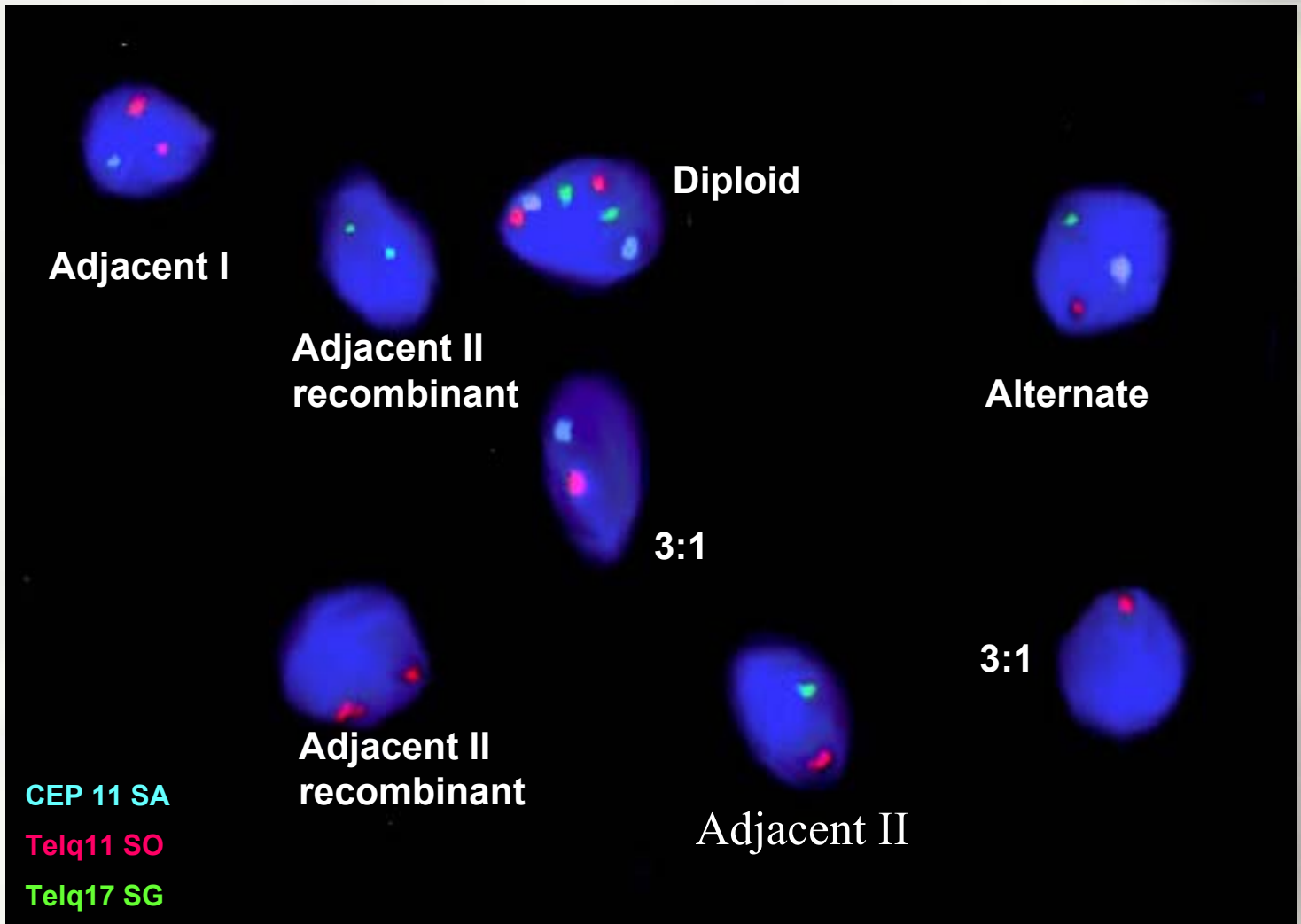
# FISH on decondensed sperm nuclei: Example

**46,XY,t(11;17)(q13.1;p11.2)**

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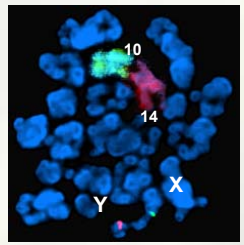
# Segregation analysis methodologies

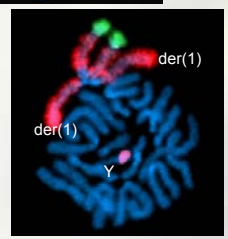
## Males:

- FISH on testicular tissue

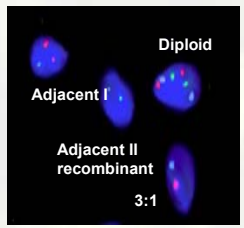



- Heterologous fertilization of hamster oocytes

(Sperm chromosome complements)



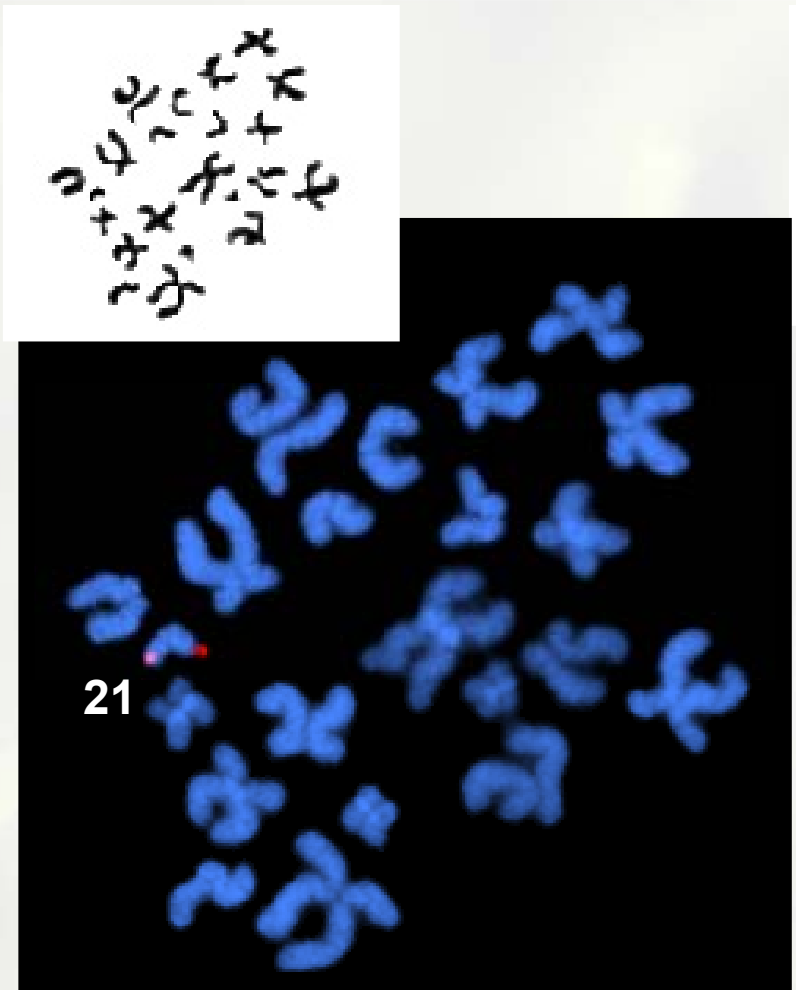

- FISH on decondensed sperm nuclei



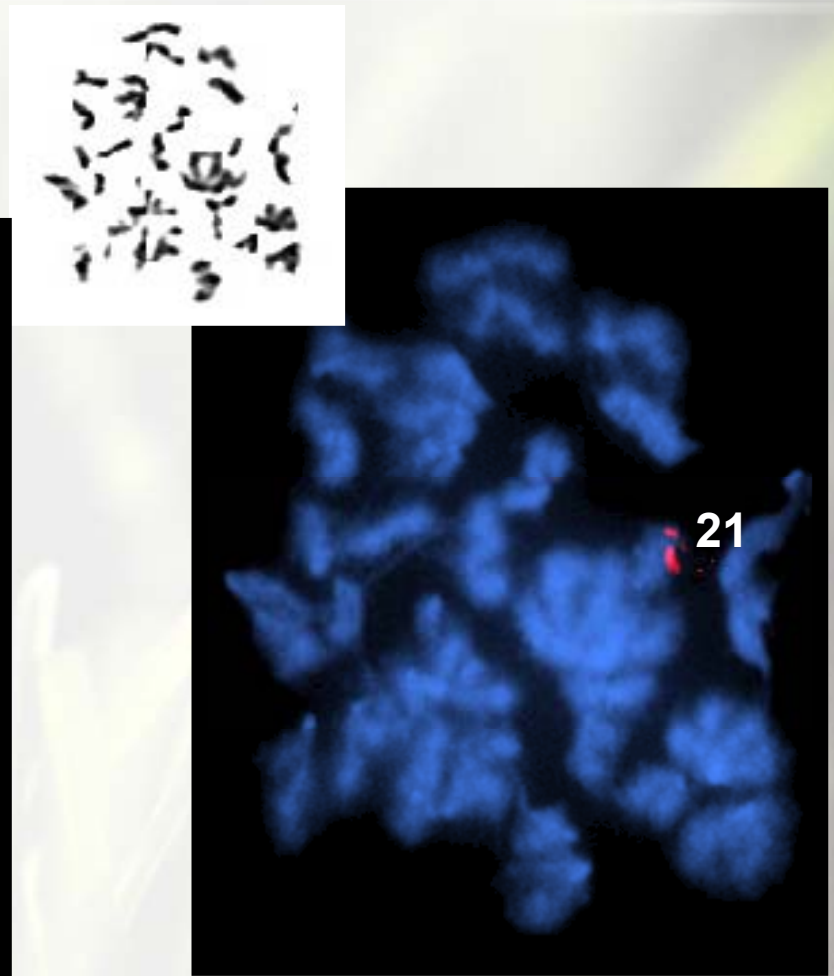

## Females:

- Analysis of the chromosome complement of oocytes and polar bodies

# FISH on oocytes and polar body: Example




Metaphase I I
Chromosome Complement




First Polar Body
Chromosome Complement

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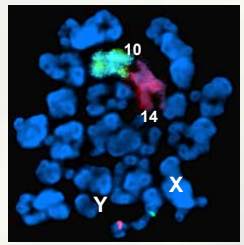
Images provided by M. Durban

# Segregation analysis methodologies

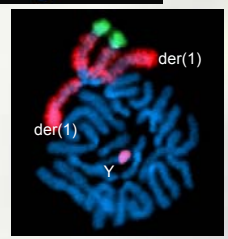
## Males:

- FISH on testicular tissue

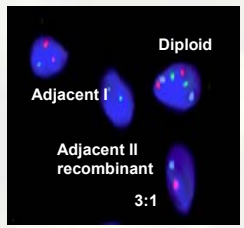



- Heterologous fertilization of hamster oocytes

(Sperm chromosome complements)

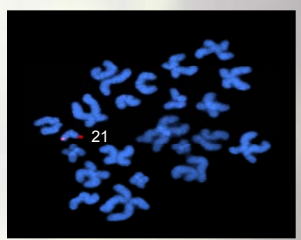



- FISH on decondensed sperm nuclei




## Females:

- Analysis of the chromosome complement of oocytes and polar bodies




- FISH on ovarian tissue from foetuses

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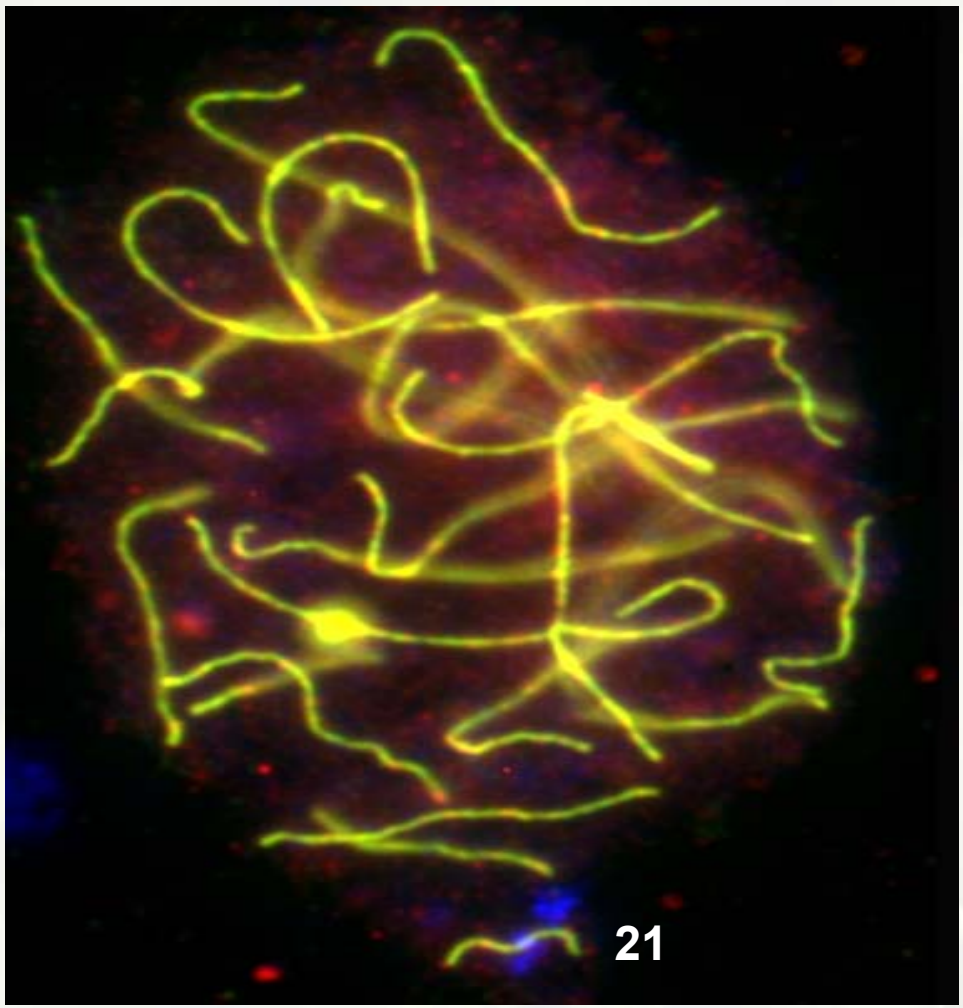
# FISH on ovarian tissue from foetuses : Example




**Pachytene configuration**

— **Cohesin Complex**

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**Images provided by P. Robles and R. Garcia**

# Reciprocal translocations

- Interchange of genetic material between nonhomologous chromosomes
- Any chromosome can be involved in a reciprocal translocation
- Associated with gametogenic failures. Found in:

  0.3-1.2% of infertile males (depending on the study)
  1.2-1.4% of couples asking for IVF by ICSI (depending on the study)

- Largest group in couples ascertained for repeated pregnancy loses
- Genetic risk depends on the chromosomes involved, breakpoints and parental origin

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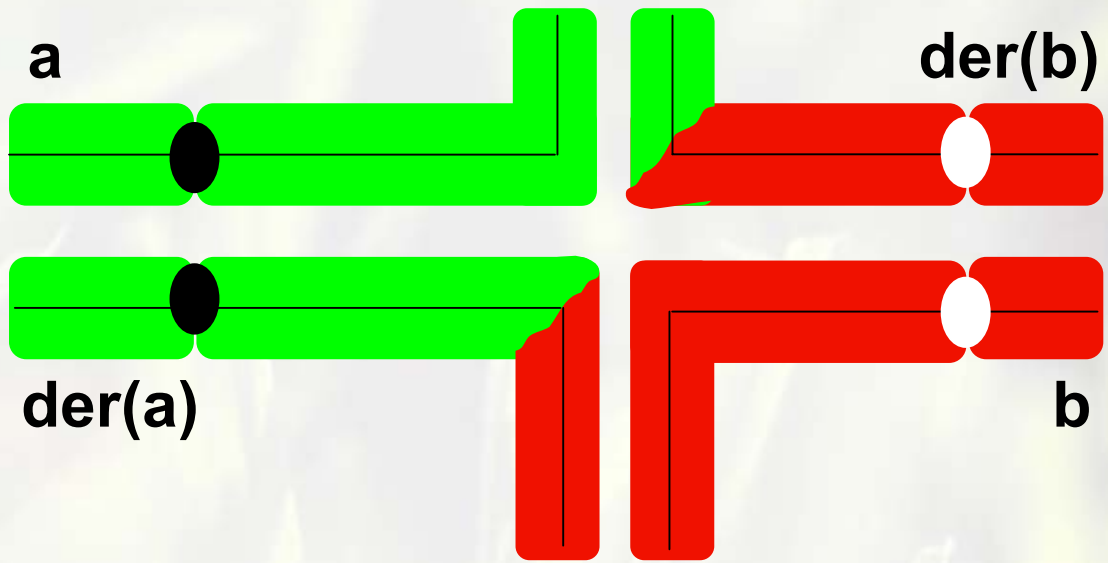
# Reciprocal translocations: meiotic behaviour

## Quadrivalent (pachytene configuration)




Production of balanced and unbalanced gametes depends on the segregation of the quadrivalent

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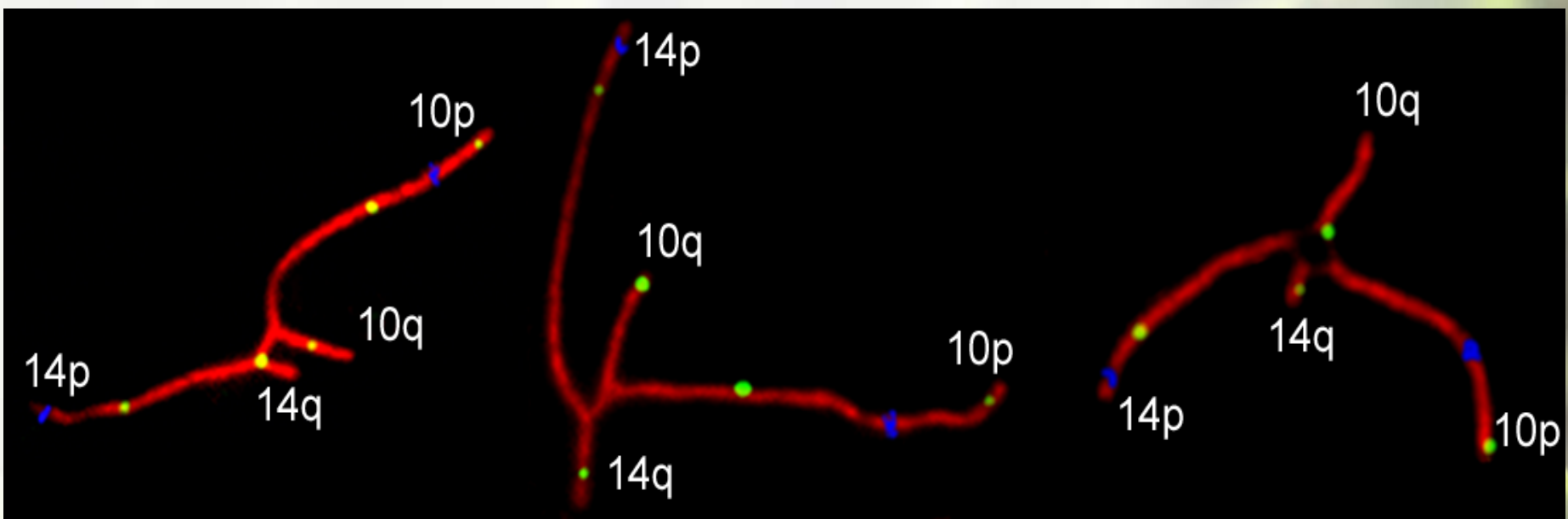
# Reciprocal translocations: meiotic behaviour

## Quadrivalent in a t(10;14)




Synaptonemal Complex (meiotic chromosome “scaffold”)

Centromere

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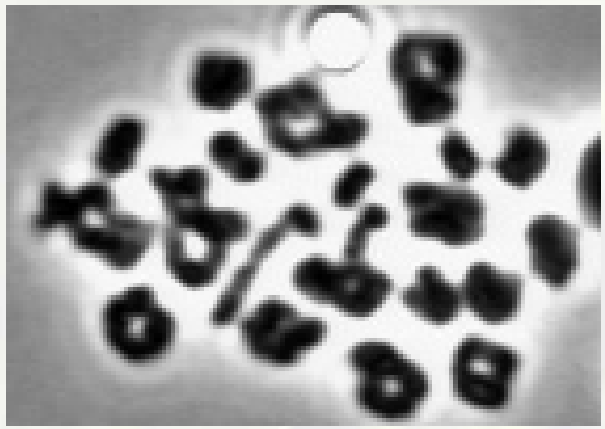
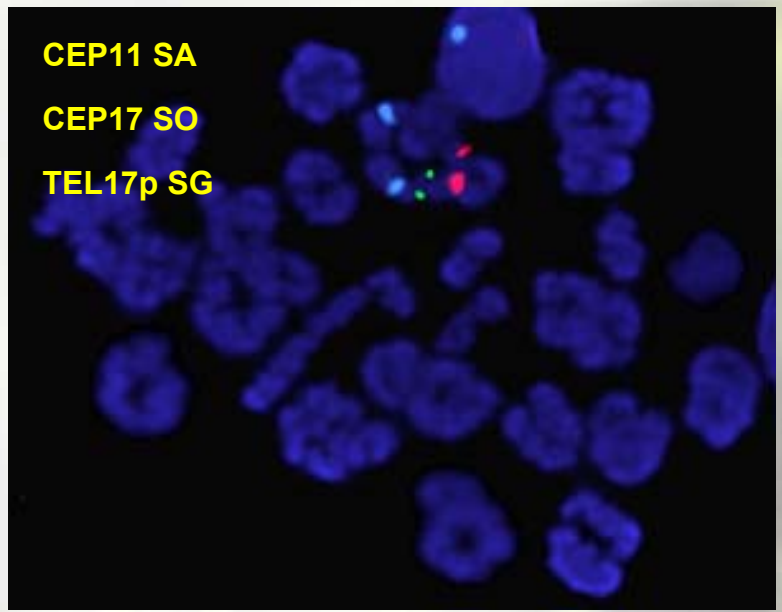
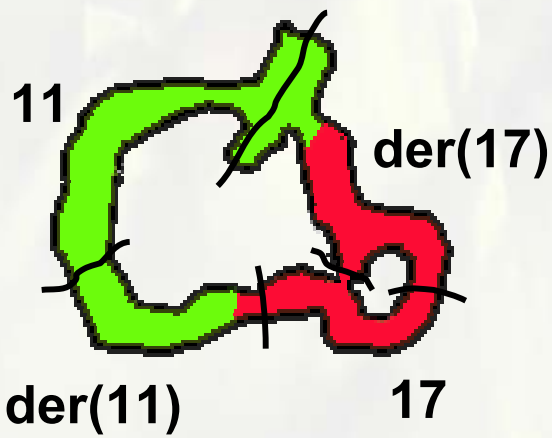
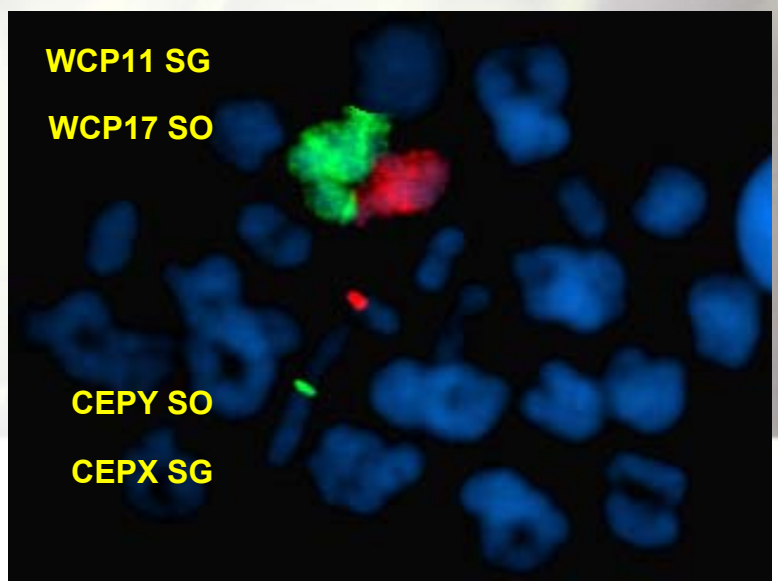
# Reciprocal translocations: meiotic behaviour

**46,XY,t(11;17)(q13.1;p11.2)**



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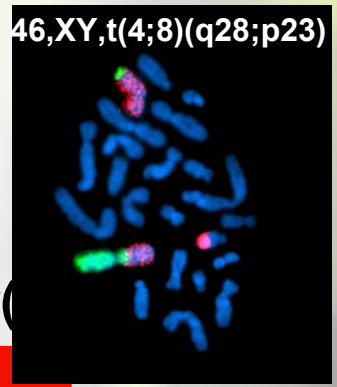
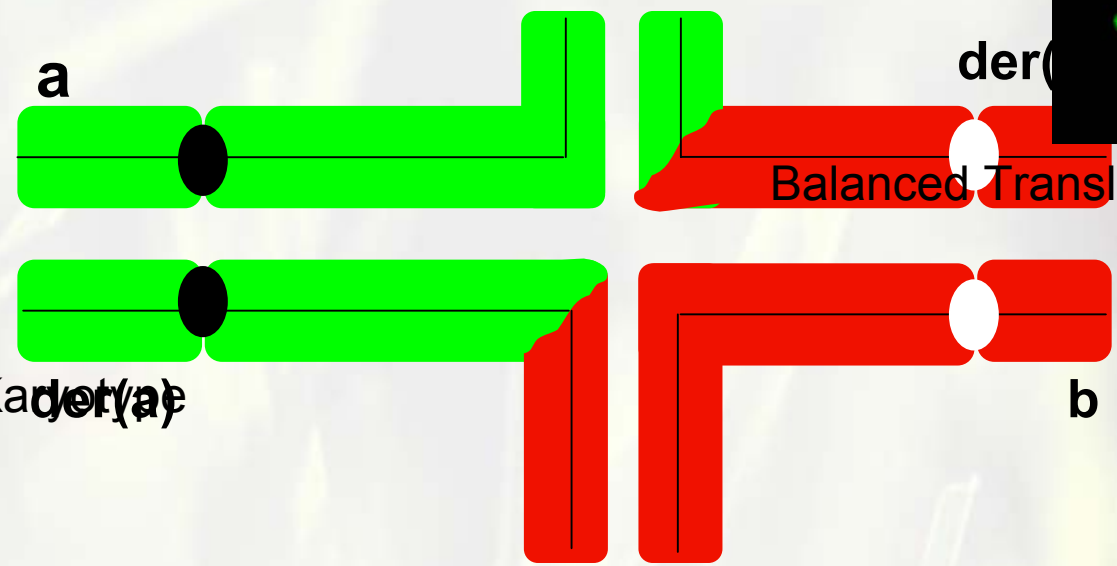
# Reciprocal translocations: meiotic behaviour

2:2 segregation

Alternate




Balanced Translocation Carriers

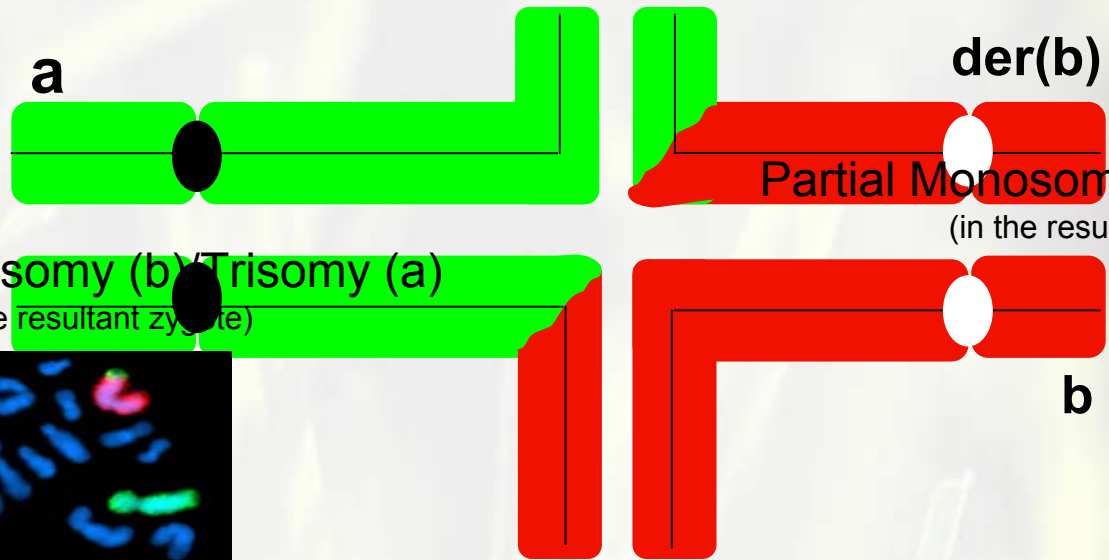
Normal Karyotype

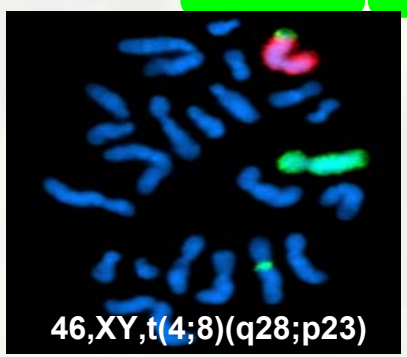
der(a)

# Reciprocal translocations: meiotic behaviour

2:2 segregation

Adjacent 1

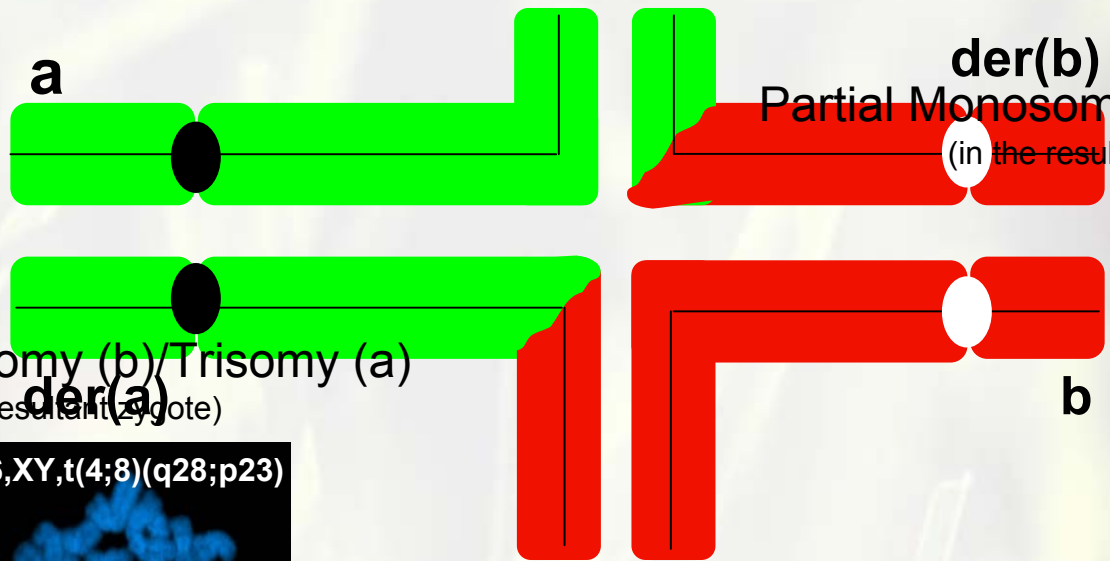


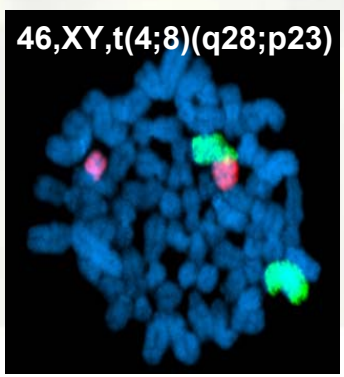



# Reciprocal translocations: meiotic behaviour

2:2 segregation

Adjacent 2

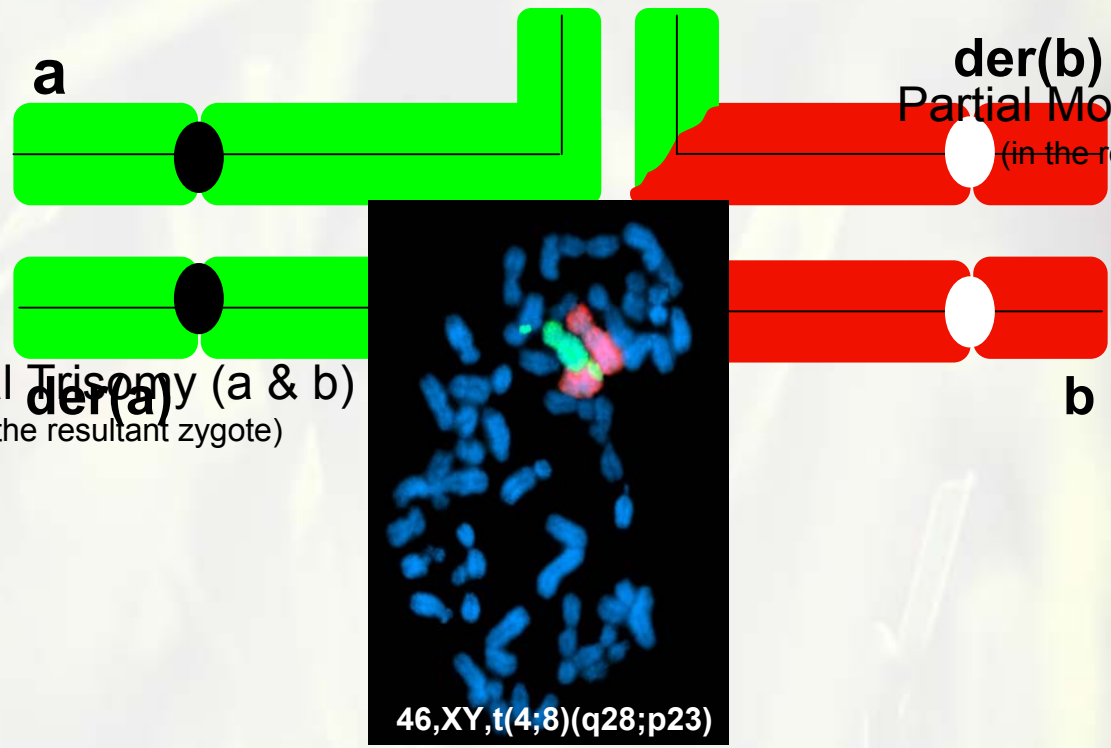


# Reciprocal translocations: meiotic behaviour

3:1 segregation

(example)



# Meiotic segregation in reciprocal translocation carriers

## Percentages of the different segregation modes (average in males)

| Methodology | Alternate | Adjacent 1 | Adjacent 2 | 3:1 |
|---|---|---|---|---|
| Sperm karyotyping | 43.9% | [redacted] | 13.6% | [redacted] |
| FISH on decondensed nuclei | 40.5% | [redacted] | 12.4% | [redacted] |
| Range | 18.6%-77.9% | 3.7%-63.4% | 1.9%-40.1% | 0-46.8% |

Adapted from Benet et al, 2005

**Differences observed between the two methodologies:**

**Technical limitations??**

**Differences in statistical resolution?**

**Sperm selection??**

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# Meiotic segregation in reciprocal translocation carriers

## Segregation modes in females (embryo analysis)

| *Karyotype* | *Alternate** | *Adjacent-1** | *Adjacent-2* | *3 : 1* | *4 : 0* | *cycles* |
|---|---|---|---|---|---|---|
| Females | | | | | | |
| 46,XX,t(1;13)(q23;p11) | 0 | 1 | 0 | 1 | 0 | 1 |
| 46,XX,t(1;19)(q32.1;q13.1) | 11 | 1 | 3 | 6 | 0 | 4 |
| 46,XX,t(2;4)(p22.2;q33) | 2 | 5 | 0 | 0 | 0 | 1 |
| 46,XX,t(3;5)(p12;q14.2) | 2 | 1 | 0 | 0 | 0 | 1 |
| 46,XX,t(4;15)(q26;q13) | 0 | 0 | 0 | 1 | 0 | 1 |
| 46,XX,t(5;14)(p15.1;q32.1) | 4 | 2 | 0 | 3 | 0 | 1 |
| 46,XX,t(8;18)(p21.1;q21.1) | 2 | 0 | 0 | 0 | 0 | 1 |
| 46,XX,t(9;20)(q34.2;q11.2) | 5 | 3 | 0 | 0 | 0 | 1 |
| 46,XX,t(11;17)(p15.5;p13) | 9 | 7 | 0 | 5 | 1 | 3 |
| 46,XX,t(11;22)(q23.3;q11.2) | 0 | 0 | 0 | 2 | 1 | 1 |
| 46,XX,t(12;17)(p13;p13) | 11 | 4 | 0 | 2 | 0 | 2 |
| 46,XX,t(14;22)(q11.2;q13.3) | 6 | 0 | 3 | 1 | 0 | 1 |
| | 52 | 24 | 6 | 21 | 2 | 18 |
| | | | 5.7% | 20.0% | 1.9% | |
| Total embryos | 105 | | | | | |

Adapted from Ogilvie and Scriven, 2002

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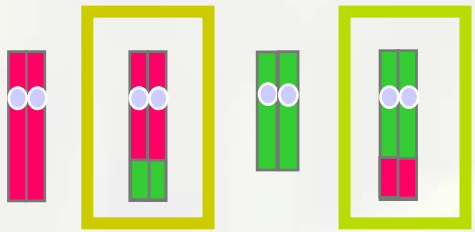
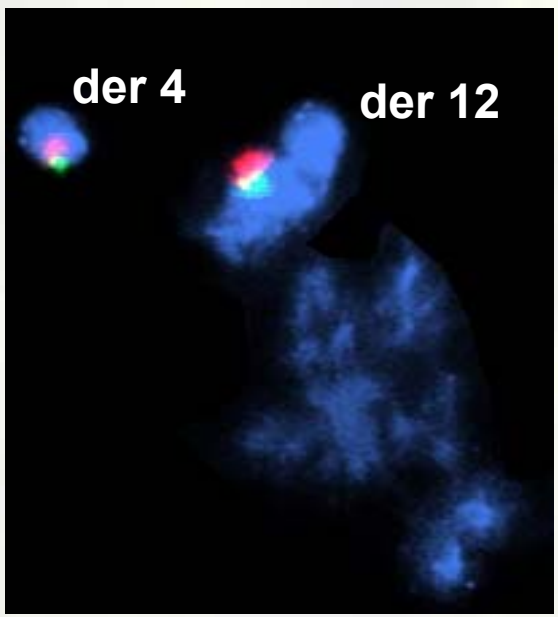
# DGP-1CP in a female carrier of a reciprocal translocation

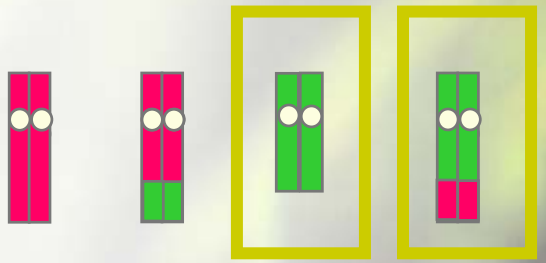
**46,XX,t(4;12) (q22;q23)**



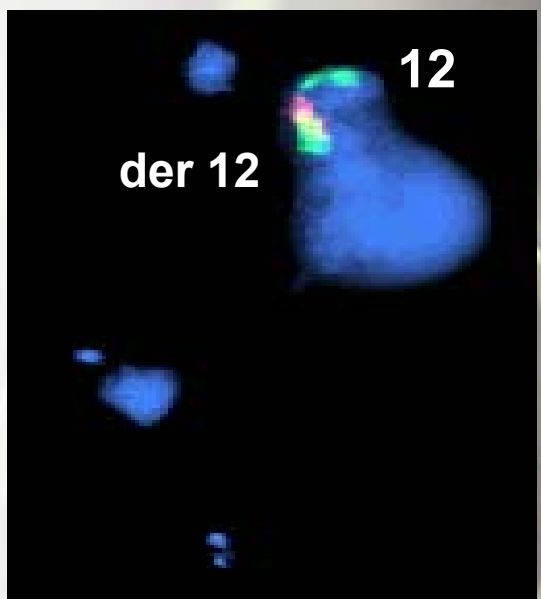
4 der4 12 der 12




Alternate



4 der 4 12 der 12




Adjacent 2

Durban et al, 2001

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# Genetic counselling: Reciprocal translocation carriers

- Cytogenetic characteristics of each translocation will determine the percentage of unbalanced gametes
- Monosomies and trysomies produced will determine the risk for the offspring
- Number and position of recombination events within the interstitial segments determines the presence of dimorhic chromatids (increased risk)
- Integration of meiotic analysis results in genetic counselling (personalized risk assesment)
- PGD or other antenatal diagnosis can also be proposed to patients

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# Robertsonian translocations

- Translocation between acrocentric chromosomes. Long arms fuse at centromere and short arms are lost

- Balanced translocation carriers have only 45 chromosomes (normal phenotype)

- Most common combinations are t(13;14) and t(14;21)

- Represent  aprox. 5% of Down syndrome cases

- Male translocation carriers have often fertility problems (oligoasthenoteratozoospermia)

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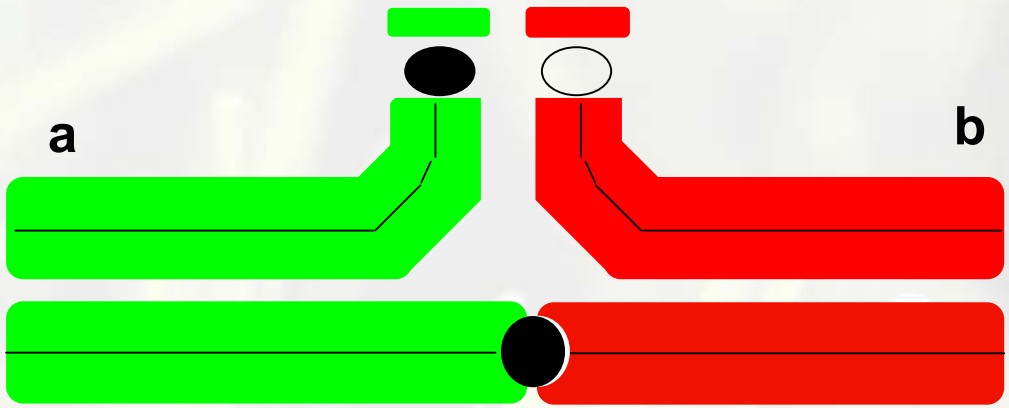
# Robertosnian translocations: meiotic behaviour

## Trivalent (pachytene configuration)




der (a;b)

# Robertsonian translocations: meiotic behaviour

## Trivalent in a 45XY t(13;14)

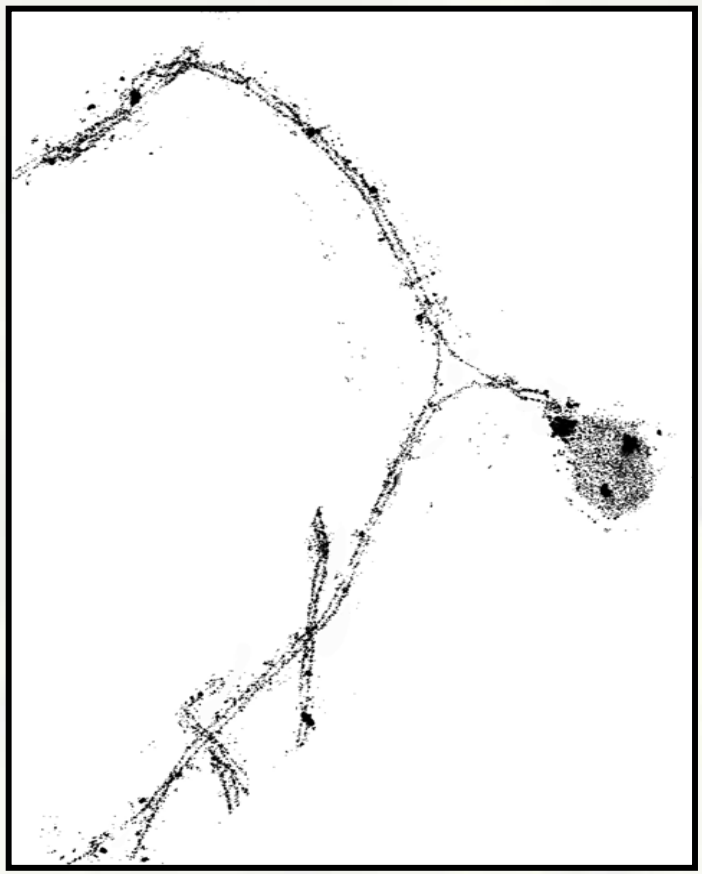
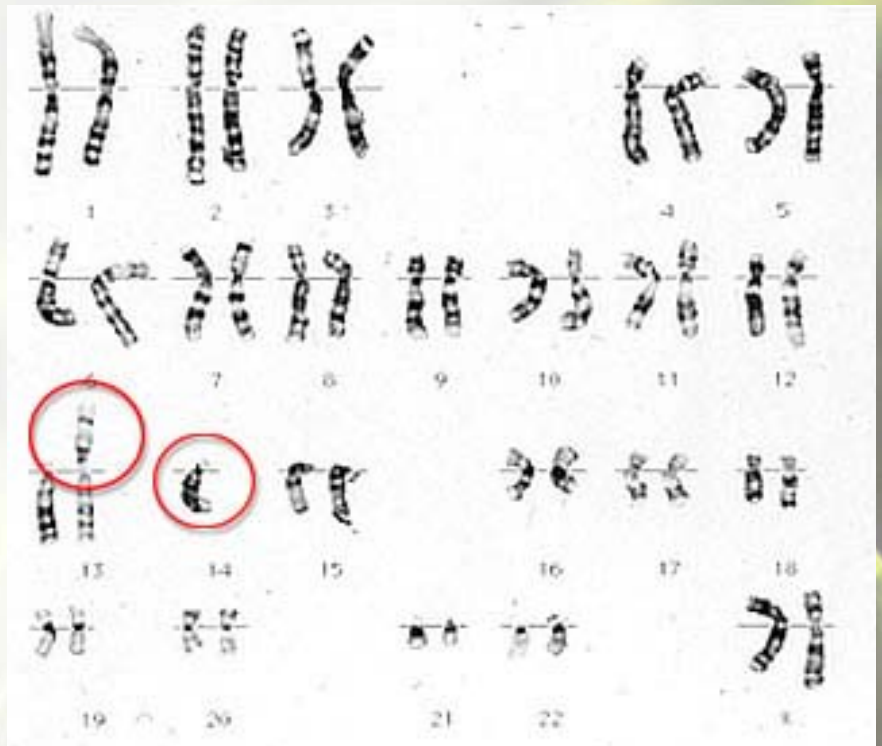


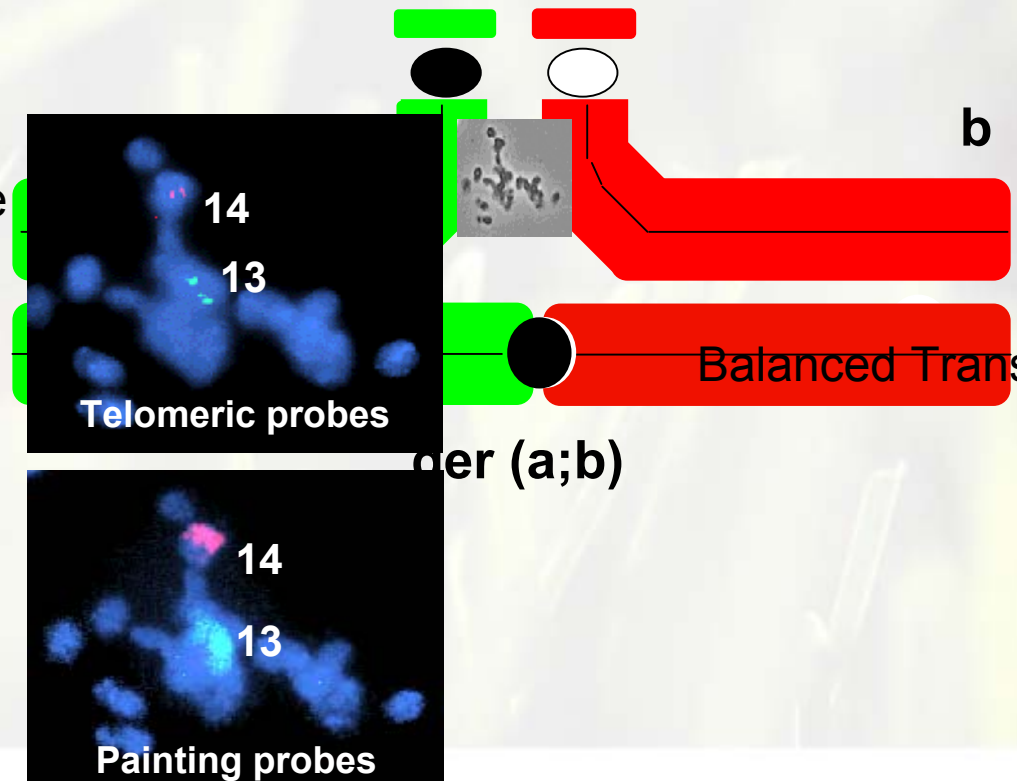
Image provided by J. Navarro



# Robertsonian translocations: meiotic behaviour

2:1 segregation

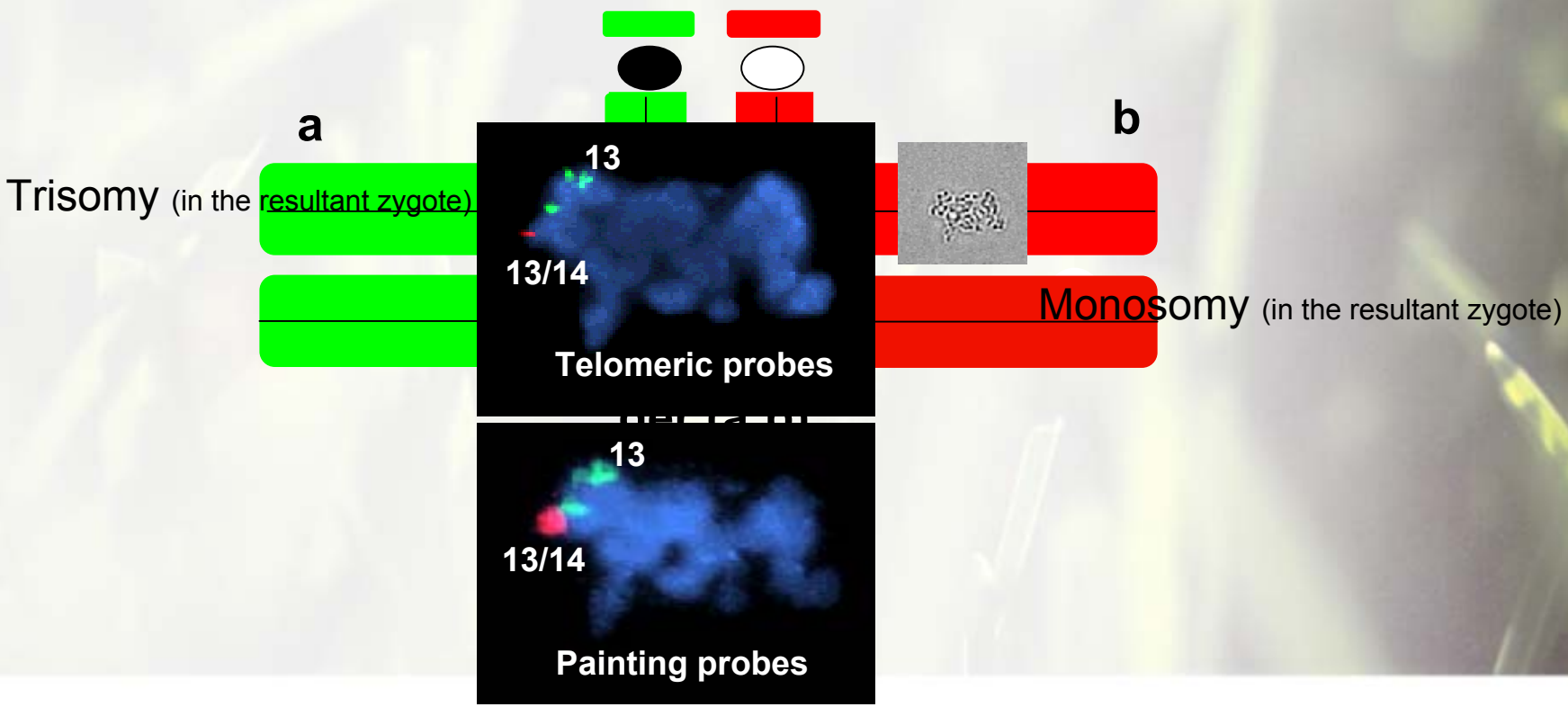
Alternant



# Robertsonian translocations: meiotic behaviour

2:1 segregation

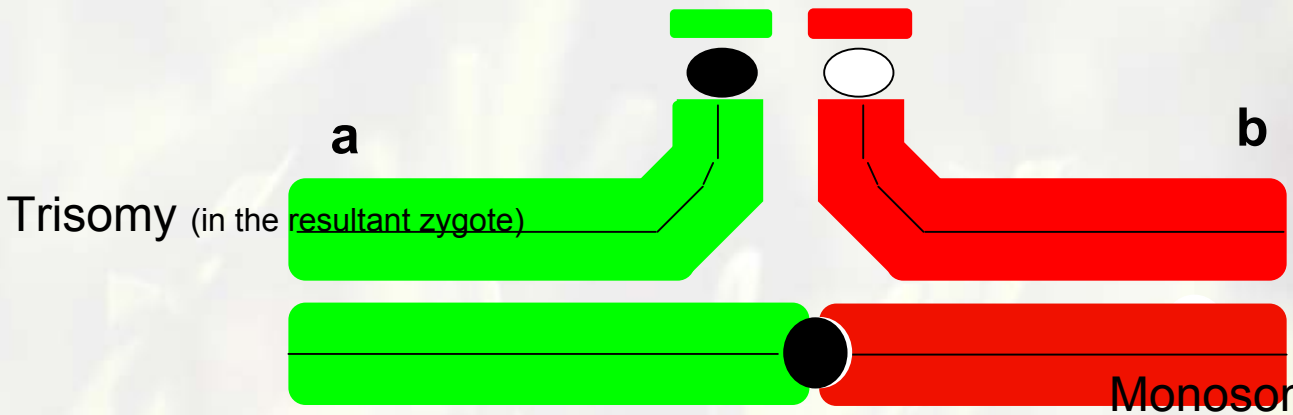
Adjacent



# Robertsonian translocations: meiotic behaviour

2:1 segregation

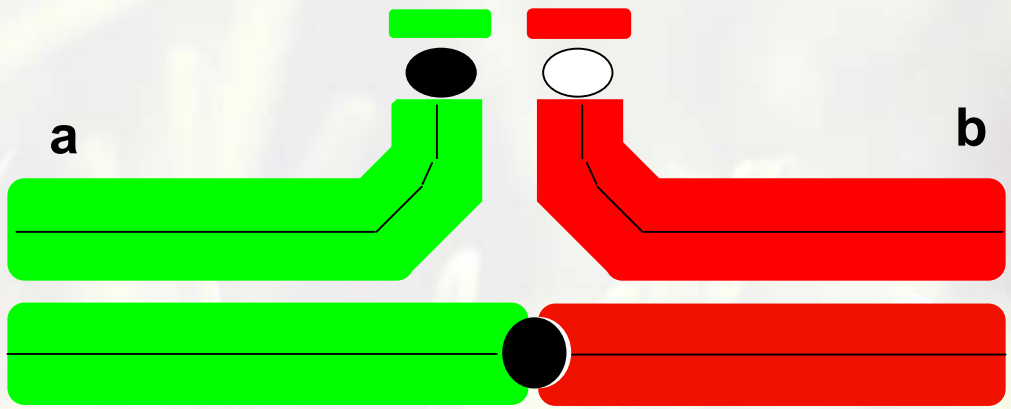
Adjacent




**der (a;b)**

# Robertsonian translocations: meiotic behaviour

3:0 segregation




**der (a;b)**

Trisomy (in the resultant zygote)

# Meiotic segregation in Robertsonian translocation carriers

## Percentages of the different segregation modes (average in males)

| Translocation | Alternate | Adjacent | 3:0 (or diploid) |
|---|---|---|---|
| t(13;14) | 85.1% | 14.2% | 0.7% |
| t(14;21) | 87.5% | 10.4% | 0.5% |
| t(14;22) | 81% | 18.3% | 0.6% |
| Total | 84.3% | 14.3% | 0.6% |

Adapted from Roux et al, 2005 and Moradkhani et al, 2006

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# Meiotic segregation in Robertsonian translocation carriers

**Percentages of the different segregation modes (average in females)**

| | Number of cases | Normal | Unbalanced |
|---|---|---|---|
| Robertsonian translocations | 15 | 60.6% | 39.4% |

Adapted from Durban et al, 2001

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# Genetic counselling: Robertsonian translocation carriers

- Similar behaviour in all types of Robertsonian translocation
- Strong prevalence of alternate segregation
- Rare Robertsonian translocations, like t(14;22), t(13;15) or t(15;22), seem to have a higher propensity for producing imbalances through meiosis
- Implications in maternal and parental UDP (imprinting):

  risk estimated around 0.5%
- Integration of meiotic segregation analysis results in genetic counselling (personalized risk assesment)
- PGD or other antenatal diagnosis can also be proposed to patients

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# Inversions

- Consist of two breaks in one chromosome. The area between the breaks is inverted (turned around), reinserted and the breaks are repared

- This reorentation does not usually affect the function of the genes present in the area (normal phenotype)

- Reproductive capacity can be compromised (meiotic disturbances due to the presence of the inversion)

- If the inverted area includes the centromere it is called a PERICENTRIC inversion

- If it does not, it is called a PARACENTRIC inversion

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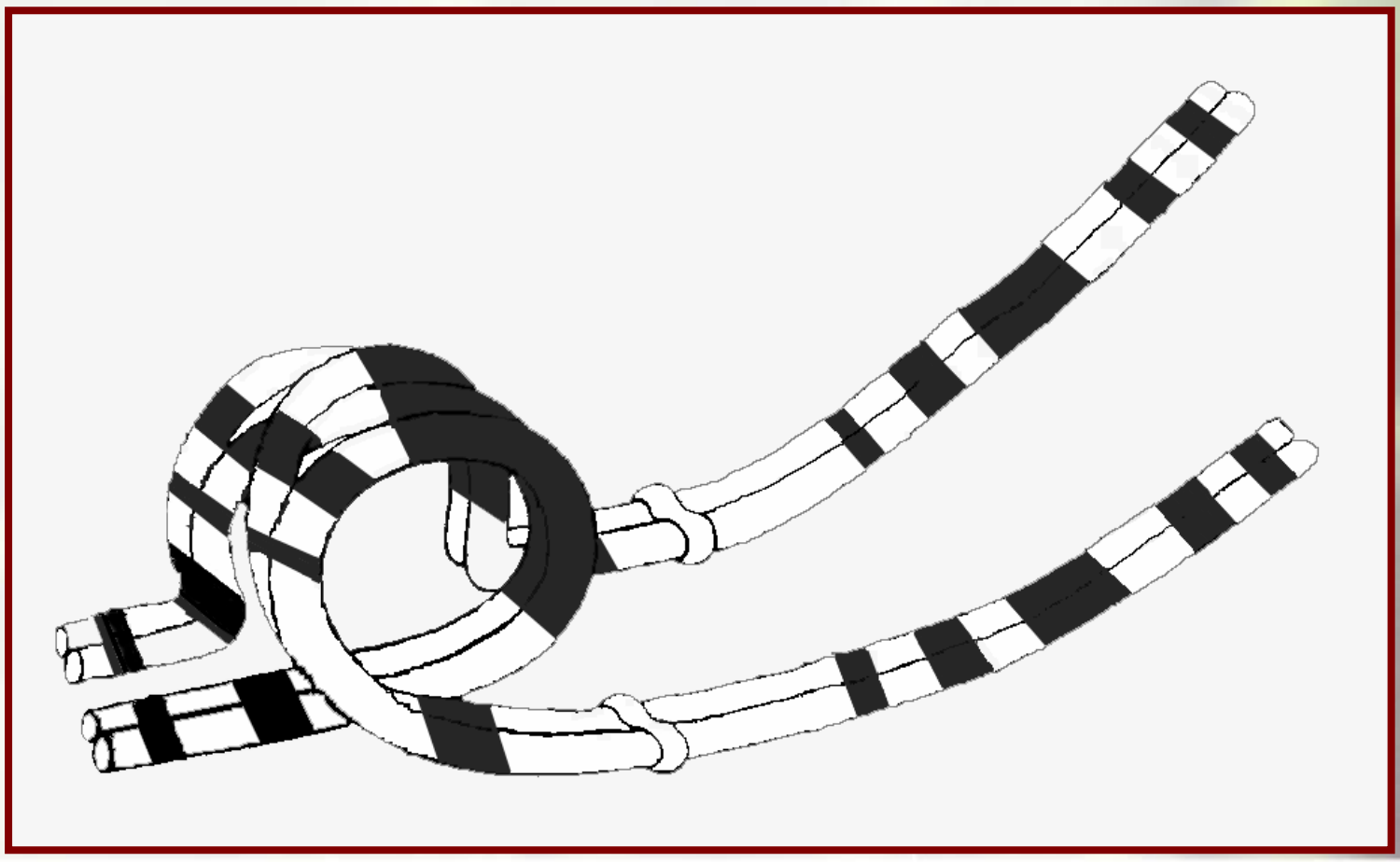
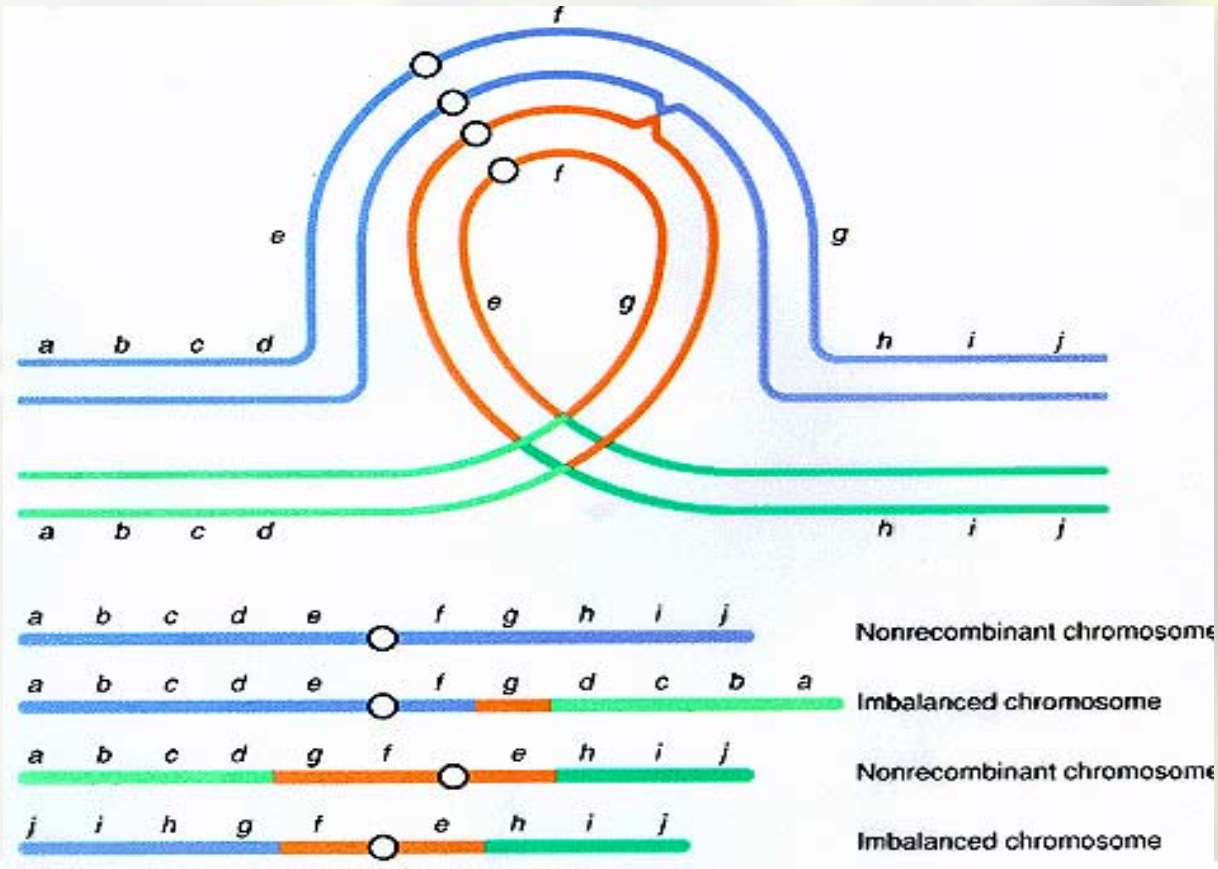
# Inversion: meiotic behaviour

## Inversion Loop
(not always)



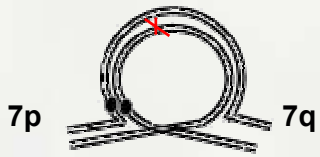
# Pericentric inversion: meiotic behaviour

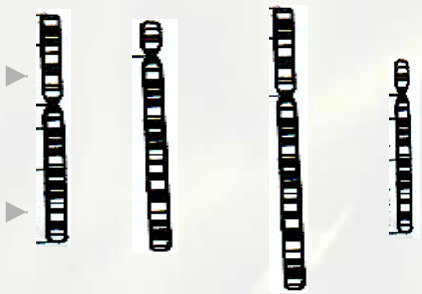
**In a PERICENTRIC inversion, a crossover within the inversion loop results in the formation of recombinant chromosomes with duplication/deletions of the material distal to the inversion breakpoints**

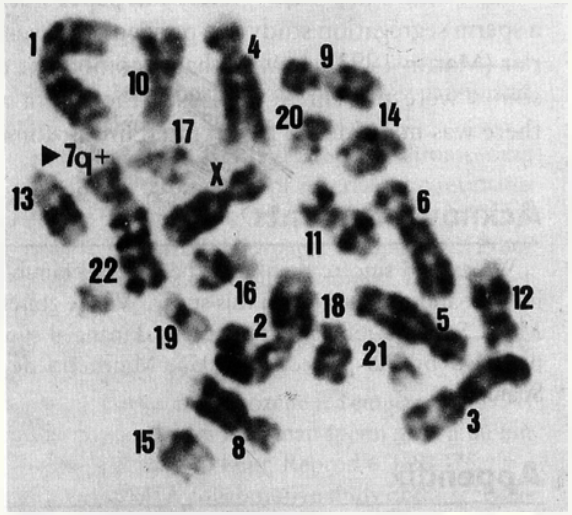


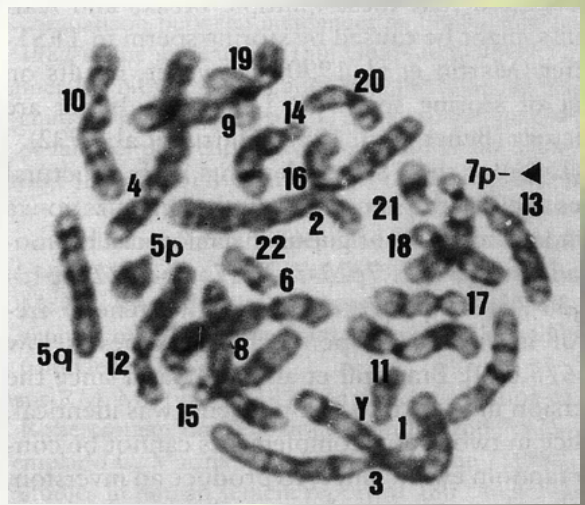
# Pericentric inversion: meiotic behaviour

46XY inv(7)(p13;q36)



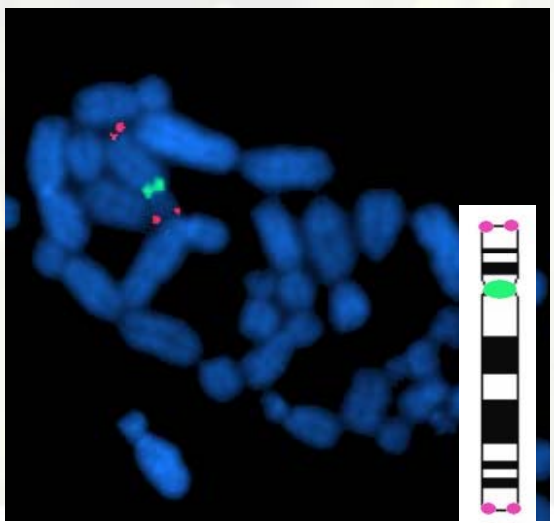



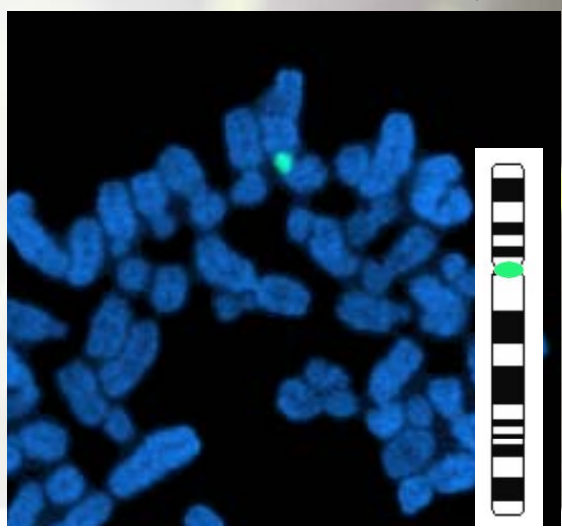




Navarro et al, 1993

CEP 7

Tel 7q





Images provided by P. Cifuentes

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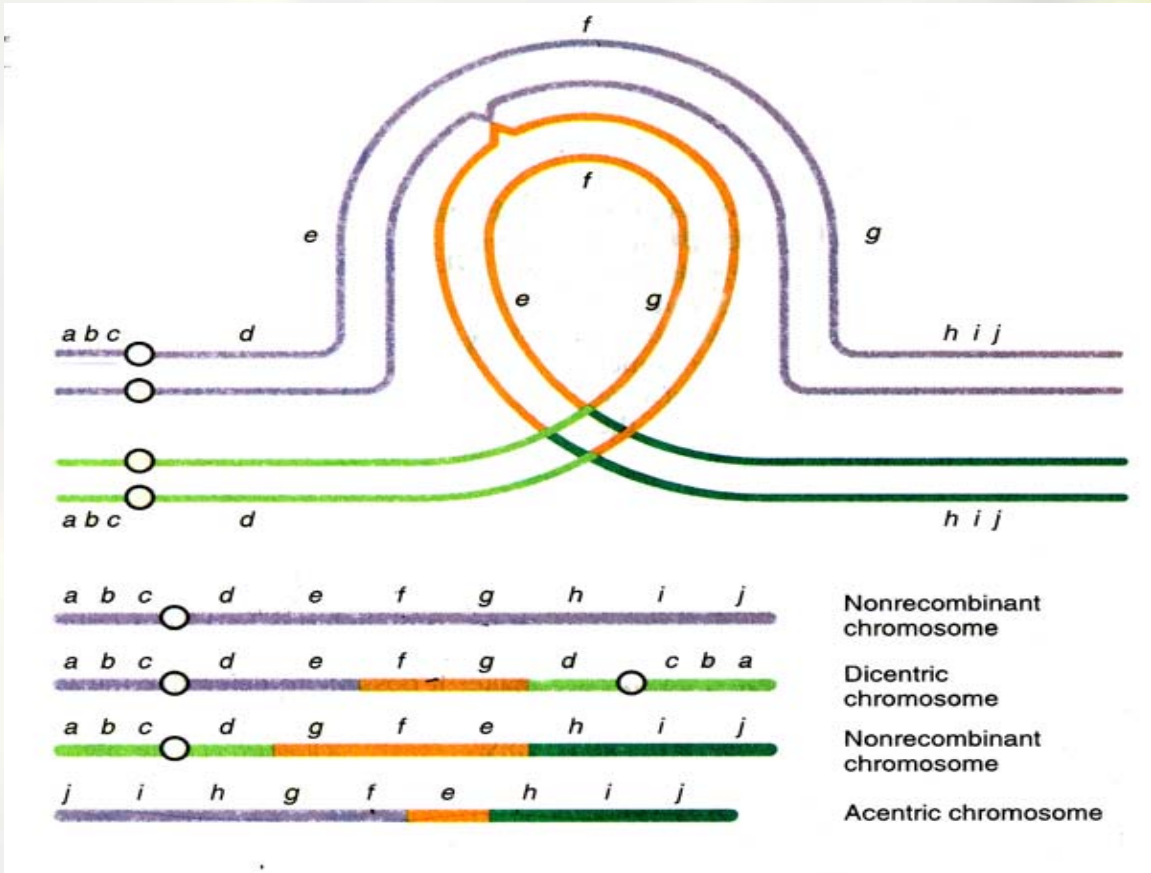
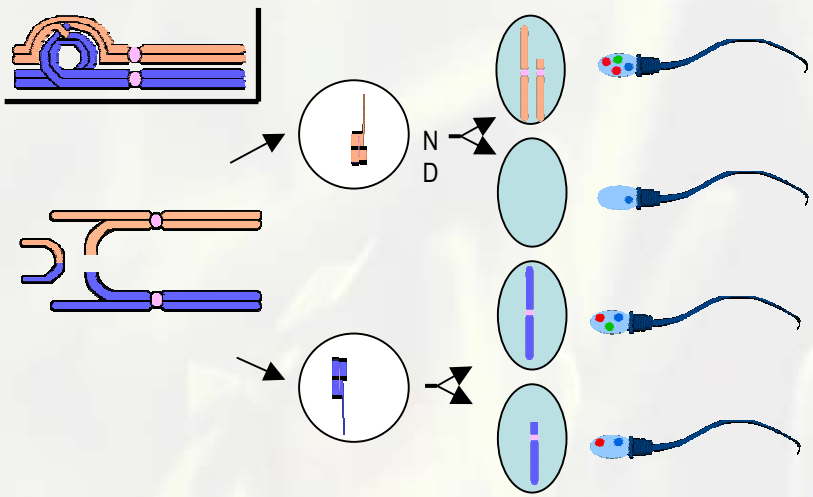
# Paracentric inversion: meiotic behaviour

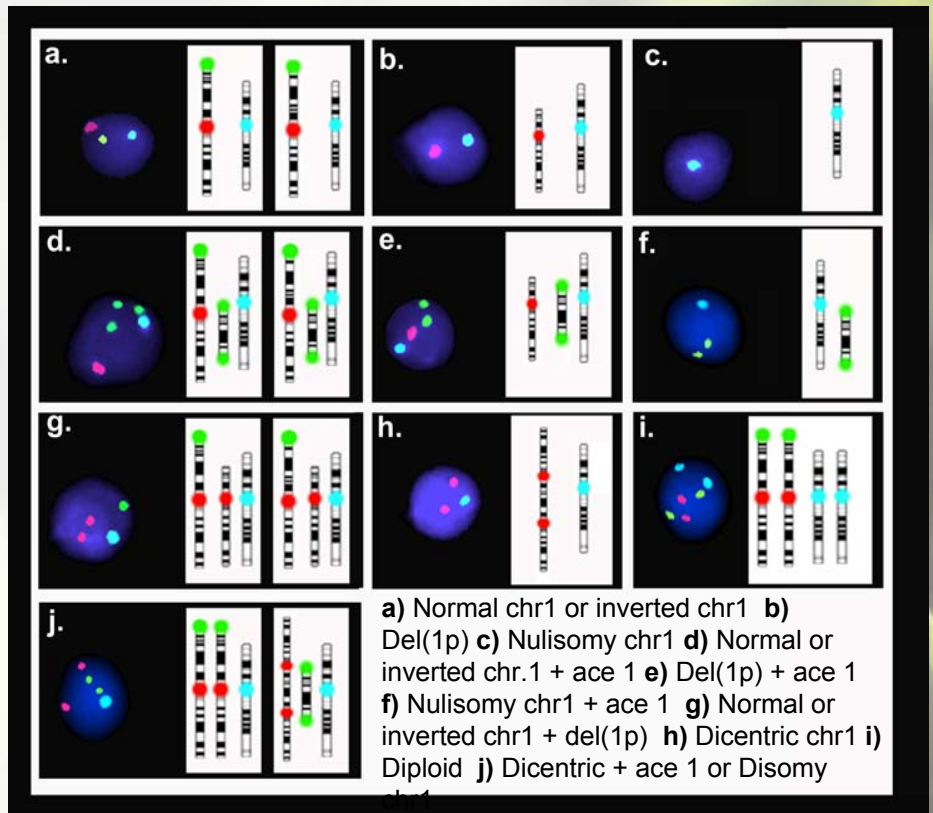
**In a PARACENTRIC inversion, a crossover within the inversion loop results in the formation of an acentric fragment and a dicentric recombinant chromosome**



# Paracentric inversion: meiotic behaviour

**46XY inv(1)(p22;p34.3)**




**a)** Normal chr1 or inverted chr1 **b)** Del(1p) **c)** Nulisomy chr1 **d)** Normal or inverted chr.1 + ace 1 **e)** Del(1p) + ace 1 **f)** Nulisomy chr1 + ace 1 **g)** Normal or inverted chr1 + del(1p) **h)** Dicentric chr1 **i)** Diploid **j)** Dicentric + ace 1 or Disomy chr1

**Images provided by M. Costa**

# Meiotic segregation in pericentric inversion carriers

| | Inversion | nº Sperm | % rec. | Crom. Length (Mpb) | Inv. Length (Mpb) | Inv. Length % | Analysis |
|---|---|---|---|---|---|---|---|
| Balkan et al, 1983 | (3)(p11q11) | 111 | 0 | 200 | 7 | 3.5 | Humster |
| Martin, 1991 | (3)(p25q21) | 144 | | | | | Humster |
| Martin, 1993 | (8)(p23q22) | 166 | 11.4 | 146 | 105 | 71.92 | Humster |
| Jenderny et al, 1992 | (20)(p13q11.2) | 26 | 0 | 63 | 37 | 58.73 | Humster |
| Navarro et al, 1993 | (7)(p13q36) | 140 | | | | | Humster |
| Martin et al, 1994 | (1)(p31q12) | 159 | 0 | 246 | 75 | 30.49 | Humster |
| Colls et al, 1997 | (9)(p11q13) | 314 | 0 | 136 | 20 | 14.71 | Humster |
| Jaarola et al, 1998 | (1)(p31q12) | 6006 | 0.38 | 246 | 75 | 30.49 | FISH |
| | (8)(p23q22) | 3168 | 13.10 | 146 | 105 | 71.92 | FISH |
| Anton et al, 2002 | (6)(p23q25) | 10049 | 37.85 | | | | FISH |
| Yakut et al, 2003 | (1)(p34q21) | 1636 | 16 | 246 | 118 | 47.97 | FISH |
| Mikhaail-Philips et al, 2004 | (2)(p23q33) | 1005 | | | 180 | | FISH |
| Anton et al, 2006 | (2)(p11.2q13) | 5460 | 0 | 243 | 29 | 11.93 | FISH |
| | (4)(p16q21) | 6406 | 0.78 | 191 | 85 | 44.50 | FISH |
| | (10)(p13q22.3) | 10723 | 3.41 | 135 | 65 | 48.15 | FISH |

**Adapted from Anton et al, 2005**

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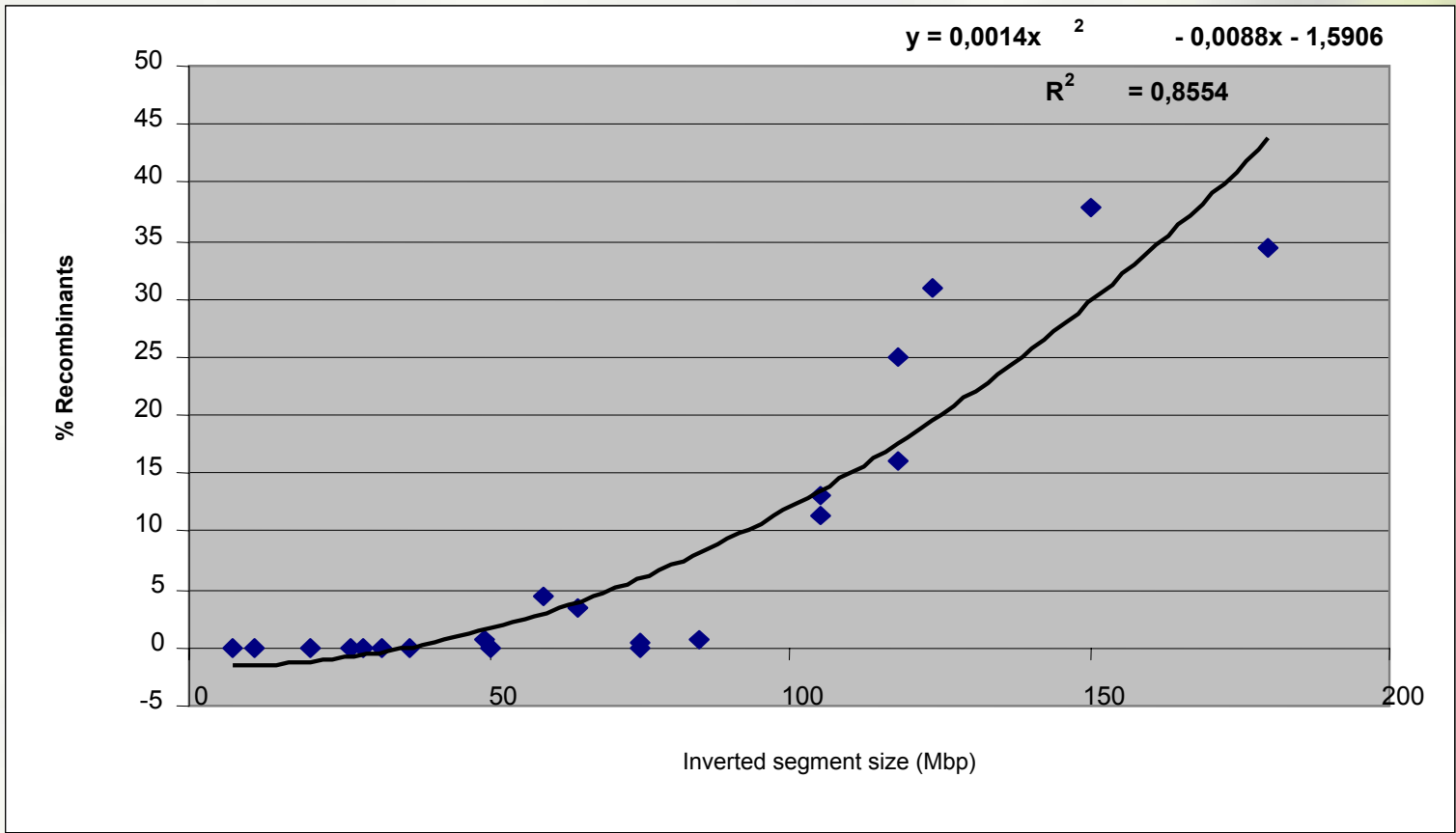
# Meiotic segregation in pericentric inversion carriers




Adapted from Anton et al, 2005

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# Meiotic segregation in paricentric inversion carriers

| Paracentric Inversions | **Inversion** | **nº Sperm** | **% rec.** | **Crom. Length (Mpb)** | **Inv. Length (Mpb)** | **Inv. Length %** | Analysis |
|---|---|---|---|---|---|---|---|
| Martin, 1986 | (7)(q11q22) | 94 | 0 | 158 | 50 | 31.65 | Humster |
| Brown et al, 1998 | (9)(q32q34.3) | 282 | 0 | 136 | 32 | 23.53 | sperm-typ. |
| Martin, 1999 | (14)(q24.1q32.1) | 120 | 0 | 105 | 27 | 25.71 | Cariotip |
| Devine et al, 2000 | (2)(q14.2q24.3) | 496 | 0.81 | 243 | 49 | 20.16 | FISH |
| Anton et al, 2006 | (4)(p14p15.3) | 8158 | 0.03 | 191 | 11 | 5.76 | FISH |
| Costa et al, (unpublished) | (1)(p22p34.3) | 11076 | 4,5 | 246 | 59 | 24 | FISH |

**Adapted from Anton et al, 2005**

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# Genetic counselling: Inversion carriers

- Cytogenetic characteristics of each inversion will determine the percentage of unbalanced gametes
- Percentage of unbalanced gametes depends on the inverted segment size
- Genetic content of the non inverted segments (deletions and duplications)
- Paracentric inversions have a better prognosis (recombination products are highly deleterious)
- Integration of meiotic segregation analysis results in genetic counselling (personalized risk assesment)
- PGD or other antenatal diagnosis can also be proposed to patients

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# Interchromosomal Effects (ICE)

- Influence from structural chromosome reorganizations in the segregation of other chromosomes
- Related to the presence of unpaired regions during pachytene
- They have been described in:
  - Reciprocal translocation carriers
  - Robertsonian translocation carriers
  - Inversion carriers (1 study)
- Two studies relate the presence of ICE with poor semenograms (Pellestor et al, 2001 and Van Asche et al, 2002)

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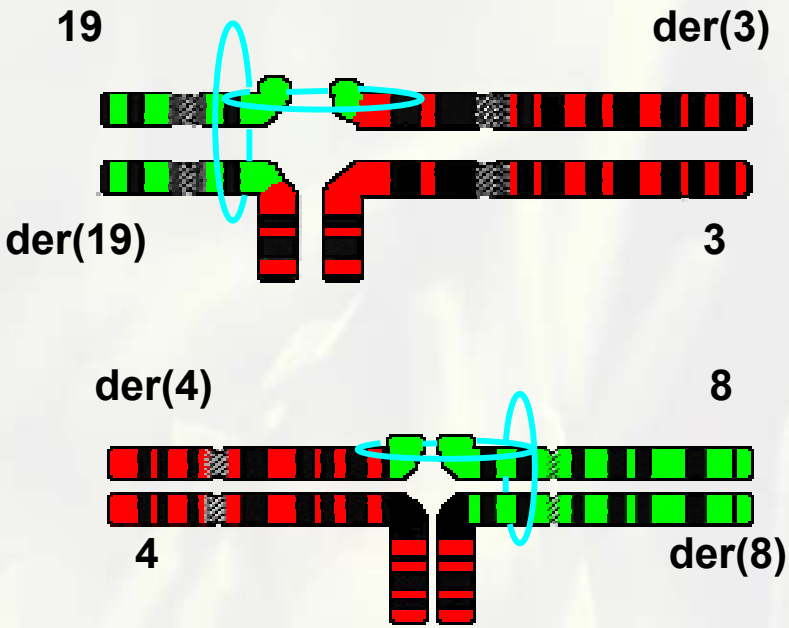
# Interchromosomal Effects (ICE)

## Morphological characteristics




Short interstitial segments

Small translocated fragments

- Positive ICE results should be considered in the genetic counselling

# Conclussions

- The techniques developed so far allow for an exhaustive study in infertile patients (males and females)

- The variability observed in the meiotic behaviour of the chromosome reorganizations makes necessary an individual risk assesment previous to the genetic counselling

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# Acknowledgements

## Unitat de Biologia Cel·lular i Genètica Mèdica