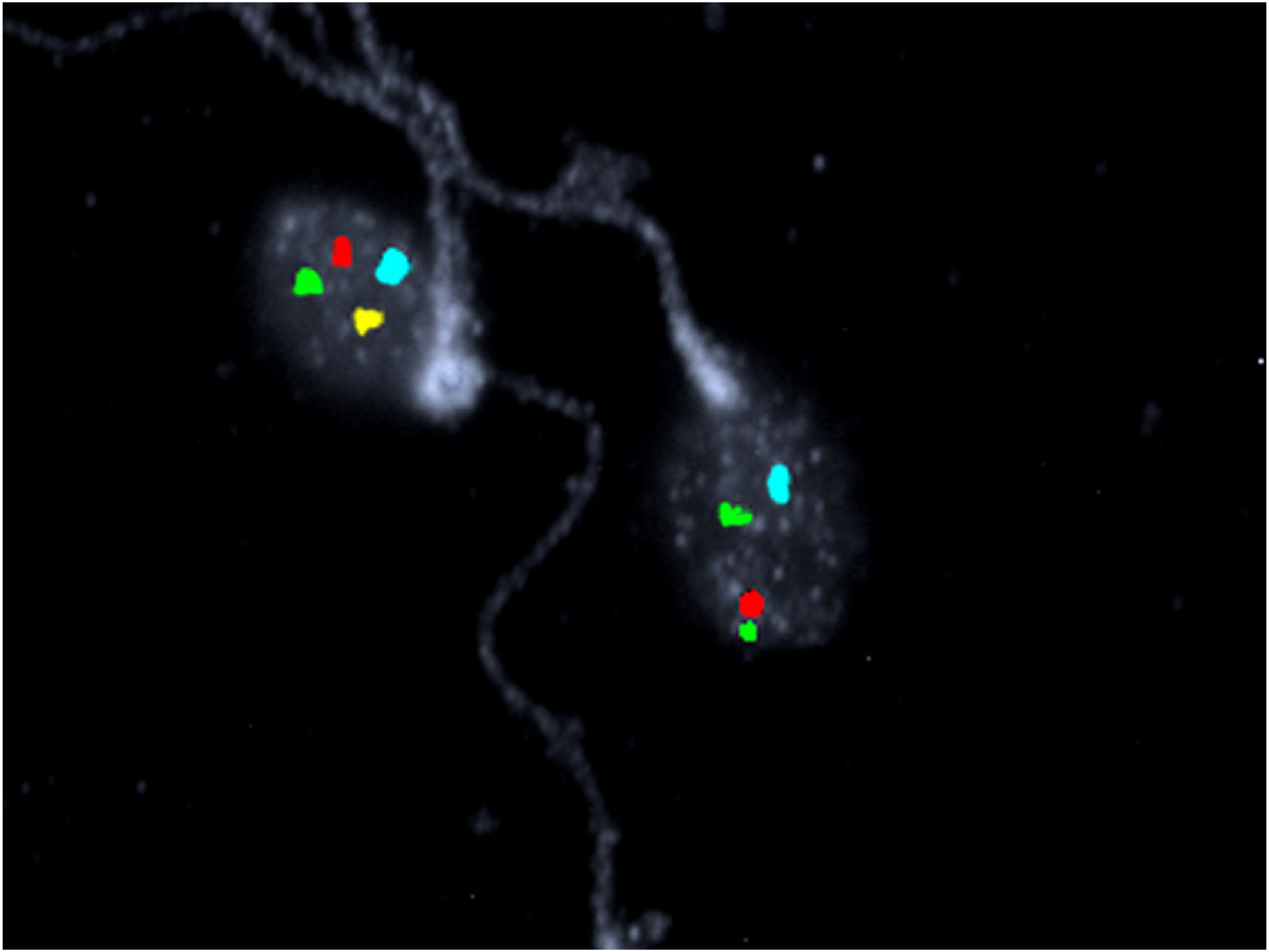
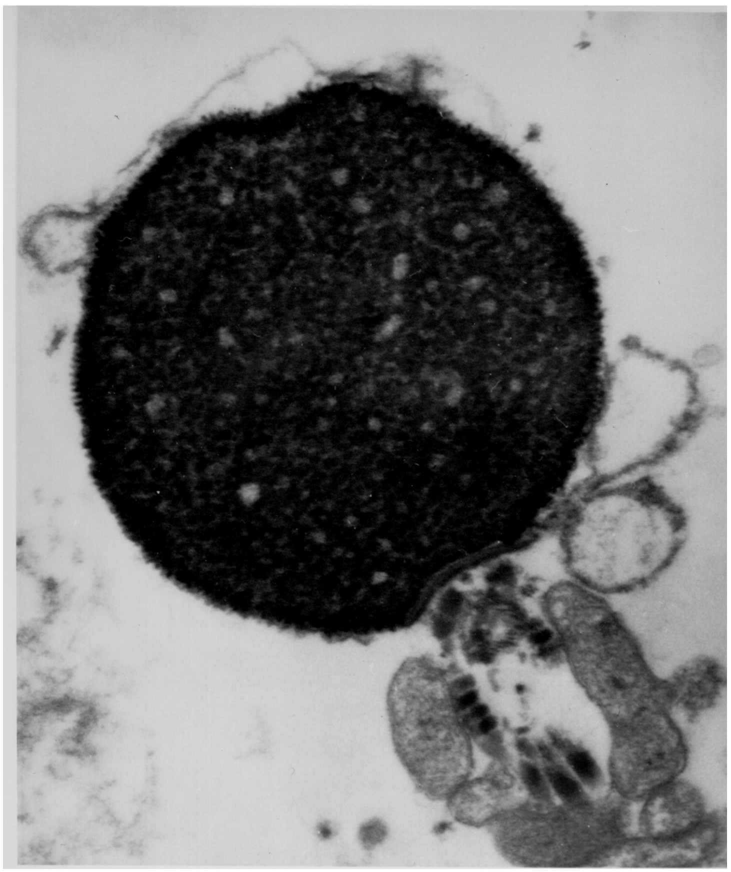


Patient #1



- 26 year old healthy
- Normal sperm count and motility. 0% normal morphology
- TEM: Type 2 Round Head Syndrome
- Brother: Type 1 Round Head Syndrome
- Brother: 48% total aneuploidy with 5 probes
- ???

Patient #2

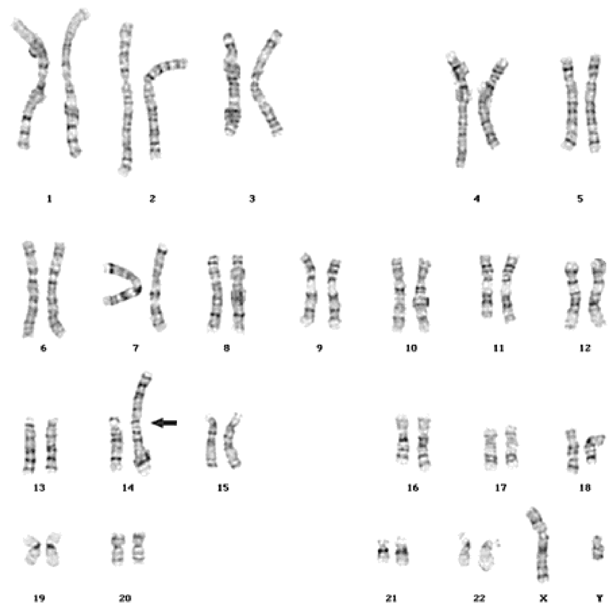
Level 1 Embryo

Level 2 Embryo

Level 3 Embryo

- 29 year old, 5 years of unexplained infertility.
- 8 AI cycles w/o pregnancy.
- IVF cycle 1: 7/10 oocytes fertilized. One level 2- embryo, six level 3 (fragmented) embryos.
- IVF cycle 2: 8 embryos, all level three.

Patient #3



- 28 year old, 2 years primary infertility
- OAT (3.5 M/mL)
- 13/14 Robertsonian Translocation
- IVF/ICSI/PGD for Translocation
- 9/9 embryos unbalanced

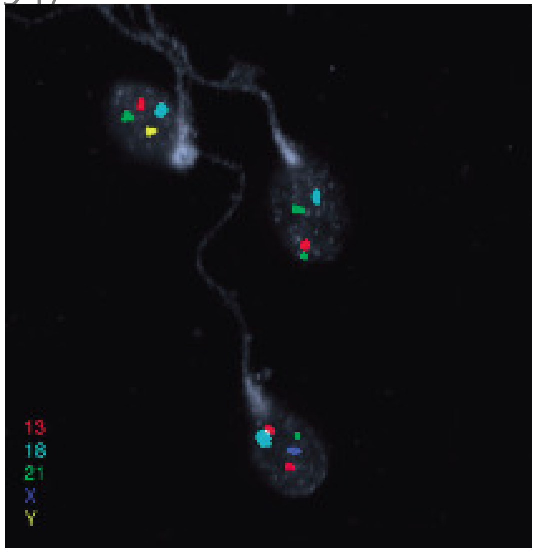
Objectives of Lecture

- Is it technologically feasible to implement aneuploidy analysis in a clinical setting?
- Is sperm aneuploidy clinically relevant?
- What is the incidence of elevated aneuploidy?
- What causes sperm aneuploidy?
- Can therapy lower the sperm aneuploidy rate?
- What are reasonable guidelines for testing?

History of Aneuploidy Testing in Sperm

- Karyotype of human sperm from decondensation and metaphase arrest in hamster ova. Rudak and Yanagimachi, 1978.
- Interphase FISH in decondensed sperm nuclei. (Martin, 1993; Wyrobek, 1994)

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

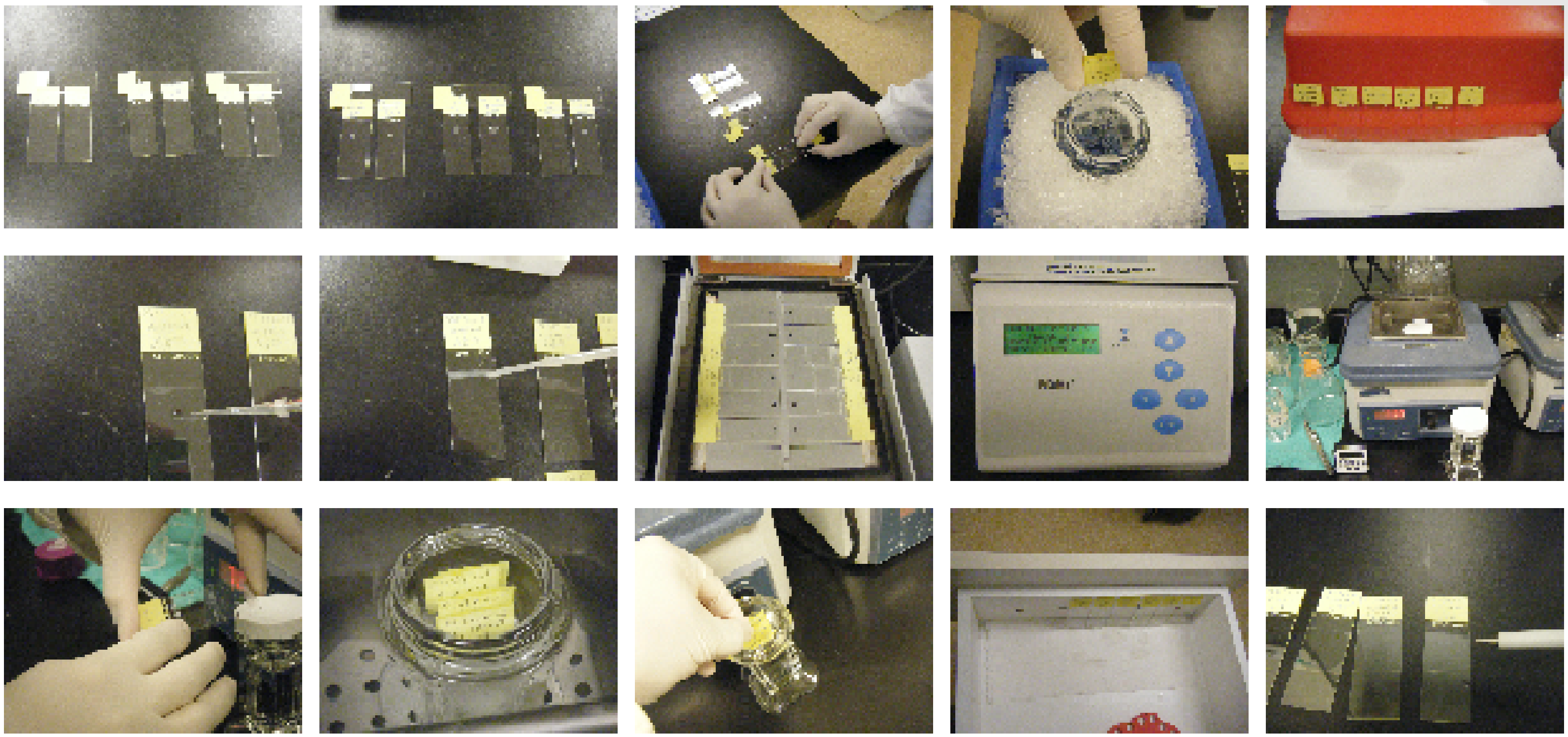


Rudak et al, 1978

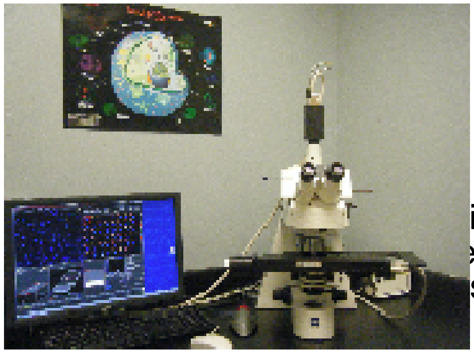
Constraints:

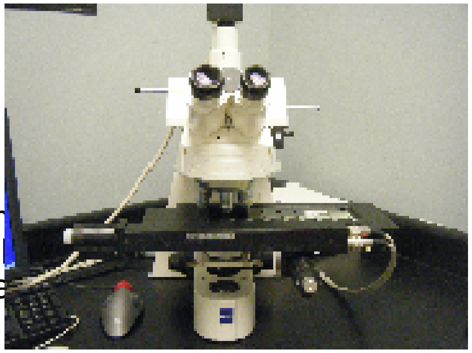
1) Development and Standardization of Good Hybridization Techniques and Counting Criteria
2) Costs – Probes/Tech time
3) Counting Time – 10,000 sperm, 5+ probes
4) Low sperm counts in some samples (i.e. biopsies)

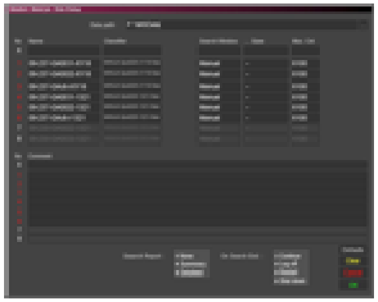
Result: Lack of Proper Validation and Large-Scale Studies

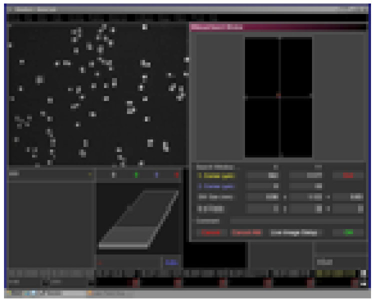


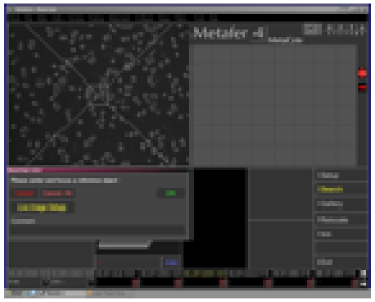
Automation of Chromosome Enumeration

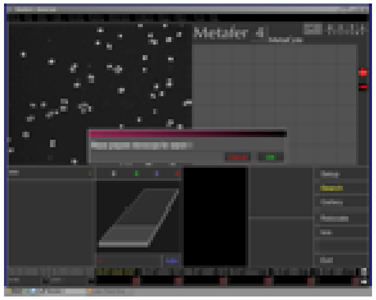




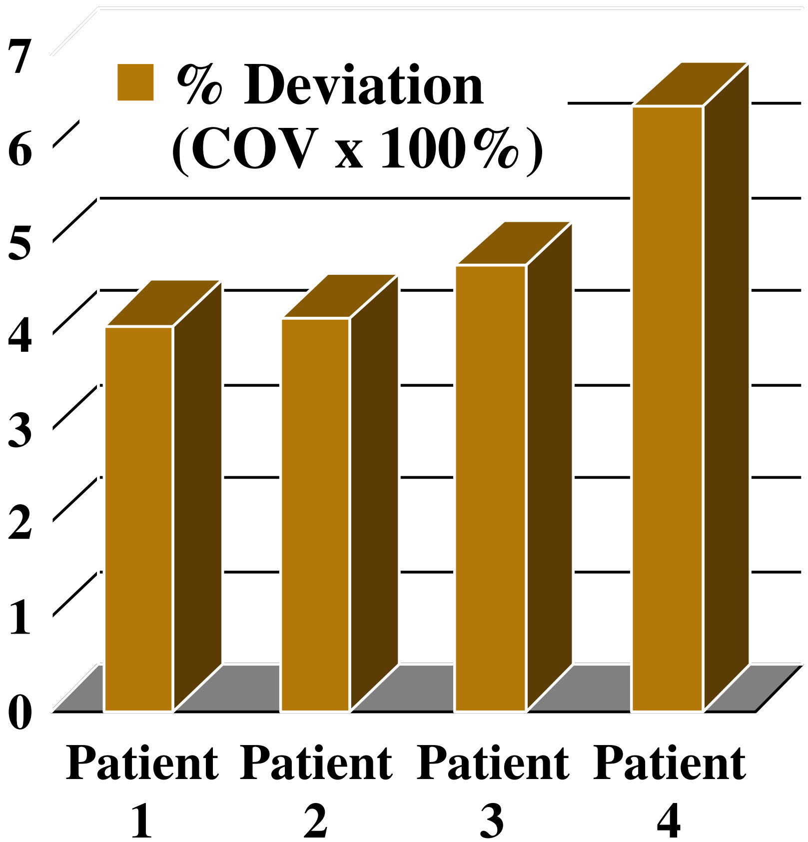


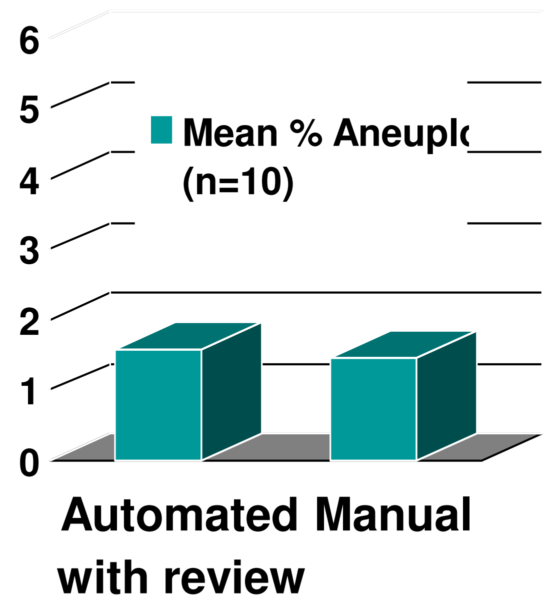






Manual vs Automated Enumeration





Carrell & Emery, 2008

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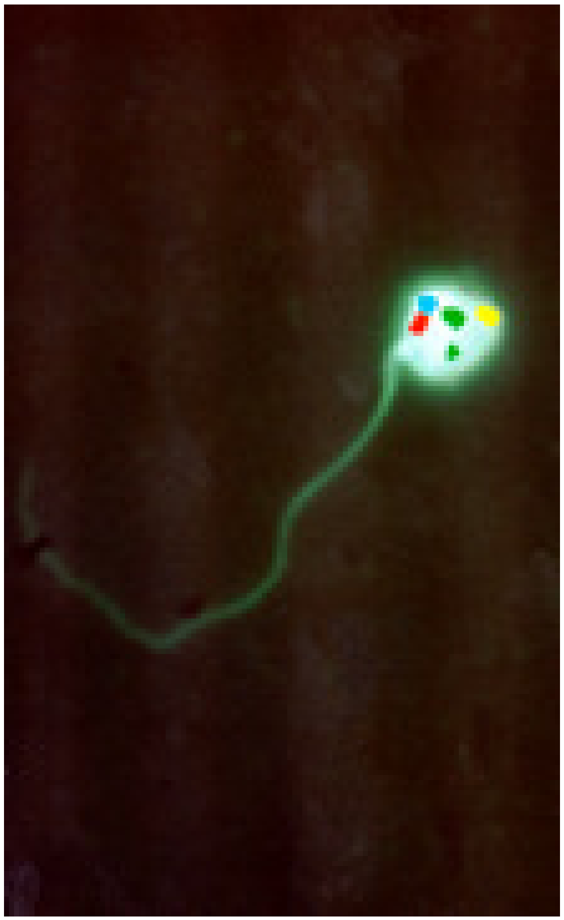
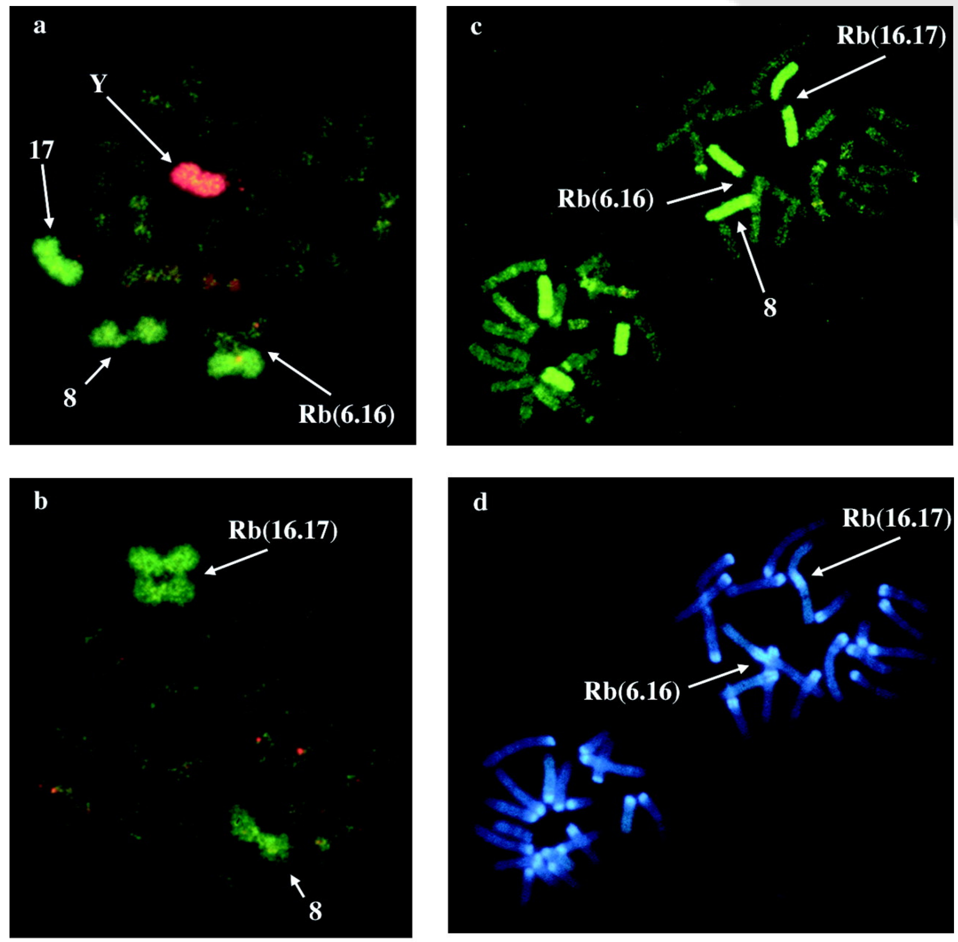
Is Sperm Aneuploidy Relevant?



- 15% of recognized pregnancies result in SAB
  - 50% of these are chromosomally abnormal
    Hassold & Jacobs, 1984
- Aneuploidies in gametes are predominately from errors of meiosis during oogenesis.
- Is sperm aneuploidy clinically relevant?

Fertilization Does Not Select for Euploid Sperm

- Marchetti et al. (Wyrobek lab), 1999
  - Double beterozygous mice for 2 Robertsonian translocations Rb(6.16)24Lub and Rb(16.17)7Bnr.
  - Chromosome painting of chrss 8, 16, 17, Y of sperm and blastomeres.
  - Aneuploidy rates equal in sperm and first cleavage blastomeres.
  - No interchromosomal effects noted.



Clinical Relevance of Aneuploidy

- Sperm aneuploidy does not influence fertilization capacity. (Marchetti, 1999)
- Correlation between sperm and PGS aneuploidy rates. (Tempest et al, 2009)
- The prevalence of sperm aneuploidy is higher in cases of repeated IVF failure with male factor infertility. (Magli, 2009)
- Screening for the prevalence of a known translocation and/or associated aneuploidies in the male germline prior to ICSI. (Munne, 2005; Carrell, 2008)

Anecdotal Evidence of Anomalies

- Elevated chrss 15 aneuploidy transmitted to fetus. Carrell et al., 2001
- 4 consecutive trisomic pregnancies with elevated frequency of associated sperm. Thomascik-Cheeseman et al., 2006
- Other reports: 21, 18, X

Sperm Aneuploidy and the Resulting Embryo

- Study design:
  - 32 Couples enrolled in the study (mean mat age 32)
  - Tested chromosomes: 13, 16, 18, 21, 22, X & Y
  - t-test

Sperm aneuploidy	# embryos	# blastomeres	% chr abnormality
<2.2%	35	628	58
2.2%-4.4%	25	318	63.5
>4.4%	23	354	73.45

Significant correlation between increased sperm aneuploidy and chromosome abnormalities in blastomeres

Tempest et al, 2009

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Sperm Aneuploidy and the Resulting Embryo
(Univ of Utah Ongoing Study)

- Study design:
  - 85 couples, Mat Age = 34.4
  - Tested chromosomes: 13, 18, 21, X & Y

Sperm aneuploidy	# embryos	# blastomeres	% chr abnormality
< 3.0	58	812	53.2
3.0 – 5.0	35	396	66.8
>5.0	37	362	81.4

Chromosomal Abnormalities Reported in IVF Embryos are Correlated to Sperm Parameters

	Normozoospermia	*Oligoasthenoter-atozoospermia*	*Obstructive azoospermia*	*Non-obstructive azoospermia*
Embryos diagnosed	594	695	127	133
FISH abnormal (%)	328 (55)[a,b]	431 (62)[a]	80 (63)	92 (69)[b]
Monosomies and trisomies (%)	147 (45)[c]	160 (37)[d]	36 (45)[e]	23 (25)[c,d,e]
Haploidy and polyploidy (%)	30 (9)	60 (14)	5 (6)	6 (7)
Complex abnormalities (%)	151 (46)[f]	211 (49)[g]	39 (49)[h]	63 (68)[f,g,h]
No. day-3 embryos with 7–8 regular cells, no fragmentation (%)	237 (40)[i]	243 (35)[j]	37 (29)[k]	21 (16)[i,j,k]

Values with same superscript letter are significantly different: [ahk] $P < 0.025$; [bg] $P < 0.005$; [cd] $P < 0.05$; [e] $P < 0.01$; [fij] $P < 0.001$.
FISH = fluorescence in-situ hybridization.

Magli, 2009

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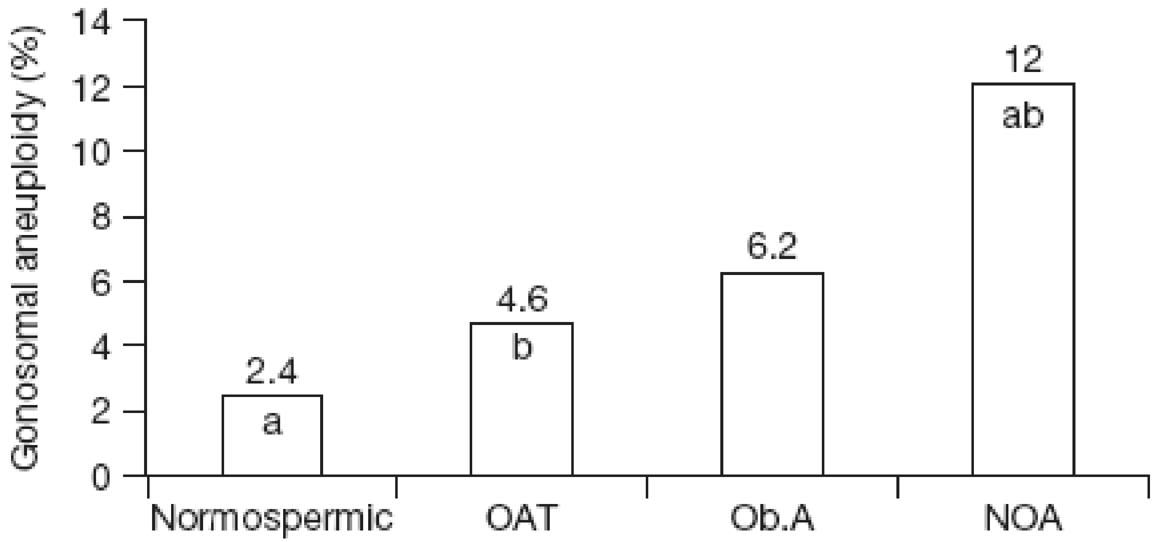
Gonosomal Aneuploidies in 1549 Embryos



Magli,
2009

Recurrent Miscarriage

	n	XY	13	18	21	Total
RPL patients	24	0.77 ± 0.11*	1.02 ± 0.10†	0.51 ± 0.05*	0.47 ± 0.06*	2.77 ± 0.22†
General population	16	0.40 ± 0.05	0.44 ± 0.06	0.33 ± 0.04	0.28 ± 0.03	1.48 ± 0.12
Fertile donors	10	0.31 ± 0.06	0.39 ± 0.03	0.25 ± 0.02	0.24 ± 0.02	1.19 ± 0.11

RPL = recurrent pregnancy loss.

Data are expressed as mean ± standard error. Total aneuploidy indicates the percentage of sperm with one or more aneuploid chromosomes. Greater than 5000 sperm were analyzed in all samples.

* $P < .05$ compared with general population and fertile donors.

† $P < .005$ compared with general population and fertile donors.

Carrell et al, 2003

How Common is Elevated Sperm Aneuploidy? What is its Clinical Relevance?

QuickTime™ and a decompressor are needed to see this picture.

1. What is Normal?
2. Few Systematic Analyses
3. Standardization
4. Improper QC

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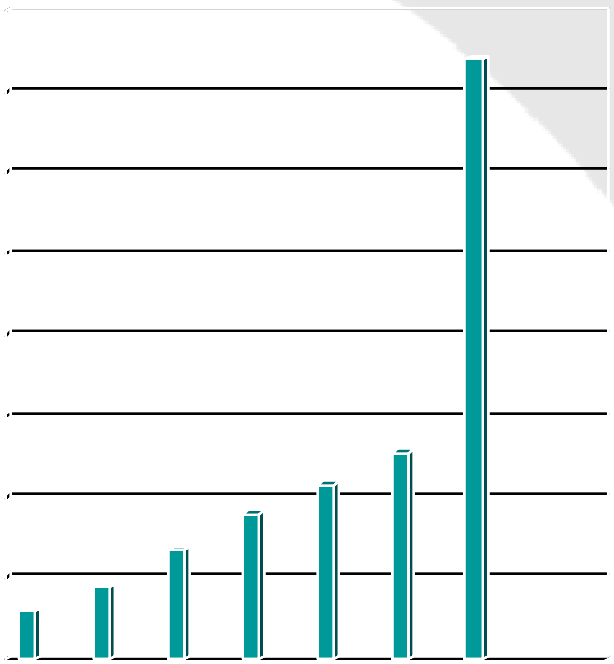
Reported Incidences of Aneuploidy

Syndrome	Aneuploidy (%)	Reference
Klinefelter syndrome (mosaic)	1.5Š7	Kruse et al, 1998; Lim et al, 1999
Klinefelter syndrome (nonmosaic)	2Š25	Rives et al, 2000; Estop et al, 1998
Robertsonian translocation	10Š23 unbalanced	Ogur et al, 2006
	1Š19 aneuploid	Ogur et al, 2006
	7Š36 unbalanced*	Fryndman et al, 2001
Reciprocal translocation	19Š77 unbalanced	Martin and Spriggs, 1995
Severe morphology defects	15Š100	Benzacken et al, 2001
Multiflagellar, macrocephalic		Devillard et al, 2002
Tail agenesis		Carrell et al, 2004; InÕt Veld et al, 1997
Round headŠonly syndrome	15Š60	Carrell et al, 1999, 2001
Nonobstructive azoospermia	1Š51	Burrello et al, 2005
Unexplained recurrent pregnancy loss	1Š34	Bernardini et al, 2004; Carrell et al, 2003
Repeated IVF failure	2Š7	Petit et al, 2005

Mean Aneuploidy Rate

- 5 Chromosomes (13, 18, 21, X, Y)
- A – Fertile Normozoospermic: 1.2% (n= 59)
- B – General Population:1.8% (n= 238)
- C – General Infertile: 2.7% (n= 364)
- D – Teratozoospermic: 3.6% (n= 176)
- E – Poor Embryogenesis: 4.3% (n= 51)
- F – Recurrent Miscarriage: 5.1% (n = 86)
- G – Severe Ultrastructure Defects:14.8% (3-78) (n= 65)



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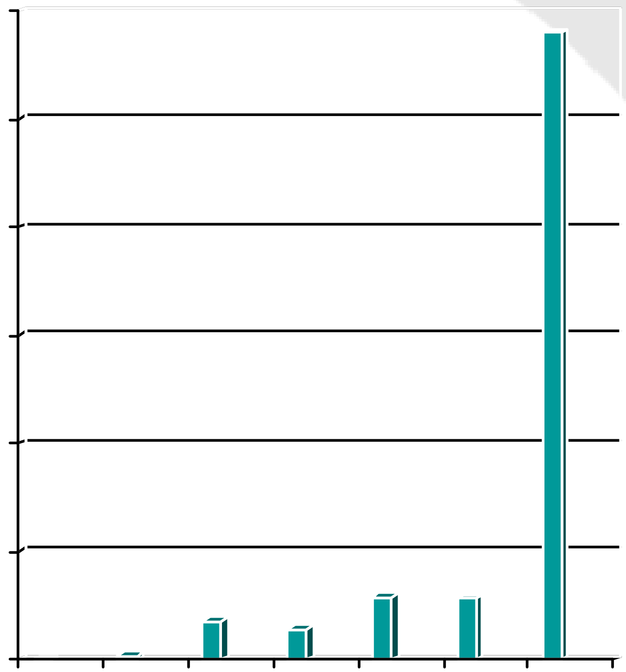
Incidence of Elevated Aneuploidy
(% of Samples >3% Total Aneuploidy for 5 Probes)

- 5 Chromosomes (13, 18, 21, X, Y)
- A – Fertile Normozoospermic: 0% (n= 59)
- B – General Population: 0.4% (n= 238)
- C – General Infertile: 3.7% (n= 364)
- D – Teratozoospermic: 3.9% (n= 176)
- E – Poor Embryogenesis: 5.8% (n= 51)
- F – Recurrent Miscarriage: 5.7% (n = 86)
- G – Severe Ultrastructure Defects: 58% (n= 65)



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Intra-Individual Variation

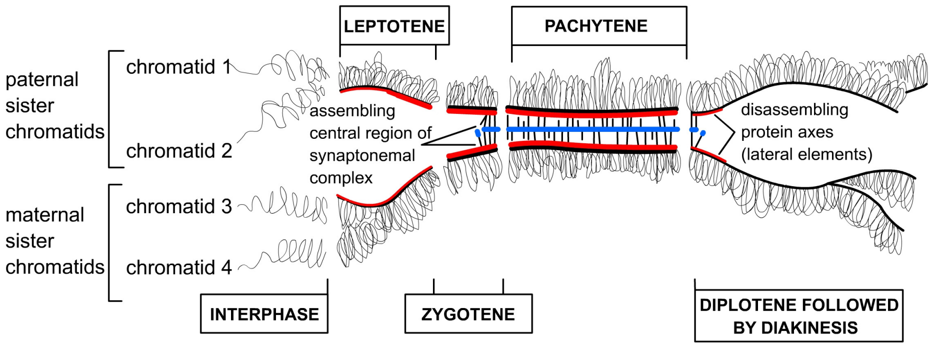
- Tempest et al., 2009
  - Sporadic variation in all 10 subjects over time.
  - Single sample variable over trial.
- Rubes et al., 2005
  - Interchromosomal differences.
  - Generally consistent at 2 years, some variability at 5 years.

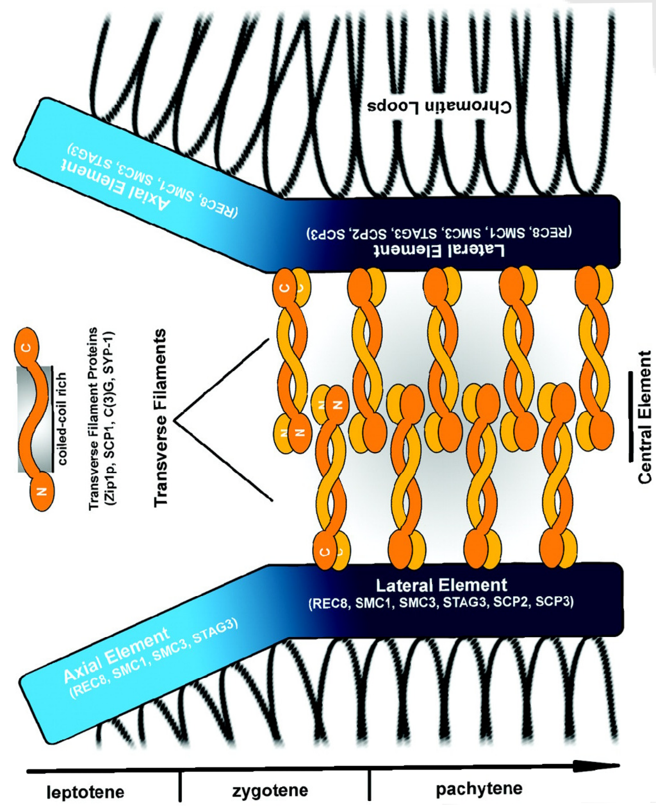
Segregation Analysis for Translocation Patients

- Vozdova et al., 2008: Different segregation patterns and unbalanced sperm rates even with similar translocations.
- Different rates with similar Robertsonian translocations. Chen et al., 2007
- Perrin et al., 2009: Different segregation patterns for similar sex/autosome translocations affecting chance of IVF success.
- Yakut et al., 2006: Sperm FISH unbalanced rate related to blastomeres.
- Wiland et al., 2008: Suggest high unbalanced rate (>60%) is still conducive to IVF pregnancy. *Note: High miscarriage rate.
- Breakpoint variability related to the variability in segregation patterns and subsequent unbalanced rates.

What Causes Aneuploidy?

Prophase 1: Synaptonemal Complex





Axial Elements: SCP 2&3, Rec 8

Hold sister chromatids together

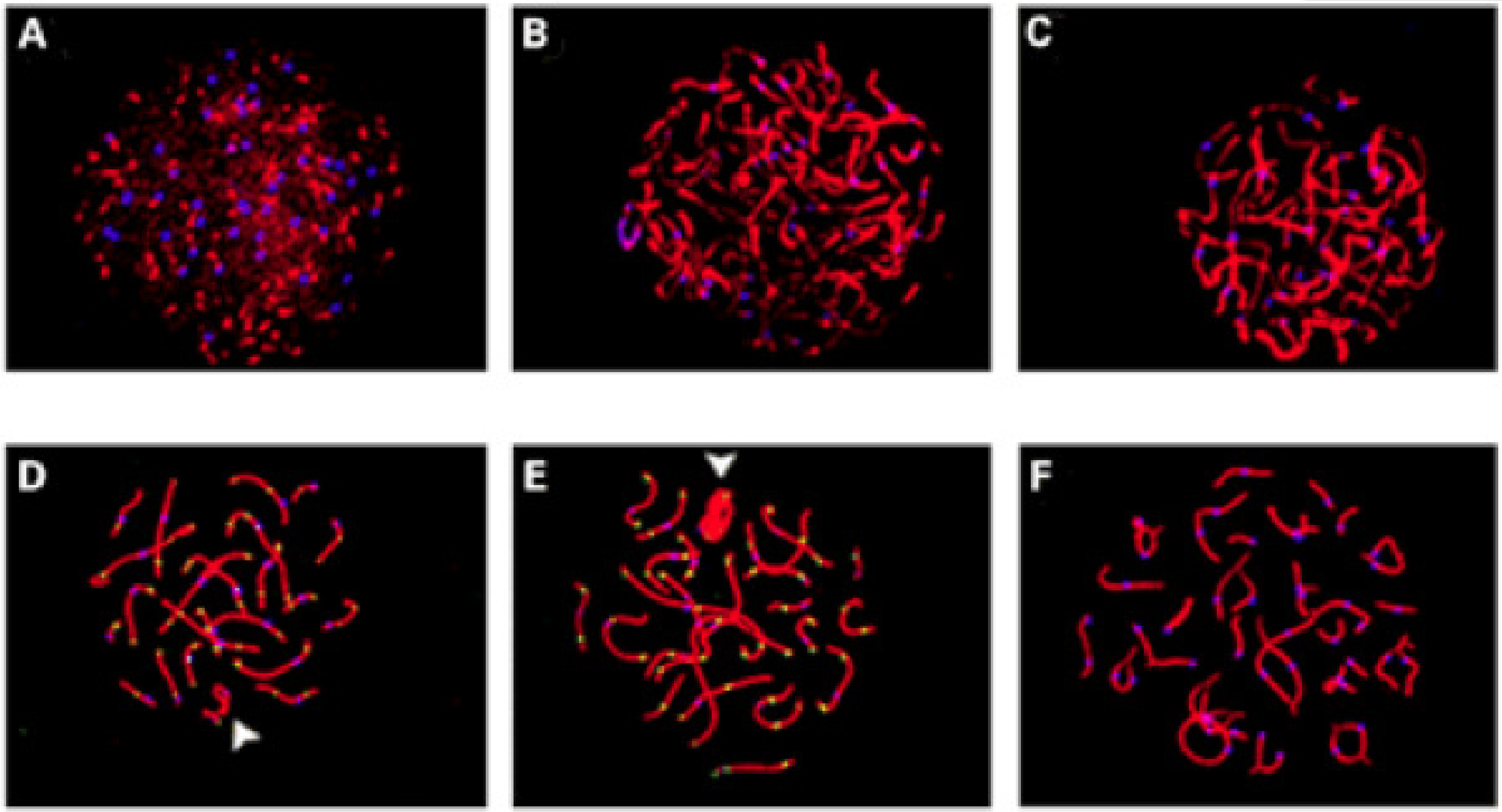
Transverse Filaments: SCP1

Holds pairs of sister chromatids together

Axial Elements become Lateral Elements of SC.

http://219.221.200.61/ywwy/zbsw(E)/edetail11.htm

Progression of Meiosis During Spermatogenesis



Gonsalves et al., (2004)

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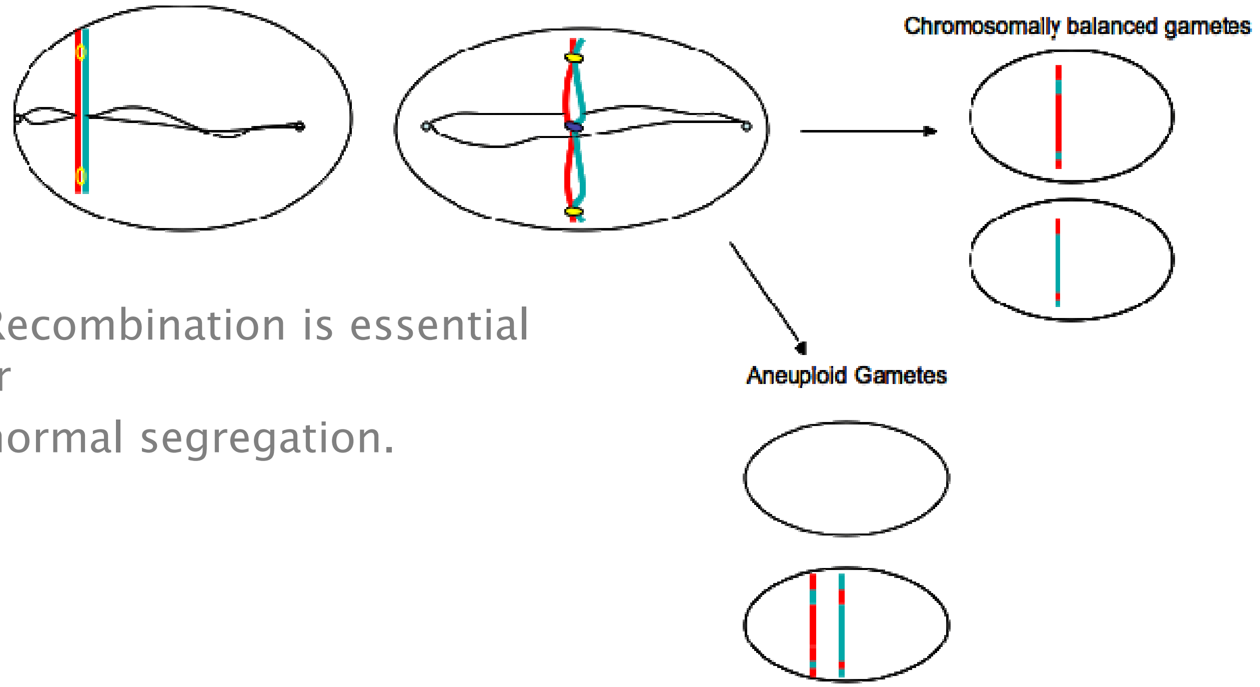
Chromosomal Non-Disjunction May Result in Aneuploidy



- Recombination is essential for
  normal segregation.

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What Causes Nondisjunction?

Recombination Mechanics – Biopsy Samples

QuickTime™ and a
decompressor
are needed to see this picture.

- Number of crossovers (MLH foci)
- Positioning in subtelomeric regions
- Fidelity of the synaptonemal complex

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The Frequency of Crossovers and MLH Foci

- "Normal" Spermatogenesis
  - Mean 49.8 ± 4.8 (S.D.) recombination sites
  - Range 46.2 ± 3.3 to 55.3 ± 3.7 (Hassold et al 2004)

Number of MLH Foci

Hassold, et al (2004)

NOA Infertile Men

- Mean 40.4 ± 6.1 recombination sites
- Range of 32.3 ± 15.1 to 48.9 ± 7.4 (Sun et al., 2006)

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Meiotic Errors are Associated with Male Infertility

The formation of synaptonemal complexes without a crossover event is increased in infertile men.

Groups	Mean No. of autosomal SCs where No. of foci is						MLH1 foci	
	0	1	2	3	4	5	Mean No. ± SD	Range
Controls	0.1	3.5	12.3	4.9	1.1	0.2	48.0 ± 4.7	21–65
Obstructive azoospermia	0.3	3.8	12.3	4.5	1.0	0.1	46.3 ± 6.3[a]	4–64
Nonobstructive azoospermia	1.9	5.3	10.6	3.7	0.7	0.1	40.4 ± 6.1[a]	1–61

[a] $P < 0.0001$, nested ANOVA.

Sun et al. 2005

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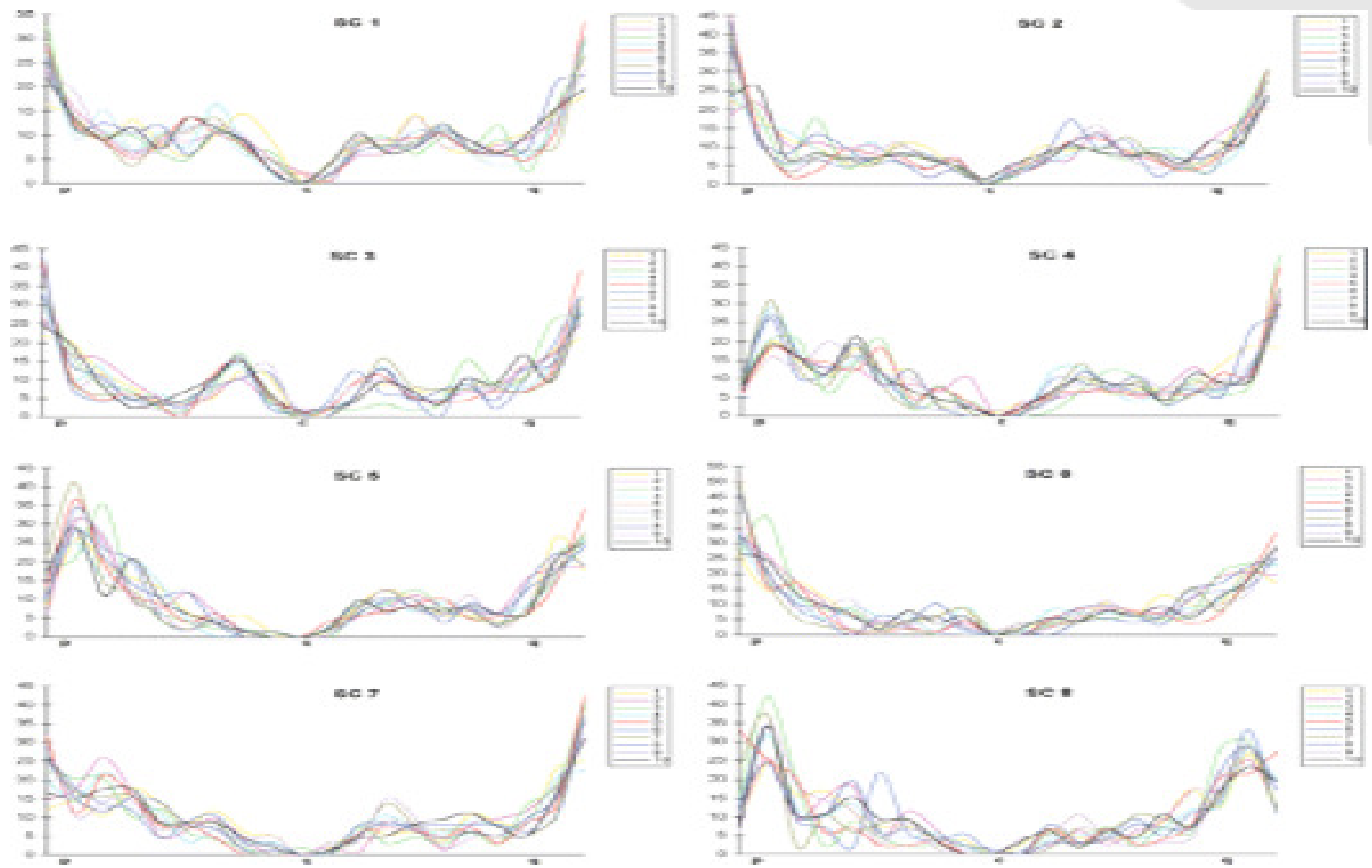
Crossovers Occur in Subtelomeric Regions



Sun et al. 2006

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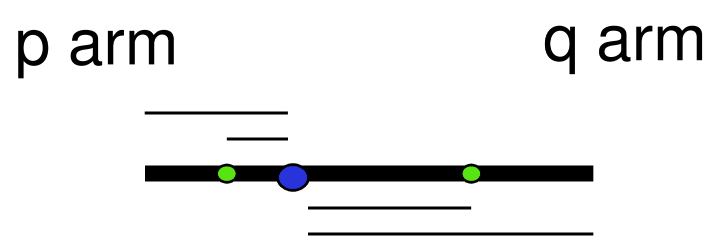
Meiotic Errors are Associated with Male Infertility



Mean Distance from the Centromere to the Proximal Crossover Sight on the p arm

- Patients
  - Mean Distance 2.84 ± 0.096 µm
- Controls
  - Mean Distance 3.20 ± 0.105 µm

P= 0.01

Mean Distance from the Centromere to the Proximal Crossover Sight on the q arm

- Patients
  - Mean Distance 4.23 ± 0.11 µm
- Controls
  - Mean Distance 4.58 ± 0.129 µm

P= 0.04

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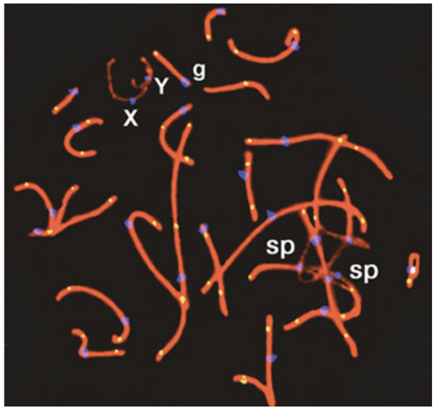
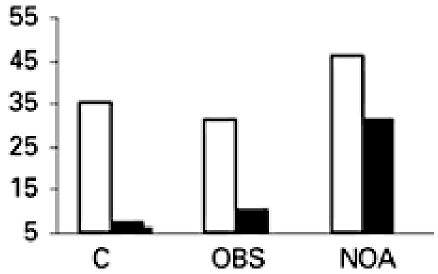
Meiotic Errors are Associated with Male Infertility

Gaps and splits of the synaptonemal complex are associated with infertile





- Gaps
  - 35% controls
  - 45% NOA patients
- Splits
  - 7.5% controls
  - 35% patients
    - Sun et al. 2005

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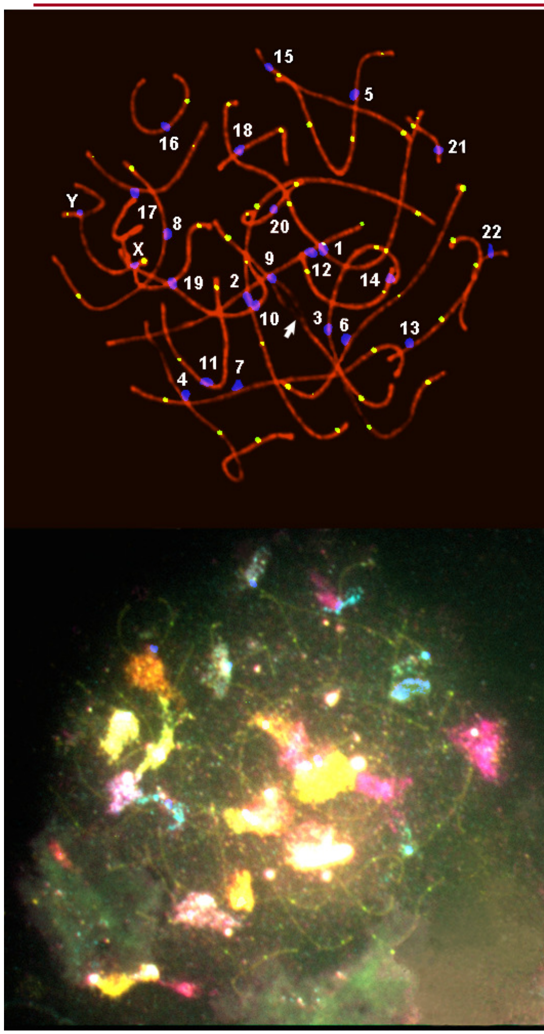
Conclusion: Analysis of Recombination



Martin, 2007

- The mechanics of meiosis affect non-disjunction.
  - Number of crossovers (MLH foci)
  - Positioning in subtelomeric regions
  - Fidelity of the synaptonemal complex
- Recombination is not just important for genetic variation, but also to assure proper segregation. (Hassold, 2007)

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Sequencing of Genes Involved in Meiosis

Azoospermic and Oligozoospermic Patients

- SPO-11 – Low frequency of Novel SNPs, Carrell et al., 2006
- Rec 8 – No Difference from Controls, Carrell et al., 2008
- MMRs – Early Data- Low level SNPs Carrell and Sanderson, 2009
- Genomewide Analysis – No significant SNPs, Aston and Carrell, 2009 J Androl

Increased Aneuploidy Patients

- Ongoing Studies Only

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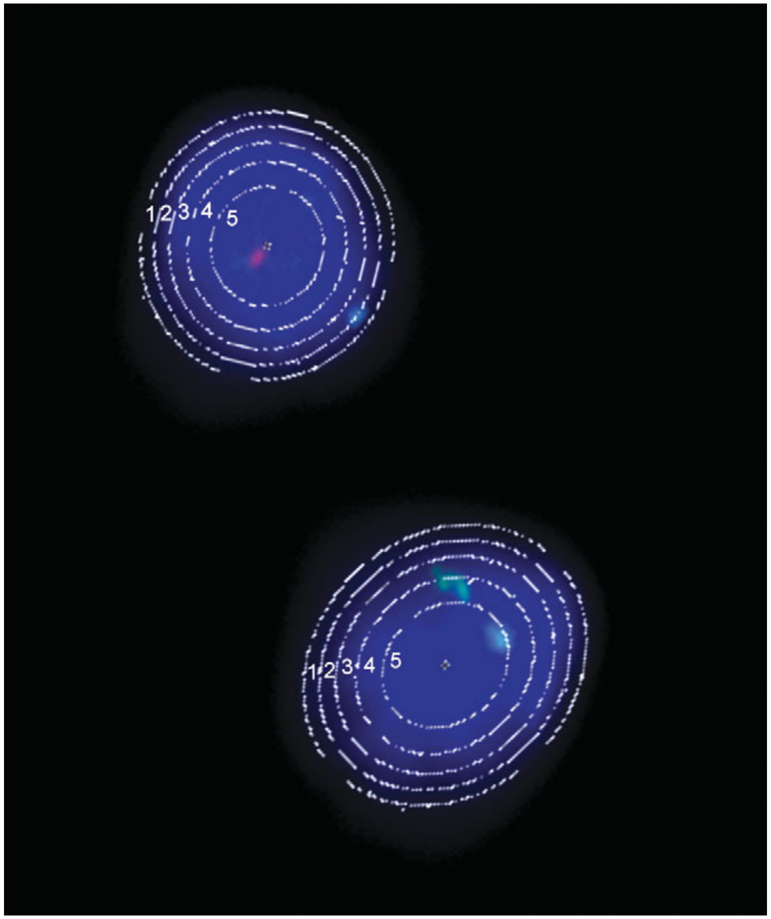
Position of Chromosomes in Nucleus



Finch et al., 2008

- Preliminary evidence for centromeric postion in center. Luetjens et al. 1999; Zalenskaya, 2004; Finch et al, 2008.
- Centromeres shifted from center in patients with disomy, compared to fertile controls. Olszewska et al., 2009

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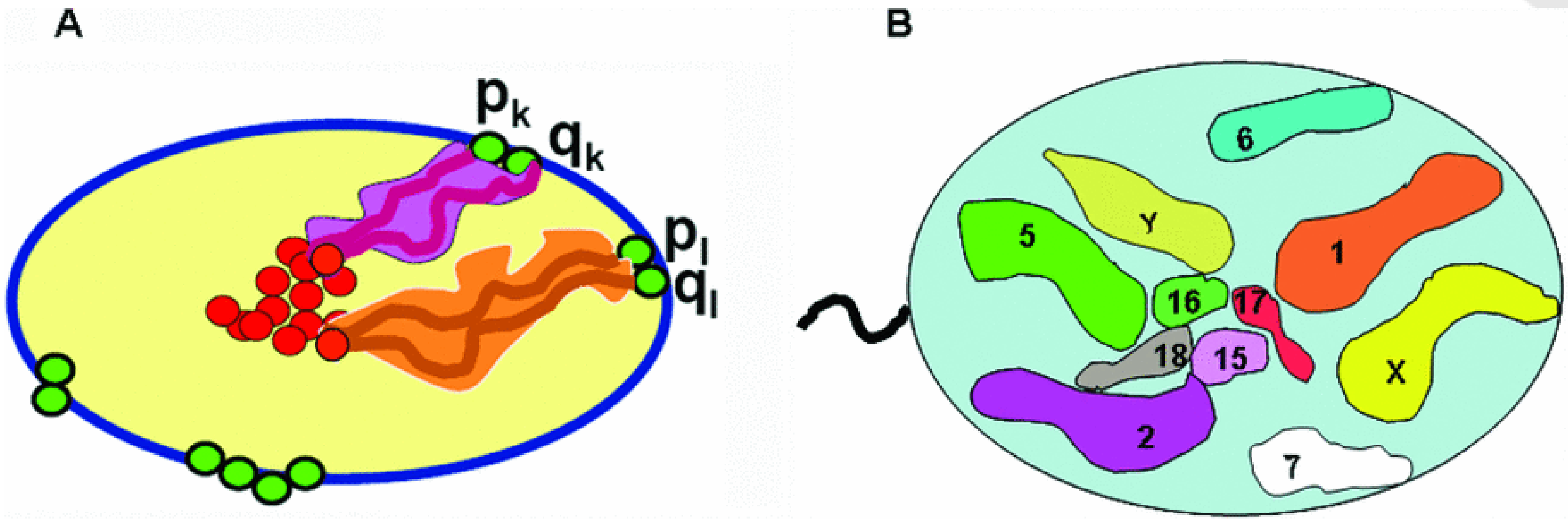
Positioning of Chromosomes in Sperm



Zalensky and Zalenskaya, 2007

Therapy and “Sperm Selection”

Sperm Selection

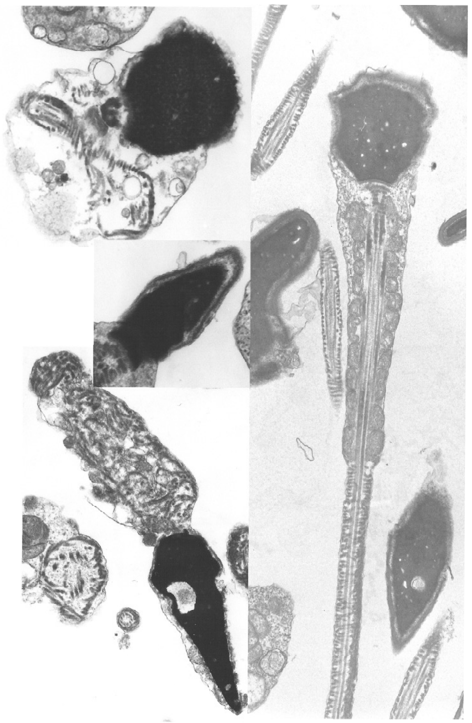
Sperm Preparation:

- Swim-up lowers aneuploidy rate. Jakab et al., 2003
- Selection of motile sperm (DG, SU, GW) does not lower aneuploidy rate. Samura et al., 2001
- Mosaic Translocation – Unbalanced chromosome increased following Density Gradient. Iwarsson et al., 2009

Sperm Selection:

- Lower aneuploidy in sperm selected by high power magnification and w/o vacuoles. Garolla et al.
- HA-mediated selection resulted in 4-6 fold reduction in disomy. Huszar et al., 2007, 2008; Paasche, 2009

Relationship of Morphology and Aneuploidy



- Normal strict criteria morphology does not predict euploidy. Ryu et al, 2001; Sun et al., 2006.
- Severe abnormal morphology is associated with an elevated aneuploidy rate. Tang et al., 2009; Carrell et al, 2004; Prissant, 2007; Collodel et al., 2006; Perrin et al., 2008

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Medical Therapy

- 90 days of FSH Therapy lowered total aneuploidy rate. Piombi et al., 2009
- Traditional Chinese Medical Therapy – Tempest et al., 2005 (not RCT)

Varicocele Repair

- Elevated rate of 17, 18 aneuploidy improved by repair. Acar et al., 2009
- Animal Model: No elevation in aneuploidy (Carrell, Unpub)

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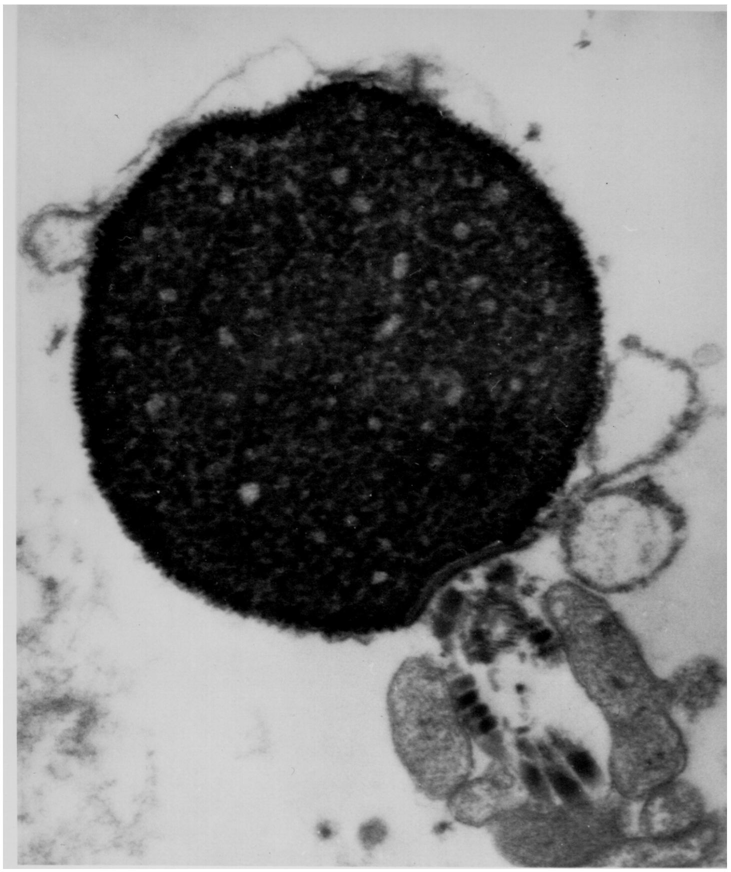
Patient #1



- 26 year old healthy
- Normal sperm count and motility. 0% normal morphology
- TEM: Type 2 Round Head Syndrome
- Brother: Type 1 Round Head Syndrome
- Brother: 48% total aneuploidy with 5 probes
- ???
- Aneploidy Analysis:
  - 2.1% Aneuploidy for 5 chromosomes

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Patient #2

Level 1 Embryo

Level 2 Embryo

Level 3 Embryo

- 34 year old, 5 years of unexplained infertility.
- 8 Cycles w/o pregnancy.
- IVF cycle 1: 7/10 oocytes fertilized. One level 2– embryo, six level 3 (fragmented) embryos.
- IVF cycle 2: 8 embryos, all level three.
- Aneploidy Analysis:
  - 17.9% Aneuploidy for 5 chromosomes

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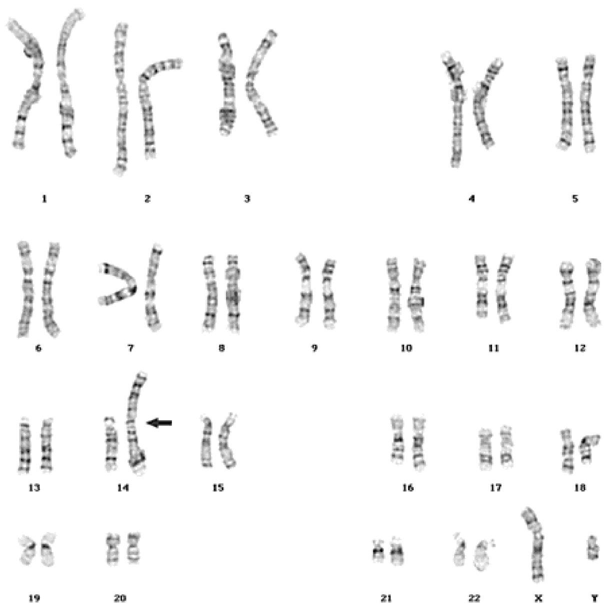
Patient #3



- 28 year old, 2 yeaars primary infertility

- OAT (3.5 M/mL)

- 13/14 Robertsonian Translocation

- IVF/ICSI/PGD

- 9/9 embryos unbalanced

• Aneploidy Analysis:
  - – 74% Unbalanced 13/14
  - –– 8.9% Aneuploidy for 5 Chromosomes

Conclusions and Clinical Recommendations

- New advances facilitate of sperm aneuploidy testing analysis in a clinical setting; however, more data are needed in establishing reference ranges, etc.
- Further studies are needed on sperm separation techniques to select euploid sperm.
- Further data are needed on specific sperm aneuploidy rates and embryo aneuploidy to assess relative risks.
- Recombination is essential for normal segregation (Quantity and Quality)
- Clinical screening may be useful in certain pathologies, which include:

When To Use Sperm Chromosome Testing

Clinical syndromes for which sperm chromosome aneuploidy testing may be advisable

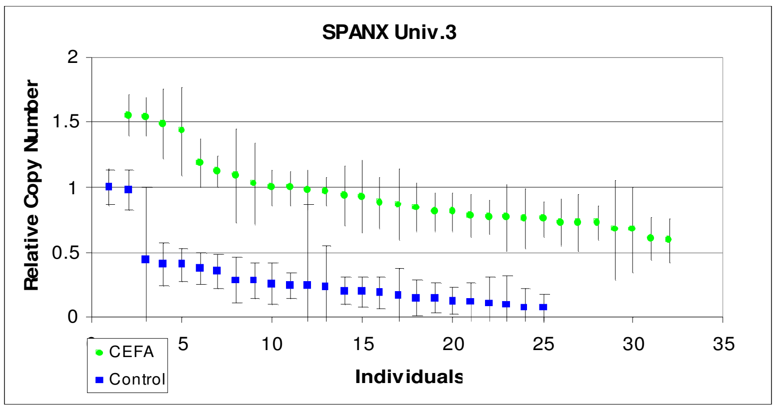
Syndrome	Aneuploidy (%)	Reference
→ Klinefelter syndrome (mosaic)	1.5Š7	Kruse et al, 1998; Lim et al, 1999
→ Klinefelter syndrome (nonmosaic)	2Š25	Rives et al, 2000; Estop et al, 1998
→ Robertsonian translocation	10Š23 unbalanced	Ogur et al, 2006
	1Š19 aneuploid	Ogur et al, 2006
	7Š36 unbalanced*	Fryndman et al, 2001
→ Reciprocal translocation	19Š77 unbalanced	Martin and Spriggs, 1995
→ Severe morphology defects	15Š100	Benzacken et al, 2001
Multiflagellar, macrocephalic		Devillard et al, 2002
Tail agenesis		Carrell et al, 2004; InÕt Veld et al, 1997
Round headŠonly syndrome	15Š60	Carrell et al, 1999, 2001
Nonobstructive azoospermia	1Š51	Burrello et al, 2005
→ Unexplained recurrent pregnancy loss	1Š34	Bernardini et al, 2004; Carrell et al, 2003
→ Repeated IVF failure	2Š7	Petit et al, 2005

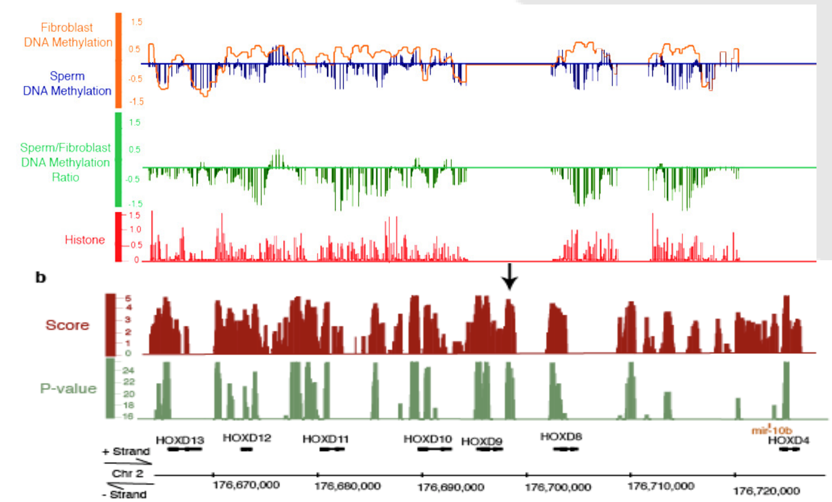
Carrell, 2008

Technical and Logistical Considerations

- Which chromosomes should be evaluated? (All?)
  - Most commonly tested Chromosomes:
    - X, Y, 13, 16, 18, 21, 22
  - Most Predictive of Recurrent Miscarriage:
    - 1, 15, 17, 21, 22
- Number of sperm to be counted for relevant data?
- Standardization and automation of hybridization and enumeration protocols?
- Relative risk?

Caveat: Non-chromosomal Aneuploidy





- Copy Number Variation (CNV) of genes/alleles.
  J Androl, 2009; Sys Biol Reprod Med (In Press)
- Functional Aneuploidy via Epigenetic Markings (Silencing)
  Nature, 2009

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