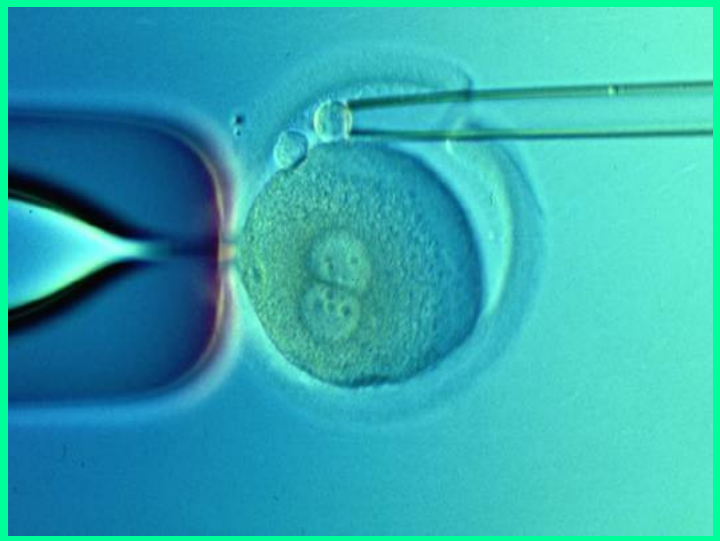
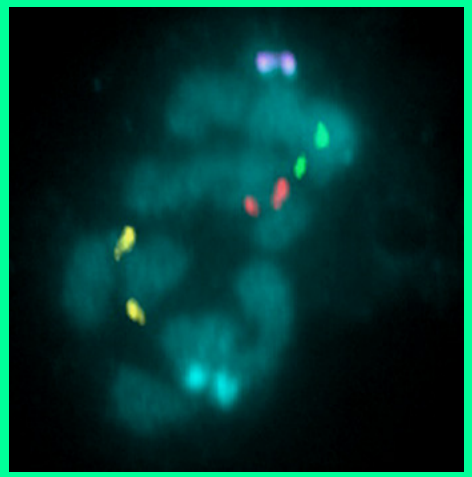
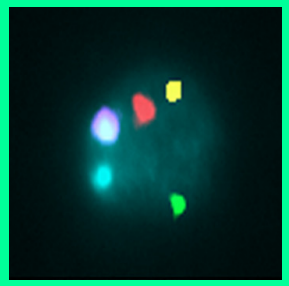






Polar Body Approach to PGD

Anver KULIEV

Reproductive Genetics Institute

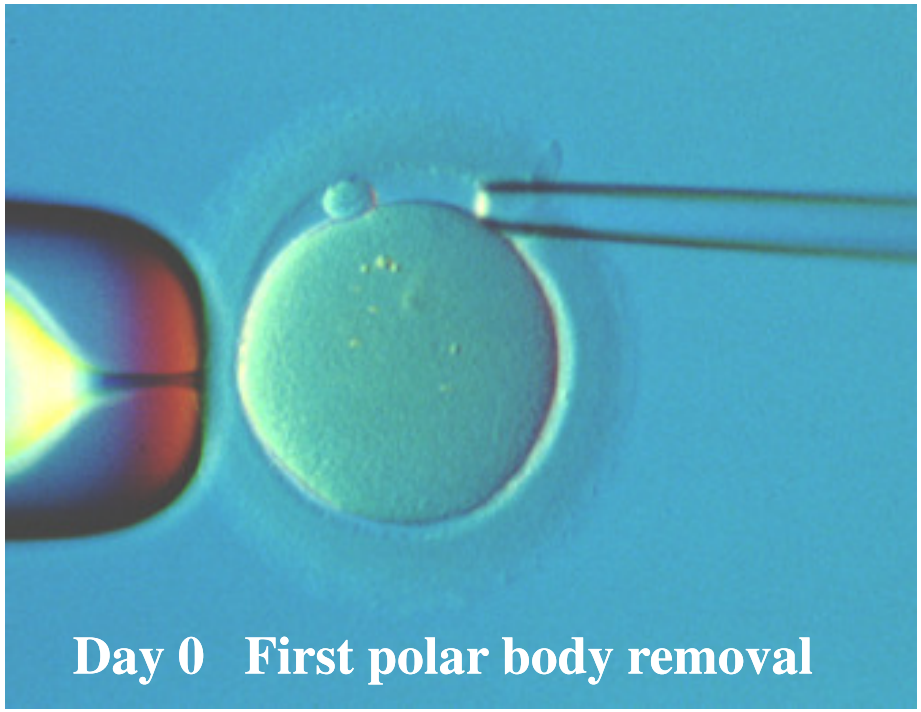
DISCLOSURE

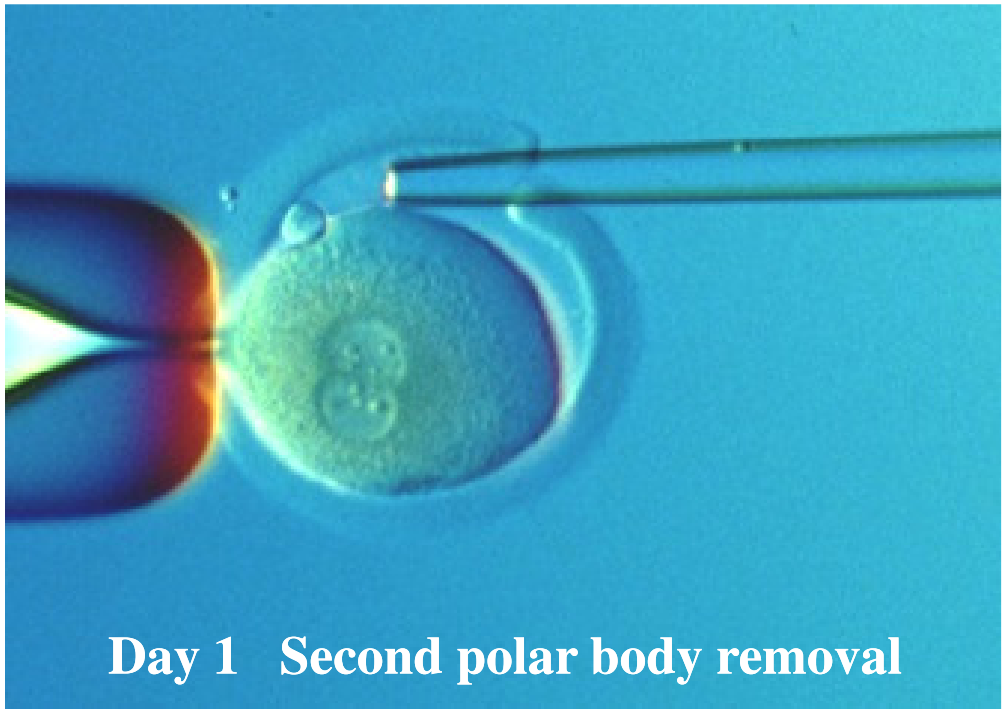
Nothing to disclose

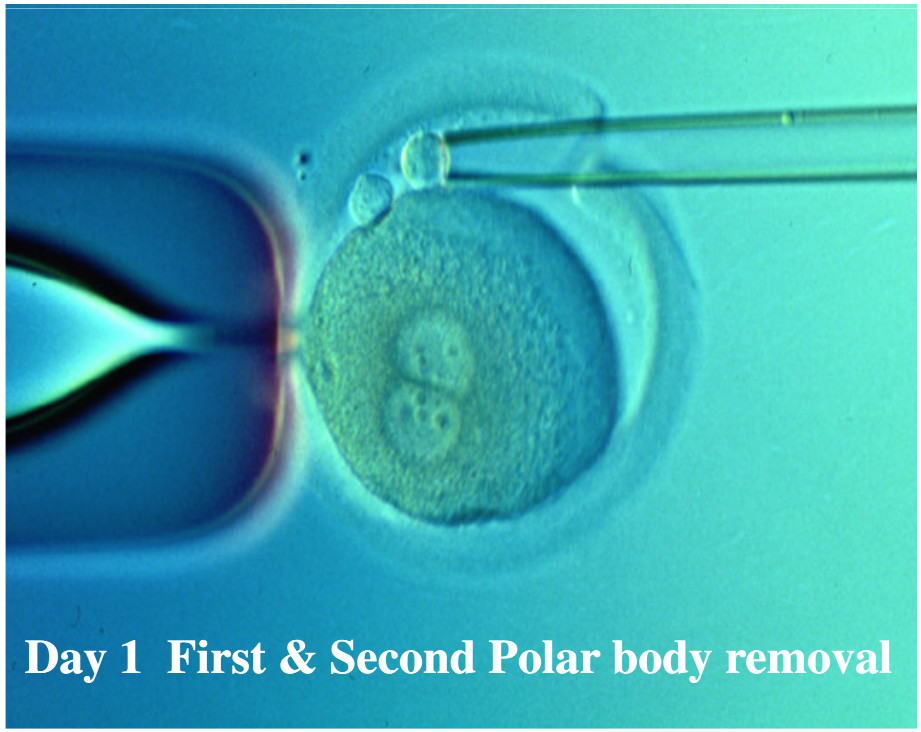
History of Polar Body Approach

- ***1984** First proposed in World Health Organization's Document "Perspectives in Fetal Diagnosis" (Kuliev et al, Ares-Serono Symposia, Rome, Italy, 1985, p. 47)*

- ***1990** First introduced by Dr. Verlinsky (Human Reproduction, 1990, 5:826-9)*







Microsurgical Techniques For Polar Body Biopsy

Present RGI Experience -

2028 PGD cycles for 221 CONDITIONS &

4428 PGD cycles for Chromosomal Disorders

Current indications:

Single Gene Disorgers

*Autosomal Recessive and Dominant Disorders

* X-linked Disorders

* Cancer predisposition genes

* Adult-onset disorders

* Infertility-causing genes

* Maternal-fetal incompatability

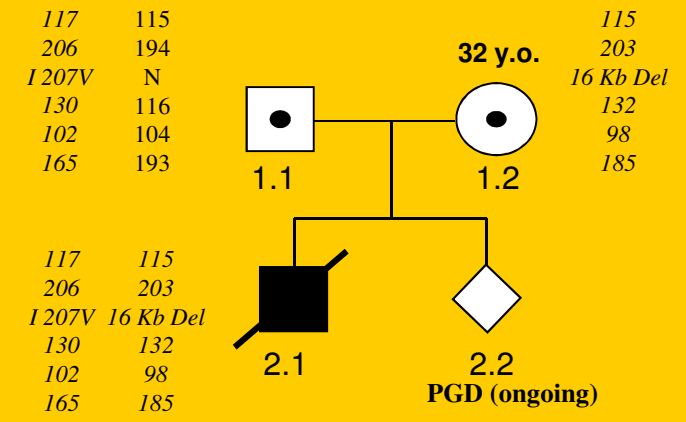
HLA genotyping

Aneuploidy testing

Pre-Embryonic Diagnosis for Sandhoff Disease

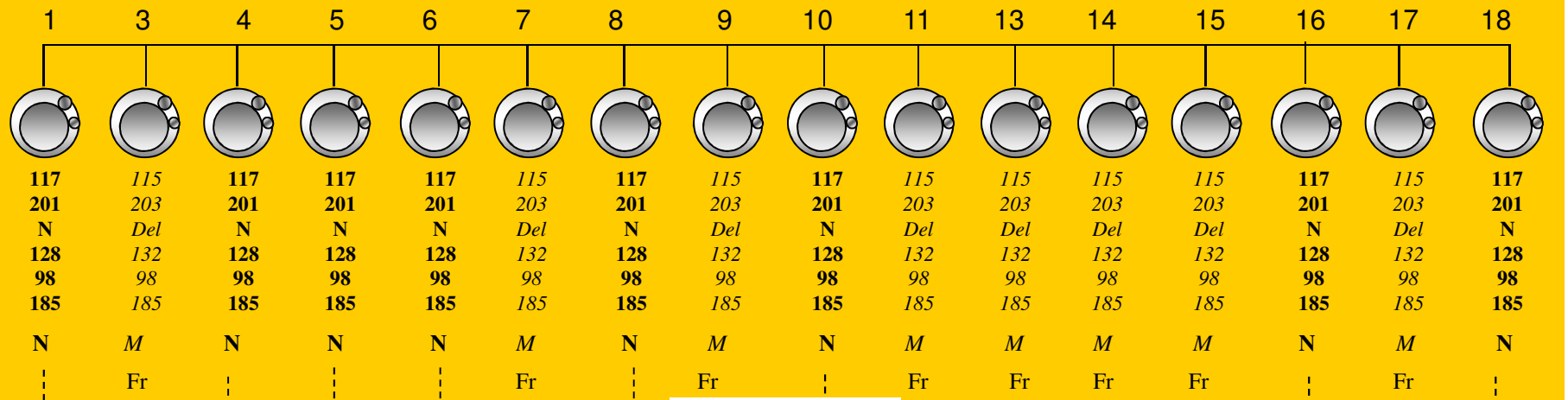
A.

Markers order:
D5S1982
D5S1988
HEXB 16Kb Del
HEXB I207V
D5S2003
D5S349
D5S1404



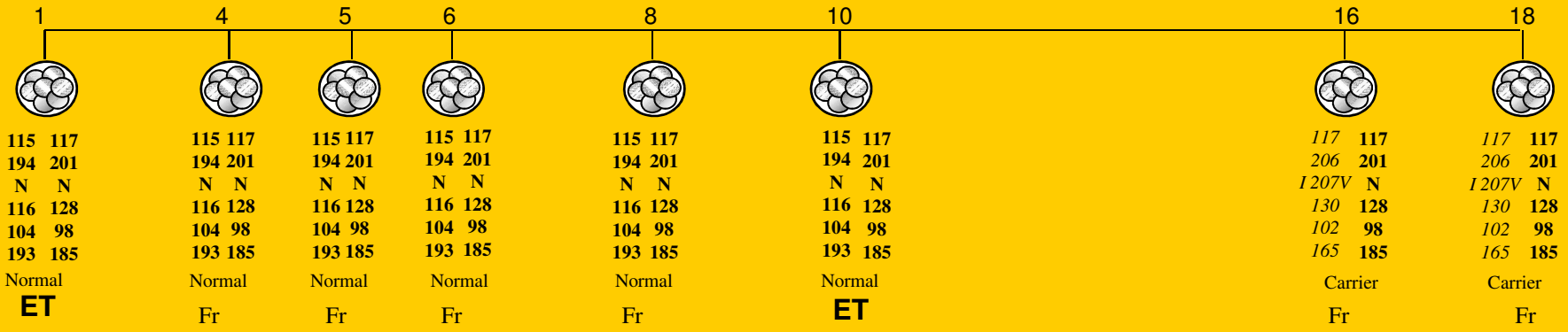
B.

Sequential Polar Body Analysis



C.

Blstomeres Analysis



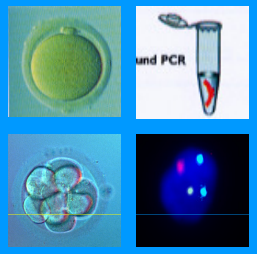
Time-table of Preembryonic Diagnosis of Sandhoff Disease –Type I

24 hrs	18 hrs	35 hrs	hCG	- 8 pm
			Aspiration	- 7 am (on the second day)
		3.5 hrs	PB1 removal	- 11.30 am
		6.5 hrs	ICSI	- 12 pm
			PB 2 removal	- 6.30 pm
		9 hrs	DNA testing	- 7 pm – 4 am
			Fertilization control	- 12.30 am & 6 am
			Freezing (of mutant oocytes at pronuclear stage)	- 6.30 am
			Blastomere biopsy (of embryos free of maternal mutation)	- day 3
			Transfer of unaffected embryos	- day 5

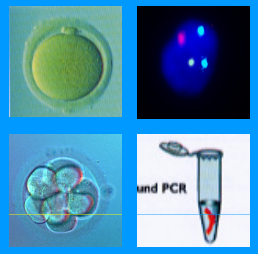
Reproductive Genetics Institute

Current Strategy

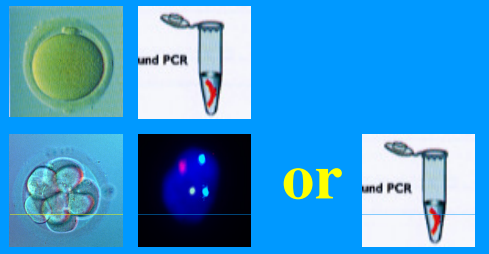
Maternal Dominant Mutation & Aneuploidy



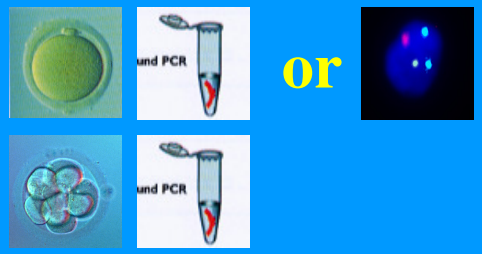
Paternal Dominant Mutation & Aneuploidy



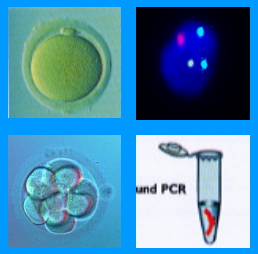
Recessive Disorder & Aneuploidy

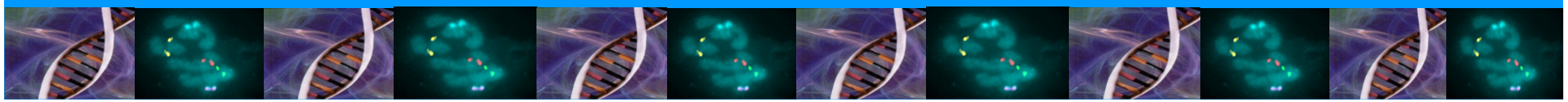


Mutation & HLA & Aneuploidy



HLA & Aneuploidy

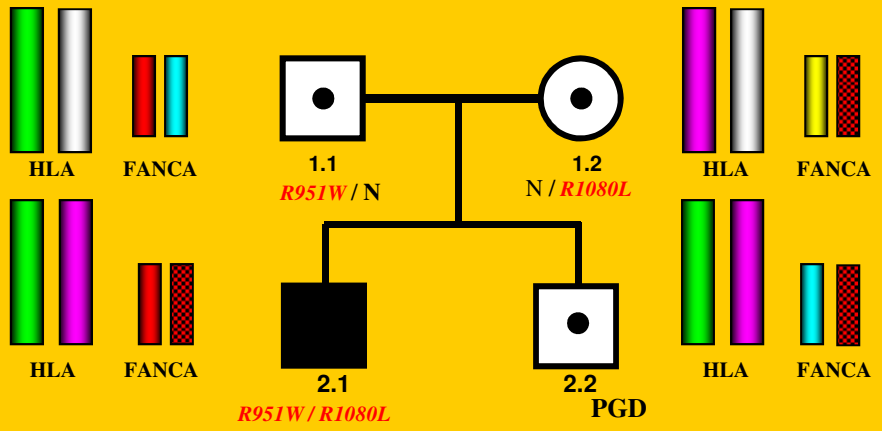




PGD for Fanconi A, HLA & Aneuploidy Testing

HLA Markers order:

D6S1568
D6S1560
TAP1
MIB
D6S265
RF
D6S306



FANCA Markers order:

D16S520
D16S3026
FANCA Intron 1
FANCA Arg951Try)
FANCA Arg1080Leu)
FANCA Intron 27 SNP
D16S3407

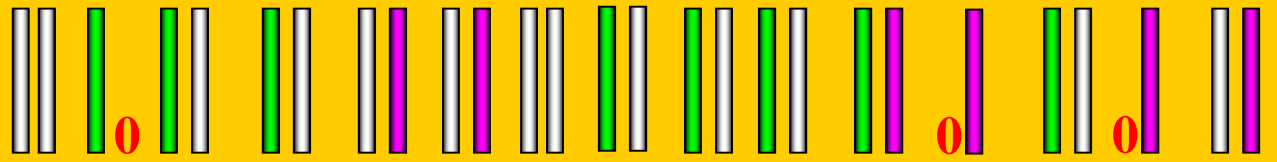
Sequential Polar Bodies Analysis

Oocyte #	1	2	3	4	5	6	7	8	9	10	11	13	14	16	18
Predicted Genotype:	*161* *160* *R1080L*	*161* *160* *R1080L*	*161* *160* *R1080L*	152 158 N	FA	*161* *160* *R1080L*	152 158 N	*161* *160* *R1080L*	152 158 N	152 158 N	*161* *160* *R1080L*	*161* *160* *R1080L*	152 158 N	*161* *160* *R1080L*	*161* *160* *R1080L*

Blstomeres Analysis

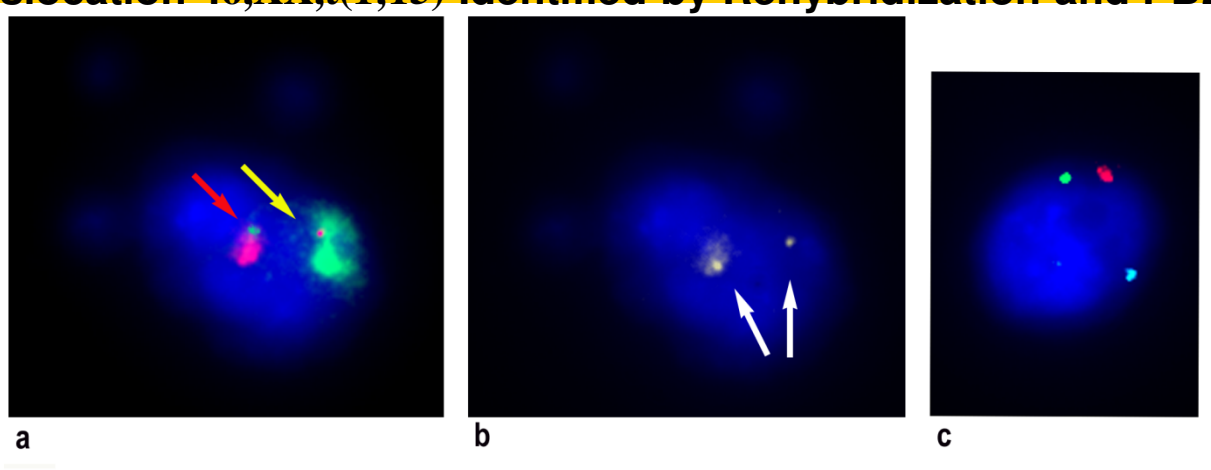
Embryo #	1	2	3	4	5	6	7	8	9	10	11	13	14	16	18
Predicted Genotype: FANCA	*R951W / R1080L*	*R1080L*	*R951W / R1080L*	*R951W / N*	N / N	N / *R1080L*	N / N	*R951W / R1080L*	N / N	*R951W / N*	N / *R1080L*	*R951W / R1080L*	N / N	FA	*R951W / R1080L*

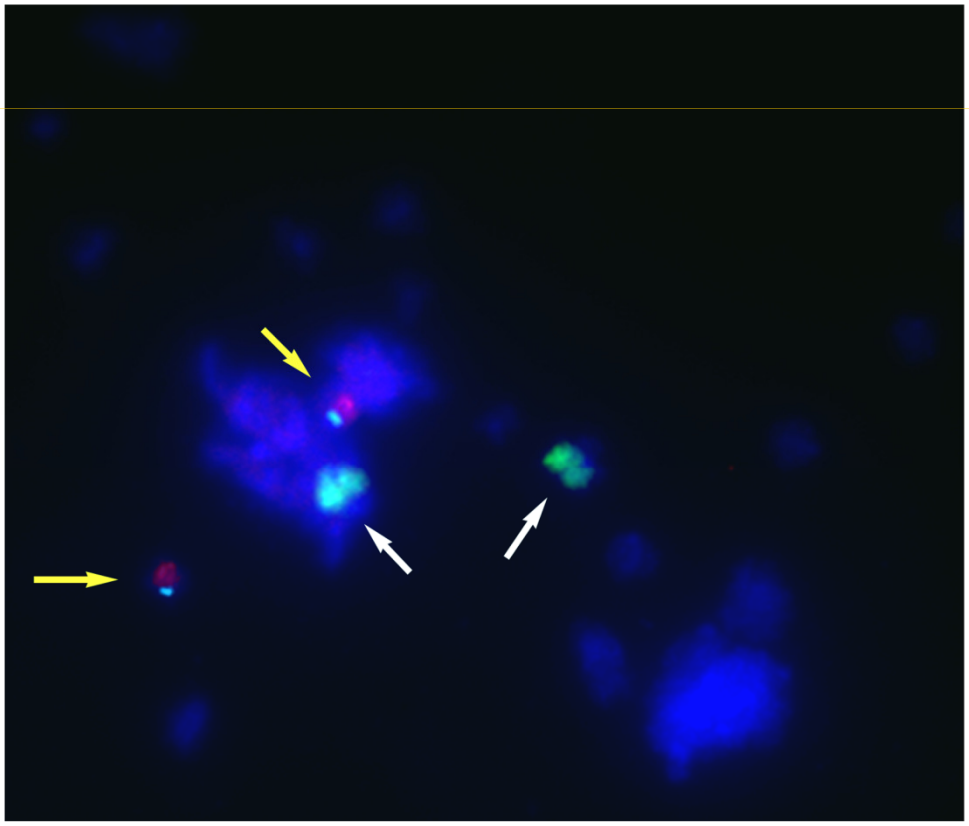
HLA Markers:
D6S1568
D6S1560
TAP1
MIB
D6S265
RF
D6S306



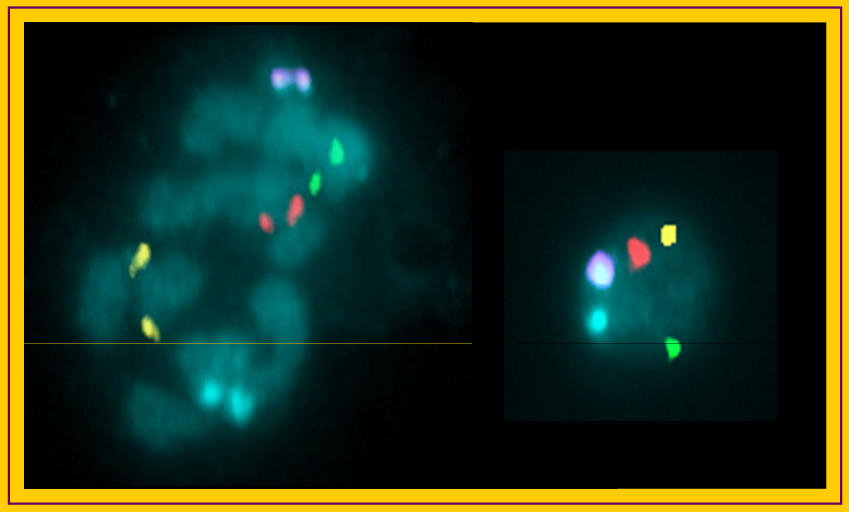
Chromosome testing															
	6, 6; 13,13; 16,16; 18,18; 21,21; 22,22; X X	6, 0 ; 13 ,0 ; 0 ,16 ; 18,18; 0 ,21 ; 22,22; Y 0	6, 6; 13,13; 16,16; 18,18; 21,21; 22,22; X Y	6, 6; 13,13; 16,16; 18,18; 21,21; 22,22; X X	6, 6; 13,13; 16,16; 18,18; 21,21; 22,22; X Y	6, 6; 13,13; 16,16; 18,18; 21,21; 22,22; X X	6, 6; 13,13; 16,16; 18,18; 21,21; 22,22; X Y	6, 6; 13,13; 16,16; 18,18; 21,21; 22,22; X X	6, 6; 13,13; 16,16; 18,18; 21,21; 22,22; X X	6, 6; 13,13; 16,16; 18,18; 21,21; 22,22; X X	6, 6; 13,13; 16,16; 18,18; 21,21; 22,22; X Y	0 , 6; 13,13; 16,16; 18,18; 21,21; 22,22; X Y	6, 6; 13,13; 16,16; 18,18; 21,21; 22,22; X X X	0 , 6 ; 0 ,13 ; 16,16 ; 18,18; 0 ,21 ; 22,22; 0, X	6, 6; 13,13; 16,16; 18,18; 21,21; 22,22; X X
	Affected, *Non-match*	Monosomy 6,13,16,21 & Y 0	Affected, *Non-match*	Carrier, *Non-match*	Normal, *Non-match*	Carrier, *Non-match*	Normal, *Non-match*	Affected, *Non-match*	Normal, *Non-match*	Carrier, *Non-match*	Carrier, Match ET	Affected, Monosomy 6	Normal, *Non-match*, Trisomy X	Monosomy 6,13,21 & X 0	Affected, *Non-match*

Chromatic Exchange of Both Normal and Derivative Chromosomes in Meiosis I in PGD for translocation 46,XX,t(1;15) Identified by Rehybridization and PB2 FISH analysis



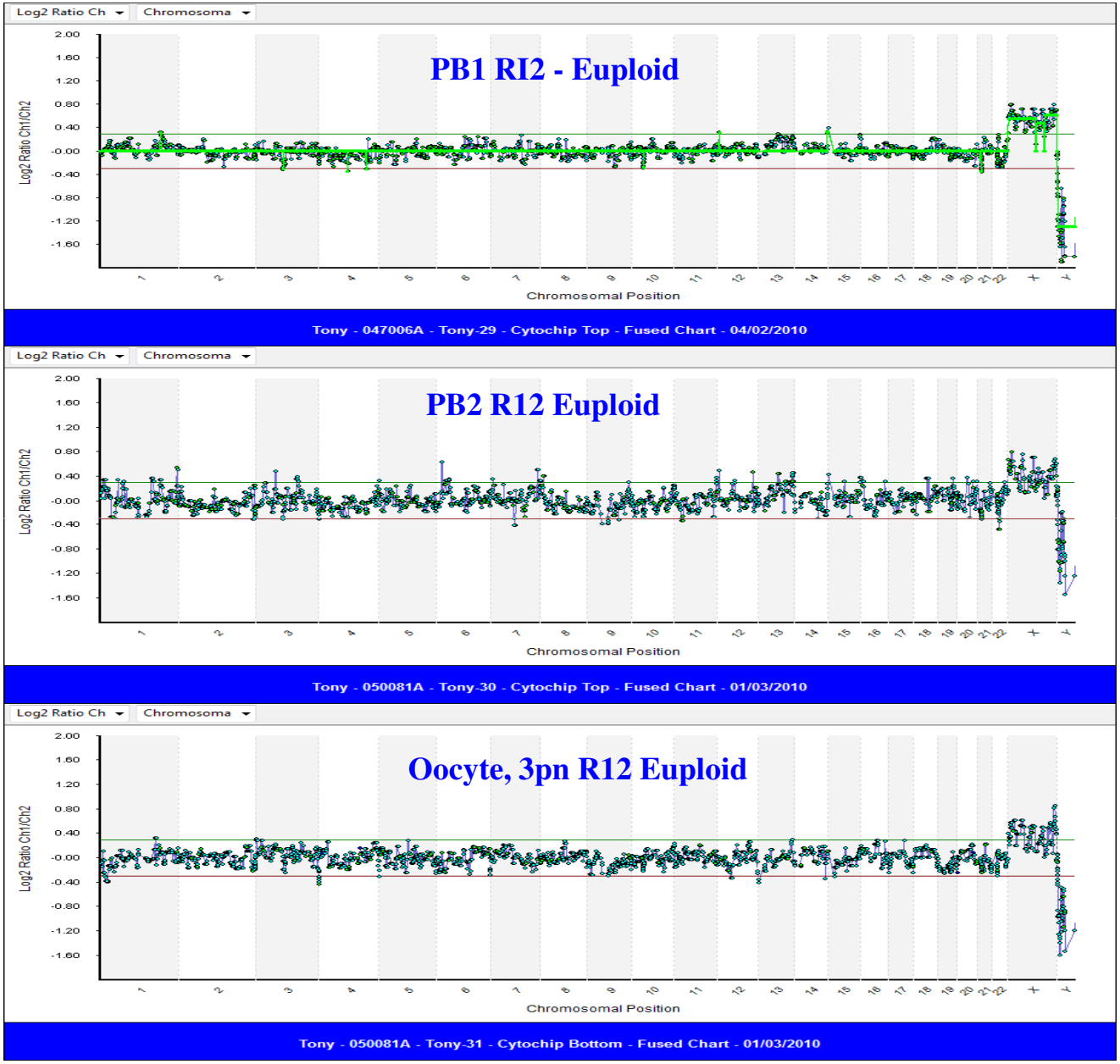


Aneuploidy testing starts with 1st & 2nd Polar Bodies

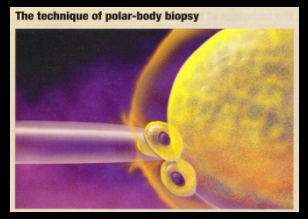


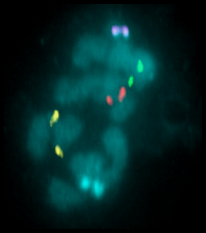
Focuses on the oocyte - the major contributor of aneuploidy in AMA due to meiotic nondisjunction

Testing is being extended to 24 chromosomes
Example: Error Free MI and MII Resulting in Euploid Oocyte

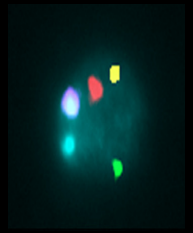


Current Aneuploidy Strategy





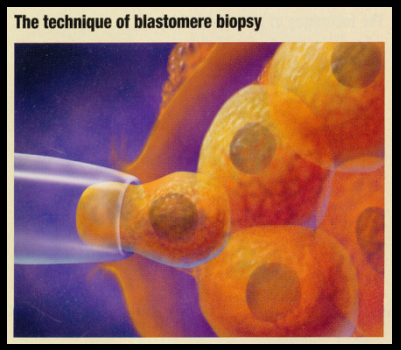
1st PB



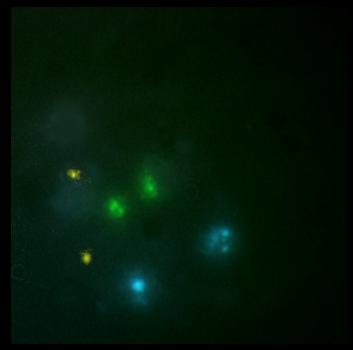
2nd PB

Polar bodies analysis for chromosomes 13, 16, 18, 21, 22

Single Blastomere analysis for chromosomes 13, 16, 18, 21, 22 followed by rehybridization for chromosomes X, Y, 15, 17



1st hybridization



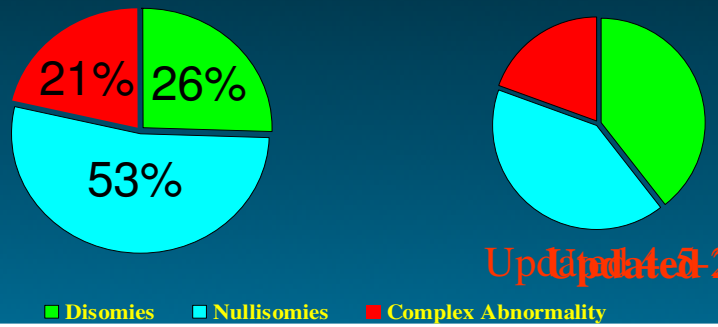
2nd hybridization

Half of Oocytes are Aneuploid (FISH), which should be detected and avoided

# Patient Cycles	# Oocytes with Results	Abnormal Oocytes
3953	20946	9772 (47.0%)

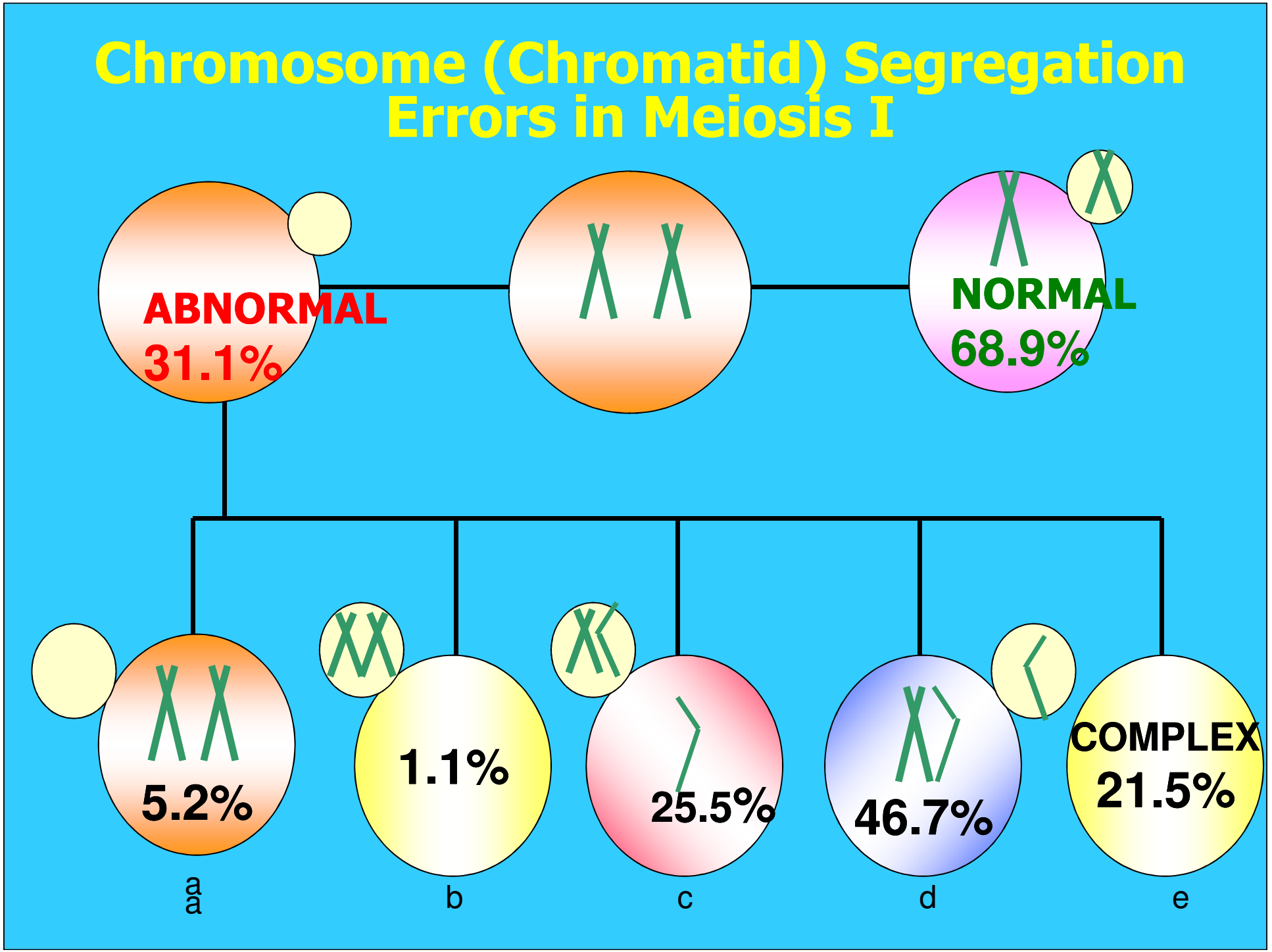
Comparable Error Rates observed in the First and Second Meiotic Divisions, so both PB1 and PB2 should be tested

FISH Results	I PB		II PB	
Normal	13097	69.0%	13635	66.0%
Abnormal	5921	31.0%	6938	34.0%
Total	19018	100%	20573	100%

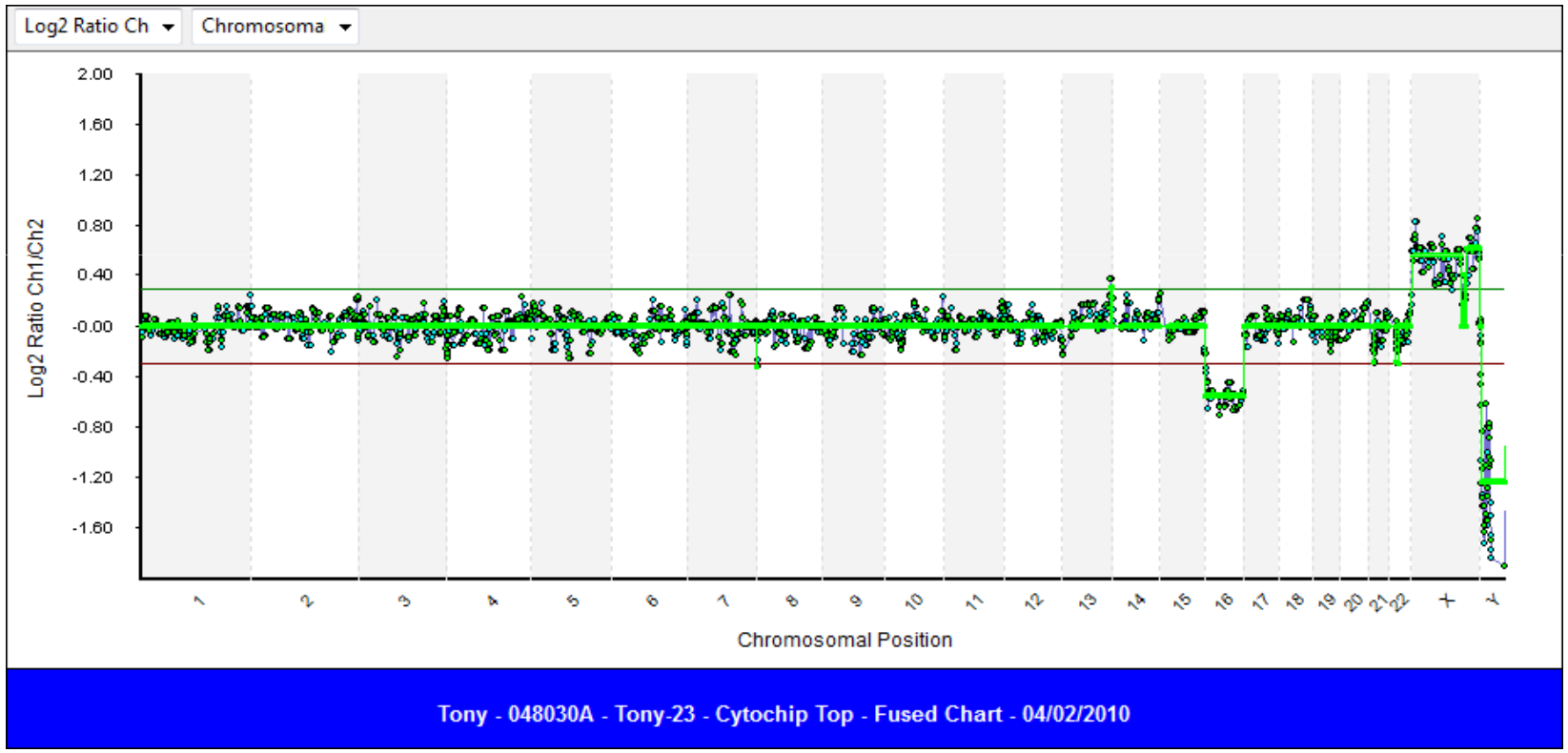


Updated 5-2009

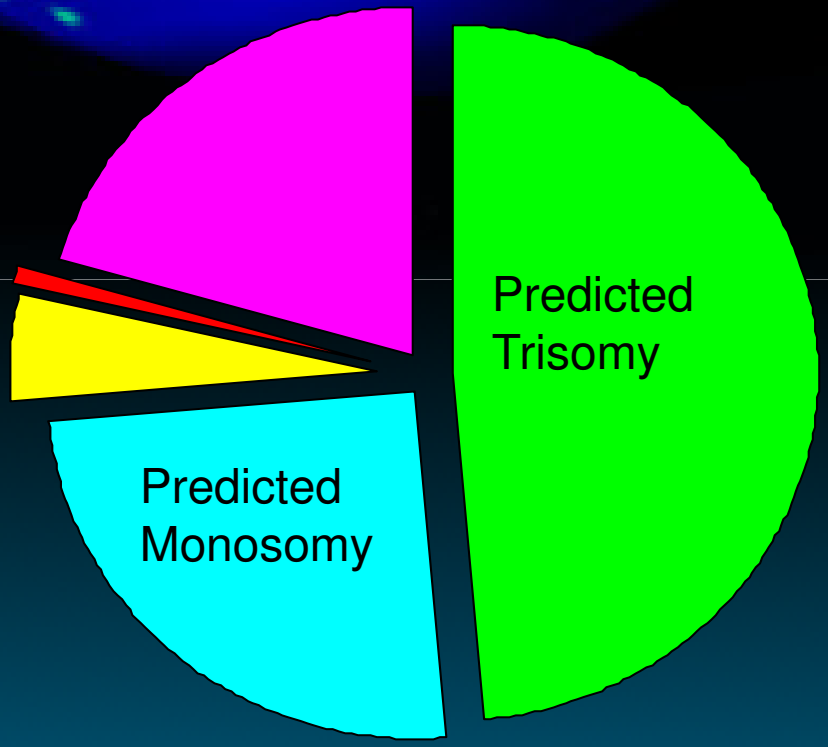
Chromosome (Chromatid) Segregation Errors in Meiosis I



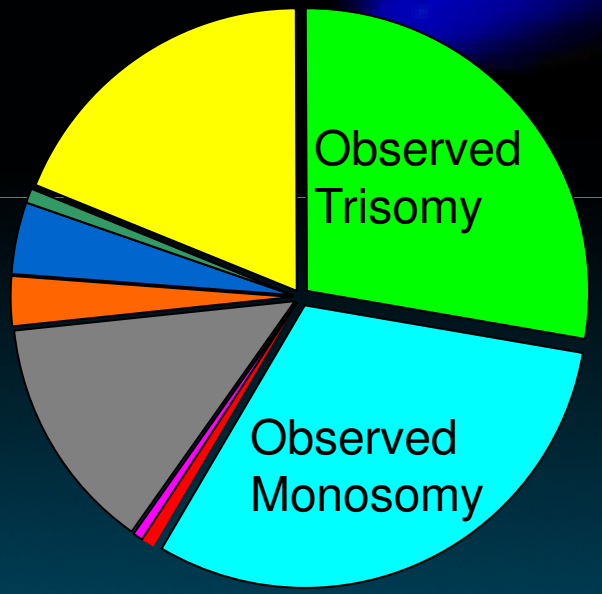
PB1 NH11 – Missing Chromatid 16



Predicted and Observed Types of Aneuploidies Based on Testing of 1st Polar Bodies and Blastomeres

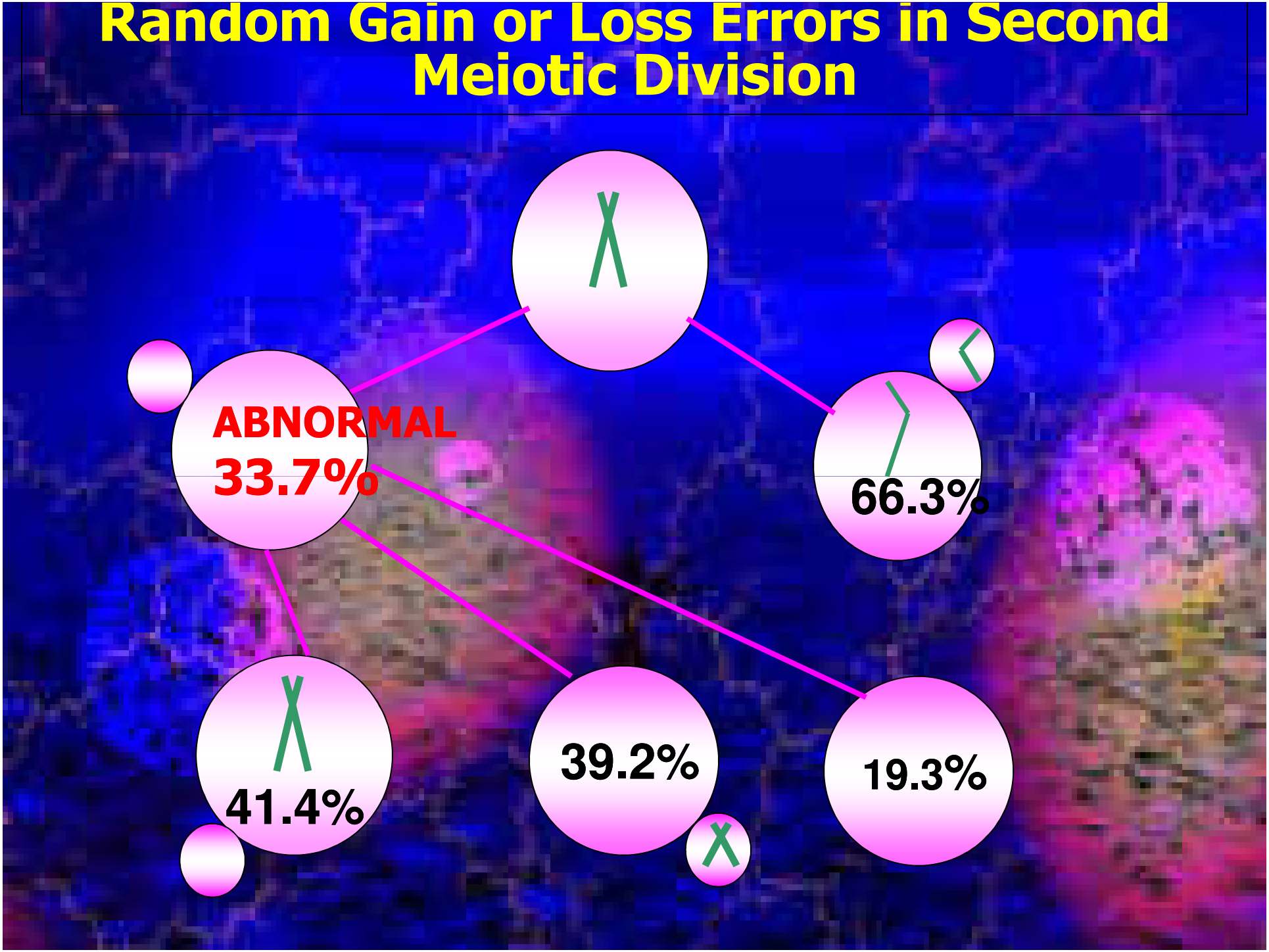


Polar Bodies

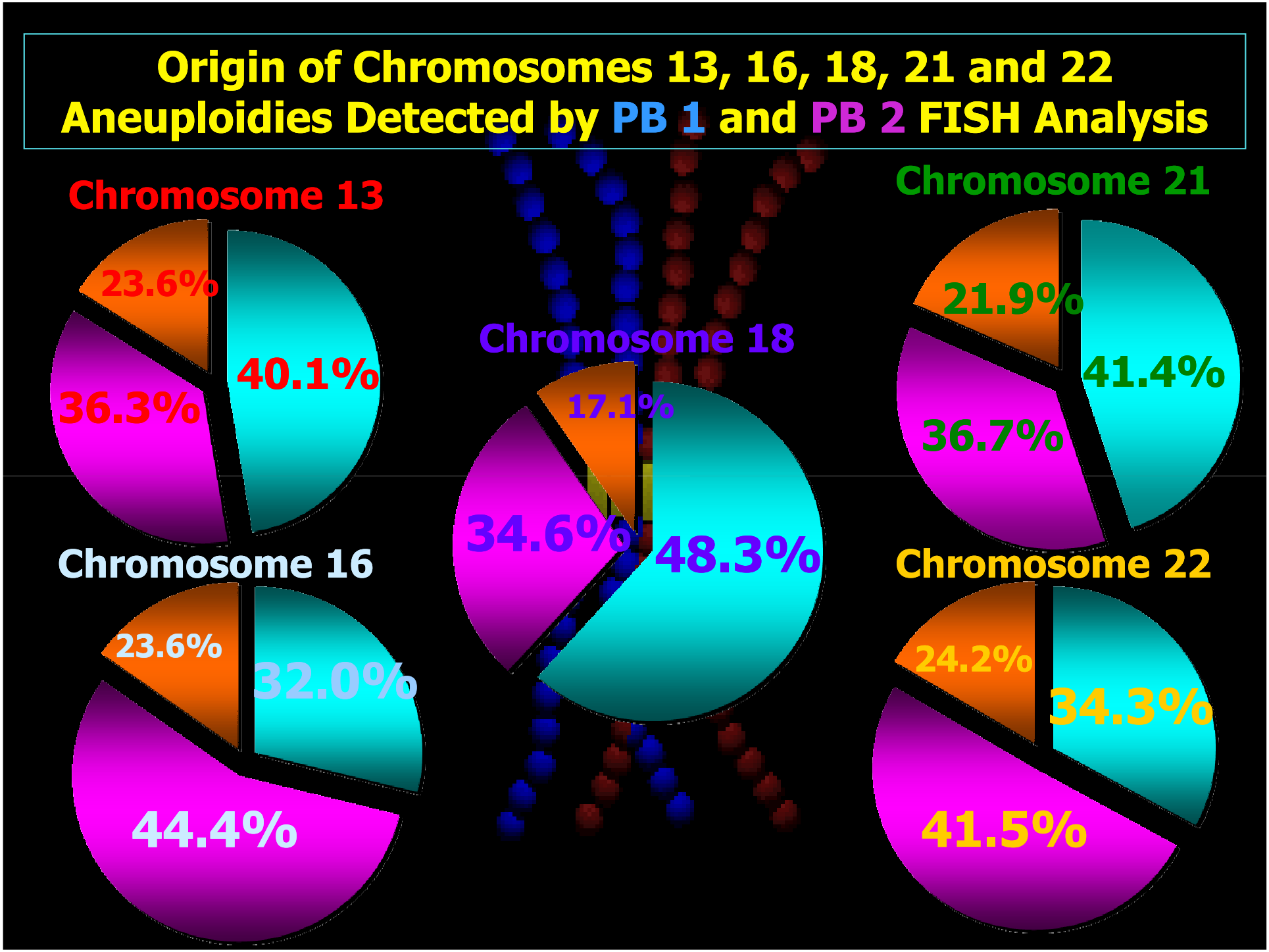


Blatomeres

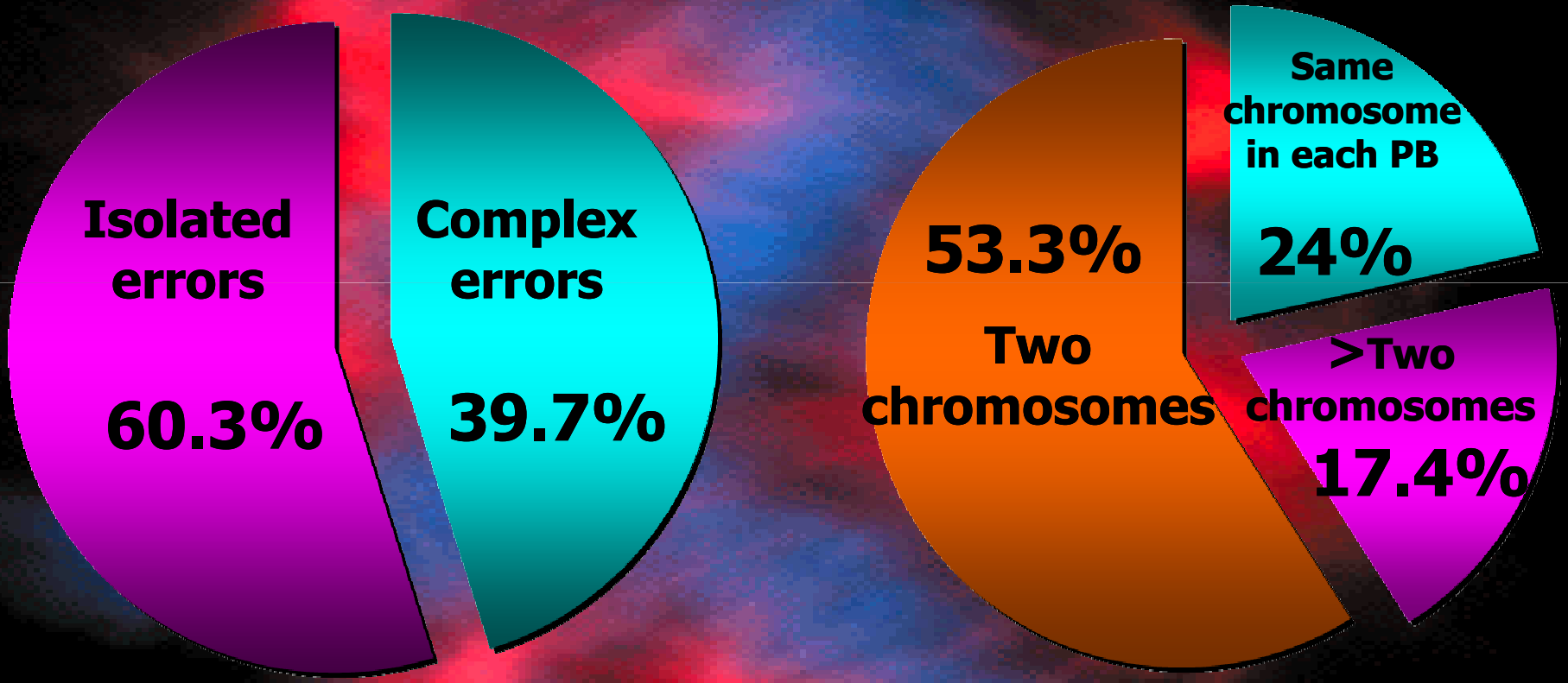
Random Gain or Loss Errors in Second Meiotic Division



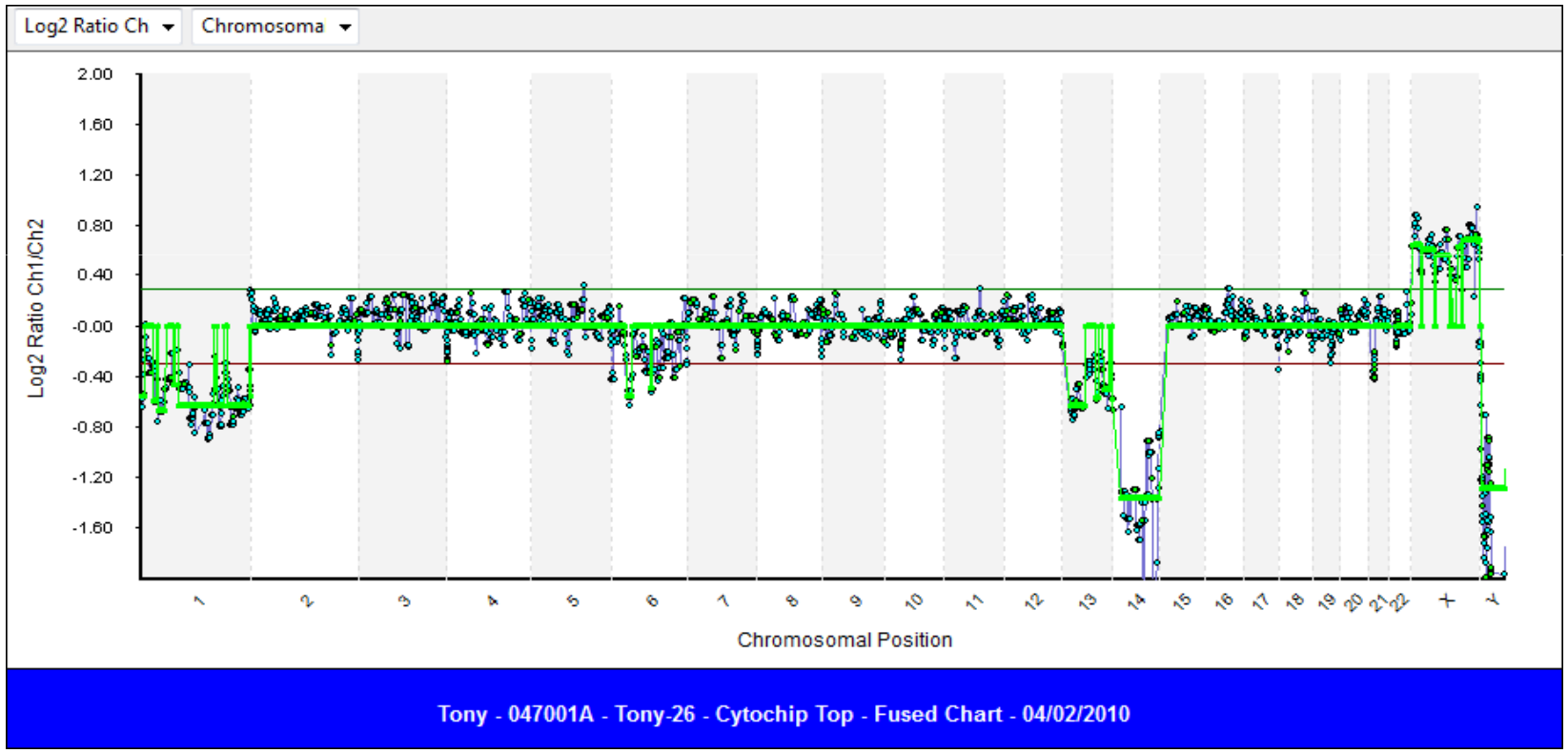
Origin of Chromosomes 13, 16, 18, 21 and 22 Aneuploidies Detected by PB 1 and PB 2 FISH Analysis



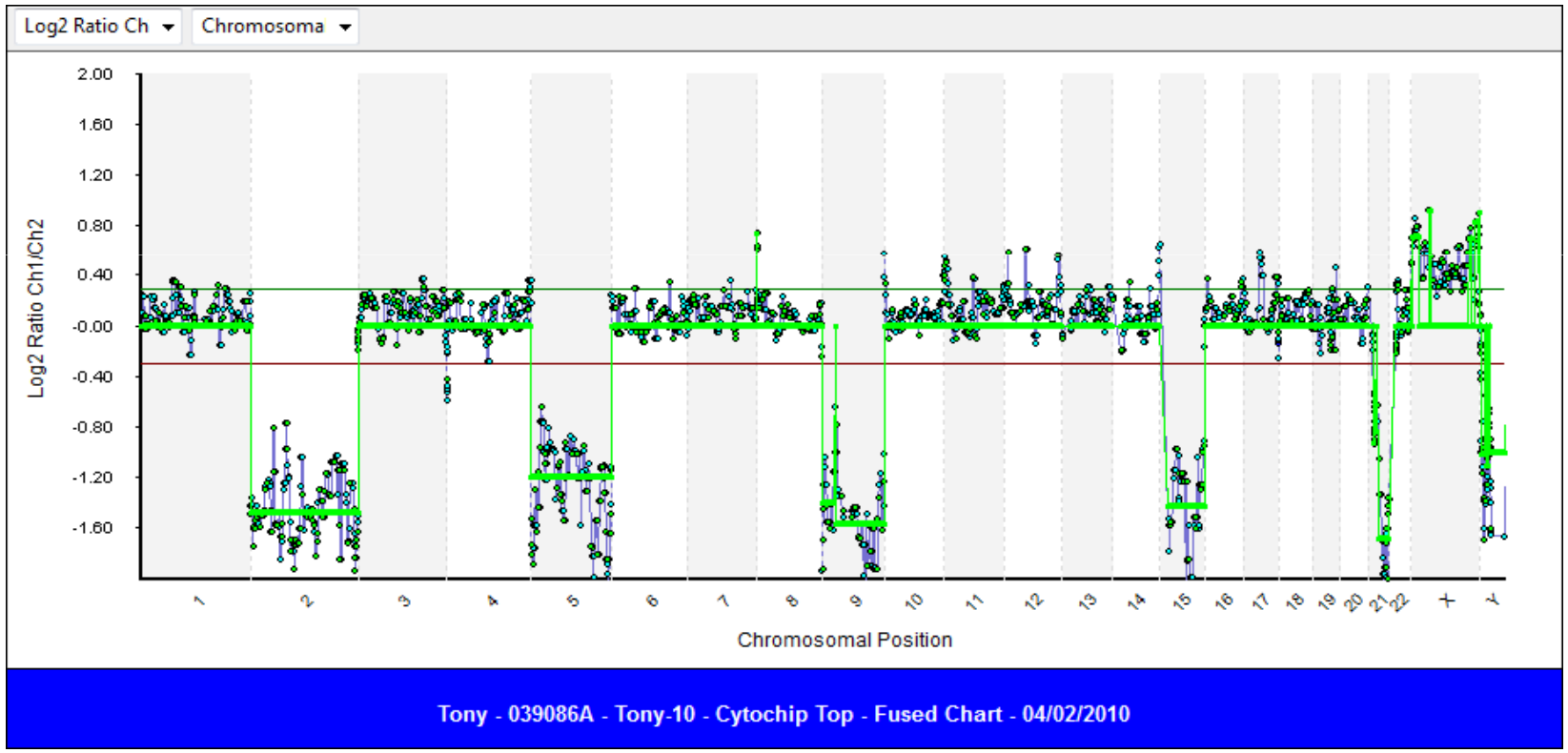
Complex Aneuploidies



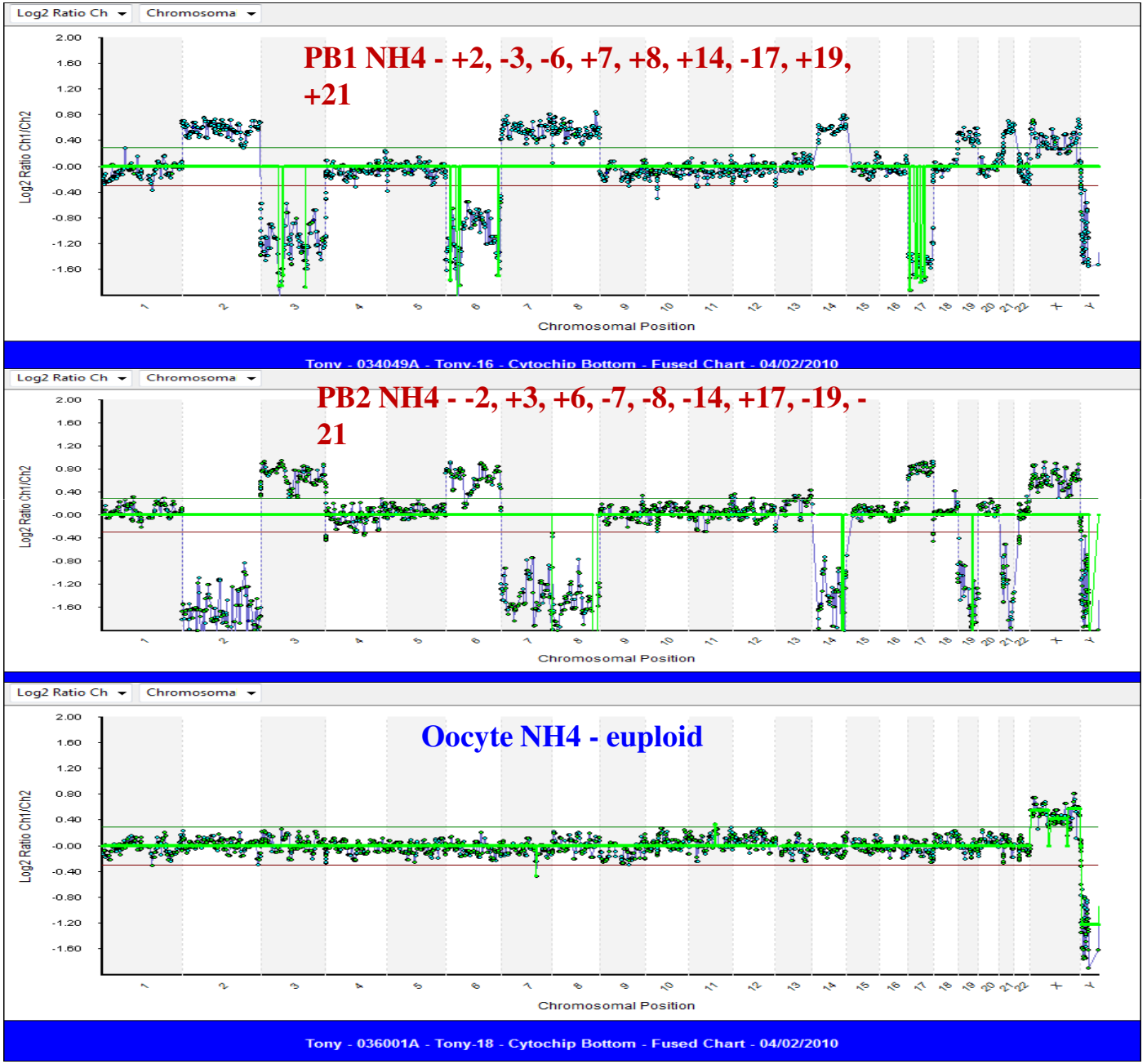
PB1 NH13 - -1, -6, -13, -14



PB2 KA6 - -2, -5, -9, -15, -21



Complimentary errors in MI and MII resulting in Euploid (Balanced) Oocyte Karyotype

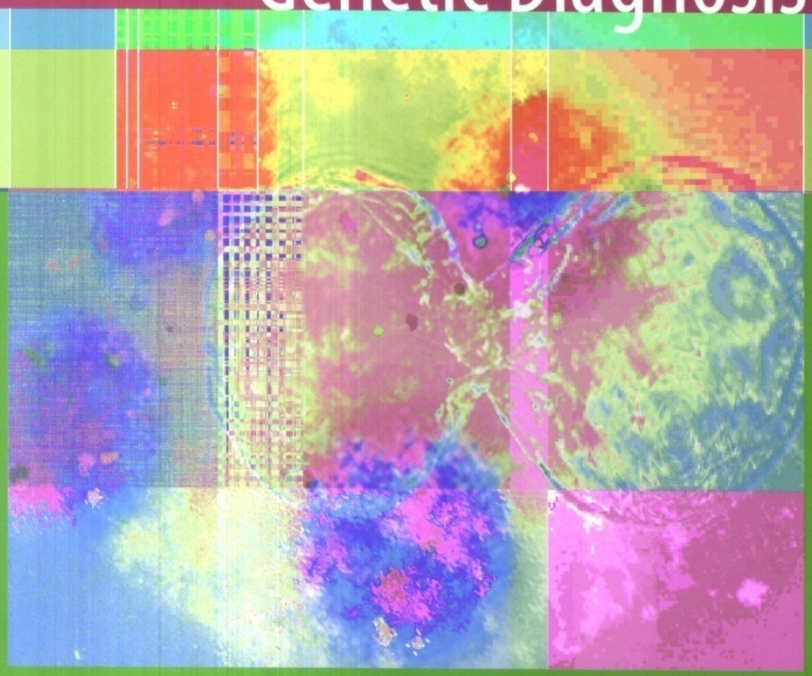


CONCLUSIONS

- ***Polar Body Approach to PGD is applicable both to Chromosomal and Single Gene Disorders, involving testing of PB1 and PB2 prior to singamy***
- ***While providing possibility for Pre-embryonic diagnosis for those couples who cannot accept embryo biopsy and discard, PB approach is also part of comprehensive strategy in cases of complex indications for PGD***
- ***There is comparable prevalence of MI & MII errors, one third of which are isolated events not detected by PB1.***
- ***Random nullisomy or disomy in MII contrasts to 2:1 ratio of missing and extra chromatid/chromosome finding in MI***
- ***Predominance of predicted trisomy by polar body analysis contrasts with the observed predominance of monosomic embryos, suggesting a limited diagnostic value of blastomere analysis***
- ***Over one third of meiotic errors are complex, indicating to an overall disturbances in female meiosis, which may possibly be detected by testing of limited number of chromosomes, despite current feasibility of 24 Chromosome testing by microarray analysis***
- ***Accurate assessment of embryo genotype requires a combined oocyte and embryo testing, particularly in testing for chromosomal disorders***

Yury Verlinsky
Anver Kuliev

Practical Preimplantation Genetic Diagnosis



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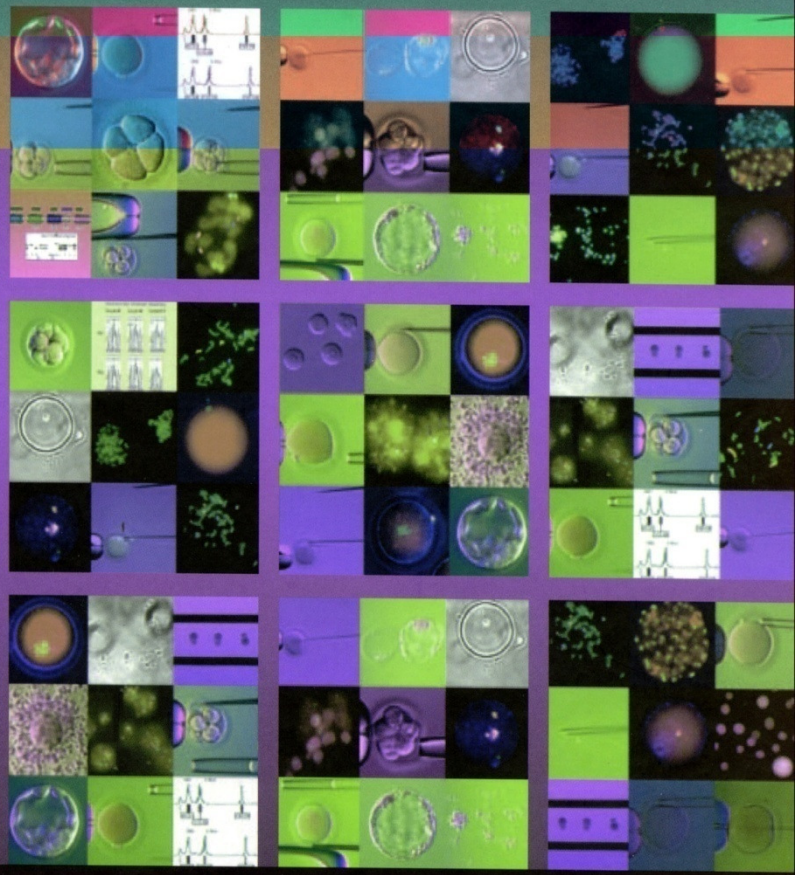
Atlas of

PREIMPLANTATION GENETIC DIAGNOSIS

Second Edition



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