ADVANCES AND PITFALLS IN PGT: WHAT THE IVF LAB SHOULD KNOW

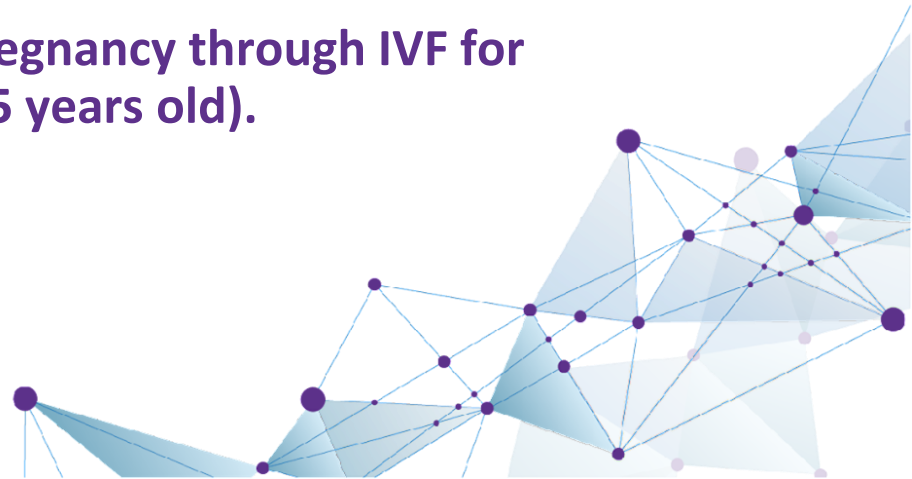
Svetlana Rechitsky, PhD[1,2]

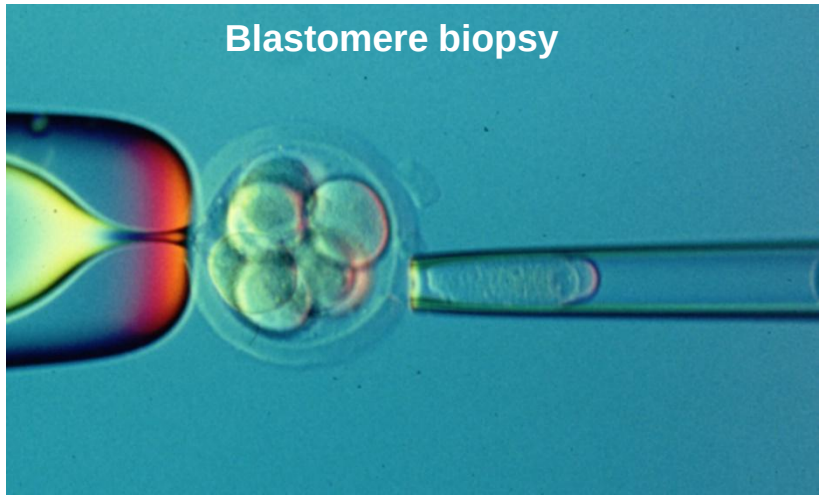
[1]Reproductive Genetics Innovation (RGI),Northbrook, Illinois

[2]Florida International University Miami, Florida

Advantages of PGT

- Reduces the risk for a known single gene disorder in a pregnancy
- Provides an acceptable option for at-risk couples that would not consider terminating an affected pregnancy detected by CVS or amniocentesis.
- Decreases the chance of miscarriages and unbalanced offspring in patients who carry balanced translocations.
- Increases the chance of a successful pregnancy through IVF for women with fertility problems (over 35 years old).

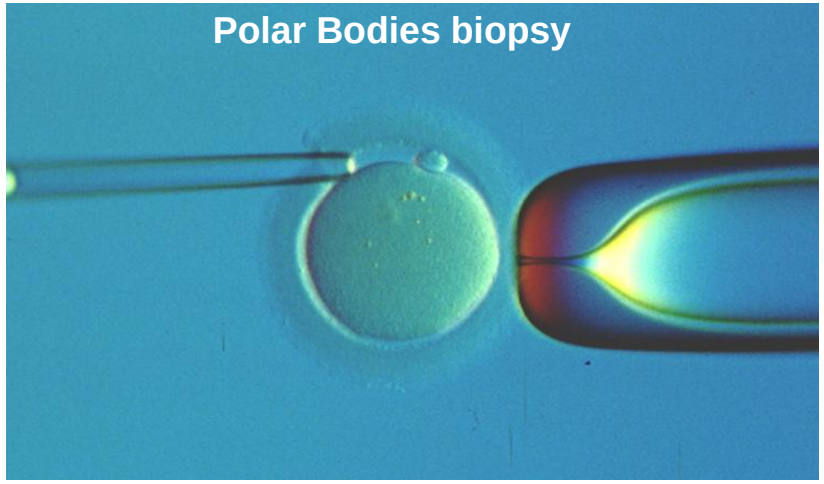




Alan Handyside

Hammersmith Hospital, London

PGT for X-linked Condition



Yuri Verlinsky

Reproductive Genetic Institute, Chicago

PGT for Autosomal Recessive Condition

RGI
REPRODUCTIVE GENETIC INNOVATIONS LLC

PGT-M: Traditional and Novel Indications

- **Autosomal recessive & dominant disorders**
- **X-linked diseases**

- **Late onset diseases:**
  - **Cancer predisposition**
  - **Inherited cardiac diseases**
  - **Neurodegenerative conditions**
  - **HLA genotyping**

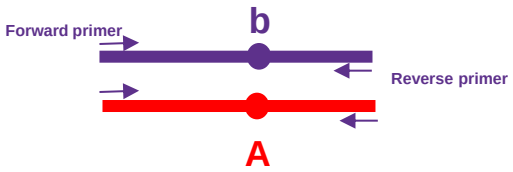
Biological Factors That Affect PGT-M Accuracy

- **Allele drop out (ADO)**
- **Failed amplification**
- **Presence of a Pseudogene**
- **Recombination: Crossover between the two copies (alleles) of a given gene**
- **Aneuploidy and Uniparental disomy**
- **Consanguinity (Difficulty obtaining unique markers)**

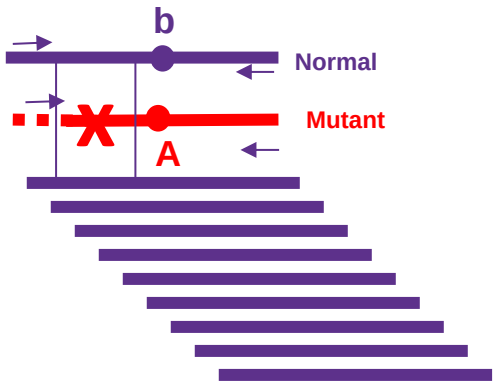
Clinical Consequences of Allele Drop Out in Autosomal Dominant Conditions

A/b	b/b
50%	50%

Heterozygous Embryo

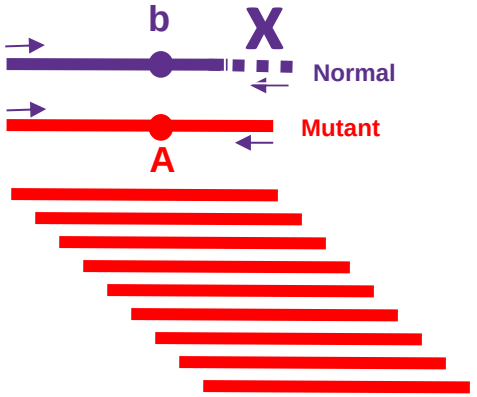


Incorrect Prediction: Normal (b/b) –ADO of A
Should be: Affected (A/b)



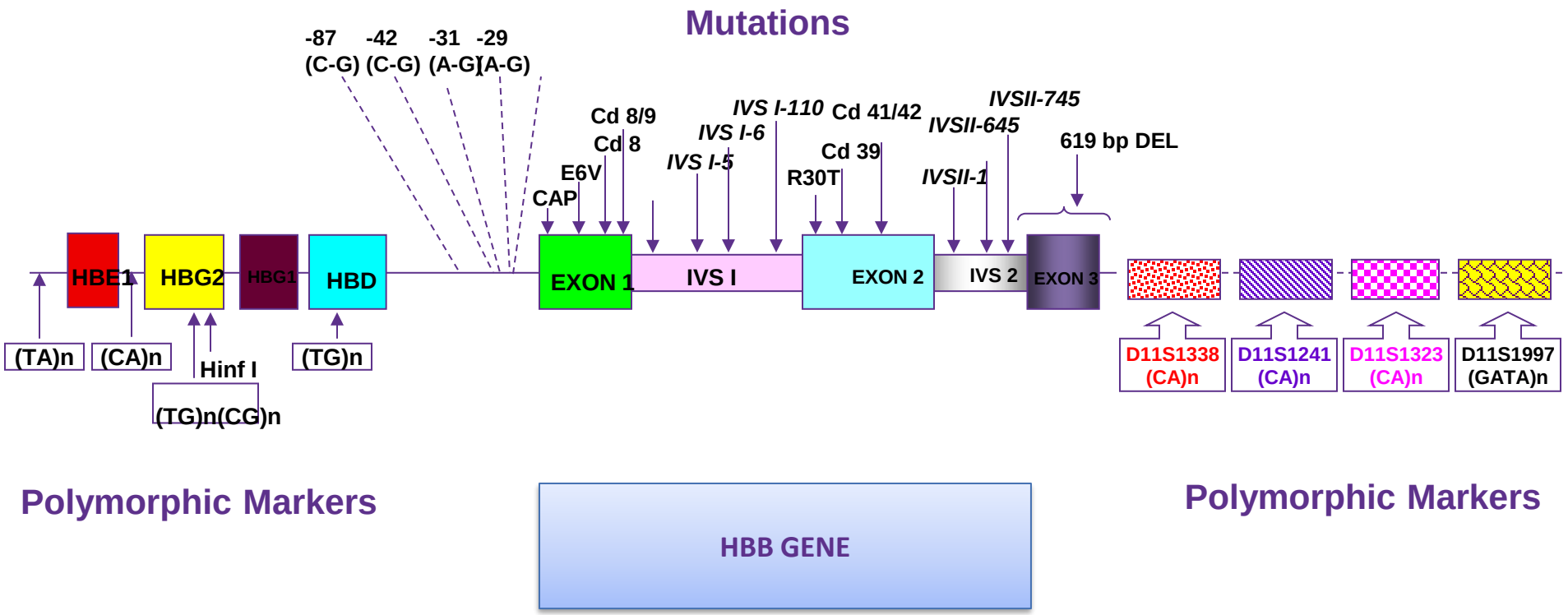
Outcome:
CLINICAL MISDIAGNOSIS!

Mutant (A/ADO of Normal allele)



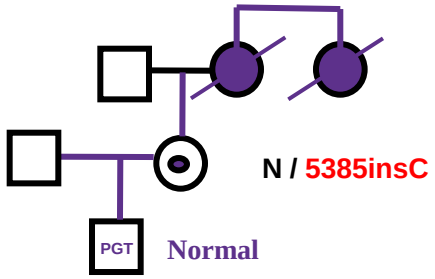
Outcome:
Misdiagnosis without clinical consequences

Mutations In Beta-globin Gene And Polymorphic Markers Used In Multiplex PCR Analysis



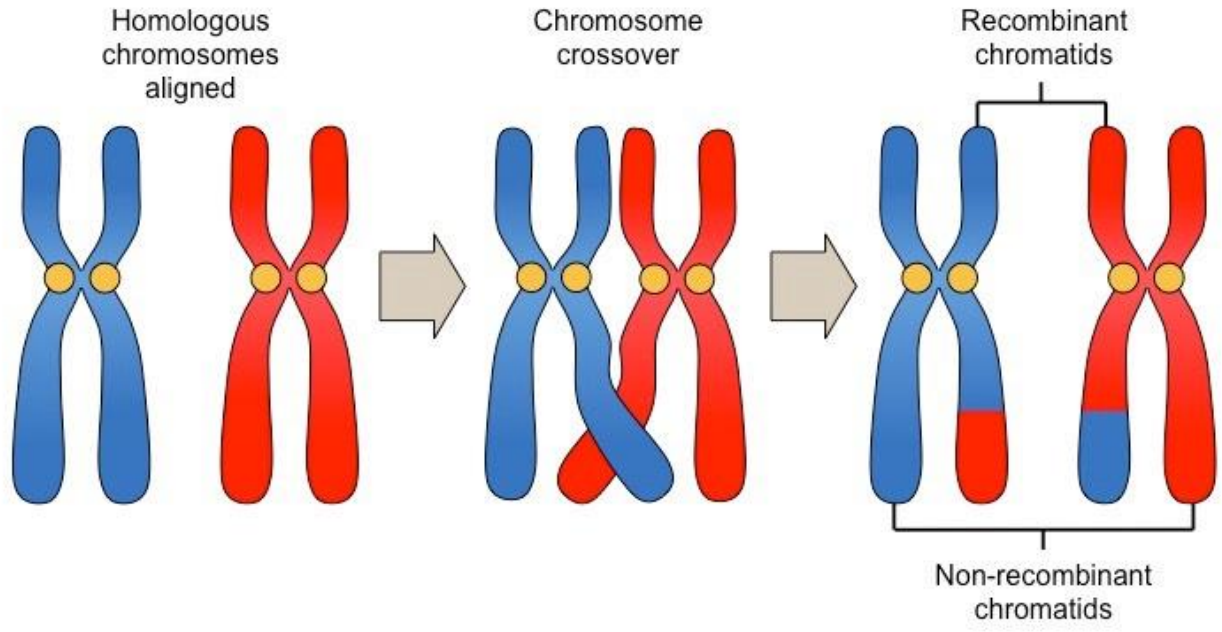
ADO Of Dominant Mutant Allele Detected By Linked Markers

PGT-M for BRCA1 mutation



Embryo #	D17S 1801	D17S 932	BRCA1	8(CA)	3'end	D17S 934	Embryo #	Predicted Genotype	TC	Comments
01	FA	99 / 118	N / 5385insC	84 / 104	177 / 173	159 / 167	01	AFFECTED	NO	
02	ADO/ 129	110 / 120	N	96 / 106	173 / 175	161 / 152	02	NORMAL	YES	
03	137 / 127	110 / 118	N / ADO	96 /104	173	161 / ADO	03	AFFECTED	NO	
04	125 / 129	99 / 120	N	84 / 106	177 / 175	159 / 152	04	NORMAL	YES	
05	125 / 129	99 / 120	N	84 / 106	177 / 175	159 / 152	05	NORMAL	YES	
06	137 / 127	110 / 118	N / 5385insC	96 / 104	173	ADO / 167	06	AFFECTED	NO	
08	ADO/ 127	110 / 118	N / 5385insC	96 / 104	173	161 / 167	08	AFFECTED	NO	
09	137 / 127	110 / 118	N / 5385insC	96 / 104	173	161 / 167	09	AFFECTED	NO	
10	137 / 127	110 / 118	N / 5385insC	96 / 104	173	161 / 167	10	AFFECTED	NO	
	125 / 137	99 / 110	N / N	84 / 96	173 / 177	159 / 161	PARTNER			
	129 / 127	120 / 118	N / 5385insC	106 / 104	175 / 173	152 / 167	PATIENT			

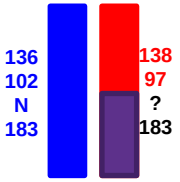
RECOMBINATION AS A SOURCE OF MISDIAGNOSIS



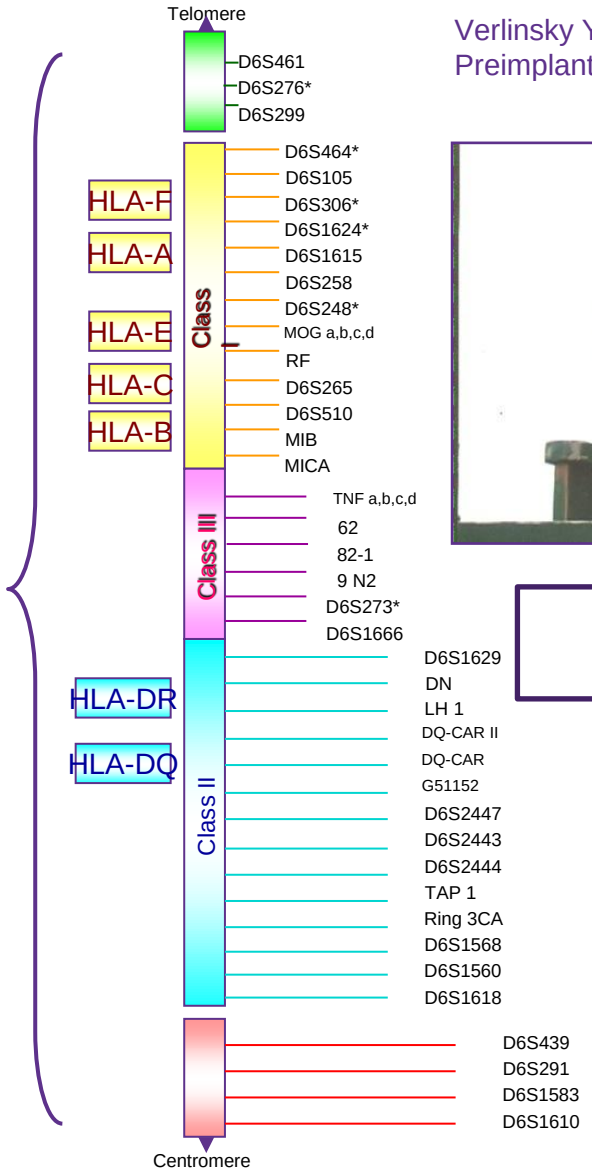
Recombination: crossover between the two copies (alleles) of a given gene

Recombination: Crossover Between The Two Copies (Alleles) Of A Given Gene

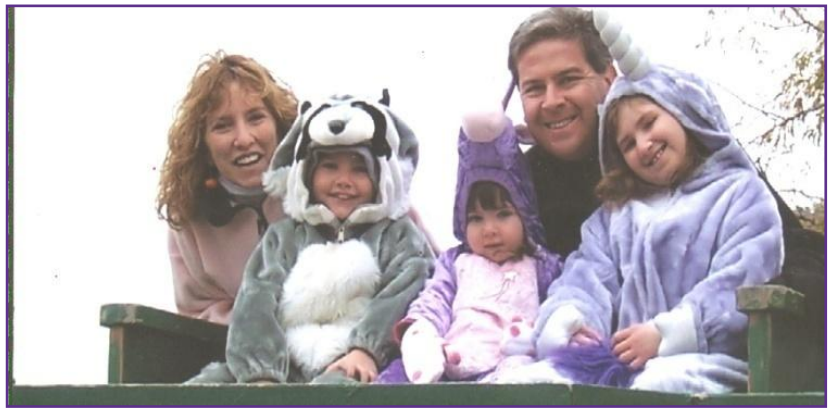
Embryo #	D17S1795	D17S588	COL1A1	D17S1869	Predicted Genotype
3	134/136	100/80	N	185/183	NORMAL
4	136/138	102/97	N/c.2685	183/181	AFFECTED
5	134/138	100/97	N/c.2685	185/181	AFFECTED
7	136/138	102/97	N	183	Recombinant
OI-121	134/136	100/102	N/N	185/183	PARTNER: (NORMAL)
OI-122	136/138	80/97	N/ c.2685	183/181	PATIENT: (AFFECTED)



PGT-HLA STRATEGY FOR HLA HAPLOTYPING



Verlinsky Y, **Rechitsky S**, Verlinsky O, Schoolcraft W, Strom C, Kuliev A.
Preimplantation diagnosis for Fanconi anemia combined with HLA matching. ***JAMA*, 2001**; 285:1-4



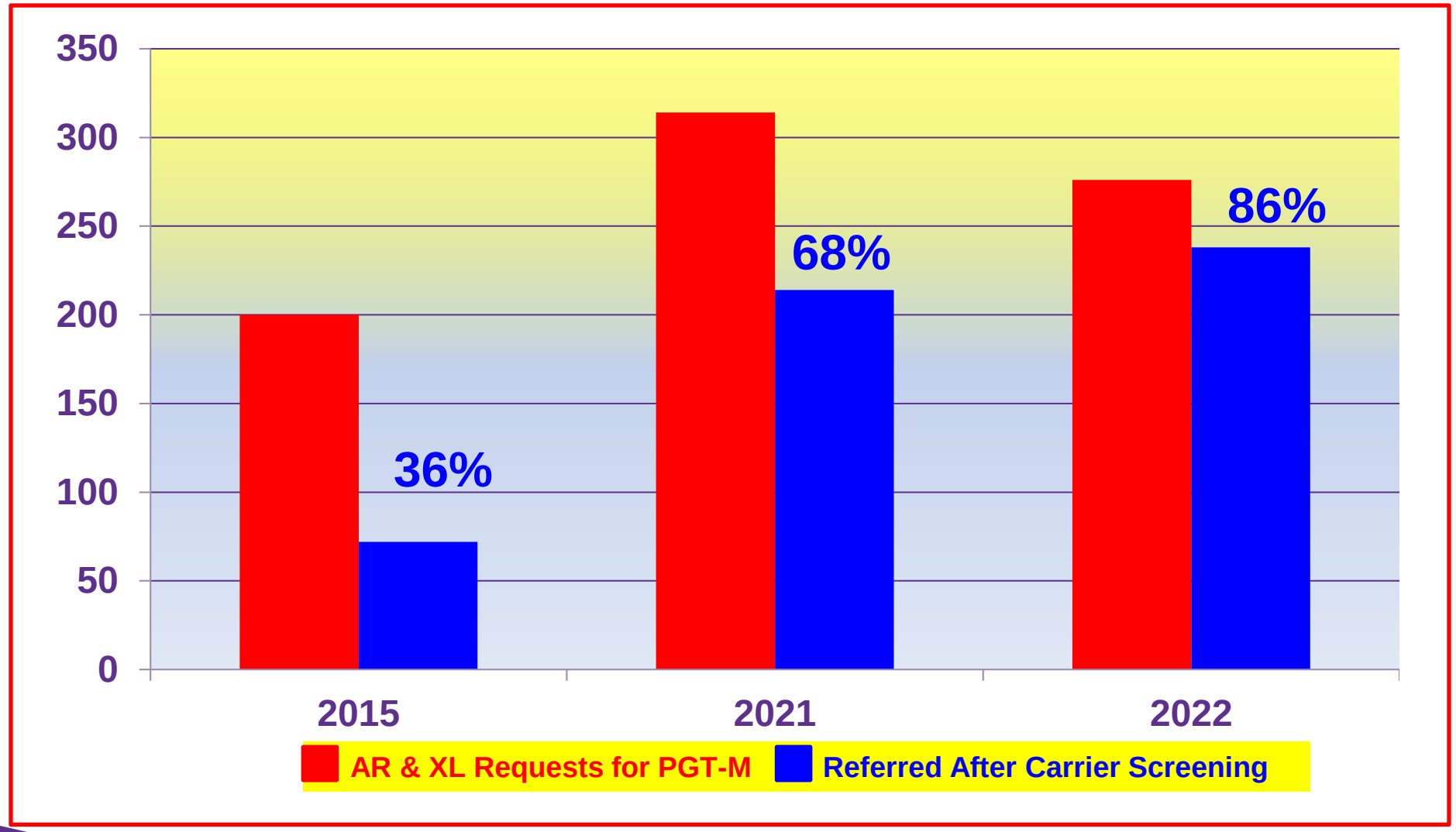
$\frac{1}{4} \times \frac{3}{4} = 3/16$

MHC class I	
locus	#
Major Antigens	
HLA A	**767**
HLA B	**1,178**
HLA C	**439**
Minor Antigens	
HLA E	**9**
HLA F	**21**
HLA G	**43**

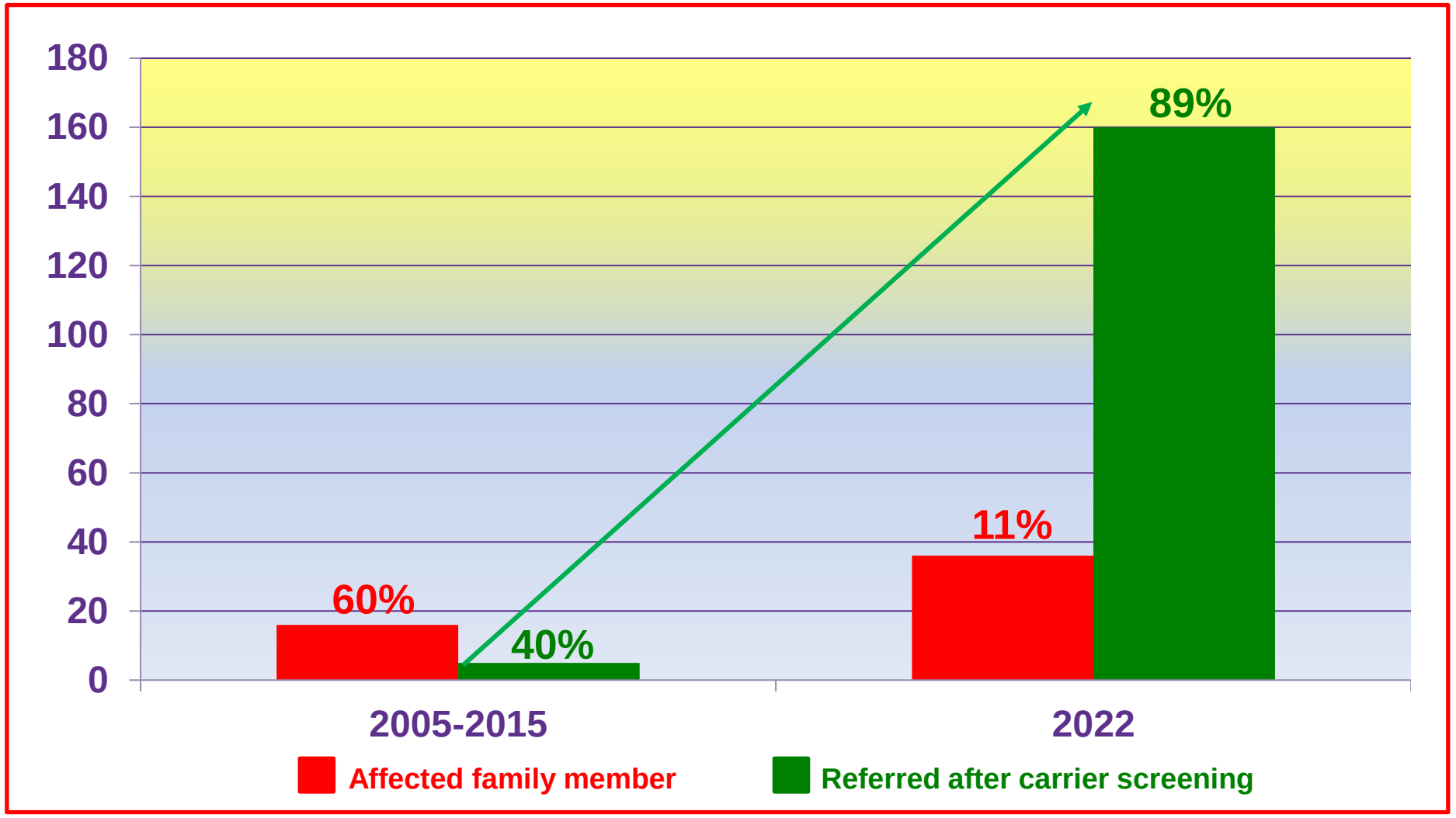
MHC class II				
HLA	**-A1**	**-B1**	**-B3 to -B5**[1]	Potential
locus	#	#	#	Combinations
DM-	**4**	**7**		28
DO-	**12**	**9**		72
DP-	**27**	**133**		3,591
DQ-	**34**	**96**		3,264
DR-	**3**	**618**	**82**	2,121
[1]DRB3, DRB4, DRB5 have variable presence in humans				

Changes Of Indication Profile Since Incorporation ECS:

INCREASED UTILIZATION OF PGT-M THROUGH PAN-ETHNIC EXPANDED CARRIER SCREENING



Increase in PGT-M Requests for Hereditary Deafness (*14* gene) After Carrier Screening



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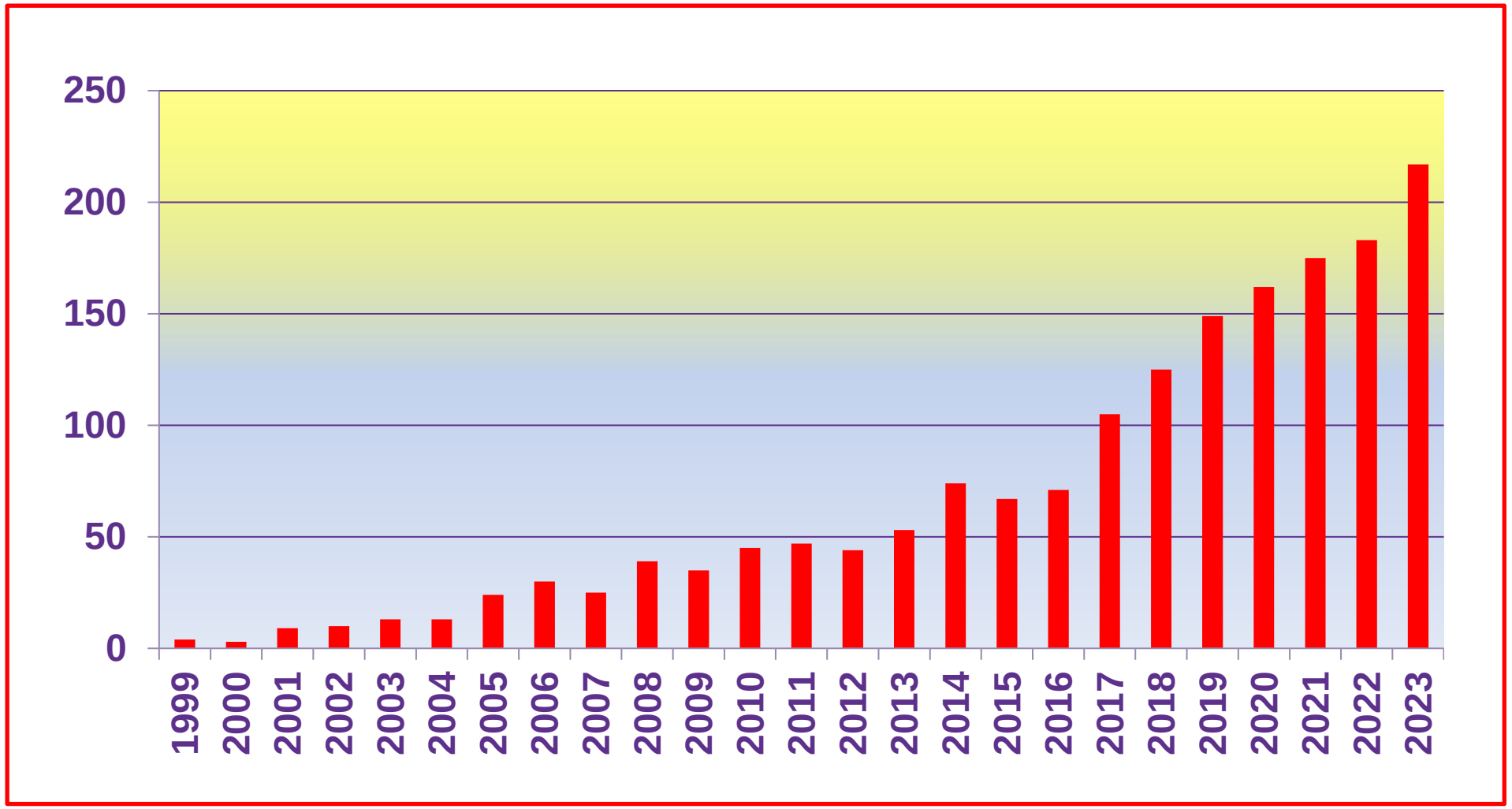
Steady Increase Of PGD Cycles For Cancer Predisposition After First Description In 1999



PGT-M for BRCA1, Fragile X and Aneuploidy by NGS

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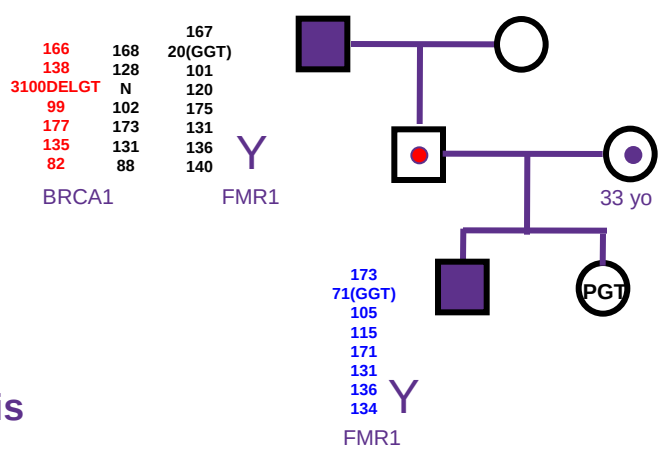
A. Family pedigree

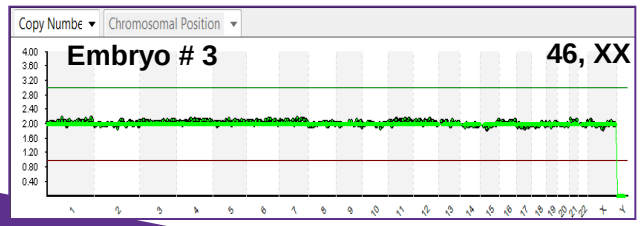


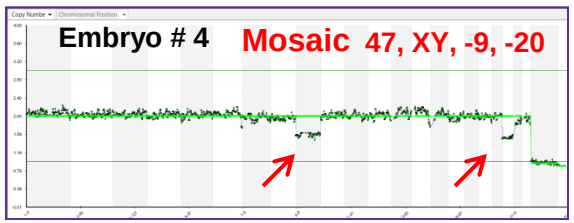
TOTAL 10
Normal BRCA1- 2
Normal FMR1- 6
SUTABLE FET - 1

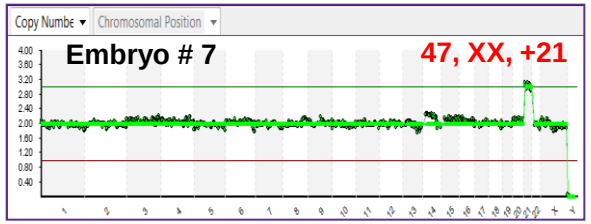
B. Trophectoderm Analysis

Embryo #	1	2	3	4	5	6	7	8	9	10
BRCA1	166/138/3100DELGT/99/177/135/82 & 174/138/N/108/175/131/96	166/138/3100DELGT/99/177/135/82 & 174/138/N/108/175/131/96	168/128/N/102/173/131/88 & 174/138/N/108/175/131/96	166/138/3100DELGT/99/177/135/82 & 174/138/N/108/175/131/96	166/138/3100DELGT/99/177/135/82 & 174/138/N/108/175/131/96	166/138/3100DELGT/99/177/135/82 & 168/130/N/104/173/131/93	168/128/N/102/173/131/88 & 168/130/N/104/173/131/93	166/138/3100DELGT/99/177/135/82 & 174/138/N/108/175/131/96	166/138/3100DELGT/99/177/135/82 & 174/138/N/108/175/131/96	166/138/3100DELGT/99/177/135/82 & 168/130/N/104/173/131/93
FMR1	171/30(GGT)/103/117/177/135/140/124 Y	171/30(GGT)/103/117/177/135/140/124 Y	167/20(GGT) & 171/30(GGT)/101/103/120/117/175/177/131/135/136/140/140/124	173/71(GGT)/105/115/171/131/136/134 Y	173/71(GGT)/105/115/171/131/136/134 Y	167/20(GGT) & 171/30(GGT)/101/103/120/117/175/177/131/135/136/140/140/124	167/20(GGT) & 171/30(GGT)/101/103/120/117/175/177/131/135/136/140/140/124	167/20(GGT) & 173/71(GGT)/101/105/120/115/175/171/131/131/136/136/140/134	167/20(GGT) & 171/30(GGT)/101/103/120/117/175/177/131/135/136/140/140/124 Y	167/20(GGT) & 171/30(GGT)/101/103/120/117/175/177/131/135/136/140/140/124
NGS	46, XY	46, XY	46, XX	47, XY, -9,-20	46, XY	46, XX	47, XX, +21	46, XX	47, XXY	46, XX

C. FET

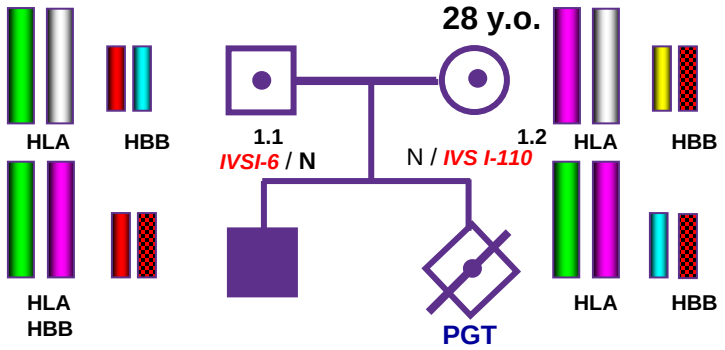




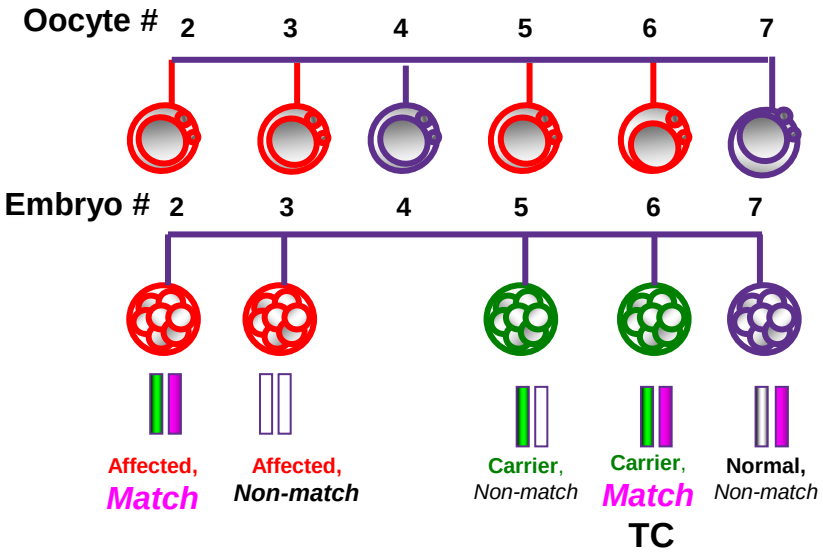


PRENATAL INCIDENTAL FINDINGS AFTER PGT-M

PGT for Thalassemia & HLA Genotyping

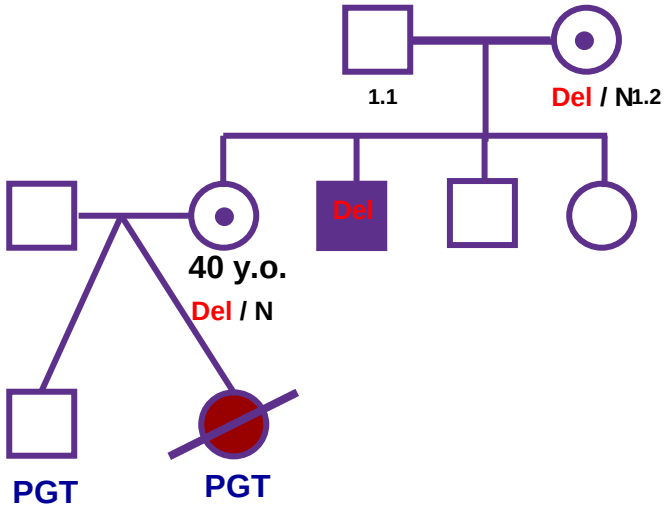


Carrier
Match XXY



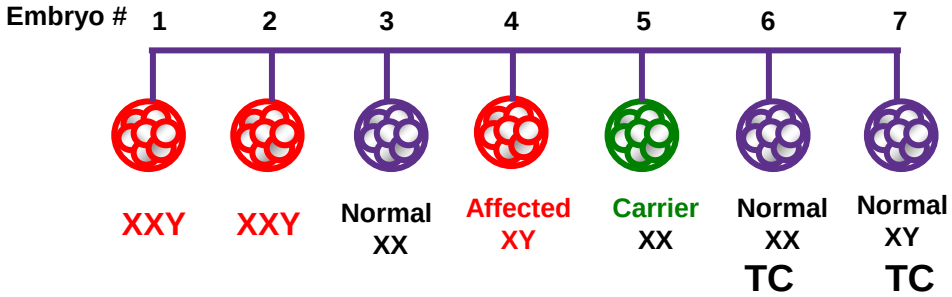
TC

PGT for DMD



Normal
46 XY

Normal for DMD
47 XX +13



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FLOW CHART FOR COMBINED TESTING

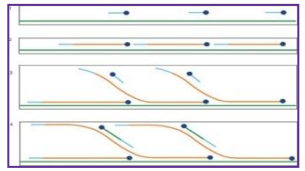
A biopsy is placed in lysis buffer and spun

Combined testing: PGT-M + PGT-A

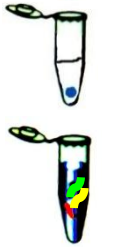
WGA:
NGS + Mutation: PEP, DOP

Karyomapping: MDA

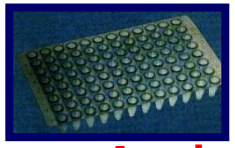
NGS: DOP,MDA or MALBAC



Mutation & Linkage (Haplotyping):
PCR Mix with multiple outside primers,

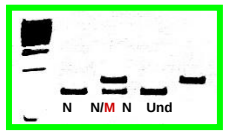


Second Round started as a separate PCR reaction for each locus

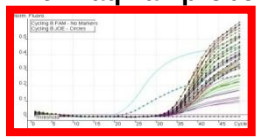


Single Gene Analysis:

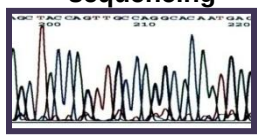
RFLP



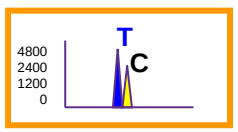
Real Time PCR with TaqMan probe



Fluorescent sequencing

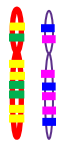


Mini-sequencing

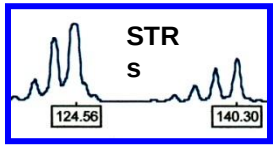


NGS

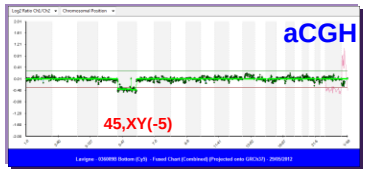
Kryomapping

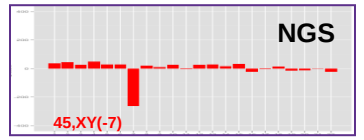


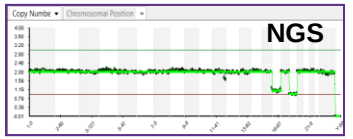
STR
s



Aneuploidy testing

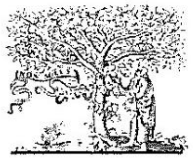






STRATEGY FOR de-novo MUTATIONS DETECTION

Reproductive BioMedicine Online (2011) 22, 350 361



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ARTICLE

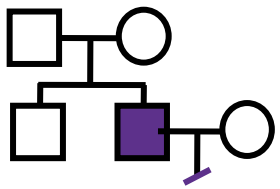
First systematic experience of preimplantation genetic diagnosis for de-novo mutations

Svetlana Rechitsky, Ekaterina Pomerantseva, Tatiana Pakhalchuk, Dana Pauling, Oleg Verlinsky, Anver Kuliev *

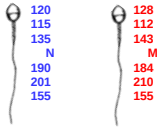
Reproductive Genetics Institute, 2825 N Halsted St., Chicago, IL 60657, USA
* Corresponding author. *E-mail address:* anverkuliev@hotmail.com (A Kuliev).

Dr. Svetlana Rechitsky is a graduate of Kharkov University's Genetics Faculty, and received her PhD in experimental molecular embryology from the second Moscow Medical Institute in 1986. She moved to the Reproductive Genetics Institute in 1989, where she heads the DNA laboratory, which has performed the largest preimplantation genetic diagnosis (PGD) series for single gene disorders, with PGD design for the majority of them developed for the first time. She has published more than 40 papers in the field of PGD, including a key contribution to the *Atlas of Preimplantation Genetic Diagnosis*.

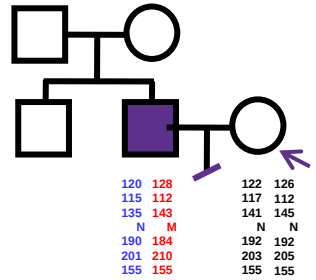
PGT-M Strategy for De Novo PATERNAL Mutations



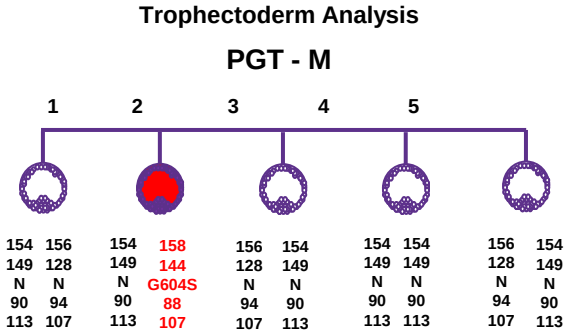
MUTATION CONFIRMATION and STR analysis
ON DNA FROM BLOOD,
BUCKLE SWABS or TOTAL SPERM



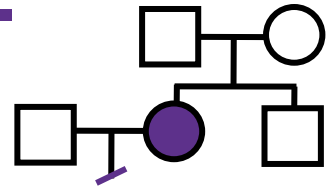
SINGLE SPERM
HAPLOTYPNG (15-50)



COUPLE GENOTYPING



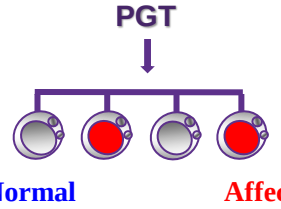
PGT-M Strategies for de novo MATERNAL Mutations



198	198	(198 201)
125	129	(125 127)
167	169	(167 165)
N	N	(N M)
117	117	(115 112)
132	138	(132 136)
176	178	(176 176)

MUTATION CONFIRMATION AND
POLYMORPHIC MARKERS EVALUATION
ON DNA BUCCAL SWABS

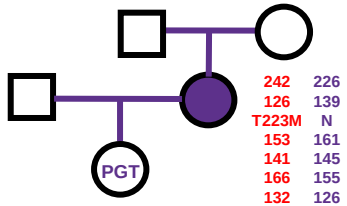
MUTATION TESTING ON
SINGLE CELLS



POLAR BODY ANALYSIS IS HIGHLY RECOMMENDED
TO ESTABLISH
NORMAL & AFFECTED HAPLOTYPES

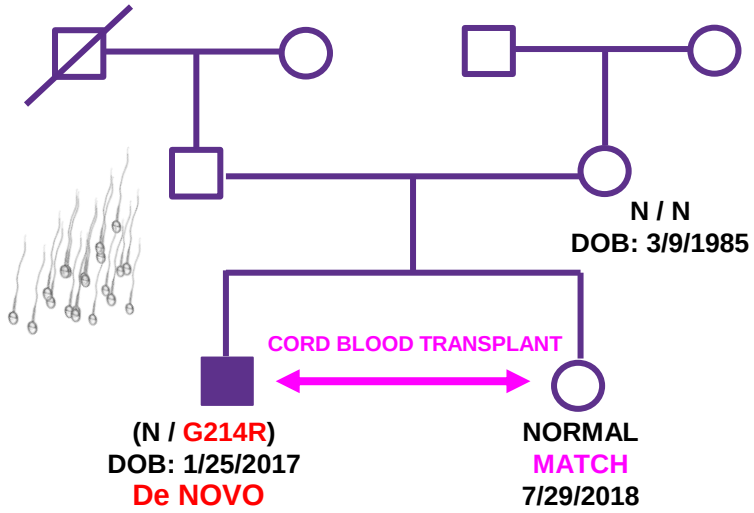
TROPHECTODERM ANALYSIS FOR HOLT-ORAM SYNDROM and ANEUPLOIDY by NGS
(de novo T223M mutation in TBX5 gene)

MARKERS ORDER:				
D12S811	228	254	242	226
D12S1341	143	135	126	139
TBX5	N	N	T223M	N
D12S354	153	159	153	161
D12S359	141	125	141	145
D12S79	160	158	166	155
D12S1665	122	122	132	126



EMBRYOS	2	4	6	7	8	10	19
	228 242	226 242	254 242	254 242	228 226	228 242	228 226
	143 126	139 126	135 126	135 126	143 139	143 126	143 139
	N T223M	N T223M	N T223M	N T223M	N N	N T223M	N N
	153 153	161 153	159 153	159 153	153 161	153 153	153 161
	141 141	145 141	125 141	125 141	141 145	141 141	141 145
	160 166	155 166	158 166	158 166	160 155	160 166	160 155
	122 132	126 132	122 132	122 132	122 126	122 132	122 126
	AFFECTED	UPD 12	AFFECTED	AFFECTED	NORMAL	AFFECTED	NORMAL
NGS	47,XXY	46,XX	45,XY -21	46,XY	46,XX	46,XY	46,XX
					TC		FROZEN

PGT FOR DE NOVO SEVERE CONGENITAL NEUTROPENIA1 (*ELANE* GENE) DETECTED IN CHILD, HLA TYPING AND ANEUPOLIDY BY NGS

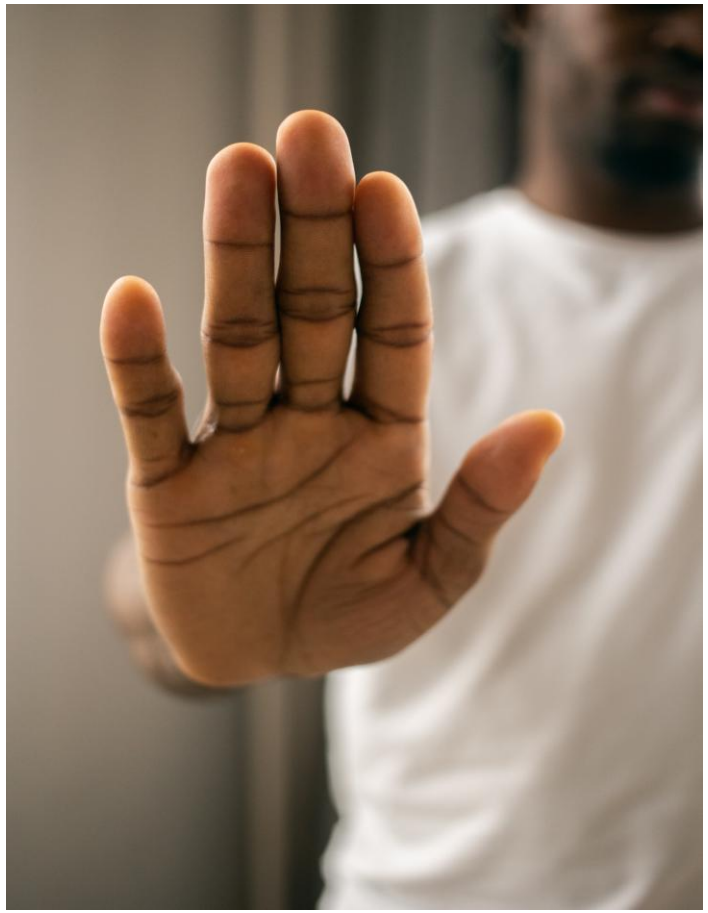


Embryo #	GENE Mutation	D19S 878	D19S 565	D19S 424	D19S 209	Embryo #	Predicted Genotype	Predicted HLA Type	TC
1	N	157/152	132/119	110/108	108/106	1	NORMAL*	MATCH	Per PT consent*
2	N	152/146	128/119	110/106	104/98	2	NORMAL*	MATCH	Per PT consent*
3	N	157/152	132/119	110/108	108/106	3	NORMAL*	NON-MATCH	Per PT consent*
4	G214R / N	157/146	132/119	110/106	108/98	4	AFFECTED	NON-MATCH	NO
HLA 1231	N / N	157/152	132/128	110/110	108/104	PARTNER (NORMAL)			
HLA 1232	N / N	152/146	119/119	108/106	106/98	PATIENT: (NORMAL)			
HLA 1236	G214R / N	157/146	132/119	110/106	108/98	CHILD: AFFECTED (de novo)			

NON-DISCLOSURE TESTING STRATEGIES:

1. Direct non-disclosure testing:
   direct mutation, linkage analysis and NGS

2. Indirect non-disclosure testing:
   Linkage analysis against affected family member

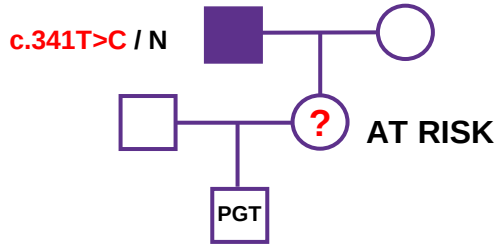


DIRECT NON-DISCLOSURE (PGT-M)

1. Number of oocytes and Embryos is not Disclosed to a patient.
2. PGT-A is mandatory to prevent calculations based on number of embryos recommended for transfer.
3. IVF Center and the patient receive recommendations for transfer only, rather than genetic status of all embryos.

NON-DISCLOSURE PGT-M for ALS (c.341 T>C mutation in *SOD1* gene)

D21S1913
D21S1909
D21S2049
D21S1888
SOD1 gene
D21S262
D21S1413
D21S2039
D21S1254



Preimplantation Genetic Testing: Summary of Recommendations

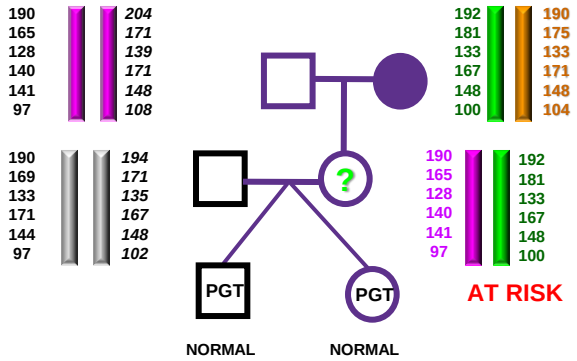
Recommended for transfer	• Based on direct non-disclosure testing for ALS and aneuploidy Embryos #1-3, #1-4, #1-6, #1-7, #1-10, #2-1, #2-2, #2-4, #2-5, #2-7, #2-8 and #2-9 are recommended for transfer. • These embryos are recommended for transfer at 98% accuracy.

INDIRECT NON-DISLOSURE

1. TESTING PERFORMED AGAINST A HAPLOTYPE FROM THE AFFECTED RELATIVE.
2. NO DIRECT MUTATION ANALYSIS.
3. DRAWBACK : NORMAL EMBRYOS ARE ELIMINATED FROM THE TRANSFER IF PATIENT IS NORMAL

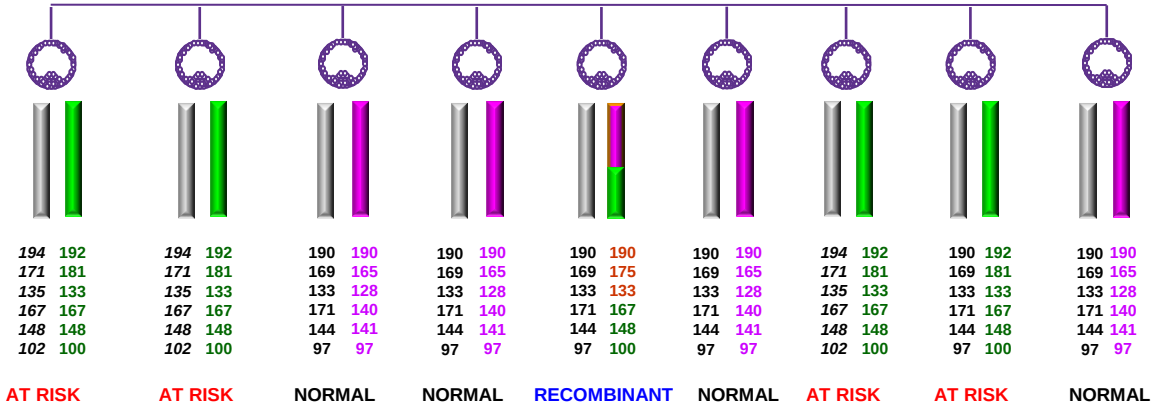
PGT-M for Early Onset Alzheimer Disease

A. Family Pedigree



THE WALL STREET JOURNAL.
Oct.13, 2014

B. Trophectoderm Analysis



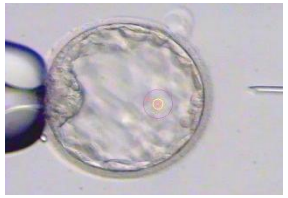
The Significant Majority of PGT is for Chromosome Assessment (PGT-A)

PGT-A has many goals:

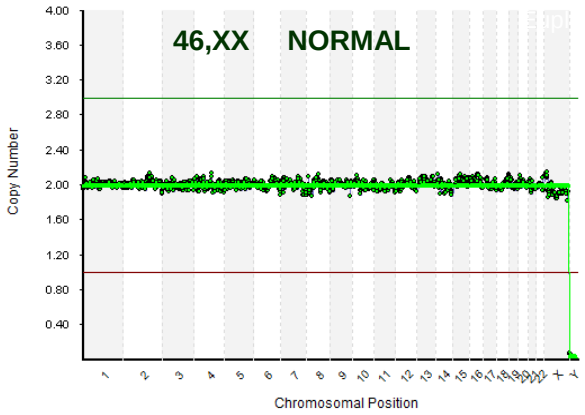
- Increase pregnancy rates
- Reduce chance of chromosomally abnormal pregnancies
- Provide an opportunity for single euploid embryo transfer
- Decrease pregnancy loss rates
- Reduce time to conceive
- Save money

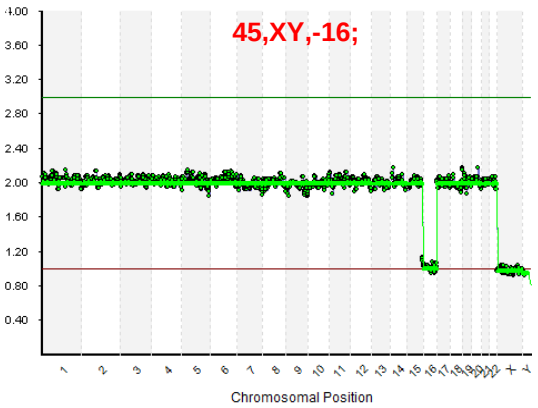
Contemporary Methods (2010 - Present)

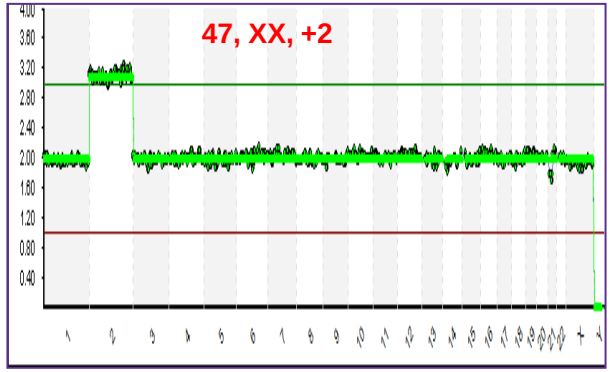
- Trophectoderm biopsy rather than blastomere biopsy.
- All 24 chromosomes tested
- Next Generation Sequencing (NGS)
  - Measures DNA content but not number of cells.
  - DNA content includes damaged cells and cells still undergoing DNA replication.
  - Result per embryo derived by proportion normal (euploid) vs. abnormal (aneuploid) DNA.



Next Generation Sequencing (NGS)





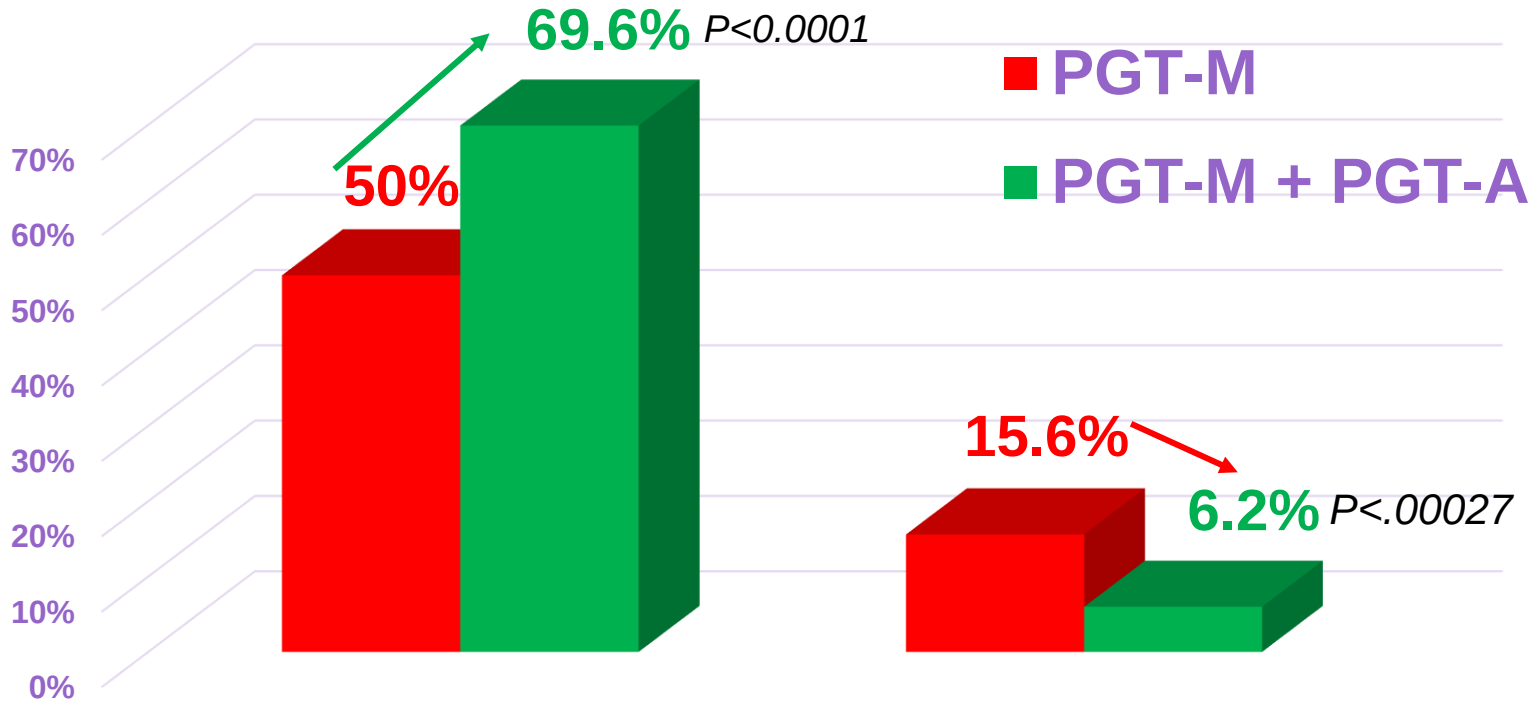


Effect of blastocyst biopsy and comprehensive chromosome screening technology on preimplantation genetic screening: a systematic review of randomized controlled trials

Elias M Dahdouh [a,b,c,*], Jacques Balayla [c], Juan Antonio García-Velasco [d]

Implantation rates	Control	PGT-A
Yang et al. 2012 (aCGH)	46%	69%
Scott et al. 2013 (qPCR)	63%	80%
Forman et al. 2013 (qPCR)	40%	58%
Rubio et al, 2016 (aCGH)	20%	50%
TOTAL	43%	65% P<0.001

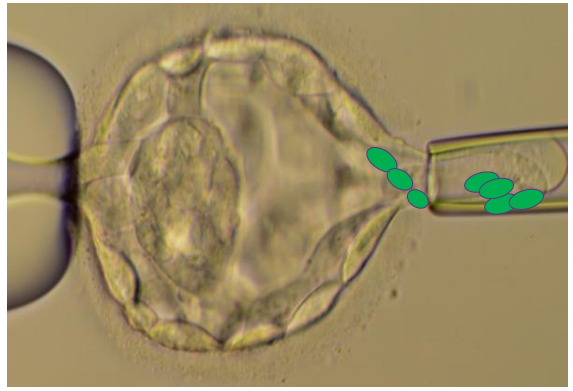
Aneuploidy Testing Impact On Pregnancy And Miscarriage Rates In Combined PGT-A + PGT-M



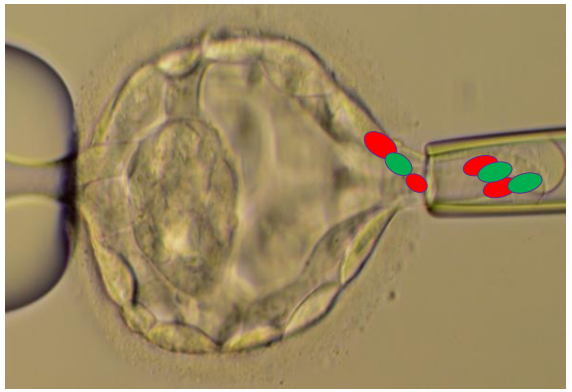
Pregnancy Rate **SAB**

Groups of Mosaic Embryos

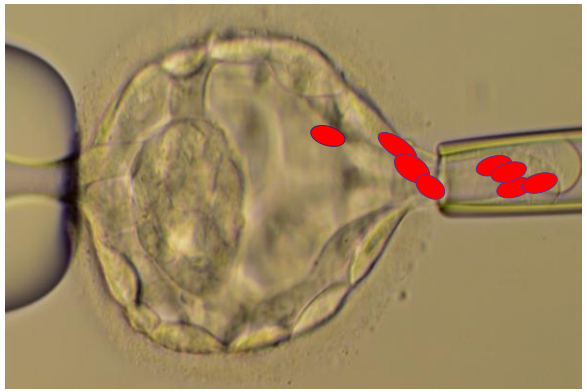
Percentage of Aneuploid Cells: 20 - 80%



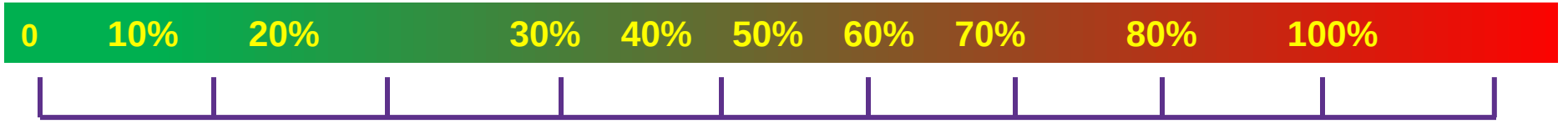
EUPLOID



MOSAIC



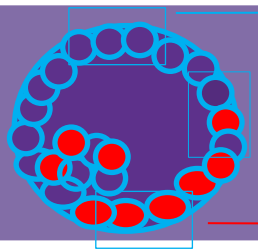
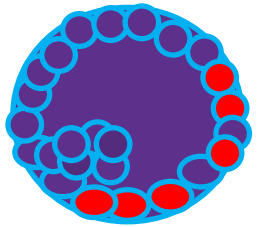
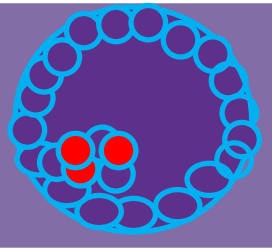
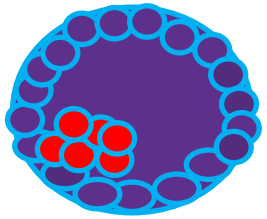
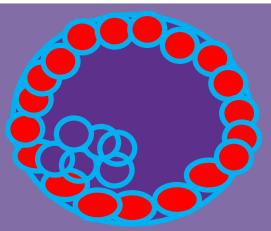
ANEUPLOID



Type of chromosomal mosaicism:

G. Harton, et al 2017

- Single and Double Monosomy
- Single and Double Trisomy
- Complex aneuploidies (>2 aneuploidies, trisomy and monosomy, segmental and whole chromosome)
- Segmental aneuploidies (one or more chromosomes)

Mosaicism Type	Biopsy location	Possible TE Prediction
Total Mosaic		EUPLOID MOSAIC ANEUPLOID
TE Mosaic		EUPLOID MOSAIC ANEUPLOID
ICM Mosaic		EUPLOID
ICM / TE Mosaic Type I		EUPLOID
ICM / TE Mosaic Type II		ANEUPLOID

Based on M.Vera-Rodriguez, C.Rubio, 2017 Fert. & Steril

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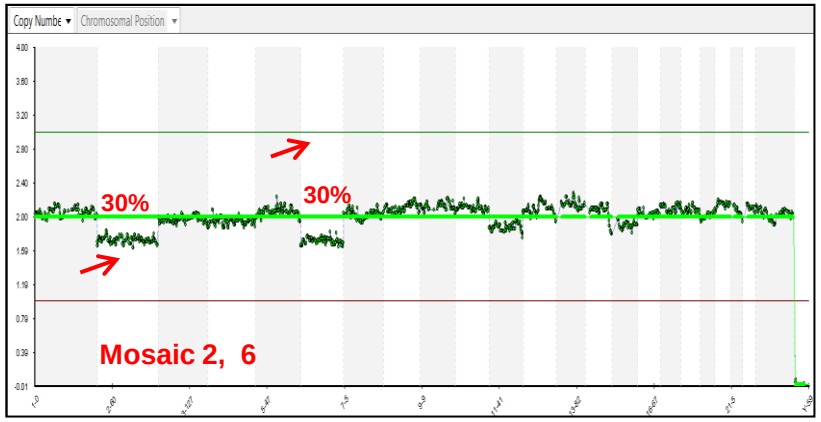
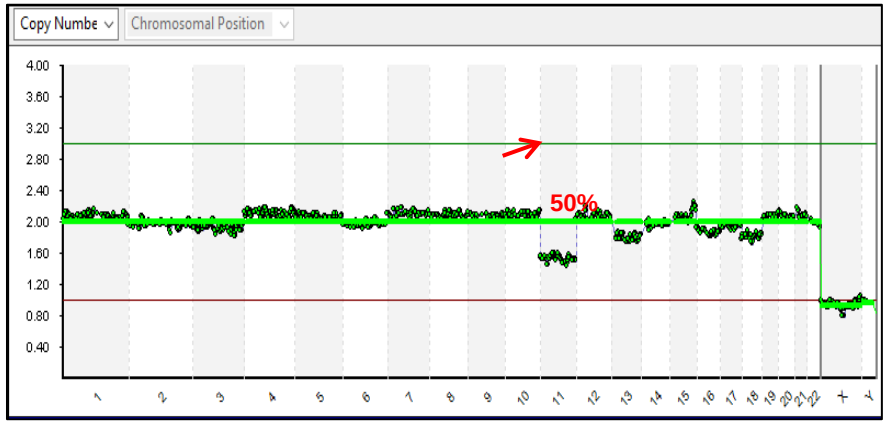
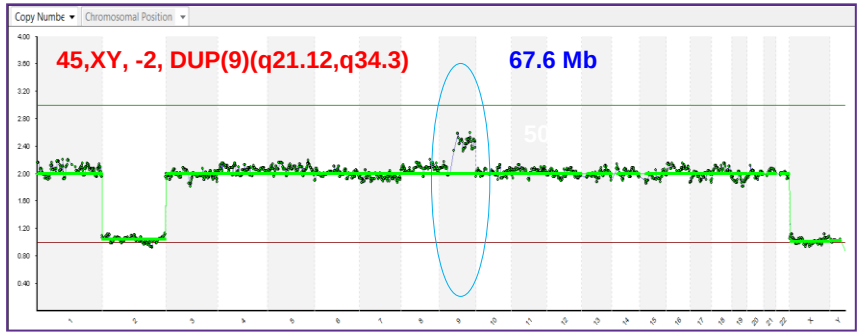
Typical NGS Profiles of Mosaic Embryos

Diploid Mosaic

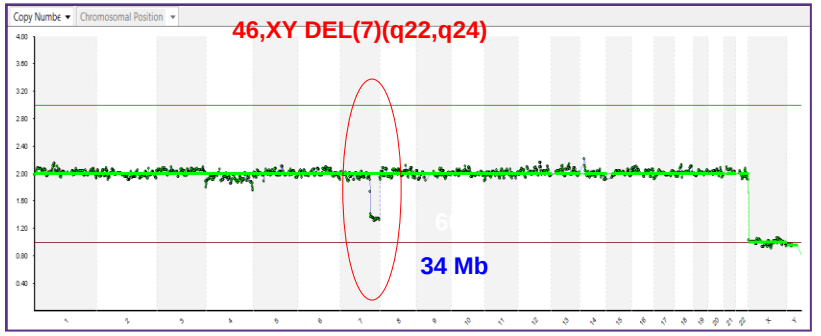




Aneuploid Mosaic

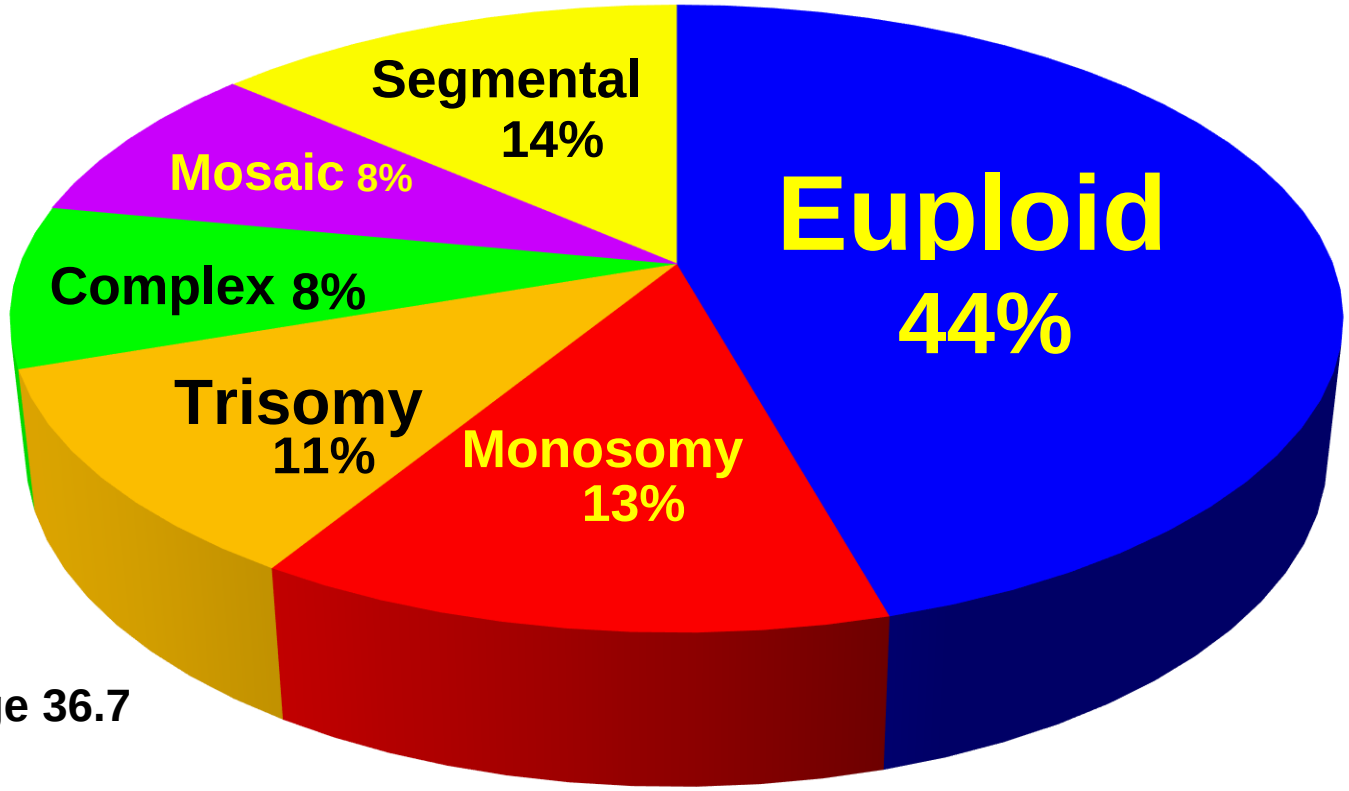


Segmental Mosaic



Proportion of Euploid And Aneuploid Embryos Detected By NGS (RGI)

N=24,922



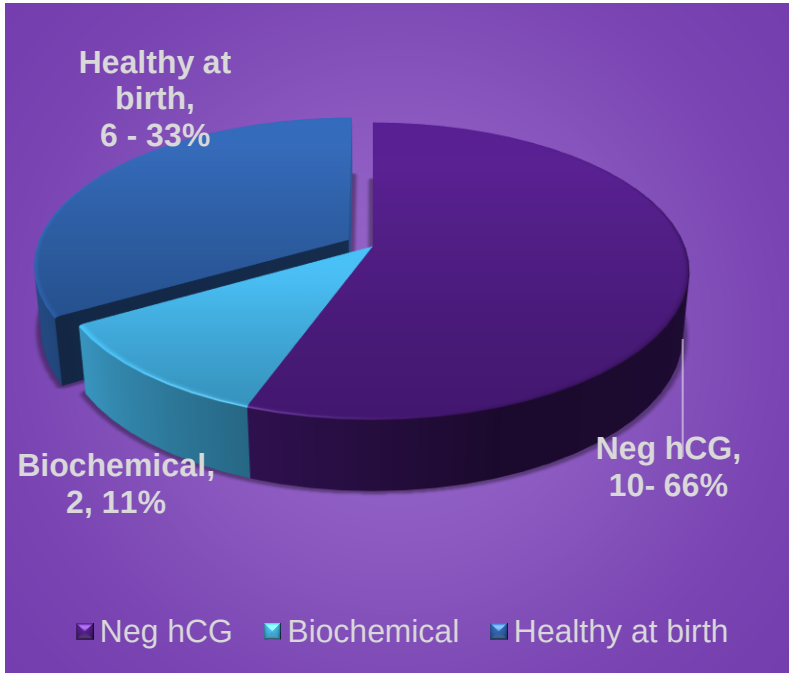
Av. Age 36.7

Mosaic Embryos Can Develop Into Healthy Newborns

The NEW ENGLAND JOURNAL of MEDICINE

Table 1. Clinical Outcomes of Single Mosaic Blastocysts Transferred.*

Patient No.	Chromosomal Constitution	Mosaicism†	Karyotype‡	Clinical Outcome
		percent		
1	arr(4)x1,(10)x1	40	46,XX	Baby healthy at birth
2	arr(6)x1,(15)x1	50	46,XX	Baby healthy at birth
3	arr(2)x1	40	46,XX	Baby healthy at birth
4	arr(2)x1	35	46,XY	Baby healthy at birth
5	arr(5)x1	50	46,XX	Baby healthy at birth
6	arr(5)x1,(7)x1	40	46,XX	Baby healthy at birth
7	arr(11)x1,(20)x3,(21)x3	30	NA	No pregnancy
8	arr(1)x1,(6)x3,(10)x3,(12)x3,(13)x3,(14)x3,(21)x3	50	NA	No pregnancy
9	arr(3)x1,(10)x3,(21)x3	35	NA	No pregnancy
10	arr(1)x3	50	NA	Biochemical pregnancy
11	arr 9p21.2q34.3(26,609,645-140,499,771)x3	45	NA	Biochemical pregnancy
12	arr(15)x3	30	NA	No pregnancy
13	arr(18)x1	50	NA	No pregnancy
14	arr(18)x1	50	NA	No pregnancy
15	arr(18)x1	40	NA	No pregnancy
16	arr(4)x1	50	NA	No pregnancy
17	arr(5)x3	40	NA	No pregnancy
18	arr 10q21.3q26.3(67,216,644-134,326,648)x3	50	NA	No pregnancy



Greco E, Minasi MG and Fiorentino F. New England Journal of Medicine 2015; 373:2089-90

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Clinical Outcome After Transfer Of Mosaic Embryos In Different Studies

STUDY	PGT-A PLATFORM	'MOSAIC ' EMBRYOS TRANSFERRED	SUCCESS RATE (%)	CONFIRMED MOSAIC
GRECO et al 2015	aCGH	18	33	0
MAXWELL et al 2016	NGS	18	33	0
FRAGOULI et al 2017	NGS	44	27	0
MUNNE et al 2017	NGS	99	45	0
INOUE et al 2017	aCGH	1	100	0
LLEDO et al 2017	aCGH	52	25	0
SPINELLA et al 2018	aCGH and NGS	77	30	0
ZORE et al 2019	aCGH	20	30	0
ZHANG et al 2019	aCGH	102	27	0
VICTOR et al 2019	NGS	100	30	0
LIU et al 2019	NGS	2	100	0
BESSER et al 2019	NGS	29	38	0
HONG and HAO 2020	aCGH and NGS	28	18	0
MUNNE et al 2020	NGS	253	37	0
KAHRAMAN et al 2020	**NGS**	**1**	**100**	**1**
TIEGS et al 2021	NGS	16	69	0
LEE et al 2020	NGS	83	47	0
ALKSERE et al 2020	aCGH and NGS	70	43	0
LIN et al 2020	NGS	108	41	0
ZHANG et al 2020	aCGH and NGS	137	47	0
CHUANG et al 2020	NGS	20	55	0
VIOTTI et al 2021	**NGS**	**1000**	**37**	**0**
YANG et al 2021	NGS	8	38	0
LI et al 2020	NGS	60	38	0
CAPALBO et al 2021	NGS	413	43	0
TOTAL (25)		**2759**	**38 (%)**	**1 (0.04%)**

Treff et al. 2021

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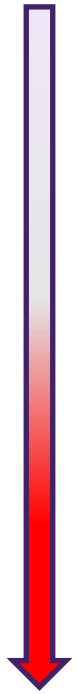
Clinical Outcomes of Euploid vs. Mosaic Embryo (2491) Transfers

International Registry of Mosaic Embryo Transfers (IRMET)

	IMPLANTATION RATE	ONGOING PREGNANCY / BIRTH	SPONTANEOUS ABORTIONS
EUPLOID	57.2%	52.3%	8.6%
MOSAIC ALL TYPES	46.5%	37%	20.4%
MOSAIC WHOLE CHROMOSOME	41.8%	30.8%	25%

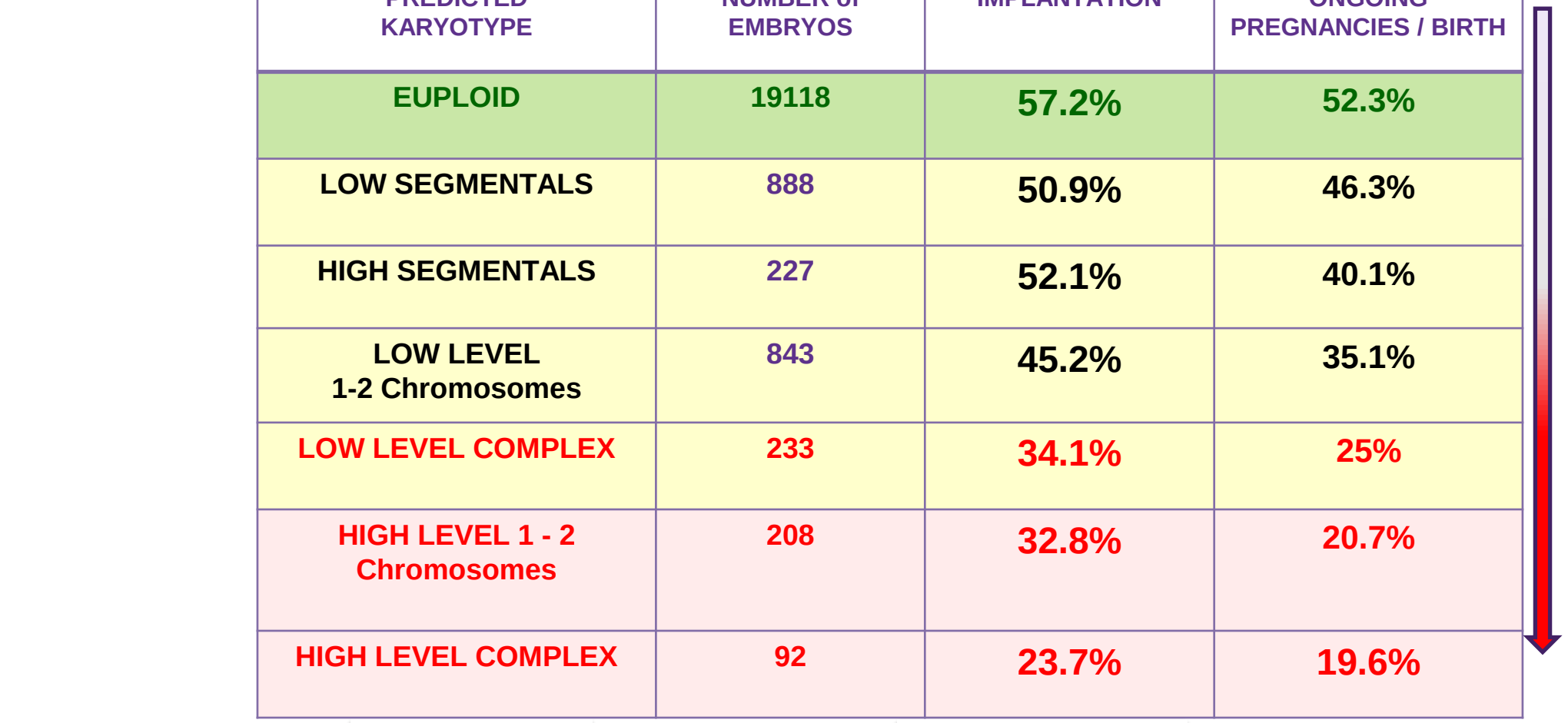


E.Viotti et al, PGDIS 2023

Clinical Outcomes of Euploid vs. Mosaic Embryo (2491) Transfers

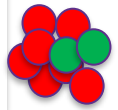
International Registry of Mosaic Embryo Transfers (IRMET)

PREDICTED KARYOTYPE	NUMBER of EMBRYOS	IMPLANTATION	ONGOING PREGNANCIES / BIRTH
EUPLOID	19118	57.2%	52.3%
LOW SEGMENTALS	888	50.9%	46.3%
HIGH SEGMENTALS	227	52.1%	40.1%
LOW LEVEL 1-2 Chromosomes	843	45.2%	35.1%
LOW LEVEL COMPLEX	233	34.1%	25%
HIGH LEVEL 1 - 2 Chromosomes	208	32.8%	20.7%
HIGH LEVEL COMPLEX	92	23.7%	19.6%



Mosaic low level <50%

Mosaic high level >50%



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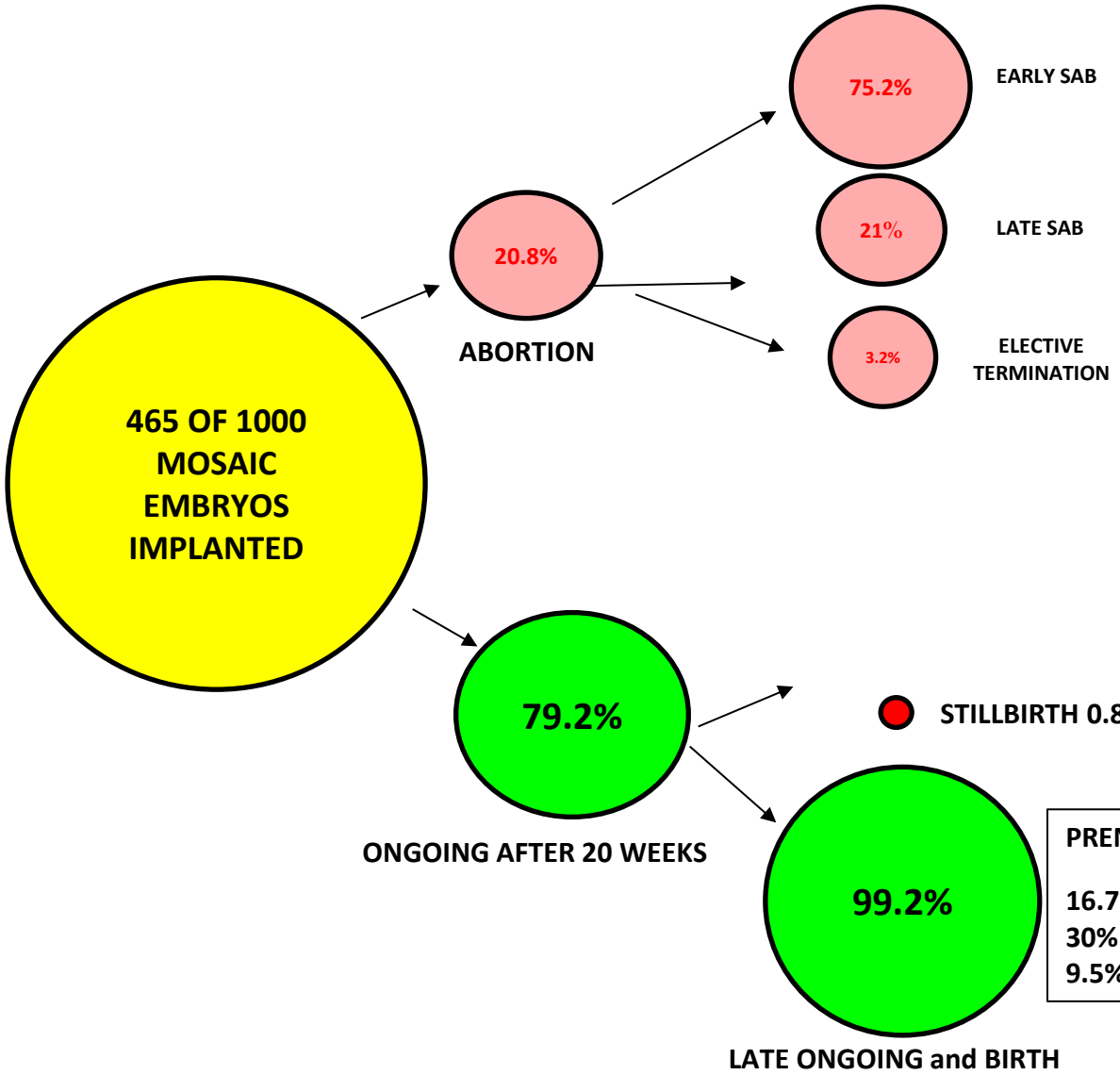
CLINICAL OUTCOME of EUPLOID vs. MOSAIC EMBRYO TRANSFER

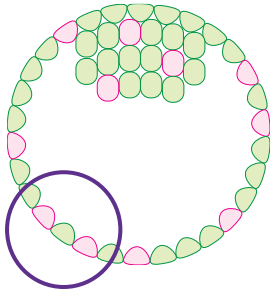


E.Viotti et al, ASRM 2020

Persistence of Mosaicism During Pregnancy

International Registry of Mosaic Embryo Transfers (IRMET)

582 Pregnancies with Chromosomal Testing



Mosaic

7 Cases

Mosaicism PGT-A = Mosaicism in Pregnancy

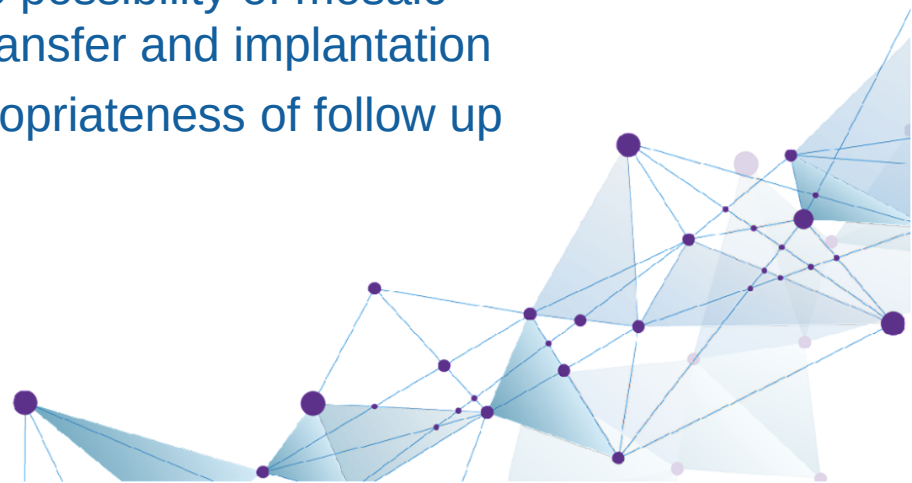
PGT-A	Prenatal Testing	POC	Ultrasound Abnormalities
mos -2 (low level)	mos +2 (Amnio)	n/a [*mos -2 in postnatal]	No
mos +1q,-7,-8,+9,-19,-20,+21 (low level)	mos +21 (CVS+Amnio)	n/a	Yes
mos -1p36.33p31.1 (low level)	mos -1p36.33p31.1 (Amnio)	mos -1p36.33p31.1	No
mos +21 (low level)	mos +21 (CVS+NIPT)	mos +21	Yes
mos +15 (high level)	mos +15 (NIPT)	mos +15 (placenta)	Yes
mos +17 (low level)	n/a	mos +17	Yes
mos +4q32.3q34.3,-Xq27.3q28 (low level)	mos +4q32.3q34.3 (CVS)	n/a	No

E.Viotti et al, PGDIS 2024

RGI REPRODUCTIVE GENETIC INNOVATIONS LLC

2021 PGDIS Recommendations:
(for the clinician)

- If available, it is suggested that euploid embryos be given priority for transfer (PGDIS statement)
- Mosaic embryos may produce successful pregnancies
- High levels of mosaicism (>50%) may decrease implantation potential and increase the risk for miscarriage.
- If a mosaic embryo is considered for transfer, the patients should be counseled on the likely impact on implantation and miscarriage rates.
- Consent form should be modified to include the possibility of mosaic results and any potential risks in the event of transfer and implantation
- Patients should receive counseling on the appropriateness of follow up prenatal testing



PGDIS Recommendations

Sample Report:

Embryo #	NGS Results	Diagnosis	Transfer Considerations	Comments
1	46, XY / NGS(1-22)x2,(XY)x1	EUPLOID MALE	YES	
2	46, XY / NGS(1-22)x2,(XY)x1	EUPLOID MALE	YES	
3	46, XX / NGS(1-22,X)x2	EUPLOIDL FEMALE	YES	
4	45, XY, -4	ABNORMAL	NO	
6	46, XX / NGS(1-22,X)x2	EUPLOID FEMALE	YES	
7	45, XX, -11	ABNORMAL	NO	
9	46, XX / NGS(1-22,X)x2	EUPLOID FEMALE	YES	
10	46, XX / NGS(1-22,X)x2	EUPLOID FEMALE	YES	
11	Complex Chromosomal Abnormalities	ABNORMAL	NO	
12	FA	NA	NO	Re-biopsy Recommended
13	Mos45, X0/ 46, XX	MOSAIC	See PGDIS Recommendations	35% MOSAIC
14	46, XY, Del(18)(q22.3,q23)	ABNORMAL	NO	Deletion (18) - 8Mb

CONCLUSIONS

- LINKAGE ANALYSIS is instrumental in avoiding misdiagnosis due to ADO, RECOMBINATION and in presence of a pseudogene.
- PGT-M is reliable for De Novo mutations.
- PGT-M is possible in the absence of additional family members.
- Single Sperm Analysis and/or Polar Body Testing is required for the accuracy of the De Novo mutation testing.

Combined PGT-M and PGT-A is performed on the same sample and can result in:

- Improving pregnancy and live birth rates
- Reducing chance of miscarriage
- Achieving a single embryo transfer

Present RGI Experience (1990-2023)

Over 34,000 PGT cases performed for 796 conditions

11,437 for Mendelian disorders (PGT-M)

Including:

650 PGT cycles for DE NOVO mutations
1651 for Cancer Predisposition
1247 for Neurodegenerative Conditions
147 for Inherited Cardiac Conditions
532 for HLA genotyping

5157 PGT-M combined with PGT-A

23,167 for Aneuploidy (PGT-A)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SRechitsky@RGIscience.com

Thank You!