Preimplantation Genetic Diagnosis (PGD) for Fanconi Anemia and HLA

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OBJECTIVES

- Preimplantation Genetic Diagnosis (PGD)
- In Vitro Fertilization (IVF)
- Fees / Insurance
- Common Questions
- Success / RGI experience with FA
- Advantages / Disadvantages / Limitations

Definitions:

Preimplantation Genetic Diagnosis (PGD):

Diagnosis of Genetic Disease Before Pregnancy

In Vitro Fertilization (IVF):

Assisted Reproductive technology procedure where oocytes (eggs) are removed from a woman's ovary and are fertilized by the male's sperm outside the body in a controlled setting. The fertilized eggs develop into embryos which can be implanted in a woman's uterus to achieve pregnancy.

Current indications for PGD:

*Autosomal Recessive, Dominant and X liked Disorders

* HLA matching

* Cancer predisposition genes

* Adult-onset disorders

* Infertility-causing genes

* Maternal-fetal incompatibility

* Chromosomal Aneuploidy

* Combined Single gene, HLA, and aneuploidy testing

Testing Techniques utilized in PGD:

- PCR (polymerase chain reaction)
  - Single gene (Mendelian) disorders like FA
    - Mutation(s) with linked markers and HLA matching
- FISH (fluorescent in-situ hybridization)
  - Aneuploidy (abnormal # of chromosomes)
  - Translocations/structural rearrangements

Getting started….

- PGD Set-up
  - Create a testing system for the specific FA mutations and HLA
- Prepare for IVF

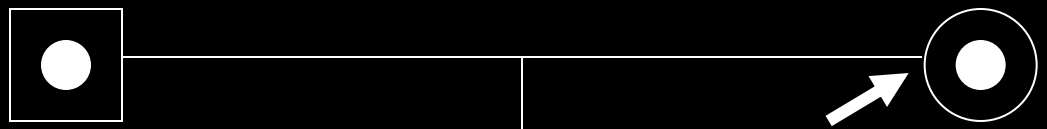
PGD SET-UP for FA/HLA

- **MEDICAL RECORDS**: FA mutation reports, HLA reports
- **PGD CONSULT** (phone or in person) with a PGD genetic counselor. Discuss the benefits, risks, limitations, accuracy (95-98% for FA and HLA) etc…..
- **DNA SAMPLES** (blood or cheek swabs) from parents and children
- **CONSENT FORMS**
- **FEES**
- **SET-UP SYSTEM** takes 4-6 weeks to complete
  - Testing for mutations and **linked markers**
- **TESTING STRATEGY** (PB/BB vs. BB)
- **IVF CYCLE DATES!!!!!!**

FANCONI ANEMIA-A
PGD SET-UP

LINKED MARKERS

1. D16S520
2. D16S3026
3. FANCA Intron 1
4. FANCA (Paternal Mutation :M)
5. FANCA (Maternal Mutation: M)
6. D16S3407



Husband (carrier)

Wife (carrier)

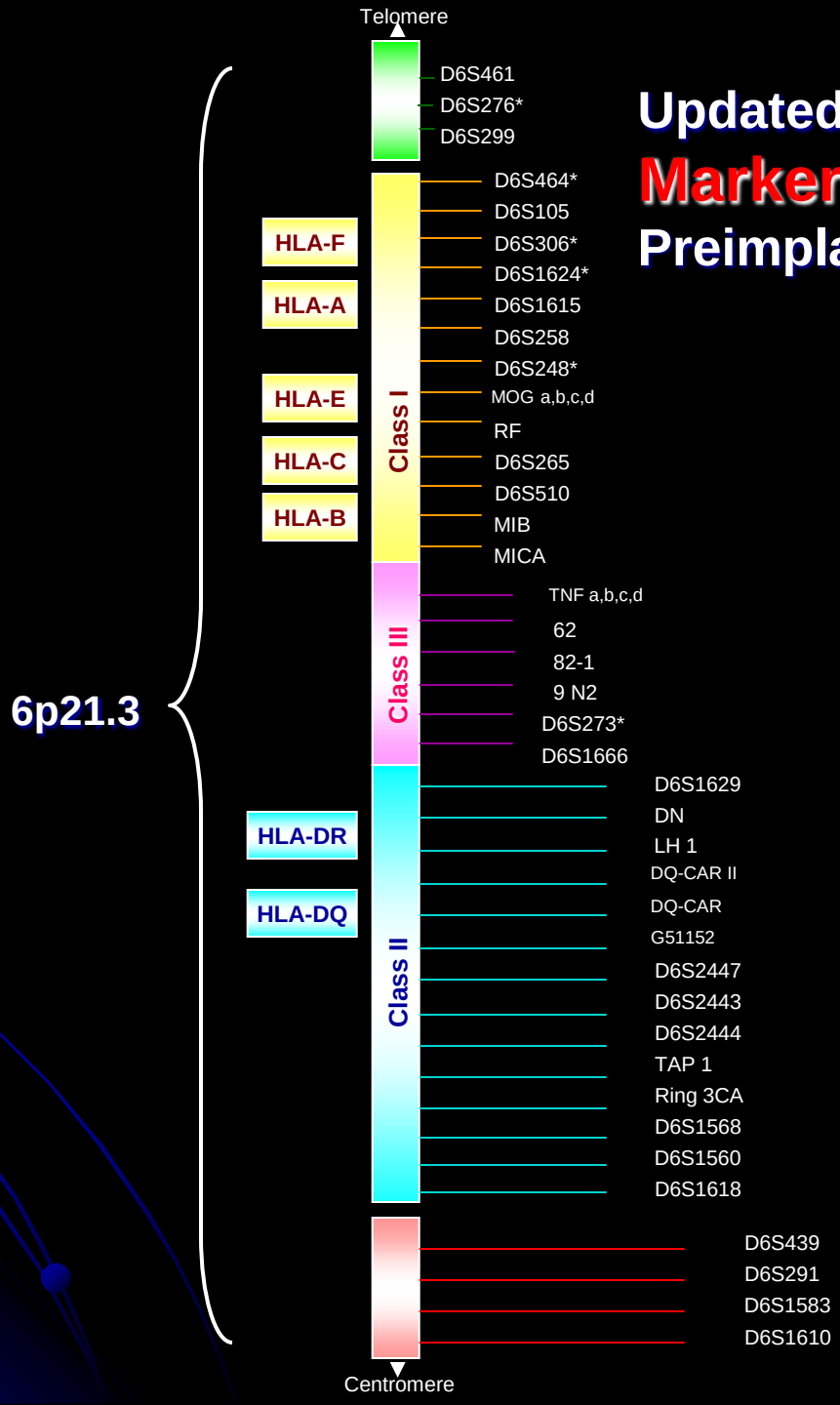
1. 10 / a
2. 20 / b
3. 30 / c
4. M / N
5. N / N
6. 60 / f

Child with FA

1. 10 / 70
2. 20 / 80
3. 30 / 90
4. M / N
5. N / M
6. 60 / 120

1. g / 70
2. h/ 80
3. i / 90
4. N / N
5. N / M
6. K / 120

Updated (June 2007) List of Markers in HLA Area for Preimplantation HLA Typing



Why are Linked Markers so important?

* Detection of Allele Drop Out (ADO)
* Detection of Recombination
* Detection of contamination
* Detection of chromosomal aneuploidy
* Embryo identification

In vitro fertilization (IVF)

- SELECT IVF CENTER
- IVF CONSULTATION
- FERTILITY WORKUP (required even if otherwise fertile)
  - Female: infectious disease panel, day 3 hormone levels, uterine cavity assessment, other genetic screening if needed, etc
  - Male: infectious disease panel, semen analysis, other genetic screening, etc
- IVF CYCLE DATES (PGD SET-UP MUST BE COMPLETE)
- BEGIN IVF CYCLE

IVF CYCLE

Menstrual cycle begins

↓

BCP for ~14 days

↓

Start Lupron injections

~10-14 days

↓

Baseline ultrasound

Start Stimulation Medication injections

~10-14 days

with monitoring throughout

↓

Egg Retrieval (Day 0)

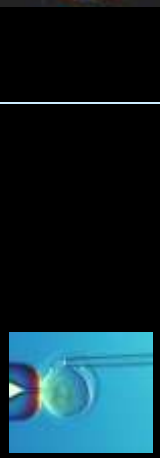
↓

PGD Testing (PB and/or BB)

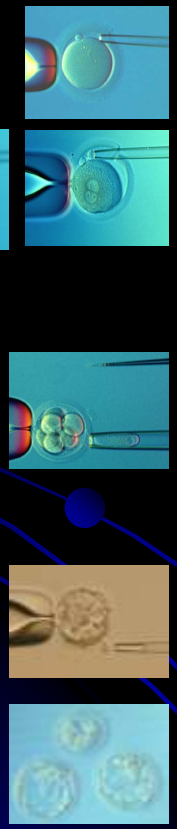
↓

Embryo Transfer (ET) on (Day 5)

Chronology of PGD Procedures



Day	
0	Egg Retrieval. 1st Polar Body removal. ICSI (fertilization)
1	Check Fertilization. 2nd Polar Body removal.
2	Embryos undisturbed. Polar Body testing in progress.
3	PB results. Blastomere Biopsy
4	Embryos undisturbed. Blastomere testing in progress.
5	Final Results and Embryo Transfer Freezing
6	Freezing of Residual Normal Embyros



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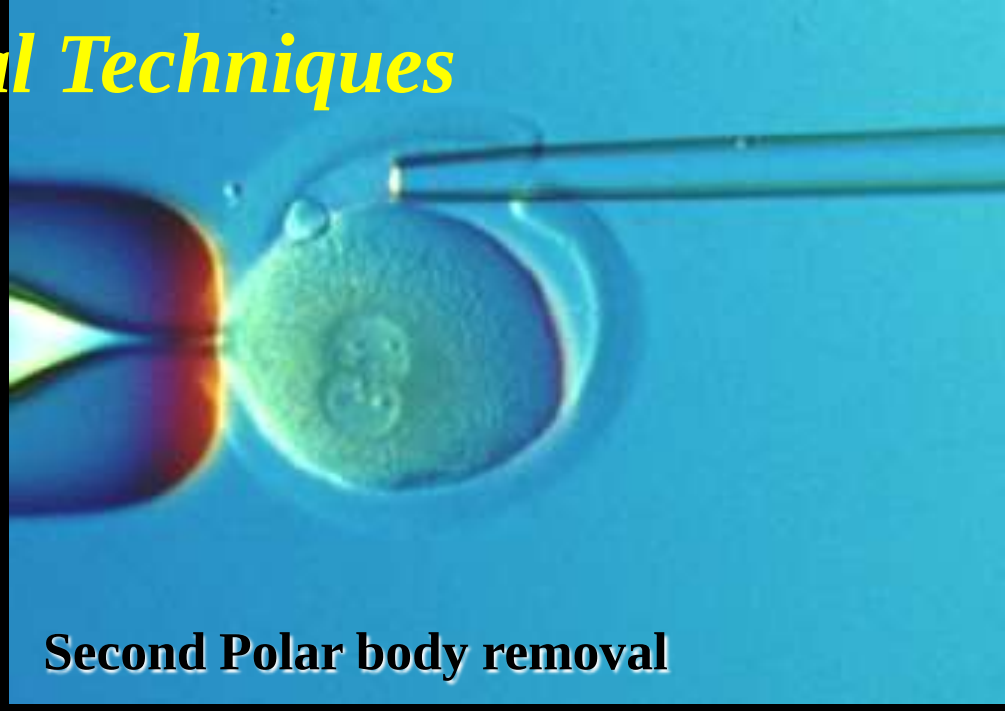
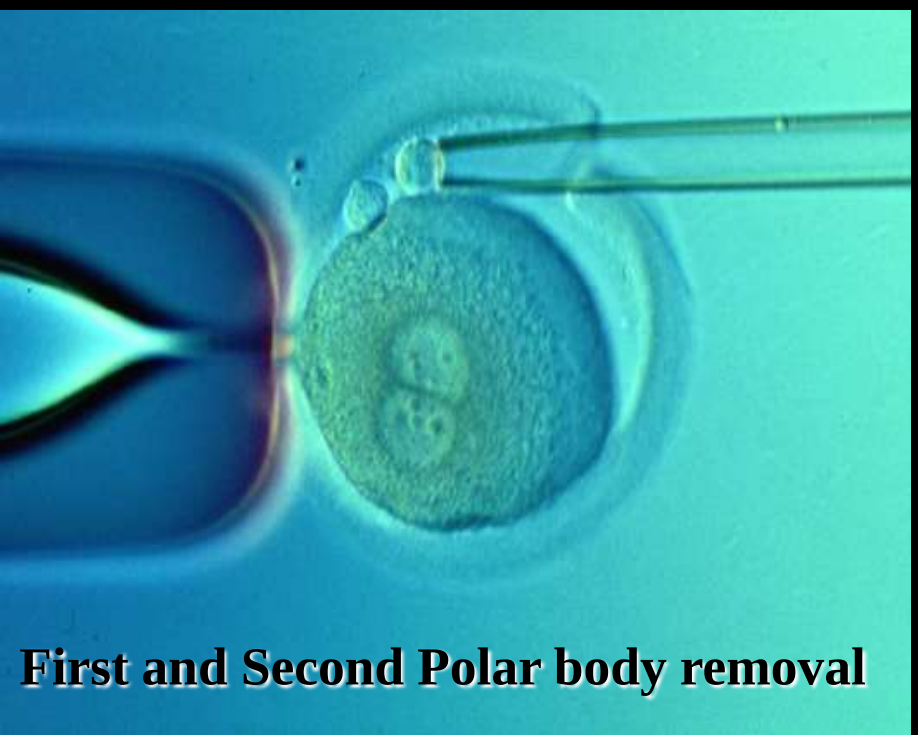
Microsurgical Techniques

First Polar body removal



Second Polar body removal



First and Second Polar body removal

Embryo biopsy

Chances for Disease Free and HLA matched embryo

Chance of normal or healthy carrier of FA	+	Chance for HLA match

3 / 4	x	1 / 4	=	3 /16

Sample PGD/IVF Cycles

CYCLE #1

- 8 eggs aspirated
- 6 mature
- 4 fertilize
- 1 FA Normal, HLA match
- Transfer 1 embryo
- Positive Pregnancy test!

CYCLE #2

- 25 eggs aspirated
- 18mature
- 13 fertilize
- 2 HLA matches
- 1 FA Affected, HLA match
- 1 FA Carrier, HLA match
- Both embryos arrested
- No embryo transfer

SAMPLE RESULTS

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Blastomere Analysis for Fanconi Anemia A and HLA

Embryo #	D11S 1759	Fanconi A Mutation	D11S 915	Predicted Embryo Genotype	ET	Comments
1	10/70	M/M	20/80	AFFECTED NON-MATCH	NO	
2	FA	FA	FA	NA	NO	
3	14/70	N / M	FA	CARRIER NON-MATCH	NO	Can be frozen
4	14/70	N / M	12/80	CARRIER NON-MATCH	NO	Can be frozen
5	14/13	N / N	12/16	NORMAL MATCH	YES	
FA 58	10/14	M / N	20/16	Husband		
FA 59	13/70	N / M	12/80	Wife		
FA 60	14/13	N / N	12/16	Normal child		
FA 61	10/70	M / M	20/80	Affected child		

Factors Affecting PGD Accuracy

- Cell quality/cell type
- Allele-drop-out
- DNA contamination
- Failed amplification -> inconclusive results
- Human error

PGD involves a modification of risk – not the elimination of risk. PGD does not replace prenatal diagnosis.

Common Questions

1) How may embryos are transferred?

1-3 (typically 2)

2) What are the chances of getting pregnant?

Age dependent, center dependent, per ET!!

<35 ~50%, 35-37 ~40%, 38-42 ~30%, >43 ~10% or less

3) What is the accuracy?

~95-98% (NOT 100%). Prenatal testing recommended.

4) Is there a minimum number of embryos required?

No (RGI)

Common Questions

8) Does the biopsy harm the embryo?

Risk is low. 0.3-0.5% risk of harming the embryo.

9) Does PGD lower the pregnancy rates?

No, similar pregnancy rates for IVF alone IF there is an ET

10) Can I use my younger sister as an egg donor when looking for an HLA match?

Possibly. If you are looking for an HLA match your sister would first have to be tested to see if she is a ½ match to the child with FA.

RGI IVF/PGD Fees

- IVF
  - IVF (aspiration, monitoring, transfer etc)
    - $9,500-$12,000**
  - IVF Medications
    - $2,000-$5,000
- PGD
  - Set up fees (one time fee)
    - $5,000
  - PGD testing fees (per IVF cycle)
    - $2,500-$3,000 for FA (waived 1st cycle if IVF at RGI)
    - $1,000-1,500 for HLA in conjunction with FA (waived 1st cycle if IVF cycle at RGI)
  - Other possible fees
    - $1,500 Biopsy fee (if performed by RGI)
    - $2,000 Embryologist travel fee (if IVF at outside center)
    - $2,000 Chromosome aneuploidy testing (optional)

Will insurance cover IVF/PGD?

- Rarely. ~90% of the time insurance does NOT cover PGD/IVF. Coverage varies from plan to plan. Some states have IVF coverage, but only if the couple is infertile.
- PGD set-up and testing for FA and HLA unlisted CPT code 84999, aneuploidy is 88299
- Biopsy codes are 89290-89291
- Can submit letter requesting coverage, then go through appeal process

Present RGI Experience -

2028 PGD cycles for 221 CONDITIONS

297 HLA cases

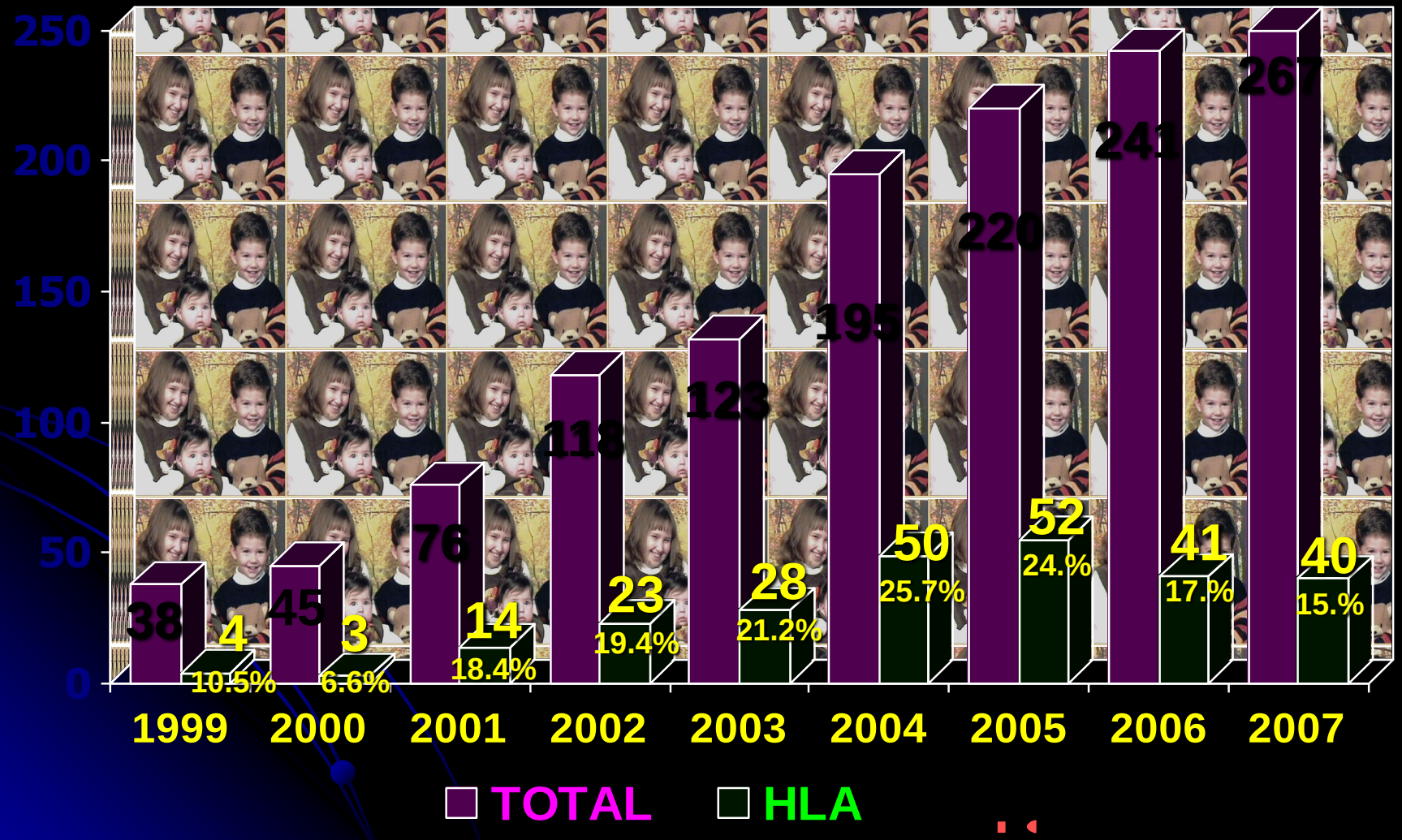
Overall Results and outcome of PGD for Single Gene Disorders & Preimplantation HLA testing

Testing	Patient/ Cycle	# of Transfer	# Embryos Transferred	Pregnancy / Birth
HLA	127/297	194	301	58/47 (3)* 30 % PR per ET
Single Gene Disorders	1012/1731	1490	2958	619/592 (51)* 41.5% PR per ET
TOTAL	1139/2028	1684	3259	677/639 (54)* 40.2% PR per ET

5/2010

* Ongoing pregnancies

Preimplantation HLA Typing in Overall RGI PGD Experience (1999-2007)



PGD for Single Gene Disorders & Preimplantation HLA testing

Testing	Patient/ Cycle	# of Transfer	# Embryos Transferred	Pregnancy / Birth
HLA + MUTATION	81/199	129	202	34/27(3)* 28% PR per ET
HLA	46/98	65	99	24/19 39% PR per ET
TOTAL	127/297	194	301	58/47(3)* 30 % PR per ET

* Ongoing pregnancies

RGI Experience in Preimplantation HLA Testing

	Patient	Cycle	ET	# embryos	*Pregnancy*	Birth
Thalassemia	48	113	74	115	*17*	11 (2)*
Sickle cell anemia	2	4	3	5	1	1
FANCA,FANCC, FANCD2,FANCF, FANCI, FANCJ	15	48	30	48	*6*	4
WAS	1	1	1	1	*0*	0
ALD	2	5	1	1	*0*	0
Hyper IgM	5	8	6	9	*3*	2
HED+ID; IP	2	9	6	8	*2*	3
DBA	3	5	3	6	*2*	2
Krabbe	1	1	1	2	1	2
DM	1	2	1	2	1	2
Chronic Granulomatous Disease	1	3	3	5	1	(1)*
TOTAL	**81**	**199**	**129**	**202**	**34**	**27 (3)**

RGI Experience in Fanconi Anemia/HLA Testing

Disease	Patient / Cycle	ET/ Cycles	Pregnancy / Birth
FANCA / HLA	9 / 34	19/ 34	5 / 3
FANCC / HLA And FANCC ONLY	4 / 8	7 / 8	2 / 3
FANCF / HLA	1 / 3	2 / 3	0
FANCJ / HLA	1 / 3	1 / 3	0
FANCI/HLA	1/2	2/2	0
FAND2/HLA	1/1	0/1	0
TOTAL	17/51	32 / 51 63% ET per cycle	7 /6 22% PR per ET 14% PR per cycle 12% THBR/cycle

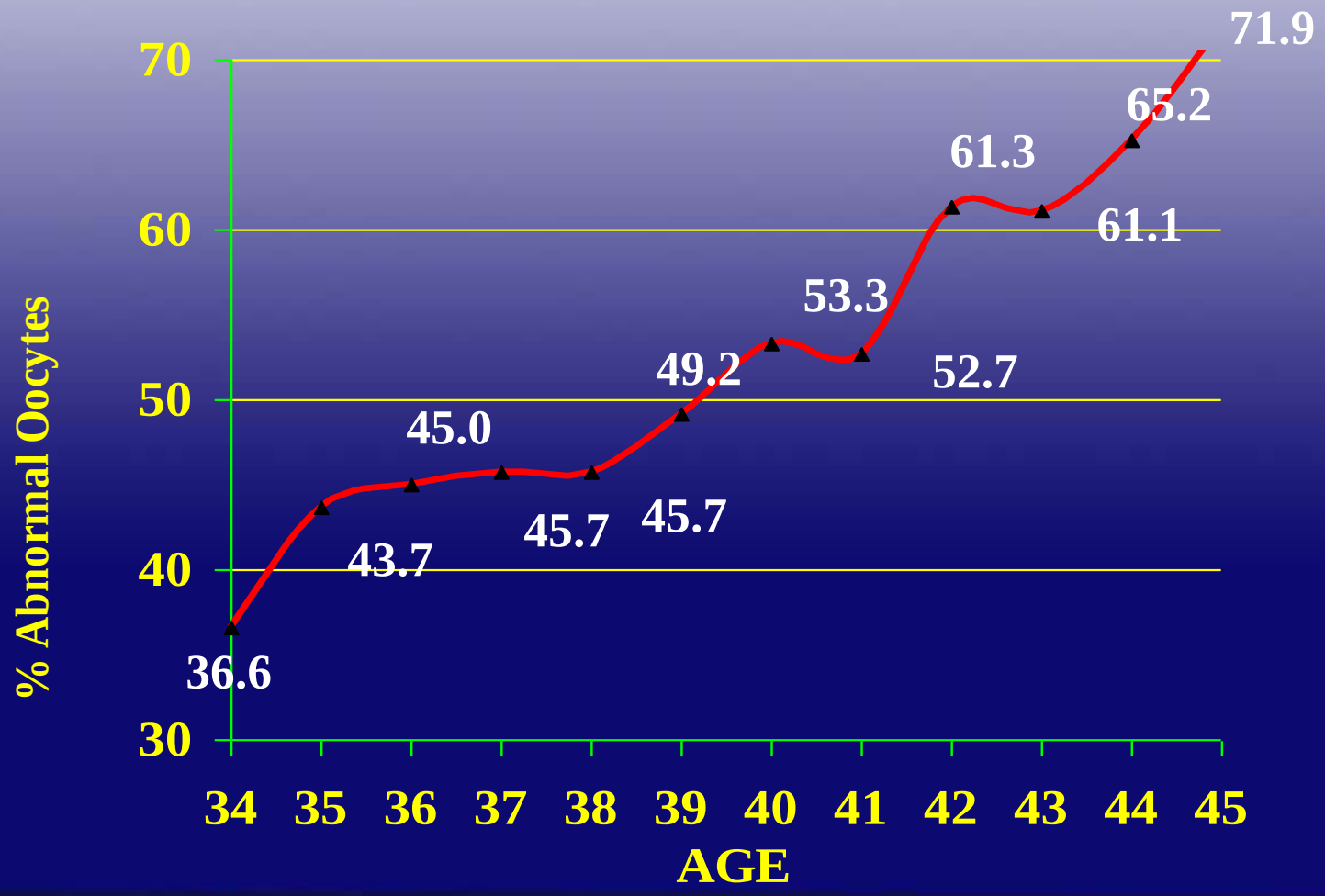
How do I choose a PGD center?

- Experience with FA and FA/HLA testing? Any misdiagnosis?
- Do they test linked markers or just mutations?
- Can they also test for chromosome aneuploidy?
- Can they test polar bodies and blastomeres?
- How often are there inconclusive results? Can they perform Day 5 blastocyst biopsy?
- Is prenatal testing (CVS or amnio) required?
- Who is the contact person? Do they have genetic counselors?

How do I choose an IVF center?

- Does the center have experience working with PGD centers?
- Will they work with any PGD center?
- Can they do their own biopsies?
- Location
- Pregnancy rates
- Exclusion criteria
- Day 5 embryo culture success

% Aneuploid Oocytes in relation to Maternal Age



Objectives of PGD for Chromosomal Disorders

- Prevent Chromosomal Disorders
- Reduce Spontaneous Abortions
- Improve Effectiveness of IVF

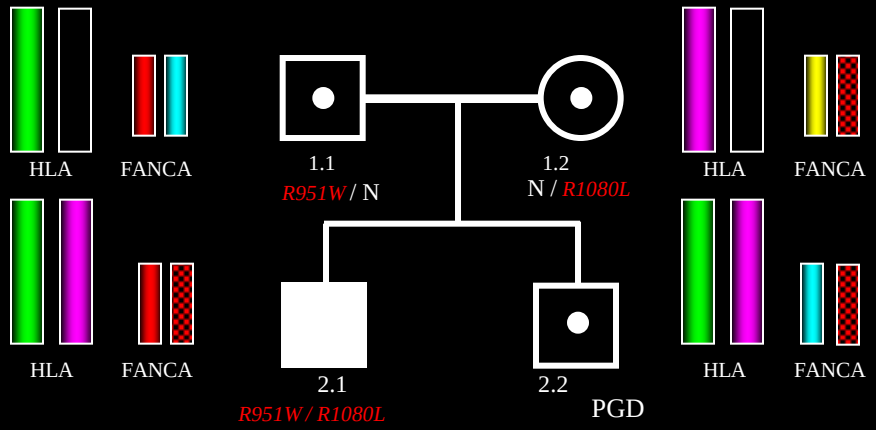
PGD for Fanconi A, HLA & Aneuploidy Testing

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

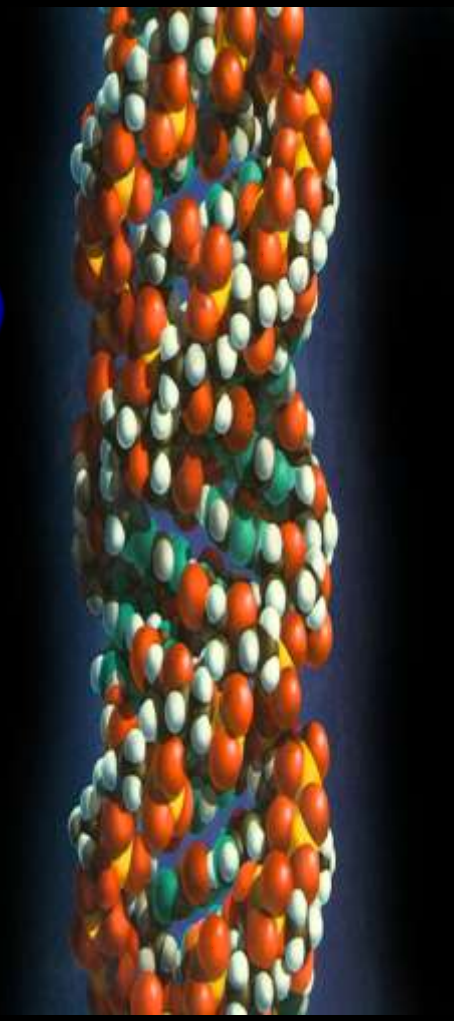
Oocyte #	1	2	3	4	5	6	7	8	9	10	11	13	14	16	18
Predicted Genotype:	*161 160 R1080L*	*161 160 R1080L*	*161 160 R1080L*	152 158 N	FA	*161 160 R1080L*	152 158 N	*161 160 R1080L*	152 158 N	152 158 N	*161 160 R1080L*	*161 160 R1080L*	152 158 N	*161 160 R1080L*	*161 160 R1080L*

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		0											0	0	
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	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*	

Drawbacks/limitations of PGD

- Risk of micromanipulation
- Fewer embryos for transfer
- Patient response can’t be predicted
- Pregnancy rates (no guarantee of pregnancy)
- Cost, availability
- ICSI required
- PGD involves a modification of risk – not the elimination of risk
- PGD does not replace prenatal diagnosis
- Fanconi Anemia E is on the same chromosome as HLA



Advantages of PGD

- An acceptable option for at-risk couples that would not consider terminating an affected pregnancy after CVS or Amniocentesis.
- Increase the chance of a successful pregnancy through IVF for women with fertility problems, especially those who are AMA.
- Can test for HLA matched/unaffected HLA matched embryos for couples with a sick child needing a stem cell transplant.

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