Preimplantation Genetic Diagnosis (PGD) for Fanconi Anemia and HLA matching

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RGI
Reproductive Genetics Institute

Outline

- PGD overview
- In vitro fertilization (IVF) and PGD process
- Accuracy of PGD
- Cost
- Frequently asked questions
- RGI's PGD experience

Definitions

- In Vitro Fertilization (IVF):
  - Assisted reproductive technology where eggs are removed from a woman's ovaries and fertilized by a man's sperm outside the body in a laboratory
  - The fertilized eggs develop into embryos which can be transferred into a woman's uterus, with the hopes of implantation and pregnancy

- Preimplantation Genetic Diagnosis (PGD):
  - Diagnosis of a genetic disease before pregnancy
  - Embryos are created through IVF and tested prior to transfer/implantation

Common indications for PGD

- Autosomal recessive, dominant and X-linked genetic disorders
  - Childhood and adult-onset disorders
  - Cancer predisposition genes
  - Maternal-fetal blood incompatibility
- HLA matching
- Inherited chromosomal rearrangements
- Spontaneous chromosomal aneuploidies (incorrect chromsome number)

Getting started

- PGD
  - Submit FA and HLA genetic reports to PGD laboratory to determine feasibility
  - Consultation with genetic counselor (phone or in-person)
    - Discuss process, next steps, accuracy, limitations, costs and timeline
  - PGD set-up
- IVF
  - Consultation with fertility center/physician (Reproductive Endocrinologist or REI)
  - Evaluation (blood tests, ultrasounds, semen analysis) to evaluate feasibility of IVF
    - Required even if couple is fertile!

PGD set-up

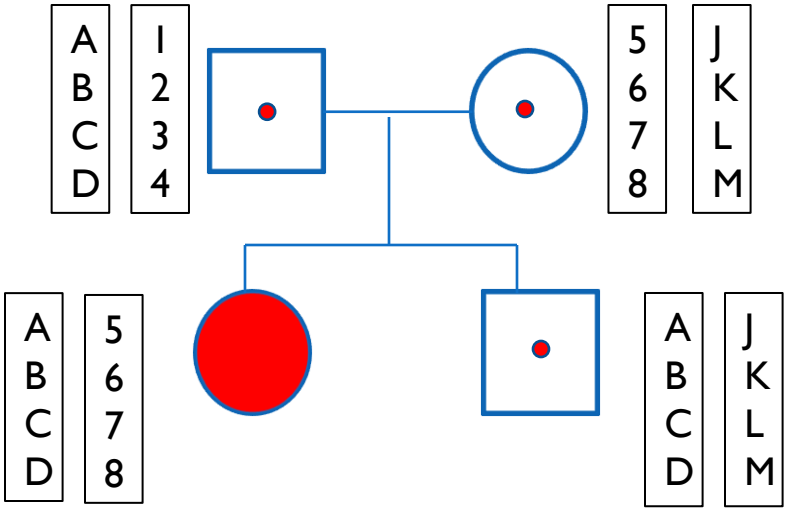
- What is needed to begin?
  - Genetic reports on couple & children
  - DNA samples (blood or cheek swabs) from couple & children
  - Consent forms
  - Set-up fee

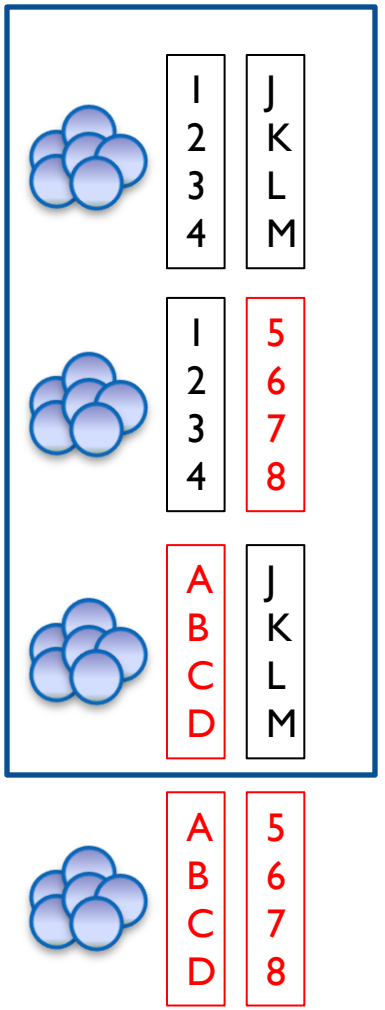
Takes 4-8 weeks to complete

Cannot start IVF medications/cycle until notified that set-up is complete!

“Establishing linkage”

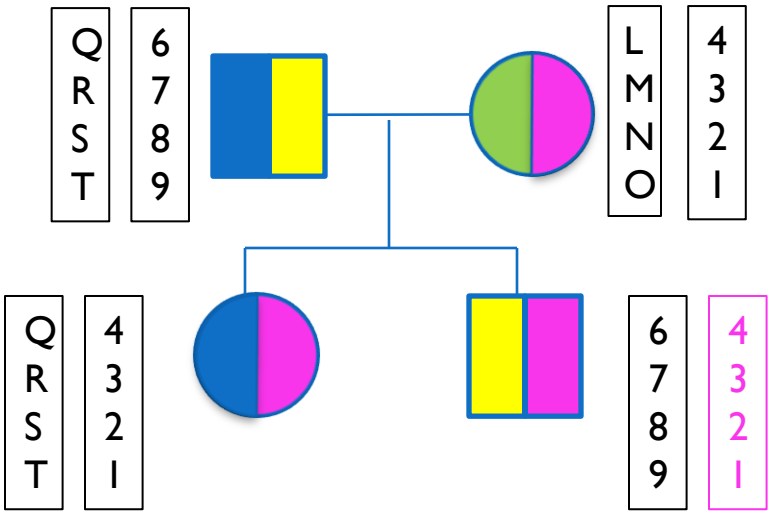
PGD set-up for Fanconi Anemia

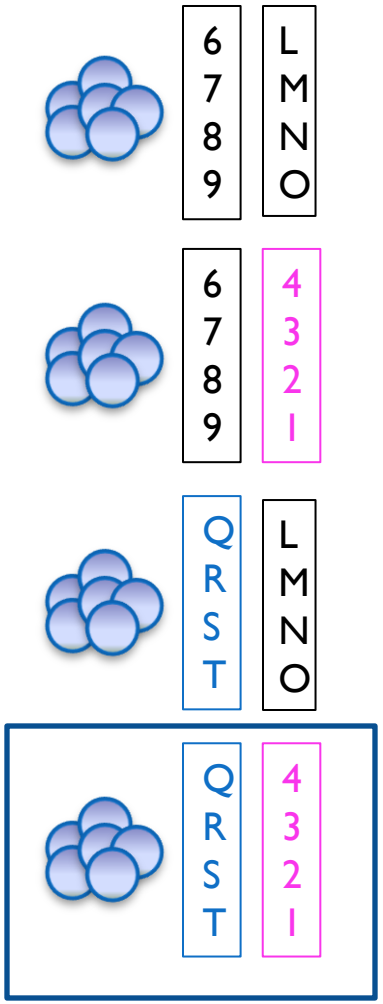




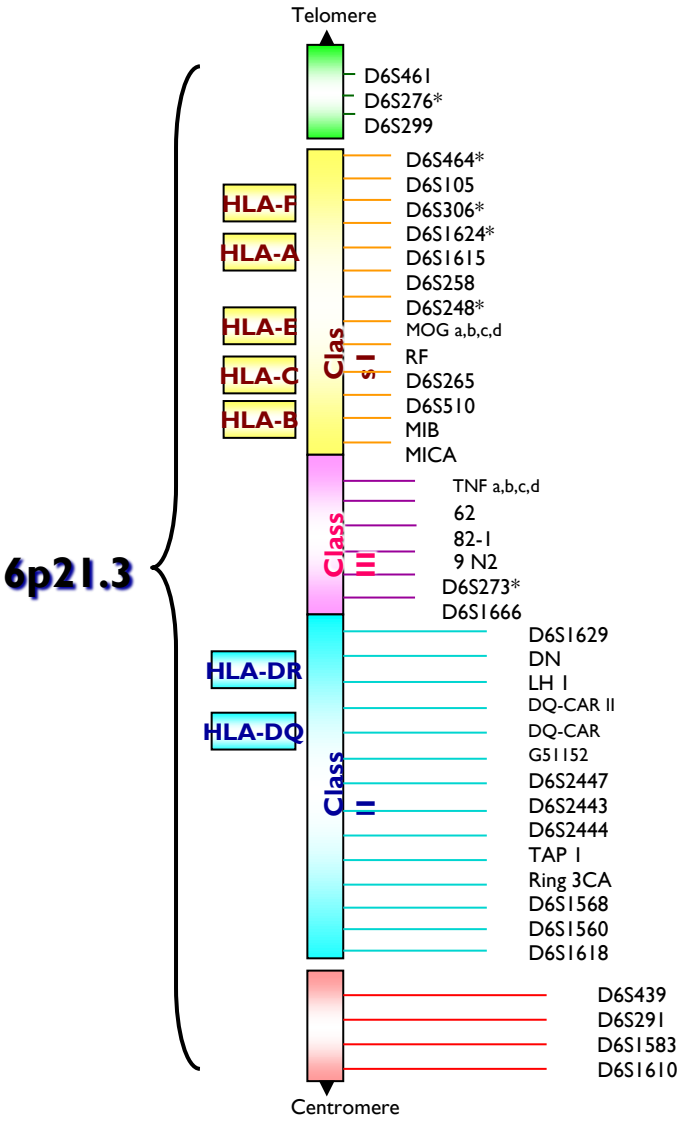
“Establishing linkage”

PGD set-up for HLA

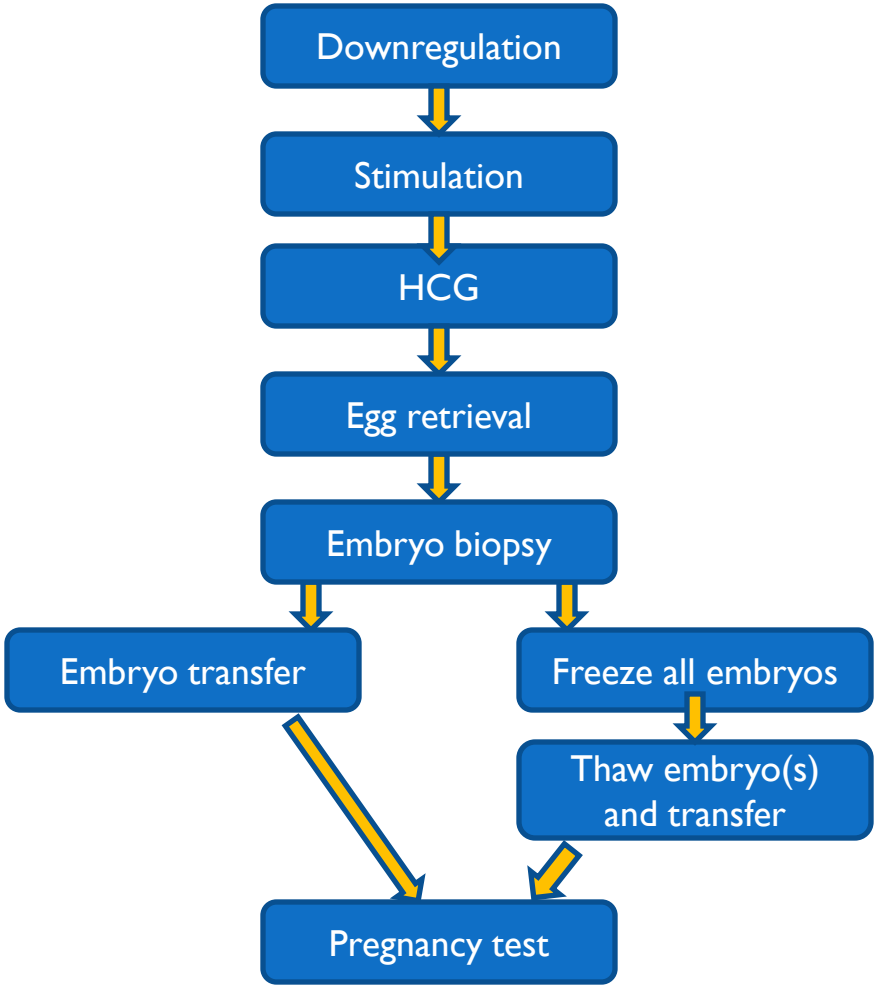




Markers in HLA complex



IVF/PGD timeline overview



IVF/PGD timeline

Downregulation (~3+ weeks)

- Shut off ovaries
- Birth control, Lupron

Stimulation (~1.5 weeks)

- Stimulate ovaries to produce many eggs
- Hormone injections

HCG (one time)

- "Trigger shot"
- Causes eggs to mature

IVF/PGD timeline

Day 0

- 36 hours after HCG shot
- Egg retrieval
  - Outpatient procedure (~20 min)
  - "Twilight" anaethesia
- Fertilization of eggs with partner's sperm (ICSI)
  - Required: reduces contamination

IVF/PGD timeline

Option #1: Day 3 (blastomere) biopsy

Day 3

- Each embryo is ~4-8 cells in size
- Remove (biopsy) one cell (blastomere) from each embryo
- Biopsied cells undergo genetic testing

Day 5

- Results from genetic testing are available
- Embryo development checked by laboratory
- 1-3 healthy/developing embryos transferred into woman's uterus

IVF/PGD timeline

Option #2: Day 5 (blastocyst) biopsy

Day 5/6

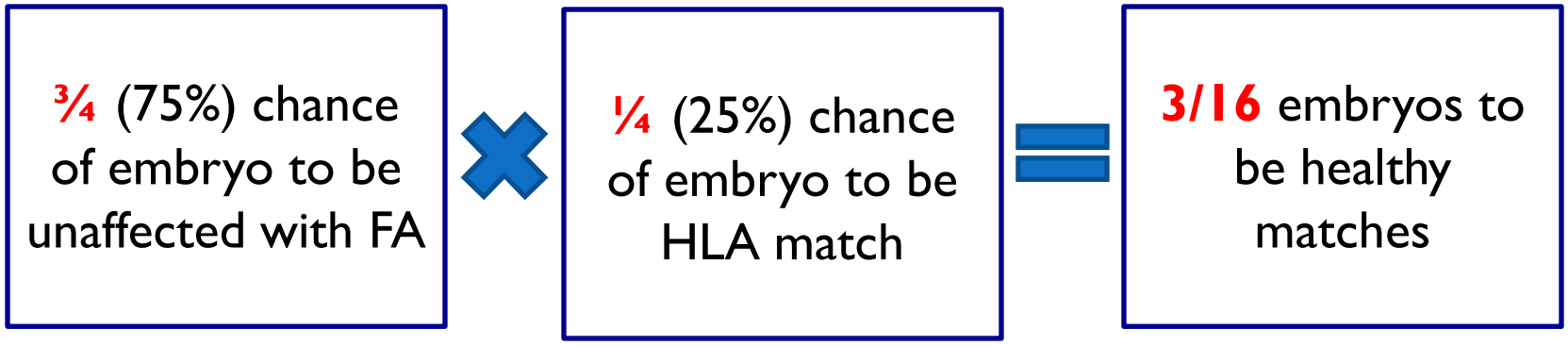
- Embryos are ~100 cells in size
- Remove (biopsy) trophectoderm cell(s) from each embryo
  - Trophectoderm: part of embryo that will become placenta during pregnancy
- Biopsied cells undergo genetic testing
- Embryos are frozen to provide enough time to obtain results

6+ weeks later

- Medications are taken to thicken the lining of the uterus
- Healthy embryo(s) thawed and transferred

Embryo (Day 3) biopsy

PGD results



~19% of embryos will be a healthy match

PGD results

Embryo number	FA status	HLA status	Embryo transfer recommendation
1	Carrier	Non-match	Can be frozen
2	Affected	Non-match	No
3	Affected	Match	No
4	Carrier	Non-match	Can be frozen
5	Carrier	Match	YES
6	N/A	N/A	No, re-biopsy if possible
7	Non-carrier	Non-match	Can be frozen*
8	Carrier	Non-match	Can be frozen

*reduced accuracy

IVF/PGD timeline

~1.5 weeks after transfer

- Blood test to determine if pregnancy occurred
- If positive, follow up every few days with additional blood tests (make sure hormone levels are increasing)

~3.5 weeks after transfer

- 1st ultrasound to confirm presence of amniotic sac (4 weeks gestation)

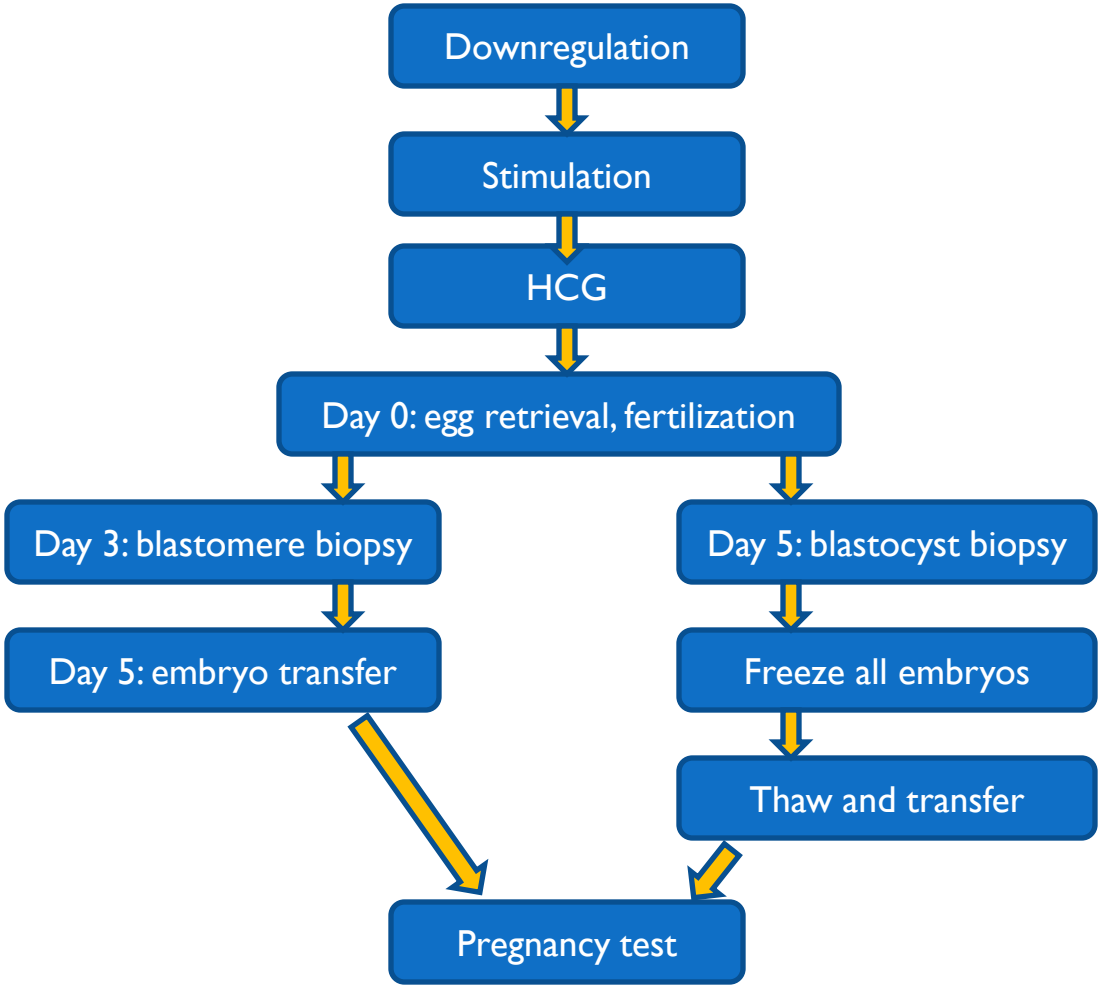
~5.5 weeks after transfer

- Ultrasound to confirm heartbeat (6 weeks gestation)

~7-9 weeks after transfer

- Released to regular OBGYN (8-10 weeks gestation)
- Followed the same as a natural pregnancy

IVF/PGD timeline summary

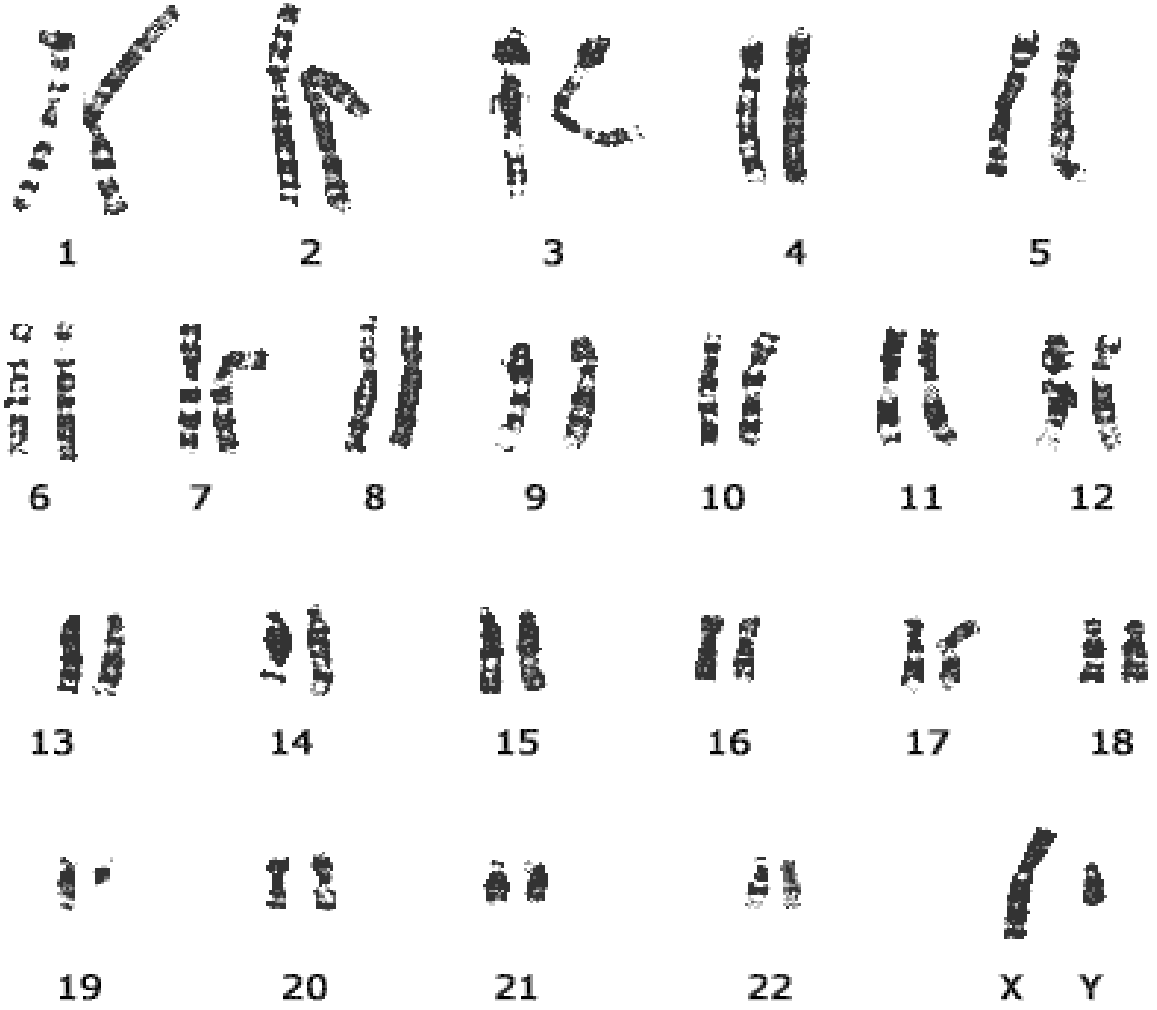


Sample IVF/PGD cycles

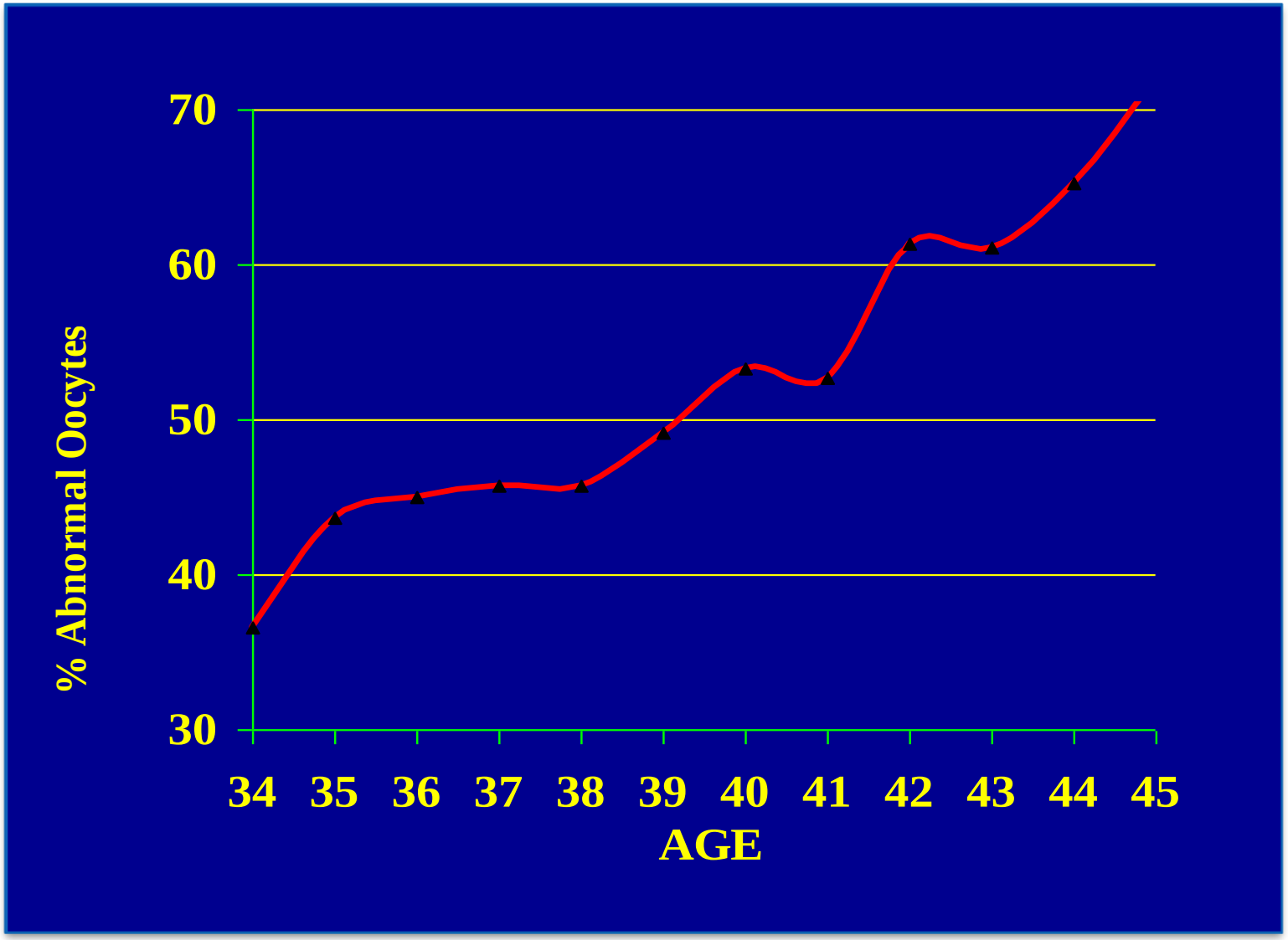
Patient #1	Patient #2
8 eggs retrieved 6 mature 4 fertilize 3 biopsied 1 healthy HLA match – developing 1 embryo transferred Positive pregnancy test!	25 eggs retrieved 18 mature 13 fertilize 10 biopsied 3 HLA matches 2 affected with FA 1 healthy – arrested No embryo transfer

Statistics are often skewed with small sample sizes

Additional testing options (chromosomes)



Percentage of aneuploid eggs by age



Why screen for chromosome problems?

- Reduce chance of live birth with chromosome disorder
- Reduce risk of miscarriage
  - 50% of first trimester miscarriage caused by incorrect chromosome number
- Improve effectiveness of IVF
  - Failed implantation often associated with incorrect chromosome number

Aneuploidy/chromosome testing

- Can be considered by women of any age, ethnicity, family history
  - Chromosome problems NOT related to family history!
- Testing options
  - Common chromosomes (5-7)
  - All chromosomes
    - 24-chromosome microarray
- Limitations
  - Imperfect test
  - Rule out more embryos

PGD accuracy

- FA/HLA
  - Typically 95-98%
  - May be reduced for some embryos
- Chromosomes
  - 90-98% depending on type of test and sample type

Factors affected accuracy of PGD

- Single cell testing
  - Cell type being tested & quality of cell
  - Allele drop-out (ADO)
  - Failed amplification of DNA
  - Chromosomal mosaicism
- DNA contamination
- Human error

PGD involves a modification of risk – not the elimination of risk

PGD does not replace **prenatal** diagnosis (CVS, amniocentesis)

PGD cost

- IVF
  - Initial evaluations ($3,000 - $4,000)
  - Procedures/monitoring ($9,000 - $13,000)
  - Medications ($2,000 - $5,000)
- PGD
  - Set-up ($3,500 - $5,000) – one-time
  - Testing ($2,500 - $3,000)
  - Biopsy ($1,500) – if at RGI
  - Embryologist travel ($1,500 - $2,000) – if needed
  - Chromosomes ($2,000 - $3,500) – optional

FAQ

1. How to choose an IVF center?
   - Location
   - Can they do their own biopsies?
   - Will they work with any PGD lab?
   - Exclusion criteria
   - Day 5 embryo culture success, pregnancy rates
2. How to choose a PGD lab?
   - Experience with FA/HLA testing
   - Any misdiagnoses?
   - Availability of chromosome testing options
   - Is prenatal testing required?
   - Availability of genetic counselors

FAQ

3. How many embryos are transferred?
   - Typically 1 or 2, sometimes 3
   - Up to patient and physician
   - More embryos transferred = higher chance of multiple gestation
4. Is there a minimum number of embryos required?
   - NO
   - Possibility of batching cycles

FAQ

5. What if I have extra embryos?
   - Freeze, discard, donate to research or other couples
6. Is there a risk to removing a cell from an embryo?
   - Low risk of embryo arrest (<0.5%)
   - Have not seen increased risk of birth defects, miscarriage, etc

FAQ

7. Do I have to come to Chicago?
   - NO – we can work with any IVF center
8. What are the chances of getting pregnant?
   - Age dependent, center dependent, does NOT depend on prior ability to conceive!
   - <u>If embryo transfer</u>:
     - <35 ~50%
     - 35-37 ~40%
     - 38-42 ~30%
     - >43 ~10% or less

RGI's experience with PGD

Testing performed	# Patients	# Cycles	Pregnancies	Live births
Single Gene	1206	2158	731	683
Aneuploidy	3205	4429	894	702
Translocation	367	539	150	119
TOTAL	**4778**	**7126**	**1775**	**1504**

As of 06/2011

Over 250 genetic conditions tested in 22 years

RGI's experience with PGD for HLA

Testing performed	# Patients	# Cycles	Pregnancies	Live Births
HLA only	46	98	24	19
HLA with genetic disease	81	199	34	27
TOTAL	**127**	**297**	**58**	**47**

As of 06/2010

RGI's experience with PGD for FA/HLA

Testing performed	# Patients	# Cycles	# Embryo Transfers	Pregnancies	Live births
FA-A only	1	3	2	1	2
FA-C only	2	5	4	1	2
FA-A + HLA	11	39	22	6	4
FA-C + HLA	2	4	4	1	1
FA-D2 + HLA	1	3	2	1	1
FA-F + HLA	1	3	2	0	0
FA-I + HLA	1	2	2	0	0
FA-J + HLA	1	3	1	0	0
TOTAL	**20**	**60**	**39 (65%)**	**10 (26%)**	**10**

As of 05/2012

Summary: Pros vs. Cons of IVF/PGD

Pros	Cons
Dramatically reduces risk of having affected offspring	Extremely expensive if insurance doesn't cover (~$25,000/cycle)
Testing occurs prior to implantation in order to avoid difficult decision-making during pregnancy	Physically and emotionally difficult
Availability of HLA testing for couples with a sick child needing a stem cell transplant	Low pregnancy rates with IVF, regardless of prior fertility
	Not a perfect technology (95-98% accurate, prenatal diagnosis is still recommended to confirm results

Thank you!

- Questions?
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