FAMILY PLANNING

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RYR1 International Family Conference

Pittsburgh, PA

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No Disclosures

Family Planning in the Setting of a Genetic Diagnosis

- Get your specific genetic / molecular diagnosis
- Understand your genetic risk
- Complex for *RYR1*-related myopathies as:
  - pathogenic variants can be:
    - dominant acting
    - recessive acting
  - pathogenic variants can have:
    - variable expressivity
    - decreased penetrance

Family Planning in the Setting of a Genetic Diagnosis

- Affected individuals with *RYR1*-related myopathies:
  - should encourage their unaffected partner/spouse to have full *RYR1* gene sequencing
  - partner's carrier status will affect the recurrence risk

For individuals with bi-allelic / autosomal recessive pathogenic variants

Partner is NOT carrier:

	r	r
R	Rr	Rr
R	Rr	Rr

Partner is a carrier:

	r	r
R	Rr	Rr
r	rr	rr

Family Planning in the Setting of a Genetic Diagnosis

- Affected individuals with *RYR1*-related myopathies:
  - should encourage their unaffected partner/spouse to have full *RYR1* gene sequencing
  - partner's carrier status will affect the recurrence risk

For individuals with monoallelic / autosomal dominant pathogenic variants

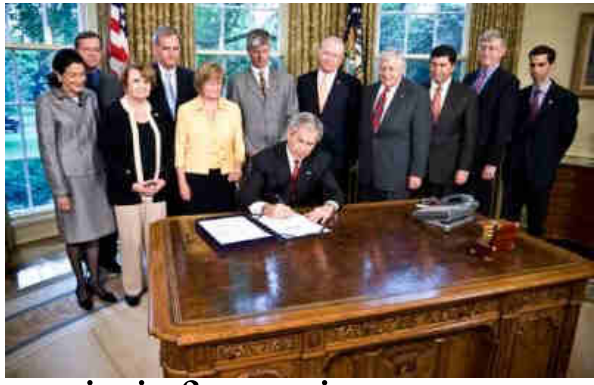
Partner is NOT carrier:

	R	r
r	rR	rr
r	rR	rr

Partner is a carrier:

	R	r
r	rR	rr
r	rR	rr

Genetic Testing – Legal Issues



Genetic Information Nondiscriminaton Act (GINA):

- H.R. 493, signed 5/21/2008 by president G.W. Bush
- Generally prohibits discrimination in health coverage and employment on the basis of genetic information.

What does GINA do?

- Prohibits requiring genetic information for decisions regarding coverage
- Prohibits from using genetic information for employment decisions

What GINA does NOT do?

- Protections do not extend to life insurance, disability insurance and long-term care insurance.
- Employment provisions do not apply to employers with fewer than 15 employees, military, federal employees, Indian health service.
- Health coverage:
  - does **not** prohibit determining eligibility or rates based on the manifestation of a disease
  - **permits** the overall premium rate for an employer to be increased because of the manifestation of a disease

Genetic Testing – Legal Issues

Affordable Care Act:

- Signed by president B. Obama on 3/23/2010
- Health coverage:
  - ends pre-existing condition for health insurance coverage
- AFA pre-existing condition protections do not extend to:
  - life insurance
  - disability insurance
  - long-term care insurance

Family Planning in the Setting of a Genetic Diagnosis

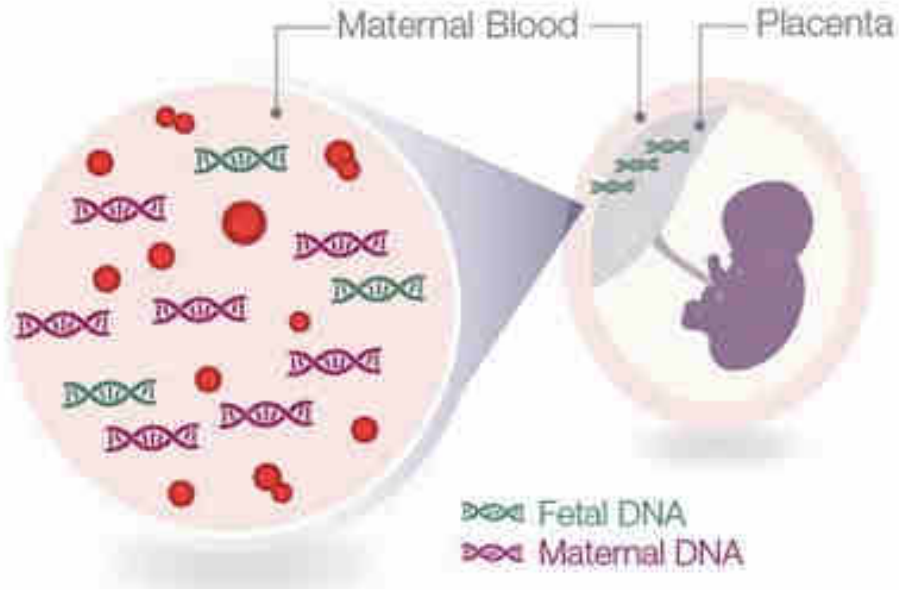
MADE TO
MEASURE

Family Planning Considerations

- Accepting your genetic risk and having children
- Adoption
- Using an egg or sperm donor
- Natural conception followed by prenatal diagnosis: CVS or amniocentesis
- *In-vitro* fertilization (IVF) with preimplantation genetic testing (PGT)

Prenatal Genetic Screening

- cfDNA (cell-free fetal DNA) **screening** test:
  - testing fetal DNA circulating in maternal blood
  - a.k.a. NIPT (non-invasive prenatal test)



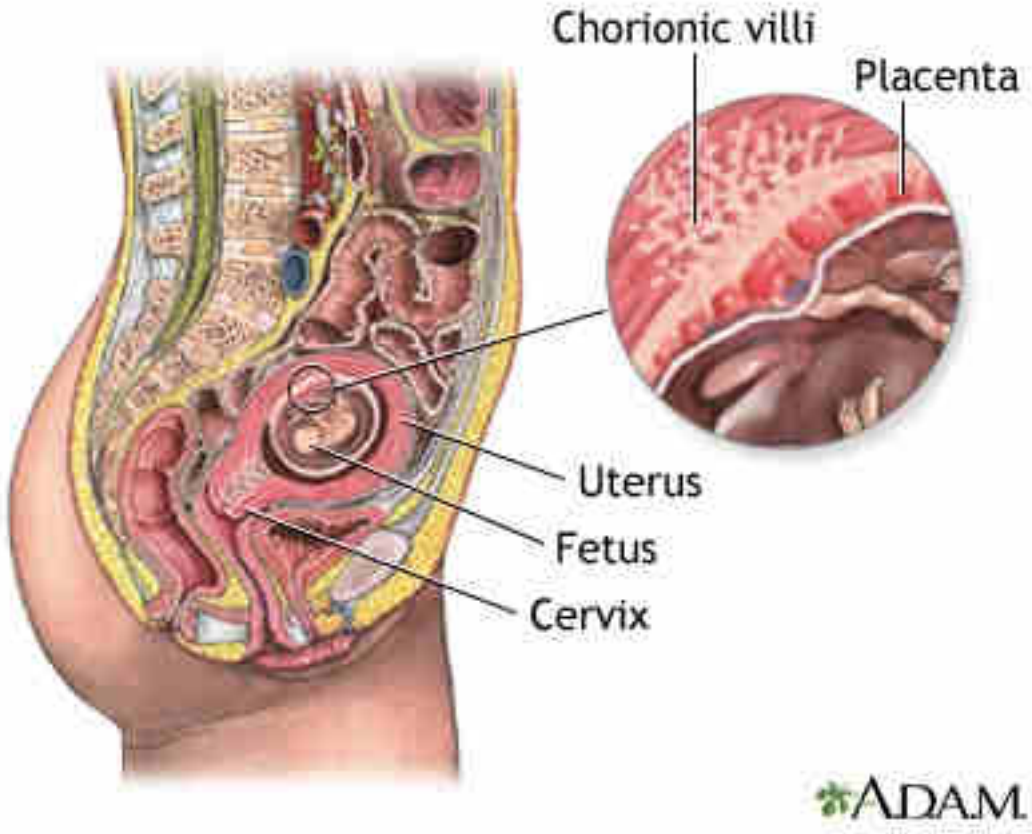
- **CANNOT** be currently used for single gene variant identification!
- Good *screening* (not diagnostic) test for common chromosomal abnormalities:
  - trisomy 21: 99.4% detection rate - trisomy 18: 96.6% detection rate
  - trisomy 13: 86.4% detection rate - monosomy X: 89.5%

Van den Veyer, F1000Research 2016, 5:2591

Family Planning Considerations

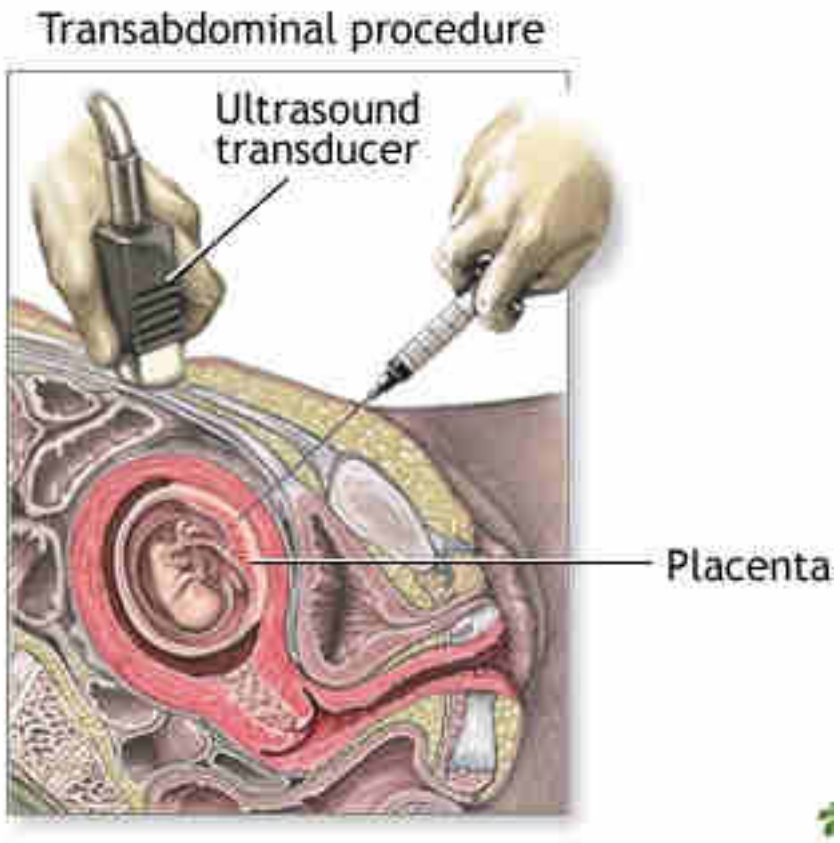
- Accepting your genetic risk and having children
- Adoption
- Using an egg or sperm donor
- Natural conception followed by prenatal diagnosis: **CVS or amniocentesis**
- *In-vitro* fertilization with preimplantation genetic diagnosis: **IVF + PGD**

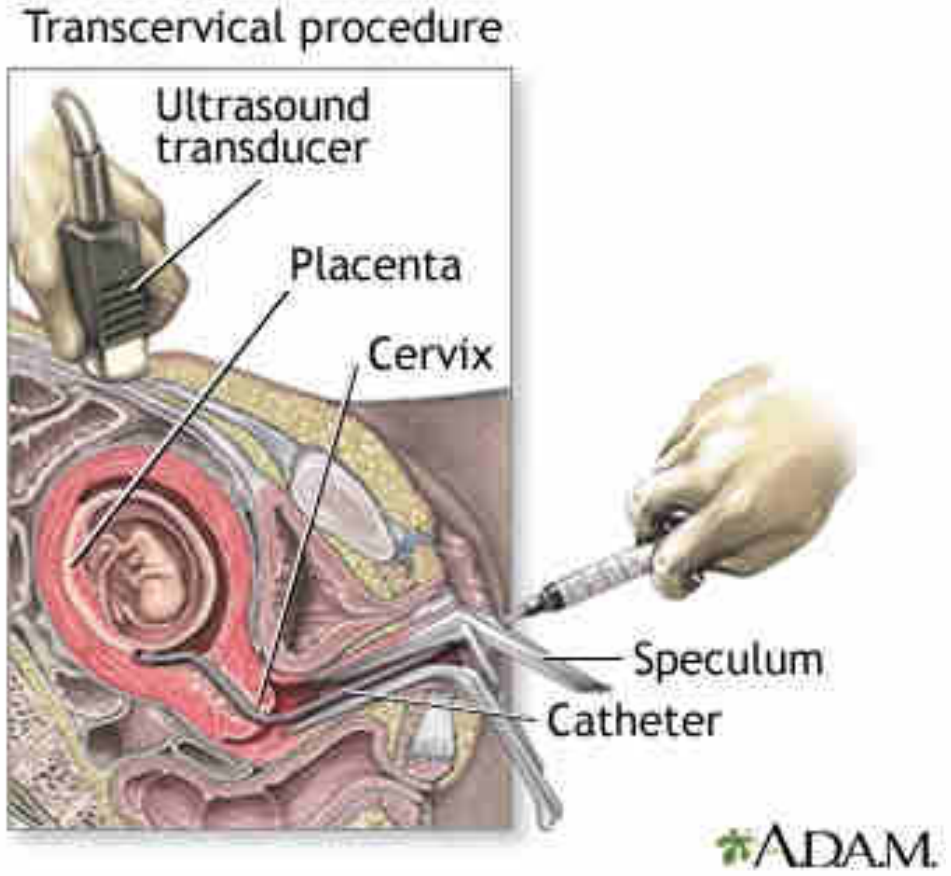
Chorionic Villus Sampling (CVS)



www.pennmedicine.adam.com

Chorionic Villus Sampling (CVS)





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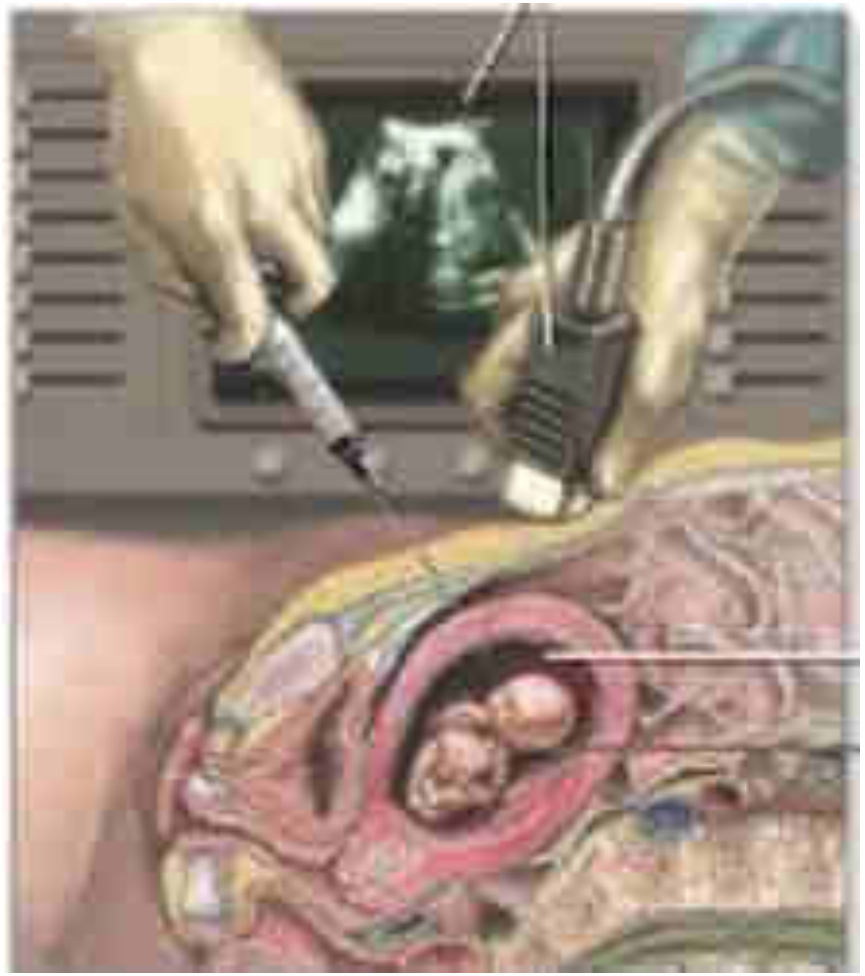
Amniocentesis



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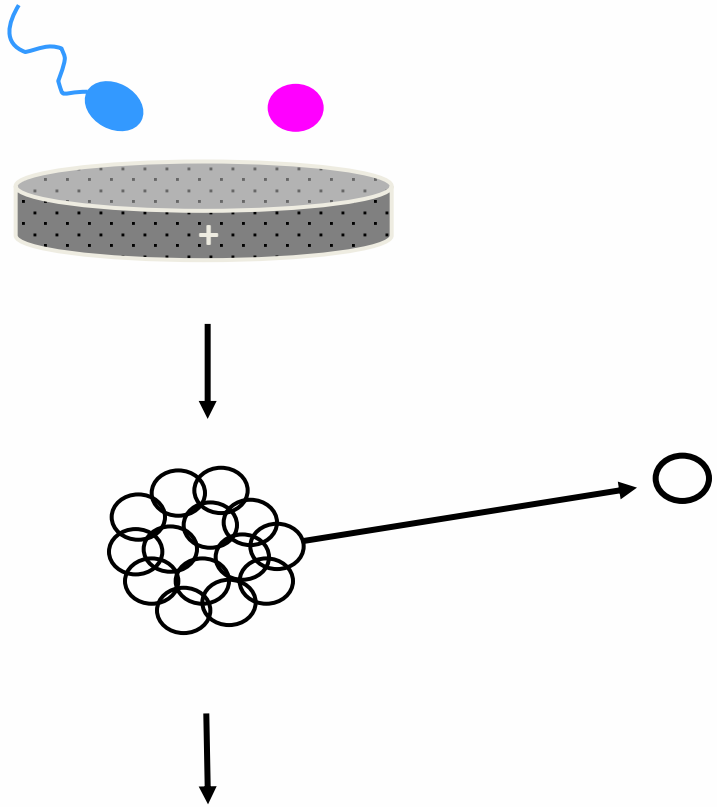
Prenatal Diagnosis

	Chorionic Villus Sampling (CVS)	Amniocentesis
Timing	10 – **12** wk GA	15 – 18 wk GA
Method	Transabdominal or transvaginal	Transabdominal
Tissue tested	Chorionic villi (placenta)	Fetal skin and GI tract cells
Result TAT	Direct: ~3 days Cultured: 2-3 weeks	Cultured: 2-3 weeks
Diagnostic Sensitivity	Excellent (with a good sample) - maternal cell contamination	Excellent

Prenatal Diagnosis – miscarriage rate

	Chorionic Villus Sampling (CVS)	Amniocentesis
ACOG	1/300 – 1/1000 (0.1 – 0.3%)	1/300 – 1/1000 (0.1 – 0.3%)
CDC	1/100 – 1/200 (0.5 - 1.0%)	1/200 – 1/400 (0.25 – 0.5%)
UK NHS	1/50 – 1/100 (1-2%)	1/100 (1%)
Recent meta-analyses		
- Akolekar et al, 2015	1/455 (0.22%)	1/909 (0.11%)
- Beta et al, 2018	1/286 (0.35%)	1/286 (0.35%)

IVF (in-vitro fertilization) + PGT (preimplantation genetic testing)



testing done on DNA from a few cells

Embryos without the pathogenic variant are implanted

What is IVF?

In Vitro Fertilization (IVF):

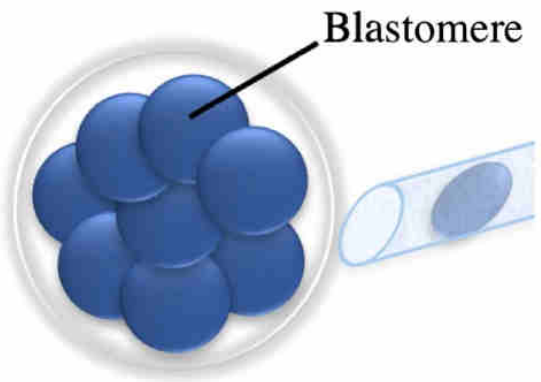
Process of fertilization by manually combining an egg and sperm in a laboratory dish, followed by transfer of a resulting embryo to the uterus

- **First successful IVF birth:** Louise Brown born 7/25/78 in the UK

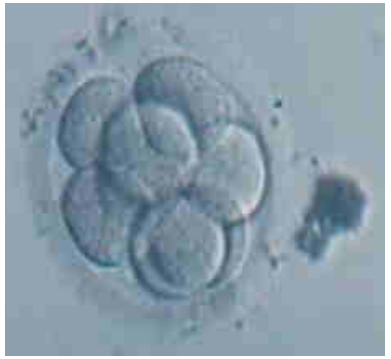
What is IVF?

PGT requires IVF with or without ICSI, embryo biopsy for DNA sampling, genetic testing, and selected embryo transfer

Preimplantation Genetic Testing – day 3 biopsy

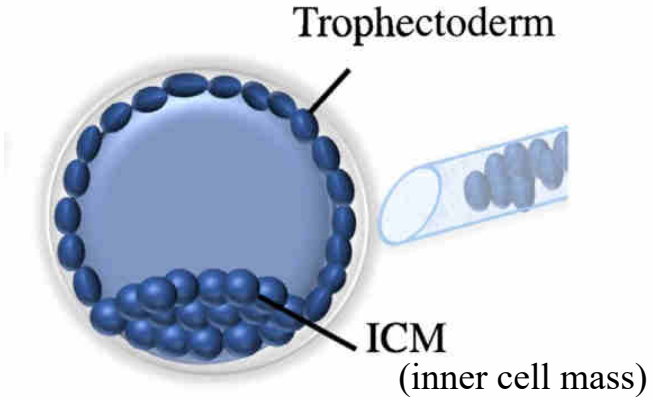


Cleavage stage

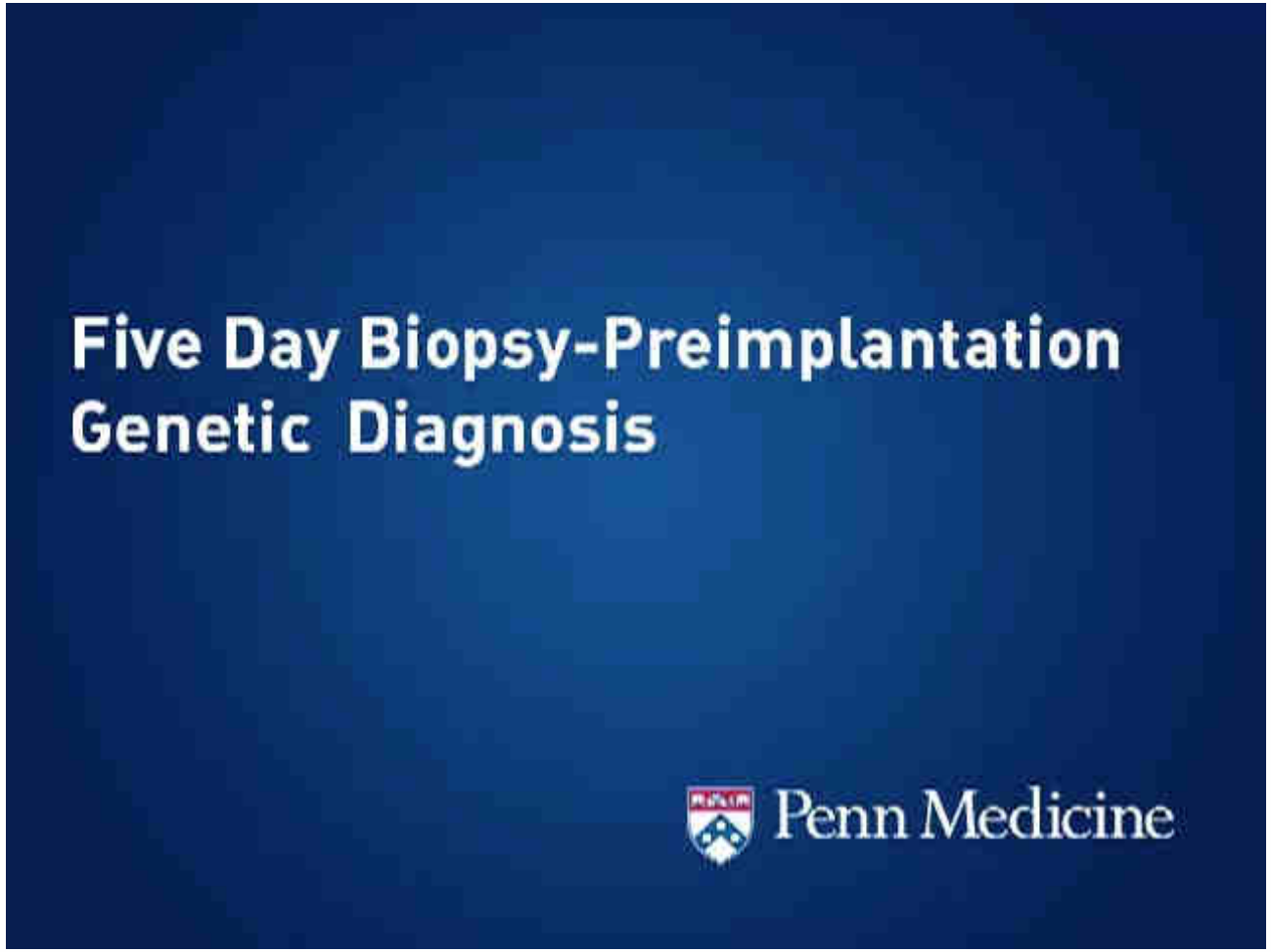


Leaver & Wells. *Hum Repr Update 26(1):16-42,2020*
Zhang et al. *PLoS ONE* 4(11): e7844, 2009

Preimplantation Genetic Testing – day 5-6 biopsy



Blastocyst stage



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Preimplantation Genetic Testing

- PGT-M: for monogenic disorders or single gene defects
- PGT-SR: for chromosomal structural rearrangements
- PGT-A: for aneuploidy detection

Preimplantation Genetic Testing

- **PGT-M: for monogenic disorders or single gene defects**
- PGT-SR: for chromosomal structural rearrangements
- PGT-A: for aneuploidy detection

- combined PGT

Maternal Age-Related Risk for Chromosomal Aneuploidy

Table 2. Risk of Trisomy 21 or Any Chromosomal Abnormality at Delivery.*

Maternal Age at Term	Trisomy 21	Any Chromosomal Abnormality†
	number/ total number	
20 yr	1/1480	1/525
25 yr	1/1350	1/475
30 yr	1/940	1/384
35 yr	1/353	1/178
40 yr	1/85	1/62
45 yr	1/30	1/18

Driscoll & Gross, *N Engl J Med,* 357:61-63, 2007

Maternal Age-Related Risk for Chromosomal Aneuploidy Trophectoderm (TE) Biopsy (5-6 day)

Maternal Age	# of Cases	# of Embryos	Avg # of Embryos/Case	Euploid Rate	Aneuploid Rate
<35 years	3,786	20,572	5	62.6%	37.4%
35-37 years	3,095	15,023	5	53.0%	47.0%
38-40 years	3,497	14,849	4	39.5%	60.5%
41-42 years	1,807	6,506	4	23.5%	76.5%
>42 years	838	2,637	3	15.6%	84.4%
OVERALL	13,023	59,587	5	48.1%	51.9%

Data from TE samples screened at Natera through July, 2017. Excludes egg donor cycles.

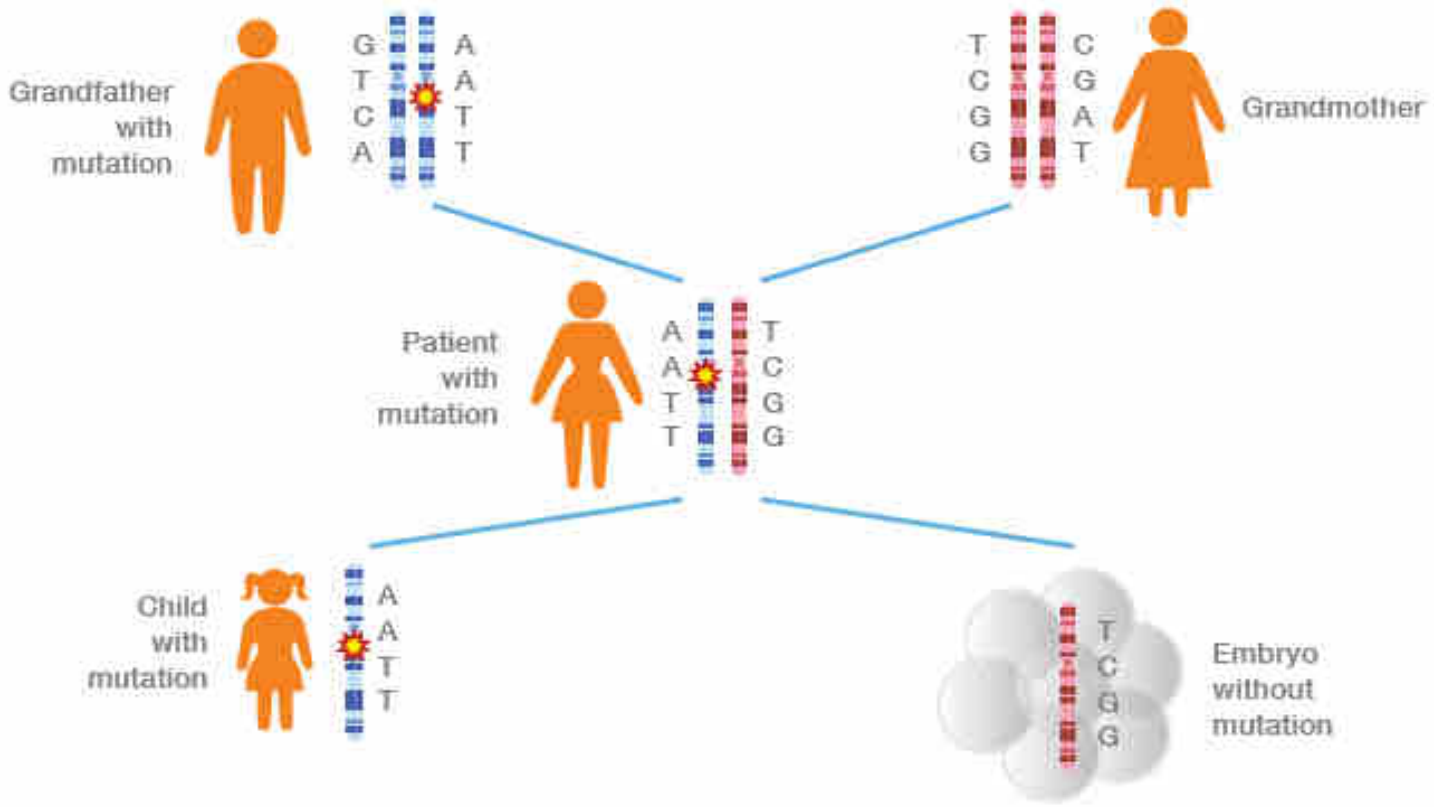
Slide provided by M. Maisenbacher Natera

Preimplantation Genetic Testing

- Develop primers for directly detecting the pathogenic gene variant and/or determine which chromosome homolog carries the mutation by looking at informative linked markers (i.e. SNPs, STRs, etc.)
- Homolog phasing requires samples from parents and often other relatives
  - Family samples are evaluated for informative markers
  - Clinical information (genetic status, affected/unaffected individuals) is referenced to determine which markers/homolog are associated with the familial mutation and which are not
- PGT can be made with a combination of direct variant analysis and homolog phasing OR in some cases by homolog phasing only
  - Embryos that inherit the linked markers/homolog associated with the disease gene pathogenic variant(s) are not recommended for transfer

Slide provided by M. Maisenbacher Natera

Homolog Phasing - PGT



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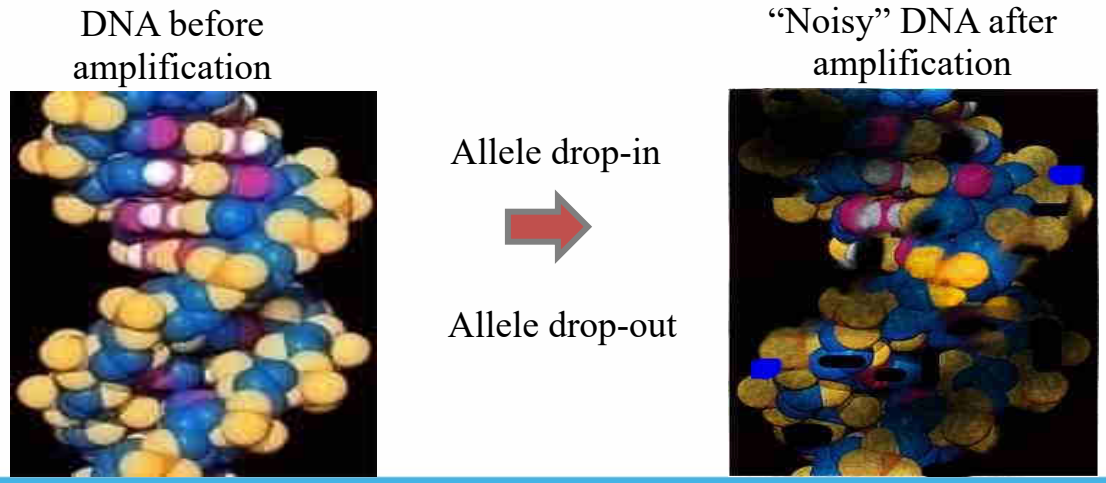
Homolog Phasing - PGT

- A large number of amplification cycles are required in order for the pathogenic variant to be visualized
- DNA amplification can lead to a high risk of ADO (regions of DNA fail to amplify) or contamination



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Homolog Phasing - PGT

- A large number of amplification cycles are required in order for the pathogenic variant to be visualized
- DNA amplification can lead to a high risk of ADO (regions of DNA fail to amplify) or contamination
- Amplifying linked markers (similar to DNA fingerprinting) close to the pathogenic variant of interest is critical

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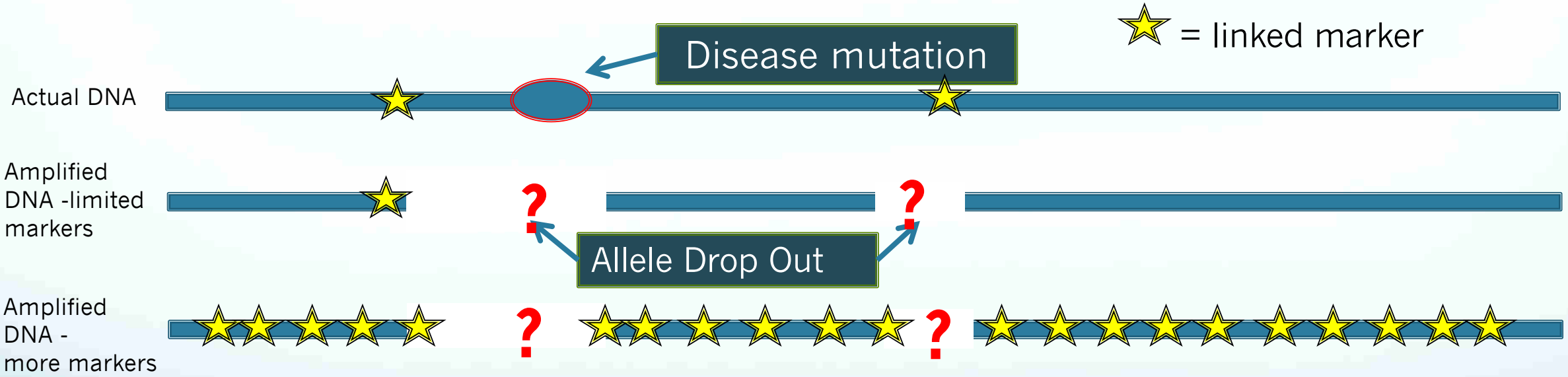
Allele Drop Out (ADO)

- Amplification of only one of two alleles present in a heterozygous single cell



Evaluating numerous markers surrounding the disease gene is important for decreasing the risk of misdiagnosis due to ADO.

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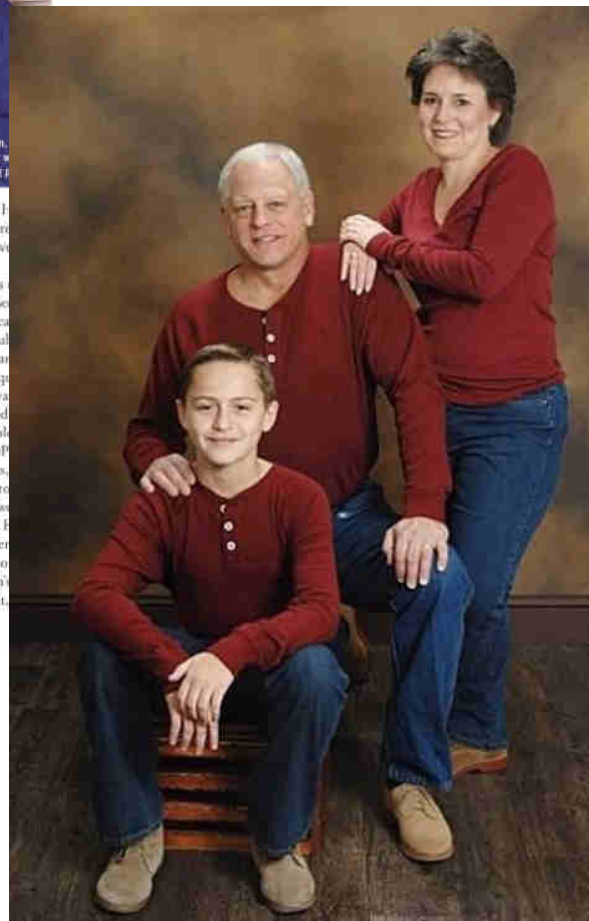
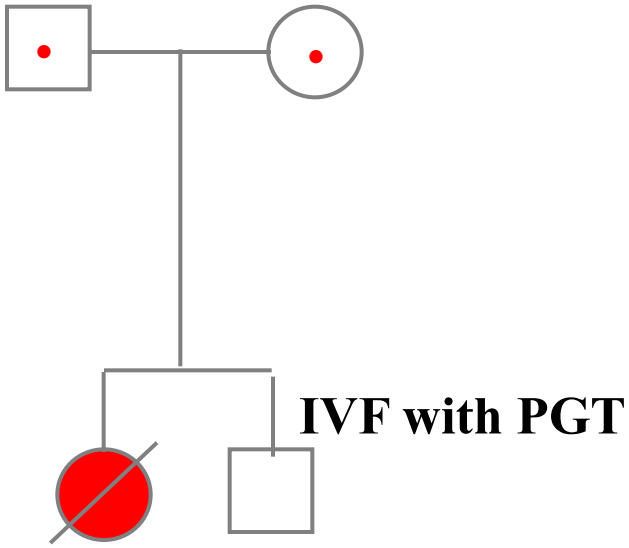




IVF with PGT

IVF + PGT path:

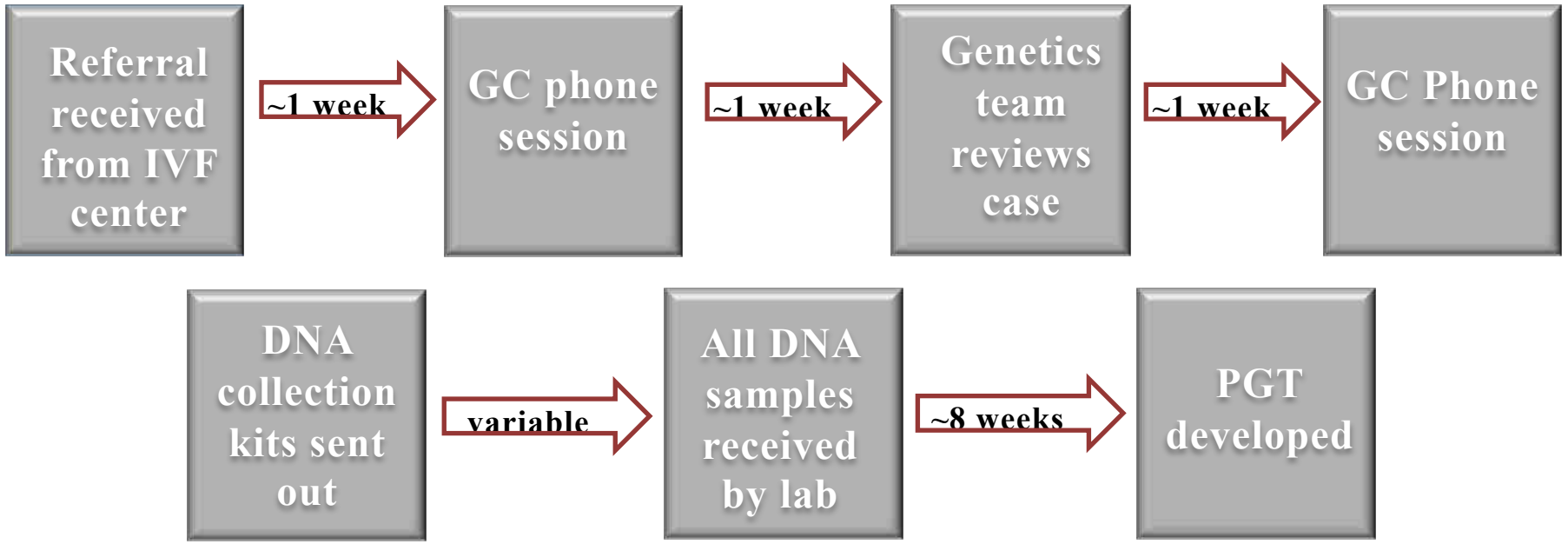
- 10 eggs retrieved
- 6 embryos biopsied
- 4 embryos had an informative genetic test result
  - 2 were unaffected
  - 2 were affected
- 2 unaffected embryos transferred
- singleton pregnancy

Points of Consideration re: IVF + PGT

- IVF related risks:
  - fertility Rx related risks
  - procedure related risks associated with egg retrieval
  - risks of multiple pregnancies and associated complications
  - slightly increased risk of imprinting disorders (epigenetic changes) for IVF + ICSI
  - no guarantee that a birth will occur
- Understanding possible residual risk:
  - PGD is not viewed as 100% sensitive
  - Prenatal diagnosis by amniocentesis is routinely recommended

Points of Consideration re: IVF + PGT

- Understanding PGT Test Development Timeline (*variable depending on PGT lab*)



Patients are instructed not to begin their IVF cycle until the PGT development process is completed

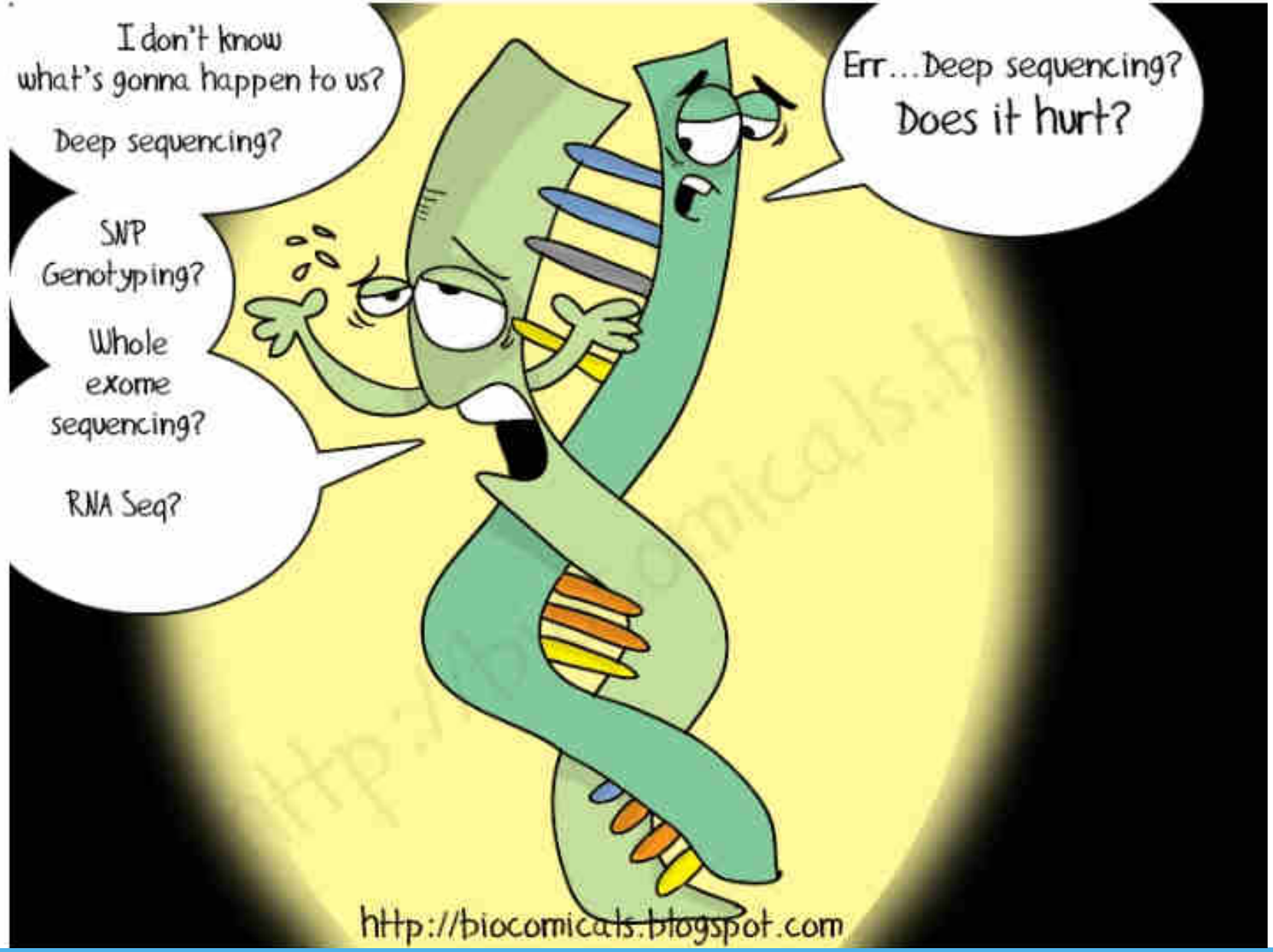
Slide provided by M. Maisenbacher Natera

Points of Consideration re: IVF + PGD

- Emotional and financial decisions re: number of cycles to pursue
- Understand the risk of having an affected child and the clinical implications:
  - what if all embryos are affected?
  - would you still consider embryo transfer?
  - will the IVF clinic let you transfer an affected embryo?
- One should find out / participate in discussion what will happen to embryos that are not transferred:
  - disposition of embryos (affected, unaffected, inconclusive results)
  - cryopreservation
  - embryo donation to other couples
  - embryo donation for research

Points of Consideration re: IVF + PGD

- Costs:
  - Emotional
  - Physical
  - Fiscal:
    - IVF cycle ~$12,000- $17,000 + FET cycle ~$3,000-5,000 + PGT ~$3,000
- Investigating insurance coverage:
  - some plans have lifetime ART benefit (set $ amount)
  - some plans cover IVF and some do not cover IVF
  - LMNs from your clinicians:
    - be ready to appeal denials multiple times
    - work with your employer's HR personnel


I don't know what's gonna happen to us?
Deep sequencing?
SNP Genotyping?
Whole exome sequencing?
RNA Seq?
Err...Deep sequencing? Does it hurt?
http://biocomicals.blogspot.com

Acknowledgements

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