Reproductive & Prenatal Genetics

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Qs

What role does infertility play in a couple's risk for a child with a genetic disorder?

What modalities exist for screening for genetic disease during pregnancy?

What are the risks and benefits of prenatal diagnostic studies?

Case Presentation

A couple in their 30s come for preconception genetic counseling:

They are both HEALTHY.

The couple is concerned about a pregnancy complicated by a genetic or congenital anomaly.

What Qs would you ask the pts…

Case Presentation

A couple, in their 30s, are referred for preconception genetic counseling:

“History is notable for 3 years of unexplained infertility”

The couple is concerned about a pregnancy complicated by a genetic or congenital anomaly:

Could the cause of their infertility increase this risk?
Does assisted reproduction technology (ART) increase this risk?

Genetic Evaluation of Infertility

1. Thus far unrecognized disorders of sexual differentiation

2. Genetic conditions associated with impaired fertility

1. Disorders of Sexual Differentiation

Level 1 - Genetic sex 46 XX vs 46, XY

Level 2 - Gonadal sex
Undifferentiated gonad – testicular development dependent on presence of TDF

Level 3 - Phenotypic sex
1. Internal reproductive structures (dependent on Mullerian inhibiting factor)
2. External reproductive structures (dependent on testosterone exposure)
3. Secondary sex characteristics at puberty

1. Disorders of Sexual Differentiation

	FEMALE INFERTILITY	MALE INFERTILITY	
GENETIC SEX	45,X 47,XXX	47,XXY 46,XY/45,X	Lack of gonadal development
GONADAL SEX	delXp FRAX premutation	DAZ deletion	Lack of gonadal support
PHENOTYPIC SEX	Late-onset CAH	Androgen insensitivity	Effects on secondary sex characteristics

Karyotype Abnormalities with Azoospermia or Oligozoospermia

Azoospermia 10-15%
Oligospermia 5 %
Normal population <1%

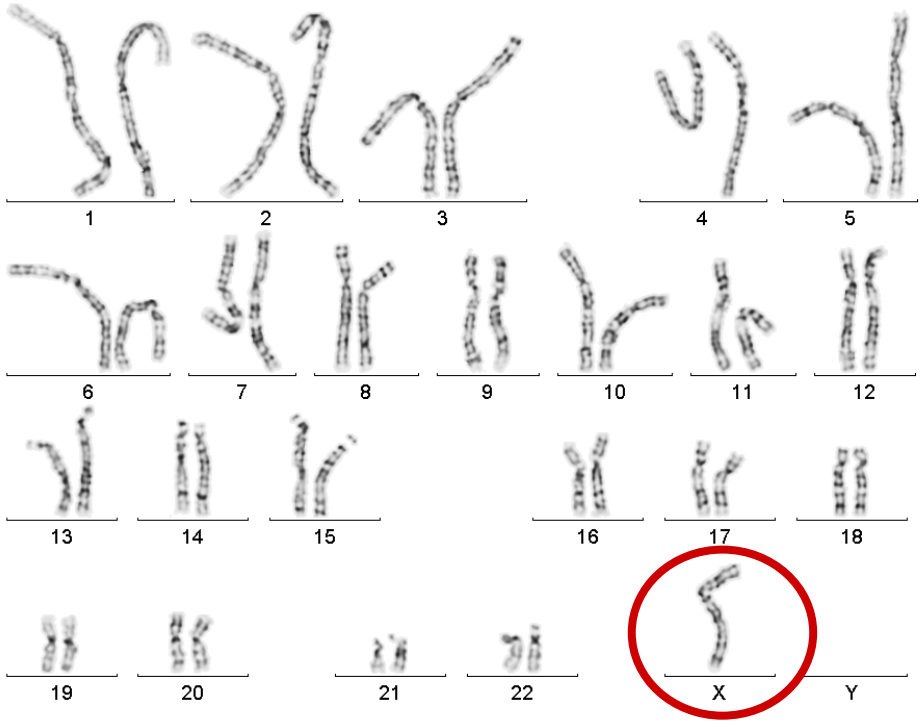
Unbalanced translocations more common with oligospermia

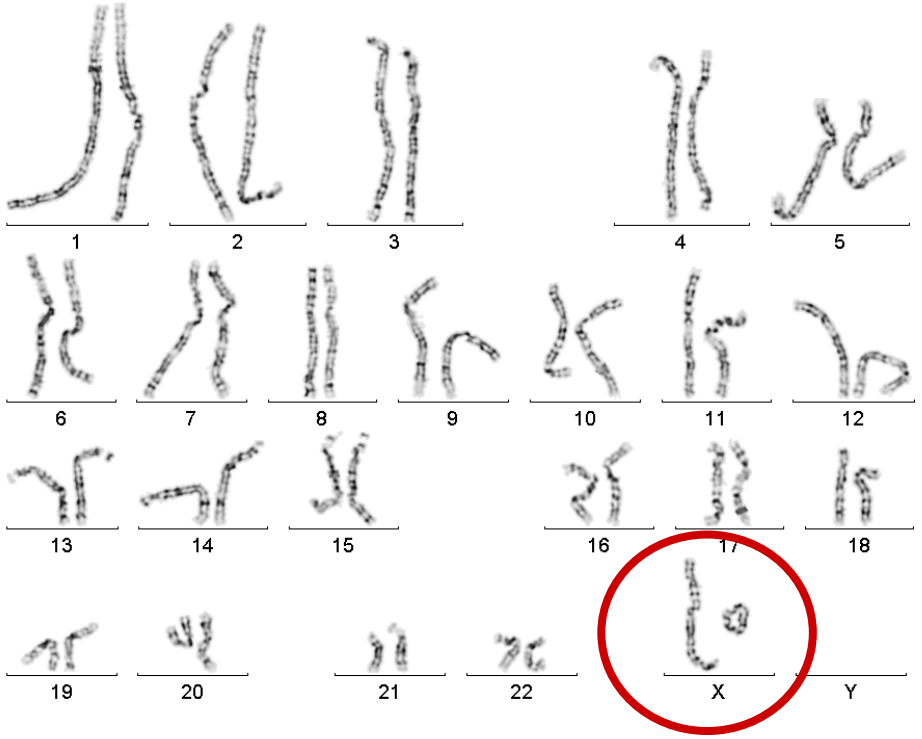
Sex chromosome anomalies increase with decreasing sperm count

Turner Syndrome

Responsible genes: X genes that escape inactivation, *SHOX*
Proteins: SHOX: Short stature homeobox protein
Cytogenetic locus: SHOX: Xpter-p22.32
Inheritance: sporadic
Clinical Features and Diagnostic Criteria: congenital lymphedema, growth failure, normal intelligence (10% dev delays), coarctation of the aorta, bicuspid aortic valve, HLHS, hyperlipidemia, gonadal dysgenesis (10% 45,X go into puberty), hypothyroidism, diabetes, strabismus, recurrent OM, SNHL, Crohns, renal malformation, osteoporosis.
Clinical Tests: echo, renal US, TFTs, GH testing, FISH SRY
Molecular Tests: Karyotype
Disease Mechanism: SHOX: thought to act as a transcription regulator with many down-stream targets that modify growth and stature. SHOX protein has been identified in the growth plate from 12 weeks to late childhood.
Treatment/Prognosis: GH, HRT, gonadectomy if Y chromosome mosaicism (risk for gonadoblastoma). Need lifelong cardiac follow-up, at risk for aortic dilation and dissection with bicuspid aortic valve.

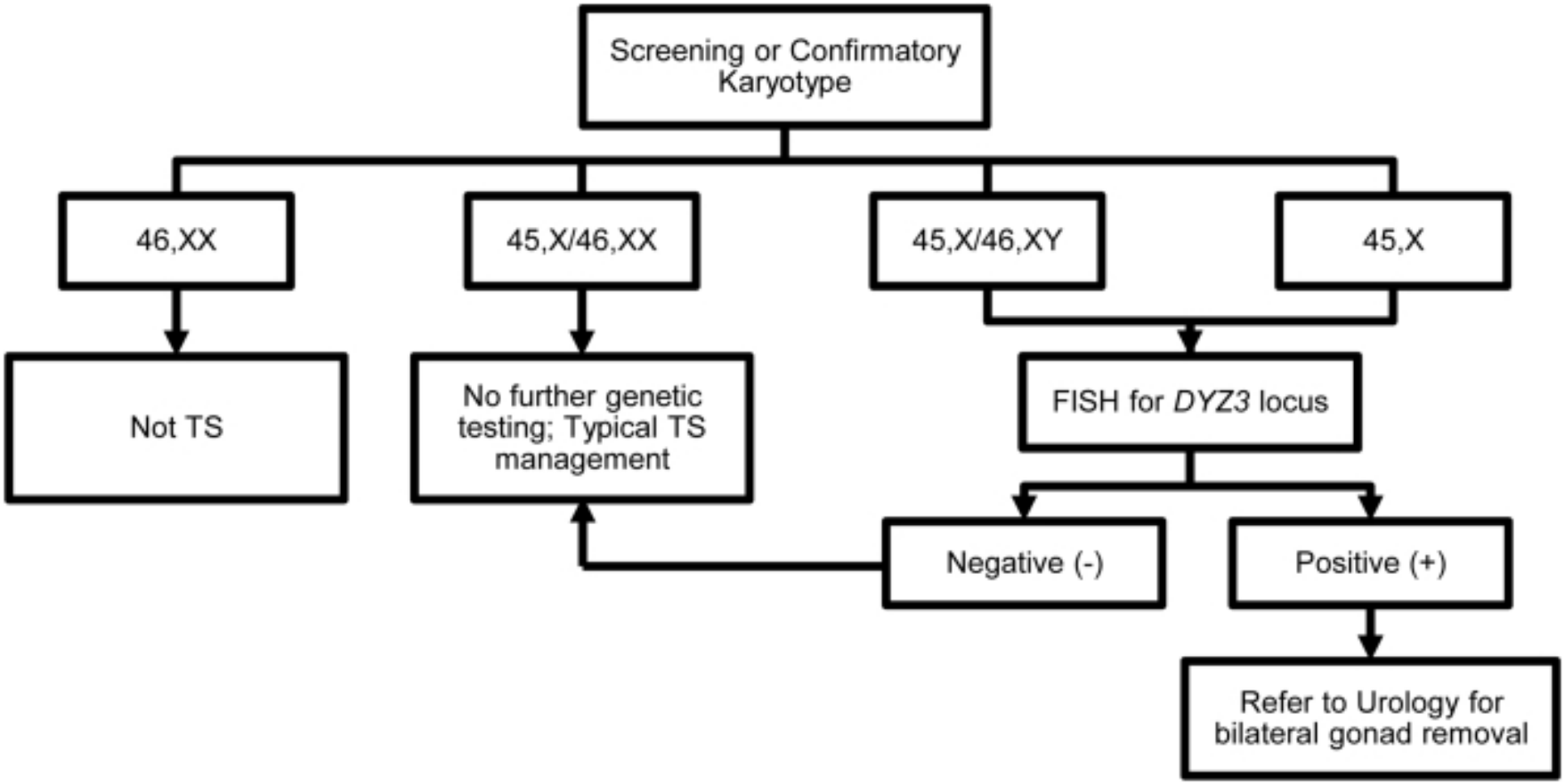
Chromosomal aberrations





mos 45,X/46,X,r(X)

Turner syndrome


Screening or Confirmatory Karyotype
46,XX
45,X/46,XX
45,X/46,XY
45,X
Not TS
No further genetic testing; Typical TS management
FISH for DYZ3 locus
Negative (-)
Positive (+)
Refer to Urology for bilateral gonad removal

Turner X0/XY

- Indications for prophylactic bilateral adnexectomy
- Increased (up to 30%?) risk of dysgerminoma arising from gonadoblastoma

Klinefelter Syndrome

Clinical Features and Diagnostic Criteria: tall stature, slightly delayed motor and language skills, learning problems, testosterone plateaus age 14, small fibrosed testes, azoospermia and infertility, gynecomastia, increased cholesterol, slightly increased risk of autoimmune disorders and mediastinal germ cell tumors (1% risk)

Molecular Tests: karyotype, at least one extra chromosome to a 46,XY

Disease Mechanism: 1st or 2nd meiotic division nondisjunction of either parent. Maternal>paternal origin + advanced maternal effect

Treatment/Prognosis: Testosterone in mid-late adolescence for bone density, Secondary sex characteristics development, muscle mass, cholesterol, increase libido, improved energy. Can do testicular biopsy and use any retrieved sperm for ICSI (increased risk sex chrom abnormality so follow with PGD)

AZF region deletions

Genetic basis of male infertility (chr Y)
Three loci: AZFa, AZFb, AZFc
Male pattern of transmission
Only Sertoli cells on testicular biopsy
Changed sperm pH(!)

2. Genetic conditions associated with impaired fertility

Cystic fibrosis

Fragile X syndrome

Myotonic dystrophy

Kennedy disease

Kartagener syndrome

2. Genetic conditions associated with impaired fertility – Cystic Fibrosis (CF)

Congenital absence of the vas deferens (CBAVD)
- clinical diagnosis, associated with azoospermia
- 98% of adult males with classic cystic fibrosis
- related to mucosal changes in developing vas

CBAVD - obstructive azoospermia; 1-2% of infertile men
- respiratory assessment supports inclusion of CBAVD in spectrum of cystic fibrosis

CBAVD with renal abnormalities – normal sweat chloride test, low rate of *CFTR* mutations

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2. Genetic conditions associated with impaired fertility – Cystic Fibrosis (CF)

MUTATIONS	severe/severe	severe/mild	mild/mild
CLASSIC CF pts	87.8%	11.3%	0.9%
CBAVD pts	0.5%	87.9%	11.6%

Common 23 mutation panel + IVS8-5T
- second mutation is frequently a polyvariant (5T variant of intron 8)
- 47.63% (double heterozygotes); 24.63% (heterozygotes)

Sequencing
- 86.15% have at least one CF mutation

2. Genetic conditions associated with impaired fertility – Fragile X Syndrome (FRAX)

- **Gene**: *FMR-1* **Protein:** FMRP ***Locus***: Xq27.3
- **Inheritance**: trinucleotide repeat expansion
- **Clinics**: developmental delay, ID (moderate/severe in boys, milder degree in girls), hyperactivity, autistic traits, premutation female carriers: OCD, depression, 20% POF, premutation male carriers: intention tremour, ataxia, parkinsonism, autonomic dysfunction (=FXTAS: >30% male carriers and <5% female carriers; 1,5% ♂ and 3%♀ late-onset ataxia;
  1/3000 ♂ two other *loci*: FraXE: only ID, FraXF: lack of phenotype
- **Diagnostics**: CGG triplet detection PCR: fast testing, small premutations; Southern: all mutation classes + normal alleles, mosaics, costly. Normal allele: 5-44 repeats, median: 45-58 repeats (grey zone), premutation: 59-200 repeats, mutation: >200 repeats
- **Mechanism**: >200 repeats = methylation = inactivation = lack of FMRP. POF and FXTAS (59-200 repeats) gain of function mutation

2. Genetic conditions associated with impaired fertility – Myotonic Dystrophy

- Most common form of adult muscular dystrophy

  adult myotonia - distal weakness, myotonic grip, frontal baldness, cardiac arrhythmias, infertility

- Male infertility

  oligoazoospermia - sclerosis of seminiferous tubules

- Congenital myotonia – polyhydramnios, neonatal hypotonia, respiratory dependency, mental retardation

  maternal transmission of expanded DNA

  dependent on size of expansion

2. Genetic conditions associated with impaired fertility – other disorders

- Kennedy Disease (spinal and bulbar muscular atrophy)

  similar to amyotrophic lateral sclerosis

  X-linked form of motor neuron disease that affects adult men, onset mid 40s, slow progression

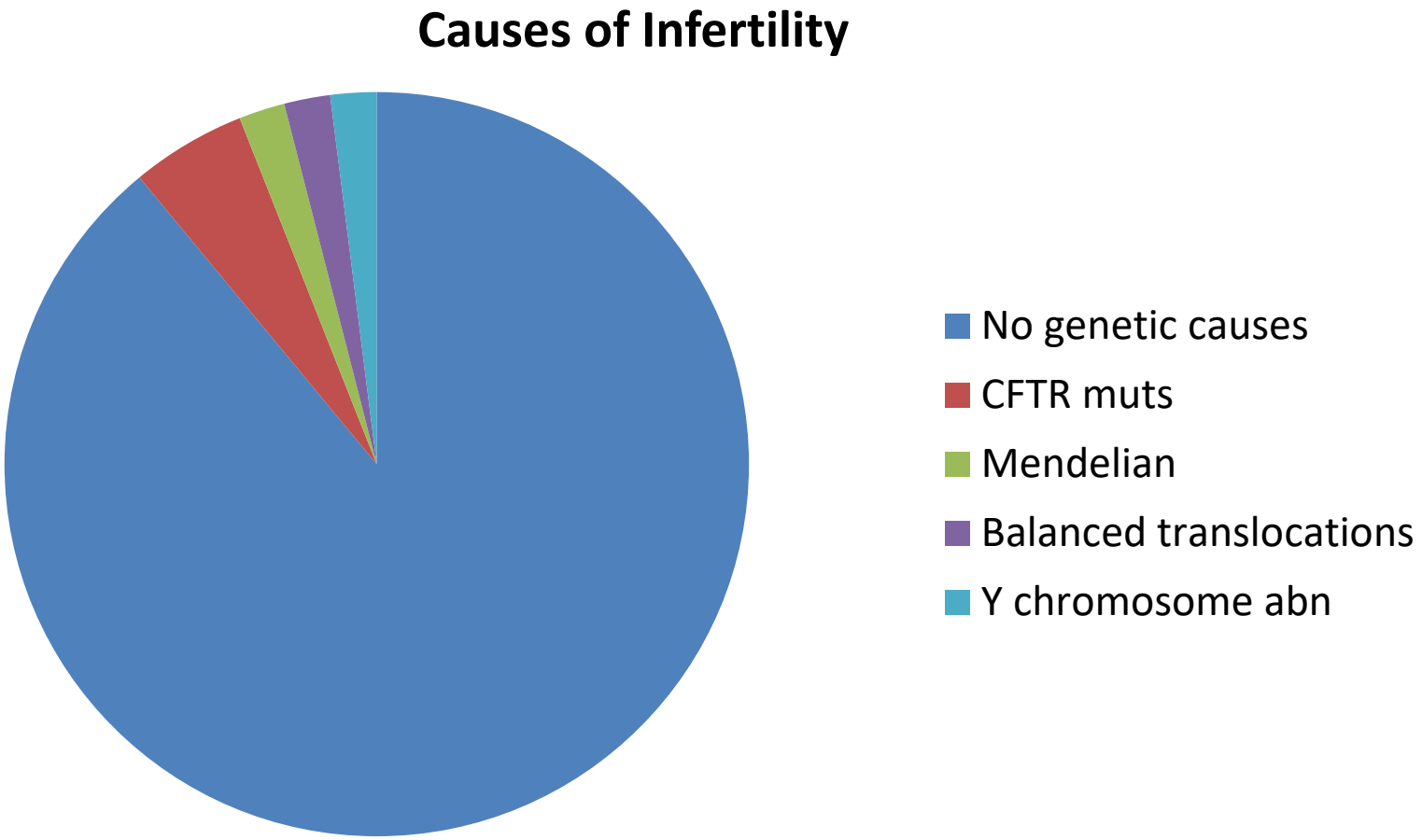
- Kartagener Syndrome (primary ciliary dyskinesia)

  both autosomal recessive and dominant forms

  male infertility - immotile cilia

  female infertility – fallopian tube motility

Genetic assessment prior to IVF (10-25% of causality is genetic)



Assisted Reprod Techniques (ART)

- Classic IVF (incubation of sperm w/oocytes): when male infertility excluded
- ICSI (intracytoplasmic sperm injection): in male infertility or immunologic disorders, or after unsuccessful IVF
- IMSI (ICSI w/morphological analysis under microscope)
- Other modifications of IMSI or ICSI

3. Genetic risks associated with ART

- Risk of congenital malformations following assisted reproduction technologies (ART)

  What is the evidence of an increased risk ?

  What type of genetic conditions are suspected ?

  Is the rate of congenital malformations increased among infertile populations irrespective of route of conception?

Assisted Reproduction Techniques(ART)

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Meta-analysis: IVF and congenita anomalies

	Highest quality (N=7)	Meta-analysis (N=25)
All anomalies	OR=1.40 (95%CI: 1.28-1.53)	1.29 (1.21-1.37)
Only major	2.01 (1.49-2.69)	1.32 (1.20-1.45)
Only singletons	1.35 (1.20-1.50)	1.31 (1.17-1.48)
Only following IVF	1.90 (1.41-2.54)	1.94 (1.50-2.50)
Only following ICSI	2.00 (1.30-3.20)	1.28 (1.14-1.43)

Hum Reprod 2005; 20:328-338

3. Genetic risks associated with ART

- New research
- Davies, 2012: Australian study (large groups) as well as Norwegian and Danish analyses, 2014
- Current ART outcome vs Current spontaneous but Former pregnancy ART: no difference
- Current ART outcome vs Infertility but no ART vs Fertile spontaneous: higher risk ICSI only
- **Concl: the risk is unlikely to be dependent on ART but would rather be linked to the fact of the pair's infertility problem, ovarian hyperstimulation or embryo cryopreservation**

Type of Birth Defect Increased in the ART Population?

- Gametogenesis and preimplantation are important times for epigenetic mechanisms
- Alterations in DNA structure rather than sequence

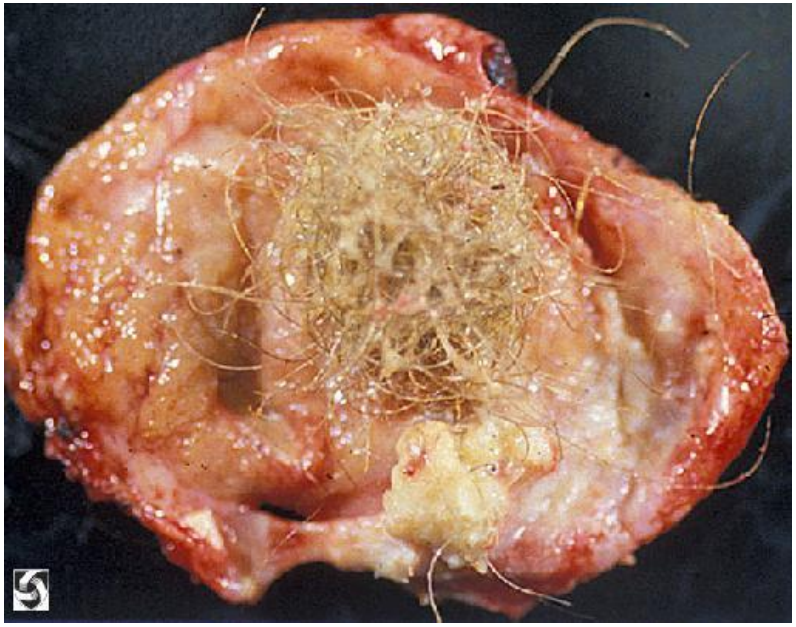
  - epigenetic states established early in development and passed intact in mitosis
- Imprinting is one type of epigenetic change

  - results in gene transcription from only one parent

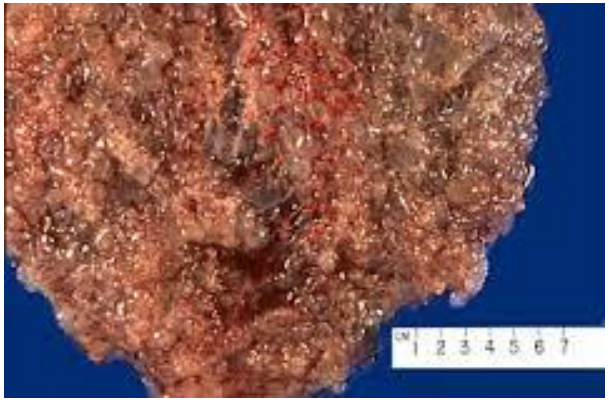
  - genes of growth and differentiation

  - forces biparental contribution to embryo and biologic diversity

Common Imprinting



Ovarian dermoid
46,XX **All maternal**



Trophoblastic disease –
complete mole
46,XX **All paternal**

ART and human epigenetic/imprinting disorders?

- Beckwith Wiedemann Syndrome - LGA, macroglossia, omphalocele

  - several causes – about 50% due to imprinting error on chromosome 15

- Six fold increase in ART among children with BWS (DeBaun, 2003)

- (Maher, 2003; Gicquel, 2003): about 5 % conceived by ART vs < 1.0% general population

Why Worry About Imprinted Genes ?

- BWS/AS following ART is rare (1/4000)

- Further roles in implantation and early embryo development

- Additional concerns regarding imprinted genes:
  - Association with cancer
    - Retinoblastoma in children from ART
  - Association with neurodevelopment

Genetic Evaluation for Infertile Couple

- Sexual differentiation disorders may present with infertility and convey specific genetic risks

  - oligoazoospermia (translocations) and poor ovarian reserve (fragile X premutation carrier)

- Some inherited disorders present with infertility with associated risks to offspring

  - cystic fibrosis and CBAVD

- Risk of congenital malformation following assisted reproduction is increased

  - imprinted genes suspected based on animal LOS studies and human studies of Beckwith-Weidemann Syndrome

The Case

- Couple returns 3 yrs later
- 37yo G3P0 three miscarriages following ART (none karyotyped)
- Balanced translocation carrier?
- What are the numbers:

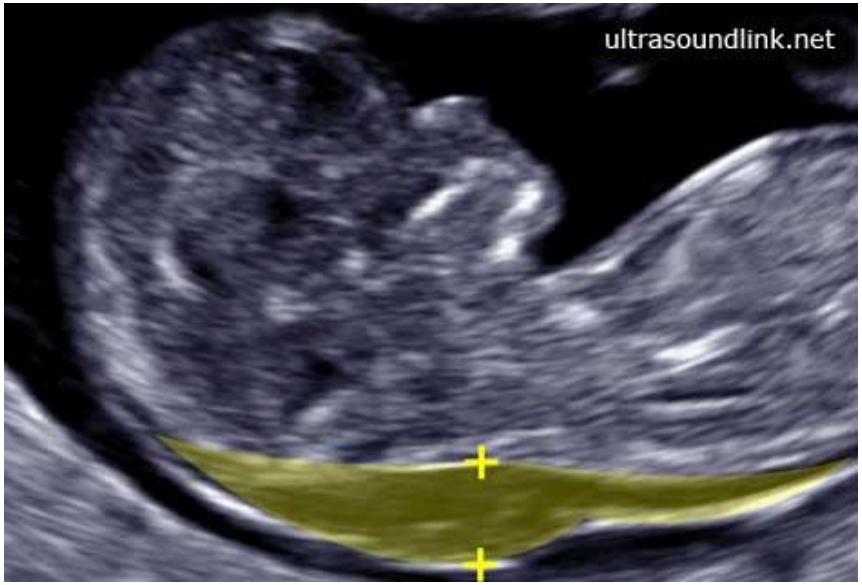
  9.2% if three miscarriages or more

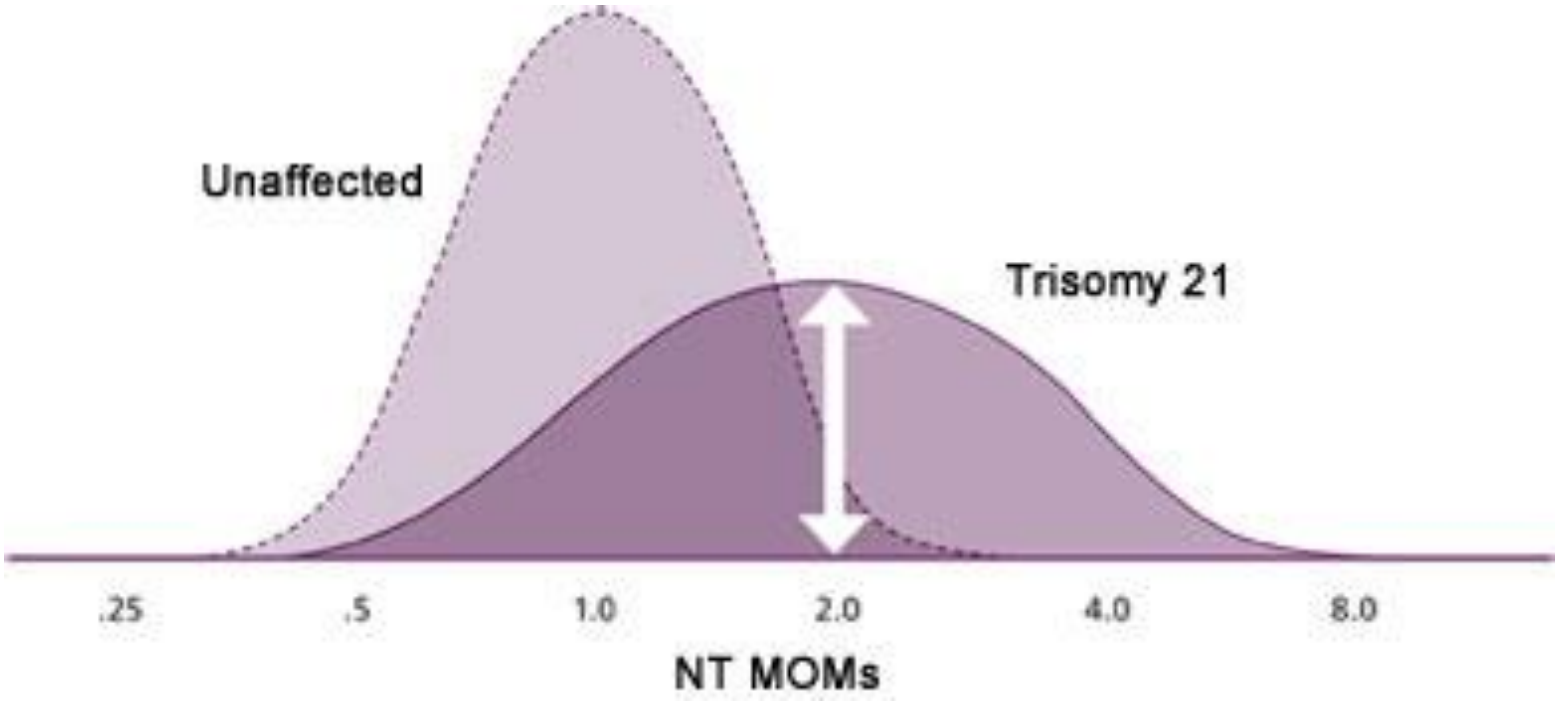
The Case

- 46, XY; 46, XX, t(5q;8q)
- Reciprocal
  10-15% abnormal conception (either parent)
- Robertsonian
  10-15% abnormal conception maternal origin vs 0-5% paternal
- Correlation with increased size of segments and rate of loss
- 1 year later, she is now 38 yo and pregnant

The Case

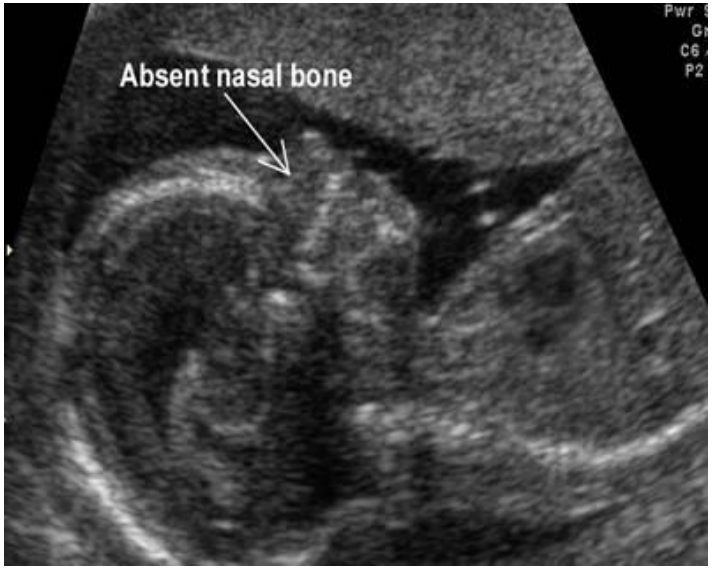
- She has her 11 week US obtained and a NL of 4.0 is detected
- How does this happen?
- Should she have the serum testing ?
- Can US look at anything else?
- What are her options for diagnostic testing?
- What other concerns are present?



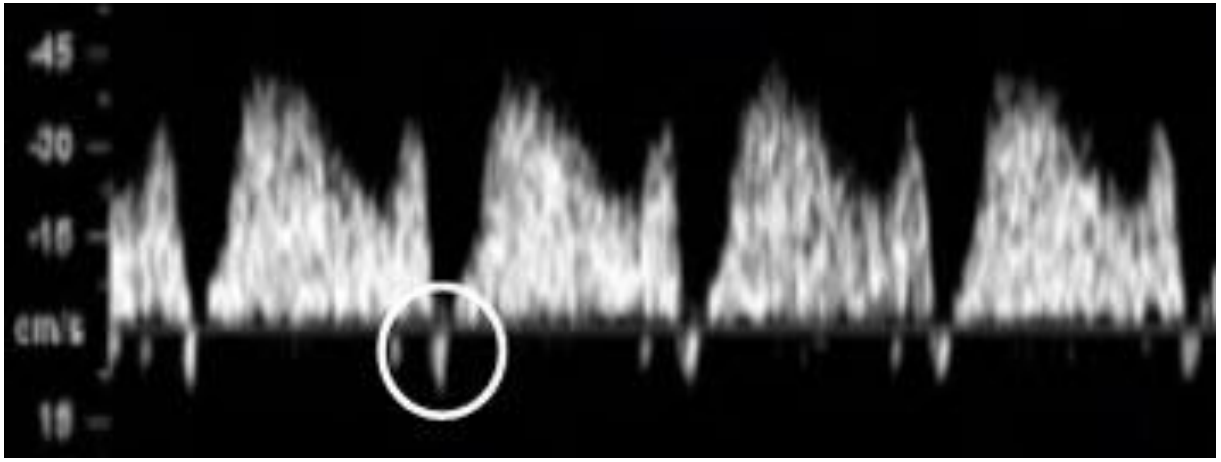

Unaffected
Trisomy 21
.25
.5
1.0
2.0
4.0
8.0
NT MOMs

Additional markers - Nasal Bone (NB) and Ductus Venosus Flow (DV)

Absent NB 7.05



DV abnormal flow 6.42



Structural Anomalies and Genetic Disorders

- Cardiac anomalies 15% risk if NL>5.5mm
- Increased venous pressures, mediastinal compression
  - congenital diaphragmatic hernia
  - narrow thorax skeletal dysplasia
- Altered extracellular matrix
  - collagen disorders (chondrodysplasias)
- Impaired fetal movement
  - fetal akenesia sydromes
- Noonan and Smith Lemli Opitz Syndromes

Do the Serum Screening or Not?

- For NL>4.0 - highly unlikely to screen negative
  - 0.09% of population
  - 33 % aneuploidy
- For NL 3.0 to 4.0 – 8% have negative serum screen result
  - 0.3% of population
  - 17 % aneuploidy

Or rather: Do the amnio- or not?

Do the NIPT?

Ultrasound Obstet Gynecol. 2015 Jan 19. doi: 10.1002/uog.14792.

Noninvasive Prenatal Testing for Trisomy 21, 18 and 13 – Clinical Experience from 146,958 Pregnancies. Zhang H et al

NIPT was performed on 146,958 samples, of which outcome data were available in 112,669 (76.7%). 3,213 cases required repeat blood sampling, 145 had no report. Aneuploidy was confirmed in 720 of 781 T21-positive cases, 167 of 218 T18-positive cases, and 22 of 67 T13-positive cases. There were 9 false negative identified, including 6 T21 and 3 T18 cases. The overall sensitivity of NIPT was 99.17% for T21, 98.24% for T18, and 100% for T13, and the specificity was 99.95% for T21, 99.95% for T18, and 99.96% for T13. There was no significant difference in test performance between 72,382 high-risk and 40,287 low-risk subjects (sensitivity 99.21% vs. 98.97%, $p = 0.82$; specificity 99.95% vs. 99.95%, $p = 0.98$). The major factors contributing to NIPT false positive and false negative results were maternal copy number variant (CNV) and fetal/placental mosaicism, but not fetal fraction.

Diagnostic choices in pregnancy

Chorionic villus sampling

Amniocentesis

Percutaneous umbilical blood sampling

Free fetal DNA

Risks involved

I MISCARRIAGE

II FETAL MORBIDITY
Rupture of membranes
Malformation
Infectious
Isoimmunization

III TECHNICAL
No sample obtained
Misdiagnosis
Malformation

Amniocentesis risks of miscarriage

1 in 200

But exact risk probably lower than 1 in 200, but unlikely as low as 1 in 1600 (randomized control trials not possible)

Focus of counseling on best estimates of loss rate, contributors, limitations of testing, accuracy of test, disease being tested

ACOG Jan 2007 removed of age 35 as a cutoff “to determine who is offered screening versus who is offered invasive testing.”

Amniocentesis – other risks

Premature rupture of membranes: 1% (91% survival rate post PROM)

Isoimmunization (special attention to isoimmunized patients and number of invasive procedures)

Hepatitis B carriers
- no increase in HepB carriers, possible increase if HBeAg+
- HIV+ patients – risk low if viral load low

Direct fetal trauma (rare with ultrasound guidance)

Chorionic Villus Sampling – other risks

Miscarriage: 0.6%-1% above standard amniocentesis risk

Limb reduction defects:
5.2 to 5.7/10,000 vs 4.8 to 5.97 general population (NSD)
- recommended CVS > 9 weeks only
- vasoconstrictive with resultant ischemia?
- immunoreactive against fetal cells with apoptosis?

Hemangiomas
3 fold increase (7.4% amniocentesis vs 21.1% CVS) confined mostly to transcervical, no relationship to sample size, gestation at sampling, bleeding

Chorionic Villus Sampling – misdiagnoses

Evaluation of solely direct or cultured cells

Maternal cell contamination

Confined placental mosaicism
- 1-2% of CVS samples
- 1/3 reflect true fetal mosaicism, 2/3 are confined to placenta
- risks of uniparental disomy
- risks of fetal growth restriction

Twins

Early Amniocentesis = before 14wks

Post-procedure loss: 4.2%

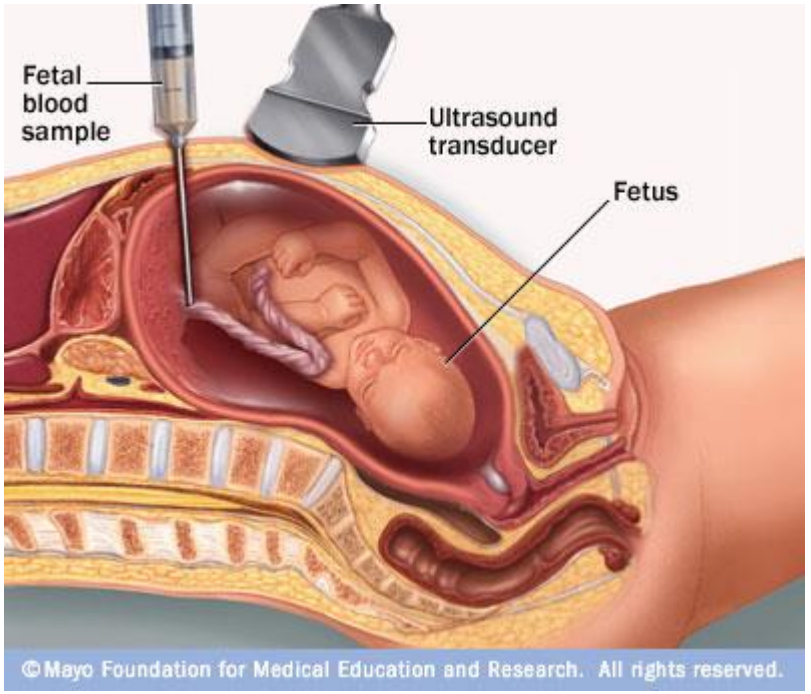
Talipes: 1%

Percutaneus Umblical Sampling (PUBS)

Ultrasound guidance, fetal umbilical vessel sampled with 22 gauge needle

Advantages:
full fetal karyotype in 48 hours
all fetal hematology and serology
utility in assessing CVS mosaicism

Disadvantages:
1-2% risk of fetal loss
later in gestation (>18 wks)



Comparison of tests

	TIMING	RISK OF LOSS	FETAL RISKS	TECHNICAL ISSUES
AMNIOCENTESIS	15WKS AND MORE	0.5%		EASIEST
EARLY AMNIOCENTESIS	14WKS AND LESS	2-3%	INCR CLUBFOOT	INCR CULTURE FAILURE
CVS	10WKS AND MORE	1%	HEMAGIOMA?	PLACENTAL MOSAICISM
PUBS	18WKS AND MORE	1-2%		HARDEST

Free fetal DNA (ffDNA) in the mother

Shorter fragments than maternal free DNA
- detection by 6 -7 weeks from LMP

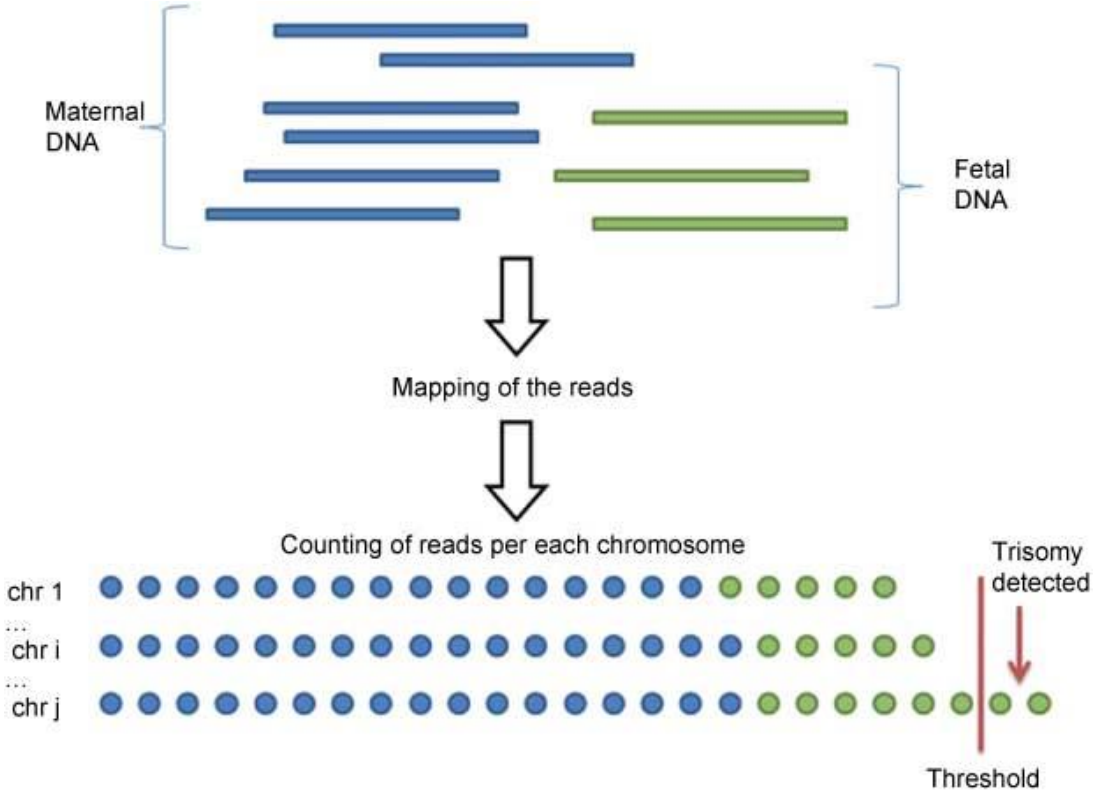
Increases with gestational age
- 3% of total free DNA in 1st trimester, 6% in 3rd trimester

Sources
- fetal circulation
- placental apoptosis
- fetal cells in maternal circulation

Cleared from maternal circulation in 2 hours

Noninvasive Diagnostics – NGS for aneuploidy

New work refocusing on massively parallel massively parallel sequencing (MPS)
"shotgun sequencing",
"next generation seq"
excess of fragments
chromosome 21 = tris 21
addition of multiplexing



The Case

- She returns with her husband two weeks later. They decided not to proceed with serum screening and present for CVS

- NL=2.0mm

- “How can this happen?” Unknown currently
- “How often does it happen?” 5/6 trisomy 21 fetuses resolve increased NT by 2nd trimester
- “Does it change her risk of aneuploidy?” NO since resolution occurs both in aneuploid and normal fetuses

The Case

- She remains concerned about a problem with the pregnancy.

- What should she do next? What concerns remain?

  Risk of a karyotype abnormality

  Abnormal second trimester ultrasound

  Risk of abnormality detected in newborn or child

Outcomes of NL>4.0mm

- Abnormal karyotype 50%
- Normal karyotype 50%:

  - 25% abn 2nd trimester fetal US (cardiac and other anomalies)

  - fetal demise

Always send for fetal echocardiogram!

US in 2nd trimester

- Structural anomalies
  - risk of aneuploidy greatest for multiple anomalies (18.8 %)
  - smaller but present risk for isolated anomalies (9.3 %)

- Issue of an “isolated” ultrasound anomaly – minor features and dysmorphia difficult to appreciate

US in 2nd trimester – soft markers

- Individually seen in 1-5% of normal fetuses
- Isolated - increases risk of aneuploidy 1.5 to 3 fold
- >2 present - increases risk 10 fold or more “soft signs”

  nuchal fold

  echogenic bowel

  echogenic cardiac focus

  shortened femur/humerus

  renal fullness

  choroid plexus cysts (trisomy 18)

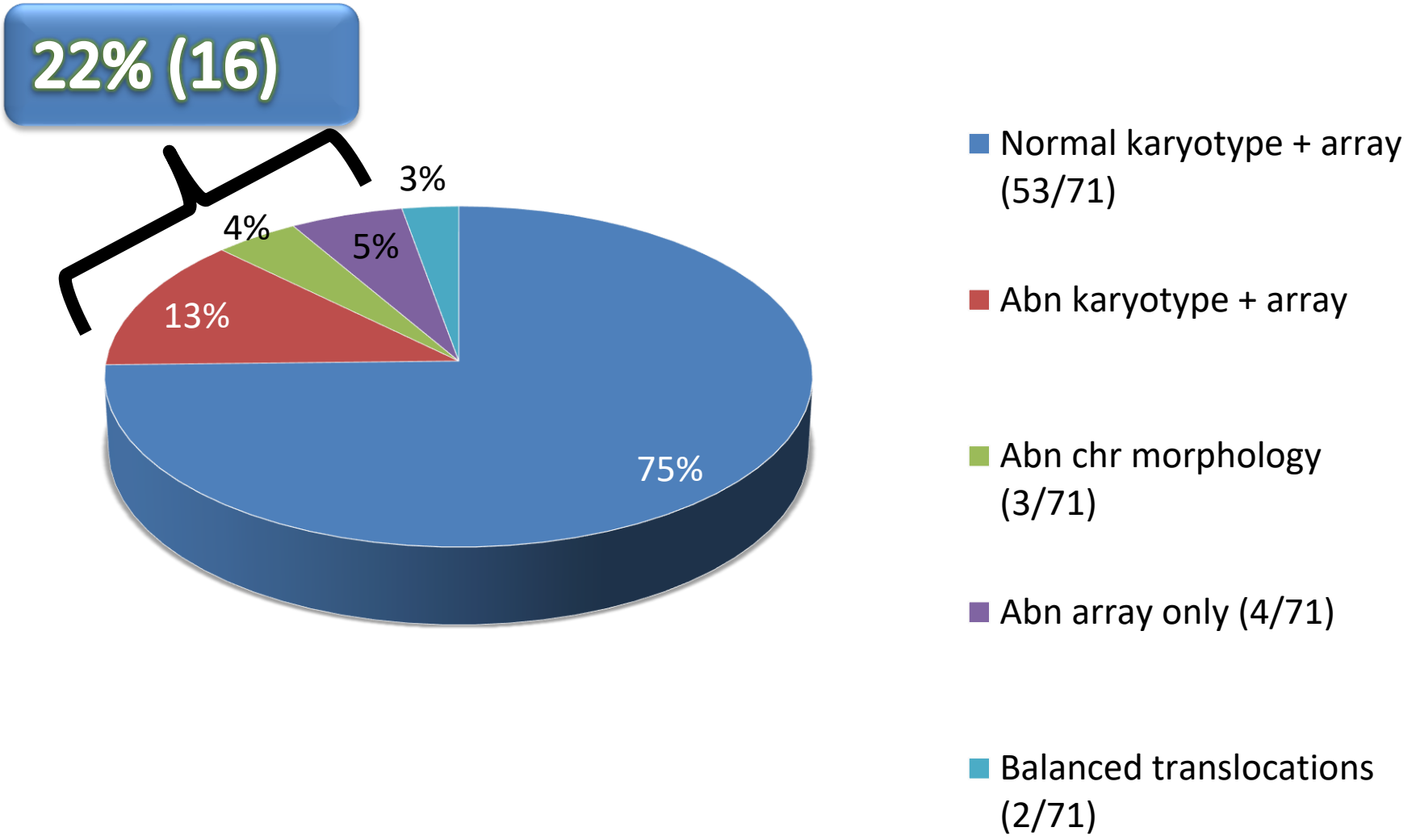
Array CGH in prenatal diagnostics

Table 1. Recent Estimates of Detectable Pathogenic CNVs in High- and Low-Risk Pregnancies.

Indication	Lee *et al.*, 2012		Armengol *et al.*, 2012		Other Studies [a]		Fiorentino *et al.*, 2013		Wapner *et al.*, 2012		Combined	
	(n = 3171)		(n = 906)		(n = 1000)		(n = 3000)		(n = 3822)		(n = 11899)	
	# Samples	# Pathog-enic CNVs (%)	# Samples	# Pathoge nic CNVs (%)	# Samples	# Pathog-enic CNVs (%)	# Samples	# Pathog-enic CNVs (%)	# Samples	# Pathog-enic CNVs (%)	# Samples	# Pathog-enic CNVs (%)
All High Risk Pregnancies (Primarily abn US)	245	19 (7.8)	173	6 (3.5)	507	13 (2.6)	120	7 (5.8)	755	45 (6.0)	1800	90 (5.0)
All Low Risk Pregnancies	2926	15 (0.5)	733	8 (1.1)	493	5 (1.0)	2880	17 (0.6)	3067	50 (1.6)	10099	95 (0.9)
Low Risk Subgroups												
AMA	1911	10 (0.5)	273	3 (1.1)	253	1 (0.4)	1118	6 (0.5)	1966	33 (1.7)	5521	53 (1.0)
Parental Anxiety	989	5 (0.5)	60	1 (1.7)	93	-	1675	11 (0.7)	372	5 (1.3)	3189	23 (0.7)
Abn Serum Screen	26	-	235	1 (0.4)	11	1 (9.1)	29	-	729	12 (1.6)	1030	14 (1.4)
Total	3171	34 (1.1)	906	14 (1.5)	1000	18 (1.8)	3000	24 (0.8)	3822	95 (2.5)	11899	185 (1.6)

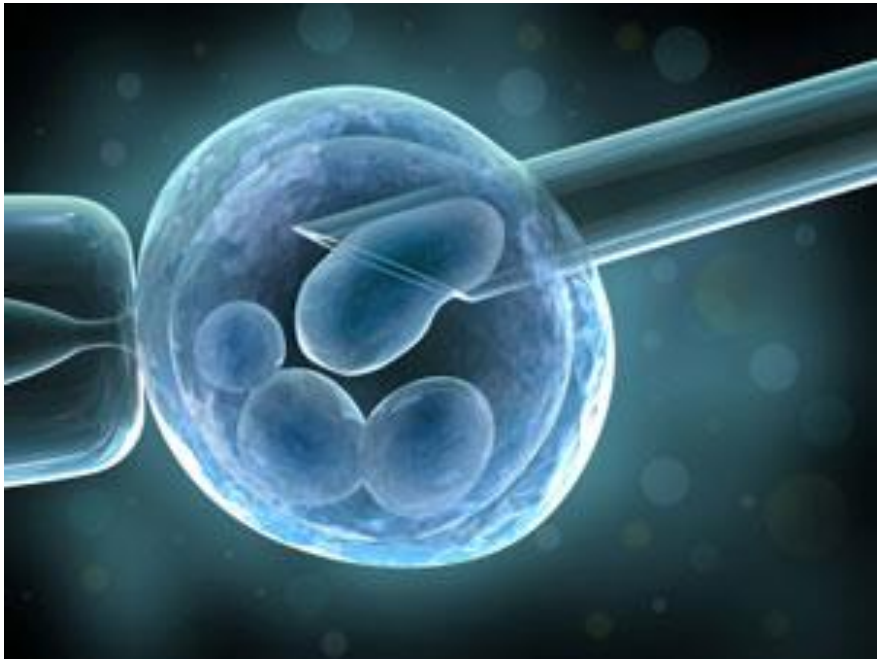
Note: a, compiled by Fiorentino *et al.*, 2013; including Coppinger *et al.*, 2009; Shaffer *et al.*, 2008; Maya *et al.*, 2010; Van den Veyver *et al.*, 2009; Sahoo *et al.*, 2006.

aCGH results - example



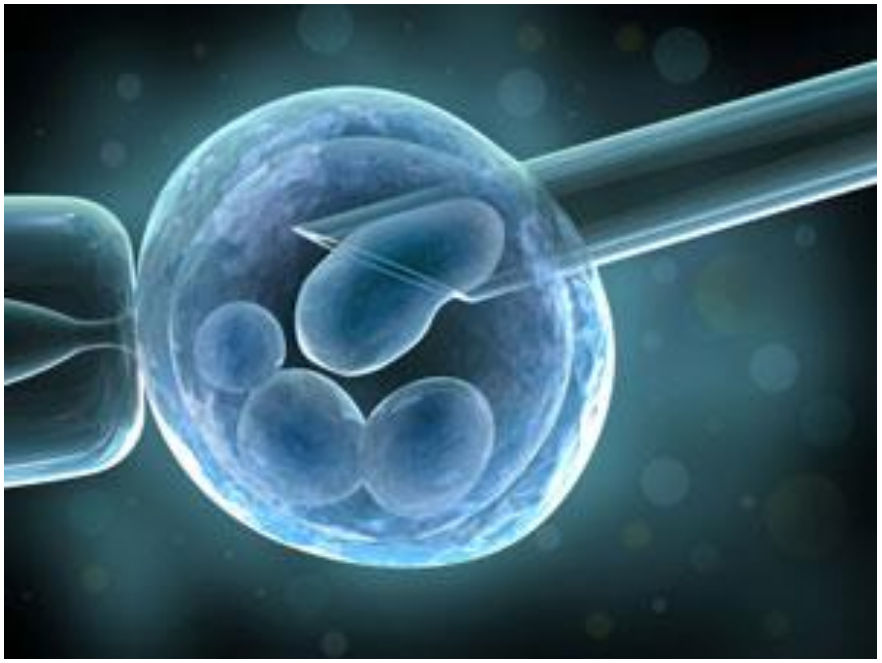
The Case

- The couple deliver a healthy son.
- They return to the geneticist two years later with about preimplantation genetic testing (PGD)



Preimplantation Genetic Diagnosis

- Screening modality that decreases the chances of transferring an "affected" embryo
- Screens only for disorder in question
- Effects of biopsy
- Possible decrease in implantation rate?



Preimplantation Genetic Diagnosis

- PGD for chromosome abnormalities
- Translocation carriers
- No longer advocated for AMA or habitual
- Miscarriage - role for aCGH?
- “More is not better”
- Increased number of probes increases the technical risk and risk of “false positive” biopsy

IVF – ways of „bypassing” the genetic problem

- Triparental embryos
- Oocyte (or sperm) adoption

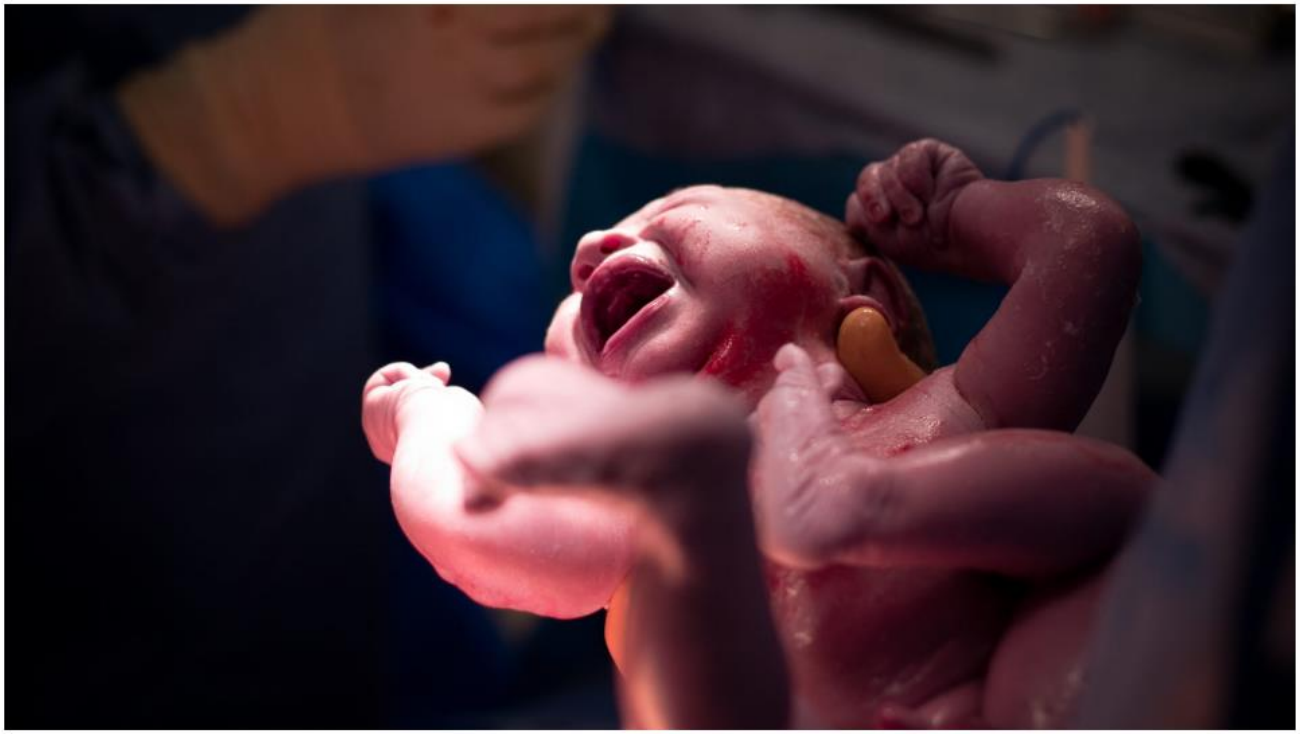
IVF – triparental embryos

IN THE LAB

World's first baby born with novel three-parent embryo technique

By Andrew Joseph Sept. 27, 2016

Reprints

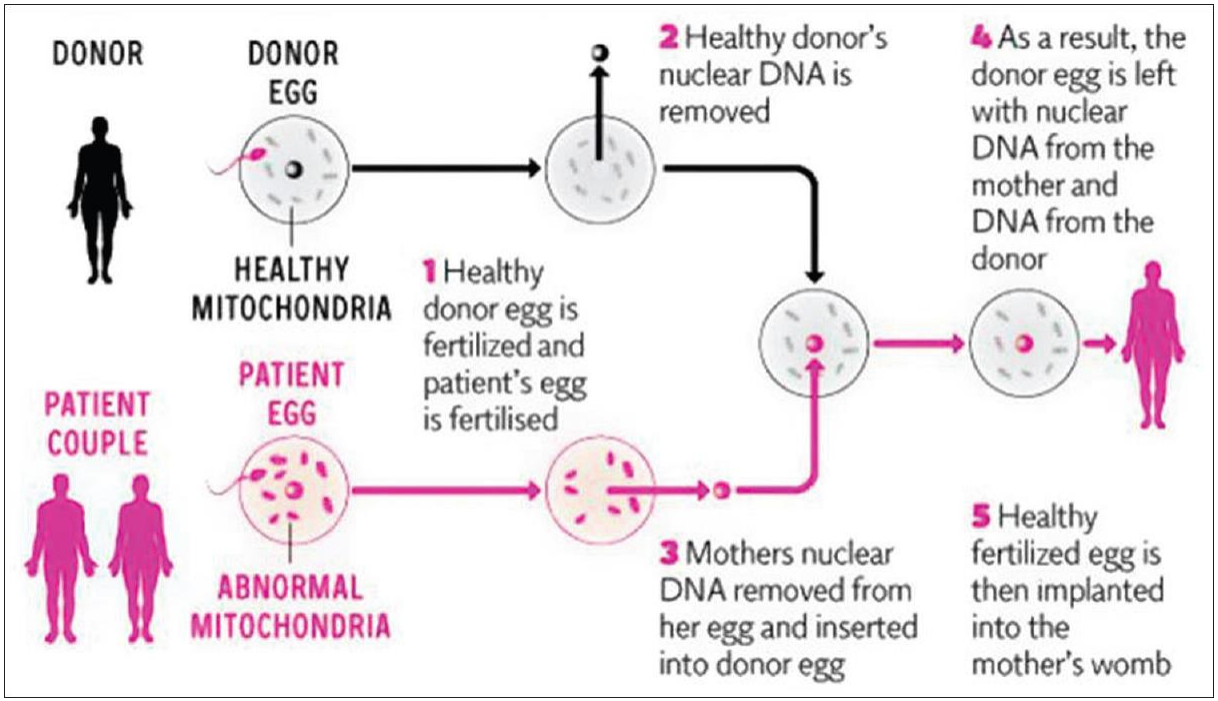


APSTOCK

IVF – triparental embryos

- Mitochondrial replacement therapy (MRT)

1. Removing nucleus from an oocyte of a healthy woman
2. Introducing nuclear DNA of a woman with mtDNA mutation into this oocyte
3. Fertilizing this oocyte w/sperm



IVF – oocyte adoption

- When do we use it?

1. A woman is a carrier of mtDNA mutation
2. A woman is a carrier of X-linked (autosomal dominant?) mutation
3. Loss of fertility due to radiation treatment of cancer
4. High FSH, low estradiol and very low AMH