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State-of-the-art methods in postconceptional diagnostic of genetic disease prior to implantation

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Importance of postconceptional diagnostic

- Approximately 3% to 5% of pregnancies are complicated by birth defects or genetic disorders [PMID18185492];
- Chromosomal abnormalities are present in approximately 1 in 150 live births [PMID 28499534];
- congenital malformations remain the leading cause of infant death and a leading cause of childhood death [PMID22291121]. Congenital heart defects (CHDs) are among the most common birth defects, affecting approximately 1 in 100 births [PMID: 31270117, PMID: 21321151, PMID: 18657826]

Incidence of common aneuploidies

Trisomy 21	1 in 800 live births
Trisomy 18	1 in 7500 live births
Trisomy 13	1 in 15,000 live births
Monosomy X (Turner syndrome)	1 in 5000 girls
Trisomy X	1 in 1000 girls
XXY (Klinefelter syndrome)	1 in 1000 boys
XYY	1 in 1000 boys

Data from Nussbaum RL, McInnes RR, Willard HF. Thompson & Thompson genetics in medicine. 7th edition. Philadelphia: Saunders/Elsevier; 2007.

Prenatal detection of diseases depends on several factors

- Technology (the availability of adequate equipment);
- Training the medical staff in the newest tehnologies;
- National screening policies;
- Access to prenatal screening services (in terms of location and costs)

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Postconceptional diagnostic of genetic disease after implantation

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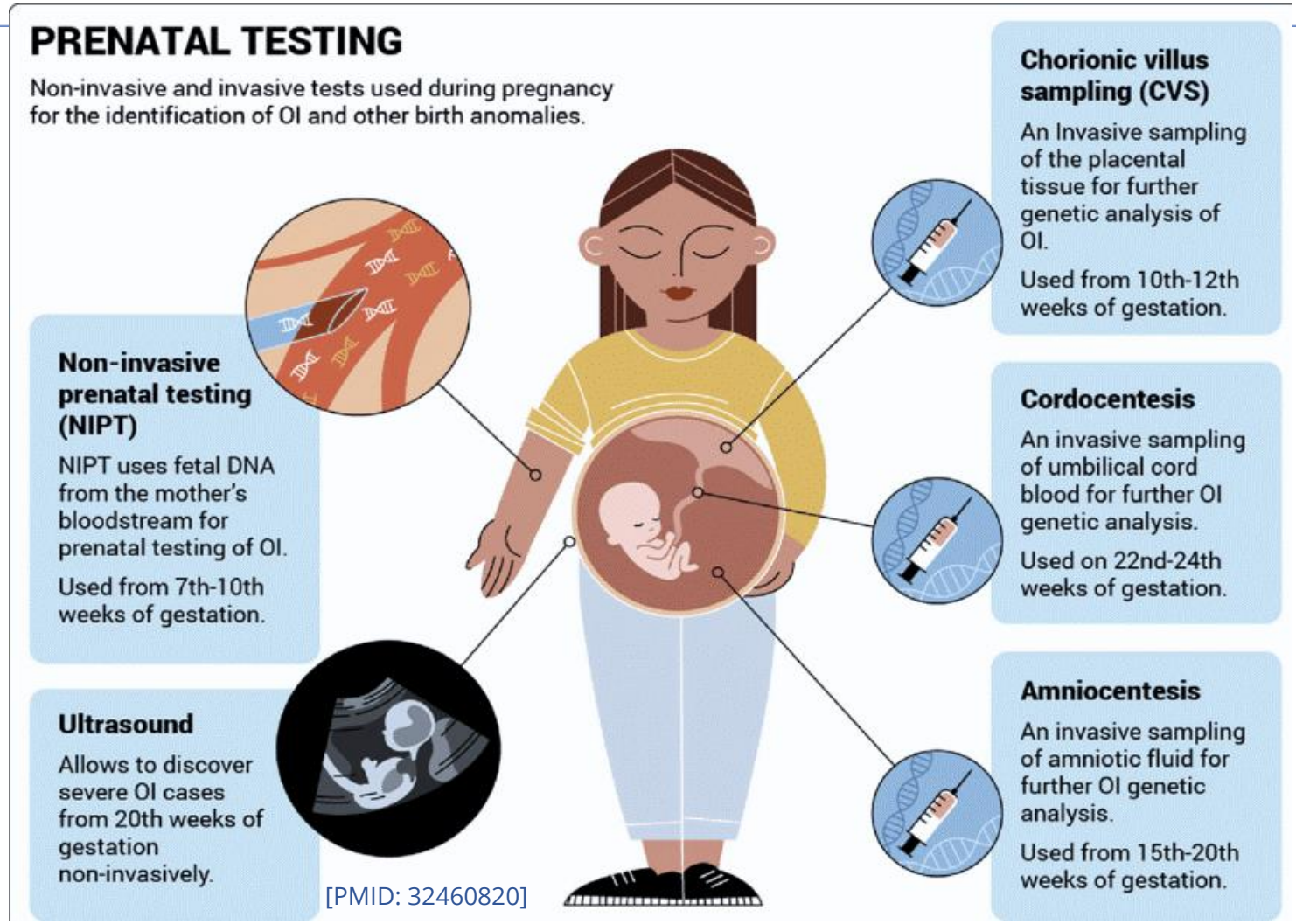
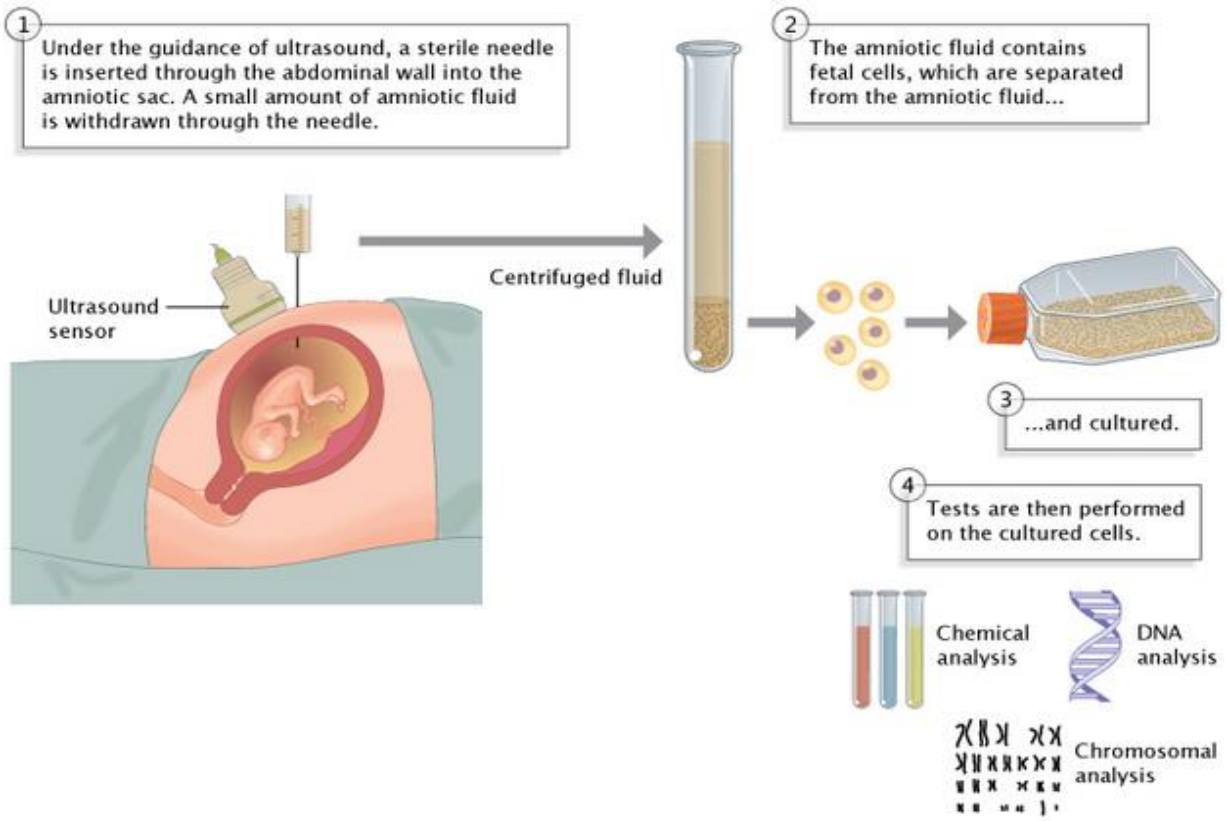


[PMID: 32460820]



- Aneuploidies
- Karyotype
- Chromosomal translocations
- Fluorescence in situ hybridization (FISH)
- DNA analysis

Major disadvantage is long time (2-3 weeks) until results are available and if result are negative a pregnancy termination must be performed

Cell-free fetal DNA

- Noninvasive prenatal screening is available from 2011 based on maternal serum, from which cell-free fragments of DNA from the pregnancy are isolated. (performed at 10 weeks' gestation)

- Cell-free DNA is then evaluated by one of 2 techniques
  - massive parallel shotgun sequencing, targeted massive parallel sequencing
  - interrogation of single nucleotide polymorphisms.

- Results are typically reported with aneuploidy detected or no aneuploidy detected or as higher low-risk for aneuploidy and with sex chromosome information if desired

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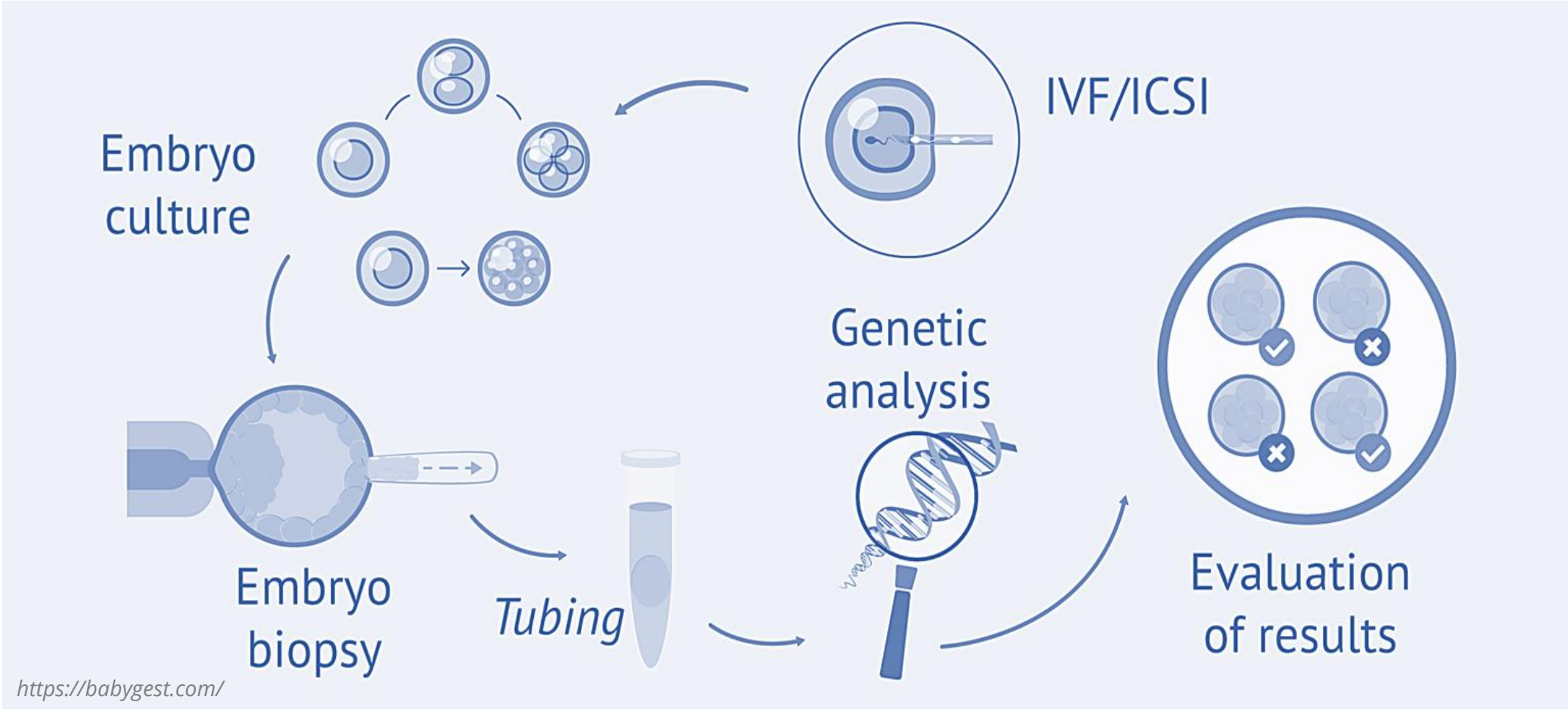
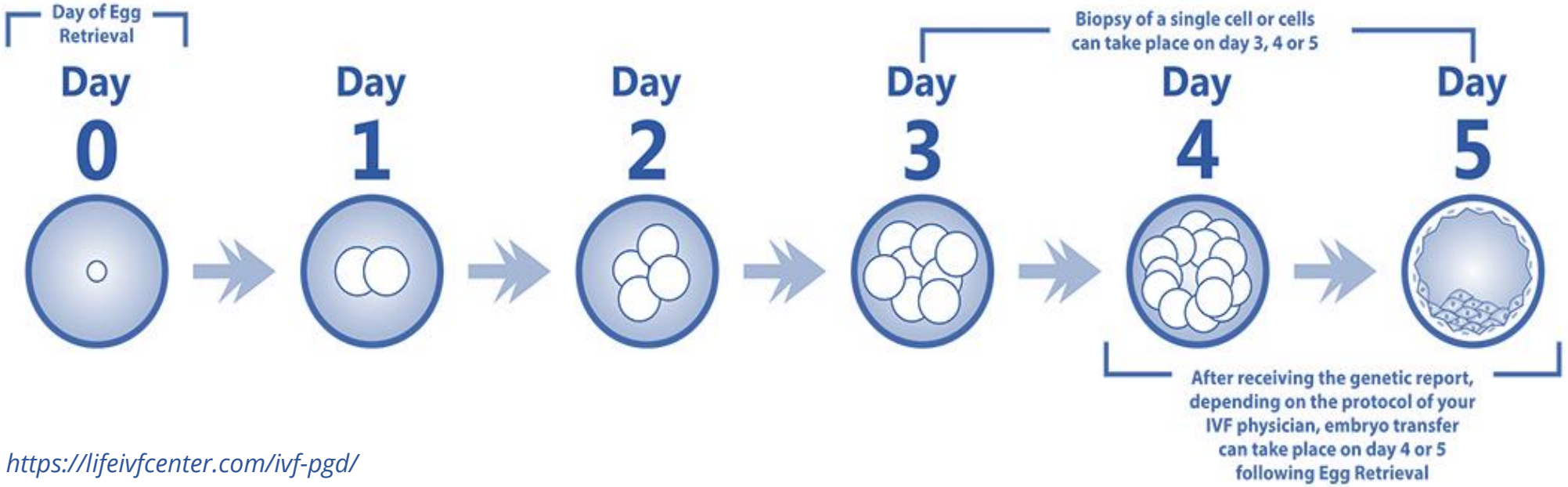


https://babygest.com/



https://lifeivfcenter.com/ivf-pgd/

Preimplantation Genetic Screening (PGS)

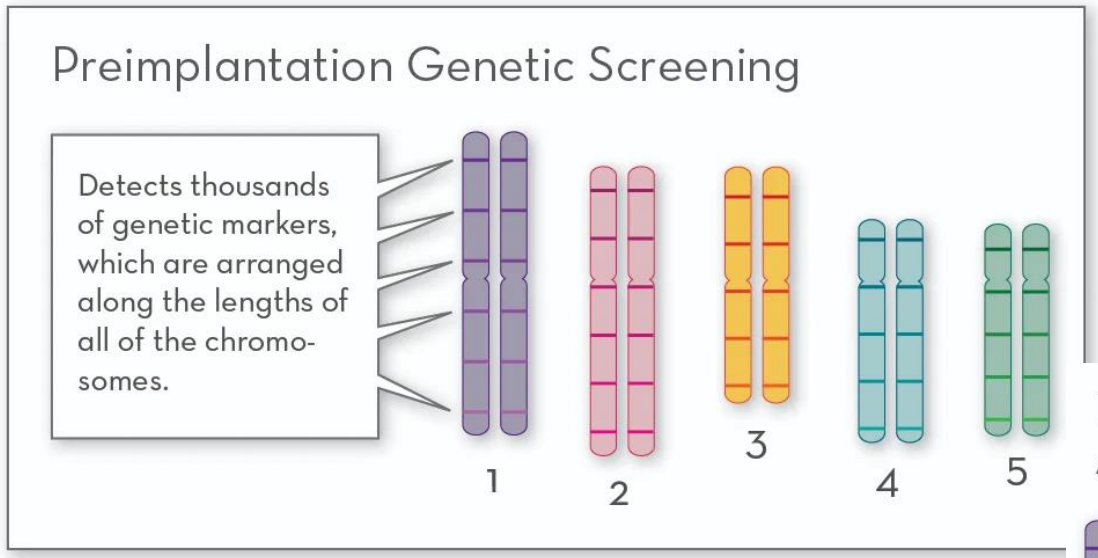
PGS, preimplantation genetic screening, refers to removing one or more cells from an in vitro fertilization embryo to test for chromosomal normalcy.

Several studies have shown that overall about 50% of human preimplantation embryos from IVF are chromosomally abnormal. Most recent studies prove that PGS increases the chance for getting pregnant in 55-70% of cases.

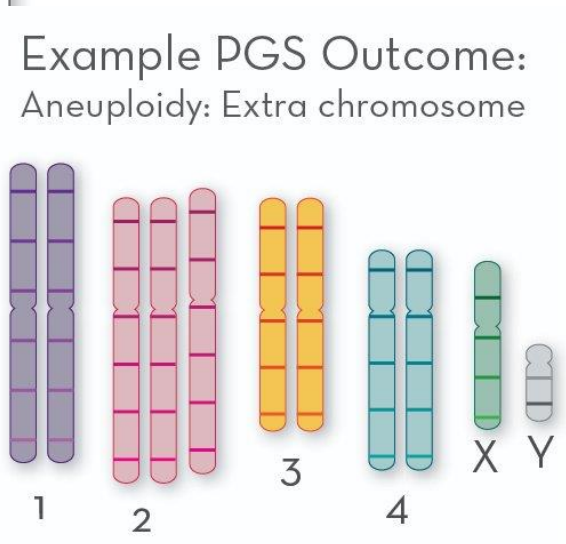
There are currently 4 technologies that can be utilized for assessment of normality of all 23 chromosomes:

- Quantitative real time polymerase chain reaction (qPCR)
- Array Comparative Genomic Hybridization (aCGH)
- Single nucleotide polymorphism microarrays (SNP)
- Next Generation Sequencing (NGS)

Quantitative real time polymerase chain reaction (qPCR)



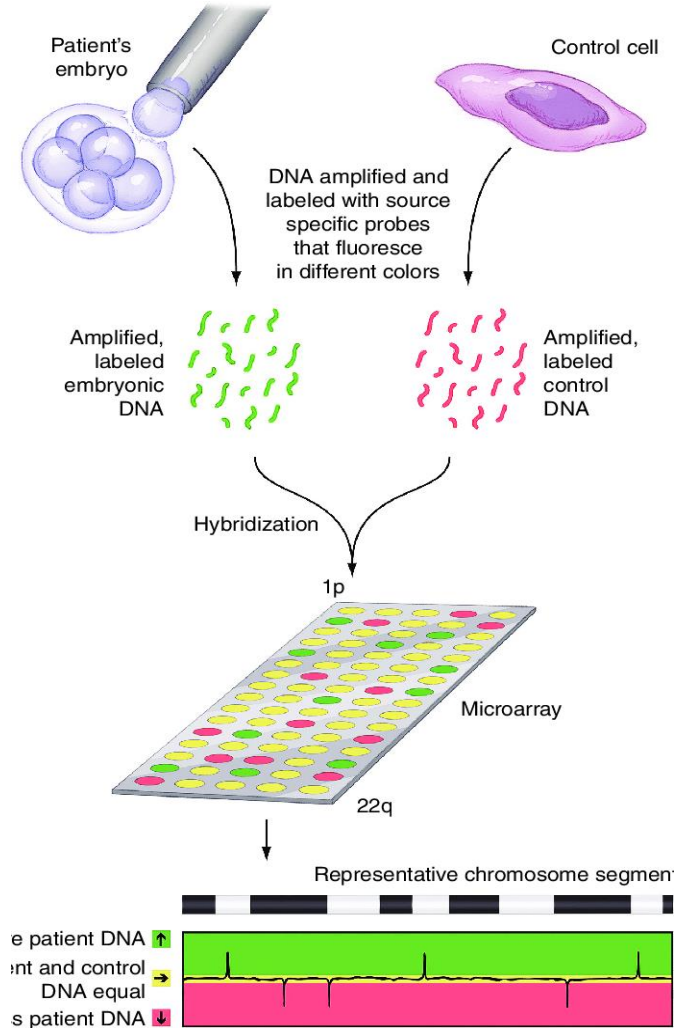
It detects the presence, absence, and copy number for large genetic regions that can include many genes



Marker	Copies	Marker	Copies
Chr 1	2	Chr 3	2
	2	(cont.)	2
	2		2
	2	Chr 4	2
	2		2
	2		2
Chr 2	3		2
	3		2
	3	X Chr	1
	3		1
	3		1
	3		1
Chr 3	2	Y Chr	1
	2		1

https://learn.genetics.utah.edu/

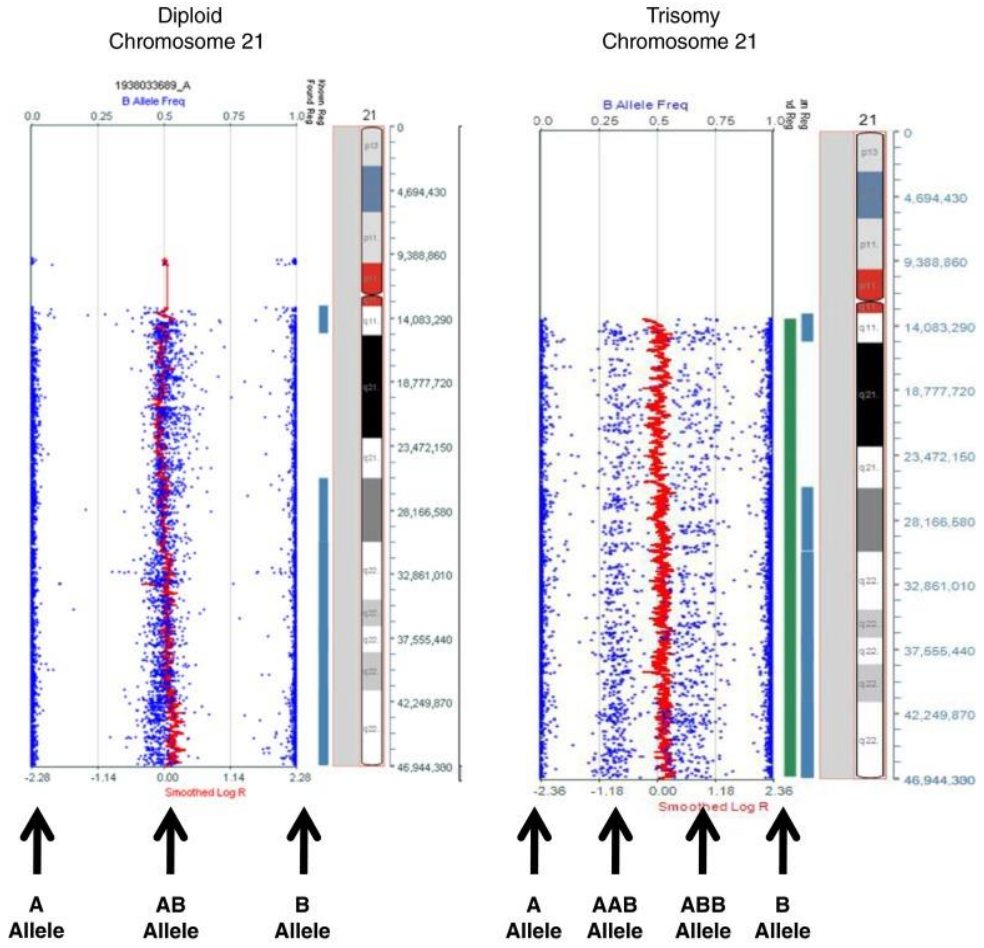
Array Comparative Genomic Hybridization (aCGH)



- For complete genomic hybridization, these spots represent the entire genome, from the beginning of the short arm of chromosome 1 (1p) to the end of the long arm of chromosome 22 plus the X and Y chromosomes
- In aCGH, DNAs from a single test cell (e.g., a blastomere) and from a control cell are amplified and labeled with colored fluorescent probes;
- Imbalances in the amounts of DNA will be seen as red or green Areas of balance and imbalance can be mapped across the complete genome.

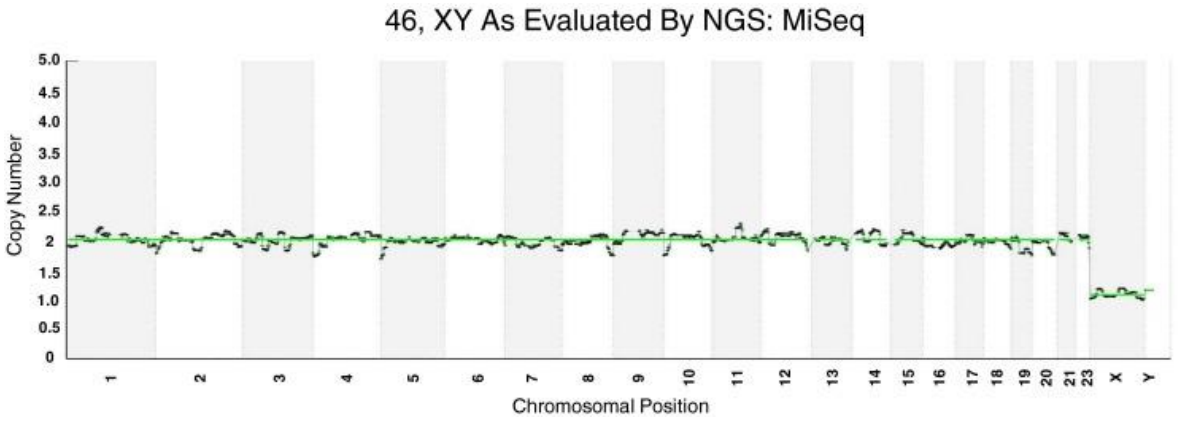
[PMID: 25659378]

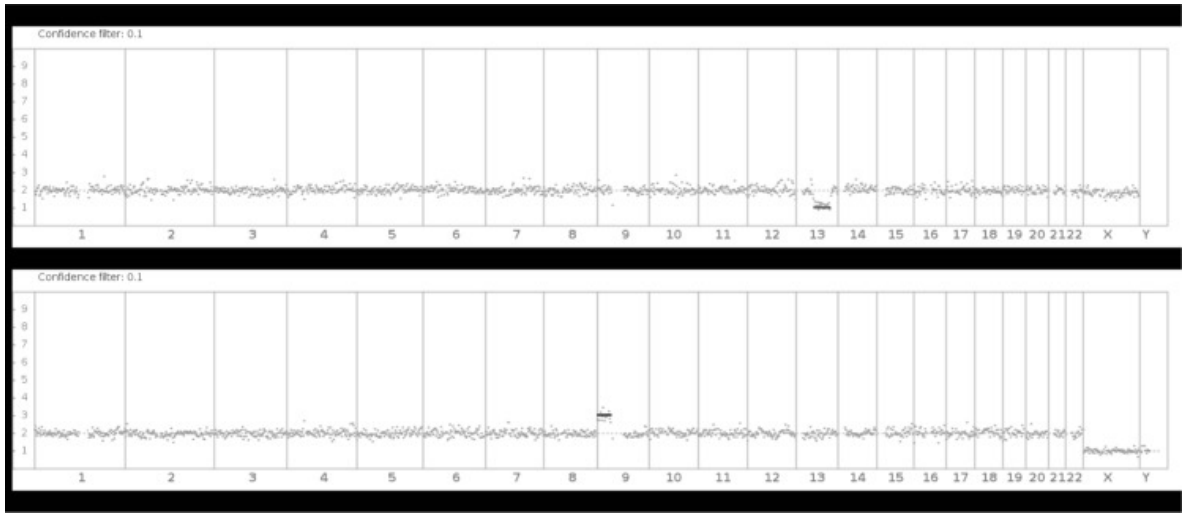
Single nucleotide polymorphism microarrays (SNP)



- SNPs (pronounced snips) are pairs of single nucleotides (A, T, C, or G) in genomic DNA that are highly variable within a given species
- SNP microarrays in PGS typically evaluate approximately 300,000 SNPs spaced throughout the genome
- SNP arrays provide a genotype (AA, AB, or BB) for each sample analyzed and compare these results to a human hap map reference genome.
- One limitation of the SNP arrays used for PGS by some reference laboratories is the inability of their algorithm to identify copy number when husband and wife are related (consanguinity).

Next Generation Sequencing (NGS) &PGS





46, XX, del(13q) and 46, XX, dup(9p) as evaluated by NGS: PGM.

Cell-Free Human Embryo Aneuploidy Testing (miPGT-A) Utilizing Combined Spent Embryo Culture Medium and Blastocoel Fluid –Towards Development of a Clinical Assay [PMID: 32350403]

Two platforms exist currently

1. **MiSeq from Illumina**
- whole chromosome aneuploidy
- not be used for detecting any structural chromosome abnormalities

2. **Personal Genome Machine (PGM) from Thermo-Fisher Scientific**

- identify whole chromosome aneuploidy, large dels or dups, and clinically significant dels or dups down to a resolution of approximately 800 kb to 1 Mb

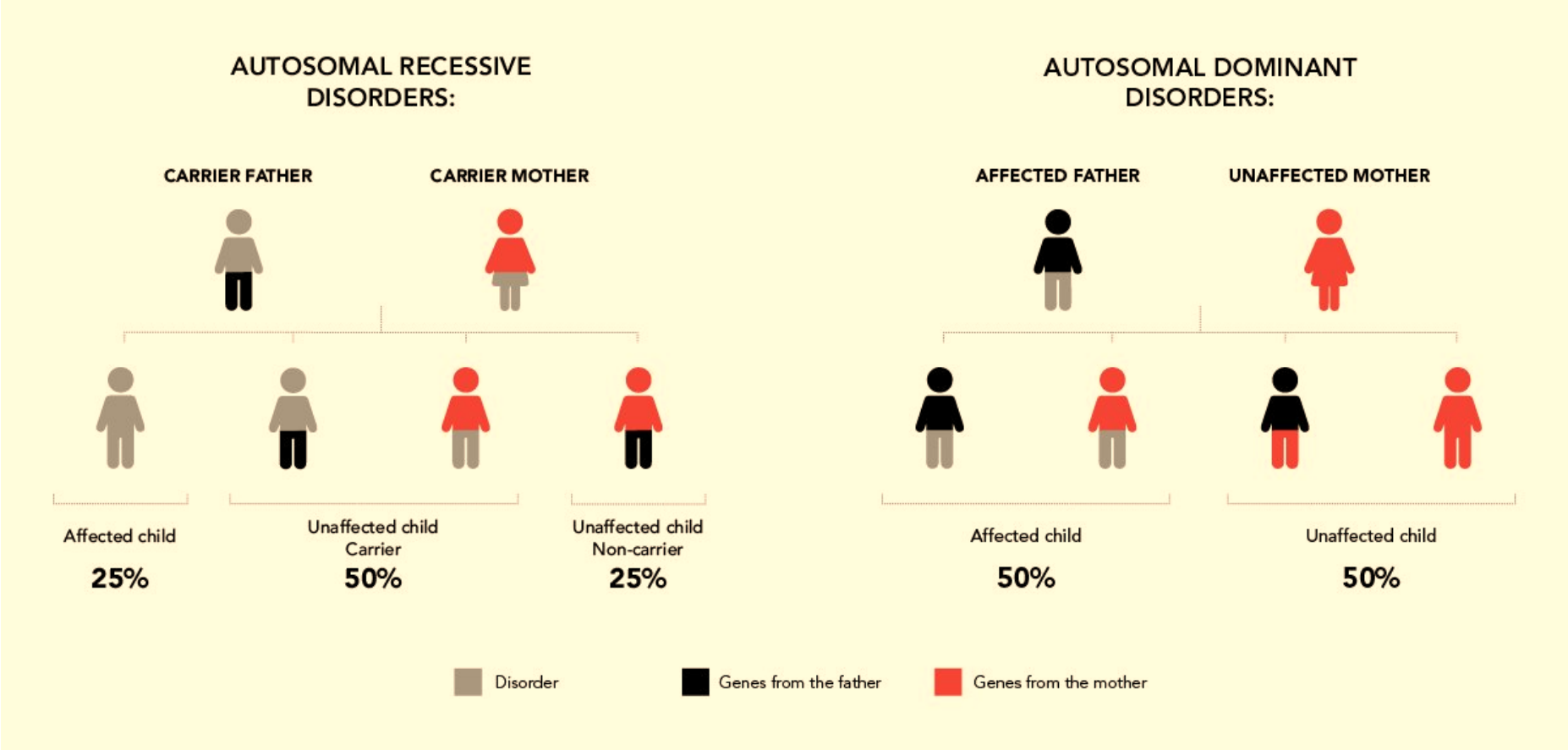
Preimplantation genetic diagnosis (PGD)

Technique that allows couples, known to be carriers or affected with an inherited condition, to avoid passing on that disorder to their offspring. It allows screening embryos for the disorder before implantation in the womb (uterus).

Performed after in vitro fertilization (IVF) by manipulation of the embryo to either remove a polar body or to remove a single cell from the blastocyst;

Refers specifically to when one or both genetic parents has a known genetic abnormality and testing is performed on an embryo to determine if it also carries a genetic abnormality

PRE-IMPLANTATION GENETIC TESTING FOR DETECTING MONOGENIC DISORDERS



Indications and applications

Monogenic disorders - PGD is available for a large number of monogenic disorders—that is, disorders due to a single gene only (autosomal recessive, autosomal dominant or X-linked)—or of chromosomal structural aberrations (such as a balanced translocation).

Pregnancy chances- Preimplantation genetic profiling (PGP) has been suggested as a method to determine embryo quality in in vitro fertilization, in order to select an embryo that appears to have the greatest chances for successful pregnancy. PGP has inherent limitations as the tested cell may not be representative of the embryo because of mosaicism. Technical drawbacks, such as the invasiveness of the biopsy, and chromosomal mosaicism are the major underlying factors for inefficacy of PGP.

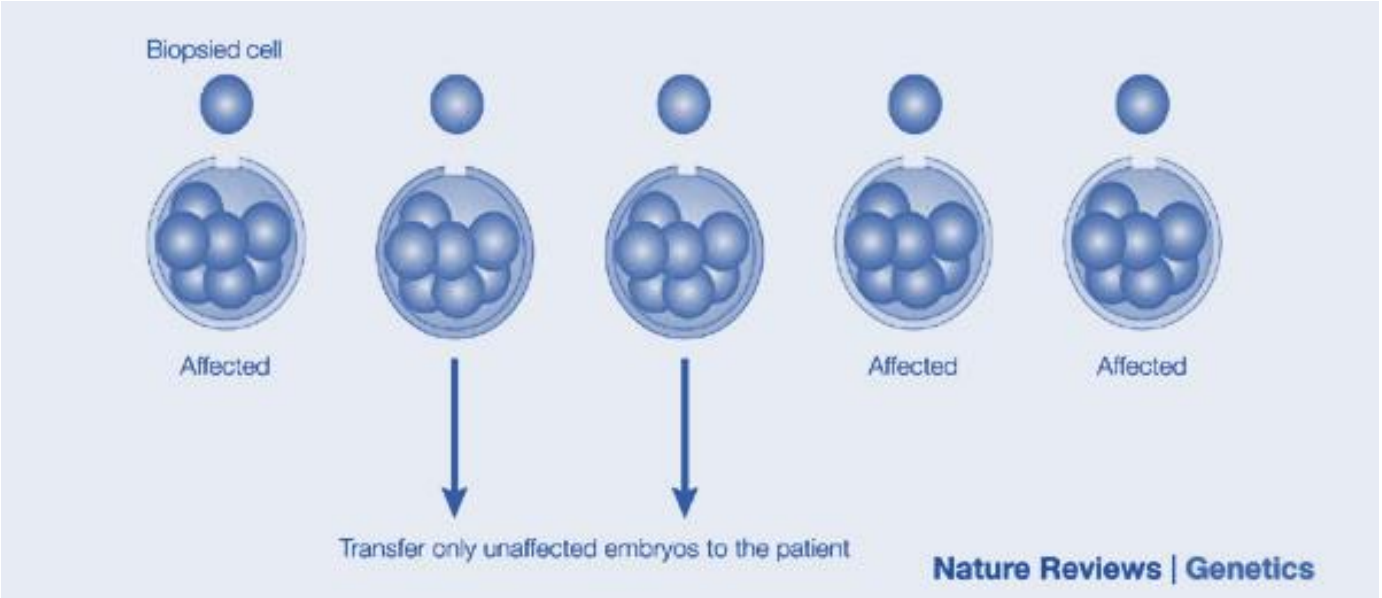
HLA matching - Human leukocyte antigen (HLA) typing of embryos, so that the child's HLA matches a sick sibling, availing for cord-blood stem cell donation. The main ethical argument against is the possible exploitation of the child, although some authors maintain that the Kantian imperative is not breached since the future donor child will not only be a donor but also a loved individual within the family.

Cancer predisposition - Diagnose late-onset diseases and (cancer) predisposition syndromes. Since affected individuals remain healthy until the onset of the disease, frequently in the fourth decade of life, there is debate on whether or not PGD is appropriate in these cases. Considerations include the high probability of developing the disorders and the potential for cures. For example, in predisposition syndromes, such as BRCA mutations which predispose the individual to breast cancer, the outcomes are unclear.

Sex discernment - In the case of families at risk for X-linked diseases, patients are provided with a single PGD assay of gender identification. Gender selection offers a solution to individuals with X-linked diseases who are in the process of getting pregnant.

Diseases most commonly diagnosed with PGD [PMID: 31317806]

1. Cystic fibrosis
2. Huntington disease
3. b-Thalassaemia
4. Sickle cell disease
5. Fragile-X syndrome
6. Myotonic dystrophy
7. Spinal muscular atrophy
8. Duchenne muscular dystrophy
9. Haemophilia A
10. Familial adenomatous polyposis
11. Dravet syndrome
12. Early onset Alzheimer's
13. Hurler syndrome
14. Becker muscular dystrophy
15. Nail patella syndrome
16. Charcot–Marie–Tooth disease
17. Myotonic dystrophy



Thank you for your attention!

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