# PGT IN ASSISTED REPRODUCTIVE 'TECHNOLOGY

NOVEMBER 2021

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# "GENETIC TESTING

Chromosome Numbers

Chromosome Structures

Single Gene Disorders

# CHROMOSOME NUMBER

FISH

aCGH

NGS

# 'STRUCTURAL ABNORMALITIES

FISH

aCGH

NGS

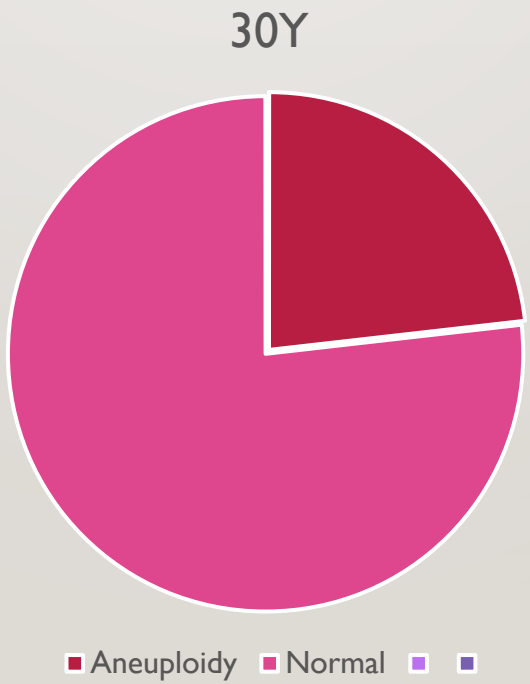
# SINGLE GENE 'DISORDERS

PCR

SNP?

NGS

# THE RISK OF OOCYTE ANEUPLOIDY "INCREASES WITH MATERNAL AGE



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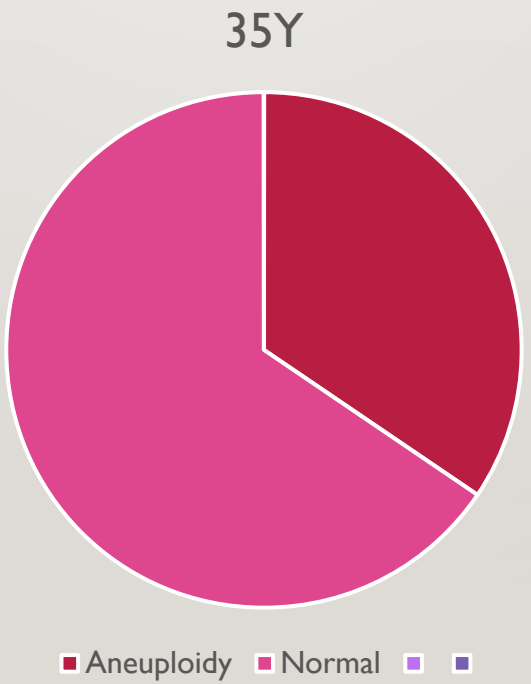

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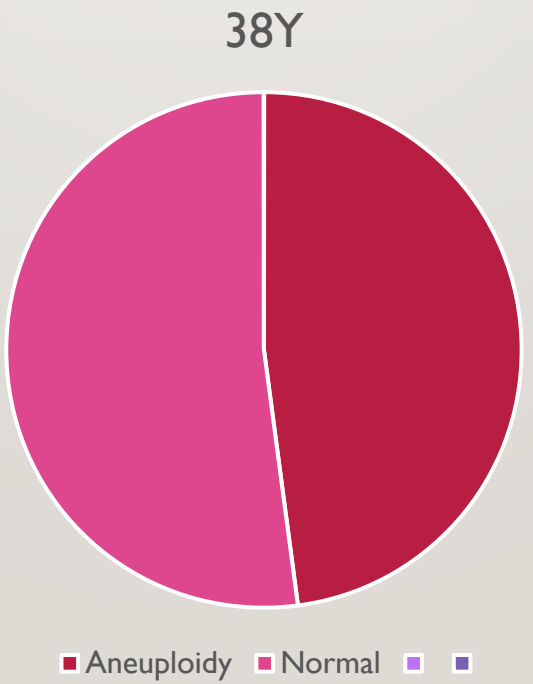

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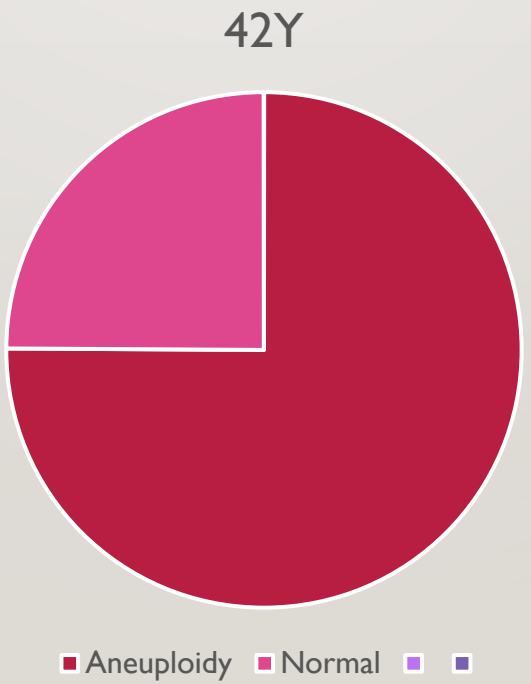

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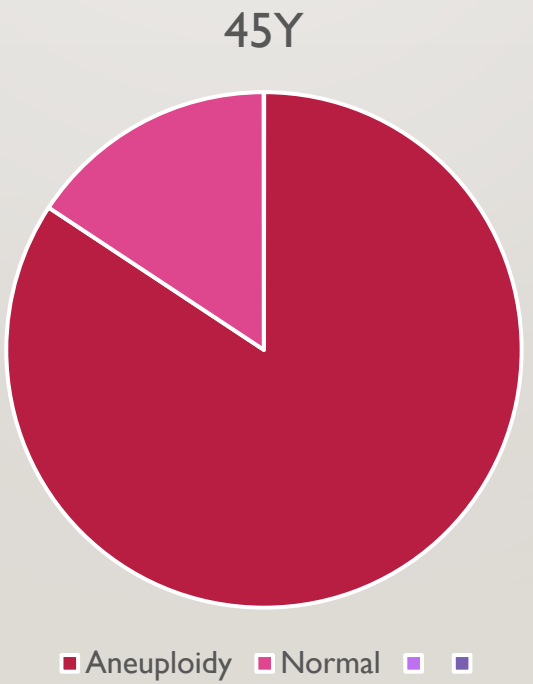

- Aneuploidy is most frequently observed for chromosomes 13, 15, 16, 18, 19, 21, and 22,

- 3%–5% of sperms  are aneuploid
- Infertile men: Chromosomes 21, 22, X, and Y more aneuploid
- In embryos, aneuploidy is paternally derived in up to 5%–10% of autosome aneuploidies and 5%–100% of sex chromosome aneuploidies

'PGT TYPES:

PGT-A

PGT-M

PGT-SR

**Potential indications for PGT-A, PGT-M, and/or PGT-SR.**

| Indications for PGT | PGT-A | PGT-M | PGT-SR |
|---|---|---|---|
| Advanced maternal age | X | | |
| Recurrent pregnancy loss | X | | |
| Repeated IVF failure | X | | |
| Family history of lethal X-linked disorder in males | | X | |
| Known alteration in an X-linked gene | | X | |
| Carriers of autosomal dominant or recessive conditions | | X | |
| Family history of gene defect for late-onset autosomal dominant disorder | | X | |
| Parent with Robertsonian translocation | | | X |
| Parent with other translocation/ structural rearrangement | | | X |
| Parent with inversion | | | X |
| Parent with deletion | | | X |
| Parent with duplication | | | X |

*Note:* PGT-M and PGT-SR are usually done in conjunction with PGT-A. IVF = in vitro fertilization; PGT-A = preimplantation genetic testing for aneuploidy; PGT-M = PGT for monogenic/single-gene abnormalities; PGT-SR = PGT for chromosomal structural rearrangements.

Harris. PGT: review of current modalities. *Fertil Steril Rev* 2021

# TESTING CHARACTERISTICS : FISH VS ACGH VS 'QPCR VS NGS

- aCGH detected 42% more abnormalities and 13% more abnormal embryos than FISH and had an error rate of only 1.9%
- aCGH and qPCR showed a sensitivity of 98.2% and 98.8%, between the two methods, respectively, and a specificity of 99.9% and 99.6%, respectively, with the use of qPCR compared with aCGH
- NGS has been shown to be the most accurate method with sensitivity and specificity of 100%

# 'WHY IS THE ERROR RATE IMPORTANT?

- False-positive results can lead to the discarding of normal embryos, which can significantly affect pregnancy outcomes
- The false-positive rate of aCGH is estimated to be 9%
- Tfalse-positive rate associated with SNP array is similar at ~10%
- The false-positive and false-negative rates of NGS are both 0%

## TABLE 2

**Comparison of testing characteristics and detectable abnormalities of each preimplantation genetic testing technique.**

| Technique | Time, h | Sensitivity/ specificity | False-positive rate | Whole-chromosome aneuploidy | Triploidy | Uniparental disomy | Mosaicism | Mitochondrial copy number | Large deletions and duplications >50 Mb |
|---|---|---|---|---|---|---|---|---|---|
| FISH | – | – | – | Yes, but does not test all chromosomes | No | No | No | No | No |
| aCGH | 12–15 | 98.2%/99.6% | 9% | Yes | No | No | Limited | No | No |
| qPCR | 4–12 | 98.8%/99.9% | – | Yes | Yes | No | – | Yes | No |
| SNP microarray | 30–40 | – | 10% | Yes | Yes | Yes | Yes | No | Yes |
| NGS-MiSeq | 13–16 | 100%/100% | 0% | Yes | Yes | Yes | Yes (down to 50%) | Yes | No |
| NGS-PGM | 13–16 | 100%/100% | 0% | Yes | Yes | Yes | Yes (down to 20%) | Yes | Yes (800 kb–1 Mb) |

*Note:* aCGH, array comparative genomic hybridization; FISH, fluorescent in situ hybridization; NGS, next-generation sequencing; PGM, Personal Genome Machine; qPCR, quantitative real-time polymerase chain reaction; SNP, single-nucleotide polymorphism.

*Harris. PGT: review of current modalities. Fertil Steril Rev 2021.*

# 'PGT-A LIMITATIONS

- ~1.2%–5.7% possibility of “no read” or “no result”
- Several groups have attempted to rewarm and rebiopsy these embryos and transfer resulting euploid embryos.

# PGT-A TESTING MAY ALSO BE UNSUCCESSFUL "IN CASES WHERE:

- Accidental damage to the embryo
- Loss of embryos
- Unsuccessful biopsy
- Failure to amplify DNA
- Failure to identify any normal embryos, requiring the patient to undergo another IVF cycle or to choose to transfer an abnormal embryo with uncertain prognosis
- The benefits of improved embryo selection may be counterbalanced by the loss of those embryos during testing.

# "PGT-A IS FURTHER LIMITED BY:

- A lack of standardization because of the multiple available platforms.
- Significant variation in laboratory techniques and training among different centers
- In a study of 44 samples from aneuploid embryos (30 diagnosed with the use of aCGH and 14 with the use of NGS) that were rebiopsied and retested by another laboratory with the use of NGS, 16 samples (36%) had discrepancies between the two labs. Five samples (11.4%) were actually shown to be euploid on retesting

# TRANSFERRING MOSAICS

- At the early stages of embryonic development, mosaicism is very common, with up to 60% of embryos having at least one aneuploid cell at the 8-cell
- At the cleavage stage up to 15%–75% of embryos are mosaic
- At the blastocyst stage ~5%–30% of embryos are mosaic
- Based on more recent NGS analyses, the prevalence of mosaicism in blastocysts ranges from 2% to 13%

- A blastocyst diagnosed with mosaicism can only be considered “at risk” of being mosaic
- Mosaicism most commonly occurs as confined placental mosaicism, in which the fetal karyotype is normal but the placental tissue is abnormal.
- This is estimated to occur in 1%–2% of viable gestations

# IS THERE CLINICAL EVIDENCE SUPPORTING THE USE OF PGT-A?

- Although open to debate, PGT-A not being advantageous in women younger than 35 years, but likely increasing the live birth rate per transfer in women 35 years of age and older.
- PGT-A does not change the cumulative live birth rate.

# COST-EFFECTIVENESS

- Whereas it may be cost-effective to use PGT-A in older women, young women do not experience the same benefit.
- Cost-analysis studies are mostly applicable to societal costs, in contrast to the individual patient.
- They do not take into account third-party payment systems or nonmonetary costs such as pain and suffering and lost work from treatment.

# PGT-M

- Benefits of DX of Single gene disorders
- Ethics
- Late Onset
- HLA

# ADVANTAGES, RISKS, AND LIMITATIONS OF PGT-M

- Cleavage Stage
- TE

# PGT-SR

- Techniques
- Abnormalities
- Benefits vs Risks

# PRENATAL GENETIC SCREENING AND TESTING AFTER PGT

- PGT-A
- PGT-M
- PGT-SR

# ETHICAL CONCERNS OF PGT

# FUTURE PROSPECTS