Next-generation Sequencing Techniques in Preimplantation Genetic Screening

Berta Font Cunill – Biotechnology degree

Universitat Autònoma de Barcelona

INTRODUCTION

Preimplantation Genetic Diagnosis (PGD) is a form of prenatal diagnosis that is performed on early embryos created by in vitro fertilization (IVF) to discover single gene mutations or chromosome abnormalities. The latter is known as Preimplantation Genetic Screening (PGS) since it is a non-invasive, rapid and sufficiently low cost procedure.

It has been seen that in many cases IVF failure is due to aneuploidy, which causes the embryo not to be able to implant and develop. For this reason, PGS is becoming a rutinary technique in many clinics to prioritise embryos for transfer, which leads to improved implantation rates and decreased number of miscarriages.

Nowadays, the widely used PGS technique to look for aneuploidies is Array-CGH but quicker, cheaper and more efficient techniques are needed. New techniques such as Next-generation Sequencing (NGS) are being developed and validated for its application.

OBJECTIVES

- Discuss the need for PGS.
- Compare the techniques used nowadays for PGS to NGS
- Review different methodologies for NGS techniques
- Evaluate NGS's current situation.

METHODOLOGY

This project is a bibliographical review. In order to find articles and reviews related to the topic, a search is done using pubmed and key words like "Preimplantation genetic diagnosis", "Aneuploidy screening" and "Next-generation sequencing" within an interval of five years.

PREIMPLANTATION GENETIC SCREENING (PGS) METHODOLOGY

EMBRYO BIOPSY

The first step in PGS is embryo biopsy. Genetic material from the oocyte or embryo must be obtained to analyse it. Embryo biopsy can be performed at different stages and every stage has its advantages and limitations making it a better or worst method depending on the case. After the embryo's biopsy, the cells are subjected to lysis and the genetic material is analyzed.

Biopsy stage	Advantages	Limitations
Polar Bodies (Day 0)	- Less invasive - No need for vitrification	- Only mother's material is analyzed - Not many genetic material (single cells)
Cleavage Stage (Day 3)	- No need for vitrification	-Invasive technique → can harm embryos - Not many genetic material (single cells)
Blastocyst Stage (Day 5)	-Doesn't harm embryos - More genetic material (multiple cells)	- Need for vitrification

PGS TECHNIQUES

There are different techniques used in PGS to look for aneuploidies.

- FISH
- CGH
- Real-time quantitative PCR
- SNP microarray
- Array CGH → Widely used technique
- Next generation sequencing (NGS)

WHOLE GENOME AMPLIFICATION (WGA)

WGA is the most critical step in NGS since the genome has to be amplified from a single cell or a few cells. There are different methodologies for WGA based on:

- → **PCR methods**: degenerated oligonucleotides that act as primers are used to amplify the entire genome in the same PCR reaction.
- → **Multiple Displacement Amplification (MDA):** random primers and an isothermal DNA polymerase are used to amplify the entire genome.

NEXT-GENERATION SEQUENCING (NGS)

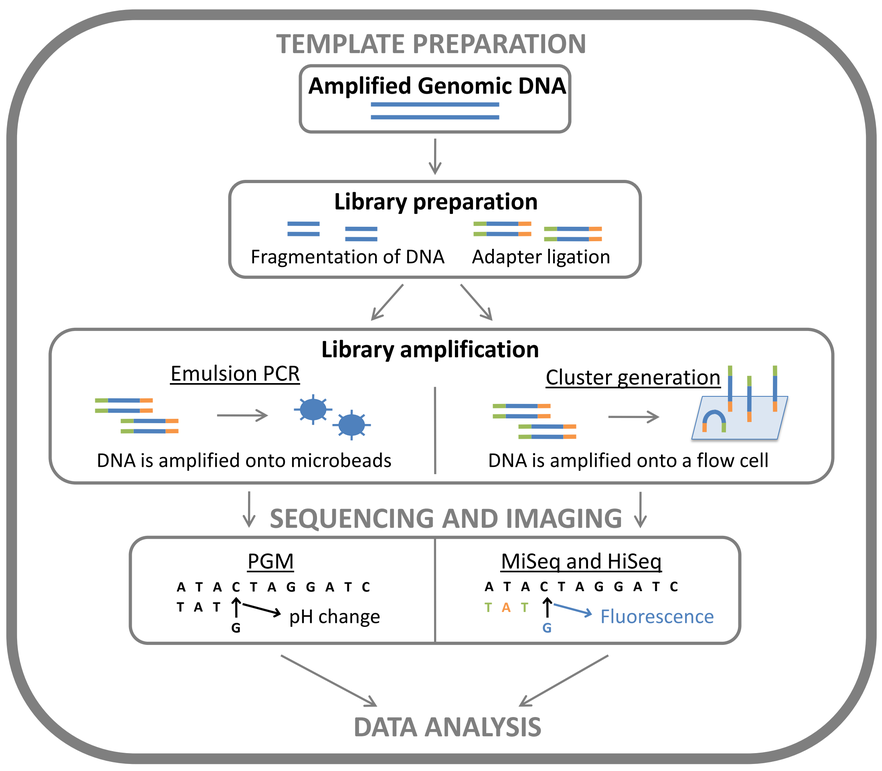
NGS techniques are a range of different sequencing techniques that allow millions of small fragments of DNA to be sequenced at once. The most commonly used NGS platforms are LifeTechnologies' Ion Torrent Personal Genome Machine (**PGM**) and Illumina's **MiSeq** and **HiSeq** platforms.

Nowadays, NGS techniques are being validated for their implemantiation in PGS to look for aneuploidies and improve the clinical outcome of embryos in IVF procedures.

In order to validate NGS, studies can:

- → compare NGS to previous methodologies in order to know NGS's sensitivity and specificity,
- → use NGS to study the clinical outcome of the embryos undergoing PGS.

NGS techniques are faster (<15 hours, compared to >24h with Array-CGH), cheaper (2/3 of Array-CGH's cost) and as accurate as Array-CGH.



CONCLUSIONS

-PGS is important due to the high rates of aneuploid embryos seen in patients undergoing IVF procedures. When the embryos are screened, euploid embryos can be prioritised for transfer and higher implantation rates are seen, leading to more successful pregnancies.

-Old PGS methods, like FISH, could only evaluate some chromosomes at the same time. Other newer methods allow 24-chromosome analysis but are expensive and too slow to allow fresh transfer of embryos after biopsy at blastocyst stage. Next-generation Sequencing is a faster, cheaper and accurate method for PGS.

-There are many NGS techniques with different methodologies. Both semiconductor sequencing and fluorescent sequencing are good techniques that allow sequencing of millions of small fragments of DNA and can be used to detect aneuploid embryos.

-Currently, NGS techniques are being validated and all the studies carried out conclude that it is a reliable technique that will most likely become the widely used technique for PGS in the near future.

REFERENCES

-Fiorentino F, Bono S, Biricik A, Nuccitelli A, Cotroneo E, Cottone G, et al. (2014). Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. *Human Reproduction*, **29**, 2802-13.

-Fiorentino F, Bricicik A, Bono S, Spizzichino L, Cotroneo E, Cottone G, et al. (2014) Development and validation of a next-generation sequencing–based protocol for 24-chromosome aneuploidy screening of embryos. *Fertil Steril*, **101**, 1375-82.

-Fragouli E, Alfarawati S, Spath K, Jaroudi S, Sarasa J, Enciso M, et al. (2013). The origin and impact of embryonic aneuploidy. *Hum Genet*, **132**, 1001-13.

-Grada A, Weinbrecht K. (2013). Next-Generation Sequencing: Methodology and Application. J Invest Dermatol, **133**.

-Handyside A. (2013) 24-chromosome copy number analysis: a comparison of available technologies. *Fertil Steril,* **100**, 0015-0282.

-Li N, Wang L, Wang H, Ma M, Wang X, Li Y, et al. (2015) The performance of whole genome amplification methods and next-generation sequencing for pre-implantation genetic diagnosis of chromosomal abnormalities. *J Genet Genomics* Accepted Manuscript.

-Scott KL, Hong KH, Scott RT Jr. (2013) Selecting the optimal time to perform biopsy for preimplantation genetic testing. *Fertil Steril,* **100**, 608-14.

-Stern H. (2014) Preimplantation Genetic Diagnosis: Prenatal Testing for Embryos Finally Achieving Its Potential. *J. Clin. Med.*, **3**, 280-309

-Wells D. (2014) Next-generation sequencing: the dawn of a new era for preimplantation genetic diagnostics. *Fertil Steril*., **101**, 1375-82.