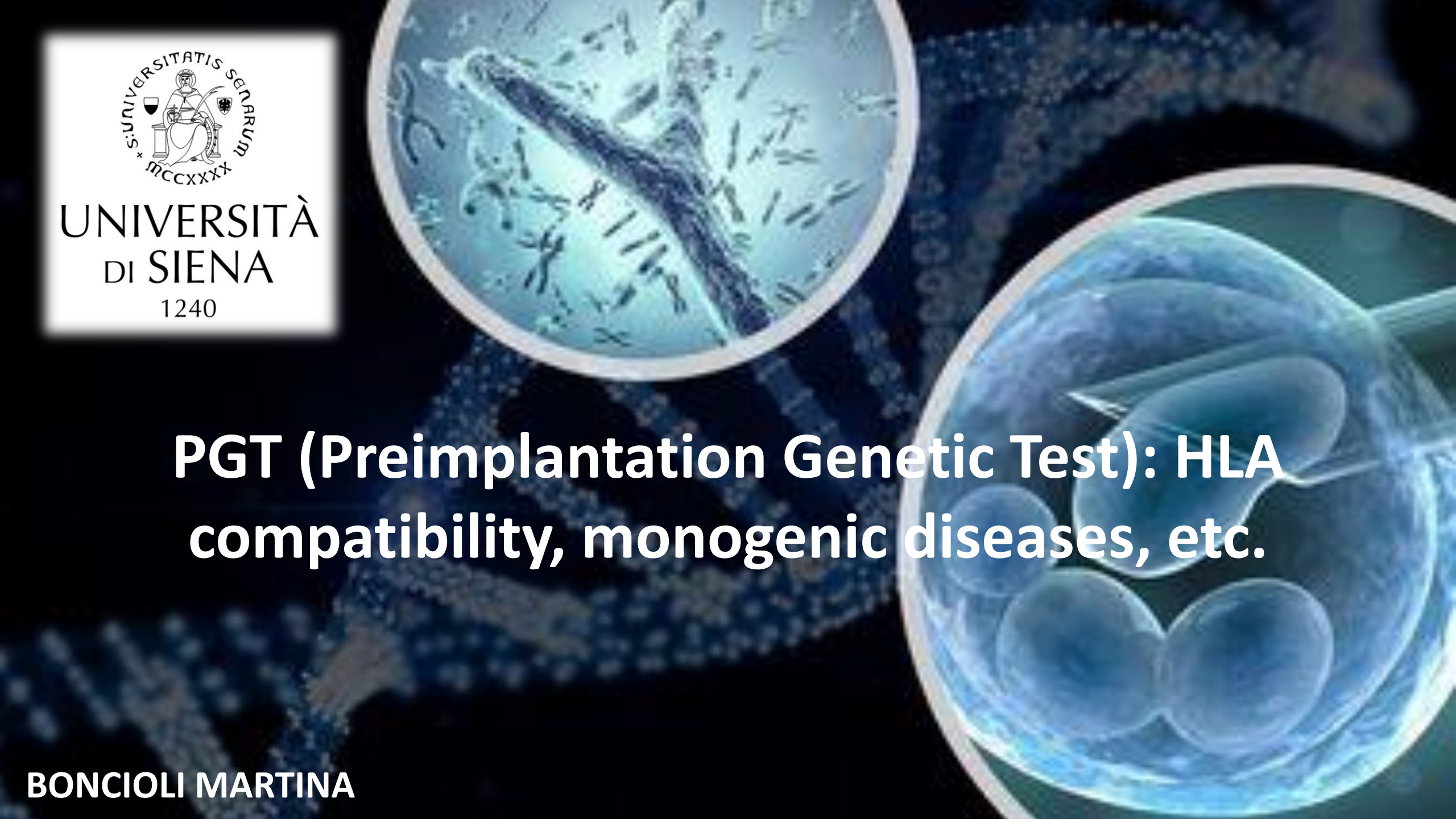


PGT (Preimplantation Genetic Test): HLA compatibility, monogenic diseases, etc.

BONCIOLI MARTINA

TABLE OF CONTENT

BACKGROUND

1967: Edwards and Gardner: Described the use of PGD for sexing of rabbit blastocysts

Nature **214**, 576 - 577 (06 May 1967)

Sexing of Live Rabbit Blastocysts

R. G. EDWARDS & R. L. GARDNER
Physiological Laboratory, University of Cambridge

1986: Marilyn Monk: demonstrated the use of PGD in a murine model

1978 : The first human IVF birth

1990: Handyside et al.: First PGD for X-linked disease

1992: Handyside et al.: Baby after PGD for Cystic Fibrosis

Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification

A. H. Handyside, E. H. Kontogianni, K. Hardy & R. M. L. Winston
Institute of Obstetrics and Gynaecology, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 ONN, UK

OVER 200 recessive X chromosome-linked diseases, typically affecting only hemizygous males, have been identified. In many of these, prenatal diagnosis is possible by chorion villus sampling (CVS) or amniocentesis, followed by cytogenetic, biochemical or molecular analysis of the cells recovered from the conceptus. In others, the only alternative is to determine the sex of the fetus. If the fetus is affected by the defect or is male, abortion can be offered. Diagnosis of genetic defects in preimplantation embryos would allow those unaffected to be identified and transferred to the uterus[1]. Here we report the first established pregnancies using this procedure, in two couples known to be at risk of transmitting adrenoleukodystrophy and X-linked mental retardation. Two female embryos were transferred after *in vitro* fertilization (IVF), biopsy of a single cell at the six- to eight-cell stage, and sexing by DNA amplification of a Y chromosome-specific repeat sequence. Both women are confirmed as carrying normal female twins.

The important success of PGD in predicting embryos that didn't present genetic pathologies has led to a wider application as a selective tool for all embryos in a particular cohort. So as to identify those with normal chromosomal complements and therefore a greater chance of success per cycle. This practice became known as **Preimplantation Genetic Screening (PGS).**

2001: Verlinsky et al.: first 'saviour baby'

Preimplantation Diagnosis for Fanconi Anemia Combined With HLA Matching

Yury Verlinsky, PhD
Svetlana Rechitsky, PhD
William Schoolcraft, MD
Charles Strom, MD, PhD
Anver Kuliev, MD, PhD

PREIMPLANTATION GENETIC DIAGnosis (PGD) was introduced for couples at high risk for producing progeny with genetic disorders to avoid prenatal diagnosis followed by pregnancy termination.[1,2] Preimplantation genetic diagnosis has become an alternative to traditional prenatal genetic diagnosis and also an integral part of assisted reproduction, providing an important tool for improving the efficiency of in vitro fertilization (IVF) through avoiding the transfer of aneuploid embryos.[3,4] Preimplantation genetic diagnosis has been performed in more than 2500 clinical cycles for at-risk couples, resulting in approximately 600 clinical pregnancies and births of at least

Context The advent of single-cell polymerase chain reaction (PCR) has presented the opportunity for combined preimplantation genetic diagnosis (PGD) and HLA antigen testing. This is a novel and useful way to preselect a potential donor for an affected sibling requiring stem cell transplantation.

Objective To perform in vitro fertilization (IVF) and preimplantation HLA matching combined with PGD for Fanconi anemia (FA).

Design DNA analysis for the IVS 4+4 A→T (adenine to thymine) mutation in the FA complement C (*FANCC*) gene in single blastomeres, obtained by biopsy of embryos, to identify genetic status and HLA markers of each embryo before intrauterine transfer.

Setting In vitro fertilization programs at large medical centers in Chicago, Ill, and Denver, Colo.

Participants A couple, both carriers of the IVS 4+4 A→T mutation in the *FANCC* gene with an affected child requiring an HLA-compatible donor for cord blood transplantation.

Main Outcome Measures DNA analysis of single blastomeres to preselect unaffected embryos representing an HLA match for the affected sibling.

Results Of 30 embryos tested in 4 IVF attempts, 6 were homozygous affected and 24 were unaffected. Five of these embryos were also found to be HLA-compatible, of which 2 were transferred in the first and 1 in each of the other 3 cycles, resulting in a pregnancy and birth of an unaffected child in the last cycle.

Conclusion To our knowledge, this is the first PGD with HLA matching, demonstrating feasibility of preselecting unaffected embryos that can also be an HLA-compatible source for stem cell transplantation for a sibling.

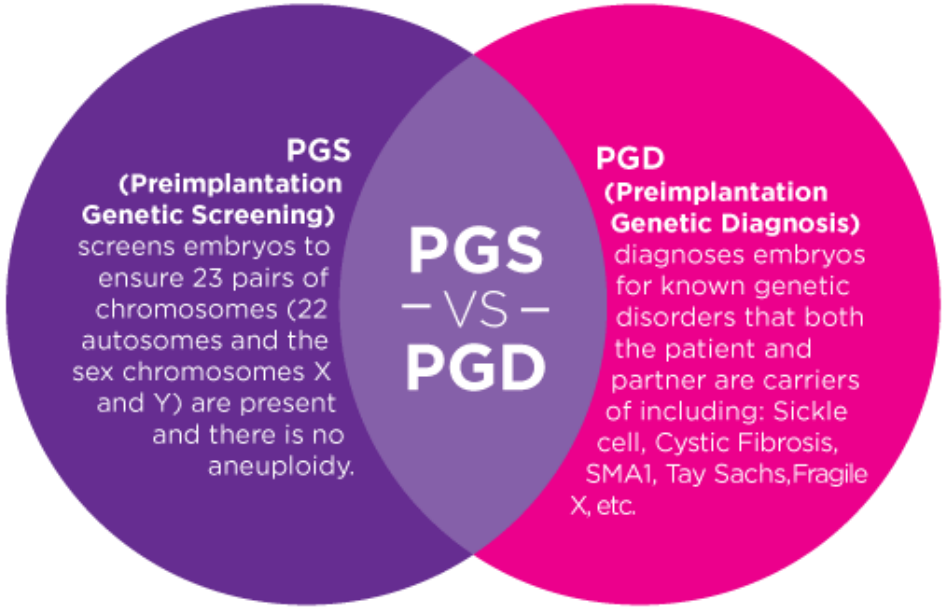
JAMA. 2001;285:3130-3133 www.jama.com

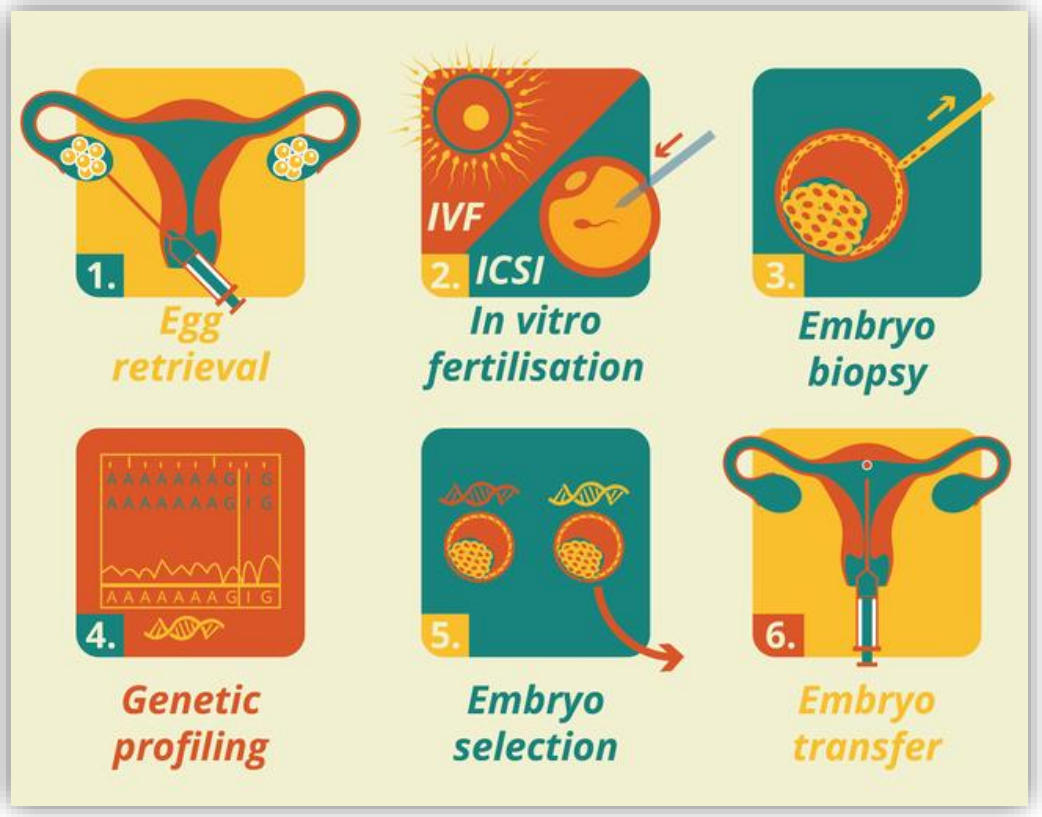
- Originally, couples were identified due to a history of a poor pregnancy outcome or a strong family history of disease
- The circumstances under which PGD is now utilized include an extensive list of sex-linked and autosomal single gene disorders, HLA typing, and translocations.
- Now, expanded carrier screening has become more widely utilized and in many circumstances allows for detection of a transmissible genetic anomaly before it has been phenotypically apparent in patients.

PREIMPLANTATION GENETIC TEST (PGT)

Recent scientific progresses allowed the identification of inheritable genetic diseases and/or chromosomal abnormalities already in the early phases of embryo development, before a pregnancy is established

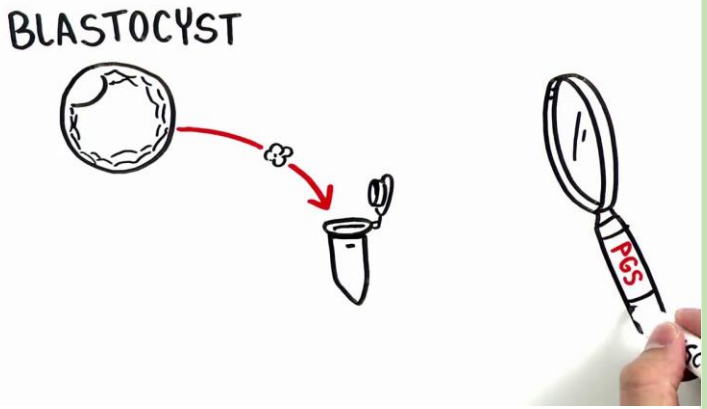
The procedure is known as **PRE-IMPLANTATION GENETIC TESTING (PGT)**and is performed during a cycle of in-vitro fertilization (IVF)





Preimplantation Genetic Screening (PGS)

PGS is a preventative measure used **to identify chromosomal abnormalities in the embryo**, even if there's no known evidence of a genetic abnormality in either parent

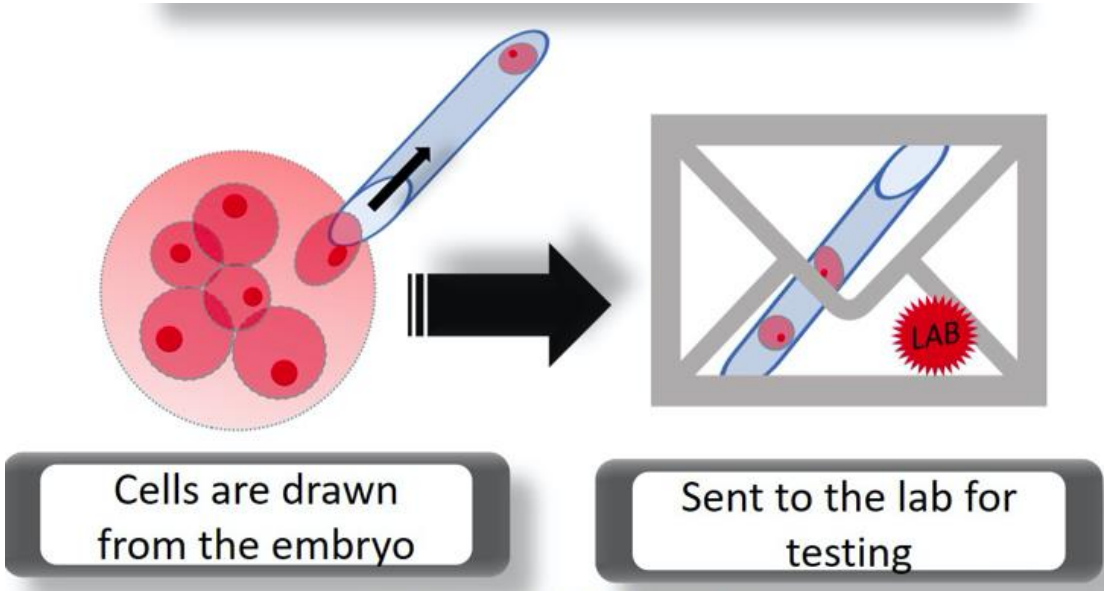


PGS is recommended for parents who have no known genetic abnormalities, as well as patients who meet any of the following conditions:

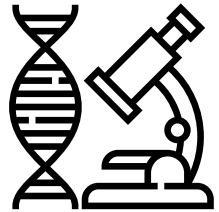
- **Female partner age 38 or older**
- **Couples interested in a single embryo transfer**
- **Couples interested in gender selection**
- **History of pregnancy loss**
- **History of failed IVF/implantation failure**

Couples who undergo an IVF cycle may require to be informed about the state of health of the embryos produced (***Article 14, Italian Law No. 40/2004***).
In these cases, and depending on each specific indication, they can proceed with PGT.

The diagnosis is performed starting from a small cellular biopsy from the embryo obtained during the cycle.

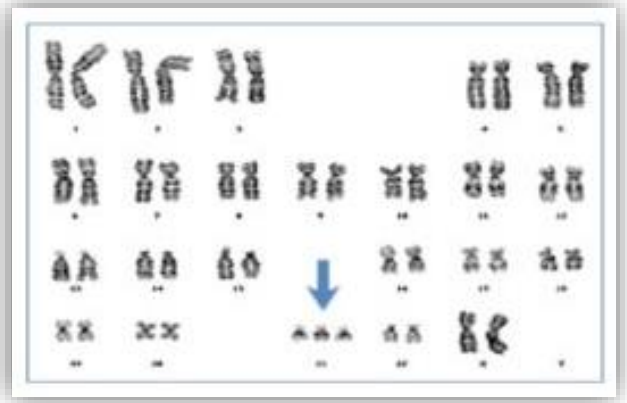


Preimplantation Genetic Diagnosis involves **genetic testing of an embryo for a specific gene mutation** when one or both biological parents is already aware of the existence of a genetic abnormality

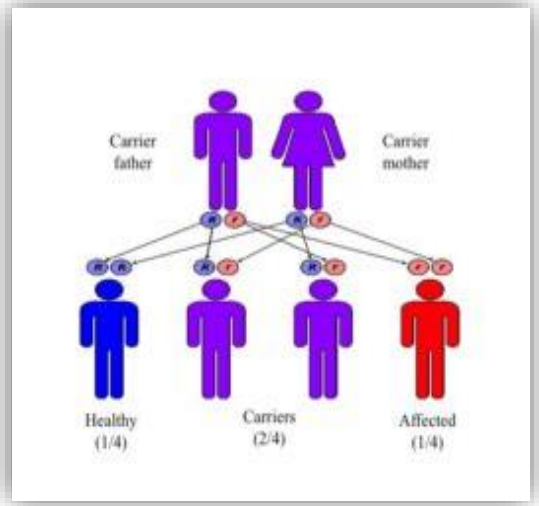


PGT is recommended for couples that have a genetic predisposition and/or have any probability of passing down **a known genetic abnormality**. Any couple with a family history of aneuploidy (abnormal number of chromosomes) which results in miscarriage, birth defects, or Down Syndrome can be screened

PGT-A (**Preimplantation Genetic Testing for Aneuploidies**), which is applied to identify de novo chromosomal abnormalities and that represents the broadest indication to preimplantation genetics at present. In the case of PGT-A, the method used for the chromosomal analysis is identical for all the patients.

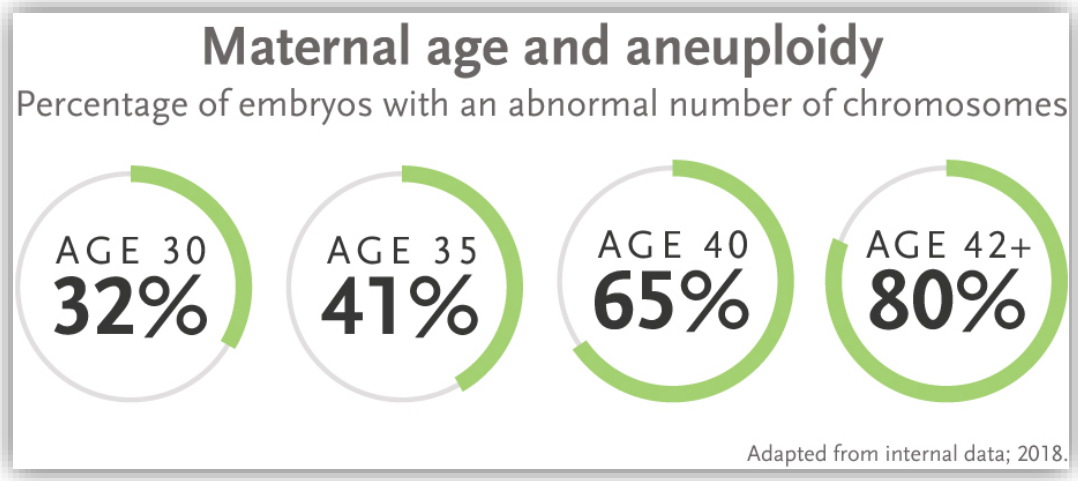


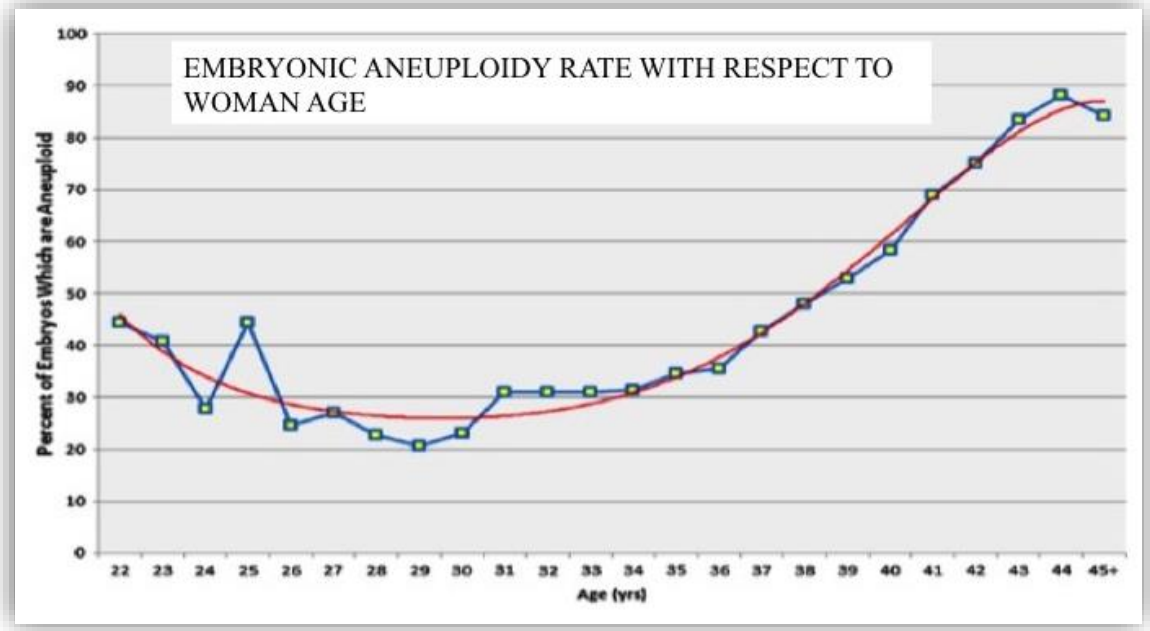
PGT-M (**Preimplantation genetic testing for Monogenic Diseases**) in the embryo is presence of a specific mutation in the parental genome which predisposes to an increased risk of transmitting a genetic disease to the offspring (e.g., cystic fibrosis, beta-thalassemia, etc.).



PGT-SR: Robertsonian translocations and Reciprocal translocations

PGT-A is the main application of genetics technologies in the preimplantation era. Its aim is the improvement of the efficiency of an IVF cycle by providing a safer IVF treatment, especially in terms of lower miscarriage and multiple pregnancy rates, as well as no risk for chromosomally abnormal pregnancies





DGR n.4183 7th April 2017 of ART-PGT

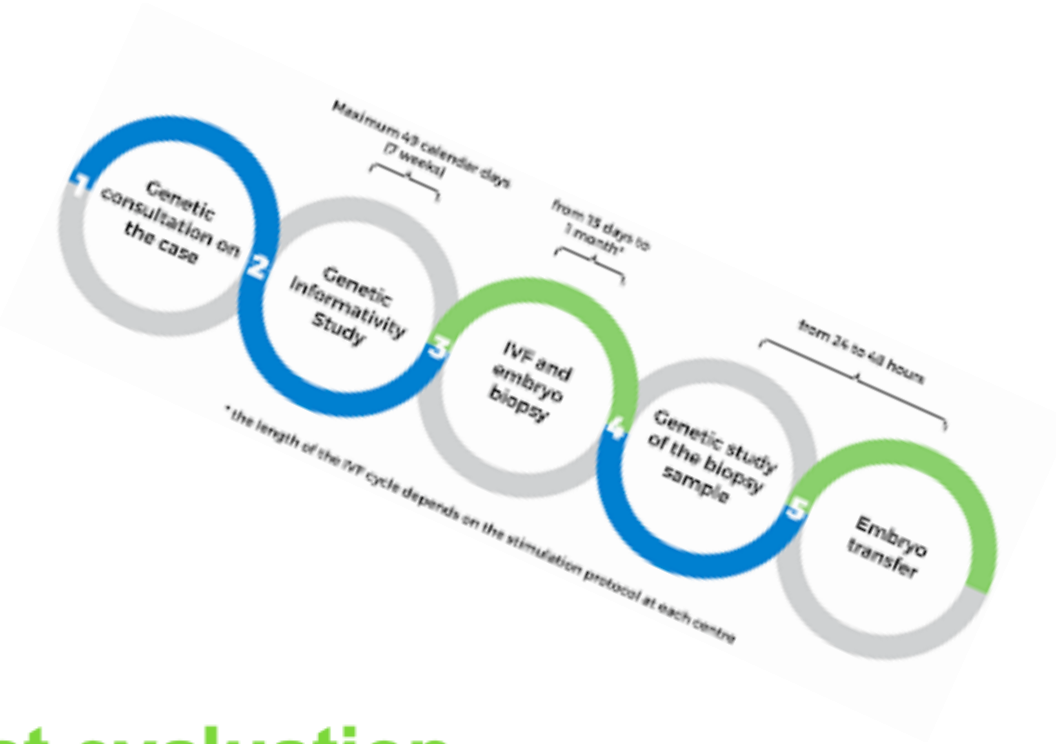
First of all, the couple must make an appointment with the PMA center, after which all the documentation will be sent to a medical genetic center

The center will assess whether it is possible or not to carry out an analysis and advise on psychological counseling

After multidisciplinary regional group approval

✓Preclinical Set-up

Conclusion of preclinical set-up
Ovulation induction
Pick-up
ICSI
Embryo growth monitoring
Embryo biopsy (day 5: blastocyst)
Tubing
Sample shipping to the PGT center
Embryo freezing waiting for PGT result

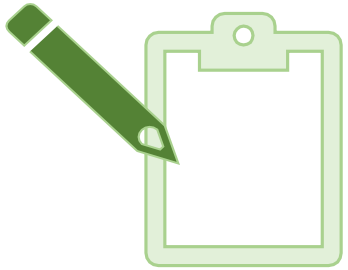


✓ PGT results

✓Multidisciplinary post-test evaluation

Thawing suitable embryos
Transfer of suitable embryos in utero

PGT Informed Consent



Mode: Pre-testing genetic counselling
set up

Post-testing genetic counselling

Time: *set up* 1 month

PGT from embryo biopsy to the eventual transfer at the following useful menstrual cycle

Purpose: To inform about the health status of embryos generated in vitro.

Technique aimed at analyzing the pathology of which the couple is a carrier, does not exclude that the embryo may be the carrier of other anomalies (numerical, structural or genetic) different from those sought

Risks and problems related to the technique:

False positives, False negatives, Allele Drop Out

Invasive Prenatal Diagnosis recommended

Risks for the child: Specific risks related to the disease from which it could be affected

Bioethical issues: Risk for embryo damage

Eugenic purpose in the use of the technique

Success rates: Establishment of pregnancy in 22.5% -29% depending on the pathology (Data PGD Consortium 2004).

Couple Choices:

The couple will declare to PROCEED with:

☐ Preimplantation Genetic Test for monogenic desease

☐ Preimplantation Genetic Test for monogenic desease
+ Preimplantation Genetic Test for chromosomal anomalies

☐ Preimplantation Genetic Test for chromosomal anomalies

The couple will declare to CONSENT or not to use
of residual biological waste material
for quality controls, as required by the relevant regional regulations

Embryo biopsy Informed Consent

Time: Embryo biospy will be performed after IVF of ovocytes and before their trasfer at the stage of blastocyst (5-6 day)

Method: Through an opening in the zona pellucida, a suitable number of cells are taken and will be analyzed

Purpose: Embryo biopsy is finalized to the pre-implantation genetic test

Risks and problems related to the technique: :

No risk for women compared to standard ART

A minimal reduction in the probability of engraftment of the embryo cannot be excluded

Risks for the child: Specific risks related to the disease from which it could be affecte

Failure to perform: reduction of the chances of success of ART

Bioethical issues: Risk of embryo damage

Eugenic purpose in the use of the technique

Success rates: Establishment of pregnancy in 22.5% -29% depending on the pathology (Data PGD Consortium 2004).

Couple Choices:

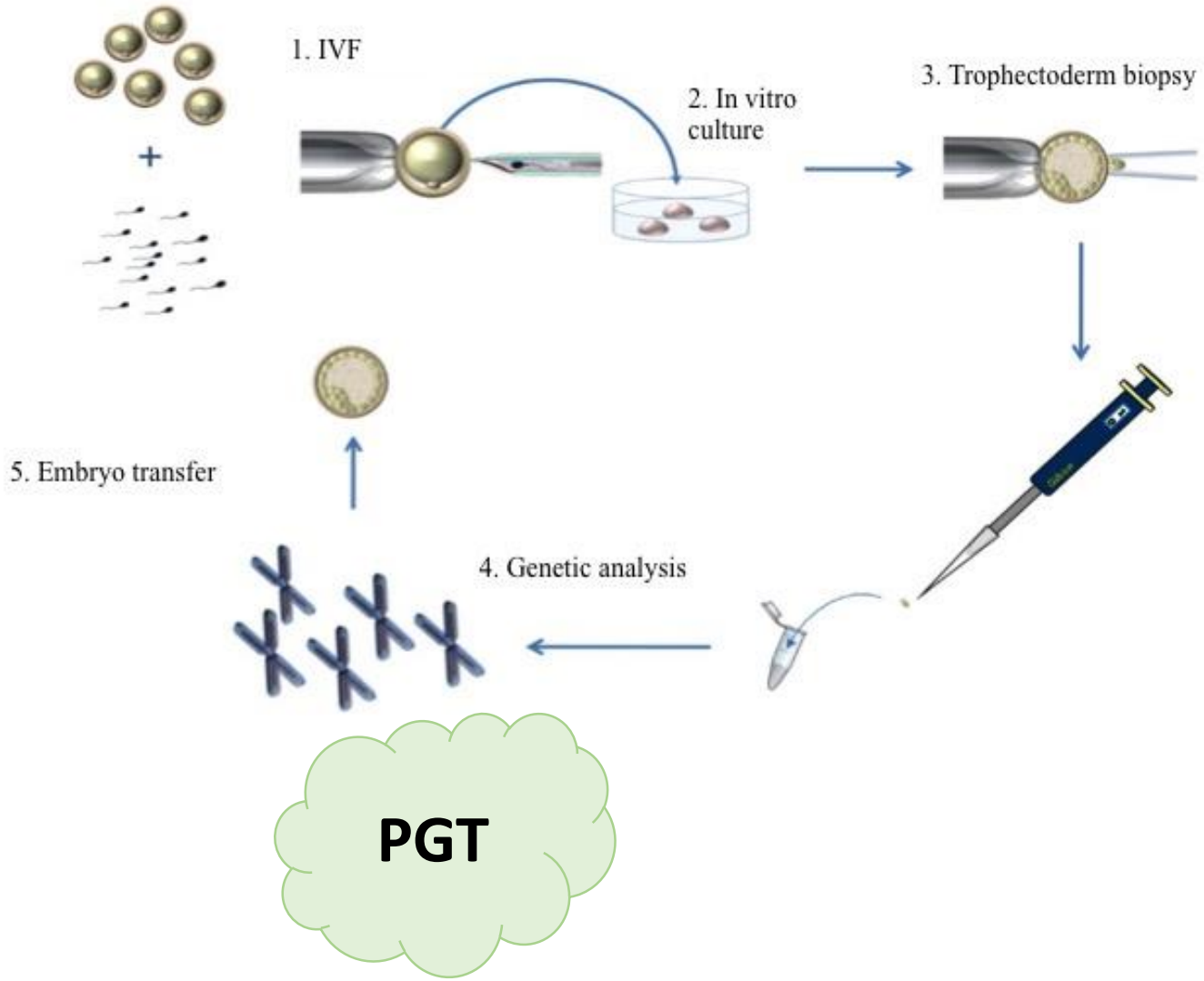
NO

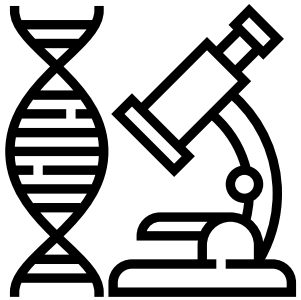
Pathological embryos will **NOT** be destroyed,
as destruction is expressly prohibited
art. 14 paragraph 1 Law 40/04,
and will be subject to cryopreservation if considered vital.

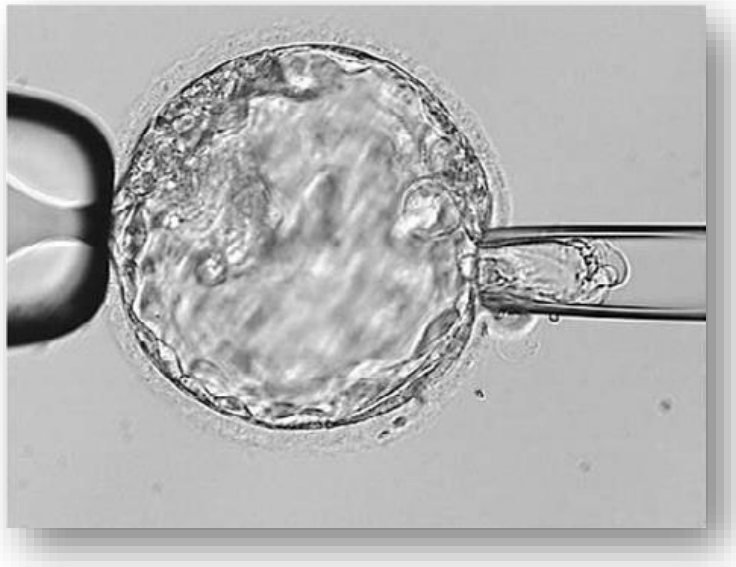
The couple will DECLARE their willingness to abandon embryos results affected by:

- ❑Anomalies incompatible with post-natal life
- ❑Anomalies compatible with post-natal life that they believe to be invalidating

THE PHASES OF PREIMPLANTATION GENETIC DIAGNOSIS

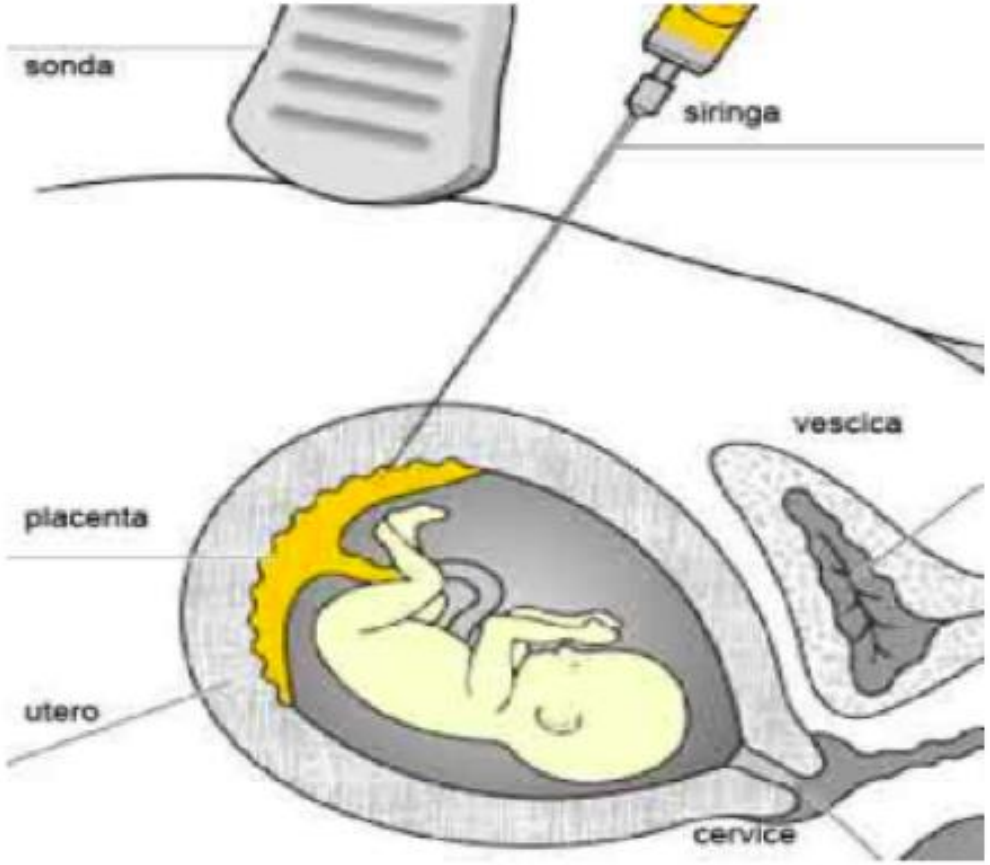


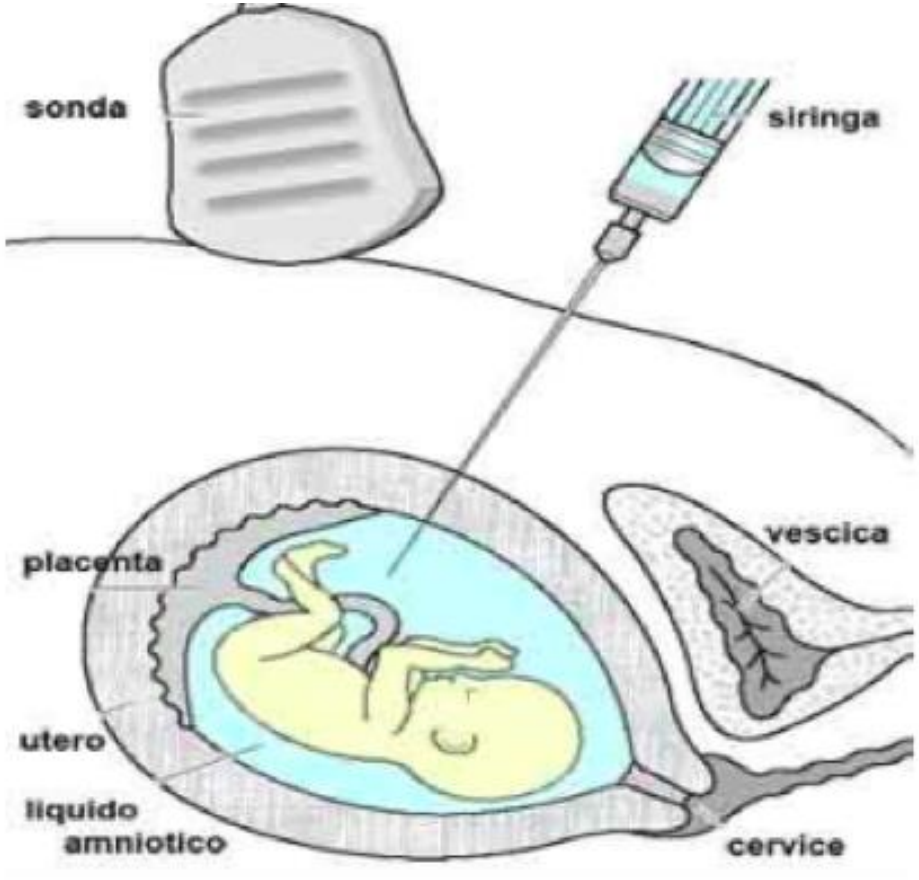




DAY 6 BLASTOCYST BIOPSY

In every case after ART-PGT is recommended to do the **Prenatal Invasive Diagnosis**





PGT-M

The principle of it is the haplotyping, that is determination of group of allele in a genetic segment that segregate together

It’s important because in this haplotypes you can find genetic markers from which can distinguish each chromosome as the embryo inherited in particularly want to see the embryo inherited the mutated chromosome from father or mother

So, you need a pre-clinical work up done in parent to determine this genetic markers (STR and/or SNP)

>99%

PGT-M CAN BE PERFORMED FOR >99% OF INHERITED SINGLE GENE DISORDERS

>95%

PGT-M IDENTIFIES AFFECTED AND UNAFFECTED EMBRYOS WITH >98% ACCURACY[1]

SNP:

- Are bi-allelic and more easy to interpret
- Informative marker in which the wildtype allele is unique
- Also, are able to detect the monogenic disorder and because of this are used in this case

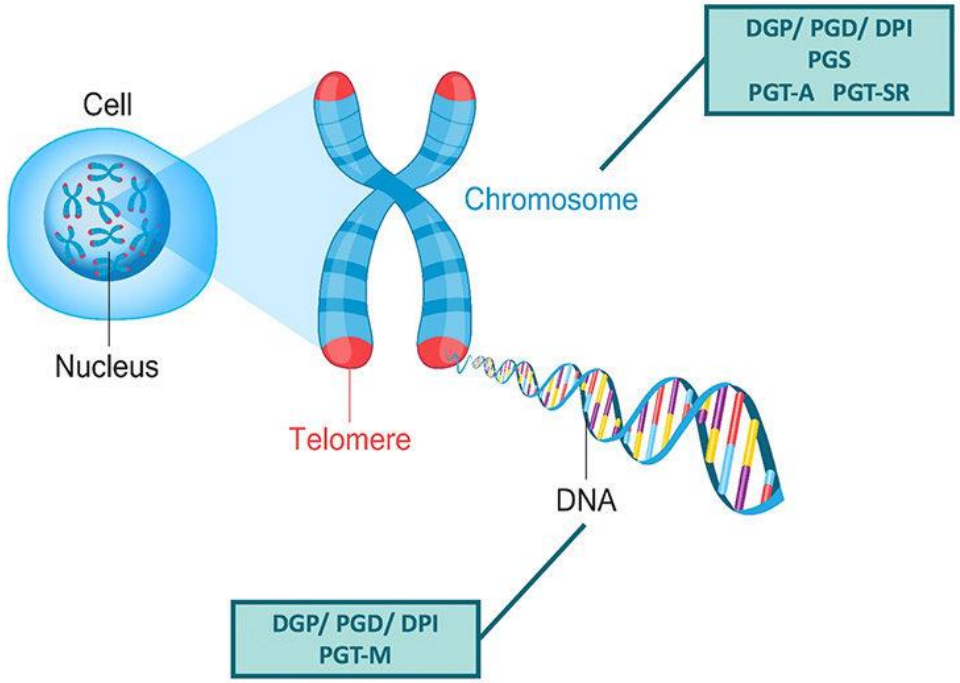
STR:

- Are short tandemly repeat of DNA sequences
- Are higher polymorphic and involve many allele
- They are targeted with fluorescence, labelled primers and co-amplify with multiplex PCR

The most use method is the Target Amplification

Direct

Indirect

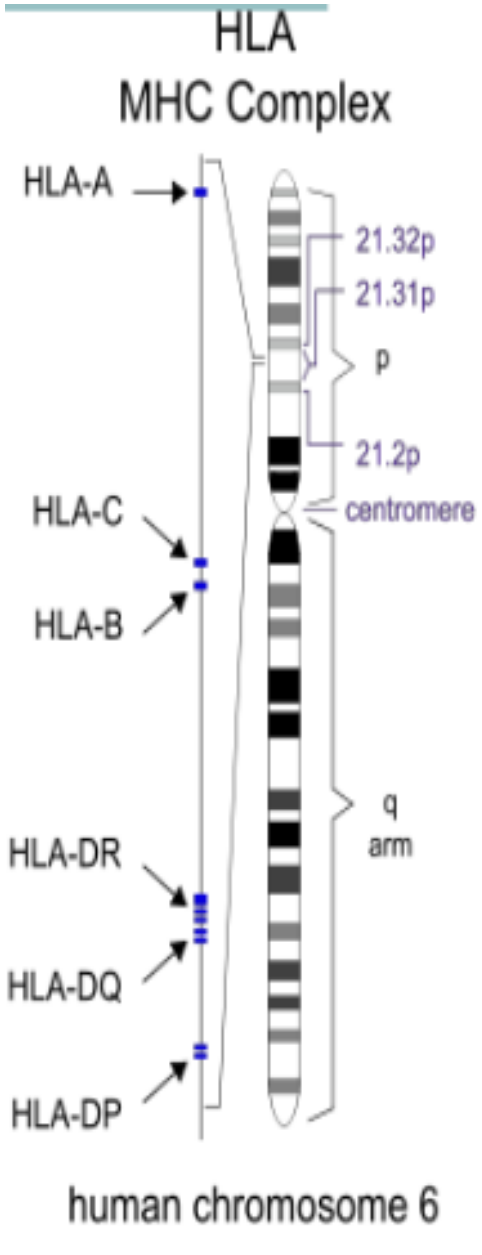


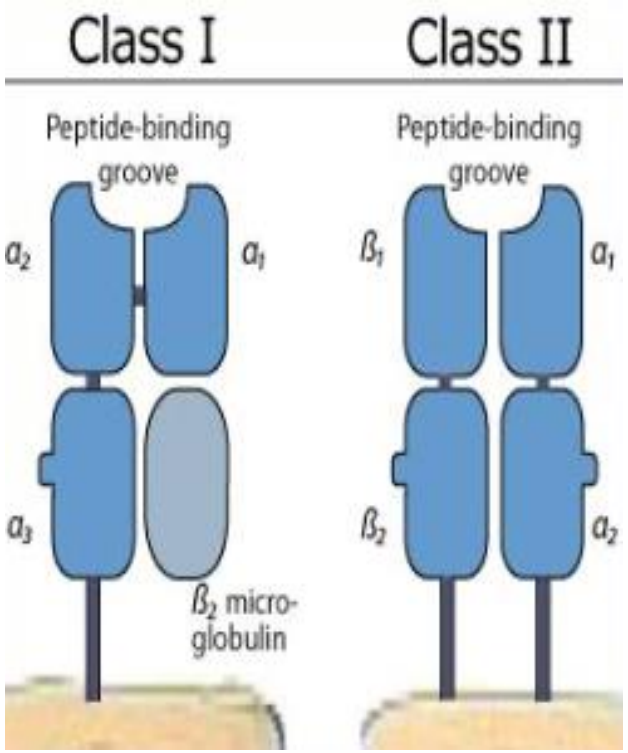
Human Leukocytes Antigens (HLA)

Cell surface molecule assists recognition of antigens by T-lymphocytes and is important in determines individual tissue typing

HLA gene:

- The genes that code the synthesis of HLA maps on chromosome 6
- Two main classes of it:
  - HLA class I which includes 3 loci A, B, C
  - HLA class II which includes the genes DP, DQ, DR





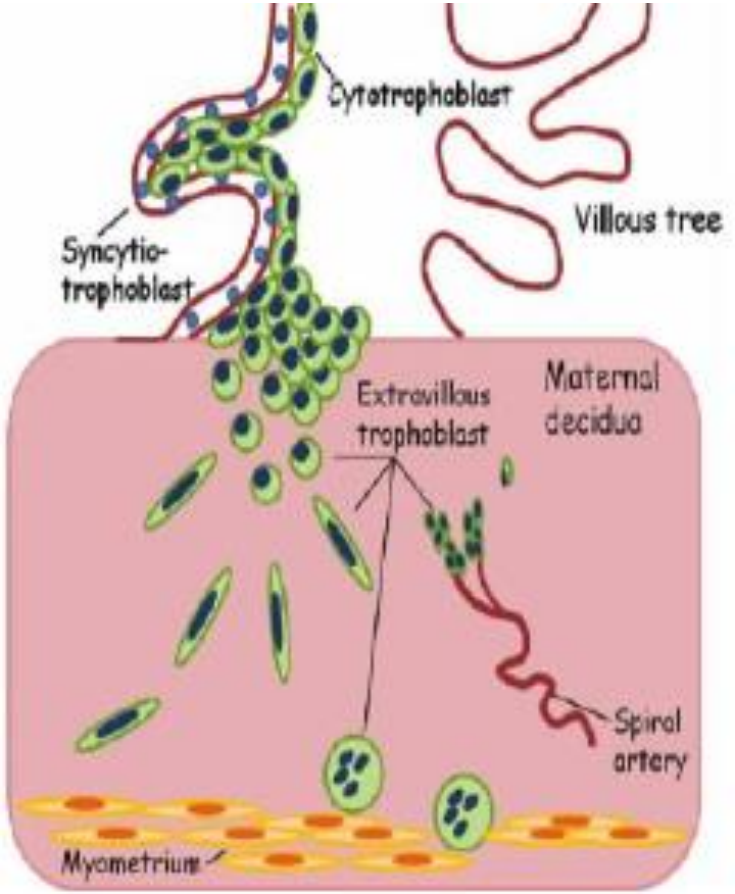
HLA molecules

- HLA class I molecules are expressed on all organs and leucocytes
- HLA class II molecules are expressed on certain leucocytes (APC), B cells, monocytes/macrophages

Extravillous trophoblast expresses only four types of HLA class I:
HLA-C, HLA-E, HLA-F and HLA-G

Villous trophoblast (syncytiotrophoblast) expresses no HLA antigens on its surfaces

HLA-G: shows very low level of polymorphism, play an important role in immune tolerance induction at the maternal-foetal interface and also is the universal NK cell inhibitor.



The HLA typing in the pre-implantation phase (Preimplantation HLA matching) has recently been proposed as an option for couples with a child suffering from a genetic disease, whose treatment requires stem cell transplantation from a HLA-compatible subject (Verlinsky et al., 2001, Fiorentino et al., 2004, 2005)

PGT represents a strategy that allows to identify and transfer the resulting embryos, to genetic analysis, both not affected by the specific disease and HLA compatible with the sick child. At the birth of the child, the stem cells (HSC) present in the umbilical cord of the unborn child can be isolated and transplanted into the couple's sick child, to allow their recovery.

This technique is suitable for genetic diseases such as

- Beta-thalassemia,
- Sickle cell anemia,
- Fanconi’s anemia
- Other hemogoblinopathies,

Indication			Cure (%)
As a rule	Congenital	Thalassemia major	70-90
		Sickle cell anemia	80-90
		Fanconi’s anemia	80-90
		Immunodeficiencies	70-90
		Blackfan-Diamond anemia	>50
	Acquired	Severe aplastic anemia	80-90
As an exception	Acquired	Acute lymphoblastic leukemia	30-50
		Chronic myeloid leukemia	40-80
		Non Hodgkin lymphoma	30-50
		Myelodysplastic syndrome	30-50

Which can be cured by transplanting HLA-compatible stem cells

Establish a pregnancy which is HLA compatible with a sick sibling, so that at birth, cord blood can be collected and used for hematopoietic stem cell transplantation and cure of the sick child

The PGT associated with HLA typing will now be able to avoid the use of prenatal diagnosis, allowing the selection and subsequent transfer into utero of only the healthy and HLA-matched embryos compatible with the child affected by the couple

In comparison, PGT offers more than obvious advantages:

- It allows to test a high number of embryos for each cycle
- Increasing the chances of finding those with the characteristics suitable for donation

Above all it allows to identify these embryos before a pregnancy is started.

There are limitations for this procedure:

- A minimal time period is needed for the procedure, a delay of 12-18 month may be necessary between the decision and treatment (related with the success of IVF treatment)
- A high n° of embryos (and several IVF cycles) may be necessary to obtain a pregnancy and a live birth
- The low chance of finding an HLA matched unaffected embryo
- The chance of obtaining a pregnancy in IVF is limited by the advancing age of the mother

HLA typing in the pre-implantation phase is a very complex procedure. It is not surprising, that this technique was applied in only 5 centres in the world (United States, Italy, Australia, Belgium and Turkey).

Italy has made a substantial contribution to this series of cases, applying the procedure in 82 pairs, obtaining 29 clinical pregnancies, 13 of which have already been completed, while 8 pregnancies are still in the gestation phase.

Ponchiroli's family

The couple has a son affected by a sporadic form of Blackfan-Diamond anemia, characterized by an inability to produce red blood cells. The only cure available was HSC transplantation. No genetic risk, HLA matching as primary indication.

Three years of attempts:

- 7 PGT cycles
- 2 different IVF centres

The son is now completely cured after HSC transplantation

SUMMARY

- PGT-HLA matching is used to identify embryos that are HLA compatible with a child who is in need of a bone marrow or cord blood transplant. PGT-M for HLA can be performed in conjunction with testing for a single gene disorder if needed, in order to recommend embryos that are both HLA-matched to an affected sibling and free of the inherited condition

- PGT-M for HLA includes the analysis of at least eight polymorphic (unique) markers throughout the HLA complex, and has been helpful in detecting potential recombination that may compromise the likelihood of a match. This approach permits HLA testing of embryos to be combined with analysis of a known single gene disorder.

Human Reproduction, Vol.33, No.12 pp. 2302–2311, 2018
Advanced Access publication on October 31, 2018 doi:10.1093/humrep/dey325

human reproduction

ORIGINAL ARTICLE *Reproductive genetics*

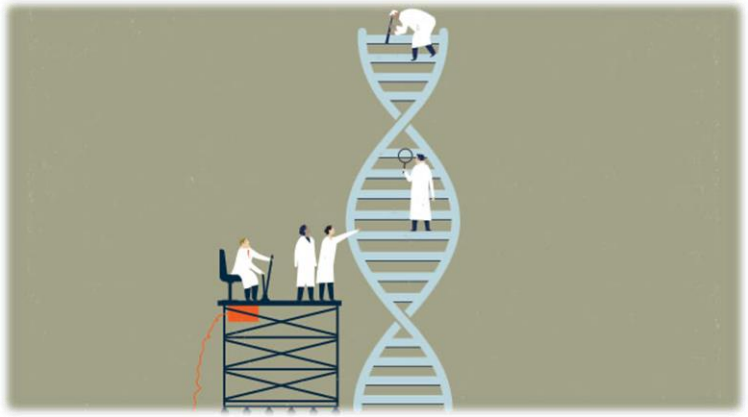
Genome-wide haplotyping embryos developing from 0PN and 1PN zygotes increases transferrable embryos in PGT-M

Aspasia Destouni[1,†], Eftychia Dimitriadou[2,†], Heleen Masset[1,†], Sophie Debrock[3], Cindy Melotte[2], Kris Van Den Bogaert[2], Masoud Zamani Esteki[2,4], Jia Ding[1], Thiery Voet[5,6], Ellen Denayer[2], Thomy de Ravel[2], Eric Legius[2], Christel Meuleman[3], Karen Peeraer[3], and Joris R. Vermeesch[1,2,*]

- Zygotes with phenotypes from the normal morphometric standards, combined with the presence/absence of the second polar body (PB2) or the fragmentation of both polar bodies, are treated as evidence of failed or abnormal fertilization and as proxies of suboptimal gamete quality

- More importantly in cycles where only 0PN-derived embryos were available their transfer yielded pregnancies, which resulted in the birth of healthy babies. Similarly to 0PNs, successful development to term has been reported following transfers of 1PN-derived embryos

This globally adopted practice aims to avoid the risk of adverse developmental outcomes including abnormal fetal development, molar pregnancy, miscarriage, neonatal death and congenital anomalies which are associated with triploid and 2n/3n mixoploid embryonic development



Genome-wide technologies such as array comparative genomic hybridization (aCGH) or low pass next generation sequencing (NGS) which are generally used for the PGT-A cannot map ploidy anomalies because normalization eliminates ploidy information. So, recent studies opted for complementary strategies such as the combined use of aCGH/NGS

Recently, the development of single-cell haplotyping methods has enabled genome-wide linkage analysis in embryos from couples who are carriers of pathogenic variants

Genome-wide haplotyping can close the existing gap between routine clinical practice whereby 0 and 1PNs are discarded and the knowledge that a substantial proportion of 0PN and 1PN zygotes can develop into normal diploid embryos

- Participants received counselling and signed an informed consent in compliance with the hospital, local and federal regulatory requirements, prior to the initiation of the cycle
- PGT-M in 0PN and 1PN-derived embryos was performed in 42 cycles from 38 couples, embryos of adequate quality for both biopsy and subsequent blastocyst vitrification
- On Day 1 (16–20 h after injection) the number of pronuclei was assessed. Zygotes with 2PN as well as 0PN and 1PN were further cultured

A single blastomere was aspirated from each embryo, and whole-genome amplified (WGAed). The embryos were immediately transferred to fresh medium and then cultured to Day 5/6 post injection. Those that formed blastocysts of sufficient quality were vitrified

RESULT

Confirm that the embryonic bi-parental diploidy is a prerequisite for accurate PGT-M.
Since the absence of a pronucleus (0PN) or the presence of one pronucleus (1PN) are treated as indications of abnormal parental ploidy.

First investigated the presence of both parental haplotypes along the embryonic genome, and score these embryos as diploid bi-parental (DBP).

Embryos with genome-wide, gross chromosome anomalies were scored as ‘abnormal’

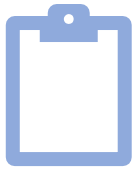
The majority of the analyzed 0PN embryos are DBPs allowing genetic diagnosis.
But this was not performed in the 0PN embryos that were scored as abnormal

In this study is demonstrate that Genome-wide haplotyping of developmentally competent 0PN and 1PN embryos allows their routine inclusion in PGT-M cycles. The diagnostic accuracy of the method is confirmed by the live-birth of unaffected babies for the monogenic disorder

The inclusion of 0PN and 1PN embryos into the diagnostic pipeline is beneficial for PGT-M couples whose transferrable pool of embryos is significantly reduced by the embryonic monogenic disorder associated genotype.

The observed increase of transferrable embryos in the 42 PGT-M cycles is a function of the high diagnostic efficiency of the method and the high blastocyst rate

The use of 0PN and 1PN zygotes in IVF practice is still not recommended by ESHRE accumulating evidence demonstrating that a significant subset can be euploid and developmentally competent



The reason for preserving these recommendations is the current lack of procedures which would accurately and comprehensively detect genome-wide parental ploidy in clinical routine such as PGT-M, PGT-A, PGT for chromosomal structural rearrangements (PGT-SR) and IVF cycles.

This study provides evidence that the haplotype can determine the parental ploidy in the whole genome in the 0PN and 1PN embryos and consequently eliminate their current loss due to the incorrect static classification of the PN

CONCLUSIONS

- Haplotyping requires DNA from relatives for phasing the parental genotypes while, this is a standard procedure for PGT-M cycles, it currently limits a more generalized adoption for PGT-A or for replacing static fertilization check in IVF cycles.

The propose is that genome-wide haplotyping holds promise as a personalized medicine feature in PGT with a potential revision of the ESHRE guidelines to achieve optimal outcomes in the management of reproductive problems.

*Take home message

- PGT is a procedure, complementary to prenatal diagnosis techniques, which allows to identify the presence of genetic diseases or chromosomal alterations in embryos in **very early stages of development.**

- Preimplantation HLA matching (PGT-HLA) has recently been proposed as an option for couples with a child suffering from a genetic disease, whose treatment requires stem cell transplantation from a HLA-compatible subject. For these patients, PGT is used not only to identify healthy embryos, but also to select those that are HLA compatible with the sick child.

This specific feature of PGT has proved to be extremely useful for this category of patients: for the first time a method of genetic diagnosis becomes a "**therapy tool**"

thank you!