HUMANITAS
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Pre-implantation genetic testing for monogenic diseases: single center study

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Sponsor (Institution): Humanitas Research Hospital

Coordinating Investigator: Paolo Emanuele Levi Setti, MD

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Contract Research Organization:	NA
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COORDINATING INVESTIGATOR

I have approved this Protocol entitled "***Pre-implantation genetic testing for monogenic disease: single center experience.***" and I agree to conduct the study as detailed herein and according to the current version of the World Medical Association Declaration of Helsinki, Good Clinical Practice guideline and applicable regulatory requirements. I will provide all study personnel under my supervision with all information needed to perform the study and I will inform them about their responsibilities and obligations.

Printed name	Paolo Emanuele Levi Setti
Role	Coordinating investigator
Department	Department of Gynecology, Division of Gynecology and Reproductive Medicine, Humanitas Fertility Center
Signature	
Date	29/06/2023

STATISTICIAN

I have approved this Protocol entitled "***Pre-implantation genetic testing for monogenic disease: single center experience***."

Printed name	Emanuela Morenghi
Signature	
Date	29/06/2023

CENTRE SIGNATURE – PRINCIPAL INVESTIGATOR

I have read this Protocol Amendment relevant to the study entitled "**Pre-implantation genetic testing for monogenic disease: single center experience."** and I agree to conduct the study as detailed herein and in compliance with guidelines for Good Clinical Practice and applicable regulatory requirements. I will provide all study personnel under my supervision with all information provided by the Coordinating Investigator/Sponsor and I will inform them about their responsibilities and obligations.

Printed name	Paolo Emanuele Levi Setti
Role	Principal investigator
Department	Department of Gynaecology, Division of Gynaecology and Reproductive Medicine, Humanitas Fertility Center
Signature	
Date	29/06/2023

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1. SUMMARY

Study Title	**"Pre-implantation genetic testing for monogenic disease: single center experience."**		
Study code	GIN2/OSS_001/2023	**Acronym:**	PGT-M experience
Version and Date	Version No.1 (29/06/2023)		
Sponsor (Institution)	Humanitas Research Hospital, Vía Alessandro Manzoni, 56, 20089 Rozzano MI		
Coordinating Investigator	Paolo Emanuele Levi Setti, MD		
Product Name	NA		
Study indication	Efficacy of pre-implantation genetic testing for monogenic diseases in a single center scenario.		
Background and rationale	From the early 1990s, preimplantation genetic diagnosis (PGD) and embryo biopsy became available: over the years, with the evolution of the technique, PGT-M has revolutionized the landscape of clinical genetics by decreasing the risk of transmitting a serious genetic disorder to patients offspring and allowing to potentially eliminate the disease from the population. With the term PGD, we include three different tests: pre-implantation genetic testing for monogenic disease (PGT-M), Pre-implantation genetic testing for aneuploidy (GT-A), and Pre-implantation for structural rearrangements (PGT-SR). PGT-M is used in patients who have or are carriers of a congenital disease that can be passed down to their offspring. Pre-implantation genetic testing for aneuploidy (PGT-A) is a procedure that screens embryos before transfer to reduce the risk of aneuploidy and chromosomal abnormalities. Pre-implantation for structural rearrangements (PGT-SR) is an option for patients with an inversion, reciprocal translocation, or Robertsonian translocation. These approaches, when combined with in vitro fertilization (IVF), enable us to differentiate between affected and unaffected embryos. To achieve these biopsy results, many IVF laboratories perform trophectoderm cell biopsies (TE), which is the development of embryos up to the blastocyst stage, which occurs 5-7 days after fertilization. Prior to trophectoderm cell biopsies (TE), biopsies were performed on polar bodies or cleavage embryos.		

	It is still open to debate the disadvantage discussed in the scientific literature that affects the technique's efficacy due to the presence of mosaic embryos and whether it should be paired with PGT for aneuploidy (PGT-A) for more concrete results.
Study Objectives	The primary objective of this retrospective observational single-center study is to describe the efficacy of PGT-M in a real-life setting. Efficacy will be evaluated in terms of live birth rate (LBR), simple and cumulative live birth rate (CLBR) per couple, and abortion rate (AR). Furthermore, investigators will consider how many cycles the participants need to undergo to achieve a viable blastocyst. The secondary objective is to evaluate the incidence of aneuploidy in tested embryos, in order to understand the need for PGTA in addition to PGTM.
Study Endpoints/Outcomes	For the evaluation of the study objectives, all patients that underwent PGTM from 2016 to 2022 will be included in the study. The results obtained from the PGT-M will be calculated as: **Live birth rate (LBR)**: defined as the "the number of deliveries that resulted in at least one live birth, divided by the number of cycles." Embryology and Alpha Scientists in Reproductive Medicine. Electronic address [1] **Cumulative live birth (CLBR) per couple**: defined as the "delivery of at least one live-born infant (>24 weeks of gestation) in the fresh or in the subsequent frozen-thawed cycles in relation to the number of oocytes retrieved per couple." Polyzos, Drakopoulos [2] **Abortion rate (AR)**: is defined as the "spontaneous demise of a pregnancy before the foetus reaches viability. The term therefore includes all pregnancy losses (PLs) from the time of conception until 24 weeks of gestation." Group, Vlaisavljevic [3] **Aneuploid embryo rate:** "the most common genetic abnormality

	resulting in embryonic demise, pregnancy loss, and congenital birth defects. At the preimplantation stage, ~50% of the IVF-derived embryos have chromosomal abnormalities that are incompatible with implantation or normal fetal development." Hindi E. Stohl [4]
Study design	Monocentric retrospective observational study
Product/Intervention	PGT-M is an option for all patients, fertile and infertile, who are at risk of transmitting a monogenic disease to their offspring. PGT-A is only performed on unaffected embryos that have undergone PGT-M; therefore, investigators do not know the percentage of aneuploidy in affected embryos.
Eligibility Criteria	Patients fertile or infertile that have undergone preimplantation genetic testing for monogenic diseases (PGTM). No exclusion criteria will be considered.
Study Procedures	The information collected for the database will be retrieved from the Fertility Center internal web-based registry where data about pregnancy follow up are noted. The study database includes all PGTM cycles performed between 2016-2022 at the Humanitas Fertility Center.
Number of patients (planned)	All 76 patients who underwent PGTM testing in the Fertility Center starting from the beginning of 2016 and going through the end of 2022 will be included in the study.
Investigational Sites (planned)	University affiliated 3rd level Fertility Center
Sample size and statistical consideration	Data will be described as number and percentage, if categorical, or mean and standard deviation, if continuous and approximately Gaussian, or with median and range.
Study timetable	• 7 years of inclusion

GCP Statement:	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki and applicable guidelines as well as all national legal and regulatory requirements.

2. STUDY FLOW-CHART

Procedures	**Blastocysts trophectoderm biopsy**
PGTM analysis	567 embryos

3. BACKGROUND

Handyside et al. were the first to publish research on pre-implantation genetic testing for cystic fibrosis in 1992. The study aimed to take cells from a cleavage-stage embryo and use DNA amplification to look for the cystic fibrosis mutation. A total of 13 embryos were biopsied from the three patients. Only one of the four suitable for transfer resulted in a pregnancy and the subsequent birth of a healthy baby. According to the study, couples have a high chance that their pregnancy will be unaffected by cystic fibrosis. As a result, pre-implantation diagnosis was considered clinically accurate, despite it taking several years to confirm its accuracy.[5]

A 2005 study published by **McArthur et al.** aimed to compare trophectoderm biopsies with their previously published experience with day-3 cleavage-stage embryos. They found that blastocysts have a lower percentage of chromosomal monosomies than cleavage-stage embryos. Meanwhile, performing a biopsy in the cleavage stage can arrest further development by reducing the inner cell mass, which is not the case for trophectoderm biopsies. Concluding that trophectoderm biopsies are a practical addition to IVF and great progress for PGD practices. [6]

A review done by **Harper et al.** in 2017 explained the advantages and disadvantages of the different biopsy methods for pre-implantation genetic screening (PGS) and pre-implantation genetic diagnosis (PGD). Three stages are available for biopsy: polar bodies from oocytes, blastomeres from cleavage-stage embryos, and trophectoderm cells from blastocysts. The polar body is the earliest stage that can be biopsied but is limited by the fact that only maternal errors can be tested. The second stage is cleavage embryos, which until recently lost their popularity since these cells are more mosaic and the number of cells biopsied is limited. The majority of pre-implantation is now done at the blastocyst stage, which is already a method of selection as only good-quality embryos reach the blastocyst stage. The advantages of a trophectoderm biopsy are that the cells are less mosaic and more abundant to analyze, making the results more accurate. This review agrees with the outcomes of the study above: that trophectoderm biopsies are the best technique for pre-implantation genetic testing.[7]

In 2018, a study done by **Zanetti et al.** described the cases of PGT-M in fertile couples in a Brazilian IVF center and compared their results to those reported by the European Society of Human Reproduction and Embryology (ESHRE) Consortium PGD XIV–XV. They mentioned the prevalence of trophectoderm biopsies and how they have become a highly viable option due to the excellent survival rates in embryo culture. They obtained a 22.1% implantation rate, a 26.8% pregnancy rate, and a 7.1% miscarriage rate. These results were comparable with ESHRE consortium cycles. They conclude that PGT-M is a very feasible option for families affected by monogenic disease. [8]

Goldman et al. published a study in 2016 with the goal of understanding the benefits of concurrent testing for both monogenic disorders and aneuploidy in a population of patients undergoing blastocyst culture, trophectoderm biopsy, and single gene testing, with and without aneuploidy screening.There are two goals: first, to quantify the incidence of aneuploidy in a population of patients undergoing trophectoderm biopsy and PGD for a single gene disorder, to better understand the benefits of dual-testing and the potential risk of not performing concomitant aneuploidy screening, and second, to determine the risk of not performing concomitant aneuploidy screening. Most patients (53.2%) had at least one blastocyst that was unaffected by monogenic disease but were affected by aneuploidy. Without screening, these unaffected but aneuploid embryos would have been transferred, leading to implantation failure, pregnancy loss, or a pregnancy affected by chromosomal aneuploidy. Therefore, the obstetrical outcomes, such as the live birth rate, were higher and the abortion rate was lower for those who underwent concomitant aneuploidy screening. When performed together with PGD, aneuploidy screening offers useful information for selecting embryos and significantly improves the rate of single embryo transfer. This study concluded that a significant increase in single embryo transfer rates between the dual-screening group and the monogenic disease-tested group shows that concurrent screening significantly benefits embryo selection. [9]

A retrospective case-control study done by **Shen et al.** had the objective of determining the application value of next-generation sequencing (NGS)-based pre-implantation genetic testing for aneuploidies (PGT-A). They performed NGS-based aneuploidy screening on multiple displacement amplification (MDA) products of embryonic trophectoderm biopsy samples that were frozen after PGT-M with known transfer outcomes. A total of 76 embryos, which resulted in live birth after transfer, showed that if the patients had PGT-A during their PGT-M cycle, 17.1% of the embryos would have been wasted due to being diagnosed as affected. It can then be understood that Shen et al. along with their data**,** believe that PGT-A might cause more harm than benefit for patients undergoing IVF. [10]

In a review published in 2022, **Scriven et al.** explored the potential harmful effect of combining PGT-M with PGT-A. The most recent ESHRE PGT Consortium data were incorporated. Combining testing for unrelated sporadic chromosomal abnormalities (PGT-A) and excluding embryos with chromosomally abnormal results from transfer offers the opportunity to reduce the risk of miscarriage and the number of embryo transfers, but it also risks excluding healthy embryos from transfer due to abnormal test results that do not reflect the embryo's true potential. The data was divided into two categories in the review: high-success clinics and typical-success clinics. The clinic success groups are subjective and are based on the number of live births per embryo transplanted (high success is 60% PGT-MA vs. 50% PGT-M) and typical success is 45.6% PGT-MA (38.0% compared. PGT-M). Both success groups had identical miscarriage rates per clinical pregnancy. The PGT-MA live birth rate per embryo transplanted is higher (45.6% vs. 38.0%), demonstrating that testing for unrelated chromosomal gain or loss can help determine if an embryo is viable. All scenarios reveal that PGT-A reduces the chance of miscarriage just slightly at the expense of healthy live births. This study concludes that PGT-M without PGT-A is preferable for achieving an undamaged live birth. [11]

3. Rationale

From the early 1990s, preimplantation genetic diagnosis (PGD) and embryo biopsy became available: over the years, with the evolution of the technique, PGT-M has revolutionized the landscape of clinical genetics by decreasing the risk of transmitting a serious genetic disorder to patients offspring and allowing to potentially eliminate the disease from the population. With the term PGD we include three different tests: pre-implantation genetic testing for monogenic disease (PGT-M), Pre-implantation genetic testing for aneuploidy (PGT-A) and Pre-implantation for structural rearrangements (PGT-SR). PGT-M is used in patients who have or are carriers of a congenital disease that can be passed down to their offspring. Pre-implantation genetic testing for aneuploidy (PGT-A) is a procedure that screens embryos before transfer to reduce the risk of aneuploidy and chromosomal abnormalities. Pre-implantation for structural rearrangements (PGT-SR) is an option for patients with an inversion, reciprocal translocation, or Robertsonian translocation.

These approaches, when combined with in vitro fertilization (IVF), enable us to differentiate between affected and unaffected embryos. To achieve these biopsy results, many IVF laboratories perform trophectoderm cell biopsies (TE), which is the development of embryos up to the blastocyst stage, which occurs 5-7 days after fertilization. Prior to trophectoderm cell biopsies (TE), biopsies were performed on polar bodies or cleavage embryos.

It is still open to debate the disadvantage discussed in the scientific literature that affects the technique's efficacy due to the presence of mosaic embryos and whether it should be paired with PGT for aneuploidy (PGT-A) for more concrete results.

4. STUDY OBJECTIVES

The **primary objective** of this retrospective observational single-center study is to describe the efficacy of PGT-M in a real-life setting. Efficacy will be evaluated in terms of live birth rate (LBR), simple and cumulative live birth rate (CLBR) per couple, and abortion rate (AR). Furthermore, investigators will consider how many cycles the participants need to undergo to achieve a viable blastocyst.

The **secondary objective** is to evaluate the incidence of aneuploidy in tested embryos, in order to understand the need for PGTA in addition to PGTM.

3.1. Endpoints

For the evaluation of the study objectives, all patients that underwent PGTM from 2016 to 2022 will be

included in the study.

The results obtained from the PGT-M will be calculated as:

Live birth rate (LBR): defined as the "the number of deliveries that resulted in at least one live birth, divided by the number of cycles."

Embryology and Alpha Scientists in Reproductive Medicine. Electronic address [1]

Cumulative live birth (CLBR) per couple: defined as the "delivery of at least one live-born infant (>24 weeks of gestation) in the fresh or in the subsequent frozen-thawed cycles in relation to the number of oocytes retrieved per couple." Polyzos, Drakopoulos [2]

Abortion rate (AR): is defined as the "spontaneous demise of a pregnancy before the foetus reaches viability. The term therefore includes all pregnancy losses (PLs) from the time of conception until 24 weeks of gestation." Group, Vlaisavljevic [3]

Aneuploid embryo rate: "the most common genetic abnormality resulting in embryonic demise, pregnancy loss, and congenital birth defects. At the preimplantation stage, ~50% of the IVF-derived embryos have chromosomal abnormalities that are incompatible with implantation or normal fetal development." Hindi E. Stohl [4]

4. STUDY POPULATION

4.1. Inclusion criteria

The study will include all fertile or infertile patients undergoing PGT analysis for monogenic diseases.

4.2. Exclusion criteria

No group will be excluded from this study.

4.3. Recruitment

The study sample will include retrospectively all patients who have undergone PGTM, matching the inclusion and exclusion criteria cited above. Data of children included in the study are retrieved from the database of Reproductive Medicine Unit (Fertility Center) in which their mothers information about the IVF cycle and results of PTGM are recorded.

5. STUDY ASSESSMENT

The study is not associated with any adverse effects or risks.

6. STATISTICAL CONSIDERATIONS

6.1. Sample size

For the purpose of the study all the children born from single thawed embryos in Fertility Center from the beginning to 2016, will be included in the study, and the sample is 567.

6.2. Analysis

Data will be described as number and percentage, if categorical, or mean and standard deviation, if continuous and approximately Gaussian, or with median and range, otherwise. Adherence to Gaussian distribution will be checked with Shapiro Wilks test.

7. QUALITY ASSURANCE AND CONTROL

7.1. Data handling and record keeping /archiving

The investigator must keep the documents on file for at least 7 years after completion or discontinuation of the study. After that period, the documents may be destroyed, subject to local regulations. Before proceeding to documents' destruction, sites must inform the Coordinating Investigator/delegate in writing.

Should the investigator wish to assign the study records to another party or move them to another location, the Coordinating Investigator/delegate must be notified in advance.

7.2. Case Report Forms

Data will be collected and included in a Microsoft Excel sheet. For each patient negative or affirmative answer will be registered for each of the 567 cycles. The investigator at each site will ensure the accuracy, completeness, and timeliness of the data. The data will be collected anonymously, and patients will be identified in the database with successive numbers. Participants will not be identified in the CRF by name or initials and birth date and appropriate coded identification will be used.

7.3. Source documents

Source data must be available at the site to document the existence of the study participants. Source data include the original documents relating to the study, as well as the medical treatment and medical history of the participant.

8. CONFIDENTIALITY OF PATIENT RECORDS

The investigator assures that patients' anonymity should be maintained and that their identities are protected from unauthorized parties. Particular attention should be paid whenever patient data are supplied to third parties and may be autonomously processed.

The investigator should keep in a confidential way a patient identification log recording both patient code and name. The investigator should also maintain patients' written consent forms, in strict confidence (i.e., not for submission to the Coordinating Investigator).

Any investigator and/or research staff member who has a conflict of interest with this study (such as patent ownership, royalties, or financial gain greater than the minimum allowable by their institution) must fully disclose the nature of the conflict of interest.

9. ETHICAL CONSIDERATIONS

The responsible investigator ensures that this study is conducted in agreement with this protocol, the Good Clinical Practice, the current version of Declaration of Helsinki and the applicable regulations.

The protocol and any amendments are subject to review and approval by the competent Independent Ethics Committee(s) ("IEC").

10. INFORMED CONSENT

Documented informed consent to access patients' information after fertility treatment for research purposes has been obtained before fertility treatment. Informed consent signed by the patient allows the staff of Fertility Center to recontact telephonically patients to collect further information about pregnancy evolution and follow up. The written informed consent form has been signed and personally dated by the patient or by the patient's legally acceptable representative, and by a medical doctor employed in the Fertility Center.

Moreover, during recruitment, all patients will be informed of the aims of the study. Verbal consent will be collected to gather information about the children considered; the patient is free to deny consent to answer all or any question. Participation is voluntary and does not prejudice the patient's subsequent care.

Patients will be informed as to the strict confidentiality of collected data.

11. DATA OWNERSHIP

Istituto Clinico Humanitas is the owner of the data resulting from the study. All centers and investigators participating in the study should be made aware of such circumstances and not disseminate information or data without the prior written consent by Istituto Clinico Humanitas.

12. PUBLICATION POLICY

After completion of the study, the Coordinating Investigator prepares a draft manuscript containing results of the study based on the statistical analysis. The manuscript is delivered to the co-authors for comments and then sent to a scientific journal for publication.

All publications, abstracts, presentations, manuscripts, and slides - issued by the Investigators of the collaborative sites and including data from the present study- should be submitted to and reviewed by the Coordinator Investigator at least 3 (three) weeks in advance the planned date for the submission to the scientific journal.

13. FUNDING AND SUPPORT

NA

15. REFERENCES

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11. Scriven, P.N., *Combining PGT-A with PGT-M risks trying to do too much.* J Assist Reprod Genet, **2022**. 39(9): p. 2015-2018.