Non-invasive preimplantation genetic testing (niPGT) - haplotyping by sequencing embryo spent culture medium

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1. Develop non-invasive PGT based on cfDNA in SCM (i.e. waste material) of IVF embryos generated for PGT-M and PGT-SR.2. Develop non-invasive PGT for aneuploidy origin (niPGT-AO) to assess the rate of aneuploidies and the origin and assess the...

Ethical review	Approved WMO
Status	Recruiting
Health condition type	Chromosomal abnormalities, gene alterations and gene variants
Study type	Observational non invasive

Summary

ID

NL-OMON51854

Source
ToetsingOnline

Brief title
non-invasive preimplantation genetic testing (niPGT)

Condition

- Chromosomal abnormalities, gene alterations and gene variants
- Pregnancy, labour, delivery and postpartum conditions

Synonym
chromosomal anomalies, monogenic diseases

Sponsors and support

Primary sponsor: Medisch Universitair Ziekenhuis Maastricht
Source(s) of monetary or material Support: Ministerie van OC&W

Intervention

Keyword: aneuploidies, chromosomal anomalies, monogenic disorders, preimplantation genetic testing (PGT)

Outcome measures

Primary outcome

1. Developing non-invasive PGT based on cfDNA in SCM for monogenic disorders or structural rearrangements.

• Study parameters are haplotypes present in SCM of human preimplantation embryos.

• Endpoints are percentage of invalid results, concordance rates, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) for genetic indication of interest and the haplotypes in niPGT compared to haplotypes in conventional PGT.

2. Developing niPGT-AO to assess the rate of aneuploidies and its origin and assess the predictive value of aneuploidy origin for a healthy baby using pregnancy outcomes.

• Study parameters are aneuploidy and its origin in the SCM and in the routinely taken TE biopsy i.e. cellular (ICM/TE) and segregational (mitotic/meiotic) origin, clinical pregnancy outcomes e.g. implantation rate, live birth rate.

• Endpoints are percentage of invalid results, concordance rates between SCM and TE biopsy, rate of mitotic and meiotic aberrations, the origin of the SCM, aneuploidy origin profiles for healthy babies and aneuploidy origin profiles

for implantation failures.

Secondary outcome

not applicable

Study description

Background summary

The rationale for this study is to develop a new methodology for preimplantation genetic testing (PGT) that enables non-invasive PGT for monogenic disorders (PGT-M) and structural rearrangements (PGT-SR) and enables PGT for aneuploidy origin (PGT-AO) for embryo transfer ranking. We propose to further develop our haplotyping-based PGT for cell-free DNA (cfDNA) from spent culture medium (SCM) from an embryo. By developing this non-invasive PGT (niPGT) method further, it has the potential to eventually replace the current PGT methodology where an (invasive) embryo biopsy is needed. A non-WMO approval by the METC aZM/UM (2020-1611) was previously obtained for reanalysis of only the affected embryos (as inferred from conventional PGT) and for chromosomes involved in the PGT indication. Here, we will further validate our genome-wide haplotyping niPGT approach using SCM from both unaffected and affected embryos and test for aneuploidy origin in the SCM and in biopsies from conventional PGT cases.

Moreover, we will develop a method for PGT-AO in the SCM determining the rate of meiotic/mitotic aneuploidies. Importantly, the proposed strategy is non-invasive and makes use of data and materials (SCM and clinical data from pregnancy) that are obtained in routine care. To develop and test the PGT-AO approach, we will merely use embryos that have undergone PGT-M using the haplotyping-based sequencing analysis or PGT-SR using the VeriSeq analysis. Sequencing data is already generated in conventional PGT, but here only the indication of interest is investigated. We will generate the aneuploidy origin information from this sequencing data from TE biopsies obtained in conventional PGT and from the sequencing data from the SCM. We will evaluate the predictive value of the aneuploidy origin in TE and SCM for a healthy pregnancy and baby. Based on the knowledge generated in this study, we could eventually create an embryo transfer ranking system which could help to increase the success rate per embryo transfer by first selecting the *best* embryo based on aneuploidy origin.

Study objective

1. Develop non-invasive PGT based on cfDNA in SCM (i.e. waste material) of IVF

embryos generated for PGT-M and PGT-SR.
2. Develop non-invasive PGT for aneuploidy origin (niPGT-AO) to assess the rate of aneuploidies and the origin and assess the predictive value of aneuploidy origin using pregnancy outcomes like implantation, miscarriage, live birth rate.

Study design

This is an exploratory, diagnostic study that makes use of IVF waste material, i.e. SCM samples, and IVF data of human preimplantation embryos (d3 and d5/6) from couples that undergo an IVF/PGT treatment and clinical information about resulting pregnancies. Following routine PGT, we will collect SCM samples and store them at -20 ºC in the IVF laboratory, MUMC+. At the time of processing, the SCM will be thawed and whole genome amplification (WGA) will be performed. The amplified products will be whole genome sequenced and analyzed using our haplotyping-based PGT approach. To this end, we will compare the genome of preimplantation embryos that underwent conventional PGT with SCM-derived niPGT. We will examine the genetic indication of interest and we will examine origin of aneuploidies. Furthermore, epigenomic profiling will be performed to allocate the origin of the cfDNA to either inner cell mass (ICM) or trophectoderm (TE) and to account for maternal contamination. In addition, we will link the epigenomic to genomic information of SCM of those embryos. The data generated will be linked to clinical pregnancy outcomes, such as implantation rate and miscarriage rate, live birth rate.

Study burden and risks

Participation in the study will not involve any risk or additional burden. No additional visits, physical examinations, tests, blood samples other than required for IVF/PGT are needed. There are no physical risks as the SCM will be collected after conventional PGT biopsy procedures are performed and SCM is considered as waste material. In this study we do not create or destroy any IVF embryo other than for conventional PGT treatment.

With our niPGT approach, we will investigate whether the result for PGT-M or PGT-SR in the SCM is concordant with conventional PGT. In a previous study, we only investigated the affected embryos (non-WMO 2020-1611). Here, we will investigate the SCM of both affected and unaffected embryos as has been assessed by conventional PGT. Besides, we will also investigate the genome-wide aneuploidy origin. Moreover, we will perform the genome-wide aneuploidy origin as an extra chromosomal test on the TE biopsy compared to standard care. In this study, we will develop PGT-AO that will elucidate the cellular origin of aneuploidies and the segregational (meiotic/mitotic) origin. Since the technique we will develop for non-invasive PGT is new and not yet well-established, all samples and data will be provided with a non-traceable code. Moreover, the results from the PGT-AO and niPGT will not be discussed

with the PGT couples. Moreover, the clinical significance of finding mitotic/meiotic aneuploidies in TE biopsies as well as the representative value of cfDNA in the SCM for the embryos* genetic constitution remains unclear. This is to be explored and falls within the scope of this study.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Inclusion criteria

Group inclusion criteria:
To be eligible to participate in this study, a couple must meet all of the following criteria:
Couples that have or are carrier of a known severe genetic disorder that choose to do in vitro fertilization (IVF) following preimplantation genetic testing (PGT-IVF). Inclusion criteria of couples are similar to inclusion criteria from PGT that are the following:
o The offspring have a high risk on inheriting a severe genetic disorder (as nationally determined by national indication committee); PGT-M.

o There is a high risk of miscarriage due to an unbalanced translocation or a high risk of an ongoing pregnancy of a child with an unbalanced translocation (PGT-SR).
• Couples meet the requirements for IVF.
• The comprehensive, sequencing-based haplotyping analysis will be used for analysis of PGT-M
• The VeriSeq PGS analysis method is used for PGT-SR.

Sample inclusion criteria:
• Oocytes are successfully fertilized and develop into d3 embryos or blastocysts (d5/6/7 embryos).
• Embryos are successfully biopsied and the conventional PGT result is conclusive.
• Spent culture medium samples from the PGT-IVF embryos could be collected and stored.

Exclusion criteria

Group exclusion criteria:
A potential subject who meets any of the following criteria will be excluded from participation in this study.
• Couples unable to give informed consent to any of the study aspects or unable to comply with the protocol
o When participants are < 18 years
o Couples that do not speak Dutch will be excluded from participation in this study.
• IVF/PGD treatment for a mitochondrial disorder (linked to mtDNA).
• The analysis of the embryos is done using a day 3 biopsy and subsequent fluorescence in situ hybridization (FISH) analysis.
• The analysis of the embryo is done using the PCR-based STR marker analysis method.
• It is technically not possible to test the preimplantation embryo for the genetic disorder, as determined by the preparation tests for PGT.

Sample exclusion criteria:
A potential sample who meets any of the following criteria will be excluded from participation in this study.
• A specific sample will be excluded for analysis when not enough (< 10 µl) or no spent culture medium of the embryo could be collected. In case of no spent culture medium, the other SCM samples from the same PGT couple will still be analyzed.

Study design

Design

Study type: Observational non invasive

Masking:	Open (masking not used)
Control:	Uncontrolled
Primary purpose:	Diagnostic

Recruitment

NL

Recruitment status:	Recruiting
Start date (anticipated):	19-07-2023
Enrollment:	317
Type:	Actual

Ethics review

Approved WMO Date:	23-01-2023
Application type:	First submission
Review commission:	CCMO: Centrale Commissie Mensgebonden Onderzoek (Den Haag)

Approved WMO Date:	09-09-2024
Application type:	Amendment
Review commission:	CCMO: Centrale Commissie Mensgebonden Onderzoek (Den Haag)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL80866.000.22