DISCLOSURE RELATING TO THE PERFORMANCE OF NON-INVASIVE PRE-IMPLANTATION GENETIC TESTING FOR THE SCREENING OF CHROMOSOMAL ANEUPLOIDIES (niPGT-A)

Definition and purpose of non-invasive Preimplantation Genetic Testing (niPGT-A)

Non-invasive Preimplantation Genetic Testing for Aneuploidies (niPGT-A) is aimed at assessing embryonic chromosomes prior to transfer, without the need for an embryonic biopsy. A set of human chromosomes usually consists of 46 chromosomes divided into 23 pairs: 22 pairs are represented by autosomes (non-sex chromosomes which are the same for males and females) and one pair of sex chromosomes consisting of two XX chromosomes in females and one X chromosome and one Y chromosome in males. Alterations in the number and structure of chromosomes can have an immediate impact on embryos, leading to failure of implantation or stunted development. Occasionally, however, an embryo affected by severe chromosome abnormalities is able to implant itself in the uterus and result in a pregnancy that subsequently miscarries. Screening for embryonic chromosomal abnormalities aims to increase the effectiveness of medically assisted procreation (MAP) and reduce the risk of chromosomal disorder in pregnancies and after birth.

Non-invasive Preimplantation Genetic Testing for Aneuploidies (niPGT-A), recently introduced into in vitro fertilisation, is performed by analysing free embryonic DNA fragments (cfeDNA) released into the culture medium during the cleavage and blastocyst stages (Hammond et. al. 2016, Kuznyetsov et. al. 2020, Rubio et al 2019). This investigation is an alternative to the traditional standard (invasive) PGT methods performed on embryonic DNA obtained through biopsy at the cleavage stage (day 3) or blastocyst stage (day 5).

Couples who are candidates for niPGT-A will first undergo a MAP cycle in which female and male gametes (oocytes and sperm) are collected and fertilised in vitro where the first stages of embryonic development take place. The test is performed by taking the culture medium of the embryo at the blastocyst stage (on day 5, 6 or 7 after fertilisation) and then the cfDNA inside is analysed following a specific protocol in order to determine the presence of any aneuploidies.

The aim of niPGT-A is to assess the set of chromosomes, identifying embryos that are more likelyto be euploid and those that are more likely to be aneuploid. The data supplied by literature indicate that the transfer of euploid embryos can reduce the time required to achieve pregnancy, although it does not increase the overall likelihood of pregnancy occurring.

Indications

niPGT-A can be performed in every MAP cycle without specific medical indication and in order to optimise the MAP cycle.
niPGT-A is indicated under certain circumstances, particularly in the following cases:

- Advanced maternal age (AMA): couples in which the female partner is conventionally older than 35. This specific indication is suggested by the numerous studies carried out on blastocysts analysed in groups of women of different ages undergoing MAP with PGT-A, in which the correlation between advanced maternal age and the number of aneuploid embryos was proven (Franasiak et al. 2014; Munné et al. 2017).
- Repeated implantation failure (RIF): couples who have undergone the transfer of three or more morphologically good quality embryos without successful implantation (absence of gestational sac visible with ultrasound five or more weeks after embryo transfer).
- Recurrent abortions: couples who have experienced repeated (three or more) first-trimester miscarriages in the presence of a normal karyotype and in the absence of "mechanical" causes such as abnormalities of the uterus or other recognised causes.
- Sperm abnormalities: couples in which the male partner has severe oligoasthenoteratospermia, cryptospermia or non-obstructive azoospermia, factors that require the use of MESA (Microsurgical Epididymal Sperm Aspiration) and TESE (TEsticular Sperm Extraction) techniques to retrieve sperm from the seminal tract.
- Patients attempting their first MAP cycle can use niPGT-A to increase the probability of implantation and reduce the time to pregnancy.

Diagnostic methodology

Collection of the culture medium.
Unlike the standard PGT procedure, niPGT-A does not require any manipulation of the embryo, but uses the culture medium in which the embryo grows during development *in vitro* and into which it releases fragments of DNA. The medium is collected in the embryology laboratory of the MAP centre on the fifth, sixth or seventh day of development.
Whole Genome Amplification (WGA)
niPGT requires total amplification of cfeDNA (known as whole genome amplification - WGA), which allows the amplification of the embryonic genome millions of times in order to obtain a suitable amount of DNA for subsequent analysis. For the application of PGT, WGA is performed using the Ion SingleSeq Kit (Thermo Fisher Scientific).

Massively Parallel Sequencing
The analysis of the entire chromosomal copy number of the embryo is performed by massively parallel sequencing (MPS) using the Ion GeneStudio S5 Plus instrument (Thermo Fisher Scientific) with the Ion Reproseq protocol

(Thermo Fisher Scientific). The chromosomal sequences obtained through MPS are then quantified by means of bioinformatic analysis, which allows screening for aneuploidies on all 24 chromosomes, and the data obtained are analysed using the Ion Reporter Software platform (Thermo Fisher Scientific).

Clinical validation of the niPGT-A method

niPGT-A was developed within the Eurofins Genoma laboratory and the clinical validation of the method was conducted on more than 500 embryos by comparing the results obtained on embryo cfeDNA samples with the results obtained from trophoectoderm (TE) biopsy. The comparison resulted in a concordance of up to 86.1% between the traditional PGT-A method and the niPGT-A method (Table 1).

TABLE 1. PERFORMANCE OF NIPGTA VS PGT-A ANALYSIS WITH EMBRYO TROPHECTODERM BIOPSY COMPARISON.

Culture times for cfeDNA release into the medium	Day 3-5	Day 3-6	Day 4-6, 7
Media analysed (Number of samples)	154	180	185
cfeDNA ploidy concordance* with TE biopsy (%)	72.6%	84.8%	86.1%
Sensitivity	83.61%	90.91%	93.04%
Specificity	52.94%	65.71%	63.89%

*CONCORDANCE: Concordance is simply the fraction of data pairs that behave as expected (assuming there are no links in the relative risk values between pairs of observations).

This is one of the highest concordance values obtained in relation to the concordance rates published so far in literature (Rubio et. al. 2020).

Results of niPGT-A

niPGT-A makes it possible to classify embryos in such a way as to determine their priority for transfer to the maternal uterus. Priority is defined on the basis of the probability of having a euploid set of chromosomal after analysis of the embryonic cfDNA in the culture medium. Embryos that are more likely to be euploid will be given priority for transfer. niPGT-A can result in the following outcomes:

- **PRIORITY 1:** Embryos with a high probability of having a normal or euploid set of chromosomes and consequently a high probability of implantation. These embryos are prime candidates for transfer.
- **PRIORITY 2:** Embryos with a high probability of having an altered or **partial aneuploid** set of chromosomes, so with a low probability of implantation and a high risk of miscarriage. These embryos are second in line as candidates for transfer.
- **PRIORITY 3:** Embryos with a high probability of having an altered or **single monosomy**, so with a low probability of implantation and a high risk of miscarriage. These embryos are third in line as candidates for transfer.
- **PRIORITY 4:** Embryos with a high probability of having an altered or **Trisomy/complex aneuploid (aneuploidy of ≥ 3 chromosomes)** so with a low probability of implantation and a high risk of miscarriage. These are the last embryos considered for transfer.

- **No Result or Inconclusive Result**: This result is obtained when there is insufficient cfeDNA in the culture medium or when the laboratory procedure has yielded results of doubtful interpretation. In the event of failed diagnosis, an embryo biopsy or assessment of the transfer of the embryo based on its morphology is indicated.

Reporting times

The test results are available within 7-10 working days of acceptance of the sample. These deadlines are not, however, peremptory and it may take longer to obtain the results in the case of further diagnostic or interpretative doubts. For niPGT-A to be carried out, the test request sheet and informed consent must be duly completed and signed. In the event of missing information, the laboratory will contact the forwarding doctor/clinic or the persons concerned to obtain this information. This may change the time for processing the sample and issuing the report.

The benefits of niPGT-A:

- niPGT-A helps to identify embryos that are more likely to be euploid, and therefore have a higher probability of implantation with the aim of improving the success rate of in vitro fertilisation.
- The niPGT-A method does not require embryo biopsies and could reduce the costs of MAP improving application times.
- It is a harmless method as it requires no embryo manipulation.
- It offers a further opportunity to classify embryos beyond simple morphological assessment.

Limits of the procedure:

- The concordance rate between PGT-A and niPGT-A performed on days 4/6 is 86.1%. International literature reports a concordance rate of 78.2% (Rubio et al 2020).
- niPGT-A is a screening test, not a diagnostic test. Consequently, it does not exclude genetic testing during pregnancy, which should be discussed during dedicated prenatal genetic counselling.
- The procedure requires the change of the drop in the medium (either sequentially or using single-formulation media) on day 4 and the ideal period of medium collection is from day 4 to day 6.
- niPGT-A can only be performed in the case of deferred MAP cycles, with freezing after sample collection.
- niPGT-A is not applicable if there are indications for performing PGT-SR or PGT-M.
- If the MAP laboratory uses a time-lapse system, single-well plates must be used for each embryo culture.
- Contamination (maternal or external origin) of the medium analysed could lead to failure of the test as well as diagnostic error if the contamination is not detected.
- Due to the phenomenon of mosaicism, an embryo could present both chromosomally normal and altered cells. As a consequence of this phenomenon, the sample analysed and the embryo it comes from could be misdiagnosed as normal in the case of aneuploidy or as abnormal in the case of euploidy.

- niPGT-A may provide no result or an inconclusive result if the quantity or quality of cfeDNA does not reach the required standard. In the case of failed diagnosis, a PGT-A using the traditional method on an embryo biopsy may be suggested. It is possible that PGT-A/SR testing will not identify any euploid and/or balanced embryos.
- The test is unable to highlight an increased risk of:
  - balanced chromosomal rearrangement
  - uniparental disomy
  - unbalanced chromosomal rearrangement if pseudoautosomal regions of X and Y chromosomes or heterochromatic regions are involved (e.g., pericentromeric regions, short arms of acrocentric chromosomes, etc.)
  - chromosomal regions that are not represented in the platform
  - chromosomal rearrangements (except rearrangements involving the whole arm [long arm or short arm] of a chromosome)
  - DNA sequence (point) variants
  - Methylation defects
  - Polyploidies
- The niPGT-A protocol requires the use of the entire sample of the medium. Consequently, in the event of failed diagnosis, the medium cannot always be recovered and an embryo biopsy or assessment of the transfer of the embryo based on its morphology is indicated.

Genetic Consultation

Eurofins Genoma offers a genetic consultancy service with its team of pre- and post-niPGT-A geneticists for both patients and IVF centres.

References:

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CONSENT TO THE PERFORMANCE OF NON-INVASIVE PRE-IMPLANTATION

GENETIC TESTING (niPGT-A)

*I, the undersigned (male partner)	
*Place of birth	*Date of birth
*Tax Code:	
*Resident in:	*Address:
*Phone:	Email:
*Document	*No.
*Issued on	*by
*I, the undersigned (female partner)	
*Place of birth	*Date of birth
*Tax Code	
*Resident in:	*Address:
*Phone:	
*Document	*No.
*Issued on	*by

*The information marked with an asterisk is compulsory

With a view to undergoing an ICSI cycle (in vitro fertilisation with intracytoplasmic sperm injection) at the Centre for medically assisted procreation (MAP), with subsequent collection of culture medium for the purpose of embryonic cfeDNA testing, we hereby declare that we have read the disclosure annexed to this consent form in its entirety, that we have fully understood its contents, and that we have received all the detailed information, both on the methods and on the diagnostic success and error rates.

We declare that we have already had one or more interviews with the staff of the MAP centre and/or the Eurofins Genoma Group laboratory during which all the points of the above-mentioned disclosure were explained to us and we were able to ask the necessary questions and receive the corresponding answers.

I / WE (tick the box as appropriate) **CONSENT**
to the performance of the following analysis: ☐ **niPGT-A** on the culture medium in which our embryos are grown.

We also declare that we have been informed of the following aspects:

Definition of the roles and scope of the collaboration

The Eurofins Genoma laboratory is a laboratory specialised in molecular biology and genetics, authorised by the Municipality of Rome, prot. no. 14965 dated 19.03.2003, to carry out molecular genetic diagnoses.
The Eurofins Genoma laboratory does NOT perform Medically Assisted Procreation techniques, regulated by Italian law no. 40 dated 19 February 2004.
The Eurofins Genoma laboratory is organised to carry out specialised genetic examinations for third party facilities, operating as a reference service provider.
The MAP Centre requested the specialised support of the Eurofins Genoma laboratory for the optimisation and performance of genetic tests on culture medium in which the embryos derived from the MAP cycle were grown.

Limits of liability

The Eurofins Genoma laboratory does not produce embryos using Medically Assisted Procreation techniques. This procedure will be carried out in the laboratories of the Medically Assisted Procreation Centre, by technicians/biologists employed by said centre.
The Eurofins Genoma laboratory is responsible exclusively for the results of the genetic test carried out on the culture medium, and has no responsibility for the work of the MAP Centre.

Cost of the activity performed

The cost of the activity relating to niPGT-A is indicated in the quote presented to patients by Eurofins Genoma staff or by the staff of the Medically Assisted Procreation Centre.
The cost of medically assisted procreation techniques (MAP cycle and harvesting of the culture medium) are to be agreed in full with the Centre for Medically Assisted Procreation to which reference should be made.

Place and date_____

Name of the female partner _____

Signature_____

Name of the male partner_____

Signature

_____

The Specialist who obtained the consent:

Name: _____Surname:_____

Tel._____E-Mail_____

Signature and stamp of the Specialist: _____