**Instructions and Informed Consent with Preimplantation Genetic Analysis**

*Dear Clients,*

*Thank you for your trust and we are pleased that you have decided to undergo preimplantation genetic analysis in conjunction with your IVF cycle. Please read these instructions carefully and sign this informed consent form in witness of your consent to the information stated below.*

1. **Identification data**

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| --- | --- |
| **Patient:** |  |
| Name and surname: |  |
| Date of birth/insurance No. (DOB): |  |
| Address: |  |
| **Partner:** |
| Name and surname: |  |
| Date of birth/insurance No. (DOB): |  |
| Address: |  |

1. **Reason for the analysis:**

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1. **Type of preimplantation genetic analysis**

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| * **PGT-A of 24 chromosomes** | **NGS** |  |
| * **PGT-A of 5 chromosomes (13, 18, 21, X, Y)** | **FISH** |  |
|  | | |
| * **PGT-SR for familial chromosomal aberration + PGT-A of 24 chromosomes** | **NGS** |  |
| * **PGT-SR for familial chromosomal aberration** solely or withsupplementary **PGT-A of 2 chromosomes (13, 21)** or with supplementary **PGT-A of 3 chromosomes (18, X, Y)** | **FISH** |  |

**Abbreviations: PGT-A**- ***P****reimplantation* ***G****enetic* ***T****esting of numerical chromosomal changes (****A****neuploidies);* **PGT-SR**- ***P****reimplantation* ***G****enetic* ***D****iagnosis/****T****esting for familial* ***S****tructural chromosomal aberration/****R****earrangement –* ***diagnosis can be performed only upon prior consideration/performance of SET-UP****;* **FISH** – ***F****luorescent* ***I****n* ***S****itu* ***H****ybridisation;* **NGS** *–* ***N****ext* ***G****eneration* ***S****equencing.*

1. **Diagnosed material**

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| --- | --- | --- | --- | --- |
| 🞎 | Trophectoderm (blastocyst) – day 5/6 |  | Other: | ................................................ |

1. **Purpose of the preimplantation genetic analysis**

Preimplantation genetic analysis is a set of laboratory methods which make it possible to analyse the genetic makeup of embryos arising from in vitro fertilisation (IVF) before their introduction (transfer) into the uterus. Currently there is no method of analysing the genetic makeup of embryos and detecting any chromosomal abnormalities which could just be based on the morphological analysis of embryos. Genetic analysis is performed after the extraction (biopsy) of one or more cells in the early stages of the development of the embryo. Preimplantation genetic analysis involves the analysis of the number and structure of chromosomes or a targeted analysis of individual genes/sequence of DNA. The chromosomes are stored in a cell nucleus and are made up of a spiral molecule of deoxyribonucleic acid (DNA), which carries the unique genetic information (genes) of each individual. Each cell body contains a set of 23 pairs of chromosomes (46 chromosomes). Any change in the number and/or structure of the chromosomes can mean a change in the amount of genetic material resulting in physical and/or mental development disorders and/or further health problems. Changes in chromosomes or individual genes can be inherited from parents (carriers of chromosomal aberrations or gene mutations), but can also newly (so-called *“de novo”)* appear in a sex cell of one of the parents or more rarely in the developing embryo.

1. **Characteristics of the sample collection, type of analysis and analysis methods** 
   1. **Sample collection (biopsy)**

During the biopsy non-differentiated cells whose removal will not affect the further development of the embryo are extracted from the developing embryo.

The biopsy can be performed:

* 3rd day of embryonic development, when 1-2 cells (blastomeres) are carefully extracted out of a 6-10 cell embryo,
* 5th-6th day of embryonic development (blastocysts), when several trophectoderm cells are carefully extracted. From the trophectoderm the placenta later develops during pregnancy so this is not an intervention into the tissue of the future foetus.

Once the biopsy is performed the embryo is immediately returned to the incubator. A sample of the extracted cells is not independently viable and is immediately submitted for genetic analysis.

* 1. **Preimplantation Genetic Testing/Screening for Aneuploidy (PGT-A)**

PGT-A is complex analysis which can rule out the deviations in the number of whole chromosomes or their parts (aneuploidies), and reduce the risk of miscarriage or birth of a child with a genetic defect. In some couples the increased risk of conception of embryos with abnormal chromosomal complement significantly reduces the chance of getting pregnant and birth of a healthy child.

PGT-A is suitable especially for couples with the following indications:

* advanced maternal age (> 35 years);
* recurrent miscarriages (twice and more);
* delivery or abortion of a foetus with a chromosomal abnormality;
* repeated failure of implantation after previous embryotransfers (twice and more),
* significantly worse spermiogram parameters (presence of pathological forms of sperm) in the partner,
* use of sperm after TESE (Testicular Sperm Extraction) or MESA (Microsurgical Epididymal Sperm Aspiration) for IVF,
* condition after cancer treatment by chemotherapy or radiation in one or both partners.

Currently we usually perform PGT-A using next generation sequencing (NGS), but it might be performed also by fluorescent *in situ* hybridization (FISH).

* 1. **Preimplantation Genetic Diagnosis (PGT-M, PGT-SR)**

PGT-M and PGT-SR is a targeted diagnosis/testing of a specific familial genetic “disorder” – either of familial monogenetic disease (PGT-M) or familial structural chromosomal aberration/rearrangement (PGT-SR). These disorders are passed on with a varying degree of risk from generation to generation and can result in several family members being affected or in recurrent miscarriages. For this reason preimplantation genetic diagnosis is always performed only upon indication of a clinical geneticist after careful examination of the family history.

Referral reason for PGT for familial chromosomal rearrangement (PGT-SR) is:

* A carrier status of so-called “balanced” chromosome rearrangement (usually translocation) in one or both partners. In the sex cells of the carriers of balanced chromosomal changes there is a risk of formation of so-called “unbalanced” forms of these rearrangements. Unbalanced rearrangements then result in recurrent miscarriages or the birth of an affected child. Embryos without unbalanced rearrangement (i.e. healthy or balanced) are recommended for transfer.

Usually is among PGT-SR also included analysis for:

* The presence of numerical changes of sex chromosomes (gonosomes), including the mosaic form, in one or both partners. The mosaic form marks the state when two (or more) cell lines with a different chromosomal complement appear in an individual, in this case a line with a disorder in the number of gonosomes together with a line with the normal number of gonosomes. Numerical changes in gonosomes can result in unbalanced gametes, i.e. in miscarriages or the birth of an affected child. Embryos with a normal complement of sex chromosomes are recommended for transfer.

Indication for PGT for monogenic disease (PGT-M) is:

* A carrier status, i.e. also a risk of transfer, of a serious genetic disease which is caused by the disorder of a single gene (so-called “monogenic disease”).

Currently we perform PGT for chromosomal aberrations (PGT-SR) using next generation sequencing (NGS) or by the Fluorescent *In Situ* Hybridisation (FISH). Examination of monogenic diseases (PGT-M) is organized in cooperation with referral laboratories.

* 1. **Next Generation Sequencing (NGS) Method**

NGS method can be used for preimplantation genetic screening of numerical chromosomal changes (PGT-A) or for preimplantation genetic testing of unbalanced forms of familial rearrangements (PGT-SR) associated with the screening of numerical changes of other chromosomes. NGS is the newest method of genetic testing, with great potential for further development and wide use for all types of genetic testing, including the possibility of combining them in a single experiment.

If analysis is done on trophectoderm sample, NGS due to its higher sensitivity of quantification, improved dynamic range of changes and better determination of signal vs noise can more reliably assess mosaic chromosomal findings (than previously used analysis by array comparative genomic hybridization (aCGH)). Finding of chromosomal aberration/s in mosaic signifies simultaneous presence of euploid (normal) and aneuploid (abnormal) cell lines in the corresponding embryo. Such findings are always related with *de novo* arisen (sporadic) chromosomal aberration in frame of PGT-A analysis. In situation when there is no (left) embryo with normal finding and after appropriate genetic counselling and upon written patient consent, transfer of embryo with mosaic aberration/s might be considered.

Before the examination by NGS method, DNA is isolated from the collected cells and amplified by whole genome amplification (WGA). From successfully amplified samples are enzymatically prepared so-called "libraries", suitable for simultaneous "reading" of many DNA sequences. Each sample / library has a unique label which allows to analyse in one experiment DNA of more subjects (embryos) at the same time. Samples / libraries are mixed in the same ratio and the resulting library is then ready for final sequencing. Proper sequence reading (sequencing) is carried out by stepwise synthesis of new DNA strands complementary to the read fragments. By special software, read sequences are compared with normal human genome and their genomic position is determined. After allocating the sequences to the individual samples, final quantitative assessment of all chromosomes is performed.

Used type of NGS (low-pass whole genome sequencing) is limited by the size of chromosomal rearrangements. Small chromosomal losses or gains cannot be detected and also any other disorders or foetal developmental defect that are not caused by a change in the number of diagnosed chromosomes or their greater parts cannot be ruled out.

* 1. **Fluorescent *In Situ* Hybridisation (FISH) Method**

Analysis by the FISH method is currently used for preimplantation genetic screening (PGT-A) of selected chromosomes (usually for 5 the most common whole chromosomal aberrations) and for preimplantation genetic diagnosis of unbalanced forms of familial rearrangements (PGT-SR) of a small extent where the NGS method cannot be applied.

Cell nuclei fixed to microscopic glass slide are analysed. The principle of the method is the labelling of several specific chromosomal regions by a fluorescently marked probe complementary to the DNA section concerned. The evaluation is performed by a fluorescence microscope. The method can be used to determine only numerical changes of selected chromosomes, their parts respectively, marked by a chosen fluorescence probe. When carrying out PGT-SR to detect unbalanced forms of familial chromosomal rearrangement it involves a part of the chromosomes participating in this aberration. PGT-SR using the FISH method usually precedes the chromosomal analysis of parents to verify the accuracy of the proposed combination of the fluorescence probes (so-called “set-up”).

FISH cannot rule out any other diseases or foetal developmental defects not caused by a change in the number of diagnosed chromosomal regions.

1. **Risks of preimplantation genetic analysis**
2. Basic conditions, above all a sufficient number of good quality and developing embryos, must be observed for performing preimplantation analysis. The definitive conditions for performing an analysis are determined individually depending on the nature of the analysis (screening, specifically analysed disease or abnormality).
3. If there is a proven abnormal genetic finding in all analysed embryos, no embryo will be recommended for transfer to the uterus.
4. A series of experiments on an animal and human model, as well as the long-term application of the method in clinical practice have not shown that a careful biopsy of polar bodies, 1-2 cells (blastomeres) or biopsy of the trophectoderm in good quality well developing embryos in itself had caused bad embryonic development or a further developmental defect and/or diseases in the foetus. Currently there are no known scientific studies showing increased risk of these defects after preimplantation diagnosis. The state when a biopsy stops embryonic development still during laboratory cultivation occurs with a frequency of less than 1%. However given the biological nature of reproduction, it cannot be ruled out that a biopsy can potentially affect the embryo.
5. Despite observance of all procedures of good laboratory practice and application of appropriate technical equipment during analysis, the result of preimplantation genetic analysis need not provide information about the embryonic genetic makeup with one hundred percent certainty (risk of incorrect diagnosis). However the risk of failure of preimplantation genetic analysis is significantly less than the risk of the presence of genetic defects after IVF without the use of genetic testing or than the common risk of congenital genetic defect in natural conception. Incorrect diagnosis can be the result of the following causes:

* cell/cells extracted from the embryo need not, under all circumstances, represent a finding in the cells which were left in the embryo (risk of mosaicism; in this case there can be a difference between the result of preimplantation analysis and karyotype of the foetus detected by further prenatal diagnosis),
* random overlapping of fluorescence signals for the given chromosomal region in the cell nucleus (FISH),
* inaccuracy of whole genomic amplification of DNA due to degraded DNA in a sample or lack of collected material (NGS),
* contamination of samples of extraneous DNA, which is very rare, but cannot be ruled out,
* unforeseeable technical problems.

1. When evaluating the results of preimplantation genetic analysis situations may arise when a diagnostic conclusion cannot be reached, i.e. when the result of the analysis is not clearly evaluable or does not achieve the reference values determined by the test laboratory for the given method. The most common causes are:

* collected embryonic cell/cells do not contain a nucleus, therefore this is not enough genetic material for analysis,
* failure or inaccuracy of whole genomic amplification of DNA due to degraded DNA in the sample or lack of collected material (NGS),
* contamination of samples of extraneous DNA, which is very rare, but cannot be ruled out,
* unforeseeable technical problems.

In embryos where genetic analysis does not bring a result for the above reasons, and depending on the individual situation, a repeat of a biopsy (rebiopsy) of embryonic cells can be recommended and the entire process of genetic analysis.

1. Pregnancy from embryo/embryos, which were recommended for transfer based on preimplantation genetic analysis, can end with spontaneous miscarriage, ectopic pregnancy or foetal death with about the same likelihood as pregnancy achieved by spontaneous conception or by assisted reproduction without preimplantation genetic analysis.
2. Congenital developmental defects, mental retardation and/or other possible deviations from normal development in children born from pregnancies after IVF, cell biopsy and preimplantation genetic testing can appear just as in children conceived naturally. Likewise, a normal finding detected during preimplantation genetic analysis does not rule out that a transferred embryo or embryos can be affected by another abnormality at chromosomal or gene level than for which tests were carried out.

**Given the above risks (especially c), d), e), g)), we recommend that each foetus after preimplantation genetic analysis is properly analysed during following pregnancy by non-invasive (ultrasound scanning to search for congenital developmental defects, prenatal biochemical screening from the mother’s blood, etc.) and invasive (collection of amniotic fluid, foetal blood or chorionic villi sampling) methods of prenatal diagnosis.**

1. **Clients statement**

**We confirm that we were provided with counselling for preimplantation genetic analysis and that we fully understand the information provided. We were informed by the clinician of the following:**

* purpose, nature and expected benefit of preimplantation genetic analysis,
* possible impact of the results of genetic diagnosis on your health and the health of your offspring and genetically related persons,
* risks of unexpected findings which are findings that are not the purpose of genetic analysis, but genetic analysis detects this. Unexpected findings could be, for example, findings that differ from common findings, but their specific impact on the present and/or future health condition of the analysed person or genetically related person cannot be determined based on present knowledge,
* the required analysis does not fully ensure pregnancy, child birth or child birth without a genetic abnormality.

We understand what hitherto known benefits and risks preimplantation genetic analysis brings and we voluntarily request that it is performed. We are also aware that after completion of preimplantation genetic analysis there is no reason to rule out standard prenatal screening tests or karyotyping of the foetus from the cells in the amniotic fluid, from the chorionic villi or from the foetal blood.

We had the chance and sufficient amount of time to consider everything properly and calmly. We have been instructed in the applied methods of preimplantation genetic analysis, its course, conditions of implementation and the possible risks. We had the chance to ask the clinician about everything we considered fundamental and necessary and everything we did not fully understand. We received a clear and intelligible answer to all our questions. We were informed that we have opportunity to consult the circumstances of performing of preimplantation analysis, including detailed interpretation of the results, with clinical geneticist.

We give our consent to the assisted reproduction centre being informed of the further progress of the pregnancy, the results of prenatal diagnosis, course of the birth and health condition of the foetus. These data continue to be confidential and the assisted reproduction centre undertakes to protect this information from being abused. Such obtained data and records of the examinations will serve to monitor the results of preimplantation genetic diagnosis and to anonymous referral of results to the register of the consortium of preimplantation genetic diagnosis of the European Society of Human Reproduction and Embryology (ESHRE) and to the National Register of Assisted Reproduction (NRAR).

* 1. **Consent to storage of DNA samples used for preimplantation genetic analysis**
  2. We consent to the fact that, if it is possible and/or expedient, the samples are stored for further analysis performed for my benefit or the benefit of biological relatives. Prior to genetic analysis which would be performed for different purposes than those stated above we will be properly instructed and this analysis will not be performed until the new informed consent is signed. The samples will be stored usually for 1 year (however no more than 50 years).
  3. We consent to the use of the stored samples to check the quality of the DNA diagnosis (samples will be used as a control during the analysis of other related persons or when analysing other patients).
  4. We consent to the anonymous use of the analysed samples in medical research (focused particularly on the improvement of the treatment of infertility).

If you do not consent to any of the above statements, please specify to which one(s):

* 1. **Statement on information about the results of the analysis**

1. We wish to be informed of the results of the laboratory tests, including potential unexpected findings.
2. We wish the following persons to be informed of the results of the laboratory tests and/or unexpected findings:
3. We consent to the use of the results of genetic diagnosis and the relevant information about the health condition for scientific or educational purposes provided that these data are presented and published in full anonymity.

If you do not consent to any of the above statements, please specify to which one(s):

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | |  | | | | |  | |
| **Based on these instructions we state that we consent to the collection of the respective biological material and the performance of the above described preimplantation genetic analysis under the conditions stated above.** | | | | | | | | | | | |
| We are aware that we can revoke our consent in writing, however only up to the time of the start of the analysis. | | | | | | | | | | | |
| In |  | | | | | Date |  | | | | |
| **Patient’s signature:** | |  | | | | **Partner’s signature:** | |  | | | |
|  | | | | | | | | | | | |
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| I confirm that I appropriately instructed the applicants about preimplantation genetic analysis and about all the above mentioned facts, and presented them this statement for their signature once they fully understood it. | | | | | | | | | | | |
| **Clinician’s  name and surname:** | | |  | | | **Clinician’s signature:** | |  | | | |
|  | | | |  | |  | | |  | | |