ESHRE PGT Consortium good practice recommendations for the organisation of preimplantation genetic testing

AUTHORS

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GENERAL INTRODUCTION

The previous terms of preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) have been replaced by the term ***preimplantation genetic testing (PGT)***, following a revision of terminology used in infertility care (Zegers-Hochschild *et al.*, 2017). PGT is defined as a test performed to analyse the DNA from oocytes (***polar bodies***) or embryos (***cleavage stage*** or ***blastocyst***) for HLA typing or for determining genetic abnormalities. This includes PGT for aneuploidy (PGT-A), PGT for monogenic/single gene defects (PGT-M) and PGT for chromosomal structural rearrangements (PGT-SR) (Zegers-Hochschild *et al.*, 2017). PGT for chromosomal numerical aberrations of high genetic risk are included within PGT-SR in the data collections of the ESHRE PGT consortium.

PGT began as an experimental procedure in 1990 with polymerase chain reaction (PCR)-based methods used for the detection of monogenic diseases. Interphase fluorescence *in situ* hybridization (FISH) was introduced a few years later and became the standard method for ***sexing*** embryos and for detecting numerical and structural chromosomal aberrations. Genome-wide technologies began to replace the gold standard methods of FISH and PCR over the last decade and this trend was most apparent for PGT-A. PGT-A has been carried out mainly for ***in vitro fertilization (IVF)*** patient groups with original aims of increasing pregnancy rates and decreasing miscarriage rates. Other outcome measures such as increasing elective single embryo transfer and reduced time to pregnancy have been added more recently. Cited indications for PGT-A include ***advanced maternal age (AMA)***, ***recurrent implantation failure (RIF)***, ***severe male factor (SMF)***, and couples with normal karyotypes who have experienced ***recurrent miscarriage (RM)***. The value of the procedure for all IVF patients and/or appropriate patient selection remains an ongoing discussion, but this is outside the scope of this manuscript.

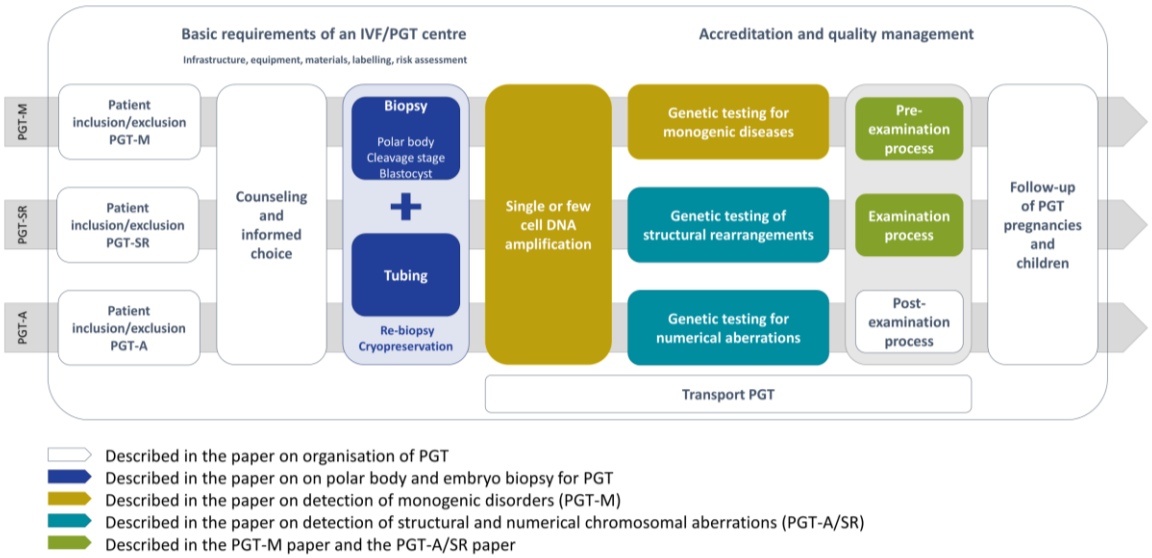
The goal of this series of papers is to bring forward best practices to be followed in all types of PGT services, offering PGT-A as well as PGT-M and PGT-SR.

In order to take PGT to the same high-quality level as routine genetic testing, guidelines for best practice have been designed by several societies. The PGD International Society (PGDIS) has drafted guidelines (The Preimplantation Genetic Diagnosis International Society (PGDIS): Guidelines for good practice in PGD, 2004, Guidelines for good practice in PGD: programme requirements and laboratory quality assurance, 2008) while the American Society for

Reproductive Medicine (ASRM) reviewed PGT practice in the USA (Practice Committee of the Society for Assisted Reproductive Technology and Practice Committee of the American Society for Reproductive Medicine, 2008) and published several opinion papers (on blastocyst culture, embryo transfer and on PGT-A). The first guidelines of the European Society for Human Reproduction and Embryology (ESHRE) PGT Consortium were published in 2005, as one of the missions of the Consortium was to bring overall standardisation and improve quality standards (Thornhill *et al.*, 2005). In collaboration with the Cytogenetics European Quality Assessment (CEQA) and the UK National External Quality Assessment Service (UKNEQAS), now together in Genomics Quality Assessment (GenQA), the ESHRE PGT Consortium also initiated External Quality Assessment (EQA) schemes to provide an independent evaluation of laboratories and help them improving their techniques and reports. A review of the original guidelines yielded four sets of recommendations on different aspects of PGT: one on the organization of PGT and three relating to the methods used: ***embryo biopsy***, amplification-based testing and FISH-based testing (Harton *et al.*, 2011a, Harton *et al.*, 2011b, Harton *et al.*, 2011c, Harton *et al.*, 2011d). These four guidelines are now being updated and extended, taking into account the fast changes in the provision of PGT services. In these guidelines, the laboratory performing the diagnosis will be referred to as the PGT centre and the centre performing the IVF as the IVF centre.

General aspects of PGT, including patient selection, counselling, pregnancy and children follow-up and transport PGT, will be covered in the paper on organisation of PGT. Technical recommendations for embryo biopsy and tubing will be covered in the paper on embryo biopsy. Recommendations for genetic testing will be covered in the papers on detection of numerical and structural chromosomal aberrations, and on detection of monogenic disorders. The content of the different papers is aligned with the IVF/PGT clinical procedure in figure 1.

Figure 1: Overview of the IVF/PGT process, and how all aspects are covered by one of the 4 recommendations papers.



The ESHRE PGT Consortium recognizes that owing to variations in local or national regulations and specific laboratory practices, there will remain differences in the ways in which PGT is

practiced (from initial referral through IVF treatment, genetic testing to follow-up of pregnancies, births and children). This does not preclude a series of consensus recommendations for best practice based on experience and available evidence. These recommendations are not intended as the only approved standard of practice, nor are they legally binding. The unique needs of individual patients may justify deviation, and the recommendations must be applied according to individual patient's needs using professional judgement. However, recommendations and opinions may be used to frame laws and regulations, and practitioners should ensure that they comply with statutory requirements or clinical practice guidelines in their own countries. To keep the papers concise, repetitions have been excluded as much as possible and many cross-references were included. Therefore, it is recommended to not consult the papers independently but always as a set when one is seeking guidance on a PGT issue.

METHODS

The PGT-Steering Committee assessed the previous guidelines (Harton *et al.*, 2011a) and deducted an outline for the current paper. All members of the Steering Committee according to their expertise, wrote a section that was later discussed in depth with the entire Steering Committee until consensus. Seven online meetings were organised for discussion. The final draft of the paper was checked for consistency with the other papers of the series. The draft was then submitted for stakeholder review; it was published on the ESHRE website between XX and XX, and ESHRE members were invited to send in comments. All comments were checked by the PGT-Steering Committee and incorporated in the final version where relevant. A review report is published on the ESHRE website.

For easier use of the recommendations, terms in bold and italic are explained in a glossary (Supplementary data 1) and abbreviations are listed (Supplementary data 2)

1. PATIENT INCLUSION/EXCLUSION CRITERIA

The decision to accept or decline patients in PGT services should be undertaken by a team of dedicated healthcare professionals (including clinical geneticists or genetic counsellors, molecular biologists/cytogeneticists, clinical IVF specialists and embryologists), based on well-defined inclusion/exclusion criteria. PGT requests should also be considered by local ethics boards, national legislation, or local/national regulatory agencies. Local regulations will vary from centre to centre as will criteria for inclusion and exclusion of patients. The following recommendations should be considered as a starting point for discussion.

1.1 General: inclusion/exclusion

It is recommended that PGT is only applied when genetic diagnosis is technically feasible, and the reliability of the diagnosis is high. Current procedures in most IVF/PGT centres allow for overall error rates as low as 1 to 3%. Each centre should be aware of their error rates and include this information in their ***informed consents*** and reports in an open communication with the patient.

When considering PGT, the following criteria should be considered: chance of success, safety issues, female age, impossibility to retrieve male or female gametes, body mass index (BMI)

and other contraindications for IVF.

Furthermore, exclusion from PGT should be considered if the woman has serious signs and symptoms of an autosomal dominant or X-linked disorder (for which PGT is requested) which could introduce medical complications during ovarian stimulation, oocyte retrieval or pregnancy, or put a child born at risk of harm. PGT may also be inappropriate if one of the partners has serious physical or psychological problems. Each specific case will need to be evaluated by the IVF/PGT centres and may be subject to local, state, or federal law.

Selection of embryos based on sex for social reasons is not acceptable.

1.2 PGT-M, mitochondrial disorders and HLA: inclusion/exclusion

PGT-M refers to testing for DNA mutations causing (combinations of) monogenic disorders, X-linked, autosomal dominantly or recessively inherited, for which the disease-causing loci (nuclear or mitochondrial) has been unequivocally identified. In this respect, HLA typing of embryos is an exceptional indication.

PGT-M

PGT-M testing can be carried out for germline genetic variant(s) (class 4-5) (Richards *et al.*, 2015), hereafter termed ***mutations***, proven to be disease causing with serious health effects that may manifest at birth, in childhood or in adulthood. Further research (e.g. functional studies, family studies) may be indicated to prove the clinical significance of genetic variants. Cases of genetic variants of unknown significance that are not predictive of a phenotype should be excluded from PGT. PGT testing is inappropriate in case of uncertain genetic diagnosis (for example genetic/molecular heterogeneity), in case of uncertain mode of inheritance, in case of low recurrence risk (e.g. <10%).

For autosomal recessive disorders, where a single mutation has been diagnosed in the proband and only one parent, it is acceptable to offer PGT if the pathogenic genotype is attributed to a single gene and sufficient evidence from the family pedigree allows identification of the disease-associated haplotypes. Similarly, it is acceptable to offer PGT for X-linked recessive single gene disorders where no mutation was found in the proband, but low- and high-risk haplotypes can be identified based on the family history.

Exclusion testing can be indicated for late-onset disorders, such as Huntington's disease, to avoid pre-symptomatic testing of the partner with a family history of the disease.

PGT with non-disclosure of the direct test results to the couple is not recommended as it requires extreme confidentiality and raises moral and ethical issues (Shenfield *et al.*, 2003).

PGT for mitochondrial disorders

PGT for mitochondrial disorders caused by mitochondrial DNA (mtDNA) mutations allows to select for embryos with an mtDNA mutation load below the threshold of clinical expression, providing an effective risk reduction strategy for heteroplasmic mtDNA mutations. As this threshold is often not known for rare or private mutations, a meta-analysis was performed for all mtDNA mutations, showing that embryos with a mutation load of less than 18% have a likelihood of more than 95% of being unaffected, irrespective of the mtDNA mutation and can be considered for transfer. For all mtDNA mutations tested so far, the mutation load in individual blastomeres is representative for the entire embryo, which was expected due to

the absence of mtDNA replication in the cleavage stage. Whether the same is true for blastocysts remains to be established, as mtDNA replication has started in this stage, leading to increased variation. Therefore, it is warranted to assess the variation in mutation load within embryos (for instance in arrested embryos of the first cycle).

PGT is not indicated in case of ***homoplasmy***. However, it is acceptable to carry out sexing to reduce the clinical risk of the disease in the case of homoplasmic mutations showing sex dependent penetrance. It should be noted that PGT in both instances is a risk reduction strategy, it does not eliminate it. It could be for future generation, when only male embryos are being transferred.

In cases where the causative mutation of the mitochondrial disease is encoded by nuclear DNA, testing is the same as for other monogenic disorders.

HLA typing

When all other clinical options have been exhausted, selection of HLA-matched embryos via PGT is acceptable for couples who already have a child affected with a malignant, acquired disorder or a genetic disorder where the affected child is likely to be cured or life expectancy is substantially prolonged by transplantation with stem cells from an HLA-matched sibling. Testing can be performed for HLA typing alone, if the recurrence risk of the disease is low, or in combination with autosomal dominant/recessive or X-linked disorders.

Consideration should be given to the time required for PGT workup, cycle(s) application and for an HLA-matched sibling to be born. Therefore, cases in which the affected child has an acute medical condition prohibiting safe stem cell transplantation or an extremely low life expectancy should be excluded from PGT. Any request for HLA typing in the absence of any specific disease to create a future donor for a sibling should be refused.

1.3 PGT-SR: inclusion/exclusion

PGT for chromosome structural rearrangements (PGT-SR) is an accepted and routine procedure in most IVF/PGT centres. It has been developed for patients, unable to achieve a pregnancy or at high risk of pregnancy loss and of abnormal live born births, resulting from inheritance of unbalanced products of the rearrangement.

Depending on the technology used (fluorescence in situ hybridization (FISH), quantitative real-time PCR (qPCR), comprehensive testing methods [array-based comparative genomic hybridization (aCGH), single nucleotide polymorphism (SNP) array or next generation sequencing (NGS)]), different inclusion/exclusion criteria may apply. In general, PGT-SR is only recommended if the technique applied is able to detect all expected unbalanced forms of the chromosomal rearrangement. When comprehensive testing strategies are applied, it is acceptable to use information on copy number of non-indication chromosomes to refine embryo transfer strategies.

1.4 PGT-A: inclusion/exclusion

Although PGT-A remains controversial in clinical practice, the following indications for its use have been reported:

- Advanced female/maternal age (AMA)

- Recurrent implantation failure (RIF)
- Recurrent Miscarriage (RM). It should be noted that couples with a history of RM have a high chance of successfully conceiving naturally.
- Severe male factor (SMF)

The exact definition (e.g. age limit, number of losses) of these factors should be determined by each centre. International definitions are provided in the glossary (See Supplementary data 1. Glossary).

For all, but in particular for RIF, RM and SMF couples, a previous karyotype of both partners is recommended, since there is a higher chance of structural rearrangements for these indications. If an abnormal karyotype is identified, the technology for the detection of unbalances can differ from the regular PGT-A.

2. COUNSELLING AND INFORMED CHOICE

2.1 Relevant documents

The following documents should be available before starting PGT:

- original or copy of results of genetic testing, karyotypes, or other specific testing of the index patient, spouse or partner, children or other family members (when appropriate).
- female reproductive history, gynaecological and fertility status.
- male reproductive history, andrological history, fertility status, results of sperm analysis (especially in cases where the genetic disorders for which PGT is desired has effects on sperm parameters, e.g. monogenic diseases, such as myotonic dystrophy and cystic fibrosis/congenital bilateral absence of the vas deferens and some Robertsonian translocations).
- reports on health problems of female and male partners, that may affect genetic diagnosis, or the outcome of IVF and pregnancy (when appropriate). Health status may need to be re-evaluated over time.
- for PGT-M, PGT-SR: a genetic counselling report together with full pedigree and family data.
- for HLA testing: a medical report of the affected child, current situation, prognosis, options for treatment other than PGT, suitability for stem cell transplantation, results of previous HLA typing (serologic and/or DNA markers) in affected child, parents and siblings.

As laws and regulations on PGT vary internationally, the legality of undertaking PGT in a particular country for a specific indication should be verified. If required, licenses or approval to carry out PGT should be obtained prior to the start of ovarian stimulation.

2.2 Counselling: General issues

- All information, oral and written, should be in language that can be understood by a layperson as technical terminology may lead to patient misunderstanding.
- Written information about treatment should be available prior to a consultation.

- As PGT involves the treatment of a couple, both partners should, when possible, attend consultations.
- An independent interpreter should be present when necessary, although a family member could act as translator in the absence of an alternative.
- Counselling should be offered both before and after each IVF/PGT cycle.
- Genetic counselling should be provided by a qualified clinical geneticist or genetic counsellor.
- A specialist in reproductive medicine should provide information regarding the IVF cycle.
- The counselling provided should be non-directive and include all reproductive options available to the couple, enabling them to reach their own conclusion about the suitability of treatment.
- Costs and timelines should also be discussed to ensure that patients are fully informed of all aspects of IVF and PGT before treatment starts. The social and psychological impact needs to be considered, especially in couples already responsible for the care of affected children.
- Additional counselling may be needed after completion of the laboratory work-up.
- Individualised post-consultation letters should contain a summary of the information discussed.

2.3 PGT-related counselling

PGT counselling includes counselling related to the IVF treatment on one side and genetic counselling on the other side.

Related to the IVF treatment

Counselling should include discussion of:

- the risk of medical complications for women during ovarian stimulation or oocyte retrieval.
- the risk of spontaneous pregnancy in the waiting time or during IVF treatment, and the need for contraception.
- the number of oocytes to be retrieved and the need to maximize this within the safe limits of medical practice. Different options for pooling oocytes or embryos before biopsy should be considered, when appropriate.
- the expected number of embryos for biopsy, the biopsy stage, the number of cells to be biopsied and the percentage of embryos expected to survive.
- the possibility that some embryos remain undiagnosed. In specific cases, re-biopsy is acceptable to achieve diagnosis. If no diagnosis is obtained, selection of these embryos for transfer is not acceptable. An exception can be made for PGT-A but requires patients' fully informed consent.
- the number of embryos to be transferred and the policy on elective single-embryo transfer in the centre. The risk of conceiving a multiple pregnancy should also be discussed.
- the possibility of having no embryos for transfer if all the embryos are morphologically and/or genetically unsuitable.
- the chance of pregnancy/live birth per cycle started and per embryo transfer, taking into account maternal age and indication.
- the risk of miscarriage and the importance of re-analysis of fetal tissue, as a tool to assess false negative rates and to advise the couple for further treatment.

- cryopreservation following PGT and the predicted success of pregnancies from biopsied and cryopreserved embryos.
- follow-up of pregnancies and children born from PGT.
- options for embryos not transferred or frozen for future use, including donation to research.

Related to the genetic analysis

Counselling should include discussion of:

- an updated review of the genetic risk and molecular or cytogenetic confirmation of the diagnosis when appropriate, the severity and variability of the condition, and presence or absence of genotype/phenotype correlation.
- the principle of the test; it should be explained that depending on the indication, biological samples and genetic reports from the couple and relevant family members may be required for the laboratory work-up.
- the condition(s) tested for, the testing method and the limitations of the test.
- the expected time-frame for the laboratory work-up and the treatment.
- decision-making about which embryos are acceptable for transfer/vitrification; this should be discussed with the patients before a treatment cycle begins and may need to be revisited. The fate of undiagnosed embryos and non-transferable embryos also needs to be addressed. It is acceptable to use non-transferable embryos for test optimisation.
- chromosomal mosaicism as an inherent biological phenomenon in human preimplantation embryos and when appropriate, how this may affect diagnosis and the centre's embryo transfer policy.
- the possibility of a misdiagnosis; error rates expressed as false negative or positive results should be based on 'in-house' work-up and follow-up analysis for specific diagnostic tests or strategies.
- the option of prenatal diagnosis for confirmation of the PGT result.

Depending on the condition, and test to be used, the following issues should also be addressed in counselling:

- for structural chromosomal rearrangements, it is important to discuss that the applied technology may not allow to discriminate between normal and balanced results.
- for autosomal recessive, as well as for X-linked recessive disorders, the transfer of carrier embryos should be discussed, according to the local regulations.
- for X-linked diseases where specific mutation detection is not possible, the pros and cons of embryo ***sexing*** should be discussed: all male embryos, affected or unaffected, will be discarded and carrier females cannot be distinguished from unaffected female embryos.
- the option of revealing the sex of the embryo should be discussed within the local legal constraints.
- for monogenic disorders caused by dynamic mutations where testing involves repeat size determination, the couple should be fully informed on the threshold of repeat expansions below which embryos can still be transferred.
- for HLA typing, the theoretical number of embryos suitable for transfer should be discussed. The fate of unaffected non-HLA-matched embryos should be discussed, taking local and national regulations into consideration. Due to the complexity of the procedure

it is recommended to maintain close collaboration between specialists of the IVF, PGT and transplant units, and to minimize the time of the whole procedure.

- All potential limitations should be communicated to the couple, including the chance of finding a transferable embryo and hematopoietic stem cell transplantation issues (potential stem cell source, timing, expected success rate).
- It is recommended to counsel prospective parents on the genetic chance of identifying a transferable embryo:
  - 25% (1 out of 4) of biopsied embryos are genetically transferable when performing preimplantation HLA-typing only;
  - 18.8% (3 out of 16) when concurrently excluding an autosomal recessive or X-linked recessive disease;
  - 12.5% (1 out of 8) when concurrently excluding an autosomal dominant disease.

2.4 Psychological support and evaluation

Psychological support should be offered to every couple before, during and after PGT, including unsuccessful cycles.

Psychological evaluation should be considered for the following patients:

- couples with a history of reproductive failure.
- patients with past traumatic experiences.
- couples for whom the geneticist, gynaecologist or other member of the IVF/PGT team has doubts regarding the welfare of existing or future children or the psychological physical wellbeing or mental capacity of future parents.
- couples who actively request psychological intervention.
- couples in whom one of the future parents is the carrier of an autosomal dominant disorder and may have signs and/or symptoms of this disorder as determined by the appropriate specialist physician (e.g. neurodegenerative/psychiatric diseases).
- couples who are undergoing PGT HLA-typing to evaluate their 'child wish' and the extent to which the new child is welcomed, not only as a donor but also as a full family member, appreciated for whom s/he is.

3. BASIC REQUIREMENTS OF AN IVF/PGT CENTRE

A close collaboration between the IVF centre and the PGT centre is essential, particularly in complex cases.

Oocyte retrieval, fertilization, culture, biopsy and transfer of embryos and PGT diagnosis should be undertaken in a centre with suitable laboratory infrastructure, equipment and trained staff, in accordance with the European Union Tissue and Cells directive or other local laws. Adherence to published best practice guidance on PGT is recommended.

The following recommendations apply to the preclinical work-up and testing of clinical cases.

3.1 Laboratory infrastructure, equipment and materials

Laboratory infrastructure

Oocyte and/or embryo biopsy should be performed in a specifically designated laboratory setting. Collection of the biopsied samples and initial steps of genetic testing procedures should be carried out in laboratory settings dedicated for single and/or few cell processing. Appropriate precautions should be taken both to prevent contamination of samples by physical isolation, and to detect any such contamination. Licenses for offering embryo biopsy procedures and/or genetic testing by the centre may be obtained, according to local regulations.

Equipment

- All clinical equipment should meet the criteria set for the intended application, be appropriately calibrated, maintained and serviced, with all aspects supported by written ***standard operating procedures (SOPs).*** Equipment used for critical steps should have uninterrupted power supply (UPS).
- For areas within the IVF centre, whether it is a dedicated area or a room, all equipment should comply with "Revised Guidelines for good practice in IVF laboratories (2015)", section 3 "laboratory safety" (ESHRE Guideline Group on Good Practice in IVF Labs *et al.*, 2016). Prior to the biopsy procedure, work surfaces, equipment and hoods should be cleaned and decontaminated with disinfectants with proven compatibility and efficacy for use in an IVF laboratory.
- For areas within the PGT centre, prior to each use, work surfaces and equipment should be cleaned and decontaminated with DNA ***decontamination*** solutions or 10% bleach, or by UV-C irradiation or autoclaving (when applicable, for example tube racks). It is not recommended to use 70% ethanol solution only, as it does not decontaminate DNA.
- Multichannel pipettes or automated systems are recommended to minimize the risks of mislabelling or misallocation of samples during the different steps of the protocol.

Materials

- To prevent contamination, protective clothing for single or few cell DNA amplification work should be worn, including full surgical gown (clean, not sterile and changed regularly), hair cover/hat, face mask (covering nose and mouth) and preferably shoe covers or dedicated shoes. Gloves should be worn at all times and changed frequently. These should be well-fitting (e.g. nitrile, but not vinyl examination gloves). For areas within the IVF centre, protective clothing, preferably with low particle-shedding and non-powdered gloves and masks should be considered.
- The pre-amplification materials and reagents should be kept away from any DNA source and preferably stored in the pre-amplification area.
- Whenever possible, all solutions or reagents should be purchased 'ready to use' and should be of 'molecular biology' grade or equivalent. All reagents (purchased and in-house) should be tested and validated. All plastic-ware used, including filter tips, should be certified DNA-free and DNase-free.
- Batch- or lot numbers should be recorded for traceability, according to the quality standards in the laboratory.
- Whenever possible, solutions or reagents should be split into small aliquots and no aliquot should be re-used for a clinical case.
- It is recommended to avoid repeated freeze-thaw cycles of all reagents.

- Reagents and solutions can be DNA decontaminated by UV-C irradiation. Alternatively, reagents and solutions made in-house can be autoclaved, preferably using a PGT-dedicated autoclave.
- Careful handling of all reagents employed must be ensured with regards to storage temperature and working conditions, following manufacturer's recommendations. Vortexing and quick temperature changes should be avoided for the most sensitive components.

Specific issues for handling of reaction tubes:

- It is recommended to avoid touching the inside or the lid of the tubes with your fingers.
- It is recommended to avoid touching the outside or the cap of the tubes with the tip of the pipette. If this happens, the pipette tip should be changed immediately.
- It is recommended to not keep the reaction tubes open longer than necessary and to open only one tube at a time.

Laboratory documentation

Well-structured (electronic and/or paper) laboratory forms should be available for recording wet-laboratory details of work-up and PGT cycle procedures.

Further specific requirements with respect to infrastructure, equipment, materials and documentation are discussed separately in the papers on embryo biopsy and PGT techniques (refer biopsy paper, PGT-M paper and PGT-A/SR paper).

3.2 Training and personnel

- It is recommended that laboratory personnel performing clinical work should be supervised by an appropriate person.
- Staff training and competence: embryo biopsy procedures and genetic testing should be performed by competent and adequately trained laboratory staff, according to national legislation. Training programs exist for embryology and PGT procedures. All staff should document their competence level and continuous professional development. The number of laboratory staff should reflect the number of cycles performed per year and also consider other duties such as administration, quality management and communication. More than one individual should be trained to avoid difficulties with absence.
- It is recommended for a member personnel with abstinence from a specific technique to demonstrate laboratory skills before working again with clinical cases.
- When the interpretation of results includes specific software, personnel may also be trained in management and interpretation of the software.
- Good laboratory practice and good scientific judgement are always required.

3.3 Labelling and witnessing

- It is recommended that an adequate labelling system, written or barcoded (electronic), with two unique patient and embryo/cell(s) identifiers is used to match the sample's diagnostic result with the embryo from which that sample was taken. This should ensure traceability throughout the IVF and PGT process up until reporting of the final results.

- The labelling system should be comprehensible and practical for both the IVF and the PGT centre. Printed sticker labelling may be superior to pens as labelling should be legible and inedible.
- Labelling and sample identification should be confirmed for critical and high-risk steps by an independent observer and signed off (see figure 2). These critical steps are detailed in the technical papers for the various methods (refer biopsy paper, PGT-M paper and PGT-A/SR paper).
- After biopsy, the sample may be analysed in house, or sent for genetic testing in another centre (see section 5. Transport PGT).

Figure 2: outline of the biopsy and genetic testing procedure with indications of the 7 critical steps where labelling and sample identification should be confirmed

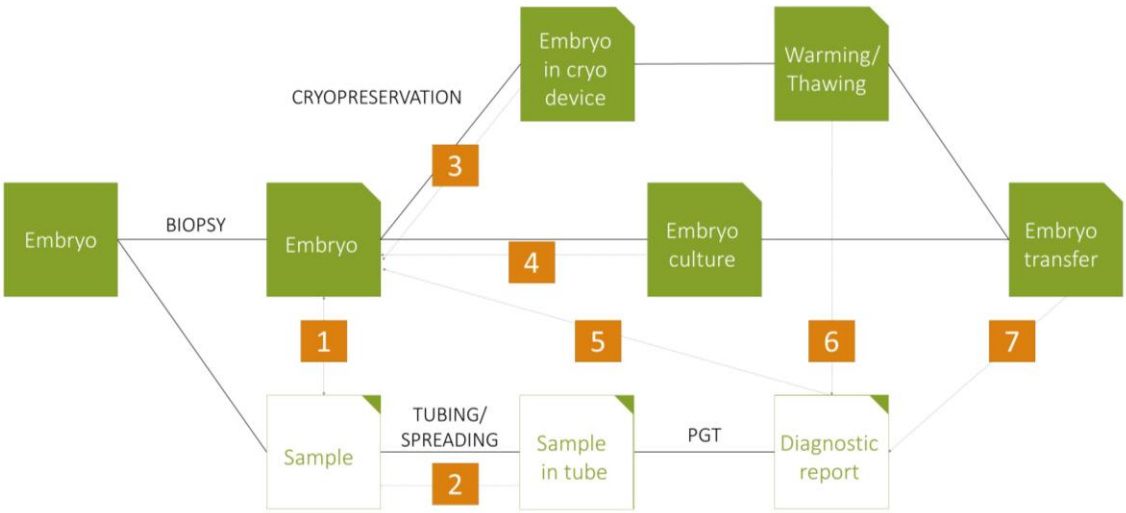


Figure legend; Witnessing is recommended during the following stages: (1) Immediately after biopsy to confirm the embryo and sample number match; (2) During spreading or tubing, to confirm that the sample identification matches the labelling on the relevant slide or tube, respectively; (3) In case of cryopreservation, immediately after biopsy before acquiring the genetic analysis results, at placing and labelling the embryo into the cryopreservation device; (4) For further embryo culture, at placing and labelling the embryo into the culture dish, and (5) When diagnostic results are issued to ensure accuracy and correlation with the correct sample and/or embryo identification; (6) During the thawing/warming procedure to ensure accuracy and correlation with the correct embryo diagnostic result; (7) At the time of selecting the embryo(s) for embryo transfer.

4. PRECLINICAL WORK-UP, EXAMINATION AND POST-EXAMINATION PROCESS

4.1 Preclinical work-up and validation

The PGT work-up report should contain at least the following information (Claustres *et al.*, 2014):

– administrative information:

    - title or name of the report,
    - number of the report (as used for document control, when available),
    - pagination including the actual and total number of pages (The patient identifier and report name/number must be present on each additional page),
    - full date of the report,
    - name and address of the physician referring the patient,
    - identification of the person(s) performing the diagnosis/authorizing the release of the report and their signature,
    - identity of the IVF/PGT centre with full contact details.
  - patient (male and female)/sample identification
    - full given name(s) and surname, or unique patient identification code
    - unequivocal date of birth
    - gender
  - specific for the preclinical work-up report:
    - date of DNA sample collection,
    - date of DNA sample arrival in the laboratory.
  - restatement of the clinical question, i.e. the indication(s) being requested for analysis, the type of required testing, the referral reason.
  - specification of genetic tests used
    - brief information on the methods used in the analysis,
    - full details of the extent of the tests, including software, where appropriate,
    - where a commercially available kit is used, this should be clearly identified in the report, including the reference and version of the kit.
  - a clear summary of the result
  - error rates/limitations of the test/misdiagnosis (a general figure should be stated for the overall cycle/treatment)
- It is recommended that all reporting based on haplotyping clearly states that the accuracy of the results is based on the assumption that samples received were correctly identified, family relationships are true and the clinical diagnosis of relatives is correct.
- It is recommended that any particularity of the protocol (e.g. specifying type of biopsy, number of cells) is clearly indicated and communicated to both the patient and the IVF centre, if needed.

4.2 Examination process

- The examination process and reporting of results, must comply with local guidelines or law, or with the ISO 15189 standard.
- Before starting a clinical PGT cycle, relevant documents should be available, labelling of samples should be checked, and genetic counselling provided to the couple.
- It is recommended that the PGT laboratory has clearly documented procedures for all steps of the examination process (explicit instructions and a summary of validation results) and release of results (diagnosis, reporting, embryos transfer policy). These procedures are preferably covered in a service level agreement between the PGT and IVF centres.

- Many different methods have been published and all appropriately validated methods are acceptable for clinical cases. The method used should have been previously implemented, tested and validated in the PGT centre.

Scoring of clinical results

- It is recommended that results are reviewed and signed or electronically validated by a suitably qualified person (name, qualification, date).

Clinical cycle report

The PGT cycle report contains interpretation of the clinical results and guidance on which embryos are genetically transferable. The same recommendations apply as specified for the preclinical work-up report (see section 4.1), together with the following items:

  - unique cycle/treatment code
  - date of oocyte retrieval
  - date of biopsy
  - date of biopsy sample arrival in the laboratory
  - information on the sample type (including number of samples and controls)
  - unique ID number for each cycle and/or biopsy sample tested
- When scoring results from PB testing, it is recommended to report what was detected in each polar body and then infer the embryo diagnosis. It is recommended to test both PBs.
- When scoring results from blastomere/TE testing, it is recommended to report what was detected in the sample and then infer the embryo diagnosis.
- When results are reported from "pooling" of embryos, it is advisable to refer to each oocyte and sample collection date and clearly differentiate the embryo number between cycle/treatment.
- Reporting of clinical results to the IVF centre must follow local regulations or international accreditation guidelines, including general data protection regulations (GDPR).
- The embryo transfer policy should be agreed upon between stakeholders (IVF centre, genetic centre, genetic counsellors, clinicians and patients). In PGT-M and PGT-SR cases, embryos with no or inconclusive results are not recommended for transfer. Depending on local rules and following adequate counselling of the prospective parents, the carrier status of embryos (for autosomal recessive or X-linked recessive disorders) may be taken into consideration for embryo selection. In case of PGT-A in addition to PGT-M or PGT-SR, it is crucial to agree on a clear embryo transfer policy and a possible embryo ranking.
- A written or electronic report should be securely transmitted to the IVF centre to ensure transfer and/or cryopreservation of the correct embryos. Results should not be communicated orally.
- Reporting time should be kept as short as possible, and when fresh transfer is intended, the timing of the IVF centre should be taken into account.
- It is recommended that the report is clear, concise, accurate and easily understandable by non-geneticists.
- It is recommended that the overall result and interpretation is presented per embryo, preferably in tabulated form.

- In case of no diagnosis and re-biopsy to try and obtain a result, this should be included in the report.
- The final clinical cycle report must be signed by appropriately qualified (authorized) personnel (name, qualification, date).
- It is recommended that the clinical cycle results are discussed with the couple before embryo transfer.
- It is recommended that the report is stored in the patient file in the PGT centre, according to local regulations.
- It is recommended to include a disclaimer in the report to address limitations of the test and any other information that may be of significance to the addressee.
- It is acceptable to indicate in the report the need for prenatal testing to confirm the result in case of pregnancy.

Further details on the specific reporting of the results and interpretation of results are outlined in the technical recommendations papers (refer PGT-M paper and PGT-A/SR paper)

4.3 Post-examination process

PGT cycle follow-up

For quality purposes, it is recommended to confirm the PGT diagnosis on embryos not transferred or cryopreserved following diagnosis, in line with local regulations. Such confirmation aims to provide ***quality assurance (QA)*** as well as accurate and up to date misdiagnosis rates to prospective PGT patients. It is recommended that this is performed on as many embryos as is practicable. It is acceptable to perform this periodically.

When a pregnancy ensues following PGT testing, it is recommended that parents are (again) made aware of the chance and risks of a misdiagnosis and be informed on the possibilities for prenatal testing. PGT and IVF centres should make special efforts to follow-up with the parents following prenatal testing or birth, especially if confirmatory testing is not possible.

Follow-up data should be used both for internal ***quality control (QC)***/QA purposes and documented in the ESHRE PGT Consortium online database for international data collection.

It is recommended that laboratories follow local regulations or accreditation schemes on storage of clinical samples and patient records.

If no local regulations or guidelines exists, it is recommended that:

- If embryos have been transferred and/or frozen, all relevant material (e.g. FISH slides, DNA amplification products) from the case should be retained and appropriately stored. Samples should be stored for at least 1 year. Prolonged sample storage could be considered taking into account the availability of information on delivery and the duration of embryo cryopreservation.
- If there is no genetically suitable embryo for transfer or cryopreservation, it is not necessary to keep the samples.
- If there is no pregnancy after transfer of all genetically suitable embryos, samples can be discarded.

Misdiagnosis rate

- It is recommended that each PGT centre performs a prospective risk analysis in order to prevent and/or eliminate possible causes of ***misdiagnosis***.
- It is recommended that misdiagnosis rates should be calculated for each type of method and for all methods from a particular centre. Misdiagnosis rates include those clinical cases in which affected pregnancies arose and cases for which re-analysis results were discordant with the biopsy result.
- It is recommended that confirmatory testing should be performed at least periodically as a QA.
- It is recommended that the published and in-house estimates of misdiagnosis rates should be available on request to prospective patients along with pregnancy rates to allow informed consent for PGT.
- Following a misdiagnosis, the IVF/PGT centre should investigate the possible causes of the misdiagnosis and make changes to protocols to eliminate the risk in the future. Many of the causes of misdiagnosis are avoidable by taking preventative actions and following the principles of quality management.
- Misdiagnosis should be reported, for instance through the ESHRE PGT Consortium online database.

Baseline IVF pregnancy rates for PGT

- Setting appropriate baseline (ongoing) pregnancy rates should be left up to the individual centres. However, it is recommended that each IVF centre should compare PGT pregnancy rates and matched non-PGT (routine IVF or ***intracytoplasmic sperm injection (ICSI)***) pregnancy rates within that IVF centre.
- Comparison of pregnancy rates with those reported by the ESHRE PGT Consortium or comparable peers can also be carried out to set benchmarks for continual improvement of the PGT centre.

5. TRANSPORT PGT

- When in house genetic analysis is not feasible, transport PGT can be an option providing patients with IVF treatment (oocyte retrieval, embryo culture, biopsy and transfer, pregnancy follow-up) at their local IVF centre, which collaborates with a PGT centre with significant experience in genetic testing.
- The IVF centre and PGT centre should have in place an official agreement (Service Level Agreement) dealing with legal, insurance and accountability issues about the Transport PGT procedures.
- The IVF centre and PGT centre should make arrangements to ensure that patients have had adequate PGT counselling.
- The IVF centre and PGT centre should have in place a set of clinical/laboratory validated protocols, including tubing/spreading protocols, and shipment protocols specifying approximate transportation time and ensuring cell and/or DNA integrity.
- In addition, practical and logistic arrangements on who will be responsible for the various stages of the PGT treatment should be clearly established.

- The IVF centre and PGT centre should delineate clear and sufficient lines of communication as documented in written procedures and complying to the General Data Protection Regulation GDPR during all stages of a transport PGT treatment.
- *Preclinical Runs*: before sending/receiving clinical samples from the treatment cycles, one or more 'preclinical runs' are recommended. This practice may detect issues related to the quality of biopsy, handling and labelling of biopsied samples, and the transport. Negative control specimens should be included in preclinical runs to assess contamination. The sensitivity and specificity of genetic testing should be evaluated and compared to in house samples and/or samples received from other IVF centres. The reporting of the results should be agreed upon.
- The IVF/PGT centres should agree on the feasibility, the number and the timing of transport PGT cycles, and define a schedule.
- It is recommended that all diagnostic results and reports are sent in written form (complying to the GDPR).
- The IVF centre and PGT centre should agree on who is responsible for the collection of PGT data and follow-up of PGT children (www.eshre.eu/data collection).

6. Follow-up of PGT pregnancies and children

6.1 Prenatal diagnosis

Prenatal diagnosis should be offered to all women who become pregnant following PGT. The discussion about the tests available should be undertaken by a suitably qualified professional to ensure that all available options are presented, including invasive tests such as chorionic villus sampling and amniocentesis, ultrasound scanning or non-invasive prenatal tests such as cell-free fetal DNA testing.

As an alternative to prenatal diagnosis, patients could choose to have postnatal confirmation by cord blood sampling. However, testing of minors for late-onset conditions in the absence of a clinical benefit is not recommended (Clarke, 1994).

6.2 Follow-up of PGT pregnancies and children

There have been concerns about the health of children after assisted reproductive technologies (ART), and in particular after embryo biopsy techniques and prolonged culture to blastocyst.

So far there is no indication that embryo biopsy causes an increased risk for adverse neonatal outcome. However, PGT includes ART for which there is evidence that ART singleton children differ from spontaneously conceived children. It is unclear whether this difference is due to the infertility status of the couple and/or the ART procedure itself.

There is uncertainty about the long-term impact of ART and/or PGT and IVF/PGT centres should be encouraged to obtain follow-up data on babies born after treatment, preferably in collaborative prospective and retrospective studies. If this is not possible, the suggested minimum data set to collect should include:

- date of birth,
- singleton versus multiple pregnancy + chorionicity status,
- gestational age at birth,

- delivery mode,
- birthweight and length,
- sex,
- congenital abnormalities

Neonatal complications and APGAR score can additionally be recorded.

7. ACCREDITATION AND QUALITY MANAGEMENT

7.1 Accreditation

Accreditation, along with proficiency testing through internal (IQA) and external quality assessment (EQA), provides a means to achieve and maintain the highest quality standards. Accreditation is the formal recognition that an authoritative body gives to a laboratory/department/centre when it demonstrates competence to carry out defined tasks and involves all aspects of management, along with technical requirements.

Where possible, IVF/PGT centres should be accredited/certified, even when it is not legally required.

Because PGT is of a multidisciplinary nature, the various units involved should each be accredited/certified for their defined tasks and according to the most appropriate quality standards. For each unit, responsibilities should be clearly outlined/described and transition of responsibility from one unit to the other during the PGT process should be well defined and guaranteed.

IVF/PGT laboratories should strive for accreditation conform the latest version of ISO15189 or equivalent international/local standards and work with international diagnostic laboratory accreditation schemes, if available.

IVF/PGT clinical units should strive for accreditation conform the latest version of ISO9001 or equivalent international/local standards and work with medical/clinical peer review, if available.

7.2 Quality management

It is recommended that a ***quality management system*** is integrated to the IVF/PGT centre. Quality management ensures that an IVF/PGT centre and the PGT service it provides, is of consistent quality. It has four main components: quality planning, QA, QC and quality improvement. To most if not all accreditation/certification schemes, QA and QC are prerequisites.

Aspects of quality management to be implemented include amongst others, quality policy, quality manual, document control, compliance to SOPs, risk management, continual improvement, audits and management review. Technical requirements include personnel, laboratory conditions and environment, laboratory equipment, all stages of examination procedures, results reporting and QA.

- It is recommended that PGT centres participate on a regular basis in EQA schemes; GenQA offers schemes for PGT that cover all types of analysis performed (https://www.genqa.org/).
- Validation of all methods used is recommended.

- Written SOPs should be available for all steps of the PGT procedure. Laboratory staff should have profound knowledge of the SOPs as these are the fundamental backbone of the service. Deviations from protocols should be recorded.
- ***Risk assessment*** is part of the QC system and required for every stage of the PGT process. It should be integrated into the SOPs.
- Laboratories should perform a risk assessment analysis to estimate the probability of a putative hazard, the severity of their consequences, as well as the chances for detection of error. A collaborative and multidisciplinary approach between the different operators involved in the management of a PGT cycle would lead then to the prevention of any putative procedural risk and implementation of specific corrective measures.

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SUPPLEMENTARY DATA 1. GLOSSARY

This glossary defines the most important terms used in the PGT papers and was developed to more easily understand the recommendations. Where appropriate, definitions were used as previously published, sometimes with adaptations to the context of PGT. Other definitions were derived from explanations within the context of the recommendations papers.

Term	Definition	Reference
Advanced maternal age (AMA)	Maternal age between 36-40 years	(Verpoest *et al.*, 2018)
Amplification bias	Artefactual duplications or deletions due to local concentration differences in guanine and cytosine bases (% G+C bias), as well as the prevalence of chimeric DNA molecules, allele drop outs (ADOs), preferential allelic amplifications and nucleotide misincorporations during amplification vary substantially between different WGA approaches. A major challenge in single-cell genome analysis is discriminating such WGA artefacts from genuine genetic variants present in the cell before WGA.	(Vermeesch *et al.*, 2016)
Allele drop out (ADO)	The failure to detect an allele in a sample or the failure to amplify an allele.	(Vermeesch *et al.*, 2016)
Allele drop in (ADI)	An allele which is detected, but is not actually part of the genotype, like a false positive.	
Assisted hatching	An ART procedure in which the zona pellucida of an embryo is either thinned or perforated by chemical, mechanical or laser methods.	(Zegers-Hochschild *et al.*, 2017)
B allele frequency (BAF)	The signal intensity of the B allele over the total signal intensity (A+B) for a SNP	
Blastocoele	Fluid-filled inner cavity of the blastocyst.	Adapted from (Zegers-Hochschild *et al.*, 2017)
Blastocyst	The blastocyst contains a fluid filled inner cavity (blastocoele), an outer layer of cells (trophectoderm) and an inner group of cells (inner cell mass). This stage of preimplantation embryo development occurs around Day 5–6, and occasionally Day 7 after ICSI.	Adapted from (Zegers-Hochschild *et al.*, 2017)
Blastomere	A cell from a cleavage-stage embryo.	Adapted from (Zegers-Hochschild *et al.*, 2017)
Cleavage-stage embryos	Embryos beginning with the 2-cell stage and up to, but not including, the morula stage.	(Zegers-Hochschild *et al.*, 2017)
Cryopreservation	The process of slow freezing or vitrification to preserve biological material (e.g. gametes, zygotes, cleavage-stage embryos, blastocysts or gonadal tissue) at extreme low temperature.	(Zegers-Hochschild *et al.*, 2017)
***De novo* segmental chromosome aberration in embryo**	A segmental or partial chromosome deletion/ duplication arising in embryos of couples with normal karyotypes.	
Decontamination	The process of removing or neutralizing contaminants.	(Guide to the quality and safety of tissues and cellls for human application, 2017)

Denudation	The removal or stripping of the cumulus and corona cells from the oocyte.	Adapted from (Guide to the quality and safety of tissues and cellls for human application, 2017)
Sequencing read depth	A crucial parameter in sequencing referring to the number of times a particular read was found independently at a given genomic position.	
Developmental competence	Ability of an oocyte/embryo to complete preimplantation development to the blastocyst stage.	
Electronic witnessing system	Any automated electronic system that assists the traceability of the gametes/embryos in an IVF centre.	
Embryo biopsy	The removal of polar bodies, blastomeres or trophectoderm cells from the embryo for the purpose of genetic analysis.	(Guide to the quality and safety of tissues and cellls for human application, 2017)
Diploidy/euploidy	The condition in which a cell has two haploid sets of chromosomes. Each chromosome in one set is paired with its counterpart in the other set. A diploid embryo has 22 pairs of autosomes and two sex chromosomes, the normal condition.	Adapted from (Zegers-Hochschild *et al.*, 2017)
Exclusion testing	An optional add-on to genetic testing, where DNA from parents and grandparents is compared with the DNA of the embryo or fetus. Exclusion testing means that the at-risk parent doesn't have to have a presymptomatic genetic test to have disease-free children.	Adapted from https://huntingtonstudygroup.org/glossary/exclusion-testing/
Freeze-all cycle	An ART cycle in which, after oocyte aspiration, all oocytes and/or embryos are cryopreserved, and no oocytes and/or embryos are transferred to the uterus of a woman in that cycle.	Adapted from (Zegers-Hochschild *et al.*, 2017)
Genome coverage	the percentage of the target genome covered (in amplification or in sequencing)	
Hatching	The process by which an embryo at the blastocyst stage extrudes out of, and ultimately separates from, the zona pellucida.	(Zegers-Hochschild *et al.*, 2017)
High-order multiple gestation	A pregnancy with three or more embryos or fetuses.	(Zegers-Hochschild *et al.*, 2017)
Homoplasmy	A basic genetic state of mitochondria, in which all of the hundreds to thousands of mitochondrial (mt)DNA copies within a cell or an individual have the same nucleotide-sequence.	(Shibata and Ling, 2007)
Informativity testing	Genotyping of different loci within or flanking the region of interest using DNA from the couple and other relatives prior to segregation analysis for PGT-M preclinical process.	
Informed consent	A person's voluntary agreement based upon adequate knowledge and understanding of relevant information, to donate, to participate in research or to undergo a diagnostic, therapeutic or preventive procedure.	(Guide to the quality and safety of tissues and cellls for human application, 2017)
Inner cell mass (ICM)	A group of cells in the blastocyst that give rise to the embryonic structures and the foetus, the yolk sac, the allantois and the amnion.	(Guide to the quality and safety of tissues and cellls for human application, 2017)

Intracytoplasmic sperm injection (ICSI)	A procedure in which a single spermatozoon is injected into the oocyte cytoplasm.	(Zegers-Hochschild *et al.*, 2017)
In vitro fertilization (IVF)	A sequence of procedures that involves extracorporeal fertilization of gametes. It includes conventional in vitro insemination and intracytoplasmic sperm injection (ICSI)	(Zegers-Hochschild *et al.*, 2017)
Log2 of fluorescence ratios	The log2 transformed value of the normalized signal intensity of a SNP. *(A logR of 0 and BAF values of 0, 0.5 and 1 represent a normal copy number (n=2). A single copy yields a logR of -1 and BAF values of 0 and 1, while 3 copies yield a logR of 0.58 and BAF values of 0, 0.33, 0.66 and 1)*	
Misdiagnosis	When a technical procedure has failed, is inaccurate or has been incorrectly interpreted. Misdiagnoses may be sample- or technique-specific.	Adapted from (Wilton *et al.*, 2009)
Monosomy	The absence of one of the two homologous chromosomes in embryos.	Adapted from (Zegers-Hochschild *et al.*, 2017)
Morula	An embryo formed after completion of compaction, typically 4 days after ICSI	Adapted from (Zegers-Hochschild *et al.*, 2017)
Chromosomal Mosaicism	A state in which there is more than one karyotypically distinct cell population arising from a single embryo.	(Zegers-Hochschild *et al.*, 2017)
Mutation	A permanent change in the nucleotide sequence, proven to be disease causing (class 4-5) and usually with a frequency below 1%.	(Richards *et al.*, 2015)
Negative control	The IVF laboratory negative control is a negative control with sample collection buffer, biopsy media, or washing media to control for contamination during each step of cell sample collection. The genetic laboratory negative control is a negative control with amplification mixture only to control for contamination during set-up of amplification reactions.	
Perivitelline space	The space between the cytoplasmic membrane enclosing the oocyte and the innermost layer of the zona pellucida. (This space may contain the first and second polar bodies and extracellular fragments)	(Zegers-Hochschild *et al.*, 2017)
Polar bodies (PBs)	The small bodies containing chromosomes segregated from the oocyte by asymmetric division during telophase. The first polar body is extruded at telophase I and normally contains only chromosomes with duplicated chromatids (2c); the second polar body is extruded in response to fertilization or in response to parthenogenetic activation and normally contains chromosomes comprising single chromatids (1c).	(Zegers-Hochschild *et al.*, 2017)
Polyploidy	The condition in which a cell has more than two haploid sets of chromosomes (f.x. a triploid embryo has three sets of chromosomes; a tetraploid embryo has four sets)	(Zegers-Hochschild *et al.*, 2017)
Preimplantation genetic testing (PGT)	A test performed to analyze the DNA from oocytes (polar bodies) or embryos (cleavage-stage or blastocyst) for HLA-typing or for determining genetic abnormalities. These include PGT for aneuploidies (PGT-A), PGT for monogenic/single gene defects (PGT-M), and PGT for chromosomal structural rearrangements (PGT-SR).	(Zegers-Hochschild *et al.*, 2017)
Quality assurance (QA)	The actions planned and performed to provide confidence that all systems and elements that influence the quality of the product are working as expected, both individually and collectively.	(Guide to the quality and safety of tissues and cellls for human application, 2017)

Quality control (QC)	The part of quality management focused on fulfilling quality requirements. In terms of preparation, it concerns sampling specifications and testing; for an organization, it relates to documentation and release procedures, which together ensure that the necessary and relevant tests have actually been carried out and that materials have not been released for use until their quality has been judged to be satisfactory.	(Guide to the quality and safety of tissues and cellls for human application, 2017)
Quality management system	The organizational structure, with defined responsibilities, procedures, processes and resources, for implementing quality management, including all activities that contribute to quality, directly or indirectly.	(Guide to the quality and safety of tissues and cellls for human application, 2017)
Recurrent implantation failure (RIF)	Three or more failed in vitro fertilization-embryo transfer cycles involving high-quality embryos	Adapted from (Harper *et al.*, 2010)
Recurrent miscarriage (RM) / Recurrent pregnancy loss	Two or more pregnancy losses until 24 weeks gestation (including chemical pregnancy)	(RPL *et al.*, 2018)
Risk assessment	Identification of potential hazards with an estimation of the likelihood that they will cause harm and of the severity of the harm should it occur.	(Guide to the quality and safety of tissues and cellls for human application, 2017)
Segregation testing	Phasing or haplotyping (determination of the group of alleles within a genetic segment on a single chromosome being inherited together) after informativity testing to establish the high-risk and low-risk haplotypes	
Severe male factor (SMF)	Sperm sample with a combination of oligospermia (low sperm concentration), asthenozoospermia (poor sperm motility) and/or teratozoospermia (abnormal sperm morphology) or azoospermia.	Adapted from (World Health Organization, 2010)
Sexing	Selection of embryos based on sex	
Standard operating procedure (SOP)	Written instructions describing the steps in a specific process, including the materials and methods to be used and the expected result.	(Guide to the quality and safety of tissues and cellls for human application, 2017)
Sub-optimal environmental conditions	Any deviation from the theoretical physiological pH, temperature, oxygen level, or osmolarity.	
Supernumerary embryos	Excess embryos after embryo transfer.	(Guide to the quality and safety of tissues and cellls for human application, 2017)
Trisomy	An abnormal number of chromosome copies in a cell characterized by the presence of three homologous chromosomes rather than the normal two.	Adapted from (Zegers-Hochschild *et al.*, 2017)
Trophectoderm	Cells forming the outer layer of a blastocyst that have the potential to develop into the placenta and amniotic membranes.	(Zegers-Hochschild *et al.*, 2017)
Time-Lapse imaging	The photographic recording of microscope image sequences at regular intervals in ART, referring to gametes, zygotes, cleavage-stage embryos or blastocysts.	(Zegers-Hochschild *et al.*, 2017)
Uniparental disomy (UPD)	The presence of two copies of (part of) a chromosome, from one parent and no copy from the other parent.	(Vermeesch *et al.*, 2016)

Validation	Documented evidence giving a high degree of assurance that a specific process or system, including pieces of equipment or the environmental conditions, will perform consistently to deliver a product meeting its pre-determined specifications and quality attributes, based on intended use.	(Guide to the quality and safety of tissues and cellls for human application, 2017)
Vitrification	An ultra-rapid cryopreservation procedure that prevents ice formation within a cell whose aqueous phase is converted to a glass-like solid.	(Zegers-Hochschild *et al.*, 2017)
Zona pellucida	The glycoprotein coat surrounding the oocyte and the developing embryo up to the blastocyst stage	Adapted from (Zegers-Hochschild *et al.*, 2017)

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768 # SUPPLEMENTARY DATA 2. LIST OF ABBREVIATIONS

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Abbreviation	Full term
aCGH	Array-based comparative genomic hybridization
ADI	Allele drop in
ADO	Allele drop out
AF	Amplification failure
AMA	Advanced maternal age
BACs	Bacterial artificial chromosomes
BMI	Body mass index
bp	Base pairs
DAPI	4′,6-diamidino-2-phenylindole, is a fluorescent stain that binds strongly to adenine–thymine rich regions in DNA
D-ARMS	Double amplification refractory mutation system
dNTPs	Deoxyribonucleotide triphosphates
EQA	External quality assessment
FISH	Fluorescence in situ hybridization
FM	Flanking marker
GDPR	General data protection regulations
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (buffering agent)
HGVS	Human genome variation society
HLA	Human leukocyte antigen
HS dsDNA	High sensitivity double stranded DNA
ICM	Inner cell mass
ICSI	Intracytoplasmic sperm injection
IQA	Internal quality assessment
ISCN	International system for human cytogenetic nomenclature
IVF	In vitro fertilization
Mb	Mega-bases
MDA	Multiple displacement amplification
MOPS	3-(N-morpholino) propane sulfonic acid (buffer)
mtDNA	Mitochondrial DNA
NGS	Next generation sequencing
PB	Polar body
PCR	Polymerase chain reaction
pg	Picogram
PGD	Preimplantation genetic diagnosis
PGS	Preimplantation genetic screening
PGT	Preimplantation genetic testing
PGT-A	PGT for aneuploidy
PGT-M	PGT for monogenic/single gene defects
PGT-SR	PGT for chromosomal structural rearrangements
QA	Quality assurance
QC	Quality control
QMS	Quality management system
qPCR	Quantitative real-time PCR

RIF	Recurrent implantation failure
RM	Recurrent miscarriage
SBR	Signal to background noise ratio
SMF	Severe male factor
SNP	Single nucleotide polymorphism
SOP	Standard operating procedure
STR	Short tandem repeat (markers)
TE	Trophectoderm
UPD	Uniparental disomy
UPS	Uninterrupted power supply
UV-C	Ultraviolet C
WGA	Whole genome amplification
ZP	Zona pellucida

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