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ESHRE PGD consortium best practice guidelines for organization of a PGD centre for PGD/preimplantation genetic screening†

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ABSTRACT: In 2005, the European Society for Human Reproduction and Embryology (ESHRE) PGD Consortium published a set of Guidelines for Best Practice PGD to give information, support and guidance to potential, existing and fledgling PGD programmes. Subsequent years have seen the introduction of new technologies as well as the evolution of current techniques. Additionally, in light of recent advice from ESHRE on how practice guidelines should be written/formulated, the Consortium believed it was timely to update the PGD guidelines. Rather than one document that covers all of PGD, the new guidelines are separated into four documents, including one relating to organization of the PGD centre and three relating to the methods used: DNA amplification, fluorescence *in situ* hybridization and biopsy/embryology. Here, we have updated the sections on organization of the PGD centre. One area that has continued to expand is Transport PGD, in which patients are treated at one IVF centre, whereas their gametes/embryos are tested elsewhere, at an independent PGD centre. Transport PGD/preimplantation genetic screening (PGS) has a unique set of challenges with respect to the nature of the sample and the rapid turn-around time required. PGS is currently controversial. Opinions of laboratory specialists and clinicians interested in PGD and PGS have been taken into account here. Current evidence suggests that PGS at cleavage stages is ineffective, but whether PGS at the blastocyst stage or on polar bodies might show improved delivery rates is still unclear. Thus, in this revision, PGS has been included. This document should assist everyone interested in PGD/PGS in developing the best laboratory and clinical practice possible.

Key words: European Society for Human Reproduction and Embryology / PGD centre organization / preimplantation genetic screening / training / counselling

Introduction

The rapidly changing nature of PGD/preimplantation genetic screening (PGS), specifically the technologies associated with its use and increasing patient access, has necessitated review and revision of the original European Society for Human Reproduction and Embryology (ESHRE) PGD Consortium guidelines (Thornhill *et al.*, 2005). As a result, the ESHRE PGD Consortium (hereafter referred to as the Consortium) has prepared four sets of guidelines: one relating to the organization of the PGD centre and three relating to the methods used: amplification-based PGD, fluorescence *in situ* hybridization (FISH)-based PGD and PGS and embryology, including embryo biopsy (Harton *et al.*, 2010a,b,c). The method guidelines should be read in conjunction with this guideline. In this guideline, the laboratory performing the diagnosis will be referred to as the PGD/PGS centre and the centre performing the IVF as the IVF centre. Topics covered in this guideline include personnel, inclusion/exclusion criteria, genetic counselling and informed consent, setting up an IVF or PGD centre, Transport PGD, Quality Assurance/Quality Control (QA/QC) and accreditation (which is further discussed in the paper by Harper *et al.*, 2010a).

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PGS, called 'low-risk PGD' in the original guidelines, has been carried out for infertile patients undergoing IVF with the aim of increasing the IVF pregnancy and delivery rates. Cited indications for PGS include advanced maternal age (AMA), repeated implantation failure (RIF), severe male infertility and couples with normal karyotypes who have experienced recurrent miscarriages (RM). To date, 11 RCTs have been performed looking at PGS for various indications that have failed to show an improvement in delivery rates for poor prognosis (Staessen *et al.*, 2004; Stevens *et al.*, 2004; Mastenbroek *et al.*, 2007; Blockeel *et al.*, 2008; Hardarson *et al.*, 2008; Schoolcraft *et al.*, 2009; Debrock *et al.*, 2010) and good prognosis patients (Jansen *et al.*, 2008; Mersereau *et al.*, 2008; Staessen *et al.*, 2008; Meyer *et al.*, 2009). These publications have led to an open discussion of PGS and its role in IVF (Mastenbroek *et al.*, 2008; Simpson, 2008; Harper *et al.*, 2008, 2010b; Hernandez, 2009). The general consensus is that since 10 of the RCTs have shown no benefit of cleavage stage biopsy/PGS (possibly owing to the high levels of mosaicism at cleavage stages and the limitations of FISH), further PGS RCTs should concentrate either on polar body or trophectoderm biopsy and a full chromosome analysis (Harper and Harton, 2010; Harper *et al.*, 2010b). An ESHRE PGS task force is supporting a two-centre pilot RCT to determine whether polar body PGS can improve IVF outcome in patients of AMA using array comparative genomic hybridization (Geraedts and De Wert, 2009). Should this RCT indicate its appropriateness, a multicentre randomized trial is planned. Since PGS is still being practiced by some IVF and PGD centres despite the PGD Consortium call that this should only take place in the context of a properly constructed trial rather than an observational study (Harper *et al.*, 2010b), the Consortium felt it was important to set forth our opinion on the best practices that should be followed in a PGS laboratory as well as those for PGD.

PGD/PGS is still relatively unregulated and lacks standardization compared with other forms of diagnostic testing; however, more federal, state and local governments are beginning to take an interest in PGD and some have begun accrediting laboratories that offer PGD (Harper *et al.*, 2010a). This is a logical step considering the comparative difficulty in achieving the highest levels of accuracy and reliability with single cells as part of PGD/PGS compared with more routine genetic testing. Many regulations, laws and voluntary networks exist in the mainstream diagnostic community to maintain the highest quality in diagnostic testing. For example, the European Quality Molecular Network has attempted to improve and standardize molecular diagnostic testing across Europe (Dequeker *et al.*, 2001). One step towards higher quality overall and standardization for PGD/PGS is to build consensus opinion on best practices within the PGD/PGS community; a component of the mission of the Consortium (ESHRE PGD Consortium Steering Committee, 1999, 2000, 2002).

The Consortium recognizes that owing to variations in local or national regulations and specific laboratory practices, there will remain differences in the ways in which PGD/PGS are practiced (from initial referral through IVF treatment, single-cell analysis to follow-up of pregnancies, births and children). However, this does not preclude a series of consensus opinions on best practice based on experience and available evidence. Indeed, the American Society for Reproductive Medicine (ASRM) published a practice committee report for PGD in 2008 (American Society for Reproductive Medicine and Society for Assisted Reproductive Technology. Practice Committee Report, 2008) essentially reviewing PGD practice in the USA. The PGD International Society (PGDIS) has also drafted guidelines that were recently updated, and although more in-depth than the ASRM report, they are concise and remain so in their recent revised edition (PGDIS, 2004, 2008). The consensus opinions provided in our document and the accompanying guidelines not only reflect the current use of PGD but also offer a consensus-based specific guidance regarding how best to practice clinical PGD based on clinical experience and both published and unpublished data.

The Consortium hopes that a minimum standard might be achieved across all centres providing PGD clinically. Achieving this goal ultimately should ensure that all patients receive optimum care regardless of the centre in which they are treated. Rather than a drift towards the lowest common denominator, established and fledgling centres alike can learn from global experiences and be guided by consensus opinion.

These opinions are not intended as rules or fixed protocols that must be followed, nor are they legally binding. The unique needs of individual patients may justify deviation from these opinions, and they must be applied according to individual patient's needs using professional judgement. However, guidelines 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.

1. Personnel

Staffing

1.1. In order to ensure that the embryo biopsy and diagnosis for PGD is performed by competent staff, the following **recommendations** are made (Geraedts *et al.*, 2001; Thornhill *et al.*, 2005; Harper *et al.*, 2010a):

1.1.1. Biopsy should be performed by a clinical embryologist who performs embryology on a day-to-day basis and holds the relevant certification for their own country, and/or where none exist uses the ESHRE certification for clinical embryologists (www.eshre.com).

1.1.2. FISH should be performed by qualified personnel with knowledge of cytogenetics or under the supervision of a cytogeneticist competent or certified to perform clinical diagnosis; most importantly, personnel performing diagnostic testing should have appropriate documented training in single-cell diagnosis. Spreading or fixing cells for FISH can be performed by an embryologist or FISH personnel, provided that they have had specific training and assessment in order to do so.

1.1.3. DNA amplification procedures should be performed by qualified personnel with knowledge of molecular biology or under the supervision of a molecular biologist competent or certified to perform clinical diagnostics; most importantly, personnel performing the diagnostic testing should have appropriate documented training in single-cell diagnosis. Placing cells into PCR tubes for amplification can be performed by the embryologist or molecular biology PGD personnel provided that they have had specific training and assessment to do so.

1.1.4. It is **recommended** that the PGD laboratory should be directed by a person or persons who have executive accountability and the competence to assume responsibility for the services

provided. It often is the national professional group that decides what the competence level should be (Harper *et al.*, 2010a).

Staff training and competency

1.1.5. Laboratory staff performing clinical work should have a recognized training and assessment programme. Established training programmes exist for embryology but currently there are no official training programmes for single-cell diagnosis. A defined training and QA programme needs to be developed by the PGD centre to encompass all areas of work, including the use of all equipment, all relevant standard operating procedures, data protection and training in the IVF centre, for example.

1.1.6. Training should be supervised by an appropriate person and records kept in the individual member of staff's own log book. All staff should keep a log of their continued professional development to ensure continual recording and updating of their competencies.

1.1.7. It is **acceptable** to train staff in both disciplines (FISH-based and amplification-based PGD/PGS) as long as each discipline has a training schedule and assessment programme to demonstrate proficiency with all the necessary skills and techniques and ability to work independently in either or both sections.

1.1.8. All personnel should demonstrate competency before being allowed to handle clinical specimens. Once trained, staff should undertake competency assessment at least once per year in all aspects of clinical procedures that they are trained to perform. Establishing internal continuous training programmes and competency assessment of staff is **recommended (**Thornhill *et al.*, 2005; PGDIS, 2008; Harper *et al.*, 2010a).

2. Inclusion/exclusion criteria for patient referrals: general

The decision to include or exclude referrals should be undertaken by a team of dedicated scientists and clinicians, including clinical geneticists or genetics counsellors, molecular biologists/cytogeneticists, clinical IVF specialists and embryologists. Referrals could also be considered by local ethics boards, national legislation or local/national regulatory agencies.

Inclusion

2.1. The Consortium understands that local regulations will vary from centre to centre as will criteria for inclusion and exclusion of patients. These recommendations are made as a general starting point for discussion.

2.2. For inclusion into PGD, it is recommended that cycles be undertaken where diagnosis is technically possible in principle and the reliability of the diagnosis is high (each clinic should understand their error rates and communicate this with the patient). Current technology in most PGD centres allows for error rates as low as 1–2%. Further, it is recommended that patients with infertility or subfertility only have PGD when IVF/ICSI is likely to overcome the fertility issue.

Exclusion

2.3. Patients should be excluded from the PGD programme if diagnosis of disease state is not technically feasible with current technology or if the PGD centre does not offer a test that can reliably diagnose the disease state of embryos from the patient. In addition, consideration should be given by each IVF centre relating to exclusion criteria for PGD patients on the basis of likelihood of success or safety; their age, reduced ovarian reserve, contraindications for IVF/ICSI and patients with an unhealthy BMI.

2.4. Exclusion from PGD should be considered if the woman has serious signs and symptoms of an autosomal dominant or X-linked disorder (for which PGD is requested) which could introduce medical complications during ovarian stimulation, oocyte retrieval (OR) or pregnancy, or put a child born at risk of harm. Each specific instance will need to be evaluated by the IVF and PGD centres and may be subject to local, state or federal law.

2.5. PGD may be inappropriate if an affected spouse has serious physical/mental/psychological/psychiatric problems related to the genetic disorder for which PGD is requested.

Inclusion/exclusion criteria specific to amplification-based PGD

Inclusion

2.6. Testing can be carried out for confirmed pathogenic germline mutation(s) that have been identified in one parent for dominantly inherited diseases or in each parent for recessively inherited disorders giving a disease recurrence risk of 50 or 25%, respectively.

2.7. The germline mutation(s) is known to be causative of serious health effects that may manifest at birth, in childhood or as an adult.

2.8. For recessive and some X-linked (e.g. Duchenne muscular dystrophy) disorders, where a single germline mutation has been diagnosed in the proband and only one parent, it is **acceptable** to offer diagnosis if the pathogenic genotype can be attributed to a single gene and there is sufficient family history to identify a haplotype linked to the germline mutation.

2.9. Exclusion testing can be carried out for late-onset disorders, such as Huntington's disease to avoid presymptomatic testing of the partner with a family history of the disease (Sermon *et al.*, 2002; Moutou *et al.*, 2004; Jasper *et al.*, 2006; Peciña *et al.*, 2009).

Exclusion

2.10. Where the genetic diagnosis is uncertain, for example, owing to genetic/molecular heterogeneity or uncertain mode of inheritance and recurrence risk is low (e.g. $<10\%$).

PGD for mitochondrial disorders

PGD testing for mitochondrial disorders may be difficult as the genetic diagnosis can be uncertain.

Inclusion

2.11. In cases where the causative mutation of the mitochondrial disease is encoded by nuclear DNA, testing is the same as for other single disorders (Altarescu *et al.*, 2008; Unsal *et al.*, 2008).

2.12. It is **acceptable** to carry out sexing to reduce the clinical risk of the disease in the case of mutations that show homoplasmy, but where the penetrance of the mutation is sex-dependent

(Bickerstaff et al., 2001). It should be noted that this use of testing only **reduces** the risk to offspring, it does not eliminate it.
2.13. PGD may also be carried out for rare cases where there is skewed meiotic segregation of particular mutations with good correlation of mutational load and disease severity, e.g. NARP 8993T → G (Steffann et al., 2006).

HLA typing

Inclusion

2.14. When all other clinical options have been exhausted, PGD is acceptable for couples who already have a child affected with a malignant disorder or a genetic disorder, if the affected child is likely to be cured or life expectancy is substantially prolonged by stem cell transplantation with cord blood from a HLA-matched sibling (Samuel et al., 2009).
2.15. Testing can be carried out for HLA typing alone where the recurrence risk of the disease is low or in combination with mutation detection in the case of autosomal recessive or X-linked recessive disorders (Verlinsky et al., 2001, 2007; Fiorentino et al., 2006; Van de Velde et al., 2009).

Exclusion

2.16. Consideration should be given to the time required for the PGD test to be developed and applied 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. A request for HLA typing in the absence of a specific genetic disease to create a future donor for a sibling should be excluded (Simon and Schenker, 2005).

Inclusion criteria specific to PGS

Although PGS remains controversial in clinical practice (see Abstract and Introduction), the following indications for its use have been reported:

2.17. AMA (>36 completed years—exact age to be determined by each centre).
2.18. RIF (e.g. ≥3 embryo transfers with high-quality embryos or the transfer of ≥10 embryos in multiple transfers—exact numbers to be determined by each centre). Implantation failure is defined as the absence of a gestational sac on ultrasound at 5 or more weeks post-embryo transfer.
2.19. RM (≥3 miscarriages—exact number to be determined by each centre). It should be noted that patients with a history of RM have a high chance of successfully conceiving naturally (Brigham et al., 1999; Carp et al., 2001).

Special considerations for PGS patients

The following **recommendations** are made:

There is a three step decision-making process by the gynaecologist in cooperation with the embryologist and the geneticist, after consultation with the patients:

2.20. Before start of controlled ovarian stimulation, there should be discussion about whether PGS is appropriate for the couple.
2.21. After OR, there should be discussion about whether PGS of oocytes or embryos should be performed and/or after review of fertilization and embryo developmental progress whether PGS of embryos should be performed.
2.22. There should be discussion after review of the genetic results as to which oocytes or embryos should be selected for culture and transfer.

3. Genetic counselling and informed choice

Referrals for PGD testing

3.1. Relevant documentation to begin PGD testing includes:

3.1.1. Genetic counselling report (Vendrell et al., 2009).
3.1.2. Original results of DNA testing or other specific testing of the index patient, spouse or partner, children or other family members (when appropriate).
3.1.3. Full pedigree and family data (Solomon et al., 2008).
3.1.4. Data on health problems of female and male partners, and specialist consultations which may impact on genetic diagnosis or IVF success and pregnancy (when appropriate).
3.1.5. Female reproductive history, gynaecological and fertility status.
3.1.6. Male reproductive history, andrologic history, fertility status, results of sperm analysis (especially in cases where the genetic disorders for which PGD 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).
3.1.7. For HLA testing, a medical report of the affected child, current situation, prognosis, options for treatment other than PGD, suitability for stem cell transplantation, results of previous HLA typing (serologic and/or DNA markers) in affected child, parents and siblings.
3.1.8. Regulation of PGD varies internationally (Soini, 2007; Dickens, 2008). The legality of undertaking PGD in a particular country should be verified and, if required, licenses or approval to carry out PGD for specific disorders or HLA typing should be obtained prior to the start of IVF stimulation.

3.2. General issues relating to counselling:

3.2.1. As PGD treatment involves both partners of a couple, both partners should, where possible, attend consultations.
3.2.2. 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 to ensure that patients are fully informed of all aspects of PGD before treatment starts.
3.2.3. Provision should be made to ensure patients have access to an independent interpreter where possible, although a family member could act as translator in the absence of an alternative.

3.2.4. Written information about treatment should be available prior to a consultation and personalized post-consultation letters should contain the information given orally during the meeting.
3.2.5. Written information must be in language that can be understood by a layperson as technical terminology may lead to patient misunderstanding.
3.2.6. The counselling provided should be non-directive, enabling patients to reach their own conclusion about the suitability of treatment (Kessler, 1997).
3.2.7. Counselling should be offered both before and after the IVF/PGD cycle, whereas additional counselling may be needed after completion of the pre-examination laboratory work-up to discuss the expected efficiency limitations of the test design, or during pre- or post-natal follow-up.

Psychological evaluation

3.3. Psychological evaluation should be considered for the following patients:

3.3.1. Patients with a history of reproductive failure.
3.3.2. Patients with a history of traumatic experiences.
3.3.3. Couples for whom the geneticist, gynaecologist or other member of the IVF/PGD team has doubts regarding welfare of existing or future children/psychological physical wellbeing/mental capacity of future parents.
3.3.4. Couples who actively ask for psychological intervention.
3.3.5. 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).
3.3.6. Couples who are undergoing HLA-matching PGD in order to evaluate their 'child wish'.
3.3.7. Counselling should be offered both before and after the IVF/PGD cycle.

Genetic risk assessment

3.4. Patient discussions should include:

3.4.1. A contemporaneous review of the genetic risk and molecular or cytogenetic confirmation of the diagnosis where appropriate. This should be undertaken by a qualified clinical geneticist or genetic counsellor (www.eurogenetest.org).
3.4.2. The risk of recurrence, and this should also be documented.
3.4.3. The severity and variability of the condition and the limitations of genotype/phenotype correlation.

Reproductive options

3.5. Alternative options to PGD should be discussed including prenatal diagnosis, gamete donation, adoption, acceptance of risk and having no (additional) children. These should be discussed in the context of the success and limitations of PGD.
3.6. The couple should be asked about their reasons for considering PGD to ensure that they have realistic expectations of what can be offered.
3.7. The risk of spontaneous pregnancy and consequent genetic risk to that offspring should be discussed if contraception is not used prior to and during treatment.

IVF-related counselling

3.8. A specialist in reproductive medicine should consider discussion of the following items:

3.8.1. Description of and details regarding the IVF/ICSI procedure.
3.8.2. Risk of medical complications for women during ovarian stimulation or OR.
3.8.3. Use of fresh or cryopreserved sperm or sperm retrieved by techniques such as percutaneous sperm aspiration or testicular sperm extraction.
3.8.4. Additional short- or long-term medical risks of the procedures and pregnancy for women affected with an autosomal dominant or X-linked genetic disorder (e.g. risk of haemorrhage during OR and parturition for haemophilia carriers; thrombosis in women with clotting disorders, such as factor V Leiden mutation).
3.8.5. Uncertainty about future fertility and health of women after PGD treatment (Winston and Hardy, 2002).
3.8.6. Chance of spontaneous pregnancy in waiting time or during IVF treatment, and need for contraception.

PGD counselling

3.9. A qualified clinical geneticist or genetic counsellor should consider discussion of the following items:

3.9.1. The number of oocytes to be retrieved and the need to maximize this within the safe limits of medical practice.
3.9.2. The number of embryos to be biopsied and error rates, the number of cells to be biopsied and the percentage of embryos expected to survive the biopsy.
3.9.3. That some embryos may be unsuitable for biopsy and some embryos may not survive the biopsy.
3.9.4. A diagnosis may not be possible for all biopsied embryos and there is a possibility of some embryos being undiagnosed or giving unclear results.
3.9.5. Likelihood of transferring unaffected embryos and the possibility that all embryos may be affected.
3.9.6. The possibility of having no embryos for transfer if all the embryos are genetically and/or embryologically unsuitable.
3.9.7. Which condition(s) are being tested for and what is not able to be detected by the test.
3.9.8. The reliability of the PGD diagnosis, the risk of misdiagnosis or adverse outcome. 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 (Dreesen *et al.*, 2008; Goossens *et al.*, 2008a,b).
3.9.9. The method of testing and sample requirements to develop the test should be clearly explained.
3.9.10. For structural chromosomal rearrangements, an adequate FISH probe combination should be used and validated to be able to detect unbalanced rearrangements (see Guidelines on FISH-based PGD for more information).
3.9.11. For specific monogenic or mitochondrial mutations, confirmation of the mutation in the family alongside the informativity of

linked markers (see Amplification-based PGD Guidelines, Harton *et al*., 2010b).

3.9.12. For conditions where the mutation has variable repeat sizes, e.g. Huntington's disease, fragile X, testing for informativity of the normal alleles.

3.9.13. The reliability of the test results should be designed to achieve the highest possible accuracy. Current technology allows for test performance in the range of 98–99% accuracy. Any limitations of testing should be clearly explained to the couple. The patients should understand that a misdiagnosis is possible, and the options that are available to them should a pregnancy occurs, including prenatal testing and genetic counselling. Each PGD centre must report an expected and observed rate of misdiagnosis (see Amplification-based PGD Guidelines and FISH-based PGD Guidelines for more details).

3.9.14. The expected time-frame for the set up of the test and the start of treatment should be achievable and applicable to the couple. Constraints, such as female age, should be considered if the time-frame for test development would have implications for the success of treatment. If specific single-cell analysis is not available 'in-house' and the set-up time is significant, there should be discussion about referring the couple to another centre.

3.9.15. If the requested genetic analysis is not performed 'in-house', the possibility of referral to another centre where testing is available or the option of 'Transport PGD'.

3.9.16. The risk of spontaneously conceiving a child affected by the genetic disorder if no contraception is used prior to PGD (Wilton *et al*., 2009).

3.9.17. Costs.

3.9.18. Cancellation policy if pre-existing fertility problems or requirement for IVF exists irrespective of need for PGD.

3.9.19. For X-linked diseases, the pros and cons of sexing (with subsequent transfer of females assumed to be unaffected) as opposed to a specific mutation detection which allows for the transfer of unaffected males and females.

Embryo choice

3.10. Decision-making about which embryos are acceptable for transfer should be discussed with the patients before a treatment cycle begins and may need to be revisited during the cycle.

3.11. A discussion should be held regarding the number of embryos to be transferred and policy on elective single-embryo transfer in the centre.

3.12. For autosomal recessive and X-linked recessive disorders, the transfer of carrier embryos should be discussed. Couples who choose not to replace carrier embryos would, in theory, have fewer embryos available for transfer, which may lower their chance of success in any given cycle.

3.13. In X-linked recessive disorders, where sex selection only is available, couples need to be informed that all male embryos, affected or unaffected, will be discarded and carrier females cannot be distinguished from unaffected female embryos. The availability of alternative direct mutation testing (possibly in another centre) should always be discussed with the patient before decisions about treatment are finalized.

3.14. For X-linked recessive disorders, where the phenotype of all embryos will be known, the issue of selection of the sex of the embryo in addition to phenotype should be discussed within the legal constraints of the relevant jurisdiction. Patients should be clear about what results are available and given a choice in terms of how much information they wish to know. The option of not knowing the sex of the embryo should be discussed.

3.15. For dynamic mutations where mutation size may have been measured and may have a phenotype/genotype correlation, this issue should be discussed with the patient before treatment starts so that they are fully informed before decisions on which embryos to replace are made.

PGD follow-up

The following issues should be discussed with patients prior to undertaking a PGD cycle:

3.16. Chance of (ongoing) pregnancy/live birth per cycle started and per transfer, related to maternal age and to specific disorder, as well as the risk of miscarriage.

3.17. Cyropreservation following PGD and the predicted success of pregnancies from cryopreserved and biopsied embryos.

3.18. The fate of unaffected, and/or non HLA-matched embryos.

3.19. The fate of non-diagnosed or non-transferable embryos.

3.20. Confirmation of PGD diagnosis on non-transferred, non-cryopreserved embryos and availability of these results for the individual couple.

3.21. Options for embryos not transferred or frozen for future use, including donation to research.

3.22. Decision-making about prenatal diagnosis and follow-up of pregnancies and children born from PGD.

Impact of PGD

Multiple pregnancies

3.23. The risk of conceiving a multiple pregnancy should be discussed with couples prior to the start of a treatment cycle. They should be made aware of both the maternal and paediatric risks (Braude, 2006; El-Toukhy *et al*., 2006). Factors that should also be raised with the couple are those relating to the family dynamics, the presence of affected children within the family unit, the health of the parents (dominant disorders) and the economic and psychosocial issues related to managing a multiple pregnancy.

3.24. Consideration should be given to the chance of successful pregnancy versus the risks of multiple pregnancies after the transfer of one or multiple embryos, taking into account relevant factors including the patient's age, reproductive history and other factors impacting implantation (Khalaf *et al*., 2008).

Affected children

3.25. Couples requesting PGD are often responsible for the care of affected children who may require nursing care. The impact of PGD treatment cycles, the travelling, time away from home, potential sequelae of OHSS in the mother and the impact of siblings in special care as the result of a multiple birth should be discussed ahead of treatment. Couples should be encouraged to ensure that they have support for current children before undertaking a treatment cycle.

Paediatric follow-up of PGD babies

3.26. The paediatric risks associated with PGD, including low birthweight, perinatal mortality and congenital abnormalities, should be discussed with patients who should be informed that these risk factors are similar to those in children born following IVF/ICSI (Lambert, 2003; Goossens *et al.*, 2008a,b, 2009; Manipalviratn *et al.*, 2009). In addition, although there is now evidence of longer term wellbeing in PGD children, and normal growth and development parameters (Banerjee *et al.*, 2008; Desmyttere *et al.*, 2008; Nekkebroeck *et al.*, 2008a,b), the uncertainty about the long-term impact of PGD should be raised and centres offering PGD should be encouraged to obtain follow-up data on babies born following treatment. The suggested minimum data set should include:

- date of birth
- birthweight
- gestation
- neonatal problems
- congenital abnormalities (Simpson and Liebaers, 1996)

3.27. Longer term follow-up should be considered by centres offering treatment and participation in collaborative prospective and retrospective studies is encouraged.

Prenatal diagnosis

3.28. Prenatal diagnosis should be offered to all women who become pregnant following PGD. 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.

3.29. If prenatal diagnosis is declined, the patients could choose (or be encouraged to think about) cord blood sampling at delivery to confirm the karyotype or genotype. Clear arrangements should be made with the patients for the sharing of the results as the implications of an adverse result could have a major impact for the parents and child.

3.30. Prenatal testing or cord blood sampling for late-onset conditions raises ethical concerns where local regulations differ widely. Testing of minors for late-onset conditions where there is no clinical benefit is not recommended (Clinical Genetics Society UK, 1994).

Counselling issues specific to monogenic disorders

3.31. The principle of the diagnostic test should be explained either using specific genetic analysis to target the mutation and/or closely linked markers.

3.32. An explanation should be given for the need to study family members to determine phase alleles in linked markers or sperm in males with *de novo* mutations.

3.33. Decision-making about transfer of carrier embryos (for autosomal recessive and X-linked recessive disorders) and the fate of affected embryos or undiagnosed embryos, taking local and national regulations into consideration.

Counselling issues specific to HLA

3.34. For HLA typing alone, an average of 25% of embryos will be suitable for transfer.

3.35. If HLA typing is combined with a specific PGD diagnosis for an autosomal recessive disorder, an average of only 3 of 16 (18.8%) embryos will be suitable for transfer.

3.36. If HLA typing is combined with sexing for an X-linked disorder, an average of only one of eight (12.5%) embryos will be suitable for transfer.

3.36.1. Couples should be referred to a centre for stem cell transplantation to obtain full information on the chances of success of stem cell transplantation of cord blood, available alternative treatments, and possible complications of stem cell/bone marrow transplantation and optimal timing of stem cell transplantation.

4. Basic requirements of an IVF and/or PGD centre

4.1. Egg retrieval, fertilization and culture of embryos should be undertaken in an establishment which has suitable laboratory premises, equipment and trained staff, in accordance with the European Union Tissue and Cells directive or other local laws.

4.2. Since polar body, cleavage stage or blastocyst biopsy is required for genetic testing, these techniques, which may not necessarily be available in standard IVF units, should be undertaken by appropriately trained individuals whose competence is regularly assessed as part of the required inspection processes. More than one individual should be trained to avoid difficulties with absence or holidays. Local approval for embryo biopsy should be obtained if necessary.

4.3. Appropriate precautions should be taken both to prevent contamination of samples by extraneous cells or DNA, by physical isolation (working in clean air hoods or isolators), and to detect any such contamination, e.g. paternal and maternal DNA markers.

4.4. A quality management system should be in place which should assure appropriate allocation of results to the embryo (egg) from which the diagnostic sample was taken (Harper *et al.*, 2010a).

4.5. Once the biopsy is taken, it may be analysed in a unit closely affiliated with or in geographical proximity to the IVF/PGD unit, but alternatively may be sent to a genetic testing unit some distance away or in another country. A system for accurate allocation of samples from particular embryos should be established and documented, and standard operating protocols for sending samples, acknowledgment of receipt, and for transferring results after testing should be established.

4.6. A close working relationship should be established with a genetics department where appropriate genetic counselling (to be distinguished from infertility counselling) is readily available and where the staff are familiar with the technique, potential and limitations of preimplantation testing.

4.7. It is essential that an adequate labelling system is used to match the cell diagnostic result with the embryo from which that cell was biopsied.

4.8. Labelling and sample identification should be confirmed for critical and high risk steps. It is recommended that the unique patient identifier and embryo/cell number should be witnessed and signed by two scientists during the following stages:

4.8.1. Immediately after biopsy to confirm the embryo and cell number match.

4.8.2. At fixation/spreading or placing cells into tubes to confirm that the cell identification matches the labelling on the relevant slide or tube.
4.8.3. When diagnostic results are recorded to ensure accuracy and correlation with the correct cell and/or embryo identification.

Baseline IVF centre pregnancy rates for PGD

4.9. It should be recognized that the outcomes of a PGD programme are dependent on the success rates of the relevant IVF centre. Minimum acceptable pregnancy rates should be determined by the individual centre.
4.10. Laboratories performing genetic testing on embryos should compare pregnancy rates, categorized by the type of genetic disorder (e.g. chromosome rearrangements, autosomal dominant, autosomal recessive, X-linked), with published data (ESHRE PGD Consortium data) and with their own data for IVF/ICSI without biopsy.
4.11. Centres performing PGS should compare pregnancy rates with equivalent groups undergoing IVF/ICSI.

Specifically for the PGD centre

4.12. Accreditation by a local or national body if possible.
4.13. Local or national approval for genetic embryo analysis, if applicable.
4.14. Adherence to published guidelines on PGD/PGS (Thornhill *et al.*, 2005; PGDIS, 2008).
4.15. Constant awareness of possible causes of misdiagnosis and precautions in place to protect against them (Wilton *et al.*, 2009)

5. Transport PGD

5.1. It is **recommended** that the IVF and PGD centres have in place an official agreement dealing with legal, insurance and accountability issues.
5.2. The IVF and PGD centres should agree on a set of clinical/laboratory protocols prior to shipment of any clinical samples. It is **recommended** (as far as feasible) that the same protocols are being used for all referring IVF centres. Special attention should be paid to who is responsible during the various stages of a transport PGD treatment. It is **recommended** that the IVF and PGD centres use combined informed consent.
5.3. It is **recommended** that the IVF and PGD centres schedule a site visit back and forth prior to shipment of any clinical samples.
5.4. The PGD centre should, for each referring IVF centre, validate the shipment protocols being used to assess approximate transportation time and ensure that transport of samples does not compromise cell morphology, FISH hybridization or DNA integrity.
5.5. IVF centres sending out cells fixed onto microscope slides should be trained in biopsy and fixation procedures according to PGD centre specified procedures. If this is not possible, the referring IVF centre should arrange to have a suitably qualified and trained embryologist to perform the biopsy and blastomere preparation.
5.6. IVF centres sending out cells in PCR tubes should be trained in procedures for embryo biopsy and placing cells into tubes according to the PGD centre specified procedures. If this is not possible, the referring IVF centre should arrange to have a suitably qualified and trained embryologist to perform the biopsy and blastomere preparation.
5.7. Practice Runs—PGD procedures should be evaluated before sending/receiving actual clinical specimens by scheduling at least one, and preferably multiple, 'practice runs' with the referring IVF centre. This practice should assess the quality of biopsy and handling of blastomeres/nuclei, proper (unique) labelling of specimens and shipment (including number of transported samples), as well as prove the ability of the testing centre to produce PGD results from biopsied and subsequently transported nuclei. In addition, the practice run will assess contamination potential and the DNA cleanliness of the IVF centre by the way of negative control specimens.
5.8. It is **recommended** that the PGD centre is in charge of the timing of transport PGD cycles (both initial start and actual cell transport). Referring IVF centres should adhere to the rules set for the maximum number of transport cycles the PGD centre can take on a given day or in a given period.
5.9. It is **recommended** that the IVF and PGD centres delineate clear and sufficient lines of communication (as documented in written procedures) during all stages of a transport PGD treatment.
5.10. The IVF and PGD centres must both ensure that patients have had adequate PGD counselling (see elsewhere) and precycle work-up (see elsewhere).
5.11. It is **recommended** that all diagnostic results and reports are sent in written form (fax or email/safe line) and no detailed results are communicated by telephone. Reports should have a fixed format and be clear and user-friendly (Harper *et al.*, 2010a). It is **recommended** that results are reviewed by a qualified professional in the IVF centre prior to the discussion of results with the patient.
5.12. It is **recommended** that the IVF and PGD centres agree on who is responsible for the collection of PGD data (ESHRE PGD Consortium) and follow-up of PGD children.

6. QC, QA and accreditation

QC and QA are an essential aspect of PGD services.

Accreditation

Accreditation, along with proficiency testing through external quality assessment (EQA), provides a means to achieve and maintain the highest laboratory 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 aspect of management, along with technical requirements.

6.1. The ESHRE PGD Consortium **recommends** that, where possible, PGD laboratories should be accredited or working towards accreditation within a defined period of time (Harper *et al.*, 2010a).
6.2. PGD laboratories should conform to ISO 15189 or equivalent local standards and work with national diagnostic laboratory accreditation schemes, if available.
6.3. To this end, the Consortium has prepared a 'beginners guide' to PGD laboratory accreditation (Harper *et al.*, 2010a).
6.4. In accordance with laboratory accreditation, it is essential that the PGD laboratory is run to the highest standards, as with other mainstream diagnostic laboratories, with standard operating procedures in place and suitably trained staff.

Quality management

Assuring patient safety through the quality of results should be the aim of all medical laboratories. This can only be achieved by establishing quality management in the laboratory setting. Quality management includes all the systems and procedures needed to maintain and improve quality.

6.5. QC, QA and quality improvement all form a part of total quality management and it is **recommended** that this system is integrated to the PGD centre [Soini *et al*., 2006; Directive 2004/23/EC of the European Parliament (on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of Human tissues and cells)].
6.6. Aspects of quality management include document control, quality manual, quality policy, resolution of complaints, continual improvement, audits and management review.
6.7. Technical requirements include personnel, laboratory conditions and environment, laboratory equipment, all stages of examination procedures, results reporting and QA (Harper *et al*., 2010a).

Internal QC and QA

Internal QC/QA should be an ongoing process (Thornhill *et al*., 2005; PGDIS, 2008). Internal QA/QC should also be maintained and documents and audit should involve confirmation of diagnosis and evaluation of misdiagnosis rates.

6.8. Protocols:

6.8.1. Clinical testing protocols should include explicit instructions including a summary of results from the validation steps of assay development, scoring criteria, reporting procedures as well as a framework for counselling patients regarding the diagnostic results.
6.8.2. All protocol documents should be controlled to ensure that the most recent version is being used.
6.8.3. All protocols should be readily accessible to relevant staff.
6.8.4. Deviations from protocol should be recorded.
6.8.5. If frequent deviations occur, there should be a mechanism in place to change procedures accordingly.
6.8.6. All protocols should be reviewed and updated at least annually and all relevant staff notified of any protocol modifications.

External quality assessment

EQA forms an important part of quality management and is essential for accreditation schemes.

6.9. The Consortium **recommends** that each centre becomes part of an EQA scheme. The ESHRE PGD Consortium has set up EQAs for PCR (in collaboration with UK National EQA Scheme and for FISH with Cytogenetic European Quality Assessment).
6.10. Voluntary participation in EQA at least once per year is **recommended** (Thornhill *et al*., 2005; Wilton *et al*., 2009; Harper *et al*., 2010a).

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