



Background document on preimplantation and prenatal genetic testing

Clinical Situation

Legal situation¹

¹ Part II of this document presents the legal situation in Council of Europe member states and will be regularly updated (last update 7 May 2015). The updated text is highlighted in green.

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Introduction

The Steering Committee on Bioethics (CDBI) elaborated a draft Additional Protocol to the Convention on Human Rights and Biomedicine concerning Genetic Testing for Health Purposes which it approved in June 2007. The scope of this protocol excludes genetic tests carried out on human embryos and fetuses including tests on components of embryonic and foetal origin such as foetal cells or DNA or RNA present in maternal blood.

The CDBI entrusted the Secretariat with the elaboration of the present background paper in consultation with the Chairperson of the Working Party on the Protection of the Human Embryo and Foetus (CDBI-CO-GT3) and the Chairperson of the Working Party on Human Genetics (CDBI-CO-GT4) to provide information on preimplantation and prenatal diagnosis and legal and ethical questions arising from them. This paper is intended to provide a basis for the CDBI to discuss how to deal with the questions relating to such tests.

In 2003, the Working Party on the Protection of the Human Embryo and Foetus (CDBI-CO-GT3) drafted a report on the Protection of the Human Embryo *in vitro*². This report provides an overview of ethical questions and positions in Europe concerning the embryo *in vitro* including a chapter on preimplantation diagnosis. The present paper extends the discussion to genetic testing carried out on the foetus *in vivo* and presents updated information on clinical practice and existing national regulations and guidelines concerning preimplantation and prenatal testing.

In vitro fertilisation has been performed since the late '70s to help couples with fertility problems. Advances in reproductive medicine have opened new possibilities to avoid genetic disease by selective transfer of embryos. At the beginning of the '90s, preimplantation genetic diagnosis (PGD) was introduced as a possible alternative to *prenatal genetic diagnosis* (PND) for couples at risk of transmitting a particularly severe genetic defect, avoiding the difficult decision of whether or not to terminate a pregnancy.

Preimplantation and prenatal genetic diagnosis aim specifically at identifying genetic characteristics which have been inherited from one or both parents or acquired during early prenatal development. PGD and PND involve chromosomal analysis or DNA or RNA analysis carried out on biological material originating from an embryo or a foetus.

How this background paper was prepared

For the preparation of this background paper, recent scientific publications and relevant documentation by national or international institutions or societies were studied alongside with the collection of existing laws, practices and professional guidelines mainly from European countries. The Secretariat also benefited from the contributions and comments of experts in the fields concerned in particular from Prof. Jacques Montagut, Prof. Tal Anahory and Dr Elisabeth Carles (France). Furthermore, a questionnaire was sent to CDBI delegations to inquire about the legal situation regarding preimplantation and prenatal genetic diagnosis in each country (see part II).

² CDBI-CO-GT3 (2003) 13

Part I. Preimplantation (PGD) and Prenatal (PND) Genetic Diagnosis. Clinical practice, Trends and Technological Developments

Part I, after short general information on genetic diseases (chapter 1), describes the techniques used for preimplantation and prenatal genetic diagnosis. In principle, 'preimplantation genetic diagnosis' refers to two main techniques involving the analysis of genetic material contained in embryonic cells or polar bodies removed *in vitro* with a view to obtain information on genetic characteristics prior to the transfer and implantation of embryos in the uterus. Use of these techniques in the clinical practice will be presented below in separate chapters:

1. Preimplantation genetic diagnosis
2. Polar body diagnosis

Prenatal genetic diagnosis is carried out during a pregnancy to investigate the genetic characteristics of a foetus *in vivo*. It will be presented in the fourth chapter of part I.

Any of these tests will be accompanied by an offer of an appropriate genetic counseling aiming to enable the parents concerned to make informed choices with regard to the test considered. Such decisions should be based on good knowledge and understanding of the aim and consequences of the test, the procedure to be followed, and the different kinds of risks involved.

1. Genetic diseases

In general³, the term « genetic disease » refers to diseases that are related to defect on one or more genes. They are classified as monogenic, polygenic or multifactorial, chromosomal and mitochondrial diseases.

1.1 Monogenic diseases

They result from the impairment (mutation) of a single gene. These diseases may be classified into three sub-groups:

***Dominant autosomal diseases.** They result from the impairment of a gene carried by one of the 22 autosomal pairs, and produce a clinical phenotype visible if at least one of the two alleles of the same gene is impaired (transmission by one of the two parents).

***Recessive autosomal diseases.** These diseases only appear if both alleles of the gene in question are impaired (homozygous individual). This presupposes that both parents of the affected individual are carriers of the impaired gene.

***Diseases linked to the sex chromosomes.** Genes on the sex chromosomes are transmitted in the same way as genes on an autosome. The difference in pattern of inheritance depends on the difference in gene content between X and Y chromosomes, where the Y chromosome has almost no genes while the X

³ Genetic disease may also be linked to chromosome aberrations. (See section 1.3)

chromosome contains genes in the same manner as autosomes. Recessive mutation on the X chromosome in males will therefore be expressed in the phenotype because there is one disease causing allele on the X chromosome and no allele in the Y chromosome. In females there are two alleles because they have two X chromosomes. Therefore in females recessive mutations situated on the X chromosome behave in the same way as in other chromosomes. In rare cases there is also dominant inheritance for genes situated on the X chromosome.

1.2 Polygenic diseases or multifactorial diseases

Unlike monogenic diseases, in the case of polygenic or multifactorial diseases, the persons inherit a genetic modification which “predisposes” them to a disease which will only be expressed if other mutations on other genes or if other factors so called environmental factors will also be present. The diseases resulting from the impact of several modified genes (each with limited impact on the expression of the disease) are referred to as “polygenic diseases”. Those resulting from the interaction between genetic modification(s) and environmental factors are called “multifactorial diseases”.

These diseases are the most frequent and also the least understood. Epidemiological studies as well as investigations using genome wide amplification study have made it possible to progress in the identification of genetic factors involved.

1.3 Chromosomal diseases

Chromosomal diseases occur when the entire chromosome, or large segments of a chromosome, is missing, duplicated or otherwise altered.

There are two types of chromosomal aberrations : numerical (when an entire chromosome is missing or duplicated) and structural. Numerical aberrations can occur in one of the autosomal chromosomes (e.g. Down syndrome) or in sex chromosomes (e.g. Turner syndrome). The structural aberrations can include almost any chromosome.

1.4 Mitochondrial diseases

Mitochondria are small corpuscles in the cytoplasm of the cells containing a small number of important genes. At fertilization the embryo will receive all mitochondria from the egg and non from the sperm and therefore in mitochondrial inheritance the embryo always receives the genes situated in the mitochondria from the mother and non from the father.

2. Preimplantation genetic diagnosis on embryonic cells

2.1 General description of the procedure

A “PGD cycle” comprises the following steps: ovarian stimulation, oocyte retrieval, *in vitro* fertilisation of several mature oocytes, by intracytoplasmic sperm injection (ICSI), removal of 1 or 2 embryonic cells, genetic analysis of nuclear material from those cells and lastly selection and transfer of embryos not carrying the abnormal genetic characteristics in question.

Removal of the embryonic cells (called blastomeres) is performed by most teams three days after fertilisation. The embryo then has 6-10 blastomeres. Depending on the indication, 1 or 2 blastomeres will be removed. Transfer of the embryo(s) analysed, and not carrying the genetic abnormality concerned, takes place on the 4th

or 5th day of embryonic development. Genetic analysis begins immediately following the biopsy of the embryo, and the results are known during the following morning depending on the techniques used.

Where the genetic indications of the PGD are concerned, some teams perform the blastomere biopsy on the 5th day after fertilisation (blastocyst stage) when embryos contain about 150 cells⁴. Unlike removal from a day 3 embryo, biopsy at the blastocyst stage allows the removal of more than two cells for diagnosis. Removal can be from the trophoblast, leaving intact the inner cell mass (embryoblast) from which the foetus develops⁵. On the other hand, removal at this stage leaves limited time available for genetic analysis since the embryo has to be transferred at the latest on day 6.

Regarding the indications for PGD concerning chromosomes analysis (cytogenetic), very few teams perform the embryo biopsy on day 5 because a marked mosaicism is observed at this stage, making it impossible to interpret the results.

If removed, cells show a specific genetic defect, then the associated embryo is known to be a carrier of the defect and is therefore withheld from transfer.

2.2 PGD uses

2.2.1 Main uses of PGD for medical indications

Use of PGD for medical indications has been offered to couples at high risk of transmitting a specific genetic disease of particular gravity (structural chromosomal aberration or monogenic disease, and untreatable at the time of diagnosis). The risk was often identified on the basis of family history or the birth of affected children. Numerous monogenic indications currently meet these criteria justifying application of PGD, such as cystic fibrosis, Duchenne Muscular Dystrophy, myotonic dystrophy, Huntington's disease, spinal muscular atrophy in infants and haemophilia.

PGD is most frequently used to detect mutations linked to monogenic diseases that are rare, severe, even lethal, with close to 100% penetrance, i.e. a person with the genetic defect will inevitably display symptoms (penetrance is the percentage of individuals with a given mutation and showing symptoms of the corresponding disease).

The same technique may also be used to identify embryos that could have a greater chance of being implanted. It is especially aimed at patients, who have experienced several miscarriages⁶, as well as patients at an advanced maternal age, patients whose implantations have repeatedly failed, and patients with azoospermia. This is often called preimplantation genetic screening (PGS), as its objectives are substantially different from PGD. It involves looking for an aneuploidy (abnormal number of chromosomes) for 6-9 different chromosomes.

⁴ Inside the Consortium. Joyce Harper. Newsletter ESHRE, January 2007.

⁵ Birth of a healthy infant following trophectoderm biopsy from blastocysts for PGD of β -thalassaemia major: Case report: [Human Reproduction](#), Volume 20, Number 7, July 2005, pp. 1855-1859(5).

⁶ The interface between assisted reproductive technologies and genetics: technical, social, ethical and legal issues. *European Journal of Human Genetics* (2006) 14, 588-645.

Taking account of these commonest uses, the European Society of Human Reproduction and Embryology (ESHRE) clearly distinguishes between two different procedures and has published best practice guidelines for centres offering medically assisted procreation and PGD⁷:

- high risk (referred to as PGD, high risk of transmission of a genetic disorder, such as single gene defects or chromosomal structural abnormalities), and;
- low risk (Preimplantation Genetic Screening (PGS) for aneuploidies).

2.2.2 Use of PGD for the benefit of the health of an existing sibling: preimplantation testing for Human Leucocyte Antigen (HLA) tissue typing

In this particular situation, the aim is to treat an existing sibling suffering from a disease which affects the haematopoietic and / or immune system with stem cells donated by a sibling or immediate family member.

However, transplantation can only be carried out if donor and recipient have matching tissue types (genetic component of Human Leucocyte Antigen) so that the donor material is not recognised as foreign material by the recipient's immune system.

The chance of siblings being HLA-identical to the recipient is theoretically 25%. Where no compatibility within the family is found, a search is made in registers of bone marrow donors. But the success of bone marrow transplantation is significantly greater where the donor is related.

The alternative offered by PGD lies in the dual selection of an embryo which would be both free of the disease and HLA-compatible to the sick elder sibling. At donor child's birth, umbilical cord blood is used as a source of stem cells to transplant into the sick sibling.

2.2.3 Use of PGD to detect mutations that predispose to onset of disease

Gene mutations that predispose to certain cancers are of variable penetrance, meaning that states of predisposition to cancer differ greatly from each other: some cause a major risk, close to 100%, of developing a severe cancer in childhood or young adulthood (e.g. Li Fraumeni's syndrome), others cause a comparatively moderate but not insignificant risk of developing a cancer with a favourable prognosis if diagnosed very early (e.g. *CDKN2A* gene and melanoma), others are associated with high risk of frequent cancers occurring in young adults with a generally favourable prognosis after early diagnosis, but not consistently so (e.g. *BRCA1* gene and breast and ovarian cancer).

On balance, nearly sixty genes have been implicated in some forty genetic predispositions to cancer.

In May 2006, the UK Human Fertilisation and Embryology Authority (HFEA) approved PGD for disease with a lower penetrance like some breast and colon

⁷ 'Best practice guidelines for clinical preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS)'. Human Reproduction Vol.20, No.1 pp. 35–48, 2005 ESHRE PGD Consortium.

cancers⁸. Before this decision, the procedure had only been approved for genetic characteristics that carried at least a 90 per cent risk of a disease that usually strikes in childhood.

2.2.4 A possible future use of PGD for extended medical indications: multifactorial disease

Monogenic disorders as described above are rare. Many widespread diseases are multifactorial meaning that their development is subject to a large number of genetic and environmental influences (see chapter 1). Examples of such diseases include many cancers, cardiovascular diseases or metabolic diseases (such as diabetes) and neurodegenerative diseases (e.g. Alzheimer disease). Onset of symptoms often occurs later in life. The influence of the genetic component may be significantly less than that of environmental factors.

For such diseases, the presence of the genetic component is usually considered insufficient to provide for a reliable prognosis.

Currently, in the technical sense, the quantity of genetic material obtained from one or two cells constitutes a limiting factor for detection of mutations in several implicated genes. According to the data collected in Europe, PGD is but very seldom used for multifactorial diseases⁹.

The *Human Fertilisation and Embryology Authority* (HFEA) recently authorised a fertility clinic to carry out preimplantation (PGD) screening in the case of a rare, early form of Alzheimer's disease. In that form, the illness is genetically caused and generally appears at about 35 years of age (often defined as a dominantly inherited form of Alzheimer disease).

2.2.5 Exceptional use of PGD: selection of an embryo with the same genetic characteristics as the parent(s) who suffer from a disabling condition

Cases have been reported¹⁰ where parents have sought PGD to select an embryo for transfer carrying a mutation in the GJB2 gene associated with deafness, because the parents desire to have a child "just like them". In other cases, parents have requested use of PGD to select an embryo that carried the mutation linked to achondroplastic dwarfism.

2.2.6 Extension of PGD use to non-medical sex selection

⁸ [HFEA](http://www.hfea.gov.uk/516.html), Authority decision on the use of PGD for lower penetrance, later onset inherited conditions, <http://www.hfea.gov.uk/516.html>

⁹ If this may change in the near future, there are currently two main technical limitations to the applications of PGD to multifactorial diseases:

- At present it is not possible to detect many different genetic variants in one or two cells;
- According to the present results of genomic studies, the real value of genetic variants to predict the risk to develop multifactorial diseases is low.

¹⁰ http://www.hfea.gov.uk/cps/rde/xbcr/hfea/PGD_document.pdf
<http://journals.cambridge.org/action/displayAbstract;jsessionid=A7D5EBECF6202473153E043E9F0E7182.tomcat1?fromPage=online&aid=6899868> , <http://www.biopsychiatry.com/misc/genetic-defects.html>

The selection of embryos for one sex may be carried out for medical purposes in the case of a sex-linked genetic disease where the actual mutation linked to the disease is not known. However, PGD is also used in some countries to select the sex of a future child for reasons of personal preference, i.e. non-medical reasons.

The aim may be to select an embryo of the preferred sex or to achieve “family balancing” by a choice of either sex for the unborn child depending on the sex of the already existing children. Prospective parents may prefer one sex to another for personal, social or cultural reasons.

This PGD application is highly controversial and it should be noted that Article 14 of the Convention on Human Rights and Biomedicine states that “the use of techniques of medically assisted procreation shall not be allowed for the purpose of choosing a future child’s sex, except where serious hereditary sex-related disease is to be avoided”.

2.3 Techniques of analysis

To identify genetic defects, PGD uses the same methods for analysis as genetic testing carried out on genetic material derived from a foetus or a person after birth. The most common approaches, which are well established and validated, are direct mutation testing or cytogenetic analysis.

PCR (Polymerase Chain Reaction)

Direct mutation testing is a molecular technique based on knowledge of the specific DNA sequences concerned; PCR is used for selective amplification of the specific sequences, which can then be analysed to determine whether the mutation associated with the disease is present.

FISH (Fluorescent in Situ Hybridization)

Cytogenetic analysis consists in examination of the chromosomes. The FISH technique allows the partial determination of the chromosomal content of a nucleus. The principle is based on hybridisation of a DNA fragment (probe) specific to a chromosomal region and marked by nucleotides coupled with a fluorochrome, on the DNA of interphase chromosomes denatured beforehand. The probe is made visible by the signal which the fluorochrome emits and appears as a fluorescent signal whose colour depends on the fluorochrome used. The FISH technique allows diagnosis of chromosomal imbalances in the nuclei of cells in interphase. Application of this technique to detect chromosomal imbalances in blastomeres or polar bodies makes it possible to perform a PGD of constitutional chromosomal imbalances either inherited or linked with the maternal age.

CGH (Comparative Genomic Hybridization)

Another method, comparative genomic hybridisation (CGH), is a global technique of DNA analysis for detecting small genomic imbalances in the whole genome (genomic segments gained or lost). But this technique proves very time-consuming and expensive and so is not routinely used.

However, even though these methods have been analytically validated, it should be pointed out that the possibility of misdiagnosis (false positive or false negative results) remains possible and has been reported to the ESHRE PGD Consortium.

Analysis of two embryonic cells rather than one is, in that case, recommended as it may reduce the risk of misdiagnosis¹¹.

The range of disease-causing genetic defects that can be identified has dramatically increased and now includes most common autosomal monogenic disorders as well as numerical and structural chromosomal abnormalities. Work on the current molecular or cytogenetic techniques is continuing in order to increase reliability of tests, reduce the time needed to reach diagnosis and boost the number of diseases laboratories can test for.

WGA (Whole Genome Amplification)

The representation of a cell's entire genome is called "whole genome amplification" (WGA). New WGA technology is based on multiple displacement amplification (MDA) which makes it possible to amplify nanogram quantities of DNA several thousandfold in only a few hours¹². The advent of highly effective whole genome amplification methods has paved the way for new technologies such as DNA microarrays (also referred to as DNA chips) and preimplantation genetic haplotyping (PGH), thus increasing yearly the number of genetic defects that can be identified in preimplantation embryos.

DNA microarrays consist of a solid support on which many specific DNA fragments are arrayed and can be used to test for a large number of different mutations, rapidly and simultaneously. The same microarray could thus be used to test the embryo for many different genetic diseases.

In PGH, blood samples are taken from family members, including at least one person affected by the disease in question. Comparing strands of DNA from affected and unaffected family members makes it possible to find markers that are associated with the disease, and thus to identify the region of a chromosome that contains the mutation. Amplified DNA from biopsied embryos can then be checked to identify those embryos that have inherited the genetic defect. Such a procedure is less time-consuming to apply, as no specific test method for a disease-causing mutation needs to be developed and it allows testing for predispositions to genetic diseases for which the specific genetic mutation is not known. It thus widens the scope and availability of preimplantation genetic diagnosis¹³. In the case of most X-linked disorders, sex determination of the embryo and selection excluding male embryos was the only available measure. It is now possible to identify unaffected female and male embryos without mutation-specific testing and thus to increase the number of embryos that can be implanted.

2.4 Database on the clinical practice of PGD in Europe

The European Society of Human Reproduction and Embryology (ESHRE) collects detailed data from specialised medical centres offering medically assisted

¹¹ Genetic Testing. Serena Emiliani, Eric Gonzalez-Merino, Yvon Englert, Marc Abramowicz. 2004, 8(1): 69-72. doi:10.1089/109065704323016058.

¹² BioTechniques® January 2007, Volume 42, Number 1: pp. 77-82.

¹³ Proof of principle and first cases using preimplantation genetic haplotyping : a paradigm shift for embryo diagnosis, Reproductive Biomedicine Online, ISSN 1472-6483, 2006, vol. 13, n°1, pp. 110-119.

Available at: http://www.pgd.org.uk/resources/aboutus/research/pamelarenwick_research.pdf

procreation and PGD across the world. The data collection has resulted in nine publications so far; information is available on 3530 PGD cycles for monogenic defects, 3524 cycles for chromosomal aberrations, and 1057 for sexing linked to X-linked diseases¹⁴ (see Appendix I).

The ESHRE database also collects information on cases of social sex selection and cases of misdiagnosis which can be reported anonymously. The ninth data collection has now been published. It is based on records from 57 centres submitted on 5858 cycles performed in 2006 which led to 1437 pregnancies and 1206 births.

According to these data, PGD was most frequently used to search for mutations linked to β -thalassaemia, sickle cell anaemia, cystic fibrosis, spinal muscular atrophy, Huntington disease, myotonic dystrophy, Duchenne and Becker muscular dystrophy, fragile X and haemophilia A and B (See appendix 2).

In the case of Huntington disease, some centres offer a pre-implantation diagnosis called "exclusion testing" to select an embryo not carrying the genetic defect without revealing his or her status to the parent who comes from a family where Huntington disease has already occurred.

The following changes in practice have been observed by the ESHRE Consortium¹⁵: use of new (laser) technologies to remove cells from the embryo, the development of polar body biopsy in countries where PGD as described above is not permitted (see part II), the increase in the number of diseases amenable to diagnosis as well as the number of cycles performed for aneuploidy screening.

Pregnancy rates following PGD seem to be lower than those observed for a regular in vitro fertilisation (IVF) i.e. without PGD. So far, children born after PGD do not show any more congenital malformations than children born after IVF without PGD¹⁶; but, according to the British Fertility Society, there were no conclusive data to link IVF with any specific abnormality. The results of a study from Université libre de Bruxelles concerning 583 children born after PGD are reassuring (work presented at ESHG meeting on 16 June 2007).

3. Polar Body Diagnosis

First and second polar bodies retrieved from oocytes in vitro are examined for chromosomal aneuploidies as well as monogenic disorders. The analysis of polar bodies provides information only about the maternal contribution to the future embryo's genotype, so that maternally transmitted mutations linked to autosomal and X-linked disorders can be identified.

¹⁴ ESHRE PGD Consortium data collection IX: cycles from January to December 2006 with pregnancy follow-up to October 2007, (<http://humrep.oxfordjournals.org/cgi/content/full/dep059v1?maxto show=&hits=10&RESULTFORMAT=1&author1=goossens&andorexacttitle=and&andorexacttitleabs=and&andorexactfulltext=and&searchid=1&FIRSTINDEX=0&sortspec=relevance&fdate=//&resourcetype=HWCIT>)

¹⁵ Inside the PGD Consortium. Joyce Harper. Newsletter ESHRE January 2007.

¹⁶ Obstetrics, Gynaecology & Reproductive Medicine [Volume 17, Issue 1](#), January 2007, pages 17-21.

3.1 Procedure and techniques of analysis

Shortly after ovulation occurs, the oocyte undergoes meiotic division to reduce its double chromosome set (46 chromosomes) to a single set of 23 chromosomes and releases the first polar body, which contains the other set of 23 maternal chromosomes. After sperm penetration, the two chromatids of each chromosome separate. One set of chromatids remains in the oocyte, whereas the 2nd set of chromatids is discharged from the so called “activated oocyte” by forming the 2nd polar body (before fusion of the two pronuclei). Polar bodies can be retrieved *in vitro* by non-invasive methods (aspiration¹⁷). Polar bodies do not play a role in successful fertilisation or normal embryonic development.

When testing for mutations linked to a monogenic disorder such as cystic fibrosis, first and second polar bodies are retrieved separately. When testing for chromosomal aneuploidies such as Down’s syndrome, both polar bodies are removed together. As for PGD, chromosomal analysis of polar bodies is done using fluorescence in situ hybridisation (FISH) for certain chromosomes or using comparative genomic hybridisation (CGH) for the analysis of all chromosomes. The main difficulty is the retrieval of the polar bodies without damaging the oocyte. Polar bodies analysis seems to provide results as reliable as those obtained through analysis of embryonic cells (see chapter 2).

3.2 Clinical practice of polar body analysis in Europe

Certain countries prohibit PGD on biopsied embryos, but allow the analysis of polar bodies.

Polar body analysis is a way to obtain information on genetic characteristics of the oocyte before the embryo is formed.

In Italy, where selection of in vitro embryos has been prohibited since 2004, researchers have tested the first polar body for chromosomal abnormalities that might make an embryo less likely to implant successfully or more likely to miscarry at a later stage¹⁸.

4. Prenatal Testing (in vivo)

4.1 General description of the procedure

4.1.1 Sampling

In order to carry out prenatal diagnosis, foetal cells must be obtained.

3 methods of retrieval are possible, depending on the stage of pregnancy at which the question of performing PND is raised, and on the type of testing required.

¹⁷ Preimplantation genetic diagnosis : Polar body biopsy. Markus Montag Eppendorf, Application Note 140, March 2007.

¹⁸ Medical Research News 19-Jun-2006

- Choriocentesis, i.e. chorionic villi sampling, is done usually between 10 and 18 weeks of pregnancy, either with forceps via the transvaginal route or transabdominally by needle insertion.

- Amniocentesis, sampling of amniotic fluid containing foetal cells, usually between 14 and 16 weeks of pregnancy, transabdominally by needle insertion. This is the sample most commonly taken.

- Cordocentesis, percutaneous foetal blood sampling from the umbilical vein, is performed by needle insertion after 20 weeks of pregnancy.

All these samples are taken under ultrasound monitoring.

There is a risk of contamination by maternal tissue, and their purity must be verified.

These acts are invasive with an ever-present risk of spontaneous abortion assessed, depending on the type of sample and the team, at 0,5-3%.

4.2 Techniques of analysis

Techniques of analysis are similar to those described for PGD (see chapter 2)

Genetic testing is divided into 2 categories:

a. chromosome analysis: determining their number (aneuploidy testing) or structural modification (translocation, inversion). Generally, except in cases of parents who carry a balanced abnormality, the diseases involved are random and not hereditary.

b. DNA analysis, looking for a structural modification (mutation) linked to genetic diseases, usually transmitted by the couple.

4.2.1 Chromosome abnormalities

This corresponds to 80% of the PNDs performed.

Five major indications can be discerned:

a. Mother's age: the risk of trisomy 21 (Down syndrome) is known to increase throughout a woman's period of fertility. The curve is exponential. The risk is 1/1500 between ages 20 and 24, increases to 1/170 at age 35, and is above 1/50 after age 40. Many European countries offer this test to all pregnant women aged over 36.

b. Higher risk irrespective of age is detected by examining (serum markers in maternal blood). Several markers are examined, the oldest being AFP (Alfa-Feto Protein), HCG (Human Chorionic Gonadotropin) and oestriol. They are measured between 14 and 18 weeks of amenorrhea. More recently, PaPPA (Pregnancy Associated Plasma Protein A) and HCG measurements at 12 weeks of amenorrhea have demonstrated their effectiveness, with fewer false positives and better detection.

Maternal age is always included in calculations of risk. The risk threshold accepted as calling for a foetal karyotype is set at 1/250 in most countries.

c. Ultrasound warning signs: thickness measurement at the back of the foetal neck (nuchal translucency) at 10-12 weeks of amenorrhea, if increased, points to trisomy 21. Risk must be calculated having regard to maternal age and

results of measuring the serum markers in the mother's blood, hence the value of PaPPA and HCG measurement at the same stage of pregnancy. Any other malformation detected by ultrasound examination in the 1st, 2nd and 3rd trimester of pregnancy justifies suspicion that a chromosomal abnormality may be present, particularly cardiac disorders, certain foot postural, cleft lip, etc.

- d. A male or female individual may be a carrier of a balanced chromosomal abnormality, involving a fragment of chromatin, a component of the chromosome, not in its proper position. For example, an acrocentric chromosome 21 (with the two top arms missing) is affixed to a chromosome 13, itself acrocentric. The person is in possession of his or her entire genetic inheritance and is normal, but when producing gametes the distribution of half the genome in each gamete (meiosis) is likely to occur in an unbalanced way. A gamete may contain both the translocated chromosome and the other unattached chromosome, which is a source of trisomy in the zygote resulting from this gamete. It can be appreciated that the risk of an unbalanced defect occurring in the embryo is increased for a couple in this situation. While most of these unbalanced transmissions of defects cause a spontaneous termination of pregnancy, some imbalances may allow the pregnancy to run its course, giving birth to a child afflicted with a usually severe disability.
- e. The latest indication from the study of foetal chromosomes is family history of a child carrying an unbalanced chromosomal abnormality (trisomy 21 for example). Not all studies agree about the risk of this type of defect recurring among the siblings. Nevertheless the study of foetal karyotype was perfected about 1965 precisely for this indication when a couple distressed by the birth of a disabled child could only contemplate a further pregnancy if assured that a child not carrying the defect would be born.

4.2.2 Genetic diseases

At present the genetic diseases prompting the offer of prenatal diagnosis are the monogenic diseases (see chapter 1).

This leads to 2 situations where:

- a disorder is known in the family or either the mother or the father carries it,
 - a first child of the couple is affected at birth.
- a. For a prenatal diagnosis to be contemplated, the disorder in the family or one partner of the couple must be determined, the suspected mutation must be known, and must be discovered in either one (dominant disorder) or both partners (recessive disorder).
Points to be discussed with the couple are the seriousness of the complaint, the lack of treatment for it, and the family experience of it.
 - b. The same applies after the birth of an affected child for whom DNA examination has allowed the causative mutation to be identified.
The problem is particularly painful where the first child has died before the investigations were conducted and the couple has a family history but there is no possibility of screening. This situation should no longer occur today; the neonatology teams caring for these babies need simply remember to keep a DNA sample for subsequent testing.

4.3 New procedures

4.3.1 Sampling procedures

New techniques for diagnosis on the foetus are favouring non invasive methods without risks for the foetus.

- a. analysis of foetal cells in maternal blood (from 6 to 8 weeks of pregnancy)
- b. analysis of free DNA/RNA circulating on the mother's blood.

Detection of foetal cells in maternal blood raised great hopes. Alas, the number of cells in circulation seems negligible. Their isolation from maternal cells is difficult. Moreover, certain foetal lymphocytes can linger protractedly in the mother's blood and could cause errors of diagnosis as lymphocytes from an earlier pregnancy could be examined during a subsequent pregnancy.

Analysis of DNA circulating in maternal blood, called free DNA, already allows sex to be determined so as to obviate invasive removal in connection with X linked disorders which concern male foetuses only. Analysing free DNA also allows Rhesus blood group typing in serious Rh incompatibilities carrying the risk of damage to the foetus. Other pathologies might eventually benefit from this technique for obtaining foetal DNA.

5. Conclusions

In those countries where preimplantation genetic diagnosis (PGD) is performed, it has become an established clinical method to analyse genetic characteristics of embryos created by in vitro fertilisation, and to obtain information which is used to select the embryos to be transferred. The use of PGD is mainly requested by couples carrying genetic conditions linked to severe disorder or premature death of their offspring who wish to avoid initiation of a pregnancy that may not come to term or that may entail the difficult question of terminating the pregnancy in case of a detected particularly severe genetic defect.

Children born so far after PGD do not show any more congenital malformations compared to children born after IVF without PDG. But scientists say that epigenetic changes may develop late in life or in the next generations. The oldest PGD children are approaching 20 years¹⁹. To look at the long term outcome would take several decades²⁰.

Finally, PGD is a technically difficult procedure that is offered only in a few specialised centres worldwide and requires the use of assisted reproduction techniques [in vitro fertilisation (IVF) by incubation or intracytoplasmic sperm injection (ICSI)] in couples even without fertility problems. The effectiveness of aneuploidy screening (PGS) to achieve higher implantation and pregnancy rates and a reduced miscarriage rate remains to be established²¹.

¹⁹ European Society of Human Genetics June 2007, BBC News Health 17 June 2007

²⁰ Isolated reports of imprinting defects: Beckwith and Angelman syndromes, associated with IVF, particularly ICSI in certain cases where spermatozooids or spermatids obtained by testicular biopsy; other imprinting problems are associated to diabetes, obesity etc. (Comment made by Prof. Jorge Sequeiros, Portugal.)

²¹ Twisk M, Mastenbroek S, van Wely M, Heineman MJ, Van der Veen F, Repping S. Preimplantation genetic screening for abnormal number of chromosomes (aneuploidies) in in

Growing use of polar body diagnosis, which is carried out before the embryo is formed, can be observed especially in countries where PGD on the embryo is prohibited. The advantage of this method is that polar bodies are naturally extruded from the oocyte so that their retrieval can be achieved without the use of invasive methods. While limited to the detection of genetic abnormalities of maternal origin, indications for polar body diagnosis include a wide range of common monogenic as well as chromosomal anomalies. It is to be noted that this type of diagnosis is based on the postulate that nuclear genetic material of the polar body is identical to the nuclear material of the oocytes - a similarity which is not absolute.

Prenatal genetic diagnosis focuses on the same monogenic disorders and chromosomal anomalies as PGD and is often used to clarify an observation using ultrasound or biochemical analysis of maternal blood. To obtain foetal cells which contain the genetic material, the use of invasive methods like amniocentesis is necessary and there is a risk of miscarriage due to the intervention. Safer methods are under development where foetal cells or free DNA circulating in the maternal blood are used for analysis. These procedures are based on a sample of maternal blood and do not pose the risk of miscarriage.

Part II. Preimplantation and Prenatal Genetic Diagnosis. Legal situation in Council of Europe member states

Introduction

In Europe, regulation of preimplantation genetic diagnosis (PGD) and prenatal genetic diagnosis (PND) varies considerably between countries. Specific legal provisions can be found in most countries for both PGD and PND. Some countries prohibit the use of PGD, some authorise a very restricted use and define the conditions, and others have not yet adopted a clear policy.

In this chapter, an overview will be presented of the legal situation in Europe, based on responses to a questionnaire with respect to preimplantation and prenatal genetic testing.

1. Legal situation in Council of Europe member states

1.1 Collection of information

In July 2007, a questionnaire was submitted to delegations of the Steering Committee on Bioethics (CDBI) to collect information on national legal frameworks for PGD and PND and specific regulations for their use. CDBI delegations were invited to fill in the questionnaire and to provide English or French versions of statutory Acts, government decrees or other binding regulatory texts.

An analysis of these responses and results in tabular form are presented in the following chapters.

1.2 Responses to the questionnaire

32 delegations responded to the questionnaire: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Ireland, Italy, Latvia, Luxemburg, Malta, the Netherlands, Norway, Poland, Portugal, Romania, the Russian Federation, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine and the United Kingdom.

Canada (observer to the CDBI) also submitted information regarding its legal situation.

Some delegations chose not to fill in the questionnaire but rather to submit information in a free text format.

1.2.1 Section A: The legal framework for preimplantation and prenatal diagnosis

Europe's legal landscape

Twenty countries reported having a legal framework in place for prenatal genetic diagnosis (PND) and twenty countries for preimplantation genetic diagnosis (PGD). The remaining countries stated that they had not adopted any regulations in these fields.

The English or French title of the corresponding legal text is given in the tables below together with an indication if testing is authorised or prohibited. Non-legally binding texts have also been reported (e.g. Codes of Good Practice).

Regulation of prenatal genetic diagnosis (PND)

According to the information submitted, prenatal genetic diagnosis may be performed during pregnancy in all the replying countries even if no specific regulation exists - with the exception of Ireland.

In Ireland, the "right to life of the unborn" is protected by the Constitution (whether or not 'unborn' includes in vitro embryos prior to implantation is a pending question).

In some of the replying countries, prenatal genetic diagnosis is governed by a regulation in the Public Health Code (e.g. France) or "Genetic Engineering Act" (Austria). Greece has adopted a law on "family planning and other arrangements" and set out specific conditions for prenatal diagnosis in a ministerial decision.

In the Russian Federation, specific provisions can be found in an Order of the Ministry of Health on the "improvement of prenatal diagnostics and prevention of hereditary and congenital diseases".

With the "Law on the Rights of Patients" adopted in May 2000, Georgia regulates prenatal genetic diagnosis non-specifically, as the above law refers to "tests which serve to identify a gene responsible for a disease or to detect a genetic predisposition to a disease".

Regulation of preimplantation genetic diagnosis (PGD)

Eighteen countries indicated that certain uses of PGD were possible, based on specific regulation in most of them. Four countries generally prohibit PGD.

Two out of the eleven countries that do not have any legal framework indicated current developments: Cyprus and Malta. In Cyprus, actual use of PGD has been reported and a draft bill authorising the use of PGD is currently under review. Malta had no specific regulations but a recommendation was made in 2005 by the Permanent Social Affairs Committee of the Maltese parliament to prohibit PGD and to support polar body diagnosis.

PGD authorising countries

Sixteen countries have specific regulations that authorise certain uses of PGD: **Austria**, Belgium, Czech Republic, Denmark, Finland, France, Greece, the

Netherlands, Norway, Portugal, the Russian Federation, Serbia, Slovenia, Sweden, Spain and the United Kingdom.

In some cases, countries provide for general regulation in national Health Care Acts (e.g. Serbia) or patients' rights (e.g. Georgia). The "Law on the Rights of Patients" adopted by Georgia in May 2000 and which also regulates this issue, prohibits the use of techniques of medically assisted procreation for the purpose of sex selection with the exception of cases of hereditary sex-linked diseases (cf. principle of Article 14 of the Oviedo Convention on the Protection of Human Rights and Biomedicine, 1997).

Other countries have adopted PGD-specific provisions as part of national laws on medically assisted procreation (e.g. Portugal, **Austria**).

PGD prohibiting countries

The use of PGD has been explicitly prohibited in Switzerland. Prohibition has been based on:

- the protection of the status of the embryo (Italy)
- a risk of extension of embryo selection (for its sex or other characteristics), risk for potential harm to the embryo following the intervention and a risk of misdiagnosis (Switzerland)

It has been noted that Switzerland currently reexamining the relevant Article of the Federal Constitution as well as the existing legislation concerning PGD to allow certain indications and to define the conditions.

When the Act regulating pre-implantation genetic diagnosis entered into force in Germany on 21st November 2011 (Federal Law Gazette I, p. 2228) a general prohibition of pre-implantation genetic diagnosis was firmly established in the German Act on the Protection of Embryos. At the same time, the legislator stipulated narrow limits for those cases, where pre-implantation genetic diagnosis shall be permitted by way of an exception, in spite of this general prohibition. **On 1st February 2014 the ordinance having the force of law regulating pre-implantation genetic diagnosis entered into force. This ordinance lays down in detail the organisation and the procedure to be followed for carrying out a lawful pre-implantation genetic diagnosis. The Länder are responsible for the enforcement of this ordinance.**

With respect to Ireland, Article 40.3.3 of its Constitution acknowledges the right to life of the unborn and, "with due regard for the equal right to life of the mother, guarantees in its laws to respect and as far as practicable by its laws to defend and vindicate that right." In November 2006, the Irish High Court decided that embryos in vitro were not "unborn" within the meaning of that Article of the Constitution. This judgment was appealed to the Supreme Court of Ireland (hearing in February 2009) and a judgment is awaited.

1.2.2 Section B: Specific provisions for preimplantation genetic diagnosis (PGD)

Specific provisions for PGD are made in national legislation to define acceptable purposes and to set criteria for its application (see questionnaire section B). In the United Kingdom, a national code of practice provides guidance to medical centers

about the proper conduct of authorised activities including PGD; in Bulgaria, such a code of practice is currently being prepared.

Where authorised, PGD is used:

- to benefit the health of the child to be born :
- indications include monogenic diseases, chromosomal abnormalities, and X-linked disorders. Four countries also declared that the use of PGD would also be allowed for multifactorial diseases.

Most laws characterise further the diseases (or genetic condition) for which PGD is permitted, i.e.:

- if the disease (or genetic condition) is serious and
- if there is no appropriate treatment

For the decision in individual cases of specific disorders, the law in most countries prescribes:

- authorisation of a competent authority
- prior evaluation of the individual case by a medical team

In addition to the above, French legislation makes it obligatory to identify the genetic defect in question in a parent before proceeding with PGD and allows access to medically assisted procreation only if certain criteria are met.

In almost all countries where PDG is permitted (with the exception of Slovenia), there is no exhaustive list of approved indications. In the United Kingdom, the Human Fertilisation and Embryology Authority (HFEA) publishes examples of diseases for which PGD is authorised. In several countries, PGD is only performed in very few specialised and licensed medical centers.

Human leucocyte antigen (HLA) typing is authorised in nine countries. The purpose of HLA typing is to find an embryo with a tissue type that matches an existing sibling who suffers from a blood disorder like thalassaemia, Wiskott-Aldrich syndrome or Fanconi anemia (see I. Clinical part). Several countries specified that HLA typing could be carried out only if the child to be born is also at risk of being affected by a severe genetic disease for which the corresponding mutation can be tested using PGD. HLA typing is then carried out in combination with PGD for this mutation.

None of the replying countries authorise the use of PGD:

- to select the sex of a future child for family balancing²²
- for any other non-medical purposes.

1.2.3 Section C: Regulation of polar body diagnosis

Country prohibiting PGD by specific law is Switzerland (under revision). The use of polar body diagnosis is not prohibited in Germany. Switzerland has authorised the use of polar body diagnosis.

²² By « family balancing » one understands a family comprising of boys and girls

As mentioned above, Malta might authorise polar body diagnosis based on a recommendation made to the parliament in 2005, but does not yet have any corresponding regulation.

According to guidelines released by the German Federal Physician Association on Assisted Reproduction and a Recommendation by the Permanent Social Affairs Committee of the Maltese Parliament, polar body diagnosis may be carried out only to benefit the health of the child to be born (see Guidelines). Switzerland specifies that polar body diagnosis may only be performed to avoid the transmission to the descendants of a serious disorder for which no treatment exists. In addition, it may only be prescribed by a medical doctor.

1.2.4 Specific provisions for prenatal genetic diagnosis

Purpose of prenatal genetic diagnosis

Prenatal genetic diagnosis may be carried out only for purposes linked to the health of a child to be born (eleven countries)

The aim of prenatal diagnosis is to detect any particularly serious disease in the embryo or foetus in utero (six countries) for which no an appropriate treatment does not exist (four countries).

It should be noted that the Social Affairs Committee of the Parliament in Malta stated in its report of 2005 that PND should be allowed only when therapeutic options exist for the correction of embryonic abnormalities.

Latvia stated that, as for PGD, there was no commonly used list of disorders for which PND was accepted.

Latvia specified further that PND must not be used in the case of multifactorial disorders; whereas Poland explicitly allows PND to be performed for such indications.

Switzerland set down in legislation testing purposes that are prohibited:

it is prohibited to test for characteristics of the embryo or foetus that do not directly affect its health

it is prohibited to determine the sex of an embryo or foetus for other than diagnostic purposes

Prerequisites for prenatal genetic testing

In Malta, the above mentioned report of the Parliament's Social Affairs Committee recommends that PND should be allowed only when:

the purpose cannot be achieved by other means

and the benefits to both embryo /foetus and the mother outweigh the risks

Prior “risk assessment” must be based on ultrasound scan and biochemical testing of maternal blood and invasive procedures may be performed only if non-invasive procedures produce no information on the condition of the foetus, (Norway)

In Norway and Poland, a minimum age has been laid down for pregnant woman who may benefit from certain applications of PND.

Further details on performance of the test

The countries that replied stated the following:

- The test(s) must be prescribed by a medical doctor (e.g. Switzerland, France);
- Acceptability in the individual case sometimes to be evaluated by a medical team (e.g. Austria, Latvia);
- Analysis must be carried out in authorised laboratories (e.g. Switzerland, France) or specifically licensed medical centers (e.g. Austria);
- Poland imposes a time limit for the performance of PND in the course of the pregnancy (22nd week at the latest, Poland).

Section A: Legal framework for preimplantation and prenatal diagnosis

1) Are the following regulated in your country?

Country / Pays	PGD	PND	Country	PGD	PND
Austria	yes	yes	Malta	no	no
Belgium	yes	no	The Netherlands	yes	yes
Bulgaria	no	yes	Norway	yes	yes
Cyprus	no	no	Poland	no	yes
Czech Republic	yes	yes	Portugal	yes	
Denmark	yes	no	Romania	non	non
Estonia	no	yes	Russian Federation	yes	yes
Finland	yes		Serbia	yes	yes
France	yes	yes	Slovakia	no	no
Georgia	yes, 'indirect'	yes, 'indirect'	Slovenia	yes	yes
Germany	yes	yes	Spain	yes	no ²³
Greece	yes	yes	Sweden	yes	yes
Ireland	no, 'not by statute'	no, 'not by statute'	Switzerland	yes	yes
Italy	yes, but no explicit regulation	yes	Turkey	no	no
Latvia	no	yes	Ukraine	no	yes

²³ All the genetic diagnostic test (pre and postnatal) are regulated in their general terms by the LIB 14/2006

Lithuania	non	yes	United Kingdom	yes	yes
Luxembourg	no	No			
Australia	yes				

2) Which legal instrument regulates?

Country	PGD	PND
Austria	Genetic Engineering Act (process) and Reproductive Medicine Act (definition)	Genetic Engineering Act
Belgium	Law of 6/7/07 concerning medically assisted procreation and the use of supernumerary embryo and gametes (Moniteur Belgique du 17/07/2007)	
Bulgaria		Health Act
Cyprus	Draft bill of law prepared. Has ratified the "Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine, Oviedo Convention" with the Ratification Law N.31(III)/2001.	
Czech Republic	Act no. 227/2006 Coll. and its implementing regulations	
Denmark	Law on artificial procreation 1997, revised 04/09/2006	
Estonia		General organizational problems of special labs regulated by Health Service Organisation Act (2002). No special law for PND.
Finland	Act on Assisted Conception Treatments 22 December 2006/1237	
France	Law (articles L. 2131-4-1 of the Code of Public Health) ; Decree (articles R.2131-22-1 a R. 2131-34 of the Code of Public Health)	Law (articles L. 2131-1 of the Code of Public Health) ; Decree (articles R.2131-1 a R. 2131-9)
Georgia	Law on the Rights of Patients (no283-IIs)	
Germany	The Embryo Protection Act of 13th December 1990, amended by the Act regulating pre-implantation genetic diagnosis of 21st November 2011 (Federal Law Gazette I, p. 2228); Ordinance having the force of law regulating pre-implantation genetic diagnosis of 21 st February 2013 (Federal Law	Act on Human Genetic Testing (Genetic Diagnosis Act – GenDG) of 31 st July 2009

Country	PGD	PND
	Gazette I, p. 323)	
Greece	Law 3305/05 Medically assisted reproduction	Law 1036/1980 "Family planning and other arrangements" Ministerial decision 1561/1980 Termination of pregnancy/congenital abnormalities Law 1609/1986 Artificial termination of pregnancy/protection of women Ministerial Decision 2799/1987 Conditions for prenatal diagnosis
Ireland	Constitution of Ireland 1937, Art. 40.3.3. High Court decided in November 2006: "unborn" in Article 40.3.3 does not include embryos in-vitro. Judgment was appealed to the Supreme Court of Ireland (hearing before Supreme Court in February 2009) and a judgment is awaited."	
Italy	Law 40/2004 on medically assisted procreation In Italy there is no explicit legal regulation for the preimplantation genetic diagnosis. ²⁴ Therefore, it is not possible to say with absolute certainty whether or not the legislation prohibits the diagnosis and whether it allows it within certain limits. There is only an explicit prohibition of 'eugenic selection'. It is possible to find the rules and reminders in Law 40/2004 (Rules on medically assisted procreation), in addition to various juridical decisions that have occurred on the occasion	Legal decree no. 281 du 28/08/1997, Agreement between the State and the Religions 15/07/2004. 104.28/VIII/1997 no. 281

²⁴ In particular, the Article 13 of Law 40, concerning the "Experimentation on human embryos", states in paragraph 1 that "there shall be no testing on each human embryo" and in paragraph 2 states that "the clinical and experimental research on each embryo is permitted provided that consideration be given to therapeutic and diagnostic purposes which are exclusively associated with it for the protection of health and development of the embryo itself, and if there are no alternative methods." With respect to paragraph 1, the paragraph 2 is an exception to the general prohibition of experimentation in the case of research aimed at protecting the health of the embryo. It should also be considered the art. 14 (Limits on the application of the techniques on embryos) stating in paragraph 5 the right of parents to request a study on the health status of the embryo and the obligation of the physician to inform them about the conditions of the same. This information along the path of the MAP procedure is also provided under the Article 6 of the Law (Informed Consent).

Country	PGD	PND
	of the practical application of this legislation. Moreover, it has been necessary a jurisprudential intervention since the regulatory provision was not sufficiently clear in terms of terminology and content. There has not been a uniform jurisprudential line ²⁵ .	
Latvia	Regulations No.611 of the Cabinet of Ministers of July 25, 2007 "Birth assistance guaranteeing procedure"; Regulations No.1046 of the Cabinet of Ministers of December 19, 2006 "The organizing and financing of health care"	
Lithuania		The following legal acts are relevant for prenatal genetic diagnosis (PND): <ul style="list-style-type: none"> • The Decree No. V-1135 of the Ministry of Health on the Prenatal care to pregnant women, dated: 29

²⁵ During these years doctrine and jurisprudence have been divided on the interpretation of the provisions in question, which were read in opposite ways. The first judgments (Catania, 2014; Lazio, 2015) have gathered from art. 13 of the Law the unlawfulness of the PGD, referring to the protection of the health and development of the embryo, risks of eugenic selection of embryos). It is on this basis that the former guidelines (2004), issued by the Higher Institute of Health and detailing the procedures and techniques of medically assisted procreation (article 7 of Law 40/2004) stated that it was possible to ascertain any abnormalities of the embryo only through a morphological analysis under the microscope, a technique that is considered non-invasive and not "dangerous" for the embryo.

The thesis that the preimplantation diagnosis is prohibited under Articles 13 and 14 of Law 40 was dropped from the next and most recent jurisprudence, which has made its interpretation in favor of the legality of PGD (Cagliari, 2007; Constitutional Court, N. 369/2006; Firenze 2007; Lazio, 2007-2008). It is emphasized the right of parents to request a study on the health status of the embryos and the obligation of the physician to inform them, in order to enable the mother withdrawing the consent to implant. In contrast, the prohibition of all pre-implantation diagnosis, invoking the next and possible eugenic purposes, could condemn the woman taking an uninformed decision and being not aware when deciding upon the transfer of all developed embryos or the rejection of such transfer. In addition, according to this second interpretation, under the law (art. 13, paragraph 2), the diagnosis should also be done taking into account the best interests of the embryo. Only through the PGD can be detected chromosomal abnormalities (triploidy and tetraploidy) and allowed therapeutic and diagnostic purposes which are exclusively associated with it for the protection of health and development of the embryo itself. The purpose of art. 13, paragraph 2, would therefore be emptied as regards the legitimacy of the "clinical and experimental research on each embryo for therapeutic and diagnostic purposes", since the restriction to the mere observational diagnosis would affect negatively on the right, making it not executable. Even with the impulse given by the decisions of Courts and the opinions of Council of the federal Board of Health, the Ministry of Health issuing the following guidelines (2008) deleted those paragraphs of the preceding Lines which allowed only the observational study.

Country	PGD	PND
		<p>December, 2006 (amended on 11 July, 2011);</p> <ul style="list-style-type: none"> • The Decree No. V-220 of the Ministry of Health on the Medical Norm MN 56:2003 for the Clinical Geneticist (MD). Rights, obligations, competence and responsibility, dated: 14 April, 2003; • The Decree No. V-522 of the Ministry of Health on the Human genetic services, compensated from the State Patient Fund budget, dated: 23 June 2005.
Luxembourg	<p>Currently, Luxembourg does not have any regulation in this field. However, the Parliament keeps on requesting the elaboration of a legislation on medically assisted procreation (MAP). The issue of preimplantation genetic diagnosis should be addressed in this framework.</p> <p>However, pending a legislation on MAP, the Health Minister has authorised the setting up of a MAP department which is the only one in the country. The authorisation granted by the Minister specifies that the Department is not entitled to carry out PGD.</p>	
Malta	No legislation, but a recommendation to prohibit it and support polar body testing.	No legislation.
The Netherlands	Regeling Preïmplantatie Genetische Diagnostiek	
Norway	The act relating to the application of biotechnology in human medicine etc. (Biotechnology Act)	
Poland		<ul style="list-style-type: none"> - The Law of 7 January 1993 on family planning, protection of the human foetus and the conditions for abortion admissibility. - The Regulation from the Health Ministry of 21 December 2004 on medical services, including screening tests and the periods during which they can be carried out. - Code of Medical Ethics of 20 September 2003
Portugal	Law no. 32/2006 of the Parliament, Art. 28	
Romania	Only information published in the Law 17/on 22 February 2001 (Romania has ratified the Convention on Human Rights and Biomedicine)	
Russian	Order of the Ministry of Health	Order of the Ministry of Health of

Country	PGD	PND
Federation	of Russian Federation N67 (26.02.2003) on medically assisted reproductive technologies.	Russian Federation N 457 (28.12.2000) on improvement of prenatal diagnostics and prevention of hereditary and congenital diseases.
Serbia	Regulated by statutory Act; Health Care Act of Serbia (2005) provides special health care for all women concerning family planning, prenatal care, delivery and motherhood (Art 11)	Art 11 of Health Care Act also applicable as general provision (special health care for all women concerning family planning, prenatal care, delivery and motherhood); Art. 45 of Health Insurance Act (2005) Ordinance on content and scope of rights to health care through compulsory insurance and participation (2007) anticipates as accepted procedures: PGD and PND (Art 10) Prof. & methodology Instruction for implementation of Decree about Health care of women, etc. (Ministry of Health, 1997)
Slovenia	The Law on Treatment of Infertility and on Biomedically Assisted Procreation, 2000	The Law on Medical Measures to Exercise the Right to Free Choice in Procreation, 1977
Spain	Law 14/2006, May 26: Assisted Human Reproduction Techniques	
Sweden	Act on Genetic Integrity (SFS 2006:351) (Act decided by Parliament)	
Switzerland	Federal Law of 18 December 1998 on medically assisted procreation (LMAP) (currently re-examined)	Federal Law on human genetic analysis of 8 October 2004 (LAGH)
Ukraine		Joint Act no. 641/84-31.12.2003 of Ministry of Health Care and Academy of Medical Science of Ukraine "The Improvement of Medico-genetic Help in Ukraine" (addition 4-10)
United Kingdom	The Human Fertilisation and Embryology Act 1990 (the HFE Act)	no legislation
Australia		

3) Are the following authorized or prohibited?

Country	PGD	PND	Country	PGD	PND
Austria	authorised	authorised	The Netherlands	authorised	authorised
Belgium	authorised	authorised ²⁶	Norway	authorised	authorised
Bulgaria		authorised	Poland		authorised
Czech Republic	authorized	authorised	Portugal	authorised	
Denmark	authorised		Russian Federation	authorised	authorised
Estonia		authorised	Serbia	authorised	authorised
Finland	authorised		Slovakia	authorised	authorised
France	authorised	authorised	Slovenia	authorised	authorised
Georgia	authorised 'indirectly'	authorised 'indirectly'	Spain	authorised	(see footnote) ²⁷
Germany	prohibited as a rule	authorised	Sweden	authorised	authorised
Greece	authorised	authorised	Switzerland	Prohibited (currently re-examined)	authorised
Ireland		prohibited	Ukraine		authorised
Italy	Neither prohibited nor authorized, explicitly	authorised	United Kingdom	authorised	Authorized
Latvia		Authorized			
Lithuania	not regulated	Authorized			
Australia	authorised				

4) If any relevant legal instrument is currently at the draft stage or subject to revision, please provide the reference and a commentary regarding the proposed provisions:

Please see question 2

²⁶ Not explicitly authorised but definitely implicitly. For example, amniocentesis is an invasive obstetric procedure that is included in the list of authorised procedures of the sickness-invalidity insurance scheme.

²⁷ Not namely authorized but not prohibited and included in the Social security provisions as a part of pregnancy care

5) If there is no legally binding text, are there other documents that provide guidance for the use of preimplantation or pre natal diagnosis (e.g. Code of Practice)? Please state reference:

Country	PGD	PND
Denmark		Guidance from the National Board of Health
Estonia		Guidance by relevant professional societies, published in local gynecologist newsletter (2005)
Poland	Code of Medical Ethics	Opinion of the Polish Association of Obstetricians concerning MAP techniques used in therapy against infertility
Slovakia	General Acts about Health Care 2004- 2006	Decree of the Ministry of Health on Methodic coordination in prenatal multimaker screening Text of Consensus of Slovak Society of Medical genetics and Slovak Society of Gynecology and Obstetrics
Turkey	Declaration of the Ethics Committee of the Turkish Medical Association (ECTMA), 1994; Declaration of the NGO of Genetics, 1994.	
Australia	The NHMRC <i>Ethical Guidelines on the use of Assisted Reproductive Technology in Clinical Practice and Research, 2007</i> (the ART guidelines)	

Section B: Specific regulations for preimplantation genetic diagnosis (PGD)

I Objectives and criteria according to legislation

6) Is preimplantation genetic diagnosis limited to specific purposes? If so, please specify:

- a. For the health of the child to be born
- b. To treat fertility problems
- c. For the health of a sibling (HLA-typing)
- d. Family balancing
- e. Other non-medical purposes – please specify

Country	a	b	c	d	e	Country	a	b	c	d	e
Austria	Yes	no	no	no	no	Portugal	yes	no	yes	no	no
Belgium	yes	yes	yes	no	no	Russian Federation	yes	yes	no	no	no
Czech Republic	yes	yes	no	no	no	Slovakia	yes	yes			
Denmark	yes	no	yes	no	no	Slovenia	yes	yes	no	no	no
Finland	yes	no	no	no	no	Spain	yes		yes	no	no
France	yes	no	yes	no	no	Sweden	yes	yes	yes	no	no
Greece	yes	no	yes	no	no	United Kingdom	yes	yes	yes	no	no
The Netherlands	yes	no	no	no	no	Australia	yes	no	no	no	no
Norway	yes	no	yes*	no	no						

*HLA-typing is allowed when there is also the risk of serious inheritable diseases in the child to be born, and the disease of the sibling can be successfully treated/cured by bone marrow transplantation. PGD for HLA-typing alone is prohibited

7) Where the permitted use of PGD for the health of the child to be born is not limited to monogenic diseases, is it allowed for the following?

- a. Multifactorial disorders
- b. Chromosomal aneuploidies
- c. Chromosomal translocations
- d. Embryo sexing for X linked disorders

Country	a	b	c	d	Country	a	b	c	d
Austria	no	yes	yes	yes					
Czech Republic	no	yes	yes	yes	Portugal	no	yes	yes	yes
Denmark			yes	yes	Russian Federation	no	yes	yes	yes
Finland	yes	yes	yes	yes	Slovakia		yes	yes	yes
France					Slovenia	no	yes		yes
Greece	yes	yes	yes	yes	Spain		yes	yes	yes
Netherlands	yes	no	no	yes	Sweden	no	yes	yes	yes
Norway	no	no	yes	yes	United Kingdom	yes	yes	yes	yes
Australia									

8) Are any of the following criteria prerequisites for allowing PGD?

- a. Severity of disorder
 - b. Absence of appropriate treatment
- Other, please specify

Country	a	b	Country	a	b
Austria	yes	no			
Czech Republic	no	no	Portugal	yes	no
Denmark	yes	no	Russian Federation	yes	yes
Finland	yes	no	Slovakia	yes	
France	yes	yes	Slovenia	yes	yes
Greece	yes	yes	Spain	yes	yes
The Netherlands	yes	no	Sweden	yes	no
Norway	yes	yes	United Kingdom	yes	Yes
Australia					

9) Is there a commonly used list of disorders for which PGD is accepted? (please provide a copy or state reference)

Country		Country	
Austria	no		
Belgium	no ²⁸	Norway	no
Czech Republic	no	Portugal	no
Denmark	no	Russian Federation	no
Finland	no	Slovakia	no
France	no	Slovenia	Yes. Listed in the code of practice to the law of 2000
Georgia	no	Sweden	no
Greece	no	United Kingdom	The HFEA publish a list of example conditions for which PGD is licensed. This list is not an exhaustive list http://www.hfea.gov.uk/docs/PGD_list.pdf
The Netherlands	no		
Australia			

II Procedural aspects of PDG

10) Who evaluates the acceptability of performing PDG in the case of a specific disorder?

- a. A medical team
- b. A competent authority (please specify the cases and the authority)

²⁸ The legislation lays down the general framework and leaves the management of PGD to the fertility and human genetics centres

Country	a	b	Country	a	b
Austria	yes	yes			
Belgium	yes	No, except for cases where PGD is carried out for the therapeutic benefit of the born child or the parties to the parental project : in this case, a favorable opinion of the authorized human genetics center must be attached to the file. The fertility centre consulted must verify that the parental project is not simply for the therapeutic benefit of the born child or the parties to the parental project, and this assessment must be confirmed by the human genetics centre consulted.	Norway	yes	yes
Czech Republic	yes	yes	Portugal	yes	
Denmark	yes	yes	Russian Federation	yes	
Finland	yes	no	Slovakia	yes	
France	yes	yes	Slovenia	yes	yes
Greece	yes	yes	Sweden	yes	
The Netherlands	yes	yes	United Kingdom	yes	Yes
Australia					

11) May PGD be carried out only in specifically licensed medical centers? (If yes, please state number of centers in your country)

Country		Country	
Austria	yes		
Belgium	Yes (18 + 7) ²⁹	Georgia	yes
Czech Republic	yes	Greece	yes
Denmark	no	The Netherlands	Yes (1)
Finland	yes	Slovakia	no
France	yes (3)	Spain	Yes
Australia			

Section C Polar bodies diagnosis

This section concerns only countries where PGD is prohibited.

²⁹ 18 authorised fertility centres. 7 human genetic centres that have drawn up a specific collaboration agreement on this subject.

12) If preimplantation diagnosis is prohibited in your country, is polar bodies diagnosis allowed?

Country	
Germany	yes
Malta	Recommendation that it should be allowed, by the Report presented to the Maltese Parliament in 2005, produced by the Permanent Social Affairs Committee of the Maltese Parliament, considering Biotechnology, including Assisted Reproduction in Malta.
Switzerland	yes

13) If polar bodies diagnosis is regulated specifically, please describe the main points (in the light of the questions asked on PGD).

Country	
Germany	The Federal Physicians Association has released Guidelines on Assisted Reproduction from the 17th February 2006. Polar bodies diagnosis is restricted to the health of the child to be born.
Malta	Medical reasons only - not for family balancing. Definitely for the health of the child to be born No mention about treating fertility problems or for the health of a sibling – issue not regulated. There is no available list of diseases.
Switzerland	This diagnostic may only be used with a aim of excluding a risk for the children to be affected by a severe and untreatable disease (see art. 33 LMAP). It can only be prescribed by a medical doctor (see art. 13, al. 1, LAGH).

Section D: Specific regulations for prenatal genetic diagnosis (PND)

14) If PND is regulated specifically, please describe the main points (in the light of the questions asked on PGD).

Country	answer
Austria	PND is limited to diagnosis or predisposition of an illness. The acceptability is decided by a medical team. PND is carried out only by specifically licensed medical centers.
Bulgaria	No specific regulations apart from the established criteria of the European Society of Human genetics
Cyprus	
Estonia	Medical genetics centers are licensed for invasive PND, mainly for the health of the child to be born, for example, for chromosomal aneuploides testing, chromosomal translocations testing and embryo sexing for X-linked disorders. The prerequisites for allowing PND are severity of disorders and absence of appropriate treatment as well.
France	- The aim of prenatal diagnosis is to detect any particularly serious disease in the embryo or foetus in utero. - Cytogenetic and biological tests carried out in order to make the prenatal diagnosis must be preceded by a medical consultation appropriate to the disease being looked for. - These tests may be conducted only in authorised public health establishments and medical biology laboratories. The authorisations concerned are issued for a five-year period by the regional hospitals agency (ARH). - Practitioners are approved by the Biomedicine Agency.

Country	answer
	- Pregnant women, couples and family doctors may request advice from multidisciplinary PND centers (authorised by the Biomedicine Agency).
Germany	The German Genetic Diagnosis Act of 31st July 2009 foresees that prenatal genetic diagnosis may only be carried out for medical purposes and only if the diagnosis is relevant to the health of the embryo or the fetus during pregnancy or after birth or the diagnosis is relevant for the treatment of the embryo or the fetus with medicines. The prenatal genetic diagnosis may only be carried out by a medical doctor and requires prior informed consent and comprehensive genetic counseling of the mother concerned before and after the genetic diagnosis.
Greece	<p>In general, legislation concerning Prenatal Diagnosis provides, among other things, for :</p> <ul style="list-style-type: none"> - the necessary prerequisites for abortion in case of indications for genetic disorders, after prenatal diagnosis is completed. - the conditions under which prenatal diagnosis is being carried out. - the aim of prenatal diagnosis, i.e. the detection of traces of serious abnormalities in the embryo that may result in the birth of a neonatal with serious pathology, - issues concerning the dissemination and application of contemporary knowledge in genetic issues and family planning, as well a issues of education in these matters. - written consent.
Latvia	<p>PND is limited: for the health of the child to be born when there is a risk of serious inheritable disease in the child that can be detected by PND</p> <p>PND is allowed for: chromosomal aneuploidies chromosomal translocations embryo sexing for X linked disorders</p> <p>PND is not allowed for: multifactorial disorders</p> <p>Criteria prerequisites for allowing PND: severity of disorder absence of appropriate treatment</p> <p>There is no commonly used list of disorders for which PND is accepted. In the case of a specific disorder the acceptability of performing PND is evaluated by: a medical team a competent authority (geneticists-gynecologists)</p> <p>PND may be carried out only in the State center for medical Genetics.</p>
Lithuania	<p>For prenatal genetic diagnosis (PND) the following legal acts are relevant:</p> <ul style="list-style-type: none"> • The Decree No. V-1135 of the Ministry of Health on the Prenatal care to pregnant women, dated: 29 December, 2006 (amended on 11 July, 2011), where requirements for prenatal care of pregnant women are provided. <p>The Decree is available (in Lithuanian) at: http://www3.lrs.lt/pls/inter3/dokpaieska.showdoc_l?p_id=290266&p_quer</p>

Country	answer
	<p>y=&p_tr2=</p> <ul style="list-style-type: none"> • The Decree No. V-220 of the Ministry of Health on the Medical Norm MN 56:2003 for the Clinical Geneticist (MD). Rights, obligations, competence and responsibility, dated: 14 April, 2003. The Decree provides the definition of prenatal diagnostics of congenital diseases and malformations, as well as regulates national standards for genetic counseling and professional responsibilities of clinical geneticists. The Decree is available (in Lithuanian) at: http://www3.lrs.lt/pls/inter3/dokpaieska.showdoc_l?p_id=210412 • The Decree No. V-522 of the Ministry of Health on the Human genetic services, compensated from the State Patient Fund budget, dated: 23 June 2005. The Decree regulates the procedure of patient reference to the Center of Medical Genetics for PND and provides the indications for PND. According to this Decree, tests are carried out on the first trimester of pregnancy in the licensed medical genetics centres. The decision to perform prenatal testing shall be made by general practitioner or other physician. Indications for the prenatal genetic diagnostics are: mother's age (over 35); history of chromosomal abnormalities in foetus or chromosomal diseases or congenital malformations in child of previous pregnancy; family history of chromosomal diseases in mother's or father's family; ultrasound warning signs; female may be a carrier of a balanced chromosomal abnormality, exposure to the mutagenic or teratogenic factors on the early stage of pregnancy. The Decree is available (in Lithuanian) at: http://www3.lrs.lt/pls/inter3/dokpaieska.showdoc_l?p_id=260062
Malta	<p>Recommendation in the Report of the Parliament's Social Affairs Committee that PND should be allowed only when:</p> <ul style="list-style-type: none"> there are therapeutic options for the correction of embryonic abnormalities; for the sole benefit of the embryo; the purpose cannot be achieved by any other means; the benefits to both the embryo/fetus and to the mother outweigh the risks; there is consent of both parents.
The Netherlands	<p>PND is legally permitted if there is a high risk of a genetically determined severe disease</p>
Norway	<p>PND can be offered in the following situations:</p> <ul style="list-style-type: none"> when the pregnant woman will be 38 years or older at the expected term/time of birth if the pregnant woman or the (genetic) father of the child previously has experienced that their fetus or child had/has a serious disease or anomaly if there is a high risk that the fetus/child will be affected by a serious disease, and the condition can be revealed by PND if the pregnant woman use medicines that may harm the fetus if ultrasound examination revealed signs of anomaly in the fetus in special situations, if the pregnant woman is in a difficult situation and will not be able to take care of a child with a serious disease or anomaly. <p>A risk assessment - ultrasound scan and if appropriate, maternal serum biochemistry – shall be performed first, and may be followed by an invasive procedure. In cases where the non-invasive procedures will offer no information on the condition, only invasive procedures are performed.</p>
Poland	<p>Poland has only general regulations relating to prenatal genetic diagnosis:</p>

Country	answer
	<p>- the Family Planning Act requires administrative bodies and local authorities to provide free access to information and to prenatal diagnosis, particularly in high-risk cases in which a genetic defect, a foetal development defect or an incurable disease which threatens the life of the foetus is presumed to exist.</p> <p>- the Ministry of Health regulations implementing the State Medical Services Act list the services provided to women presenting a higher than average risk of disease or defect, genetic or other, as well as the periods for which these are available. Thus prenatal genetic intervention consists of examinations and tests carried out during the first six months of pregnancy, and during the 22nd week of pregnancy at the latest. These comprise:</p> <ol style="list-style-type: none"> 1. non-invasive examinations: <ol style="list-style-type: none"> a. ultrasound scans of the foetus with a view to detecting genetic abnormalities and diseases, b. biochemical examinations of the mother's blood, serum markers: <ul style="list-style-type: none"> - plasma protein A (PAPP-A), - alpha-foetoprotein (AFP), - unconjugated oestriol (E3), - beta-human chorionic gonadotrophin (B-hCG) ; 2. invasive examinations: <ol style="list-style-type: none"> a. trophoblast biopsy, b. amniocentesis, c. cordocentesis, d. analysis of umbilical cord blood; 3. cytogenetic and molecular analyses: <p>Main indications for prenatal screening:</p> <ul style="list-style-type: none"> - mother aged 40+, - chromosomal abnormalities in the foetus or in the child of the previous pregnancy, - structural chromosomal abnormalities confirmed in the pregnant woman or in the child's father, - confirmed risk of the birth of a child affected by a monogenic or multifactorial disease, - scan result and/or result of biochemical tests indicating a high risk of chromosomal or foetal abnormality.
Slovenia	According to the code of practice to the law of 1977, with the approval of the Hospital Ethics Committee.
Spain	Laboratories are not specifically licensed for genetic (molecular and cytogenetic) studies; accreditation of professionals and procedures for genetic tests are not regulated in Spain. Quality Control of prenatal genetic tests (monogenic disorders) is not regulated at a national basis. But recommendations and guidelines are published and not mandatory Quality control procedures exist. They both come from the Scientific Societies: <i>SEGO</i> (Sociedad Española de Ginecología y Obstetricia), <i>AEDP</i> (Asociación Española de Diagnóstico Prenatal) & <i>AEGH</i> (Asociación Española de Genética Humana).
Switzerland	For various reasons, there has been no attempt in Switzerland to set down in legislation the admissible indications for prenatal testing. The solution adopted indicates what such tests should not look for. Thus, it is prohibited to carry out prenatal tests with a view to determining characteristics of the embryo or foetus not directly influencing its health, or to determining gender for a purpose other than a diagnostic one (cf. Art. 11 of the Federal Law on the Genetic Testing of Humans, LAGH). A deliberate decision was taken not to define the concept of "characteristics not directly influencing its health". Use of the word "directly"

Country	answer
	<p>excludes psychosocial indications. A prenatal genetic analysis can only be prescribed by a medical doctor with an appropriate postgraduate training or by a medical doctor who, in the framework of a postgraduate training works under the supervision of a medical doctor having appropriate postgraduate training (cf. art. 13, al. 2, LAGH).</p> <p>The analysis can only be carried out in a laboratory duly authorised by the Public Health Federal Office (cf. art. 8, al. 1, LAGH).</p>
Ukraine	<p>Joint Act _#641/84 from 31.12.2003 of Ministry of Health Care and Academy of Medical Science of Ukraine Special Centres for PND-6</p>
Australia	

APPENDIX 1

PGD for monogenic diseases ³⁰

Type of monogenic disease	Name of monogenic disease	Number of cycles (out of 931 cycles)	Percentage (%)
autosomal recessive	β -thalassemia and/or sickle cell syndromes	82	8,8
	β -thalassemia/sickle cell with human leucocyte antigen (HLA) typing ³¹	28	3
	Cystic fibrosis	78	8,37
	Spinal muscular atrophy	56	6
autosomal dominant diseases	Myotonic dystrophy type I (DM1)	98	10,5
	Huntington disease	98	10,2
	Neurofibromatosis	21	2,26
	Charcot-Marie-Tooth	14	1,5
specific diagnosis of X-linked diseases	Fragile X syndrome	75	8,05
	Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy	43	4,61
	Haemophilia A and B	11	1,18

As for previous years, data IX showed that PGD for reciprocal translocations³² was performed more often than for Robertsonian translocations³³ or other types of chromosome abnormalities.

Finally, there were also reported 82 cycles for social sexing.

³⁰ V. Goossens, G. Harton, C. Moutou, J. Traeger-Synodinos, M. Van Rij and J.C. Harper (2009) ESHRE PGD Consortium data collection IX: cycles from January to December 2006 with pregnancy follow-up to October 2007, Journal of Human Reproduction,

³¹ Besides the 28 cycles for β -thalassemia and/or sickle cell syndromes with HLA typing, there were 12 cycles for HLA compatibility typing plus a further 17 cycles for HLA typing along with a specific disorder including one each for adenosine deaminase deficiency, hyper IgM syndrome and Wiscott–Aldrich syndrome, two each for Blackfan–Diamond and Duncan syndrome, three for granulomatous disease and seven for Fanconi anaemia.

³² Chromosome rearrangement involving the exchange of chromosome segments between two chromosomes that do not belong to the same pair of chromosomes. (MedTerms Dictionary, <http://www.medterms.com/script/main/hp.asp>)

³³ Type of chromosome rearrangement that is formed by fusion of the whole long arms of two acrocentric chromosomes (chromosomes with the centromere near the very end) (MedTerms Dictionary)

APPENDIX 2

Overview of main diseases for which PGD is performed³⁴ (according to data collected by ESHRE – IX compilation)

β-thalassemia (BT)

Frequency: estimated at 100,000 cases/year.

Characteristics and types of BT : Beta-thalassaemia (BT) is marked by deficiency (B+) or absence (B0) of synthesis of the beta globulin chain of the haemoglobin (Hb) protein. The disease was initially described in the Mediterranean basin but severe forms of BT occur throughout the Middle East, South East Asia, India and China. Three types of BT have been described. 1) *Thalassaemia minor* (BT-minor) is the heterozygous form and is usually asymptomatic. 2) *Thalassaemia major* (Cooley anemia; BT-major) is the homozygous form and is associated with microcytic and hypochromic anemia. Onset occurs from 6-24 months of age. The severe anemia requires systematic transfusions to maintain Hb levels within the range of 90-100 g/L and allow normal activity. Transfusion of red cell concentrates results in iron overload which hampers the vital prognosis (due to cardiac involvement) and causes significant morbidity (due to endocrinal and hepatic manifestations). 3) *Thalassaemia intermedia* (BTI) groups together around 10% of homozygous disease forms with numerous compound heterozygous forms. The degree of anemia in BTI is variable, but is less severe and is diagnosed later than that in BT-major. Patients with BTI may or may not require occasional transfusions.

Treatment : There are two lines of treatment for BT. 1) A combination of transfusions and chelators (early and regular parenteral deferoxamine has led to increased survival during the last 30 years). 2) Haematopoietic stem cell transplant is the only curative treatment for BT: results are very favorable for children with HLA-identical familial donors.

Genetic background: Transmission is autosomal recessive and around 200 mutations (B0 or B+) have been identified.

Sickle cell anaemia

Frequency: Estimated in 25 European states 1/150 individuals. In central and western Africa (15-25%), in the French West Indies (10-15%) and in Mediterranean areas (1-15%). A high prevalence is observed in areas that are or have been affected by malaria, because the trait offers protection against pernicious malaria.

Characteristics: Sickle cell anemias are chronic hemolytic diseases that may induce three types of acute accidents: severe anemia, severe bacterial infections, and ischemic vasoocclusive accidents (VOA) caused by sickle-shaped red blood cells obstructing small blood vessels and capillaries. Many diverse complications can occur.

³⁴ Source: Orphanet, available at : <http://www.orpha.net/consor/cgi-bin/index.php?Ing=EN>

Orphanet provides more detailed and specific information on these diseases.

Symptoms: Clinical manifestations are extremely variable between individuals and at different times. In addition to anemia and bacterial infections, VOAs cause hyperalgetic focal ischemia (and sometimes infarction) when they occur in the muscles or skeleton. Over the course of time, VOAs may compromise the integrity of tissues or organs.

Treatment: An orphan drug based on hydroxycarbamide (hydroxyurea) has obtained European marketing authorization for the severe forms of the disease. Regular or occasional transfusions remain an essential therapeutic method.

Genetic background: Transmission is autosomal recessive. Sickle cell anemia is determined by combinations of two abnormal alleles of the beta globin gene among which at least one carries the beta 6 glu-val mutation (Hb S).

Cystic fibrosis

Frequency: Estimated in Europe between 1/8 000 and 1 in 10 000 individuals.

Characteristics: Cystic fibrosis is the most common potentially fatal genetic disorder among Caucasian children. It is characterized by alterations in the CFTR protein leading to changes in the characteristics of exocrine excretions. An absence of functional CFTR in the epithelial cell membrane leads to the production of sweat with a high salt content (associated with a risk of hyponatremic dehydration) and mucus secretions with an abnormal viscosity (leading to stasis, obstruction and bronchial infection).

Symptoms: The disease is chronic and generally progressive, with onset usually occurring during early childhood or, occasionally, at birth (meconium ileus). Virtually any internal organ may be involved but the principle manifestations concern the breathing apparatus (chronic bronchitis), pancreas (pancreatic insufficiency, adolescent diabetes and occasionally pancreatitis) and, more rarely, the intestine (stercoral obstruction) or liver (cirrhosis). The most common form of cystic fibrosis is associated with respiratory symptoms, digestive problems (steatorrhea and/or constipation) and staturoponderal growth anomalies. Mortality and morbidity depend on the extent of the bronchopulmonary involvement. Male sterility is a constant feature. Late-onset forms, which are usually only mildly or monosymptomatic, have also been reported.

Treatment: Treatment of cystic fibrosis remains purely symptomatic, revolving around bronchial drainage, antibiotics for respiratory infections, pancreatic analysis and administration of vitamins and calorific supplements for digestive and nutritional problems. These cost-effective treatments have significantly improved the prognosis for cystic fibrosis patients: in the 1960's the majority of patients died before 5 years of age, whereas the current average life-span exceeds 35 years and life-expectancy is 40 years.

Genetic background: Cystic fibrosis is a monogenic autosomal recessive disease caused by mutations in the CFTR gene (chromosome 7).

Spinal muscular atrophy (SMA)

Frequency: 1-9 / 100 000 individuals

Characteristics and types: Spinal muscular atrophies are a group of neuromuscular disorders characterized by progressive muscle weakness resulting from the

degeneration and loss of the lower motor neurons in the spinal cord and the brain stem nuclei. Four subtypes have been defined according to the age of onset and severity of the disease: type 1 (PSMA1/SMAI), the most severe form, with onset before six months of age; type 2 (PSMA2/SMAII), with onset between 6 and 18 months of age, type 3 (PSMA3/SMAIII), with onset between childhood and adolescence, and type 4 (PSMA4/SMAIV), the least severe form, with adult onset. The weakness is almost always symmetric and progressive.

Symptoms: The prognosis depends on the severity of the disease, which generally correlates with the age of onset: earlier-onset forms are generally associated with a poor prognosis, whereas life expectancy may be close to normal in later-onset forms. Death may occur due to respiratory insufficiency and infections.

Treatment: Management remains symptomatic, involving a multidisciplinary approach that aims to improve quality of life. Physiotherapy and occupational and respiratory therapies are necessary. Noninvasive ventilation and gastrostomy may be required. Antibiotic therapy is used in case of pulmonary infection. The scoliosis and joint manifestations may require surgical correction. Patients may require a wheelchair, or use a corset/back brace for support.

Genetic background: The spinal muscular atrophies are the second most common autosomal-recessive inherited disorders after cystic fibrosis.

Myotonic dystrophy type I (DM1)

Frequency: estimated at 1/20,000 individuals

Characteristics: Steinert disease, also known as myotonic dystrophy type 1, is a muscle disease characterized by myotonia and by multiorgan damage that combines various degrees of muscle weakness, arrhythmia and/or cardiac conduction disorders, cataract, endocrine damage, sleep disorders and baldness. It is the most frequent myotonic dystrophy in adult.

Symptoms: Disease course is usually slowly progressive but rapid deterioration may sometimes be observed. Life expectancy is reduced by the increased mortality associated with the pulmonary and cardiac complications. Neonatal and infant presentation can occur. The first is life threatening due to respiratory and cardiac problems and it occurs among children of affected mothers. The second could appear from any affected parent and its clinical course is similar to other infantile or juvenile muscular dystrophies.

Genetic background: The disease is associated with abnormalities at the 19 chromosome. Transmission is autosomal dominant, and anticipation may occur, that is, disease may be more severe and occur earlier in offspring. Based on the genetic test results on the affected parent, an accurate prognosis on the child is not possible.

Huntington disease (HD)

Frequency: 1/16 000 individuals

Characteristics: HD is a neurodegenerative disorder of the central nervous system, mainly affecting the basal ganglia. The disease affects females and males indifferently and usually occurs in adults, although at variable ages. Fewer than 10% of cases have juvenile HD, with onset before the age of 20.

Symptoms: Onset is often insidious, either with motor abnormalities (choreic syndrome), or with personality or behavioural changes, and even psychiatric disturbances (depression). Alongside the progression of motor disorders leading to falls, dysarthria and difficulties to swallow, dementia sets in. The association of motor and intellectual disorders in patients who are often young adults makes the disease very difficult to manage at home or in institutions. HD progresses slowly and leads to loss of autonomy.

Treatment: Treatment is symptomatic only (neuroleptics for abnormal movements, psychotropic drugs if needed, physiotherapy). Treatments with implants of genetically modified or embryonic cells are currently being assessed.

Genetic background: The mutation is the expansion of a trinucleotide repeated in gene IT15 (chromosome 4). HD is transmitted as an autosomal dominant trait, with penetrance increasing with age.

Neurofibromatosis (NF)

Frequency: 1/4500 individuals for type 1, 1/60000 individuals for type 2

Characteristics and types: Neurofibromatosis type 1 (NF1) is an inherited, multi-system, neurocutaneous disorder that predisposes to the development of benign and malignant tumors. Neurofibromatosis type 2 (NF2) is a tumor-prone disorder characterized by the development of multiple schwannomas and meningiomas. Neurofibromatosis type 6 (NF6), also referred as café-au-lait spots syndrome, is a cutaneous disorder characterized by the presence of several café-au-lait (CAL) macules without any other manifestations of neurofibromatosis or any other systemic disorder. Isolated CAL lesions do not require medical care. CAL spots are benign and may resolve with age, usually after the age of 2.

Symptoms: Patients with neurofibromatosis type 1 have a better prognosis, with fewer CNS tumors, than patients with neurofibromatosis type 2. Malignant peripheral nerve sheath tumors and vascular disease are major causes of mortality in NF1. Affected individuals from NF2, inevitably develop schwannomas, typically affecting both vestibular nerves and leading to hearing loss and deafness. NF2 represents a difficult management problem with most patients facing substantial morbidity and reduced life expectancy.

Treatment: Treatment is symptomatic and can include surgery for symptomatic neurofibromas, progressive scoliosis and pseudoarthrosis for type 1. For type 2 surgery remains the focus of current management although watchful waiting with careful surveillance and occasionally radiation treatment have a role.

Genetic background: Neurofibromatosis is a dominantly inherited tumor predisposition syndrome. NF1 gene is located in chromosome 17 and NF2 in chromosome 22.

Charcot-Marie-Tooth (CMT)

Frequency: 1-5 / 10 000 individuals

Characteristics and types of CMT: CMT is a common inherited neurologic disorder. CMT is characterized by inherited neuropathies without known metabolic

derangements. Charcot-Marie-Tooth disease (CMT) is actually a heterogeneous group of genetically distinct disorders with a similar clinical presentation. There are several different types of CMT.

Treatment: There is no treatment, however, preventive measures must be taken: physiotherapy, splints, orthopedic surgery and various technical aids.

Fragile X syndrome

Frequency: 1-5 / 10 000 individuals

Characteristics: Fragile X syndrome is the most frequent cause of inherited mental retardation.

Symptoms: The clinical features other than mental retardation include subtle dysmorphism, behavioral abnormalities and macroorchidism in postpubertal males. The phenotype being subtle, clinical diagnosis may be difficult especially in young children. Hence, testing all cases with mental retardation without obvious cause for fragile X syndrome is often the only way to identify fragile X syndrome cases.

Treatment: Although there is no causal treatment of syndrome X-fragile, medical care, educational, psychological and social support could be offered, improving their prognosis and social inclusion. Approximately 6% of children with learning disabilities, tested in institutions, are suffering from this syndrome.

Genetic background: Mutation located in X chromosome.

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD)

Frequency: 1/3,300 male births for DMD and 1/18,000 to 1/31,000 male births for BMD

Characteristics: DMD and BMD are neuromuscular diseases characterized by progressive muscle wasting and weakness due to degeneration of skeletal, smooth and cardiac muscle. DMD is more frequent, occurs earlier and is more severe than BMD.

Symptoms: In DMD, walking is often delayed. Cognitive functions can be altered. Diagnosis is generally made at the age of 5 when children present with a waddling gait and talipes equines with calf hypertrophy (positive Gower's sign). Inability to walk appears by 10 to 12 years of age. Scoliosis, cardiomyopathy and restrictive respiratory failure progressively appear. BMD appears later, between the ages of 5 and 15 years with a proximal motor deficiency of variable progression. Heart involvement can be the initial sign. Other clinical forms also exist. Progression is severe with end-stage cardiorespiratory failure in the young adult with DMD; it is slower and life span is subnormal to normal for BMD.

Treatment: is symptomatic and multidisciplinary: orthopedic (prevention and treatment of retractions, physiotherapy, fitting with prosthesis, spinal arthrodesis (at 12-15 years of age), and technical support), respiratory (prevention and treatment of infections, respiratory physiotherapy, and ventilation), and cardiac (ACE inhibitors and heart protection). Corticotherapy helps to stabilize motor abilities.

Genetic background: Both X-linked recessive diseases are caused by dystrophin deficiency in skeletal and heart muscles, leading to progressive necrotizing lesions.

Haemophilia A and B

Frequency: 1-9 / 100 000 individuals

Characteristics and types: Hemophilia is a genetic disorder characterized by spontaneous hemorrhage or prolonged bleeding due to factor VIII or IX deficiency. Hemophilia primarily affects males, but female carriers of the disease-causing mutations may also manifest generally milder forms of the disease.

Symptoms: In general, onset of the bleeding anomalies occurs when affected infants start to learn to walk. The severity of the clinical manifestations depends on the extent of the coagulation factor deficiency.

Treatment: revolves around substitution therapy with plasma derivatives or genetically engineered recombinant alternatives. Treatment may be administered after a hemorrhage or to prevent bleeding (as a prophylactic treatment). The most frequent complication is the production of inhibitory antibodies against the administered coagulation factor. Surgical interventions, most notably orthopedic surgery, may be carried out but should be conducted in specialized centers. Current treatment approaches now allow the prognosis to be favorable: the earlier the substitutive therapy is received and the more adapted the treatment is to the clinical status of the patient, the better the prognosis.

Genetic background: Hemophilia is transmitted in an X-linked recessive manner and around 70% of hemophiliacs have a positive family history of the disease.