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human reproduction update

The ESHRE PGD Consortium: 10 years of data collection

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BACKGROUND: Since it was established in 1997, the ESHRE PGD Consortium has been collecting data from international preimplantation genetic diagnosis (PGD) centres. Ten papers have been published, including data from January 1997 to December 2007.

METHODS: The data collection originally used a hard-copy format, then an excel database and finally a FileMaker Pro database. The indications are divided into five categories: PGD for chromosome abnormalities, sexing for X-linked disease, PGD for single gene defects, preimplantation genetic screening (PGS) and PGD for social sexing. The main end-points are pregnancy outcome and follow-up of deliveries.

RESULTS: In data collection I, 16 centres contributed data, which increased to 57 centres by data X (average of 39 centres per data collection). These centres contributed data on over 27 000 cycles that reached oocyte retrieval. Of these cycles, 61% were for aneuploidy screening, 17% for single gene disorders, 16% for chromosomal abnormalities, 4% for sexing of X-linked disease and 2% for social sexing. Cumulatively, 5187 clinical pregnancies gave rise to 4140 deliveries and 5135 newborns (singletons: 3182, twins: 921, triplets: 37).

CONCLUSIONS: In this paper, we present an overview of the first 10 years of PGD data, highlighting trends. These include the introduction of laser-assisted biopsy, an increase in polar body and trophectoderm biopsy, new strategies, methodologies and technologies for diagnosis, including recently arrays, and the more frequent use of freezing biopsied embryos. The Consortium data reports represent a valuable resource for information about the practice of PGD.

Key words: PGD / PGS / biopsy / Consortium / ESHRE

Introduction

Since the first report of preimplantation genetic diagnosis (PGD) by Handyside *et al.* (1990), the technique has been widely applied in IVF and PGD centres around the world and the technology has rapidly developed (Harper and SenGupta, 2011). It is important to monitor the use and safety of PGD as it involves manipulation of the embryo and genetic testing of human embryos, both of which have safety and ethical issues. Besides the reports of the PGD Consortium, there have been three main publications on international PGD data collection (Harper and Handyside, 1994; Harper, 1996; Verlinsky *et al.*, 2004). Recent data collections have been local or regional.

The ESHRE PGD Consortium was established in 1997. The aims of the Consortium are: (i) to survey the availability of PGD; (ii) to collect prospectively and retrospectively data on accuracy, reliability and effectiveness of PGD; (iii) to initiate follow-up studies; (iv) to produce guidelines and recommended PGD protocols; (v) To formulate a consensus on the use of PGD; (vi) to educate in the science of genetics and reproduction.

The Consortium has been collecting annual PGD data since 1997 from its members. The data contained here encompass data published in each of the last 10 years (PGD cycles performed from 1997 to 2007) (ESHRE PGD Consortium Steering Committee, 1999, 2000, 2002; Sermon *et al.*, 2005, 2007; Harper *et al.*, 2006, 2008, 2010; Goossens *et al.*, 2008, 2009). No other data of this magnitude exists in the literature. This paper will review the 10 sets of data collections in terms of overall trends, highlighting how the practice of PGD has evolved over the years.

Methods

Data collection

The first data collection (ESHRE PGD Consortium steering committee, 1999) was a preliminary assessment of PGD practice around the world. It involved the use of individual printed forms that were submitted to the ESHRE PGD steering committee members assigned to co-ordinate the evaluation and analysis of the data. The fields collected included information related to the stage of PGD referrals (patient history, PGD indication, reason for PGD, decision of centre and patients), PGD cycles (ART data, and PGD/PGS analysis and results), pregnancies (ultrasound observations, prenatal diagnosis and outcome, pregnancy evolution and complications) and baby delivery after PGD/PGS (neonatal parameters, congenital defects and short-term follow-up of children). This mode of data collection was rather crude and became unmanageable as the numbers of PGD centres and cycles increased. Thus subsequent data collections were transferred to an electronic format. Initially these involved the use of Microsoft Excel databases, but from data collection IV (PGD cycles for 2001) data were collected using FileMaker Pro databases designed by C. Moutou. From data V onwards, cycle data were collected for an entire calendar year, along with resulting pregnancies through to the October of the following year. This allowed a complete follow-up of every cycle reported through to delivery, and furthermore supported the electronic interlinking of each data subset (i.e. referral, cycle data, pregnancy and frozen embryos).

Database fields were updated to take into account technological evolutions (i.e. whole genome amplification, HLA typing, microarray-based testing) and also modified to facilitate automatic calculation of specific data parameters within each data set. Each year, the data submitted were thoroughly checked to identify omissions and any inconsistent data. Clarifications of ambiguous data were requested from the appropriate participating centre. Any case records with insufficient or spurious data, e.g. missing cycle or patient identification, missing disease-indication or inappropriate dates were excluded from subsequent data calculations. Following editing and correction of all data, the entire collection was separated into the five categories listed above and sent to expert co-authors for further checking and analysis.

The ever-increasing number of cycles performed by the many centres participating each year has promoted the development of a more advanced method of data collection. A database working group under the auspices of the ESHRE PGD Consortium is currently developing a web-based system, using Silverlight technology. Amongst the many advantages foreseen is the capability to apply prospective data collection. The launch of a pilot database is planned mid-2011 (after the ESHRE annual meeting) to evaluate the system, and, if approved, it will replace all data entry methods used so far, supporting manipulations and calculations for all future data collections.

Results

Centres who submitted data

The PGD Consortium has full and associate membership from all parts of the world (Fig. 1). Full members are centres that submit full data from oocyte retrieval (OR) through pregnancy data and associate members are centres that submit summary data about PGD cycles, but without the details of every case, or are IVF units that work with an external PGD centre. Over the 10 years of data collection, the number of centres submitting annual data has steadily increased (Fig. 2).

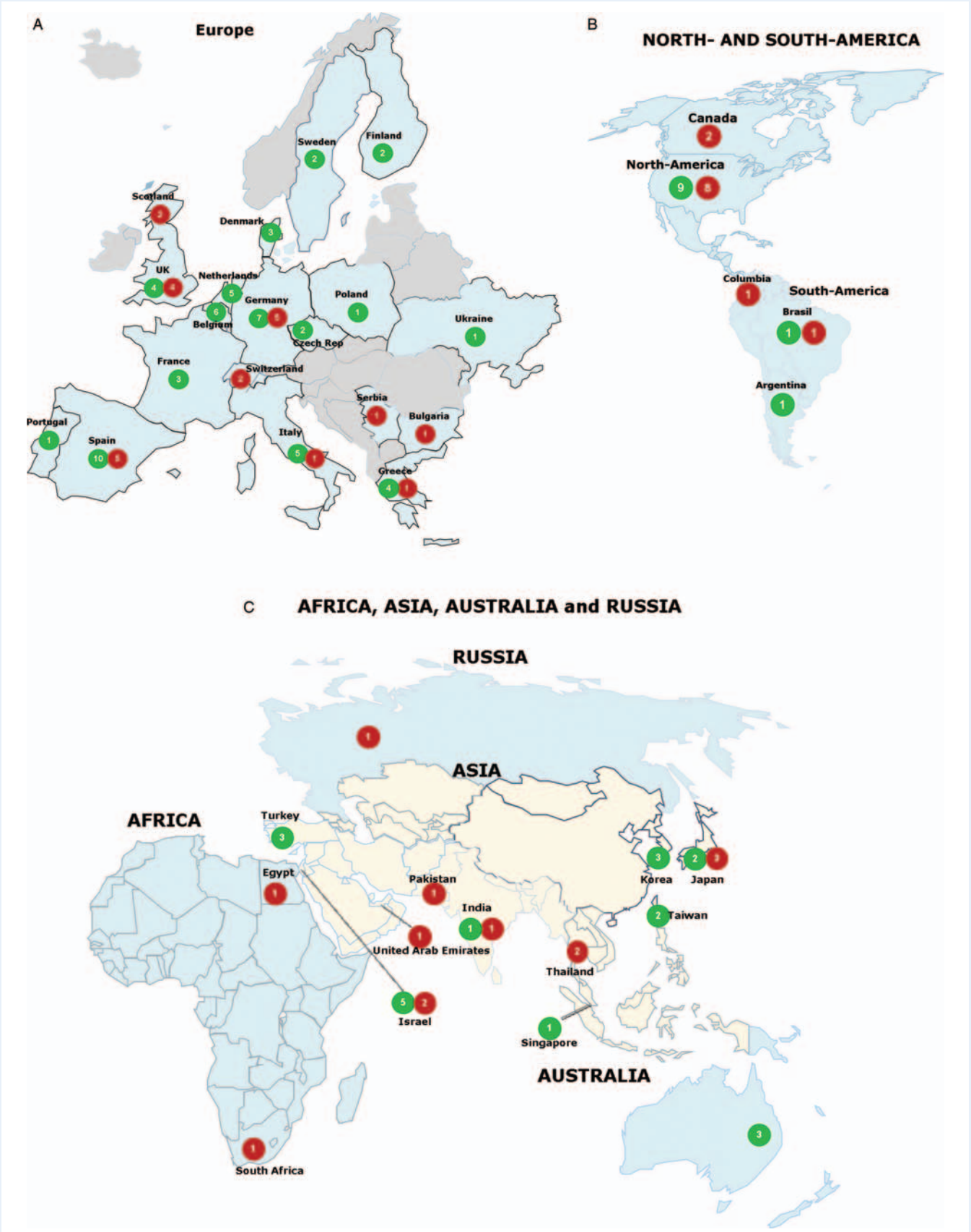

Figure 1 Full and associate member centres by location. (**A**) Europe, (**B**) North and South America and (**C**) Asia, Africa, Australia and Russia. Green are centres that are or have been members during data collections I–X (for at least 1 year). Red are centres that became members after data X or never sent in data.

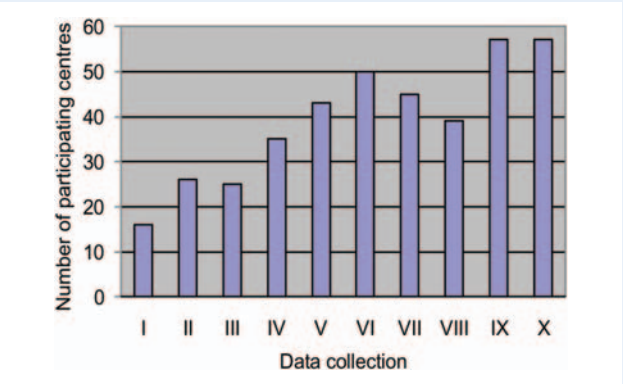

Figure 2 The number of centres that submitted verified data for each data collection I–X.

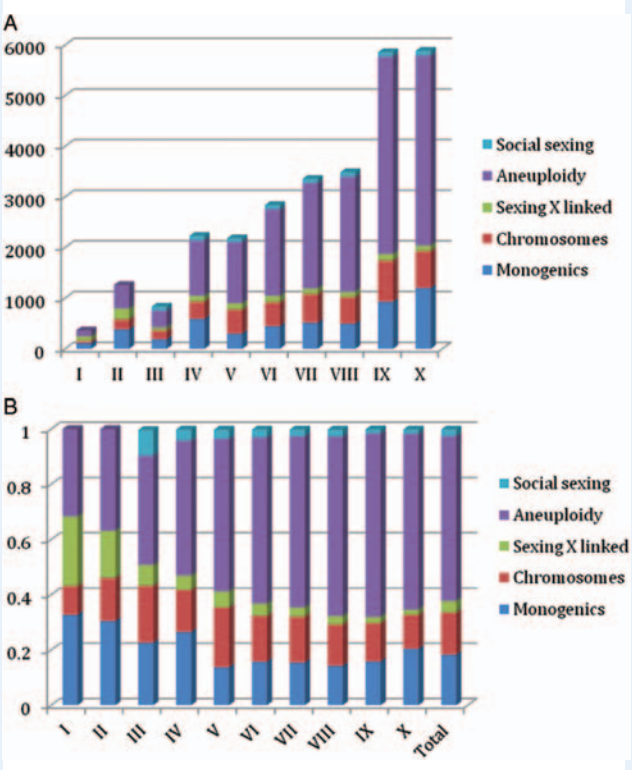

Figure 3 Ten years of cumulative data for each group of indications. (**A**) Number of cycles for each indication and (**B**) Proportion of cycles for each indication.

Indications

The indications for PGD have shown dramatic change throughout the data sets. Figure 3A shows the number of cycles for each indication per data set and Fig. 3B shows the different groups of indications as a proportion of all the cycles reported. Overall there have been 27 630 cycles to OR reported, 61% have been for aneuploidy screening, 17% for single gene disorders, 16% for chromosomal abnormalities, 4% for sexing of X-linked disease and 2% for social sexing. In Data I, the proportion of cycles for single gene disorders (33%), sexing for X-linked disorders (25%) and aneuploidy screening (32%) were comparable. Only 10% of cycles were for chromosomal disorders and social sexing was not reported. The proportion of cycles for PGS has increased with each set of data, except in Data X where there was a small decrease. The actual number of cycles for social sexing has remained constant throughout and from Data IV onwards so has the number of cycles for sexing for X-linked disease. For single gene disorders and chromosomal abnormalities, there has been an increasing trend upwards in the number of cycles with each data set.

The overall pregnancy rates for each indication are shown in Table I. The pregnancy rates were highest for PGD for single gene defects and lowest for chromosomal abnormalities. This is consistent with the trend that patients undergoing PGD for single gene defects often do not have fertility problems and only undergo IVF as part of the PGD procedure, whereas patients with chromosome rearrangements tend to be infertile or subfertile due to the chromosome anomaly. Furthermore, based on Mendelian genetics, ~50–75% of embryos in a single gene disorder PGD are expected to be unaffected for the at-risk disease, whereas only ~10–15% of embryos are expected to be transferrable in PGD cycles applied to exclude chromosome rearrangements.

Clinical IVF and ICSI data

In data collections I–VI, case referral details, as well as data on cycles initiated but cancelled prior to the OR were requested. However, since only a few centres contributed this data, it was discontinued from data VII onwards. Throughout the 10 data collections, data were collected on cycles that had an OR but were cancelled prior to insemination (only 20/27 630 ORs) or cancelled after insemination but prior to the embryo biopsy (1003/27 630 ORs). Most of these cases were mainly due to insufficient oocyte/embryo numbers or quality.

From the 10 years of data collected, 27 630 ORs resulted in the collection of 339 966 oocytes, 202 357 fertilized oocytes and 35 944 embryos transferred in 19 901 embryo transfer procedures.

ICSI was used in the majority of cases (23 830 cycles) compared with conventional IVF (3113 cycles). For PGD for single gene defects, the use of ICSI is recommended (Harton *et al.*, 2011c) to prevent paternal contamination from excess sperm lodged in the zona pellucida. ICSI was also the method of choice for FISH-based diagnosis as many centres felt it ensured good fertilization rates. In 9 out of the 10 data collections, a number of cases for single gene defect PGD were reported as having been fertilized by conventional IVF rather than ICSI. This situation was noted and discussed in the relevant reports. Over the entire 10-year data collection period, the overall fertilization rate was 60% (202 357/339 966), which is comparable to IVF/ICSI cycles not destined for biopsy and genetic testing.

Overall, from the 26 609 cycles that reached the diagnosis stage, 19 901 cycles resulted in a transfer procedure (74%). In the remaining 6708 cycles that did not result in a transfer were usually reported to have all embryos abnormal or untransferable due to embryonic arrest or inconclusive results.

Reporting of the 'fertility status' and maternal age of each patient was attempted from Data III onwards. Over the ten data collections, 17 900/26 730 (67%) cycles were reported to involve an infertile

Table I Ten years of PGD Consortium data.

	Cycles to OR	No. embryos biopsied	No. embryos transferred (mean/ET)	Embryo transfer procedures	Clinical pregnancy rate (per OR and per ET)
Single genes	4733	27 980	7035 (1.9)	3727	22% per OR 29% per ET
Structural chromosome abnormalities	4253	27 068	4775 (1.7)	2731	17% per OR 26% per ET
Sexing X-linked	1167	7317	1598 (1.8)	880	19% per OR 26% per ET
Aneuploidy	16 806	90 404	21543 (1.8)	12071	19% per OR 27% per ET
Social sexing	671	4285	993 (2.0)	492	21% per OR 29% per ET

OR, oocyte retrieval; ET, embryo transfer procedure.

patient. This is higher than expected but is probably skewed due to the sheer numbers of cycles involving preimplantation genetic screening (PGS) where a large proportion of patients would be expected to be infertile. Broken down by indication, 83% of PGS patients, 37% of single gene defect patients and 15% of social sexing patients were classified as infertile. However, in the absence of specific criteria to support the evaluation and reporting of fertility status, these numbers may not reflect the true levels of infertility in patients undergoing PGD. The average maternal age was 35 years, being lowest in the single gene defect group (33 years) and highest in the PGS group (37 years).

Throughout the 10 years of data collection, attempts have been made to collect information on cycles with embryos frozen prior to embryo biopsy and PGD due to IVF-related complications (e.g. ovarian hyperstimulation), or cycles with frozen surplus embryos following PGD. The collection and evaluation of this information has always been problematic as embryos from different ORs are often used together in subsequent transfer cycles. These data will continue to present a challenge, since more and more embryos will likely be frozen following testing due to embryo banking (undergoing multiple IVF cycles and collecting a number of embryos for testing at one time), plus the tendency of more cycles to culminate in single embryo transfer. It is hoped that the new web-based database will allow more flexibility and the tracking of individual embryos, both fresh and frozen.

Biopsy

In looking at the entire set of data, the most common method of zona breaching used was laser drilling 15 467/26 609 (58%), however, this has not always been the case. In the first data collection, acid Tyrodes (AT) drilling was the most common method of breaching the zona (115/120; 96%), the remaining cases were performed using mechanical methods. By data collection III, laser drilling was being used almost as much as AT drilling (48 versus 51%) and by data collection VI, laser drilling had overtaken AT drilling (50 versus 43%) with the remaining cases being done by mechanical methods. By data VI and onwards, laser drilling was the dominant method for zona breaching, being used in 60% of the cases in data VI and in 72% of the cases by data X.

In addition to the method of zona breaching, the stage of biopsy and method of cell removal were also recorded. In general, cleavage stage biopsy has predominated data collections I–X. Polar body biopsy was first reported in data collection II, and blastocyst biopsy was first reported in data VI. For cleavage stage biopsy, multiple methods of cell removal have been reported including cleavage aspiration (most common), cleavage extrusion and cleavage flow displacement. Cleavage stage aspiration is the predominant method of cell removal in data collections I–X.

PGD for chromosome abnormalities

There have been 4253 cycles of PGD for inherited chromosome abnormalities that have reached the stage of oocyte collection reported during the first 10 years of data. In data I, only 40 cycles for chromosome abnormalities were performed and by data X, 729 cycles were reported.

In data III, for the first time the chromosome abnormality data were separated into Robertsonian, reciprocal and other chromosome abnormalities, and in data IV it was further divided into Robertsonian and reciprocal, male and female, other chromosome abnormalities and sex chromosome abnormalities.

Overall, PGD was performed for male Robertsonian translocation carriers (742 cycles), female Robertsonian translocation carriers (471 cycles), male reciprocal translocation carriers (1156 cycles), female reciprocal translocation carriers (1257 cycles), sex chromosome abnormalities (337 cycles) and other chromosome abnormalities (290 cycles) (Fig. 4). There has been very little difference in the number of cycles for each type of translocation between the sets of data over 10 years. In all years, PGD for reciprocal translocations was performed more often than for Robertsonian translocations.

PGD for chromosome abnormalities has shown that a high number of embryos are not suitable for transfer due to chromosome imbalance in the embryo. Overall, from 58 817 oocytes retrieved only 11% were suitable for transfer and, from 24 773 embryos that were successfully diagnosed, only 26% were suitable for transfer. These data are summarized in Fig. 5 for each type of translocation.

The low number of transferable embryos resulted in a low number of cycles that had a transfer (64%; 2731/4253), compared with 79% for single gene disorders (3727/4733). Robertsonian translocations

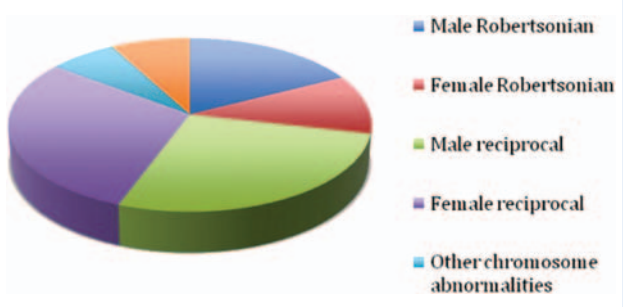

Figure 4 Breakdown of the number of cycles for PGD for chromosome abnormalities.

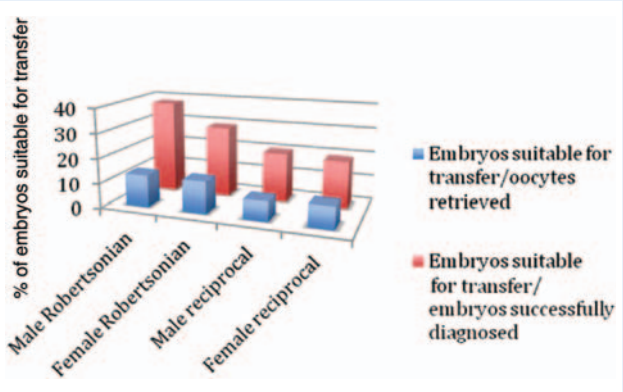

Figure 5 PGD for chromosome abnormalities; embryos suitable for transfer.

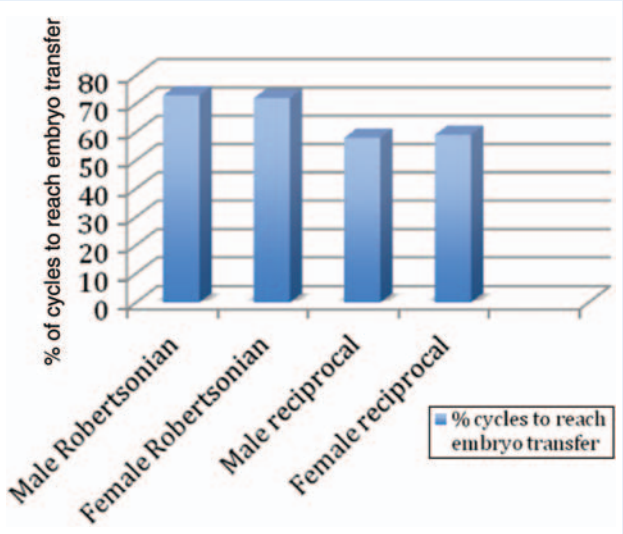

Figure 6 PGD for chromosome abnormalities; % cycles to reach an embryo transfer procedure.

resulted in more cycles with transfers compared with reciprocal translocations (male Robertsonian: 73%, female Robertsonian: 72%, male reciprocal: 58% and female reciprocal: 59%; Fig. 6).

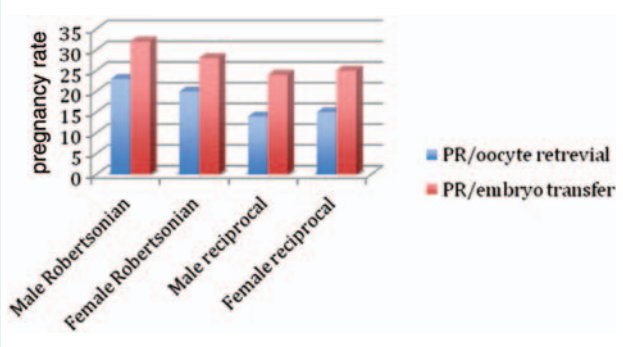

Figure 7 PGD for chromosome abnormalities: pregnancy rate per OR and per embryo transfer procedure.

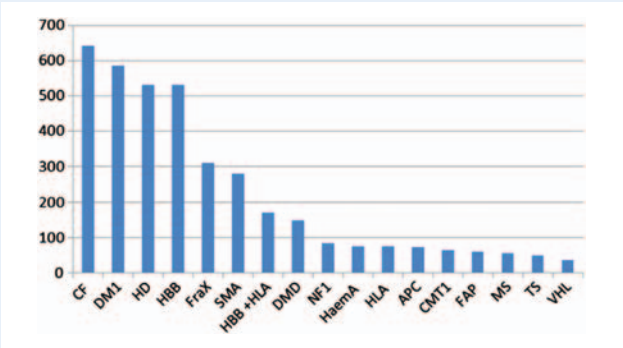

Figure 8 Number of cycles of most commonly tested disorders. CF, cystic fibrosis; DM1, myotonic dystrophy type 1; HD, Huntington disease; HBB, β thalassaemia / sickle cell anaemia; FraX, fragile X; SMA, spinal muscular atrophy; HBB +HLA, β thalassaemia / sickle cell anaemia + human leukocyte antigen; DMD, Duchenne muscular dystrophy; NF1, neurofibromatosis type 1; HaemA, haemophilia A; HLA, human leukocyte antigen for acquired diseases; APC, familial adenomatous polyposis; CMT1, Charcot-Marie-Tooth disease type 1; FAP, familial amyloidotic polyneuropathy; MS, Marfan syndrome; TS, tuberous sclerosis; VHL, Von Hippel Lindau.

The relatively low pregnancy rate (17% per oocyte collection and 26% per transfer) reflects the low proportion of embryos considered to be chromosomally normal and available for transfer. Robertsonian translocations had a higher pregnancy rate compared with reciprocal translocations (Fig. 7).

In summary, there was little difference in outcomes between male and female translocation carriers but there was a difference in outcome according to the type of translocation being tested. Robertsonian translocations showed a higher number of normal/balanced embryos available for transfer leading to a higher pregnancy rate compared with reciprocal translocations, irrespective of whether they were male or female carriers.

PGD for single gene diseases

Over the past 10 years, data have been collected for 4733 PGD cycles that reached OR for single gene defects. Figure 8 summarizes the most common indications, listed according to the number of cycles

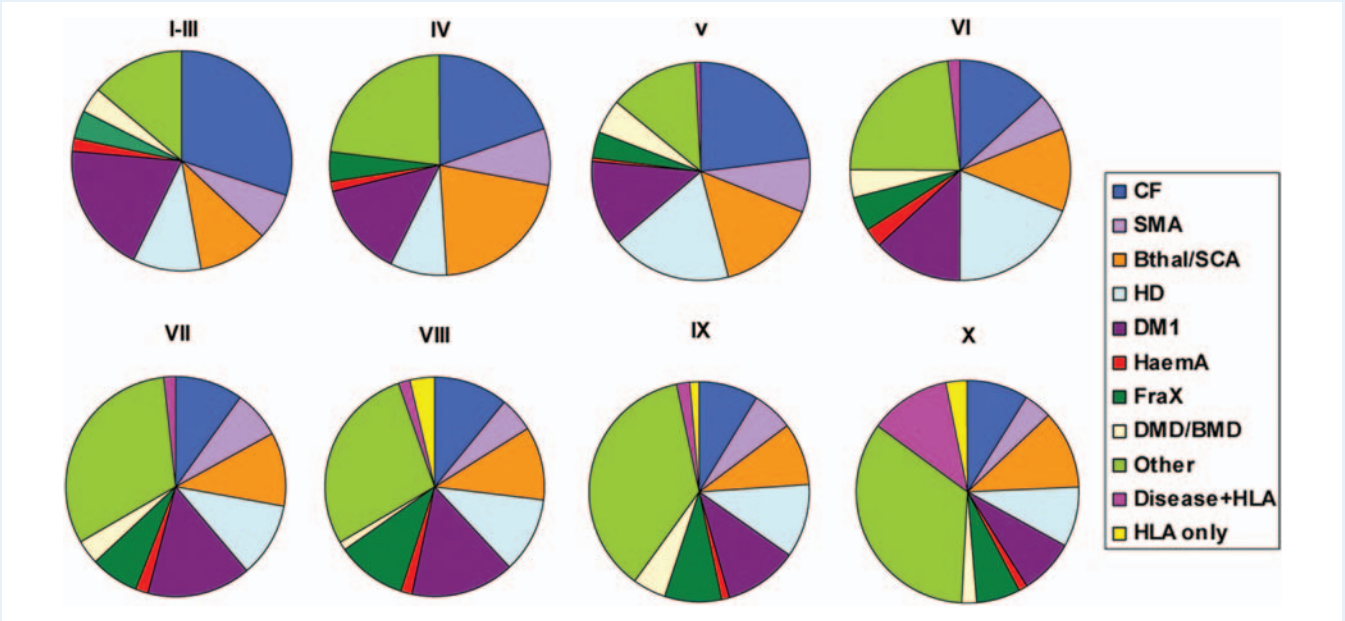

Figure 9 PGD for single gene disorders: proportional representation of main indications in each data set. CF, cystic fibrosis; SMA, spinal muscular atrophy; Bthal, beta thalassaemia; SCA, sickle cell anaemia; HD, huntington disease; DM1, myotonic dystrophy; HaemA, haemophilia A; FraX, fragile X syndrome; DMD, Duchenne muscular dystrophy; BMD, Becker muscular dystrophy; HLA, human leukocyte antigen; Other, all other indications tested in data set.

performed. The most common indications shown in Fig. 9 do not generally reflect the typical work load of most PGD centres due to some regional idiosyncrasies. For example, beta-thalassaemia and sickle cell syndromes are more common in Mediterranean countries; familial amyloid polyneuropathy is mainly observed in Portugal and Sweden. Equally the majority of the HLA typing is being undertaken by one PGD centre. However, there is an evident set of 'core' diseases offered.

Autosomal recessive

Until recently, the most common indication was cystic fibrosis (643 cycles). However, when in more recent data collections, the cycles for HLA typing to select a histocompatible sibling to facilitate a bone marrow transplant in thalassemia major patients, (data collections IX and X, 170 cycles) are added to the cycles testing beta-thalassaemia and sickle cell syndromes alone (530), diseases caused by mutations in the HBB gene become the most requested (700 cycles). The next most common autosomal recessive indication was spinal muscular atrophy (280), which is in keeping with respective population carrier frequencies of the diseases.

Autosomal dominant

The largest number of cycles have been performed for myotonic dystrophy type I (586) and a similar number of cycles for Huntington disease (HD) (530). HD cycles include direct testing where an individual knows their HD status, as well as exclusion testing and non-disclosure, which are two approaches allowing individuals at risk of developing HD, who do not wish to know their status, to have children free of the burden of this late onset, untreatable disease.

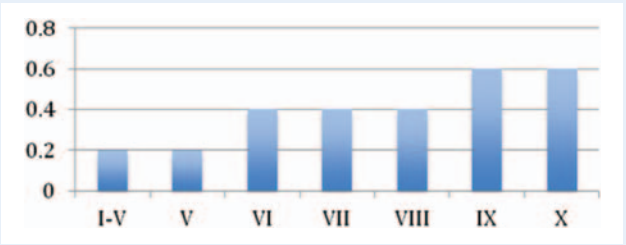

Figure 10 Proportion of all cycles for X-linked disease that had a specific diagnosis (rather than embryo sexing).

X-linked disorders

Fragile X syndrome (311) was the most common indication followed by Duchenne muscular dystrophy (DMD) (148) and haemophilia (75). Historically, these indications have been categorized into X-linked dominant and X-linked recessive inheritance, respectively, but such boundaries are blurring due to the increasing awareness of X-inactivation on the presenting clinical phenotype resulting in manifesting female carriers. None of the other indications had >35 cycles each throughout the 10 years of data collection.

Overall

Figure 9 shows the change in proportional representation of indications in each data collection. While the overall number of cycles has increased, the absolute number of cycles for each of the main indications has stayed remarkably steady per year. The major rise in cycles is attributed to 1–2 cycles of PGD being carried out for an increasing

number of new indications within each data set. 'Other' indications accounted for 14% of cycles in data sets I–IV and this percentage has more than doubled to represent 34% of the cycles in data set X. Overall, PGD for an additional 173 'other' single gene diseases were initiated in 910 cycles. Only two cycles of PGD have been reported for the diagnosis of MELAS mutations in the mitochondrial genome. Also of note is that the proportion of X-linked disorders with specific diagnosis in PGD compared with sexing alone has increased with each data set (Fig. 10). The increase in the spectrum of different indications offered is likely due to the availability of countless databases for human genetic information (NCBI, ENSEMBL, UCSC) along with the availability of improved reagents, especially DNA polymerases (e.g. multiplex DNA polymerase kits or whole genome amplification kits), all of which support a more rapid optimization of linkage-based protocols in PGD (Renwick and Ogilvie, 2007; Spits and Sermon, 2009; De Rycke, 2010). Additionally in data VIII, FISH was first reported for the diagnosis of single gene disorders caused by microdeletions, and in data X the use of PCR for translocations was reported.

In addition to HD, other adult onset neurodegenerative disorders to which PGD has been regularly applied includes the spinal cerebellar ataxia types 1, 3 and 7.

Of interest is the cumulative increase in the number of cycles for a variety of cancer predispositions with high penetrance including neurofibromatosis type I, familial adenomatosis polyposis, von HippelLindau and retinoblastoma. PGD for cancer predispositions of lower penetrance such as BRCA1 are emerging. PGD for Charcot Marie Tooth (CMT1A), a neuropathy with variable severity of symptoms, is routinely undertaken, although in some countries this condition is not considered to warrant the use of PGD. Furthermore, PGD cycles for non-life threatening conditions such as non-syndromic deafness have also been reported.

The selection of a histocompatible sibling to facilitate a bone marrow transplant is now a widely acceptable application of PGD, through HLA haplotyping. Overall, there were 225 cycles of HLA typing along with exclusion of a specific disorder. The majority (170) were for beta haemoglobinopathy syndromes, but other indications include Fanconi anaemia, Gaucher disease, adrenoleukodystrophy and osteopetrosis. HLA compatibility testing alone, not in conjunction with testing for a specific disorder, is an indication for acquired disease, and 72 cycles of PGD were undertaken for HLA compatibility only.

The data show an increasing trend in the proportion of embryos with a successful diagnosis following testing; the early data sets achieved an 83% diagnosis rate, versus a 90% diagnosis rate in data set X. If more embryos are successfully diagnosed, then more embryos will be available to select for transfer, which should increase the chance of a successful PGD cycle in couples. The increased ability to diagnose embryos is likely a reflection of improvement over the years of molecular techniques applied to single cell testing. There have been landmark advances in DNA amplification over the past decade including the introduction of fluorescent PCR, whole genome amplification and multiplexed PCR amplification of loci, which allows inclusion of linked markers to achieve diagnosis even in the presence of allele drop out; an inherent problem of testing single cells (Renwick and Ogilvie, 2007; Spits and Sermon, 2009; De Rycke, 2010).

Overall PGD for single gene disorders have shown the highest pregnancy rate (23% per OR, 29% per ET) compared with all other indications. Testing for HLA compatibility on its own or with specific diagnosis for another disorder apart from the HBB gene showed the greatest difference between the pregnancy rate per OR (16%) and pregnancy rate per ET (29%). This reflects the small chance of identifying an HLA match using PGD based on genetic likelihood.

Sexing for X-linked disease

The first PGD cycles, reported by Handyside *et al.* (1990), were carried out in families with X-linked diseases. Initially, PCR was used to amplify a specific repeat on the Y chromosome, but soon FISH became the standard method for gender determination as it is a more robust and accurate method for this. In data I–III, 15% of cycles were still PCR-based, but this number decreased from data IV onwards till data VIII, when FISH became the only method used.

A total number of 1167 cycles for sexing for X-linked disease were reported during the first 10 years of data collection leading to a clinical pregnancy rate of 26% per transfer. From data IV onwards, the number of cycles for sexing for X-linked disease has remained stable (Fig. 3B) although the total number of cycles using a specific diagnosis for an X-linked disease gradually increased due to the fact that more specific tests have become available (Fig. 10). Specific DNA diagnosis has two important advantages: firstly, healthy male embryos are not discarded and secondly, female carriers can be identified and excluded from transfer or not, according to the patients' wishes and the centre's policy. The most common indications in the sexing only cycles were for DMD and haemophilia A and B. When the results for these diseases are compared between specific X-linked cycles and sexing only cycles, there are few differences. The specific X-linked cycles rely mainly on ICSI as the fertilization method to avoid contamination during PCR, whereas in sexing only cycles, IVF is still applied in 26% of cycles. Another difference is that because of the more stringent genetic selection, fewer genetically transferable embryos are available in the sexing only cycles, which may limit the choice of embryos to transfer with a good morphology. Nevertheless, the clinical pregnancy rates per transfer for sexing only cycles (26%) and specific diagnosis (26% for DMD and 27% for haemophilia) are very similar.

Preimplantation genetic screening

The application of embryo biopsy and preimplantation genetic testing to identify numerical chromosome errors in embryos, known as PGS, was first reported by the PGD Consortium in data collections I–III for 787 cycles when it constituted 36% of all reported PGD. At this time, it was applied to embryos from women of advanced age (AMA) and those who had suffered repeated implantation failure (RIF) and repeated miscarriage (RM) (Fig. 11). The application of PGS continued to grow in subsequent reports. For the first time since the data collection began, the number of PGS cycles dropped in data collection X to 3753 (63% of all PGD cycles). AMA has always been the predominant indication with almost 50% of all PGS cycles being performed for this reason, meaning that the mean age of women undergoing PGS was ~38 years, higher than the mean age of women having PGD for single gene disorders or chromosomal rearrangements. Whilst the primary indications have continued to be AMA, RIF and RM, in data collection V severe male factor was added as an indication. In subsequent data collections it was realized that these indications were often an over-simplification of the patients' true aetiology, and participating

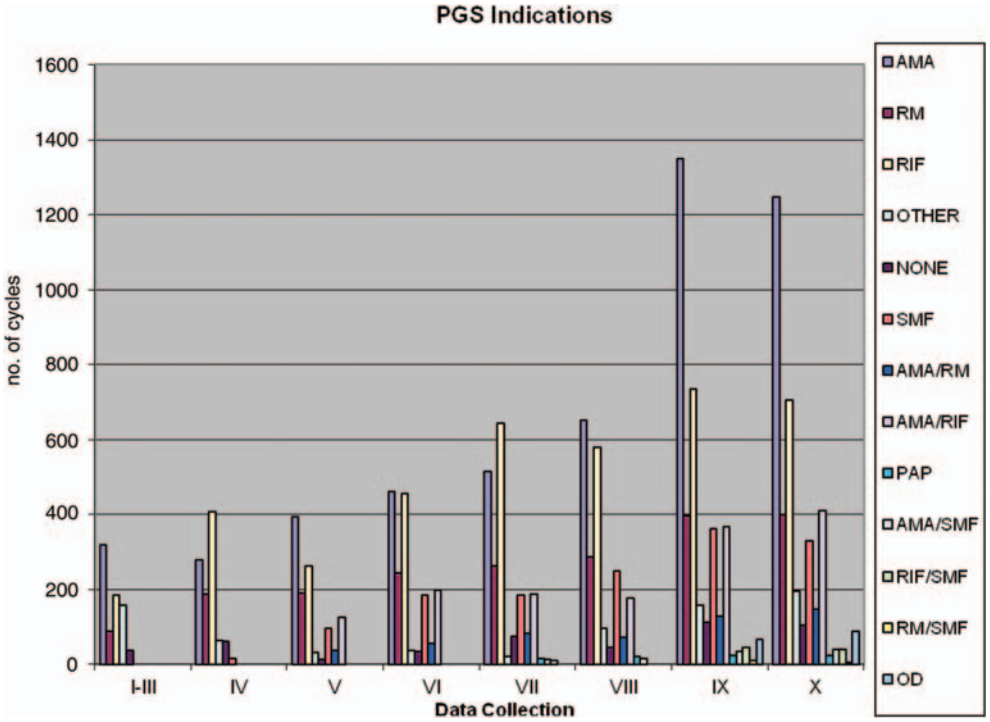

Figure 11 The evolution of PGS indications over the 10 sets of data. AMA, advanced maternal age; RM, repeated miscarriage; RIF, repeated implantation failure; SMF, severe male factor; PAP, previous abnormal pregnancy; OD, oocyte donation.

centres were able to enter data where the patient had multiple indications, e.g. AMA + RM.

In total, 16 806 cycles of PGS have been reported to the Consortium during the first 10 years of data collection. During this time 89 373 embryos have been successfully biopsied and 21 543 embryos have been transferred to give a clinical pregnancy rate per transfer of 27%. Of the biopsied embryos, only 29 278 (33%) were diagnosed as genetically suitable for transfer, which meant that 25% of patients had no suitable embryos and no transfer took place.

Chromosomal enumeration in PGS cycles has predominantly been achieved using fluorescence *in situ* hybridization (FISH). Data on which chromosomes were tested for using FISH have not been collected by the Consortium, but a search of the literature shows that most centres test for ~8–10 chromosomes usually including at least chromosomes X, Y, 13, 16, 18, 21 and 22. Other chromosomes that may be included are 4, 14, 15 and 17.

It seems intuitive that identifying embryos that harbour lethal aneuploidies and selecting against them for transfer and, conversely, selecting embryos that are apparently euploid for a number of chromosomes for transfer should have a positive effect on cycle outcomes. The application of PGS has become controversial in recent years after the publication of eleven randomized controlled trials that failed to demonstrate a benefit to pregnancy rates and live birth outcomes (Staessen *et al.*, 2004, 2008; Stevens *et al.*, 2004; Debrock *et al.*, 2010; Mastenbroek *et al.*, 2007; Blockeel *et al.*, 2008; Hardarson *et al.*, 2008; Jansen *et al.*, 2008; Mersereau *et al.*, 2008; Meyer *et al.*, 2009; Schoolcraft *et al.*, 2009). Consequently many centres reduced or stopped PGS treatment and this probably explains the drop in PGS cycles reported to the Consortium in data collection X.

Many experts agree that possible reasons for the failure of PGS to show a benefit include the well-documented chromosomal mosaicism which exists in early human embryos, meaning that the biopsied cell may not be truly representative of the rest of the embryo (Harper *et al.*, 1995) Additionally, it is most likely that, because FISH is limited to the detection of only about one-third of the chromosomes, embryos diagnosed as euploid by FISH may harbour aneuploidies of other chromosomes that were not tested for (Voullaire *et al.*, 2000). This has in fact been demonstrated using complete molecular karyotyping methods such as comparative genomic hybridization (CGH) where all chromosomes can be analysed in a single cell (Voullaire *et al.*, 2000; Wells and Delhanty, 2000). Array-based CGH is now being trialled by a number of PGD centres around the world and there is significant interest in whether this approach will result in improved outcomes for PGS. The ESHRE PGS task force has completed a pilot on the feasibility of using array-CGH and polar body biopsy (Geraedts *et al.*, 2011; Magli *et al.*, 2011), and a multi-centre randomized controlled trial has been set up.

Social sexing

When social sexing data were first submitted to the Consortium, there was an ethical debate about whether these data should be reported as it is banned in the EU and other countries. It was agreed that these data should be included.

There have been 671 cycles for social sexing reported during the first 10 years of the ESHRE PGD Consortium data collection. In data I–II, there were no social sexing cases reported. From data collections III-X, 671 cycles were started, and 655 went on to PGD testing, with 9329 oocytes retrieved, 4285 embryos biopsied and 3709 embryos diagnosed. Diagnosis of embryos was performed by both PCR and FISH, with PCR-based testing dominating the early data collections and FISH-based testing dominating the later data collections. A total of 492 cycles ended in a transfer of embryos, resulting in 197 positive hCG tests and 143 cycles with a positive fetal heart beat. Overall, this resulted in a 40% pregnancy rate per embryo transfer, and a 29% clinical pregnancy rate per transfer.

It was not until data collection VII that the 'desired gender' was included in the data collected. Looking at data collections VII–X, more than 66% of the couples desired a male (162/246). This may say something about social sexing and the potential for skewing sex ratios one way or the other. However, as noted in data collections VII–X, the vast majority of the cases for social sexing originated from one centre in the USA where MicroSort sperm selection was available to the general public. As noted in data collection VII 'Although the initial requests for males and females is reported to be 50:50, those requesting a female may opt for MicroSort® sperm selection only as the average sort purity is >90% for X-bearing sperm, compared with an average sort purity of just over 70% for Y-bearing sperm (Schulman and Karabinus, 2005). This disparity in sort percentage is the most likely cause for the uneven distribution of social sexing PGD cycles' (Harper *et al.*, 2008).

Social sexing remains illegal in most countries (i.e. Australia, China, Europe), however, preferential selection of one sex over another most likely occurs and is not reported. Most, if not all, chromosome screening panels used for PGS contain probes for the sex chromosomes and, therefore, many couples may be aware of the sex of the embryos ahead of transfer and may select or lean towards one gender or the other when choosing embryos for transfer. The data presented above barely scratches the surface; the true nature and depth of social sexing will probably never be known.

Pregnancies and babies

The PGD Consortium has given special emphasis to the outcome of pregnancies achieved following PGD and the follow-up of these babies.

In data I, 82 pregnancies and 110 fetal sacs were reported. By data X, there were 1516 pregnancies and 1609 fetal sacs reported. Cumulatively, in the 10 data collections, 6458 fetal sacs were observed in a total of 6111 pregnancies. These 5187 clinical pregnancies gave rise to 744 pregnancy complications and resultant 4140 deliveries, and 5135 newborns (singletons: 3182, twins: 921, triplets: 37).

Overall, 48% of the deliveries were by Caesarean section (1986/4140). In 483 cases the method of delivery was not known. In data collections I–III, 39% of the deliveries were done by Caesarean section. In the following data collections Caesarean section was performed more often than vaginal delivery (54.2% in data collections IV–X).

Cumulatively, of the 5187 cycles ending in a pregnancy with a positive heartbeat, follow-up data on 4994 pregnancies were reported. Of these pregnancies, a total of 854 (17%) pregnancies resulted in first- and second-trimester losses (739 and 115 losses, respectively).

In data collections I–VIII, of 2604 clinical pregnancies, 12% resulted in first-trimester and second-trimester loss. In data collections IX and X, these values were higher, being 23 and 19%, respectively. Data on 4047 newborn characteristics were reported.

Confirmation of PGD cases in cumulative data I–X were performed prenatally in 2462 cases (2014 FISH and 448 PCR cases) and postnatally in 2049 cases (1727 FISH and 322 PCR cases). At birth, a total of 157 malformations were detected in 4021 newborns. Of these, 84 were major malformations (2%). Neonatal complications were reported in 402 of 3917 cases (10%). These cumulative data again confirm that pregnancies and babies born after PGD are similar to the pregnancies obtained and babies born after ICSI treatment (Bonduelle *et al.*, 2002).

Misdiagnosis

In most forms of laboratory testing, a misdiagnosis results from some sort of technical or systemic failure. The estimates of misdiagnosis after PGD are complex. Misdiagnosis can be underestimated because many transferred embryos do not result in a pregnancy, some spontaneously abort and others that are mistakenly predicted to be affected are discarded and results not confirmed. Conversely, misdiagnoses can be overestimated as pregnancies are always assumed to result from the embryo or embryos that were transferred. Many patients, particularly those having PGD for single gene disorders, will be fertile and there is the possibility that a pregnancy could result from a natural conception rather than an embryo that was transferred. Despite these limitations, the PGD Consortium has always encouraged reporting of misdiagnoses by making the data collection and publication anonymous.

Misdiagnoses after PGD have been described as taking two different forms, that is, adverse and benign (Wilton *et al.*, 2009). Adverse misdiagnoses are those that result in a severe adverse outcome for the patient, such as the birth of an affected child or the termination of an affected pregnancy. Benign misdiagnoses are those where the outcome is less severe, such as birth of a carrier of an autosomal recessive condition when the embryo was predicted to be unaffected.

In addition to the paper by Wilton *et al.* on misdiagnosis in PGD, the ESHRE PGD Consortium recently published an updated set of Best Practice Guidelines in PGD (Harton *et al.*, 2011a, b, c, d). These guidelines include specific recommendations for best practice in the set-up of a PGD program, amplification-based and FISH-based PGD/PGS and embryo biopsy and freezing as it relates to PGD. In addition, the Consortium is currently analysing data from a large set of embryo follow-up studies undertaken at a number of participating centres. These data will include follow-up data on amplification-based PGD for single gene defects and FISH-based PGD for chromosome rearrangements and aneuploidy screening. These publications will be a useful resource for all professionals in the IVF/PGD field.

Misdiagnosis after FISH testing

In data collections I–X there have been 21 829 cycles performed where FISH was used to perform the diagnosis. These cycles consisted of testing for chromosomal rearrangements, PGS, sexing for X-linked disease and social gender selection, and resulted in 15 981 embryo

transfer procedures. A total of 16 misdiagnoses have been reported giving a misdiagnosis rate after embryo transfer of 0.1%. For chromosomal rearrangements the reported misdiagnosis rate is 3/2731 transfers or 0.1% [the misdiagnosis after PGD for a chromosomal rearrangement reported in Wilton *et al*., 2009 is incorrectly reported as 46,XY,der(15)t(13;15)(q25.1;q26.3)pat. It should read 46,XY,der(15)t(3;15)(q25.1;q26.3)]. For PGS it is 10/12071 (0.08%), for X-linked disease testing it is 4/825 (0.5%) and for social gender selection it is 1/354 (0.3%).

There are many causes of misdiagnoses that are specific to single cell preimplantation FISH testing. There are limitations of the technology as well as biological factors relating to the embryo. Technical limitations include overlapping FISH signals, hybridization failure, non-specific hybridization and the difficulty of interpreting closely adjacent signals. Strategies to minimize the impact of these limitations are discussed in detail in Wilton *et al*. (2009). The inherent complexities of the biology of the embryo also probably contribute to misdiagnosis after FISH. It is well known that preimplantation embryos can be chromosomally mosaic and that different cells may have a different chromosomal constitution. This could lead to adverse misdiagnoses when some cells are aneuploid and others are euploid.

PGD testing for chromosomal rearrangements using FISH is affected by additional difficulties. An individualized panel of FISH probes must be devised to detect all possible segregants of the translocation. At least one misdiagnosis reported to the Consortium occurred when the FISH protocol was unable to detect some unbalanced forms of the translocation (Wilton *et al*., 2009).

Misdiagnosis after PCR testing

Allele dropout (ADO) and contamination are inherent pitfalls of single cell PCR and each can lead to an adverse misdiagnosis. Strategies to minimize the impact of these limitations are discussed in detail in Wilton *et al*. (2009).

A total of 12 adverse misdiagnoses were reported: 10 for single gene disorders and 2 following PCR-based sexing for X-linked disease.

For single gene disorders, in data collections I-X, 4534 cycles used PCR-based assays to perform diagnosis, resulting in 3727 embryo transfer procedures. The 10 reported adverse misdiagnoses give a misdiagnosis rate of 10/3727 (0.27%) after embryo transfer. Of the misdiagnoses, four of the indications were autosomal dominant, five autosomal recessive and one sex-linked dominant. Two of the misdiagnoses were identified following birth and eight were detected by prenatal diagnosis with four elective terminations subsequently undertaken, the other four pregnancies were delivered.

A total of 65 PGD cycles, mainly undertaken in data sets I–IV, used PCR to determine sex for X-linked disease. These cycles resulted in 55 embryo transfers from which 2 misdiagnoses arose (data sets III and IV), giving a 3.6% misdiagnosis rate per transfer procedure. Both misdiagnoses were detected by prenatal diagnosis with one elective termination performed (Wilton *et al*., 2009). From data set VIII, determination of sex transferred wholly to FISH-based analysis, being technically more robust than a simple PCR assay.

Subsequent investigations of the 12 misdiagnoses concluded the cause of misdiagnosis as contamination in 2 cases and ADO in 5 cases, which reflects the technical limitations of single cell analysis. A further case was attributed to a laboratory sample switch and two cases to incorrect feasibility segregation analysis, which would have been avoided by quality management systems; four remain undetermined (Wilton *et al*., 2009).

The majority of the misdiagnoses (9/12) occurred within the first 5 years I–V (2 were reported in VII but had occurred earlier), and after such time PCR assays became technically advanced with the introduction of fluorescent PCR and amplification of multiple loci. For the last 2 years (IX–X) there were no PCR misdiagnoses reported in 2061 PGD cycles for single gene disorders. These advances mean that PCR for disease-associated alleles in conjunction with sex determination is now the preferred approach for identifying sex-linked specific disorders as unaffected males can be identified.

Discussion

The Consortium data represent a unique collection. From these data, the evolution of PGD and PGS can be seen, such as the introduction of laser-assisted biopsy, an increase in polar body and trophectoderm biopsy, new strategies, methodologies and technologies for diagnosis, including most recently arrays, and the more frequent use of freezing biopsied embryos. The Consortium data reports represent a valuable resource for information about the practice of PGD.

Statistical analysis of data

The merging of 29 336 cycle data from data I–X has just been completed and is in the process of being analysed by a statistician using a multivariate analysis in order to evaluate success rates according to different factors such as indication, maternal age, ART methods and results, etc. These data will be published soon.

Future data collections

As we look back on the first 10 years of data collection, we can see that a large amount of work has gone on in the PGD field over these last few years, and a large amount of change has occurred during this time. Changing testing methodologies and biopsy methods (AT to laser), the increase and fall in cycles for aneuploidy screening, all show that the field has been and will continue to evolve. What is not obvious from these papers is the amount of work that goes into submitting data, and the amount of work that goes into cleaning and presenting the data in a meaningful way. We are hopeful that the data collected and published have been a help to clinics, clinicians and perhaps patients as PGD cycles continue to grow worldwide.

Looking forwards, significant changes are underway to make data collection and analysis simpler and more straightforward. A new, online data submission system is being developed at this moment and should be operational later this year. This new system will allow for real-time entry of data rather than one data entry exercise at the end of each collection period. The online database will be searchable, should aid in data analysis and publication, and should allow for more ways to access and manipulate the massive amounts of data submitted each year. We are hopeful that this significant change in data collection will lead to a much more useful and open database for the next 10 years and beyond.

The Consortium will continue to proactively monitor new applications of PGD and new technologies that could be applied to PGD. Of immediate interest is the use of BAC microarrays to detect aneuploidy

of all chromosomes in PGS (Fragouli et al., 2011; Gutierréz-Mateo et al., 2011) and segmental errors in PGD for chromosomal rearrangements (Alfarawati et al., 2011; Fiorentino et al., 2011). Other technologies on the horizon include SNP arrays to detect single gene disorders, without the need for specific, individualized test development in many cases, and next generation sequencing which, if successfully applied to biopsies from embryos, could enable differentiation between balanced and completely unaffected forms of reciprocal and Robertsonian translocations.

Authors' roles

All authors were involved in the final editing and have been involved in data analysis over the last 10 years. All have been authors on previous data collection papers. J.C.H. wrote the outline of paper, the sections on chromosome abnormalities and IVF/ICSI, the abstract, etc. L.W. wrote the sections on PGS, misdiagnosis and the future of PGD. J.T.-S. wrote the section on single gene defects. V.G. was involved with data collection and prepared some of the figures. C.M. was involved in data collection, wrote sections on data collection and analysis, and designed filemaker Pro database. S.S. wrote the sections on single gene disorders and indications. T.P.B. wrote the sections on biopsy and pregnancies and babies. P.R. wrote the sections on single gene defects and misdiagnosis. M.D.R. wrote the section on sexing for X-linked disease. J.G. contributed towards the introduction and discussion. G.H. contributed towards the introduction, discussion, social sexing and single gene defects.

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