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ESHRE Preimplantation Genetic Diagnosis (PGD) Consortium: preliminary assessment of data from January 1997 to September 1998

ESHRE PGD Consortium Steering Committee*

The first clinical application of preimplantation genetic diagnosis (PGD) was reported almost a decade ago. Since then, the range of genetic defects that can be detected at single cell level has increased dramatically. At the 13th Annual Meeting of ESHRE in Edinburgh in 1997, a PGD Consortium was formed to undertake the first systematic and long-term study of the efficacy and clinical outcome of PGD. We report here the first data collection covering the period of January 1997 to September 1998. Referral data on 323 couples have been collected for a variety of monogenic and chromosomal disorders, providing information about which patients, at risk for which genetic diseases, are interested in PGD. Data were collected on 392 PGD cycles, resulting in 302 embryo transfers and 66 clinical pregnancies. Because of the importance of follow-up of the children born after PGD, participating centres were asked to contribute data on the pregnancies achieved and the children born after PGD since the start of their PGD programme. Data on 82 pregnancies and 110 fetal sacs were collected, and information was available on 79 children. Finally, biopsy, fluorescence in-situ hybridization and polymerase chain reaction protocols were collected, clearly showing that no consensus exists on technical aspects such as which culture medium to use, and emphasizing the role the PGD Consortium could play in setting up guidelines for good laboratory practice. In conclusion, it is clear that the effort of gathering data on PGD cycles is worthwhile and will be continued in the future, preferably using electronic data collection.

Key words: fluorescence in-situ hybridization/in-vitro fertilization/polymerase chain reaction/preimplantation genetic diagnosis

*The ESHRE PGD Consortium Steering Committee:
Joep Geraedts, Department of Molecular Cell Biology and Genetics, University of Maastricht, J. Bechlaan 113, Maastricht, The Netherlands, e-mail joep.geraedts@gen.unimaas.nl.
Alan Handyside (Chair, SIG in Reproductive Genetics) School of Biology, University of Leeds, Leeds, UK,
email a.h.handyside@bmb.leeds.ac.uk.
Joyce Harper, Dept of Obstetrics and Gynaecology, University College London, 86–96 Chenies Mews, London WC1E 6HX, UK, e-mail joyce.harper@ucl.ac.uk.
Inge Liebaers, Centre for Medical Genetics, Dutch-speaking Brussels Free University, Laarbeeklaan 101, 1090 Brussels, Belgium, e-mail lgenlsi@az.vub.ac.be.
Karen Sermon, Centre for Medical Genetics, Dutch-speaking Brussels Free University, Laarbeeklaan 101, 1090 Brussels, Belgium, e-mail lgensnk@az.vub.ac.be.
Catherine Staessen, Centre for Reproductive Medicine, Dutch-speaking Brussels Free University, Laarbeeklaan 101, 1090 Brussels, Belgium, e-mail lriasnc@az.vub.ac.be.
Alan Thornhill, Department of Obstetrics and Gynaecology, 7th Floor North Wing, St Thomas's Hospital, Lambeth Palace Road, SE1 7EH London, UK, e-mail a.thornhill@umds.ac.uk.
Anna Vanderfaeillie, Centre for Medical Genetics, Dutch-speaking Brussels Free University, Laarbeeklaan 101, 1090 Brussels, Belgium, e-mail lgensnk@az.vub.ac.be.
Stephane Viville, BP 63, IGBMC, 1, Rue Laurent Fries, 67404 Illkirch-Strassbourg, France, e-mail viville@titus.u-strasbg.fr.

Background

The first clinical application of preimplantation genetic diagnosis (PGD) using in vitro fertilization (IVF), cleavage stage embryo biopsy and single cell genetic analysis to identify unaffected female embryos in a series of couples at risk of X-linked disease, was reported almost a decade ago (Handyside *et al.*, 1990). Since then the number of centres offering PGD has increased slowly, but steadily, mainly because of the difficulties of single cell analysis and the need to combine expertise in assisted reproduction and molecular genetics. In contrast, the range of genetic defects causing disease that can be detected at the single cell level has increased dramatically and now includes most common autosomal dominant and recessive single gene defects as well as structural chromosome abnormalities and aneuploidy screening using various polymerase chain reaction (PCR) or fluorescence in-situ hybridization (FISH) methods (Handyside and Delhanty, 1997; Lissens and Sermon, 1997).

During the First International Symposium on Preimplantation Genetics in Chicago in 1990, the International Working Group for Preimplantation Genetics was established, chaired and organized by Verlinsky and colleagues. The IWG meets annually to review developments and reports on progress world-wide (Verlinsky, 1999). In addition, while the number of centres remained manageably low, it was possible to report detailed summaries of diagnostic methods, cycles, pregnancies and births (Harper and Handyside, 1994; Harper, 1996). As the number of centres world-wide now exceeds 40 and since there has never been any systematic registration of PGD cycles, this has now become impossible. At the 13th Annual Meeting of ESHRE in Edinburgh in 1997, therefore, members of the Special Interest Group in Reproductive Genetics decided to form a consortium of centres actively involved in PGD, the ESHRE Preimplantation Genetic Diagnosis (PGD) Consortium, to undertake the first systematic and long term study of the efficacy and clinical outcome of PGD. Data collection and organization of the Consortium would be by a Steering

Committee who would report to the Consortium members on a regular basis and hold a meeting each year at the ESHRE Annual Meeting.

Membership and aims of the ESHRE PGD Consortium

Membership of the Consortium is open to all centres actively involved in the clinical application of PGD. (Centres at a preclinical stage of PGD development or with a general interest in the area are encouraged to become members of the Special Interest Group in Reproductive Genetics.) As an ESHRE sponsored activity, the Consortium members are mainly based in Europe though several centres in Australia and the US have already joined. However, it should be emphasized that it is not the aim of the Consortium to comprehensively survey PGD on a global basis. Rather, the overall aim is to collate detailed data on a long-term basis from a number of centres to increase the statistical significance of any outcome analysis and to promote exchange of information between active clinics. Members therefore need to be committed to the work involved in this process and be prepared to provide the resources to gather relevant data, for example, in following up children after birth. In return for this effort, members have access to the anonymous data, protocols and publications and can participate in regular ESHRE sponsored meetings on progress in clinical PGD within the Consortium.

The main aims of the Consortium are summarized as follows: To survey the availability of PGD for different conditions facilitating cross-referral of patients. To collect prospectively and retrospectively data on the accuracy, reliability and effectiveness of PGD. To initiate follow-up studies of pregnancies and children born. To produce guidelines and recommended PGD protocols to promote best practice. To formulate a consensus on the use of PGD.

Data collection

Each centre participating in the Consortium is assigned a centre code following completion of a centre registration form. This can be obtained from ESHRE Central Office or printed from the ESHRE web site (www.eshre.com). A centre pack is then sent to the centres containing master copies of eight forms for duplication as necessary. A brief description of the information requested on these forms is as follows:

Form (1)

Clinical referral data: Patient history (pregnancies, miscarriages, termination of pregnancies), indication for PGD. Reason for PGD (e.g. objection to abortion, concurrent infertility). The basis of the centre's decision to offer PGD (or not), and the reasons for patient's declining PGD.

Form (2)

Cycle data: Stimulation, embryology and biopsy data. Number of cumulus oocyte complexes, oocytes inseminated, normally fertilized and biopsied. Results of genetic analysis, transfer data and pregnancy outcome.

Form (3)

Pregnancy data: Ultrasound observations, prenatal diagnosis and outcome, pregnancy evolution and any complications, and mode of delivery.

Form (4)

Follow-up data: Neonatal parameters, congenital defects and long term developmental follow-up of children.

In addition, on an optional and voluntary basis, forms (5)–(8) are provided for supplying information on embryo biopsy, PCR and FISH protocols and also any relevant publications. At present, hard copies of completed forms are mailed to ESHRE Central Office for collation by members of the Steering Committee. It is hoped that submission of data can soon be via the Internet initially in spreadsheet form by e-mail but later directly through a dedicated web site accessed from the main ESHRE site and suitably password protected.

Twenty-five centres had registered by the time of the first annual Consortium meeting in Gotëborg in June 1998. At that meeting, it was decided to collect data retrospectively for cycles started in 1997 and the Steering Committee undertook the responsibility of preparing a report based on these data for the meeting the following year. More recently, data collection has been extended to 30 September 1998. These data are reported here. In future, it is hoped to extend this further both prospectively on an ongoing basis but also retrospectively to include all of the PGD cycles within the Consortium. By June, 1999, 16 centres had provided most of the requested data but many had not provided data in all of the areas requested, e.g. several centres had provided data on the PGD cycles, but not the referral data of their patients. As the Consortium is an active collaboration, membership of those centres which have failed to provide any data will be deemed to have lapsed subject to the statutes of the PGD Consortium installed at the last ESHRE meeting in Tours in June, 1999. In those cases where only partial information has been provided, centres will be encouraged to complete their submissions and the data accumulated as it becomes available. To some extent, this failure has been caused by ambiguities in the original forms and misunderstanding of the precise information requested and the problem may be eliminated as it becomes clearer what data are needed.

Preliminary assessment of data for January 1997 to September 1998

As the information obtained from the 16 centres actively participating in the Consortium is not yet complete, we are able only to present a preliminary assessment of the data. However, some clear features are already emerging that may have important practical implications for the future development of clinical PGD.

Form (1): Referrals

A total of 323 referral sheets have been collected over this 2 year period for a wide variety of monogenic (Table I) and chromosomal disorders (Table II) (a number of which are not technically feasible at the present time). This is undoubtedly

Table I. Indication for referral for monogenic diseases

Autosomal recessive	No. of referrals	Autosomal dominant	No. of referrals	X-linked	No. of referrals
CF (13 mutations, 8 CBAVD)	38	Myotonic dystrophy	20	Duchenne and Becker's muscular dystrophy	29
β-Thalassaemia	15	Huntington's disease	12	Haemophilia	14
Spinal muscular atrophy	8	Charcot–Marie–Tooth disease	11	Fragile-X syndrome	13
Tay–Sachs disease	8	Neurofibromatosis type 1	2	Mental retardation	7
Rh isoimmunization	3	Marfan syndrome	1	Wiskott–Aldrich syndrome	5
Gaucher disease	2	Osteogenesis imperfecta	1	Charcot–Marie–Tooth	3
Sandhoff disease	2			Coffin–Lowry syndrome	2
Sickle cell anaemia	2			Granulomatous disease	2
Adrenoleukodystrophy	1			Hydrocephalus	2
Dystonia	1			FG syndrome	2
Factor V Leiden	1			Agammaglobulinaemia	1
Familial hypophosphataemia	1			Anderson–Fabry disease	1
Fanconi anaemia	1			Ataxia	1
Friedreich ataxia	1			Autism	1
Medium chain AcylCoA deficiency	1			Barth syndrome	1
Methylmalonic acidaemia	1			Goltz syndrome	1
Ornithine transcarbamylase deficiency	1			Hunter syndrome	1
Pyruvate dehydrogenase deficiency	1			Hypohydrotic ectodermal dysplasia	1
Polycystic kidney disease	1			Incontinentia pigmenti	1
				Kennedy disease	1
				Lowe syndrome	1
				Pelizaeus–Merzbacher syndrome	1
				Proliferative disease	1
				Retinitis pigmentosa	1
				Retinoschisis	1
				Vit-D resistant rickets	1

Other referrals: mitochondrial encephalopathy ($n = 1$), none/unknown ($n = 2$).

Table II. Referrals for chromosomal disorders

Structural chromosomal aberrations:	
Reciprocal translocation	36
Inversion	1
Deletion	3
Aneuploidy risk:	
Aneuploidy risk	27
Klinefelter syndrome	9
Sex chromosomal mosaicism	4
Male meiotic abnormalities	3

Table III. Reproductive history of the patients requesting PGD

	0	1	2	3	4	5	6	Unknown
Pregnancies	105	53	50	41	31	17	12	14
Pregnancies >28 weeks	186	74	34	8	2	1	1	14
Healthy children	247	42	8	4	0	1	0	18
Affected children	214	75	16	1	0	1	0	16
Stillborn	291	7	0	0	0	1	0	19
Spontaneous abortions	229	27	22	4	9	6	5	16
Termination of pregnancies	208	43	29	12	8	1	1	13

Table IV. Reasons for PGD. Values in parentheses are percentages

Genetic risk and previous TOP	92/323 (28)
Genetic risk and objection to TOP	207/323 (64)
Genetic risk and sub-or infertility	103/323 (32)
Genetic risk and sterilization	2/323 (0.6)
Age related aneuploidy	19/323 (6)
Other	15/323 (5)
Unknown	5/323 (1.5)

an underestimate of the overall demand for PGD, however, as many prospective referrals are rejected at a preliminary stage on the basis that specific tests are not available and the details of the couples are not recorded. The group indicated as 'aneuploidy risk' consists of patients with previous trisomy or triploidy pregnancies, age related aneuploidy or recurrent abortion. In these cases neither of the two partners had a constitutional chromosomal abnormality. Interestingly, in no less than four cases, there was a combination of two indications: Klinefelter syndrome/translocation (11;22), translocation (2; 4)/translocation (11;22), translocation (1;3)/sickle cell anaemia and fragile-X syndrome/Duchenne muscular dystrophy.

The reproductive history of the couples requesting PGD is presented in Table III as the number of couples having 0, 1 or more previous pregnancies etc. For example, 105 couples had never had a previous pregnancy, while 53 couples had had one pregnancy. It is clear that most couples have had one or more pregnancies and only a minority have had healthy children; whereas most had personal experience of affected children and termination of pregnancy (TOP) after prenatal diagnosis. The mean maternal age was 33.7 ($n = 283$, range 23–54 years), while the mean paternal age was 35.4 ($n = 223$, range 24–47 years).

Table IV summarizes the reasons why the patients requested PGD. As expected, the most important reasons are previous TOP, objections to TOP and concurrent sub- or infertility. Table V shows the decisions reached by the centres in accepting or rejecting the request for PGD. The most frequent reason for rejecting a PGD is the technical feasibility of the test,

Table V. Centre decision

	Yes	No	Undecided	Unknown
Suitable for IVF	305	18	–	–
Technically possible	266	55	–	2
Ethically acceptable	295	26	–	2
PGD accepted	249	71	2	2

Table VI. Reasons for declining

Low success rate	5
Cost	8
Inaccuracy	0
Spontaneous pregnancy	5
Inconvenience	9
Age related risks	1
Donor spermatozoa needed	1
Donor oocytes needed	1
Funding refused	1
Hormonal treatment	1
Postponed	5
Lost for follow-up	11
PND instead of PGD	2
Other centre	4
Undecided	8
Unknown	2

followed by the ethical acceptability. Thirty-six couples declined for a variety of reasons (Table VI).

Form (2): Cycles

Tables VII–X summarize the PGD cycle data from 16 centres and 392 cycles.

The largest number of cycles for a specific application were for age-related aneuploidy ($n = 116$) at four centres using multicolour FISH. The next most frequent was identification of sex in X-linked disease ($n = 112$) by either multicolour FISH ($n = 104$) or PCR methods ($n = 8$). Fifty-one cycles were performed for cystic fibrosis (CF) for patients carrying various mutations, but mainly DF508. This included cycles from one centre still using IVF rather than intracytoplasmic sperm injection (ICSI) which is normal practice to avoid possible sperm DNA contamination. Forty PGD cycles were for chromosome abnormalities, mainly translocations, again using multicolour FISH. Other autosomal recessive diseases with several reported cycles were β-thalassaemia, Rhesus-isoimmunization, spinal muscular atrophy, Tay–Sachs disease, adrenogenital syndrome, as well as one cycle for sickle cell anaemia. A large number of cycles were also performed for autosomal dominant diseases: 31 cycles for myotonic dystrophy, 10 for Huntington's chorea, and several cycles for Marfan's syndrome, osteogenesis imperfecta and Charcot–Marie–Tooth disease type 1A. For X-linked diseases, specific PCR diagnoses were performed for Duchenne's and Becker's muscular dystrophy, as well as several cycles for fragile-X.

Of the 392 cycles reported for the period January 1st, 1997 to September 30th, 1998, 26 (7%) were cancelled, mainly due to a poor response, and 366 cycles reached oocyte retrieval. In most cases, acid Tyrode's was used for zona drilling, while three centres used laser drilling and two used mechanical means. All centres performed the biopsy at cleavage stages and used aspiration to remove the blastomeres. A total of 4837 cumulus oocyte complexes were retrieved (average 13.2 oocytes per retrieval) and 4473 oocytes were inseminated resulting in 3046 (68%) normally fertilized embryos.

Of the normally fertilized oocytes 2395 (77%) embryos were biopsied (average 6.5 embryos biopsied per oocyte retrieval), 2330 (97%) successfully. Single cell genetic analysis and diagnosis was successful for 2086 embryos (90% of those successfully biopsied, average of 5.7 embryos per oocyte retrieval). Overall, therefore, a diagnosis was achieved in 43% of oocytes retrieved. Of those embryos diagnosed, 919 (44%) embryos were suitable for transfer, i.e. not at risk of genetic disease and 659 (32%) were transferred (range: 1–9 embryos per transfer, most in the range 1–3). Eighty-six cycles did not result in a transfer (22% of cycles started). One embryo was transferred in 72 cycles (9 clinical pregnancies), two embryos in 116 cycles (17 clinical pregnancies), three embryos in 95 cycles (32 clinical pregnancies), four embryos in 17 cycles (six clinical pregnancies) and five or more (maximum nine) in six cycles (three clinical pregnancies). These results are summarized in Table X. In one cycle, sperm selection was used and in one cycle the embryos were frozen due to equipment failure which prevented the diagnosis taking place. In two cycles undiagnosed embryos were transferred at the request of the patient. In total pregnancies were detected in 83 cycles from a total of 306 embryo transfers by raised serum human chorionic gonadotrophin (HCG) and confirmed by the presence of a fetal heart beat using ultrasonography in 67. Clinical pregnancy rates at the fetal heart stage were therefore 22% per embryo transfer, 18% per oocyte retrieval or 17% per cycle. Two cases have been lost to follow-up. One hundred and thirty-seven of the remaining embryos were cryopreserved but no frozen embryo cycles or transfers were reported.

Form (3): Pregnancies

Because the Consortium is especially interested in the outcome of the pregnancy and the babies born after PGD, the centres were asked to report all pregnancy and baby data since the start of their PGD programme, thus the cycle data and pregnancy data do not entirely concern the same cycles. Data on 82 pregnancies from 12 PGD centres were reported (Table XI). Most pregnancies were reported in cycles employing FISH protocols for diagnosis (54/82; 66%) while the remainder involved PCR analysis. The mean age of the pregnant women for which data are available ($n = 44$) was 32 years 4 months ± 4 years. A detailed analysis of pregnancy outcome is presented in Table XII. The initial number of pregnancies was 82, including a total of 110 fetal sacs: 58 singleton pregnancies (71%), 21 twin pregnancies (25%), two triplets (2%) and one quadruplet pregnancy (1%). Eleven fetal sacs were lost due to a first-trimester miscarriage. During the second trimester, one miscarriage (13 weeks) and one stillbirth (24 weeks) occurred. Reduction of fetal sacs was performed in the two triplets and in the quadruplet pregnancy. One pregnancy had to be terminated because of a misdiagnosis (see also below).

Information on ultrasound follow-up was available for 96

Table VII. Summary of PGD cycles using PCR based genetic analysis (16 centres)

	Autosomal recessive							Autosomal dominant					Sex-linked			Total	
	CF	β-Thal	Rh-inc	SMATS		AGS	SS	DM	HD	MS	OI	CMT	Sex	FRAXA	DMD		
Total cycles	51	6	4	3	2	2	1	31	10	2	1	2	8	5	4	132	
Cancelled	6	0	1	1	0	0	0	4	0	0	0	0	0	0	0	12	(9%)
Cycles to OR	45	6	3	2	2	2	1	27	10	2	1	2	8	5	4	120	
IVF	4	0	1	0	1	0	0	0	0	0	0	0	3	0	0	9	
ICSI	41	6	2	2	1	2	1	27	10	2	1	2	5	5	4	111	
AT drilling	45	6	2	2	2	2	1	27	10	2	1	2	4	5	4	115	
Laser drilling	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Mechanical	0	0	1	0	0	0	0	0	0	0	0	0	4	0	0	5	
Cleavage aspiration	45	6	3	2	2	2	1	27	10	2	1	2	8	5	4	120	
PCR	45	6	3	2	2	2	1	27	10	2	1	2	8	5	4	120	
COC	595	60	44	21	26	22	6	374	161	19	32	22	110	29	59	1580	
Inseminated*	561	58	44	21	24	22	5	363	156	19	29	22	103	29	59	1515	
Fertilized	361	33	40	12	11	13	3	206	108	14	24	18	70	18	47	978	
(%)	64	57	91	57	46	59	60	57	69	74	83	82	68	62	80	65	
Biopsied	269	24	28	11**	11	12	3	162	56	8	8	15	68	16	40	731	
Successfully	267	23	26	11	11	12	3	160	54	8	8	15	64	15	40	717	
biopsied (%)	99	96	93	100	100	100	100	99	96	100	100	100	94	95	100	98	
Diagnosed	225	16	20	9	9	11	3	142	39	6	8	13	39	12	38	590	
(%)	84	70	77	82	82	92	100	89	72	75	100	87	61	80	95	82	
Failed	42	7	6	2	2	1	0	18	15	2	0	2	25	3	2	127	
(%)	16	30	23	18	18	8	0	11	28	25	0	13	39	20	5	18	
Transferable	159	9	13	8	7	9	1	68	16	6	6	2	33	1	28	366	
(%)	71	56	65	89	78	82	33	48	41	100	75	15	85	8	74	62	
Transferred	94	13	11	4	7	6	1	45	14	5	3	2	24	1	9	239	
(%)	42	81	55	44	78	54	33	32	36	83	38	15	62	8	24	41	
Cycles to embryo transfer	40	5	3	2	2	2	1	23	9	2	1	1	7	1	4	103	
(%)	89	83	100	100	100	100	100	74	90	100	100	50	88	20	100	77	
Frozen	29	0	0	0	2	0	0	12	0	1	0	0	12	0	17	73	
HCG positive	12	0	0	1	2	1	0	7	1	0	0	1	2	0	1	28	
Positive heart beat	11	0	0	1	2	1	0	5	1	0	0	1	1	0	1	24	
%	22	0	0	33	100	50	0	16	0	0	50	13	0	25	18	18	
Lost to follow-up	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Abbreviations used in the table: CF = cystic fibrosis (various mutations), β-Thal = β-thalassaemia, Rh-inc = rhesus incompatibility, SMA = spinal muscular atrophy, TS = Tay–Sachs disease, AGS = adrenogenital syndrome, SS = sickle-cell anaemia, DM = myotonic dystrophy, HD = Huntington's disease, MS = Marfan's syndrome, OI = osteogenesis imperfecta, CMT = Charcot–Marie–Tooth disease, FRAXA = fragile-X syndrome, DMD = Duchenne's muscular dystrophy (specific), OR = oocyte retrieval, AT = acidic Tyrode's, COC = cumulus oocyte complexes

*The number inseminated is not accurate as some centres completed the old forms which did not include this field.

% transferable and transferred is of the total diagnosed.

out of 110 fetal sacs. The pregnancy evolution of these 110 fetal sacs is shown in Table XII. Eighty-one showed a normal development, five of which were reduced because of multiple pregnancy. Eleven fetal sacs were lost during the first trimester. A morphological abnormality was found in four fetuses. In one of the triplet pregnancies, two of the fetuses were a monochorial-monoamniotic twin. One of these twins was an acardiacus, while the other twin suffered intrauterine growth retardation (IUGR) due to twin-twin transfusion. In another twin pregnancy, one twin was shown to have an exencephaly. The fourth abnormality was a stillbirth at 24 weeks gestation.

Information on pregnancy complications was available for 59 pregnancies (Table XIII). Eight pregnancies were still ongoing at the time of reporting. No information was available for four singleton pregnancies. Most pregnancies were uneventful (49/59; 83%). Complications of pregnancy occurred in 17% (10/59); 6/43 (14%) singleton pregnancies and 4/16 (25%) twin pregnancies. The most frequent complication was preterm labour (3/59, 5%). Caution must be given for this number: there is far less preterm labour reported than prematurity in the children. This is due to underreporting of preterm labour as a maternal complication.

At the time of reporting, 63 women had delivered 79 children, while eight pregnancies (four singletons and four twins) were still ongoing. No further data are available on these ongoing pregnancies (Table XIV). In total, 47 (75%) singletons and 16 (24%) twin pregnancies were delivered. Fifty-one percent (24/47) of the singleton pregnancies were delivered spontaneously, while 38% (18/47) were delivered by Caesarean section. No method of delivery was reported for 5/47 singleton deliveries. Only 3/16 twin pregnancies (19%) were delivered spontaneously, while 10/16 (62%) were delivered by section. The mean gestational age at delivery was 37 weeks and 3 days (calculated excluding one twin pregnancy for which information is not available). There was no correlation between the maternal age and the gestational age at birth. Seventeen out of 62 (27%) deliveries were premature: 13% (6/47) of the singletons and 73% (11/15) of the twins.

Table VIII. Summary of PGD cycles using FISH based analysis of chromosomes

	Aneuploidy screening	Chromosome abnormalities	Sexing	Total FISH
Total cycles	116	40	104	260
Cancelled	0	2	12	14
(%)				5
Cycles to OR	116	38	92	246
IVF	40	12	39	91
ICSI	76	26	53	155
AT drilling	111	32	80	223
Laser drilling	5	6	7	18
Mechanical	0	0	5	5
Cleavage aspiration	116	38	92	246
FISH	116	38	92	246
COC	1544	557	1156	3257
Inseminated*	1333	485	1140	2958
Fertilized	935	353	780	2068
(%)	70	73	68	70
Biopsied	748	272	644	1664
Successfully biopsied	735	259	619	1613
(%)	98	95	96	97
Diagnosed	703	231	562	1496
(%)	96	89	91	93
Failed	32	28	57	117
(%)	4	11	9	7
Transferable	282	84	187	553
(%)	40	36	33	37
Transferred	249	58	149	456
(%)	35	25	27	30
Cycles to embryo transfer	101	27	75	203
(%)	87	68	72	78
Frozen	11	10	43	64
HCG positive	35	9	11	55
Positive heart beat	29	7	7	43
(%)	25	18	7	17
Lost to follow up	0	2	0	2

*The number inseminated is not accurate, as some centres completed the old forms which did not include this field.

Table IX. Summary of all PGD cycles

	Total PCR	Total FISH	Total
Total cycles	132	260	392
Cancelled	12	14	26
(%)	9	5	7
Cycles to OR	120	246	366
IVF	9	91	100
ICSI	111	155	266
AT drilling	115	223	338
Laser drilling	0	18	18
Mechanical	5	5	10
Cleavage aspiration	120	246	366
FISH	0	246	246
PCR	120	0	120
COC	1580	3257	4837
Inseminated*	1515	2958	4473
Fertilized	978	2068	3046
(%)	65	70	68
Biopsied	731	1664	2395
(%)			77
Successfully biopsied	717	1613	2330
(%)	98	97	97
Diagnosed	590	1496	2086
(%)	82	93	90
Failed	127	117	244
(%)	18	7	10
Transferable	366	553	919
(%)	62	37	44
Transferred	239	456	659
(%)	41	30	32
Cycles to embryo transfer	103	203	306
(%)	77	78	78
Frozen	73	64	137
HCG positive	28	55	83
Positive heart beat	24	43	67
(%)	18	17	17
Lost to follow-up	0	2	2

*The number inseminated is not accurate, as some centres completed the old forms which did not include this field.

Form (4): Children

Care must be taken when evaluating the following data, since a large proportion of the data were not reported (see also the right column of Table XV). In total, 79 children were born, of which 28 were premature (35%) (Table V). The sex ratio at birth was heavily skewed towards females reflecting the selective transfer of mainly females in X-linked disease: 43 females and 27 males. Mean birth weight, length and head circumference were 2850 ± 740 g, 48.3 ± 5.3 cm and 32.9 ± 2.7 cm respectively. The Apgar score was excellent in 41 children (data were unavailable for 36 children). A bad Apgar was observed twice: one child in the triplet pregnancy with the acranius twin which delivered prematurely at 24 weeks and died of intracranial bleeding and one term child (singleton) was observed for 24 h but showed normal development.

Only two malformations were observed at birth (Table XV): one pes equinovarus, which normalized spontaneously, and one exencephaly which was seen prenatally by ultrasound in a twin pregnancy. This child died within hours following the delivery. There were no neonatal complications in 44/73 children (60%), while complications were observed in 29 (40%) cases. No information was available for six children.

The most frequent complication at birth was prematurity *per se* in 38% (28/73), 22 of which were twins (Table XV). Of these premature children, 20 had a low birth weight (<2500 g), 17 of which were twins. Only two of these 20 children had a birth weight below 1000 g (twin born at 24 weeks gestation), while one child of this group weighed between 1000 and 1500 g. In eight children, the prematurity was accompanied by other problems.

There were three perinatal deaths (one stillbirth, two neonatal deaths; see above). Perinatal mortality rate was 3.8%. At the time of reporting, three children were still on the neonatal ward. No follow-up data are available (Table XV).

Pre- and postnatal confirmation of diagnosis

Prenatal testing was performed in 61/110 fetal sacs (Table XVI): in 21 cases CVS was used (11 FISH and 10 PCR cases), while in 38 cases amniocentesis was used (29 FISH and nine PCR cases) and for two cases the method of prenatal testing is unknown. No miscarriages were reported following prenatal testing. Confirmation of the PGD diagnosis was performed postnatally in 11 babies, four of which already had a PND, and two miscarriages: four karyotypes, five sweat tests and four genetic tests, twice for Tay–Sachs disease, once for Charcot–Marie–Tooth disease and once for cystic fibrosis

Table X. Summary of pregnancy rate related to the number of embryos transferred

	Number of embryo transfers	Raised HCG % per embryo transfer	Positive fetal heart % per embryo transfer
One embryo	72	13	9
(%)		18	13
Two embryos	116	22	17
(%)		19	15
Three embryos	95	37	32
(%)		39	34
Four embryos	17	8	6
(%)		47	35
Five embryos or more	6	3	3
(one pregnancy obtained after the transfer of eight embryos) (%)		50	50

Table XI. Indications and methods used for pregnancies after PGD

FISH		PCR	
X-linked disease:			
Haemophilia A	5	CBAVD*/cystic fibrosis	13
Duchenne muscular dystrophy	1	Myotonic dystrophy	6
Pelizaeus–Merzbacher	1	Duchenne/Becker muscular dystrophy	3
Incontinentia pigmenti	1	Charcot–Marie–Tooth disease	2
Retinitis pigmentosa	1	Adrenogenital syndrome	1
Hunter syndrome	1	Tay–Sachs disease	2
Mental retardation	2	Spinal muscular atrophy	1
Hydrocephaly	1		
Kennedy disease	1		
Klinefelter syndrome	2		
Velocardiofacial syndrome	2		
Male meiotic abnormality	1		
Aneuploidy, abnormal karyotype	11		
Aneuploidy, age	15		
Repeated cycle	5		
Recurrent miscarriage	2		
Unknown indication	2		
FISH total: 54/82 pregnancies (66%)		PCR total: 28/82 pregnancies (34%)	

CBAVD = congenital bilateral absence of the vas deferens.

Table XII. Evolution of the PGD pregnancies

Clinical pregnancies:	82	Fetal sacs: 110
Singleton	58/82 (71%)	
Twin	21/82 (25%)	
Triplet	2/82 (2%)	
Quadruplet	1/82 (1%)	
no heartbeat:(4 S, 1 Tw)		5/110
blighted ovum:		1/110
first trimester miscarriage		2/110
vanishing twins:		2/110
ectopic pregnancy:		1/110
Ongoing clinical pregnancy (>12 weeks):	74	Fetal sacs: 99
second trimester miscarriage (13 weeks)	1/99	
stillbirth (24 weeks) induced reduction		1/99
triplet→twin at $18^{2/7}$ weeks triplet→singleton at 12 weeks quadruplet→twin		5/99
termination of pregnancy (misdiagnosis)		1/99
Normal evolution of pregnancy	71	Fetal sacs: 91
Singleton:	51/71 (72%)	
Twin:	20/71 (28%)	
Ongoing pregnancy (4 S, 4 Tw)	8/71	
Delivery:(47 S, 16 Tw)	63/71	

*S = singleton pregnancy, Tw = twin pregnancy.

(miscarriage) were performed. On seven occasions, the postnatal testing was the first confirmation of the PGD. In total, PGD was confirmed through prenatal diagnosis or postnatal examination in 45/76 (59%) conceptuses after PGD using FISH and in 25/34 (74%) conceptuses after PGD using PCR. This is a total of 70/110 (64%) conceptuses (61 with PND, two in miscarriage material and seven postnatally); four conceptuses had prenatal as well as postnatal confirmation of the PGD.

The results confirmed the unaffected status of the fetus or baby in 68/70 cases. One trisomy 16 was detected following a miscarriage where the indication for PGD had been X-linked incontinentia pigmenti (chromosome 16 had not been analysed), and one fetus was detected as affected for myotonic dystrophy by amniocentesis and the couple elected to terminate the pregnancy. Overall, therefore, there was only a single reported misdiagnosis in the Consortium out of 70 cases (1.4%) for which confirmation has been carried out.

Table XIII. Complications of pregnancy

No complications	49/59 (83%)	
Complications	10/59 (17%)	
AHT	2/59	(1 single, 1 twin)
gestational diabetes	1/59	(1 single)
preterm contractions	2/59	(1 single, 1 twin)
preterm labour	3/59	
abruptio placentae	1/59	(single)
PROM	2/59	(2 twins)
pre-eclampsia	1/59	(single)
eclampsia	1/59	(twin)
HELLP	1/59	(single)
Complications in singleton pregnancies	6/43 (14%)	
twin pregnancies	4/16 (25%)	
Complications if mother's age		
<35 years	7/28 (25%)	
⩾35 years	3/13 (23%)	

AHT = arterial hypertension; PROM = premature rupture of the membranes; HELLP = haemolysis elevated liver enzymes low platelet syndrome.

Table XIV. Delivery

	Total	Singleton	Twin
Method of delivery	27/63 (43%)	24/47 (51%)	3/16 (19%)
Vaginal			
Caesarean section	28/63 (44%)	18/47 (38%)	10/16 (62%)
Unknown	8/63 (13%)	5/47 (11%)	3/16 (19%)
Gestational age at delivery			
Preterm	17/62 (27%)	6/47 (6 children)	11/15 (22 children)
A term	45/62 (73%)	41/47 (41 children)	4/15 (8 children)

Forms (5–8): PGD protocols

On a voluntary basis, centres were invited to submit their protocols for embryo biopsy and single cell genetic analysis subdivided into FISH and PCR protocols. Fifteen centres responded, with eight providing protocols in each of these areas. The basic reason behind this initiative was to identify critical differences in the protocols of the most successful centres that could be shared with the Consortium through incorporation into standardized protocols with the ultimate goal of promoting the use of best practice. In addition, it is hoped to establish baseline standards for embryo biopsy (similar to those commonly in use for intracytoplasmic sperm injection) and single cell analysis both in terms of preliminary work up and clinical application.

It was clear that most centres were still using the original approach of day 3 post-insemination biopsy at cleavage stages following zona drilling by acid Tyrode's solution (Handyside *et al.*, 1990). However, there were significant differences in the media used (reflecting the practice of individual centres) and about half of the responding centres were now using calcium- and magnesium-free media to decompact embryos prior to biopsy. The criteria (if any) for cancelling a cycle because too few follicles, oocytes or embryos were available varied widely. Working practice in terms of the number of blastomeres removed varied widely, as did whether or not it was considered worthwhile to re-biopsy embryos.

Most centres employed FISH primarily for identification of sex in X-linked disease. Over half, however, were also attempting the detection of unbalanced karyotypes in translocation carriers and three centres used multicolour FISH to screen for the common aneuploidies causing miscarriage and congenital abnormality. Surveying the PCR protocols, it is clear that most are still based on nested protocols and conventional methods for mutation detection. However, half of the responding centres were now using fluorescence PCR for mutation detection and/or linkage analysis. Strikingly, there remains no consensus over optimal lysis buffers to use for single cell PCR and this is a critical aspect of PCR protocols which could be addressed by the Consortium.

Conclusions

This first preliminary report and assessment of the current clinical status of PGD within the ESHRE PGD Consortium has taken a lot of time and effort from the Steering Committee and members of the Consortium reporting their data. It is already clear, however, that this effort is worthwhile and that the Consortium can play an important role in the future development of the use of assisted reproduction in prevention of inherited disease. Initial problems with the forms for reporting different categories of data have been overcome largely and suggestions for further improvements are welcome. However, the scale of the data base will soon require alternative strategies and it is hoped to switch to electronic submission of data initially by email and later directly to dedicated pages of the ESHRE web site before the next annual report is produced. Using electronic data collection, prospective data gathering should become routine and this will greatly enhance the quality of the data.

There has been considerable debate over how the data should be collected, particularly over the issue of when to report each cycle, with some arguing that this should not be until the diagnosis has been confirmed and the pregnancy outcome is known. In practice, as data from each cycle are collected from each centre and given a unique identifying code (determined by each centre), it is hoped that these data can be continuously accumulated including follow up of the children at birth and later in development. However, there is still much to be learned from the initial stages of each cycle and centres are therefore encouraged to submit the referral and cycle data as soon as possible.

Given the diversity of single gene defects, it is perhaps not surprising that many referrals were for conditions for which PGD is not yet available and conversely that the highest referral rates should be for common diseases and/or those that are currently available (Tables I and II). The number of referrals for aneuploidy screening is not representative and will certainly increase as more centres offer this form of PGD to women of advanced maternal age, repeated IVF failure and other indications.

The reproductive history of couples requesting PGD (Table III) confirms that many have had affected children and only a minority have healthy children. Most couples have had one or more previous pregnancies and about a third have had a termination of an affected pregnancy. This is reflected in the average maternal and paternal ages (33.7 and 35.4 respectively). Although pregnancy rates following IVF in women of this age are reasonable, it is clear that if younger women elected to have PGD at an earlier stage, possibly as a result of screening programmes for common diseases, the prospects for a successful outcome could be improved.

PGD should be regarded as an option for couples at high risk of having a genetically abnormal child. PGD is an extra alternative, since couples can also opt for prenatal diagnosis, donor insemination or refrain from having children. The major differences between PGD and prenatal diagnosis during established pregnancy are the avoidance of selective termination on the one hand and the use of IVF or ICSI on the other. In this respect, it is interesting to note that about one third of the couples from this series have had personal experience with termination of pregnancy which was one of the reasons for referral (Table IV). From the data presented in Table II, it is clear that about 50 couples had multiple (up to six) terminations

Table XV. Data on live born children

			Not available
Sex	43/70 female 27/70 male	sex ratio (m/f) 0,6	9/79
Birth weight	mean 2850 ± 740 g		7/79
Birth length	mean 48.3 ± 5.3 cm		32/79
Head circumference	mean 32.9 ± 2.7 cm		54/79
Apgar	good in 41/43, bad in 2/43: 1 premature child (24 weeks) 1 term, single child	Evolution: neonatal death 24 h neonatal observation	6/79
Malformations at birth	none in 71/73 present in 2/73: 1 pes equinovarus 1 excencephaly	Evolution: spontaneous normalization neonatal death	6/79
Neonatal complications	none in 44/73 (60%) present in 29/73 (40%) 28/73 prematurity (<37 weeks) 8/73 prematurity + complications 3 neonatal observations 2 artificial respirations (1× <24 h, 1× <7 days) 1 PDA ($24^{5/7}$ weeks, twin). Normal evolution 2 neonatal death 1 intracranial bleeding ($24^{5/7}$ weeks), twin with IUGR 1 exencephaly (35 weeks) 1/73: neonatal observation in aterm (bad Apgar)		6/79

of pregnancy. A much larger group (two out of every three couples) was referred because of objection to abortion on moral or religious grounds.

PGD also offers opportunities to couples with sub- or infertility and a concurrent genetic risk (Pembrey, 1998): for example, for couples where the woman had a tubal ligation because PND was not available at the time or for couples undergoing IVF treatment and who have a concurrent genetic risk. The first group is very small (only two patients) and will eventually disappear. The second is much larger, about one-third of the couples in our series, and its proportion will most probably increase. The patients referred for age-related aneuploidy screening belong to a separate group, although they also need infertility treatment, because they have a much lower risk of abnormal children. The screening is mainly carried out to increase the pregnancy rates and this is why it has to be considered whether this indication needs a separate registry. Indeed, it has been suggested that aneuploidy screening should be referred to as preimplantation genetic screening (PGS) to distinguish it from PGD. In the near future, the numbers of patients in this group will rapidly increase.

From the centre decisions (Table V) and the reasons for declining (Table VI), one might get the impression that the vast majority of all cases are suitable for IVF, technically possible and clinically acceptable. However, it should be noted that the picture which emerges from these data is most likely only incompletely representative of the true situation. As stated above, there must have been many more referrals since the Consortium data base contains more cycles than referrals and some centres fail to provide referral data for their patients. The prospective data collection, which will start in the near future, will circumvent this problem. Only then will it become clear what the true number of patients is that have declined from treatment because of aspects connected to IVF or ICSI treatment.

In this series, only centres performing PGD at the cleavage stage have participated, so no data are available on the results of polar body biopsy to make any comparison between both methods. Similarly the reported experience with other variations of the standard protocol are too few to make solid conclusions. However, it is clear that one of the most important contributions that the Consortium can make is to take initiatives proactively on a multicentre basis to provide the statistical power to investigate all aspects of methodology to establish best practice in all aspects of PGD.

Overall, the pregnancy rates are disappointing (Tables VII–X). The clinical pregnancy rate for all reported PGD cycles was 71/403 (17.6%). This compares to world-wide pregnancy rates of 25% per cycle ($n = 134$) and 30% per transfer reported in 1994 (Harper and Handyside, 1994), 25% per cycle ($n = 197$) and 29% per transfer to February, 1995 (Harper, 1996) and, most recently, 20% per cycle ($n = 569$) and 26% per transfer to January, 1997 (Harper, personal communication). These rates appear even lower when compared to pregnancy rates after ICSI in infertile couples (28% per cycle) (Tarlatzis and Bili, 1998). The reasons for this low success are manifold and complex but worthy of detailed consideration since without a reasonable prospect of establishing a pregnancy, at risk couples, who are generally fertile, are unlikely to choose PGD to avoid the risk of having affected children.

Table VII summarizes the Consortium experience (16 centres) with PCR detection of single gene defects. For most genetic defects, only a handful of cycles ($n = 1$–10) have been reported and technical aspects of these tests can only be evaluated with more experience. For cystic fibrosis (CF) ($n = 51$) and myotonic dystrophy (DM) ($n = 31$), however, the

Table XVI. Pre- and postnatal confirmation of diagnosis

Confirmation	Prenatal			Postnatal			Total
	Method		Results	Method		Results	
FISH	by CVS	11		by karyotype:	4		45/76 (59%) fetal sacs after FISH
	by Amnio	29		2×first testing postnatal		3/4 normal karyo	
	method NA*	2		1×confirmation prenatal		1/4 47,XY,+16 (miscarriage)	
				1×karyotype of miscarriage			
	Total	42	39/42 normal karyotype 3/42 NA				
PCR	by CVS	10		by sweat test (CF)	5	5/5 normal	25/34 (74%) fetal sacs after PCR
	by Amnio	9		4×first testing			
	method NA*	0		1×confirmation			
	Total:	19	18/19 normal	by genetic testing (TS, CMT, CF)	4	9/9 normal	
			1/19 myotonic dystrophy 1×testing on miscarriage	1×first testing 2×confirmation			
	Total prenatal testing	61/110	Total prenatal results:	Total postnatal testing:	13	Total postnatal result	70/110: 64%
			57/61 normal	7×first testing			12/13 normal
			1/61 affected	4×confirmation prenatal			1/13 abnormal karyo
			3/61 NA	2×testing of miscarriage			

*NA = not available.
Amnio = amniocentesis.
CVS = chorion villi sampling.

series are larger. Pregnancy rates with CF (22% per cycle, 24% per OR and 27.5% per embryo transfer) are comparable to a previous report (for the ΔF508 deletion only) of global figures for six centres of 29% per OR and 33% per embryo transfer (Ao *et al.*, 1996). Also, there may be scope for improvement since although 71% of diagnosed embryos were available for transfer, this was only 44% of the original cohort of 2PN embryos in those cycles because of a relatively high diagnostic failure rate of 16%. There is clear evidence in conventional IVF cycles that pregnancy rates are better when a fixed number of embryos can be selected for transfer from a larger number of morphologically good quality embryos. This restricted choice on genetic criteria must therefore have significant impact on pregnancy rates and extra efforts to improve diagnostic efficiency are obviously needed. The same comments are also true of the DM series with the added problem that as an autosomal dominant only 50% of embryos on average will be unaffected. From that perspective, a pregnancy rate of 22% per embryo transfer is encouraging.

Table VIII summarizes the Consortium experience using FISH to identify sex in X-linked disease and to detect various chromosomal abnormalities. The pregnancy rates with aneuploidy screening (25% per cycle and 29% per embryo transfer) are excellent considering that all of these couples had a poor prognosis for conventional IVF mainly because of advanced maternal age. Nevertheless, there is a case to be made that this form of PGD should be considered separately (see discussion above). Again there is good news for detection of chromosomal abnormalities mainly in carriers of translocations though the proportion of cycles reaching transfer is relatively low (68%), possibly indicating that some couples may not be suitable even for preimplantation screening because too few karyotypically normal embryos are present. The most striking feature of the Consortium data to date, however, is the poor results with identification of sex in X-linked disease with multicolour FISH using X and Y chromosome specific probes along with a variable number of autosomal probes. In these cases (n = 104), the pregnancy rate was only 7% per cycle, 8% per OR and 9% per embryo transfer. This is a major application of PGD, since it is a generic approach to over 300 X-linked recessive diseases and has been in development clinically for almost 10 years. Even in the early years, pregnancy rates of 22% per cycle and 28.5% per embryo transfer were reported (Griffin *et al.*, 1994). If these cycles are removed (Table IX), the overall pregnancy rates for the Consortium increase to 20.5% per cycle, 21.5% per OR and 25.5% per embryo transfer which is more comparable to previous global rates (see above).

This clearly needs detailed investigation and it is already clear that, for whatever reason, some centres are simply not identifying a sufficient proportion of female embryos. The reasons for this could be a combination of the increased number of chromosome probes analysed in addition to the X and Y chromosomes and some centres are analysing two cells where possible to avoid errors resulting from chromosomal mosaicism. The problem is that this may be leading to the

rejection of some female embryos for transfer. Finally, these data show results obtained during 21 months, whereas several of the leading centres have now performed PGD for >5 years. The complete data set, from the start of PGD to the current situation, should give a more balanced picture.

Perhaps the most important new contribution from the Consortium is in following the pregnancy outcome, births and development of the children following PGD. The results of this initial survey have not shown any large increase in congenital abnormalities as reported previously. Bonduelle *et al.* (Bonduelle *et al.*, 1998) have reported a malformation rate of 2.3% in 1987 children born after ICSI, as compared to 2/79 (2.5%) in the current series. However, they certainly do not give grounds for complacency with a high incidence of biochemical and early pregnancy loss, a high proportion of multiple pregnancies, a high incidence of prematurity and preterm delivery and neonatal complication in 40% of pregnancies (Tables XI–XV).

Finally, a single misdiagnosis in a case of myotonic dystrophy has been reported (Table XVI). As a proportion of the number of pregnancies tested either by postimplantation procedures or at birth, the misdiagnosis rate would be 1/70 or 1.4%. Together with three other misdiagnoses (one for sexing done with PCR, and two for cystic fibrosis; Lissens and Sermon, 1997), this is the fourth reported misdiagnosis. Again, this experience urges us to be cautious and to continue to improve our techniques, building in extra safeguards to prevent more events like these. Indeed, the Consortium plans to investigate any errors reported in the future and to share any conclusions that may have general relevance to other centres. In the past, there has been little or no evaluation of how misdiagnoses have arisen and although some problems may be specific to certain tests, it is vital for the progress of PGD to learn as much as possible about what might have gone wrong.

Over the last 10 years, we have progressed from experimentation in animal models to demonstrate feasibility, to research on human embryos *in vitro*, to the first tentative clinical application and then to increasingly sophisticated embryological and diagnostic approaches. The challenge that lies ahead is to evaluate the accuracy, safety and value of PGD as an alternative form of prenatal diagnosis and to ensure that we can deliver this as a robust and reliable clinical service to patients. The ESHRE PGD Consortium with the support of the Society and Consortium membership is well placed to make an important contribution to this process alongside the IWG on Preimplantation Genetics. We also hope that through making progress with PGD accessible and in the public domain through regular reports like this one and through the ESHRE web site that legislation, regulation and provision of service in different countries can be informed by an accurate assessment of the current clinical status of PGD.

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