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ESHRE Preimplantation Genetic Diagnosis Consortium: data collection III (May 2001)

ESHRE PGD Consortium Steering Committee*

The ESHRE PGD Consortium was formed in 1997 to undertake a long-term study of the efficacy and clinical outcome of preimplantation genetic diagnosis (PGD). Here, the third report of the ESHRE PGD Consortium is presented, collating data received from 25 centres on referrals, cycles, pregnancies and babies born after PGD. The second report, published in December 2000, reported on 886 referrals, a total of 1318 started cycles (of which 465 for aneuploidy screening, 386 for fluorescence in-situ hybridization (FISH) and 385 for PCR going beyond oocyte retrieval), 163 pregnancies and 162 children born. This year, 675 referrals from 12 centres were added giving a total of 1561 referrals, 370 regular PGD cycles, 334 PGD-aneuploidy screening (PGD-AS) cycles and 78 cycles for social sexing from 24 centres and 215 pregnancies and 117 babies from 12 centres. Because more in-depth information was asked for the cycles, this year's data will be shown separately as well as cumulatively. One striking feature of this year's data collection is the appearance of the first data for gender screening on preimplantation embryos for social reasons. The ethical concerns regarding social sexing will be discussed, as well as the forthcoming changes in timing of data collection. When the data collection was discussed at the last meeting of the members of the ESHRE PGD Consortium in Lausanne, Switzerland (June, 2001), it appeared that the current system of data collection, although yielding results very quickly, showed fundamental flaws. The ESHRE PGD Consortium Steering Committee intends to remedy to these problems, on the one hand by introducing a new type and timing of data collection, and on the other hand by re-analysing and correcting the data which have already been sent in during the past 4 years.

Keywords: Consortium data collection/FISH/gender screening/PCR/PDG

Introduction

Since the introduction of preimplantation genetic diagnosis (PGD) (Handyside *et al*., 1990), the number of cycles and centres performing PGD world-wide has been steadily increasing. New technologies have been added to the arsenal at the disposition of the PGD-performing scientist: after the introduction of single-cell PCR for sexing (Griffin *et al*., 1993), fluorescence in-situ hybridization (FISH) was introduced and quickly shown to be a better alternative for sexing (Staessen *et al*., 1999). The advent of commercially available probes labelled with more different fluorochromes led to the possibility and spreading of PGD for aneuploidy screening (PGD-AS) (Munné *et al*., 1993; Gianaroli *et al*., 1999) and translocations (Munné *et al*., 2000). The accuracy of nested PCR (Ray *et al*., 1998) was increased by the introduction of fluorescent PCR (Goossens *et al*., 2000) and multiplex PCR (Dreesen *et al*., 2000; Apessos *et al*., 2001; Piyamongkol, *et al*., 2001). Although regular reports appear in the literature on the results and outcome of PGD, the ESHRE PGD Consortium was the first to attempt a data collection from several centres in order to attain higher significance through higher numbers. The two previous reports of the ESHRE PGD Consortium (1999, 2000) have been used extensively not only as standard reference in the literature, but also in the policy making of individual

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centres concerning PGD and the counselling of patients willing to embark on PGD. This third report is a supplement of these two, as well as a transition to a new type of report. It is a supplement because more data are collected on the same topics, although the appearance of sex selection of embryos for social reasons has generated extensive discussion within the Consortium. It is a transition because the data collection as it is performed now allows for a quick collection and report of data, but there are some fundamental flaws—not least the fact that the outcome of cycles resulting in pregnancy cannot always be followed up in their respective pregnancies and babies. The opinion of Consortium members towards social sexing will be discussed, as well as the intended changes to the way that data are collected.

Materials and methods

Membership

Before January 2000, centres interested in becoming a member of the ESHRE PGD Consortium were asked to complete a centre registration form containing information on current PGD practices, IVF results, cost of treatment, etc. Upon receipt, a centre code was assigned and a centre pack containing eight forms was sent. Since then, registration has been essentially after contact with a member of the Steering Committee, after which the interested centre was sent a registration form and an information pack by e-mail. A consortium number is assigned to new members upon receipt of the completed and signed registration form and blank Excel spread sheets (one for referral data, one for cycles and one for pregnancies and babies) have been sent to the joining centres.

Data collection

For information on the content of the different forms (referral, cycle, pregnancy, baby, biopsy protocol, FISH protocol, PCR protocol) we refer to the first PGD Consortium report (ESHRE PGD Consortium Steering Committee, 1999). Before March 2000, data were collected on hard-copy forms that were then sent to ESHRE Central Office. Since then, centres have used directly the blank Excel spreadsheets which were used by the Steering Committee members for processing of the data and which have been distributed through e-mail. All participating centres have received the complete spreadsheets after they had been corrected by the Steering Committee.

Results

Referral data

Referral data (i.e. information on the patient gathered at the first consultation) were obtained from 12 centres. The highest number of referrals per centre was 129, and the lowest five. The number of referrals included in this year's data collection (675) is higher than in previous years (323 and 563, respectively).

With respect to the reproductive histories for a total of 1561 cycles, it is clear that the majority of patients has had one or more pregnancies already (Table I), although the vast majority has no healthy children. About one-quarter of the couples has one or more affected children, while an even larger percentage of the couples have experienced spontaneous abortions or terminations of pregnancy after prenatal diagnosis.

In comparison with previous data collections, it is clear that the number of referrals for aneuploidy screening is increasing. Accordingly, the other reasons have decreased relatively (Table II). Genetic risk and objection to termination of pregnancy are still the most important reasons for PGD. About one-quarter of the couples need artificial reproductive treatment and want to combine this with PGD. Other reasons for PGD were recurrent abortion and objection to presymptomatic testing in case of Huntington's disease. Two couples wanted an HLA-identical donor for a child suffering from Fanconi anaemia.

Table III lists the referrals according to indication. What is new in comparison with the past is the first group of referrals for social sexing or family balancing. These couples have no medical indication for sex determination, and therefore it is inappropriate to use the term 'diagnosis'. The genuine medical indications can be divided into two broad groups: chromosomal, and monogenic. Mitochondrial disease is the indication in $<0.5\%$ of cases.

Table IV shows that the referrals for chromosomal disorders can also be divided into two groups: structural, and numerical abnormalities. Reciprocal translocation remains the most important abnormality among the first group. All these rearrangements are private, i.e. they have a sporadic nature, except the reciprocal translocation (11;22) which is known to be recurrent. There are four times more reciprocal than Robertsonian translocations. Inversions and deletions are reasons for referral in $<1\%$ of the cases each. Aneuploidy screening is performed for different reasons: maternal age, repeated IVF-failure (i.e. implantation failure), recurrent spontaneous abortion, or combinations of these.

The relative proportions of the monogenic disorders in Table V have changed slightly in comparison with previous years. There is a relative increase of dominant disorders, and a decrease in referrals for X-linked disease. The top three in each disease group remains constant over the years: cystic fibrosis, thalassaemia and spinal muscular atrophy as autosomal recessive diseases; myotonic dystrophy, Huntington's disease and Charcot–Marie–Tooth disease as autosomal dominant; and Duchenne's muscular dystrophy, Fragile-X syndrome and haemophilia as X-linked disorders.

The centres' decisions given in Table VI show that, if the unknowns are not taken into account, $>80\%$ of the cases were technically possible, about 90% of the patients can be accepted for IVF or ICSI, and that only a few cases are rejected on ethical grounds. The two most important reasons for not accepting women for IVF were high FSH concentration and increased maternal age. In one centre, women with myotonic dystrophy were not accepted for IVF because of the risks involved in the treatment. For the same reason, the hormonal treatment of patients with β-thalassaemia major was conducted with special care.

This year, only 13 referrals were not accepted on ethical grounds. The reasons were the cases looking for an HLA-identical donor mentioned above, low risk of affected progeny, AZFc deletion and achondroplasia. In one case a single woman was not accepted. Finally, the request of an Islamic couple, of which the husband did not want daughters was dishonoured.

The most important reasons for declining remained

Table I. Reproductive histories of the patients requesting PGD

	0	1	2	3	4	5	6	Unknown
Pregnancies	505	314	239	163	134	62	62	82
Pregnancies >28 weeks	860	346	149	37	6	2	2	159
Healthy children	1108	235	60	14	1	1	0	142
Affected children	1056	307	61	4	0	1	0	90
Stillborn	1359	30	4	1	0	1	0	166
Spontaneous abortions	1029	137	95	60	56	23	38	123
Termination of pregnancies	1098	184	74	27	11	3	1	163

Table II. Reasons for preimplantation genetic diagnosis

Genetic risk and previous TOP	330/1561 (21.1)
Genetic risk and objection to TOP	565/1561 (36.2)
Genetic risk and sub- or infertility	400/1561 (25.6)
Genetic risk and sterilization	16/1561 (1.0)
Aneuploidy screening	222/1561 (14.2)
Other	100/1561 (6.4)
Unknown	91/1561 (5.8)

Values in parentheses are percentages.
TOP = termination of pregnancy.

Table III. Referrals (*n*) according to indication

Chromosomal	647
X-linked	294
Autosomal recessive	290
Autosomal dominant	254
Mitochondrial	6
Two indications	9
Y-chromosome deletion	2
Social sexing	30
Unknown	29

Table IV. Referrals (*n*) for chromosomal disorders

Structural chromosomal aberrations	
Reciprocal translocation	252
Robertsonian translocation	61
Inversion	12
Deletion	6
Aneuploidy risk	
Aneuploidy risk	249
47,XXY;47,XYY	25
Sex chromosomal mosaicism	16
Male meiotic abnormalities	14
Other	4
Unknown	8

unchanged (Table VII): inconvenience or burden of IVF or ICSI, spontaneous pregnancy and low success rate. The costs of the procedure was the reason for declining in only a few cases.

Cycles

Last year, the cumulative data from 1999 and 2000 for PGD and PGD-aneuploidy screening (PGD-AS) totalled 1318 cycles. As for last year, the Consortium members felt it important to separate the PGD-AS from the PGD cycles for specific diagnosis of familial disease. Table VIII summarizes the PGD data for this year, last year's data collection (1999 and 2000), and the cumulative data for all three data collections (no PGD-AS or PGD for social sexing). The data for 2001 are from 25 centres. Additionally, over the past year we asked whether patients were fertile or infertile. For the 426 cycles in 2001 which reached oocyte retrieval (OR) for PGD, 164 cycles were for infertile patients. By analysing only the 2001 data, as in previous years, the majority of PGD cycles used ICSI to achieve fertilization. This year, the number of cycles where a laser was used for zona drilling was nearly as high as those using acid Tyrode's (199 versus 211). Only three cycles used mechanical means for zona breaching; the majority of centres used cleavage-stage biopsy and aspiration of the blastomere(s). Only two polar body biopsies were conducted (followed by cleavage-stage biopsy) and no blastocyst biopsies. From a total of 5985 oocytes collected, 3703 fertilized (62%), 2874 were suitable for biopsy (48%), and 2844 of these were successfully biopsied (99%). The diagnosis was successful in 2452 cases (85% of the number of embryos biopsied), and 997 embryos were diagnosed as suitable for transfer (17% of the oocytes collected). Of these, 708 were transferred in 349 embryo transfer procedures. Seventy-seven cycles did not result in an embryo transfer procedure. Eighty-one clinical pregnancies resulted, which gives a pregnancy rate of 19% per OR and 23% per embryo transfer procedure.

Examination of the cumulative data shows that a total of 1197 cycles went on beyond OR, with a clinical pregnancy rate of 17% per OR and 22% per embryo transfer procedure. The biopsy was successful in 97% of cases and the diagnosis obtained in 86% of successfully biopsied blastomeres.

PGD for chromosomal abnormalities

Table IXA shows the data for this year and previous years, and the cumulative data for all three years. For the first time it has been possible to break down the data for 2001 into the type of chromosome abnormality, i.e. Robertsonian or reciprocal translocation or other chromosome abnormality. This data could be further analysed, e.g. sex of the carrier, etc., and it is hoped that this will be completed in the next PGD Consortium report.

For the Robertsonian translocations, a total of 51 cycles reached the OR stage, and the average female age was 34 years. A high number of patients were also infertile. ICSI was undertaken in most cases. All embryo biopsies were performed at the cleavage stage, using blastomere aspiration. Laser and

Table V. Referrals for monogenic diseases

Autosomal recessive	No. of referrals	Autosomal dominant	No. of referrals	X-linked	No. of referrals
Cystic fibrosis	109	Myotonic dystrophy	88	Fragile-X syndrome	75
Thalassemia	53	Huntington's disease	73	Duchenne/Becker's muscular dystrophy	69
Spinal muscular atrophy	50	Charcot–Marie–Tooth disease	20	Haemophilia	26
Other	78	Other	73	Other	124
Total	290	Total	254	Total	294

Table VI. Centre decision

	Yes	No	Undecided/unknown
Suitable for IVF	1160	115	260
Technically possible	1184	240	137
Ethically acceptable	1272	61	228
PGD accepted	1070	292	199

PGD = preimplantation genetic diagnosis.

Table VII. Most important reasons for declining

Inconvenience/burden of IVF or ICSI	60
Spontaneous pregnancy	38
Low success rate	36
Cost	17
Donor spermatozoa needed	6
Donor oocytes needed	5

Table VIII. Overall data for PGD only (no PGD-AS or social sexing)

Indication	PGD 2001	1999 + 2000	PGD cumulative All 3 years
Cycles to OR	426	771	1197
Female age	34 years	Not known	–
No. infertile	164	Not known	–
IVF	64	154[a]	218
ICSI	363[b]	619	982
Cancelled post OR	13	13	26
Cycles to PGD	413	758	1171
FISH	232	381	613
PCR	181	377 + 9[c]	558
AT drilling	211	602	813
Laser drilling	199	146	345
Mechanical	3	10	13
PB biopsy	2[d]	4[d]	6
Cleavage aspiration	378	755	1133
Cleavage extrusion	35	0	35
COCs	5985	10 267	16 252
Inseminated[e]	5274	9090	14 364
Fertilized	3703	6465	10 168
Biopsied	2874	5224	8098
Successfully biopsied	2844	5041	7885
Diagnosed	2452	4323	6775
Transferable	997	1838	2835
Transferred	708	1340	2048
Cycles to embryo transfer	349	639	988
Frozen	130	360	490
HCG-positive	107	174	281
Positive heart beat[f]	81 (19)	141 (16.5)	222 (17.3)

[a]Some had IVF and ICSI
[b]Two FISH cycles had IVF and ICSI.
[c]Nine cycles involved PCR and FISH diagnosis.
[d]One cycle PCR diagnosis had polar body biopsy and cleavage-stage aspiration; diagnosis involved PCR and FISH.
[e]Number of oocytes inseminated not accurate as some centres did not record this information. In cycles where data were not recorded, the figure entered was the same as the number of oocytes collected.
[f]Values in parentheses are % per oocyte retrieval.
AT = acid Tyrode's; COC = cumulus–oocyte complex; OR = oocyte retrieval.

AT (acid Tyrode's) drilling were used in almost an equal number of cases. From 768 oocytes, 414 fertilized (54%), 314 were suitable for biopsy, and 312 of these were successfully biopsied (100%). The diagnosis was successful in 252 cases (81% of embryos successfully biopsied), and 85 embryos were diagnosed as transferable (11% of oocytes collected). Thirty-eight embryo transfer procedures were conducted (74% of OR), and 11 clinical pregnancies resulted (22% of OR and 29% of embryo transfer).

For the reciprocal translocations, a total of 96 cycles reached the OR stage, and the average female age was 34 years. In this case, only 25 patients were infertile, which was much lower than in the case of Robertsonian translocations. ICSI was undertaken in most cases. All embryo biopsies were performed at the cleavage stage, using mostly blastomere aspiration. The majority of clinics used acid Tyrode's for zona drilling, which is also different to the cases of Robertsonian translocations. From 1570 oocytes, 1016 fertilized (65%), 866 were suitable for biopsy and 856 of these were successfully biopsied (99%). The diagnosis was successful in 789 cases (92% of embryos successfully biopsied), and 195 embryos were diagnosed as transferable (13% of oocytes collected). Seventy-three embryo transfer procedures were conducted (76% of OR), and 17 clinical pregnancies resulted (18% of OR and 23% of embryo transfer). The karyotypes of the chromosomal rearrangements for which PGD has been performed are listed in Table IXB.

Twenty-five cases of PGD were performed for other chromosome abnormalities, with four clinical pregnancies.

The cumulative data for all three years show that 368 cycles have now been performed, with 689 embryos being suitable for transfer (13% of the oocytes collected), 290 embryo transfer procedures and 62 clinical pregnancies (17% per OR and 21% per embryo transfer).

Table IXA. Preimplantation genetic diagnosis (PGD) for structural chromosomal abnormalities

Chromosome abnormality	Robertsonian translocation	Reciprocal translocation	Other chromosome abnormality	Total 2001	1999+2000	Cumulative for 3 years
cycles to OR	51	96	25	172	196	368
Female average age	34	34	32	34	Not known	–
No. Infertile	41	25	19	85	Not known	–
IVF	7[a]	29	5	41[a]	45[a]	86
ICSI	45[a]	67	20	132[a]	152	284
Cancelled after OR	0	2[b]	0	2	3	5
AT drilling	23	78	9	110	157	267
Laser drilling	28	14	16	58	36	94
Mechanical	0	2	0	2	0	2
Polar body biopsy	0	0	0	0	3	3
Cleavage aspiration	37	90	23	150	190	340
Cleavage extrusion	14	4	2	20	–	20
COCs	768	1570	307	2645	2732[b]	5377
Inseminated	656	1389	274	2319	2327	4646
Fertilized	414	1016	182	1612	1722[c]	3334
Biopsied	314	866	143	1323	1471	2794
Successfully biopsied	312	856	140	1308	1393	2701
Diagnosed	252	789	128	1169	1254	2423
Transferable	85	195	60	340	349	689
Transferred	75[c]	155	39	269	308	577
Cycles to embryo transfer	38	73	20	131	159	290
Frozen	5	30	5	40	13	53
HCG positive	14	22	5	41	40	81
				23.8%	20.4%	22.0%
Positive heart beat	11	17	4	32	30	62
				18.6%	15.3%	16.8%
lost to follow up	0	0	0	0	3	3

[a]One cycle with IVF and ICSI.
[b]One cycle with 23 embryos frozen (OHSS), one cycle with only three embryos which were transferred without biopsy.
[c]Two cycles where embryos without diagnosis were transferred (failed diagnosis).
AT = acid Tyrode's; COC = cumulus–oocyte complex; OR = oocyte retrieval.

Sexing by FISH for X-linked disease

Almost all clinics performing sexing for X-linked disease use FISH. Table X shows the FISH results of sexing for X-linked disease and other non-specific X-linked conditions, e.g. autism. For sexing for X-linked disease in 2001, a total of 64 cycles reached the OR stage. The patients had an average age of 35 years, and only 17 cycles were for infertile patients. ICSI was used in most of the cases (n = 42). For all cycles, cleavage-stage biopsy was performed using aspiration to remove the blastomere, and equal numbers used acid Tyrode's or a laser for zona drilling. A total of 852 oocytes was collected, 535 fertilized (63%), 413 were successfully biopsied (98%) and 356 of these were successfully diagnosed and 153 considered transferable (18% of oocytes retrieved). Embryos were transferred in 57 cases, with a clinical pregnancy rate of 20% per OR and 23% per embryo transfer.

The cumulative data show that 254 cycles have reached the stage of OR, 219 embryo transfers been performed, and a clinical pregnancy rate of 16% per OR and 19% per embryo transfer has been achieved.

PCR diagnosis for single gene disorders

Last year it was disappointing that the exact diseases for which some centres had performed PGD using PCR could not be evaluated; hence, only one overall table of PCR PGD was reported, without a list of diseases that had been diagnosed. Due to alterations in the data collection to ensure that centres made it clear which disease they were diagnosing, this year it was possible to present a breakdown of the data (Table XIA) and a list of diseases diagnosed (Table XIB).

The data have been divided into autosomal recessive, autosomal dominant, X-linked recessive-specific, X-linked dominant-specific, autosomal recessive combined with X-linked disease (i.e. patients carrying two abnormalities) and mitochondrial disease. For the autosomal recessive diseases, 98 cycles were started, and 91 reached the stage of OR. The average maternal age was 32 years, and 47% were infertile. In one cycle, IVF was performed. As the Consortium has confirmed in previous publications, ICSI should be used in all cases of PCR-PGD to avoid sperm contamination. Most cycles used acid Tyrode's for drilling, 79 cycles used cleavage-stage aspiration, and 10 used cleavage-stage extrusion. From 1221 oocytes collected, 749 fertilized (61%), embryo biopsy was performed on 607 embryos (and was successful on 600 of these; 99%), 490 embryos were successfully diagnosed (82%) and 294 were diagnosed as transferable (normal or healthy carriers)(60%). From 98 cycles started, 86 had embryo transfer procedures (88%) and 20 resulted in a clinical pregnancy (22% per OR and 23% pregnancy rate per embryo transfer procedure).

For the autosomal dominant diseases, 72 cycles were started, and 69 reached the stage of OR. The average maternal age was 31 years, and only 8% were infertile. Most cycles used the laser for drilling, 64 cycles used cleavage-stage aspiration and three used cleavage-stage extrusion. From 929 oocytes

Table IXB. Translocations analysed, 2001 only

Female chromosomal abnormalities	No of cycles	Male chromosomal abnormalities	No of cycles
45XX,t(13:14)	16	45XY, t(13;15)	1
45XX,t(14;21)	6	45XY,t(13:14)	14
45XX,t(15;21)(q10;q10)	1	45XY,t(14;15)(q10;q10)	3
46X,der(X)t(X;Y)(?p22.13;?q11.2)	1	45XY,t(14;21)	7
46XX,t(4;5)	1	46XY(12;16)(p11.2;p11.2)	1
46XX,t(1;10)(q44;q11.2)	2	46XY,t(1;06)(p22;p21.3)	1
46XX,t(1;13)	2	46XY,t(1;11)(p36.3;q13)	2
46XX,t(1;15)(p35;q22.1)	1	46XY,t(1;13)	1
46XX,t(1;16)(q42;q24)	1	46XY,t(1;15)	1
46XX,t(1;19)	2	46XY,t(1;17)(p34;q25)	2
46XX,t(1;4)(q23;q13)	1	46XY,t(1;2)(q31;p23)	1
46XX,t(1;7)(q32;q36)	1	46XY,t(11;12)	11
46XX,t(10;11)		46XY,t(11;22)	3
46XX,t(10;11)(p11.2;q23.3)	3	46XY,t(12;15)(q13;q26)	1
46XX,t(11;17)	2	46XY,t(13;15)(q13;p22)	1
46XX,t(11;22)	1	46XY,t(15;17)(q21;p12)	1
46XX,t(11;22)(q23.3;q11.2)	2	46XY,t(18;14)	1
46XX,t(12;13)(q21.3;q32)	2	46XY,t(18;21)	1
46XX,t(13;22)	2	46XY,t(2;13)(q35;q14)	1
46XX,t(14;18)(q11.2;q21.2)	3	46XY,t(3;12)(p14;q24.3)/46,XY	1
46XX,t(19;22)(q22;q11)	1	46XY,t(3;15)(q24;q25)	1
46XX,t(1p;20q)	1	46XY,t(3;19)	1
46XX,t(2;11)(q21.3;q25)	1	46XY,t(3;4)(q12;p15.2)	1
46XX,t(2;19)(q37.3;q13.1)	1	46XY,t(3;7)	2
46XX,t(2;3)	1	46XY,t(4;11)(q35.1;q15)	2
46XX,t(2;7)(q37.3;q34)	2	46XY,t(5;13)(p10;p10)	1
46XX,t(2;8)(p23;q24.1)	1	46XY,t(5;15)(q35;q22)	1
46XX,t(3;14)	1	46XY,t(5;8)	1
46XX,t(3;5)(p13;q22)	1	46XY,t(6;11)(q23;p13)	2
46XX,t(5,11)(q34,p25)	2	46XY,t(6;13)(p22.2;q14.2)	1
46XX,t(5;14)	1	46XY,t(6;7)(q25.2;q21.3)	1
46XX,t(6;10)(p23;q24)	1	46XY,t(7;10)(q15.3;q23)	3
46XX,t(6;22)(q23;q13)	1	46XY,t(7;16)(q22;q22)	1
46XX,t(8;10)(q12;p1)	1	46XY,t(8;12)(q24.1;q22)	3
46XX,t(8;14)(p21;q22)	1	46XY,t(8;14)	1
46XXt(12;13)	1	46XY,t(8;14)(q21.3;q31),inv(9)	3
46XXt(6;7)(p25;q11.2)	2	46XY,t(8;22)(q24.2;q11.2)	1
46XXt(9;13)(q12;p13)	2	46XY,t(9;17)(q21;p11.2)	2
46XXt(9;16)(q34.3;p12)	3	46XY,t(Y;18)(q12;p11.2)	2
History of molar pregnancies —select males	1	abnormal male meiosis	1
pt has mosaic Turner's	1	DiGeorge syndrome	1
pt is XXX	1	Klinefelter	10
Pseudocentric chromosome trisomy	8	45,XY,psu dic(15;22)(p12;p12)	2

collected, 604 fertilized (65%), embryo biopsy was performed on 381 embryos and was successful in all cases. In total, 324 embryos were successfully diagnosed (85%) and 154 were diagnosed as transferable (normal only)(48%). As expected, this is lower than for autosomal recessive diseases, as for dominant diseases embryos are either normal or affected. From 72 cycles started, only 55 had embryo transfer procedures (76%) which reflects the high number of cycles with only affected embryos, while 11 resulted in a clinical pregnancy (16% pregnancy rate per OR and 20% per embryo transfer procedure).

Nine cycles were undertaken for a specific diagnosis of an X-linked recessive disease, and 15 for X-linked dominant. Three pregnancies were obtained for the X-linked recessive disease and only one for the X-linked dominant. Four cycles were cancelled in the X-linked dominant group and these were all for Fragile X patients. Three cycles were performed where the patients carried two diseases; in all cases this involved an autosomal recessive and an X-linked disease. Two cycles were performed for mitochondrial diseases; in these cases polar body was combined with cleavage-stage biopsy, but no embryos were diagnosed as transferable.

The cumulative data for all three years show that there have been 575 cycles to OR, with 36 cycles having IVF (all should have had ICSI). The majority of cases used cleavage-stage biopsy with aspiration, and 479 cycles resulted in embryo transfers (83%) and 119 clinical pregnancies (21% per OR and 25% per embryo transfer procedure).

PGD-AS

The Consortium discussed which indications were now being performed for aneuploidy screening, and the following groups were identified: (i) age >35 years; (ii) recurrent IVF failure, defined as at least three failed IVF attempts (in Australia, the government pays for unlimited IVF cycles and their definition would be more than 10 embryos transferred); (iii) more than

Table X. FISH sexing

Reason for sexing	X-linked disease 2001	Non-specific X-linked 2001	Total for 2001	1999+2000	Cumulative for all 3 years
Cycles to OR	53	11	64	190	254
Female average age (years)	35	35	35	Not known	Not known
No. infertile	6	11	17	Not known	Not known
IVF	22	0	22	74	96
ICSI	31	11	42	117	159
Cancelled after OR	0	2	2	2	4
AT drilling	27	3	30	145	175
Laser drilling	26	6	32	38	70
Mechanical	0	0	0	5	5
Polar body biopsy	0	0	0	0	0
Cleavage aspiration	53	9	62	188	250
COCs	701	151	852	2412	3264
Inseminated	637	139	776	2281	3057
Fertilized	421	114	535	1603	2138
Biopsied	356	64	420	1364	1784
Successfully biopsied	349	64	413	1317	1730
Diagnosed	304	52	356	1180	1536
Transferable	122	31	153	441	594
Transferred	87	19	106	327	433
Cycles to embryo transfer	48	9	57	162	219
Frozen	21	1	22	96	118
HCG-positive	17	3	20	38	58
Positive heart beat[a]	13 (24)	0 (0)	13 (20)	28 (15)	41 (16)

[a]Values in parentheses are % per oocyte retrieval.
AT = acid Tyrode's; COC = cumulus–oocyte complex; OR = oocyte retrieval.

Table XIA. Cycles performed for single-gene disorders using PCR

Type of disease	AR	AD	X-linked recessive-specific	X-linked dominant-specific	AR+XL	Mitochondrial	Total for 2001	1999 +2000	Cumulative data for all 3 years
Total cycles	98	72	9	15	3	2	200	–	–
Cycles to OR	91	69	9	15	3	2	190	385	575
Female average age (years)	32	31	32	33	36	35	32	Not known	Not known
No. infertile	46	6	1	6	0	2	62	Not known	Not known
IVF	1	–	–	–	–	–	1	35	36
ICSI	90	69	9	15	3	2	189	350	539
Cancelled after OR	2	2[a]	0	4[b]	0	0	9	8	17
AT drilling	52	14	2	1	2	0	71	300	371
Laser drilling	36	53	7	10	1	2	109	72	181
Mechanical	1	0	0	0	0	0	1	5	6
Polar body biopsy	0	0	0	0	0	2[c]	2	1	3
Cleavage aspiration	79	64	8	11	2	2	166	377	543
Cleavage extrusion	10	3	1	0	1	0	15	0	15
COCs	1221	929	120	140	66	7	2488	5123	7611
Inseminated	1054	821	109	119	65	6	2179	4482	6661
Fertilized	749	604	76	74	45	5	1556	3140	4696
Biopsied	607	381	52	43	43	5	1131	2389	3462
Successfully biopsied	600	381	51	43	43	5	1123	2331	3454
Diagnosed	490	324	41	36	31	5	927	1889	2816
Transferable	294	154	30	17	9	0	504	1048	1552
Transferred	187	110	18	13	5	0	333	705	1038
Cycles to embryo transfer	86	55	8	9	3	0	161	318	479
Frozen	45[d]	11	7	2	3	0	68	251	319
HCG-positive	29	12	3	1	1	0	46	96	142
Positive heart beat[f]	20[e] (22)	11[e] (16)	3 (30)	1 (7)	1 (30)	0 (0)	36 (19)	83 (22)	119 (21)

[a]One cycle cancelled because of infection.
[b]All Fragile X syndrome cycles.
[c]All embryos with polar body biopsy also had cleavage-stage biopsy.
[d]Some embryos frozen before biopsy.
[e]One twin each.
[f]Values in parentheses are % per oocyte retrieval.
AD = autosomal dominant; AR = autosomal recesssive; XL = X-linked.

Table XIB. Indications for PCR diagnosis for 2001 only

Mode of inheritance	Indication	No. of cycles
Autosomal dominant	Central core disease	1
	Charcot–Marie–Tooth 1A	4
	Charcot–Marie–Tooth 2A	2
	Crouzon syndrome	1
	FAP–Gardner	1
	HD-exclusion	1
	Huntington's disease	21
	Marfan's syndrome	1
	Myotonic dystrophy	33
	Neurofibromatosis	2
	Osteogenesis imperfecta I	1
	Osteogenesis imperfecta IV	1
	Stickler syndrome	2
	Tuberous sclerosis	2
Autosomal recessive	Beta-thalassaemia	21
	CDG1C	3
	Cystic fibrosis	49
	Epidermolysis bullosa	2
	Gaucher's disease	2
	Hyperinsulinaemic hypoglycaemia PHH1	1
	Sickle cell	2
	Spinal muscular atrophy	17
	Tay–Sachs disease	1
Sex-linked recessive	Agammaglobulinaemia	1
	Alport syndrome	1
	Duchenne's muscular dystrophy	5
	Hunter's syndrome MPSII	1
	Spinal and bulbar muscular atrophy	1
Sex-linked dominant	Alport syndrome	1
	Fragile X syndrome	13
	Oro-facial-digital syndrome type 1	1
Mitochondrial	MELAS	2
Mixed	CF+FRAXA	2
	CF+XL mental retardation	1

CF = cystic fibrosis; FRAXA = Fragile X syndrome; XL = X-linked.

two miscarriages with the parents having a normal karyotype; and (iv) other, which would include patients with two indications, and other reasons for performing PGD-AS that do not fit into the above categories.

This year, a total of 334 PGD-AS new cycles were included in these data, from 11 centres (Table XII). For 2001, 101 cycles were performed for age alone, 45 for recurrent miscarriage with the parents having normal karyotypes, 117 cycles for recurrent IVF failure, and 71 cycles for other indications. For all indications the majority of patients were infertile. Cleavage-stage aspiration was used in all cases for 2001. Overall, only 40% of embryos were suitable for transfer, and only 75% of cycles had an embryo transfer procedure. Sixty-six cycles resulted in a clinical pregnancy (20% per OR and 26% per embryo transfer procedure). The only striking result was the low pregnancy rate for patients with recurrent IVF failure (7% per OR and 11% per embryo transfer procedure). Analysis of the spare embryos which were not transferred showed that in 8% the original result could not be confirmed. This may have been due to chromosomal mosaicism, as the majority of clinics performing PGD-AS take only one cell, but FISH failures cannot be ruled out.

The cumulative data for the three years show that 796 cycles reached the stage of OR, and the majority of these used acid Tyrode's for zona drilling and cleavage-stage aspiration. Some 39% of embryos were diagnosed as transferable, and 618 embryo transfer procedures were performed, resulting in 199 clinical pregnancies (25% per OR and 32% per embryo transfer).

PGD for social sexing

For social sexing, the majority of patients were fertile (Table XIII). The average age was 36 years. All cycles except one used FISH for the PGD. One cycle used PCR, and from five oocytes collected, three were fertilized but no embryos were biopsied. A total of 78 cycles reached the OR stage, with the majority having IVF, laser drilling and cleavage-stage aspiration. From 1003 oocytes, 735 were fertilized, 623 embryos were biopsied, and 579 were successfully biopsied, of which 241 (41%) were diagnosed as transferable. A clinical pregnancy rate of 35% per OR was achieved, which is higher than any other PGD pregnancy rate. No information was given regarding the sex selected for.

Pregnancies and babies

Data on pregnancies and babies were obtained from 12 centres this year. The number of pregnancies per centre varied between one and 39. Since the start of the data collection up to May 2001, data on 451 pregnancies, including 25 subclinical pregnancies were collected (Table XIV). In comparison with the previous data collection, the relative number of pregnancies resulting from FISH cycles (from 55 to 68%) has increased compared with PCR cycles (from 45 to 32%). This change is mainly due to the fact that in the data collection 2001, 82% of the pregnancies resulted from FISH diagnosis, 50% because of a pre-existing genetic risk (X-linked disease or parent carrier of a chromosomal anomaly) and the other 50% for aneuploidy screening.

The total number of clinical pregnancies registered is now 309, with 426 fetal sacs. A clinical abortion in the first trimester occurred in 47/426 (11%) of the cases. Three pregnancies were terminated after misdiagnosis at prenatal testing. One of the misdiagnoses reported this year was a social sexing cycle, where a female fetus was found at amniocentesis and subsequently the pregnancy was terminated. In a fourth misdiagnosis (a twin pregnancy) a selective reduction of one male fetus was performed; this cycle of sexing with PCR for X-linked disease was already 5 years old, but was reported for the first time in 2001. In the group of 256 pregnancies with normal evolution, 184 were singletons, 64 were twin pregnancies and eight were triplets.

In 157 of the 215 pregnancies which went to delivery, data on possible complications during pregnancy were completed (Table XV). In the other cases the field 'complications in pregnancy' was left blank. Presumably, there were no complications in these latter pregnancies, but for scientific correctness these blank fields were not included. Complications were present in 52/157 (33%) of the pregnancies. As expected, the number of complications was higher in the multiple pregnancies compared with singletons. It is obvious that premature contractions and premature rupture of membranes were the commonest complications and were closely related to multiplicity. Other

Table XII. Cycles performed for PGD-AS for 2001 and cumulative data

	Age 2001 only	Recurrent miscarriage 2001 only	Recurrent IVF failure 2001 only	Other*** 2001 only	Total for 2001	1999 + 2000	Cumulative data for all 3 years
Cycles	101	45	117	71	334	465	799
Cycles to OR	101	43	116	71	331	465	796
No. infertile	96	40	117	66	319	465	784
IVF	38	13	30	40	121	123	244
ICSI	63	30	92*	36	221*	342	563
Cancelled post OR	1	0	10	3	14	0	14
AT drilling	61	30	76	28	195	432	627
Laser drilling	39	13	30	38	120	11	131
Mechanical	0	0	0	2	2	22	24
PB biopsy	0	0	0	0	0	26[a]	26[a]
Cleavage aspiration	100	43	106	68	317	440	757
COCs	1418	593	1674	821	4506	6025	10 531
Inseminated	1281	522	1459	766	4028	5432[b]	9460
Fertilized	914	392	1040	540	2886	3755	6641
Biopsied	786	337	726	476	2325	2994	5319
Successfully biopsied	777	337	709	452	2275	2950[c]	5225
Diagnosed	739	300	650	397	2086	1859	3945
Transferable	250	133	255	208	846	676[d]	1522
Transferred	184	85	142	157	568	908	1476
Cycles to embryo transfer	78	38	74	60	250	368	618
Frozen	10	15	79	36	140**	7	147
Misdiagnosis of spares	11	4	14	5	34	–	–
HCG-positive	32	13	20	22	87	23[e]	112
Positive heart beat[f]	28 (28)	12 (28)	8 (7)	18 (25)	66 (20)	133 (28)	199 (25)

*Some cycles had IVF and ICSI.
**Some no result but frozen.
***Previous trisomy, two indications, male meiotic abnormalities, no indication.
[a]One cycle had polar body and cleavage-stage biopsy.
[b]Number of oocytes inseminated not accurate as some centres did not record this information. In cycles where the data are not recorded, the figure entered was the same as the number of oocytes collected.
[c]One centre (116 cycles) did not record the number of successful biopsies; hence the number recorded was the same as the number of embryos biopsied.
[d]One centre (116 cycles) did not record the number of embryos diagnosed as transferable.
[e]One centre (116 cycles) did not perform a HCG test and so this value was not available.
[f]Values in parentheses are % per cycle started.

Table XIII. Summary of social sexing data (77 cycles by FISH, one by PCR)

	Social sexing, 2001 only
Cycles to OR	78
Female average age	36 years
No. infertile	19
IVF	65
ICSI	13
Cancelled after OR	2
AT drilling	6
Laser drilling	70[a]
Mechanical	0
Polar body biopsy	0
Cleavage aspiration	76[a]
COCs	1003
Inseminated	996
Fertilized	735
Biopsied	623
Successfully biopsied	579
Diagnosed	519
Transferable	241
Transferred	133
Cycles to embryo transfer	64
Frozen	75
HCG-positive	28
Positive heart beat[b]	28 (35)

[a]Natural cycle.
[b]Value in parentheses is % per oocyte retrieval.

complications observed were bleeding, hypertension and (pre)-eclampsia. These data are comparable with those obtained in the previous data collection.

At birth, the incidence of multiple gestations was 27% (Table XVI). Caesarean section was more common in twin pregnancies (61%) and triplets (100%), while the Caesarean section rate in singletons was 37%. Prematurity (birth before or at 36 weeks) was observed in 6% of the singletons, 44% of the twin pregnancies, and 60% of the triplets. The male/female sex ratio was 0.65. The mean birthweight for the total group was 2856 g (n = 237), mean length was 48 cm (n = 132), and mean head circumference was 33.3 cm (n = 89), which is comparable with the values in the previous data collection. Mean birthweight was lower in the twins and triplets than in the singletons. Apgar scores were normal in the majority of children (Table XVII). This year, only one major malformation was reported, phocomelia and pulmonary deficiency in one child of a triplet pregnancy. In total, major malformations were found in seven children (Table XVIII), leading to neonatal death in two cases. The total perinatal mortality rate was 3/180 (16 per 1000). Prematurity was the most frequent neonatal complication noted, and occurred mainly in the multiple pregnancies.

Table XIX provides an overview of the confirmations of diagnosis. The PGD diagnosis was confirmed in 226/451 (50%) of all fetal sacs. Prenatal testing was performed in 122/

Table XIV. Evolution of pregnancy

	n		*n*
Pregnancies			451
FISH cycles			305
PCR cycles			146
Subclinical pregnancy[a]			25/451 (5.5)
Clinical pregnancies	309	Fetal sacs	426
Singletons	212/309 (69)		212
Twins	78/309 (25)		156
Triplets	18/309 (6)		54
Quadruplets	1/309 (0.3)		4
First trimester fetal loss			47/426 (11)
No heartbeat			23
Extrauterine pregnancy			4
Miscarriage			19
Artificial abortion			1
Vanishing twins/triplets			20/426 (5)
Ongoing pregnancies (⩾12 weeks)	266	Fetuses	359
Second trimester pregnancy loss	10/266		14/359
TOP for misdiagnosis	3		4[b]
TOP after amniocentesis	1		1[c]
Second trimester miscarriage	3		5
Stillbirth	2		2
Premature rupture of membranes	1		2
Reductions of multiple pregnancies (no pregnancy loss)			9/359
Triplet → twin			3
Triplet → singleton			4
Quadruplet → twin			2
Normal evolution	256/309 (83)	Fetuses	336
Singletons	184/256 (72)		184
Twins	64/256 (25)		128
Triplet	8/256 (3)		24
No follow-up	9	Fetuses	14
Singletons	5		5
Twins	3		6
Triplets	1		3
Ongoing	32	Fetuses	43
Singletons	23		23
Twins	7		14
Triplets	2		6
Deliveries	215	Babies born	279
Singletons	156 (73)		156
Twins	54 (25)		108
Triplets	5 (2)		15

Values in parentheses are percentages.
[a]Subclinical pregnancy defined as pregnancy without any other clinical signs, but positive serum HCG.
[b]One misdiagnosis for sexing, FISH, female fetus, indication social sexing; one misdiagnosis for sexing, PCR, indication Duchenne, twin pregnancy, selective termination of male fetus. Cycle done in 1996, Y-specific amplification only, now improved by using X and Y amplification; one misdiagnosis beta thalassaemia, PCR; one misdiagnosis myotonic dystrophy, PCR.
[c]Trisomy 18 after amniocentesis, indication for PGD parent carrier of reciprocal translocation not involving chromosome 18.
TOP = termination of pregnancy.

Table XV. Complications in clinical pregnancies (n = 157)

Complication	Incidence
Total complications	52/157 (33)
Singletons	33/112 (27)
Twins	16/45 (35)
Triplets	3/3 (100)
Nature of complications[a]	
Hypertension	6 (3 singleton, 3 twins)
HELLP	2 (2 singletons)
Pre-eclampsia	4 (3 singletons, 1 twin)
Eclampsia	1 (1 twin)
IUGR	2 (2 singletons)
Polyhydramnios	1 (1 twin)
Oligohydramnios	2 (2 singleton)
Preterm contractions	19 (7 singletons, 9 twins, 3 triplets)
Premature rupture of membranes	6 (1 singleton, 5 twins)
Bleeding	11 (8 singletons, 3 twin)
Cervical insufficiency	1 (1 singleton)
Cerclage	1 (1 singleton)
Chorioamnionitis	1 (1 singleton)
Septic abortion	1 (1 twin)
Placenta accreta	1 (1 singleton)
Retroplacental haematoma	(1 singleton)
Diabetes	3 (3 singletons)
Idiopathic thrombocytopenia	1 (1 singleton)
Toxoplasmosis	1 (1 singleton)

[a]More than one complication reported in some pregnancies.
HELLP = Haemolysis, elevated liver enzymes, low platelet count syndrome; IUGR = intrauterine growth retardation.

Table XVI. Method of delivery and gestational age (n = 215)

	Total	Singleton	Twin	Triplet
No. delivered	215	156	54	5
Method of delivery				
Vaginal	101 (47)	88 (56)	13 (24)	–
Caesarean section	95 (44)	57 (37)	33 (61)	5 (100)
Unknown	19 (9)	11 (7)	8 (15)	–
Gestational age at delivery				
Preterm	37 (17)	10 (6)	24 (44)	3 (60)
At term	163 (76)	140 (90)	21 (39)	2 (40)
Unknown	15 (7)	6 (4)	9 (17)	–

Values in parentheses are percentages.

305 (40%) of the FISH group and in 65/146 (44%) of the PCR group. Until now, a total of six misdiagnoses have been reported after prenatal diagnosis, one in the FISH group (female karyotype after social sexing) and five (of which two for sexing with PCR) in the PCR group. Four of these six were terminated while two went to term (one for cystic fibrosis, one for X-linked retinitis pigmentosa). In addition, two misdiagnoses have been recorded post-natally. One was a 47,XX,+der t(11;22)(q23;q21) in a miscarriage of a parent with a balanced translocation (11;22); the other misdiagnosis was a trisomy 21 in an aneuploidy screening cycle. The total rate of misdiagnoses can now be calculated as 8/451 (1.8%), and is higher in the PCR group (5/145, 3.4%) than in the FISH group (3/305, 0.9%).

Discussion

Referrals

It is encouraging to see that the quantity of referral data, which contain specific information about the patient's reproductive history, reasons why they choose PGD, and centre's decision concerning the acceptance of the patient for treatment, sent in this year has increased, as it has been particularly difficult to obtain these data. Many centres receive most of their patients through referrals via other centres and have very few if

Table XVII. Data on live-born children

	Data available (*n*)	Data not available (*n*)
Total children born	279	
Sex		12/279
Male	105	
Female	162	
Mean birthweight (g)	2856 (*n* = 237)	42/279
Singletons	3196 (*n* = 141)	
Twins	2412 (*n* = 84)	
Triplets	1987 (*n* = 12)	
Mean birth length (cm)	48.0 (*n* = 132)	147/279
Mean head circumference (cm)	33.3 (*n* = 89)	190/279
Apgar scores		148/279
Good[a]	125	
Poor	6	

[a]Good Apgar score is ⩾8.

Table XVIII. Congenital malformations and neonatal complications at birth

Malformation	Data available (*n*)	Data not available (*n*)
No malformations	168/180	99/279
Malformations	12/180 (6.6)	
Major[a]	7/180 (3.9)	
Phocomelia and pulmonary deficiency, one child of triplet pregnancy		
Chylothorax, neonatal death		
Congenital hip dislocation		
Cystic mass in abdomen		
Pes equinovarus: 2		
Exencephaly, one child of twin pregnancy, neonatal death		
Minor	5/180 (2.7)	
Syndactyly, mother also		
Hydrocele testis		
ASD		
Mongolian spot		
Sacral dimple		
No neonatal complications	104/180	99/279
Neonatal complications	76/180 (42)	
Premature	67/180	
Prematurity and complications	10/67	
Neonatal observation: 4		
Artificial respiration: 2		
PDA: 1		
Neonatal deaths: 3 (1 intracranial bleeding, 1 exencephaly, 1 chylothorax)		
Dysmature	6/180	
Pneumothorax	1/180	
Respiratory problems unspecified	1/180	
Neonatal observation because of poor Apgar score, in term baby	1/180	

Values in parentheses are percentages.
[a]Major malformation defined as malformation that generally causes functional impairment or require surgical correction (Holmes, 1976).
ASD = atrium septum defect; PDA = persistent ductus arteriosus.

no information on the patient themselves, but only on the embryological and genetic data of a particular cycle. The Steering Committee has always seen this part of the data as an important means of understanding the motives for patients to accept PGD, and the choices of the centres as to which patients will be accepted. Although it is still suspected that most referral data are from patients who actually came through for PGD cycles—as can be concluded from the fact that most patients are accepted—interesting information is gathered nevertheless. Most PGD patients have experienced a catastrophic reproductive history, with repetitive miscarriages, the birth of affected children and/or serial terminations of pregnancy. Clearly, the indications are mirrored by the indications for the actual cycles. The Steering Committee will in future consider changing the referral data collection as some founded criticism has been vented, for example regarding the minimal data needed to call a request for information an actual referral, and the way in which the referrals are handled if PGD for this particular disorder is not possible at the moment of consultation, but where development of the test will be undertaken in the foreseeable future.

Cycles

Currently, a growing number of PGD cases are being performed. This year, it was useful to have details of the patient's infertility and the female's age. However, the Consortium need to clarify what is meant by infertility, as some groups feel that patients carrying chromosome abnormalities who are experiencing repeated miscarriages are infertile. There is still one case where IVF was used in a PCR cycle instead of ICSI, but this is an improvement on last year's data. The pregnancy rates are slowly improving. The use of the laser is increasing, there are few polar body biopsies (both backed-up by cleavage-stage biopsy for mitochondrial diseases), and no blastocyst biopsies. As discussed earlier, blastocyst biopsy may not be a viable option for PGD as a large number of embryos are required. Therefore, the majority of cases used cleavage-stage biopsy, with aspiration to remove the blastomere. The debate of whether to take one or two cells for the diagnosis is still under discussion. A growing number of embryos are being frozen in PGD cycles, but no clinical pregnancies have been reported from frozen–thawed embryos except one from comparative genomic hybridization (CGH) (Wilton *et al*., 2001). Another Australian group has reported good results for cryopreservation after embryo biopsy (Lalic *et al*., 2001), while other authors have found it to be detrimental (Magli *et al*., 1999).

It has been useful in this report to have a breakdown of the PCR and translocation data, and it is hoped to continue this in the future and apply this to all the collected data.

A very high number of PGD cycles was for patients carrying chromosomal abnormalities, as these patients often experience repeated miscarriages and many feel that PGD is their only hope of having a normal child (Munné *et al*., 2000). Current protocols using either a combination of telomeric and centromeric probes (Scriven *et al*., 1998; Munné *et al*., 2000) or cell conversion (Verlinsky and Evsikov, 1999), makes this test much more simple than previous protocols. Another problem is that a high number of abnormal embryos are often found in these cases, reflected by the low number of transferable embryos and reduced number of cycles with an embryo transfer procedure. A separate publication on this matter is on the agenda of the Steering Committee.

The number of PGD cycles for sexing for X-linked disease has decreased as compared with previous years. This might be explained by the fact that, as more X-linked diseases are characterized at the molecular level, patients would opt for

Table XIX. Confirmation of diagnosis per fetal sac

Prenatal diagnosis				Post-natal diagnosis			
Method	Result			Method	Result		
	n	Normal	Abnormal		*n*	Normal	Abnormal
FISH				*FISH*			
CVS	30	–	–	Karyo miscarr.	7	2	5[c]
Amnio	90	–	–	Karyo post-natal	16	15	1[d]
Unknown	2	–	–				
Total	122	115	2[a]	Total	23	17	6
PCR				*PCR*			
CVS	37	–	–	DNA test miscarr.	2	2	–
Amnio	26	–	–	DNA test post-natal	10	10	–
Unknown 2	–	–		Sweat test	4	4	–
Total	65	60	5[b]	Total	16	16	–
FISH fetal sacs tested 122/305 (40%)				FISH fetal sacs/babies tested 23/305 (7.5%)			
PCR fetal sacs tested 65/146 (44%)				PCR fetal sacs/babies tested 16/146 (11%)			
Total prenatal testing 187/451 (42%)				Total post-natal testing 39/451(9%)			
Total confirmation of diagnosis 226/451(50%)							

[a]One trisomy 18, indication for PGD reciprocal translocation; one misdiagnosis for sexing, female fetus, social sexing.
[b]One misdiagnosis XL Duchenne (selective termination, twin pregnancy, cycle in 1996); one misdiagnosis beta thallassaemia (terminated); one misdiagnosis cystic fibrosis (ongoing); one misdiagnosis XL retinitis pigmentosa (ongoing); one misdiagnosis myotonic dystrophy (terminated).
[c]One trisomy 16; one trisomy 22; one mosaic trisomy 22; one monosomy X; one misdiagnosis, 47,XX,+der t(11;22)(q23;q21), parent carrier balanced translocation.
[d]One misdiagnosis, trisomy 21, aneuploidy screening
CVS = chorionic villus sampling; FISH = fluorescence in-situ hybridization.

specific DNA diagnosis rather than simple sexing. However, the drop in sexing with FISH is not reflected in a rise of specific PCR-PGD diagnoses. It was reassuring to see that the low pregnancy rate achieved in the first report (only 7%) has now risen to 20% per OR, which is comparable with the pregnancy rate for social sexing.

As for the other FISH and PCR cycles, the centres were requested to specify the indications for PGD-AS more clearly. As this was done at a later stage in the data collection, it was still not possible to break down the data this year. In the next data report it is hoped that it might be possible to separate out those patients who have had a previous abnormal pregnancy, but this could not be done in the present report as some centres had not made this clear. The strikingly low pregnancy rate in the group of recurrent IVF failure (7%) raises certain comments. First, the Consortium here fulfils one of its roles, namely to identify problems which may not be apparent when smaller groups are considered. Second, as was emphasized by one of the members, these patients had 0% success before treatment, and even though 7% is still low when compared with other types of PGD-AS, it is still an improvement over these patients' original prognoses. In addition, many centres use this procedure to give some closure to these patients, by showing them that the reason for IVF failure is, in many cases, a lack of chromosomally normal embryos.

What can be remembered from the analysis of the PCR cycles, is that autosomal recessive diseases have a better outcome than autosomal dominant diseases. This is of course very simply explained by the fact that in the first situation 75% of the embryos are expected to be without the disease in question, while in the latter there are only 50%. This basic fact of Mendelian inheritance also explains why patients carrying an autosomal dominant disease will more rapidly turn to PGD, since their risk for an affected fetus at prenatal diagnosis is higher. This fact is even more to the point for patients who are at a combined risk for an autosomal recessive disease and an X-linked disease (in this case Fragile X). It can be calculated that these patients have one chance in four for a pregnancy affected with the autosomal recessive disease, and one chance in two for a pregnancy affected with Fragile X, leading to the chance of five out of eight that a pregnancy would be affected with either or both of the two genetic diseases. The high number of cycles which had to be cancelled for Fragile X, is altogether not surprising. It is known that carriers of premutations suffer from premature ovarian failure, and the fact that these patients are difficult to stimulate may be a reflection of this (Sherman, 2000). A new category of disease for which PGD has been performed are the mitochondrial diseases, which are especially challenging because of their complex inheritance patterns which are difficult to predict. Further results and developments for mitochondrial diseases are awaited with much interest.

For the first time, three centres submitted data after having performed PGD for social sexing. A survey among the Consortium members showed that from the 21 centres that replied, 15 were against social sexing, while four were in favour (only one of the three centres performing sexing replied). Two centres did not clearly mention their opinion. Among the arguments for sex selection, the right to self-regulation of countries was mentioned. One of the respondents, who

answered in a personal capacity and not for his/her centre and who was in favour of sexing, attached a few conditions in that: (i) sexing should be used for family balancing; (ii) there should be a balance in the sex ratio within one centre and within one year (as many cases for boys as for girls); (iii) healthy embryos (i.e. embryos screened for aneuploidy as well as gender) of the unwanted sex should be donated to other couples; and (iv) patients should themselves pay for the treatment. The main argument put forward by the centre which is performing social sex selection and which responded, was that elimination of embryos of the unwanted sex was better than to perform abortion. As an argument against sexing, it was mentioned that PGD and prenatal diagnosis (PND) should only be used for serious genetic diseases and not for eugenics. One respondent also called social sexing child rights abuse, another argument was the cost to society of social sexing, and finally the influence of social sexing on the sex ratio balance was mentioned.

The Consortium members were also asked if they felt that the data from the three centres should be published in this report. Seventeen of the responding centres were in favour of publishing the data on the cycles for social sexing, and four were against. The following arguments in favour of publishing were given: (i) data on efficiency (of PGD in general) are much needed; (ii) not publishing these results would amount to censorship; (iii) PGD for social sexing should be put into context in relation to the other forms of PGD; (iv) the suppression of data would lead to much criticism if it was found out; and (v) reporting of the data should allow open debate. Some centres requested conditions to the publishing of the data. The first request was to include sex ratio at birth; this was not be possible for the current data collection, but could be added in a later data collection round. The second request (made by two centres) was to add the names of the centres performing social sexing. As the PGD Consortium collects data on an entirely voluntary and anonymous basis, this is not possible. However, as it seems that the main fear of the centres with this second request is to be associated with the centres performing social sexing, a clear statement of the general opinion within the PGD Consortium should alleviate this fear. Four centres were against publication because: (i) the publication could have a negative effect on the decision-making in their home country (or other countries where PGD is still being discussed); (ii) the PGD Consortium should not support, approve or include these centres, and hence their data should not be published; (iii) publishing these data would attract unnecessary controversy; (iv) publishing data on social sexing was akin to using data from nazi experiments; and (v) some centres do not want to be associated with social sexing. Two French centres have taken a very radical view against social sexing, and even against the publication of the results. Their statement can be found in a Letter to the Editor in this issue (Ray *et al*., 2001).

In conclusion, although most PGD Consortium members are against PGD for social sexing, most are in favour of publishing the data collected through the centres that are performing social sexing. As the fear was raised that social sex selection in embryos may be associated with ESHRE, the chairman of ESHRE as well as representatives from the Special Interest Group on Law and Ethics were consulted on the issue. Both agreed to the publication of results because selective withholding of data would be unethical. ESHRE has not yet issued a statement or guidelines concerning social sex selection, in contrast to the ASRM (Ethics Committee of the American Society of Reproductive Medicine, 1999), but will hopefully do so in the near future.

Pregnancies and babies

This year, there has been a shift in the indications towards more pregnancies after PGD using FISH, and this is due to the larger number of babies born after aneuploidy screening. This was to be expected as the same shift is present in the cycle data. With regard to the complications during pregnancy and at birth and the data of the children at birth, no changes can be noted compared with former data collections and comparable groups of ICSI children (Bonduelle *et al*., 1999; ESHRE PGD Consortium Steering Committee, 2000). Again, the high proportion of multiplets, leading to an important number of the pregnancy complications and premature babies is something which much be warned against. This year again, a number of misdiagnoses, both prenatally and post-natally, are to be regretted. The Steering Committee feels that the time is now ripe to draw conclusions from the data gathered over 3 years, and that a start should be made with the formulation of guidelines specific for the practice of PGD.

Future prospects

This year there has been much discussion within the ESHRE PGD Consortium about two aspects of the data: social sexing (as discussed above); and the follow-up of the cycles resulting in pregnancy. It has been noted that from the first two Consortium reports, the clinical pregnancies reported in the cycle data could not be followed through in the pregnancy and baby data. This has mainly been due to centres not completing pregnancy and baby sheets for all their pregnancies reported in the cycle data. Two novelties will be introduced to eliminate this problem. First, Moutou and Viville (Strasbourg, France) have developed a database based on FileMaker Pro 5, which will allow the Consortium to collect the same data as collected hitherto, as well as an automatic calculation of results and linking of the referral sheet, cycle sheet and pregnancy sheet of one and the same patient. The second innovation is the timing at which the data will be collected. Until now, cycles for which the outcome was known as well as pregnancies and babies were collected from May to May of the next year. From now on, cycles collected during a given calendar year will only be collected in October of the next year, so that any ensuing pregnancies should be completed by the time of data collection and could also be reported at the same time as the relevant cycle. The first method allowed for a quick collection of data, which was the first aim of the PGD Consortium, while the second method will hopefully yield more complete results, albeit at a later time. Therefore, this will be the final year where the data are reported in this manner, and from next year onwards all cycles resulting in pregnancies will be completed with the data on the children born. The Steering Committee

intends to spend the coming year cleaning up the existing data and filling any gaps left so that, for the next report, a more complete and accurate overview will be given of PGD and its outcome.

As the use of a more efficient database will give the Steering Committee more time, the conduction of retrospective studies will be taken up again. To this end, all members have received a list of possible studies to which they were invited to participate. We hope to be able to present the first results of these studies in the next report.

Finally, a 'PGD-mail' was set up, which is similar to the broadly known 'embryo mail', and which would serve the same aims, i.e. to be a forum for exchange of information and for discussion on PGD. J. Harper is currently coordinating PGD mail, and so registration to PGD mail should be addressed to her (joyce.harper@ucl.ac.uk).

In conclusion, the data collected this year confirmed the trends that were already apparent in the two former reports. The appearance of sex selection for social reasons has generated quite a debate within the Consortium, and we expect this to continue with the publication of this report. The Steering Committee hopes to be able to implement a series of improvements to the data already gathered and the data to be collected in the future, and ultimately to offer complete and reliable data to PGD practitioners and their patients alike.

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