

ESHRE PGD Consortium data collection IV: May–December 2001

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The ESHRE PGD Consortium was formed in 1997 to survey the practice of preimplantation genetic diagnosis (PGD). Since then, three reports have been published giving an overview on PGD from an ever-increasing number of centres and reporting on an increasing number of PGD cycles and pregnancies and babies born after PGD. After these initial influential publications, important shortcomings were identified primarily on the method of data collection, i.e. with Excel spreadsheets, and in the timing of the collection (cycles were collected in a different time frame from pregnancies and babies, making the follow-up of cycles very difficult). This is why the Steering Committee has made a major investment in developing and implementing a new database in FileMaker Pro 6. It was also decided that cycles would be collected from one calendar year, as well as the pregnancies and babies ensuing from that particular calendar year. This gave us the opportunity to take a closer look at the data collected earlier, and to attempt to improve their quality. This is a report on the corrected data from the first three data collections (I–III) as well as the result of the last data collection (IV) that was completely carried out using the new database.

Key words: data collection/ESHRE/PGD

Introduction

Preimplantation genetic diagnosis (PGD) is a very early form of prenatal diagnosis. Polar bodies or blastomeres are retrieved from fertilized oocytes or embryos and genetically analysed, followed by the selective transfer of embryos free of the disease under consideration. Two types of PGD can be distinguished: PGD for patient couples with a high *a priori* risk of an abnormal conceptus (e.g. carriers of monogenic diseases or chromosomal abnormalities) (Sermon *et al.*, 2004) and PGD for aneuploidy screening where the number of a selection of key chromosomes is checked aiming to increase the pregnancy rates in IVF patient groups with a poor prognosis (Wilton, 2002). This latter type of PGD will be called preimplantation genetic screening (PGS) throughout this manuscript.

One of the most prominent aims of the ESHRE PGD Consortium, that was formed at the ESHRE Annual Meeting in 1997 in Edinburgh, has been to collect detailed data on the practice of PGD. Therefore, PGD centres were asked to

become members and to give data voluntarily on the patient referrals, PGD cycles, ensuing pregnancies and babies. These data were collected, first on paper forms, and later on Microsoft® Excel spreadsheets (ESHRE PGD Consortium Steering Committee, 1999, 2000). Because the Steering Committee judged that speed was of importance in the early phase, data on cycles and on pregnancies and babies were collected from the same time frame. Thus, by the time the cycles were collected, the ensuing pregnancies had not reached term, and the pregnancies collected were from cycles from previous collections. This, and the limitations inherent to the spreadsheet, made it very difficult to link any given cycle to its pregnancy and further outcome (ESHRE PGD Consortium Steering Committee, 2002). To remedy this problem, two important decisions were made: the first was that a more user-friendly database would be developed, that would also allow the easy link between the different stages of a PGD treatment. The second decision was that the cycles from a complete calendar year would be collected, as well as the ensuing pregnancies

and babies in the following year until the end of October. The shift in timing led to the fact that no data would be collected the year following the decision, and this gave the Steering Committee the opportunity to take a closer look at the existing databases and to identify and correct shortcomings in these data.

In this report, two sets of data are presented. The first set, referred to as data I–III, concerns an updated corrected version of the data presented in the former reports (ESHRE PGD Consortium Steering Committee, 2002). The second set, referred to as data IV, was collected using the FileMaker Pro 6™ (FP6) database. It concerns cycles carried out between May 2001 and December 2001, and the pregnancies and babies ensuing from these cycles (i.e. born before October 2002).

Materials and methods

Data I–III

The data obtained during the former rounds of data collection (ESHRE PGD Consortium Steering Committee, 2002) were stored in three different spreadsheets: one for the referrals, one for the cycles and one for the pregnancies and babies. The data were divided into several data subsets, and a member of the Steering Committee corrected each subset. Mistakes and missing data were identified in the Excel spreadsheets and centres were re-contacted to correct or complete the data. Most importantly, a significant effort was made to link the cycles obtained during the three rounds of data collection with the pregnancies and babies.

Referral data were not corrected and re-analysed.

Data IV

A database was designed in FP6 by C.M., which contained the following forms. (i) A referral form with information on the indication and the patient's reproductive history, as well as the centre's and the patient's decision to go ahead with PGD. (ii) A form with information on the PGD cycle, such as cycle and patient identification, indication, type of ART and biopsy methods, and details on the biological material available. Relevant data entered in the referral form were automatically transferred to the cycle form. (iii) Frozen cycle form: this form is linked to the fresh cycle and can be introduced at several points in the fresh cycle, such as freezing before or after biopsy. (iv) Pregnancy form: this form also automatically takes over relevant data from the cycle form, and contains information about ultrasound data, prenatal diagnosis, possible miscarriages or other reasons for pregnancy loss, and date and gestational age at birth. (v) Baby form: contains data on the first month after birth and records basic data such as the birth weight of the baby, as well as possible malformations or neonatal complications. Each of these five forms is linked, allowing for the easy identification of, for example, the PGD cycle that led to a given pregnancy and birth. The PGD centres were sent these five forms along with a clear instruction manual, and were asked to fill in the referrals and cycles covering the period from May 1, 2001 until December 31, 2001 (except for the newly joined centres who were asked to give all their data from the beginning of their activities) and the data on the pregnancies and babies ensuing from these data. The total data were again divided into subsets, and the data were checked for mistakes and missing data. The corrected FP6 files were sent to the participating centres.

Results and discussion

The results are shown in the tables, and only highlights and important trends will be discussed in the text. Thirty-six centres sent in their data, while 15 failed to do so. Eleven centres were new members of the Consortium and/or had not started their PGD activity in 2001 and it is anticipated that they will send in data for the next data collection.

As mentioned above, the referral data I–III were not re-analysed.

Referral data IV

Due to the change in the way the data collection was carried out in the study period, a direct comparison between the absolute numbers of previous data collections and this round are only valid for a few items.

Looking at the referrals according to indication, it is clear that chromosomal indications are the main reason for referral during the present data collection (Table I).

From Table II, it is clear that referrals for chromosomal disorders changed drastically. The structural disorders showed a ratio of reciprocal:Robertsonian translocations of ~4:1 in the past, which changed to ~3:2 on the most recent occasion. Furthermore, the number of referrals for Robertsonian translocations showed an absolute increase. The referrals for numerical chromosome abnormalities and PGS changed even more. The number of indications tripled. Table III shows that genetic risk and previous termination of pregnancy (TOP) or objection to TOP have both decreased as a reason for the PGD. On the other hand, it is clear that PGS is at present the major indication for PGD.

Table I. Referrals according to indication

	III	IV
Chromosomal (numerical/structural)	647	1056
X-linked	294	103
Autosomal recessive	290	84
Autosomal dominant	254	62
Mitochondrial	6	0
Two indications	9	2
Y chromosome deletion	2	1
Social sexing	30	38
Unknown	29	45

Table II. Referrals for chromosomal disorders

	III	IV
Structural chromosomal aberrations		
Reciprocal translocation	252	120
Robertsonian translocation	61	80
Inversion	12	8
Deletion	6	0
Numerical chromosome aberrations		
Aneuploidy risk	249	811
47,XXY; 47,XYY	25	3
Sex chromosomal mosaicism	16	17
Male meiotic abnormalities	14	6
Other	4	2
Unknown	8	9

Table III. Reasons for PGD

	III	IV
Genetic risk and previous TOP	21.1%	8.3%
Genetic risk and objection to TOP	36.2%	20.6%
Genetic risk and sub- or infertility	25.6%	18.0%
Genetic risk and sterilization	1.0%	0.2%
Age-related aneuploidy	14.2%	53.7%
Other	6.4%	19.7%

This rise in the number of PGS cycles is reflected in the reproductive histories. The percentage of couples having experienced one or more TOPs has dropped from 21 to 11%. The percentage of couples with spontaneous abortions has increased from 28 to 40%. The combined results show that 22% of all couples had healthy children at the time of referral in previous data collections. This percentage has dropped to 14%. These changes are due mainly to the large percentage of referrals for PGS: if these numbers are calculated for the PGD cases excluding PGS (i.e. including sexing, monogenic and chromosomal aberrations), the following figures are obtained: 26% of the couples have experienced at least one TOP, 40% have experienced at least one spontaneous abortion (this is due mainly to the reciprocal translocations) and only 17% of the couples have at least one healthy child.

Because of the increase in referrals for chromosomal disorders, the proportion of referrals for monogenic disease has decreased, but the numbers of referrals have remained more or less constant if one takes into account that the study period comprised only 8 months. The most frequent referrals for X-linked disease were fragile X syndrome, Duchenne's or Becker's muscular dystrophy and haemophilia.

For autosomal recessive diseases, the most frequent referrals were similar between data collections: cystic fibrosis/congenital bilateral absence of vas deferens, β -thalassaemia and spinal muscular atrophy (SMA). Also among autosomal dominant disease, myotonic dystrophy and Huntington's chorea remained the two most common reasons for referral.

Due to the bias in the data collection (see below), it is easily explained that centres accepted the patients for PGD in almost all cases. In nearly every case it was concluded that the patient was suitable for IVF, and that a diagnosis was technically possible and ethically acceptable.

The most important reason for the couples to refrain from treatment has changed from inconvenience and/or burden of the ICSI or IVF procedure to the low success rate of the procedure. The occurrence of a spontaneous pregnancy and the treatment costs were minor reasons for declining.

The change in the method of data collection that was introduced between collections III and IV has resulted in a significant shift in the completeness of the referral data. Previously, most referrals were reported at the time of initial contact between the couple and the centre. Since the retrospective data collection was introduced, all data are collected at one point in time, which sometimes is separated by more than a few years from the time of referral. Furthermore, retrospective data collections are directed preferentially towards those referrals which have resulted in treatment. Therefore, a

number of the changes observed can be explained by the different methods of data collection. This partly includes the reason for referral. However, it is clear that the group of patients is changing dramatically. The number of chromosomal indications is growing exponentially. This is due primarily to PGS, mainly for advanced maternal age. Therefore, the data collection is split up into PGD for high-risk situations and PGS.

Data on cycles (Tables IVa and b)

Tables IVa and b summarize the cycle data for collections I–III and IV. The data for I–III, which were published previously (ESHRE PGD Consortium Steering Committee, 2002), differ slightly from the original report as some errors were found.

Chromosomal abnormalities I–III and IV (Tables Va and b). Tables Va and b summarize the 393 and 340 cycles collected for data collection I–III and IV, respectively. Overall there was very little difference between the two data collections. Reciprocal translocation was the most frequent class of chromosome aberration; ICSI was the predominant mode

Table IVa. Overall cycle data collection I–III

Indication	PGD	PGS	PGD-SS	Total
Cycles to OR	1337	801	93	2231
Female age	32	37	33	34
Number infertile	286	772	19	1077
ART method				
IVF	222	240	72	534
ICSI	1111 ^a	554	21	1686 ^a
IVF + ICSI	2	7	0	9
Frozen	2	0	0	2
Cancelled post-OR	46	14	5	65
Cycles to PGS/PGD	1292	787	88	2167
FISH	671	787	78	1536
PCR	621	0	10	631
Zona breaching				
AT drilling	927	618	19	1564
Laser drilling	352	145	69	566
Mechanical	13	24	0	37
Biopsy method				
Polar body biopsy	5 ^b	26	0	31 ^b
Cleavage aspiration	1272 ^b	763	88	2123 ^b
Cleavage extrusion	17	0	0	17
Embryology				
COCs	18 330	10 569	1185	30 084
Inseminated	16 103	9456	1153	26 712
Fertilized	11 533	6675	826	19 034
Biopsied	8986	5345	708	15 039
Successfully biopsied	8766	5246	647	14 659
Diagnosed	7596	3966	569	12 131
Transferable	3139	1637	254	5030
Transferred	2255	1491	146	3892
Cycles to ET	1078	626	73	1777
Frozen	530	284	93	907
HCG positive	317	212	28	557
Positive heart beat (% per OR)	252 (19%)	164 (20%)	28 (30%)	444 (20%)

OR = oocyte retrieval; AT = acidic Tyrode's; COC = cumulus–oocyte complex.

^aThree cycles were cancelled after OR and before ICSI (immature oocytes or no sperm found on TESE).

^bOne cycle had polar body biopsy and cleavage stage biopsy for a combination of cystic fibrosis and PGS.

Table IVb. Overall cycle data collection IV

Indication	PGD	PGS	PGD-SS	Total
Total cycles	707	1094	89	1890
Cycles to OR	660	1075	84	1819
Female age	33	37	37	36
Number infertile	299	1002	0	1301
ART method				
IVF	56	115	37	208
ICSI	570	909	46	1525
IVF + ICSI	0	3	0	3
Frozen	12	4	5	21
ICSI + frozen	3	1	1	5
Unknown	19	43	0	62
Cancelled post-OR	38	63	9	110
Cycles to PGS/PGD	610	1012	80	1702
Unknown cycle outcome	11	0	0	11
FISH	399	1012	37	1448
PCR	222	0	43	265
Zona breaching				
AT drilling	364	522	0	886
Laser drilling	155	388	37	580
Mechanical	83	37	43	163
Unknown	15	0	65	80
Biopsy method				
PB biopsy	6	45	0	51
Cleavage aspiration	559	756	37	1352
Cleavage extrusion	34	143	43	220
Cleavage flow displacement	2	16	0	18
Unknown	16	52	0	68
Embryology				
COCs	9067	13 805	1104	23 976
Inseminated	7518	11 474	877	19 869
Fertilized	5555	8327	585	14 467
Biopsied	4019	5663	485	10 167
Successfully biopsied	3942	5514	462	9918
Diagnosed	3510	5079	447	9036
Transferable	1235	1988	215	3438
Transferred	956	1441	158	2555
Cycles to ET	492	716	58	1266
Frozen	103	152	35	290
HCG positive	141	219	28	388
Positive heart beat (% per OR)	110 (16%)	172 (16%)	16 (19%)	298 (16%)

OR = oocyte retrieval; AT = acidic Tyrode's; COC = cumulus–oocyte complex.

of fertilization; and acidified Tyrode's with cleavage aspiration was the predominant sampling method. A global average of 15 cumulus–oocyte complexes (COCs) per oocyte retrieval (OR) cycle was collected for both the I–III and IV data collections; the fertilization rate (72 and 75%), the proportion of biopsied embryos diagnosed (88 and 89%), the proportion of biopsied embryos with a transferable result (25 and 25%), and the HCG-positive (21 and 19%) and positive heart beat (16% and 14%) pregnancy rate per OR were similar.

The relatively low pregnancy rates are likely to reflect that the low proportion of embryos with a transferable result limits the choice of embryo for transfer (approximately one in four embryos biopsied for all classes of chromosome aberration, and only one in five embryos for reciprocal translocation cycles in particular). Tables with the full list of karyotypes for which PGD was offered can be found in the electronic version of this paper available at *Human Reproduction Online* (Tables Vc and d).

Sexing (Table VIa and VIb). The data of sexing only (I–III) using fluorescence *in situ* hybridization (FISH) or PCR are presented in Table VIa. The most common disorders for which sexing only was performed were haemophilia A and Duchenne's muscular dystrophy with 71 cycles each, and X-linked mental retardation with 22 cycles. The centres performing sexing mainly used FISH for diagnosis (85%). A total of 350 cycles reached OR with an average of 13.4 oocytes per retrieval, 71% fertilized and 96% successfully biopsied. Two cycles with thawed embryos were also included. A diagnosis was possible for 88% of embryos successfully biopsied, and 80% of the cycles with OR had an embryo transfer with an average of 2.0 embryos transferred per cycle. A clinical pregnancy of 19% per OR was achieved. The number of embryos frozen includes 13 cycles where embryos that were not biopsied or where the diagnosis failed were cryopreserved.

Table VIb presents the data from data collection IV for sexing only using FISH and PCR. Sexing only was performed mostly for haemophilia A (27 cycles), Duchenne's muscular dystrophy (16 cycles) and Becker's muscular dystrophy (12 cycles). A total of 106 cycles had oocyte retrieval with an average of 14 oocytes. Three cycles on thawed embryos were included in the tables. The biopsy was successful in 96% of embryos biopsied and 90% could be diagnosed. There was a transfer in 70% of the cycles with OR with an average of 2.0 embryos transferred per cycle. The overall clinical pregnancy rate was 17% per OR. The number of cycles analysed is too small to draw conclusions. A list of indications for which PGD with sexing was performed can be found in the electronic version of the article (Tables VIc and d).

Monogenic disease (Tables VIIa and b). Tables VIIa and b summarize the data for the specific diagnosis performed using PCR. A breakdown is only presented for the most common disorders. Concerning the data collection I–III (Table VIIa), for autosomal recessive disorders, the most common indications for treatment were cystic fibrosis (197 cycles), β -thalassaemia (55 cycles), SMA (45 cycles), sickle cell anaemia (11 cycles) and epidermolysis bullosa (eight cycles). For the dominant disorders, myotonic dystrophy was the most common disorder treated (125 cycles), followed by Huntington's disease (66 cycles which included three cycles of exclusion testing), Charcot–Marie–Tooth disease (11 cycles) and Marfan's syndrome (10 cycles). Specific diagnosis for X-linked disease was performed most commonly for fragile X (27 cycles), Duchenne's muscular dystrophy (25 cycles) and haemophilia A (12 cycles).

Important points to note from these data are, for some of the recessive disorders, only half of the embryos were diagnosed as transferable, such as β -thalassaemia in which all of the 90 transferable embryos were transferred. This is disappointing as for these conditions it is estimated that 75% would be transferable. If fewer embryos are diagnosed as normal per cycle, it will reduce the choice of morphologically good quality embryos that can be transferred. A similar finding is observed for the X-linked disorders.

Table Va. PGD for chromosomal abnormalities, data collection I–III

Indication	Robertsonian translocations, male carrier	Robertsonian translocations, female carrier	Reciprocal, male carrier	Reciprocal, female carrier	Sex chromosome abnormalities	Other	Total
Cycles to OR	51	63	107	100	38	34	393
Female average age	32	32	33	32	33	33	32
Number Infertile	46	16	29	14	25	9	139
ART method							
IVF	3	10	18	39	5	13	88
ICSI	48	52	89	61	33	22	305
IVF + ICSI	0	1	0	0	0	0	1
Cancelled after OR	0	0	0	0	1	0	1
Cycles to biopsy	51	63	107	100	37	34	392
Zona breaching							
AT drilling	34	45	90	94	21	26	310
Laser drilling	17	18	16	5	16	8	80
Mechanical	0	0	1	1	0	0	2
Biopsy method							
Polar body biopsy	0	1	0	2	0	0	3
Cleavage aspiration	51	62	107	98	37	34	389
Embryology							
COCs	670	931	1761	1545	402	512	5821
Inseminated	587	775	1512	1390	357	476	5097
Fertilized	380	563	1107	1055	268	316	3689
Biopsied	250	482	904	936	192	252	3016
Successfully biopsied	244	471	878	913	190	241	2937
Diagnosed	189	434	809	841	174	213	2660
Transferable	76	118	187	192	96	84	753
Transferred	67	107	158	171	69	58	630
Cycles to ET	35	54	75	77	33	25	299
Frozen	2	5	7	0	7	2	23
HCG positive	10	17	17	18	9	11	82
Positive heart beat (% per OR)	9 (18%)	14 (22%)	13 (12%)	15 (15%)	4 (11%)	9 (26%)	64 (16%)

Overall there was an average of 13.2 oocytes collected per oocyte retrieval, the biopsy was successful in 99% of embryos, the diagnosis was possible in 83% of the embryos successfully biopsied, 84% of cycles to OR had an embryo transfer and 20% of these patients achieved a clinical pregnancy.

The following points can be noted from data collection IV (Table VIIb). The most common indications either for autosomal recessive, dominant or X-linked disorders have not changed. Of notice is the relative decrease in the number of cycles performed for cystic fibrosis since it represented two-thirds of the cycles for recessive disorders in data collection I–III and only one-third in the data collection IV. A similar observation can be made for the dominant disorders, where there is a relative decrease of the number of cycles for myotonic dystrophy. In this category, amyloid polyneuropathy appears for the first time, with 10 cycles performed by one centre. No cycles are reported for Duchenne's muscular dystrophy in the specific diagnosis for X-linked disease. This is quite surprising since 25 cycles were reported in the previous report and this method has the important advantage of allowing the transfer of male unaffected embryos.

Again, for some recessive disorders (cystic fibrosis and β -thalassemia), only about half of the diagnosed embryos are transferable. Such a distortion is also observed for myotonic dystrophy since only a third of the diagnosed embryos are transferable. A possible explanation could be the use of multiplex PCR that includes polymorphic markers

allowing the detection of different (chromosomal) abnormalities.

Overall there was an average of 12.9 oocytes collected per OR, the biopsy was successful in 99% of embryos, the diagnosis was possible in 86% of the embryos successfully biopsied, nearly 80% of cycles going to OR had an embryo transfer and 21% of these patients achieved a clinical pregnancy.

A list of monogenic diseases for which PGD was performed can be found in the electronic version of the article (Tables VIIc and d).

PGD with aneuploidy screening (Table VIIIa and b). Outcomes for PGS cycles are presented in Table VIIIa (collection I–III) and VIIIb (collection IV). The use of PGS has increased substantially, with almost 35% more cycles reported in data collection IV than in data collections I–III combined. The mean number of oocytes collected was 13.2 per retrieval in both data collections. The fertilization rate was 71 and 73% and the proportion of embryos successfully biopsied was 98 and 97% in data collections I–III and IV, respectively. In collection IV, a diagnosis was obtained in 92% of all embryos successfully biopsied. As described in Table VIIIa, it was not possible to calculate this figure accurately from data collection I–III.

There are a number of indications for PGS, and in both data collections the majority of patients fell into the categories of advanced maternal age, recurrent miscarriage or recurrent IVF failure. In data collection IV, there

Table Vb. PGD for chromosomal abnormalities, data collection IV

Indication	Robertsonian translocation, male carrier	Robertsonian translocations, female carrier	Reciprocal, male carrier	Reciprocal, female carrier	Sex chromosome aneuploidy	Other	Total
Total cycles	69	47	75	62	65	22	340
Cancelled	4	2	5	3	4	2	20
Cycles to OR	65	45	70	59	61	20	320
Cancelled after OR	0	0	1	0	3	0	4
Female average age	33	33	34	32	34	33	33
Number infertile	50	22	37	21	65	11	206
ART method							
IVF	4	4	10	11	4	6	39
ICSI	59	40	58	47	54	14	272
Frozen	0	0	1	1	0	0	2
ICSI + frozen	2	1	0	0	0	0	3
Cancelled after IVF/ICSI	3	2	6	1	2	3	17
Cycles to PGD	62	43	63	58	56	17	299
Zona breaching							
AT drilling	51	29	47	40	28	10	205
Laser drilling	11	11	10	11	4	3	50
Mechanical	0	3	6	7	24	4	44
Biopsy method							
Polar body biopsy	1	3	0	1	0	0	5
Cleavage aspiration	60	40	59	49	56	16	280
Cleavage extrusion	1	0	3	7	0	1	12
Cleavage flow	0	0	1	1	0	0	2
Embryology							
COCs	1044	721	1045	980	672	203	4665
Inseminated	865	593	892	799	519	159	3827
Fertilized	619	442	698	611	377	110	2857
Biopsied	397	318	545	484	287	91	2122
Successfully biopsied	386	309	539	478	285	89	2086
Diagnosed	333	274	495	451	246	86	1885
Transferable	120	81	102	95	96	39	533
Transferred	98	64	91	80	71	30	434
Cycles to ET	58	34	45	43	41	16	237
Frozen	2	8	0	0	6	0	16
HCG positive	16	10	9	9	12	4	60
Positive heart beat (% per OR)	13 (20%)	7 (16%)	7 (10%)	6 (10%)	8 (13%)	4 (20%)	45 (14%)

OR = oocyte retrieval; AT = acidic Tyrode's; COC = cumulus–oocyte complex.

were 65 cycles that were for male indications [abnormal FISH results in spermatozoa, male meiotic abnormalities, male infertility, e.g. oligo-, terato- and azospermia, with or without testicular sperm extraction (TESE) or micro-epididymal sperm aspiration (MESA)].

The overall pregnancy rate per OR in data collection I–III was 20% with the subgroups of advanced maternal age, recurrent miscarriage and recurrent IVF failure having pregnancy rates of 24, 30 and 10%, respectively. In data collection IV, the overall pregnancy rate per OR was slightly lower at 16%. The pregnancy rate for the recurrent IVF failure patients was 15%, which was slightly higher than in data collection I–III. However, in data collection IV, patients with advanced maternal age had a pregnancy rate of only 9%. This may be partly explained by the reduced number of oocytes recovered per patient in the advanced maternal age group in data collection IV compared with data collection I–III. In the first three rounds of data collection, advanced maternal age patients had an average of 12.7 oocytes collected, which resulted in an average of 8.2 embryos and there was a mean of 2.4 embryos per transfer. In data collection IV, advanced maternal age patients had an average of 9.4 oocytes collected which resulted in 5.9 embryos with a mean

of 1.9 embryos per transfer. The lower number of oocytes collected cannot be explained by a difference in maternal age, as this was 40 years in both data collections.

Social sexing (Table IXa and b). Table IXa shows the data of PGD for social sexing (data collection I–III). The centres performing PGD for social sexing mainly used FISH for diagnosis. The female average age was 33 years. A total of 93 cycles reached oocyte retrieval with an average of 12.7 oocytes per OR. IVF was used in 77% of the cycles. The embryos were biopsied using the laser in 88% and all embryos were cleavage stage aspirated. Out of 91% successfully biopsied embryos, 88% were diagnosed. There was an embryo transfer in 78% of the cycles with OR, which resulted in a clinical pregnancy rate of 30% per OR. An average of 2.0 embryos was transferred. This pregnancy rate is high compared with other PGD pregnancy rates. No information was provided regarding the sex selected for.

The results of PGD for social sexing (data collection IV) are presented in Table IXb. The female average age was 36.6 years. A total of 84 cycles had an OR with an average of 13.1 oocytes per retrieval. A diagnosis was possible in 97% of the successfully biopsied embryos and 69% of the cycles with OR had an embryo transfer, with an average of 2.7

Table VIa. Sexing only for X-linked disease using PCR or FISH, data collection I–III

	FISH	PCR	Total
Cycles to OR	297	53	350
Female average age	32	33	32
Number infertile	26	8	34
ART method			
IVF	102	10	112
ICSI	196	43	239
IVF + ICSI	1	0	1
Frozen	2	0	2
Cancelled after OR	20	0	20
Cycles to PGD	279	53	332
Zona breaching			
AT drilling	183	49	232
Laser drilling	91	0	91
Mechanical	5	4	9
Biopsy method			
Cleavage aspiration	276	53	329
Cleavage extrusion	3	0	3
Embryology			
COCs	3965	716	4681
Inseminated	3676	551	4227
Fertilized	2563	446	3009
Biopsied	2005	375	2380
Successfully biopsied	1942	339	2281
Diagnosed	1750	255	2005
Transferable	619	133	752
Transferred	459	103	562
Cycles to ET	235	44	279
Frozen	120 ^a	56 ^b	176 ^c
HCG positive	72	20	92
Positive heart beat (% per OR)	54 (18%)	14 (26%)	68 (19%)

OR = oocyte retrieval; AT = acidic Tyrode's; COC = cumulus–oocyte complex.

^aEleven cycles with embryos frozen without biopsy or after failed diagnosis included.

^bThirteen cycles with embryos frozen without biopsy or failed diagnosis included.

^cTwenty-four cycles with embryos frozen without biopsy or after failed diagnosis included.

embryos transferred. The pregnancy rate is 10% lower than for data collection I–III for social sexing and is comparable with other PGD pregnancy rates. Only 89 cycles are analysed and only two centres are involved. Consequently, it is difficult to draw conclusions from these data. There was no information regarding the sex selected for.

Pregnancies and babies data I–III

A significant effort was made to link the cycles with a positive HCG in the cycle database with the pregnancies and babies in the database. Five hundred and thirty-seven pregnancies were recorded, either in the cycle database or in the pregnancy database, or in both. In 204 of these, we only know that the cycle ended in a pregnancy, but no further data on the pregnancies and babies born were obtained. Data on 333 pregnancies were collected, of which 57 had no cycle data. This means that complete data concerning the cycle, the ensuing pregnancies and the babies born were collected for 276 pregnancies. The 333 pregnancies led to the birth of 315 babies (175 singletons, 128 twins and 12 triplets) or 243 pregnancies leading to birth.

The 333 pregnancies for which data are available are represented in Tables Xa, XIa, XIIa, XIIIa, XIVa and XVa.

Table VIb. Sexing only for X-linked disease using PCR or FISH, data collection IV

	FISH	PCR	Total
Total cycles	109	6	115
Cancelled before OR	6	0	6
Cycles to OR	102	4	106
Female average age	33	29	33
Number infertile	25	0	25
ART method			
IVF	13	0	13
ICSI	88	4	92
Frozen	1	2	3
Unknown	1	0	1
Cancelled post-OR	3 ^a	1 ^b	4
Cycles to PGD	100	5	105
Zona breaching			
AT drilling	53	1	54
Laser drilling	28	0	28
Mechanical	19	4	23
Biopsy method			
Cleavage aspiration	98	1	99
Cleavage extrusion	2	4	6
Embryology			
COCs	1389	92	1481
Inseminated	1143	76	1219
Fertilized	857	55	912
Biopsied	574	37	611
Successfully biopsied	548	37	585
Diagnosed	501	31	532
Transferable	149	19	168
Transferred	129	16	145
Cycles to ET	70	4	74
Frozen	37	0	37
HCG positive	20	2	22
Positive heart beat (% per OR)	16 (15%)	2 (33%)	18 (17%)

OR = oocyte retrieval; AT = acidic Tyrode's; COC = cumulus–oocyte complex.

^aTwenty-seven embryos from two cycles frozen before biopsy due to hyperstimulation;

^bTwenty embryos frozen before biopsy.

13 cycles sexing only with unknown indication.

As in previous reports, the data on the pregnancies and babies concerning pregnancy and birth parameters such as complications, malformations and birth weights are very comparable with ICSI babies (Bonduelle *et al.*, 2002; Van Steirteghem *et al.*, 2002). Of note is of course the high number of multiples (30%), leading to a high rate of prematurity (19% overall; 46% in the twins and triplets) and a low average birth weight (2885 g overall; 3279 g for singletons, 2500 g for twins and 1361 g for triplets).

The misdiagnoses have already been discussed in ESHRE PGD Consortium Steering Committee, 2002 (Table XVa). However, more information has become available on the affected pregnancy with chromosome imbalance 47,XX,+ der(22)t(11;22)(q23.3;q11.2)mat following PGD for the 'common' reciprocal translocation between chromosomes 11 and 22 (Kyu Lim *et al.*, 2004). This is a well-studied reciprocal translocation, and tertiary trisomy for the derivative chromosome 22 (as found in this pregnancy that resulted in spontaneous abortion) is the only form of this translocation with chromosome imbalance found in recognizable pregnancies and live born children and is associated with multiple congenital abnormalities (Gardner and Sutherland, 2004). The probe strategy chosen by this centre did not include

Table VIIa. Cycles performed for single gene disorders using PCR, data collection I–III

Indication	Autosomal recessive					Autosomal dominant				Specific sex-linked			Other	Total
	CF	β -thal	SMA	SC	EB	DM1	HD	CMT	MS	FRAXA	DMD	Haem		
Total cycles	197	55	45	11	8	125	66 ^b	11	10	27	25	12	65	657
Cancelled	22	11	3	0	0	11	5	1	0	1	3	0	6	63
Cycles to OR	175	44	42	11	8	114	61	10	10	26	22	12	59	594
Female average age	33	33	32	36	32	32	31	33	32	35	33	26	32	32
Number infertile	56	22	2	2	0	3	2	0	0	12	1	0	13	113
ART method														
IVF	11	0	1	0	0	0	0	0	0	0	2	6	2	22
ICSI	164	44	41	11	8	111 ^{aa}	61	10	10	26	20	6	57	569
Cancelled after OR	6	0	2	1	0	7	3	0	0	4	0	0	2	25
Cycles to PGD	169	44	40	10	8	107	58	10	10	22	22	12	56	568
Zona breaching														
AT drilling	130	39	29	7	8	66	35	2	8	7	15	12	27	385
Laser drilling	39	5	10	3	0	41	23	8	2	15	7	0	28	181
Mechanical	0	0	1	0	0	0	0	0	0	0	0	0	1	2
Biopsy method														
Polar body biopsy	1 ^c	0	0	0	0	0	0	0	0	0	0	0	1 ^c	2 ^c
Cleavage aspiration	161 ^c	44	37	10	8	104	58	10	10	22	22	12	56 ^c	554 ^c
Cleavage extrusion	8	0	3	0	0	3	0	0	0	0	0	0	0	14
Embryology														
COCs	2393	558	483	182	145	1357	842	148	112	229	336	122	921	7828
Inseminated	2065	504	411	133	119	1184	749	123	97	207	282	109	796	6779
Fertilized	1430	319	280	100	106	852	543	103	79	138	198	79	608	4835
Biopsied	1116	278	248	60	83	601	356	50	64	86	160	57	431	3590
Successfully biopsied	1101	273	247	60	83	594	351	49	63	85	160	56	426	3548
Diagnosed	932	195	194	51	62	499	303	40	58	75	135	30	357	2931
Transferable	607	90	110	35	34	225	140	16	36	34	91	17	199	1634
Transferred	322	90	79	27	23	172	92	11	25	24	53	15	130	1063
Cycles to ET	151	41	37	10	8	87	52	6	10	15	20	9	54	500
Frozen	146	6	7	24	0	18	25	3	4	5	31	4	58	331
HCG positive	44	17	9	1	3	22	11	2	2	3	6	3	20	143
Positive heart beat (% per OR)	34 (19%)	14 (32%)	8 (19%)	0 (0%)	1 (13%)	19 (17%)	9 (15%)	2 (20%)	2 (20%)	3 (12%)	6 (27%)	2 (17%)	20 (34%)	120 (20%)

CF = cystic fibrosis (various mutations); β -thal = β -thalassaemia; SMA = spinal muscular atrophy; SC = sickle-cell anaemia; EB = epidermolysis bullosa; DM1 = myotonic dystrophy; HD = Huntington's disease, MS = Marfan's syndrome; CMT = Charcot–Marie–Tooth disease; FRAXA = fragile-X syndrome; DMD = Duchenne's muscular dystrophy (specific); Haem = haemophilia; OR = oocyte retrieval; AT = acidic Tyrode's; COC = cumulus oocyte complex.

^aThree cycles had no ICSI (two cycles with immature oocytes only, one cycle no sperm found after TESE).

^bIncludes three HD with exclusion.

^cTwo cycles had both polar body biopsy and cleavage stage biopsy: one for cystic fibrosis combined with PGS, and one for MELAS.

Table VIIIb. Cycles performed for single gene disorders using PCR, data collection IV

Indication	Autosomal recessive				Autosomal dominant					X-linked		Others	Total
	CF ^a	β-thal	SMA	SC	HD	DM1	AP	CMT	ACH	Haem	FRAXA		
Total cycles	50	48	21	4	21	35	10	7	3	4	11	38	252
Cancelled before OR	6	2	1	0	1	5	0	0	0	0	1	2	18
Cycles to OR	44	46	20	4	20	30	10	7	3	4	10	36	234
Female age	33	32	36	35	33	33	28	33	33	30	36	33	33
Number infertile	19	25	3	1	3	8	7	0	0	0	2	0	68
ART method													
IVF	1	0	0	0	0	0	1	0	0	0	2	0	4
ICSI	38	46	16	4	17	27	9	7	3	3	6	30	206
Frozen	0	1	4	0	0	0	0	0	0	1	0	1	7
Unknown	5	0	0	0	3	3	0	0	0	0	2	5	18
Cancelled after OR	1	2	4	0	0	5	0	2	0	0	0	3	17
Cycles to PGD	42	38	16	4	18	25	10	5	3	2	10	33	206
Unknown	1	6	0	0	2	0	0	0	0	2	0	0	11
Zona breaching													
AT drilling	18	39	4	2	0	12	10	0	0	3	4	13	105
Laser drilling	17	1	6	2	17	11	0	5	3	0	4	11	77
Mechanical	3	0	6	0	0	0	0	0	0	1	0	6	16
Unknown	5	0	0	0	3	2	0	0	0	0	2	3	15
Biopsy method													
Polar body	1	0	0	0	0	0	0	0	0	0	0	0	1
Cleavage aspiration	33	39	10	4	17	23	10	5	3	3	8	25	180
Cleavage extrusion	3	1	6	0	0	0	0	0	0	1	0	5	16
Unknown	6	0	0	0	3	2	0	0	0	0	2	3	16
Embryology													
COCs	508	661	269	65	223	371	111	61	20	54	67	511	2921 ^b
Inseminated	463	546	214	56	186	337	93	53	15	45	63	401	2472
Fertilized	332	341	160	39	130	264	82	34	11	34	54	305	1786
Biopsied	297	195	116	35	95	165	78	17	10	15	46	217	1286
Successfully biopsied	294	190	116	34	92	163	78	17	10	15	46	216	1271
Diagnosed	256	160	105	32	77	135	61	15	8	11	42	191	1093
Transferable	134	84	75	19	37	47	32	7	4	11	18	66	534
Transferred	83	69	42	10	19	41	25	6	4	10	12	56	377
Cycles to ET	36	37	16	4	16	20	9	3	3	2	8	27	181
Frozen	15	14	1	4	8	1	0	0	0	0	1	6	50
HCG positive	14	12	6	2	4	3	3	2	0	1	3	9	59
Positive heartbeat (% per OR)	12 (27%)	10 (22%)	6 (30%)	1 (25%)	3 (15%)	2 (7%)	1 (10%)	2 (28%)	0	1 (25%)	3 (30%)	6 (20%)	47 (20%)

CF = cystic fibrosis (various mutations); β-thal = β-thalassaemia; SMA = spinal muscular atrophy; SC = sickle-cell anaemia; DM1 = myotonic dystrophy; HD = Huntington's disease; AP = amyloid polyneuropathy; CMT = Charcot–Marie–Tooth disease; ACH = achondroplasia; FRAXA = fragile-X syndrome; DMD = Duchenne's muscular dystrophy (specific); Haem = haemophilia; OR = oocyte retrieval, AT = acidic Tyrode's; COC = cumulus oocyte complex.

^aOne cycle for two indications: cystic fibrosis and fragile X syndrome.

^bEight cycles with missing data.

Table VIIIa. Cycles performed for PGS, data collection I–III

Indication	AMA	Recurrent miscarriage	Recurrent IVF failure	No indication	Other	Total
Cycles to OR	322	87	196	37	159	801
Female age	40	36	35	33	36	37
Number infertile	311	78	197	36	150	772
ART method						
IVF	109	18	51	10	52	240
ICSI	213	68	140	27	106	554
IVF + ICSI	0	1	5	0	1	7
Cancelled post-OR	2	0	11	0	1	14
Cycles to PGD	320	87	185	37	158	787
Zona breaching						
AT drilling	262	64	150	30	112	618
Laser drilling	45	20	34	4	42	145
Mechanical	13	3	1	3	4	24
Biopsy method						
Polar body biopsy	17 ^a	3	1	3	2	26
Cleavage aspiration	304 ^a	84	184	34	156	763
Embryology						
COCs	4084	1242	2717	481	2045	10 569
Inseminated	3732	1099	2414	449	1762	9456
Fertilized	2650	775	1674	338	1238	6675
Biopsied	2214	666	1192	274	999	5345
Successfully biopsied	2183	649	1172	262	980	5246
Diagnosed	1569 ^b	392 ^b	1054 ^b	151 ^b	800 ^b	3966 ^b
Transferable	53 ^b	183 ^b	425 ^b	151 ^b	342 ^b	1637 ^b
Transferred	596 ^b	206 ^b	297 ^b	87 ^b	305 ^b	1491 ^b
Cycles to ET	245	79	143	34	125	626
Frozen	63	16	124	7	74	284
HCG positive	92	29	34	11	46	212
Positive heart beat (% per OR)	77 (24%)	26 (30%)	20 (10%)	11 (30%)	30 (19%)	164 (20%)

OR = oocyte retrieval; AT = acidic Tyrode's; COC = cumulus–oocyte complex; AMA = advanced maternal age.

^aOne cycle had cleavage-stage biopsy and polar body biopsy.

^bSeveral cycles from one centre had no information on the number of embryos diagnosed or number of embryos diagnosed as transferable, but patients did have embryos transferred. In these cases, undiagnosed or abnormal embryos were transferred.

Table VIIIb. Cycles performed for PGS, data collection IV

Indication	AMA	Recurrent miscarriage	Recurrent IVF failure	Male indication	No indication	Other	Total
Total cycles	303	194	445	65	17	70	1094
Cycles to OR	302	190	434	64	16	69	1075
Female age	40	36	36	32	32	34	37
Number infertile	285	151	440	65	17	44	1002
ART method							
IVF	19	21	69	1	1	4	115
ICSI	277	167	336	63	15	51	909
Frozen embryos	0	0	4	0	0	0	4
IVF + ICSI	1	1	1	0	0	0	3
ICSI + frozen	0	0	1	0	0	0	1
Unknown	5	1	23	0	0	14	43 ^a
Cancelled post-OR	23	3	27	4	0	6	63
Cycles to PGD	279	187	407	60	16	63	1012
Zona breaching							
AT drilling	92	134	244	7	8	37	522
Laser drilling	164	48	117	46	6	7	388
Mechanical	10	4	9	7	2	5	37
Unknown	13	1	37	0	0	14	65 ^a
Biopsy method							
PB biopsy	35	4	6	0	0	0	45
Cleavage aspiration	167	167	315	33	15	59	756
Cleavage extrusion	57	14	42	26	1	3	143
Cleavage flow displacement	7	1	6	1	0	1	16
Unknown	13	1	38	0	0	0	52 ^a
Embryology							
COCs	2849	2892	5991	952	218	903	13 805 ^a
Inseminated	2411	2368	5065	735	179	716	11 474
Fertilized	1777	1720	3721	464	129	516	8327

Table VIIIb. Continued

Indication	AMA	Recurrent miscarriage	Recurrent IVF failure	Male indication	No indication	Other	Total
Biopsied	1366	1250	2303	326	91	327	5663
Successfully biopsied	1340	1178	2268	320	84	324	5514
Diagnosed	1246	1069	2132	263	64	305	5079
Transferable	529	344	794	129	44	148	1988
Transferred	375	259	584	104	26	93	1441
Cycles to ET	195	144	273	51	14	39	716
Frozen	29	28	72	10	0	13	152
HCG positive	38	45	84	21	7	24	219
Positive heart beat (% per OR)	26 (9%)	35 (18%)	66 (15%)	19 (30%)	6 (38%)	20 (29%)	172 (16%)

OR = oocyte retrieval; AT = acidic Tyrode's; COC = cumulus–oocyte complex; AMA = advanced maternal age.

^aSeveral cycles had incomplete results.

a probe on the derivative 22, and therefore this unbalanced form of the translocation was not detectable using their probe set (Mackie Ogilvie and Scriven, 2004). This affected pregnancy underlines the need for thorough assessment of each reciprocal translocation referred for PGD (Scriven *et al.*, 1998; Ogur *et al.*, 2002; Scriven, 2003), preferably in collaboration with a cytogenetics department with the necessary expertise (Mackie Ogilvie and Scriven, 2001).

Taking the misdiagnoses discovered both pre- and postnatally, a total of three misdiagnoses were reported after PGD

using FISH (one for sexing, one trisomy 21 after PGS and one for a reciprocal translocation), leading to a misdiagnosis rate of three out of 145 concepti tested (2%). After PGD using PCR, seven misdiagnoses were reported: two for sexing (for retinitis pigmentosa and Duchenne's muscular dystrophy), one for β -thalassaemia and for myotonic dystrophy, and three for cystic fibrosis (one discovered prenatally, and twins where it was found after birth that both were carriers instead of homozygous normal). This gives a misdiagnosis rate after PCR of seven out of 85 concepti tested (8%). The overall misdiagnosis rate is then 10 out of 230 or 4%.

Table IXa. PGD for social sexing, data collection I–III

	Method for sexing			Total
	FISH	PCR	Unknown	
Cycles to OR	78	10	5 ^a	93 ^a
Female average age	33	31	35	33
Number infertile	18	0	1	19
ART method				
IVF	65	4	3	72
ICSI	13	6	2	21
Cancelled after OR	0	0	5	5
Cycles to PGD	78	10	0	88
Zona breaching				
AT drilling	9	10	0	19
Laser drilling	69	0	0	69
Mechanical	0	0	0	0
Biopsy method				
Cleavage aspiration	78	10	0	88
Embryology				
COCs	1038	124	23	1185
Inseminated	1021	113	19	1153
Fertilized	754	61	11	826
Biopsied	649	59	0	708
Successfully biopsied	598	49	0	647
Diagnosed	534	35	0	569
Transferable	246	8	0	254
Transferred	138	8	0	146
Cycles to ET	67	6	0	73
Frozen	79 ^b	14	0 ^c	93
HCG positive	28	0	0	28
Positive heart beat (% per OR)	28 (36%)	0	0	28 (30%)

OR = oocyte retrieval; AT = acidic Tyrode's; COC = cumulus–oocyte complex.

^aOne natural cycle included.

^bEleven cycles with embryos frozen without biopsy or failed diagnosis included.

^cThree embryos frozen without biopsy were not included.

Table IXb. PGD for social sexing, data collection IV

	Method for sexing		
	FISH	PCR	Total
Total cycles	40	49	89
Cancelled before OR	0	0	0
Cycles to OR	37	47	84
Female average age	36	37	37
Number infertile	0	0	0
ART method			
IVF	37	0	37
ICSI	0	46	46
Frozen	3	2	5
ICSI + frozen	0	1	1
Cancelled after OR	3	6	9
Cycles to PGD	37	43	80
Zona breaching			
AT drilling	0	0	0
Laser drilling	37	0	37
Mechanical	0	43	43
Biopsy method			
Cleavage aspiration	37	0	37
Cleavage extrusion	0	43	43
Embryology			
COCs	341	763	1104
Inseminated	322	555	877
Fertilized	218	367	585
Biopsied	191	294	485
Successfully biopsied	168	294	462
Diagnosed	166	281	447
Transferable	41	174	215
Transferred	28	130	158
Cycles to ET	23	35	58
Frozen	14	21	35
HCG positive	9	19	28
Positive heart beat (% per OR)	7 (19%)	9 (19%)	16 (19%)

Table Xa. Evolution of pregnancy, data collection I-III

	N pregnancies	N fetal sacs
Pregnancies		333
Fish cycles	212/333	416
PCR cycles	121/333	266/416
Subclinical pregnancies ¹	27/333	150/416
Clinical pregnancies		306
Singletons	213/306	416
Twins	77/306	213/416
Triplets	15/306	154/416
Quadruplet	1/306	45/416
First trimester loss	34/306	4/416
Miscarriage	31/306	60/416
Vanishing twins		32/416
Vanishing triplets		15/416
Ectopic pregnancy	3/306	10/416
Ongoing pregnancies		272
Second trimester loss	9/272	356
Miscarriage (> 13w)	2/272	13/356
Late miscarriage (20–24w)	3/272	2/356
TOP after misdiagnosis ²	3/272	6/356
TOP after amniocentesis ³	1/272	3/356
Reduction ⁴		1/356
Reduction of multiple pregnancies		5/356
Quadruplet to twin		2/356
Triplet to twin		2/356
Triplet to singleton		2/356
Ongoing		263
Lost to follow-up	20/263	338
Deliveries		243
Singletons	175/243	315
Twins	64/243	175/315
Triplets	4/243	128/315

¹Subclinical pregnancy defined as pregnancy without any other clinical signs, but positive serum HCG.

²One misdiagnosis for sexing, FISH, female fetus, indication social sexing; one misdiagnosis for β -thalassaemia, PCR; one misdiagnosis for myotonic dystrophy, PCR.

³Trisomy 18 after amniocentesis, indication for PGD parent carrier of reciprocal translocation not involving chromosome 18.

⁴One misdiagnosis for sexing, PCR, indication Duchenne, twin pregnancy, selective termination of male fetus. Cycle done in 1996, Y-specific amplification only.

TOP = termination of pregnancy

Table Xb. Evolution of pregnancy, data collection IV

	N pregnancies	N fetal sacs
Pregnancies		315
FISH cycles	273/315	387
PCR cycles	42/315	
Subclinical pregnancies	26/315	
Clinical pregnancies		289
Singletons	204/289	387
Twins	72/289	204/387
Triplets	13/289	144/387
First trimester loss	33/289	39/387
Miscarriage	31/289	51/387
Extrauterine pregnancy	2/289	39/387
Vanishing twins/triplets		2/387
Ongoing pregnancies (> 12 weeks)	256	10/387

Table Xb. Continued

	N pregnancies	N fetal sacs
Second trimester loss	7/256	10/336
Miscarriage	5/256 ¹	8/336 ¹
TOP ²	2/256 ³	2/336
Reduction of multiple pregnancies		5/336
Triplet to twin		4/336 ¹
Twin to singleton		1/336
Normal evolution	249	321
Singletons	183/249	183/321
Twins	60/249	120/321
Triplet	6/249	18/321
Lost to follow-up	13/249	19/321
Singletons	8/183	8/183
Twins	4/60	8/120
Triplets	1/6	3/18
Deliveries	236	302
Singletons	175/236	175/302
Twins	56/236	112/302
Triplets	5/236	15/302

¹One triplet: fetal reduction, followed by amniocentesis and loss of remaining twin at 16 weeks (one fetal sac counted in reduction, two in miscarriage, one second trimester pregnancy loss after miscarriage counted).

²One polymalformation, one misdiagnosis (45,X after PGS).

³TOP = termination of pregnancy.

Pregnancies and babies data IV

The number of pregnancies submitted has increased, and reflects the increased number of cycles submitted by a larger number of centres. Because of the use of the FP6 software, it has been much easier to control the submission of pregnancies and baby data. This means that most cycles ending in a positive heartbeat also had a pregnancy sheet and, vice versa,

Table XIa. Complication in clinical pregnancies, data collection I-III

Complication	Incidence
Total complications	40 patients
Singletons	27 patients
Twins	13 patients
Triplets	None reported
Nature of complications	
Preterm contractions	13 (8 singletons, 5 twins)
Preterm labour	13 (8 singletons, 5 twin)
Pre-eclampsin and hypertension	9 (6 singletons, 3 twin)
Bleeding	5 (4 singletons)
Diabetes mellitus	3 (3 singletons)
Premature rupture of the membranes	3 (3 twins)
Cerclage	2 (2 singleton)
Pleacenta praevia	2 (2 singletons)
Abruptio pleacenta, retroplacental hematoma	2 (2 singletons)
HELLP syndrome ¹	2 (2 singleton)
Pleacenta accreta	1 (1 singleton)
Intrauterine growth retardation	1 (1 singletons)
Pyelonephritis	1 (1 singletons)
Idiopathic thrombocytopeny	1 (1 singleton)
Toxoplasmosis maternal problem of asphyxia and shock lung	
Polyhydramnios	1 (twin)
Oligohydramnios	1 (1 singleton)
Total	59

¹HELLP = haemolysis, elevated liver enzymes, low platelet count.

Table XIb. complication in clinical pregnancies, data collection IV

Complication	Incidence
Total Complications	52 patients
Singletons	38 patients
Twins	11 patients
Triplets	3 patients
Nature of Complications	
Preterm Contractions	12 (10 singletons, 2 twins)
Bleeding	10 (9 singletons, 1 twin)
Intrauterine growth retardation	6 (5 singletons, 1 twin)
Preterm labour	7 (5 singletons, 2 twins)
Diabetes mellitus	3 (1 singletons, 2 twins)
Preterm dilatation	3 (1 singletons, 2 twins)
Cerclage	3 (1 singletons, 2 triplets)
Pre-eclampsia and hypertension	3 (2 singletons, 1 twin)
Chorioamnionitis	2 (2 singletons)
Placenta praevia	2 (2 singletons)
Placenta accreta	2 (2 singletons)
Premature rupture of the membranes	2 (2 twins)
Intrauterine death	2 (2 twins)
Abruptio placentae, retroplacental hematoma	2 (singletons)
Gastrointestinal problems	2 (1 singleton, 1 triplet)
Anemia	1 (singleton)
HELLP syndrome ¹	1 (singleton)
Psychological problems	1 (singleton)
Abortion risk	1 (singleton)
Edema	1 (singleton)
OHSS ²	1 (singleton)
Oligohydramnios	1 (twin)
Twin to twin transfusion	1 (twin)
Extrauterine pregnancy followed by salpingectomy	1 (singleton)
Total	70

¹HELLP = haemolysis, elevated liver enzymes, low platelet count.

²OHSS = ovarian hyperstimulation syndrome.

that most of the pregnancies and babies had a cycle in the cycle database. Of the 296 cycles ending in a positive heartbeat, 18 (9%) had no pregnancy file. Conversely, of the 315 pregnancies, 14 had no concurring cycle. Eight of these pregnancies were from cycles before April 2001, and these cycles can be found in the I–III cycle database, while for six of these (2%) no cycle was found. The characteristics of the pregnancies (evolution, complications Table Xb, XIb),

Table XIIa. Method of delivery and gestational age, data collection I–III

	Total	Singletons	Twins	Triplets
No. delivered	243	175	64	4
Method of delivery				
Vaginal	116	99	17	0
Caesarian	95	57	34	4
Unknown	32	19	13	0
Term at delivery				
Preterm	46	15	28	3
Term	168	144	23	1
Unknown	29	16	13	0

Table XIIb. Method of delivery and gestational age, data collection IV

	Total	Singleton	Twin	Triplet
No. delivered	236	175	56	5
Method of delivery				
Vaginal	88	78	10	0
Caesarean section	132	88	40	4
Vaginal + Caesarean section	1	0	1	0
Unknown	15	9	5	1
Gestational age at delivery				
Preterm	70	31	36	3
At term	153	136	16	1
Unknown	13	8	4	1

Table XIIIa. Data on live-born children, data collection I–III

Total children born	315	
Sex		
Male	111	111/286
Female	176	176/286
Unknown	28	
Mean birth weight (gr)		<i>n</i> = 268
Singletons	2885	<i>n</i> = 158
Twins	3270	<i>n</i> = 103
Triplets	2500	<i>n</i> = 7
Unknown	1361	<i>n</i> = 47
Mean birth length (cm)		<i>n</i> = 179
Singletons	48	<i>n</i> = 116
Twins	49,8	<i>n</i> = 63
Triplets	47	
Unknown	No data	<i>n</i> = 136

Table XIIIb. Data on live born children, data collection IV

Total children born	293	
Sex		
Male	130	
Female	156	
Unknown	7	
Mean birth weight (g)		
Singletons	3262	(<i>n</i> = 161/170)
Twins	2450	(<i>n</i> = 91/108)
Triplets	1954	(<i>n</i> = 14/15)
Mean birth length (cm)		
Singletons	50	(<i>n</i> = 103/170)
Twins	47	(<i>n</i> = 34/108)
Triplets	44	(<i>n</i> = 6/15)
Mean head circumference		
Singletons	34.5	(<i>n</i> = 60/170)
Twins	33.8	(<i>n</i> = 22/108)
Appar scores		
Good	81 singletons, 16 twins	
Poor	1 twin (neonatal death)	

Numbers in parentheses indicate the number of newborns for whom information is available out of the total number of newborns.

deliveries (multiple gestations, type of delivery Table XIIb) and babies (e.g. birth weight, complications at birth Tables XIIIb and XIVb) are very comparable with the data collection I–III, and with large series of ICSI pregnancies and babies (Bonduelle *et al.*, 2002; Van Steirteghem *et al.*, 2002).

Unfortunately, three misdiagnoses have again occurred: one after FISH (45,X seen on prenatal diagnosis after PGS) and two after PGD for monogenic diseases (amyloid

Table XIVa. Congenital malformations and neonatal complications at birth, data collection I-III

No data available	95/315
No malformations	206/220
Total malformations	14/220
Major malformations at birth	
Congenital hip luxation	1 singleton
Bilateral clubfoot	1 singleton
Cystic mass abdomen	1 twin
Pes equinovarus	1 singleton
Phocomelia and pulmonary deficiency	1 singleton
Chylothorax	1 twin
Exencephalia	1 twin
Minor malformations	
Cryptorchid	1 singleton
Syndactyly digit iv-v	2 singletons
Mongolian spot	1 singleton
Sacral dimple	1 singleton
Bilateral hydrocoele	1 singleton
ASD	1 twin
Neonatal complications	
No data available	112/315
No neonatal complications reported	175/203
Neonatal complications reported	32/203
Stillborns	2 twins, 2 singletons ¹
Neonatal deaths <7days	3 ²
Dysmature	6 twins (3 preg)
Prematurity and intubation (<7d)	2 twins (1 preg)
Prematurity 4 weeks neonatal care	2 twins (1 preg)
Prematurity 3 days neonatal care	1 twin
Pneumothorax	1 twin
Respiratory problems (unspecified)	1 singleton
	1 twin
Feeding problems	2 twins (2 pregn)
Apnea	1 twin
Observation poor apgar	1 singleton
Prematurity recorded as neonatal complication	3 triplets
	3 Singletons
	1 twin

¹One twin stillborn after chorioamnionitis, one twin after ruptured membranes, one singleton stillborn after polyhydramnios, and one singleton no reason given.

²One intracranial bleeding (term 24 weeks), one exencephaly, one chylothorax (term 32 weeks); three twins from three pregnancies.

Table XVa. Congenital malformations and neonatal complications at birth, data collection IV

No data available	20
No malformation	
Singletons	156
Twins	39
Triplet	2
Babies with malformation	
Major	
Unilateral intra-uterine torsio testis	1 singleton
Large cavernous haemangioma	1 singleton
Cleft lip and palate	1 singleton
Hydrocephaly	1 singleton
Fryns syndrome, neonatal death	1 singleton
Stillborn at 28 weeks (no details)	1 twin
Prune belly syndrome and stillbirth	1 twin
Minor	
Capillary haemangioma	2 singletons, 2 twins
Unumbilical artery	1 singleton
Pyelourethral junctional stenosis	2 twins

Neonatal complications	
24 h neonatal ward at 34 weeks	1 singleton
< 1 week neonatal ward with torsio testis at 36 weeks	1 singleton
Gastro-oesophageal reflux	1 singleton
Prematurity with long hospitalization	2 singletons
Fryns syndrome and neonatal death	1 singleton
Respiratory distress and 1 week hospitalisation	1 twin
3 weeks neonatal ward	1 twin
Prune belly syndrome in one twin and prematurity in the other twin (28 weeks)	2 twins
Stillborn at 21 weeks, no further information	1 twin
Stillborn at 26 weeks	1 twin
1 month hospitalization for prematurity and low birth weight	3 triplets from 1 pregnancy
1 intra-uterine death at 31 weeks, prematurity in two other children	3 triplets from 1 pregnancy

Table XVb. Confirmation of diagnosis per fetal sac, data collection I-III

Method	Result		
	<i>n</i>	Normal	Abnormal
Prenatal diagnosis			
FISH			
CVS	28		
Amniocentesis	85		2
Unknown	3		
Total	116	114	2 ^a
PCR			
CVS	40		
Amniocentesis	27		5
Unknown	2		
Total	69	64	5 ^b
Postnatal diagnosis			
FISH			
Karyo miscarriage	7	2	5 ^c
Karyo postnatal	22	21	1 ^d
Total	29	23	6
PCR			
DNA test miscarriage	2	2	0
DNA test postnatal	10	9	1 ^e
Sweat test	4	4	0
Total	16	15	1

Prenatal diagnosis: FISH, fetal sacs tested 116 out of 266 (47%); PCR, fetal sacs tested 69 out of 150 (46%); total prenatal testing, 185 out of 416 (44%). Postnatal diagnosis: total FISH sacs/babies tested 29/266 (11%); total PCR sacs/babies tested 16/150 (11%); total postnatal testing 45/416 (11%); total checking of diagnosis 230/416 (55%).

^aMisdiagnosis for sexing, female fetus, social sexing, one trisomy 18 after PGD for reciprocal translocation.

^bOne XL Duchenne (selective reduction of one affected embryo of twin pregnancy), one β -thalassaemia (terminated), one myotonic dystrophy (terminated), one cystic fibrosis (ongoing pregnancy with live born), one misdiagnosis XL retinitis pigmentosa (ongoing pregnancy with live born).

^cOne trisomy 16, one trisomy 22, one mosaic trisomy 22, one monosomy X, one misdiagnosis 47,XX, + der(22)t(11;22)(q23.3;q11.2)mat; parent carrier balanced translocation.

^dOne misdiagnosis, trisomy 21 after aneuploidy screening.

^eOne cystic fibrosis carrier twin pregnancy; on PGD both diagnosed as homozygote normal.

Table XVb. Confirmation of diagnosis per fetal sac, data collection IV

Method	Result		
	<i>n</i>	Normal	Abnormal
Prenatal diagnosis			
FISH			
CVS	12	12	0
Amniocentesis	94	93	1 ^a
Ultrasound	8	7	1 ^b
Total	114	112	2
PCR			
Amniocentesis	13	13	0
Ultrasound	5	4	1 ^c
Ultrasound	4	3	1 ^d
Total	22	20	2
Postnatal diagnosis			
FISH			
Karyo miscarriage	9	5	4 ^f
Karyo postnatal	9 ^g	9	0
Physical examination	97 ^g	95	2 ^h
Total	115 ^g	109	6
PCR			
DNA test miscarriage	0	0	0
Physical examination	2 ⁱ	1 ⁱ	1 ⁱ
DNA test postnatal	7	7	0
Karyotype	2	2	0
Karyo + DNA	1	1	0
Unknown	1	1	0
Total	13	12	1

Prenatal diagnosis: FISH fetal sacs tested, 114 out of 336 (34%); PCR fetal sacs tested, 22 out of 51 (43%); total prenatal testing: 136/387 (35%).

Postnatal diagnosis: FISH fetal sacs/babies tested, 114/336 (34%); PCR fetal sacs/babies tested, 13/51 (25%); total postnatal testing, 127/387 (33%); total checking of diagnosis, 225/387 (58%)^e.

^aMisdiagnosis after PGS: 45,X, terminated.

^bPolymalformation on ultrasound, normal karyotype, terminated.

^cMisdiagnosis for amyloid polyneuropathy, born.

^dEchogenic bowel at ultrasound, misdiagnosis for CF, born.

^eThirty-eight of the 387 fetal sacs (four after PCR, 34 after FISH) had both prenatal and postnatal checking of PGD.

^f47,XX, + 16,inv(18)(p11.23q11.2) after PGD for inversion, 47,XX, + 15 after PGS for recurrent miscarriage, 47,XY, + 3 after PGS for abnormal FISH result in spermatozoa, 47,XY, + D(3) after PGS for repeated IVF failure and advanced maternal age.

^gOne baby born with Fryns syndrome had a karyotype (normal result).

^hOne baby with Fryns syndrome, one baby with Prune belly syndrome (both normal karyotype).

ⁱOne twin after PGD for cystic fibrosis: one misdiagnosis, one healthy.

polyneuropathy, born, and cystic fibrosis, born). This gives a total misdiagnosis rate of three out of 136 (2.2% of the fetal sacs tested); one out of 114 (0.9% of the fetal sacs tested) for FISH and two out of 22 for PCR (9.1% of the fetal sacs tested).

General remarks

The original aim of collecting the referral data was to evaluate the reasons why patients opt for PGD, and how centres offering PGD respond to patients' requests. However, it is now clear that within the Consortium many centres only send in those referrals that are followed by a PGD cycle, most probably because many centres do not take care of the patient directly, but are a referral centre for the diagnostic material only. The ESHRE PGD Consortium will continue to collect these valuable data, but will focus on the data from those centres that take care of the complete PGD cycle, from patient intake to transfer and follow-up of the babies.

As with every new report, new indications in PGD or PGS appear: noteworthy is the increasing number of PGS cycles performed for male indications and for previous aneuploid pregnancies.

A new piece of information that was added when the FP6 was introduced was the number of cycles cancelled before OR. A comparison of the number of cancellations between categories and with regular IVF could yield important information on the correct management of PGD patients, who have a lower chance of achieving a pregnancy in all categories. From the low number of cycles cancelled (149 out of 1990 or 7%), it is difficult to draw conclusions, except that the bias which distorted the referral data is also at play here: diagnostic centres who only receive a fixed blastomere have no access to information concerning cycle cancellation before OR.

The ESHRE PGD Consortium will continue to collect PGD data, and this collection will be expanded to the follow-up of children born after PGD. The next data collection (V) will include data from January 1, 2002 until December 31, 2002 and is currently being prepared for publication. The Consortium have also written PGD guidelines (Thronhill *et al*, in press).

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Appendix I.

List of participating centres (Contact person, affiliation, city, country)

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Chamayou Sandrine: HERA-UMR, Catania, Italy

Chen, Chun-Kai: Chang Chung Memorial Hospital and Medical College, Tao-Yuan, Taiwan

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Emiliani, Serena: Hopital Erasme, Université Libre de Bruxelles, Brussels, Belgium

Fernandez, Esther: Fundacion Jimenez Diaz, Madrid, Spain

Gianaroli, Luca: SISMER, Bologna, Italy

Gitlin, Sue: Jones Institute for Reproductive Medicine, Norfolk, Virginia, USA

Hanson, Charles: Sahlgrenska Hospital, Goteborg, Sweden.

Harper, Joyce: University College London, London, UK

Harton, Gary: Genetics and IVF Insitute, Fairfax, Virginia, USA

Hindkjaer, Johnny: Aarhus University Hospital, Aarhus, Denmark

Hussey, Nicole: University of Adelaide, Department of Obstetrics and Gynaecology, Adelaide, Australia

Hyden-Granskog, Christel: Helsinki University Central Hospital, Helsinki, Finland

Inn Soo Kang: Samsung Cheil Hospital, Seoul, Korea.

Kahraman, Semra: Istanbul Memorial Hospital, Istanbul, Turkey

Kontogianni, Elena: IVF and Genetics, Athens, Greece

Lavery, Stuart: Hammersmith Hospital, London, UK

Malcov, Mira: Tel-Aviv Sourasky Centre, Tel-Aviv, Israel

Manor, Dorit: Rambam Medical Centre, Haifa, Israel

Marshall, Jim: Sydney IVF, Sydney, Australia

McAdoo, Sallie: Baylor College of Medicine, Houston, Texas, USA

Montag, Marcus: University of Bonn, Bonn, Germany

Rubio, Carmen: Instituto Valenciano de Infertilidad, Valencia, Spain.

Salin, Paivi: AVA-Clinic, Tampere, Finland

Santalo, Josep: Unitat de Biologia Cellular, Universidad Autonoma Barcelona, Barcelona, Spain

Sermon, Karen: Centre for Medical Genetics Vrije Universiteit Brussel, Brussels, Belgium

Traeger-Synodinos, Joanne: Medical Genetics, Athens University, St. Sophia's Children's Hospital, Athens, Greece.

Van de Elst, Josiane: Infertility Centre, Ghent University Hospital, Ghent, Belgium

Vandamme, Brigitte: Leuven Insitute for Fertility and Embryology, Leuven, Belgium

Veiga, Anna: Instituto Dexeus, Barcelona, Spain

Vesela, Katerina: Sanatorium Repromeda, Brno, Czech Republic

Viville, Stéphane: Service de la Biologie de la Reproduction, SIHCUS-CMCO, Strasbourg, France

Wilton, Leeanda: Melbourne IVF, Melbourne, Australia

List of centres that failed to send in data

Alberola, Trinidad: Sistemas Genomicos SL, Valencia, Spain

Decherney, Alan: Department of Obstetrics and Gynecology, UCLA School of Medicine, Los Angeles, USA

Frydman, Nelly: Service de Biologie Génétique de la Réproduction, Hopital Beclère, Paris, France

Gadou, Moutaz: Elaj Medical Center, Jeddag, Saudi Arabia

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