

Clinical Value of Preimplantation Genetic Diagnosis

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The clinical application of preimplantation genetic diagnosis (PGD) has provided an alternative approach for the prevention of affected pregnancies in couples at high reproductive risk. The frequent contribution of genetic factors to infertility problems makes PGD of particular value for assisted reproductive practices. In addition, the selection of euploid embryos for transfer has a strong impact on IVF efficiency as aneuploidies are the main cause of spontaneous abortions and implantation failures. In this study, the clinical outcome in PGD cycles is presented. The list of monogenic disorders for which PGD is performed is rapidly extending and the safety of the procedure has led to an increasing interest among couples at high reproductive risk. Following PGD for aneuploidy, a higher implantation rate and a lower incidence of spontaneous abortions are obtained in patient categories where aneuploidy is a prominent cause of reproductive failure. In view of these findings, PGD has become an integral part of assisted reproductive techniques for the prevention of affected pregnancies and improvement of IVF efficiency.

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INTRODUCTION

As science progresses, the study of DNA and DNA-related processes has firmly entered the field of clinical reproductive medicine. Identification of the genes involved in many diseases affecting reproduction has permitted the development of new treatment strategies for the approach to infertility.

In the general framework of genetic medicine, the definition of criteria that control the selection of healthy, viable embryos has become increasingly important. Since the development of amniocentesis in the early 1970s, couples at risk for a genetic disease have been recommended to undergo prenatal diagnosis with the possibility of terminating an affected pregnancy and giving birth to only healthy children. More recently, preimplantation genetic diagnosis (PGD) has been proposed as an extremely early form of prenatal diagnosis based on the analysis of a single cell: a blastomere biopsied from regularly cleaving day 3 embryos, or the polar bodies in the case of oocytes (Handyside et al., 1990, 1992; Verlinsky et al., 1990). The original idea was proposed in 1967 when sexing of rabbit blastocysts was followed by the birth of healthy pups of the predicted sex (Edwards and Gardner, 1967). In the following years, the introduction of in vitro fertilization (IVF) techniques in human reproduction (Stephens and Edwards, 1978) and the refinement of methods for single cell analysis made the clinical application of PGD possible. The merging of these two scientific technologies represents a new approach in the prevention of genetic disorders and its application is increasing proportionally to the growing knowledge of the human genome.

Basically, PGD is recommended to couples with high genetic risk or other severe problems related to recurrent miscarriages or failed implantation. Two situations can be envisaged: the screening of single-gene disorders or the analysis of chromosomal abnormalities, both numerical (aneuploidies) and structural (translocations and inversions). The application of PGD is especially relevant in reproductive medicine since genetic alterations are often associated with infertility (mutations in the gene responsible for cystic fibrosis, translocations, Klinefelter's or Turner's syndromes). Moreover, the selection of embryos for transfer based on their chromosomal status has a positive effect on the clinical outcome in patients at risk of generating aneuploid embryos. This is probably due to the relevance of chromosomal abnormalities in spontaneous abortions and, theoretically, in implantation failures (Gianaroli et al., 1997; Munné et al., 1998).

The aim of this study was to evaluate the clinical value of PGD in couples at high reproductive risk.

MATERIALS AND METHODS

Patients

From September 1996 to December 2002, 667 couples attended the S.I.S.Me.R. Reproductive Medicine Unit for PGD. In 77 of them, single gene defects were the indication to treatment for which a preliminary phase of research on the possibility of applying PGD was performed. Twenty couples underwent 30 PGD cycles for cystic fibrosis (15 cycles), thalassemia (5 cycles), haemophilia (4 cycles), Duchenne

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muscular dystrophy (3 cycles), Lesch-Nyhan (1 cycle), Steinert myotony (1 cycle) and retinoblastoma (1 cycle).

PGD for aneuploidy was indicated to 590 couples which performed 828 cycles for aneuploidy screening (AS): in 435 cycles the maternal age was 36 years or more, 97 cycles had previously performed at least three unsuccessful attempts, 145 cycles had an altered karyotype due balanced translocations (75 cycles), inversions (9 cycles), gonosomal mosaicism (61 cycles), 47 XYY (1 cycle) or 47 XXY (1 cycle), 67 cycles had sperm obtained by micro-epididymal sperm aspiration or testicular sperm extraction and failed at least one cycle, and 84 cycles had other indications which are still under study.

Embryo biopsy

Day 3 embryos with regular morphology and development were selected for embryo biopsy that was performed at 62–64 h after insemination. After loading acidic Tyrode's solution (pH 2.35) in a 12 µm diameter pipette, an opening was made in the zona pellucida of approximately 20 µm diameter. One nucleated blastomere (aneuploidy screening) or two (PGD for monogenic disorders and translocations) were aspirated into a 30–40 µm diameter glass pipette with extreme care to avoid damage to either the biopsied cell or the surrounding blastomeres. After biopsy, the embryos were thoroughly washed, put in fresh medium and incubated until the time of transfer.

PGD for monogenic diseases

The cells were lysed in alkaline lysis buffer at 65°C for 10 min followed by neutralization with 5 µl of neutralization buffer (900 mM Tris-HCl, 300 mM KCl, 200 mM HCl). The PCR strategy included an initial multiplex external amplification followed by nested PCR. Amplification was assessed by loading 5 µl of PCR products on 2 per cent agarose gel in 1 × Tris-Borate/EDTA buffer stained with 0.5 µg/ml ethidium bromide and running electrophoresis for 5' at 150 V. In each step, positive and negative controls were included.

Mutation analysis was performed on positively amplified blastomeres using the minisequencing technique (Fiorentino et al., 2003). Two nanograms of the purified PCR products were used for the minisequencing reaction, using ABI PRISM® SnaPshot Multiplex Kit (Applied Biosystems, Foster City, CA, USA). The sample detection was achieved by 15 min (for each blastomere) of capillary electrophoresis on automatic DNA sequencer ABI PRISM® 310 (Applied Biosystems, Foster City, CA, USA) with POP-4™ polymer, 47 cm × 50 µm capillaries.

PGD for aneuploidy

Fluorescent probes were used for the simultaneous detection of different chromosomes in successive rounds of FISH

Table 1. PGD for single-gene disorders

No. cycles	30
No. patients	20
Age (M ± SD, years)	34.4 ± 2.6
No. retrieved oocytes (M ± SD)	10.8 ± 5.2
No. generated embryos	211
No. Analyzed embryos	177
No. diagnosed embryos	157 (89)
Wild type (%)	56 (36)
Wild type+no result*	2
Healthy carriers (%)	59 (39)
Affected (%)	40 (25)
No. transferred embryos (M ± SD)	1.3 ± 0.9
No. transferred cycles (%)	25 (86)
No. clinical pregnancies (%)	6
Implantation rate (%)	18.9
Take-home baby rate per transfer (%)	30

* In two heterozygous carriers, one allele was wild type and the second mutation failed to be diagnosed.

(fluorescence in situ hybridization). In all, 141 embryos were analysed for 5 chromosomes (XY, 13, 18, 21), 345 for 6 chromosomes (XY, 13, 16, 18, 21), and all the others for 8–9 chromosomes including those implicated in the most frequent aneuploidies detected in spontaneous abortions and trisomic pregnancies (XY, 13, 15, 16, 18, 21, 22) (Gianaroli et al., 1999a). In the case of translocations, the chromosomes involved in the translocation were screened using enumerator probes (robertsonian translocations) or a combination of centromeric and telomeric probes (reciprocal translocations); embryos resulting normal or balanced were also screened for the chromosomes involved in conventional aneuploidy analysis (Gianaroli et al., 2002).

Clinical outcome

Clinical pregnancies were defined by the presence of a gestational sac with fetal heart beat at ultrasound scanning. The implantation rate represented the percentage of gestational sacs with fetal heart beat divided by the total number of embryos transferred. All pregnant patients were recommended to undergo prenatal diagnosis to confirm the results from PGD.

Statistical analysis

Data were analyzed by Fisher's exact test and chi-square analysis applying the Yates' correction.

RESULTS

PGD for monogenic diseases

A total of 177 embryos generated by 30 cycles underwent genetic analysis (Table 1). A diagnosis was obtained for 157 of

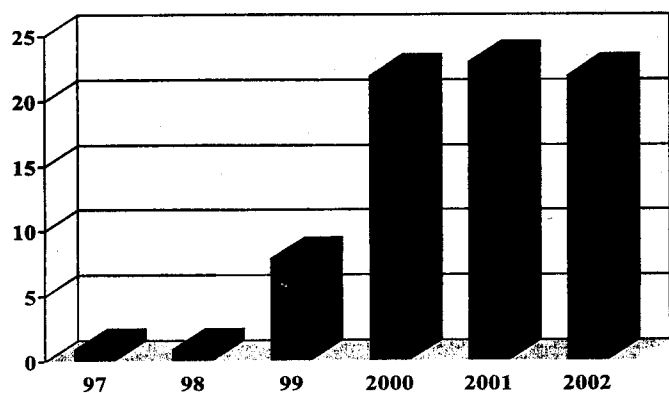


Figure 1. Referrals from 1997 to December 2002 for PGD for single gene disorders.

them (89 per cent): 56 were wild type, 40 were affected, 59 were healthy carriers of the pathology and two embryos with a normal allele had no result obtained for the second mutation investigated. Embryo transfer was possible in 25 cycles (1.3 ± 0.9 embryos per transferred cycle) and generated six clinical pregnancies with an implantation rate of 20.0 per cent. In all cases, PGD results were confirmed by prenatal diagnosis and by the birth of six healthy, unaffected infants. Four of the six children were born from carriers of cystic fibrosis mutations, and two from carriers of thalassemia mutations. Following the transfer of two embryos, one patient with PGD for thalassemia had two gestational sacs with three fetal heart beats. The couple decided to undergo selective fetal reduction of the monozygotic twins.

The referrals throughout the years for PGD of single gene disorders are shown in Figure 1. The request seems to have reached a plateau during the last 3 years.

PGD for aneuploidy

A total of 5082 embryos were generated from 828 cycles and 4244 of them were selected for embryo biopsy (Table 2). A diagnosis was obtained in 4213 embryos (99 per cent); 1381 had a normal chromosomal complement (33 per cent) and made the transfer possible in 552 cycles (67 per cent). An average of 1.8 ± 0.7 euploid embryos were replaced yielding 160 clinical pregnancies (29 per cent) and an implantation rate of 20.4 per cent. The take-home baby rate was 23 per cent per patient.

The number of cycles undergoing PGD for aneuploidy from 1996 to 2002 are reported in Figure 2.

Maternal age

PGD for aneuploidy was performed in 435 cycles with a maternal age ≥ 36 years and a normal karyotype. A total of 2231 embryos were diagnosed resulting in 688 chromosomally normal (31 per cent) whereas the remaining 1543 (69 per cent)

Table 2. PGD for aneuploidy: overall results in 828 treatment cycles

No. cycles	828
No. patients	590
Age ($M \pm SD$, years)	37.1 ± 4.5
No. retrieved oocytes ($M \pm SD$)	8.7 ± 4.1
No. generated embryos	5082
No. FISH analyzed embryos	4244
No. FISH diagnosed embryos	4213
FISH normal (%)	1381 (33)
FISH abnormal (%)	2832 (67)
No. transferred embryos ($M \pm SD$)	1.8 ± 0.7
No. transferred cycles (%)	552 (67)
No. clinical pregnancies (%)	160 (29)
Implantation rate (%)	20.4
Take-home baby rate per patient (%)	23

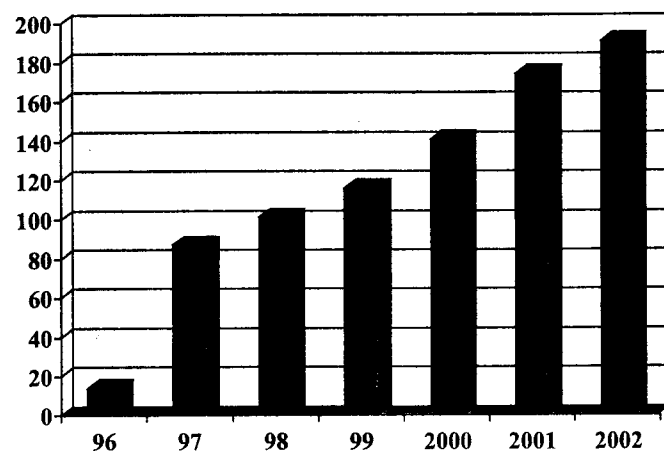


Figure 2. Number of cycles with PGD for aneuploidy throughout the years.

carried chromosomal abnormalities which were not compatible with a healthy implantation. Euploid embryos were replaced in 279 cycles generating 74 clinical pregnancies (27 per cent) and an implantation rate of 18.3 per cent. The take-home baby rate was 20 per cent per patient. The percentage of chromosomally abnormal embryos increased proportionally to age, ranging from 63 per cent in women of 36–37 years to 81 per cent in patients aged 43 years and older. Pregnancy and implantation rates varied accordingly with a significant decrease starting at 43 years where the take-home baby rate per patient was 7 per cent compared to 30 per cent in the group 36–37 years.

Table 3 describes the chromosomal abnormalities detected in the group of advanced maternal age with especial attention paid to the monosomies and trisomies that have an implantation potential. Twenty percent of total abnormalities were monosomies and 42 of them were compatible with implantation: nine embryos were X0, the condition responsible of Turner syndrome, and 33 were monosomy 21. Trisomies were detected in 336 embryos representing 22 per cent of total abnormalities; the trisomic conditions compatible with life, trisomy 21, 13, 18 and trisomy of gonosomes were diagnosed in 147 embryos: 63 had trisomy 21 (Down's syndrome), 39 had trisomy 13 (Patau's syndrome), 25 had trisomy 18 (Edwards'

Table 3. Chromosomal abnormalities in the group of patients with advanced maternal age

No. retrieved oocytes (M ± SD)	8.8 ± 4.5
No. FISH diagnosed embryos	2231
No. FISH normal embryos (%)	688 (31)
No. FISH abnormal embryos (%)	1543 (69)
No. monosomies (%)	315 (20)
Monosomy X (X0)	9
Monosomy 21	33
No. trisomies (%)	336 (22)
Trisomy 21	63
Trisomy 13	39
Trisomy 18	25
Trisomy 15	45
Trisomy 16	45
Trisomy 22	53
No. monosomies and trisomies (%)	34 (2)
No. haploidies (%)	72 (4.7)
No. polyploidies (%)	83 (5.4)
No. complex abnormalities (%)	703 (46)

Table 4. Frequency of abnormalities for the chromosomes analyzed by FISH according to maternal age

Chromosomes	No. observations	Maternal age (years)				P
		36–37	38–39	40–42	≥43	
21	2159	27 ^a	33	37	47 ^a	<0.001
22	1988	30 ^a	34	38	44 ^a	<0.001
15	1899	32 ^a	37	40	44 ^a	<0.001
13	2191	29 ^a	32	34	38 ^a	<0.01
16	2155	32 ^a	37	36	39 ^a	<0.05
1	195	30	51	49	43	
14	261	25	36	28	38	
17	192	36	45	54	52	
18	2192	29	33	33	32	
XY	2219	25	29	28	31	

Values with same superscript are significantly different.

syndrome), 13 had three copies of X chromosome and seven had the constitution XXY (Klinefelter's syndrome). Trisomy of chromosomes 22, 15 and 16, which are frequently found in spontaneous abortions, were diagnosed in 143 embryos: 53 had trisomy 22, 45 trisomy 15 and 45 trisomy 16. In 34 embryos, monosomy of one chromosome was associated with trisomy for another chromosome; haploidy and polyploidy accounted for approximately 5 per cent each over total abnormalities, whereas 703 embryos had complex abnormalities.

The frequency of single chromosome aneuploidies in relation to maternal age demonstrated different rates in relation to age, suggesting that some chromosomes are more prone than others to segregation errors. According to the results presented in Table 4, aneuploidies of chromosomes 15, 21 and 22 have a strong increase in relation to age; the same tendency is observed for chromosomes 13 and 16, although at a lower

extent. Conversely, chromosomes 1, 14, 17, 18, X and Y show similar variations irrespective of age.

Repeated IVF failures (≥3 cycles)

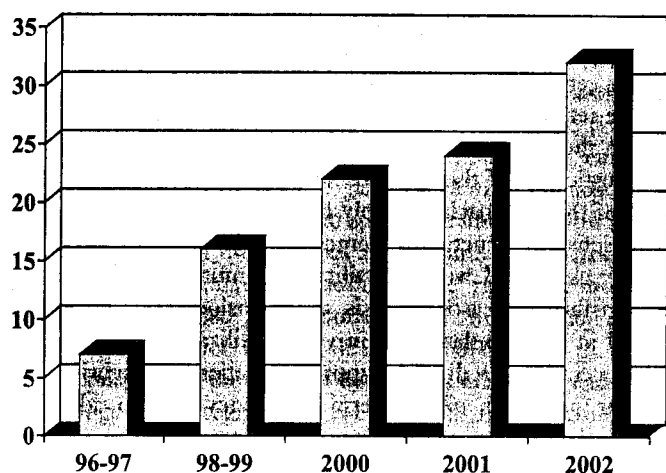
Approximately 59 per cent of the embryos generated by 70 young patients in 99 PGD cycles were chromosomally abnormal. Embryo transfer was performed in 77 cycles (78 per cent) yielding 27 clinical pregnancies (35 per cent) and an implantation rate of 25.2 per cent. The take-home baby rate per patient was 34 per cent.

The distribution of chromosomal abnormalities was significantly different from that characterized in the group of maternal age patients with monosomy and trisomy representing 35 per cent of total abnormalities vs 44 per cent detected in older women ($P < 0.005$). Other defects such as haploidy, polyploidy and complex abnormalities had the highest incidence in this category of PGD patients, evoking a dysfunction in the processes or structures entering cell division, including sperm centrosome. In addition, the high proportion of complex abnormalities suggests that other chromosomes, different from those tested, could have an important role in the viability of these embryos (Gianaroli et al., 1997).

Altered karyotype. Ninety-five couples with an altered karyotype underwent 145 PGD cycles. A total of 734 embryos were diagnosed by FISH and 212 were classified as normal; embryo transfer was performed in 91 cycles (63 per cent) yielding 40 clinical pregnancies (44 per cent) with an implantation rate of 30.6 per cent and a take-home baby rate of 33 per cent. Forty-six carriers of balanced translocations, 22 with robertsonian translocations and 24 with reciprocal translocations, performed 35 cycles and 40 cycles respectively. Six carriers of reciprocal translocations did not have their embryos screened for the translocation due to the impossibility of having the correct probes available in a short time; therefore they decided to perform 11 conventional PGD cycles with the screening for the chromosomes XY, 1, 13, 15, 16, 18, 21, and 22. Five of these couples returned for a further attempt and had the probes ready to detect the chromosomes involved in the translocation. Table 5 shows the results derived from PGD of translocation on 35 cycles with robertsonian translocations and 29 cycles with reciprocal translocations. For carriers of Robertsonian translocations, a normal or balanced chromosomal complement was diagnosed in 43 embryos of the 175 analyzed (25 per cent); the transfer of euploid embryos was possible in 22 cycles (63 per cent) with 13 clinical pregnancies (59 per cent per transfer) and an implantation rate of 44.4 per cent. The take-home baby rate per patient was 41 per cent. In the case of reciprocal translocations, the proportion of chromosomally normal or balanced embryos was significantly lower (12 per cent, $P < 0.005$); only 11 cycles were transferred (38 per cent) with three clinical pregnancies and an implantation rate of 20 per cent. The take-home baby rate was 4 per cent. As shown in Figure 3, a constant increase has been observed in the number of cycles treated with PGD for translocation.

Table 5. Patients with an altered karyotype: robertsonian and reciprocal translocations

	Translocations		P
	Robertsonian	Reciprocal	
No. cycles	35	29	
No. patients	22	24	
Age (M ± SD, years)	35.5 ± 3.7	34.0 ± 5.3	
No. retrieved oocytes (M ± SD)	9.3 ± 4.3	9.7 ± 4.6	
No. generated embryos	206	187	
No. FISH analyzed embryos	175	154	
No. FISH diagnosed embryos	175	154	
FISH normal (%)	43 (25)	18 (12)	<0.005
FISH abnormal (%)	132 (75)	136 (88)	<0.005
No. transferred embryos (M ± SD)	1.6 ± 0.9	1.4 ± 0.5	
No. transferred cycles (%)	22 (63)	11 (38)	=0.03
No. clinical pregnancies (%)	13 (59)	3 (27)	
Implantation rate (%)	44.4	20.0	
Take-home baby rate per patient (%)	41	4	=0.03

**Figure 3.** Number of cycles with PGD for translocation throughout the years.

Forty-one carriers of gonosomal mosaicism underwent 61 PGD cycles. FISH diagnosis was obtained in 321 embryos, resulting in 117 normal (37 per cent). Euploid embryos were transferred in 44 cycles (72 per cent) generating 22 clinical pregnancies (50 per cent) and an implantation rate of 35.1 per cent. A take-home baby rate per patient of 49 per cent was obtained.

PGD for aneuploidy was applied to embryos generated by patients with a karyotype 47,XXY and 47,XXY respectively. In the first of these cases, three embryos were analyzed and one was found to be normal and two abnormal (one was X0). The euploid embryo was transferred, but no pregnancy occurred. Four embryos generated by the patient with the Klinefelter's syndrome were analyzed by FISH: two were normal and two abnormal (one was X0). Both euploid embryos were transferred and one implanted.

MESA-TESE

Table 6 shows the data of 67 PGD cycles performed by 52 azoospermic patients who had failed at least one conception cycle. A high percentage of chromosomally abnormal embryos was detected and was similar in sperm retrieved by MESA and TESE (75 per cent and 69 per cent respectively). Only 61 per cent of the cycles were transferred with eight clinical pregnancies (20 per cent) and an implantation rate of 14.9 per cent. Forty-four per cent of the analyzed embryos were monosomic and trisomic and 6.8 per cent of the abnormal embryos exhibited gonosomal aneuploidies. The results obtained in TESE patients were also divided for the cause of azoospermia, obstructive in eight cycles and non-obstructive in 42 cycles. The proportion of chromosomally abnormal embryos was 55 per cent and 70 per cent respectively; 9.7 per cent of the embryos in the non-obstructive category showed aneuploidy of gonosomes.

Recurrent abortions

PGD was performed in 14 patients younger than 36 years and with a normal karyotype who had a history of repeated abortions. In all, 89 embryos were diagnosed and 30 resulted with a normal chromosomal constitution. In six cycles no euploid embryos were detected and embryo transfer was cancelled. Four clinical pregnancies were obtained in eight transferred cycles with an implantation rate of 50.0 per cent. No abortion occurred yielding a take-home baby rate of 29 per cent per patient. The analysis of chromosomal abnormalities demonstrated that 40 per cent of the abnormalities were due to monosomy and trisomy. Chromosomes 15 and 16 were involved in 5 and 3 monosomies respectively; of the 14 trisomies, 4 were due to chromosome 16 and 4 to chromosome X, 3 to chromosome 13, 2 to chromosome 21 and 1 to chromosome 18.

Table 6. Azoospermic patients with sperm retrieved by MESA and TESE: FISH and clinical results

	MESA	TESE	Total
No. cycles	17	50	67
No. patients	13	39	52
Age (M ± SD, years)	32.5 ± 4.6	35.3 ± 4.4	34.6 ± 4.6
No. generated embryos	93	267	360
No. retrieved oocytes (M ± SD)	11.8 ± 4.0	10.0 ± 4.7	10.3 ± 4.6
No. FISH analyzed embryos	77	216	293
No. FISH diagnosed embryos	77	215	292
FISH normal (%)	19 (25)	67 (31)	86 (30)
FISH abnormal (%)	58 (75)	148 (69)	206 (70)
No. embryos transferred (M ± SD)	1.5 ± 0.8	1.7 ± 0.7	1.6 ± 0.7
No. cycles transferred (%)	9 (53)	32 (64)	41 (61)
No. clinical pregnancies (%)	3 (33)	5 (16)	8 (20)
Implantation rate (%)	28.6	11.3	14.9
Take-home baby rate per patient (%)	23	10	14

Others

Other indications to PGD of aneuploidy are still under investigation in order to evaluate categories of patients that can derive benefit from resorting to this technique. Among them are: poor responders to hormonal stimulation, the extremely severe male factor, previous cycles with irregular embryo development, embryos generated by thawed oocytes.

DISCUSSION

The results obtained worldwide demonstrate that PGD provides a valuable option for couples at high reproductive risk which desire to minimize the possibility of an affected pregnancy and the consequent need to decide whether to undergo therapeutic abortion (ESHRE PGD Consortium Steering Committee, 2002; Kuliev and Verlinsky, 2002).

The diagnostic importance of PGD has reached a point that makes this approach more and more requested worldwide. The majority of fertile patients who decide to undergo PGD for the screening of monogenic disorders either have strong objections to therapeutic abortion after conventional prenatal diagnosis or have previously experienced termination of affected pregnancies. A similar situation characterizes carriers of an altered karyotype, especially reciprocal translocations, where unbalanced segregation occurs at very high rates. Recurrent abortions are the most common consequences due to total or partial monosomy or trisomy in the resulting embryo. The selection of normal embryos by PGD can alleviate the poor prognosis associated with these conditions, but a good response to follicular hyperstimulation is a crucial requisite especially when considering that embryos are selected for transfer not only on the basis of their genetic content but also on the basis of their morphology and developmental rate (Vandervorst et al., 1998). In other words, PGD is an additional criterion that makes embryo selection even stricter in consideration of the expected incidence of the genetic abnormality that is 25 per cent in the case of monogenic disorders and much higher in

selected categories of patients. Accordingly, the pregnancy rate has been proven to be closely dependent on the number of oocytes generated (Gianaroli et al., 2000a, 2002). Nevertheless, in our center, the rate of cancellation of PGD cycles after the oocyte retrieval is only dependent on embryo development and not on the number of oocytes retrieved in consideration of the diagnostic role of the chromosomal analysis. The major drawback of this approach probably resides on the fact that genetic mutations and structural chromosomal abnormalities which are mainly associated with infertility (i.e., mutations in the gene responsible for cystic fibrosis and translocations) are maintained in the population by in vitro fertilization techniques.

In a completely different approach, patients' categories with a poor prognosis of full term pregnancy due to advanced maternal age or repeated unsuccessful attempts have now the reasonable certainty of undertaking an assisted conception cycle with the same chances of young, normal karyotyped couples, provided that embryo transfer is performed. A continuous effort is made to widen the range of PGD indications, as well as to improve the accuracy and reliability of the results.

The clinical application of PGD for aneuploidy has confirmed the hypothesis of a strong association between chromosomal abnormalities and reproductive failure. Couples with PGD indications have chromosomal abnormalities in more than 50 per cent of their embryos, confirming not only that the frequency of chromosomal abnormalities is higher than expected by considering the sum of aneuploidies observed in oocytes and spermatozoa, but also that a strong selection against chromosomal abnormalities in the initial phases of human life occurs. This is suggested by the frequency of chromosomal abnormalities in spontaneous abortions, approximately 50 per cent, and in livebirths, 0.5 per cent–1 per cent with the highest incidence (7.3 per cent) in women over 40 years. Therefore, implantation failure and spontaneous abortions are frequently due to an altered number of chromosomes as confirmed by the higher implantation rate and lower incidence of spontaneous abortions after PGD in patients' categories where aneuploidy is the main cause of reproductive

failure (Gianaroli et al., 1999b; Munné et al., 1999). Nevertheless, there is no increase in the take-home baby rate per patient due to the high proportion of cycles where no transfer is performed as no euploid embryos are detected (Egozcue et al., 2002).

The possibility of investigating the chromosomal condition of preimplantation embryos also provided a great contribution to the general knowledge in reproductive medicine. Gonadal failure both in males and females has been identified as a condition at risk of generating aneuploid embryos; these findings substantiate the low implantation rate that characterizes these patients' categories (Gianaroli et al., 2000a,b). The treatment of extremely severe male factor infertility has notably increased after the introduction of ICSI at the beginning of the 1990s (Van Steirteghem, Liu and Joris, 1993; Tournaye et al., 1994; Devroey et al., 1995; Gianaroli et al., 1999c). The follow-up of the infants born after ICSI has shown an increased incidence of inherited and de-novo chromosomal abnormalities, with most of the aneuploidies accounting for sex chromosomes (Liebaers et al., 1995; Bonduelle et al., 1998). In agreement with this finding, the frequency of aneuploid sperm has been found to be higher in cases of severe oligoasthenoteratospermia or azoospermia due to testicular failure (Levron et al., 2001). The FISH analysis of preimplantation embryos revealed no variation in the percentage of chromosomally abnormal embryos according to sperm parameters. However, a higher incidence of monosomies and trisomies was detected in MESA-TESE embryos; in addition the rate of gonosomal aneuploidy increased proportionally with the severity of the male factor condition (Gianaroli et al., 2000b).

In conclusion, the clinical value of PGD has made this technology an integral part of assisted reproduction and represents a valuable tool for couples to minimize the risk of having an affected child. At the same time, the screening for aneuploidy improves the clinical outcome in reproductive medicine by avoiding the transfer of chromosomally abnormal, non-viable embryos. This may contribute in a near future to a general improvement in assisted reproductive techniques in the framework of preventive medicine.

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