

Safety, Accuracy and Reproductive Outcome of Preimplantation Genetic Diagnosis

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Abstract

PGD is now performed for 357 different conditions, with over 99.5% accuracy in the leading PGD centers. There is also no restriction in provision of PGD, which may presently be performed for any genetic condition, even in cases that the genetic condition is first identified in one of the parents or only in the affected child. Indications for PGD are steadily expanding, with current wider application of PGD to diseases with genetic predisposition, such as different cancers and cardiovascular disorders, so PGD centers will face a forthcoming increase of PGD requests from this highly sensitive group of at risk couples. Despite recent controversy in PGD for chromosomal disorders, the present progress in improving the accuracy of the procedure through adequate choice of biopsy material and microarray analysis for 24 chromosomes demonstrated the obvious clinical impact of avoiding the transfer of aneuploid embryos. A major breakthrough has been in the development and application of microarray technology for PGD of chromosomal disorders, allowing a highly improved detection of chromosomally abnormal oocytes and embryos, resulting in a higher accuracy and improved clinical outcome following the application of 24-chromosome aneuploidy testing coupled with blastocyst biopsy.

Keywords: PGD; Single gene disorders; Chromosomal disorders; PGD accuracy; Reproductive outcome

Introduction

After more than two decades of the application to clinical practice PGD is no longer a research tool, but an established procedure, providing a realistic option for at-risk couples to reproduce responsibly without facing risk of having an affected pregnancy. With such option available, these couples can achieve the desired family size with no much difference from the couples without any known inherited risk. As will be described below, the available data of, approximately, hundred thousand of PGD cycles performed by the present time suggest that the procedure is safe, accurate and reliable, and should be offered to those at need for the procedure, who will otherwise not reproduce because of fear of affected pregnancy or prenatal diagnosis followed by termination of pregnancy [1,2].

Although the majority of PGD cycles are still performed in the USA and Western Europe, increasing numbers are reported from other areas of the world, where the prevalence of some of the common genetic disorders is particularly high, such as hemoglobinopathies in Eastern Mediterranean and South East Asia. Indications for PGD are also expanding, with more PGD cases being performed for the conditions that have never been practiced in traditional prenatal diagnosis, such as preimplantation gender determination for social reasons [3-5], common late onset diseases with genetic predisposition, and preimplantation HLA typing. However, the majority of PGD cycles are still performed for chromosomal disorders, with the ratio of PGD cycles for chromosomal and single gene disorders 3:1.

Approaches and Indications

PGD was first applied for pre-existing Mendelian diseases, such as Cystic Fibrosis (CF), thalassemia and sickle cell disease [1,2,6]. Wider application was associated with the introduction of Fluorescent *In Situ* Hybridization (FISH) analysis for PGD of chromosomal disorders, due to importance of identification of embryos with higher potential to result in pregnancy in assisted reproduction practices. Of further practical significance was the ability of PGD to detect translocations, initially with the use of locus-specific and subtelomeric FISH probes, and presently by microarray technology (see below). Because many

carriers of balanced translocations have a poor chance of having an unaffected pregnancy, PGD has a clear advantage over the traditional prenatal diagnosis in assisting these couples to establish an unaffected pregnancy and deliver a child free from unbalanced translocation.

The application of PGD has further expanded with its introduction to late-onset diseases with genetic predisposition, such as cancer and heart disease, indications never previously considered for the traditional prenatal diagnosis [7]. For the patients with inherited pathological predisposition to common diseases of public health importance PGD provides a realistic reason for undertaking pregnancy, with a reasonable chance of having an unaffected offspring. Prospective parents at such risk should be aware of this emerging option, especially when there is no opportunity to diagnose the disease until it is fully realized, such as in cases of inherited cardiac diseases leading to premature or sudden death.

Another unique option that can presently be considered, although involving ethical debate, is preimplantation HLA typing as part of PGD, which has never been considered in traditional prenatal diagnosis. In this application PGD offers not only preventative technology to avoid affected offspring, but also a new method for treating (older) siblings with congenital or acquired bone marrow diseases, for which there is still no available therapy. This may in future be applied for any condition that can be treated by embryonic stem cell transplantation.

Preimplantation HLA typing was first applied to couples desiring an unaffected (younger) child free from the genetic disorder in the

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Received November 05, 2013; Accepted December 20, 2013; Published December 23, 2013

Citation: Kuliev A, Verlinsky O, Rechitsky S (2013) Safety, Accuracy and Reproductive Outcome of Preimplantation Genetic Diagnosis. J IVF Reprod Med Genet 1: 116. doi:10.4172/2375-4508.1000116

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older sibling. In addition to diagnosis to assure a genetically normal embryo, HLA matched, unaffected embryos were replaced. At delivery cord blood (otherwise to be discarded) was gathered for stem cell transplantation. This approach has been also used without testing of the causative gene, with sole purpose of finding a matching HLA progeny for a source of stem cells transplantation for affected siblings with congenital or acquired bone marrow disease or cancer.

There are three main approaches to PGD, including Polar Body (PB), blastomere or blasocyst biopsy based analysis [7-9]. For PB analysis the first and second polar bodies (PB1 and PB2) are removed from the matured and fertilized oocytes. For the cleavage stage PGD a single blastomere is biopsied from the eight-cell embryo, while for blastocyst biopsy analysis up to dozen trophoctoderm cells are removed. The biopsied material is tested for single gene disorders using PCR analysis, or used for PGD of chromosomal abnormalities by Fluorescent In-Situ Hybridization (FISH), or microarray analysis (see below). Each of these PGD methods has advantages and disadvantages, and their choice depends on circumstances, however, in some cases combination of two or three methods might be required [7,8]. Despite a possible embryo cell number reduction, which might have a potential influence on the embryo viability, blastomere biopsy allows detecting paternally derived abnormalities. Removal of PB1 and PB2, on the other hand, should not have any effect on the embryo viability as they are naturally extruded from oocytes as a result of maturation and fertilization, but they provide no information on the paternally derived anomalies, even if this constitutes less than 5% of chromosomal errors in preimplantation embryos.

To perform PGD for single gene disorders PB1 and PB2 are removed separately in sequence and tested by PCR analysis, while for testing chromosomal aneuploidies both PB1 and PB2 are removed simultaneously, next day after insemination of the matured oocytes or ICSI, and analysed by FISH or microarray technology [7,8]. The method of blastomere biopsy was much more extensively used in PGD practice, despite its limitation due to high rate of mosaicism in cleaving embryos. This is currently shifting to the blastocyst analysis, which has an advantage of analyzing not one but group of cells, obviating the problem of mosaicism at least to some extent. The aneuploidy testing in blastocyst biopsy was further improved by the application of microarray technology (see below), which may pick up mosaicism of 10% and higher. Overall, these methods were used now in a total of almost hundred thousand clinical cycles, and as will be described below, resulted in birth of dozens of thousands unaffected children, showing a comparable prevalence of congenital abnormalities to that in general population.

Safety of PGD

The clinical outcome data were reported for 7126 PGD cycles performed in this center, and for 15885 PGD cycles collected from 39 different centers by ESHRE PGD Consortium [5]. These resulted in 1775 (25%) and 2881 (18%) clinical pregnancies per initiated cycles, respectively, and birth of 4227 healthy children, overall (1504 and 2723, respectively), with the multiple pregnancies observed in over one third of the cases. The overall congenital malformation rate was under 5%, which is not different from the general population prevalence, of which only half were attributable to the major abnormalities.

No difference was found in the report on a longstanding systematic follow up study from the other world's leading PGD center either [6], which presented the physical findings at birth and up to 2 months of age for 995 children born after PGD, in comparison to 1507 children born after ICSI. Comparison was made for prematurity,

mean birth weight, very low birth weight (<1500 g), perinatal death, major malformations and neonatal hospitalizations in singletons and multiples born following PGD versus ICSI. Compared with ICSI, fewer multiples born following PGD presented a low birth-weight.

The detailed testing of consecutive series of 581 children, born after cleavage stage based PGD showed even lower major malformations (2.13%) compared to that in 2889 ICSI children (3.38%), despite significantly higher overall perinatal death rate in the post-PGD children. A thorough systematic study of PGD offspring based on a 2 month exam also supported the above data. There were a total of 563 PGD live-berths, 18 stillborns, and 9 neonatal deaths. Among these were 300 live-born babies after PGD for single gene disorders, 7 stillborns and 4 neonatal deaths; the other cases were after PGD for aneuploidy testing. No differences were found in structural malformations between PGD and ICSI offspring - 2.13 vs. 3.38%, respectively. There further were no differences between offspring resulting from PGD for single gene disorders and PGD for aneuploidy. There proved to be no differences in singleton in respect to stillborns, live-borns, or neonatal deaths. In multiple gestations PGD offspring showed increased perinatal deaths. This is in agreement with our data, showing similar major congenital anomaly rate, 1.9% in 1230 babies born after PGD [7]. Average maternal age in this retrospective study was 34.8 years with multiple pregnancies in 24% of cases, with 22.4% twins and 1.6% triplets. Spontaneous delivery was only in 49.1% of pregnancies, with cesarean section performed in the remaining of cases. Intrauterine Growth Retardation (IUGR) was found in 3%. Only 33.1% of couples followed recommendation for prenatal diagnosis and 66.9% declined the confirmation testing. Only one imprinting disorder, Beckwith Weidermann syndrome, was observed (1/1230).

In no study have anomalies been disproportionately clustered in any given organ system in either cohort. So PGD does not seem to introduce any extra risk to the overall medical condition of newborn children, and the differences are due to multiple pregnancies [5-7].

Diagnostic Accuracy of PGD

At least 20,000 PGD cycles have been performed for single gene disorders, with the outcome data available for hundreds of clinical pregnancies and babies, suggesting an extremely high accuracy of PGD. Both Polar Body (PB) and embryo biopsy methods have been used extensively, and were shown to be extremely accurate in the leading PGD centers. One of the reports on the PGD accuracy, involved the testing of a total of 9036 oocytes by sequential first (PB1) and second (PB2) PB removal, demonstrating 97% amplification efficiency with embryo transfer in 84.2% of the initiated cycles [8]. As a result of this approach, only two misdiagnoses were described in PB testing of over 9,000 oocytes, including one in PGD for fragile-X syndrome, and the other in PGD for myotonic dystrophy. Both of these misdiagnoses were due to undetected ADO, as only one, or two linked markers, respectively, were available for testing, which otherwise is insufficient for accurate diagnosis, but the risk of misdiagnosis was accepted by the couple due to unavailability of other embryos for transfer. Assuming that these misdiagnoses were observed in 790 PB based PGD transfer cycles, the accuracy rate of this approach was as high as 99.7% per transfer.

Similar accuracy rate per transfer was reported in this center's overall experience, which is the world's largest PGD series for Mendelian disorders, with the majority of PGD cycles performed by embryo biopsy [8]. The overall series of 3592 PGD cycles, performed for 357 genetic conditions, resulted in 1298 (48%) unaffected pregnancies

and birth of 1291 apparently healthy children, with a total of only four misdiagnoses (including the above two cases, mentioned). One of the misdiagnoses was observed at the very initial stage of introduction of PGD and caused by undetected ADO in PGD for Cystic Fibrosis (CF) in a mutant double heterozygous embryo, erroneously diagnoses as unaffected carrier [9], and the others were due to transfer of embryos with predicted low accuracy in PGD for fragile-X, muscular dystrophy and beta-thalassemia, when the couples opted to transfer the embryo tested normal based on insufficient number of linked markers, leaving the probability for ADO, as mentioned above.

A high accuracy rate was also reported in the above mentioned world's leading PGD experience for monogenic disorders, mentioned, which documented only 0.6% misdiagnosis rate in PGD of 1443 PGD cycles, one in PGD for myotonic dystrophy and 3 in PGD for Charcot-Marie-Tooth disease (CMT1A), due to errors in linkage analysis in preparation to PGD [6,10,11]. However, the accuracy is much lower in a collection of PGD data from many centers, because of differences in the experiences and expertise.

Reproductive Outcome of PGD

As mentioned above, a high pregnancy rate of 48% was observed in PGD for single gene disorders, despite transferring of only 1.8 embryos per cycle on the average (4902 embryos were transferred in 2698 cycles). This may be explained by the fact that these are fertile couples of younger reproductive age under 35 years, compared to the poor prognosis IVF patients referred for aneuploidy testing. It is also of interest that the pregnancy rate in PGD cycles for HLA typing, combined with aneuploidy testing was significantly higher, compared to that in PGD for HLA typing without aneuploidy testing, as the average maternal age in these patients are much higher than in those presented for PGD of single gene disorders [12].

In PGD for chromosomal disorders the indications are also expanding, with further obvious interest in PGD for translocations, because of a strong impact PGD on reducing the spontaneous abortion rate in the carriers of balanced translocations [13,14]. The majority of these cycles have been performed in the two largest US centers [13-16], with increasing number of PGD for translocations in other centers worldwide as well. The available experience demonstrates a clear advantage of PGD for translocations over traditional prenatal diagnosis, attributable to a poor meiotic outcome, particularly in reciprocal translocations. As mentioned, the accuracy of PGD for translocation has been improved by the introduction of increasing number of subtelomeric probes and the technique of blastomere nucleus conversion to metaphase, currently performed by chemical methods [16], which allows a reliable testing for any complex chromosomal rearrangement. In addition, PCR based approaches for translocation detection had been introduced, including haplotyping [17] and microarray technology, which will further improve the accuracy of the procedure [18]. The experience of over 5000 PGD cycles for chromosomal rearrangements accumulated at the present time worldwide further confirms the previous observations on at least five-fold reduction of spontaneous abortion rate in translocation carriers, making PGD a preferred option for chromosomal translocations over traditional prenatal diagnosis. Although, the proportion of PGD cycles with detected balanced or normal embryos for transfer was not sufficiently high, especially in reciprocal translocations, the transfer of these normal or balanced embryos resulted in pregnancy rates comparable to those PGD cycles performed for Mendelian disorders.

More than half of the preimplantation embryos are chromosomally abnormal from the onset [19,20], so have to be avoided from transfer

in IVF patients of advanced reproductive age. These biological data provide the background for clinical application of aneuploidy testing, making it obvious that the recent controversy about PGD application in IVF is not about its benefit, as the transfer of chromosomally abnormal embryos should obviously be avoided, but solely concerns the accuracy and reliability of testing. The high aneuploidy prevalence in oocytes and embryos makes it clear that without the detection and avoidance of chromosomally abnormal embryos, there is a 50% chance of transferring the abnormal embryos, destined to be lost during implantation or post-implantation development. So in addition to benefit of avoiding aneuploid embryos from transfer, which contributes to the improvement of the pregnancy outcome of poor prognosis IVF patients, this should improve the overall standard of medical practice, upgrading the current selection of embryos by morphological criteria to include the testing for aneuploidy.

The expected obvious benefit of avoiding aneuploid embryos from transfer may explain the widespread application of aneuploidy testing, representing over 75% of the PGD cycles performed worldwide in the effort to preselect the embryos with highest developmental potential. It is not surprising that most of systematic studies have demonstrated the clinical benefit of aneuploidy testing, in terms of the improved IVF outcome through the improved implantation rates and reduction of spontaneous abortions in poor prognosis IVF patients, including those of advanced reproductive age, repeated IVF failures and recurrent spontaneous abortions [21-26]. However, the majority of these studies were not randomized, nor had sufficient case numbers to detect a significant increase in live-birth rates. Randomized clinical trials in the US (and many other countries) have been difficult to perform because of the high associated cost and the self-pay nature of IVF, and the lack of sufficient funding for human embryo research.

On the other hand, PGD it is still a highly sophisticated procedure, involving the oocyte and/or embryos biopsy, which may have detrimental effect on embryo development if not performed up to the standard (1). This implies also to the genetic testing applied on a single cell, including FISH or microarray-based testing, also requiring sufficient training and experience, due to the well known limitations of genetic analysis in a single cell. So the failure of observing a positive effect of aneuploidy testing on reproductive outcome in a few smaller studies may be due to methodological shortcomings [27-31]. This may first of all due to potential detrimental effect of removing two blastomeres instead of one [28,29], according to the present PGD guidelines (1), which could definitely have reduced the implantation potential of the biopsied embryos to the extent that could not be bridged by preselection of aneuploidy-free embryos [32]. Overall, the potential improvement in ART outcome caused by selecting against abnormal embryos through PGD should far outweigh the potential damage caused by the biopsy procedure when one cell is removed. Without taking into consideration the technical details, the results may erroneously be misinterpreted as the lack on PGD impact, although even the absence of the differences between PGD and non-PGD groups may suggest the beneficial effect of preselection of aneuploidy-free embryos, in terms of compensating a detrimental effect of one or two cell biopsy at the day 3.

Significant technical problems were present also in the other randomized controlled study which was based on a single blastomere biopsy [30]. First of all, as high as 20% of no results in biopsied embryos was reported, in contrast to acceptable 5% [1], with as low as 6% of implantation rate in these cycles. With 14.7% implantation rate in the control non-PGD group, this may have suggested an over 50%

reduction in implantation potential due to the biopsy procedure, due to a detrimental effect the embryo biopsy that could have compromised the embryo survival. Another factor contributing to the outcome of the study is the exclusion from testing of the chromosomes 15 and 22, accounting for approximately 10% of aneuploidies in the cleavage-stage human embryos, which could have further reduced the selection potential of the technique. Finally, the acceptance in the study of patients with average number of 5 and less available embryos for biopsy, could have affected an appropriate pre-selection of embryos for transfer. Obviously, biopsying only 2-3 embryos, even if biopsy and diagnosis were done optimally, would have little beneficial effect.

Despite the above methodological shortcomings, which have been heavily criticized [32-36], this have been misinterpreted in favor of transferring embryos without aneuploidy testing [37], which may suggest the alternative of incidental transfer of chromosomally abnormal embryos, as every second oocytes or embryos obtained from the poor prognosis IVF patients is chromosomally abnormal, and if not avoided from transfer are destined to be lost before or after implantation. In fact, only one in ten of the chromosomally abnormal embryos survive to the recognized clinical pregnancy, 5% survive to the second trimester, and only 0.5% reach the birth, suggesting that the majority of them are eliminated before or during implantation, being a major cause of a miserable implantation rate in poor prognosis IVF patients, and explaining a high fetal loss rate in these patients without PGD. This has been demonstrated by testing of products of conception from poor prognosis non PGD IVF patients, which confirmed the high prevalence of chromosomal aneuploidy in the absence of PGD [38].

So there seems to be actually no controversy in the uselessness of aneuploid embryos transfer the major issue being the safety and reliability of aneuploidy testing, which have recently been improved (see below). In the absence of sufficient data of the well designed randomized controlled studies, the beneficial impact of PGD have been also demonstrated by the comparison of reproductive outcome in the same patients with and without PGD, with the assumption that the previous reproductive experience of the patients may serve an appropriate control for PGD impact [39,40].

In the lights of these data, the current IVF practice of selection of embryos for transfer based on morphologic criteria may hardly be an acceptable procedure for poor prognosis IVF patients. In addition to an extremely high risk of establishing an affected pregnancy from the onset, this will significantly compromise the very poor chances of these patients to become pregnant, especially with the current tendency of limiting the number of transferred embryos to only two, leaving only a single embryo on the average with a potential chance of reaching the term.

Introduction of Preimplantation 24 Chromosome Aneuploidy Testing

One of the major limitations of previously applied methods for preimplantation aneuploidy testing was that only a limited number of chromosomes were tested, so the transfer of embryos with undetected chromosome anomalies could have contributed to a poor reproductive outcome. This has been overcome by the application of microarray-based technology for 24-chromosome aneuploidy testing, and most recently Next Generation Sequencing (NGS), which combined with the blastocyst stage biopsy procedure allowed improving the accuracy of preimplantation aneuploidy testing to quantify the actual impact of pre-selecting of aneuploidy-free embryos for transfer on the IVF efficiency.

Recent reports on the application of 24 chromosome testing have already demonstrated a strong beneficial impact. There were two major approaches, one based on PB biopsy, and the other performed on blastocyst biopsy samples, as it provides sufficient material not only to identify the loss or gain of specific chromosomes, but also detect mosaicism exceeding 10%. One approach was undertaken to determine whether PB1 and PB2 biopsy approach followed by 24 chromosome testing enables a reliable, timely and accurate identification of maternal contribution to the chromosomal status of the corresponding zygote [41-43]. For this purpose, array-based CGH analysis was used, which allowed completing both PB 1 and PB2 analysis within 12 hours, on Day 2 of preimplantation development to perform fresh transfer in participating centers. Accurate identification of the maternal contribution to the chromosomal status of the zygote was achieved in more than 90% of cases, the remaining showing the diagnostic problem mainly due to amplification failure or high noise of the signals.

Follow up testing of corresponding abnormal zygotes showed concordance rate of aneuploidies to the PB1 and PB2 results in 130 of 138 (94%) zygotes, one of which appeared to be with different aneuploidy compared to prediction, while the remaining seven were euploid, despite the expected aneuploid results. The latter was explained by possible compensation of aneuploidy of PB1 by corresponding aneuploidy in PB2 or sperm. As expected the aneuploidy rate was higher than that detected by FISH, which could have missed 23.7% of the corresponding aneuploidies.

Although at least one zygote was predicted to be affected in 41 of 42 cycles, euploid zygotes were available for transfer in 23 cycles. Overall, 39 euploid embryos were transferred (1.6 per cycle on the average), resulting in eight clinical pregnancies (33% per transfer), and birth of seven unaffected children (the implantation rate was 26% per embryo transferred). Although, the data is not sufficient for conclusions, the results of this study suggest that the array CGH analysis of PB1 and PB2 for 24 chromosome testing is accurate and reliable and allows performing PGD in time for fresh transfer, resulting in an acceptable clinical outcome.

A number of reports on the outcome of preimplantation 24 chromosome aneuploidy testing based on embryo biopsy were reported during the last 3 years, including two randomized controlled studies [44-50], all showing positive results of such comprehensive aneuploidy testing. Furthermore, some of the randomized control trials [50,51], showed that even young patients with only 45% abnormal embryos (compared to >60% in patients 35 and older), significantly benefit from blastocyst biopsy and array CGH, resulting in higher pregnancy rates. This means that the older patients should also benefit, provided sufficient number of embryos are available for testing, when 24-chromosome aneuploidy testing is applied combined with blastocyst biopsy. This is also significant in achieving a single embryo transfer, to avoid multiple pregnancies.

Preimplantation 24-chromosome aneuploidy testing is of special value in PGD for single gene disorders (Table 1). As mentioned, aneuploidy testing improved significantly pregnancy rates in preimplantation HLA typing, as the majority of patients in this group is of advanced reproductive age. As seen from Table 1, pregnancy rates was significantly improved with introduction of array-CGH based 24 chromosome aneuploidy testing, combined with PGD for 91 different single gene disorders, which was as high as 65.5% in contrast to 47.2% without aneuploidy testing. In addition, spontaneous abortions rate was reduced from 15.9% without aneuploidy testing to 5.3% in a combined 24 chromosome aneuploidy testing [52].

Testing	Patient/Cycle	# ET	# Embryos	Pregnancy	SAB	# Babies
SG (INCLUDING HLA)	1599/2602	1995	3706 (1.8)	943(49)* 47.2%	150 (15.9%)	939
SG+A by PCR or FISH	387/ 730	561	992 1.76	261(9)* 46.5%	22 (8.4%)	271
SG + aCGH For 91 conditions (INCLUDING HLA)	155 / 260	143	204 1.6	94 (20)* 65.7%	5 (5.3%)	81
TOTAL	2141/3592	2698	4902 (1.8)	1298 (78) (48%)	177 (13.6%)	

SG: Single Gene Disorders; A: Aneuploidy Testing; sCGH: arrayCGH
ET: Emryo Transfer; SAB: Spontaneous Abortions

Table 1: Results and clinical outcome of 3592 pgd cycles for single gene disorders and hla typing combined with aneuploidy testing by pcr, fish and array-cgh approaches.

While it may be predicted that preimplantation 24 chromosome aneuploidy testing will soon become a standard practice for IVF patients of advanced maternal age, it cannot be excluded that pre-selection of aneuploidy free embryos may appear even of higher value for younger IVF patients, as demonstrated in the randomized controlled studies above. So the IVF patients will need to be informed about the availability of PGD so they could be able to use the option of preimplantation aneuploidy testing. This will definitively contribute to improving the standards of the assisted reproduction practices, substituting the presently practiced “blind” selection of embryos for transfer using morphological parameters by the pre-selection of chromosomally normal embryos with the highest possible potential to result in pregnancy. As a tool for a reliable pre-selection of aneuploidy free embryos, PGD will potentially contribute not only to prevention of the birth of children with chromosomal disorders, but will also be a useful tool for the improvement of the efficiency and standards of assisted reproduction.

Conclusions

PGD has become a practical tool of detecting and avoiding from transfer the embryos with genetic abnormalities as an alternative to the embryo transfer based on morphological criteria in the current IVF practice. Although PGD was originally introduced for preexisting genetic conditions as an alternative option to prenatal diagnosis, it has become of special value for assisted reproduction practices, because genetic factors contribute considerably to the infertility problems. With the majority of the IVF patients being of advanced reproductive age, PGD provides an obvious option for a pre-selection and avoidance from transfer of the embryos with the age-related aneuploidies, the major contributors to spontaneous abortions and implantation failure. It has recently reported that the most notable effect can be observed in group of PGD patients of 40 year of age or older, provided that sufficient embryos are available for testing.

Based on the evaluation of the outcomes of thousands of PGD cycles, it was demonstrated that PGD is safe in comparison to non PGD cycles and those in which ICSI was applied. The diagnostic accuracy of PGD is extremely high, exceeding 99.5% per transfer in leading PGD centers. Of special value was the recent introduction of PGD for single gene disorders and HLA typing, together with 24 chromosome aneuploidy testing, resulting in significant improvement of pregnancy rates in patients with advanced reproductive age.

It may be predicted that, with the future improvement of the safety and accuracy, PGD may definitely contribute to improving the overall standards of the assisted reproduction practices, by pre-selection of chromosomally normal embryos with the higher potential to result in pregnancy.

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Citation: Kuliev A, Verlinsky O, Rechitsky S (2013) Safety, Accuracy and Reproductive Outcome of Preimplantation Genetic Diagnosis. *J IVF Reprod Med Genet* 1: 116. doi:[10.4172/2375-4508.1000116](https://doi.org/10.4172/2375-4508.1000116)

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