Clinical Management of in Vitro Fertilization with Preimplantation Genetic Diagnosis

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Abstract

Patients who undergo in vitro fertilization (IVF) because of preimplantation genetic diagnosis (PGD) require different clinical management than those who come in because of infertility alone. PGD adds a "fourth dimension" to the emotional aspect of a patients' assisted reproductive technology treatment. It significantly decreases the number of embryos available for transfer by 25 to 81%, and therefore ovarian stimulation for IVF with PGD should be tailored individually, taking into account patients' safety and estimated ovarian reserve. Recent studies showed that with increased number of oocytes retrieved, the higher the chance to have an embryo transfer and normal cryopreserved blastocysts. With adequate ovarian stimulation, there is no cutoff for the numbers of oocytes/embryos needed to start PGD with, especially for younger patients. Patient-friendly protocols, such as those based on gonadotropin-releasing hormone antagonist and vaginal progesterone support may be used. Elective single embryo transfer and blastocysts cryopreservation to avoid multiple pregnancies may be offered with PGD. The benefit of adding preimplantation genetic screening to IVF treatment is still controversial, and evidence-based data on 24-chromosome testing of polar bodies or trophectoderm is needed before it may be implemented into routine patient care. This review discusses the clinical management of IVF with PGD based on the best available data and my personal clinical experience as a reproductive specialist with >1000 IVF/intracytoplasmic sperm injection-PGD cycles. The information provided here will assist reproductive specialists, nurses, geneticists, genetic counselors, and embryologists to better counsel and treat couples who wish to conceive with a healthy child through IVF with PGD. It is time for PGD to be viewed as a modern modality of preventive medicine. As such, it should be incorporated into national health-care systems and be covered by medical insurance.

Keywords

- preimplantation genetic diagnosis (PGD)
- preimplantation genetic screening (PGS)
- assisted reproductive technology (ART)
- IVF outcome
- single embryo transfer (SET)
- nondisclosure PGD
- hematopoietic stem cell transplantation (HSCT)
- HLA typing

The use of genetic technology to avoid the birth of a child with a genetic disorder is in accordance with the ethical principles associated with physicians' therapeutic role.[1] Preimplantation genetic diagnosis (PGD) using in vitro fertilization (IVF) represents a major scientific advance for couples at risk of having children with heritable and debilitating genetic diseases.[2–5] PGD for couples who carry a balanced chromosomal translocation significantly decreases the risk of spontaneous miscarriage (up to <20%) and significantly increases live-birth rates.[2,6–10] The practice committees of the Society for Assisted Reproductive Technology and of the American Society for Reproductive Medicine published their opinion on PGD in 2008 and stated that "because the birth of the healthy child validates the efficacy of PGD, randomized controlled

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trials are not necessary."[2] At the same time it is highly recommended both in Europe and North America that couples be informed with detailed counseling on IVF/intracytoplasmic sperm injection (ICSI) procedures and risks, as well as the technical limitations of PGD. They should also be provided with realistic expectations for the number of the embryos they will have available for transfer and/or cryopreservation, as well as their chance to conceive and deliver a healthy infant.[2,3,11–13]

Several reproductive options are available for a high-risk couple to prevent passing on a genetic disorder to their offspring, so it is very important to examine how many couples would prefer PGD over other alternatives. It is clear now that most couples who carry a serious genetic disorder prefer to conceive with healthy embryos tested in vitro before implantation and thus avoid the very difficult dilemma of whether or not to terminate a pregnancy or deliver a sick child.[3] Musters et al[14] recently investigated the attitude of 960 couples with different genetic disorders toward PGD. Of the couples who wished to conceive, and if PGD would be performed without any significant delay, 80% preferred PGD over natural conception and prenatal testing. Even when delay in treatment of up to 1 to 2 years may occur (as may often may happen in Europe), because of lack of resources and time needed for PGD genetic setup, 74% of them still preferred to use PGD.

Most women would also prefer preimplantation genetic screening (PGS) as an alternative to prenatal testing for Down syndrome.[17] The benefit of adding PGS to IVF treatment is still controversial, and evidence-based data on 24-chromosome testing (of polar bodies or trophectoderm) is needed before it may be implemented into routine patient care.[2,12,14–16,18–28]

Since PGD was introduced in 1990 by Verlinsky et al[15] in Chicago with polar body biopsy and in London by Handyside et al that same year[16] with blastomere biopsy, its indications have expanded rapidly. Nevertheless, there are still questions to be answered regarding its clinical practice. This article discusses the safety of PGD procedures and children's outcome and reviews how to optimize ovarian stimulation and PGD success. The daily routine of clinical management of IVF for PGD, as well as special clinical dilemmas and protocols such as nondisclosure PGD, are examined.

The information provided here will assist reproductive specialists, nurses,[29] geneticists, genetic counselors, and embryologists to better counsel and treat couples who wish to conceive a healthy child through IVF with PGD. The potential for PGD to become a modern modality of preventive medicine is advocated.

Is PGD Safe? Embryo Development, Pregnancy Rates, and Children Born after PGD

Couples who wish to conceive with a healthy child want to know whether PGD affects the clinical outcome of IVF/ICSI. It is needless to say that any procedure performed on oocytes/embryos may damage a specific embryo. The experience of the assisted reproductive technology (ART) center in ovarian stimulation for IVF with PGD and in embryo micromanipulation, the technique used for biopsy, and the numbers of cells removed from the embryo may affect embryo development, implantation rate, and the pregnancy outcome.[30–33] The outcome of the study published by Mastenboek et al in 2007[34] raised similar concerns.[33,35]

Magli et al[36] investigated implantation rates of embryos after combined polar bodies and embryo biopsies compared with blastomere only. They found similar implantation rates (26% versus 25%, respectively) regardless of the number of micromanipulations performed. We have looked at whether one, two, or three micromanipulations for PGD for polar bodies and/or blastomere biopsies affects blastocysts development rate.[37] Comparing the development of 9925 embryos biopsied for PGD to 28,126 nonbiopsy ICSI embryos from the same time period revealed similar blastocysts development rates, irrespective of the number of biopsies performed. The PGD technique performed at our center was by mechanical breaching of the zona pellucida and by removing only one blastomere from day 3 embryos.[37] Removing two cells from a day 3 embryo has been shown to affect embryo development significantly, and this practice should be stopped.[30–32]A recent prospective cohort study demonstrated that the live-birth rate was significantly higher after one-cell biopsy on day 3 compared with that of two-cell biopsy, as commonly performed in Europe.[32] Whether the holes created in the zona pellucida for PGD increase the incidents of monozygotic twins was investigated by Verpoest et al.[38] They found 1.5% monozygotic twins in the clinical pregnancies established in the PGD group ($n = 1992$ cycles) compared with 2.1% in the 2429 IVF/ICSI cycles (p = not significant [NS]). This demonstrates that multiple micromanipulations on oocytes and embryos can be performed safely for PGD at experienced centers.[39]

PGD may, however, decrease the β-human chorionic gonadotropin (hCG) levels measured when patients conceive. Two groups have demonstrated initial lower β-hCG levels with pregnancies obtained after PGD compared with controls.[40,41] At the same time, the biochemical pregnancy rate, clinical miscarriage rate, and the take-home infant rate were similar in both groups. The lower β-hCG levels may result from the blastomere biopsy, which may decrease the β-hCG-producing activity of the trophoblast, especially at early pregnancy, or a delayed implantation that may occur in biopsied embryos. A delayed implantation may also be related to the type of controlled ovarian hyperstimulation (COH) used.[42] Although this finding poses no significant clinical concern, a lower cutoff value of serum β-hCG level for predicting successful pregnancy outcome following PGD procedure may be clinically implemented.

The data collected by the European Society of Human Reproduction and Embryology (ESHRE) PGD consortium suggests a lower pregnancy rate for cycles with PGD compared with regular ART.[43,44] However, comparing their results to those of some centers in the United States demonstrate this is not necessarily the case.[45–47] During a 4-year period (November 2002 to November 2006), the author performed 166 consecutive oocyte retrievals for IVF for PGD for 51 different monogenic disorders (women $\leq$42 years of age) at IHR/RGI in

Chicago.[46,47] The outcome of these cycles was compared with the outcome of the 520 oocyte retrievals from 45 centers reported by the PGD ESHRE consortium for 2004.[43] The mean women's age was 33 years in both groups. The mean number of oocytes retrieved, the fertilization rate by ICSI, and the percentage of embryos biopsied out of those fertilized were all significantly higher at IHR/RGI in Chicago compared with those in Europe (15.7 versus 13.7, 81% versus 71%, and 95% versus 71%, respectively; $p < 0.0001$). The mean number of embryos transferred was comparable, 1.7 in Chicago versus 1.9 in Europe. The implantation rates were 35% in Chicago versus 16% in Europe, and the clinical pregnancy rates per oocyte retrieval and per embryo transfer (ET) were 38% and 47% in Chicago, compared with 20% and 26% in Europe, respectively ($p < 0.0001$). When comparing data from Chicago to three leading centers in Europe, the percentage of live births per ET, of cycles performed at a similar time period for patients of mean age 30 to 33 years, was 44% in Chicago, which was significantly higher than the 32% in Brussels,[48,49] 29% in London,[50] and 23% in Paris.[51] The percentage live birth per couple reached 52% in Chicago. These data suggest that a team of reproductive specialist experienced in IVF with PGD, together with skilled embryologists and PGD laboratory (including removal of only one cell from day 3 embryos), may obtain high pregnancy rates.

Most importantly, parents undergoing PGD should expect similar developmental outcomes for their children as seen in those born following IVF/ICSI without PGD.[11,51–54] Children born after PGD do not demonstrate a higher rate of malformation or neonatal problems.[11,55–60] In fact, because infertility has recently been shown to be an independent factor affecting children's health with or without ART,[61] studies should investigate whether children born to couples who underwent PGD without infertility problems demonstrate an even lower rate of malformation or neonatal problems than IVF/ICSI-only children.

Several cohort studies demonstrated similar mental and psychomotor development at age 2 to 4 for children born after PGD compared with children born after natural conception or IVF/ICSI. No adverse effects of PGD/PGS on growth, congenital malformations, neonatal intensive care admissions, behavior, or mental and psychomotor development were found.[43,54,55,57–60,62,63] Middelburg et al[63] demonstrated that although scores on all mental, psychomotor, and behavioral tests were within the normal range, PGS children showed an unexplained lower neurological optimality scores compared with the control children. An increased rate of stillbirths in multiple pregnancies following PGD was reported from one center that used to remove two cells for biopsy, which needs further attention.[60] Long-term prospective follow-ups on PGD children should be continued.[11,60]

In summary, PGD in experienced laboratories seems to be safe, and couples may expect similar clinical outcomes as with regular ART. When biopsy is performed on day 3, only one cell should be removed for PGD/PGS. Embryo biopsy does not add risk factors to the health of singleton children born after PGD or PGS. Further prospective follow-up studies on biopsy safety and on children born should be encouraged.

How to Maximize IVF-PGD Outcome

A woman's chances of having a pregnancy and a live birth by using ART are influenced by many factors, some of which are patient related and outside a fertility clinic's control such as the woman's age, infertility diagnosis, history of previous births, previous miscarriages, and previous failed ART cycles.[64] When PGD is added to IVF, additional factors may affect treatment outcome. PGD decreases the numbers of embryos available for transfer by 25 to 81%. Twenty five percent of the embryos are expected to be affected with recessive single-gene disorders, 50% with dominant mutations, 30 to 70% (depending on women's age) will be aneuploid, 75% will demonstrate unbalanced translocation, and 81% will not be suitable for ET when PGD is performed for HLA matching together with recessive mutation. Moreover, the genetic status of the woman may affect her response to ovarian stimulation. The additional high cost and the technical complexities associated with PGD should also be considered when deciding on the number of embryos to be transferred after PGD.[46,48,65,66]

Infertility Diagnosis May Affect IVF-PGD Results

PGD was originally offered to fertile couples who delivered a child with a genetic disorder.[15,16] A recent study of the cumulative reproductive outcome of 1498 couples who underwent PGD found that fertility status and parity had no significant effect on PGD outcome.[49] However, in 10 to 45% of the cases, one may find in the evaluation prior to the IVF procedure that these couples have other conditions that cause infertility and may affect the IVF outcome.[23,67] For example, severe male factor, endometriosis, hydrosalpinx, and low ovarian reserve may be diagnosed. Embryo development after ICSI performed to avoid DNA contamination when molecular genetics is planned may be different when ISCI is performed because of severe male factor. Aneuploidy rate in embryos may also depend on the sperm quality.[68] Furthermore, the couples may have social habits such as smoking, drinking, and drug use that can also affect the ART results.

Thus, couples who come for IVF because of PGD should be routinely investigated for possible infertility factors independent of their genetic diagnosis. Evaluation of the ovarian reserve should be performed by using age, antral follicle count and/or anti-Müllerian hormone, and day 3 follicle-stimulating hormone (FSH) levels to allow individual tailoring of the ovarian stimulation protocol.[46,47,65,69–71] An investigation of the uterine cavity should also be done to check for polyps, submucosal fibroids, or intrauterine adhesions. Unsuspected uterine cavity abnormalities may be found in 11 to 22% of patients.[72–74] Appropriate treatment and counseling should be performed.

Ovarian Stimulation

Is There an Optimal Number of Oocytes to Start ART?

Any benefit of PGD can be demonstrated only when enough oocytes/embryos are available for biopsy.[75] ART success, even without PGD, was demonstrated to be strongly dependent on women's age and the number of oocytes retrieved. Sunkara

et al[76] published their analysis of 400,135 IVF/ICSI cycles performed in the United Kingdom. They found a strong association between the number of oocytes retrieved and live-birth rate (LBR) adjusted for age. The number of oocytes that maximized the LBR was 15. LBR rose with an increase in numbers of oocytes retrieved up to 15, plateaued between 15 and 20 oocytes, and steadily declined beyond 20 oocytes. Others have shown that the optimal number of retrieved oocytes to conceive in their center was 13.[77] They reached this conclusion after analyzing 7422 ART cycles and calculating the pregnancy rates per ET and per started IVF cycle. This optimal number of 10 to 15 oocytes may explain why mild ovarian stimulation is associated with decreased pregnancy rates.[78]

Is There a Minimal Number of Oocytes to Start PGD?

If the optimal number of oocytes to succeed in ART is ~10 to 15 and the PGD results will significantly decrease the number of embryos available for transfer by 25 to 81%, one may ask if there is a minimum number of oocytes with which to start PGD. The statistical uncertainties with the hidden variables important to the outcome of PGD and PGS are very high.[75] In 1998 Vandervorst et al[79] suggested that if fewer than six oocytes are expected to be retrieved, oocyte retrieval for IVF-PGD treatment should be canceled. Furthermore, if fewer than nine oocytes were to be retrieved, patients should be counseled on the poor prognosis for this cycle. This article has been quoted internationally for many years as a basis for canceling PGD when fewer than eight oocytes were expected/retrieved. New data challenge this cutoff practice.

To better counsel patients on IVF-PGD outcome, Tur-Kaspa et al[46,65,69] investigated PGD's efficiency to produce embryos suitable for ET and pregnancy, with low and high number of oocytes at different age groups. During a 4-year period (November 2002 to November 2006), 560 consecutive IVF-PGD cycles were performed at IHR/RGI in Chicago. A total of 251 PGD cycles were for aneuploidy screening (AS), 166 cycles for 51 single-gene disorders (SGD), 99 cycles for HLA-matched embryos with or without SGD with or without AS, and 44 for translocations with or without AS. Patients' ovarian reserves were estimated before treatment by age, antral follicle count, and day 3 FSH levels, and changes in the stimulation protocol or increases in medication dosage to try to improve oocyte yield were implemented accordingly. Data were analyzed for all cycles by the indication for PGD and by age. The availability of normal/unaffected embryos diagnosed by PGD, as well as the likelihood of ET and pregnancy, increased with the number of oocytes retrieved. Nevertheless, a low number of oocytes (fewer than seven) was still associated with a fair chance for ET and pregnancy, especially in young patients (<35 years of age). Therefore, Tur-Kaspa et al concluded that the practice of canceling of cycles when low number of oocytes/embryo are anticipated should be reconsidered.[46,65,69]

Verpoest et al,[49] from the same center as Vandervorst et al,[79] reevaluated the 1998 findings and summarized in 2009 the cumulative reproductive outcome of 1498 couples who underwent PGD. They reached a similar conclusion to the one recommended by Tur-Kaspa. The number of oocytes collected at retrieval significantly contributed to the reproductive outcome as an independent factor. At the same time, their analysis revealed there should be no cutoff of oocyte numbers below which PGD should be canceled. This new conclusion was in contrast with the previous report that Vandervorst et al[79] published in 1998. The larger number of cycles analyzed and the more comprehensive statistical analysis performed may explain this change.

A 2006 study on PGS for recurrent pregnancy loss also demonstrates that the recommendation to cancel PGD testing because of low numbers of embryos is not justified.[66] The authors investigated whether PGS should be continued in such patients when five or fewer embryos were achieved by IVF. All patients who consented for PGS and had five or fewer embryos on day 3 were given the option to cancel the PGS and have ET. When comparing the group that decided to cancel the PGS to the group that continued with it, the author demonstrated that the implantation and delivery rates were significantly higher in the group that continued with the PGS, even though fewer embryos were available for transfers. Unal et al[80] analyzed results of PGS cycles when one blastomere was biopsied from a total of 6098 day 3 embryos with at least seven blastomeres. Although ET and pregnancy were less frequent when fewer than six oocytes were aspirated, there were no significant difference in terms of implantation rates and clinical pregnancy once a patient had 6 to 10, 10 to 20, or >20 oocytes retrieved.

In summary, with optimal ovarian stimulation and when the patient wishes to conceive with her own eggs, IVF with PGD may be continued as long as we have at least one embryo for testing. Patients should be reminded that with lower oocyte yield, their chances of ET and pregnancy are lowered but not impossible. The practice of canceling PGD when fewer than eight oocytes were retrieved or fewer than four embryos are available for biopsy may be abandoned in most cases.

Ovarian Stimulation and Aneuploidy

Achieving maximally effective ovarian stimulation while avoiding ovarian hyperstimulation syndrome (OHSS) is a foremost concern in ART. COH is required to provide enough matured oocytes for insemination. The possible impact of exogenous gonadotropins with gonadotropin-stimulating hormone (GnRH) analogs on oocytes aneuploidy is crucial in determining the preferred COH protocol for IVF with PGD. The use of GnRH antagonists was suggested as a more patient-friendly protocol.[81,82] Munné et al[83] were first to suggest an association between exogenous FSH administration and the risk of human embryonic aneuploidy. This finding was supported by some animal models[84] as well as human in vitro studies[85] and some PGS studies.[86,87]

One randomized controlled trial (RCT)[87] suggested that mild COH using 150 IU of rec-FSH with GnRH antagonist protocol resulted in a significant lower embryonic aneuploidy rate compared with the conventional protocol of midluteal long agonist protocol with 225 IU of rec-FSH. Although this is a RCT (evidence level 1), one should be very cautious in the

interpretation of this study. Although 111 women, age 22 to 37 years, were randomized, 40% (27 of 67) of the cycles of the antagonist group and 25% (11 of 44) of the long agonist cycles did not reach PGD. Only 57% of the embryos that were generated were actually tested by PGS. When only one blastomere was biopsied for PGS, 34% (16 of 47) of the embryos in the antagonist group and 33% (20 of 61) in the agonist group were normal. When two cells were biopsies from day 3 embryos for PGS, 39% (37 of 96) of the embryos were diagnosed as normal in the antagonist group compared with 28% (27 of 98) in the agonist group. The ongoing pregnancy rates per started cycles were 19% in the antagonist group and 17% in the agonist group. Although this RCT was well designed, because of the low numbers of embryos that were actually tested for PGS, its result must be validated by other RCTs.

However, several studies from leading centers in the United States[88] and Europe[80,89,90] could not demonstrate a significant association between COH and aneuploidy rates. They retrospectively analyzed a combined sum of >14,000 oocytes/embryos and failed to find any significant effect of the type of medication used (pure FSH versus preparations with luteinizing hormone activity), the use of GnRH agonist versus antagonist, or the numbers of oocytes retrieved on aneuploidy rate.

Tur-Kaspa et al[88] analyzed 221 consecutive PGS cycles of patients <43 years of age with 2132 oocytes/embryos tested. The average rates of aneuploidy for women <35 years were 53 to 56% whether 1 to 7, 8 to 15, 16 to 20, and >21 oocytes were retrieved compared with 68 to 72% aneuploidy rates for women 36 to 42 years, respectively ($p < 0.05$). The number of oocytes retrieved, type of gonadotropins or GnRH analog used, antagonist or agonist, did not affect the aneuploidy rate. Kahraman's group have summarized aneuploidy rates from a total of 6098 day 3 embryos and found similar aneuploidy rate irrespective of the number of oocytes retrieves.[80] A recent retrospective study from an experienced center in Brussels[89] specifically looked at the possible effect on aneuploidy rate of long GnRH agonist use versus antagonist protocol. The study involved 694 consecutive PGS cycle for women <37 years of age. The aneuploidy rates were 50% in both groups, whether agonist or antagonist were used. Multivariate analysis showed that the type of stimulation adjusted for age, total gonadotropins dosage, and the numbers of oocytes retrieved did not influence the aneuploidy rate. Gianaroli et al,[90] from another leading European center, investigated aneuploidy rates in 3816 first polar bodies in 706 cycles and found no significant correlation between the proportions of normal oocytes and the type of stimulation protocol used, agonist or antagonist. An inverse and significant correlation was found between the proportion of normal oocytes and the number of FSH units used for COH per oocytes.

Poor responder patients are usually treated with a maximal dose of gonadotropins to try and increase the number of oocytes retrieved. Even in this specific challenging group, the aneuploidy rate was similar to control, suggesting that women responding poorly to COH are not at higher risk of producing aneuploid embryos in vitro.[91,92] The Brussels group[93] have retrospectively investigated factors affecting the outcome of a total of 2753 PGD and PGS cycles. Although age had a significantly negative effect on outcome, as expected and similar to regular ART, mode of inheritance, fertility status, and type of ovarian stimulation protocol did not influence the PGD success rates.

If COH will increase oocyte aneuploidy, an increased aneuploidy rate is also expected in the miscarriages after COH/intrauterine insemination (IUI) and/or IVF treatments. Massie et al[94] recently answered this question by examining the rate of aneuploidy in missed abortions among infertile couples after conceiving either through COH for IUI or IVF, or by natural conceptions. The rate of abnormal results in the cytogenetic analysis of the product of conceptions in pregnancies conceived with COH (63%) was similar to the rate in the spontaneous conceptions (70%). Moreover, despite a significant higher dosage of FSH used in the IVF group compared with the IUI patients, the miscarriage aneuploidy rate was 63% in both groups. This suggests that exogenous FSH used for COH does not increase the risk of aneuploidy.[89] Even in chromosomally normal infertile individuals, there appears to be an increased propensity to meiotic errors leading to aneuploidy.[95] Munné et al[96] compared aneuploidy rates in PGD embryos from infertile patients and normal oocytes donors (ages 18 to 34 in both groups) and found a significantly higher frequency of normal embryos in the donor group (43%) compared with the infertile group (34%), suggesting that infertile women produce more aneuploid embryos. Verpoest et al[97] demonstrated a 36.4% (95% confidence interval, 10.9 to 69.2) aneuploidy rate in embryos obtained with unstimulated IVF cycles (mean women's age: 31.4 years). Thus, infertile women should be counseled that infertility by itself could increase the probability of aneuploidy.

In summary, based on considerable data obtained from retrospective studies (>14,000 oocytes/embryos) from experienced European and U.S. centers, there is insufficient evidence to suggest that the type of gonadotropins and GnRH analogs used for ovarian stimulation protocols or the number of oocytes retrieved affect embryos' aneuploidy rate. However, inadequate stimulation and/or poor PGS/PGD techniques may indeed affect cycle outcome.

Genetic Status of the Woman and Her Response to Controlled Ovarian Hyperstimulation

The genetic diagnosis of the PGD patient may sometimes affect ovarian response to stimulation and thus may decrease the numbers of oocytes retrieved. One must remember that if these mutations, such as fragile X syndrome or myotonic dystrophy (DM), had been associated with infertility, they would not have become as frequent as they are. Polar bodies–based PGD may be performed for these or other mutations.[98,99] These young women may further demonstrate that there is no direct relationship between oocyte quantity and embryo quality.[100] Whether DM patients and female carriers of balanced translocations demonstrate lower response to COH is still debatable.

Fragile X Syndrome

Platteau et al[101] were the first to demonstrate that PGD for fragile Xa syndrome may be difficult but not impossible with regard to ovarian responsiveness to COH. Because women with a permutation are at increased risk of premature ovarian failure, it was no surprise that ~20 to 30% of these women responded poorly to COH. Nevertheless, once they achieved ET, their chance of conceiving is comparable with other patients. Infertility is usually not a presenting symptom of fragile X syndrome carriers, and it seems that the decrease in the numbers of oocytes retrieved represents a quantity issue rather than a quality problem with regard to implantation.

Myotonic Dystrophy

DM was also suggested to decrease ovarian response to COH.[102] Oligomenorrhea, miscarriages, and early menopause have been reported in women with myotonic dystrophy type 1 (DM1).[103] Male DM1 patients are known to have reduced sperm quality as a result of gonadal atrophy, and they have an increased risk of infertility.[104] Feyereisen et al[102] compared the ovarian response of carriers of X-linked disorders to those with DM. Although patient characteristics and the number of oocytes retrieved were similar, the numbers of days on COH were significantly prolonged and the prevalence of poor quality embryos was higher in the DM group. Sahu et al[105] also demonstrated a reduced ovarian reserve and ovarian response to COH for these women.

In contrast, Verpoest et al[106,107] found no evidence of decreased ovarian response to stimulation in women with DM1. They further demonstrated that the reproductive outcome for these women undergoing PGD was not affected by the size of the expanded CTG repeats. It seems that although some of these patients may have low response to COH, it is from resistance to stimulation and thus represents a quantity issue rather then poor oocytes quality.

Female Carriers of Balanced Translocation

Female carriers of balanced translocation may also have lower ovarian response to COH. Chen et al[108] demonstrated a significantly higher proportion of female carrier of balanced translocation who responded poorly to ovarian stimulation compared with women whose male partner had the translocation. They suggested that given the significant embryo attrition due to chromosomal imbalance, aggressive stimulation should be considered if the patient is not at risk for OHSS. We have looked at the clinical outcome of all consecutive oocyte retrievals that were performed at our center in a 4-year period for PGD for reciprocal translocations (Tur-Kaspa, unpublished data). When the translocation was of female origin, compared with cycles with male origins, even at age <35 years and with higher total dose of gonadotropins used (4787 ± 1646 versus 2971 ± 1280 IU), significantly fewer oocytes were retrieved (13.5 ± 3.5 versus 18.7 ± 6.6), fewer cycles resulted in ET (25% versus 77%), and fewer embryos were transferred (1.0 ± 0 versus 1.9 ± 0.5). Here again, once the patients had an ET, the clinical pregnancy rate per ET was good and reached 44%. Because PGD for translocations decreases the number of embryos available for ET by ~75%, we considered female carriers for translocation as potential low responders, and we increase the initial dosage of gonadotropins by 75–150 IU of rec-FSH, compared with other age-matched women, to try to improve oocyte yield.

However, Benner et al[109] found no differences in ovarian stimulation parameters and cycle outcomes when the translocation was of female origin compared with cycles with male origins. Their conclusion was that female carriers of balanced translocations have no diminished ovarian reserve.

In summary, the numbers of oocytes retrieved is a significant predictor of IVF-PGD cycle success, similar to regular ART cycles. Optimal oocyte yield seems to be ~15, 10 to 15 for the younger patients, and 15 to 20 for the older ones. Nevertheless, once patients were adequately stimulated, and wish to conceive with their own eggs, oocyte retrieval and PGD may be continued even with a very low number of oocytes or embryos (one to seven). When a young patient responds poorly to COH, there is no risk for OHSS, and gonadotropins dosage may be safely increased to the maximal dosage used by the center. Patients with fragile X, DM, or balanced translocation should be informed that although the ovarian response to COH may be decreased, the outcome of ETs is not affected. The possible role of mild stimulation for patients who come for IVF because of PGD remains to be investigated.

Elective Single Embryo Transfer and PGD

Multiple pregnancies are a major iatrogenic complication of ART, and elective single embryo transfer (eSET) has been suggested and implemented as the best strategy to prevent it. In some countries it is imposed by legislation and in others by guidelines and regulations of professional societies. Compared with double embryo transfer (DET), eSET is effective in significantly lowering twin pregnancy rates by 94%. However, it also significantly reduces the likelihood of live birth by 38%.[110,111] Nevertheless, evidence from RCTs suggests that increasing the number of eSET attempts (fresh or frozen) results in a cumulative LBR similar to that of DET.[110]

When postthaw survival and implantation rates of cryopreserved surplus blastocysts biopsied for PGD are comparable with the postthaw survival and implantation rates of nonbiopsied embryos, eSET with potential future frozen ETs should be recommended to young women undergoing PGD. These results were demonstrated for embryos that were biopsied either on day 3 or day 5 regardless of whether they were cryopreserved by slow freezing methods or by vitrification.[112–117] We at IHR/RGI[112] have been offering eSET to all patients <36 years since 2004, and when top-quality embryos are available for transfer, pregnancy rates were not compromised.

The group of de Boer et al,[118] from Sydney IVF, was the first to suggest moving to blastocyst biopsy for PGD/PGS with SET to reduce the rate of multiple pregnancies. They have shown that blastocyst biopsy on day 5 or 6 with cryopreservation allowed embryos to be electively thawed and transferred one at a time later on.

Donoso et al[48] demonstrated in a retrospective study that SET after PGD does not significantly reduce the deliver rate for

women <36 years of age. They compared the outcome of SET PGD cycles from 2003 to 2005 versus DET PGD cycles from 2001 to 2003. Although only 37% of patients undergoing SET had more than one embryo available for transfer (eSET), the delivery rates were 27.4% in the SET group and 34% for the DET group (p = NS). Although the SET pregnancy rates were from a later period and may have been affected by improved ART outcome generally observed during these years, this study still demonstrated that implementing SET for PGD significantly reduced the multiple pregnancies without affecting the delivery rate. El-Toukhy et al[115] described similar findings, experiencing a significant decrease in multiple pregnancies in the fresh PGD cycles (from 36% to 10%) with no reduction in pregnancy rates after implementing an eSET policy and cryopreservation of the extra embryos.

In summary, vitrified cryo-thawed biopsied embryos after PGD may reach a survival rate and implantation rate comparable with embryos with no biopsy. eSET should be offered to young women undergoing PGD or PGS, especially when top-quality embryos are available.

Clinical Expertise in IVF-PGD Treatments

Another fundamental factor in PGD success is the clinical experience and expertise of a particular ART center's staff and the quality of the IVF and PGD laboratories. Because the decision to undergo ART treatment is a very personal decision, requiring a commitment of time, effort, emotional energy, and money, couples considering an ART procedure should meet personally with an infertility specialist to discuss their specific medical situation and their likelihood of success at their center.[64]

An infertility specialist should have the experience to tailor the appropriate ovarian stimulation protocol for each patient undergoing IVF for PGD to prevent a genetic disorder, for translocation, or for HLA typing. Most ART centers offer PGD to their patients,[119] but the Centers for Disease Control and Prevention reported that in 2006 only 5 of 426 U.S. centers performed >50 cycles with PGD, and among them >10 cycles specifically for the purpose of prevention of genetic disorders.[64] Three of these centers are located in New York, one in Colorado, and one in Chicago, Illinois (IHR). The expertise of the embryologists in performing biopsies for PGD, as well as the type of procedure used to breach the zona pellucida[120] and the type (polar bodies, blastomere, and/or trophectoderm)[39] and number of cells biopsied,[30–33] may all affect embryonic survival and development.[36,37] Furthermore, the experience and the expertise of the PGD laboratory are important in providing reliable genetic results for each embryo.[11,25,33,121,122] The rate for misdiagnosis at experienced PGD laboratories has been reported to be <1.0% (0.3 to 0.6%).[18,25,54,123]

When PGD is not performed routinely at an ART center, collaboration with another more experienced institution should be established to provide optimal care for the couple. Both ESHRE and the PGD International Society (PGDIS) have recently updated their guidelines on the minimal requirement for ART and PGD centers and on the collaboration needed between those two centers.[11,13] This type of collaboration will ensure discussions on the preferred ovarian stimulation protocol and optimal location for the patient's monitoring, oocyte retrieval, and embryology work including embryo biopsy. A reliable communication system for reporting the PGD results should be established. Collaboration like this may be performed nationally or internationally as they are routinely being performed at IHR/RGI as well as at other leading centers, even if the couple resides where PGD procedures are banned.[124]

In summary, ART centers should evaluate their experience and outcome of IVF for PGD and decide whether they wish to offer it locally to patients or to collaborate with another more experienced center. Centers involved in PGD should follow the guidelines published by ESHRE and/or PGDIS. The monitoring for the stimulation may be performed locally, and the oocyte retrieval, embryology and biopsy, and PGD testing may be performed at a more experienced center. Alternatively, the IVF may be completed locally, including embryo biopsy by an experienced local or traveling embryologist, and the PGD performed at another location. Patients should be counseled on the experience and results of the center for such treatments.

Special PGD Challenges for Clinical Management

Nondisclosure PGD

A center's clinical experience is particularly important for special PGD cases, such as nondisclosure PGD. Most potential carriers of late-onset autosomal dominant diseases, such as Huntington disease (HD) and amyotrophic lateral sclerosis (ALS), decide that knowing their actual status as a carrier or not is significantly detrimental to their well-being, and therefore, they elect not to be tested. At the same time, they wish to assure that their children will not carry the disease and have to go through similar emotional turmoil. Nondisclosure PGD, in which mutation results are not disclosed to the couple, can achieve these goals simultaneously.

The way nondisclosure PGD originated, with direct mutation testing, has raised several ethical concerns. The first and most important concern was the difficulty in assuring full nondisclosure, especially in some leading PGD centers in Europe.[125,126] The need for nondisclosure brought on other ethical concerns for fear that patients would guess their genetic status based on their treatment results. These included the possible use of fake transfers for patients who did not have unaffected embryos for transfer, collecting payment for PGD that was not necessary in 50% of the time (for non-carriers), as well as avoiding embryo cryopreservation so that patients will not be able to guess their genetic status based on how many embryos were available for it. The consensus is that performing "unnecessary IVF" for ~50% of the couples to maintain the nondisclosure cannot be avoided. Exclusion testing for nondisclosure PGD was suggested as a way to resolve some of these issues. It is based on a linkage analysis with polymorphic markers when parental and grandparental origins of the chromosomes can be established, and thus the

genetic status of the potential carrier remains unknown both to the medical staff and to the patients. However, its main disadvantage is that it results in discarding also potentially unaffected embryos, decreasing the numbers of viable embryos available for transfer, and thus decreasing the patient's chance to conceive.

Tur-Kaspa and Najeemuddin[127] described a revised nondisclosure PGD protocol that we believe eliminates the ethical and practical dilemmas of the patients and of the medical staff. This protocol was approved by the institutional ethical board of IHR/RGI and was applied successfully in patient care. These are the main principles of this revised protocol[127]: (1) Direct mutation testing will be performed, but to maintain the nondisclosure, all medical and administrative staff in direct contact with the couple will not be aware of the genetic status of the patient. (2) General PGS will be added to decrease the risk of nonrelated fetal chromosomal abnormalities (thus the issue of payment for "unperformed PGD" will be avoided). (3) Because aneuploidy or embryo development may cause to have no embryos for transfer, regardless of the patients' genetic status, performing sham transfers will not be needed. (4) For the same reason, embryo cryopreservation will be performed when possible. (5) Because direct mutation testing will be performed, no unaffected embryos will be discarded. And (6), to avoid multiple pregnancies, the couple will decide in advance whether they wish for one or two (if possible) blastocysts to be transferred.

Patients with a family history of HD or ALS who underwent such nondisclosure IVF-PGD treatments successfully, delivered healthy children, and some have cryopreserved blastocysts. All patients and medical staff involved in their care were very comfortable with this protocol.[127] Although many different professionals were involved in the direct care of these couples, including collaboration with other national and international ART centers, ensuring nondivulgence of the carrier status of the patients was easy to maintain.

In summary, potential carriers of late-onset autosomal dominant diseases have the right not to know their genetic status. PGD can be performed effectively in selected centers while assuring nondisclosure for these couples.

PGD for HLA typing

Another unique PGD procedure is PGD for HLA typing, which has generated an ongoing international controversy on the issue of "savior siblings." IVF with PGD for HLA typing is a well-established procedure to conceive with a child who may donate matched hematopoietic stem cell transplantation (HSCT) to an ill sibling.[128] HSCT from an HLA matched donor is the best therapeutic option for children with nonmalignant disorders such as genetic diseases affecting the hematopoietic and/or the immune system (e.g., thalassemia, Fanconi anemia, Wiskott-Aldrich syndrome, sickle-cell disease) or acquired diseases like aplastic anemia. HLA-matched transplant from biological siblings are preferred as donors because of the reduced risks of transplant-related complications as compared with unrelated donors.[129,130] It can also be offered for the treatment of malignant diseases like leukemia. Match-related HSCT for leukemic patients has less posttransplant mobility and mortality rates compare with unrelated matched transplants.[131,132] About 70% of patients do not have a match-related donor in their immediate and extended family. IVF with PGD can be used to select unaffected HLA-matched embryo for ET and to give birth to a healthy matched sibling that can donate cord blood or bone marrow to its affected brother or sister.[133–138]

Ethical concerns of the medial staff and the lack of availability of this technology in many countries have significantly limited its use. To assist families when PGD for HLA was not available locally, IHR and RGI have established clinical collaborations with >100 different centers worldwide to provide these advanced services. Other leading centers have done the same.[137,138] Recently, Bellavia et al[124] described a setup for an international collaboration when PGD for HLA is not permitted locally.

Families should be informed about this option of PGD for HLA sooner than later to allow them ample time to consider it, especially because women's age is a major factor in ART success. Because we have encountered several occasions when PGD for HLA was never discussed with families that may need it, Tur-Kaspa and Najeemuddin[139] recently proposed clinical guidelines on when to offer PGD for HLA typing to parents of a sick child: PGD for HLA should be offered to families when a related matched donor is not available, within weeks of the diagnosis (even for diseases with a good prognosis), and when HSCT is not urgent and clinically can be postponed by at least 9 to 12 months. Moreover, if PGD for HLA is not available locally or nationally, than collaboration with another center should be established to have this treatment available for the family.

In summary, conceiving with a healthy infant that is HLA matched to his sibling is a unique opportunity for parents to act independently to save their sick child with HSCT. Clinical guidelines will improve the care of children in need of matched HSCT. Once future advances lead to improved outcomes of unrelated transplants, this rationale should be revised.

The Future of PGD

PGD may now be offered to patients with all known single-gene disorders, chromosomal rearrangement, for HLA-matched siblings, cancer predisposition genes, and for late-onset disorders with or without disclosure.[4,23,121,122,129,133,140,141]

PGS for advanced maternal age, repeated pregnancy loss, repeated IVF failures, and severe male infertility was on the rise until 2007.[25,45,119,122,142–147] So far RCTs have failed to demonstrate the suggested benefit of PGS for advanced maternal age in routine IVF/ICSI treatments when one to two blastomeres were biopsied on day 3 and were analyzed by fluorescent in situ hybridization.[23] New studies analyzing either polar bodies or blastocysts for 24 chromosomes are ongoing in Europe and the United States, and they may shed new light on the use of PGS.[24,26–28] Two leading U.S. ART groups recently achieved outstanding results with PGS. Scott et al[28] conducted the first RCT on 24-chromosome PGS by

quantitative polymerase chain reaction and fresh ET on day 5 and 6 in a small group of patients age <43 years. They achieved 92% clinical pregnancy rates in the PGS group compared with 60% in the control. Schoolcraft et al[27] performed PGS by single-nucleotide polymorphism–based microarray after trophectoderm biopsy, cryopreservation by vitrification, and later FETs (mean patient age was 38 years). They achieved a 56% LBR per oocyte retrieval and 71% per ET. The possibility to test for monogenic disorders or translocations together with aneuploidy makes PGD methods even more attractive.[4,136,148,149]

In 2006 Tur-Kaspa et al[150] and Offit et al[140] were the first to suggest that PGD should be viewed as a modern modality of preventive medicine.[5] Taking into account medical and psychological consideration as well as cost-benefit analysis, health-care professionals can contribute to the translation of ART and PGD into the practice of preventive medicine. This can be done by introducing PGD into routine care for families affected by childhood-onset genetic disorders, late-onset diseases, and hereditary cancer, and for children who are in need of HSCT.[4,5,140] Every couple of reproductive age who carry a genetic disorder may clinically benefit from PGD and should be counseled on it.

PGD should be an option not only for the few couples that can afford it.[4,5] IVF-PGD should be offered to all carrier couples that wish to conceive a healthy infant instead of one affected with a genetic disorder. Recently, two independent cost-benefit analyses, by Tur-Kaspa et al[5] and Davis et al,[151] demonstrated that offering IVF with PGD to all carrier couples of cystic fibrosis (CF), as an example, is highly cost effective and can save billions of dollars in direct medical costs. The implications of implementing a national IVF-PGD program for all carriers who wish for this procedure are remarkable not only from an economic perspective but also from a moral and personal perspective for families that carry a genetic disorder. Delivering a healthy infant instead of one affected with a debilitating and life-shortening disease means avoiding not only the direct medical treatment expenses and the significant loss of productivity for the patients and their caregivers over a lifetime but also gaining a normal quality of life. Therefore, Tur-Kaspa et al[5] have suggested that a large-scale national IVF-PGD program as a novel preventive medical strategy for diseases like CF may have a profound potential in modern health-care systems. PGD should be encouraged to become an integral part of any health-care system and should be covered by medical insurances. Once given the option, the final decision is of course for the couple to make. When a cure is found to a specific disease, this rationale should be reevaluated.

Biopsy for PGD may affect implantation potential, but in general seems to be safe, except when two cells are removed on day 3. Children born after PGD have similar outcomes to regular IVF/ICSI infants. Optimization and individualization of ovarian stimulation and collaborations with experienced centers should allow PGD to continue with any number of oocytes/embryos available. SET may be offered to young patients after PGD or PGS for 24 chromosomes[152] with good quality blastocysts to avoid a multiple pregnancy. Better clinical management of IVF-PGD treatments will improve its outcome.

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