Reproductive BioMedicine Online (2010) 21, 280–282

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COMMENTARY

Preimplantation genetic diagnosis after 20 years

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Abstract Preimplantation genetic diagnosis (PGD) should not be an option only for the few couples at risk of serious genetic conditions who can afford it. We appear to have lost sight of the original driving force behind the development of PGD, which is that most couples who carry a serious genetic disorder find it more acceptable to choose to conceive with healthy embryos tested in-vitro at preimplantation stages of development within the first week following fertilization, even if that means discarding those diagnosed as affected. It has been shown using cystic fibrosis as an example, that the cost savings to the US healthcare system of providing free IVF-PGD to all carrier couples compared to the lifetime costs of medical treatment for patients affected by this disease, run to dozens of billions of dollars. With the increasing emphasis in medicine on early diagnosis and prevention of disease together with the availability of new molecular genetic diagnostic tools, a national IVF-PGD programme seems to be the next step in modern health care.

KEYWORDS: aneuploidy, cost-benefit analysis, preimplantation genetic diagnosis, single gene defect

Preimplantation genetic diagnosis (PGD) should not be an option only for the few couples at risk of serious genetic conditions who can afford it. We appear to have lost sight of the original driving force behind the development of PGD, which is that most couples who carry a serious genetic disorder find it more acceptable to choose to conceive with healthy embryos tested in-vitro at preimplantation stages of development within the first week following fertilization, even if that means discarding those diagnosed as affected. Thus, they can avoid the very difficult dilemma of whether or not to terminate a pregnancy or to deliver a sick child. This is particularly so if the condition is not immediately life-threatening, but from their own experience of affected children or relatives (such as with cancer predisposition genes or Huntington disease), they consider it as serious enough to undergo IVF treatment with PGD to avoid the birth of affected children.

Professor Ilan Tur-Kaspa and colleagues, including the late PGD pioneer Yury Verlinsky, argue persuasively, using cystic fibrosis as an example, that the cost savings to the US healthcare system of providing free IVF-PGD to all carrier couples compared to the lifetime costs of medical treatment for patients affected by this disease, run to dozens of billions of dollars (Tur-Kaspa et al., 2010). While the cost of medical care for such patients can be lower in Europe or in other continents, the IVF-PGD treatment will cost also less. Therefore, such a national IVF-PGD programme will still be cost effective. Given that new microarray-based testing for common mutations in a range of inherited disease is now possible and population screening is actively being considered, PGD could play a major role in reducing the incidence of inherited disease. Tur-Kaspa and co-workers suggest that IVF-PGD, if covered by national healthcare services or other providers and available to all that are in need, and not only to those who can afford it, will be highly cost-effective. While medical, ethical and economic discussions will continue, this is could be the new way of modern preventive medicine.

IVF with PGD represents a major scientific advance for couples known to be at risk of having children with a herita-

1472-6483/$ - see front matter
doi:10.1016/j.rbmo.2010.07.007

ble and debilitating genetic disease. In addition, for couples with chromosomal translocations, PGD increases significantly the likelihood of achieving a live birth. Twenty years ago, Robert Winston and I, leading a small group at Hammersmith Hospital in London, published a report on the first pregnancies following IVF, cleavage-stage embryo biopsy and genetic testing for inherited disease, now known as PGD (Handyside et al., 1990). For the first time, at-risk couples could start a pregnancy knowing from the beginning it would be unaffected and avoid the possibility of terminating an affected spontaneous pregnancy following conventional invasive prenatal tests at later stages of development. Looking back over 20 years and thousands of healthy children born worldwide following IVF-PGD, what have been the major developments and what are the challenges for the future?

Twenty years ago, application of the polymerase chain reaction (PCR) for amplification of specific target fragments of DNA from single cells was still in its infancy. Nevertheless, we were able to demonstrate that amplification of a highly repetitive Y chromosome-specific sequence enabled the identification of male embryos, allowing selective transfer of females in several couples at risk of having children with various X-linked conditions, including Duchenne muscular dystrophy, which typically only affect males. However, for rapid detection of the amplified fragment on minigels, high numbers of PCR cycles were required and both contamination with extraneous DNA and, conversely, amplification failure limited the accuracy of the diagnosis. As a result, one of these first pregnancies was confirmed to be male by chorion villus sampling and was terminated. This stimulated the development of alternative more reliable methods including multicolour fluorescence in-situ hybridisation (FISH) to interphase nuclei for identification of gender with X-, Y- and autosomal-specific control probes. Further development and extension of the number of probes, which could be analysed in a single nucleus, using sequential rounds of FISH was later extensively used for detection of chromosome aneuploidy and translocation imbalance.

Another development was the use of two rounds of PCR using nested primers, so-called nested PCR, which reduces the possibility of errors caused by amplification of previously amplified fragments and allows the amplification of unique sequences from single cells. Working with Mark Hughes and his team, this led in 1992 to the report of the first birth of a healthy child following specific diagnosis of a single gene defect, in this case the common delta F508, three base pair deletion causing cystic fibrosis (Handyside et al., 1992). Since then many groups have contributed to the further development of increasingly accurate strategies for a seemingly ever expanding range of genetic defects including all of the common and many rare Mendelian single gene defects, late onset conditions, predisposition to cancer and HLA matching for so called 'saviour siblings' which was pioneered by the late Yury Verlinsky and his colleagues. Now the use of fluorescent multiplex PCR combined with automated sequencer-based fragment analysis allows the successful amplification of multiple closely linked polymorphic markers combined with minisequencing for direct mutation detection. This minimises the risk of errors caused by undetected allele dropout and has provided a highly accurate and widely applicable method for PGD of any single gene defect for which markers can be identified in the families (Fiorentino et al., 2006). Furthermore, this has been greatly facilitated by completion of the sequencing of the human genome and would not have been possible 20 years ago because the sequence information was not freely available.

Other more recent developments include the use of whole genome amplification (WGA), using either improved PCR-based protocols or isothermal multiple displacement amplification (Handyside et al., 2004), which can generate micrograms of DNA from single cells. Although the resulting DNA still has 'gaps' and does not eliminate allele dropout, the amount of DNA available allows extensive testing under conventional conditions not requiring a specialist laboratory to exclude contamination. This has allowed the development of generic approaches like preimplantation genetic haplotyping using large panels of markers applicable to most families at risk of specific conditions. Also, with the availability of larger quantities of DNA, genome-wide analysis using various types of microarrays has become possible. Microarrays of DNA probes spaced evenly across the genome, for example, can be used for comparative genomic hybridisation and detection of aneuploidy for all 24 chromosomes and for translocation imbalance. High density single nucleotide polymorphism (SNP) genotyping arrays can also be used for high resolution molecular cytogenetics and linkage analysis (Johnson et al., 2010; Treff et al., in press). Furthermore, karyomapping by Mendelian analysis of the SNP genotypes of the parents and appropriate family members provides a universal approach for genome-wide linkage-based analysis of any genetic abnormalities, combined with detection of chromosomal aneuploidy and other abnormalities (Handyside et al., in press).

PGD is now considered as a well-established clinical service in many centres around the world and there are also a number of laboratories specialising in developing patient- and disease-specific tests and performing the single cell analysis on samples sent to them from remote clinics. Cleavage-stage embryo biopsy and single cell analysis has proved to be efficient and reliable for PGD and remains the most widespread general approach. However, the discovery that human embryos have a relatively high incidence of chromosome aneuploidy of post-zygotic origin which arises during the early cleavage divisions may partly explain the variable clinical outcomes following aneuploidy screening. For this reason, biopsy of the first and/or second polar bodies or trophectoderm biopsy at the blastocyst stage are being used increasingly to detect inherited meiotic aneuploidies affecting the whole embryo (Cieslak-Janzen et al., 2006).

The European Society of Human Reproduction and Embryology (ESHRE) PGD Consortium was set up in 1997 and maintains a detailed database of PGD treatment cycles and their outcome in all of the major centres in Europe and several in other countries (ESHRE PGD) (Goossens et al., 2009). Most importantly, it has also established detailed follow-up of the children born following PGD. When compared with children born following intracytoplasmic sperm injection, the Consortium data show no significant increase in serious congenital abnormalities. The main complication during pregnancy is prematurity, often associated with

multiple pregnancies, but this is similar to pregnancies following IVF generally. A recent report of an increased incidence of still-births in multiple pregnancies following PGD in one centre warrants further investigation (Liebaers et al., 2010). Reducing the proportion of multiple pregnancies therefore remains a priority. The possibility of using less destructive methods for cryopreserving biopsied embryos by vitrification, particularly at the blastocyst stage, should encourage patients to elect for single embryo transfer.

What of the future for PGD? Considering the significant improvements in IVF pregnancy rates and diagnostic accuracy over the last 20 years, it is disappointing that conventional prenatal diagnostic procedures continue to far outnumber PGD cycles. PGD was initially pioneered outside the mainstream of clinical genetics and reports of misdiagnoses, together with the widespread belief that IVF pregnancy rates were low, discouraged clinicians from recommending PGD as an option and diagnostic laboratories from becoming involved. In addition, the requirement to set up clean areas free from potential DNA contamination meant that it was impossible for many laboratories even to consider it. Furthermore, many patients could not afford IVF treatment with the additional cost of testing and those that could often had to wait long periods, in some cases years, for bespoke tests to be developed and tested on single cells. PGD also rapidly acquired the pejorative epithet of 'designer baby', despite the obvious untruth of this description, and in the UK has become over-regulated, causing additional unnecessary delays in treatment, with the preimplantation embryo being better protected than the fetus (Handyside, 2010). It is important to note that couples are not encouraged in any way to undergo PGD if it is against their moral perspectives or religious concerns. But as Tur-Kaspa and colleagues have shown, even if only 50% of the carrier couples will undergo IVF-PGD, such a programme will still be very cost-effective and will save billions of dollars for any healthcare system.

Today, with pregnancy rates for women under the age of 35 approaching 50% per cycle and many clinics encouraging single blastocyst transfer to avoid multiple pregnancy, the prospects for a successful clinical outcome, particularly in younger couples, have improved considerably. Furthermore, by adopting WGA strategies and microarray-based testing, specialised DNA-free labs are no longer essential and a broad range of conditions can be diagnosed without the need for labour-intensive patient- or disease-specific test development. In my view, it is time that national healthcare systems reconsidered the contribution that PGD could make to clinical genetics. In the UK, funding from the National Health Service is often available but only after consideration at a local level on a case-by-case basis and some years ago, when PGD was considered by the Department of Health, it was concluded that PGD was still an experimental treatment.

With an increasing emphasis in medicine on early diagnosis and prevention of disease together with the availability of new molecular genetic diagnostic tools, a national IVF-PGD programme seems to be the next step in modern healthcare. While celebrating the 20th birthdays of the first PGD children, the cost-benefit analysis by Tur-Kaspa and colleagues suggests that from both a medical and an economic point of view, PGD is now ready for 'prime time'.

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Declaration: the author reports no financial or commercial conflicts of interest.