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Preimplantation genetic diagnosis and embryo research – human developmental biology in clinical practice

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ABSTRACT Research on human preimplantation embryos *in vitro* is controversial. Yet it has been the cornerstone for the development important clinical assisted conception techniques. Preimplantation genetic diagnosis which has developed out of this assisted reproductive technology for the first time provides a realistic alternative to prenatal diagnosis and abortion for couples who are at substantial risk of conceiving a pregnancy affected by a known genetic disorder. It also provides the first real hope of therapy for couples who have suffered repeated miscarriages due to chromosome translocations. However, the ability to test very early embryos *in vitro* presents new and unusual ethical challenges for clinicians and developmental biologists.

KEY WORDS: *Preimplantation genetic diagnosis, human embryo research, embryo biopsy, PGD.*

Preimplantation Genetic Diagnosis (PGD) is an emerging alternative to prenatal diagnosis (PND), being suitable for a couples who are at substantial risk of conceiving a pregnancy affected by a known genetic disorder (Taylor and Braude, 1994). A single cell can be removed as a biopsy from cleavage stage embryos, or a larger number of cells removed at the blastocyst stage (Summers *et al.*, 1988; Veiga *et al.*, 1997). Alternatively the first and/or second polar body can be removed for analysis (Rechitsky *et al.*, 1999; Verlinsky *et al.*, 1998). A diagnostic genetic test is then performed on the biopsied material and only embryos believed unaffected by the genetic disorder are made available for replacement into the uterus.

PGD has been facilitated as a direct consequence of a number of groundbreaking techniques: the ability to fertilise a human egg *in vitro* and culture the zygotes sufficiently to reach at least day 4 of preimplantation development (Gardner and Lane, 1997); the ability to remove safely a single blastomere from a cleavage stage embryo and not to compromise further development substantially (Hardy *et al.*, 1990; Tarin and Handyside, 1993); the ability to amplify minuscule quantities of DNA using PCR so that current methods of genetic analysis can be applied (Muggleton-Harris *et al.*, 1995; Sermon *et al.*, 1996; Strom *et al.*, 1994; Tsai, 1999); the ability to achieve fertilisation *in vitro* by the injection of a single sperm into an egg in order to prevent contamination of biopsied embryonic sample with sperm DNA(Van Steirteghem *et al.*, 1993). Of greatest importance is the fact that many of the embryological techniques in common use today could not have been developed without the ability to research on human embryos *in vitro* (Bock and O'Connor, 1986; Braude and Johnson, 1989). It is not often appreciated quite how much Anne McLaren was instrumental in maintaining that ability (McLaren, 1988; McLaren, 1989; McLaren, 1990).

The Report of the Committee of Inquiry into Human Fertilisation and Embryology (DHSS, 1984) was one of the most important documents for human developmental biology this century (McLaren, 1985b; Warnock, 1985). Not only did it influence profoundly the way that assisted conception is practised in the United Kingdom - the Human Fertilisation and Embryology Act (1990) being passed as a direct consequence of its recommendations - but its impact has been felt internationally because of its unique inclusion of regulation of research on human embryos. Being the first country to legislate comprehensively in this area, the HFE Act has become a model for the deliberations of many other countries worldwide. Of fundamental importance was the recognition that research on human embryos needed to continue, but needed to continue in a way that would ensure public confidence. The statutory regulatory body the Human Fertilisation and Embryology Authority (HFEA), formation of which was required by the Act, licenses and inspects all assisted conception treatment in the UK, including research projects which use human preimplantation embryos. Being the only embryologist on the Committee of Inquiry, Anne was required to communicate quite complex issues about embryological development to many who were not scientists but in whose gift lay decisions about the degree and direction of progress. As is still the case, many were opposed to research on human embryos for

Abbreviations used in this paper: HFE, human fertilisation and embryology; PGD, Preimplantation genetic diagnosis.

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religious reasons, but for those who were less certain of the morality of so doing, Anne's solid scientific knowledge and rational thinking must have been a great help. The reintroduction of the term pre-embryo to make clear the distinction between those stages where multipotentiality and plasticity were still present, from those later stages where differentiation into embryo proper and trophectodermal derivatives had occurred was important (McLaren, 1986; McLaren, 1987). However, this definition drew criticism, both from opponents of embryo research, who felt this was just a ploy to fudge the issue of what were just experiments on "persons", and from those in the scientific community who opposed it for being scientifically invalid or unnecessary, not quite understanding the need and usefulness of this distinction for those who were struggling with developmental concepts. However it had a certain logical imperative which was helpful for those focussed on analysing the development of soul and individuality (Ford, 1998; Johnson, 1989). In making this distinction a time could be proposed as to when the conceptus was no longer capable of twinning and hence could be regarded as an individual (McLaren, 1984). Anne's solid scientific background, profound embryological knowledge and quiet but measured manner must have been a very persuasive positive influence on the interchanges that took place on that committee which led directly to what is generally considered sensible yet cautiously permissive legislation.

It is now 10 years since the passage of that legislation, yet the subject of embryo research is still the subject of intense media attention and for many is still highly controversial. It is comforting that despite the huge changes that have taken place in the practice of assisted conception treatment, in our understanding of development of the human embryo and in genetics in general, most of those recommendations are still valid.

That continued ability to examine human embryos *in vitro* has allowed important information to be gathered about gene activity (Braude *et al.*, 1988; Tesarik *et al.*, 1988), DNA replication (Capmany *et al.*, 1996), chromosome complement (Munne *et al.*, 1994; Plachot *et al.*, 1988; Van Blerkom, 1989), and cytoarchitecture (Johnson *et al.*, 1990; Van Blerkom and Davis, 1995) as well as metabolic activity (Leese *et al.*, 1993) of the early human preimplantation embryo. The advent of preimplantation genetic diagnosis and the frequent examination of embryos by molecular and cytogenetic techniques are providing more information daily (Munne *et al.*, 2000). Although we have a clearer idea about the natural milestones *in vitro* in the human, it is the differences from other mammalian embryos seemingly unique to the human that are most surprising (Tesarik, 1988). Why are so many human embryos of such a "poor quality" when one compares them to development of other mammalian embryos *in vitro*? Why are fragmentation and multinucleation (Pickering *et al.*, 1995) such a frequent and frustrating feature of human *in vitro* development? Why is there such a high level of aneuploidy and mosaicism? The reasons for these occurrences are speculative but it would not be surprising if the quality of the media in which embryos are fertilised and grown should prove relevant. Certainly the impact of newly developed sequential media allows a greater proportion of embryos to reach the blastocyst stage and also to reach each milestone more consistently (Gardner and Schoolcraft, 1998) although a substantial improvement in pregnancy rates following transfer is yet to be shown convincingly. Apoptosis is a putative cause of fragmentation (Jurisicova *et al.*, 1996). However why this should be a feature so persistent in human development *in vitro* is unclear. We have so little comparative information about *in vivo* fertilised naturally developing embryos (Buster *et al.*, 1985; Croxatto *et al.*, 1972) that it is difficult to decide whether these are features of *in vitro* culture, or whether they are hallmarks of the human embryonic development in general. The extraordinary level of chromosomal abnormality in human oocytes and embryos might be attributed to the fact that most of the embryos examined have come from women with a fertility problem. However, examination of embryos from couples where the only defect seems to be the extraordinarily low sperm count in the male, demonstrates similar levels of chromosomal chaos. It is also present in embryos where the chromosomes have been examined by FISH for sexing for X-linked disease, suggesting that aneuploidy and mosaicism are features of embryos from the fertile as well as the infertile. Perhaps the way these oocytes are recruited and gathered also plays a part. The concept that superovulation recruits and possibly rescues from atresia follicles that were never destined to ovulate may be relevant (Braude, 1998; Van Blerkom *et al.*, 1997). The parameters on which we base the decision to harvest oocytes for IVF are so crude (the size of the follicle on ultrasound and consistently rising estradiol level), that it is not surprising that many of the oocytes are not appropriately matured (Moor *et al.*, 1998; Van Blerkom, 1990). However the fact that superovulation regimes in mice and other mammals seem to produce embryos of a uniform and acceptable quality, would tend to counter this.

Many of the practical aspects of PGD were developed in Anne's laboratory or following her enthusiastic ideas (Braude *et al.*, 1989; Monk *et al.*, 1988; Monk *et al.*, 1987; Monk and Holding, 1990). Indeed she was writing about its potential use in principle many years before the technique was established as even feasible let alone useful (McLaren, 1985a; Penketh and McLaren, 1987). PGD has been an important therapeutic development in the diagnosis and prevention of genetic disease and has given couples who carry genetic disorders a realistic alternative reproductive option to gamete donation, adoption or continuing to play "reproductive roulette". Conventional prenatal genetic diagnosis requires the couple first to achieve a pregnancy naturally and then to be faced with the difficult decision as to whether or not to terminate a wanted pregnancy. It is also an alternative for those women who may be opposed to abortion on religious or moral grounds yet face a 25% chance, or in dominant conditions, a 50% chance of having an affected child. It has been our experience that this latter reason for requesting PGD is relatively infrequent – rather they are couples who have had at least one affected child and who have terminated one or more affected pregnancies who then call "enough" and look to PGD as their only reasonable alternative option (Bickerstaff *et al.*, 2000).

Preimplantation genetic diagnosis was first applied to perform sex selection using PCR for X linked disease (Handyside *et al.*, 1990). It now may be offered for a variety of single gene defects (Wells and Sherlock, 1998), chromosomal imbalances (Conn *et al.*, 1998; Munne *et al.*, 1998; Scriven *et al.*, 2000) and for sex selection for X-linked disease. Initially very few tr*et al.*, 1998*et al.*, 1998*et al.*, 1998eatment cycles were performed and treatment methods and results were published for individual cases. A large body of literature about PGD now exists which reflects the huge research potential and commitment associated with the investigation and detection of genetic diseases at the single cell level. Although the exact number of cases being performed annually is

difficult to assess precisely, from the early days of PGD development, attempts have been made to gather data and share information by an international working group which reported annually (Editorial, 1999). More recently, a European consortium (ESHRE PGD Consortium Steering Committee) has undertaken the first long term study of the efficacy and outcome of PGD in Europe (Geraedts *et al.*, 1999).

For the most part the reasons for requesting PGD are clear – that of a lethal disorder which has already claimed one or more members of the family, or where the woman repeatedly miscarries or has a handicapped child due to an unbalanced translocation (the most frequent reason for referral to our unit), or where there is a child severely handicapped by an inheritable genetic or chromosomal disorder. However as is often the case with the availability of new technology, it presents difficult and often unanticipated scenarios as to its appropriate use. How, and should one limit its use for genuine medical purposes? Where and how do we draw the line between genuine prevention of inherited genetic defect, when does PGD become screening and when is its use inappropriate eugenics? How severe does the disease have to be for the request for PGD to be reasonable? Does society feel comfortable about its use for "family balancing" – choosing the desired sex of offspring for social reasons? What of its use for genetic screening as part of a range of conditions apart from the main genetic one to be tested – e.g. sexing in addition to diagnosing cystic fibrosis, or screening for Down syndrome while looking for translocations. It is not surprising that the spectre of designer babies has been raised, and that there are strident calls for clear guidelines to be issued. Throughout most of the world there are no rules and the decisions are left to individual clinicians or those teams practising their art. This is not the case in the UK. The HFEA requires that each condition for which PGD is performed must be licensed, and in that application for a licence, a clear case must be given of the medical reason for its request. In addition, it must be demonstrated that the unit has the skills to perform the test and has taken cognisance of the need for quality control and accuracy within its laboratory. However, who judges how severe the disease needs to be? Many disability groups would contend that testing for and selecting out affected embryos sends the message that disability is unacceptable in our society and reduces tolerance of those who have non-treatable or non-inheritable disabling conditions. In the UK, under the terms of the Human Fertilisation and Embryology Authority's Code of Practice, each clinic performing PGD is required to take into account the welfare of any child born as a result of a licensable treatment (IVF, ICSI and DI)[1] . This presents novel difficulties in the diagnosis of late onset disease and when there is one or more handicapped or affected children in the family unit.

Three examples, which have rattled the heads of some of my own students, may help in understanding the new dilemmas that have been created by the use of PGD.

Case 1: Request for replacement of affected embryos

Jeff and Sarah are both aged 32 and both suffer from achondroplasia, a dominantly inherited condition characterised by extreme short stature but without any problems of mental retardation. As they are both affected the chance of them conceiving an affected child by normal conception is 50%, and an unaffected child is 25%, and with a double dominant (lethal in utero) 25%. They wish to have PGD to ensure that they do not have a child with the double dominant but unusually they request to have heterozygotes replaced rather than the homozygote unaffected to guarantee them a child with achondroplasia like them. To their way of thinking, achondroplasia is a positive advantage in life. Both feel that their lives have been enhanced by the disorder, partly through having to cope with the disability and coping with other people's perceptions of them. Furthermore they feel that an unaffected child may feel excluded and stigmatised amongst their family and friends through "suffering" normal stature. Lastly they are worried about obstetric complications if Sarah were to be pregnant with an unaffected child.

The issue of who should make the choice as to which embryos are replaced is a vexed one. Patient autonomy should be primary but under terms of the HFEAct, the welfare of the child must be taken into account before agreeing to provide treatment. Who is it who decides what appropriate quality of life should be? Is it reasonable to assist a couple to have a child with a medical disorder? However, it is noteworthy that although the couple would have a 25% risk of having a dead baby, and an equal risk of having one without achondroplasia by natural conception – not their ideal - the odds of live child being affected with achondroplasia without medical interference are 2:1. It may be argued that since there are no Acts to stop them so doing on their own, why not help them avoid a lethal condition at their request, and comply with their wishes. Would replacement of embryos where only the double dominant is selected out, but where heterozygotes could be replaced with homozygote normals as would occur naturally be an acceptable "quasi-natural' option?

Case 2: Non-disclosure PGD for Huntington's Disease

Huntington's disease (HD) is a progressive neuropsychiatric late onset genetic disorder characterised by involuntary movements (chorea), cognitive deterioration and affective symptoms. There is no cure for the illness, the only treatment being symptom relief and support. The mean age of onset is 40 years. Symptoms progress slowly with death occurring an average of 15 years after the start of disease process. The mode of inheritance is autosomal dominant with variable age related penetrance.

HD is a triplet repeat disorder. The expansion of a repeat array of CAG on the short arm of chromosome 4 disturbs the normal function of the gene, which codes for a protein called huntingtin. The normal gene has around 20 repeats of the CAG trinucleotide. Expansion of the unstable trinucleotide repeats beyond 36 results in disease.

Linda and John request PGD for Huntington's disease. Linda aged 34, found out about HD 6 years ago when her father's condition was diagnosed. He is now requiring large amounts of nursing care, and suffers from depression. Linda knows that she is at 50% risk of carrying the gene, but after counselling has declined to undergo predictive testing. She was already married to John at the time of her fathers' diagnosis. The couple had always wanted children and after long consideration decided to try to conceive. No grandparental DNA was available for use in linkage testing for conventional prenatal diagnosis – thus they have opted for PGD. However Linda has specified that she did not wish to know the

[1] A woman shall not be provided with treatment services unless account has been taken of the welfare of any child who may be born as a result of treatment...and of any other children who may be affected by the birth – HFE Act (1990).

result of the diagnosis under any circumstances (non-disclosure) as long as only unaffected embryos are replaced. During the PGD cycle, 15 embryos are tested and all are found to be unaffected making it highly unlikely that Linda has Huntington's disease. However because of her specific request this news (which we may feel is good news) may not be given to her. Despite the replacement of three embryos, Linda does not fall pregnant and she requests a further IVF cycle, which her clinicians know she does not need and has difficulty in affording.

This case illustrates the some of the difficulties of PGD for late onset disease and the pitfalls of complying with the request for non-disclosure (Braude *et al.*, 1998). Is it justified to perform PGD and exclude embryos for a disorder where affected individuals will have 40 or 50 years of normal life ahead of them? Is it in the interests of the child to deliberately be brought into a family where one parent (perhaps the mother) will require increasing amounts of nursing care and will become progressively more deranged and debilitated? Besides the difficulty of maintaining complete confidentiality with the number of people who are involved in making a PGD diagnosis, the commitment to non-disclosure leaves the clinician vulnerable in having to undertake potentially hazardous treatment when it is not needed. Furthermore should all the embryos have been found to be affected, or for some other non-genetic reason there were no embryos for transfer, they are placed in the invidious position of having to undertake a mock embryo transfer or to explain the reason for no transfer without inferring the diagnosis.

Case 3: PGD to provide a life-saving bone marrow match

A couple have 3 children aged 10, 3 and 2. The 3-year-old son, Fabian suffers from acute myeloid leukaemia (AML). His chance of survival is only 50% without a bone marrow transplant within the next 18 months. Even if he does receive a transplant, his chances of long-term survival are still only between 10% and 30% because neither his siblings nor the rest of his family are an appropriate HLA match. Fabian's parents have decided to have another child but need to have IVF because she was sterilised by salpingectomy after her third child. The couple are hopeful that the new baby will be an HLA match for Fabian and that cells from the placenta or from the bone marrow can be used to save Fabian's life. Since the probability of having an HLA matched baby is only 25%, the couple have requested PGD during their IVF attempt in order to find an HLA match for their son Fabian.

Is the generation of individual to save the life of another an appropriate use of PGD? What might be the possible effects on either child should the transplant succeed or fail? Does the fact that the couple needed IVF anyway influence the decision to provide the PGD or not?

It is clear that the technology that we now have in early human reproduction is powerful and profound. The ability to research on human embryos and to make genetic diagnoses at very early embryonic stages is providing increasing amount of surprising information, and equally producing taxing questions. Our ability to select embryos genetically has provided society with a powerful tool that requires careful handling. The ethical difficulties raised by PGD and embryo research are not particularly new or unexpected, they stem from the inevitable and inexorable progress of scientific inventiveness and technological progress in answer to the tantalising question "Can we really?" In every age large leaps in invention and initiative have been followed by the question "should we?" The answer to that question, especially in developmental biology, is often species dependent. Applications that may be may be highly prized and lauded for plants, or domestic or laboratory animal species may be questionable or wholly unacceptable in humans. It is the application rather than the discovery itself that seems to be the root of debate. Since in a free society enquiry and discovery are the cornerstones of progress, the question next put is usually "How do we control the effects of technological change?" Although this may be achieved by rules, regulation, or legislation in individual states or countries, as in the UK, it is unlikely that this will be achieved globally. Although scientific progress is indeed inexorable, the pace can be modulated to ensure public confidence. Responsible progress requires a sensitive and responsive scientific and medical community that is prepared to consider public opinion before proceeding along a particular course. Anne has demonstrated that taking the time to inform at a level that law makers, politicians and an educated public can follow is likely to pay dividends in finding a consensus of what is acceptable in each society.

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