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PEPing up preimplantation testing

In the mid-1980s, the prospects of a successful pregnancy and birth for an infertile couple enrolled in an *in vitro* fertilization (IVF) programme were bleak. The reasons for success or failure were poorly understood at the time and an infertile couple seeking IVF could expect only a one in ten chance of success, despite a commitment to a protocol that could last many months. Robert Winston, Alan Handyside and their colleagues at the Institute of Obstetrics and Gynaecology of the Hammersmith Hospital in London were fast establishing a reputation as world leaders of IVF as a treatment for infertility. At the time, the polymerase chain reaction (PCR) was heralding a revolution in the molecular genetics laboratory. With the first successful one-day PCR-based prenatal diagnosis (for cystic fibrosis)[1] and the demonstration that PCR could be used to amplify DNA from a single haploid or diploid cell[2], some of the promises were realized and the implications for genetic diagnosis were evident. Handyside and Winston were well placed to combine their IVF skills with the emerging, and much more straightforward, PCR genetic diagnostic methods and soon reported the first successful biopsy of a preimplantation embryo and subsequent PCR-based sexing of the biopsy[3]. This landmark paper, published in *Nature*, came at a crucial time during the UK debate on legislation for embryo research. Following the parliamentary vote in favour of limited embryo research, the scene was set for developing genetic diagnosis of single embryonic cells.

But although the possibility of pre-implantation genetic diagnosis was apparent, it was far from clear to whom this high-technology service might be made available. For fertile couples at risk of giving birth to children with genetic conditions, the option of a natural pregnancy followed by chorionic villus sampling (CVS) and a late first or early second trimester termination was the more likely, owing to the poor success rate and very limited resources available for IVF. For the rare cases of an infertile couple who were coincidentally at risk of giving birth to a genetically abnormal infant, adding a PCR diagnostic step to the IVF procedure was clearly called for. However, the limited success rate of IVF procedures meant that the idea of preimplantation genetic diagnosis seemed, to many, to be a case of misguided ambition[4].

On the other side of the Atlantic and at about the same time, Mark Hughes, director of the Prenatal Genetics Center at Baylor, was investigating molecular genetic approaches to embryo diagnosis. As part of a group with little experience of manipulating human embryos, he needed to collaborate with those versed in IVF treatment. Aiming high, he approached Handyside and Winston who in turn were happy to collaborate with Hughes' experienced prenatal diagnosis group. Although on the face of it this was "a collaboration made in heaven", as Hughes puts it, it had one obvious drawback. In light of the US government's policy not to fund any embryo research, Hughes was faced with regular international travel that, given his limited research funding, was a significant financial burden. Not one to be beaten, it seems, Hughes pleaded his case with the US carrier Continental Airlines,

which yielded a number of complimentary return flights between Texas and London.

The collaboration has been fruitful. The group developed techniques to allow single blastomeres to be removed from a number of embryos at the four-to-eight cell stage, following superovulation and IVF. Each sampled embryo is stored to await genetic testing of the removed blastocyst using carefully controlled PCR assays tailored to the specific mutation under investigation. Embryos considered to be affected are discarded, while a number of unaffected embryos are transferred to the prospective mother. These techniques have resulted in a number of successful pregnancies and births of healthy infants where one or both parents were carriers of mutations causing cystic fibrosis, Lesch–Nyhan syndrome, adrenoleukodystrophy and X-linked disorders.

Something surprising then happened to the harmonious collaboration. Handyside, from an essentially 'embryos and development' background, became interested in looking for aneuploidy (such as trisomy 21) in the biopsied embryos. The PCR was not well suited to this, and so Handyside started working on fluorescent *in situ* hybridization (FISH) to examine chromosome numbers and gross structure. Hughes, on the other hand, was more interested in testing for specific DNA mutations, for which PCR was ideal. Thus the two groups diverged and even developed a degree of healthy competition. Interestingly, they recently submitted very similar manuscripts to the same journal, each advocating their favoured approach to pre-implantation sexing and each pointing out the failings of the alternative approach[5,6].

Both approaches are clearly restricted by the availability of just a single cell. The problem with the PCR approach was that, working with minute amounts of target DNA, the reaction conditions had to be optimized for a limited set of amplimers such that the fine multiplex reactions developed to test multiple exons of the Duchenne muscular dystrophy (DMD) or cystic fibrosis genes, for example, were of no use. To get around this limitation, Hughes has taken the PCR technique one step further and incorporated the whole genome amplification method[7], developed by Norman Arnheim and colleagues, into the procedure. After sampling the embryo, DNA from the single cell is subjected to PCR amplification using a battery of random 15-mer amplimers. This results in a near-complete amplification of the genome (a procedure referred to as primer extension preamplification or PEP). On page 19 of this issue, Kristjansson *et al.*[8] successfully apply this method to the single cell diagnosis of deletions in the DMD gene. The PEP product was used to seed five individual DMD exon amplifications, although Hughes claims that each PEP provides ample target DNA for up to twenty reactions, allowing a minimum of twenty loci or specific mutations to be tested for.

But will these techniques ever become commonplace? Although progress in IVF procedures means that the best centres can now boast a much more reasonable one-in-three chance of pregnancy, it may be that this bottleneck in the system can be avoided altogether. For fertile couples seeking to avoid the risk of a second or subsequent child with a particular genetic disorder, a natural pregnancy could be achieved. With careful monitoring, the early stage embryo can be removed, a single cell biopsied and the embryo replaced only following a negative genetic test result. With this protocol, the cost and length of the procedure would be significantly reduced. Hughes points out that given this sort of streamlining, the demand for the technique would be high — not only from couples who, on religious or moral grounds, wish to avoid the risk of a termination following a positive test result on a CVS, but also from couples against termination because of the distress it would cause their older and more aware, living affected children (who may interpret the termination of an affected fetus as a sign of their parents' unhappiness with their own condition).

Hughes also anticipates technical improvements that will soon allow a quantitative PCR-based detection of aneuploidy in couples at an increased risk of whole chromosome disorders. Thus it seems likely that the competition between the FISH and the PEP communities is set to run its course. It will be interesting to see which approach wins out in the long run. □

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