The genetic revolution in artificial reproduction: a view of the future*

Carol Brenner1 and Jacques Cohen

The Institute for Reproductive Medicine and Science of Saint Barnabas, West Orange, New Jersey, USA

*To whom correspondence should be addressed at: 101 Old Short Hills Road, Suite 501, West Orange, New Jersey 07052, USA. E-mail: carol.brenner@embryos.net

With the completion of the human genome project, micro-array technology offers the potential to open up a whole new vista in assisted reproduction. In the next 10–20 years we will be able to screen each human embryo for all numerical chromosomal abnormalities as well as many genetic diseases. Micro-array analysis may permit the screening of multiple alleles for monogenetic diseases and polygenic diseases, including diabetes, hypertension and schizophrenia. In the near future, it may be possible to assess an individual’s genetic predisposition for cardiovascular disease, all types of cancer and infectious diseases. In the distant future, it may even be possible to screen for any genetic trait, e.g. stature, baldness, obesity, hair colour, skin colour or even IQ. Although it is still uncertain what molecular genetic tools may be available, we can be sure that some of these trends will have major consequences on the future of assisted reproduction and society at large.

Key words: DNA micro-arrays/genetic predispositions/human genome project/reproductive markers/RNA expression profiling

Introduction

Predictions of clinical progress in assisted reproduction are usually confined to reviews of fairly recent work, mainly because a look further down the road ahead may appear arrogant, misleading or biased by the author’s specialism. Admitting that our short treatise may fit all three presumptions, we feel compelled to give it a try, since it is likely that some of the emerging technologies will leave their mark on every human in centuries to come.

What we are witnessing is a merging of multiple scientific fields. This has already occurred in areas of biophysics, since cryobiology and optical imaging have had such a major influence on reproductive biology. The next major breakthrough is coming from the bioinformatics field of molecular genetics. Although most IVF centres do not appear to need or desire such technology, this will not be the case for much longer. Here, we discuss the integration of molecular genetics with reproductive science, and its most important first step, that of diagnostic assessment. We will leave the aspects of genetic manipulation of the germline to other authors for now. Doubtless, there are many other exciting areas of assisted reproduction for making predictions, but we believe that major progress will come from the greater integration of embryology and genetics.

With the Human Genome Project now almost completed, micro-array technology offers the potential to open up a whole new vista in the field of human reproduction. In the next 10–20 years, we will be able to screen each human embryo simultaneously for all numerical chromosomal abnormalities and many genetic diseases. In addition, genotyping micro-arrays will permit allele
determination at hundreds of different loci from the human genome. Applied to expression analysis, this approach will facilitate the measurement of RNA levels for the complete set of transcripts of the human embryo. Arrays offer the first great hope for such global views in reproduction by providing a systematic way to survey DNA and RNA variation. It is still too early to predict what the ultimate impact of micro-arrays (or similar but even more complex technology) will be on our understanding in clinical embryology.

DNA micro-arrays are tiny chips made of silicon glass a couple of centimetres across, dotted with thousands of DNA snippets of oligonucleotides from either the coding region of a gene or its variants associated with a particular disease. One can pour on a sample containing pieces of fluorescently-labelled cDNA that have been expressed in a particular oocyte or embryo, and the pieces will stick to the genes that have matching sequences. The bright spots then show which genes are being turned on or expressed. The patterns of expression will offer clues to the developmental potential of an individual oocyte or embryo. Also, the analysis of a single cell in a cleavage stage embryo may eventually reveal the genetic predisposition of each individual embryo.

Analysis of chromosomal abnormalities using DNA micro-array technology

Assisted reproductive technologies have allowed us to study the occurrence of chromosomal abnormalities in human oocytes and embryos. The association between reduced reproductive function and assessment of numerical chromosomal abnormalities in women of increasing maternal age is important in Western societies, since women tend to postpone childbearing. Fluorescence in-situ hybridization (FISH) on single cells has been used with great efficiency (85–95%) to study the chromosomal constitution of cleavage-stage human embryos (Munné et al., 1995). Multiprobe FISH of common aneuploidies (at least X,Y, 13, 18, 21) has been applied to preimplantation genetic diagnosis (PGD), testing either oocyte polar bodies or blastomeres from cleavage-stage embryos (Munné and Cohen, 1998). In a multi-centre IVF study, two conclusions have been reached: (i) PGD of aneuploidy reduces embryo loss after implantation; and (ii) the implantation rates were not significantly improved but the proportion of ongoing pregnancies and delivered babies increased per cycle (Munné et al., 1999). The major limitation of FISH is that fewer fluorochromes are available than the 24 needed to assay all chromosomes. So, by analysing a limited number of probes it is quite possible that one may be looking at the wrong chromosomes. In a recent article, we suggested that the chromosomes most frequently involved in aneuploidy events were different from those classically affecting fetuses after amniocentesis or at birth (Bahce et al., 1999).

One alternative to FISH is the use of spectral karyotyping (SKY) which employs 24 chromosome-specific painting probes for each human chromosome. SKY technology has been able to karyotype poor quality metaphases of polar bodies, oocytes and blastomeres (Marquez et al., 1998). Unfortunately, techniques such as SKY require metaphase chromosomes, which are technically difficult or nearly impossible to obtain from blastomeres. One approach is to alter a cell’s cell-cycle by fusing it with cells that are naturally progressing towards metaphase and arresting, e.g. unfertilized eggs (Willadsen et al., 1999). In the future, embryologists may inject small amounts of cell-cycle regulating factors in biopsied cells imitating the approach now made feasible after fusion with eggs.

Other approaches, e.g. comparative genome hybridization (CGH) and quantitative polymerase chain reaction (PCR), have recently been performed on single cells and show great potential for the future (Wells et al., 1999). For example, one of the applications of genome technology will be the design of a microchip that will contain all the numerical chromosomes. Large DNA clones from each of the 22 pairs of chromosomes (plus X and Y) can be immobilized on glass slides and screened using a CGH approach with amplified DNA from blastomeres biopsied from preimplantation embryos (Harper and Wells, 1999). It will be possible, simultaneously, to hybridize, screen and analyse the micro-array targets in a timely fashion to provide results for IVF procedures. At present, however, a limitation to the application of this
technology is the large amount of DNA (and therefore numbers of cells) required for hybridization. For adequate fluorescence, the total DNA currently required per target, per array, is 50–200 μg or the equivalent of $>10 \times 10^6$ cells. One solution to the problem is first to amplify the whole genome, thus providing enough DNA for subsequent experiments. We are testing different whole genome amplification (WGA) methods and modifications of techniques such as primer extension preamplification (PEP) (Zhang et al., 1992) and degenerate oligonucleotide primed PCR (DOP–PCR) (Harper and Wells, 1999; Wells et al., 1999). We, and others, are bound to solve this problem in the next few years; and this will become a powerful tool with many applications.

**PGD for human genetic predispositions**

A major goal in human genetics is to understand the role of common genetic variants in susceptibility to common diseases. This will require characterization of the nature of gene variation in human populations, assembling an extensive catalogue of single-nucleotide polymorphisms (SNPs) for candidate genes and performing association studies for particular diseases. At present our knowledge of human gene variation remains rudimentary.

Over the last 10 years, PGD by cleavage-stage embryo biopsy and single cell genetic analysis has become established as an alternative to conventional prenatal diagnosis for single gene defects (Handyside et al., 1998). By the end of 1996, ~600 cycles of PGD had been reported worldwide, resulting in $>100$ pregnancies and babies born (Handyside et al., 1998; ESHRE PGD Consortium Steering Committee, 1999). To date, most important PGD centres are already using or are in the process of switching to fluorescent PCR for genetic diagnosis. Use of fluorescent primers and detection of the resultant PCR products on fluorescent DNA sequencing apparatus may reduce the incidence of allele drop-out (ADO) since the products are detected at 100 times greater sensitivity. Furthermore, many inherited genetic diseases, e.g. cystic fibrosis, thalassaemias, sickle cell anaemia, Duchennes’ muscular dystrophy, Huntington’s chorea, etc, have been successfully screened by preimplantation genetics and resulted in normal births (Wells and Sherlock, 1998). Such screening has involved expensive customized patient-specific DNA probes, which must be developed each time a couple presenting a new mutation comes for a clinical IVF procedure.

In the near future, micro-array analysis (Figure 1) will be used to profile the common monogenic diseases, examine the more complex diseases, as well as discover new disease-related genes. In the first generation, these new genomic assays will contain all the DNA oligonucleotides coding for genetic aberrations often screened for in prenatal
testing. The advantage of these oligonucleotide micro-arrays for mutation analysis is that they will be able to screen simultaneously for all possible sequence variants of the disease genes in the population. For example, arrays of 1480 nucleotide probes were designed to detect 37 known mutations as well as the most common ΔF508 in the coding region of the CFTR gene (the gene for cystic fibrosis), plus all possible single-nucleotide substitutions (Ramsey, 1998). The complex mutation spectrum of most disease genes in the general population reflects the allelic heterogeneity of the human genome. Another pertinent example is the inherited breast and ovarian cancer gene, BRCA1, for which >400 distinct mutations have been reported (Hacia et al., 1996). Re-programming genes, e.g. the telomerase catalytic subunit, may affect both early embryonic health and the future prospects of an individual (Brenner et al., 1999). Although one can use various PCR techniques to amplify the whole genome from a single cell, a problem that frequently arises when examining heterozygous loci in single cells is ADO. ADO results from the preferential amplification of one of the pairs of heterozygous alleles, whereby the other allele fails to be amplified (Lissens and Sermon, 1997). As a result, basing a clinical evaluation on a single cell may lead to a misdiagnosis. Consequently, in order to use oligonucleotide micro-arrays for heterozygous mutation screening in preimplantation genetics, accuracy must be improved.

A greater challenge in both the genetic and reproductive community will be the ethical issues raised by the genomics screening. It will soon be possible to screen for polygenic diseases including diabetes, hypertension and schizophrenia. Consequently, this will be applied to pre-implantation embryos providing a screening potential that goes considerably further than criteria that just determine implantation potential. In the near future, we will be able to assess an individual’s predisposition for cardiovascular disease, all types of cancers and infectious diseases. In the distant future it will become possible to screen for any genetic trait, e.g. stature, baldness, obesity, hair colour, skin colour, potential intelligence, etc. A couple will be able to select an embryo based on an assortment of genetic and phenotypic traits. As a result, will we be selecting for a population of genetically fit individuals? Especially disconcerting to some individuals and groups, may be the general advances in clinical efficiency that will be achieved in the coming years. Once assisted reproduction guarantees a single baby in >90% of attempts, coupled with a simplified surgical approach, prospective, but perfectly fertile parents may commonly opt for IVF to review and select their embryos (Gosden, 1998; Silver, 1998). The old reproductive method may, one day, be considered too uncertain a process. Although such philosophical and ethical questions will not be resolved until we see what tools are available, we can be sure that some of these trends will have major consequences on the future of assisted reproduction and society at large.

**Gene expression and molecular markers**

Now the Human Genome Project has nearly been completed, it will be possible to survey 100 000 expressed human genes (Landers, 1999). This will provide the molecular reproductive biologist with a unique challenge. So far in human embryology, we have tended to examine genes and gene families individually. We have looked at <100 genes in human oocytes and embryos; genes coding for cell cycle regulators and checkpoints, apoptosis, cytoskeleton, metabolism, and others with developmentally related consequences. It is now time to gain a global perspective on the human oocyte and embryo by asking genome-wide questions, and in many cases by studying all 100 000 genes and gene products together. This is the current aim of the new emerging area of gene expression informatics.

Expression profiling using cDNA micro-arrays will simultaneously monitor the expression of all genes (Figure 2). The mRNA levels may reflect the developmental state of the cell, and its ability to respond to its environment. To decipher the logic of gene regulation, we will need to be able to monitor the expression level of all genes simultaneously in a single cell, with an adequate quantitative sensitivity level; and a qualitative sensitivity sufficient to distinguish all alternatively-spliced forms. This is quite a tall order but abso-
The genetic evolution in ART

Gene Expression Analysis in Single Cells

- Single cell RT-PCR
- cDNA amplification
- Hybridization to chip
- Scan/Analysis

Figure 2. Gene expression analysis in single cells.

...olutely necessary for its future application to the reproductive field. Recent technological advances in DNA micro-arrays augur well for the eventual feasibility of this goal. Micro-array analysis will be performed to identify clinically useful reproductive markers from human granulosa cells, cumulus cells, oocytes and embryos. Ultimately, we expect to be able to determine which reproductive markers are critical indicators for oocyte and embryo quality and the differing factors between good and poor responders to follicular stimulation.

Quantification of transcript copy number in human oocytes, embryos and cytoplasmic samples

Deciphering the complex series of regulatory events that occur during early development depends partly on the ability to quantify stage-specific mRNA species accurately. However, the paucity of biological material coupled with the lack of sensitivity and/or reproducibility of the currently available quantitative methods have been a severe limitation of single cell analysis. Fluorescent probes have recently been added prior to DNA amplification to monitor product accumulation during the PCR process. Rapid cycle DNA amplification is a highly sensitive technique for the amplification of specific DNA sequences. With the addition of fluorescent probes, we have recently shown that it is possible to monitor the log-linear phase of amplification, i.e. the most useful parameter for obtaining quantitative data. Rapid temperature light cycling was used to examine expression levels of the housekeeping genes, β-actin and HPRT, in individual murine and human oocytes and/or embryos. The results obtained compared favourably with those attained by others using large pooled samples and followed the predicted temporal pattern of expression (Steuerwald et al., 1999). Other experiments indicate that this is a useful method to quantify mitochondria in individual oocytes and embryos for assessment of oxidative phosphorylation and mitochondrial mutations (N.Steuerwald, unpublished results). Such alterations may affect fertility because the mitochondria must have stored all the energy required for the resumption of meiosis II, fertilization and development.

Once informative reproductive molecular markers are identified by micro-array analysis, minimally invasive techniques can be developed to biopsy cytoplasm and/or polar bodies for clinical evaluation using the rapid cycle methods. Subsequently, benign sub-cellular analysis methods can be used as clinical IVF diagnostic tools. However, this presumption may be reflective of our times, since most cell microsurgery is detrimental, at least to some extent. Eventually, invasive micro-surgical cell techniques will be replaced, for instance by the use of surface markers that are painted over viable cells. Such markers may monitor cell development as well as gene expression...
and preview later development. The scientific bridge between such cell monitoring devices and observers has yet to be developed. The how and when of this feasibility is unpredictable but ultimately, all these problems will be solved; and patients will inevitably one day ask Lee Silver’s famous question ‘Why can’t my children have genes that other children have naturally’ (Silver, 1998).

References