

MINIREVIEW SERIES FOR THE 50TH VOLUME

Preface to minireviews Innovations in assisted reproduction: a view of the future

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It is now over 20 years since the birth of Louise Brown and in the meanwhile there has been a dramatic increase in the number of infertility clinics around the world that can offer *in vitro* fertilization (IVF) and newer, related assisted reproduction technologies. IVF techniques have given embryologists the extraordinary possibility of exploring the first events which preside at the beginning of a human life. There has been a gradual increase in the success rates achieved using these techniques, but it is still necessary to make qualitative judgments about early embryos. The evaluation of embryo viability in clinical IVF relies on morphological criteria which are assessed by light microscopy. What should be considered normal and abnormal?

Blastomere number and size, cytoplasmic appearance, presence of fragments in the perivitelline space, and the cleavage rate in relation to the time frame of development are the most non-invasive parameters to evaluate embryonic competence. However, what we define as morphologically 'abnormal' may only represent different physiological aspects of oocyte and embryo development. Conversely, a 'normal' appearance is not necessarily synonymous with viability. As a consequence, it is possible that the limited efficiency of IVF techniques could be partially due to our poor ability to identify a viable embryo.

Opportunities have now been opened to replace human embryos at various stages into their mothers in attempts to alleviate infertility. It is thus possible to plan the preimplantation diagnosis of genetic diseases by excising a blastomere(s) at 4 cell to 8 cell stage-embryos. Studies on outgrowths of embryonic cells from blastocysts are also possible now. This would enable embryonic stem cells, or differentiating tissues, to be grown *in vitro* and used for study or, one day, for grafting into sick adults.

The use of ovulation stimulation techniques to improve and simplify human IVF led to the recovery of large numbers of oocytes and consequently embryos. In order to limit the risks of multiple pregnancies arising

from the transfer *in utero* of numerous embryos and to avoid the wastage of supernumerary embryos, the development of embryo cryopreservation was initiated in humans. Mammalian embryos have been successfully frozen and stored since 1972. The human embryo freezing spread rapidly in the world and became an indispensable extension of IVF, with the maintenance of optimized and simplified biological procedures.

Cryopreservation of immature oocytes, supposed to be less sensitive to cryo-injury, could be an advantage and could offer an opportunity to store the gametes of young patients requiring anti-cancer therapy for whom urgency of treatment may preclude the whole stimulation protocol used in mature oocyte collection. Numerous experimental studies on immature human oocytes have been carried out according to the embryo freezing procedure. Nevertheless, no advantage of immature oocyte freezing could be drawn from these results, despite the birth of a normal female baby after cryopreservation of prophase I oocytes with subsequent *in vitro* maturation. Improvements in the technique of oocyte *in vitro* maturation should precede the extension of immature oocyte freezing to reach satisfactory results.

Intracytoplasmic sperm injection (ICSI) is a highly effective therapeutic intervention used to assist fertilization with sperm from an infertile male, and its innovation offers a previously unavailable opportunity for couples with male factor infertility to now produce children. Its inception has revolutionized the management of male infertility. However, critics have suggested that the use of ICSI for the treatment of male infertility has the potential to negatively impact the genetic composition of the human population for future generations. ICSI is a technique that bypasses the effective biologic mechanisms of sperm selection that were set in place during the evolution of the human reproductive process. The application of ICSI to human reproduction has not been preceded by an extensive research trial in other mammals. Consequently, the human experience with ICSI is the experimental record.

The application of ICSI foregoes an understanding of the underlying etiology of the individual male's infertility, which may be of a transmissible genetic basis. The use of ICSI to alter the reproductive performance of a male with infertility resulting from a specific genetic constitution or genotype changes the fitness of his genotype. The application of ICSI in assisted reproductive technology provided the ability to dramatically improve the fitness of many mutant male genotypes and potentially alter the genetic composition of a population. It is therefore essential to have an understanding of the genetic risks and possible consequences that are inherent when ICSI is used to assist fertilization in the population of infertile males.

Genetic diagnostic testing of infertile males should be strongly recommended when there is a significant risk of transmitting identifiable chromosomal abnormalities or genetic mutations to the conceptus that will severely impact its future health and well being. All couples in which a male has been identified as having a chromosomal anomaly or a clinically significant genetic mutation should be referred to a certified genetics counselor and/or a clinical or prenatal geneticist for preconception counseling and possible prenatal diagnostic evaluation.

With the Human Genome Project now almost com-

pleted, 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. The patterns of expression will offer clues to the developmental potential of an individual oocyte or embryo. The analysis of a single cell in a cleavage stage embryo may eventually reveal the genetic predisposition of each individual embryo.