Intracytoplasmic sperm injection and other aspects of new reproductive technologies

Louise Brown was 21 in 1999. Since her birth, in vitro fertilisation (IVF) has become a widely used treatment for the subfertile couple. Currently about 1% of births in the United Kingdom follow conceptions in vitro. Certain forms of subfertility, largely those derived from male problems (affecting up to 40% of subfertile couples), cannot be treated by conventional IVF, and the development of intracytoplasmic sperm injection (ICSI) has allowed some of these couples to conceive.

What is ICSI?

ICSI was developed in humans in Belgium in 1992.[1] The procedure involves injecting a single sperm into an egg using a micropipette one fourteenth the diameter of a human hair. The spermatozoa can be obtained either after ejaculation or after aspiration (directly) from the testis or epididymis (percutaneous epididymal sperm aspiration). The spermatozoa are prepared by washing away seminal plasma and, where possible, separating the progressive (most) motile sperm from cellular debris. Poorly motile or abnormally shaped sperm are not usually selected for injection, unless no normal appearing sperm are available in the preparation. Progressive motile sperm are slowed down in polyvinylpyrrolidine, which increases viscosity of the medium and permits a better spermatozoon selection. Immobilisation is performed by crushing the tail of the spermatozoon with the injection pipette. This disturbs the membrane potential, appears to improve fertilisation, and prevents the tail of the sperm damaging the ovum cytoskeleton. If apparently normal fertilisation occurs, up to three of the resulting embryos are transferred to the uterus 48 hours after egg collection using a standard procedure in which a fine flexible catheter containing the embryos is passed through the cervix into the uterine cavity, and the embryos are expelled in a minimal quantity of medium.

Use of ICSI

ICSI is a major adjunct to conventional IVF and has been rapidly introduced world wide. More than 100 centres in the United Kingdom and more than 750 centres in the European Union are now performing ICSI (figures from the Human Fertilisation and Embryology Authority/European Society of Human Reproduction and Embryology).

On the basis of 1997 birth rates and assuming that 25% of IVF procedures involve ICSI, at present, there are some 10 000 "ICSI births" a year in the European Union. The use of ICSI is increasing so much that, in Belgium, as many as 60% of IVF cycles involve an ICSI procedure. One of the main reasons for the popularity of ICSI is that couples who are paying for treatment believe that their "take home baby rate" will be higher if ICSI is performed (for male factor problems), although this has not been confirmed by a randomised controlled trial.[2] Increasingly ICSI is used for "non-male factor" problems such as tubal malfunction or "unexplained infertility" where fertilisation was poor or failed with normal IVF. This is in addition to the standard indication of oligozoospermia (often with coincident asthenozoospermia (poorly motile sperm) and teratozoospermia (abnormal forms)). More recently other advances in reproductive technologies have resulted in still further potential applications for ICSI (discussed below).

Why are there concerns about the safety of ICSI?

There is no suitable animal model—that is, an infertile primate—on which to test the technique, so the safety of ICSI could not be assessed on animal models before introduction. ICSI involves bypassing sperm natural/competitive selection by the use of a single spermatozoon.

The following concerns have arisen.

(*a*) The risks of using sperm that potentially carry genetic abnormalities: it is thought that oligozoospermic males carry a higher rate of genetic defects.[3]

(*b*) The risks of using sperm with structural defects: although there is no absolute evidence that teratozoospermia (abnormal phenotype) represents an abnormal sperm genotype, these sperm would not normally be those that fertilise.

(*c*) The potential for chemical and mechanical damage: chemical damage could arise from agents injected into the egg within the medium, including sperm slowing agents—for example, polyvinylpyrrolidine—or there could be mechanical damage to the ovum from the injection process.

(*d*) The risk of introducing foreign material into the oocyte: some culture media may contain heavy metals known to be toxic to sperm.[4] The description of mammalian transgenesis by ICSI[5] has shown the most convincing evidence (so far) that inadvertent transfer of exogenous DNA into the ova by ICSI could occur. Perry and colleagues[5] co-injected unfertilised mouse oocytes with sperm heads and exogenous DNA encoding a green fluorescent protein, with 20% of offspring expressing the integrated transgene. The risk of infection by exogenous gene expression or integration into ICSI embryos has also been inferred by the work of Chan and colleagues[6] using rhesus monkeys. They have shown that exogenous DNA bound to sperm before insemination could be transferred to rhesus ICSI embryos, but was excluded from IVF embryos because of the sperm-egg interactions before sperm penetration.

Recent concerns

There are new as well as continuing concerns. For example, Dowsing *et al*[7] have suggested the greater possibility of the transmission of trinucleotide repeat sequences from ICSI treated fathers to future generations. Excessive amplification of these trinucleotide repeat sequences is associated with the increased risk of neurodegenerative disease.[8]

Equally disturbing are reports by Schatten and colleagues[9] in Oregon using ICSI in rhesus macaque monkeys. In a standard ICSI procedure, the injection pipette is polarised at 90° to the (visible) first polar body. This is to avoid damage to the (invisible) first meiotic spindle, to which it has been assumed there is a fixed relation. Schatten has dismissed this assumption. Using fluorescent markers, he has shown that the relation between the first meiotic spindle and the first polar body is not fixed. Thus the injecting micropipette may damage the first meiotic spindle (with unknown consequences). It is possible that injection into the region containing the spindle could result in chromosome damage or chromosome misalignment.

What is known about outcome?

Most early ICSI programmes started in 1994–1995, and the eldest children are now only 5–6 years old. However, there are several early outcome studies on ICSI offspring. The large series by Bonduelle and colleagues[10 11] has provided some reassurance. However, most of Bonduelle's reports lack a control group. Her work has suggested an increase in sex chromosome abnormalities in ICSI offspring,[11] but this needs to be confirmed in a larger sample. Other reports[12 13] about perinatal outcome of ICSI conceived children have been reassuring and include the recent report by Loft *et al*,[12] which involved all Danish born ICSI children. Interim findings of a United Kingdom based population study[14] have suggested that ICSI conceived toddlers are healthy in relation to a normally conceived control group. Less reassuring was the report by Bowen and colleagues[15] suggesting that a single centre Sydney born cohort of children were developmentally delayed at the age of 1 in relation to a normally conceived control group. This study had a number of limitations including lack of power, multiple observers, unstandardised testing systems, and failure to allow for confounders.[16]

Severe idiopathic oligozoospermia (about 60% of ICSI treated patients in the United Kingdom) is now recognised in 10% of cases to be associated with specific gene deletions on the Y chromosome. Such deletions occur in the AZFc (azoospermia factor) region of the Y chromosome[17] and other related genes. ICSI conceived boys from these fathers will inherit these Y chromosome microdeletions and will need ICSI themselves to become fertile unless there are further advances (as will their male offspring).

Future studies

A European collaborative group involving Belgium, Denmark, Greece, Sweden, and the United Kingdom is performing a developmental study examining child and family welfare at school entry.

The best way to deal with the issue of congenital abnormalities is through a birth registry of ICSI children. In the United Kingdom, a birth registry is planned, and agreement in principal has been obtained from 98% of United Kingdom ICSI centres to collaborate.[18]

More recent developments in new reproductive technology

ICSI appears to be useful for other recent developments in fertility treatment where there may be a shortage of gametes.

EXTENDED EMBRYO CULTURE

In standard IVF, embryo transfer normally takes place at 48 hours, but embryo implantation rates may improve if the in vitro culture period is extended to five days—that is, with transfer taking place at the blastocyst stage.[19]

IN VITRO MATURATION OF OOCYTES/TOWARDS SINGLE EMBRYO REPLACEMENT

In another development, immature oocytes[20] are being harvested and matured in vitro and then fertilised. This in vitro maturation may produce eggs of more certain quality than by the present practice of hormonally stimulated polyovulation producing ova of uncertain maturity. Better oocyte quality results in better embryo quality.[21] At present, after hormonally stimulated polyovulation, these variable quality/maturity oocytes are harvested. These are then fertilised and typically two apparently normal embryos are replaced. In vitro maturation may obviate the need to replace two such embryos with the replacement instead of one better quality embryo.

Alternatively there are increasing advances in embryo scoring,[22] which will allow the selection of a single better quality embryo after a standard procedure. These advances in turn may solve a fundamental problem of current IVF treatment, namely the birth of twins, triplets, and other higher order births.

ADVANCES IN FREEZING

Cryopreservation of oocytes is a technique developed to preserve oocytes of patients undergoing cancer treatment or for oocyte donors. Cryopreserved oocytes require ICSI for fertilisation (after thawing) because the cryopreservation process brings about changes in the zona pellucida preventing sperm penetration.[2] Only cryopreservation of mature oocytes has been successful. There has been a first report of successful cryopreservation of postpubertal ovarian tissue and reimplantation into the same patient[23] after previous oophorectomies.

IMMATURE GERM CELLS

This has led to speculation about the possibility of cryopreservation and subsequent reimplantation of immature, prepubertal germ cell tissue after children have been treated for cancer and also the separate possibility of the in vitro maturation of primordial germ cells. These are of unknown risk.

A mouse named Eggbert has been born after the first successful maturation from a primordial germ cell. Eggbert died young and was obese, diabetic, and had developed intestinal lymphosarcoma.[24] Critically these problems developed after full physical maturity was attained.[24]

Conclusions

Although evidence to date suggests that ICSI conceived children are healthy, it is unsafe to draw any conclusions about their long term wellbeing. Caution needs to be exercised when considering the implications for potential children whose parents have conceived with help from these new reproductive technologies. Although infertility sometimes has devastating effects on a person's sense of completeness and self worth, the health of the child should be paramount in further developments of these new techniques.

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Public health

Is the ethos of medical practice in community paediatrics compatible with that in public health?

Public health and community paediatrics go back a long way together. At times, in their history, the two have been so closely linked as to be indistinguishable. Two early "public health" initiatives in the UK—the establishment of the school health services, and of maternal and child welfare clinics—bear witness to early awareness that measures to improve children's health may be important for the health of adults. Infant mortality rates have long been regarded as a key indicator of the overall health of a nation in international comparisons, and in the UK doctors working in community child health services were first based in departments of public health. At other times the two specialties have seemed very separate. The 1974 NHS reorganisation and the concomitant development of two separate medical specialties—community child health (as community paediatrics was then called) and community medicine (as public health medicine was then called) pulled them apart. Several different forces are encouraging the two back together again: the political glasnost on social inequalities in health, and recognition at professional level that these inequalities have their most noxious impact on children[1 2]; the need to join forces, in the face of powerful financial interests, to advocate for a healthier environment for children (against the tobacco industry, the motor industry, and baby milk manufacturers); the need to maintain high levels of immunisation and the need to modernise the child health surveillance programme[3]; the rediscovery of the "life course approach to health"[4 5] and of "cycles of disadvantage"[6]; and the publication of research which shows that it is possible to have an impact on intractable adult public health problems by intervention in early childhood.[7 8] Some have proposed that the development of a new specialty—child public health—is the best way to have an impact on some of these problems. This article looks at some of the similarities and differences between medical practice in public health and community paediatrics. It also looks at some of the aspects of medical practice that make improving health a challenge for both specialties.

Principles of public health practice

An enduring definition of public health is that of Acheson in 1988: "The science and art of preventing disease, prolonging life, and promoting health, through the organised efforts of society". This definition springs from an essential premise of public health practice, that health is determined by social and environmental factors, and that health improvement depends primarily on interventions made outside clinical practice. Public health doctors have, in the past, had greater resources at their disposal than they do now, but they have never been in a position to "organise society". What they have achieved in this respect has been achieved through persuasion. They have gathered evidence, made speeches, written reports, identified collaborators, established coalitions. By the time that their proposals are implemented, their initial involvement may be forgotten. The main benefits of public health interventions are often felt when they have become an accepted and invisible part of the social fabric. Public health is therefore a specialty in which people need to be able to derive job satisfaction from playing a small part. Heroism and personal acclaim are rarely on offer.

Principles of community paediatric practice

Community paediatrics is primarily a clinical specialty, revolving around the suffering of individual children and their parents. The essential premise of clinical practice is that doctors can help sick people get better, and disabled people have a better quality of life. When clinicians' interventions work, their patients' lives are made easier in a way that is often clearly attributable to the intervention of the individual clinician. The relationship, when it works well, is a very personal one of appropriate and timely support, and appropriate and rewarding gratitude. Community paediatrics has some similarity with public health in that the interventions are not necessarily "clinical" and delivery is often the responsibility of a group of people. The doctor may have acted as an advocate for the provision of services, which are not under their control—housing, or environmental modification of a school—but the intervention is still made on behalf of, and felt by a single individual or family, and is attributable to the team leader, who is most often the doctor.

These are stereotypes, and reality is rarely so clear cut. There are plenty of examples of public health doctors needing and seeking personal acclaim for their achievements, and there are an equal number of examples of unsung heroism in community paediatrics. When the previous government was in power, public health doctors spent most of their time on NHS purchasing, focusing, like their clinical colleagues, on clinical interventions. At the same time some community paediatricians have taken a lead in intersectoral initiatives to develop, for example, accident prevention or parent support programmes. Many have worked with head teachers and schools to develop policies on medication, which mean that all children with asthma can have access to their inhalers when needed. So it is more helpful to view the two specialties as covering a spectrum of approaches, where the means differ, but the confidence intervals overlap.

The promotion of health in clinical practice

Public health and community paediatrics therefore share many goals. Tensions between the two specialties, in so far as they exist, arise from the clinical practice component of community paediatrics and they do so because clinical practice has very different goals from public health. Public health has the goal of preventing disease and enabling health improvement, clinical practice of enabling recovery from ill health, and mitigating the impact of disability. These goals need to be met in different ways. Problems arise because medical education is tailored primarily to enable doctors to treat sick people and, at undergraduate level, provides little in the way of support to developing doctors whose practice will in future include the promotion of health (public health, community paediatrics, and general practice).

In clinical practice doctors are required to take decisions, often under pressure, on behalf of sick patients,