Human Reproduction vol.9 no.7 pp.1214–1219, 1994

OPINION

Treating male infertility needs more clinical andrology, not less

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Key words: andrology/genetics/health/male infertility/micro-manipulation

We propose to define clinical andrology as the examination and evaluation of the infertile male. The examination includes taking his clinical and genetic history and attempting to diagnose the cause of his infertility. It includes making proposals for his treatment and evaluation of the prognosis of that treatment.

It is a sadly neglected subject. Clinicians who treat infertility are, traditionally, gynaecologists. They have generally had little or no training in the clinical examination of the male genital tract, nor in any aspect of the pathology of the testis and its excurrent ducts. The study of the male thus has acquired low priority. This is no inherent failing in the profession: it is an accident of medical history and perspective.

The treatment of male infertility thus tends to be based for the most part on the examination of seminal fluid alone. The clinical history and examination of the man may be ignored because the clinician lacks the skills, background and resources to pursue these important clues. Furthermore, semen quality can vary widely in quality even within fertile men (World Health Organization, 1992), and the semen analysis *per se* may have little or no relation to the underlying aetiology (Jequier, 1993). No diagnosis as to the cause of his infertility can therefore be made. As a consequence, laboratory scientists without training in any area of patient management may now define and indeed determine the treatment given to these men. In addition, lack of depth in clinical understanding of male infertility may lead, paradoxically, to costly and redundant pathology testing, such as investigations of antisperm auto-antibodies and endocrine disorders when in reality these present only rarely as causes of infertility (Jequier and Holmes, 1993). The stunning recent advance in our capacity to overcome male infertility by what Fishel *et al.* (1993) have termed 'micro-assisted techniques' emphasizes that the treatment of the semen rather than the man is the focus of attention in infertility clinics today. This success, in particular that of intracytoplasmic sperm injection (ICSI), has led some to remark (facetiously, one hopes) that the discipline of clinical andrology is now redundant. In our opinion this is improvident: in the majority of male patients that present with subfertility, the underlying cause of their problem is not understood (Jequier and Holmes, 1993), and it may be inappropriate to intervene to produce children in such a state of ignorance. We stress that we do not advocate the banning of micro-assisted techniques; however, we wish to act as devil's advocates and to argue that there are profound ethical, scientific and medical reasons to proceed with caution, and to redouble our efforts to understand the causes of male subfertility that may lie behind repeated fertilization failure.

Over the last 5 years, techniques for assisting fertilization using spermatozoa from severely subfertile men have progressed from pharmaceutical enhancement of sperm function (Yovich *et al.*, 1990; Cummins and Yovich, 1993; Yovich, 1993), to micro-assisted approaches (Fishel *et al.*, 1993). These include zona splitting (Laws-King *et al.*, 1987), subzonal sperm microinjection (Ng *et al.*, 1988), electroporation (Palermo *et al.*, 1992), and now, direct ICSI (Palermo *et al.*, 1993; Van Steirteghem *et al.*, 1993a,b,c). This latest technique has clearly become the most successful means of overcoming severe male subfertility. In such cases, the man's spermatozoa are either incapable of fertilizing or are available in such low numbers as to make fertilization extremely improbable even *in vitro*. One advantage is that the technique avoids the need to expose oocytes and embryos to high and potentially toxic concentrations of spermatozoa, to maximize the chances of fertilization.

The persistent clinical myth that there is no treatment for male infertility has thus now largely been dispelled, at least judging from recent results using the ICSI technique. Although micro-assisted fertilization involves the use of in-vitro culture and embryo transfer, pregnancy rates approaching those for natural conception are now being reported for couples in whom the husband's sperm numbers and vitality are extremely low.

The technique of ICSI, in which a technician chooses an individual spermatozoon and injects it directly into the cytoplasm of an oocyte, circumvents all sperm selection processes that have developed during the evolution of fertilization mechanisms. Indeed, the most successful approach is to immobilize—and possibly disrupt—the spermatozoon mechanically before drawing it into the injection miocropipette (Payne *et al.*, 1993). It must be remembered, however, that this technique is merely a device to overcome subfertility due to low sperm numbers or poor sperm function: it does nothing to resolve underlying problems in the man, nor does it help identify the cause of his subfertility.

Treatment of male subfertility is thus usually based upon the examination of semen alone, and little or no attempt is made by many clinics to understand the underlying pathophysiology that has caused an abnormal semen analysis. For example, primary

testicular disease probably makes up ~20–50% of all subfertility seen in the male, but in >60% of men with this condition no cause for these changes to the testis can be elucidated from either the history or the clinical examination (Jequier and Holmes, 1993). Obstruction of the epididymis is also a frequent cause of infertility in the male, and is an obvious indication for micro-assisted fertilization, yet no aetiology can be identified in more than half of the patients with this condition (Jequier, 1986; Handelsman, 1992). More alarming still is that this form of subfertility is now being 'treated' by techniques such as ICSI, frequently without any attempt being made by clinicians to discover the cause of the problem, let alone to identify its aetiology.

There are ethical pitfalls in treating spermatozoa in isolation from the individual: men are not mere gamete producers and should have the right to the most accurate information and diagnosis of their problems. There may indeed be many men being steered towards micro-assisted fertilization for whom a more careful clinical evaluation might raise doubts about the suitability of this approach. Moreover, careful attention to in-vitro fertilization (IVF) technique may circumvent the need for micro-assisted fertilization in many cases (Ord *et al.*, 1993) The long-term psychological stresses to a man that may result from the recognition that his child can only be produced by this type of technological intervention can, at present, only be guessed at. In addition, the very real risks to the wife (who may have nothing wrong with her) must not be ignored. It is essential that we acknowledge that conception is a mutual affair that engages two sentient human beings. In reducing treatment to mere gamete manipulation we run the risk of dehumanizing the treatment and understanding of infertility. Side issues of economics may also colour medical decision-making: clinics that have invested in expensive equipment and in training technicians are likely to encourage couples to consider micro-assisted approaches to justify that investment. In addition, there are pressures on entrepreneurial clinics to develop micromanipulation for reasons of commercial competitiveness: they need to be seen to practise the 'latest' glamorous techniques.

Although protagonists of micromanipulation do indeed recognize that this area contains the seeds of ethical dilemmas and controversies (Ng *et al.*, 1992; Fishel *et al.*, 1993), in general any risks inherent in these techniques have been very much played down. The ability to produce conceptions for couples with long-standing infertility would seem to make any criticism of these techniques redundant. By contrast, we suggest that the apparent short-term success of this technology may be blinding us to some of its possible long-term dangers. To proceed with treatment and with the production of human embryos, without even attempting to make a clear appraisal of the possible clinical risks, is in our opinion foolhardy. With this in mind let us now examine some potential areas of concern.

Cryptic genetic defects as causes of infertility

There is no evidence in a general sense that the phenotype of an individual spermatozoon reflects the genotype it carries. Rare examples of post-meiotic gene expression affecting sperm function, such as the T locus in mice, are the exception rather than the rule (Hurst, 1993). Indeed, reflection on the evolution of fertilization, and the importance of random recombination of genotypes in Mendelian genetics, suggests that there are probably fundamental reasons why this is so (Cummins, 1990). However, severely dysfunctional spermatozoa are usually the products of a severely dysfunctional reproductive tract that may in turn have its basis in genetic anomaly. It is relevant to ask whether it is wise to manipulate such spermatozoa to achieve syngamy, given that they are incapable of doing so on their own.

As we have stressed, we are largely ignorant as to the aetiology of male infertility. The condition of congenital bilateral absence of the vasa deferentia (CBAVD) is an obvious indicator for micro-assisted fertilization. However, we now know that this is associated with the presence of mutations in the gene complex controlling cystic fibrosis (CF) (Bienvenu *et al.*, 1993; Patrizio *et al.*, 1993a,b). Mutations in the CF trans-membrane regulator gene complex are highly significantly associated with reduced fertilization rates using epididymal spermatozoa in men with CBAVD (Patrizio *et al.*, 1993b). Several pregnancies have been generated in this way before the genetic association with CF was established. Reproductive technology thus has the potential to be abused: it can preserve and even increase the incidence of genetic disorders. It is clear, by simple Mendelian genetics, that the CF gene complex mutations will be present in half of the embryos produced, so that half of the male babies born may, like their fathers, suffer from CBAVD or epididymal blockage. While screening for CF is possible in embryos (Liu *et al.*, 1993) and first trimester fetuses (McIntosh *et al.*, 1989), this serves to multiply the technological intervention load and raises the additional ethical dilemma of selective termination of pregnancy. Genetic screening of husbands (and wives) should be mandatory in all such cases.

Besides clear-cut cases such as CF and CBAVD, it is likely that, as understanding advances, other genetic causes of 'unexplained' male infertility will emerge. For example, Young's syndrome is a poorly characterized combination of obstructive azoospermia and chronic respiratory problems (Young, 1970). In such cases spermatogenesis, duct development and sperm ciliary function appears normal, but blockage occurs through the accumulation of inspissated epididymal secretions (Handelsman *et al.*, 1984). While classically this clinical picture has been considered distinct from CF proper, Hirsh *et al.* (1993) recently demonstrated that two of seven infertile men with obstructive azoospermia were carriers of the ΔF508 CF mutation. This is normally only found in between 1 and 25 to 1 in 30 of the N. European population, and the authors advocate that CF screening and genetic counselling should be considered for men with Young's syndrome. Turning to other genetic anomalies, it is well established that infertile men show an approximately 10-fold increase in the incidence of chromosomal anomalies as compared with normal controls (Chandley *et al.*, 1972; de Kretser *et al.*, 1972; van Zyl *et al.*, 1975; Chandley, 1979; Dutrillaux *et al.*, 1982). This is a powerful argument for increasing rather than decreasing the rigour of andrological investigation. We cannot rely simply on 'natural selection' (a euphemism for embryonic mortality) to weed out genetic anomalies that may be associated with human subfertility. While it is reassuring that analysis and follow-up of 119 children born as a result of micro-assisted fertilization techniques have revealed no significant problems to

date (Van Steirteghem *et al.*, 1993), this is no excuse to relax vigilance, as many of the genetic associations with infertility, both male and female, may only appear at puberty or even later in life.

Genomic imprinting

We are only just beginning to comprehend the nature of genomic imprinting, in which certain genes transmitted through the male germ cell lineage differ in expression from those derived through the female, by differential DNA methylation (Surani *et al.*, 1993). On the whole, the imprinted genes derived from the male appear to be concerned with placental formation, as exemplified by loss of the maternal genome in benign trophoblast disease (hydatidiform mole). By contrast, female-derived genes are more concerned with embryo development, as seen in the development of ovarian teratomas through spontaneous activation of ovarian oocytes.

It is not clear when imprinting is complete in sperm formation: fusion between round spermatids and oocytes has been demonstrated in hamsters and mice but no successful embryo development has yet been achieved (Ogura *et al.*, 1993). The clear links between sperm dysfunction and imperfect nuclear condensation (discussed below) give rise to concern for using immature gametes for ICSI, at least until we can unequivocally determine the timetable for imprinting in spermatogenesis.

Testicular ageing

The links between advanced paternal age and several genetic conditions in humans have been recognized for many years (Lints, 1978; Auroux, 1993). These include the diverse dominant clinical conditions achondroplasia, myositis ossificans, Apert's syndrome, Marfan's syndrome, Duchenne's muscular dystrophy, haemophilia A; and the sex-linked recessive condition bilateral retinoblastoma. We have argued elsewhere that there is a strong associative link between unexplained male infertility and accelerated testicular ageing, as manifest in ischaemic disorders, mitochondrial DNA deletions, disordered oxidative phosphorylation and loss of control over free radical production (Cummins *et al.*, 1994). It is entirely conceivable that the increased risk of genetic disorders associated with increased paternal age may also apply to male infertility. However, several hundreds or even thousands of births may be required, statistically, to demonstrate any such increased incidence.

DNA breakage

Human semen is known to carry oxidized DNA products, and amounts may be increased in those individuals with an enhanced risk of oxidative damage, such as cigarette smokers (Fraga *et al.*, 1991). In such cases dietary anti-oxidants such as ascorbate can reduce the seminal values and may also improve semen quality in some men (Dawson *et al.*, 1992). Simple incubation of spermatozoa *in vitro* can also result in significant increases in DNA breakage both in humans (Martin *et al.*, 1992) and laboratory animals (Estop *et al.*, 1993). Breakage of DNA in turn can lead to chromosomal damage (Marder and Morgan, 1993). This can be accelerated by mutagenic agents such as ethyl methanesulphonate and potentiated by depletion of free-radical scavenging molecules such as glutathione (Evenson *et al.*, 1993). These changes are consistent with the general view that most sperm-derived genetic damage to embryos occurs through chromosomal breakage, whereas most oocyte-derived damage occurs through chromosomal rearrangements (Plachot *et al.*, 1988; Plachot, 1992). While we are unaware of any reports that yet address this question directly, the potential amounts of oxidized DNA, and thus the risk that spermatozoa carry chromosomal breakages, are likely to be elevated in infertile men for several reasons.

(i) Spermatozoa in subfertile men are exposed to abnormal amounts of reactive oxygen species and altered amounts of scavenging enzymes that control them, such as superoxide dismutase (Aitken *et al.*, 1991; Kobayashi *et al.*, 1991; Iwasaki and Gagnon, 1992). These highly reactive molecules are well-known mutagens.

(ii) In men with impaired spermatogenesis as well as epididymal lesions, it is likely that increased epididymal transit times will lead to ageing of gametes *in vivo* (Johnson, 1989). Artificially ageing spermatozoa in the male tract of rabbits is known to result in increased chromosomal anomalies in resulting embryos (Martin-De Leon *et al.*, 1973) and in increased embryonic mortality (Tesh and Glover, 1969).

(iii) There are significant associations between male subfertility and imperfect spermiation and nuclear condensation. This can lead to poor DNA protection through incomplete protamine packing (Silvestroni and Fabrizio, 1976; Evenson *et al.*, 1980; Bianchi *et al.*, 1993). In such cases single-stranded or denatured DNA can be detected by acridine orange fluorescence microscopy (Claasens *et al.*, 1992; Kosower *et al.*, 1992).

(iv) The failure to control amounts of transition metals in culture media used in IVF is likely to increase oxidative risk to DNA through catalysis of free-radical production (Aitken *et al.*, 1993; Miesel *et al.*, 1993). Many clinics use commercially prepared media such as Ham's F10 for convenience. This contains copper and iron: it should be strongly discouraged in human IVF and especially in cases dealing with spermatozoa that may already be rendered dysfunctional through the generation of excessive reactive oxygen species or the impairment of scavenging systems.

Recommendations

We do not wish to deny the undoubtedly excellent results that have been published from several major laboratories that have pioneered the use of techniques such as ICSI to overcome male subfertility. Nor are we arguing that micro-assisted fertilization should be abandoned. However, we believe that there is genuine cause for concern when all natural selective barriers to fertilization are circumvented without a sound clinical knowledge concerning the cause of the sperm dysfunction. Concerns will grow as these techniques are taken up by other groups less firmly grounded in good IVF laboratory practice and without the resources for good genetic screening and andrological investigation. Potential

environmental causes of male infertility, such as exposure to pesticides, heavy metals and abnormal amounts of oestrogens need to be evaluated thoroughly (Carlsen *et al.*, 1992; Giwercman and Skakkebaek, 1992; Sharpe, 1993; Sharpe and Skakkebaek, 1993). There may be links between the environment and the genome that result in subfertility in both men and women.

We would like to make the following recommendations.

(i) Wherever possible, spermatozoa with fully condensed nuclei of normal shape (Katz *et al.*, 1982; Menkveld *et al.*, 1990) should be selected for ICSI. Parallel samples should be screened for the incidence of single-stranded (denatured) DNA by acridine orange fluorescence microscopy (Claasens *et al.*, 1992; Kosower *et al.*, 1991) or similar suitable techniques.

(ii) Infertile men with a history of failed IVF, and who are considering assisted fertilization by micromanipulation, should be screened for the presence of oxidized DNA products in semen and urine. If these are elevated the couple should receive appropriate counselling concerning the risk of transmitting DNA breakage defects to embryos.

(iii) All men with infertility due to congenital absence of the vasa deferentia should be screened (together with their wives) for mutations to the cystic fibrosis gene complex. They should be warned of the possible consequences to any potential fetus generated.

(iv) Good animal models are urgently needed so that the pathogenesis of male infertility can be better understood. These will need to take into account the common factors of ischaemia, disordered oxidative phosphorylation and elevated amounts of reactive oxygen species that seem to be so prevalent in male subfertility (Cummins *et al.*, 1994).

(v) Long-term genetic and developmental evaluation of all children born as a result of micro-assisted techniques should be mandatory.

(vi) Lastly, careful clinical and genetic assessment of every subfertile man contemplating techniques such as ICSI is essential if our understanding of male infertility is to improve.

We recognize that these concerns could be raised against any form of assisted conception. However, we suggest that in using spermatozoa that cannot achieve fertilization by themselves, we may have reached a critical ethical threshold. With the advent of micro-assisted fertilization, a more thorough approach to clinical andrological investigation is needed: now, more than ever before. We are not alone in this concern (Fishel *et al.*, 1993; Maschiach *et al.*, 1993). The lessons of the diethylstilboestrol experience in causing delayed reproductive disorders in men (Sharpe and Skakkebaek, 1993) and women (Epelboin and Bulwa, 1993) should give us all cause for reflection.

Clinics should ponder these questions. Who wants to be the first to bring about a child with cystic fibrosis, or other genetic disorder, who would not exist without the expensive (and life-threatening) intervention of micro-assisted fertilization? Who will call them to account in 20 or 30 years when consequent problems may emerge? What a rich potential harvest for medical malpractice lawyers! Can we honestly say that we have no cause for concern? We suggest not. Reproductive technology is already under attack by feminist groups as being ineffective, unnecessary and expensive. They argue that it is not cost-effective for society to divert scarce medical resources away from the poor and needy, to richer couples not experiencing life-threatening illness (Hawthorne and Klein, 1991; Gennaro *et al.*, 1992). We do not endorse this position, but by approaching micro-assisted fertilization in a state of profound ignorance about the causes of male infertility, we may be sitting on an ethical time bomb.

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Received on January 17, 1994; accepted on March 30, 1994