The future of male infertility management and assisted reproduction technology

David Mortimer

Genesis Fertility Centre, #550, 555 West 12th Avenue, Vancouver, BC V5Z 3X7, Canada
E-mail: david@oozoa.com

Intracytoplasmic sperm injection (ICSI) is undoubtedly a powerful, and sometimes the only effective, form of infertility treatment. Nonetheless, it is a non-specific treatment that, combined with increasingly heroic techniques to recover male germinal cells, has led to perceptions of men as just providers of gametes in the infertility equation. In response to this nihilist attitude, where women are investigated extensively and scant attention is paid to men, there is a re-emerging awareness of andrology—particularly in countries with limited healthcare resources. Structured management strategies, using diagnostic information to recognize causative factors amenable to simpler, even systemic, therapies with reasonable chances of pregnancy rather than resorting prematurely to assisted reproduction technology, represent rational, cost-effective approaches to infertility management. Furthermore, genetic testing (particularly cystic fibrosis gene defects and Y-chromosome microdeletions) is essential for couples to make fully informed decisions on their options. Recognition that free radical-induced damage to the sperm genome (e.g. from smoking or in-vitro sperm manipulation) underlies deleterious paternal effects on preimplantation development promotes further synergy between andrology and embryology. Although societies strike different balances between considerations of affordability and cost-effectiveness of assisted reproduction technology, ICSI represents a last resort, to be used when less-invasive, lower-cost treatments have been deemed inappropriate or have failed. Consequently, rather than assisted reproduction technology eliminating the need for andrology, the future will see increasingly tighter integration of multidisciplinary infertility care, embracing careful diagnosis and patient education before obtaining truly informed consent and embarking upon cost-effective treatment.

Key words: andrology/assisted reproduction/cost-effective treatment/infertility management/male infertility

Introduction

Infertility is a problem manifest by couples, not individuals. A given man or woman might be sterile, or have a form of reproductive dysfunction that causes them to have reduced fertility potential (‘be infertile’), but such attributes can only be expressed when the individual is part of a reproductive unit, i.e. a couple attempting to achieve a pregnancy. When considering the contribution of the male partner of a couple to the aetiology of their infertility, the impact of a given problem such as a low sperm count will depend upon not only the severity of the problem per se but also upon the female partner’s relative fertility potential. Hence ‘male infertility’ is at best a misnomer, although we might consider the existence of ‘male factor infertility’—but not to the exclusion of possible (likely?) coexistent female factor infertility.
Male infertility treatment

ity in a given couple. These principles have been discussed, and their application described, many times (e.g. Comhaire et al., 1987; Cummins and Jequier, 1994; Hargreave, 1994a; Rowe et al., 1994; Irvine, 1998; Kamischke and Nieschlag, 1998, 1999a) and whereas in the past failures to apply them were more often due to patriarchal social beliefs, the development of assisted reproduction technology now constitutes a frequent reason for their abandonment. This review considers recent developments in the relationship between andrology and the ‘modern’ application of clinical assisted reproduction technology.

It is certainly true that the development of our understanding of the regulation of the male and female reproductive processes advances asynchronously. While great progress has been made in elucidating the neuroendocrine regulation of gametogenesis in the female, spermatogenesis remains even today much less well understood. The existence of multiple spermatogenic waves occurring simultaneously, even within a single seminiferous tubule, represents a far more complex system to unravel than the 28-30 day ovarian cycle. Consequently, for the male partner of an infertile couple we still have little more in our laboratory armamentarium than the traditional descriptive semen analysis and a range of poorly standardized, and often laborious or technically complex, sperm function tests. Whereas specific endocrine treatments exist for a variety of dysfunctions of the ovarian cycle, there is no proven successful treatment for defective spermatogenesis beyond the extreme case of hypogonadotrophic hypogonadism (Wu, 1994). Sperm quality is rarely investigated in any depth, being considered only in terms of visual appraisal of sperm motility and sperm morphology using light microscopy, and problems of gamete approximation (insemination, sperm transport through the female tract, sperm storage and longevity in vivo) are almost entirely ignored.

Hence sperm transport, as well as various aspects of sperm-oocyte interaction, anti-sperm antibodies (ASAB) are often ignored because of confusion as to their detection and the interpretation of test results, despite extensive evidence that they can affect sperm-cervical mucus interaction (Bronson, 1999; Hjort, 1999). Indeed, ASAB are an excellent example of how a pathological process impacts upon fertility: their effects are specific at the cellular level, but their expression is variable at the organism level. Therefore, rather than taking exceptions as evidence to disprove a rule (e.g. ‘I’ve had patient with ASAB who impregnated his wife’), they should be recognized as the extreme of a distribution of effects arising as a complex product of the severity (and intrinsic variability) of a pathological process in the man and the relative fertility of his partner. Indeed, such understanding is central to how clinical medicine and infertility care should be practised.

Infertility management and assisted reproduction technology

The possible causes of, and approaches for the treatment of, male factor infertility are summarized in Table I. Clearly certain problems are amenable to surgical intervention, and sexual dysfunction is frequently remedied by medical or psychological therapy (e.g. Hargreave, 1994b; Kamischke and Nieschlag, 1999b; Shah, 1999). Exposure to reproductive toxins is finally being recognized as an environmental, lifestyle or workplace hazard, the impact of which can be alleviated by avoidance (e.g. de Jager and Bornman, 1999). However, the fundamental problems of making more, or better, spermatozoa remain devoid of effective treatment (e.g. Hargreave, 1994b; Kamischke and Nieschlag, 1998, 1999a). Consequently, the majority of therapeutic modalities for male factor infertility have been founded upon the simple principle of bypassing the problem(s):

- pericervical artificial insemination of homologous spermatozoa (AIH) for problems of insemination;
- various forms of epididymal sperm aspiration to obtain spermatozoa in cases of agenesis or inoperable blockage of the excurrent ducts;
- intrauterine insemination (IUI) to bypass problems of sperm transport at the cervical level;
- gamete intra-Fallopian transfer (GIFT) or IVF to bypass more severe problems of sperm transport or less serious problems of poor sperm quantity or quality;
- IVF to compensate for more serious problems of poor sperm quantity or quality;
<table>
<thead>
<tr>
<th>Cause of infertility</th>
<th>Treatment approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterility</td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td></td>
</tr>
<tr>
<td>Spermatogenic arrest</td>
<td>Endocrine?</td>
</tr>
<tr>
<td>Sterilizing defect</td>
<td>None</td>
</tr>
<tr>
<td>Tract agenesis</td>
<td>None</td>
</tr>
<tr>
<td>Tract dysgenesis</td>
<td>Surgical?</td>
</tr>
<tr>
<td>Tract blockage</td>
<td>Surgical?</td>
</tr>
<tr>
<td>Functional</td>
<td></td>
</tr>
<tr>
<td>Sperm dysfunction</td>
<td>None</td>
</tr>
<tr>
<td>Subfertility</td>
<td></td>
</tr>
<tr>
<td>Sperm production</td>
<td>?</td>
</tr>
<tr>
<td>Too few spermatozoa</td>
<td>IVF or ICSI</td>
</tr>
<tr>
<td>Abnormal spermatozoa</td>
<td>?</td>
</tr>
<tr>
<td>Extrinsic factors</td>
<td>(Immunosuppression)</td>
</tr>
<tr>
<td>Antisperm antibodies</td>
<td>ICSI</td>
</tr>
<tr>
<td>Free radicals (ROS)</td>
<td>Antioxidants?</td>
</tr>
<tr>
<td>Toxicological exposure</td>
<td>Reduce exposure</td>
</tr>
<tr>
<td>Sperm delivery</td>
<td>Surgical</td>
</tr>
<tr>
<td>Partial obstruction</td>
<td>Medical or surgical</td>
</tr>
<tr>
<td>Ejaculatory disorders</td>
<td>Psychological and/or</td>
</tr>
<tr>
<td>Impotence</td>
<td>pharmacological</td>
</tr>
</tbody>
</table>

ART = assisted reproduction technology; TESE = testicular sperm extraction; ICSI = intracytoplasmic sperm injection; MESA = microepididymal sperm aspiration; ROS = reactive oxygen species.

- sub-zonal sperm insertion (SUZI) to bypass sperm transport and penetration of the oocyte-cumulus complex (although an acrosome reaction was still required—but could be induced artificially — and sperm-oocyte fusion had to occur); and
- intracytoplasmic sperm injection (ICSI) as the ultimate solution that bypassed all considerations of sperm physiology and function by delivering the male partner’s haploid genome contribution directly into the oocyte.

Most recently (i.e. during the second half of the 1990s) we have gone even further and, in cases where no mature spermatozoa were available even from the epididymis, spermatozoa are sought from within the tests. For such men, who are otherwise medically sterile, various workers have reported ICSI treatment using testicular spermatozoa [extracted from the lumen of the seminiferous tubule post-spermiation (TESE)] or more immature spermiogenic stages such as elongated or elongating spermatids (ELSI) and even round spermatids (ROSI) recovered or extracted from the Sertoli cells, and ICSI has even been attempted using isolated round spermatid nuclei (ROSNI). However, the competence of these progressively earlier germinal cells as gametes is compromised both when assessed using in-vitro sperm function testing (Aslam and Fishel, 1999) and when used clinically (Silber and Johnson, 1998; Al-Hasani et al., 1999; Aslam et al., 1999; Ghazzawi et al., 1999; Prapas et al., 1999; Vanderzwalmen et al., 1999; also see Table II), raising doubt as to the real value of such heroic feats of sperm retrieval, and giving concern that the man is now seen as little more than a source of (any) gametes in the infertility equation.

In a traditional specialist infertility centre the female and male partners of each couple receive a careful and thorough investigation, including comprehensive medical histories, physical examinations, and a work-up using a range of medical and surgical diagnostic tests. The basic standards for such infertility care were formalized by the World Health Organization in its publication the WHO Manual for the Standardized Investigation and Diagnosis of the Infertile Couple (Rowe et al., 1994) which constitutes the de-facto minimum standards worldwide. Because of the asynchronous development of treatment options for male factor infertility compared to female factors, a revised edition considering advances for managing the infertile male has been prepared recently (Rowe et al., 2000). However, many infertile couples now present (or are referred by their family doctor)
Male infertility treatment

Table II. Spermatids as gametes. Outcomes from intracytoplasmic sperm injection (ICSI) using either elongated spermatids (ELSI), round spermatids (ROSI) or round spermatid nuclei (ROSNI) according to a recent literature survey by Aslam et al. (1999)

<table>
<thead>
<tr>
<th>Outcome measure</th>
<th>ELSI</th>
<th>ROSI</th>
<th>ROSNI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment cycles (with embryo transfer)</td>
<td>55 (55)</td>
<td>80 (75)</td>
<td>9 (9)</td>
</tr>
<tr>
<td>Fertilization (2 pronuclei)</td>
<td>57.5%</td>
<td>31.3%</td>
<td>64.0%</td>
</tr>
<tr>
<td>Embryos per transfer</td>
<td>2.1</td>
<td>3.6</td>
<td>&lt;3.3</td>
</tr>
<tr>
<td>Implantation rate per embryo transferred (%)</td>
<td>6.1</td>
<td>6.5</td>
<td>0</td>
</tr>
<tr>
<td>Clinical pregnancies</td>
<td>12</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Live births</td>
<td>11</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Deliveries per embryo (%)</td>
<td>5.6</td>
<td>5.8</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Note that the implantation rates per embryo transferred are substantially lower than those achieved by modern IVF/ICSI units (~20–25%).

more-or-less directly to an ‘IVF Clinic’ where, typically, the female partner might have already undergone, or be expected to undergo, a WHO-compatible comprehensive work-up, including history and physical, extensive endocrine testing, ultrasonographic examination of the ovaries, hysterosalpingography and/or laparoscopy (with tubal patency test) and perhaps hysteroscopy. In contrast, the male partner will typically receive little attention beyond a perfunctory history and semen analysis. Only rarely are the male partners of assisted reproduction technology couples examined physically or undergo such procedures as scrotal thermography, ultrasound and vasography comparable to their partners’ investigations. While this situation might be explained as due to the limited availability of specialist clinical andrologists, or to poor perceptions of treatment availability for problems that might be identified in the male partner, the need for and value of extensive work-up of the female partner should also be reconsidered if treatment is pre-ordained to be IVF-based.

Another problem is the widespread consideration of poor quality semen as a diagnosis. For the male partner of an infertile couple a finding of ‘oligoasthenoteratozoospermia’ at semen analysis is not a diagnosis but rather a symptom of some unknown problem(s). Basing treatment upon such a non-specific symptom can only be considered arbitrary (even presuming, for the sake of argument, that the semen analysis laboratory was run to an adequate standard to minimize technical error and was therefore able to provide accurate results, see below). Of even greater concern is the trend in some centres to use ICSI as the first-line treatment, often regardless of the aetiology of a couple’s infertility.

Consequences of ICSI

In some centres, and indeed in some regions or countries, there is a growing tendency for ICSI to be used as the first choice of treatment for infertile couples. In some circumstances this has arisen in response to simplistic management by health insurance providers, in others as a consequence of misplaced concerns for avoiding treatment failure by using a technique that is presumed to solve all problems, and in others because ICSI is seen as the most successful form of treatment. Overall, this has been considered ‘insidiously lazy’ (Cummins, 1999) and a dangerous loss of control over the clinical decision-making process (Cummins and Jequier, 1994; Jequier and Cummins, 1997). However, consider those couples whose infertility is amenable to treatment using, for example, intrauterine insemination (IUI). A success rate of ~20% per treatment cycle can be achieved by IUI combined with mild ovarian stimulation (e.g. Branigan et al., 1999) giving, over three sequential cycles, a cumulative pregnancy rate of ~50%. This is greater than that achieved in many centres using IVF or ICSI, of which only a single cycle would typically be performed in a given 3 month period. Furthermore, the cost of three IUI cycles is less than one of IVF or ICSI, and the risk of side-effects lower (provided that appropriate care is taken to control the stimulation—and limit the number of IVF or ICSI embryos transferred—to
minimize the risks of multiple pregnancy). A similar comparison can be made for couples where the man’s spermatozoa are perfectly capable of achieving fertilization in vitro and so for whom IVF will have the same success rate as ICSI, but typically at a lower cost per treatment cycle. Further arguments can be made to undermine the perceived greater success of IVF compared to IUI, or ICSI compared to IVF (or IUI) when allowance is made for the expected better prognosis of those patients with less severe problems: IUI-suitable patients treated using IVF will certainly show better fertilization rates (and perhaps embryo quality, see below) than patients who were unsuitable for IUI due to decreased sperm quality, and their inclusion in the IVF population will create an overall increase in the outcome of the population treated using IVF. This same argument was used to explain the higher success rate of GIFT, and the often poorer IVF success rates seen in centres that treated a significant proportion of their patients using GIFT.

Beyond these perceptions of improved success rates using ICSI as the first choice option, there is a range of more critical concerns (e.g. Cummins, 1999; Lamb, 1999), including reports that ICSI in rhesus monkeys produces offspring with an increased prevalence of chromosomal abnormalities (Hewitson et al., 1999a) and can facilitate transmission of foreign DNA into embryos (Chan et al., 2000). ICSI as the panacea for (male) infertility leads to many couples having an incomplete, or no, diagnosis of the cause of their infertility and significant factors can go unnoticed, for example systemically induced sperm chromatin damage caused by infections of the male genital tract (e.g. Ochsendorf, 1999), by smoking (e.g. Fraga et al., 1994), or in cases with oligoasthenozoospermia (e.g. Aitken et al., 1998). Furthermore, the tendency to use therapeutic ‘overkill’, and failing to use appropriate medical or surgical treatments, especially if more cost-effective, might be considered poor practice. From the woman’s perspective, unnecessary ovarian stimulation has potentially serious side-effects, not to mention the risks associated with ovarian hyperstimulation syndrome.

Preferential use of ICSI to avoid possible poor or failed fertilization covers two failings: (i) failure to identify specific causes, or even the risk, of sperm dysfunction during diagnostic work-up by the physician responsible for the couple, or (ii) a tendency to tolerate lower technical standards within the laboratory that themselves lead to poor/no fertilization at IVF, but which are obviated by using ICSI. Accepting either situation must be considered unprofessional at best.

Finally, there are genetic sequelae of treating many cases by ICSI, a topic that is the subject of heated debate (e.g. Tournaye et al., 1997; Barratt et al., 1999; Cummins, 1999; Lamb, 1999; Schlegel, 1999). However, this does not imply that ICSI per se causes genetic abnormalities of the embryos and resulting offspring (Bonduelle et al., 1999), nor does it concern the increased prevalence of sex chromosome aneuploidy in men with severe oligozoospermia (e.g. Pfeffer et al., 1999), but rather recognizes a series of genetic, and hence heritable, disorders:

- The high risk of Y chromosome microdeletions in men with severe oligozoospermia or azoospermia (Patrizio, 1997; Vogt, 1999), which will be transmitted to any sons created using assisted reproduction technology and who will, in their turn, be sterile (Kleiman et al., 1999; Page et al., 1999).
- The risk of transmitting cystic fibrosis to the offspring in couples where the man has congenital bilateral absence of the vasa deferentia (CBAVD), which is due to mutations of the cystic fibrosis transmembrane conductance regulator gene (Schlegel and Shin, 1999), and where the female partner is a AF508 carrier.
- The growing number of single gene defects that cause male infertility or sterility and can be inherited by sons produced using assisted reproduction technology (e.g. Vogt, 1997, 1999; Cummins, 1999).

In the most extreme cases, assisted reproduction technology can now be considered to have made male sterility a heritable condition (Tournaye et al., 1997; Barratt et al., 1999). While couples who have been fully informed of their diagnosis and likely outcome often chose still to proceed with treatment, failing to inform them of all pertinent information can be considered to negate their ability to provide informed consent to treatment (e.g. Cummins, 1999).
Role of diagnostic laboratory andrology

The semen analysis remains the initial laboratory investigation for all male partners of infertile couples. After decades of effort to improve the value and reliability of this fundamental test, we are finally seeing significant improvements in semen analysis standards in many countries as a result of greater adherence to established methods (e.g. Mortimer, 1994a,b; Mortimer and Mortimer, 1999; World Health Organization, 1999), the development of coherent training courses by organizations such as the British Andrology Society, the European Society for Human Reproduction and Embryology and the Nordic Association for Andrology (Mortimer, 1994c; Bjørndahl and Kvist, 1998; Punjabi and Spiessens, 1998; Vreeburg and Weber, 1998), recognition of the value of internal quality control (e.g. Cooper et al., 1992; Clements et al., 1995), and the appearance of external quality assurance programmes (e.g. Neuwinger et al., 1990; Matson, 1995).

Laboratory investigation of the male partners of infertile couples can be viewed in two opposing perspectives: (i) trying to predict fertility, and (ii) trying to identify pathology that will cause infertility or sterility. Although the former, prognostically motivated attitude is by far the more common, in reality the second is more useful—and readily applicable. There are far too many confounding factors that prevent reliable prognosis, short of stating that achieving a pregnancy (or fertilization in vitro) is, for the majority of men, the most likely outcome and can therefore be expected to correlate with most measurements made during diagnostic tests. The most useful information is obtained when a test gives an abnormal result that is predictive of failure—but not simply failure of the overall process (i.e. conception), rather failure of a specific component or step in the process that can be used to identify a treatment strategy. In this way the information is not used to generate an overall prognosis for the couple, but to direct the management of their treatment. These concepts, which have been described as 'structured management', are discussed in detail elsewhere (Mortimer, 1999) and have been considered in the WHO’s updated recommendations for management of male factor infertility (Rowe et al., 2000).

A ‘modern’ view of sperm testing in relation to the functional life of the spermatozoon (Figure 1) is shown in Table III. In these terms a semen analysis is not simply descriptive, using a series of counts to determine the adequacy of sperm production in abstract terms, but rather it is performed in an attempt to identify the likelihood of the man being able to achieve colonization of his partner’s cervical mucus by his spermatozoa and hence have at least a chance of establishing a pregnancy in vivo. It is possible that in-vitro sperm-mucus interaction tests can help achieve this determination more directly, but their performance is confounded by access to the partner’s cervical mucus — although other tests of sperm function such as the hyaluronate migration test (Mortimer et al., 1990; Neuwinger et al., 1991;
Table III. Laboratory assessment of the infertile male

<table>
<thead>
<tr>
<th>Type of testing</th>
<th>Aspect being assessed</th>
<th>Tests available</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen analysis</td>
<td>Sperm production, quantitative</td>
<td>Semen analysis: concentration, total count</td>
</tr>
<tr>
<td></td>
<td>Sperm production, qualitative</td>
<td>Semen analysis: motility, vitality, morphology</td>
</tr>
<tr>
<td></td>
<td>Sperm maturation</td>
<td>Sperm morphology (creatine kinase?)</td>
</tr>
<tr>
<td></td>
<td>Autoimmunity to spermatoza</td>
<td>Antisperm antibody tests: Immunebead test, SpermMAR test (Friberg, Isojima or Kibrick tests)</td>
</tr>
<tr>
<td></td>
<td>Free radicals</td>
<td>ROS analysis: differential between WBCS and spermatozoa</td>
</tr>
<tr>
<td></td>
<td>Sperm DNA damage</td>
<td>SCSA, nick translation, chromomycin A3 staining, Comet assay, TUNEL assay</td>
</tr>
<tr>
<td></td>
<td>Suitability for ART</td>
<td>'Trial wash' (i.e. trial sperm preparation) with post-preparation assessment</td>
</tr>
<tr>
<td>Sperm function tests</td>
<td>Migration</td>
<td>Sperm-mucus interaction (e.g. Kremer test)</td>
</tr>
<tr>
<td></td>
<td>Hyperactivation</td>
<td>Hyaluronate migration test</td>
</tr>
<tr>
<td></td>
<td>Acrosome reaction</td>
<td>CASA ± pharmacological sperm stimulation</td>
</tr>
<tr>
<td></td>
<td>Zona binding</td>
<td>ARIC test (uses A23187; rhZP3 in future?)</td>
</tr>
<tr>
<td></td>
<td>Fertilizing ability</td>
<td>Hemizona assay using salt-stored zonae (artificial zonae using rhZP3 in future?)</td>
</tr>
<tr>
<td>Genetic testing</td>
<td>Numerical or structural anomalies</td>
<td>Karyotype (high resolution G-banding) (FISH for more specific analyses?)</td>
</tr>
<tr>
<td></td>
<td>CF testing in men with CBAVD</td>
<td>PCR for CFTR gene defects</td>
</tr>
<tr>
<td></td>
<td>Y-chromosome microdeletions</td>
<td>PCR for AZFa,b,c sequence-tagged sites</td>
</tr>
<tr>
<td></td>
<td>Single gene defects</td>
<td>Molecular genetics (PCR, sequencing, etc.)</td>
</tr>
</tbody>
</table>

MAR = mixed agglutination reaction; ROS = reactive oxygen species; WBC = white blood cell; SCSA = sperm chromatin structure assay; TUNEL = TDt (terminal deoxynucleotidyl transferase)-mediated dUDP nick-end labelling; CASA = computer-aided sperm analysis; ARIC = acrosome reaction after ionophore challenge; FISH = fluorescent in-situ hybridization; PCR = polymerase chain reaction; CFTR = cystic fibrosis transmembrane regulator.

Aitken et al., 1992) could resolve this difficulty. More specific evaluations of sperm fertilizing ability have been described in detail elsewhere (reviews: Mortimer, 1994a; ESHRE Andrology Special Interest Group, 1996; Oehninger et al., 1997, 1998; De Jonge, 1999; Huszar et al., 1999) and the most commonly employed tests include:

- Sperm hyperactivation as a concomitant of capacitation and functional requirement for sperm penetration of the cumulus complex and of the zona pellucida (analysed using computer-aided sperm analysis or ‘CASA’; see Mortimer, 1997). Analysis of human sperm hyperactivation has been hampered by its multiphasic and temporally variable expression, with substantial inter-individual variability, although an assay employing a single time point and stimulation of hyperactivation expression by progesterone and pentoxifylline has been reported (Mortimer et al., 1997).
- The ability to undergo the acrosome reaction in response to appropriate agonists such as the ionophore A23187 as used in the ‘acrosome reaction after ionophore challenge’ or ‘ARIC’ test (Cummins et al., 1991; ESHRE Andrology Special Interest Group, 1996; De Jonge, 1999) (although use of a powerful ionophore is seen by many to limit the value of the ARIC test because the fertilizing spermatozoon, both in vivo and in vitro, undergoes its acrosome reaction on the surface of the zona pellucida in response to binding to the sperm receptor of the zona, ZP3.)
- Sperm binding to the zona pellucida (e.g. the hemizona assay or ‘HZA’; see ESHRE Andrology Special Interest Group, 1996; Oehninger et al., 1997, 1998). However, the dependence of this test upon the availability of human zonae pellucidae does limit its widespread application.

It should be noted that the latter two tests will become more amenable to routine clinical application with the availability of bioactive recombinant...
What the Human Sperm Contributes at Fertilization

Figure 2. Illustration of the components of the human spermatozoon contributed to the zygote at fertilization.

What does the spermatozoon contribute at fertilization?

A further dimension to sperm assessment is recognized when considering what the human spermatozoon contributes to the embryo at fertilization (see Figure 2). While the chromatin contained in the highly condensed sperm nucleus is unanimously recognized as being the 'payload' of the spermatozoon, it does not influence embryonic development until around day 3 when the embryo activates its own genome; development prior to that stage being controlled by mRNA stored in the oocyte. But the spermatozoon contributes two more essential components at fertilization: (i) yet-to-be-defined cytoplasmic oocyte activation factor(s) (Dale et al., 1999; Perry et al., 1999); and (ii) the proximal centriole that becomes the centrosome of the zygote and orchestrates its first cleavage division (e.g. Hewitson et al., 1999b; Moomjy et al., 1999). It is now known that sperm mitochondria are tagged with ubiquitin (most probably during spermiogenesis) and thereby marked for destruction in the zygote (Sutovsky et al., 1999) and, although sperm mitochondria (or their DNA content) can be tracked during the first few cleavage divisions using fluorescent probes or PCR (e.g. Rinaudo et al., 1999; St. John et al., 1999), it seems most probable that they are not destined to be contributed to the next generation (Cummins, 1997, 1998; Cummins et al., 1997; Shoubridge, 1999).

Although a deleterious effect of free oxygen upon human spermatozoa was originally described by John MacLeod in the 1940s, it was not until the 1980s that the effects of free radicals (or reactive oxygen species: ROS) upon sperm function, and their role in the aetiology of male infertility, were well established (Aitken and Clarkson, 1987). Shortly thereafter, the detrimental influence of ROS generation during in-vitro sperm preparation was discovered (Aitken and Clarkson, 1988) and widespread evidence for how this phenomenon could impact upon sperm function tests and IVF was collated (Mortimer, 1991). ROS are generated by both leukocytes present in semen and the spermatozoa themselves (Krausz et al., 1992; Aitken, 1995; Whittington and Ford, 1999). However, only those spermatozoa with excess retained spermatid cytoplasm generate ROS (Aitken et al., 1994; Aitken, 1995; Huszar et al., 1998, 1999), again emphasizing an increased risk of dysfunction in semen from men with defective sperm production. It is now accepted that ROS not only affect the sperm plasma membrane by causing phospholipid peroxidation, and hence decrease membrane fluidity and induce a loss of sperm function, but that they also affect the sperm DNA, causing strand breaks that can be revealed by various tests of sperm DNA integrity such as nick translation (Sakkas et al., 1997) and the sperm chromatin structure assay (SCSA: Evenson, 1999; Evenson et al., 1999).

Of particular importance in the practice of assisted reproduction technology is a recent report...
that sperm preparation using colloidal silica-based density gradient separation (Percoll; Pharmacia Biotech, Uppsala, Sweden; and PureSperm; Nidacon International AB, Göteborg, Sweden) permits the recovery of selected populations of ‘better’ spermatozoa with a lower incidence of DNA nicks (Sakkas et al., 2000). Parallel swim-up migration from semen did not achieve comparable selection of the genetically less compromised cells, although other studies using the SCSA (which assesses the susceptibility of sperm DNA to in-vitro pH- or temperature-induced damage) have shown that swim-up (Spanò et al., 1999) and glass wool filtration (Larson et al., 1999) can also select spermatozoa with better chromatin stability. The relevance of these observations is that while many assisted reproduction technology laboratories employ density gradient preparation for their IVF spermatozoa, many still use simple washing for ICSI since the risk of ROS-induced damage to sperm dysfunction arising from simple centrifugal washing (Mortimer, 1991) was not considered important because sperm function is believed to be unimportant for ICSI. However, since those men deemed as requiring treatment by ICSI would be more likely to have increased proportions of abnormal spermatozoa, they would be most susceptible to ROS-induced sperm DNA damage. Since it is well established that ROS-induced damage to spermatozoa involving substantial degradation of their DNA does not necessarily affect their fertilizing ability (Aitken et al., 1998), colloidal silica density gradients must be considered the most appropriate generally applicable technique of sperm preparation (Mortimer, 2000).

Paternal effect on preimplantation development

During the last few years a paternal influence upon mammalian preimplantation development has been recognized and at least indirect evidence found for its action in human embryos (review: Sakkas, 1999). For example, Janny and Ménézo (1994) found that more embryos stopped development when zygotes were produced by IVF from men with abnormal semen quality. Also, when culturing supernumerary embryos remaining after day 2 embryo transfer it was found that many fewer ICSI-derived embryos (i.e. embryos produced from men with poorer semen quality) reached the blastocyst stage compared to IVF-derived embryos (Shoukir et al., 1998). The relative significance of pre-existing damage to the sperm DNA (i.e. that occurring in the male tract, perhaps arising from infections: Ochsendorf, 1999) compared to what might be induced during in-vitro manipulation for assisted reproduction technology remains unknown.

Provision of infertility health care

The management of male infertility in future will continue to be greatly affected by ‘non-scientific’ issues which cannot be ignored. The availability of, and access to, medical services are controlled by complex interactions between political, economic and social pressures, and payment for infertility treatment is a balance between government, insurance and personal funding sources. For example, in Australia, a highly effective national infertility support organization has ensured strong government support for IVF so that all infertile women receive substantial coverage towards the medical and laboratory costs of IVF. Moreover, the gonadotrophins are provided under the Pharmaceutical Benefits Scheme. However, the availability of subsidized gonadotrophins does not apply to artificial insemination cycles, and so IUI is not covered by Medicare — making IVF the cheapest option for patients in Australia. Yet in nearby New Zealand, there is no government support for IVF and the provision of infertility health care is strongly controlled by resource allocation issues. In Canada, a country that prides itself upon the universal provision of health care by the state, only one province (Ontario) has partial government support for IVF, and in some provinces, all infertility treatment is confined to private or academic practitioners.

With cost-effectiveness becoming more integral to health care provision, funding decisions in future will be based increasingly upon outcomes analysis and affordable technology. In some places it might now appear financially sensible to provide one cycle of ICSI as the primary (and sometimes sole) treatment for all infertile couples because it saves extensive and costly diagnostic investigations, but more careful testing, increased success rates (e.g. resulting from improved embryo culture systems),
and complete analysis of the true cost of treatment (e.g. including the associated costs arising out of a multiple pregnancy resulting from the transfer of more than two embryos) will lead to changes in funding patterns, and to changes in patient expectations.

Conclusions
A continued increasing recognition of the clinical importance of sperm dysfunction, and a growing awareness of the importance of the cost-effectiveness of infertility treatment, foreshadow changes in infertility treatment during the coming decade. There will be increasing focus on genetic testing of men with significantly impaired fertility and, as the general uselessness of pathology laboratory semen analyses becomes more widely recognized, there will be a continuing integration of specialized andrology laboratories into multidisciplinary infertility programmes and assisted reproduction technology units. These laboratories will provide standardized, quality-controlled semen analysis and sperm function assessments, both before and during assisted reproduction technology treatment (Mortimer, 1994c,d; World Health Organization, 1999, Rowe et al., 2000). With improved biotechnology, in particular the production of bioactive rhZP3, a new generation of sperm function tests is foreseen that will allow routine investigation of defects of sperm function, both within the diagnostic process and during progressive treatment of the infertile couple. Structured management of couples will become more widespread, especially in countries where health care resources for infertility care are more limited, and although ICSI will undoubtedly continue as a highly successful mode of assisted reproduction technology treatment, it will be seen more as a last resort for those who cannot, or did not, achieve a pregnancy by less invasive forms of treatment. Contrary to the perceptions of some, recent advances in assisted reproduction technology have not diminished the value and importance of diagnostic andrology but rather have revealed it to the perspicacious as having even greater significance than recognized previously, a perspective reflected in the revised edition of the WHO clinical manual for the infertile male (Rowe et al., 2000).

Male infertility treatment

References

107


Page, D.C., Silber, S.J. and Brown, L.G. (1999) Men with infertility caused by AZFc deletion can produce sons by intracytoplasmic sperm injection, but are likely to transmit the deletion and infertility. *Hum. Reprod.*, 14, 1722–1726.


Pfeffer, J., Pang, M.G., Hoegerman, S.F. et al. (1999) Aneuploidy frequencies in semen fractions from ten oligoasthenoteratozoospermic patients donating sperm...
D.Mortimer


