REVIEW

Using the alkaline comet assay in prognostic tests for male infertility and assisted reproductive technology outcomes

Sheena E. M. Lewis* and Ishola M. Agbaje

Reproductive Medicine, Queen's University of Belfast, Institute of Clinical Science, Grosvenor Road, Belfast, BT12 6BJ, Northern Ireland, UK

Infertility affects one in six couples in Europe during their reproductive years with dysfunctional sperm being one of the most common causes. Conventional semen analysis has proven variable and lacking in prognostic value so, over the past decade, more useful molecular fertility biomarkers have been explored. Among the tests showing most promise are those measuring sperm DNA quality. Sperm DNA damage has been closely associated with numerous indicators of reproductive health, including, fertilization, embryo quality, implantation, spontaneous abortion and childhood diseases. It therefore has great potential as a prognostic test for assisted reproductive treatment (ART), when couples are presenting with male infertility. Unlike somatic cells, sperm have a unique tightly compacted chromatin structure. Our group has modified the alkaline comet assay for use with sperm. Sperm DNA also differs from somatic cells in its high susceptibility to oxidative damage; this is largely due to the presence of abundant polyunsaturated fatty acids acting as substrates for reactive oxygen species (ROS) and its lack of repair mechanisms. Consequently, the effects of ROS and antioxidant protection on sperm DNA fragmentation have been widely investigated. In this review, the relationship between actual sperm DNA damage as determined by the alkaline comet assay and potential DNA damage as measured by DNA adduct testing will also be examined and the potential of routine clinical practices such as cryopreservation and prolonged incubation to induce further DNA damage was investigated. Finally, the usefulness of sperm DNA tests as prognostic markers and in particular, the opportunities and challenges provided by DNA testing in male fertility determination will be discussed.

Sites of DNA damage in sperm

Sperm chromatin differs from somatic cells in both constituents and arrangement. During spermiogenesis, protamines (1) which are half the size of histones (2), replace the majority of histones and the chromatin is wound into unique supercoiled doughnut structures named toroids (3,4). As the sperm pass through the epididymis, the protamines are cross-linked by disulphide bonds reducing the chromatin to one-sixth the volume taken up in somatic cell nuclei (2). This dense compaction gives protection against exogenous assault to the sperm DNA as reflected by the high levels of irradiation required to damage sperm DNA, compared with somatic cells (5) and also by the relative resistance of sperm nuclear and mitochondrial genomes to

damage when treated with hydrogen peroxide (6,7). Despite this protection, basal levels of sperm DNA damage are very high in infertile (8,9) and even fertile men (5). In addition to exhibiting higher basal levels of DNA damage, sperm from infertile men are more susceptible to damage from hydrogen peroxide, X-ray irradiation (5,10) and cryoinjury (11).

Damaged DNA has been observed in testicular, epididymal and ejaculated sperm. Sperm DNA first becomes susceptible to damage if chromatin packing is not completed during spermatogenesis (12,13). Some strand breaks may be necessary to reduce the torsional stress experienced by DNA during the rearrangement of its tertiary structure, a time when protamine replacement is occurring in elongating spermatids (14–16). However, these are temporary nicks and if they are not repaired (17, 18) increased DNA fragmentation in mature, ejaculated sperm may result. Damage can also occur as a result of suboptimal compaction (19) due to incomplete disulphide cross-linking during epididymal transit.

Although DNA repair does occur in developing sperm (20), it is terminated as transcription and translation cease post-spermiogenesis (21,22). As a result, sperm have no mechanism to repair DNA damage incurred during their transit and storage in the epididymis or post-ejaculation. In addition, the cellular machinery that allows these cells to complete apoptosis is discarded. As a result, advanced stages of germ cell differentiation into spermatocytes, spermatids and subsequently spermatozoa cannot be deleted, even though they may be defective or even partially apoptosed as evidenced by Fas expression or endonuclease activation (23,24). This may explain the large numbers of defective sperm present even in fertile mens' semen (25).

Further, DNA damage can occur as sperm pass through the epididymis. This damage can be induced in mature sperm by adjacent ROS-producing immature sperm in the epididymis, by ROS-producing epithelial epididymal cells or through toxic factors present as sperm undergo epididymal maturation. The latter hypothesis is supported by work that shows lower levels of DNA damage in testicular sperm which increases in caudal epididymal and ejaculated sperm (26–28). This has recently been confirmed in a study by Greco *et al.* (29), where sperm DNA damage was markedly lower in testicular than ejaculated sperm. Suganuma's group (30) is also in agreement showing that defective sperm experienced an increase in DNA damage during passage through the epididymis. It is acknowledged that even the proximal epididymis has substantial proportions of senescent sperm (28) releasing ROS as they age and die (31) and damaging those adjacent to them. This may at least in part explain the higher levels of sperm DNA damage in the epidydimis.

Oxidative stress, a major cause of DNA damage

As with other indications of sperm dysfunction, the importance of ROS, caused by either increased ROS generation or

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*To whom correspondence should be addressed. Tel: +44 28 9093 3987; Fax: +44 28 9032 8247; Email: s.e.lewis@qub.ac.uk

impaired antioxidant defence, as a primary instigator of sperm DNA damage is well established (32–34). Sperm are particularly vulnerable to damage from ROS because of their high polyunsaturated fatty acid content and limited ability to repair damage. Sperm from infertile men are often associated with high levels of ROS caused by either increased generation or impaired antioxidant defence (35,36). Associations between oxidative stress and sperm DNA damage have been reported in numerous studies (9,31,37–39).

The oocyte can provide limited repair to damaged sperm DNA post-fertilization (40,41). However, if inadequately repaired, such damage can predispose to mutations in the developing embryo with the potential to induce disease in the offspring (34). It is acknowledged that a greater proportion of inherited diseases have their origin in the paternal germ line (42). Furthermore, cancers arising from germ cell mutations show a much greater paternal than maternal contribution (43). This fact is further illustrated by the finding of higher rates of haematological cancers (leukaemias and lymphomas) in offspring of men who smoke (44) with the suspected causal link being the increased level of oxidative sperm DNA damage (45).

Antioxidant therapy

Although there is now consensus as to oxidative stress as a major source of sperm DNA damage, less progress has been made in developing useful antioxidant therapies. The production of oxidative stress from hydroxyl, superoxide and hydrogen peroxide radicals can be kept in check by chain-breaking antioxidants such as vitamins C and E. Vitamin C concentrations are 10 times higher in seminal plasma than blood plasma (36) emphasizing their physiological importance. Further, our group has also reported reduced levels of chain-breaking antioxidants; vitamins C and E in both sperm and seminal plasma of infertile men compared with fertile males (36,46) and protective benefits when added during sperm preparation for ART (47). Metal chelators can also be useful in reducing ROS generation and preventing lipid peroxidation of sperm membranes, thereby protecting sperm DNA [reviewed by (48)]. Paradoxically, the addition of combinations of antioxidants such as vitamins C and E can have damaging effects to DNA in vitro (37) and in vivo where it causes an increase in DNA decondensation (49) or they can be ineffective (50). Indeed, vitamin E alone has been described as 'a double-edged sword', its effects being strictly dependent on dosage (51) and ineffective if given to males whose infertility aetiology is not oxidative stress. This belief is supported by a study of couples who had had failed ART treatments (29), where the male partners had high levels of sperm DNA damage but not necessarily as a result of oxidative stress. Antioxidant treatment of vitamin C (1 g) plus vitamin E (1 g) daily for 2 months was administered before a further intracytoplasmic sperm injection (ICSI) attempt. Following treatment, significantly lower levels of sperm DNA damage concomitant with higher success rates from ICSI (48 versus 7% pregnancy rates) were observed. However, some patients did not respond to antioxidant therapy despite evidence of sperm DNA damage, perhaps suggesting the damage was occurring through either a non-oxidative pathway or other complicating factors, such as, vitamin E's non-antioxidant functions (52), preventing its efficacy. Further research is urgently needed to find the most effective antioxidant therapy for sperm DNA protection in the greatest range of patients.

Sperm DNA damage tests

Traditionally, male infertility diagnoses have depended on microscopic analyses and biochemical assays to determine human semen quality. The commonly measured parameters are sperm concentration, motility and morphology in the ejaculate. Most laboratories also include 'sperm suitability' tests where the subpopulations of fastest swimming sperm are separated by density centrifugation. These tests are essential to provide the fundamental information on which clinicians base their initial diagnosis. However, their clinical value in predicting fertility is questionable. Over the past decade, a number of laboratory tests have been developed to determine specific aspects of sperm function. These include quantitative sperm motion parameters, capacitation, basal and induced acrosome reactions and sperm–zona pellucida interactions. However, few have been proven to have strong prognostic value and thus have not become routine clinical tests. There is general acceptance that sperm nuclear DNA tests show the most promise in of the diagnosis and treatment of male infertility. This has led to the development of numerous techniques to assess sperm DNA integrity. Of these, the alkaline comet, terminal deoxynucleotidyl transferase (TdT, TUNEL) and Sperm Chromatin Structure (SCSA) assays [reviewed by (26,48,53)] have been shown to be most robust. Each of these tests determines different aspects of DNA damage.

The alkaline comet assay (originally known as the Single Cell Gel Electrophoresis assay) assesses actual DNA strand breaks and alkaline labile sites when used under alkaline conditions. It has been tested in vitro and in vivo in a wide variety of mammalian cells (54–56) employing a number of different genotoxic stimuli including UV radiation, carcinogens, radiotherapy and chemotherapy (57). It has been proven to be rapid (57,58), reproducible (10) and have higher sensitivity than alkaline elution or nick translation (NT) assays, even with prior chromatin decondensation (9,59). The alkaline comet assay can detect damage equivalent to as few as 50 single-strand breaks (SSB) per cell. One of its unique and powerful features is the ability to characterize the responses of a heterogeneous population of cells by measuring DNA damage within individual cells as opposed to just one overall measure of damaged cells versus undamaged cells as in the TUNEL. This is important, since DNA damage may be the pivotal factor in determining the sperm's capacity to achieve a pregnancy. Semen is one of the most heterogenous biological fluids in humans. ART outcomes are improved by isolating the best subpopulations for clinical use. By using the alkaline comet, the actual damage load of small cohorts of sperm may be measured. As the alkaline comet only requires 100 cells for analysis it has also been particularly useful for studies involving DNA of testicular sperm and for men with low sperm concentrations where sperm numbers are limited (60).

The most commonly used alkaline comet measures are tail DNA (percentage of DNA in the tail compared to the percentage in the 'head' or unfragmented DNA), tail length (the length of the tail measured from the leading edge of the head) or Olive tail moment (OTM) [percentage of DNA in the tail (tail DNA) times the distance between the means of the tail and head fluorescence measures]. The OTM is expressed in arbitrary units. Each of these parameters describes endogenous DNA damage corresponding to DNA strand breakage and/or alkali-labile sites. In the optimization of the alkaline comet for use with sperm, we found tail DNA to be the most reproducible

parameter (10), therefore, sperm DNA damage has been expressed as tail DNA throughout our studies.

Clinical thresholds for ART success have not yet been established for the alkaline comet assay. However, its clinical value has been shown in diagnosis of suboptimal semen profiles and associations with classic parameters of semen analyses such as sperm concentration (9), morphology (11), mitochondrial function (68) and oocyte penetration (69). The subpopulations of sperm isolated from semen by density centrifugation for ART have also been shown to have less DNA fragmentation (70). Furthermore, the predictive values of DNA damage in embryo quality (71) and pregnancy with ejaculated and testicular sperm (60) have also been reported.

A second commonly used test for sperm DNA damage is the SCSA. It is also believed to measure both SSB and DSB (72), although primarily single-stranded DNA using a DNA fragmentation index (now called DFI, formerly $COMP_{\alpha 1}$). It can also be used to measure what the authors call 'immature sperm populations' that have higher than normal stainability (high density stainability). It can be used in conjunction with light microscopy or flow cytometry, enabling very large numbers of sperm to be assayed rapidly. In addition, it benefits from individual laboratory training prior to use by its initiator Don Evenson and a standard protocol closely adhered to by all users minimizing interlaboratory variation. The SCSA is less specific than alkaline comet or TUNEL in determining DNA fragmentation in that it detects changes in protamine content and disulphide cross-linkage as well as DNA strand breaks. However, its advantages are that it is highly repeatable and clinical thresholds have been established showing that there is a greater chance of pregnancy after intrauterine insemination, *in vitro* fertilization and ICSI and if the semen has <27–30% DFI, respectively (72).

In the TUNEL assay, TdT preferentially labels the blunt 3′-OH ends of double-stranded DNA breaks, but also measures SSB (73). It has the advantage of being relatively quick and easy to perform. However, one major limitation of this assay in sperm results from the high levels of sperm DNA compaction combined with the absence of a lysis step. While the protocol may not induce further damage, it may limit accessibility of the TdT enzyme to all 3′-OH ends. This may account for the range of suggested clinical thresholds as reviewed by Tesarik *et al.* (74), showing a number with considerably lower values [12, 15, 18%, (75–78)] than that proposed by Evenson (30%). There are just a few studies comparing alternative assays within the same study (SCSA and alkaline comet (79), alkaline comet and TUNEL (68) and SCSA and TUNEL (80–82) which give surprisingly close correlations between assays despite the differences in protocol and parameters measured by each assay.

Modifications to alkaline comet assay for use with sperm

The alkaline comet assay has been extensively used to study DNA fragmentation in a number of cell types. The study of sperm DNA fragmentation using this technique requires the use of a modified protocol primarily because of the differences in DNA packaging between sperm and somatic cells as described earlier. The formation of disulphide bonds between protamines and DNA is the key in facilitating the high level of DNA compaction in sperm. In addition, it is acknowledged that following ejaculation additional bonds are formed, further enhancing chromatin stability (61). However, the extent of this DNA compaction prevents DNA strands migrating during electrophoresis in conventional alkaline comet assay protocols. To overcome this difficulty, our group has performed a number of studies modifying alkaline comet protocols for use with sperm (10,37). In early studies, the use of conventional alkaline comet protocols designed for somatic cells failed to facilitate lysis indicated by the sperm head and tails remaining intact. In subsequent studies, the protease enzyme Proteinase K was added to remove protamines, thereby allowing sperm DNA to decondense and migrate. However, this was only effective with relatively high concentrations of Proteinase K (100 mg/ml) and after prolonged (overnight) incubation. This had the concomitant adverse effect of inducing sperm DNA damage. Baseline damage for tail DNA in sperm was variable, often reaching 25% (10,47,62). In our current protocol, lysis and decondensation steps involve immersion (within agarose gels) in freshly prepared cold lysis solution (2.5 M NaCl, 100 mM ethylenediamene tetraacetic and 10 mM Tris (pH 10), with 1% Triton X-100 added just prior to use), for 1 h at 4°C. This is followed by incubation with dithiothreitol (10 mM) for 30 min at 4°C followed by lithium diiodosalicylate (4 mM) for 90 min at 20°C to reduce the disulphide bonds. The use of this modified protocol has been shown to be a reliable and reproducible method of assessing DNA damage in sperm with baseline damage stabilizing at ~10–15% in sperm with normzoospermic profiles (37).

One criticism of the alkaline version of the alkaline comet assay for sperm DNA is that it measures alkali-labile sites that are not specific for infertility (63). In addition, it has limited ability to distinguish between endogenous and induced strand breaks or between SSB and DSB (53,64). It has been suggested (65) that markers of DSB may be more important in relation to fertility because although sperm DNA damage can be repaired by oocytes between sperm entry and initiation of the next S-phase, this DNA repair capacity is limited and DSB are more difficult to repair than SSB (40,65,66). In contrast, others [reviewed by (67)] suggest that total DNA damage is a more valuable indicator. The alkaline comet assay, under alkaline conditions, measures single and double-strand DNA breaks and those alkali-labile sites, which, at high pH, are susceptible to breakage and conversion into SSB. Since all these are included in the analysis, proponents of the alkaline comet assay suggest it is the optimal assay for the assessment of overall DNA damage.

Sperm DNA adducts and their relationship with DNA fragmentation

In addition to discrete strand breaks, ROS can induce other types of DNA damage, including base loss or modification/adduct formation, the most common of which is 7, 8-dihydro-8-oxo-2'-deoxyguanosine (8-OHdG), an oxidative adduct of the purine guanosine (83). If sperm with DNA adducts are successful in achieving a pregnancy, paternally originating errors in DNA replication, transcription and translation can occur, potentially predisposing the offspring to a number of cancers and other degenerative disorders (84,85). Furthermore, given time, such base modifications may also lead to discrete DNA strand breaks (86).

In a study from our group (87), sperm from Type 1 diabetic men had significantly higher 8-OHdG 10^{-5} dG as well as DNA fragmentation (as assessed by the alkaline comet) than those of non-diabetic men. A significant association ($r_s = 0.7$) between DNA fragmentation assessed by the alkaline comet assay and

levels of 8-OHdG 10^{-5} dG was also reported. Therefore, the measurement of DNA adducts combined with fragmentation assays gives an insight to potential as well as actual DNA damage (88). The direct effects of oxidative sperm DNA adducts on pregnancy have been reported in one study where the likelihood of pregnancy occurring in a single menstrual cycle was inversely associated with 8-OHdG 10^{-5} dG (89), further emphasizing its prognostic value.

Can sperm DNA integrity predict success? Relationships with assisted conception outcomes

Aitken *et al*. (33) have reviewed the relationships between sperm DNA damage and fertilization. This group has shown that at low levels of oxidative stress, DNA damage is induced yet the fertilizing potential of the sperm is actually enhanced, reflecting the importance of cellular redox status in driving tyrosine phosphorylation events associated with functions such as sperm capacitation (33,90). These results are clinically significant since they support the studies of Ahmadi *et al*. (66,91) showing that sperm with damaged DNA can still achieve fertilization. Furthermore, the oocyte controls both fertilization and early cleavage stages of embryonic development (92) suggesting that sperm with damaged DNA can retain their fertilizing potential resulting in damaged DNA becoming part of the next generation's genome.

In clinical studies, although fertilization *in vitro* (by IVF) has been shown to be negatively correlated with DNA damage (93), this is not the case with the recent modification of IVF; namely, ICSI which now accounts for ∼50% of ART cycles. ICSI is an invaluable innovation in the treatment of infertile males with poor quality sperm (94). However, ICSI has removed many of the cellular checkpoints that prevent poor quality or immature sperm from successfully fertilizing oocytes. In addition, it has de-emphasized the importance of sperm selection and allowed the arbitrary choice of sperm for injection. For the first time in history, we have perfected a technique that circumvents all natural barriers to fertilization thus facilitating the union of potentially defective gametes.

While fertilization may be independent of sperm DNA integrity, the post-fertilization development of the embryo can be seriously disrupted by such damage. After the third stage of cleavage, the paternal genome exerts a major influence (92,95) and evidence of DNA damage is reflected in impaired embryonic development. Thus, in assisted conception cycles, preimplantation development is negatively correlated with DNA damage assessed by a variety of methods including NT (96), the alkaline comet assay (71) TUNEL (91,97) and SCSA (98,99).

Pregnancy rates have a negative correlation with high levels of sperm DNA fragmentation in artificial insemination cycles (77). An inverse relationship has also been reported between pregnancy rates with ICSI and the level of DNA fragmentation in immature (60) and mature sperm (60,75,98,100,101). In addition, even fertile couples took longer to conceive naturally, with the time to pregnancy increasing as a function of the proportion of sperm with abnormal chromatin (102).

Evenson (8) also demonstrated that the miscarriage rate was higher in couples who conceived naturally but where the partner's sperm had poor chromatin. Higher rates of pregnancy loss have also been documented in ICSI than in IVF cycles (103). As these pregnancies were almost inevitably achieved with sperm of poor quality that would have been incapable of

fertilizing an oocyte naturally, the link may well be sperm DNA damage. Poor subpopulations of sperm (discarded after density centrifugation but equivalent to those used in ISCI) (70) also have markedly higher levels of DNA fragmentation. However, because of the success of ICSI in bypassing rather than addressing the problem of poor sperm quality, these sperm with potentially damaged DNA continue to be used indiscriminately in ART.

While sperm with damaged DNA may show a reduced capacity, fertilization and implantation do occur with unknown consequences on the health of the next generation. There are a growing number of studies associating high mutational risk paternal occupations (such as exposure to metals, solvents and pesticides) and an increase in birth defects and childhood diseases (104). Animal studies have also demonstrated very clearly that sperm damaged by paternal exposure to cancer therapeutic agents can have adverse effects on the offspring (105–107). Tobacco, another source of mutagenic xenobiotics (108) that can induce sperm DNA damage has been associated with a higher incidence of childhood cancer in the next generation (44,109). Indeed, up to 14% of all such cancers have been linked to paternal smoking (109). New reports also show increases in schizophrenia, achondroplasia and Apert's syndrome in children of older men with high levels of sperm DNA damage (67). This further suggests that sperm DNA damage can impact negatively upon the health of offspring.

Clinically induced DNA damage

The advent of ISCI in 1995 has facilitated the use of immature testicular sperm that would be incapable of fertilizing an oocyte *in vivo*. The DNA of these sperm is even more vulnerable to damage than that of ejaculated sperm (29,60,86) perhaps because they have not completed the process of disulphide cross-linking. The selection of sperm for ICSI usually involves an evaluation of motility, as this gives an indication of the viability of the sperm (110). This is problematic in testicular sperm due to lack of inherent motility. The use of a non-viable testicular sperm may lead to lower fertilization rates than obtained with an ejaculated sperm (111). The technique of culturing testicular sperm *in vitro* prior to ICSI has been recommended by a number of groups (112–114) in order to promote an increase in sperm motility. A period of 24 h has been suggested as optimal for the development of motility in a sufficient number of testicular sperm to give choice in the treatment cycle (114). However, incubation of fresh testicular sperm for ICSI appears to be beneficial only in terms of the development of motility and morphology. Worryingly, this procedure also damages sperm DNA (115). In this study, from our laboratory we provided evidence that both fresh and frozen-thawed testicular sperm from men with obstructive azoospermia display substantial DNA fragmentation following routine incubation *in vitro* prior to ICSI injection (115). To avoid this, we recommended that all testicular sperm should be injected without delay in order to protect the genetic health of the resulting child.

Cryopreservation

Cryopreservation is a core technique in the preservation of male fertility before cytotoxic chemotherapy or radiotherapy and during ART. The use of frozen semen is also mandatory in donor insemination programmes where samples are stored until

donors are screened for infections such as HIV and hepatitis B. However, despite many refinements in methodology, the quality of post-thaw samples remains suboptimal and ART success rates with frozen sperm are lower than with fresh samples (116). Sperm lose most of their cytoplasm during maturation and therefore lose the enzymatic defences present in somatic cells, including chain-breaking antioxidants. This leaves them at a considerable disadvantage. However, sperm are protected during ejaculation, by the high levels of antioxidants in seminal plasma. For example, seminal ascorbate is present at 10 times the concentration of that in blood plasma (36). By returning prepared sperm to seminal plasma to prepared sperm before freezing, the DNA of those subpopulations of sperm with greatest fertility potential can be protected from cryoinjury (11). This is supported by a previous study in which similar protection of sperm DNA was observed if sperm were prepared for ART in the presence of antioxidants (47).

Cryopreservation of testicular sperm is also very important, ensuring the availability of sperm for subsequent treatment cycles without the need to perform additional invasive biopsies (117). However, cryoinjury to DNA is common in testicular sperm from fertile and infertile men. This may be due to the fact that all testicular sperm are more vulnerable to oxidative damage than ejaculated sperm since they have not undergone epididymal transit and maturation, where their DNA will be cross-linked conveying protection.

Vasectomy

Traditionally, vasectomy has been considered an irreversible form of contraception. Today, however, many post-vasectomized men wish to have a second family with a new partner. Many vasectomy reversals have been replaced by testicular biopsy performed at an outpatient clinic and subsequently used in ICSI (118). It has always been accepted that previously fertile men would suffer no impairment to sperm since the vasectomy was simply a forced blockage as opposed to defective spermatogenesis. However, it has recently been reported that the post-vasectomized men have markedly reduced sperm yields (119). In addition, significant increases in DNA damage have been observed in testicular sperm post-vasectomy compared to those from fertile men with a positive correlation between DNA fragmentation and time following vasectomy. The impairment of sperm quantity and quality is most significantly reflected in a reduction in pregnancy rates after ART (120).

A major barrier to progress

The advent of ICSI has provided a means of treatment for infertile couples with severe male factor infertility previously considered untreatable by conventional ART. However, its success has also impeded progress in the development of prognostic tests for sperm health because this therapeutic technique allows us to bypass the natural hurdles to fertilization so that even unfit sperm succeed. The consequence of this has been a further reduction in the diagnostic significance of parameters such as sperm concentration, motility and morphology but without the inclusion of more appropriate selection tests. Since short-term (i.e. pregnancy) success rates with ICSI are as good as those of IVF, where dysfunctional sperm are not the primary problem, there has been little incentive for the

development of sperm selection tests for ICSI. This approach has been described as a human experiment, since ICSI is in its 'infancy', with comparatively little long-term data on the health and well-being of subsequent generations. Given the plethora of studies associating damaged sperm DNA with impaired fertility at every milestone, a more cautious approach would be to select sperm with genomic integrity.

Opportunities and challenges—the establishment of clinical thresholds and the integration of DNA testing into clinical practice

Since ART, in particular ICSI, facilitates reproduction using sperm that would not achieve a pregnancy spontaneously, it is important to assess the quality of paternal genetic material and to establish criteria by which to choose appropriate cohorts of sperm. Assisted reproduction is expensive financially and emotionally, highly invasive and the long-term consequences remain unknown. Therefore, couples justifiably want to know the likelihood of success before embarking on a treatment cycle. These patients have a right to the fullest information that we can provide and scientists need to provide reliable tests for the clinician to give couples quantitative estimates of their chances of a pregnancy from their treatment.

Two challenges face the scientists working in the field of ART. The first is to establish robust DNA tests with high prognostic strength and the second is to persuade the clinicians and managers of fertility centres that the inclusion of such testing in male infertility will be beneficial to patient and centre alike. At present, there is no consensus as to the best test to use whether it is the alkaline comet, SCSA or TUNEL. This will require international collaboration to standardize protocols, patient groups, tests and scientific and clinical outcome parameters. Ideally this should be facilitated by the formation of a subcommittee within a recognized professional society such as European Society of Human Reproduction and Embryology (ESHRE) to oversee the validation studies. This precedence was set by the establishment of a Special Interest Group in 1996 (121) whose remit was to establish guidance on sperm function tests prior to the discovery of sperm DNA testing. The development of a similar group to assess sperm DNA tests was proposed by the working group at the Male Mediated Developmental Toxicity conference in 2003 (64); to our knowledge, it has not yet been implemented. The formation of such groups and collaborations should be considered a high priority.

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