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INVITED REVIEW

Sperm quality and its relationship to natural and assisted conception: British Fertility Society Guidelines for practice

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Abstract
Reports on the influence of semen parameters on natural or assisted pregnancy are contradictory, suggesting that the many confounding variables which contribute to outcome have not been taken into account. However, it is possible to derive some consensus for both natural and assisted conception by focussing on studies which use WHO-recommended semen analysis on relatively large populations, applying appropriate statistics and accounting for 'female factors'. The concentration of progressively motile sperm has consistently been shown to be the most predictive factor with regard to outcome. Around 64% of studies suggest that a reasonable chance of success with artificial insemination requires at least 5×10^6 motile sperm and this is supported by the WHO's revised reference range for natural conception. Sperm morphology remains controversial, with a lack of standardisation across centres, the adoption of ever-stricter scoring criteria and changing reference values. Antisperm antibodies do not appear to influence outcome independently of sperm motility and agglutination. Sperm DNA damage appears to be related to sperm quality, embryo development and pregnancy loss, yet there remains no consensus on the best testing procedures, clinical reference values and how patients with an adverse result should be managed. In conclusion, laboratories should continue to focus on reducing the uncertainty and improving the quality of their basic semen analysis.

Keywords: *Assisted conception, fertility, semen analysis*

Levels of evidence

Hierarchy of evidence
1a. Systematic review and meta-analysis of randomised controlled trials (RCTs).
1b. At least one randomised controlled trial.
2a. At least one well-designed controlled study without randomization.
2b. At least one other type of well-designed quasi-experimental study.
3. Well-designed non-experimental descriptive studies, such as comparative studies, correlation studies or case studies.
4. Expert committee reports or opinions and/or clinical experience of respected authorities

Grade Strength of evidence

A Requires at least one RCT as part of a body of literature of overall good quality and consistency addressing the specific recommendation. (Evidence levels 1a, 1b).

B Requires the availability of well-controlled clinical studies but no randomised clinical trials on the topics of recommendations. (Evidence levels 2a, 2b, 3)

C Requires evidence obtained from expert committee reports of opinions and/or clinical experiences of respected authorities, which indicates an absence of directly applicable clinical studies of good quality. (Evidence level 4)

GPP Good practice point

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These gradings are as used by the National Institute for Clinical Excellence (NICE, 2004).

Guidance

a. Studies examining the influence of semen quality on natural or assisted conception must consider the 'uncertainty' associated with their semen analysis methodology (from specimen collection to reporting) and ensure that confounding variables such as female factors and age are controlled for. **B2a**
b. The lower reference limits based on the 5th centile of a large group of patients cited in WHO (2010), of $>15\times10^6$/ml for concentration, $>32\%$ for progressive motility and $>4\%$ normal forms are based on a considerable evidence base and should be used in relation to the probability of natural conception. **B2a**
c. There is consensus on a threshold figure of 5 million motile sperm inseminated during IUI, below which significantly lower pregnancy rates are likely. **B2a**
d. The clinical value of reporting the per cent normal forms in the ejaculate is limited due in part to the limitations and subjectivity of the assay. Laboratories should focus on identifying specific morphological defects (e.g. globozoospermia, stump tail defect) associated with infertility. **B2a**
e. With different recipient populations and the use of cryopreserved sperm, the requirements for DI pregnancy cannot necessarily be extrapolated from IUI or natural pregnancy studies. **GPP**
f. There is insufficient evidence to justify the use of ICSI in cases of isolated teratozoospermia. **B2b**
g. A minimum threshold in terms of sperm quality for selecting patients for IVF over ICSI cannot be given. **B2b**
h. Hyaluronic Acid (HA) binding may be used to select sperm for injection for ICSI but is of no more value than conventional semen analysis in deciding on the mode of treatment. **B2b**
i. The use of Intracytoplasmic Morphologically Selected sperm Injection (IMSI) may improve implantation rates compared to conventional ICSI. **B2b**
j. Due to the uncertainty associated with test methods, there is no clear link between the outcome of assisted conception and the presence of antisperm antibodies. **B2b**
k. There is evidence for a relationship between sperm DNA damage and semen parameters and/or the outcome of assisted conception. However reports conflict and depend largely on the laboratory test utilised. Results are unlikely to alter patient management. **GPP**

Recommendations

The current WHO reference limits which were based on stronger evidence than previously should be adopted. A test wash (trial sperm preparation) may be used to determine whether 5 million motile sperm may be harvested.

World Health Organisation (WHO) reference limits are valid only for WHO-recommended methodology. Laboratories should focus on reducing the uncertainty associated with their semen analysis by using validated methods and reducing other sources of error; from sample collection to the reporting of test results.

IVF should be used in cases of the failure of IUI or where less than 5 million motile sperm are harvested from a 'test wash' procedure. It should not be used on the basis of isolated teratozoospermia.

ICSI should be used in cases where there is a significant risk of IVF failure: e.g., failure of surgical sperm retrieval (SSR); severe oligo/astheno/teratozoospermia (the threshold cannot be defined); specific morphological defects such as globozoospermia or stump tails.

HIC (Higher Insemination sperm Concentration) for moderate male factor infertility has been shown to be as effective as ICSI in a RCT.

Introduction

The relationship between measures of semen quality and the chance of conception has been a matter of debate for decades. For many clinics, unless the patient is diagnosed with azoospermia, the semen analysis remains no more than a rough guide for differentiating 'probably fertile' from 'probably sub fertile' patients. Prediction of natural conception over time remains difficult and selection policy of appropriate Assisted Reproductive Technology (ART) based on semen quality is, in most cases decided at a local level. Unfortunately, all studies which profess to demonstrate powerful relationships between the results of semen analysis and sperm function and natural conception are inevitably weakened by the fact that the end point in question (biochemical or confirmed pregnancy) has both a male and a female component. Many studies have examined aspects of semen composition, sperm number and function, relating them to natural and assisted conception, but all have made the significant assumption that the female recipient, the provider of the oocyte has no fertility-related pathology. This is of course a considerable assumption. Although data can be strengthened by eliminating clear female factors such as tubal abnormality, anovulation and age, the quality of the oocyte or the internal environment provided for fertilisation and implantation cannot be controlled completely.

This review aims to identify well-designed trials/studies and put forward a 'consensus view' of the relationship between both traditional semen parameters and more advanced sperm function tests and

outcome. It will attempt to avoid focussing too much on individual studies but on the overall 'body of evidence' and the consensus derived from studies demonstrating accepted good practice. Outcome measurements include: fertilisation rate, biochemical and clinical Pregnancy Rate (PR), ongoing PR and live birth rate (LBR). Pregnancy loss will be examined specifically in relation to studies involving sperm DNA damage.

Quality assurance in laboratories and clinics

Perhaps the most challenging aspect of studies examining the clinical value of semen analysis is the level of 'uncertainty' associated with variation in laboratory and clinical practice. Apart from the more obvious factors such as adequate resourcing, facilities and staff education, quality assurance in semen analysis requires the use of robust methods for the collection and analysis of specimens, reporting of results, and the application of rigorous quality control procedures (Keel, 2004; Riddell et al., 2005; Pacey, 2006, 2010; Tomlinson, 2010). This is well illustrated by quality assessment data provided by the UK National External Quality Assurance Scheme (NEQAS), showing that semen assessment, in particular the sperm count, performed in one location does not necessarily equate to that in another (Pacey, 2006; Tomlinson, 2010). Laboratory methods recommended by the WHO tend to offer reliability, reproducibility and a superior level of clinical validation over alternatives (WHO, 1987, 1992, 1999, 2010). In light of this, and where possible, only studies describing the adoption of WHO methodologies are included for review, unless the authors provide a compelling argument, with evidence, for the particular methods used.

Variation in clinical practice is also a clear obstacle to isolating the contribution of male factors to the outcome of assisted reproduction. Using artificial insemination (AI) as one example, the majority of randomised controlled trials (RCTs) describe a number of factors which may influence outcome including: stimulated versus natural cycles, clomid versus superovulation, single insemination versus double insemination, type of insemination (intra-uterine, intra-cervical or Fallopian tube perfusion) and method for timing of insemination (blood and urinary LH). To isolate the contribution of male factors would first require knowledge of which of these additional factors are important. Yet almost without exception, recent meta-analyses (Goldberg et al., 1999; Van Waart et al., 2001; Van Weert et al., 2004; Pandian et al., 2005; Verhulst et al., 2006; Bensdorp et al., 2007; Boomsma et al., 2007) have been largely inconclusive. Clearly, the determination of the male contribution to outcome is far from easy when taking into consideration female factors, variations in laboratory method, and differences in the approach to treating the patient.

Semen quality and natural conception

The relationship between natural conception and male fertility is usually examined by follow up of couples to discover whether pregnancy occurs in a given time period and assessing semen quality (Bostofte et al., 1990; Barratt et al., 1992; Bonde et al., 1998, Zinaman et al., 2000; Larsen et al., 2000; Guzick et al., 2001; Cooper et al., 2009) or the recruitment of 'fertile men' who have achieved a recent pregnancy (Cooper et al., 2009). While these approaches appear sensible, both have similar weaknesses. First, they assume that the sample provided for analysis is of similar quality to that which gave rise to a pregnancy. They also make the assumption that all couples have sufficient knowledge of the appropriate timing of intercourse in order to maximise their chance of conceiving. Despite these shortcomings, a number of studies meeting our inclusion criteria have provided meaningful data which show a direct association between a number of semen parameters and conception (Table 1). These studies have examined sufficiently large patient populations, used WHO-recommended methods for semen analysis, attempted to control for female factors and used statistical tests such as proportional hazards Cox's regression (Cox, 1958) which take into account time to pregnancy (TTP). The emerging pattern appears to show that the chance of pregnancy increases with increasing numbers

Table 1. Relationship between semen parameters and natural conception in cases where no female pathology is diagnosed – summary of appropriate studies.

Study	Couples	Statistics	Significant findings
Jouannet et al. (1988)	394	Cox's regression	Sperm concentration, % motility, % normal forms
Barratt et al. (1992)	325	Cox's regression	% motility, mean velocity, duration of infertility
Wichmann et al. (1994)	907	Cox's regression	% motility, morphology, duration of infertility
Bonde et al. (1998)	430	Logistic regression	Concentration of motile spermatozoa
Larsen et al. (2000)	430	Cox's regression	Concentration, motility, velocity
Zinaman et al. (2000)	210	Cox's regression	% motile sperm, motile sperm number, total sperm number, sperm morphology, concentration of normal sperm
Guzick et al. (2001)	1461	CART analysis, ROC curves	Concentration ($>48\times10^6$/ml), motility ($>63\%$), morphology ($>12\%$)
Garrett et al. (2003)	1191	Cox's regression	Total sperm number, % progressive motility, zona preferred morphometry, sperm velocity
Cooper et al. (2009) WHO (2010)	4500 men	Quantile regression	Concentration ($>15\times10^6$/ml), motility ($>33\%$), morphology ($>4\%$)

of motile sperm in the ejaculate and, in some studies, those with a higher proportion of morphologically normal forms.

The earliest study included is that of Jouannet et al. (1988) which followed 394 couples for up to 3 years and showed clear relationships between sperm concentration, motility, morphology and pregnancy. In this cohort, the most predictive variables were the percentage of motile sperm and the Multiple Anomalies Index (MAI), which provided a compound value for the mean number of abnormalities observed per sperm (Jouannet et al., 1988; Ducot et al., 1988). In a similar study, this time using Computer Assisted Sperm Analysis (CASA), Barratt et al. (1992) showed that progressive motility and mean progressive velocity were the most significant positive predictors, suggesting that the provision of quantitative motility measurements as opposed to the rather subjective manual assessment strengthened motility as a predictive variable. This was confirmed by Larsen et al. (2000) who also used automated semen analysis and showed that not only does cumulative conception rate (CCR) correlate with increased per cent motility but perhaps more importantly the significance is greater when high velocity (Grade a) motile sperm (velocity >25 μm/sec, WHO, 1999) are used. They examined 430 couples followed up over six menstrual cycles. Cox's regression was used to model semen parameters in relation to time to pregnancy and demonstrated a clear relationship between increasing concentration of motile sperm, sperm velocity and pregnancy. This was re-enforced in a later study by Garrett et al. (2003) who demonstrated the improved predictive value of automated measures including progressive velocity and zona-preferred morphometry. An earlier study from the same Danish group using conventional semen analysis had demonstrated a similar relationship with sperm concentration and morphology but not motility. It is interesting that without a reliable automated measurement, the clinical significance of motility appeared to be diminished (Bonde et al., 1998).

Wichmann et al. (1994) used life table analysis and Cox's regression to examine 907 couples over a three-year period. The study was weakened slightly by its approach to the semen analysis, with a delay of 2 hours in the measurement of sperm motility. However, it was demonstrated that motile sperm number, percent motility and sperm morphology were all independently predictive of pregnancy. One of the most comprehensive studies was performed by Guzick et al. (2001) involving 765 infertile (no pregnancy after 12 months) and 696 fertile (conceived within the previous 2 years) couples. This was one of few such studies to have documented the consideration of issues such as specimen variability, laboratory methods, female factors, staff competence and statistics. Multiple specimens from both groups of men and mean results were entered into a Classification And Regression Tree (CART) analysis which produced estimates for clinical thresholds to discriminate between normal and abnormal sperm. Definitely fertile men were shown to have a sperm concentration of $>48\times10^6$/ml, a motility of $>63\%$ and normal forms of $>12\%$, whereas the infertile group had a sperm concentration of $<13\times10^6$/ml, motility of $<32\%$ and normal forms of $<9\%$. Despite the power of the study, it was concluded that no individual parameter was a particularly powerful predictor of pregnancy and a huge indeterminate range of patients was to lie between the fertile and infertile groups.

It would be impossible to consider the relevance of semen parameters to male fertility without mentioning the WHO manual for the examination and processing of human semen (and previously, cervical mucus and sperm/cervical mucus interactions). The guidance provided has undoubtedly led to more aligned practice and increased standardisation across laboratories but whether practice has improved as a result is more difficult to substantiate. The reference ranges reported in the previous three versions of the manual (WHO, 1987, 1992, 1999) have been the subject of debate for many years, with many authors criticising their usefulness and the evidence base from which they were derived (Bartoov et al., 1993; Davis & Gravance, 1994; Barratt et al., 1995; Ombelet et al., 1997; McDonough, 1997; Menkveld et al., 2001; Van der Steeg et al., 2010). In response, the WHO commissioned extensive trials to provide clinical data based on semen variables obtained using their own recommended methodology (WHO, 2010). As a result, lower reference limits based on the 5th centile are greater than 15×10^6/ml for concentration, $>32\%$ for progressive motility and $>4\%$ normal forms have been produced (highlighted in Table 1).

Sperm quality and assisted reproduction

There are clear advantages in assessing the impact of sperm quality on assisted conception since: (i) there is considerably less doubt that the sample assessed is the one giving rise to the pregnancy; (ii) the timing of insemination is assured; (iii) the follow-up period is relatively short; and (iv) basic female factors should have been assessed and controlled for. However, selection bias and variation in laboratory and clinical practices remain confounding factors. Perhaps more as a precaution, many ART clinics have a tendency to 'over treat' patients rather than run the risk of treatment failure. For example, patients who might become pregnant through relatively low-tech treatments such as intrauterine insemination (IUI) may be directed towards *in vitro* fertilisation (IVF); similarly, patients may have intracytoplasmic sperm injection (ICSI) when IVF may well have been successful. Conversely, some patients maybe given, or opt for, low complexity treatment (such as IUI) on the grounds of cost, risk or a variety of other reasons. There are also those who are inappropriately given a treatment which is unlikely to succeed due to misjudgement or lack of a well-defined patient pathway.

It is impossible to have completely standardised clinical and laboratory practices, so this review has chosen to focus on studies describing a clinical service with success rates which are no lower than expected. With natural cycle AI treatment, the number of studies which have examined timing protocol, intra-cervical versus intrauterine and one versus two inseminations also complicate any review (Hurd et al., 1993; Brook et al., 1994; Goldberg et al., 1999; Carroll & Palmer, 2001). To simplify this, the studies described below (perhaps with one exception) employ single inseminations, and treatment is timed using urinary or blood LH monitoring prior to HCG administration. Intra-cervical insemination (ICI) has been included in the review of DI only because of the relative influence and power of several large studies conducted in the 1980s and 1990s.

IUI and sperm quality

Of the few IUI meta-analyses that focus on the male contribution to outcome, conclusions are made, in almost every case, with the caveat that the lack of well-controlled RCTs limits the quality of the evidence. Bensdorp et al. (2007) examined only those RCTs which examined IUI as a treatment for male sub-fertility and could not conclude whether IUI was more effective than timed intercourse (TI) with or without stimulation nor, indeed, whether IUI was an effective treatment for this group. Van Weert et al. (2004) examined 16 IUI studies and concluded that differences in practices between centres made comparison extremely difficult. They suggested that the optimal total motile sperm (TMS) count required for success could lie anywhere between 0.8 and 5 million. Cohlen et al. (2000) expressed slightly more confidence, arguing that IUI was effective if greater than 1×10^6 sperm were inseminated and no other sperm defect was present. Van Waart et al. (2001) performed a meta-analysis of eight studies examining the influence of sperm morphology on IUI outcome; however doubts remain over differences in clinical practice between the studies cited and in the elimination of female factors. With a general lack of agreement between the few meta-analyses performed and the consensus view that IUI studies lack commonality, the majority of this review focuses on some of the larger prospective and retrospective studies available, which are summarised in Table 2.

One of the largest and most notable of these was by Wainer et al. (2004), who performed a retrospective analysis on 2564 cycles from 889 couples. A total of 331 pregnancies provided an overall PR of 12.9%. The data showed improved PRs with increasing numbers of motile sperm inseminated (NMSI). Those having a NMSI of greater than 5million and normal morphology had an overall PR per cycle of 18.4%, which dropped to 13.9% if normal morphology was less than 30%. Patients with an NMSI of greater than 5million had a PR as low as 5.4% per cycle if normal forms were also less than 30%. More recent studies (Badawy et al., 2009; Merviel et al., 2010) also found higher success rates for patients with a TMS concentration of 5 million per ml in clomiphene- and gonadotropin-stimulated cycles. Spiessens et al. (2003) examined 872 IUI cycles in 440 couples and showed the PR was double for normozoospermic men compared to male factor patients (21.3% vs 9.6%) and that isolated teratozoospermia was negatively predictive of outcome. Cohlen et al. (1998) suggested that a minimum of 10 million motile sperm were required, providing that stimulation with gonadotrophins was used, though numbers of pregnancies were low. A later study by Ombelet et al. (1997) examined predictive factors in 792 clomiphene citrate (cc) stimulated IUI cycles in couples with a high rate of male factor infertility and concluded that an inseminated motile sperm concentration of less than 1×10^6 coupled to a morphology of less than 4% normal forms was predictive of failure.

The central theme running through these studies is that male factors have a significant influence on IUI

Table 2. Semen parameters of sub-fertile couples treated using IUI with superovulation.

Study	Couples (cycles)	Statistics	Findings
Tomlinson et al. (1996)	134 (260)	Logistic Regression	Follicle number Progressive motility
Ombelet et al. (1997)	373 (792)	ROC curves Chi square	NMSI $> 1 \times 10^6$ $>4\%$ normal forms
Cohlen et al. (1998)	74 (308)	Chi square Cox's regression	TMS >10 million (stimulated only)
Branigan et al. (1999)	414 (757)	Chi square Cox's regression	NMSI >10 million 24 h sperm survival
Spiessens et al. (2003)	440 (872)	Life table analysis Chi square	Normal forms $>4\%$ Male factor
Wainer et al. (2004)	889 (2564)	Chi square Cox's regression	NMSI >5 million
Van Weert et al. (2005)	290 (722)	Logistic regression ROC curves	Female factors <6 million sperm/ml in test wash
Badawy et al. (2009)	393 (714)	Chi squared	TMS >5 million teratozoospermia
Merviel et al. (2010)	353 (1038)	Chi square Logistic regression	TMS >5 million teratozoospermia

NMSI, Number of motile sperm inseminated; TMS, Total number of motile sperm.

outcome. PR increases when male factor sub-fertility is absent but clearly the interplay between female factors (age and stimulation) and male factors is complex. There is no consensus on a threshold at which one might offer the treatment, although five out of the nine studies and a total of 5,038 IUI cycles appeared to suggest that a minimum of 5×10^6 motile sperm should be inseminated in order to achieve satisfactory results.

Donor insemination and semen parameters

The results from donor insemination (DI) provide a unique opportunity to study ejaculate quality and outcome in a group of subjects whose fertility can be proven. Not only is it possible to relate semen parameters to outcome of individual treatments but the relative fecundity of individuals can be monitored over time as their samples achieve a number of pregnancies. Unfortunately, DI has the disadvantage that the sperm used in treatment have been cryopreserved. Outcome can be related directly to post-thaw sperm concentration, motility and morphology but what cannot be detected are the subtle, sub-lethal effects that cryopreservation has on the sperm membranes and ultrastructure (Mahadevan & Trounson, 1984; Henry et al., 1993; Alvarez & Storey, 1992; James et al., 1999). Extrapolation of data derived using frozen sperm to the fresh IUI situation may not therefore be entirely valid.

Another major difference between the DI and IUI models is the recipient population. As a group, DI recipients have changed dramatically over the past 20 years (Barratt & Cooke, 1993) in the sense that before the advent of ICSI, DI treatments were performed predominantly for cases of relatively moderate male factor infertility. Such treatments were more successful in couples where the male partner had azoospermia than with oligozoospermic men (Le Lannou & Lansac, 1989). Currently, DI is unlikely to be the treatment of choice unless the man is sterile, at risk of transmitting genetic disease to the offspring or cannot afford to pay for ICSI. The DI recipient is just as likely to be a fertile single woman or lesbian female and so the same relationships between frozen-thawed sperm quality and pregnancy may not hold true. Historic data from the UK show that in 1992, over 26,000 DI cycles were performed resulting a 5% LBR. In 2005, the DI cycle number was less than 6,000 with an LBR of 10.3% (HFEA, 2008).

As with IUI, many of the larger studies of DI outcome appear to be weakened by a number of confounding variables. Meta-analyses or RCTs focus largely on the route of insemination (IUI vs ICI, stimulation vs natural cycles, or the choice of stimulation (cc vs FSH)) (Besselink et al., 2008; Ferrara et al., 2002; O'Brien & Vandekerckhove, 2000; Goldberg et al., 1999; Wainer et al., 1995). All suggest an overwhelming influence of clinical practice and female factors and, as a result, the influence of the inseminated sperm tends to become lost. Not surprisingly, the route of insemination (IUI vs ICI) influences any relationship that sperm quality may exert on outcome if the technique does indeed concentrate sperm number at the site of fertilisation as has been suggested (Goldberg et al., 1999; Wainer et al., 1995). For this reason, this review considers only a small selection of prospective and retrospective studies which examined sperm quality while controlling for stimulation and insemination route.

Some of the most powerful data were generated from laboratories within the French CECOS (Centre d'Etude et de Conservation des Oeufs et du Sperme) Federation, benefitting from the member laboratories operating to the same principles for processing specimens, patient management and treatment. Le Lannou et al. (1995) showed that PR was highly dependant on sperm quality when using ICI. PR increased from 9% when the number of motile sperm inseminated was below 4×10^6, almost doubling to 17.2% when motile sperm concentration was greater than 8×10^6/ml (Table 3).

In contrast, the relationship between sperm quality and the success of donor IUI cycles appears to be less clear. Apart from using a 16 mm follicle as the threshold size for hCG, Khalil et al. (2001) showed the same relationship in 1131 donor IUI as shown by the CECOS data, provided the inseminated dose was above 2 million. Marshburn et al. (1992) analysed over 1000 IUI cycles from 191 patients and demonstrated the influence of CASA-generated sperm data independent of stimulation and female factors. In washed sperm, curvilinear velocity (VCL) and straight line velocity (VSL) had a bearing on outcome whereas the number of motile sperm inseminated did not, suggesting that conventional semen analysis was of little use in predicting the outcome of donor IUI treatment.

Diagnostic sperm preparation

Extrapolation from studies describing a clinically relevant threshold for post-preparation motile sperm number (Tables 2 and 3), naturally gives rise to the idea that this might be used diagnostically. When most therapeutic sperm preparation used the swim-up or sperm migration methods, the diagnostic version became known as the Sperm Migration Test (SMT: Makler et al., 1984; Arny & Quagliarello, 1987). A number of groups later suggested that the ability of sperm to swim against gravity could be used not only as a method for washing and preparing sperm but as a test of sperm function. Buckett et al. (1998) examined the SMT in 261 couples prior to IUI and showed a significantly higher PR in couples where greater than 5million progressively motile sperm per ml were harvested. Rather than a sperm

Table 3. Increasing PR with increasing sperm dose per straw (Le Lannou et al., 1995).

	Motile sperm number per straw (10^6) per ml		
Cycles	<5	5–10	>10
1480	513	635	341
PR	7%	13%	15%

function test per se, it may be that the ability to yield at least 5 million progressively motile sperm per ml (by whatever method available) significantly improves the chance of IUI success. The migration of sperm from seminal fluid appears to comprise a chance encounter between the sperm and semen/media interface that can be enhanced simply by increasing the surface area between the two layers, usually by tilting the tube to an angle of 45° (WHO, 1992). This method works just as readily in a horizontal plane and leads to harvesting of a motile-enriched fraction (Hossain et al., 1999). Similarly, swim-down methods have also been used and appear to provide higher sperm yields and improved morphology (Makler et al., 1993; Almagor et al., 1993). More recently, Density Gradient Centrifugation (DGC) has been shown to reduce numbers of abnormal sperm and improve DNA integrity, when compared directly to swim-up (Sánchez et al., 1994; Sakkas et al., 2000; Hammadeh et al., 2001). With the advantage that DGC also takes less time, it is now adopted in many centres as the sperm preparation and test wash method of choice.

IVF and ICSI

When considering IVF methods, the complexities apparent in separating the influence of sperm quality from confounding factors are even more pronounced. Table 4 details some of the confounding variables likely to weaken the predictive value of studies relating sperm quality to IVF/ICSI outcome. It is evident that sperm factors are just a few of many possible influences on measures such as fertilisation, embryo quality, implantation and PRs. Bearing this in mind, the importance of well-constructed RCTs to provide robust data and guide clinical decision-making cannot be overstated. It is therefore all the more surprising to discover only a few examples of such RCTs (Gerris et al., 1999; Meintjes et al., 2009) other than those dealing with clinical issues, such as drug choice and dosage, embryo transfer method, day of transfer and number of embryos transferred (Fiddelers et al., 2006; Drakeley et al., 2008; Devroey et al., 2009; Jayaprakasan et al., 2010). Hence, when seeking correlations between sperm parameters and outcomes, it is difficult to find evidence-based consensus derived from large patient populations, and weakened by variation in laboratory practices or the lack of sufficient information about techniques used for sperm assessment. It is also not surprising that with increased regulation and governance, particularly with the time burden in preparing research ethics applications that many laboratories have neither the time nor resources to conduct RCTs when changes in practice are proposed.

Standardisation of semen analysis in the IVF setting is no better than in the andrology or general pathology laboratory. Furthermore, there appears to be less support from published evidence that current clinical tests and associated thresholds relate strongly to IVF outcome (Kini et al., 2010). Bearing in mind what has been stated previously and the understandable requirement to manage risk of treatment failure and so potentially 'over-treat' patients, this should not be a surprise. Perhaps rather than predicting success, the important question with regard to IVF in particular should be: how does the laboratory avoid failure? There are patients for whom IVF is clearly the appropriate treatment of choice and would include those: (i) with tubal infertility; (ii) who fail to become pregnant through IUI; (iii) fail to meet the defined sperm quality requirements post preparation. Likewise where ICSI is concerned, clear cases would be patients with: (i) surgical sperm retrieval (SSR); (ii) severe asthenozoospermia (threshold unknown); (iii) severe oligozoospermia (threshold unknown); (iv) sterilising morphological conditions (e.g. globozoospermia, stump tail defect); and (v) a failed attempt at IVF.

Patients with cryopreserved sperm often fall between both groups, where the treatment option has to balance the chance of success with post-thaw sperm survival and the availability of what might be a scarce and sometimes irreplaceable resource. Outside these patient groups, there is significant uncertainty when the overriding treatment decision becomes based on the need to avoid failure of fertilisation. Once again, rigorous examination of this issue is rendered impossible by the tendency, in clinical practice, to take a precautionary attitude and divert patients to ICSI where there is doubt about sperm quality. A recent meta-analysis (Andersen et al., 2008) showed huge variation between centres in terms of ICSI policy and selection criteria.

Table 4. Clinical and laboratory factors which are likely to have influence on the outcome of IVF and ICSI.

Clinical	Embryology	Andrology
Patient age	Media	Sperm concentration
Body mass index	Culture conditions	Motility
Ovarian reserve	Culture consumables	Morphology
Polycystic ovarian syndrome	Equipment	Ejaculate volume
Endometriosis	Number of observations	DNA fragmentation
Number of embryos transferred	Embryo grading/selection	Surface proteins
Drugs/stimulation	Embryo stage	Sperm preparation
Luteal phase support	Assisted hatching	Capacitation/Acrosome
Oocyte quality	PGS/PGD	Aneuploidy
Operator competence	Operator competence	Operator competence

PGS, Pre-implantation Genetic Screening; PGD, Pre-implantation Genetic Diagnosis.

Nordic countries, the Netherlands and the UK adopted a conservative approach to ICSI using it in 40%–45% of IVF cycles, whereas in Mediterranean countries such as Greece, Italy and Spain, ICSI was used in 66%–81% of treatments. In the USA, only 50% of ICSI cycles were performed because of clear male factor infertility. It was also found in the USA that the predominant indications for use of ICSI are not severe impairment of sperm quality but a number of reasons such as: (i) patient age; (ii) age-related oocyte quality and (iii) levels of public funding and insurance cover. It was concluded that 'the available evidence does not support the liberal use of ICSI in couples without a clear male factor'. In these clinical settings, an attempt to define the lowest thresholds for sperm quality to attain fertilisation is probably impossible. Moreover, to attempt to do so in a prospective trial setting may be considered unethical. So the question remains: how useful is semen analysis in the planning of ART other than artificial insemination? The fundamental problem is when semen analysis is used as a guide to treatment programming, the sperm population processed for ART is often not representative of the heterogeneous population in the original specimen. Several authors have shown that in addition to sperm concentration and motility, other parameters such as morphology and DNA integrity are improved after sperm preparation, particularly using density gradients (Yao et al., 1996; Sakkas et al., 2000; Tomlinson et al., 2001; O'Connell et al., 2003). When assessing semen quality in patients receiving ART, measurements may be more relevant after this selection process (i.e. sperm preparation has taken place). Considering this, the lack of standardisation between laboratories and the confounding factors highlighted in Table 4, it is not surprising to discover that the previous WHO references ranges (WHO, 1999) are not particularly helpful for semen diagnosis in relation to fertilisation and pregnancy outcome (Chen et al., 2009; Kini et al., 2010).

Perhaps the most controversial area remains sperm morphology. It makes sense that sperm should possess the appropriate motion characteristics for fertilisation to take place, and that the significance of total motile count (observed in natural conception and artificial insemination) becomes diminished in relation to IVF as there is no requirement for the sperm to make the journey from the cervix to the site of fertilisation. It is therefore reasonable to suggest that at this point, other sperm quality measures such as morphology, DNA integrity and acrosomal status would come to the fore (Liu et al., 2007; Barratt et al., 2009). Yet sperm morphology analysis continues to be shrouded in controversy due in no small part to the technical challenges of providing a reliable, reproducible, test result (Keel et al., 2000; Keel et al., 2002; Riddell et al., 2005). Since IVF became routine clinical practice, the threshold for normal forms in a routine diagnostic test has changed from 50% (WHO, 1987) to 30% (WHO, 1992) to no agreement on a threshold in WHO (1999) and now 4% (WHO, 2010). Indeed, this latter threshold is probably a reflection of the number of centres now performing morphology testing to even stricter criteria, according to the methods first published by Kruger (Kruger et al., 1986, 1988; Menkveld et al., 1990, 1996; Coetzee et al., 1998). Although ground-breaking in that these early studies established a relationship between IVF outcome and morphology, they were carried out on relatively low numbers of IVF cycles and as a result low numbers of pregnancies in each patient group according to morphology ($<4\%$, 5–14% and $>14\%$ normal forms). In a more recent, more powerful study, Keegan et al. (2007) examined sperm morphology according to Tygerberg criteria in 495 consecutive couples and showed that isolated teratozoospermia had no influence on fertilisation, pregnancy or live births; indeed the use of ICSI did not improve the outcome. A recent meta-analysis (Hotaling et al., 2011) agreed with this conclusion, showing that isolated teratozoospermia was not associated with reduced chance of pregnancy in IVF with or without ICSI. The study covered the period between 1986 and 2009 and was severely hampered by a lack of standardisation with only four studies out of a possible 31 meeting the inclusion criteria. However, the conclusions were ultimately based upon almost 3,000 IVF/ICSI cycles and 673 men classified as having severe teratozoospermia. As the study was not an examination of upstream events such as fertilisation and embryo quality, the authors were justified in concluding that certain morphological abnormalities may yet be of clinical relevance. However, it would seem that the per cent normal forms in the ejaculate, particularly considering the small sample size ($n = 200$) routinely used in most laboratories, does not impact on the chance of pregnancy and should not therefore be used in isolation in the management of patients. Assessment of the impact of an individual morphological abnormality on such upstream events is unfortunately lacking. With the tendency to report per cent normal forms in recent years, few laboratories are able to correlate specific abnormalities with failed fertilisation or conception. This was recently highlighted by Chen et al. (2009) who conceded that the percentage of normal forms did not differ between pregnant and non-pregnant groups but showed a relationship between outcome and both the Teratozoospermia Index and the Sperm Deformity Index which suggests that laboratories ought to re-consider how sperm morphology analysis is performed. Clearly the most important use of morphology assessment is to mitigate the risks of fertilisation failure but it appears, from current data, that this not possible. Hershlag et al. (2002) divided oocytes collected and performed IVF on one half and ICSI on the other, demonstrating that ICSI rescued the cycle in 10.9% of cases while IVF resulted in fertilisation failure. An alternative strategy was proposed by Tournaye et al. (2002) who used a Higher Insemination sperm Concentration (HIC) for moderate male factor infertility and showed it to be as effective as ICSI in a RCT.

Sperm selection for ICSI

As previously mentioned, the primary selection point for ICSI is insufficient sperm numbers or quality for use in conventional IVF. Once ICSI is decided upon, the challenge becomes one of selecting sperm for injection that will optimise the chance of fertilisation. A significant amount of data suggest that sperm possessing the HA (Hyaluronic acid) receptor are more mature, have improved morphology, reduced DNA fragmentation and fewer DNA aneuploidies (Cayli et al., 2003; Jakab et al., 2005; Huszar et al., 2006) and this has led to the launch of a commercial hyaluronan binding assay (HBA). Others have since demonstrated that HA sperm selection improves embryo quality and implantation (Parmegiani et al., 2010) and is now being proposed as a test for poor prognosis with IVF (Tarozzi et al., 2009). However, the few clinical studies to date are relatively small scale and slightly contradictory. In the largest study to date of 175 patients, Ye et al. (2006) showed significant association between HA binding and conventional semen parameters and some correlation with fertilisation. However they concluded that HA gave no additional predictive information than was provided by sperm morphology. In a group of 68 patients undergoing IVF/ICSI after IUI failure, Nijs et al. (2009) showed that HBA correlated with conventional semen parameters (with the exception of morphology), embryo quality and miscarriage but did not predict fertilisation. Similarly Tarozzi et al. (2009) studied another relatively small group of 60 patients showing a relationship between HA, DNA damage and sperm morphology but no correlation to fertilisation, pregnancy or embryo quality. In the latest clinical study, Kovacs et al. (2011) conducted a prospective, controlled trial in patients undergoing split IVF/ICSI for unexplained infertility. Fertilisation with ICSI was higher than conventional IVF but HA binding was unable to predict failed fertilisation, suggesting that its use as a laboratory tool for deciding between the two treatments was limited. Clearly more work, with larger, well designed studies, is required in this area. Based on the current level of evidence it would seem that HA binding may be of use in selecting sperm for injection at ICSI but, for deciding on the mode of treatment, is of no more value than conventional semen analysis.

Although there is little evidence to suggest that traditional sperm morphology analysis is useful in predicting ICSI success (French et al., 2010), the discrimination offered by the more recent IMSI (intracytoplasmic morphologically selected sperm injection) techniques show promise. High-powered morphological examination and sperm selection was first proposed by Bartoov et al. (2001, 2003) who demonstrated that by combining ICSI with motile sperm organellar morphology examination (MSOME), PRs were significantly improved. Although these early studies included only low numbers of patients, they provided a platform for further study, and the use of IMSI has rapidly increased among IVF clinics. Berkovitz et al. (2005) compared outcome from two groups of patients: the first comprised those who satisfied all the MSOME criteria ($n = 38$), the second a matched group of patients undergoing ICSI but using sperm which did not meet those strict criteria. Fertilisation and PRs were significantly higher in the group with intact sperm nuclei. Although this report was based on only 27 pregnancies, the findings were supported by a larger-scale study by Antinori et al. (2008) who randomised patients to ICSI ($n = 219$) or IMSI ($n = 227$). IMSI demonstrated a superior clinical PR (29% vs 12.9%) and reduced miscarriage (17.4% vs 37.5%). A later meta-analysis by Souza Setti et al. (2010) which incorporated the three clinical studies above but was unsurprisingly heavily influenced by Antinori's work since this comprises by far the largest of the studies included. Nevertheless, the findings based on 357 IMSI and 349 ICSI cycles are persuasive, demonstrating no significant difference in fertilisation between IMSI and traditional ICSI but significantly improved implantation and PRs. More recent work has shown that IMSI-selected sperm not only have fewer nuclear malformations but a lower incidence of chromosomal aneuploidy (Figueira et al., 2011).

Anti-sperm antibodies

The relationship between male fertility and Anti-sperm Antibodies (ASA) has been studied for many years (Rumke & Hellinga, 1959) and demonstrated a number of associations between the presence of ASA and sperm function and, as a consequence, male infertility (Rümke, 1965; Bronson et al., 1989; Barratt et al., 1989). However, the correlation between a particular threshold for ASA and either natural or assisted pregnancy remains confused and so the indication for routine ASA testing is unclear.

A number of different assays have been used to detect ASA including: (i) enzyme linked immunosorbant assay (ELISA); (ii) tray agglutination test (TAT); (iii) gel agglutination test (GAT); (iv) immunobinding tests such as mixed antiglobulin reaction (MAR) or IBT (immunobead test); and (v) flow cytometry (Hellema & Rümke, 1976; Clarke et al., 1985; Morroll et al., 1993; Andreou et al., 1995; Nicholson et al., 1997; Lenzi et al., 1997). From this wide array of tests comes conflicting literature on their effect on natural or assisted conception as well as any consensus on the clinical thresholds required before such effects are observed. Policies for ASA testing have largely relied on evidence and recommendations provided by the WHO. Although the WHO has focussed on only a few testing methods, the evidence used to formulate its clinical reference values is remarkably thin. Its most recent guidance (WHO, 2010) continues to recommend routine ASA testing but no new data was provided for a reference range (Cooper et al., 2009). Instead they retained the 50% binding level; a partial evidence-based criterion derived from a single study. Ayvaliotis et al. (1985) followed up 108 ASA positive

men for between 6 and 46 months, arbitrarily allocated to a high ASA+ (>50% binding) and a low ASA+ (<50%) group and found that PR was lower in the high ASA+ group. However their findings were based on only 25 couples giving rise to six pregnancies. This single study appears to be the only clinical evidence used by WHO to form the basis of its recommendations for a testing strategy over the past 20 years.

Much of the evidence implicating ASA as a cause of male infertility centres around their effect on sperm agglutination and its concomitant influence on sperm concentration and motility. There seem to be very few RCTs, meta-analyses and only a few large-scale studies on clinical cases. In terms of the influence of ASA on natural conception, there are few studies of any significant power. Using the SpermMAR test, Comhaire et al. (1988) showed that 16/312 men (5%) attending an infertility clinic had more than 40% of particles bound to motile spermatozoa. In the same study none of the fertile controls tested more than 40% positive. Critser et al. (1989) on the other hand examined sera from 20 fertile and 242 infertility patients but found similar levels of ASA (measured by the IBT) in both groups and concluded that routine ASA testing was of questionable value.

Whether ASA affect fertilisation events independently of other semen parameters also remains unclear. Much of the evidence base is experimental rather than clinical. For example groups have created antibody positive sperm *in vitro* by incubation of donor sperm with immunoglobulin and then challenged sperm function in assays for the acrosome reaction, zona binding or oocyte penetration (Bronson et al., 1989; Liu et al., 1991; Francavilla et al., 1997) and many have demonstrated a significant negative impact of ASA in this artificially created situation. However, there remains little evidence for a direct association between fertilisation failure at IVF, or PR, due to ASA, independent of other sperm factors such as motility. Many studies are relatively low in power and contradictory (Table 5) and the results of a significant proportion of these were recently summarised in one of the few meta-analyses performed on the subject by Zini et al. (2011). They considered 10 IVF and 6 ICSI studies which met their inclusion criteria but failed to find any significant link between the presence or level of ASAs and pregnancy. However they did concede that the most commonly used laboratory methods (Immunobead test or Sperm Mar) were crude at best and unable to determine the exact function of the immunoglobulin being detected. There remains no protocol which is either standardised or universally accepted as the "gold standard" method. Indeed, inconsistency in interpretation of the various tests based on an indirect test prompted the UKNEQAS scheme to abandon its EQA (external quality assessment) antibody scheme (Goddard, personal communication). Fertility and pathology laboratories should therefore consider the merits of providing a routine clinical test in the knowledge that reliability and quality cannot be assured.

Sperm DNA damage

Although previous discussions have shown that conventional semen analysis, when performed properly can provide useful clinical information, that information is clearly limited in terms of its ability to predict outcome. The determination of sperm number, ability to swim and estimates of shape and size cannot determine how well the sperm is able to bind to the oocyte, initiate fertilisation or relate to the development of the embryo. These questions require a more detailed investigation of sperm structure and function. Sperm DNA damage testing has been proposed for a number of years as a useful supplementary investigation for the 'subfertile' man (Aitken & De Iuliis, 2007; Evenson et al., 2007; Lewis, 2007; Giwercman et al., 2010; Barratt et al., 2010) and has been suggested by some to be more robust than conventional semen parameters as a predictor of outcome (Lefièvre et al., 2007; Lewis, 2007; Castilla et al., 2010).

Table 5. ASA positivity, natural conception and IVF – summary of significant findings.

Author(s)	Patients (cycles)	Natural/IVF	Findings
Ayvaliotis et al. (1985)	1025	Natural	Reduced PR with >50% ASA (IBT) positive
Clarke et al. (1985)	17 couples	IVF	FR 27% when IBT>80%
Junk et al. (1986)	72 couples	IVF	FR reduced when IBT positive for both IgA and IgG
Comhaire et al. (1988)	200	Natural	>40% MAR positive associated with infertility (OR 3.59)
De Almeida et al. (1989)	15 couples	IVF	FR 14% when IBT>70% vs 60% (<70%)
Witkin et al. (1992)	67 couples	IVF	FR reduced with increasing sperm bound ASA or ASA in female sera. Serum ASA in the male not significant
Tomlinson et al. (1993)	229	Natural	ASA no predictive value
Rajah et al. (1993)	36 couples	IVF	FR 50% in ASA+ men vs 72.7% in ASA –ve. PR not significant
Lähteenmäki (1993)	33 couples (47)	IVF	FR reduced when >90% MAR positive sperm. Correlated to sperm motility
Ford et al. (1996)	183 couples	IVF	IBT level correlated to FR but not pregnancy. No predictive value
Sukcharoen and Keith (1995)	160 couples	IVF	No association between IBT positivity and FR
Culligan et al. (1998)	251 couples	IVF	No association between FR and ASA positivity
Vujisić et al. (2005)	52 couples	IVF	No association between sperm bound ASA, ASA in serum or follicular fluid and IVF outcome

The term 'DNA damage testing' is essentially an 'over-arching' description for a variety of tests with significantly different methodologies which measure a number of aspects of DNA damage from double and single strand breaks to modified bases (Barratt et al., 2010). Some tests determine DNA damage directly and under physiological conditions while others assess damage indirectly or under induced acid or alkaline conditions. All tests require differing levels of specialist knowledge and equipment to perform them and provide a variety of test outcomes. Importantly, from the perspective of this document, one form of sperm DNA testing is now available as a commercial kit and is performed by a number of clinics with little guidance on the scope, interpretation and limitations of individual tests or indeed subsequent clinical management based on the outcome of that test.

A summary of 18 studies published using either: (i) the Sperm Comet Assay; (ii) Sperm Chromatin Structure assay (SCSA); (iii) Terminal transferase dUTP nick end labelling (TUNEL) or (iv) Sperm Chromatin Dispersion (SCD) or Halo test to detect DNA damage in sperm is shown in Table 6. In summary, perhaps the most notable conclusion from all of these studies is that there are significant differences and contrasting findings not only between laboratories using different methodologies but between those purportedly using the same assay. Only half of these studies were able to show a relationship between DNA damage and pregnancy, from which it can be concluded either that the relationship is tenuous or the standardisation and reproducibility of such assays is even poorer than it is for conventional semen analysis.

Undoubtedly the largest body of evidence linking sperm function and fertility with sperm DNA damage has been obtained using the SCSA. The SCSA utilises flow cytometry to measure the fluorescence in acridine orange-stained sperm in a relatively large cell population but unfortunately relies on the laboratory having access to costly equipment which often means sending specimens to a third-party specialist laboratory. The pioneering work by Don Evenson et al. established the SCSA as a test of sperm DNA quality in a number of species including humans in the 1980s (Ballachey et al., 1988; Evenson et al., 1989, 1991, 1999) and established a relationship between DNA damage and fertility. In a group of 200 couples, those conceiving earlier within a 12-month period had significantly lower levels of DNA damage than those conceiving later or indeed those failing to conceive. Abnormally high values in the SCSA were also associated with increased risk of miscarriage (Evenson et al., 1999). Controversially, and in the largest study to date by Bungum et al. (2007), 388 IVF cycles were examined from a total of 998 which also included 387 IUI and 223 ICSI cycles. There was little evidence to suggest that IVF rates or pregnancy were influenced by the results of the SCSA. Deliveries per cycle were 28.5% in the low (< 30%) DFI group versus 25.8% in the high (> 30%) DFI group. Moreover, their conclusion was that sperm DNA damage measured in prepared sperm had no predictive value in terms of outcome. Interestingly, those couples with DFI more than 30% had significantly higher pregnancies with ICSI but not with IVF. Further, they demonstrated a significantly reduced delivery rate in a group of 387 IUI couples with high DNA damage (1.5% vs 19.0%).

Table 6. Sperm DNA fragmentation and ART outcome – summary of significant findings.

Author	ART	Patient no.	Assay	Association with ART	Prognostic threshold (%)
Tomsu et al. (2002)	IVF	40	COMET	Pregnancy	–
Morris et al. (2002)	IVF	20	COMET	Embryo cleavage	–
Simon et al. (2010)	IVF	230	COMET	Fertilisation, Pregnancy	–
Simon et al. (2010)	ICSI	130	COMET	Fertilisation, Pregnancy	–
Simon et al. (2011)	IVF	75	COMET	Fertilisation, Pregnancy	25
Zini et al. (2005)	ICSI	60	SCSA	Pregnancy loss	30
Boe-Hansen et al. (2006)	IVF	139	SCSA	Pregnancy	27
Boe-Hansen et al. (2006)	ICSI	47	SCSA	No association	27
Benchaib et al. (2007)	IVF	88	SCSA	Fertilisation, Pregnancy loss	15
Benchaib et al. (2007)	ICSI	234	SCSA	Fertilisation, Pregnancy loss	15
Bungum et al. (2007)	IVF	388	SCSA	No association	30
Bungum et al. (2007)	ICSI	223	SCSA	No association	30
Bungum et al. (2007)	IUI	387	SCSA	Pregnancy	30
Lin et al. (2008)	IVF	137	SCSA	Pregnancy loss	27
Lin et al. (2008)	ICSI	86	SCSA	Pregnancy loss	27
Henkel et al. (2004)	IVF	249	TUNEL	Pregnancy	37
Huang et al. (2005)	IVF	217	TUNEL	Fertilisation	10
Huang et al. (2005)	ICSI	86	TUNEL	Fertilisation	4
Borini et al. (2006)	IVF	82	TUNEL	Pregnancy loss	10
Borini et al. (2006)	ICSI	50	TUNEL	Pregnancy loss	10
Bakos et al. (2008)	IVF	45	TUNEL	No association	–
Bakos et al. (2008)	ICSI	68	TUNEL	Pregnancy loss	–
Muriel et al. (2006a)	IVF/ICSI	85	SCD	Fertilisation	–
Muriel et al. (2006b)	IUI	100	SCD	Semen parameters	–
Velez de la calle et al. (2008)	IVF/ICSI	622	SCD	Embryo quality Fertilisation	18
Evenson et al. (1999)	Natural	165	SCSA	Pregnancy	30
Giwercman et al. (2010)	Natural	273	SCSA	Pregnancy	20

In one of the larger recent studies of IVF ($n = 230$) and ICSI ($n = 130$) patients using the Comet assay, Simon et al. (2010, 2011) demonstrated that male partners of non-pregnant couples had significantly higher levels of DNA fragmentation in their native semen (39.5% vs 51.7%) and in the gradient-prepared (26.9% vs 36.8%) sperm sample when compared to those achieving pregnancy. Establishing a clinical threshold of 25% they showed that men with more than this level of DNA damage had reduced fertilisation rates and a reduction in embryo quality. The risk of failure to achieve a pregnancy increased when sperm DNA fragmentation exceeded a threshold value of 52% for sperm in seminal plasma and 42% for those prepared by density gradient centrifugation. LBRs were also significantly reduced (33% vs 13%) in IVF couples ($n = 203$) with high sperm DNA damage (Simon et al., 2013).

Terminal deoxynucleotidyl transferase dUTP nick end labelling, or 'TUNEL' assay, is another method for measuring DNA damage and has the advantage over the previous two that it can be used with a conventional microscope and therefore potentially within a routine andrology laboratory. A number of studies have shown an association between the number of TUNEL positive sperm and reduced fertilisation, IVF, IUI or ICSI success (Høst et al., 2000; Duran et al., 2002; Henkel et al., 2004; Huang et al., 2005; Borini et al., 2006; Bakos et al., 2008). Others have also shown clear relationships between TUNEL assay and traditional semen parameters (Tomlinson et al., 2001; Duran et al., 2002). However, most of the studies are relatively small with few pregnancies in either arm (low or high TUNEL positivity), vary in precise methodology and there is little consensus with regard to a clinical threshold which would help guide the clinic in management of the patient.

The SCD or Halo test is a relatively simple, fast and inexpensive method for detecting DNA damage and is now available in commercial kit form (Fernández et al., 2003). It can be carried out with equipment normally available in andrology laboratories, and the test endpoints (non-dispersed and dispersed nuclei) can easily be assessed by light microscopy. As a commercially available product, one might assume that the test has been adequately validated for clinical use; however, the reality is that clinical data are perhaps more lacking for Halosperm than for the non-commercial alternatives. At the time of writing, of more than 20 publications cited on the Company's own website http://www.halotechdna.com/en/research_and_development/scientific_publications), only one appears to relate Halosperm results to ART outcome (Muriel et al., 2006a), and the remainder appear to relate to DNA damage assessed using other methods. Despite a correlation with fertilisation rate, this single publication was unable to demonstrate any effect on pregnancy or pregnancy loss. In a separate study, Muriel et al. (2006b) showed that Halosperm results appear to correlate with traditional semen parameters but not the outcome of IUI treatments. A later and much larger study, on 622 IVF/ICSI couples, demonstrated that Halosperm results correlated with traditional sperm parameters and fertilisation rates (Velez de la Calle et al., 2008). However no statistical significance was shown in relation to pregnancy or LBRs.

Taken as an entire 'body of evidence' that is, the association with poor semen quality, reduced success with assisted reproduction and pregnancy loss, there appears to be merit in developing/performing some form of sperm DNA damage test. However, the overall picture with regard to patient management remains confusing (Table 6). Not only is there a lack of consensus regarding the relationship between test outcome and the ability to conceive, but the various testing methods give different reference values from which to differentiate between a normal and abnormal result. The literature to date does not clearly identify a particular clinical indication for sperm DNA testing, nor how patients should be managed if the test outcome is unfavourable. The strongest evidence to date relates DNA damage with pregnancy loss but this still does not identify clearly the most appropriate test or a clear clinical threshold at which clinics might advise patients that treatment is inadvisable. A recent position report by ESHRE's Special Interest Group in Andrology (SIGA) committee drew much the same conclusion (Barratt et al., 2010). The report also highlighted issues with a number of the protocols used for DNA testing which themselves appear to induce DNA damage, the concern being that the differences observed between methods were not biological, but iatrogenic. The SIGA concluded that a more robust clinical test was required to corroborate or refute recent study findings. In addition, it suggested that any routine clinical test procedure is validated in extensive clinical trials (Barratt et al., 2010).

Discussion

It seems that there exists a sufficiently large body of evidence showing some relationships between traditional semen quality parameters and pregnancy. Although, for the reasons discussed at length, the studies do not all agree, the overall trend is that patients with reduced sperm concentration, motility or morphology have a reduced chance of natural or assisted conception success than those in the so-called 'normal range'. In some cases the data are sufficiently powerful to provide a threshold below which conception is significantly less likely to occur, acting as an indicator to the clinician that an alternative form of ART may be more appropriate: this appears to be the case for the number of (progressively) motile sperm. Unfortunately, clear clinical thresholds are not available for other semen parameters. Of the studies mentioned earlier (Tables 2 and 3), suggesting that motile sperm

number influences outcome, five out of the eight cite greater than 5×10^6 motile sperm as the minimum requirement for a reasonable chance of success with AI (IUI). Comparing this with the WHO's lower reference (normal) limit of 32% motility (progressive) and concentration of 15 million per ml (combined giving 4.8 million motile per ml), the thresholds for natural and AI appear to concur. Five million (rapidly) progressive sperm would therefore appear to be a sensible threshold value which could be used to select patients for IUI. Below this number, it would seem appropriate that more invasive treatments such as IVF (or ICSI) should be recommended. The decision to use IVF over ICSI is probably not one which can be taken on the basis of a simple threshold for sperm quality. The level of uncertainty and error associated with assessing concentration and motility is too high to discriminate with any accuracy at a level below 5 millions. One area of doubt that remains is over the reporting of progressive motility alone (WHO, 2010) as opposed to the discrimination between the four grades of motility as described by the WHO in previous guidance (WHO, 1992, 1999). Over the years, many authors, and especially those advocating the use of CASA, have described the technical difficulty in performing a manual motility analysis and most of the studies described in this review fail to discriminate between rapidly motile (Grade a) sperm and the more sluggish (Grade b) sperm at 37°C. It is this technical difficulty alone that prompted the authors of the latest WHO manual (WHO, 2010) to recommend that the number of motility grades be reduced down to three: Progressive (P), non-progressive (NP) and immotile (I). There is however a gulf of difference in the apparent level of energy displayed by the average rapidly motile sperm often travelling at more than 40–50 microns per second compared with those sluggish Grade b sperm crawling along at speeds as low as 6 microns per second and many believe that failure to distinguish between the two could lead to inappropriate diagnosis and/or ART success. Studies using CASA (Bongso et al., 1989; Barratt et al., 1992; Marshburn et al., 1992; Larsen et al., 2000; Garrett et al., 2003) clearly demonstrate the clinical significance of sperm velocity and would seem to indicate that a sample containing mainly Grade b progressively motile sperm is more likely to be sub-fertile. If these robust studies are to be accepted then it would seem inexcusable to fail to distinguish between highly motile and less motile progressive sperm. In collating the evidence base for the 2010 manual, it is – unfortunate that the WHO chose not to analyse the significance of individual motility grades with regard to TTP and only examined the product of a + b or a + b+ c grades. If, ultimately, clinical studies qualify the WHO approach, then laboratories can simply report Grade a and Grade b sperm together as 'progressive'. If, on the other hand, the classification of progressive sperm together (with no discrimination between swimming speeds) is shown to be inadequate, many patients could be diagnosed inappropriately or go without the appropriate ART when it is required.

Sperm morphology continues to provide the most controversy, but on balance appears to have some significance with regard to AI and natural conception though possibly less with IVF and ICSI. There is very little evidence to support the use of ICSI in cases of isolated teratozoospermia. However it is highly likely that available data is weakened considerably by technical issues which reflect both the subjectivity of manual assessment and the difficulty in standardising methodology within and across centres. A rapid, reliable automated test does not currently exist, and laboratories may therefore require a more pragmatic risk-based approach which focuses on abnormalities (Jouannet et al., 1988) as opposed to one which relies on a rather subjective search for the 'normal' sperm. It is recognised that sperm with multiple heads or tails are likely to have poor motility and that those with multiple or macrocephalic heads have a higher incidence of aneuploidy and/or chromatin anomalies (Lacroix & Warter, 1982; Bianchi et al., 1996; Perrin et al., 2011). A priority of sperm morphology analysis must be to exclude these and other potentially sterilising defects such as globozoospermia or stump tail defects (Kilani et al., 2004; Ravel et al., 2006) since failing to do so could undoubtedly lead to patient dissatisfaction (and possibly litigation), but whether the search for the elusive perfect sperm form is required is highly debatable.

The basis for selecting patients for ICSI is clearly more complex and the wide geographical variation in selection criteria throughout the world demonstrates that they are often not based on male clinical factors (Andersen et al., 2008). For these, a pragmatic approach must be taken and maximising the patient's chance of success and minimising the risk should be the overriding consideration. Although the current evidence base is lacking, it is possible that in future, improvements in supplementary morphology or DNA testing or, indeed, other tests of sperm function will help with such decisions. More recently developed tests such as HA binding and IMSI may prove useful in improving success rates using ICSI but seem to contribute little towards the decision-making process pointing patients towards one particular treatment or another.

Based on available evidence, and despite previous recommendations of the WHO (WHO, 1992), there appears to be little justification for the testing of all patients attending the infertility clinic for anti-sperm antibodies. Because the deleterious effects of ASA tend to be strongly associated with other semen quality parameters such as poor motility and agglutination, the remaining compelling argument for ASA testing is to prevent those with a high risk of fertilisation failure (FF) from receiving IVF. Although the scientific justification may not be high, the risk-aware laboratory would want to screen out patients at risk of FF and the economically sensible

approach would therefore be to test only patients listed for IVF for ASAs.

The arguments which favour routine DNA damage testing over traditional semen parameters or as an additional independent parameter remain difficult to interpret. In the only meta-analysis available, Zini et al. (2008) examined a total of 808 IVF and 741 ICSI cycles from 11 studies and showed a consensus association between DNA damage and pregnancy loss but not fertilisation or pregnancy. Both the findings of the recent ESHRE position report (Barratt et al., 2010) and this current review agree that the overall impression is confusing and there is more work to be done, particularly in the standardisation of the most appropriate test, establishing clinical thresholds and working with clinical colleagues to advise on patient management. With current levels of knowledge, and assuming the clinician can interpret the test results adequately, any form of DNA damage testing is unlikely to alter patient management. Many couples will still opt for IVF/ICSI as their only realistic chance of pregnancy regardless of a heightened risk of pregnancy loss. Before adequate clinical decisions can be made on the results of DNA testing, a number of very basic, yet important, questions must be addressed: (i) which group(s) of patients requires sperm DNA damage testing; (ii) which test is most suitable and (iii) what decisions do clinics make with an adverse result?

The economic reality of providing any test of semen/sperm quality requires that test selection is carried out on the basis of its clinical value and cost effectiveness. The WHO (2010) has recently provided bold guidelines for performing the standard semen analysis with an emphasis on good laboratory practice and the need to consider the likely sources of operator error. Unfortunately, increasing the reliability and reproducibility of semen analysis comes at a price, and following these methods to the letter will add considerably to the time taken to perform a single test, a point which needs to be made clear to those responsible for setting budgets and providing funding. With this in mind, laboratories must be mindful of performing tests which add little prognostic value but could add significantly yet unnecessarily to costs to patients. Instead, they should concentrate on the measures of sperm quality which have a body of evidence to justify their use in the decision-making process. Despite all the efforts of large organisations such the WHO and ESHRE, the results of EQA schemes demonstrates clearly that standardisation of semen analysis methodology and inter-laboratory agreement remains relatively poor (Keel, 2004; Pacey 2006, 2010; Tomlinson, 2010). The aims of the ART laboratory, which are essentially the rapid and low-risk preparation of sperm for IUI, IVF or ICSI, may not be compatible with those of the pathology laboratory or indeed the requirements of the diagnostic semen analysis as laid down in WHO (2010). However, as ART centres are required to be the source of most of our method validation in relating testing to treatment outcome, efforts must be made to standardise between diagnostic and treatment arms of the service.

The take-home message to our clinical colleagues, and especially General Practitioners who are usually the first in line to examine a diagnostic semen analysis report, would be to take a holistic approach to the evaluation of semen quality. Having excluded severe morphological defects, fertility centres/Units may simply need to focus on improving the quality of measurement for semen parameters that appear to be of most use in the clinic (i.e. robust sperm count and motility analysis).

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Declaration of interest: Mathew Tomlinson is a Director/Owner of Procreative diagnostics, a new company established to develop and market the Sperminator® an automated system for measuring sperm concentration and motility. He is also a senior member of the ABA (Association of Biomedical Andrologists) Education sub-committee committed to improving standards in Laboratory andrology.

Sheena Lewis is the Chief Executive Officer and a shareholder of Lewis Fertility Testing Ltd., a spin-out company of Queen's University Belfast' that is now marketing the SpermComet test.

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