

## Unexplained Male Infertility – Looking Beyond Routine Semen Analysis

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### Abstract

Unexplained infertility is a diagnosis reserved for couples in whom routine semen analysis is within normal values and female infertility factors have been ruled out. Its reported prevalence ranges from 6–27 % depending on how exhaustive the patient work-up is. Despite differing according to urological societies, recommendation for male infertility evaluation usually considers routine semen analysis results as surrogates for fertility potential. Male infertility evaluation, however, must go far beyond counting spermatozoa and assessing motility and morphology. It has to be complemented with comprehensive history taking, proper clinical examination and relevant endocrine, genetic and/or other investigations. There is a broad spectrum of potential aetiologies of unexplained male infertility (UMI), ranging from the simple couple's miscomprehension of the concept of the female fertile window to complex molecular and functional defects involving either the male or the female, or both. Novel contemporary technologies added to the current knowledge on this subject highlight a case for careful reconsideration before clinicians make treatment decisions for patients with unexplained infertility.

### Keywords

Infertility, male, female, diagnosis, treatment, semen analysis, oxidative stress, DNA damage, genetics, assisted reproductive techniques, andrology

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Infertility is customarily defined as the inability of a sexually active, non-contracepting couple to achieve spontaneous pregnancy in one year.<sup>1</sup> It has been estimated that 15 % of couples seek medical assistance for infertility and the origins of the problem seem to be equally shared between male and female partners.<sup>1</sup> Unfortunately, the cause of fertility impairment cannot be determined in nearly half of the cases despite the availability of a relatively broad range of diagnostic tests and eventually 5 % of couples remain unwillingly childless.<sup>1–3</sup>

Infertility of unknown origin comprises both idiopathic male infertility and unexplained infertility. Men presenting with idiopathic infertility (30–40 %)<sup>1,2</sup> have reduced semen quality with no obvious abnormalities in their history of fertility problems and both physical examination and endocrine laboratory testing. The category 'unexplained infertility' is reserved for couples in whom routine semen analysis is within normal values and female infertility factors have been ruled out.<sup>4</sup> Its highly variable reported prevalence ranging from 6–27 %<sup>1–6</sup> strongly depends on how exhaustive is the couple evaluation. In countries with limited resources for diagnosis it is likely that the prevalence of unexplained infertility increases.<sup>7</sup>

Recommendations for male infertility evaluation based on semen analyses results may be confounded due to the limited validity of routine semen analysis as surrogates for the assessment of male fertility potential.<sup>7,8</sup> The prognostic fertility value of semen characteristics, such as sperm concentration, percent motility and morphology, is influenced by sexual activity, function of the accessory sex glands and other conditions. Nonetheless, routine semen analysis does not account for

sperm dysfunctions such as immature chromatin or DNA damage. In addition, the assumption that one ejaculate is representative of a given man's semen profile argues against the current knowledge of the high biological variability of semen variables from the same individuals. Lastly, criteria for semen normality vary according to the World Health Organization (WHO) laboratory manual edition for the examination and processing of human semen edition.<sup>9–11</sup> As noted, in its newly released edition the WHO has established new reference values for human semen characteristics which are markedly lower than those previously reported.<sup>8,10</sup> The utilisation of the new WHO manual reference values into clinical practice will likely result in a re-classification of many of the infertile couples. Specifically, those couples previously classified as having male factor infertility with sperm parameters above the new reference limits but below the previous values (WHO, 1999) will now be diagnosed as having unexplained infertility.

These introductory considerations highlight the current importance of unexplained infertility and the shortcomings of routine semen analysis. The male evaluation regarding fertility must go far beyond counting spermatozoa and assessing motility and morphology. It has to be complemented with a proper clinical examination comprehensive history taking and relevant endocrine, genetic and/or other investigations.

### Candidate Aetiologies of Unexplained Male Infertility

The following possibilities should be considered in the assessment of men with unexplained infertility:

- presence of a female factor;
- inappropriate coital habits;
- erectile dysfunction;
- presence of antisperm antibodies (autoimmune infertility); and
- sperm dysfunctions.<sup>12</sup>

The first three conditions can be ruled out by comprehensive history taking and through gynaecological evaluation whereas modern andrology aids in the evaluation of the last two possibilities.

## Autoimmune Male Infertility

It is defined as an improper bodily immune response, whether humoral or cellular, against sperm antigens causing sperm functional dysfunction and rendering the male infertile. Antisperm antibodies (ASA), the hallmark of autoimmune infertility, are implicated in sperm dysfunction by their direct effect on various sperm antigens. ASA may interfere with various sperm functions such as inducing acrosome reaction prematurity and activating apoptosis. ASA may also hinder fertilisation event by inhibition of cervical mucus penetration, zona pellucida (ZP) binding or sperm-oocyte fusion. Furthermore, ASA may change some macromolecular and sub-cellular function by altering chaperone function, protein folding and disulphide bonds.<sup>13</sup> These alterations ultimately result in a decrease in pregnancy rates.<sup>14</sup>

About 10 % of infertile men have ASA compared to only 2 % in fertile counterparts.<sup>15</sup> Nevertheless, elevated levels of ASA may be found in as high as 40 % of men with unexplained male infertility (UMI).<sup>16</sup> The disruption of blood-testis barriers by immunosuppression defects or genital tract insults are the primary mechanisms responsible for leakage of sperm antigens and formation of ASA. ASA can be found in the serum, seminal plasma and sperm-bound. However, it is still unknown whether ASA are locally formed within the genital tract or merely transuded from the serum. The pathogenesis of autoimmune male infertility is further complicated by the observation that 7–17 % of infertile women also produce antisperm antibodies in their cervical fluids.<sup>17,18</sup>

Elevated ASA titers can be found in men with normal conventional semen parameters.<sup>19</sup> Nonetheless, attention should be paid to sperm agglutination, which is the only finding highly suggestive of elevated ASA titers.<sup>14</sup> However, sperm agglutination is time-dependent and rarely involves a large proportion of motile spermatozoa immediately after liquefaction, even when all ejaculated spermatozoa are antibody coated.<sup>14</sup> Along the same lines, both the immobilising and the apoptogenic impacts of ASA on spermatozoa require complement activation which is prevented by potent anticomplement substances in the seminal plasma such as membrane cofactor protein (MCP) or decay accelerating factor (DAF).<sup>20,21</sup> Currently, the most popular tests to identify sperm-bound ASA are both the direct immunobead test (IBT) and the direct mixed agglutination reaction (MAR).<sup>22</sup>

The diagnosis of immunological infertility requires two conditions to be satisfied:<sup>23</sup>

- a minimum of 50 % percent of motile spermatozoa (progressive and non-progressive) have beads attached to their surface. It should be noted, however, that particle binding restricted to the tail tip is not associated with impaired fertility and can be present in fertile men;<sup>10</sup> and
- sperm-bound antibodies interfere with sperm function; this is usually demonstrated by using functional tests such as the

sperm–mucus penetration test, zona binding assays and the acrosome reaction.

## Genetic Causes of Unexplained Male Infertility

Genetic abnormalities causing male infertility include alterations in sperm chromosomal complement, sperm DNA integrity defects and gene mutation and polymorphisms.

### Alterations in Sperm Chromosomal Complement

Non-disjunction events during gametogenesis result in either extra or missing chromosome that lead to aberrations in numerical chromosomal complement; such aberration is termed 'aneuploidy'. On the other hand, structural chromosomal complement defects such as deletions, translocations and inversions may also occur in spermatozoa or oocytes due to chromosomal insults. Both structural and numerical chromosomal complement defects can give rise to dysfunctional sperm thus impacting on male fertility and pregnancy viability.<sup>24</sup>

Although sperm chromosomal aneuploidy rates are inversely correlated to sperm concentration and total progressive motility,<sup>25,26</sup> spermatozoa of normozoospermic infertile males may also harbour such defects.<sup>27–30</sup> The overall frequency of chromosomally abnormal spermatozoa in the general population is estimated to be 7 %. The mean frequency of disomic sperm (presence of two copies of a chromosome) for autosomes and sex chromosomes are 0.13 and 0.37 %, respectively, while the rate for diploid sperm (two copies of each chromosome) is 0.2 %.<sup>31,32</sup> For normozoospermic infertile males, the corresponding figures are 0.11, 0.44 and 0.3–1.0 %, respectively, but are higher in those with abnormal semen analysis results.<sup>31,32</sup> The exact causes of aneuploidy are mostly unknown, but smoking, alcohol, chemotherapy and ageing may play a role.<sup>33–35</sup>

Interestingly, a variable disomy rate has been observed with sex chromosomes and chromosomes 21 and 22; the higher rate of abnormalities related to chromosomes may be due to their lower rate of meiotic recombination which renders them more prone to non-disjunction.<sup>36</sup> Nonetheless, both morphologically normal and abnormal spermatozoa can be disomic or diploid,<sup>37</sup> or contain damaged DNA.<sup>38</sup> As such, selecting morphologically-normal spermatozoa for assisted reproductive techniques (ART) does not guarantee the absence of chromosomal abnormalities with a possible exception of spermatozoa retaining excess cytoplasm (ERC). In the latter, a greater extent of aneuploidy and diploidy has been observed regardless of the technique used for sperm processing.<sup>39–41</sup>

Similarly as chromosomal aneuploidy, structural chromosomal aberrations such as inversions, deletions, balanced or unbalanced translocations as well as Y-chromosome microdeletions are also associated with abnormal semen parameters. Moreover, such abnormalities are associated with higher miscarriage rates and presumably with higher risk genetic abnormalities in the offspring.<sup>42</sup> Surprisingly, in Y-chromosome microdeletions related infertility, the azoospermia factor c (AZFc) region is prone to many smaller subdeletions than those thought to be caused by intrachromosomal recombination.<sup>43</sup> These partial deletions produce a wide array of phenotypes, ranging from normozoospermia to azoospermia, due to factors that include the interaction of the environment and the genetic background.<sup>33</sup> Chromosomal abnormalities may be detected by using one of the following methods: sperm karyotyping, fluorescence *in situ* hybridisation analysis and quantitative polymerase chain reaction (Q-PCR).

## Sperm DNA Integrity Defects

During spermiogenesis, the haploid sperm chromatin undergoes significant changes in which most histones are replaced first by transition proteins, then by positively charged protamines.<sup>34</sup> By this remodelling process the sperm DNA condenses so tightly that it is resistant to mechanical stresses.<sup>35</sup> When there is a defect in this process or when there is single or double stranded sperm DNA breakage, the sperm DNA integrity is jeopardised and the fertilising capacity of the sperm is undermined.

Sperm DNA integrity is increasingly being distinguished as an important marker of fertilising efficiency and it is associated with better diagnostic and prognostic values than standard sperm parameters. Sperm with DNA damage are more often seen in subfertile/infertile men than in fertile ones.<sup>27-29</sup> Increase of spermatozoa with abnormal chromatin structure or DNA damage (expressed as DNA fragmentation index [DFI]) has been negatively correlated with intracytoplasmic sperm injection (ICSI) and *in vitro* fertilisation (IVF) outcomes.<sup>30</sup> It is known that about 5–8 % of men misdiagnosed as having UMF, according to the normal semen parameters on routine analyses, present sperm DNA integrity defects.<sup>44,45</sup> The aetiologies of such defects are due to variety of extrinsic and intrinsic factors. Heat, smoking, alcohol, radiation and other gonadotoxins are examples of extrinsic factors while protamine deficiency, mutations affecting DNA packaging, reactive oxygen species and ageing are the primary intrinsic factors.<sup>46</sup>

Sperm DNA damage is often assessed by the determination of chromatin compaction or DNA fragmentation, however, each measurement method has certain cut-off value. The former methods examine the accessibility of dyes (acridine blue, aniline blue and chromomycin A3) to nucleoproteins or chromatin after challenging spermatozoa with physical insults; as such, it reflects how susceptible the DNA is, or has been, to noxious agents.<sup>39</sup> On the other hand, DNA fragmentation is measured by detecting single or double strand DNA breaks. Transferase-mediated dTUP nick-end labelling (TUNEL), comet assay, acridine orange test, sperm chromatin structure assay (SCSA) and sperm dispersion test (SCD)<sup>40</sup> are clinically available methods to detect DNA fragmentation. Although they differ in costs and methods, most of the mentioned tests are clinically significant and correlates with sperm function and fertility.<sup>41</sup> For TUNEL, our group at Cleveland Clinic has reported a cut-off DFI of 19.25 % with 100 % specificity to differentiate infertile men with DNA damage from healthy men.<sup>47</sup> Evenson et al. reported 30 % as the threshold for DFI with SCSA.<sup>48</sup>

## Specific Gene Defect – Mutations and Polymorphisms

It is likely that specific genes play a role in patients with UMF since they control meiosis events, spermiogenesis, remodelling, motility, capacitation and fertilisation. In a comparative analytic study on spermatozoa from normozoospermic infertile men and fertile healthy controls using microarray technology, hundreds of gene sequences (targets) were differentially expressed between these two groups; there are few genes that are over-expressed whereas all others are under-expressed in infertile men.<sup>49</sup>

## Oxidative Stress

The imbalance between the production of reactive oxygen species (ROS) and the natural antioxidant defense system is termed as oxidative stress (OS). ROS are specifically defined as a group of oxygen derived metabolites formed by reduction of oxygen such

as superoxide anion, the hydroxyl radical and hydrogen peroxide. Normally, mammalian spermatozoa are redox cells that are able to produce reactive oxygen species and to export them to the extracellular medium to regulate several biological functions such as acrosome reaction, hyperactivation and fertilisation.<sup>50-54</sup>

However, overabundance of these unstable metabolites is harmful to the sperm, causing damage to sperm DNA in the nucleus and mitochondria, and inducing lipid peroxidation in the sperm plasma membrane as well as denaturing cellular protein.<sup>55</sup> In fact, sperm are extremely susceptible to the effect OS because of their high content of polyunsaturated fatty acids (PUFA) in plasma membranes as well as their limited antioxidant defence.<sup>56</sup>

ROS are usually associated with poor semen quality and male infertility. It has been shown that 40–88 % of non-selected infertile patients have high levels of seminal ROS.<sup>57</sup> However, recent reports found that normozoospermic infertile men have higher ROS levels and reduced total antioxidant capacity (TAC) levels than the normozoospermic fertile counterpart.<sup>58</sup> The exact prevalence of OS in normospermic infertile men is unknown. Nevertheless, a controlled study on a limited number of patients detected that 11 % of normospermic men have OS.<sup>58</sup>

The most often used methods for detecting oxidative stress include methods to measure ROS and methods to measure total antioxidant capacity (TAC). In an andrology setting, ROS measurement techniques are divided into two major categories, i.e., direct methods such as chemiluminescence and flow cytometry, and indirect methods, such as the colorimetric one, which target the final products of sperm cell membrane lipid peroxidation. For total antioxidant capacity measurements, several methods are available, such as the ones using enhanced chemiluminescence, spectrophotometry, fluorometry (e.g. ORAC assay) and electrochemistry (coulometry, voltammetry or electron spin resonance assay). Some of them are commercially available, particularly spectrophotometric and fluorometric methods.<sup>59</sup>

## Sperm Fertilisation Defects

Sperm ability to fertilise the ova is associated to their potential of undergoing capacitation, which includes the acquisition of hyperactivated motility, releasing acrosomal enzymes (acrosome reaction) to penetrate the zona pellucida and fusing with the oolema. The competent sperm can successfully achieve all these physiological processes culminating in fruitful fertilization outcome. It has been assumed that normozoospermic infertile men may have defective functional (incompetent) sperm that are unable to fertilise. This assumption is strengthened by the observation of low success rates of IVF and intrauterine insemination (IUI) in certain cases of unexplained infertility.<sup>60</sup>

Sperm fertilising performance can be examined from different angles encompassing all the stages of sperm-egg interactions.

## Zona Pellucida Binding Defects

Sperm binding to ZP3 (the proteinaceous layer that covers the oocyte) induces signal transduction pathways within the spermatozoon, involving multiple proteins, particularly protein-kinases A and C, that lead to the acrosome reaction.<sup>61</sup> Defective ZP bound sperm are present in approximately 15 and 25 % of subfertile men with a normal semen analysis and with abnormal ones, respectively.<sup>62-64</sup>

Such individuals have a reduced chance of achieving successful fertilisation when undergoing IVF.<sup>64</sup> Two tests of sperm binding to the human zona have been described:

- the hemizona assay; and
- the sperm-zona binding ratio test.

### Hyperactivation Defects

Capacitation is a phenomenon involving several physiological and molecular changes that prepare the sperm for fertilisation.<sup>65</sup> Hyperactivation (HA), considered the first step of the complex capacitation process, is characterised by typical swimming pattern of movement shown by most sperm retrieved from the oviductal ampulla at the time of fertilisation.<sup>66</sup> Such motility is essential for the sperm to detach from the oviduct wall, to penetrate into the cumulus oophorus and finally to efficiently drill zona pellucida and reach the oolema.<sup>67</sup>

Assessment of hyperactivation motility *in vitro* involves the use of computerised semen analyser (CASA) in conjunction with the kinematics module to distinguish different subpopulations of motile spermatozoa and to measure the spontaneous hyperactivated sperm and the induced hyperactivation HAmx (using pentoxifylline and progesterone). Hyperactivated spermatozoa can be distinguished from non-hyperactivated ones by their high curvilinear velocity (VCL), low linearity (calculated as straight-line velocity/VCL), and large amplitude of the lateral head displacement. The clinical significance of such data is reflected by their correlation with IVF outcomes and spontaneous pregnancy rates.<sup>68</sup> Mortimer et al. showed that HAmx >50 % is correlated with good *in vitro* fertilisation outcomes.<sup>69</sup>

Recently, it has been suggested that increased intracellular calcium entry through voltage gated calcium channels (CatSper<sup>1-3,5</sup>; Cation channel of Sperm) in the principal piece of the sperm flagellum is the prime mechanism for hyperactivation.<sup>70-72</sup> Defects in the gene coding for this protein is responsible for lack of hyperactivation, as shown in males with mutated CatSper1 gene who are infertile due to poor HA response despite having normal sperm count, morphology and even their initial motility.<sup>73</sup> Further investigation is needed to disclose the minor mutations in human CatSper genes in males with unexplained infertility.

### Acrosome Reaction Defects

The acrosome reaction (AR) is defined as the process of fusion of sperm plasma membrane with outer acrosomal membrane leading to release of exocytotic proteolytic enzymes (acrosine and hyaluronidase) in response to sperm–zona pellucida binding. Human sperm initiate primary binding to the ZP with intact acrosome.<sup>74</sup>

Some patients with unexplained infertility that have normal sperm–ZP binding have defective ZP-induced AR (AR insufficiency), which will result in reduced sperm–ZP penetration and failure of fertilisation.<sup>75</sup> Patients with this condition usually have a long duration of unexplained infertility, normal semen analysis and normal sperm–ZP binding, but show failure of ZP sperm penetration and have zero or low rates of fertilisation with standard *in vitro* fertilization (IVF).<sup>75</sup>

The diagnostic feature is that very low proportions of sperm undergo AR after binding to the ZP.<sup>76</sup> However, those patients achieve high fertilisation and pregnancy rates with intracytoplasmic sperm injection (ICSI).<sup>76</sup> Despite being higher in subfertile men with idiopathic oligozoospermia (65 %) and severe teratozoospermia (62 %, strict

normal sperm morphology  $\leq 5$  %), defective ZP-induced AR (ZPIAR) is found in 25 % of normozoospermic subfertile men.<sup>63</sup>

To assess the inducibility of AR, artificial stimuli are used *in vitro* to challenge the acrosome reaction such as calcium ionophore A23187 and progesterone. Under normal conditions, >15 % AR in response to ionophore treatment is expected.<sup>77</sup> Visualisation of acrosome reacted sperm can be achieved using different techniques as follows:

- birefringence characteristics of acrosomally-reacted spermatozoa under polarised light microscopy;<sup>78</sup>
- fluorescence microscopy after staining with fluoresceinated lectins;<sup>79,80</sup> and
- flow cytometry after addition of fluoresceinated-anti-CD-46 monoclonal antibody.<sup>81</sup>

### Fusogenic Ability Defects

The fusogenic potential of the already capacitated and acrosome-reacted human sperm is represented by their ability to fuse with the vitelline membrane of the oocyte. The fusogenic ability is usually tested by using the sperm penetration assay (SPA), also known as the zona-free hamster oocyte penetration test. It has been reported that 34.1 % of patients with unexplained infertility had <10 % oocyte penetration against 0 % in a control group of fertile men.<sup>82</sup> Various studies have evaluated the ability of the SPA to predict success or failure of IVF. Some investigators have shown no correlation with an abnormal test,<sup>83</sup> whereas others have claimed 100 % predictability.<sup>84</sup> Taking an average from different studies, a normal SPA may have 70 % predictability of normal fertilisation *in vitro*.<sup>85</sup> Semen samples which fail to fertilise hamster ova are usually unable to fertilise human ova.<sup>42</sup> Although the SPA is considered a research tool, it may be of clinical value for men with unexplained infertility with poor fertilisation rate on IVF.

### Work-Up Plan For The Management of Males with Unexplained Infertility

The initial test for couples with UMF is the post-coital test (PCT). PCT is a technically challenging test that must be appropriately timed and performed. Cervical mucus is normally hostile to sperm, except near the time of ovulation. In the peri-ovulatory period, the absence of sperm on a post-coital test in the presence of normal semen parameters suggests incorrect coital technique or failure to ejaculate into the vagina, while the presence of normal sperm numbers exhibiting reduced motility or shaking motility is suggestive of antisperm antibodies.<sup>86</sup> The finding of a normal post-coital test raises the possibility of a functional sperm defect. Assessment of sperm function can be divided into two steps. The first should be directed to check sperm competence before fertilisation by measuring the levels of ROS as well as DNA and chromatin integrity and the second step should include the assessment of sperm fertilisation potential especially for those patients with history of prior failure of conventional IVF. These tests include: sperm–ZP binding assay, capacitation, hyperactivation motility, inducibility of acrosome reaction and the ability of sperm to fuse with the vitelline membrane (zona-free hamster egg penetration test) may be used (see *Table 1*). *Figure 1* depicts an algorithm for the management of unexplained male infertility.

### Treatment

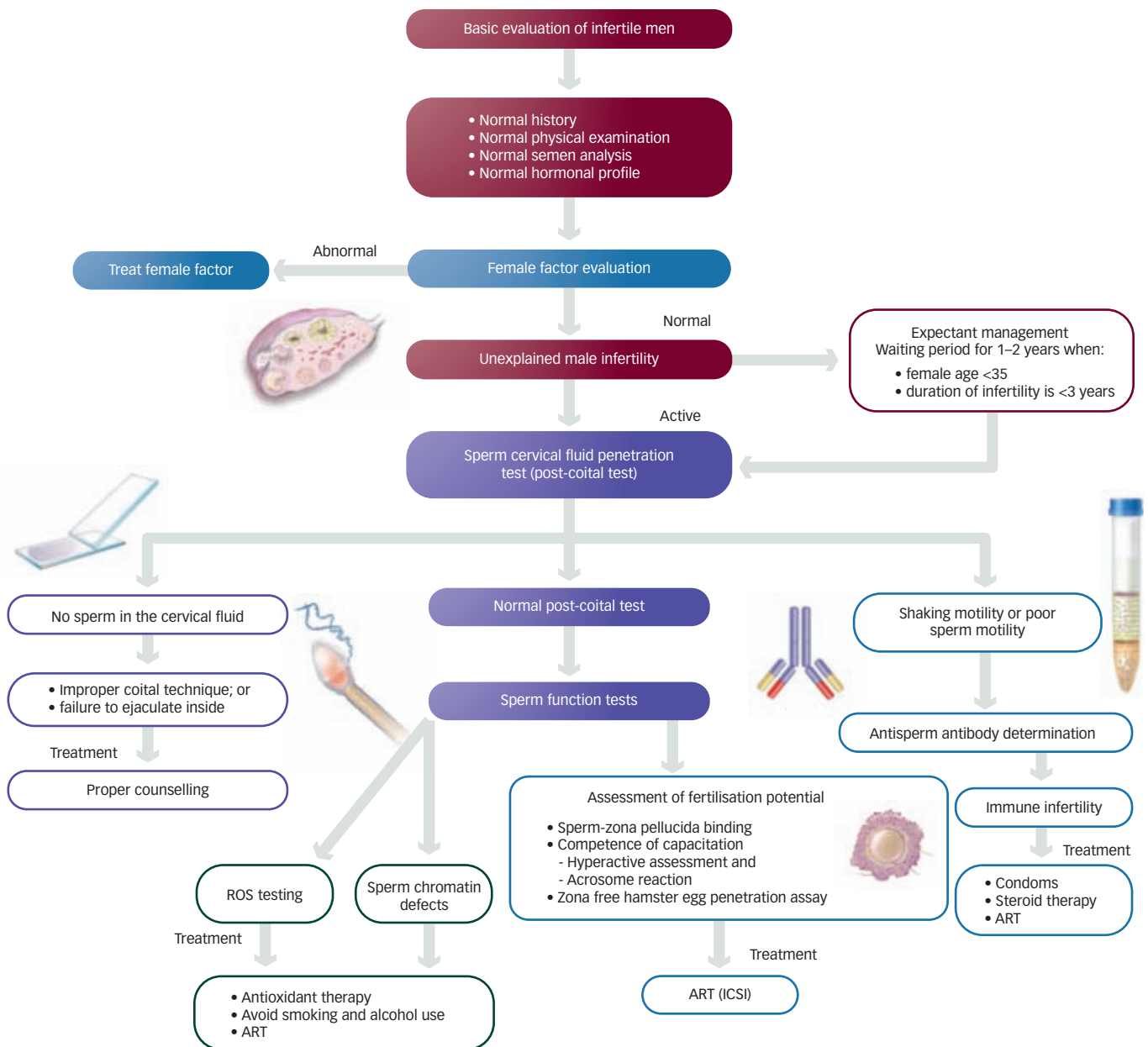
Standardised protocols for treating couples with unexplained infertility are still lacking, not only because the reasons for infertility remains

**Table 1: Diagnostic Testing for Unexplained Male Infertility**

Condition	Target	Testing
Autoimmune infertility	Antispermantibodies	Immunobead test (IBT) and mixed agglutination reaction (MAR)
Genetic causes		
<i>Sperm chromosome complement</i>	Numerical structural autosomal and sex chromosomes	Karyotype, FISH, Yq microdeletion PCR screening
<i>Sperm DNA integrity defects</i>	Sperm chromatin	Chromatin compaction or DNA fragmentation tests
<i>Specific Gene Defects</i>	DNA sequencing	DNA microarray analysis
Oxidative stress	Reactive oxygen species	Direct methods (chemiluminescence and flow cytometry)
	Total antioxidant capacity (TAC)	Direct method (e.g. enhanced chemiluminescence assay)
	Lipid peroxidation	Indirect assays (e.g. colorimetric)
Sperm fertilisation defects	Zona-pellucida binding	Hemizona assay and sperm-zona binding ratio test
	Acrosome reaction	Acrosome reaction challenge test
	Fusogenic ability	Sperm penetration assay

FISH = fluorescence in situ hybridisation; PCR = polymerase chain reaction.

**Figure 1: Proposed Work-up Plan for Unexplained Male Infertility**



ART = assisted reproductive techniques; ICSI = intracytoplasmic sperm injection; ROS = reactive oxygen species.

unrecognisable in several cases but also for the lacking of randomised clinical trials comparing different treatment modalities. However, two lines of treatment are often used based on the infertility duration and age of the female partner.

### Counselling and Watchful Waiting

Counselling is an important aspect in the management of couples with unexplained infertility, particularly with regard to orientation about the physiology of ovulation and the need to time intercourse with the peri-ovulatory period. A detailed medical history may help to disclose any hidden problems such as sexual dysfunction and inadequate coitus habits.

Watchful waiting is advised for young couples with short duration of infertility and presenting with unrecognisable infertility cause after thorough evaluation. Pregnancy may occur spontaneously without any interventions in cases of unexplained infertility.<sup>87</sup> Hull et al. found a cumulative pregnancy rate (PR) ranging from 50–80 % over a three-year period as a function of female age and 30–80 % PR as a function of infertility duration.<sup>88</sup> Cumulative pregnancy rates of 60 % may be achieved within two years.<sup>87</sup> However, infertility periods longer than three years are associated with very low PR of 1–3 % particularly if the female partner is aged 35 years or older.<sup>87</sup> For couples whose time to conceive is longer than three years, the cumulative PR decreases by 2 % for each year of age after 25.7 years.<sup>89</sup> Due to the costs of infertility treatments and given high proportion of couples with unexplained infertility who spontaneously conceive within a two-year period, it is advisable to defer treatment of couples in this time period unless the female partner is aged 35 years or older.

### Intervention

Interventions, which include medication and/or surgery or assisted conception, are justified when specific causes have been identified, or in unexplained infertility of long duration and/or advanced maternal and paternal age.

### Autoimmune Infertility

The best treatment strategy for immunological male infertility has been the microinjection of the compromised spermatozoa into the oocyte cytoplasm (ICSI), thus bypassing sperm-oocyte membrane interaction. Interestingly, the outcome of ICSI in men with autoimmune infertility is not influenced by the percentage of ASA-bound spermatozoa, by the dominant type of antibodies present, or by the location of ASA on the spermatozoa.<sup>90,91</sup>

### Excessive Oxidative Stress

Therapeutic strategies for men with unexplained infertility and elevated levels of oxidative stress markers include lifestyle habits

modification, use of antioxidants and ART. Patients are advised to quit smoking, eat antioxidant-rich food and avoid pollutant environmental conditions. Oral antioxidant therapy such as carnitine, vitamin C, vitamin E, coenzyme Q10, selenium, glutathione, N-acetyl cysteine, carotenoids therapy has attracted attention in the recent years. A recent Cochrane review on the use of antioxidants for male subfertility suggests that antioxidant supplementation may improve the outcomes of live birth and pregnancy rate for subfertile couples undergoing ART cycles, but further head-to-head comparisons are necessary to identify the superiority of one antioxidant over another.<sup>92</sup>

### Sperm DNA Damage

Successful pregnancies in IVF/ICSI cycles can be obtained using semen samples with a high proportion of DNA damage. Bungum et al. demonstrated that significantly higher clinical pregnancy rates (52.9 versus 22.2 %) and delivery rates (47.1 versus 22.2 %) were obtained after ICSI as compared to IVF when semen samples with high levels of sperm DNA damage were used, as previously suggested.<sup>93</sup>

### Fertilization Defects

Couples should be advised that significantly higher rate of successful pregnancy achieved with IVF-ICSI compared to conventional IVF and IUI in such cases.<sup>62,76,94</sup>

### Donor Insemination

Donor insemination is an alternative when all the above treatment options fail.

### Conclusions

Remarkable developments have been achieved in the field of andrology in the recent years, which significantly improved our understanding of sperm physiology. Novel diagnostic tools envision real time sperm function and may aid in revealing hidden sperm alterations possibly related to infertility. Men facing unexplained infertility are characterised by being childless despite presence of normal semen parameters and normal female partner evaluation. Obviously, classical detailed history taking and physical examination is always necessary to disclose erectile dysfunction problem or irregular coital timing with regard to the peri-ovulatory period. However, when all these measures fail, there is still need to go through more sophisticated and expensive tests to monitor sperm function in more detail. ART may solve the problem of UMF and bypass all the natural barriers that a dysfunctional sperm must face to achieve fertilisation. However, as interventional therapy, ART is not without complications and further studies are needed to refine its role and decrease its impacts on the offspring. ■

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The semen analysis is the cornerstone of the male infertility workup. A specimen is collected by masturbation into a clean, dry, sterile container or during coitus using special condoms (containing no spermicidal lubricants). The patient should be abstinent for 2-3 days prior to maximize sperm number and quality. Routine testicular ultrasonography in infertile men is controversial, but some suggest it because of the increased risk of testicular cancer in infertile men (1 of 200 versus 1 of 20,000 in the general population). [48]. Color-flow ultrasonography is used to evaluate for varicocele using a 7- to 10-MHz probe. Unexplained infertility is really many fertility issues lumped together. It's the doctors don't know why group. Testing and treatment options reviewed. Laparoscopy surgery is no longer done as part of the routine fertility workup. Therefore, we are not finding all of the causes of infertility that we used to - leaving many more couples in the unexplained category. The current rate of unexplained infertility is about 50% for couples with a female partner under age 35, and about 80% by age 40 (see discussion below about age). In reality, there are probably hundreds of "causes" of infertility. There are a lot of things that have to happen perfectly in order to get pregnant and have a baby. Hamadaa, A., Estevesb, S.C. and Agarwal, A. (2012) Unexplained Male Infertility—Looking beyond Routine Semen Analysis. *European Urological Review*, 7, 90-96. has been cited by the following article

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