Unexplained male infertility: potential causes and management
Alaa Hamadaa, Sandro C. Estevesb and Ashok Agarwala

aCenter for Reproductive Medicine, Cleveland Clinic, Cleveland, USA and bANDROFERT – Andrology and Human Reproduction Clinic, Campinas, Brazil

Correspondence to Ashok Agarwal, PhD, HCLD, Lerner College of Medicine, Director, Center for Reproductive Medicine, Cleveland Clinic, Desk A19.1, 9500 Euclid Avenue, Cleveland, Ohio 44195, USA
Tel: 216-444-9485; fax: 216-445-6049; e-mail: agarwaa@ccf.org

Received 28 February 2011
Accepted 12 March 2011

Human Andrology 2011, 1:2–16

Objective
To highlight the concept of unexplained male infertility and discuss the potential causes and its proper management.

Design
Review of literature.

Results
Male infertility of unknown origin is a condition in which fertility impairment occurs spontaneously or due to an obscure or unknown cause. It includes two categories, unexplained male infertility and idiopathic male infertility. The dividing line between them is semen analysis, which is normal in the unexplained category and abnormal in idiopathic infertility. After ruling out female infertility factors, erectile problems and coital factors, modern andrology may help to analyze the unexplained male fertility problem on the basis of cellular and subcellular mechanisms. Furthermore, this analysis may lead to the selection of proper treatment options fitting the needs of patients with unexplained infertility.

Conclusion
Despite the advances and innovation of sophisticated laboratory tests in the field of andrology, further research is still needed to solve the dilemma of infertility.

Keywords:
male infertility, normospermia, sperm function, oxidative stress, autoimmune infertility, assisted reproductive techniques

Introduction
Infertility is a common clinical problem affecting 13–15% of couples worldwide [1]. The prevalence varies throughout developed and underdeveloped countries, being higher in the latter in which limited resources for diagnosis and treatment exist [2]. A male factor is solely responsible for infertility in approximately 20% and contributory in another 30–40% of couples; as such, a male factor is implicated in more than 50% of couples attempting to conceive [3]. A reduction in the male fertility potential may be due to congenital or acquired conditions such as urogenital abnormalities, varicocele, infections of the genital tract, genetic abnormalities, endocrine disturbances, testicular failure, immunologic problems, cancer, systemic diseases, altered lifestyle, and exposure to gonadotoxic factors [4]. The cause of infertility cannot be determined in several cases, despite the advances in diagnosis by the introduction of novel sophisticated tests.

Infertility of unknown origin includes unexplained male infertility and idiopathic male infertility; it is a condition in which fertility impairment occurs spontaneously or due to an obscure or unknown cause. Infertility of unknown origin accounts for 37–58% [5–7]. The category ‘unexplained male infertility’ (UMI) is reserved for infertile men with infertility of unknown origin with normal semen and in which female infertility factors have been ruled out [8]. The reported prevalence of UMI ranges from 6 to 27% [5] and it strongly depends on how exhaustive is the evaluation of the patient. Men classified as having idiopathic male infertility have an unexplained reduction in semen quality with no history associated with fertility problems and have normal findings on physical examination and endocrine laboratory testing. Their routine semen analysis shows decreased number of spermatozoa (oligozoospermia), decreased motility (asthenozoospermia), or an increased proportion of abnormal forms (teratozoospermia). These abnormalities usually occur together and are described as the oligoasthenoteratozoospermia syndrome [6]. This category comprises approximately 31% of infertile men [6]. Table 1 clearly shows the frequency distribution of different causes of male infertility [8]. Furthermore, the frequency of male infertility of unknown origin is different between countries. In a group of 2383 subfertile male individuals attending one of the investigators’ (S.C.E.) tertiary center for male reproduction, 12.1% of the individuals were categorized as having infertility of unknown origin (Table 2).

In brief, the initial assessment of subfertile men includes history, physical examination, and at least two semen analyses after 12 months of unprotected intercourse. However, the initial male work-up may be carried out earlier, particularly in the cases of advanced female age (more than 35 years), the presence of known male...
Table 1 Distribution of final diagnostic categories found in male infertility clinic [8]

<table>
<thead>
<tr>
<th>Category</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunological</td>
<td>–</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>32.6</td>
</tr>
<tr>
<td>Varicocele</td>
<td>26.6</td>
</tr>
<tr>
<td>Obstruction</td>
<td>15.3</td>
</tr>
<tr>
<td>Normal female factor (unexplained male infertility)</td>
<td>10.7</td>
</tr>
<tr>
<td>Cryptorchidism</td>
<td>2.7</td>
</tr>
<tr>
<td>Ejaculatory failure</td>
<td>2.0</td>
</tr>
<tr>
<td>Endocrinologic</td>
<td>1.5</td>
</tr>
<tr>
<td>Drug/radiation</td>
<td>1.4</td>
</tr>
<tr>
<td>Genetic</td>
<td>1.2</td>
</tr>
<tr>
<td>Testicular failure</td>
<td>1.1</td>
</tr>
<tr>
<td>Sexual dysfunction</td>
<td>0.7</td>
</tr>
<tr>
<td>Pyospermia</td>
<td>0.5</td>
</tr>
<tr>
<td>Cancer</td>
<td>0.4</td>
</tr>
<tr>
<td>Systemic disease</td>
<td>0.3</td>
</tr>
<tr>
<td>Infection</td>
<td>0.2</td>
</tr>
<tr>
<td>Torsion</td>
<td>0.1</td>
</tr>
<tr>
<td>Ultrastructural</td>
<td>0.1</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 2 Distribution of diagnostic categories in a group of infertile men attending a male infertility clinic

<table>
<thead>
<tr>
<th>Category</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varicocele</td>
<td>26.4</td>
</tr>
<tr>
<td>Infertility</td>
<td>3.0</td>
</tr>
<tr>
<td>Hormonal</td>
<td>2.3</td>
</tr>
<tr>
<td>Ejaculatory dysfunction</td>
<td>1.2</td>
</tr>
<tr>
<td>Systemic diseases</td>
<td>0.4</td>
</tr>
<tr>
<td>Infertility of unknown origin</td>
<td>12.1</td>
</tr>
<tr>
<td>Immunologic</td>
<td>2.3</td>
</tr>
<tr>
<td>Obstruction</td>
<td>15.1</td>
</tr>
<tr>
<td>Cancer</td>
<td>0.5</td>
</tr>
<tr>
<td>Cryptorchidism</td>
<td>14.3</td>
</tr>
<tr>
<td>Genetic</td>
<td>7.9</td>
</tr>
<tr>
<td>Testicular failure</td>
<td>14.5</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
</tr>
</tbody>
</table>

infertility risk factors (such as an undescended testicle), or if a man questions his fertility potential [3]. In roughly half of the patients, the initial assessment will identify the cause of infertility, whereas many other patients will need to go through several complementary tests to find its cause.

The goals of meticulous evaluation of subfertile men are (i) identification of the cause of subfertility, which is a prerequisite for the correct indication for appropriate surgical or medical treatment, (ii) identification of inadequate lifestyle or sexual behavior to allow counseling toward an improvement in the reproductive potential and overall health status, and (iii) identification of significant medical pathologies that threaten a man’s overall health or life (endocrinopathies, testicular and prostate cancer, brain and spinal cord tumors), which can be identified in up to 6% of infertile men [9–12].

Potential etiologies of unexplained male infertility

Two vital questions come across the clinicians’ mind when dealing with a subfertile male whose medical evaluation is unrevealing. First, how predictive are the semen analyses results in anticipation of spontaneous conception? Second, what should be done next after performing a comprehensive work-up or when failing to identify the causes of infertility? Normal semen analysis results do not guarantee fecundity. A significant proportion of patients with normal semen on routine analysis remain childless over several months attempting to conceive [13]. In one study involving 430 couples, 45% of men with a sperm concentration of greater than 40 million sperm/ml were unable to impregnate their wives [14]. It had been demonstrated that the routine semen analysis was unable to detect sperm functional deficiencies in 40% of men presenting with subfertility [15]. Table 3 shows the frequency of semen analysis abnormalities in 8758 infertile patients attending the fertility clinic.

For men with unexplained infertility and normal semen analyses the following possibilities should be considered: (i) presence of a female factor, (ii) inappropriate coital habits, (iii) erectile dysfunction, (iv) the presence of antisperm antibodies (ASAs) (autoimmune infertility), and (v) sperm dysfunction [12]. To exclude the first three conditions, proper history taking as well as a thorough gynecological evaluation is needed, whereas modern andrology could be of help in managing the last two conditions.

Autoimmune infertility

Autoimmune infertility has long been postulated as one of the causes of subfertility [16]. It represents approximately 4.5% of male factor infertility [6]. Why does a man’s body treat his sperm as foreign invaders? To explain this, three theories are hypothesized. The first states that sperm are not present at the time of embryological development during which the immune system establishes tolerance to self antigens [17]. The second claims that spermatozoa are haploid and have a different chromosomal make up from the somatic cells [16]. Meanwhile, the third theory, called ‘immunosuppression theory’, postulates that T suppressor lymphocytes, which inhibit immune responsiveness, are activated by small amounts of spermatozoal antigens continuously leaked from the genital tract [18]. As soon as spermatozoa, which are considered immunologically foreign cells, are formed during puberty, they must be completely isolated from the immune system. This isolation occurs within the testis, one of the immunologically privileged sites, by the blood–testis barrier. In other regions of the male genital tract, the epithelial lining, probably supplemented by a local immunosuppressive barrier, is responsible for this isolation [19]. Despite its immune-privileged status, the

Table 3 Distribution of abnormalities of semen parameters in 8758 patients [8]

<table>
<thead>
<tr>
<th>Abnormality in semen parameters</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoospermia</td>
<td>4</td>
</tr>
<tr>
<td>Predominance of a single abnormal parameter</td>
<td>29</td>
</tr>
<tr>
<td>Motility</td>
<td>18</td>
</tr>
<tr>
<td>Volume</td>
<td>2</td>
</tr>
<tr>
<td>Morphology</td>
<td>7</td>
</tr>
<tr>
<td>Density</td>
<td>2</td>
</tr>
<tr>
<td>Defects in two or more parameters</td>
<td>37</td>
</tr>
<tr>
<td>All parameters normal (unexplained male infertility)</td>
<td>30</td>
</tr>
</tbody>
</table>
testis is clearly capable of mounting inflammatory responses, as proven by its effective cellular and humoral defense against infections. In pathological circumstances, the imbalance between the tolerogenic and the effenter limbs of the testicular immune response can lead to the development of ASAs, and in rare instances, to autoimmune epididymoorchitis [16,20–22]. Both humoral and cellular immunity have been implicated in the etiology of immune infertility.

**Humoral immune infertility**

Approximately 10% of all infertile men may have ASA (vs. 2% of fertile men) [23]. Unexplained male factor infertility may be related to ASAs. Moghissi et al. [24] confirmed that the incidence of sperm antibodies was significantly higher (42.5%) among patients with unexplained and persistent infertility. The pathogenesis of the formation of ASAs is still a matter of debate. Antisperm immune responses occur probably as a result of the disruption of the epithelial or blood–testis barrier [25,26], an immunosuppression defect [27], or as a result of an insult to the genital tract that would provide an excess of spermatozoal antigens that could override the mechanism of immunosuppression [28]. A major cause of ASA is vasectomy [29–31]. Other causes include vas obstruction [29], testicular trauma, torsion, malignancy, infection of the genital tract, semen deposition at nongenital tract sites (homosexuality), and perhaps, varicocele [31–33] and intolerance to heavy metals [34]. It is still unclear whether ASA is produced locally in the genital tract or transudated from serum. ASA can be found in serum, seminal plasma, and can be sperm bound. Among these, sperm-bound antibodies are the most clinically relevant. The antibody classes that appear to be clinically relevant include immunoglobulin G (IgG) and IgA. IgG antibody is derived from local production and from transudation from the bloodstream. IgA, in contrast, is thought to be purely locally derived [35]. The epididymis is postulated to be the production site in cases of obstruction because of the combined effect of increased intraluminal pressure and leak of spermatozoa antigens. In contrast, a woman can also produce ASAs in her cervical fluid. Such antibodies have been reported in 7–17% of infertile women, varying with the type of test performed and the population screened [36,37]. ASA impair sperm function by induction of apoptosis, or by inducing premature acrosome reaction (AR). ASA may also interfere with fertilization by inhibition of cervical mucus penetration, zona pellucida (ZP) binding or sperm–oocyte fusion. ASA may change some macromolecular and subcellular function by altering chaperon function, protein folding, and disulfide bonds [38]. The end result is that pregnancy rates may be reduced by ASA [39]. When dealing with patients with unexplained male infertility, one should keep in mind that the presence of elevated levels of ASA may occur even in the face of normal semen analysis. Sperm agglutination is the only well-established semen alteration related to the presence of ASA [40]. However, sperm agglutination, which is a time-dependent phenomenon, only rarely involves a large proportion of motile spermatozoa soon after liquefaction, even when all ejaculated spermatozoa are antibody coated. Therefore, sperm agglutination, although extremely suggestive of sperm autoimmunization, does not represent an important mechanism of the antibody interference with fertility in most cases. Apart from sperm agglutination, there is little evidence that suggests a cause/effect relationship between ASA and the abnormality of semen parameters [39]. A negative impact of ASA on sperm motility/vitality, for instance, should involve a complement-mediated sperm injury, which is prevented by anticomplementary activity in human seminal plasma [41,42]. Nevertheless, adequate amount of complement is present in the cervical fluid, which can be activated through antibody antigen reaction and can exert a toxic effect on sperm.

The diagnosis of immunological infertility requires two conditions to be satisfied [43].

1. Fifty percent or more of the motile spermatozoa (progressive and nonprogressive) have attached beads. 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;

2. 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 AR [43].

Frequently, antibody-coated sperm may appear as a poor postcoital test (PCT). Complement, which is normally found in higher amounts in the cervical mucus than in the seminal plasma, is needed to immobilize spermatozoa. The antibody complement reaction may take at least 6 h to manifest. Physicians performing a PCT within 2 h after intercourse or using in-vitro mucus penetration assays may miss the immobilizing antibodies. Consequently, these patients may appear as having an absence of male factor infertility. It is therefore advisable to perform a PCT after at least 6 h after intercourse [44]. ASA can cause infertility without obvious problems with cervical mucus penetration. Such antibodies may interfere with the AR and may inhibit sperm penetration into the zona pellucida and fusion with the oocyte [45].

Currently, the most popular tests to identify sperm-bound ASA are both the direct immunobead test (IBT) and the direct mixed agglutination reaction [46]. In the direct IBT, beads coated with covalently bound rabbit antihuman immunoglobulins against IgG or IgA are mixed directly with washed spermatozoa. The binding of beads with antihuman IgG or IgA to motile spermatozoa indicates the presence of IgG or IgA antibodies on sperm surface [43]. IBT is more time consuming but it identifies the proportion of antibody-bound sperm in a given sample, the antibody class, and the location of antibodies on the sperm surface. In contrast, the direct mixed agglutination reaction test is an inexpensive, quick, and sensitive screening test in which sheep erythrocytes are used instead of immunobeads to detect and localize antibody-bound sperm [47,48].
Cellular immune infertility
There is evidence that cell-mediated immunity may play a role in immunological infertility. Histopathological studies demonstrated the presence of inflammatory cells infiltrating the contralateral testis in animal models and patients with unilateral testicular torsion because of release of germ cell inoculums in response to ischemia and necrosis of the torsed testis [49,50]. Despite this immunologic event, ASA are rarely seen in such cases. Other supporting evidence come from immunoreactivity studies using purified macrophages isolated from individuals with repaired unilateral and bilateral cryptorchidism and exposed to homologous sperm. It had been found that 50% of patients with unilateral and 80% with bilateral surgically repaired cryptorchidism had cell-mediated immunoreactivity [51]. Sperm granuloma at the vasectomy site is another evidence of cell-mediated immunity; it represents a dynamic structure and a site of spermatozoal phagocytosis. Intraluminal macrophages, also known as spermatoocytes, absorb degradation products rather than the whole sperm. T-type lymphocytes, in addition to ASA, may cause testicular damage after vasectomy [52]. Spermatozoa exposed to cytokines such as tumor necrosis factor and interferon γ show impairment in motility and inability to penetrate hamster eggs [53,54]. Despite all this, the full-blown spectrum of cell-mediated immunity is difficult to prove using laboratory tests and its role in unexplained infertility is still speculative [47]. More sophisticated investigation is needed to detect the impact of cellular immunity in men with unexplained infertility.

Deficient sperm function
Conventional semen parameters such as sperm count, motility, vitality, and morphology are inadequate to monitor sperm function and to be used as markers of fertility potential [55]. Conversely, sperm function tests may provide more clinically useful prognostic and/or diagnostic information. Such tests may be used to distinguish between fertile and infertile men and to aid in showing the cause of male subfertility and in suggesting therapeutics. Sperm function tests available in the andrology armamentarium include assays that investigate sperm DNA integrity, seminal reactive oxygen species, AR, hyperactivated motility, and ZP binding and penetration.

Sperm chromosomal complement and DNA integrity defects
Germ cells undergo meiotic divisions to form four haploid spermatids. During meiosis, shuffling of some genes occurs between homologous chromosomes giving rise to genetic diversity. During spermiogenesis, the haploid sperm chromatin undergoes significant changes in which most histones are replaced first by transition proteins, and then by positively charged protamines [56]. By this remodeling process, the sperm DNA condenses so tightly that it is resistant to mechanical stresses such as sonication [57] and even to boiling [57], which destroy the DNA in somatic cells. The condensation of sperm DNA protects it during its transit through the male and female reproductive tracts. Cytogenetic analysis and molecular biology genetic testing may identify subfertile men misdiagnosed as having unexplained and idiopathic infertility. Abnormalities causing male infertility include:

1. Chromosomal complement changes in number (e.g., aneuploidy) or structure such as translocations or inversions.
2. Gene mutation and polymorphisms.
3. DNA integrity defects.

Chromosomal complement: the risk of sperm chromosomal aneuploidy is inversely related to sperm concentration and total progressive motility [58,59]. The overall frequency of chromosomally abnormal sperm in the general population is estimated to be 7%. The mean frequency of disomy for autosomes and sex chromosomes is 0.13 and 0.37%, respectively, whereas the rate for diploidy is 0.2%. For normospermic infertile men, correspondent figures are 0.11, 0.44, and 0.3–1%, respectively [60,61]. Increased sperm aneuploidy rates may impact male fertility and pregnancy viability. Their causes are unknown, but smoking, alcohol, chemotherapy, and aging may play a role. Interchromosomal variation in the rates of disomies has been observed with sex chromosomes and chromosomes 21 and 22; the higher rate of abnormalities related to such chromosomes may be due to their lower rate of meiotic recombination, which renders them more prone to nondisjunction [62].

Abnormal spermatozoa that retain excess cytoplasm show a greater extent of aneuploidy and diploidy than those without excess cytoplasm from the same ejaculate, whether selected by density gradient centrifugation [63], swim-up [64], or binding to hyaluronic acid [65]. In contrast, both morphologically normal and abnormal spermatozoa can be disomic or diploid [66], or contain damaged DNA [67]; as such, selecting normal-looking spermatozoa for assisted reproductive technique (ART) does not guarantee the absence of chromosomal abnormalities.

Chromosomal inversions, deletions, balanced or unbalanced translocations, and Y-chromosome microdeletions are often associated with abnormal semen parameters and higher rates of abortion, and in some cases, with a higher risk for the birth of a severely handicapped child [68]. In Y-chromosome infertility, the AZF region is prone to many smaller subdeletions that are thought to be caused by intrachromosomal recombination [69]. These partial deletions produce a wide array of phenotypes, ranging from normospermia to azoospermia, because of factors that include the interaction of the environment and the genetic background [70].

Most chromosomal abnormalities may be detected by using one of the following methods: (i) Sperm karyotyping for detection of numerical chromosomal abnormalities; (ii) Fluorescence in situ hybridization analysis, which can be used to assess numerical and structural chromosomal changes by using specific probes; and (iii) quantitative polymerase chain reaction, which is a promising technique to detect and quantify damage to nuclear and mitochondrial DNA [71].

Specific gene defect (mutations and polymorphisms): the definition of nucleotide sequence for the human genome
has facilitated the identification of human fertility-related genes and this will open the way to identify these genes in the future. Nevertheless, DNA sequence analysis is rarely performed in the evaluation of male infertility [72]. In animal studies involving mice, up to 300 null mutations and 50 conditional targeted deletions have produced models of male infertility. Not only the DNA sequence has effect on male infertility but there is also a role for epigenetic events and modifiers of gene expression. With regard to unexplained male infertility, genes certainly play a role as they control meiosis events, spermiogenesis, remodeling, motility, capacitation, and fertilization. It is now possible to monitor the expression of thousands of genes simultaneously with DNA microarray analysis. Garrido et al. [73] used the microarray technology in the analysis of spermatozoa from fertile and infertile men with normal semen analysis. They found that hundreds of gene sequences (targets) were differentially expressed between groups. Preliminary results confirmed that there are few genes that are overexpressed, whereas all others are underexpressed in infertile men.

**DNA integrity defects:** sperm DNA integrity is increasingly being recognized as an important marker of fertilizing efficiency, and it is associated with better diagnostic and prognostic values than standard sperm parameters [74]. Saleh et al. [75] reported that an increase of spermatozoa with abnormal chromatin structure or DNA damage (expressed as DNA fragmentation index) negatively correlated with intracytoplasmic sperm injection (ICSI) and in-vitro fertilization (IVF) outcomes.

Populations of sperm with DNA damage are more often seen in subfertile/infertile men than in fertile ones [76–78] Spermatozoa with damaged DNA may lead to paternal transmission of defective genetic material with adverse consequences to embryo development [79,80]. Approximately 8% of infertile men have abnormal DNA integrity despite normal semen parameters [81].

DNA damage is often assessed by the determination of chromatin compaction or DNA fragmentation. The former examines the accessibility of dyes (Toluidine 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 [82]. Toluidine and aniline blue stains bind to lightly packed chromatin and to lysine residues of histone that are not fully replaced by protamines, respectively. Chromomycin A3 binding is specific for protamine-deficient areas because of its affinity to guanine–cytosine (GC)-rich areas of DNA [83]. In contrast, DNA fragmentation is measured by detecting single-strand or double-strand DNA breaks. Transferase-mediated dTUP nick-end labeling, comet assay, acidine orange test, and sperm chromatin structure assay are methods clinically available to detect DNA fragmentation. Although they differ in costs and methods, most of the mentioned tests are clinically significant and correlate with sperm function and fertility [84].

**Reactive oxygen species**

It has been shown that 40–88% of nonselected infertile patients have high levels of seminal ROS [85]. Moreover, normospermic infertile men have higher ROS levels and reduced total antioxidant capacity levels than the normospermic fertile counterpart [86]. However, the true prevalence of oxidative stress problem among normospermic men remains to be determined. Mammalian spermatozoa are redox cells that are able to produce oxygen radicals and to export them to the extracellular medium [87–91].

The main source of oxygen radicals in spermatozoa appears to be the mitochondria, as the result of the monovalent reduction of molecular oxygen during oxidative phosphorylation [88]. ROS refer to a group of metabolites formed by reduction of oxygen, including free radicals such as superoxide anion (O2– ●), the hydroxyl radical (OH●) as well as powerful oxidants such as hydrogen peroxide (H2O2). It also includes reactants of carbon-centered radicals with molecular oxygen, including peroxyl radicals (ROO●), alkoxyl radicals (RO●), and organic hydroperoxides. ROS may also include other powerful oxidants such as peroxyxinitrite or hypochlorous acid as well as the highly biologically active free radical, nitric oxide (●NO). ROS in semen originate from immature spermatozoa and seminal leukocytes [92]. These free radicals have considerable reactivity and the ability to react with and modify the structure of many different kinds of biomolecules, including proteins, lipids, and nucleic acids. The wide range of targets that can be attacked by ROS is a critical facet of their chemistry that contributes significantly to the pathological importance of these molecules [92]. ROS in low levels have a physiological role. They are required by sperm to attain their functional maturity and are essential for capacitation, hyperactivation, and AR [93,94]. ROS also exert their effect on sperm–oocyte interaction. Low levels of lipid peroxidation cause modifications of plasma membranes facilitating sperm adhesion to the oocyte [95]. However, the physiological ROS levels are still undetermined.

A natural antioxidant defense system offers protection against ROS. It consists of radical scavengers, chain-breaking antioxidants, and ROS-metabolizing enzymes in the vicinity of the spermatozoa during their sojourn in the male reproductive tract. Radical scavengers include small molecular mass such as vitamin C, uric acid, tryptophan, and taurine [96,97]. Chain-breaking antioxidants include membrane-associated antioxidants epitomized by α-tocopherol, a hydrophobic vitamin that is capable of terminating the peroxidation chain reaction [98]. Spermatozoa also harbor antioxidant enzymes such as superoxide dismutase and those of the glutathione cycle, but very little catalase. Imbalance between the oxidant load and natural antioxidant defense system has a pathological effect on sperm function by causing damage to sperm DNA in the nucleus and mitochondria as well as by inducing lipid peroxidation in the sperm plasma membrane [99]. DNA damage includes single-stranded or double-stranded DNA breaks, DNA base-pair oxidation,
chromatin cross-linking, chromosome microdeletions, and even various types of gene mutations such as deletions, point mutations, or polymorphisms that may result in decreased semen quality [100,101]. 8-Hydroxy-2-deoxyguanosine is considered a key biomarker of oxidative DNA damage [102]. ROS may induce lipid peroxidation and hence loss of sperm motility and may even initiate a chain reaction by activating caspasess that ultimately lead to apoptosis [103].

The most often used methods for detecting ROS in an andrology setting are divided into two major categories, that is, direct methods such as chemiluminescence and flow cytometry, and indirect methods such as the colorimetric one. Chemiluminescence uses the probes lucigenin or luminol to detect ROS [104,105]. Luminol (C8H7N3O2) is a versatile chemical that shows chemiluminescence when mixed with an appropriate oxidizing agent. It can penetrate inside the cell and react with intracellular reactive oxygen species, in addition to extracellular ones. This probe may undergo a one electron oxidation before it becomes sensitized to the presence of ROS, which can be accomplished by the addition of horseradish peroxidase to promote luminol oxidation in the extracellular space. In the absence of exogenous horseradish peroxidase, the assay is dependent on the presence of intracellular peroxidase to activate the probe [104–107]. The one electron oxidation of luminol leads to the creation of a radical species (LK). The latter then interacts with ground-state oxygen to produce O2● that induces the oxygenation of LK to create an unstable endoperoxide, which ultimately breaks down with the release of light. Lucigenin is a positively charged molecule and hence it cannot enter the cell and therefore could only measure the extracellular ROS only. Photons produced are converted to an electrical signal and measured using a luminometer [108], with ROS generation being measured as counted photons per minute. The normal range is less than 0.2 × 106 cpm per 20 million spermatozoa [109]. Intracellular ROS can be measured by flow cytometry using different fluorescent probes such as 2′, 7′-dichlorofluorescein-diacetate, hydroethidine, that react with ROS to emit a red fluorescence [110]. The colorimetric technique is also widely used for indirectly measuring ROS. It is based on the principle of spectrophotometry and measures lipid peroxide end products, mainly malondialdehyde, lipid hydroperoxides, and isoprostanes [111].

Fertilization defects

The sperm fertilizing potential is related to its ability of undergoing capacitation, which includes the acquisition of hyperactivated motility, and the acrosomal reaction to penetrate the ZP and its ability to fuse with oolema. Normospermic infertile men may have defective sperm that are unable to fertilize. This assumption is based on the observation of low success rates of IVF and intrauterine insemination (IUI) in certain cases of unexplained infertility. The major cause of fertilization failure in conventional IVF [112] is due to abnormalities of sperm–ZP binding and penetration. Although most sperm–ZP binding and penetration defects are due to obvious sperm abnormalities such as asthenozoospermia and teratozoospermia, many patients have normal semen analysis and subtle sperm defects that affect sperm–ZP interaction. These defects cannot be shown by routine semen analysis but are apparent with sperm–ZP interaction tests [113].

Zona pellucida binding defects: sperm binding to the ZP is attributed to the presence of complementary binding sites or receptors on the surface of the gametes; typically, these receptors manifest a high degree of species specificity [114–116]. Human ZP (hZP) is composed of four major glycoproteins (hZP1, hZP2, hZP3, and hZP4) [117]. The ZP3 of human oocytes is believed to be the primary ZP receptor for capacitated acrosome-intact sperm binding [117,118]. In contrast, the exact nature of human sperm receptors for the ZP has not been established. Although a number of candidate sperm proteins have been found to be able to interact with either solubilized or intact ZP, it is not clear whether or not they are the primary receptors for sperm binding to the ZP [118–121]. Sperm binding to ZP3 induces a signal transduction cascade within the spermatozoon, involving multiple proteins and other factors, including protein kinases A and C pathways [122], that leads to the AR. Acrosome-reacted spermatozoa are believed to bind to ZP2, which facilitates the penetration to the zona matrix and progression into the perivitelline space [123]. Defective ZP-bound sperm are present in approximately 15 and 25% of subfertile men with a normal semen analysis and with an abnormal one, respectively [124–126]. Such individuals have a reduced chance of achieving successful fertilization when undergoing IVF [126]. Mackenna et al. [127] reported that two of 18 men with unexplained infertility showed lack of sperm binding to the zona despite having sperm morphology and hyperactivation status similar to fertile individuals. The presence of defective sperm–ZP binding in infertile men with normal semen may be due to defective signal transduction pathways upstream of protein kinases A and C. However, most defective sperm–ZP binding infertile men with normal semen and those with severe teratozoospermia are likely to have downstream disorders, structural defects, or absence of sperm receptors for binding the ZP.

Two tests of sperm binding to the human zona have been described: (i) the hemizona assay and (ii) the sperm–zona binding ratio test. In the former, a single zona is bisected and each zona half is incubated with control and patient sperm suspensions [128]. In the latter, a complete zona is incubated with equal numbers of motile spermatozoa from control and test populations, each labeled with a different fluorescent dye [129]. In each case, the number of spermatozoa from each population bound per whole or half zona is counted and the number of test sperm is expressed as a ratio of that of the control.

Capacitation defects: capacitation is a combination of concomitant processes; mainly, the sperm acquisition of a motility pattern known as hyperactivated motility, which enables efficient zona drilling and allows spermatozoa to reach the oolema, and preparation for the AR
Defects in capacitation may explain subfertility in some normospermic infertile men.

Hyperactivation is considered the first step of the complex capacitation process. It involves a typical swimming pattern of movement shown by most sperm retrieved from the oviductal ampulla at the time of fertilization [131]. Hyperactivated sperm show high-amplitude and asymmetrical flagellar bending movement. Hyperactivation is characterized by switching of sperm movement from progressive motility to more vigorous (nonprogressive) flagellar motion. The role of hyperactivation is to enhance the ability of sperm to detach from the oviduct wall, to move around in its labyrinthine lumen, to penetrate into the cumulus oophorus, and finally, to penetrate the ZP of the oocyte [132]. However, little is known about the mechanisms that lead the sperm to hyperactivation. It is speculated that specific signals appear within the oviduct shortly before ovulation. There is evidence that various components of the female reproductive tract serve as physiological stimuli of hyperactivation, such as hormones (e.g., progesterone), ions, and secretions in the oviduct luminal fluid [133].

When the oocyte enters the oviduct, it usually brings along cumulus oophorus and FF that have been shown to influence sperm motility. A number of physiological factors such as Ca²⁺, c-AMP, bicarbonate, and metabolic substrates have been found as essential for the initiation or the maintenance of hyperactivated motility in vitro [134]. Recent studies have demonstrated that increased intracellular calcium entry through voltage gated calcium channels (Cation channel of sperm; CatSper1–4) in the principal piece of the sperm flagellum is the prime mechanism for hyperactivation [135–137]. This entry is induced by intracellular alkalinization because of extrusion of H⁺ through voltage gated proton pumps, which are also located in the principal piece of the flagellum [135]. Increased intracellular pH and intracellular Ca²⁺ regulate not only the hyperactivation process but also the AR and the ability of the sperm to fertilize the egg [135]. Interestingly, molecular studies on CatSper ion channel show that it is a novel protein complex composed of six subunits. Of these, four are α subunits (CatSper1–4) with calcium-selective pore and two are transmembrane proteins with large extracellular domains, called CatSperβ and CatSperγ, of unknown functions [138,139]. For humans, hyperactivation is not well defined as it is for other species [134], and only a small proportion of the sperm population may be hyperactivated at each time. The extent of hyperactivated motility in a population is positively correlated with the extent of zona binding, the AR, zona-free oocyte penetration, and fertilizing capacity in vitro [140].

Migration of spermatozoa toward the oocyte in the oviduct’s ampulla is probably assisted by chemotaxis, which is the migration of spermatozoa toward a thermal gradient. The ampulla has a higher temperature than the isthmus of the fallopian tube and this may mediate long-range migration to the ampulla [141]. It has been shown that only capacitated spermatozoa respond to these influences [141]. The spermatozoa may also be directed to the egg within the cumulus by chemotaxis, which is the migration of spermatozoa toward a higher concentration of chemoattractant [142].

Assessment of hyperactivation motility in vitro involves the use of computerized motion analysis in conjunction to kinematics module to distinguish different subpopulations of motile spermatozoa. Hyperactivated spermatozoa can be distinguished from nonhyperactivated 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 [143]. Munir et al. [144] showed that there is a significant decrease in the percentage of hyperactivated sperm, sperm motility, progressive motility, and VCL from infertile men in comparison with sperm from fertile donors after overnight incubation with capacitating conditions, whereas linearity was increased in the former. Computerized assessment of follicular fluid (FF)-induced hyperactivation has been proved to be significantly lower in patients with unexplained infertility in comparison with normal fertile men [128]. The absence of hyperactivation after the addition of FF was observed in 39% of patients with unexplained infertility [127]; it is likely that spermatozoa from such patients have reduced ability to penetrate through the oocyte vestments and ZP as a result of this abnormal hyperactivation response to FF. In fact, Avenarius et al. [145] discovered that male patients with mutated CatSper1 gene are infertile with poor hyperactivation response despite their normal sperm count, morphology, and even their initial sperm motility. Furthermore, an animal study on mice concluded that mutation in each of CatSper (1–4) ion channel protein can lead to infertility despite normal semen parameters, normal testicular histology, size, and weight [146]. Interestingly, there are two known CatSper2 gene-related mutations in humans that cause male infertility, termed CatSper-related nonsyndromic male infertility and deafness–infertility syndrome [147]. However, both syndromes are associated with gross semen abnormalities. Further investigation is needed to show the genetic and molecular nature of fertilization in patients with defective hyperactivation response and unexplained infertility. Moreover, minor mutations in human CatSper (1–4) genes are yet to be deciphered in men with unexplained infertility.

Acrosome reaction: The AR is defined as the process of fusion of sperm plasma membrane with the outer acrosomal membrane leading to the release of exocytotic proteolytic enzymes (acrosine and hyaluronidase) in response to sperm–ZP binding. ZP3 is considered the natural stimulus for the AR, which leads to the proteolytic dissolution of the ZP. Human sperm initiate primary binding to the ZP with intact acrosome [148]. Glycoprotein (ZP3), present in the ZP, is involved in the induction of the AR [149]. Artificial stimuli used in vitro to challenge the AR are calcium ionophore A23187 and progesterone. There are two types of defective AR, which have clinical significance. The first is the AR prematurity, which is defined as a high level of spontaneous AR.
ZPIAR rates spermatozoa–ZP binding, but significantly reduced 0.5 mmol of zinc to the culture media had no effect on significantly higher in men with defective ZPIAR. Addition of shown that the seminal zinc concentration was signifi- cantly higher in subfertile men. In normozoospermic men, it has been shown overlying plasma membrane in severe teratozoospermic abnormalities, or associated abnormalities in the structural defects of the sperm head, such as small or defective ZPIAR is more likely to be related to major defects. In men with normal sperm morphology, defective ZPIAR may be caused by different mechanisms [160–163]. It is therefore apparent that defective ZPIAR; currently, only spermatozoa–ZP interaction tests using human zona-free hamster oocyte penetration test. Although this test does not assess sperm–ZP, it measures the spermatozoan’s ability to undergo capacitation, AR, fusion and penetration through the zona, and decondensation within the cytoplasm of an oocyte. The ZP is removed from a hamster oocyte, which is then incubated with human spermatozoa. In the original test, scoring is achieved by calculating the percentage of ova that are penetrated; normal sperm are able to penetrate 10–30% of hamster ova [68]. Recent refinement of this test is performed by incubating sperm in more potent capacitating media, which allow the majority of ova to be penetrated; scores are obtained by calculating the number of sperm that penetrate each ovum [68]. Aitken et al. [170] reported that 34.1% of patients with unexplained infertility had less than 10% oocyte penetration against 0% in a control group of fertile men. 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 [171], whereas others have claimed 100% predictability [172]. Taking an average from different studies, a normal SPA may have 70% predictability of fertilization in vitro [44]. Nevertheless, semen samples that fail to fertilize hamster ova usually are unable to fertilize human ova [68]. Although the SPA is considered a research tool, it may be of clinical value for men with unexplained infertility with poor fertilization rate on IVF.

A practical approach for the assessment of men with unexplained infertility

PCT, if appropriately timed and performed, can be the initial test for couples with unexplained male infertility. Cervical mucus is normally hostile to sperm, except near the time of ovulation. The absence of sperm on a PCT in the presence of normal semen parameters suggests incorrect coital technique or failure to ejaculate into vagina, whereas the presence of normal sperm numbers but reduced motility or a shaking motion on a PCT is suggestive of the presence of ASAs [173]. The finding of a normal PCT raises the possibility of a functional sperm defect. Assessment of sperm function can be divided into two steps. The first step should be to check the competence of the sperm before the fertilization event by measuring the levels of ROS and DNA integrity defects. The second step should include the assessment of the fertilization potential of sperm, especially for those patients with a history of failure of conventional IVF. These tests include sperm–ZP binding assay.
incidence of side effects [178]. Moreover, efficacy of suppressive therapy had been tried in early years but it is formation of ASA in the cervical mucus [177]. Immuno- tract and hence decrease the sensitization and for frequent exposure of sperm to the female reproductive- condoms and systemic steroid. Condoms are theoretical- bound ASA. ASA titers may be decreased by using either decrease ASA production or to remove sperm- Treatment of immune infertility includes methods to disclose any hidden problems such as sexual dysfunction and inadequate coitus habits.

**Expectant management**

Expectant management is advised for young couples with a short duration of infertility. Pregnancy may occur spontaneously without any interventions in cases of unexplained infertility [174]. Hull et al. [175] found a cumulative pregnancy rate (PR) ranging from 50–80% over a 3-year period as a function of female age and 30–80% PR as a function of infertility duration. Cumulative PRs of 60% may be achieved within 2 years. However, infertility periods longer than 3 years are associated with very low PR of 1–3%, particularly if the female partner is aged 35 years or older [174]. For couples whose time to conceive is longer than 3 years, the cumulative PR decreases by 2% for each year of age after 25.7 years [176]. Owing to the costs of infertility treatments and given high proportion of couples with unexplained infertility who spontaneously conceive within a 2-year period, it is advisable to defer treatment of couples in this time period, unless the female partner is aged 35 years or older.

**Interventional management**

Interventions, which include medication and/or surgery or assisted conception, are justified in cases of unexplained infertility of long duration and/or advanced maternal and paternal age.

**Immunological infertility**

Treatment of immune infertility includes methods to either decrease ASA production or to remove sperm-bound ASA. ASA titers may be decreased by using condoms and systemic steroid. Condoms are of theoretical benefit because they may help to lessen the chances for frequent exposure of sperm to the female reproductive tract and hence decrease the sensitization and formation of ASA in the cervical mucus [177]. Immuno-suppressive therapy had been tried in early years but it is seldom used nowadays, mainly because of the high incidence of side effects [178]. Moreover, efficacy of steroids remains unclear as most studies lack appropriate placebo controls or have used different regimens and drugs. Despite these shortcomings, two prospective and randomized placebo-controlled studies were conducted and showed conflicting results. In the study of Hendry et al. [178], 40 mg of prednisolone was given for a 6-month period from cycle days 1–10 of the female partner, and then was tapered rapidly for the next 2 days. The PR of treated and untreated groups was 31% and 9%, respectively. In another study, the investigators reported similar PR after administration of methylprednisolone for three cycles, despite a significant decrease in sperm-associated IgG (but not IgA) in the steroid treatment group [179]. It has been shown that steroids may be only effective in removing sperm-bound ASA in the presence of low antibody titer [180]. Treatment with high-dose steroids for long time is associated with side effects that include mood changes, fluid retention, dyspepsia, gastrointestinal bleeding, aseptic necrosis of the hip joint, and significant decrease of bone mineral density in up to 60% of the patients [178,181,182].

Alternatively, methods to remove ASA already bound to sperm include sperm washing and IgA protease treatment. The effectiveness of these techniques in recovering antibodies-free spermatozoa is conflicting; most reports show limited success because of the difficulty of eluting the sperm cell surface by any washing method [183]. Esteves et al. [184] demonstrated that the population of antibody-free spermatozoa was increased by 29% after discontinuous colloidal gradient centrifugation. However, the investigators observed that sperm washing was ineffective to remove ASA in approximately 30% of the cases, and advise that the potential benefit of this strategy has to be tested individually. Microinjection of the compromised spermatozoa into the oocyte cytoplasm (ICSI) bypasses sperm–oocyte membrane interaction, and ICSI has been shown to increase fertilization when compared with conventional IVF in cases of male immunologic infertility. Nagy et al. [185] analyzed the outcome of ICSI in 37 men with a proportion of antisperm antibody-bound spermatozoa of 80% or higher. They concluded that fertilization, cleavage, and PRs after ICSI were 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. However, embryo quality was lower in the ASA-positive group. In another study, similar results were observed but a higher rate of first trimester pregnancy loss was observed in the ASA-positive group [186]. Clarke et al. [187] and Check et al. [188] studied 39 patients with a strong positivity on IBT (> 80%) and 93 patients with various degrees of autoantibodies, respectively. They found that fertilization and PRs were comparable between different levels of ASA on sperm. Esteves et al. [189] analyzed a large cohort of 351 patients and confirmed that fertilization, cleavage, and PRs after ICSI were not influenced by the ASA levels on sperm. These investigators observed neither the negative impact of ASA on embryo quality and cleavage rate nor an increase in pregnancy loss, as reported by other investigators. They also compared ICSI outcomes between patients with ASA...
positivity and a group of patients in which ICSI was indicated for other reasons. Fertilization, embryo development, pregnancy success, and miscarriage rates after ICSI in men showing varying levels of autoimmune immunity against spermatozoa were within the same range as our population of ICSI patients with severely abnormal seminal parameters. The investigators conclude by suggesting that ASA may become inactive within the ooplasm after microinjection, or that a segregation process may occur during the first cleavage divisions, similar to the inactivation and segregation processes that also occur with the acrosome and sperm tail after microinjection.

Excessive oxidative stress

Men with unexplained infertility may have higher oxidative stress than controls [86,190]. Lines of therapy include lifestyle habit modification, use of antioxidants, and ART. Patients are advised to quit smoking, eat antioxidant-rich food, and avoid pollutant environmental conditions. An antioxidant therapy has attracted attention in the recent years. Antioxidants are compounds and reactants that dispose, scavenge, and suppress the formation of ROS, or oppose their actions. Various antioxidants such as carnitine, vitamin C, vitamin E, coenzyme Q10, selenium, glutathione, Α-acetyl cysteine, carotenoids, and trace metals are available. A recent Cochrane review on the use of antioxidants for male subfertility suggests that antioxidant supplementation may improve the outcomes of live birth and PR for subfertile couples undergoing ART cycles, but further head-to-head comparisons are necessary to identify the superiority of one antioxidant over another [191]. In addition, therapeutic dosing, duration, and the toxic levels of ROS are still to be determined.

DNA damage

The management of unexplained male subfertility because of DNA damage often requires ART. The probability of fertilization in vitro and by IUI seems to be low when the proportion of sperm cells with DNA damage exceeds 30 and 12%, as detected respectively by sperm chromatin structure assay or transferase-mediated dTUP nick-end labeling [192,193]. Sperm DNA damage is negatively correlated with embryo quality and blastocyst formation in IVF cycles and with fertilization rates both in IVF and ICSI cycles [194]. However, successful pregnancies in IVF/ICSI cycles can be obtained using semen samples with a high proportion of DNA damage. Bungum et al. [195] demonstrated that significantly higher clinical PRs (52.9 vs. 22.2%) and delivery rates (47.1 vs. 22.2%) were obtained after ICSI as compared with IVF when semen samples with high levels of sperm DNA damage were used, as previously suggested.

The activation of embryonic genome expression occurs at the four-cell to eight-cell stage in human embryos. Therefore, the paternal genome may not be effective until this stage and it is speculated that an elevated level of sperm DNA strand breaks seems to be of importance in the later stages of embryonic development. Aitken and Krausz [196] proposed that sperm DNA damage is promotagenic and can give rise to mutations after fertilization, as the oocyte attempts to repair DNA damage before the initiation of the first cleavage.
Mutations occurring at this point will be fixed in the germline and may be responsible for the induction of infertility, childhood cancer in the offspring and for a higher risk of imprinting diseases. So far, however, follow-up studies of children born after ICSI compared with children born after conventional IVF have not been conclusive regarding the risks of congenital malformations, imprinting diseases, and health problems, in general. IVF, in general, is associated with multiple gestations and an increased risk of congenital abnormalities (including hypospadias) [197]. ICSI, in particular, carries an increased risk of endocrine abnormalities as well as epigenetic imprinting effects [197]. Although the absolute risk of any of these conditions remains low, current data are limited and study populations are heterogenic [197–200]. It is therefore recommended that well-defined groups of couples undergoing ICSI with ejaculated sperm, ICSI with epididymal sperm, and ICSI with testicular sperm, and a control group of naturally conceived children are closely followed up.

Fertilization defects
ART is indicated for fertilization defects involving sperm capacitation, sperm–ZP interaction, or sperm–oocyte fusion. Couples should be advised that a significantly higher rate of successful pregnancy is achieved with IVF-ICSI compared with conventional IVF and IUI in such cases [124,158,201].

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

Conclusion
Clinical management of couples with unexplained infertility is usually limited to full gynecological evaluation of the female partner and to traditional clinical and laboratory assessment of the male factor infertility. As such, the work-up for men may be prematurely ended based on normal semen parameters and normal hormonal profile. This strategy has been historically supported by the high spontaneous conception rate for couples experiencing unexplained infertility, particularly when the duration of infertility is less than 3 years and the female partner is aged 35 years or less with no detectable functional abnormalities. However, proper scrutiny for uncommon male fertility problems should commence as soon as possible in couples with diminished chances of spontaneous pregnancy. Modern andrology has novel techniques and methods for the diagnosis of hidden sperm functional problems, which may tailor the subsequent application of various treatment options, including ART.

Future perspectives
The understanding of sperm physiology and fertilization is far from complete. However, molecular and genetic studies are on the pace to give a detailed and thorough perception of the entire process of human fertilization. Consequently, this perception may suggest, in the future, specific molecular therapy or even genetic target needed to be precisely modified to improve male reproductive potential. Moreover, major advances in biomolecular techniques as well as in the sensitivity and accuracy of mass spectrometry are transforming our understanding of sperm physiology. The ‘omics’ era is under way, which refers to the study of genes (genomics), transcripts (transcriptomics), proteins (proteomics), and the various metabolites (metabolomics). Diagnostic genomics may help us to identify genotypes associated with specific sperm defects, as already reported in animal models [202]. A comprehensive proteomic analysis of normal and defective spermatozoa may provide insights into the structure–function relationships [203,204]. It has been suggested that sperm DNA damage is promutagenic and can give rise to mutations after fertilization, as the oocyte attempts to repair DNA damage before the initiation of the first cleavage. Mutations occurring at this point will be fixed in the germline and may be responsible for the induction of infertility, childhood cancer in the offspring, and for a higher risk of imprinting diseases [196]. Sperm metabolomics may elucidate which metabolic defects are associated to oxidative stress and sperm damage. This novel information may be useful both to identify the causes or consequences of oxidative stress in the male germline and to tailor individualized therapeutic intervention, such as an optimized regimen of antioxidants. Moreover in the context of unexplained infertility, glycomic analyses may be useful to reveal the causes of defective sperm–Zona interaction [205]. It is then likely that future laboratory semen evaluation will move from the simple assessment of conventional semen profile into the assessment of sperm biochemistry, which may aid in the understanding of the underlying physiopathology of male infertility and in suggesting options for treatment and prevention.

References


51 Tesaki J, Greco E, Mendoza C, Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. Hum Reprod 2004; 19:611–615.


70 Van Gestel RA, Brewis IA, Ashton PR, Brouwers JD, Gadella BM. Multiple proteins present in purified porcine sperm apical plasma membranes interact with the zona pellucida of the oocyte. Mol Hum Reprod 2005; 11;1395–1403.


