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Use of Diagnostic Testing to Detect Infertility

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Abstract

The evaluation of the infertile male continues to be a clinical challenge of increasing significance with considerable emotional and financial burdens. Many physiological, environmental and genetic factors are implicated; however, the etiology of suboptimal semen quality is poorly understood. This review focuses on the diagnostic testing currently available, as well as future directions that will be helpful for the practicing urologist and other clinicians to fully evaluate the infertile male.

Keywords

Male infertility; Sperm function testing; Genetics; Semen analysis

Introduction

Infertility is a difficult and stressful condition not only for patients, but also for the treating physicians as well. The failure to conceive within 1 year occurs in about 15% of couples [1], and about 50% of problems related to conception are either caused entirely by the male or is a combined problem with the male and his female partner. Male infertility continues to be a clinical challenge of increasing significance. While the etiology of impaired semen quality is currently not completely understood, many variable causes are known to contribute, and a multitude of potential problems are being ardently investigated.

The primary goals of the evaluation of the male presenting with infertility are to identify etiological conditions that can be reversed with resulting improvement of fertility status; medically significant and potentially dangerous diagnoses underlying the male's infertility; genetic etiologies that may have implications for the patient and/or his offspring; and irreversible conditions that may be best managed with the use of assisted reproductive techniques (ARTs) or the recommendation of donor insemination or adoption. Over the past

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decade, the diagnostic capabilities available to assist in formulating a diagnosis for the infertile male have been evolving rapidly. Although the number of tests available continues to expand, not every patient needs all tests.

Male Evaluation

The workup of the infertile male should proceed as any other workup for a medical illness. A thorough history should be performed, followed by a physical examination and any appropriate diagnostic testing. The history should address the amount of time the couple has attempted conception, previous pregnancies, intercourse timing, use of lubricants, and the presence of erectile dysfunction. A query of medical conditions and surgical conditions also should be noted. Special attention should be paid to a developmental history; recent febrile illness; a history of viral orchitis, bronchopulmonary illnesses, undescended testes, childhood cancers and treatments; genitourinary tract infections; and congenital or genetic abnormalities. Scrotal, prostatic, spinal, inguinal, and retroperitoneal surgeries should be highlighted along with a family history of both infertility and genetic abnormalities. A medication history including the use of anabolic steroids should be elicited. Finally, occupational and environmental exposures such as contact with toxins, chemicals, radiation, ethanol intake, and smoking should be noted.

The physical examination should include a general description of the patient, absence or presence of facial and pubic hair, gynecomastia, and abnormalities of skeletal structures. The examination should include a complete examination of the penis, including measurement of the length of the phallus and assessment of position of the meatus. The scrotum should be examined for the size and consistency of the testes, presence and consistency of epididymis and vas deferens, and presence of a varicocele. The digital rectal examination should begin with an examination of anal position, sphincter tone, and a thorough prostate exam, evaluating its size, consistency, and presence of midline cysts or palpable dilation of the seminal vesicles.

Spermatogenesis is a complex stepwise process that relies on an adequate and functional signaling pathway. Testosterone and follicle-stimulating hormone measurements should be obtained to evaluate the hypothalamic-pituitary-gonadal (HPG) axis. If abnormal values are discovered, then more extensive blood tests, which may include estradiol (if the patient is obese), luteinizing hormone, prolactin, thyroid-stimulating hormone, sex hormone–binding globulin, and cortisol, should be implemented. Although endocrinopathies account for less than 3% of infertility cases [2], a thorough hormonal evaluation is essential when appropriate.

Integrating the history, physical examination, semen analysis, and endocrine profile are steps toward establishing the diagnosis of the infertile male. By utilizing this systematic testing, the patient can be further characterized as having primary, secondary, or tertiary testicular failure; obstructed versus nonobstructed; or having abnormalities of sperm quantity and quality. During this process, an effort is made not only to identify the underlying conditions and harmful exposures leading to male infertility, but also to determine the potential effectiveness of specific versus empiric therapies, as well as likely candidacy for ARTs.

Semen Analysis

Male infertility can be divided into three main components: inadequate sperm production, sperm function deficiency, or insufficient sperm delivery. In the absence of sperm in the ejaculate, the specimen should be centrifuged, the pellet evaluated, and a qualitative fructose assay performed. The physician also may adjunctively order one of many additional studies

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(eg, white blood cell assay, DNA integrity testing, and reactive oxygen species [ROS] quantification) if supported by abnormalities in the semen analysis (SA) or initial male evaluation.

The most important and pivotal aspect of the initial laboratory evaluation is the SA. It is recommended that at least two SAs be obtained with similar abstinence periods of 3 to 7 days. It is possible to have wide variations in semen parameters between specimens from the same patient. Reports indicate that sperm density increases 25% per day for the first 4 days of abstinence before a SA, but other parameters such as motility and morphology remain stable [3].

While it may be true that a man with azoospermia is considered sterile, there is a wide range of overlap between semen parameters of fertile men compared to infertile men [4]. The World Health Organization (WHO) has published reference values for semen including volume, pH, sperm concentration, total sperm count, motility, morphology, and other parameters (Table 1) [5,6]. Medical professionals and reproductive specialists throughout the world have increasingly utilized these reference values in the evaluation of the infertile couple.

Semen samples should be obtained correctly by either masturbation or ejaculation into a specialized, nonlatex, spermicidal-free collection condom. Collection of the sample can be performed in the office, or it may be collected at home and brought in for processing. In the latter case, the sample must be stored at room temperature and delivered within an hour of collection.

Microscopic Examination

Microscopic survey of semen must be performed before actual sperm counting to identify round cells, debris, and bacteria. Contamination of the specimen during collection, as well as the presence of urethral or prostatic bacteria, can result in the presence of bacteria in the semen. Significant infection is seen if concentration exceeds 1000 bacteria per mm [7]. The presence of round cells in the semen can represent either immature germ cells or leukocytes [8]. Of note, up to 20% of infertile men have excess leukocytes present in the semen, and of these, only 20% are associated with significant bacterial colonization [9].

Sperm Counting

The preferred method for determining sperm concentration (“counts”) is by using commercially available Neubauer hemocytometer counting-chamber slides. Despite using counting chambers, there still exists a significant variation in results between each type of chamber, whether computer based or manually determined [10]. In general, one should count at least 200 sperm for an adequate determination of sperm concentration. When using microscopes that integrate a grid for counting, all sperm is counted within the grid and the concentration is calculated as a function of a coefficient specific to each chamber [11].

Sperm Motility

The percent of total motile sperm is the most important parameter when correlating semen samples to pregnancy outcomes [12]. It also is the most difficult part of the manual evaluation of the SA and introduces a significant amount of subjectivity among technicians. One method involves a subjective estimation of motility from surveying several fields by the technician and averaging those estimates to produce a motility percentage.

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A more objective and accepted method is to count motile and nonmotile sperm in each grid and average the percentages to produce a motility value. This can be done with a manual hematology cell counter. Drawbacks of this method are that it is inherently time consuming and an overestimation of motility can occur. This "overestimation" is due to the fact that as the technician moves along the grid, very motile sperm may progress to other areas of the grid and, theoretically, be double counted.

Methods to neutralize the inherent difficulty of counting motile sperm have been proposed [11]. Once the semen sample has liquified, an aliquot is prepared in parallel for counting. In one aliquot, only the nonmotile sperm (NM) are counted. In the other, an immobilizing agent, usually water, is used, after which all sperm (T) are counted. Motility can be calculated by using the formula T-NM.

Strict Sperm Morphology

The traditional evaluation of sperm morphology classifies sperm as normal if they do not fit into one of several defined categories, although most sperm in an ejaculate are neither uniform nor symmetrical, displaying large variation in shape and size. A common classification scheme designates sperm as normal (oval), amorphous, tapered, duplicated, and immature. However, more strict criteria to identify "normal" spermatozoa have been developed [13]. Kruger et al. [13] reported that, using his strict criteria, patients with fewer than 4% normal forms had a fertilization rate of 7.6% of oocytes in comparison to over 50% in patients with 4% to 14% normal forms.

For determining strict criteria, a minimum of 200 spermatozoa are counted on a stained slide using 100X oil-immersion magnification. An eyepiece reticule initially is recommended for measuring the sperm head and tail size (length and width). Using the strict morphological criteria, a normal spermatozoon is characterized by a smooth oval head, 4 to 6 μm in length and 2.4 to 3.5 μm in width. The acrosome must be well defined, covering 40% to 70% of the sperm head. There cannot be any mid piece or tail defects. Finally, there should be no cytoplasmic droplets greater than half the size of the sperm head. Utilizing the strict criteria method, a spermatozoon that may be considered "borderline" would be classified as abnormal [14].

Although strict morphology criteria have been widely accepted, its clinical usefulness remains an area of controversy. Sperm morphology was not a reliable predictor in selecting sperm without chromosomal aberrations [15], while normal morphology was not a good indicator of genetically normal sperm [16]. Guzick et al. [4] reported the existence of significant overlap between fertile and infertile men, and neither sperm morphology, concentration, nor motility was a powerful discriminator between the two groups. Poor sperm morphology does provide important information regarding the process of spermiogenesis in the testis.

While the establishment of high-quality control standards in evaluating morphology may improve the clinical utility of morphology determination, couples should be counseled with caution to pursue ARTs based solely on an abnormal strict morphology.

Antisperm Antibodies

Antisperm antibodies (ASAs) are found in up to 12.8% of infertile couples; however, these antibodies also are present in up to 2.5% of fertile men [17,18]. There is a large body of literature examining the effects of ASAs in serum and semen as a potential cause of infertility. Risk factors for the development of ASAs include any process that may have potentially disrupted the blood–testis barrier, including obstruction of the genital tract.

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Current methods of detection include direct tests to detect the presence of ASAs on sperm, such as the mixed agglutination assay, immunofluorescence assay, and immunobead test. Overall, these studies indicate that couples with ASAs have lower pregnancy rates than couples without evidence of ASAs. These studies also bring to light evidence that ASAs have multiple effects on various aspects of fertilization, such as acrosome reaction, capacitation, and implantation, although the evidence for each of these effects remains controversial, as reviewed by Chiu et al. [19]. Clinically, this has been a useful test in determining which infertile couples should proceed to in vitro fertilization (IVF) more quickly.

White Blood Cell Assay

Leukocytospermia designates an abnormally high concentration of white blood cells in semen. The WHO has suggested a concentration of over 10^6leukocytes per mL as a valid threshold value. Although several studies have attempted to characterize the relationship between the presence of leukocytes in semen and male infertility, it still remains incompletely defined. Some infertile patients have numerous round cells in their semen, and both leukocytes and spermatocytes (immature germ cells) appear similar under microscopy. While the presence of leukocytes in the semen may be indicative of infection or inflammation, there remains controversy about the significance of true leukocytospermia. However, it is imperative to be able to differentiate between leukocytes and immature germ cells, which cannot be done without special staining techniques. Leukocytospermia long has been associated with decreased sperm concentration, motility, and morphology and defective fertilization; however, these clinical, epidemiological, and experimental studies have reported inconsistent results, leaving this relationship still controversial [9,20].

Genetic Testing

Karyotype Analysis

Identifiable chromosomal abnormalities account for about 5% of male factor infertility [21]. In patients that are found to be azoospermic, the rate increases threefold [22]. Aneuploidy occurs in instances in which there are superfluous or insufficient numbers of chromosomes. The most common syndrome caused by aneuploidy in the infertile male is Klinefelter's syndrome, which occurs in 1:500 births and is found in 15% of males with infertility. The most common chromosomal arrangement in Klinefelter's syndrome is nonmosaic 47, XXY or mosaic 47, XXY/46X. These patients commonly have some degree of spermatogenic dysfunction that ranges from severe oligospermia to azoospermia.

This technique allows geneticists to microscopically visualize chromosomes in the metaphase portion of the cell cycle. Karyotype analysis can detect chromosomal abnormalities such as a loss or gain of an entire chromosome or portions of a chromosome and translocations (particularly Robertsonian); however, many genetic abnormalities cannot be detected using a karyotype, including point mutations, frameshift mutations, and other submicroscopic deletions not visible at the cytogenetic level.

Y-chromosome Microdeletion

The Y chromosome is comprised of 60 million base pairs with a short arm (Yp) and a long arm (Yq). The sex-determining region (SRY) is located on Yp and is an essential member of the group of genes that ultimately determines the fate of the bipotential gonad [23]. The Y chromosome contains vital components needed for male differentiation and sperm function. The azoospermia factor region (AZF) on Yq of the Y chromosome is responsible for sperm development. The AZF region is subdivided by location into AZFa, AZFb, and AZFc, which correspond to proximal, middle, and distal portions of the chromosome, respectively [24].

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Deletions in these locations are responsible for varying degrees of spermatogenic dysfunction. Entire microdeletions of AZFa or AZFb regions of the Y chromosome portend an exceptionally poor prognosis in sperm retrieval, such that microscopic sperm extraction is predictably negative [25].

Depending on the severity of the deletion, a microdeletion in AZFc can result in a spectrum of spermatogenic deficiencies including oligospermia and azoospermia [25]. Deletions in the Yq are too small to be detected with a karyotype and thus are termed microdeletions. These deletions are identified using polymerase chain reaction techniques to analyze sequence tagged sites. Indications for testing AZF microdeletions are sperm concentrations less than 5 million per mL. Importantly, male offspring of patients with Y microdeletions will inherit the abnormal gene, rendering them likely to be infertile. Thus, intracytoplasmic sperm injection (ICSI) with preimplantation genetic diagnosis (PGD) should be discussed.

Fluorescent In Situ Hybridization

Since the early 1990s, fluorescent in situ hybridization (FISH) in decondensed sperm nuclei has been used to study the chromosome constitution of human spermatozoa. After proper incubation, multicolor FISH is performed: triple-color FISH for chromosome 18, X and Y, and dual-color FISH for chromosomes 13 and 21. The slides are analyzed under an epifluorescent microscope and the spermatozoa are scored according to defined criteria. The incidence of numerical chromosomal abnormalities in spermatozoa has been reported in a wide range of individuals including carriers of chromosome anomalies as well as fertile and infertile males. In fertile control patients, the percentage of aneuploid sperm is estimated to be at least 6.5% [26]. The mean frequency of disomy for the autosomes and sex chromosomes in this population are 0.13% and 0.37%, respectively. The presence of diploid sperm also can be detected in 0.06% to 0.24% of the control patients [27].

In infertile men with a normal karyotype, a significantly higher aneuploidy rate in sex chromosomes and diploid nuclei in spermatozoa has been reported [28]. Using FISH, Vegetti et al. [29] reported that the risk of chromosomal aneuploidy in spermatozoa is inversely correlated to sperm concentration and total progressive motility [29]. Studies on testicular tissue samples from infertile men confirmed to have idiopathic impaired spermatogenesis showed increased incidence of aneuploidy among the diploid nuclei [30]. This suggests that chromosome instability is a result of altered genetic control during cell division and proliferation during spermatogenesis. Because these patients are the most frequent candidates for IVF/ICSI, information on meiotic studies and sperm chromosome analysis by FISH should be considered for proper reproductive counseling.

The use of FISH with specific DNA probes also has been used to determine chromosomal segregation patterns and aneuploidy levels in sperm from carriers of chromosomal structural reorganization or translocation [31]. FISH studies have shown increased frequency of sex-chromosome hyperhaploid and diploid sperm in most patients with numeric sex-chromosome anomalies (eg, Klinefelter's syndrome) [28]. Because these patients are an important referral group for PGD, it has been suggested that FISH studies should be performed to establish a prognosis before PGD [32].

A study by Rubio et al. [33] suggests a correlation between sperm chromosomal abnormalities and couples with first trimester miscarriages. FISH studies in sperm may have both diagnostic and prognostic values for couples with a history of recurrent miscarriages.

The major drawback for a wide use of FISH in infertility evaluation, in addition to cost, is the large variability in the reported frequencies of chromosomal abnormalities from different clinical/research groups. However, technical differences in sperm-decondensing protocols,

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scoring criteria, number of sperm analyzed, and the characteristics of the probes used may be the main factors that have led to the wide variability of the reported results. Standardization and rigorous controls from each laboratory are essential in the future development of this technique. The laboratory must be able to distinguish normal from abnormal results. Nevertheless, FISH has become a useful tool in the diagnosis and understanding of genetic causes of infertility.

Reactive Oxygen Species

Oxidative stress is a recognized etiology of male infertility. ROS, in the form of superoxide anions, hydrogen peroxide, and the hydroxyl free radical, are formed as a by-product of oxygen metabolism. The presence of excess ROS can cause oxidative damage to lipids, proteins, and DNA [34•]. Abnormal ROS formation is found in up to 40% of infertile patients [35], with some reports suggesting an inverse relationship between seminal ROS levels and spontaneous pregnancy outcomes of infertile couples [36]. Many studies have attempted to define the relationship between seminal ROS and IVF [37] but have met with conflicting results. Nevertheless, a growing body of knowledge on ROS and fertility makes testing for oxidants in the semen an important part of the infertile male evaluation.

There are various methods to detect seminal ROS, including chemiluminescence, nitroblue tetrazolium test, cytochrome C reduction test, and xylenol orange–based assay.

The chemiluminescence assay utilizes a luminometer to measure chemical reactions between ROS found in human semen and a chemiluminescent probe, such as luminol or lucigenin. Luminol is an uncharged particle that is cell membrane–permeable and therefore, can react extracellularly and intracellularly with hydrogen peroxide, hydroxyl anions, and superoxide anions. In contrast, lucigenin is a positively charged particle that is membrane impermeable and reacts with superoxide anions in the extracellular space [38].

It is important to remember that most of the assays for chemiluminescence measure total oxidative stress and generally do not distinguish between ROS produced by leukocytes and those produced by spermatozoa. Moreover, there are other factors that can alter these assay results and must be considered.

Leukocyte contamination in the semen impacts negatively on fertility [9]. Leukocytes are responsible for a significant proportion of ROS activity in the semen [20]; therefore, these assays should be coupled with selective leukocyte removal strategies if leukocytospermia is present [39]. Not doing so would lead to a falsely elevated ROS value.

In contrast, prolonged time from preparation to analysis of the sample can artificially decrease the ROS identifiable in the semen [40]. For this reason, it is recommended that testing be performed within an hour of sperm preparation.

DNA Damage

High levels of sperm DNA damage can negatively impact reproduction. Sperm samples from infertile men have been shown to have significantly more DNA damage than their fertile counterparts [41]. Multiple reports have also implicated DNA damage with poor IVF results [42], although newer studies have not corroborated these findings [43]. Finally, some evidence suggests that DNA fragmentation can be a cause of early embryo death, poor embryo progression, and poor implantation [44]. In general, damage to sperm DNA occurs during intratesticular development as well as during the maturation and transport process that takes place outside the testes.

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The causes of DNA damage are largely unknown, although there is evidence to suggest that genetic defects may underlie some sperm DNA damage [45••,46]. Spermatogenesis is controlled by selective apoptosis. Abnormal sperm are tagged for apoptosis in the same manner that all other cells are marked for programmed cell death. Protamine deficiency has been identified as another primary testicular cause of sperm DNA damage, and this deficiency frequently is seen in infertile men compared to fertile counterparts [47]. In addition, certain polymorphisms in the protamine gene have been implicated in male infertility and sperm DNA damage [48]. Evidence suggests that a malfunction in this process allows sperm with DNA damage to be transported in the ejaculate, a process referred to as abortive apoptosis [49]. A variety of different tests are available: the acridine orange–staining test, the sperm chromatin structural assay, terminal deoxynucleotidyl transferase–mediated nick-end labeling, and the comet assay.

Excessive ROS in the ejaculate correlates with increasing sperm DNA damage [50]. Fortunately, there is evidence suggesting that reduction in ROS levels with antioxidant therapies can decrease sperm DNA damage [51]. Studies also have implicated clinically significant varicoceles as a cause of sperm DNA damage [52]. Recent reports suggest improvement in DNA damage after microsurgical varicocelectomy [53].

Because ARTs now are commonly used to circumvent virtually all types of male infertility, it is important to understand the differences between ejaculated, epididymal, and testicular sperm and their respective levels of DNA damage. O'Connell and colleagues [54] found that testicular sperm had fewer DNA mutations and fragmentations when compared to epididymal sperm in preparation for IVF/ICSI. When comparing testicular sperm to ejaculated sperm, Greco and colleagues [55] found that there was significantly lower DNA fragmentation in the testicular sperm. In addition, they reported improved pregnancy rates using testicular sperm compared to ejaculated sperm. If IVF/ICSI is to be performed using sperm with high DNA damage, consideration should be given to testicular sperm extraction only after less invasive treatments for known causes of DNA damage have failed.

Sperm Function Testing

Hypo-osmotic Swelling Test

The hypo-osmotic swelling test is one of the most basic tests performed in the andrology laboratory and answers the basic question of whether immotile sperm are alive with an intact membrane and merely immotile or whether they are dead. Thus, it represents a test of viability when immotility is present, and differs from a live/dead stain in that the latter only measures whether the sperm membrane is physically disrupted. The test is based upon the premise that when placed in a hypo-osmotic condition (150 mOsm/L or less), a normal live sperm maintains an osmotic gradient and absorbs fluid, resulting in a swelling of the plasma membrane. Jeyendren et al. [56] described a test based upon this principle that showed a normal ejaculated semen sample has over 60% viability. Clinically, this test may be useful in the evaluation of a patient with immotile sperm to differentiate immotile cilia syndrome from other motility defects causing necrozoospermia, as well as selecting viable sperm for ICSI from testis biopsies when the sperm are immotile.

Acrosome Reaction Assays

The acrosome, modified from the Golgi apparatus of the spermatid, is a socklike structure covering the upper 75% or so of the sperm head. The acrosome contains hydrolases such as acrosin (the predominant one), hyaluronidase, and neuraminidase. At the time of reaction, the sperm plasma membrane and outer acrosomal membrane fuse. This is an exocytotic process necessary in the spermatozoon for successful penetration of the oocyte. If the acrosome reaction does not occur, normal fertilization cannot occur (it is noteworthy that the

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acrosome generally is intact when the sperm are injected for IVF/ICSI). Measurement of acrosomal status after no preincubation (basal level), extended incubation (asynchronous/ spontaneous reaction) or after incubation and treatment with a stimulator of the acrosome reaction (induced asynchronous or synchronous reaction) may provide diagnostic information about fertilization potential. While the test usually is performed in men with globozoospermia (round-headed sperm) who have a genetic defect resulting in total absence of the acrosome, clinically, it has become increasingly limited with the development of IVF/ ICSI and the ability of basic semen analysis/strict morphology to detect this abnormality.

Sperm Zona Binding Assays

Binding to the zona pellucida is an obligatory step in the normal process of fertilization. Several assays are designed to measure this activity, but all are limited by the availability of unfertilized human oocytes. In 1988, Burkman et al. [57] designed a test that used ova that had failed to fertilize in IVF and used micromanipulation to bisect the ova resulting in two fragments of zona pellucida (hemizona), which could be stored in preservative with no measurable loss of binding activity. The test compares the ability of patient and donor sperm to bind to the zona, and these bound sperm are examined using phase-contrast microscopy. Liu et al. [58] modified this test using fluorescently labeled sperm with different fluors for the patient and fertile donor sperm that were competitively bound to the fixed zona so that creation of the hemizona was no longer required. Clinically, this test could play an important role in determining those patients who require IVF/ICSI by providing an indication of those couples who fail IVF.

Sperm Penetration Assay

In 1976, Yanagimachi and colleagues [59] observed that upon removal of the zona pellucida of hamster ova, the eggs were “promiscuous” and allowed penetration by sperm of other species. This test measures the ability of sperm to undergo capacitation, fuse with the egg membrane, and decondense the sperm head resulting in the formation of the male pronucleus. Although a number of studies showed that this test was a useful predictor of fertilization in IVF, the test was plagued by false negatives and assay variability. Johnson et al. [60] improved this assay by altering the conditions to enhance sperm penetration rates by orders of magnitude and by devising a control method to ensure assay precision and accuracy. A positive score on this test was highly predictive of a positive outcome in IVF. Unfortunately, the test rarely is ordered today despite providing useful information. A modification of this assay used in combination with ICSI into hamster oocytes accurately identified male factor patients who failed to fertilize after IVF/ICSI due to a defect in sperm head decondensation [61].

Future Directions

Cytogenetics is the part of genetics that deals with chromosomes, particularly with numerical and structural chromosome abnormalities, and their implications in congenital or acquired genetic disorders. Standard karyotyping, successfully used for the past 50 years in investigating a genetic etiology in patients with infertility, fetal abnormalities, and congenital disorders, is constrained by the limits of microscopic resolution and is not suited for the detection of subtle chromosome abnormalities. The ability to detect submicroscopic chromosomal rearrangements that lead to copy-number changes has escalated progressively in recent years with the advent of molecular cytogenetic techniques. Array-comparative genomic hybridization has emerged as a powerful new molecular tool for the high-resolution analysis of copy-number variation and breakpoint analysis.

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What else does the future hold? While semen analysis truly is the cornerstone for the evaluation of the infertile male, in this century alone, andrologists and clinicians alike will have an ever expanding armamentarium of powerful investigative "omics" that will allow the careful examination of defective sperm of infertile patients and also gain a more complete understanding of the underlying etiologies. With the development of metabolomics, proteomics, and genomics, the "omics revolution" already has begun [62]. These tools provide detailed molecular data about the underlying biochemical mechanisms of disease, such as resolving the causes of defective sperm-zona interaction with glycomic analyses, assessing the consequence of oxidative stress in the male germline with application of lipidomics to the analysis of sperm quality, and even identifying particular genotypes associated with specific defects in semen quality with advanced diagnostic genomics.

Conclusions

Over the past few decades, with the advent of IVF/ICSI, the options for treatment of infertility have been revolutionized. Similarly, the ability to evaluate and diagnose the infertile male has developed as well. The information gained from these diagnostic tests allows the clinician to decide who are the appropriate couples to proceed more rapidly to IVF/ICSI. Furthermore, developments in the ability to understand the molecular and biochemical mechanisms of infertility continue to evolve, giving us the knowledge to improve our evaluation and treatment of the infertile couple.

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Table 1

WHO semen parameter reference values

Semen parameters	4th edition [5][a]	5th edition [6][b]
Volume	≥2 mL	≥1.5 mL
Sperm concentration	≥20 M/mL	≥15 M/mL
Total sperm count	≥40 M	≥39 M
Motility	≥50% progressive (a+b); ≥25% a only	≥40% total motility; ≥28% progressive (a+b) motility
Morphology	≥15% by strict criteria	≥4% by strict criteria
Vitality	≥75%	≥58% sperm viable
WBC	<1 M/mL	<1 M/mL

WHO World Health Organization

[a] Limits of adequacy and determined by consensus

[b] Well-defined reference ranges derived under strict statistical analysis