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What does the clinician need from an andrology laboratory?

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1. ABSTRACT

What does the clinician need from an andrology laboratory? The andrology laboratory is vital for the accurate diagnosis and treatment of male factor infertility, which is contributory in 50% of infertile couples. While there are some diagnostic tests of limited clinical utility, many tests can be useful in specific clinical settings. In this chapter, we will review the basic interpretation of the semen analysis, testing for sperm vitality and motion, inflammation, semen antioxidant capacity, sperm DNA damage, antisperm antibodies, post ejaculate urine analysis as well as other functional testing. Several clinical vignettes are presented for real-life examples of interpretation of the role of the andrology laboratory in clinical infertility.

2. INTRODUCTION

Infertility, defined as the inability to conceive after one year of unprotected intercourse, occurs in approximately 15% of couples (1). Andrology is the branch of medicine concerned with male diseases and physical abnormalities affecting the male reproductive system. The term first appeared in 1887 in a lecture introducing a course in obstetrics; this defined "andrology and gynecology, as being concerned with the study of the physical and mental peculiarities of men and women respectively" (2). The role of the andrology laboratory comes into play in the diagnosis of male factor infertility; which is the sole etiology in approximately 20% of couples and contributory in another 20-40% (3). In addition, many andrology

laboratories are involved in infertility treatment with assisted reproductive technologies (ART) and intrauterine insemination (IUI). This includes: semen preparation for intrauterine insemination (IUI), semen and tissue preparation for intracytoplasmic sperm injection (ICSI), as well as sperm cryopreservation. While the emotional costs of infertility cannot be quantified, the personal and public costs of infertility treatment are significant (4). With improved technology and in-vitro fertilization (IVF), many previously infertile couples are now able to have children.

There has been the suggestion that in the era of ART, the evaluation of the male is considered unnecessary. However, there is compelling evidence that it is both safer and more cost efficient to evaluate the male as well as the female partner (5). Evaluation of the male partner allows identification of underlying non-genitourinary medical conditions associated with infertility as well as chromosomal abnormalities that have implications for future offspring. Additionally, some couples with targeted male factor treatment could conceive naturally, sparing the risks of IVF (6). The costs of IVF are significant with the first systematic analysis of achieving a pregnancy in 1994 being calculated at $66,667 for one cycle (7). With the use of ICSI, the costs are even higher with $103,940 average cost per live birth (8). The cost effectiveness of treatment of underlying pathology for male patients has been convincingly shown in patients with varicocele or obstructive azoospermia secondary to vasectomy (9). The role of the andrology laboratory is vital in the identification and diagnosis of male factor infertility. When this work-up identifies irreversible conditions that are amenable only to assisted reproductive techniques, the andrology laboratory plays a pivotal role in the identification and preparation of usable sperm.

The clinical evaluation of a patient begins with a thorough history, physical, and two semen analyses preferably separated by several weeks. This evaluation is typically triggered by the failure to conceive within one year of regular unprotected intercourse, or earlier if there are male infertility risks factors, female infertility risk factors (including advanced female age) or patient concern (10). The semen analysis is the fundamental and most important part of the evaluation of potential male factor infertility. It helps define the severity of the male factor as well as guiding the remainder of the evaluation including whether the patient may need a full endocrine evaluation, imaging studies, specialized semen tests or genetic screening. Unfortunately, conventional semen analyses are relatively non-specific for identification of the particular etiology of the male factor infertility (11). Because the semen analysis is often the gateway test from which multiple expensive and often invasive treatments are based, the importance of a reliable andrology laboratory cannot be over-emphasized.

3. LOGISTICS OF A GOOD DIAGNOSTIC ANDROLOGY LABORATORY

Many factors are critical for the function of a reliable andrology laboratory. Accuracy, the degree to which the measurement reflects the actual or true value, as well as precision, the reproducibility of the results, are both vitally important. The semen analysis comprises a battery of tests, each of which requires different techniques and skills to measure. A standard semen analysis measures pH and includes a macroscopic evaluation consisting of a viscosity, semen volume, and liquefaction analysis. In addition, a microscopic evaluation identifies various cellular components, agglutination, motility and morphology. The microscopic evaluation allows determination of sperm concentration as well as the leukocyte and possible immature germ cell count. A good laboratory will have experienced technologists as well as systems in place to ensure that basic collection tenets are either upheld or recorded as deviant. For example: ideally a semen sample is obtained after 2-6 days of abstinence and requires collection of the entire ejaculate. A good technologist can precisely measure the volume of the ejaculate, however this would be inaccurate if there was an incomplete collection and it was not noted/recorded for the clinician to interpret. Inaccuracy in this manner could lead to concerns for obstructive pathology and possible unnecessary testing in attempts to further evaluate this hypothesis. Accuracy is a challenge for the andrology laboratory as semen analysis remains one of the few modern laboratory tests which still require manual microscopic skills. The full evaluation of the specimen requires knowledge and facile use of classification systems in the evaluation of morphology as well as adept use of the microscope for counting purposes. These skills improve with daily use, use of quality control standards as well as continuing proficiency testing. A laboratory which deals with large quantities of these specimens is more likely to have employees with the experience and ability to obtain accurate and precise results. With both accuracy and precision, the clinician is able to rely upon the values provided by the andrology laboratory and can safely direct the further work-up, diagnosis and counseling of the infertile male.

The turnaround time for tests is important due to time dependent deterioration of semen parameters, such as motility, as well as the time sensitive nature for infertility evaluation. The decline in female fertility is well established, with one study reporting the incidence of infertility rising steadily with age; with 18% of women aged 35-39 years old being infertile and 28% being infertile by age 40 (12). Although the effects of age on male infertility are less pronounced, men can exhibit declines in bulk semen parameters as well as sperm DNA integrity with aging. There are also psychosocial stresses seen in both men and women with increased treatment costs and the amount of testing and treatments required (13). Timely turnaround, accurate and reproducible results allow the physician to provide adequate education on possible causes and treatment options reducing the stress, costs, and time taken to treat infertility.

In order to provide timely turnaround, it is important for an andrology laboratory to provide the full spectrum of tests that the physician routinely uses. Ideally, a laboratory should provide routine semen tests as well as

more specialized testing. Convenience factors such as hours of operation, comfortable and private collection rooms, convenient drop off locations for specimens and customer service are important in reducing the stresses associated with an infertility evaluation. Processes to ensure proper documentation about collection factors such as abstinence time, complete versus partial collection and method of collection are important as these factor influence the clinicians interpretation of the testing.

3.1. Certifications and accreditations

There are multiple certifications and accreditations for an andrology laboratory. The intricacies of andrology testing make adherence to quality control vital. Accreditation and certification demonstrate the lab's ability to meet minimum standards. In the United States, the minimum legal standards are as set out by federal law in the Clinical Laboratory Improvement Act (CLIA) to ensure proficiency testing and quality improvement programs for each laboratory.

In attempts to provide guidelines and an accreditation consistent with higher standards, the College of American Pathologists (CAP), a private organization, started collaborating with the American Society for Reproductive Medicine (ASRM) to provide voluntary accreditation which exceeds the standards set by CLIA. The results of this collaboration resulted in the Reproductive Laboratory Accreditation Program (RLAP), which provides accreditation and standards for reproductive laboratories including andrology laboratories and embryology/ART laboratories. The RLAP standards stipulate that 1) a qualified board-certified pathologist, PhD or physician direct the lab, 2) there are sufficient physical resources including equipment to perform the testing, 3) there exists quality control with proficiency testing, on-going quality improvement, validation of test systems, quality management of pre- and post-analytic processes, specimen storage and communication with clinicians and patients and 4) there are administrative standards, including periodic on-site inspections and self-assessment. About 35% of reproductive laboratories are accredited through the CAP/RLAP program (14). Differences for the accreditation and certification of ART laboratories as compared to andrology laboratories include most notably different qualifications and skill sets for the personell. An ART laboratory needs an American Board of Bioanalysis Embryology Laboratory Director certification or its equivalent and a physician with board certification through either the American Board of Obstetrics and Gynecology or the American Board of Urology (15).

Besides the CAP/RLAP accreditation, the Society for Assisted Reproductive Technology (SART) also recognizes The Joint Commission (TJC) formerly known as the Joint Commission on Accreditation of Healthcare Organizations (JCAHO) as an appropriate accrediting agency (16). TJC has accredited freestanding laboratories since 1995 and like the CAP/RLAP meets or exceeds the CLIA standards. The difference between TJC and CAP/RLAP is that TJC uses full-time surveyors who are paid to perform the inspection process instead of using professional peers. There is also the Commission on Laboratory Accreditation (COLA) accrediting program which has been approved for CLIA certification and is aimed at inspecting smaller volume andrology laboratories. Some state agencies are also legally able to accredit laboratories as meeting CLIA standards. As of 2002, there were approximately 400 ART laboratories in the United States with even more non-ART affiliated andrology laboratories. The RLAP reported certifying 220 laboratories with an additional 50 accredited through TJC, leaving a significant number unaccredited (17).

In addition to the accreditation bodies, there are also certifications which document the personnel qualifications and maintain a minimum educational level among technicians. In the United States, the American Board of Bioanalysis (ABB) is the only board that offers certification for laboratory personnel for andrology and embryology specific processes. CLIA regulations recognize ABB certification of laboratory directors and clinical consultants including bioanalyst, clinical laboratory director, high-complexity clinical laboratory director and embryology laboratory directors. The ABB documented that while national proficiency testing is practical; the technical expertise, procedural care and meticulous quality control required for semen analysis continued to show poor agreement on several factors most notably sperm morphology grading (18). Unfortunately, even with enrollment in the American Association of Bioanalysts Andrology Proficiency Testing Program, 61% of laboratories participating in ART and general andrology examinations, have a significant lack of standardization in both the performance and reporting of semen analysis (19).

While these accreditations and certifications attempt to promote accuracy and reproducibility of results, there is a lack of evidence demonstrating a positive change in fertility outcomes accompanying accreditation. A study in 2000 showed no significant differences in pregnancy rates between CAP accredited or non-accredited labs (20). Practically, accrediation can be used by physicians to provide a modicum of security that the semen analyais presented represents an accurate and precise reading of that particular semen sample, thereby negating the need for expensive multiple repetitive analyses. Unfortunately, the effects of accreditation on outcomes other than fertility, such as accurate diagnosis rates have not been published. The cost impact of accreditation is also unclear and has been suggested by some to be prohibitory (21). Documentation of improved outcomes may come with improvement in the methods of accreditation and a focus on quality improvement by the laboratories instead of merely meeting the minimum requirements for accreditation.

3.2. Quality control

The goal of the CLIA was to ensure quality control of all laboratories. Although there is a body of evidence documenting the imprecision of semen analysis, it has been shown that quality control programs with detailed protocols and rigorous education can improve the inter-laboratory variability (18). The World Health Organization

(WHO) has also attempted to improve the quality of semen analysis by providing strict guidelines (22-25). The importance and impact of both adhering to the guidelines as well as the reference ranges from the WHO manuals is clear as these have implications for clinical management. For example, although sperm morphology is one of the least precise measurements documented between laboratories, some physicians will recommend ICSI if sperm morphology falls below 4% based upon interpretation of data presented by Kruger *et al* in 1988 (26). It is clear therefore, that improvement in accuracy and precision of these parameters are paramount. For the clinician, accreditation and certification provide a secondary marker of a laboratories dedication to quality control.

3.3. Testing offered

For the tertiary referral center, it is important to offer a broad spectrum of specialized semen testing performed onsite as this provides timely results. This is not true for all centers as the benefits of quick turnaround for testing are outweighed by the cost of performing the tests, quality assurance, and maintaining trained personnel for specialized tests. Routine testing includes comprehensive semen analysis, post ejaculatory urine analysis, anti-sperm antibody testing, assessment of seminal leukocytes, and sperm viability testing. For the referring urologist, this allows for a likely preliminary diagnosis and the ability to identify those who may need further referral and or an in-depth work-up. Although routinely performed in the office, it is valuable to have a lab that can perform post vasectomy semen analyses for physicians who are not routinely examining these specimens. This can be used as definitive evidence of sterility after vasectomy. Tests of limited clinical value include the post coital test, seminal fructose assay, sperm penetration assay, and testing to determine acrosome reaction. The rationale behind these tests and why they are considered of limited clinical value will be discussed in detail during the description of each individual test. For general andrology laboratories that are not associated with ART laboratories, it is still prudent to have the ability to bank sperm as well as process sperm for IUI as well as the ability to harvest sperm from biopsy in order to facilitate further ART.

4. ALGORITHM FOR EVALUATION OF THE INFERTILE PATIENT

Laboratory testing is a cornerstone of the full evaluation of an infertile couple. The history of the patient should incorporate a detailed past reproductive history as well as current medications, past medical, surgical, family, and social histories all of which may elucidate potential etiologies of infertility. The presence of a recent febrile illness would give the physician pause on ordering a full component of semen analyses until anticipated sperm recovery, which may take up to 3 months after the event.

Semen analysis should be performed on two separate semen specimens at least 3 months after a febrile illness and separated generally by 2-4 weeks between specimens (27-28). The clinician should document proper specimen collection. This includes whether the collection was complete, i.e. the entire ejaculate was recovered. The type of container, ideally sterile and non-toxic, used to transport the specimen should be noted. The elapsed time from ejaculation to semen analysis processing in the laboratory should be documented. For specimens that were produced at home or in the laboratory it is important to document that the specimen was stored at body temperature and if it was analyzed within one hour. The length of abstinence, ideally over 48 hours and less than 7 days should also be noted. Ideally the method of collection consists of masturbation however there are special collection condoms that can be used. The components of a standard semen analysis include the total count, concentration, motility or forward progression, morphology by World Health Organization (WHO) criteria (1987, 1992, 1999, and most recently 2010), volume, pH, color, viscosity, liquefaction and number of round cells (22-25). With semen analysis, patients can be classified as being normozoospermic with normal semen counts, azoospermic, no sperm from two separate centrifuged specimens or oligozoospermic with sperm concentration < 15 million/mL (25). Abnormal sperm morphology is termed teratozoospermia and impaired motility is asthenozoospermia. Aspermia is the complete lack of ejaculate.

Based upon the history, physical, and semen analysis, some patients may be referred for genetic evaluation. In patients with either azoospermia or severe oligozoospermia, in the absence of evidence of ductal obstruction, the evaluating physician should evaluate for karyotype abnormalities and for Y chromosome microdeletions. This testing may confirm diagnoses that were suspected from history and physical exam such as Klinefelter's syndrome or congenital bilateral absence of the vas deferens (CBAVD). Klinefelter's syndrome patients have a 47, XXY karyotype with small volume firm testes, gynecomastia and poor virilization. CBAVD is associated with potential mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. In patients with genetic defects, it is important to counsel patients about the potential for transmitting these abnormalities to their children if IUI/ART is pursued as treatment. Endocrinopathies can be implicated in infertility and should be pursued if there is a low sperm concentration (< 10 million/mL), erectile dysfunction, low libido or other findings suggestive of an endocrine etiology of infertility (29). Physical exam itself may be diagnostic of other potential etiologies of infertility including the presence of varicocele, or a midline structure in the prostate suggestive of ejaculatory duct obstruction. Ultrasound is recommended currently in whom physical examination of the scrotum is difficult, uncertain, or when there is a suspicion of a testicular mass. Currently ultrasound is not recommended for varicocele screening as correction of subclinical varicoceles has not been shown to improve fertility (29).

Advanced laboratory testing beyond the semen analysis is generally reserved for cases in which diagnosis will alter treatment choices. Based upon the history,

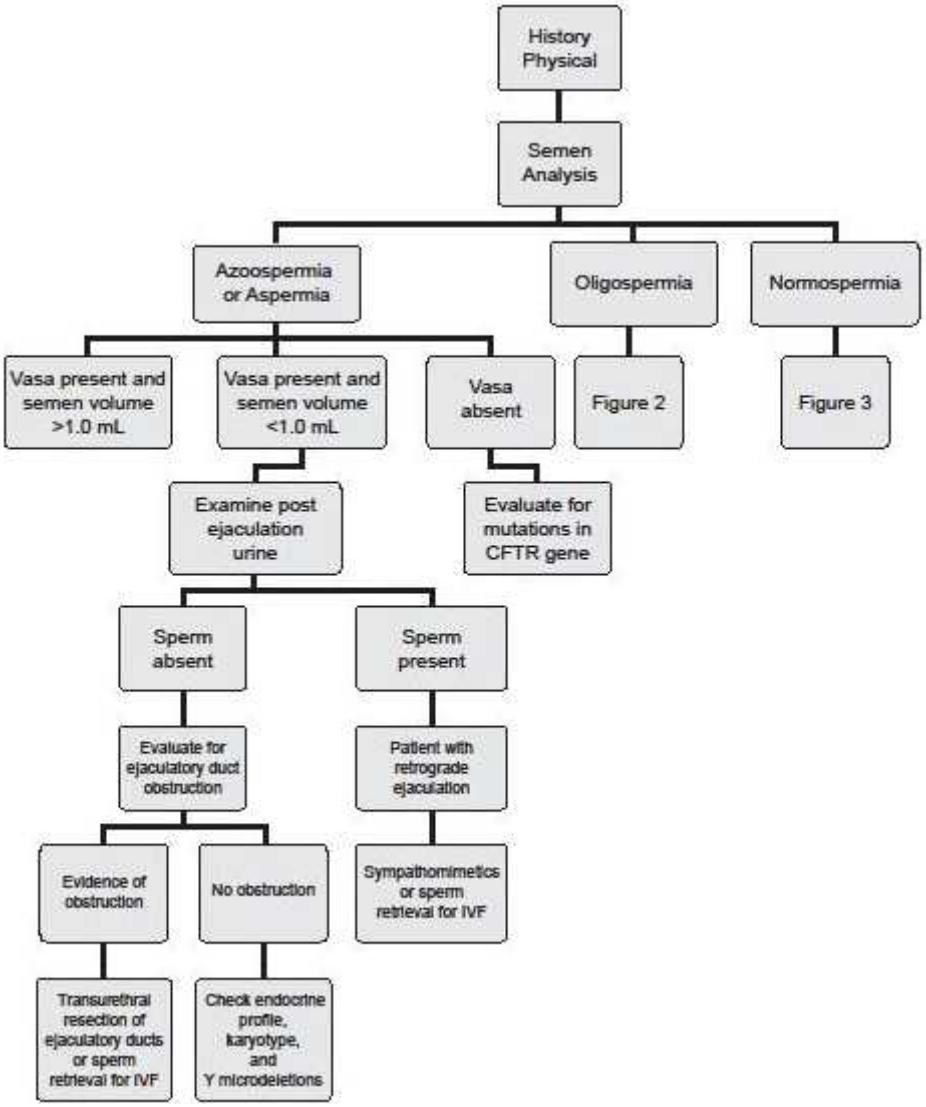

Figure 1. Evaluation algorithm: the general and asperima/azoospermia algorithm is presented.

physical, and semen analysis, further testing can be recommended (Figure 1-3). Briefly, advanced testing consists of post ejaculation urine analysis, an assessment of seminal leukocytes using techniques such as peroxidase staining microscopy (endtz test), reactive oxygen species testing, sperm DNA testing, antisperm antibodies testing, and sperm vitality testing. Some testing is of little clinical use and will be discussed for historical interest and completeness. Other testing that will be discussed, although promising, is still primarily investigational.

5. INTERPRETING ANDROLOGY LABORATORY TESTS

5.1. Semen analysis

The proper interpretation of the semen analysis remains one of the most challenging among contemporary laboratory testing. Reference values for semen analysis were previously derived from imprecisely defined reference populations as well as from multiple laboratories with potentially different methodologies. Caution must be exercised with interpretation of the semen analysis based upon the reference values as men may be infertile with "normal" semen parameters or alternatively, be fertile with markedly "abnormal" semen profiles. There is likely no upper limit of semen morphology, motility or count as pregnancy rates appear to generally increase with increasing numbers as well as improved sperm morphology and motility (30-31). In 2010, Cooper *et al* published updated reference values obtained from analyses of multi-country data with laboratories that have used the WHO standard methodology for semen analysis (32). The 95% reference intervals are commonly referenced with the lower 2.5 and 5 percentile being used as limits for two and one-sided distributions (Table 1). Methodologic challenges of semen analysis performance continue despite recently introduced methods of quality control (33-34). Even with internal and external quality controls, semen analysis is

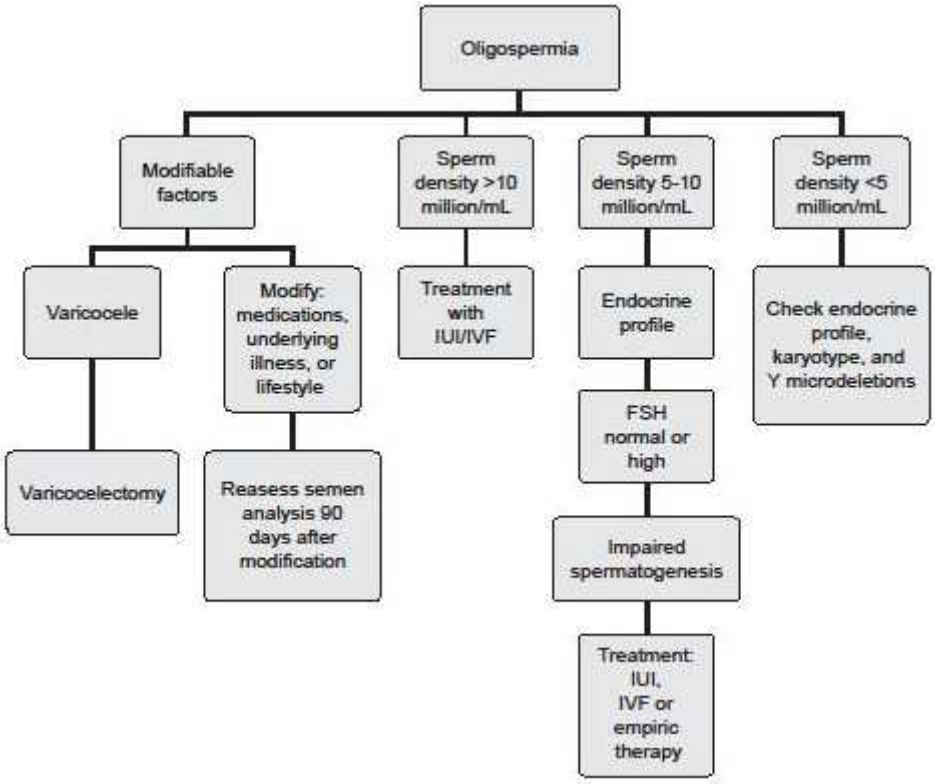

Figure 2. Evaluation algorithm: the oligospermia algorithm is presented.

operator dependent with one of the most subjective assessments being that of morphology (18).

5.1.1. Sperm count

Sperm count is typically reported both as concentration (millions of sperm per milliliter) as well as total sperm count (sperm concentration x mL of semen) in the ejaculate. Normozoospermia, oligozoospermia as well as azoospermia are diagnosed based upon total sperm count. Prior to the diagnosis of azoospermia, the sample should be centrifuged and the pellet examined for sperm. Oligozoospermia is typically defined as concentrations of sperm < 15 million/mL (25). This finding can accompany a variety of defects and has implications for the type of assisted reproductive options that can be utilized as there are significant reductions in pregnancy rates for patients undergoing IUI with counts less than 10 million (35).

5.1.2. Morphology

Morphology is a particularly challenging parameter to interpret because of the subjective nature of the classification, the presence of multiple classification systems, as well as controversy about the implications of low morphology in general. There are studies correlating fertilization rates with morphology scores and other studies which show no relationship between morphology scores and IVF results (36-37). Because of the numerous scoring methodologies, the clinician should be familiar with their particular laboratory's methodology and reporting. Although there is controversy with morphology scores overall, some findings, such as the absence of acrosomes or globozoospermia, are highly predictive of failure of fertilization (38). Because of these findings, it is beneficial for the physician to have a detailed analysis of the morphological defects in addition to the percentage of normal forms. For instance, with globozoospermia, a morphology seen in sperm without acrosomes, treatment with ICSI can be successful while IUI success rates are poor (39). For some morphologies, adoption may be the only viable option. For example, even with ICSI, patients with pin head or short tailed sperm on morphologic evaluation fail to have pronuclie fusion leading to ICSI failure (40). Overall however, there is significant difficulty with defining the relationship between morphology and pregnancy rates and therefore there has been no consensus about thresholds and management of patients with low morphology scores (29).

5.1.3. Volume, pH, liquefaction and viscosity

The complete semen analysis includes analysis of the semen volume, pH, liquefaction or non-liquefaction and viscosity. The main component of semen is a coagulated alkaline fluid that comes from the seminal vesicles. This fluid along with the sperm from the vas deferens empties through the ejaculatory duct. Prostatic fluid, the second largest component of seminal volume, generally has a relatively acidic pH of 6.5 and combines with the seminal fluid and sperm in the urethra. Prostatic fluid does not traverse the ejaculatory ducts. This is important, as normal semen has a pH of over 7.2. An acidic low volume specimen therefore is consistent with obstruction of the ejaculatory ducts. Testing for the presence of fructose, a

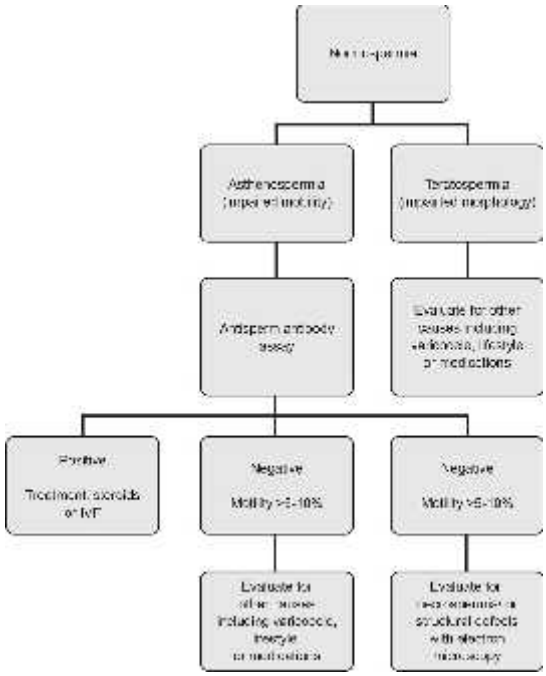

Figure 3. Evaluation algorithm: the normospermia algorithm is presented.

component of semen derived from the seminal vesicles, may be utilized to confirm obstructive causes of infertility. This test, however, is of limited clinical value as the information can be obtained simply by analysis of the clinical picture, semen pH, and volume. Isolated low volume, defined as less than 1.5 mL, could be secondary to improper collection, hypogonadism, or ejaculatory dysfunction including retrograde ejaculation or anejaculation. Liquefaction of semen depends on coagulation proteins found in the seminal fluid as well as the liquifaction by prostate specific antigen, a proteolytic enzyme, secreted by the prostate. This process may take up to 60 minutes. If complete liquefaction does not occur after 60 minutes it should be noted. Viscosity of semen is noted after liquefaction although the clinical significance of hyperviscous semen is controversial. There is evidence showing no correlation between seminal hyperviscosity and semen cultures, leukospermia, or presence of sperm antibodies; as well as evidence showing worse outcomes after IVF with seminal hyperviscosity (41-42).Sperm processing prior to IUI can be considered if there is a clinical concern for hyperviscosity.

5.2. Sperm motion and vitality

Sperm motion is determined by flagellar movement, termed motility, as well as the direction in which sperm move, termed forward progression. The new WHO manual (2010) categorizes motion as either progressive motility, nonprogressive motility or immotile/no movement (25). Computer-aided sperm analysis (CASA) can be, in principle, useful for sperm count, motility and morphology. Unfortunately, CASA initially had difficulties distinguishing spermatozoa from particulate debris making concentration and total sperm counts unreliable. These calculations have improved with fluorescent DNA stains and tail detection algorithms (42-43). In practice, the clinical significance of CASA calculated kinetic studies remains to be established (29). Low motility can be associated with ultrastructural defects or necrospermia. Primary ciliary dyskinesia and Kartagener syndrome are associated with low motility, situs inversus abnormalities, and recurrent respiratory tract infections. Oxidative stress can affect the sperm cell membrane and flagellar axonemal structure with subsequent impairment of progressive motility (44). Antisperm antibodies have also been associated with poor motility as well as with sperm agglutination (45-46). Sperm vitality testing, performed either with dye exclusion assays or hypoosmotic sperm swelling evaluation, is indicated when sperm motility is low and is used to differentiate between necrospermia or structural defects. Exclusion of either trypan blue and eosin Y stains are found only in live sperm, unfortunately this test requires air drying and thus kills the sperm during the staining process (25). For the hypoosmotic sperm swelling assay, live cells swell from the entrance of water, however, are not killed during the assay allowing the sperm to be used for ICSI (47). Of note, the hypoosmotic sperm swelling assay appears to not be suitable for identifying viable sperm in frozen-thawed specimens (48). Confirmation of axonemal structural defects can be obtained with electron microscopy.

5.3. Assessment of seminal leukocytes

The presence of round cells in the semen analysis can be caused by either spermatogenic or non-spermatogenic origin. Inflammatory cells including neutrophils, lymphocytes, and macrophages are seen as round cells and can be confused with degenerated spermatids, spermatocytes, or even epithelial cells. The WHO recommends further evaluation when there are >

Table 1. World health organization 2009 reference standards for semen parameters

Semen characteristic	2.5 %	5 %	95 %
Semen volume (mL)	1.2	1.5	6.8
Sperm concentration (10^6/mL)	9	15	213
Total number (10^6/Ejaculate)	23	39	802
Total motility %	34	40	78
Progressive motility %	28	32	72
Normal forms %	3	4	44
Vitality %	53	58	91

$1x10^6$ round cells/mL. There is controversy about the impact of leukospermia on male subfertility. Some studies have shown no association between semen parameters and leukocyte concentration, as well as poor concordiance between high numbers of leukocytes and positive cultures. Others report association of elevated seminal leukocytes with poor sperm quality, elevated levels of oxidative stress, increased sperm DNA fragmentation, and poor sperm penetration assays (49-52). In patients with a history of sexually transmitted disease or tuberculosis, the clinician must consider obstruction of the epididymis or vas deferens as a sequelae of genital infection with these organisms (53). The presence of increased inflammatory cells can be documented by testing with the endtz test, a myeloperoxidase staining which differentiates immature sperm and leukocytes. The endtz test can also be used as an indicator of excessive reactive oxygen species (54). Other methods include traditional cytologic staining and other immunohistochemical techniques (55).

5.4. Reactive oxygen species

Reactive oxygen species (ROS) are important for hyperactivation, capacitation and acrosome reactions. However, excessive ROS cause oxidative stress which has been associated with decreased sperm motility, viability and mid-piece sperm defects (56-58). There is controversy about testing for ROS, the best methods for testing for ROS, and the efficacy of therapies for treatment of excessive ROS. Testing for ROS is difficult as these molecules have a short half-life necessitating rapid testing on fresh semen specimens. There is evidence that elevated ROS levels are present in the semen of 25% of infertile men versus 0% of fertile men (59-60). Currently the American Urological Association (AUA) consensus is that there is insufficient data supporting routine testing for ROS (29).

5.5. Total antioxidant capacity

Although the testing and treatment of ROS is controversial, the presence of seminal oxidative stress has been seen in increased frequency in subfertile males (61). In addition to measurement of seminal ROS, there is movement to evaluate the total antioxidant capacity of semen. Multiple studies support the treatment of infertile men with antioxidants as this improves semen characteristics and improves assisted and spontaneous pregnancy rates (62-65). This is likely because antioxidants dispose and suppress formation of ROS which in turn reduces sperm DNA damage (66). Total antioxidant capacity provides an objective assessment of ROS scavenging capability therein allowing targeted antioxidant therapy. While not a routine test, this may provide useful clinical information that can guide treatment.

5.6. Sperm DNA damage

Significant sperm DNA damage i.e. fragmentation, abnormal packaging or protamine deficiency, impairs function and therefore fertility (67). Elevated levels of DNA fragmentation have been associated with lower spontaneous pregnancy rates as well as compromised IUI/ART outcomes (68-69). Testing can be either direct or indirect. Common direct tests include the comet assay, the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) assay, and DNA oxidation measurement. Indirect testing is performed with the sperm chromatin structure assay and the sperm chromatin dispersion assay (70). Some authors have suggested utilizing this test in infertile patients with idiopathic, or suspected oxidative stress related semen abnormalities, who are considering IUI/ART as a tool for prognosis on success of therapy (71). There has been a correlation between sperm DNA damage and clinical varicocele as well as improvement post varicocelectomy. Therefore in patients debating undergoing varicocelectomy an elevated sperm DNA damage test may facilitate counseling (72-73). The current AUA consensus is that there is insufficient evidence to support routine DNA damage testing (29).

5.7. Antisperm antibodies

Testing for antisperm antibodies is indicated in patients who have had any potential disruption of the blood-testis barrier, asthenozoospermia or sperm agglutination seen on semen analysis. Potential disruption of the blood-testis barrier is found in those with a history of genital infections, trauma, vasectomy, or prior surgery of the testicles. Assessment of direct binding of antibodies to sperm is of more clinical utility than indirect assays examining semen or serum antibodies bound to donor sperm. For these patients steroids may lower antibody titers, however, for some patients its use is controversial due to side effects (74). An alternative is to attempt semen processing for IUI/ART (75-76).

5.8. Post ejaculate urine analysis

A post ejaculation urine analysis is indicated to establish potential retrograde ejaculation in those who have low semen volume; in the absence of either obstructive etiologies, improper collection or hypogonadism. In patients with azoospermia or aspermia on semen analysis, the presence of any sperm in a post ejaculatory urine analysis would be consistent with retrograde ejaculation. However there is no expert consensus on cut-offs for diagnosis of retrograde ejaculation in patients with oligozoospermia (29). Retrograde ejaculation is seen with neuropathic disorders including patients who have had retroperitoneal lymph node dissection, diabetes mellitus, or patients who have had pelvic surgery. This can be treated with sympathomimetics or the sperm can be recovered after alkalinization of urine.

5.9. Other functional testing

5.9.1. Sperm-cervical mucus/post coital test

The post-coital test is an in-vivo assessment of sperm motility and involves the identification of motile sperm in cervical mucus during the periovulatory period. The results of this test are very subjective with low precision, making it of limited clinical value (55).

5.9.2. Capacitation

The process of capacitation takes place in the female genital tract and can be tested *in vitro* by placing spermatozoa in capacitation-inducing medium. This incubation causes hyperactivation of the spermatoza. The clinical value of this testing is currently unclear and it remains an investigative tool (29).

5.9.3. Acrosome reaction testing

The acrosome is a cap-like vesicle containing proteolytic enzymes critical for sperm-egg fusion. The function of the acrosome can be assessed with multiple tests. Using microscopy or fluorescence microscopy sperm with absent acrosomes are identified as having globe-shaped heads. The function of the acrosome is assessed with acrosome reaction testing. This assesses the percent of spermatozoa which have spontaneously deployed acrosome contents as well as the percent that can be induced in-vitro to undergo release of acrosome. The clinical relevance of this testing remains to be established. It may be helpful for counselling couples with regards to success rates of conventional IVF or IUI, although the current AUA consensus is that this is primarily an investigative tool (77-79).

5.9.4. Sperm binding tests

This class of testing examines the ability of sperm to bind to either human or zona free hamster oocytes. The zona pellucida binding assay must utilize a human zona as the binding is species specific. There are two tests to evaluate this. The hemizona assay uses nonfertilized oocytes in which the zona has been divided and one half is incubated with fertile donor sperm and the other with patient sperm. The ratio of sperm bound is then reported (80). The other test involving human oocytes, the sperm zona binding ratio test, uses sperm that has been labeled with fluorochromes and zona intact oocytes that did not fertilize during IVF (81). The zona free hamster oocyte penetration test assesses sperm egg fusion in a similar fashion to the hemizona assay tests. This test has a wide variability of performance between laboratories.

Overall, these tests are infrequently used, although they may have a limited role in identifying patients who would require ICSI rather than IUI or IVF. The AUA consensus for this category of test is that it should only be ordered for patients in whom the testing would influence treatment choice (29). Currently the couples who fail to fertilize with IVF typically proceed automatically to ICSI and therefore the clinical utility of these tests are questionable.

6. ASSISTED REPRODUCTIVE TECHNOLOGIES AND INTRAUTERINE INSEMINATION

Technologies employed to assist with pregnancy include IUI, IVF, and ICSI. In 1996, the Center for Disease Control initiated data collection for ART performed in the United States as mandated by the Fertility Clinic Success Rate and Certification Act of 1992. In 2006, 138,198 ART procedures were reported. 72% of these procedures used embryos from patient's eggs that were freshly fertilized (82). The andrology laboratory is involved with IUI and ART via semen processing for IUI, IVF and ICSI as well as sperm cryopreservation. Sperm cryopreservation is utilized for donor semen as well as in patients who are to undergo procedures or treatment which may induce sterility such as vasectomy or certain chemotherapies. Couples who need ART require fertility laboratories facile in these techniques. For some patients, medical and surgical therapy may be employed to promote natural pregnancy, while in others, therapy may be aimed at increasing the sperm count so that IUI is a reasonable option. Based upon a full evaluation, the clinician may determine that IVF or ICSI are the best options for some patients. For patients with congenital or acquired obstructions, or those with anejaculation, andrology laboratories can identify viable sperm from either percutaneous or microsurgical epididymal sperm aspiration. In addition, andrology laboratories can process testicular tissue from biopsies to identify sperm suitable for ICSI (83). The least motile sperm are obtained from testicular sperm retrieval. With ICSI, the source of sperm is less important, with one report showing a 27.4% live birth rate with ejaculated sperm, a 31.3% rate for fresh epididymal sperm, a 27.2% for frozen or thawed epididimal sperm, versus a 23.1% rate for sperm extracted from the testicle (84). The modern andrology laboratory plays a critical role in the processing and preservation of sperm for effective IUI/ART techniques.

7. CLINICAL VIGNETTES

7.1. Introduction

The diagnosis of the etiology of male factor infertility is complex as well as emotionally difficult for many otherwise healthy patients. In order to further discuss the interpretation of specific testing, clinical vignettes will be presented consisting of the history, physical, and laboratory findings of patients presenting to a tertiary referral center for evaluation of infertility.

7.1.1. Clinical vignette A: Retrograde ejaculation

40 year-old male presents to the clinic with primary infertility as well as sexual dysfunction for 6 months. He complains of poor erections, frequent anejaculation, and decreased libido. He has tried PDE-5 inhibitor therapy with minimal effect. He denies a history of mumps, testicular trauma or history of cryptorchidism. He has had diabetes mellitus type 2 for 10 years and is currently on insulin. He has not had any surgeries. He does not smoke, use alcohol or recreational drugs. On physical exam he is a well masculinized male without gynecomastia and normal testicles bilaterally measuring 18 mL each. Vas are palpable bilaterally. A grade II left sided varicocele and

Table 2. Clinical vignette A: semen analysis and post ejaculate urine analysis

	Reference Range	Semen Value	Urine Value
Days of abstinence		Unknown	
Volume	2-5 mL	0.2 mL	67 mL
Count	>20 M/mL	94	5
Motility	>40%	50%	60%
Forward progression		2.5	
Morphology kruger	Low >14%	5%	
Viscosity		Increased	
Color		Normal	
pH	7.2-7.8	8.0	6.7
Clumping		None	
Abnormal head		83%	
Abnormal tail		12%	

Table 3. Clinical vignette B: endocrine profile

	Reference Range	Value
LH	1-7.0 mU/mL	20.5
FSH	2.2 mU/mL	37.8
Testosterone	220-1000 ng/dL	111
Estrogen total		119
Prolactin	2-14 ng/mL	5.8

Table 4. Clinical vignette C: endocrine profile

	Reference Range	Value
LH	1-7.0 mU/mL	0.5
FSH	2.2 mU/mL	1.8
Free Testosterone	9.3-26.5	4.7

Table 5. Clinical vignette C: semen analysis

	Reference Range	Value
Days of abstinence		90 days
Volume	2-5 mL	0.5 mL
Count	>20 M/mL	70
Motility	>40%	76
Forward progression		2.5
Morphology kruger	Low >14%	7%
Viscosity		Normal
Color		Normal
pH	7.2-7.8	7.2
Clumping		None
Round cells	M/mL	0.8 M/mL
Leukocyte	Low <1M/mL	0.6 M/mL
Abnormal head		87%
Abnormal tail		6%

Table 6. Clinical vignette C: endocrine profile

	Reference Range	Value
LH	1-7.0 mU/mL	3.5
FSH	2.2 mU/mL	2.2
Testosterone	220-1000 ng/dL	277

The leading clinical concern for this patient is retrograde ejaculation associated with his diabetes. In addition to his retrograde ejaculation, the presence of bilateral clinical varicocele likely had an impact on his semen quality. The initial treatment plan for this patient included a trial of alpha agonist therapy in an attempt to produce antegrade ejaculation. He had a minimal amount of ejaculate (Table 2). Ultimately he was treated with alkalinization therapy for eventual sperm recovery from his urine. Adequate numbers were recovered allowing for successful IUI treatment.

7.1.2. Clinical vignette B: Klinefelter's syndrome

27 year-old male presents to the clinic with primary infertility for three years. He has had no previous work-up. He has no history of undescended testicles, mumps, or testicular trauma. He is otherwise healthy, has never had surgery and takes no other medications. He has no family history of infertility or cystic fibrosis. He smokes 1-2 packs of cigarettes per day with no alcohol or recreational drug exposure. On physical exam he is a well masculinized male with significant gynecomastia and small testicles bilaterally measuring 4 mL each. Vas are palpable bilaterally and he does not have a clinical varicocele.

The leading clinical concern for this patient is Klinefelter's syndrome. He received endocrine, semen and genetic testing. The semen analysis revealed normal volume azoospermia. Endocrine testing is summarized (Table 3). Karyotype confirmed the diagnosis with non-mosaic 47 XXY chromosomes. Patient was referred for genetic counselling and instructed that he may be candidate for ICSI with attempts at sperm extraction via microsurgical testicular sperm extraction. He started anastrozole 1 mg by mouth daily in advance of the sperm extraction as this has shown improvement in success rates (85). Improvements are theoretically seen in treatment with anastrozole as it blocks the degradation of testosterone and increases intratesticular testosterone. These patients do not respond well to luteinizing hormone releasing hormone therapy as they have low numbers of Leydig cells. Sperm was successfully recovered via biopsy and ICSI was utilized resulting in a successful pregnancy (Table 3).

7.1.3. Clinical vignette C: Kallman syndrome

30 year-old male presents to the clinic with primary infertility as well as sexual dysfunction. He states that although he was able to ejaculate in the past, he has been anejaculatory for the last 10 years. He also related marked decline in erectile function and libido. He had delayed pubertal development and denies anosmia. (Table 4). On physical exam he is poorly masculinized with decreased muscle mass and facial hair. His testicles are markedly decreased in volume at 10 mLs. He has palpable vas and no varicocele.

The leading clinical concern for this patient is hypothalamic hypogonadism or Kallmann syndrome. He was started on HCG followed by HMG therapy to induce spermatogenesis. Repeat testing demonstrated improved semen parameters and hormonal profile (Table 5, Table 6). The couple conceived via IUI.

7.1.4. Clinical vignette D: Inflammation

30 year-old male presents to the clinic with primary infertility for one year. He denies history of STDs, mumps orchitis, testicular trauma or history of cryptorchidism. He is otherwise healthy and has not had any surgeries. He denies a family history of infertility or cystic fibrosis. He does not smoke or use alcohol. On physical exam he is well masculinized without gynecomastia. He has normal testicles bilaterally measuring 18 mL each. Vas were palpable bilaterally. Rectal exam reveals a normal prostate.

He had a preliminary work-up including a complete semen analysis (Table 7). The elevated endtz test suggests the possibility of subclinical genitourinary

Table 7. Clinical vignette D: semen analysis

	Reference Range	Value
Semen volume	2.0-5.0 mL	3.50
Semen pH	7.2-7.8	7.6
Concentration	Low: >20 M/mL	27.75
% Motile sperm	50-100 %	59
Total count sperm	Low: >40 M	97.13
Total motile sperm	M	57.31
Normal oval head	14-100 %	1
% Normal sperm	30-100 %	9 %
% Tapered sperm	0-10 %	13 %
% Amorphous sperm	0-11 %	62 %
% Small sperm	0-15 %	6 %
% Megalo sperm	0-2 %	2 %
% Abnormal tails	%	8 %
Endtz	0.0-0.1 m/mL	2.4

Table 8. Clinical vignette D: semen analysis

	Reference	Value
Semen pH	7.2-7.8	7.2
Days of abstinence		3 days
Volume	2.0-5.0 mL	3.8
Count	Low: >20 M/mL	35
Motility	Low: >40 %	83
Morphology kruger	Low: >14 %	4 (L)
Viscosity		Normal
Color		Normal
Clumping		No
Undifferentiated round cells	M/mL	0.8
Endtz	0.0-0.1 m/mL	0.2
Abnormal head		96 %
Abnormal tail	%	0

Semen analysis after treatment with doxycycline for 3 weeks and antioxidant therapy.

Table 9. clinical vignette E: semen analysis

	Reference Range	Value
Volume	2-5 mL	2.7 mL
Count	>20 M/ml	19.6
Motility	>40%	30
Viscosity		Normal
Color		Opaque
pH	7.2-7.8	7.3
Round cells	M/mL	1.54
Abnormal head semen		100%
% Normal sperm	30-100 %	0
% Tapered sperm	0-10 %	13
% Amorphous sperm	0-11 %	42
% Abnormal tails	%	43
Eosin/Nigrosin Stain	Low: >75 %	16.5
Endtz	0-0.1m/mL	0.4
Total antioxidant capacity	Low: >2000 MTE	3030
Reactive oxygen species	Low: <20 RLU/sec	152
DNA damage	Low: <24 %	28

infection. The patient and his partner underwent testing for *chlamydia trachomatis*, *mycoplasma genitalium* and *neisseria gonorrhoeae*. All results were negative. Given the elevated endtz test, the patient was treated with doxycycline for 3 weeks for potential subclinical genitourinary infection. Typically the partner is only treated if there is evidence of infection. The patient was also started on antioxidant therapy. Repeat semen analysis three months later showed improved sperm concentrations and near resolution of seminal leukocytes (Table 8). The couple subsequently conceived spontaneously.

7.1.5. Clinical vignette E: Varicocele

38 year-old male presents to the clinic with primary infertility for 9-10 months. He describes normal sexual frequency and libido. He had a history of chlamydial infection 3 years ago. He denies history of mumps orchitis, testicular trauma or history of cryptorchidism. He is otherwise healthy. On physical exam he is well masculinized without gynecomastia. He has mild reduction in left testicular volume at 16 mL and a normal volume right testicle. Vas are palpable bilaterally. A grade II left sided varicocele and a grade I right sided varicocele is present. Rectal exam reveals a normal prostate.

He had a preliminary work-up at an outside clinic with semen analysis revealing oligoteratozoospermia. A subsequent scrotal ultrasound confirmed bilateral varicoceles. Endocrine evaluation with serum FSH and testosterone were normal. Advanced semen analysis showed low motility with elevated endtz test, high reactive oxygen species and high DNA damage (Table 9). He was prescribed doxycycline and recommended antioxidant therapy as well as bilateral varicocele repair. Three months after bilateral varicocele repair, a repeat semen profile showing improved concentration, motility, DNA fragmentation and reactive oxygen species although persistent teratozoospermia (Table 10). The couple pursued IUI and subsequently conceived.

7.1.6. Clinical vignette F: Y microdeletion

35 year-old male presents with primary infertility for 2 years. He states he has normal sexual frequency and libido. He denies history of mumps orchitis, sexually transmitted disease, testicular trauma or history of cryptorchidism. He is otherwise healthy and has never had surgery. He takes no medications regularly. The patient denies family history of infertility or cystic fibrosis. On physical exam, he is well masculinized with normal testicles bilaterally. Vas are palpable bilaterally. There are grade 2 varicoceles bilaterally and a normal prostate on rectal exam.

He had a preliminary work-up at an outside clinic with a semen analysis showing normal volume azoospermia, normal hormone profile, normal karyotype and scrotal ultrasound showing bilateral varicocele (Table 11). A Y microdeletion study was subsequently obtained that revealed complete AZFb deletion which is associated with poor likelihood of successful testicular sperm extraction. The couple chose to adopt.

7.1.7. Clinical vignette G: testicular tumor

38 year-old male presents to the clinic with primary infertility for 2 years. He denies history of mumps, sexually transmitted disease, testicular trauma or history of cryptorchidism. He denies recreational drug use. On physical exam he is well masculinized without gynecomastia and small testicles bilaterally measuring 8-10 mL each. Vas are palpable bilaterally. There are no palpable varicoceles and a normal prostate on rectal exam.

He had a preliminary work-up at an outside clinic with semen analysis showing normal volume oligoasthenozoospermia (Table 12). A scrotal ultrasound reveals two hypoechoic solid right intra-testicular masses. One is located in the upper pole measuring 3x2x2mm. The

Table 10. clinical vignette E: semen analysis

	Reference Range	**Value**
Volume	2-5 mL	3.0 mL
Count	>20 M/mL	21
Motility	>40%	56
Viscosity		Normal
Color		Opaque
pH	7.2-7.8	7.6
Round cells	M/mL	0.1
Abnormal head semen		95%
% Normal sperm	30-100 %	9
% Amorphous sperm	0-11 %	45
% Abnormal tails	%	11
Eosin/Nigrosin Stain	Low: >75 %	10
Endtz	0-0.1m/mL	0.0
Total antioxidant capacity	Low: >2000 MTE	4070
Reactive oxygen species	Low: <20 RLU/sec	10
DNA damage	Low: <24 %	2.57

Results after treatment with doxycycline.

Table 11. Clinical vignette F: semen analysis

	Reference	**Value**
Volume	2-5 mL	1.7 mL
Count	>20 M/mL	0 M/mL

Table 12. Clinical vignette G: semen analysis

	Reference	Value
Volume	2-5 mL	0.5 mL
Count	>20 M/mL	4.45 M/mL
Motility	>40%	30%
Morphology		68%

Table 13. Clinical vignette G: endocrine profile

	Reference Range	**Value**
LH	1-7.0 mU/mL	5.9
FSH	2.2 mU/mL	18.5
Testosterone	220-1000 ng/dL	289
Prolactin	2-14.0 ng/mL	4.3

a grade I right sided varicocele are present. On routine urine analysis sperm were noted.

second is located in the midpole and measures 3x2x3 mm. Endocrine evaluation shows elevated FSH but is otherwise normal (Table 13). Given these findings, the patient had testicular tumor markers evaluated which were in the normal ranges. He was counseled on his options including observation with serial scrotal ultrasounds, orchiectomy or possible partial orchiectomy. The patient elected to undergo partial orchiectomy. He had sperm cryopreserved preoperatively. Using intraoperative ultrasound, the masses were localized and excised. Pathology showed Sertoli only syndrome in occasional seminiferous tubules as well as two Leydig cell tumors.

8. SUMMARY

While clinicians need different testing depending on their clinical practice, all clinicians require a quality andrology laboratory capable of providing accurate and precise results for the testing offered. Referring physicians primarily need reliable semen analysis. Specialists on the other hand require an andrology laboratory capable of accurately reporting and performing a plethora of special tests. Reliability and quality are not only evidenced by accreditation and a focus on quality control, but also by attention to systems ensuring proper specimen collection, labeling and reporting.

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Abbreviations: ART: assisted reproductive technologies, IUI: intrauterine insemination, ICSI: intracytoplasmic

sperm injection, IVF: *in vitro* fertilization, CLIA: Clinical Laboratory Improvement Act, CAP: College of American Pathologists, ASRM: American Society for Reproductive Medicine, RALP: Reproductive Laboratory Accreditation program, SART: Society for Assisted Reproductive Technology, TJC: The Joint Commission, JCAHO: Joint Commission on Accreditation of Healthcare Organizations, COLA: Commission on Laboratory Accreditation, ABB: American Board of Bioanalysis, WHO: World Health Organization, CFTR: cystic fibrosis transmembrane conductance regulator, ROS: reactive oxygen species, AUA: American Urological Association, TUNEL: transferase-mediated deoxyuridine triphosphate nick end-labeling, STDs: sexually transmitted diseases

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