Semen Analysis in Laboratory Practice: An Overview of Routine Tests

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Semen analysis is a basic step in the investigation of several disturbances affecting the male genital tract. Analysis of seminal parameters provides important clinical information on the spermatogenesis and functional competence of spermatozoa, as well as on the secretory pattern of the accessory genital glands. Semen analysis is particularly useful in the evaluation of couples requiring fertility investigation (to detect genital infections and pathologies) and in verifying the influence of environmental factors, drugs, lifestyle, chemical products, and professional activities on several diseases affecting male reproductive health. Measure of semen quality is of substantial interest for diagnoses in clinical urology, andrology, and

gynecology. Currently, basic requirements for semen analysis are standardized by World Health Organization (WHO) guidelines that describe several procedures for an objective evaluation of the semen quality with diagnostic purposes. These guidelines include: parameters for the physical and biochemical evaluation of semen; parameters for the analysis of sperm characteristics; and other seminal parameters that can be easily adopted in any laboratory. This report summarizes current concepts on semen analysis and the significance of the seminal parameters for reaching a diagnosis based on the procedures recommended by WHO guidelines. *J. Clin. Lab. Anal.* 17:247–258, 2003. © 2003 Wiley-Liss, Inc.

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INTRODUCTION

Semen analysis (SA) is of clinical significance in evaluating the function of the male genital organs. Analysis of seminal parameters is particularly helpful in investigating male infertility, genital infections, and pathologies (1,2). SA can also be useful in evaluating the adverse effects of chemical products, environmental factors, professional activities, drugs, and nonsexual diseases affecting men's fertility (3,4).

It is a known fact in laboratory practice that accuracy of SA depends on the employment of proper and reliable methodologies. In this regard, one more direct measurement of semen characteristics has been made available and is now widely accepted (5,6). This criterion was standardized by the World Health Organization (WHO) protocol (7). These procedures may be performed successfully in any clinical laboratory, employing simple and inexpensive techniques.

This review focuses on current SA concepts, with particular emphasis on the standard procedures laid down in the WHO guidelines, and on the association between abnormal seminal parameters and dysfunction of the male genital organs.

STANDARDIZATION

In order to provide an objective analysis for clinical purposes, a basic SA routine must take into account the helpfulness of seminal parameters toward formulating a diagnosis in accordance with the clinical setting of the examination. Therefore, assessment of parameters that can provide information on the functional activity of the main genital organs (testicles, epididymis, seminal vesicles, and prostate) is recommended. The following is a summary of such assessment.

SEMEN PARAMETERS AND TESTICULAR FUNCTION

Testicles produce spermatozoa through the complex process of differentiation of haploid germ cells from

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diploid stem cells known as spermatogenesis (Fig. 1). Spermatozoa are produced after several steps of spermatid development (spermiogenesis) (8,9).

Testicular function is developed by stimulus to the central nervous system, specifically to the pituitary gland, which secretes follicle stimulating hormone (FSH) and luteinizing hormone (LH) under the influence of pulsatile secretions of gonadotrophin-releasing hormone (GnRH) from the hypothalamus. LH stimulates the biosynthesis of testosterone in the Leydig cells of testis, whereas testosterone and FSH modulate the spermatogenesis transduced by hormonal signals through Sertoli cells and peritubular cells.

Testosterone is essential for normal spermatogonial mitosis and completion of meiosis, as well as for spermiogenesis. FSH promotes the increase of Sertoli cells that provide support for germ cells. Under FSH stimulation, Sertoli cells still produce: compounds involved in the feedback loop of the hypothalamic-pituitary-gonadal axis (inhibin, activin, and follistatin); androgen-binding protein (ABP) that binds to the testosterone and transports it within the seminiferous tubules; and other factors required for the development of germ cells (transferrin, plasminogen activate factor, IgF1, etc.).

The current paper describes only basic spermatogenesis concepts. Comprehensive reviews of the mechanism of male germ cell differentiation have been reported previously and are suggested for further reading (10–15).

Assessment of the functional activity of the testicles through SA is based on the analysis of the following

Fig. 1. Spermatogenesis and the hypothalamic-pituitary-gonadal axis.

seminal parameters: 1) sperm count or density; 2) sperm morphology; 3) sperm vitality; and 4) sperm precursors.

Sperm density

This is the oldest parameter used in SA. It determines the amount of spermatozoa of the specimen, expressed in sperm/milliliter (mL) of semen. Table 1 shows the nomenclature for sperm density. The main features are as follows.

Polyzoospermia

Polyzoospermia is characterized by the presence of normal sperm parameters (vitality, motility, and morphology), particularly in semen with sperm densities between 250 and $350 \times 10^6/\text{mL}$ (16). However, polyzoospermia has been considered a pathological entity, not because it is a functional disorder with massive production of spermatozoa, but for its association with an impaired reproductive performance supposedly caused by chromosome anomalies (17), decreased ATP content (18), and abnormal acrosome function (19). Polyzoospermia is also strongly associated with a higher miscarriage risk that affects around 25% of the women whose husbands have polyzoospermic semen (20).

In sperm count higher than $350 \times 10^6/\text{mL}$, polyzoospermia can be associated with teratozoospermia and/or asthenozoospermia (16). The larger the sperm density is, the greater is the prevalence of these disturbances. On occasion, some semen may even reach values of up to $1 \times 10^9/\text{mL}$. However, this is not a frequent finding in SA. Since polyzoospermia only affects 0.2–4.2% of men (21), available data related to it, such as strategies of treatment and their results, are scarce in the literature.

Normozoospermia

Although situated within normal sperm density, several disorders cause teratozoospermia and/or asthenozoospermia in normozoospermic men. Varicocele, antisperm antibodies, leukocytospermia, changes in the functional activity of the prostate and of the seminal vesicles, and bacteriospermia are disturbances that impair the semen quality of normozoospermic men.

TABLE 1. Nomenclature for sperm density

Polyzoospermia: sperm count $> 250 \times 10^6/\text{mL}$
Normozoospermia: sperm count between 20 and $250 \times 10^6/\text{mL}$
Mild oligozoospermia: sperm count between 10 and $20 \times 10^6/\text{mL}$
Moderate oligozoospermia: sperm count between 5 and $10 \times 10^6/\text{mL}$
Severe oligozoospermia: sperm count $< 5 \times 10^6/\text{mL}$
Azoospermia: no spermatozoa in the ejaculate

Moderate and mild oligozoospermia

Moderate and mild oligozoospermia are particularly caused by functional disturbances of testicular origin such as varicocele and endocrine disorders, as well as nontesticular factors such as: mumps orchitis, exposure to drugs, chemical products, environmental factors, and X-rays that are deleterious to spermatogenesis (22,23).

Severe oligozoospermia

Seminal abnormalities are more intense and, as a result, decrease male fertility potential significantly. SA detects many sperm anomalies, showing the degree of the damages done to spermatogenesis. Severe oligozoospermia is often associated with microdeletions of the Y chromosome and genetic risk factors (24).

In summary, oligozoospermia is associated with poor sperm motility and abnormal sperm morphology. It reduces sperm quality and its fertilization capacity, although oligozoospermic men do fertilize naturally at times, even in severe cases (25).

Azoospermia

For diagnostic purposes, azoospermia is classified as follows: 1) nonobstructive (secretory) azoospermia that is caused by severe testicular failure and 2) obstructive (excretory) azoospermia that is a result of testis, epididymis, and excretory ducts occlusion that prevents the release of spermatozoa in the ejaculate. Differential diagnosis is based on physical examination, testis biopsy, genetic screening, and endocrine evaluation (26). A special case of azoospermia is the congenital bilateral absence of the vas deferens, a pathology associated with increased cystic fibrosis gene mutation (27), in which vas deferens, and usually the seminal vesicles, are missing due to abnormal embryonic development. The ejaculate only consists of prostate fluid. Therefore, very high levels of the biochemical markers of the prostate gland (see below) are detected. It is likely, therefore, that seminal vesicles markers (see below) are not detected in SA. Moreover, pH of the semen is <7.0 and the volume ≤ 1.0 mL (27). In spite of being rare, this pathology can be easily identified by SA.

Evidence in the literature indicates that microdeletions of the Y chromosome play a role in the development of azoospermia. The DAZ gene located in the AZFc region of the long arm of the Y chromosome is often absent in azoospermic men (24).

Some disturbances that cause obstructive and non-obstructive azoospermia are listed in Table 2.

TABLE 2. Etiology of obstructive and nonobstructive azoospermia

Nonobstructive azoospermia	Obstructive azoospermia
Spermatogenic arrest	Congenital agenesis of the vas deferens
Hypospermatogenesis	Previous inguinal and genital surgery
Hyalinization, fibrosis, sclerosis	Epididymal obstruction
Klinefelter's syndrome	Rete testis obstruction
Sertoli-cell-only syndrome	Ejaculatory ducts obstruction
Malignant tumors	Post inflammatory obstruction
Mumps orchitis	Bronchiectasia (Young's syndrome)

TABLE 3. Relationship between sperm anomalies and the diagnosis

Sperm anomaly	Associations with the diagnosis
Small	Varicocele, endocrine disorders
Large ^a	—
Amorphous	Varicocele, endocrine disorders
Round-headed	Globozoospermia (round-headed syndrome)
Double-headed ^b	—
Pin-headed	Severe teratozoospermia
Tapering	Varicocele
Angulation ^a	—
Cytoplasmic droplet	Varicocele ^c
Amorphous mid-piece ^a	—
Double tail ^b	—
Coil tail	It associates with seminal colonization of mycoplasmas
Short tail	Tail stump syndrome
Combined anomalies	Severe teratozoospermia

^aNot associated with a specific disturbance.

^bIncreases in a disturbance in which double tail sperm is also increased.

^cThis anomaly often associates with an increase of reactive oxygen species in semen.

Combined anomalies include multi-anomalies, e.g., head + mid-piece, head + tail, mid-piece + tail and head + mid-piece + tail.

Sperm Morphology

This is another time-honored SA parameter that evaluates the quality of the spermatozoa. Currently, there are two criteria to assess sperm morphology:

1. The WHO criteria (7) analysis is based on earlier reports (28–32) and standardized by the WHO's 1980, 1987, 1992, and 1999 publications. It determines the percentage of oval head sperm and of a variety of sperm anomalies found in semen. WHO criteria look for a more detailed morphologic evaluation of the sperm head, midpiece, and tail characteristics in order to detect the increase of sperm anomalies that often associate with abnormal spermatogenesis (28,33–35) and seminal pathologies (Table 3).

2. The Tygerberg strict criteria (36) analysis takes into consideration the acrosome characteristics in classifying the spermatozoa morphologically. According to strict criteria of sperm morphology analysis (SCSMA), normal spermatozoa present oval headed with well-defined acrosome occupying 40–70% of the sperm head. Semen with normal spermatozoa values >14% present good prognosis for both in vivo and in vitro fertilization (normal population). Values between 4% and 14% also present good prognosis (Pattern G), although the likelihood of fertilization is smaller than with semen found in the normal population. Semen with values <4% have poor prognosis (Pattern P).

SCSMA also considers spermatozoa with slightly elongated head and normal acrosome, midpiece, and tail. Oval shaped heads with normal acrosome and slight neck defects, e.g., debris around the neck, are also assessed in SCSMA. Both are described as slightly amorphous spermatozoa (36). When the sum of normal spermatozoa plus slightly amorphous sperm is >30%, a cutoff value termed morphologic index, expectation of conception is also favorable. Spermatozoa with no acrosome, or with acrosome smaller than 30% or larger than 70%, are classified as severely amorphous. All other sperm anomalies (small, large, round, etc.) are classified according to the WHO criteria. As can be observed, SCSMA does not seek detailed characterization of sperm anomalies in order to associate them with any disturbance. SCSMA has predictive value for patients undergoing investigation of male infertility or those who submit to in vitro fertilization (IVF).

It should be emphasized that update of the WHO's 1999 (fourth edition) guideline now describes sperm morphology, taking into account SCSMA in order to characterize normal spermatozoon. Thus, the concept of teratozoospermia has changed. Actual reference value for normal sperm morphology analysis is undefined because multicenter population-based studies are in progress. Based on assisted reproductive technology programs, a cutoff of 15% of oval heads, including normal acrosome, comprising 40–70% of the head area is recommended. It now seems that the natural trend for sperm morphology analysis is the integration between the WHO criteria and Tygerberg criteria, in order to define a unique approach to evaluate this semen parameter in the future.

Sperm Vitality

This parameter assesses percentage of live and dead spermatozoa based on the measurement of the integrity of the sperm membrane by dye exclusion. Living cells

hinder stain penetration, whereas dead cells allow cellular staining. Sperm vitality is influenced by abnormal spermatogenesis, by epididymal fluids during the storage of the gamete in the epididymis, and by vesicular and prostatic fluids during and after ejaculation. Therefore, sperm vitality can be affected by disturbances that affect any genital organ. Thus, clinical value of this semen parameter is restricted to the evaluation of the deleterious effects of disturbances that affect the survival of spermatozoa.

Sperm Precursors

In addition to the role of sperm density, morphology, and vitality to evaluate testicular function, analysis of sperm precursors (i.e., spermatogonia, primary and secondary spermatocytes, and spermatids) would be desirable for infertile men. Abnormal exfoliation of immature germ cells (IGC) is occasionally observed in SA as a result of seminiferous tubules dysfunction, hypospermatogenesis, varicocele, and other testicular disturbances, all related to male infertility (37–40).

Attempts have been made to differentiate IGC in semen (7,41–43). However, little is known about the morphological variants, frequency, and range of these cells in semen. Indeed, characterization of IGC is a procedure that calls for a skillful analyst who can identify the different cell forms present in semen. Ultimately, IGC analysis is only well practiced by a few experts worldwide and consists, therefore, of an optional examination procedure. Some methodologies for this analysis are recommended by the WHO manual (7).

SEMEN PARAMETERS AND EPIDIDYMIS FUNCTION

Spermatozoa leave the seminiferous tubules and migrate slowly, conveyed by the testicular fluids through the rete testis, until they reach the caput of the epididymis. At this stage, they neither exhibit progressive motility nor fertilize the egg. With the exposure to the epididymal microenvironment, spermatozoa undergo molecular changes, developing the ability to fertilize the egg (sperm maturation) and increased capacity for forward progressive and sustained motility (44,45). Remodeling processes of sperm involve uptake of epididymal proteins, glycoproteins, sugar and lipid determinants, and binding of epididymal proteins such as P34H, anti kcl, and FLB 12 on the sperm surface. Local modifications of sperm proteins, particularly PH-30 and fibronectin, and stabilization of nuclear chromatin and dense fibers by disulfide bonds also occur. Mature/motile sperm are then stored in quiescent state within the cauda of epididymis, being released only

at ejaculation, when they acquire an instantaneous burst of vigorous motility (44). The epididymis is still the major source of antioxidants for the semen (46).

The evaluation of the secretory activity of the epididymis in SA is a very restricted procedure, being limited to the determination of the levels of the biochemical compounds produced almost exclusively by the epididymis (α -1,4 glucosidase, glycerylphosphorylcholine, and carnitine) that play a role in sperm metabolism (44). However, the methodologies for the analysis of these epididymal markers are complex and often present conflicting results (47,48). Therefore, they are not assessed in the SA routine, although determination of neutral α -1,4, glucosidase isoenzyme activity is recommended by the WHO manual (7).

In fact, analysis of sperm motility is the only traditional SA parameter that can sometimes give additional information on epididymal function. However, this seminal parameter is a glandular marker restricted to some cases of unexplained asthenozoospermia originating in epididymal dysfunctions that are not frequent in the SA routine. Unfortunately, sperm motility also has a direct relationship with the function of the testicles (motility depends on the quality of the produced spermatozoa) and is influenced by the secretions of the seminal vesicles and of the prostate after ejaculation. Functional disorders in these genital organs can also affect sperm motility. Therefore, this analysis is of poor clinical value as an epididymal marker.

According to the WHO criteria (7), sperm motility is classified in four degrees of sperm progression (Table 4). Degree (a) $<25\%$ or progression (a)+(b) $<50\%$ is a disturbance called asthenozoospermia. Table 5 shows factors that affect sperm motility.

SEMEN PARAMETERS AND SEMINAL VESICLES FUNCTION

Seminal vesicles secrete the major fraction of the ejaculate (49) with two-thirds of its total volume. They produce compounds that play a significant role in seminal physiology, such as fructose (source of energy for sperm metabolism), semenogelin I (the predominant component of the coagulum), sperm motility stimulators (bicarbonate, prolactin, prostaglandin), seminal plasma motility inhibitor (a protein that affects sperm motility

TABLE 4. Degrees of sperm progression according to WHO manual

(a) Rapid progressive motility
(b) Slow or sluggish progressive motility
(c) Nonprogressive motility
(d) Immotility

TABLE 5. Factors affecting sperm motility

Varicocele
Endocrine disorders
Genital infections
Antisperm antibodies
Bacteriospermia
Flagellar defects (immotile cilia syndrome, tail stump defect, Young's syndrome)
Prostate and/or seminal vesicle secretory dysfunction
Cigarette smoking
Excessive alcohol consumption
Drugs
Stress
Oxidative stress

when not degraded after ejaculation), antioxidants (ascorbic acid), and antigens that prevent the production of antisperm antibodies in the female genital tract (50–52). In addition, products of semenogelin degradation bind to the spermatozoa surface in order to avoid premature sperm capacitation (53). Therefore, changes in seminal vesicles secretory patterns can modify the composition of products of the vesicular fluid and of the ejaculate, affecting sperm function.

Semen analysis evaluates the secretory activity of the seminal vesicles through the measurement of coagulation, viscosity, fructose levels, volume, and pH (7). Low and very low fructose levels are more specific for the diagnosis (52), whereas lack of or poor coagulation is also clinically significant, although less frequent in SA. Seminal hyperviscosity also suggests deficient secretory activity of the seminal vesicles (54). Although volume and pH have less clinical value, they sometimes also yield significant information.

Individually, markers of the seminal vesicles are of poor clinical value. However, when associated either with each other or with prostate markers, they help in the characterization of functional disturbances that can be clinically investigated using other resources for the diagnosis.

SEMEN PARAMETERS AND PROSTATE FUNCTION

The prostate secretes 30–35% of the ejaculate (49) and produces several compounds that are available for analysis in the seminal plasma. Assessment of citric acid, calcium, zinc, magnesium, and activities of Y glutamyl-transferase and acid phosphatase have been reported in the literature (55–59). Reduced levels of these prostate markers are indicative of glandular dysfunction that often associates with abnormal pH (>7.8), volume (decreased or increased), liquefaction (incomplete), and/or viscosity (hyperviscosity). On the other hand, SA also

detects increased levels of biochemical markers, generally associated with either abnormal volume (decreased or increased) or pH (<7.2) that are also indicative of glandular dysfunction. Analysis of prostate markers is, therefore, of clinical value in investigating glandular function, particularly in infections. In infertile men, some sperm characteristics (mainly that of sperm motility) can be affected by prostate dysfunction (57,60).

SEMEN PARAMETERS AND THE DYNAMIC INTERACTION BETWEEN PROSTATE AND SEMINAL VESICLES

Semen analysis also provides valuable information on the dynamic interaction that takes place between the secretory activities of the prostate and the seminal vesicles. Owing to the multiglandular origin of the seminal plasma, these glands interact with each other according to glandular pathophysiology, increasing or decreasing their contribution to the composition of the ejaculate. Differential analysis involves assessment of physical (coagulation, liquefaction, volume, viscosity, and pH) and biochemical parameters (fructose, inorganic phosphorus, citric acid, calcium, zinc, Y glutamyl-transferase, and acid phosphatase). SA often shows clear changes that characterize the etiology of the disturbance. However, abnormal results do not necessarily correlate to a simultaneous disturbance in both glands because secretion of one gland can be affected without concomitant abnormality of the other glandular function. For example, seminal pH >7.8 suggests secretory dysfunction of the prostate since decreased levels of biochemical markers are commonly found (60). Even so, pH increase does not indicate abnormal dysfunction of the seminal vesicle. In fact, such pH imbalance is the result of impaired prostatic secretory activity that reduces its contribution to the ejaculate, increasing the pH. Prostate secretion is slightly acid, whereas the vesicular fluid is more alkaline (61).

Conversely, pH <7.2 followed by low volume is often due to a scarcity of vesicular fluid. Although prostate markers can be increased, decreased levels of seminal vesicle biochemical markers will disclose the disturbance owing to an abnormal distribution of the glandular secretions in the ejaculate. Poor semen coagulation can also be found in such specimens.

Simultaneous changes can also occur in parallel order. For example, the presence of low levels of prostatic and vesicular biochemical markers may suggest disturbances in the secretory pattern of both glands (62).

Although it has been the subject of investigations for many years and several uncertainties remain to be solved, analysis of seminal markers of the accessory glands provides valuable information on the dynamic

interaction between the prostatic and vesicular functions. Such analysis can, therefore, reveal functional disturbances and their effects on sperm function and appears to be important for a better understanding of glandular pathophysiology and, obviously, for a more rational treatment of the disturbances. Hence, analysis of seminal markers of the accessory glands should be a mandatory part of the SA routine.

OTHER SEMEN PARAMETERS

Semen analysis can also yield valuable data on other factors affecting the male genital organs in the following ways.

Leukocyte Count

According to the WHO manual (7), leukocyte count above $10^6/\text{mL}$ exceeds the reference value. This abnormality, termed leukocytospermia, is a presumptive indicator of genital tract inflammation (63).

Clinical significance of seminal leukocytes is conflicting. One reason for this controversy is the methodologies employed for the identification of these cells in the semen. Conventional staining techniques, such as Giemsa and Papanicolaou, make the differential diagnosis between leukocytes and IGC difficult. Cytochemical methods, particularly the peroxidase stain technique, do not detect lymphocytes and monocytes (7). Methods using monoclonal antibodies identify all leukocyte cells in semen (63). However, these methods are time-consuming and high priced for routine purposes. Determination of polymorphonuclear elastase (64) is an alternative diagnostic tool, but it is also time-consuming and expensive. Therefore, the ability to precisely identify leukocytes in semen depends on the methodology applied. Today, immunocytochemistry and peroxidase stain are the methods of choice to routinely evaluate this seminal parameter (7).

Investigators have noted that the presence of leukocytes in semen is a physiological event aimed at the elimination of abnormal germ line elements from the ejaculate (65). Seminal leukocytes are found in the epididymis, prostate, seminal vesicles, and urethra (65). Due to the blood-testis barrier, leukocytes are not normally found in testicles (65).

Although controversial, negative impact of leukocytospermia on male fertility has been recognized in many studies. Since the presence of leukocyte in semen may reflect a physiological event, toxic effects of metabolites generated by these cells seem to be inhibited by seminal plasma antioxidants (66). However, high leukocyte counts increase generation of toxic metabolites that can be higher than the neutralizing capacity of seminal plasma antioxidants. Oxidative stress then develops,

affecting some seminal parameters, particularly sperm motility and chromatin integrity.

There are, however, specific infections, such as epididymitis, that affect sperm function regardless of leukocyte concentration. Furthermore, genital infections can lead to the production of antisperm antibodies induced by macrophages and lymphocytes that also affect sperm function (67). Therefore, leukocytospermia is not often the main cause of deleterious effects on sperm function in genital infections. Sperm damage depends on the site of the infection, on the characteristics of the leukocyte infiltration, and on the leukocyte subpopulation (65). Impact of leukocytospermia on the sperm function is, therefore, unclear in many aspects, but it should be considered in the investigation of male factor infertility. In this context, the association between leukocytospermia and the impairment of the organ markers is remarkable, because it can single out the infection site (57). Furthermore, in silent infection, leukocytospermia is also an essential diagnostic probe (68).

In summary, it is relevant to perform leukocyte count in SA so as to verify the association between leukocytospermia and abnormal seminal parameters in an attempt to identify genital infections, their site of origin, and their impact on sperm function.

Hypoosmotic Swelling Test

Spermatozoa with normal function and membrane integrity undergo swelling under hypoosmotic conditions, due to the influx of water from the external environment to the tail that expands the cell membrane to a balloon shape (69). Based on the sperm-swelling phenomenon, Jeyendran et al. (70) developed a single test, the hypoosmotic swelling test (HOST). Normal semen presents with more than 60% of sperm tail swelling, which associates with high rates of fertilization in vitro and in vivo (71). Patients with HOST <50%, have less conception probability, whereas values between 50% and 60% are in the grey zone. HOST is a functional test whose value is recognized clinically and is particularly used for patients undergoing investigation of infertility as well as in predicting the outcome of IVF.

Antisperm Antibodies (ASA)

Antigens are present on the sperm surface and in the seminal plasma (Table 6). These antigens are inhibited by potent seminal immunosuppressor mechanisms developed since men's fetal life (72,73). Protective mechanisms include: the blood-testis barrier in the testicles and the tight junction in the epididymis that inhibit access by immunoglobulins; complement components; and macrophages and leukocytes to those

TABLE 6. Semen compound with antigenic properties

LDH-C4 (Lactodehydrogenase C4) ^a	YMK-II ^a
PH-20 ^a	BS-63 (nucleoporin related) ^a
SP-10 (sperm protein) ^a	rSMP-B (rabbit sperm membrane protein) ^a
RSA (rabbit sperm antigens) ^a	BE-20 ^b
HAS-63 ^a	Lactoferrin ^b
FA-1 (Fertilization antigen 1) ^a	IgBF (immunoglobulin binding factor) ^b
FA-2 (Fertilization antigen 2) ^a	SAGA-1/CD52 ^b
CS-1 (Cleavage signal protein 1) ^a	Zyxin (HED-2) ^c
BS-17 (Calpastatin) ^a	

^aSperm antigens.

^bSperm-coating antigens.

^cSertoli cell compounds with antigenic properties.

genital organs. T-supressor lymphocytes (CD8+) present in the epididymis and vas deferens also hamper the humoral response to the sperm antigens by inhibiting antigenic recognition. In addition, Sertoli cells contribute to immune tolerance because they phagocyte and degrade all spermatogenesis residues that would trigger antigenic stimulus should they remain in the seminiferous tubules.

Exposure of sperm antigens elicits immune response and leads to the production of ASA. The main factors responsible for ASA production are testicular trauma or torsion, genital infections, obstruction of the genital ducts, and mumps orchitis (73). These factors decrease immune tolerance inducing macrophages to develop both phagocytosis and antigen presentation, thus eliciting immune response.

Frequency of ASA in semen depends on the origin of the disturbance. High percentage of ASA affects seminal parameters, particularly sperm motility and fertilization events, such as cervical mucus sperm penetration, ovum binding, penetration in the zona pellucida, and fusion with the oolema (74). ASA also provoke sperm agglutination, sperm immobilization, and cellular lyse (cytotoxic effect). Therefore, investigation of ASA in SA routine is recommended.

Reactive Oxygen Species (ROS)

ROS, such as hydroxyl radical, superoxide anion, and hydrogen peroxide, are generated by semen components, mainly abnormal spermatozoa and leukocytes. They form part of seminal oxygen metabolism and are needed for the acquisition of fertilizing ability by spermatozoa. Levels of ROS on the sperm surface appears to be essential for the physiological processes of sperm hyperactivation, capacitation, and acrosome reaction (75–77).

The seminal concentration of ROS is regulated by scavenging systems of spermatozoa that prevent their (ROS's) excessive generation (76). Normal balance is maintained between the amount of ROS produced and scavenged. However, human spermatozoa are sensitive to oxidative stress due to the polyunsaturated fatty acid content in the sperm plasma membrane. The presence of leukocytospermia and abnormal spermatozoa can cause lipid peroxidation of the sperm plasma membrane, which in turn leads to excessive generation of ROS and results in deleterious effects to sperm motility, DNA integrity, and fertilizing ability (75,77,78).

It is currently recognized that peroxidase damage to human spermatozoa may be an etiological factor of male infertility. Although expensive to perform in SA routine, measurement of ROS levels and semen antioxidant capacity are recommended to evaluate the influence of oxidative stress on male fertility.

Chromatin Integrity

The chromatin structure of the sperm nuclei is extremely compact and stable, consisting of DNA and nucleoproteins. The highly packaged characteristics of the sperm chromatin are acquired during spermatogenesis and completed at sperm maturation in the epididymis (79,80). Sperm chromatin packaging occurs gradually, following replacement of histones by protamines and cross-linking of protamine disulfide bonds. This chromatin organization protects man genome during transit through the male and female genital tract.

Although a considerable quantity of spermatozoa in the ejaculate exhibit compact chromatin, a granular and most heterogeneous appearance of sperm nuclei can also be found even in morphologically normal spermatozoa (81). This characteristic is indicative of chromatin immaturity, which results, for example, from abnormal protamine content, apoptotic degeneration, or oxidative stress (80,81), causing DNA strand breaks (82). Therefore, human ejaculate contains spermatozoa with normal and abnormal chromatin structure. High percentage of spermatozoa with immature chromatin is predictive of infertility.

Several assays have been developed to identify DNA damage and abnormal chromatin (83–88). Although these assays are often labor consuming, they may identify nonviable spermatozoa and, therefore, provide predictive thresholds for male infertility. Such assays can also help in the prevention of early pregnancy loss (79,80).

Computer Assisted Semen Analysis (CASA)

The development of computer-assisted semen analysis (CASA) in the 1980s made possible the measurement of

sperm characteristics in a faster and more objective way. In the CASA instruments, the image of the microscope field is converted into a digital image and kinematic values are determined for each spermatozoon from a number of images per second (frames) covering velocity of movement, head trajectory, spermatozoa number, and morphometry (89). Therefore, CASA determines "classical" sperm characteristics (sperm count, motility, and morphology), as well as sperm trajectory characteristics such as amplitude of lateral displacement, beat/cross frequency, curvilinear velocity, and straight-line velocity, which could not be determined by microscope observation. CASA is also practical to evaluate patterns of sperm hyperactivated motility, the natural event defined as nonprogressive, with high flagellar movement that has a potential role in sperm–egg interaction. Patterns of hyperactivated motility have a relationship with fertilization rates and constitute, therefore, a biological marker of sperm capacitation (90). There is considerable interest in this analysis since in the field of infertility investigation it can be useful, given the clinical settings.

As a result of its practical aspects, the use of CASA has become widespread in clinical and, mainly, in andrology laboratories.

RECOMMENDATIONS FOR A BASIC ROUTINE FOR SEMEN ANALYSIS

In the clinical laboratory, it is mandatory that methodologies employed for any analysis be carefully standardized. To that end, different approaches have also been used to identify parameters for semen measurements that are based on the WHO protocol (7). SA is widely employed to investigate disorders affecting the genital tract of men, especially infertility. Therefore, it is reasonable to establish minimum requirements to be fulfilled in order to provide valuable information for the diagnosis, as well as to establish prognosis for males seeking help in the clinical practices of gynecology, urology, and andrology.

Considering that infertility is the major clinical setting for SA (Table 7), the meaningful way to develop a standard SA routine is the choice of parameters that evaluate the functional activity of the genital organs in order to determine the influence of genital pathophysiology on the reproductive capacity of men, even if some parameters do not carry clinical value for other indications. Table 8 shows a summary of recommended and optional parameters for SA.

SEMEN ABNORMALITIES AND THE DIAGNOSIS

Abnormal seminal parameters are always indicative of disorders in the male genital tract. Table 9 shows

TABLE 7. Clinical settings for semen analysis

Infertility
Genital infections
Genital pathologies (e.g., hemospermia)
Adolescent varicocele
Recurrent miscarriage
Vasectomy
Cryptorquidism
Ongoing medical treatments (cancer chemotherapy, sulfasalazine)
Scrotal injuries such as trauma, testicular torsion
Mumps orchitis
Vasovasostomy
Antenuptial
Occupational exposures to toxicants

a summary of the main abnormalities and their relationship to the diagnosis.

CONCLUSION

SA involves the analysis of several parameters. The results can aid in the detection of disturbances affecting the male genital tract. However, because it has been undervalued in the laboratory, SA remains a poorly performed analysis and is only well practiced by few experts. Several seminal parameters are overlooked in worldwide examination, yielding disappointing results. In addition, lack of SA performance and reporting standardization among laboratories is commonplace,

TABLE 8. Overview of routine parameters for semen analysis

Physical examination	Biochemistry	Sperm characteristics	Other parameters
Coagulation	Fructose	Density	Antisperm antibodies
Liquefaction	Citric acid (optional)	Vitality	Leukocyte count
Volume	Calcium (optional)	Motility	Reactive oxygen species (optional)
Color-aspect	Acid phosphatase (optional)	Conventional morphology	
Viscosity	Magnesium (optional)	Strict criteria morphology	
PH	Zinc	Hypoosmotic swelling test	
	γ -Glutamyl-transferase (optional)	Sperm precursors (optional)	
	Carnitine (optional)	Chromatin integrity (optional)	
	Glycerylphosphorylcholine (optional)	CASA (optional)	
	Neutral α -glucosidase isoenzyme (optional)		

TABLE 9. Relationship between semen abnormalities and the diagnosis

Seminal parameter	Disturbance
Density	Several disturbances, mainly varicocele and endocrine disorders
Conventional morphology	As presented in Table 3
Strict criteria morphology	Infertility
Vitality	Disturbances that affect the survival of spermatozoa
Motility	Varicocele, endocrine disorders, ASA, genital infections
Low fructose levels	Dysfunction of the seminal vesicles
Fructose absent	Agenesis of the vas deferens
Low levels of prostate biochemical markers	Dysfunction of the prostate
Increased levels of prostate biochemical markers	Some dysfunctions of the prostate and hypofunction of the seminal vesicles
Coagulation absent	Agenesis of the vas deferens
Poor coagulation	Hypofunction of the seminal vesicles
Incomplete liquefaction	Some dysfunctions of the prostate and/or seminal vesicles
Hyperspermia	Dysfunction of the prostate and seminal vesicles
Hypospermia	Same as previous
Color-aspect	Hemospermia
Hyperviscosity	Dysfunctions of the prostate and seminal vesicles
High pH (>7.8)	Dysfunctions of the prostate
Low pH (<7.2)	Dysfunctions of the seminal vesicles
Leukocytospermia	Infections
Hypoosmotic swelling test	Infertility
Antisperm antibodies	Immune response (several causes)
Reactive oxygen species	Oxidative stress (several causes)
Chromatin integrity	Infertility

even in the United States. In spite of the significance of the disorders that affect not only the male genital tract but also men's health in general, mainly their reproductive health, SA has enjoyed limited diagnostic application. However, when basic requirements are used, clinically valuable information can be obtained. SA data help in the diagnosis, in the choice of the best option for treatment, and on assisted reproduction referral. Furthermore, there are numerous pathologies, some rare, that can be detected through SA (91–94).

It is still important to emphasize that SA is an efficient marker of the secretory activity of the prostate and seminal vesicles, a subject that it is not often debated in the literature. In fact, research development in this investigation area is promising because it can benefit the diagnosis of disturbances that affect those glands, particularly the prostate of older men. Abnormal glandular markers are frequently detected in SA routine, although seldom noted.

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