Chapter 2
Interpreting Semen Analysis and Level 2 Sperm Testing

Nabil Aziz

Introduction

Semen analysis is the first test requested when fertility potential of a man becomes questionable. The results of the semen analysis are often taken as a surrogate measure of his ability to father a pregnancy. The test provides information on the functional status of the seminiferous tubules, epididymis, seminal vesicles, and the prostate. Thus the results of the test should be interpreted in the light of a full clinical history and physical examination of the patient to determine possible causes for reduced fertility potential and of any suboptimal semen analysis result (see Chap. 1).

Although semen analysis is the most practical laboratory assessment of the male we have, it comes with many limitations. The foremost of these limitations is the limited reliability of the result of semen analysis due to variations in the method and timing of obtaining ejaculates and the lack of standardization of the semen analysis methodology [1–4]. There is also limitation of scope as semen analysis is concerned primarily with measuring the volume of the ejaculate and with assessing the number, motility, and the shape of the sperm population. The count of other cellular contents such as spermatids and leucocytes may be noted. The narrow scope of the analysis reduces its ability to identify an underlying pathology when the analysis results are substandard. This in turn contributes to the relatively low prognostic power of semen analysis as a diagnostic test. When azoospermia cases are excluded, the results of semen analyses do not always correlate with pregnancy rates or infertility [5, 6]. Nevertheless, semen analysis provides essential information on the clinical status of the man being investigated.

N. Aziz (✉)
Department of Gynecology and Reproductive Medicine, Liverpool Women's Hospital and The University of Liverpool, Liverpool, UK
e-mail: naziz@liv.c.uk

N. Aziz and A. Agarwal (eds.), *The Diagnosis and Treatment of Male Infertility*,
DOI 10.1007/978-3-319-56547-7_2

Prior to communicating the semen analysis results with patients, the clinician needs to evaluate the significance of the results by answering three questions:

1. Is the semen analysis result reliable?
2. Does it identify a specific pathology?
3. Can this man achieve a pregnancy given his female partner fertility potential?

The Reliability of Semen Analysis Result

There are several factors that may influence the reliability of the semen analysis result. The clinician needs to be aware of these factors before making a clinical judgment on the significance of the result.

Standardization of Semen Analysis Techniques

The standards of semen analysis were first conceived when animal husbandry was being studied in the nineteenth century. The primary focus was on sperm morphology. Sperm count and motility had secondary importance. These same principles were adopted for semen analysis in men in the early twentieth century [7]. This was done without paying much attention to the fact that contrary to other animals, men, as well as chimpanzees, have very high heterogeneity in sperm shape and motility in their ejaculates. Subsequently, several classification systems employing diverse laboratory techniques were proposed to evaluate the quality of semen. This has led to the inability to compare semen analysis results from different laboratories.

The World Health Organization (WHO) addressed this problem by publishing five laboratory manuals consecutively over the past 35 years to provide a standardized approach to the techniques used in collecting and evaluating ejaculated semen. In the first four manuals, cutoff points for each parameter measured were set. The latest fifth manual published in 2010 adopted a new approach by setting lower reference limits derived from examining the semen qualities of 1953 recent fathers [4]. The study population was retrieved from different countries worldwide and with the condition that the time-to-pregnancy was 1 year. The fifth centile (lower reference limit) and its 95% confidence interval (CI) for each semen parameter in these fathers was calculated and used as a guide to judge the quality of other men facing a difficulty in achieving a pregnancy (Table 2.1) [4, 8]. The adoption of these standards worldwide to promote the reliability of semen analysis has been enhanced through a diversity of local internal and external quality control measures including schemes for andrology laboratory accreditation and certification. In spite of this huge endeavor, there remain significant issues with variability of results from different laboratories because WHO methodologies are not always

Table 2.1 Lower reference limits (5th centiles and their 95% confidence intervals, *CI*) for semen characteristics discussed in this chapter

Parameter	WHO 2010 lower reference limits (95% *CI*)	WHO manual (1999) cutoff points
Semen volume (ml)	1.5 (1.4–1.7)	$\geq$ 2 ml or more
Total sperm number (10^6 per ejaculate)	39 (33–46)	$\geq$ 40
Sperm concentration (10^6 per ml)	15 (12–16)	$\geq$ 20
Total motility (PR + NP, %)	40 (38–42)	$\geq$ 50
Progressive motility (PR, %)	32 (31–34)	$\geq$ 25
Sperm morphology (normal forms, %)	4 (3.0–4.0)	Undecided
Vitality (live spermatozoa, %)	58 (55–63)	$\geq$ 50

A complete list of reference limits is found in the WHO manual 2010 [4]. The reference limits are compared to the cutoff points quoted in WHO manual 1999 [8]
WHO—World Health Organization, *CI*—confidence interval, *PR*—progressive motility, *NP*—nonprogressive motility

strictly observed, which may happen intentionally or unwittingly. Moreover, there remains an element of subjectivity in the assessment of sperm count, motility, and morphology with significant inter- and intra-observer variability [9, 10]. Computerized sperm count estimation and motility assessment have been adopted by many laboratories to eliminate the subjectivity of the assessment [11, 12].

Biological Variability

The quality of semen is influenced by many biological variables [13]. These include

- **The completeness of sample collections**: Collecting all the successive emissions (fractions) of ejaculated semen is very important because of the varying composition of the first fraction compared to the latter ones. The first fraction of ejaculated semen is sperm-rich. This is followed by the part of the ejaculate that is diluted by the seminal vesicles fluid [14]. Thus, if the first fraction of the ejaculate is lost, the sperm concentration and total sperm count will be lower than what it should be.
- **The impact of age**: With age both the number of sperm produced and the fluid component contributed by the prostate and seminal vesicles may be reduced [15]. This may give the elusion that sperm concentration in older men is maintained when in reality the total number of sperm in the ejaculate is reduced.
- **Testicular size**: The size of the testis correlates with the total number of spermatozoa in the ejaculate [16]. This is reflective of the level of spermatogenesis in the seminiferous tubules [17]. An extreme clinical example of this association between testicular size and sperm count is encountered in cases of hypogonadotropic hypogonadism and in anabolic steroid abuse cases presenting

with azoospermia and small testicular volume due to suppressed spermatogenesis. The testicular volume increases gradually as spermatogenesis resumes in response to treatment with human chorionic gonadotropin (hCG) injections and abstaining from anabolic steroid use, respectively.

- **Duration of abstinence**: The WHO recommends 2–7 days sexual abstinence prior to semen analysis as a means of standardizing semen analysis. Frequent sexual activity prior to semen analysis may result in reduced semen volume, sperm concentration, and total motile sperm count without significant change in sperm motility and normal morphology [18]. On the other hand, there is significant increase in semen volume, sperm concentration, and total sperm count when the abstinence length is increased [19, 20]. However, length of abstinence does not influence pH, viability, morphology, or total motility [17]. A short abstinence period (24 h) is associated with immature sperm DNA, whereas longer abstinence intervals may be associated with sperm DNA fragmentation [14, 15].
- **The intensity of sexual stimulation**: It has been documented that ejaculates recovered from non-spermicidal condoms used during intercourse at home can be of higher quality than those produced by masturbation and collected into containers in a room close to the laboratory [3]. However, for the sake of standardization it is recommended that ejaculates are collected in a well-equipped room next to the laboratory to reduce the impact of prolonged transport time and the exposure to nonoptimal temperature. The place of producing the ejaculate should be noted in the semen analysis report.
- **Delayed liquidation and viscosity**: Delayed liquefaction and abnormal sample viscosity after complete liquefaction may impact the result negatively. Semen samples are kept at a temperature of 20–37 °C while waiting for sample liquefaction that is normally completed within 30 min of ejaculation. Liquefaction is reported as delayed when it is not complete after an additional 30 min standing. If liquefaction is not achieved after 60 min, mechanical and enzymatic techniques are described in the WHO manual to achieve liquefaction. This should be noted in the analysis report because treatments applied to the sample to achieve liquefaction may affect seminal plasma biochemistry, sperm motility, and sperm morphology [4]. High semen viscosity after complete liquefaction can also interfere with determination of sperm motility, sperm concentration, and the detection of antibody-coated spermatozoa. High viscosity can be recognized by the elastic properties of the sample when a semen droplet is stretched for 2 cm or more between two pipettes. Again the clinician needs to take note of this when interpreting the results.
- **Reversible changes in sperm parameters**: Fever, intercurrent viral infections, medication with drugs such as nitrofurantoin, genital tract infection (epididymitis), and exposure to excessive heat may be associated with reversible deterioration in different semen parameters. When the semen analysis result is suboptimal, these factors should be excluded or corrected before making a final judgment on the quality of semen.

In view of the variability in the quality of ejaculates produced by one man it is impossible to rely on a single semen analysis to characterize a man's semen quality. The analysis of two or more ejaculates may be required to obtain a more valid baseline data to facility a sound view of man's clinical status.

External Technical Factors that May Influence Semen Analysis Result

The result of semen analysis may be impacted negatively by external factors such as the temperature at which the sample is left standing before the start of the analysis and the time elapsed between ejaculation and the start of the test. This information should be noted in the result report before making a considered judgment of its significance.

Does the Traditional Semen Analysis Identify a Specific Pathology?

The semen analysis results should be correlated with relevant medical and lifestyle issues identified during history taking, clinical examination, and other baseline assessments such as hormone profile and scrotal ultrasound scanning. In cases of unexpected azoospermia additional investigations, such as karyotyping and cystic fibrosis gene screen, may be required. Checking for Y chromosome micro-deletions may be sought in severe oligospermia (sperm count $<3 \times 10^6$) on repeated testing. If the analysis reveals teratospermia (sperm with normal morphology <3%) karyotyping is recommended because of the association between teratospermia and chromosomal abnormalities both in somatic cells and spermatozoa [21]. The presence of leucospermia (leucocytic count $>1 \times 10^6$/ml semen) is associated with poor sperm morphology and motility and sperm oxidative stress [22–24].

However, traditional laboratory testing may fail to provide an explanation for the reduced sperm parameters in other cases. This is referred to as idiopathic infertility. Level 2 sperm testing is required in these situations (see later) [25]. But initially let us consider the clinical significance of some of the traditional semen parameter:

The Significance of Semen Volume

The seminal vesicles secretions contribute up to 70% of the normal ejaculate volume. The lower reference limit for semen volume is 1.5 ml (5th percentile, 95% confidence interval 1.4–1.7) [4]. A low sperm volume is more likely to be due to the

incomplete collection of the ejaculate. It also may be due to acquired obstruction of the ejaculatory duct. In cases of congenital bilateral absence of the vas deferens (CBAVD) there is dysplasia or absence of the seminal vesicles resulting in the loss of its contribution to the semen volume [26, 27].

According to WHO standards, retrograde ejaculation should be suspected in any case when the seminal fluid volume is <1 ml [4]. The diagnosis is confirmed by finding spermatozoa in the post-ejaculatory urine sediment when all collected urine is centrifuged and pelleted. The sperm is found mostly dead due to the combined effects of osmotic stress, low pH, and urea toxicity [28]. The recovery of high-quality sperm after the induced modification of the urine composition and pH to facilitate its use in the intracytoplasmic sperm injection technique (ICSI) has been described [29, 30].

Occasionally, the orgasm is associated with a miniscule amount of ejaculate or no ejaculate at all (dry ejaculation, aspermia). This happens in a diversity of neurological diseases and subsequent to surgical procedures on the lower urinary tract [31]. It may also be the presentation of retrograde ejaculation. The explanation for the dry ejaculation is mostly apparent from the initial history taking. If not, then retrograde ejaculation needs to be excluded before referring to the urologist or neurologist for further assessment.

The Significance of Semen pH

The balance between the alkaline secretion of the seminal vesicles and the acidic prostatic secretion determines the semen pH. The importance of assessing the semen pH and its physiological reference range has been a matter of intense debate [32]. However, WHO 2010 sets the lower reference value of the pH of liquefied semen at 7.2 [4]. In CBAVD, the semen pH is characteristically lower (pH 6.8) because of the absence of the seminal vesicles' alkaline secretion. In these cases the scanty seminal plasma is formed mainly from the relative acidic prostatic secretion.

The Value of Semen Analysis in Azoospermic Cases

In the vast majority of azoospermic men, their condition is first identified when they are referred for fertility assessment. In a minority of cases, azoospermia was previously identified including men with cystic fibrosis disease, the majority of Klinefelter's syndrome and, presumably, after vasectomy. Even in these men semen analysis is required to confirm the azoospermic status. Clinically, cryptospermia has been described after vasectomy [33] and up to 10% of men with Klinefelter's syndrome have few sperm in their ejaculates [34–36]. In the andrology laboratory,

azoospermia is suspected when no spermatozoa are observed in replicate wet preparation examined according to the WHO standards [4]. Azoospermia only can be confirmed if no spermatozoa are found in the sediment when the whole ejaculate is centrifuged and pelleted. Cryptospermia describes the absence of sperm in wet preparation but sperm is then found in the sediment. This additional process of semen analysis should be described in the analysis report sent to the clinician. The benefits of a reliable diagnosis of azoospermia in the field of reproductive medicine are multiple. First, an inaccurate diagnosis may lead to an unnecessary invasive procedure to retrieve testicular sperm to treat the couple using their own genetic material. Second, when azoospermia is confirmed additional genetic testing is required to diagnosis the underlying cause. These include karyotyping for chromosomal abnormalities, testing for Y chromosome micro-deletions, and screening for cystic fibrosis gene mutations. Thirdly, the underlying genetic abnormality may have a detrimental impact on the offspring and the affected couples are required to receive adequate genetic counseling prior to undergoing treatment. Finally, the demonstration of absent motile spermatozoa in ejaculate is required after vasectomy to declare the procedure a success.

If azoospermia is secondary to the testosterone hormone or anabolic steroids abuse, periodic repeat semen analyses are required for up to 24 months after abstaining from taking these drugs to monitor the spontaneous resumption of spermatogenesis in many of these patients [37, 38]. If spermatogenesis has not resumed in an acceptable time frame or abstinence is not tolerated, alternative medications may be considered [37].

When considered together, semen volume and pH can help in determining the differential diagnosis of the cause of azoospermia. In patients with low-volume, acidic, azoospermic samples, the differential diagnosis is CBAVD or bilateral complete ejaculatory duct obstruction (EDO). Azoospermic ejaculates with a normal volume and alkaline pH indicate functional seminal vesicles and patent ejaculatory ducts. The differential diagnosis includes spermatogenic failure or an obstruction at the level of the more proximal vas deferens or epididymis, but does not include CBAVD or bilateral EDO.

Can This Man Achieve a Pregnancy Given His Female Partner's Fertility Potential?

In its successive five editions, the WHO manual offered increasingly lower cutoff points to assess sperm parameters. The reference limits for sperm count, motility, and normal morphology quoted in the latest edition [4] are significantly lower compared the cutoff points that were thought to be compatible with normal male fertility in the previous editions. As an example, the cutoff point for sperm count

quoted in the 1999 WHO manual [8] as 20×106^6/mL is almost double the 5th centile of the reference limit (12×10^6/ml) quoted by the 2010 WHO manual [4] (Table 2.1). This may cause confusion among concerned clinicians if the andrology laboratory does not indicate which WHO reference limits values are used. Another reason for the confusion is how the same semen analysis result may be classified as not compatible with normal fertility prior to 2010, but is judged as compatible with normal fertility when applying the 2010 lower reference limits.

At this point it becomes apparent that a semen analysis result is not prescriptive but descriptive of a man's clinical status, with the exception of azoospermia where spontaneous pregnancy cannot happen. This stems from the huge heterogeneity in the characteristics of semen in men compared to other animals. This has led to an extensive overlap of the distributions of semen parameters' results among fertile and non-fertile men (Fig. 2.1c) [39]. This is contrary to the assessment of other biological features where there is distinct distribution or only a minimal overlap of results among affected and unaffected populations (Fig. 2.1a, b) [39]. When that is the case, the identification of a cutoff point to reliably distinguish affected and unaffected individuals is feasible. On the other hand, the extensive overlap in semen analysis results between affected (infertile) and unaffected (fertile) populations causes significant numbers of both false-positive and false-negative cases [39] compromising the prognostic value of semen analysis.

Currently, it is not feasible to define a single useful and reliable "sharp" cutoff level for each individual semen analysis parameter that can distinguish between truly "fertile" and "infertile" men with a high degree of certainty. Although there is a highly significant statistical difference between the two groups, still the degree of the overlap will create low predictive values. For clinicians, the strict scientific interpretation of a reference limit based on "fertile men" is that a result under the limit implies a probability of less than 5% that this sample represents a sample from a "fertile" man. These reference limits, then, should not be referred to as limits of normality. Looking at this from a different perspective, a recent father and a man being investigated for infertility may have a very similar semen analysis result and the only difference may be in the fertility potential of the female partner.

Men with partially reduced fertility are potentially more likely to be infertile when the female also has a reduced fertility potential. In other words suboptimal semen analysis result may be compensated when the female partner has uncompromised fertility potential. This is a clinical judgment that can only be made after full assessment of both partners including clinical history and physical examination.

Level 2 Sperm Testing

From the previous discussion, it becomes clear that restricting semen assessment to the traditional parameters of sperm count, motility, and morphology is associated with numerous concerns. First, for a long time it has been recognized that sperm

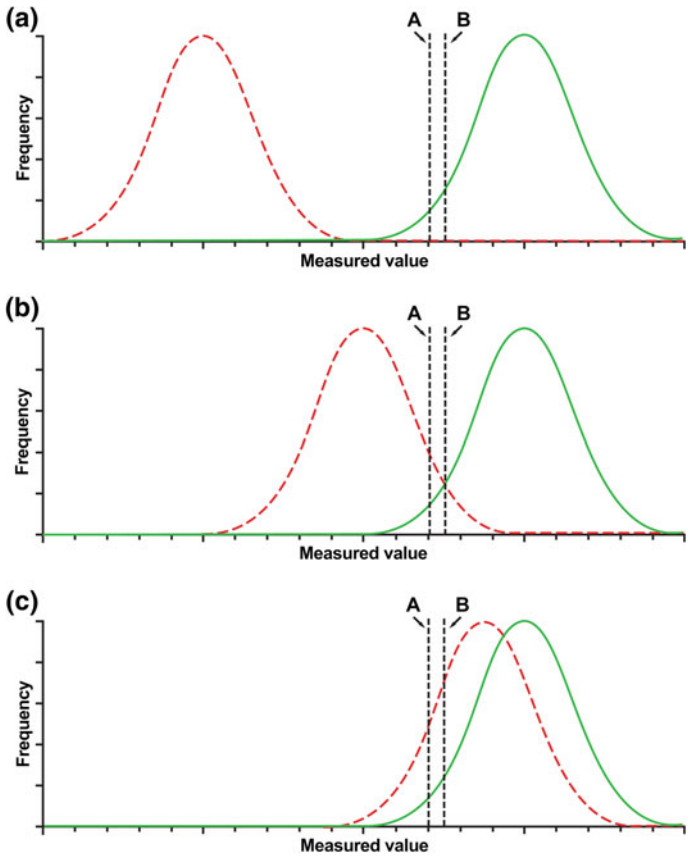

Fig. 2.1 Hypothetical distributions of results from 2 populations: unaffected ("controls") to the right (*green/solid line*) and affected ("patients") to the left (*red/hatched line*). **a** No significant overlapping of results from the 2 groups. A cutoff can be located anywhere between the 2 distributions. If the lower 2.5 percentile (*dotted vertical line A*) of the unaffected population is used as a cutoff, 2.5% of results from the unaffected population will be labeled as "affected." If the lower 5 percentile (*dotted vertical line B*) is used, 5% of the unaffected population will be labeled as "affected." **b** Some overlapping of the 2 distributions. A reasonable cutoff limit would be where the 2 curves cross (corresponding to the lower 5 percentile of the unaffected men, *line B*). Using the lower 2.5 percentile (*line A*) of the unaffected group would label more affected men as unaffected than if the crossing point is used. **c** Gross overlap between results from the groups of men. Using either the 2.5 percentile (*line A*) or the 5 percentile (*line B*) of the unaffected population as a cutoff would lead to most of the affected men being labeled as unaffected. Using the crossing point of the 2 curves would give equal low specificity and low sensitivity so that a large group of unaffected men are labeled as affected. Thus in this case, this parameter is not very useful to distinguish between the 2 groups.

count, motility, and normal morphology may fluctuate, and their assessment can be very subjective and prone to intra-observer and inter-observer variability [40]. Second, although the traditional semen analysis maintains its central role in the assessment of male fertility potential, this often is inadequate to provide a definitive diagnosis of the cause of infertility in many men [41, 42]. Conventional semen analysis cannot cover the diverse array of biological properties that the spermatozoon expresses as a highly specialized cell, such as the presence of sperm apoptosis and chromatin fragmentation [43, 44]. As a result, many infertile couples with no detectable abnormalities are labeled with the clinically convenient but vague diagnosis of unexplained infertility. Third, the predictive power of the cutoff values of the traditional sperm parameters is not absolute, because there is significant degree of overlap in the distribution of the results between fertile and infertile men for each parameter assessed. Fourth, we now have a better understanding of the impact of processes such as sperm capacitation and acrosome reaction [45], sperm oxidative stress (OS) [46], and apoptosis [47] on both sperm–egg interaction and the fertilizing ability of sperm both in vivo and in vitro. The assessment of these aspects of sperm function was included for the first time in the 2010 WHO manual only as research tools [4]. Last but not least, numerous studies in the literature have demonstrated that semen quality is declining and that the incidence of testicular cancers is rising [48, 49]. These observations have been shown to be associated with increased sperm chromatin damage. During in vivo reproduction, natural selection against infertile men limits their opportunity to pass on an infertility trait to offspring, with only rare exceptions. However, some assisted reproduction technologies bypass this natural selection process, leading to the possibility that an abnormal spermatozoon will be selected to fertilize the oocyte.

Sperm oxidative stress, apoptosis, and DNA damage are induced by disease, lifestyle issues, and environmental factors and are implicated in the pathogenesis of male infertility. In view of this current understanding and the availability of the required laboratory additional assessment of sperm damage beyond the traditional sperm parameter of count motility and morphology, this level 2 testing needs to move from the research lab to the mainstream clinical andrology laboratory. When indicated, testing for evidence of sperm OS, apoptosis, and DNA fragmentation can provide a definitive diagnosis of the underlying causes of what is clinically identified as “idiopathic” and “unexplained infertility.” This also may detect men who may perpetually propagate their genetic complement that is linked to male infertility through techniques such as in vitro fertilization using the intracytoplasmic sperm injection (IVF/ICSI). Ultimately, men should be able to find out the exact reason for their inability to father a pregnancy naturally without glossing over the problem by rushing into IVF/ICSI.

It is conceivable that the next task of the WHO is to coordinate the endeavor to determine the most appropriate laboratory tests and the associated standards to achieve this goal. Unless the WHO seeks strict standardization of these tests, the mistakes of the past in assessing sperm count, motility, and morphology will be repeated. The WHO could provide a clinically standardized two-level approach for

semen analysis to achieve a more reliable male fertility assessment. Level 1 will be adequately served by the criteria and standards of the routine semen and sperm parameter to offer an initial screening for men presenting within an infertile relationship. Level 2 testing would have the objective of addressing two different scenarios:

1. To offer a definitive etiological diagnosis for men with abnormal findings in level 1 testing with no clinical explanation (idiopathic infertility); and
2. To seek to clarify if there is an unrecognizable sperm defect on the subcellular level when investigating the male and his female partner did not identify clinical explanation for the couple's fertility (unexplained infertility).

Level 2 testing is also desirable for those who are offered IVF/ICSI. It would appear that the time is ripe for this leap forward in male fertility assessment by expanding the scope of diagnostic features in performing sperm assessment.

References

1. Alvarez C, et al. Biological variation of seminal parameters in healthy subjects. Hum Reprod. 2003;18:2082–8.
2. Pound N, et al. Duration of sexual arousal predicts semen parameters for masturbatory ejaculates. Physiol Behav. 2002;76:685–9.
3. Zavos PM, Goodpasture JC. Clinical improvements of specific seminal deficiencies via intercourse with a seminal collection device versus masturbation. Fertil Steril. 1989;51:190–3.
4. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva: World Health Organization; 2010.
5. Bonde JP, Ernst E, Jensen TK, Hjollund NH, Kolstad H, Henriksen TB, Scheike T, Giwercman A, Olsen J, Skakkebaek NE. Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. Lancet. 1998;352:1172–7.
6. Guzick DS, Overstreet JW, Factor-Litvak P, Brazil CK, Nakajima ST, Coutifaris C, Carson SA, Cisneros P, Steinkampf MP, Hill JA, Xu D, Vogel DL. Sperm morphology, motility, and concentration in fertile and infertile men. N Engl J Med. 2001;345:1388–93.
7. Williams WW, McGugan A, Carpenter HD. The staining and morphology of the human spermatozoon. J Urology. 1934;32:201–12.
8. WHO. Laboratory manual for the examination of human semen and sperm–cervical mucus interaction. 4th ed. Cambridge: Cambridge University Press; 1999.
9. Auger J, Eustache F, Ducot B, Blandin T, Daudin M, Diaz I, Matribi SE, Gony B, Keskes L, Kolbezen M, Lamarte A, Lornage J, Nomal N, Pitaval G, Simon O, Virant-Klun I, Spira A, Jouannet P. Intra- and inter-individual variability in human sperm concentration, motility and vitality assessment during a workshop involving ten laboratories. Hum Reprod. 2000;15 (11):2360–8.
10. Dunphy BC, Kay R, Barratt CL, Cooke ID. Quality control during the conventional analysis of semen, an essential exercise. J Androl. 1989;10:378–85.
11. Yeung CH1, Cooper TG, Nieschlag E. A technique for standardization and quality control of subjective sperm motility assessments in semen analysis. Fertil Steril. 1997;67:1156–8.
12. Verstegen J, Iguer-Ouada M, Onclin K. Computer-aided sperm analysis: past, present and future. Theriogenology. 2002;57:149–79.
13. Castilla JA, et al. Infl uence of analytical and biological variation on the clinical interpretation of seminal parameters. Human Reprod. 2006;21:847–51.

14. Björndahl L, Kvist U. Sequence of ejaculation affects the spermatozoon as a carrier and its message. Reprod Biomed Online. 2003;7:440–8.
15. Ng KK, et al. Sperm output of older men. Human Reprod. 2004;19:1811–5.
16. Andersen AG, et al. High frequency of sub-optimal semen quality in an unselected population of young men. Human Reprod. 2000;15:366–72.
17. Holstein AF, et al. Understanding spermatogenesis is a prerequisite for treatment. Reprod Biol Endocrinol. 2003;1:107.
18. Welliver C, Benson AD, Frederick L, Leader B, Tirado E, Feustel P, Kontio J, McAsey M, Köhler TS. Analysis of semen parameters during 2 weeks of daily ejaculation: a first in humans study. Transl Androl Urol. 2016;5:749–55.
19. Agarwal A, Gupta S, Du Plessis S, Sharma R, Esteves SC, Cirenza C, Eliwa J, Al-Najjar W, Kumaresan D, Haroun N, Philby S, Sabanegh E. Abstinence time and its impact on basic and advanced semen parameters. Urology. 2016;94:102–10.
20. De Jonge C, LaFromboise M, Bosmans E, Ombelet W, Cox A, Nijs M. Influence of the abstinence period on human sperm quality. Fertil Steril. 2004;82:57–65.
21. Lewis-Jones I, Aziz N, Seshadri S, Douglas A, Howard P. Sperm chromosomal abnormalities are linked to sperm morphologic deformities. Fertil Steril. 2003;79:212–5.
22. Aziz N, Agarwal A, Lewis-Jones I, Sharma RK, Thomas AJ Jr. Novel associations between specific sperm morphological defects and leukocytospermia. Fertil Steril. 2004;82(3):621–7.
23. Aziz N, Agarwal A, Lewis-Jones I, Sharma RK, Thomas AJ Jr. High sperm deformity index (SDI) and acrosomal damage in infertile men with leukocytospermia. Fertil Steril. 2004;82:621–7.
24. Aziz N, Saleh RA, Sharma RK, Lewis-Jones I, Esfandiari N, Thomas AJ Jr, Agarwal A. Novel association between sperm reactive oxygen species production, sperm morphological defects, and the sperm deformity index. Fertil Steril. 2004;81:349–54.
25. Aziz N, Agarwal A. Evaluation of sperm damage: beyond the World Health Organization criteria. Fertil Steril. 2008;90:484–5.
26. de la Taille A, Rigot JM, Mahe P, Gervais R, Dumur V, Lemaitre L, et al. Correlation of genitourinary abnormalities, spermiogram and CFTR genotype in patients with bilateral agenesis of the vas deferens. Prog Urol. 1998;8(3):370–6.
27. Daudin M, Bieth E, Bujan L, Massat G, Pontonnier F, Mieusset R. Congenital bilateral absence of the vas deferens: clinical characteristics, biological parameters, cystic fibrosis transmembrane conductance regulator gene mutations, and implications for genetic counseling. Fertil Steril. 2000;74(6):1164–74.
28. Mortimer D. Semen analysis and other standard laboratory tests. In: Hargreave T, editor. Male infertility. 2nd ed. London: Springer; 1994. p. 55–6.
29. Aust TR, Brookes S, Troup SA, Fraser WD, Lewis-Jones DI. Development and in vitro testing of a new method of urine preparation for retrograde ejaculation; the Liverpool solution. Fertil Steril. 2008;89(4):885–91.
30. Jefferys A, Siassakos D, Wardle P. The management of retrograde ejaculation: a systematic review and update. Fertil Steril. 2012;97(2):306–12.
31. Mehta A, Sigman M. Management of the dry ejaculate: a systematic review of aspermia and retrograde ejaculation. Fertil Steril. 2015;104:1074–81.
32. Meacham R. From androlog. J Androl. 2002;23(3):330–1.
33. Aziz N. The importance of semen analysis in the context of azoospermia. Clinics (Sao Paulo). 2013;68(Suppl 1):35–8.
34. Samplaski MK, Lo KC, Grober ED, Millar A, Dimitromanolakis A, Jarvi KA. Phenotypic differences in mosaic Klinefelter patients as compared with non-mosaic Klinefelter patients. Fertil Steril. 2014;101(4):950–5.
35. Wikstrom AM, Raivio T, Hadziselimovic F, Wikstrom S, Tuuri T, Dunkel L. Klinefelter syndrome in adolescence: onset of puberty is associated with accelerated germ cell depletion. J Clin Endocrinol Metab. 2004;89(5):2263–70.
36. Davis SM, Rogol AD, Ross JL. Testis development and reproductive options in males with klinefelter syndrome. Endocrinol Metab Clin North Am. 2015;44:843–65.

37. McBride JA, Coward RM. Recovery of spermatogenesis following testosterone replacement therapy or anabolic-androgenic steroid use. Asian J Androl. 2016;18:373–80.
38. Rahnema CD, Lipshultz LI, Crosnoe LE, Kovac JR, Kim ED. Anabolic steroid-induced hypogonadism: diagnosis and treatment. Fertil Steril. 2014;101:1271–9.
39. Björndahl Lars. What is normal semen quality? On the use and abuse of reference limits for the interpretation of semen analysis results. Human Fertil. 2011;14:179–86.
40. Keel B, Webster B. The standard semen analysis. In: Webster B, editor. CRC handbook of the laboratory diagnosis and treatment of infertility. Boca Raton, FL: CRC Press; 1990. p. 27–69.
41. Nallella KP, Sharma RK, Aziz N, Agarwal A. Significance of sperm characteristics in the evaluation of male infertility. Fertil Steril. 2006;85:629–34.
42. Tomlinson MJ. Uncertainty of measurement and clinical value of semen analysis: has standardisation through professional guidelines helped or hindered progress? Andrology. 2016;4(5):763–70.
43. Zini A, Kamal K, Phang D, Willis J, Jarvis K. Biologic variability of sperm DNA denaturation in infertile men. Urology. 2001;58:258–61.
44. Evenson D, Larson K, Jost L. Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl. 2002;23:25–43.
45. Evenson D, Larson K, Jost L. Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl. 2002;23:25–43.
46. Agarwal A, Prabakaran SA. Mechanism, measurement, and prevention of oxidative stress in male reproductive physiology. Indian J Exp Biol. 2003;43:963–74.
47. Aziz N, Said T, Paasch U, Agarwal A. The relationship between human sperm apoptosis, morphology and the sperm deformity index. Hum Reprod. 2007;22:1413–9.
48. Aitken RJ, Koopman P, Lewis SE. Seeds of concern. Nature. 2004;432:48–52.
49. Kobayashi H, Larson K, Sharma RK, Nelson DR, Evenson DP, Toma H, et al. DNA damage in patients with untreated cancer as measured by the sperm chromatin structure assay. Fertil Steril. 2001;75:469–75.

http://www.springer.com/978-3-319-56545-3

The Diagnosis and Treatment of Male Infertility
A Case-Based Guide for Clinicians
Aziz, N.; Agarwal, A. (Eds.)
2017, XIV, 304 p. 38 illus., 34 illus. in color., Hardcover
ISBN: 978-3-319-56545-3