

Critical Appraisal of World Health Organization's New Reference Values for Human Semen Characteristics and Effect on Diagnosis and Treatment of Subfertile Men

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In 2010, the World Health Organization established new reference values for human semen characteristics that are markedly lower than those previously reported. Despite using controlled studies involving couples with a known time to pregnancy to establish the new limits, the reference studies are limited with regard to the population analyzed and the methods used for semen evaluation. The present review discusses concerns related to the new reference values for semen characteristics, including the effect on patient referral, diagnosis, and treatment of recognized conditions, such as varicocele, and on the indications for assisted reproductive technologies. UROLOGY xx: xxx, xxxx. © 2011 Elsevier Inc.

Semen analysis is of paramount importance in the initial investigation of the male partner, and its results are often taken as a surrogate measure of his ability to father a pregnancy. It provides information on the functional status of the seminiferous tubules, epididymis, and accessory sex glands. The prognostic value of semen characteristics, such as sperm concentration, percentage of motility, and morphology, as surrogate markers of male fertility is confounded in several ways. The fertility potential of a man is influenced by sexual activity, the function of the accessory sex glands, and other conditions. Routine semen analysis itself has its own limitations and does not account for sperm dysfunction, such as immature chromatin or DNA damage. The results from at least 2, and preferably 3, separate seminal analyses must be obtained before a definitive conclusion can be drawn, because wide biologic variability exists, even within the same individual. Routine semen analysis should include (a) the physical characteristics of semen, including liquefaction, viscosity, pH, color, and odor; (b) the specimen volume; (c) the sperm concentration; (d) sperm motility and progression; (e) sperm morphology; (f) leukocyte quantification; and (g) fructose detection in

cases in which no spermatozoa are found and the ejaculate volume is low.

NEW REFERENCE VALUES FOR HUMAN SEMEN CHARACTERISTICS: HOW WERE THEY OBTAINED?

The World Health Organization (WHO) periodically releases manuals for the laboratory examination of human semen. The first was published in 1980, with subsequent updates in 1987, 1992, and 1999.¹⁻³ These manuals are used as a source of standard methods for laboratories performing semen analyses worldwide.

The WHO published its updated 5th edition laboratory manual in late 2010, and, for the first time, multi-country semen analysis results from recent fathers with a known time-to-pregnancy (TTP), defined as the number of months (or cycles) from stopping contraception to achieving pregnancy, were incorporated.⁴ The reference values were obtained from data involving 1953 semen samples from 5 studies in 7 countries on 3 continents.^{5,6-10} Only subjects with a TTP of ≤ 12 months were included. The semen analysis results from this group of men were pooled and analyzed to provide the reference distributions for semen characteristics. The mean \pm SD male age was 31 ± 5 years (range 18-53), and only 10 men were >45 years old. The laboratories generating the data used standardized methods for semen analysis according to the WHO manual for the examination of human semen current at the time of the original studies. In addition, combined data, used to calculate the reference distributions, were provided by laboratories that practiced internal and external quality control.⁵ One-sided lower ref-

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Table 1. Cutoff reference values for semen characteristics as published in consecutive WHO manuals

Semen Characteristics	WHO 1980	WHO 1987	WHO 1992	WHO 1999	WHO 2010*
Volume (mL)	ND	≥2	≥2	≥2	1.5
Sperm count (10 ⁶ /mL)	20-200	≥20	≥20	≥20	15
Total sperm count (10 ⁶)	ND	≥40	≥40	≥40	39
Total motility (% motile)	≥60	≥50	≥50	≥50	40
Progressive motility [†] (%)	≥2 [‡]	≥25	≥25 (grade a)	≥25% (grade a)	32 (grade a + b)
Vitality (% alive)	ND	≥50	≥75	≥75	58
Morphology (% normal forms)	80.5	≥50	≥30 [§]	14 [¶]	4
Leukocyte count (10 ⁶ /mL)	<4.7	<1.0	<1.0	<1.0	<1.0

WHO = World Health Organization; ND = not defined.

* Lower reference limit obtained from lower fifth centile value.

[†] Grade a, rapid progressive motility (>25 μm/s); grade b, slow/sluggish progressive motility (5-25 μm/s); normal, 50% motility (grade a + b) or 25% progressive motility (grade a) within 60 min of ejaculation.

[‡] Forward progression (scale 0-3).

[§] Arbitrary value.

[¶] Value not defined, but strict criterion suggested.

^{||} Strict (Tygerberg) criterion.

reference limits (the fifth centile) were generated and were proposed as the lower cutoff limits for normality. Data on sperm morphology, extracted from 4 studies, including approximately 1800 fertile men, were reported according to the “strict” (Tygerberg) method.^{5,6,8,10,11} An assessment of progressive motility according to grade, as recommended by the previous WHO manuals, was replaced by categorizing motile sperm as being “progressive” or “nonprogressive.” This simplification in motility assessment should allow a more objective evaluation because sperm velocity is often interpreted subjectively by technicians. In our own experience, clinicians tend to overestimate the importance of sperm exhibiting grade “a” motility. This simple, yet important, modification will allow clinicians to focus on the proportion of progressive motile sperm rather than on the type of progressive motility as rapid or slow. Sperm vitality data, assessed using the eosin-nigrosin method, were obtained from approximately 400 men from 2 countries.⁵ The leukocyte reference values (<1 × 10⁶/mL) remain the same as in previous manuals.

Apart from the total sperm number per ejaculate, the lower limits of these distributions are less than the values presented in previous editions (Table 1).¹⁻³

WHERE THE DEBATE STARTS

The inclusion of reference values for semen analysis from controlled studies involving fertile fathers with a known TTP represents the most important feature of the fifth WHO manual. In comparison, previous versions reported reference values based on the clinical experience of investigators who had studied populations of healthy fertile men with unknown TTPs.¹⁻³ Previous WHO manuals acknowledged the limitations of their reference values by stating that each laboratory should determine its own reference values for each variable.

The goal of the lower reference limits included in the current edition of the WHO manual is to provide evidence-based thresholds that could aid clinicians in estimating the relative fertility of a given patient. However, several concerns arise from a careful examination of the

studies that generated the current reference values.^{5,6-13} First, it should be noted that apart from a single Australian study, all others came from Northern hemisphere countries. The Australian study included 206 subjects, representing approximately 10% of the “fertile” reference population.⁶ Roughly, 55% of the data came from 4 Western European cities (Paris, Turku, Edinburgh, and Copenhagen).^{7,9,11,12} The remaining patients came from a small study from another Western European city (Oslo) and from the United States.^{8,10} According to the investigators of the original study that referenced the 5th edition WHO manual, the laboratories and data were identified through the known published data and personal communication with the investigators and the editorial group of the 5th edition of the WHO laboratory manual.⁵ Interestingly, 4 of 5 studies were from the same group of investigators or was collaborative work among them (Table 2). The semen analyses results for the group of fertile men differed among these “reference” studies. It was not clear whether these differences represented real biologic dissimilarities among the men in different regions or laboratory-dependent biases of measurement, despite their adherence to the WHO manual methods. Cooper et al⁵ stated in their report that generated the reference values that “the studies included in the present analysis were conducted in different regions of the world with some areas over-represented, such as Northern Europe, and others, such as Africa, parts of Europe and Central and South America, under-represented.” However, their reference limits for the fertile population with a known TTP came only from Northern Europe, Australia, and the United States; as such, other areas were not represented at all. Millions of fertile men living in China, India, Africa, Middle East, and South America were not incorporated into these data analyses. From these data, it seems unsound to assume, as proposed by Cooper et al,⁵ that the reference values represented the global semen characteristics of fertile men.

Second, although the co-authors of the 5th edition WHO manual claim to have included only studies using

Table 2. Characteristics of reference studies used to establish new limits for human semen characteristics

Study	Country	TTP <12 mo Stated	Sperm Morphology Evaluation Criterion	Overlapping Authorship or Collaboration Among Authors
Bonde et al, ¹³ 1998	Denmark	Yes	David	Yes
Auger et al, ^{11*} 2001	France, Denmark, United Kingdom, Finland	No	Modified David	Yes
Jørgensen et al, ¹² 2001	France, Denmark, United Kingdom, Finland	No	David	Yes
Jensen et al, ⁹ 2001	France, Denmark, United Kingdom, Finland	Yes	David	Yes
Slama et al, ⁷ 2002	France, Denmark, United Kingdom, Finland	Yes	David, Tygerberg	Yes
Swan et al, ^{8*} 2003	United States	No	Tygerberg	Yes
Haugen et al, ^{10*} 2006	Norway	Yes	Tygerberg	No
Stewart et al, ^{6*} 2009	Australia	Yes	Tygerberg	Yes

TTP = time to pregnancy.

* Studies contributing to data on sperm morphology.

the Tygerberg “strict” method for morphology assessment (Kruger et al¹⁴), they in fact included studies using another morphology classification system from 1975 (David et al¹⁵). Indeed, Auger et al,¹¹ Slama et al,⁷ Jensen et al,⁹ Jørgensen et al,¹² and Bonde et al¹³ all used the method originally described by David et al,¹⁵ which differs from the method proposed by Kruger et al.¹⁴ The consequence of including studies using 2 different classification systems is that it results in a widened distribution of “normal” morphology values: the mean “normal” morphology is ~10% using the Tygerberg “strict” method (Swan et al⁸) but is ~50% using the David classification system (Slama et al⁷). Thus, the morphology reference limits reported in the 5th edition WHO manual do not accurately represent either the Tygerberg “strict” method or the David classification.

Third, it is not easy for the reader to understand how the data from the 5 reference studies were pooled by Cooper et al.⁵ For instance, when referring to the study by Swan et al,⁸ 593 samples were tabulated, but only 512 were reported in their original study. Moreover, a TTP of ≤12 months was clearly defined as an eligibility criterion for patient inclusion in only 2 studies,^{7,10} but in all remaining studies, it must be inferred.^{6,8,9}

Finally, a single semen sample was taken to represent each man in the reference studies. The assumption that 1 ejaculate is representative of a given man’s semen profile argues against the current knowledge of the high biologic variability of semen variables from the same individuals. Several guidelines, including the WHO manual, recommend that 2, but preferably 3, semen samples should be obtained before 1 man’s fertility status is depicted.¹⁻⁴

Ideally, a systematic review of the published data on semen quality in various populations should provide the recommendation for lower reference values. However, this is not feasible owing to the variability in the methods used in assessing the sperm count, motility, and morphology, even among studies claiming that WHO standards were applied. As a result, additional well-conducted studies with standardized methods and a recognized quality

control procedure are required to confirm the validity of the global reference ranges proposed by the 5th edition WHO manual. Beyond spontaneous conception, it will be of interest to determine the success of various clinical management protocols in relation to the chosen lower reference limits. If regional differences are revealed, their mechanism and significance for fertility will need to be evaluated, before it can be decided whether there should be specific reference values for different ethnic groups or regions.

IMPLICATIONS OF THE NEW SEMEN CHARACTERISTICS REFERENCE VALUES IN CLINICAL PRACTICE

Is Male Fertility Declining?

It is tempting to suggest that the lower reference limits of semen parameters, as proposed by the 2010 WHO manual, are a part of the gradual declines in sperm count extensively reported during the past 2 decades. The hypothesis that endocrine disruptors and other environmental pollutants, such as insecticides and pesticides, are responsible for declining overall sperm quality¹⁶ have attracted supporters,¹⁷⁻²⁰ as well as critics.²¹⁻²⁵ However, 2 other explanations are possible that could explain the difference in the reference values between the current and previous WHO manuals. The first is the adherence by many laboratories to greater quality control standards, especially when assessing sperm morphology. The second is that the previous WHO reference values were obtained mainly from the clinical experience of investigators who have studied populations of healthy fertile men with an unknown TTP rather than controlled populations of fertile men, such as in the current edition.¹⁻⁴ For these reasons, one must exercise caution when concluding that the newly proposed lowered WHO reference values can be justified by the suggested decline in global sperm quality. It is more probable that such differences are instead related to a methodologic bias created by different methods of generating the reference values.

Will Referrals for Assessment of the Male Partner Decrease?

The answer to this question is not straightforward, because it will depend on the acceptability of the new WHO manual reference values. The use of the new WHO manual reference values into clinical practice will likely result in a reclassification of many infertile couples. Specifically, those couples previously classified as having male factor infertility with sperm parameters greater than the new reference limits but less than the previous values will now be diagnosed as having unexplained or female factor infertility. It is also likely that some patients previously categorized as having an abnormal semen analysis will now be considered “normal,” with referral for evaluation postponed or not undertaken. This deferment poses a potential problem, because it has been exhaustively reported that the male and female reproductive age are clearly associated with reproductive outcome. It is unclear whether this reclassification will result in a more cost-effective evaluation of the infertile couple or in a delay in the male factor evaluation, with subsequent delay in the definitive diagnosis and management of the infertile couple.

In contrast, it is important to acknowledge the limitations of the semen analysis results in predicting the health and functional capacity of the male reproductive organs and cells. The male evaluation regarding fertility must go far beyond counting spermatozoa and assessing motility and morphology. It has to be complemented by a proper clinical examination, comprehensive history taking, and relevant endocrine, genetic, and/or other investigations.

Did We Overtreat Our Male Patients Before?

According to the new reference values, a man with 6% strict morphology, 16 million sperm/mL, and 40% progressive motility is considered to have a semen analysis within the so-called normal reference values; however, the same patient would be categorized as having an abnormal analysis according to the reference values proposed by the 1999 WHO manual.³ According to preliminary results of a current study involving individuals seeking fertility evaluation, 380 (38.7%) of 982 of a group previously classified as having an abnormal semen analysis by the 1999 WHO 4th edition manual would now be within the normal range (S.C.E.; unpublished data). This contradiction raises another question: Do we, as clinicians have to correct our semen analysis reports from the previous years or contact our couples for a reassignment of the infertility diagnosis? Caution must be exercised when interpreting these new reference values, because it is obvious that the prevalence of couples facing difficulties in conceiving has not changed, despite the publication of new reference values. Every couple attempting to conceive for >1 year of unprotected intercourse, or less in the case of advanced female age or in men with a recognized fertility problem, deserves a med-

ical evaluation that must include both partners, irrespective of the semen analysis results. It is known that about 30% of men misdiagnosed as having unexplained male infertility, according to the normal semen parameters on routine analyses, present with sperm deficiencies that can be solely identified by sperm functional tests, such as the assessment of DNA integrity, oxidative stress, and anti-sperm antibodies.²⁶⁻²⁹ Semen analyses, as routinely performed, are limited in their validity as surrogates for the assessment of male fertility potential. Therefore, it has been suggested that sperm function tests should be included in the semen analysis of individuals seeking fertility evaluation.³⁰

The couples' probability to conceive is influenced by multiple factors, and our task, as treating physicians, is multifaceted. It is our responsibility to diagnose existing conditions that might compromise, now or in the future, the fertility potential of our patients. The goal is to identify potential life-threatening diseases and to treat reversible conditions, including poor lifestyle habits, sub-clinical infections, hormonal disorders, and clinical varicocele, to cite a few.

Dilemma of the Clinical Varicocele and the New Reference Values: to Treat or Not to Treat?

Approximately 8% of men of reproductive age seek medical assistance for fertility-related problems. Of these, varicocele accounts for roughly 35% of the cases.³¹ Several studies have demonstrated that surgical treatment of clinical varicoceles is highly effective in decreasing seminal oxidative stress, increasing the seminal concentrations of antioxidants, and improving the sperm quality and pregnancy rates.³²⁻³⁷ However, the current guidelines propose that varicoceles should be treated if palpable and in the presence of abnormal semen analyses.³⁸⁻⁴⁰ The application of the new WHO reference values into clinical practice will result in patients previously deemed candidates for varicocele repair now ineligible for treatment if their semen parameters are greater than the fifth centile. Health insurance companies might not grant authorization or might refuse reimbursement if treatment is performed in men with semen parameters now reclassified as “normal.” The concern is that by denying these men varicocele repair we might prevent them from achieving a substantial improvement in semen parameters and a greater chance of spontaneous pregnancy. Men with a clinical varicocele and mild oligozoospermia or normozoospermia achieve greater spontaneous pregnancy rates after varicolectomy than couples with moderate to severe oligozoospermia.^{41,42} As such, the available data would support the practice of varicolectomy for infertile men with clinical varicocele and low “normal” semen parameters according to the new WHO reference values. Nonetheless, it would be very informative to reanalyze the prospective and randomized, controlled studies on varicolectomy to determine the magnitude of sperm quality improvement and pregnancy outcomes

in the subgroup of patients now classified as having “normal” semen analysis results.

The repair of adolescent varicocele must also be re-evaluated in light of the new WHO reference values. According to several professional societies’ guidelines, varicocele repair is recommended in adolescents and young adults with clinical varicocele and ipsilateral testicular atrophy or abnormal semen parameters.³⁸⁻⁴⁰ It is still unclear whether and how the application of the new WHO reference values will affect the management of the adolescent and young adult with varicocele. Mori et al,⁴³ studying a group of 360 adolescents attending a public school in Brazil, found that 27.8% presented with a palpable grade 2 or 3 varicocele, but only one half of them had testicular asymmetry. More importantly, the semen analysis results revealed that adolescents without varicocele had significantly greater numbers of progressively motile sperm (134.1 million) than did the adolescents with grade 2 (72.7 million) or 3 (30.3 million) varicocele. Despite the marked difference in the seminal profiles between adolescents with and without varicocele, all were still within the reference range for normality according to both the fourth and fifth WHO manual editions. Given the progressive deleterious effect of varicocele on testicular function,^{44,45} the goal of treating varicoceles (in the adolescent) is to halt the deterioration of sperm quality and prevent individuals with low “normal” semen parameters from crossing into the defined infertile range. Moreover, adolescent varicocele repair can also improve sperm quality and male reproductive potential.⁴⁶ Adolescents and adults with palpable varicocele can also present with normal semen analysis results but altered sperm function, as shown by elevated DNA fragmentation rates and oxidative stress levels.^{31,34,47} Taken together, this knowledge challenges the current WHO recommendations for varicocele treatment and highlights the importance of continued debate.

Effect of New Reference Values on Assisted Reproductive Technology Treatment

At first glance, one might expect that the lower reference values in the new WHO manual would result in a lower use of advanced assisted reproductive technology (eg, intracytoplasmic sperm injection [ICSI])—a technique largely designed to treat male factor infertility), because there will be relatively fewer couples with subnormal semen parameters. However, ICSI is generally reserved for couples in whom the man has severe abnormalities in sperm count and motility; thus, ICSI use is unlikely to change because the sperm parameters of these men will certainly be less than the new reference values. Moreover, many centers are already using low morphology thresholds (<5% normal morphology by strict criteria) as an indication to proceed to ICSI rather than intrauterine insemination or conventional in vitro fertilization⁴⁸; thus, the effect on ICSI use based on morphology criteria will likely be minimal. Along the same lines, intrauterine

Table 3. Distribution of semen characteristics of fertile men whose partners had a time-to-pregnancy of ≤ 12 months, used to establish 2010 WHO manual reference limits, according to percentiles

Characteristic	Percentile		
	5%	50%	95%
Volume (mL)	1.5	3.7	6.8
Sperm count ($\times 10^6$ /mL)	15.0	73.0	213.0
Sperm count ($\times 10^6$ /ejaculate)	39.0	255.0	802.0
Motility (%)			
Total	40	61	78
Progressive	32	55	72
Normal*	4	15	44
Alive†	58	79	91

WHO = World Health Organization.

* According to the strict Tygerberg criterion.

† Eosin-nigrosin staining.

Adapted from Cooper et al.⁵

insemination candidates include not only couples with mild male factor infertility but also those with normal semen parameters and unexplained infertility. As such, it is unlikely that the new semen parameters reference values will have a profound effect on the indication of intrauterine insemination.

It is important to stress that the reference semen values proposed by the new WHO manual are not suitable to indicate a treatment modality. They merely represent the distribution of the semen profile of a small group of fertile individuals. The choice of assisted reproductive technology should be determined by the clinical features of each case, as well as on the center’s experience and reported results with different assisted reproductive technology modalities rather than on the semen analysis reports.

Expanding Interpretation of New WHO Reference Values: Focus on 50th Percentile

The 95% reference interval for semen characteristics of recent fathers included in the 5th edition WHO manual has been generated in line with clinical chemistry standards, and the fifth centile was proposed for the lower limit of semen characteristics (Table 3).^{4,5} As such, reference values are important for comparison with the values obtained from the patient being assessed. The observed values can be used to aid in the clinical decision-making process by comparing them with the reference distributions and reference intervals. Therefore, it is important, not only to compare patient results with the lower reference limit, but also with the 50th percentile, which represents a value into which 50% of the reference population of “fertile” men falls. This strategy might be more realistic and can help in understanding a patient’s seminal profile in relation to the reference group.

SHOULD THE NEW REFERENCE VALUES BE ADOPTED?

At present, whether the new reference values should be adopted remains unresolved, and more debate is needed.

It is possible that global reference values are not achievable because of geographic and racial variations. It would be ideal to have well-funded prospective studies designed to evaluate several racial and geographic populations of fertile men. From our discussion, different laboratories seeking to adopt the new standard should determine a strategy that would aid in the clear communication of the clinical significance of their results.

CONCLUSIONS

The WHO manuals for the laboratory examination of human semen have been used over the years as a source of standard methodology for laboratories performing semen analyses worldwide. For the first time since the publication of its first edition 30 years ago, the WHO has reported evidence-based reference values for the semen characteristics of fertile men that, not surprisingly, are much lower than those recommended in previous editions. Despite the notable advance of using controlled studies involving couples whose TTP was <12 months to establish the new limits, the reference studies are limited with regard to the populations analyzed and the methods used for semen evaluation. As such, it seems unreasonable to assume that the reference values represent universal cutoff points of semen characteristics of fertile samples, such as was proposed in the 5th edition WHO manual. Moreover, caution should be exercised to not overinterpret the new reference values, because they have not been shown to accurately discriminate populations of fertile and infertile men. Properly performed semen analyses, coupled with an adequate clinical examination of the male, can give valuable information related to the organs producing “semen,” a highly complex fluid, and thus help in better understanding the physiology of the reproductive organs and the causes of dysfunction. In view of the expected effect of these new values on patient referral, diagnosis, and treatment of recognized conditions, such as varicocele, and on the indications for assisted reproductive technologies, we conclude that the WHO should have allowed for an extensive debate within the scientific community before the publication of these values. The time has come for technological developments that bring robust and cost-effective clinically useful tests to assess the fertilizing potential of a semen sample, and that can replace, at least partially, the shortcomings of the standard semen analysis.

References

- World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction*, 2nd ed. Cambridge: Cambridge University Press; 1987.
- World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction*, 3rd ed. Cambridge: Cambridge University Press; 1992.
- World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction*, 4th ed. Cambridge: Cambridge University Press; 1999.
- World Health Organization. *WHO Laboratory Manual for the Examination and Processing of Human Semen*, 5th ed. Geneva: WHO Press; 2010.
- Cooper TG, Noonan E, von Eckardstein S, et al. World Health Organization reference values for human semen characteristics. *Hum Reprod Update*. 2010;16:231-245.
- Stewart TM, Liu DY, Garrett C, et al. Associations between andrological measures, hormones and semen quality in fertile Australian men: inverse relationship between obesity and sperm output. *Hum Reprod* 2009;24:1561-1568.
- Slama R, Eustache F, Ducot B, et al. Time to pregnancy and semen parameters: a cross-sectional study among fertile couples from four European cities. *Hum Reprod*. 2002;17:503-515.
- Swan SH, Brazil C, Drobnis EZ, et al. Geographic differences in semen quality of fertile U.S. males. *Environ Health Perspect*. 2003; 111:414-420.
- Jensen TK, Slama R, Ducot B, et al. Regional differences in waiting time to pregnancy among fertile couples from four European cities. *Hum Reprod*. 2001;16:2697-2704.
- Haugen TB, Egeland T, Magnus O. Semen parameters in Norwegian fertile men. *J Androl*. 2006;27:66-71.
- Auger J, Eustache F, Andersen AG, et al. Sperm morphological defects related to environment, lifestyle and medical history of 1001 male partners of pregnant women from four European cities. *Hum Reprod*. 2001;16:2710-2717.
- Jørgensen N, Andersen AG, Eustache F, et al. Regional differences in semen quality in Europe. *Hum Reprod*. 2001;16:1012-1019.
- Bonde JP, Ernst E, Jensen TK, et al. Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. *Lancet*. 1998;352:1172-1177.
- Kruger TF, Acosta AA, Simmons KF, et al. Predictive value of abnormal sperm morphology in in vitro fertilization. *Fertil Steril*. 1988;49:112-117.
- David G, Bisson JP, Czyglik F. Anomalies morphologiques du spermatozoïde humain. 1. Prognostion pour un système de classification. *J Gynecol Obstet Biol Reprod*. 1975;4:17-36.
- Carlsen E, Giwercman A, Keiding N, et al. Evidence for decreasing quality of semen during past 50 years. *BMJ*. 1992;305: 609-613.
- Ginsburg J, Okolo S, Prelevic G, et al. Residence in the London area and sperm density. *Lancet*. 1994;343:230.
- Auger J, Kunstmann JM, Czyglik F, et al. Decline in semen quality among fertile men in Paris during the past 20 years. *N Engl J Med*. 1995;332:281-285.
- Adamopoulos DA, Pappa A, Nicopoulou S, et al. Seminal volume and total sperm number trends in men attending subfertility clinics in the greater Athens area during the period 1977-1993. *Hum Reprod*. 1996;11:1936-1941.
- Irvine DS, Twigg JP, Gordon EL, et al. DNA integrity in human spermatozoa: relationships with semen quality. *J Androl*. 2000;21: 33-44.
- Younglai EV, Collins JA, Foster WG. Canadian semen quality: an analysis of sperm density among eleven academic fertility centers. *Fertil Steril*. 1998;70:76-80.
- Andolz P, Bielsa MA, Villa J. Evolution of semen quality in Northeastern Spain: a study in 22,759 infertile men over a 36 year period. *Hum Reprod*. 1999;14:731-735.
- Auger J, Jouannet P. Evidence for regional differences of semen quality among fertile French men. *Fédération Française des Centres d'Etude et de Conservation des Oeufs et du Sperme humains. Hum Reprod*. 1997;12:740-745.
- Handelsman DJ. Estrogens and falling sperm counts. *Reprod Fertil Dev*. 2001;13:317-324.
- Sadeu JC, Hughes CL, Agarwal S, et al. Alcohol, drugs, caffeine, tobacco, and environmental contaminant exposure: reproductive health consequences and clinical application. *Clinical Rev Toxicol*. 2010;40:633-652.

26. Bungum M, Bungum L, Giwercman A. Sperm chromatin structure assay (SCSA): a tool in diagnosis and treatment of infertility. *Asian J Androl*. 2010;13:69-75.
27. Agarwal A, Makker K, Sharma R. Clinical relevance of oxidative stress in male factor infertility: an update. *Am J Reprod Immunol*. 2008;59:2-11.
28. Shefi S, Turek PJ. Definition and current evaluation of subfertile men. *Int Braz J Urol*. 2006;32:385-397.
29. Kefer JC, Agarwal A, Sabanegh E. Role of antioxidants in the treatment of male infertility. *Int J Urol*. 2009;16:449-457.
30. Aziz N, Agarwal A. Evaluation of sperm damage: beyond the World Health Organization criteria. *Fertil Steril*. 2008;90:484-485.
31. CDC. Vital and Health Statistics, series 23, no. 26. Available from: <http://www.cdc.gov>. Accessed December 10, 2009.
32. Zini A, Blumenfeld A, Libman J, et al. Beneficial effect of microsurgical varicocelectomy on human sperm DNA integrity. *Hum Reprod*. 2005;20:1018-1021.
33. Esteves SC, Glina S. Recovery of spermatogenesis after microsurgical subinguinal varicocele repair in azoospermic men based on testicular histology. *Int Braz J Urol*. 2005;31:541-548.
34. Agarwal A, Deepinder F, Cocuzza M, et al. Efficacy of varicocelectomy in improving semen parameters: new meta-analytical approach. *Urology*. 2007;70:532-538.
35. Smit M, Romijn JC, Wildhagen MF, et al. Decreased sperm DNA fragmentation after surgical varicocelectomy is associated with increased pregnancy rate. *J Urol*. 2010;183:270-274.
36. Esteves SC, Oliveira FV, Bertolla RP. Clinical outcome of intracytoplasmic sperm injection in infertile men with treated and untreated clinical varicocele. *J Urol*. 2010;184:1442-1446.
37. Marmar JL, Agarwal A, Prabaskan S, et al. Reassessing the value of varicocelectomy as a treatment for male subfertility with a new meta-analysis. *Fertil Steril*. 2007;88:639-648.
38. Male Infertility Best Practice Policy Committee of the American Urological Association; Practice Committee of the American Society for Reproductive Medicine. Report on varicocele and infertility. *Fertil Steril*. 2004;82(suppl 1):S142-S145.
39. European Association of Urology. Guidelines on Male Infertility 2010. Available from: <http://www.uroweb.org/gls/pdf/Male%20Infertility%202010.pdf>. Accessed February 27, 2011.
40. Sociedade Brasileira de Urologia & Colégio Brasileiro de Radiologia; Projeto Diretrizes da Associação Médica Brasileira. Varicocele. Available from: http://www.projetodiretrizes.org.br/8_volume/40-Varicocele.pdf. Accessed February 27, 2011.
41. Kamal KM, Jarvi K, Zini A. Microsurgical varicocelectomy in the era of assisted reproductive technology: influence of initial semen quality on pregnancy rates. *Fertil Steril*. 2001;75:1013-1016.
42. Richardson I, Grotas AB, Nagler HM. Outcomes of varicocelectomy treatment: an updated critical analysis. *Urol Clin North Am*. 2008;35:191-209.
43. Mori MM, Bertolla RP, Fraietta R, et al. Does varicocele grade determine extent of alteration to spermatogenesis in adolescents? *Fertil Steril*. 2008;90:1769-1773.
44. Gorelick JJ, Goldstein M. Loss of fertility in men with varicocele. *Fertil Steril*. 1993;59:613-616.
45. Witt MA, Lipshultz LI. Varicocele: a progressive or static lesion? *Urology*. 1993;42:541-543.
46. Cayan S, Acar D, Ulger S, et al. Adolescent varicocele repair: long-term results and comparison of surgical techniques according to optical magnification use in 100 cases at a single university hospital. *J Urol*. 2005;174:2003-2006.
47. Bertolla RP, Cedenho AP, Hassun Filho PA, et al. Sperm nuclear DNA fragmentation in adolescents with varicocele. *Fertil Steril*. 2006;85:625-628.
48. Coetzee K, Kruger TF, Lombard CJ. Predictive value of normal sperm morphology: a structured literature review. *Hum Reprod Update*. 1998;4:73-82.