Will the New World Health Organization Standards for Semen Examination Change the Clinical Management of Male Infertility?

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INTRODUCTION

Semen analysis is of great value in the initial investigation of male and its results are often taken as a surrogate measure of male fecundity and pregnancy risk. It provides information on the functional status of the seminiferous tubules, epididymis and accessory sex glands. Reference ranges for semen parameters from a fertile population may provide data from which prognosis of fertility or diagnosis of infertility can be extrapolated. Nonetheless, the prognostic value of semen components such as sperm count, 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, function of accessory sex glands and other conditions. Routine semen analysis itself has its own limitations, and does not assess for sperm dysfunctions such as immature chromatin or a fragmented DNA.

The World Health Organization (WHO) periodically releases manuals for laboratory examination of human semen. The first one was published in 1980, with subsequent updates in 1987, 1992 and 1999.[1–3] These manuals are used as a source of standard methodology for laboratories performing semen analyses worldwide. The WHO published its updated 5th edition in late 2010 with important differences from previous versions.[4] The new edition contains more detailed information on how to analyze semen samples in a routine basis and how to perform advanced sperm function tests. It includes new chapters on sperm preparation techniques for assisted conception and cryopreservation. There is a completely revised chapter on quality control and, for the first time, multicountry data from recent fathers with known time-to-pregnancy (TTP) was incorporated.

The evidence-based reference ranges and reference limits for various semen characteristics are, in our opinion, the most important albeit controversial features of the new manual. Reference values were obtained from data involving 1,953 semen samples from 5 studies in 8 countries on 3 continents.[5–10] Only subjects with a TTP of ≤12 months

were included. Semen analysis results from this group of men were pooled and analyzed to provide reference distributions for semen parameters. Laboratories generating the data used standardized methods for semen analysis according to the *WHO Manual for the Examination of Human Semen* which were available at the time of the original studies. In addition, data that were combined to calculate the reference distributions were provided by laboratories that practiced internal and external quality control.[5] One-sided lower reference limits (the 5th centile) were generated and were proposed as the lower cutoff limits for normalcy. Apart from total sperm count per ejaculate, the lower limits of these distributions are lower than the previously presented 'normal' or 'reference' values (Table 1).[1–3]

Data on normal sperm morphology, extracted from 4 studies including approximately 1,800 men, were reported according to the 'strict' (Tygerberg) method.[5,6,8,10,11] Assessment of progressive motility according to grades, as recommended by the previous *WHO Manual*, was replaced by categorizing motile sperm as being 'progressive' or 'nonprogressive'. Sperm vitality data, assessed by the eosin-nigrosin method, was obtained from approximately 400 men of 2 countries.[5] Leukocyte reference values remained the same as previous manuals.

SHORTCOMINGS OF THE NEW WORLD HEALTH ORGANIZATION STANDARDS FOR SEMEN EXAMINATION

Despite the complex relationship between semen analysis results and pregnancy outcome, the reference values included in the current version of the *WHO Manual* are aimed to provide evidence-based thresholds that may aid clinicians in calculating the relative fertility of a given patient. However, several concerns arise from a detailed examination of the studies which generated the current reference values.[5–13]

Table 1
Standards for Semen Examination as Published in Consecutive World Health Organization Manuals

Semen parameters	WHO 1980	WHO 1987	WHO 1992	WHO 1999	WHO 2010[a]
Volume (mL)	—	≥2	≥2	≥2	1.5
Sperm count (10^6/mL)	20–200	≥20	≥20	≥20	15
Total sperm count (10^6)	—	≥40	≥40	≥40	39
Total motility (% motile)	≥60	≥50	≥50	≥50	40
Progressive motility[b]	≥2[c]	≥25%	≥25% (Grade A)	≥25% (Grade A)	32% (A + B)
Vitality (% alive)	—	≥50	≥75	≥75	58
Morphology (% normal)	80.5	≥50	≥30[d]	14[e]	4[f]
Leukocyte count (10^6/mL)	<4.7	<1.0	<1.0	<1.0	<1.0

[a]Lower reference limit obtained from the lower fifth centile value.
[b]Grade A: rapid progressive motility (>25 µm/s); Grade B: slow/sluggish progressive motility (5–25 µm/s); Normal: 50% motility (Grades A + B) or 25% progressive motility (Grade A) within 60 min of ejaculation.
[c]Forward progression (scale 0–3).
[d]Arbitrary value.
[e]Value not defined but strict criterion is suggested.
[f]Strict (Tygerberg) criterion.

First, it should be noted that apart from a single Australian study all others came from countries situated in the Northern hemisphere. The Australian study included 206 subjects which represented only about 10% of the 'fertile' reference population.[6] Roughly 55% of the data came from 4 western European cities (Paris, Turku, Edinburgh and Copenhagen) and we speculate that the studies from Slama et al[7] and Jensen et al[9] used the same database. The remaining patients came from a small study from another western European city (Oslo)[10] and from the USA.[8] A systematic review of the literature was not performed to identify all data on semen quality in various populations. According to the authors of the original study that referenced the 5th edition of *WHO Manual*, laboratories and data were identified through the known literature and personal communication with investigators and the editorial group of the same.[5] Interestingly, 4 out of 5 studies were from the same group of authors or collaborative work among them. Semen analyses results for the group of fertile men differed among these 'reference' studies. It was not clear if these differences represented real biological differences among men in different regions or laboratory-dependent biases of measurement, despite their adherence to the *WHO Manual* methods. Cooper et al[5] stated in their original report 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*'. The point is that their reference limits for the fertile population with known TTP came only from Northern Europe, Australia and USA; as such, other areas were not represented at all. What about millions of fertile men living in China, India, Africa, Middle East and South America? These are the areas where the vast majority of people live nowadays. From these data, it seems unsound to assume, as proposed by Cooper et al,[5] that the reference values represented global semen characteristics of fertile men.

Second, strict criterion was not the method used for assessing sperm morphology in one of the studies that had been claimed to source the reference values. Auger et al[11] used the method originally described by David et al,[14] which differs from the one proposed by Kruger et al.[15] Moreover, it should be noted that in the study by Slama et al, which sourced 46% of data, sperm morphology was also evaluated by the David's method with proper quality control. Then, morphology slides were sent to another laboratory for additional assessments of morphology according to the strict criterion by a single physician; unfortunately, no details of quality control were provided for such analysis.[7]

Third, it is not easy for the reader to understand how the data from the 5 reference studies were pooled by Cooper et al. For instance, when referring to the study by Swan et al, 593 samples were tabulated but only 512 were reported in their original study.[8] Moreover, in only 2 studies[7,10] a TTP of ≤12 months was clearly defined as an eligibility criterion for patient inclusion while in all remaining ones it has to be inferred.[6,8,9] To explain these discrepancies, it is likely that the original datasets were provided by the authors of these reference studies to the ones conducting the WHO study, and then the information was re-organized and re-analyzed.

Lastly, a single semen sample was taken to represent each man in the reference studies. The assumption that one ejaculate is representative of a given man semen profile argues against the current knowledge of the high biological variability of semen parameters from same individuals.

Further studies will be required to confirm the validity of global reference ranges as proposed by the 5th edition of *WHO Manual*. If regional differences are revealed, their mechanism and significance for fertility will need to be studied before it can be decided whether there should

be specific reference values for different ethnic groups or regions. Laboratories may have to produce their own local reference ranges for semen parameters. A confirmatory analysis including a systematic review of laboratories using highly standardized techniques (such as those presented in the 5th edition of the *WHO Laboratory Manual*) reporting participation in quality control programs, and taking geographical and ethnic origins into account, is needed. It will be of interest to determine the success of various clinical management protocols that incorporate the reference limits into research and practice guidelines.

WHY ARE THE NEW WORLD HEALTH ORGANIZATION STANDARDS LOWER THAN PREVIOUS ONES?

At a first glance, it may be concluded that the reason for such observation is a trend towards male fertility decline, as suggested by Carlsen et al.[16] The authors' findings, suggesting that endocrine disruptors and other environmental polluters such as insecticides and pesticides are responsible for declining overall male fertility, have attracted supporters[17–20] as well as critics.[21–25] However, there are other reasons that may explain the difference in the reference values between the current and previous *WHO Manual*. One is the adherence by many laboratories of higher quality control standards especially when assessing sperm morphology. Another one, not well explored by recent commentaries, is that previous WHO reference values were mainly based on the clinical experience of investigators who have studied populations of healthy fertile men of unknown TTP rather than controlled populations of fertile men 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 male fertility. It may be possible that such differences are not related to the decline in male fertility at all, but rather a methodological bias created by different ways of generating data.

IMPLICATIONS OF THE 5TH EDITION WORLD HEALTH ORGANIZATION STANDARDS FOR THE CLINICAL MANAGEMENT OF MALE INFERTILITY

Patient Referral

If the new WHO standards are accepted by most laboratories performing semen analysis, it is likely that several patients previously categorized as having abnormal semen analysis will be now considered 'normal', and referral for evaluation may be postponed or not undertaken. It poses a potential problem since it has been exhaustively reported that male and female reproductive age are clearly associated with reproductive outcome. On the other hand, it is important to acknowledge the limitations of semen analysis results to assess 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 supplemented with a proper clinical examination, a comprehensive history taking, and relevant endocrine, genetic, and/or other investigations. Every couple attempting to conceive for >1 year of unprotected intercourse, or less in the cases of advanced female age or in men with a recognized fertility threat, deserve medical 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 sperm deficiencies that can be identified by sperm function tests, such as the assessment of DNA integrity, oxidative stress and antisperm antibodies.[26,27] Sperm DNA fragmentation and elevated oxidative stress, for instance, are recognized as having great importance in males experiencing difficulties to conceive. Abnormalities in the male genome

characterized by damaged sperm DNA may be indicative of male subfertility regardless of routine semen parameters which do not reveal DNA defects. An abnormal proportion of spermatozoa with fragmented deoxyribo nucleuc acid can be found in 5–10% of infertile men with normal semen analyses but is rarely seen in fertile individuals.[28] Advanced paternal age, inadequate diet intake, drug abuse, pesticide environmental exposure, tobacco use, varicocele, medical disease, scrotal hyperthermia, air pollution, genital inflammation or infectious diseases can be cited as possible causes, some of which are reversible.[29] Deoxyribonucleic acid fragmentation can be secondary to internal factors such as apoptosis and oxidative stress, or external factors such as the presence of leukocytes. The oxidative stress-induced sperm damage has been suggested to be a significant contributing factor in 30–80% of all cases of male infertility.[27] Semen analyses results, when routinely performed, is limited in its validity as surrogate for male fertility potential. The couples' chances to conceive involve multiple factors and our goals, as treating physicians, are manifold. It is our responsibility to diagnose existing conditions that may compromise, now or in the future, the fertility potential of our patients, to identify potentially life-threatening diseases and to treat reversible conditions such as inadequate lifestyle habits, subclinical infections, hormone disorders and clinical varicocele.

Indication for Varicocele Treatment

Several guidelines propose that varicoceles should be treated if palpable and in the presence of abnormal semen analyses.[30–32] According to the new WHO reference semen values several patients will be ineligible for treatment when the new guidelines are strictly followed. Health insurance companies may not grant authorization or refuse reimbursement if treatment is performed in men with normal semen parameters. The question whether or not a man with clinical varicocele should undergo repair in the face of normal semen parameters according to the 5th edition of *WHO Manual* is not simply answered. Most important would be to know the semen parameters of the same individual if varicocele had been treated. It would be very informative to re-analyze the meta-analysis studies on varicocelectomy to determine the magnitude of sperm quality improvement in the subgroup of patients that is now classified as having 'normal' semen. This information will certainly come, but for the time being emerging evidence seems to support the indication of treatment for men with clinical varicocele and so-called 'normal' semen parameters according to the new WHO reference values.

Mori et al examined a group of 360 nonselected adolescents aged 14–18 years attending a public school in Brazil.[33] They found that 27.8% presented with a palpable grade II or III varicocele but only half of them had testicular asymmetry. Semen analysis results revealed that adolescents without varicocele ejaculated significantly higher number of progressively motile sperm (134.1 million) compared to adolescents with grade II (72.7 million) and III (30.3 million) varicocele. Despite the marked difference in the seminal profile between adolescents with and without varicocele all individuals were still within the reference range for normality according to the WHO standards. Because semen samples of this group of adolescents are still considered normal, and because testicular asymmetry will not be present in ≥50% of adolescents, treatment is not recommended according to current professional societies' guidelines, such as the ones published by the American Urological Association, American Society for Reproductive Medicine, European Association of Urology and Brazilian Society of Urology.[30–32] Thus, surgical correction of the varicocele will only be offered when adolescents have already crossed into the infertile range, even though initial evaluation had already shown that their

seminal profile were significantly lower than their counterparts without varicocele. Due to the progressive effect of varicocele,[34,35] it is expected that treatment of varicocele halts deterioration of sperm quality and prevents individuals with yet 'normal' semen analysis to cross into the defined infertile range. Moreover, it is also possible that improvements in sperm quality after varicocele repair would increase the male reproductive potential, albeit pre- and post-treatment values are within the newly proposed reference values. Adolescents and adults with palpable varicoceles may present with normal semen analysis but altered sperm function, as shown by elevated DNA fragmentation rates and oxidative stress levels.[36] Taking together, this knowledge challenges the current recommendations for varicocele treatment, and highlights the importance of a continuous debate.

Indication for Assisted Reproductive Techniques

In clinical practice, assisted reproductive techniques (ART), especially intracytoplasmic sperm injection (ICSI), are indicated according to the semen analysis results. It is unlikely that the new reference semen values will change the clinicians' practice, at least towards the use of more complex ART such as ICSI. If a reproductive center utilizes the cutoff value of 4% strict morphology for recommending ICSI, it should not be changed because of the new guidelines since sufficient data exists to support the superiority of this technique as compared to conventional in vitro fertilization (IVF) or intra-uterine insemination (IUI) in such cases.[37] Moreover, the indication of ICSI based on very poor sperm count and motility is unlikely to be changed since the thresholds will certainly be within the abnormal ranges according to the new reference values. A potential problem may arise for IUI, since several cases of mild abnormal semen analysis (that will now fit within normal reference limits) are treated by this ART modality. However, the impact of the new reference values on IUI is likely to be minimal because IUI is routinely performed in cases of unexplained infertility and normal semen analyzes. The choice of ART should be based on the clinical features of each case as well as on the Center's experience and reported results with different ART modalities rather than on semen analyses reports. It is unlikely that a clinician will modify a treatment strategy based only on the new reference values for semen parameters being considered 'normal' or 'abnormal' if his/her results with a less complex technique are poor in a particular semen scenario. It is important to stress that the reference semen values, as proposed by the WHO, cannot be over-interpreted to indicate a treatment modality. It merely represents the distribution of the semen profile of a small group of fertile individuals.

PERSONAL CLINICAL EXPERIENCE

According to preliminary results of a current study involving individuals seeking fertility evaluation, 38.7% (380/982) of the group previously classified as having abnormal semen analyzes by the WHO 4th edition (1999) guidelines are now within the normal range (unpublished data).

The 5th edition of *WHO Manual* presents semen characteristics of a population of men who had fathered a child within 1 year of trying to induce conception. The 95% reference intervals for a range of semen parameters and the lower reference limits have been generated in line with clinical chemistry standards.[4,5] For a conventional one-sided distribution, the 5th centile was proposed for the lower limit of semen characteristics. Clinical reference values are important for comparison with values obtained from the patient being assessed. The observed values may be used to aid in the clinical decision making process by comparing them with reference distributions and reference intervals. Therefore, it is important not only

Table 2.
Distribution of Values According to the 5th, 50th and 95th Percentiles for Semen Parameters from Fertile Men whose Partners had a Time-to-Pregnancy of 12 Months or Less.[5]

	Centiles		
	5	**50**	**95**
Volume (mL)	1.5	3.7	6.8
Sperm count/mL ($x10^6$)	15.0	73.0	213.0
Sperm count/ejaculate ($x10^6$)	39.0	255.0	802.0
% Motility (total)	40	61	78
% Motility (progressive)	32	55	72
% Normal*	4	15	44
% Alive†	58	79	91

*According to the strict (Tygerberg) criterion.
†Eosin-nigrosin staining.

to compare the patient results with the lower standards but also with the 50th percentile, which represents the point in the curve where 50% of the reference population fits (Table 2).

The question of whether or not the new WHO standards for semen examination would be universally adopted by andrology laboratories is still unanswered. It would be ideal to have well-funded prospective studies designed to evaluate several populations of fertile men to account for geographic and racial diversity. Our andrology laboratory decided to adopt the new WHO reference semen values. However, we elected to modify our semen analysis report form by including not only the lower reference limits but also the 50th and 95th percentiles. We believe that the presentation of reference values according to percentiles may aid the clinician who receives the semen analysis report to better understand where his/her patient fits in comparison to the reference population. Additionally, we decided to provide extra support for clinicians requesting semen analysis, including a clear explanation that the new reference limits are derived from semen samples from men whose partners conceived spontaneously and that such reference provide only a guide regarding a patient's fertility. We further go on by explaining that semen characteristics are highly variable within and among men; and these parameters are not unique.

It is important to note that semen parameters within the 95% reference interval do not guarantee fertility nor do values outside those limits necessarily indicate male infertility. It is also clear from the reported reference values that 5% of the fertile men providing the reference data had values below the 5% centile defined as the minimum threshold. A man's semen characteristics need to be interpreted in conjunction with his clinical information and the reference limits should not be used to determine the nature of that treatment.

CONCLUSION

The WHO has established new standards for semen examination in its 5th edition

manual which are lower than those previously reported. Several questions arise after a careful examination of the proposed new values, especially regarding the implications of these references for diagnosis and treatment of male infertility. Despite the notable advance of using controlled studies involving couples whose time to pregnancy was <12 months to generate the new standards, reference studies are limited with regard to the population analyzed and the methods used for semen evaluation. As such, it seems unreasonable to assume that reference values represent global semen characteristics of fertile men as proposed in the 5th edition of *WHO Manual*. Caution should be exercised to not over-interpret the new reference values as they may fail to accurately discriminate populations of fertile and infertile men. Properly performed semen analyses coupled with an adequate examination of the man can give valuable information related to the organs producing 'semen', a highly complex fluid, and thus help in better understanding of the physiology of the reproductive organs and the causes of their dysfunctions. The adoption of the proposed WHO standards for semen examination is likely to have an important impact on patient referral and treatment of certain conditions such as varicocele. Therefore, more debate is needed before the universal acceptance of the proposed WHO standards by worldwide andrology laboratories. For those considering to adopting the new standards, a better approach would be the presentation of reference values by percentiles rather than solely the lower cutoff limits. The time has come for technological developments that bring robust and cost-effective clinically-useful sperm function tests to replace, at least partially, the shortcomings of routine semen analysis.

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