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PERSPECTIVE

New WHO-reference limits—revolution or storm in a teapot?

Gerhard Haidl

Since release of the latest WHO manual with the new lower reference values of semen parameters, a lot of discussion has been raised about their usefulness and appropriateness for assessment of male fertility. As with the previous reference values the new limits do neither allow an andrological diagnosis based on nosological criteria nor clear-cut differentiation between fertility and sub-/infertility. Therefore, considering the fact that fertility is a continuum, the new lower reference limits should not be overestimated. Most probably, more sperm function tests, such as determination of DNA integrity, and—in the future—assessment of biomarkers, such as sperm proteomics will be included into andrological work-up, thus resulting in a more personalized approach of infertility management. On the other hand, the detailed instructions for standard and advanced semen analysis provided in the new manual are very much appreciated and should be adopted by each seriously working laboratory.
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INTRODUCTION

Recently the new WHO laboratory manual for the examination and processing of human semen (5th edition) has been published[1] and a Special Issue of the *Asian Journal of Andrology* to mark that occasion has highlighted in a series of papers the controversies arising from that new edition. This new version of the manual provides substantial changes and improvements compared with the previous versions and contains more detailed information and instruction, thus facilitating the work in the andrological laboratory. Along with a large series of instructive photographs, including those demonstrating normal spermatozoa according to strict criteria and respective pathomorphology, it contains very detailed and clear explanations of all the basic techniques. Moreover, new chapters on sperm preparation for assisted reproduction techniques and cryopreservation have been included. The text also incorporates a series of detailed standardized protocols for more advanced assessments of additional elements of semen analysis, such as the detection of leukocytes, the identification of precursor germ cells and the determination of antisperm antibodies. Most importantly, there is a completely revised chapter on quality control.[2] The most striking changes in this new edition of the WHO manual, however, concern the reference values for semen quality. Whereas the previous reference values were based, more or less, on expert opinion, the new ones were acquired by analysing semen samples from 1800 recent fathers (time to pregnancy of $\leqslant$12 months) living in eight countries on three continents. From these data, one-sided lower reference limits were generated from the fifth percentile of the data distribution.[3] The development of evidence-based reference ranges for semen analysis resolves one of the major concerns of previous editions. These new reference values reveal some drastic differences from the previous ones; for example, progressive motility is considered as normal when 32% of spermatozoa move forward (instead of 50%), and the lower limit of normally-shaped spermatozoa is 4%.[1]

NEW REFERENCE LIMITS

For means of simplification, progressive motility is no longer divided into rapid (WHO a) and slow (WHO b) but summarized only as progressive motility. As the fundamental problem exists that results from semen analysis are in general very poor markers of true male fertility, one can cast doubt on the usefulness of summarizing forward motility only and refrain from determining rapidly progressing spermatozoa. Reduced sperm motility—although being within the normal range according to the new reference values—may be a symptom of disorders related to male accessory gland secretion; for example, detrimental effects of male accessory gland infection would be easily overlooked when only relying on otherwise normal semen parameters. Meanwhile, it is well established that such inflammations can severely impair sperm function and other parameters important for natural conception, as well as *in vitro* fertilisation, e.g. disturbed DNA integrity.[4,5] Both with *in vivo* and *in vitro* fertilisation, rapidly progressive sperm motility has long been considered to be a useful indicator. Therefore, with the exception of treatment by intracytoplasmic sperm injection, progressive sperm motility is required for success. That is why distinguishing between slow and rapid progressive spermatozoa remains essential. To neglect such information available from the semen sample would impoverish the clinical usefulness of semen analysis.[4]

Similar concerns have to be raised regarding evaluation and assessment of sperm morphology. Is it really sufficient to focus on spermatozoa with an 'ideal' morphology?[6–8] Clinical practice shows that patients with a percentage of at least 4–5% of ideally shaped

Department of Dermatology/Andrology Unit, University of Bonn, Bonn 53127, Germany
Correspondence: Dr G Haidl (gerhard.haidl@ukb.uni-bonn.de)

spermatozoa do not show 95–96% of malformed or disturbed, non-functioning spermatozoa, but in the majority of cases have a reasonable number of spermatozoa with only slight aberrations and preserved fertilizing potential.[6,9] Reconsidering the original paper on the hemizona assay, there are many spermatozoa bound to the zona pellucida that do not meet strict criteria but show slight hyperelongations of the post-acrosomal region of the head.[10] Therefore, it seems reasonable to characterize the pathomorphology of spermatozoa and its degrees, focusing on the major categories (acrosome, postacrosomal region, midpiece and tail), as well. Beyond the low values of morphologically normal spermatozoa, it should be possible to inform both the gynaecological partner and the patient whether there is a severe or only a minor disturbance of sperm morphology.[9,11] Only focusing on the low values of morphologically normal spermatozoa, by using the strict categorisation, raises the question of its relevance.[7,8] There is a need to have precise criteria for defining ideal morphology,[12,13] but one should also note the continuum of shapes and sizes of sperm cells. Otherwise, much confusion will be caused in the future. Therefore, it has to be emphasized that the new lower reference values do not reflect cut-off values for normal fertility above which male fertility is not disturbed. This was also not the case regarding the former reference values.[14]

The definition of cut-off values to define normal ranges imposes an artificial dichotomy that limits the prognostic value of the information gained from semen analysis. We must accept that fertility is a continuum and gear diagnostic procedures to estimate a couple's chance of conception in a reasonable period of time, such as one year.[15] It has been argued that the new WHO guidelines may be less useful for practising physicians working with infertile couples, because several recent publications show that a sperm concentration of 15 million spermatozoa per ml may be too low to be associated with normal fertility in general, although some will be able to achieve a conception. In this range even the previous WHO cut-off value of 20 million spermatozoa per ml was probably too low to identify a significant group of males who need to be referred to andrological experts. A higher cut-off value of 40 million spermatozoa per ml has been suggested on the basis of a prospective study of first-time pregnancy planners. Other authors have recently proposed that even values as high as 50–60 million spermatozoa per ml should be used as lower cut-off level for full reproductive competence.[16–18]

This concern should not be neglected, because a considerable number of subfertile men may not be referred for andrological work-up by solely taking the new WHO reference values into account.

LABORATORY FINDINGS VERSUS ANDROLOGICAL DIAGNOSIS

Although reference values serve as decision criteria for clinical management, non-specific changes in semen analysis, such as impairment of sperm motility or a reduction in sperm count, do not unravel the underlying causes of male sub- or infertility.[19] By means of the conventional semen analysis, patients are classified into descriptive groups such as oligozoospermic, asthenozoospermic, etc. A single condition such as oligozoospermia may have been caused by a plethora of different aetiologies. Moreover, effective treatment needs the establishment of a nosologically sound diagnosis and this cannot be based on symptoms reflected by semen evaluation.[2] Relying exclusively on the latter has lead to conflicting results concerning the predictive value of semen analysis, e.g., the percentage of spermatozoa with normal morphology for *in vitro* fertilisation success. There is an ongoing debate whether sperm concentration, motility or morphology is most important for assessing male fertility or, more generally, whether semen evaluation is of any use at all.[8,20]

Routine semen analysis provides useful information concerning sperm production by the testes, sperm motility and viability, the patency of the male genital tract, the secretions of the accessory organs, as well as ejaculation and emission. Hence, the information obtained by this procedure is obviously useful for the initial evaluation of the infertile male. However, as far as the diagnosis of sub- or infertility is concerned, semen analysis does not represent a definitive test of male fertility. Needless to say that during andrological work-up reductions in a single semen parameter has only limited progostic value. In a comprehensive approach, the results of physical examination as well as the cumulative importance of various laboratory findings including hormone analysis, etc., have to be considered. In line with this perspective, the investigation and treatment should not focus on unspecific symptoms but on any potential underlying disorder, thus reducing or even eliminating the contribution of the male partner to couple subfertility.[7]

SPERM FUNCTION TESTS

The results obtained by basic semen analysis alone do not allow differentiation between fertile and infertile populations, unless the man is azoospermic. Therefore, sperm function tests should be included, which provide important insights into the abnormalities of spermatozoa affecting fertilisation.[15,21] Notably, the results of sperm function tests give information about the potential of the spermatozoon to undergo the different processes that are required to achieve fertilisation of an oocyte. In detail, spermatozoa must be capable of penetrating and passing through the cervical mucus, and through the uterus to the ampullae of the oviducts; furthermore, they must undergo capacitation and the acrosome reaction, bind to the zona pellucida and penetrate through it, until finally penetration of the oocyte occurs. Once the spermatozoon enters the egg, it must then undergo nuclear decondensation to deliver the appropriate haploid chromosomal complement, followed by additional, but poorly understood, events required for fertilisation and early embryo development. Defects in any of these complex events can result in male infertility. Over the last two decades, tests have been developed to identify abnormalities in these processes.[21] The new WHO manual has incorporated a series of standardized protocols for performing such tests, including assessments of cervical mucus penetration, zona-binding assays, techniques for measuring the acrosome reaction, the hamster oocyte penetration test and computer-assisted sperm analysis of sperm movement.[1]

It should be recognized that in the era of intracytoplasmic sperm injection as the major therapeutic option for severe male infertility, detailed assessments of sperm function may be largely irrelevant. Nevertheless, many studies have shown that even for this form of therapy, analysis of sperm chromatin integrity is important because DNA damage in human spermatozoa has been associated with adverse clinical outcomes, including poor fertilisation rates, impaired embryonic development, an increased risk of miscarriage and morbidity in the offspring, including childhood cancer.[2] Although many sperm function tests have been able to be superior to conventional semen analysis as predictors of both natural conception and *in vitro* fertilisation success, they have never achieved sufficient advances in discrimination to be incorporated into the andrological work-up as common practice. Tests should be able to detect underlying pathologies that can be referred to specific treatment. For example, it has been shown that factors such as oxidative stress, or inflammatory processes in the male genital tract in general can affect DNA integrity which can be

determined by comparing red and green fluorescence staining of acridine orange of the sperm nucleus by flow cytometry in the sperm chromatin structure assay.[15]

FUTURE DEVELOPMENTS

In addition to sperm function tests, defects in intracellular regulation and the developing field of 'omics' should be explored for more exact information.

By the use of investigative 'omics', molecular defects in the intracellular regulation of spermatozoa of infertile men can be examined and a deeper understanding of the underlying aetiology can be gained. A recent publication has shown a detailed proteomic analysis of human spermatozoa, including a comparison between the protein structure of normal and defective spermatozoa. However, it was pointed out that such comparisons are meaningless unless the functional defects in the spermatozoa have been precisely defined. In addition, the causes or consequences of negative influences such as oxidative stress in the male germ line may also become apparent when lipidomics are applied to the analysis of human sperm quality. Furthermore, glycomic analyses might help to resolve the causes of defective sperm–zona interaction. Modern advances in diagnostic genomics might also help identify genotypes associated with specific defects in semen quality.[2,22] Such biomarkers could potentially be related not only to the clinical manifestation of male infertility, but also to patients' response to treatment. The variability in patient characteristics of infertility necessitates proven, personalized diagnostic approaches to optimized efficacy and safety outcomes. The further development of personalized management strategies, based on individual patient characteristics, may provide real progress towards individually tailored fertility treatment.[23]

CONCLUSIONS

Semen analysis is an imperfect tool but remains the cornerstone of the investigation of male infertility; therefore, it must be performed to a consistently high standard.[14] From this point of view, the concepts of the new WHO manual are highly appreciated. The conventional semen parameters allow only a rough estimation of the fertility status. As very few treatments other than assisted conception (intrauterine insemination, *in vitro* fertilisation and intracytoplasmic sperm injection) are applied to overcome disturbed male fertility, clear characterisation of the fertility problem, including provision of a prognosis, is the most important feature in the investigation of the male factor. Therefore, the results of semen analysis should help to decide, whether a couple requires immediate treatment or should be encouraged to keep trying, and, if treatment is necessary, which assisted reproduction techniques would be appropriate.[15] In this context, the usefulness of the new lower reference limits might be questioned, because fertility is a continuum and the diagnostic procedures should help to estimate a couple's chance of conception in a reasonable period of time, such as one year. For this purpose, both male and female factors and a complex interaction between both partners have to be taken into account. For example, a patient with semen quality close to the lower reference limits can impregnate a young wife, whereas with increasing female age he will fail to do so. At this point in the discussion, it has to be emphasized that the new lower reference limits are—for the first time—evidence based. On the other hand, the published data indicate that the average values of basic semen parameters such as sperm concentration and total sperm number among a population of fertile males are much higher.[3] The new WHO manual is a laboratory manual and not an andrological textbook. Thus, it provides invaluable help to perform semen analysis at high quality-controlled standards. The conclusions drawn from the results of the semen analysis and the appropriate interpretation remain a matter for the clinician. In the future, for the assessment of male fertility, sperm parameters alone will probably no longer be given such priority; therefore, the new reference values should not be overestimated. A further question will be how health insurance authorities will handle these values regarding payment of infertility treatments. Although it has been pointed out several times, it must be stressed once more that sperm parameters are important but do not allow differentiation over a wide range of values between normal and disturbed fertility. Therefore, subfertile males may not be identified and referred to andrological work-up if one considers the lower reference values generally as normal values.[16] Such a view might even increase the number of 'idiopathic' infertility and, consequently, the number of assisted reproduction technique treatments. For prevention of such a consequence, it should be emphasized that fertility is a continuous process, where clear cut-off values may not be helpful. Alternatively, the inclusion of a 'grey' zone between infertile and normal range could be discussed. Concerning sperm concentration an area between 15 and 40 million spermatozoa per ml has been suggested.[16]

In view of the complex matter of infertility, management aiming at more individually tailored treatments, based on additional examinations such as sperm function tests or the promising new developments in the field of biomarkers may represent the most likely future in andrology and reproductive medicine.[23]

COMPETING FINANCIAL INTERESTS

The author declares no competing financial interests.

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