Editorial
Male reproductive health and infertility

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Editorial Commentary on Draft of World Health Organization Sixth Edition Laboratory Manual for the Examination and Processing of Human Semen

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INTRODUCTION

Semen examination is the cornerstone of the evaluation of male fertility potential. Despite its apparent simplicity, it is a complex series of assessments with highly variable results that are subject to interpretation. The semen analysis is used to gauge reproductive potential and guide the clinician in management of the infertile couple. Over the past 40 years, the World Health Organization (WHO) Infertility Task Force has attempted to standardize the methodology of semen examination so as to bring uniformity and relevance to the test. The 1st edition of the "Laboratory Manual for the Examination and Processing of Human Semen" was published in 1980. Since then, four more editions of the WHO manual have been published, each attempting to reflect global male population demographics, to address limitations from previous versions, and to incorporate technological and scientific evolution in the field of reproduction. Currently, the 5th edition of the WHO manual, published in 2010, is implemented worldwide, and is considered one of the most used, yet contested documents in the field of male infertility [1].

In March 2021, the WHO released a preliminary draft of the 6th edition of its manual for public review and suggestions (https://www.who.int/publications/i/item/9789241547789). Essentially, the new manual comprises three parts: semen examination; sperm preparation and cryopreservation; and quality control and assurance. The procedures for semen examination include basic (routine) examinations, extended examinations (which may be used by laboratories or clini-

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cians in certain situations), and advanced tests (which are not currently recommended for routine use and are primarily for research purposes).

The 6th edition has presented the basic examination in a step-by-step and temporal manner, so that it can be reproduced precisely in any laboratory with the required equipment and expertise. The sections on extended and advanced examinations have been completely revamped in concordance with current clinical practice, with many older tests being abandoned and new tests being adopted.

The purpose of this mini review and commentary is to familiarize fertility specialists with the principal changes proposed in the anticipated 6th edition of the WHO manual for human semen analysis. The advantages, limitations and clinical implications of these changes are discussed.

1. Use of decision limits to identify abnormal ejaculates

The most important change proposed in the 6th edition is the adoption of decision limits to differentiate normal from abnormal ejaculates. The editors of the 6th edition acknowledge that the reference ranges described in the 5th edition should be abandoned as they are of limited value in differentiating fertile from infertile men.

The 5th edition WHO manual utilized a population of 1,800 fertile men to obtain the reference distributions for semen parameters [1]. The lower 5th percentile was used to define the reference values for normal semen parameters. While the 5th percentile is commonly utilized as a statistical approach to determine cut-off norms in medical tests, this resulted in much controversy when applied to male fertility [2]. It has been argued that the 5th percentile is not applicable to assign normality in this case and proves unable to discriminate between fertile and infertile patients [3]. Several studies reported a shift of fertility status from abnormal to normal in 15% to 44% of patients by just using the 5th edition norms instead of the 4th edition [4-7]. It was therefore proposed that WHO reference ranges did not adequately reflect fertility dynamics of the male partner, with several investigators and clinicians believing that normal values were above the lower 5th percentile. With these limitations in mind, the editors of the 6th edition suggested using different reference limits because the previous reference ranges fail to differentiate fertile from infertile men [8]. Decision limits were introduced by Guzick et al [9], who proposed a two-level reference range narrative by defining an intermediate group of values. According to their data the sub-fertile population demonstrated a sperm concentration below 13.5×10^6/mL, sperm motility less than 32% and typical forms below 9%. Yet, the normal fertile subgroup had a sperm concentration greater than 48×10^6/mL, sperm motility over 63%, and normal morphology above 12%. Values between these two levels were categorized as intermediate fertility [9].

The 5th edition has been criticized for neglecting the female factor as an important confounder [3] and for both over- and under-representation of various regions of the world [4]. The editors of the 6th edition have acknowledged these limitations (Appendix 8.1 of the manual) and stipulated that semen examination cannot strictly differentiate between pathological and normal samples. Moreover, they recognize that using the lower 5th percentile is not the correct approach to identify normal or abnormal semen samples, and that semen analysis alone cannot predict fertility as this depends on multiple variables, particularly, female factors.

Hence, the "normal" reference values of the 5th edition have been replaced by "decision limits" in the 6th edition. These are classified as "normal", "borderline", and "pathological". A "normal" concentration is $\geq20\times10^6$/mL, "borderline" lies between 10 to 20×10^6/mL, and "pathological" is $<10\times10^6$/mL. For motility "normal" is defined as ≥50% progressively motile sperm, "borderline" is 35% to 49% progressively motile, while a "pathological" sample is defined as <35% progressively motile sperm. Morphology has been categorized as "normal" when typical forms are ≥14%, "borderline" is between 4% and 13% and "pathological" morphology is below 4%. Additionally, the 6th edition states that sperm antibodies are not the sole cause of agglutination as this may be caused by different pathologies. Therefore, sperm antibody binding below 50% is considered "normal"; it is "borderline" between 50% to 79%, and "pathological" when ≥80%.

The introduction of the "decision limits" concept is an attempt to emphasize that the purpose of the semen examination is not to label a man as fertile or infertile, but rather to decide next steps in terms of further evaluation and treatment. The current limits are still arbitrary and future studies with clinical outcomes in

the various groups will help refine these limits. The creation of a "borderline" group will have significant clinical implications as many men whose sample would previously have been labeled as normal using the 5th edition criteria, will now be classified as "borderline" and be eligible for therapeutic interventions. Clinicians can still offer hope for natural pregnancy in these cases before opting to pursue ART. The impact of this classification shift will likely be significant in clinical practice. If we now apply the new criteria and deem men with parameters below the new "normal" threshold ("borderline"+"pathological") as infertile, we will suddenly increase the number of infertile men in our practices.

Interestingly, the upcoming manual presents revised reference values based on combined data of fertile men (with time to pregnancy less than 12 months) from the 2010 manual (5th edition) and by including released data from studies published until 2020. The 6th edition has included data from 5 additional studies (with 1,789 more male subjects) to those used in the 5th edition. The five new studies have incorporated data from two regions in Europe, one from Africa and two from Asia, although notably 1,200 out of 1,789 participants originate from China alone, therefore skewing the reference values towards normality of specific geolocations that do not necessarily apply in different populations.

2. Change in motility grading system

A criticism of the 5th edition was the decision to eliminate the reporting of rapidly progressive motility, and the editors of the 6th edition have now reverted back to a four-category classification as follows:

Rapidly progressive: ≥25 μm/s, or at least half tail length per second.

Slow progressive: 5 to <25 μm/s, or at least one head length to less than half tail length/sec.

Non progressive: <5 μm/s, or less than one head length.

Immotile: no tail movement.

A return to the earlier classification of the 4th edition of WHO manual for sperm motility [10] is a welcome improvement as it allows a better characterization of motility and may provide additional prognostic information.

3. Sperm DNA fragmentation testing

The editors of the 6th edition should be commended for introducing tests of sperm DNA fragmentation (SDF) in the manual. The techniques of the various SDF tests (TUNEL and Comet assays, Sperm Chromatin Dispersion test, and Acridine Orange flow cytometry) have been described and notes on the clinical interpretation of these assays have been included. The editors have also provided suggested thresholds for clinical decision-making. They state that "the evaluation of SDF could constitute an important addition in the work-up of male infertility". However, the editors of 6th edition suggest that the Comet assay should not be used in clinical practice because of an important degree of inter-laboratory variation.

The application of sperm DNA tests in clinical practice remains controversial [11]. This controversy stems in part from the modest predictive value of SDF tests in reproduction and the multitude of available tests with variable thresholds and inter-lab variability.

The editors of the manual have not addressed the clinical value of these assays and have provided no guidance as to the indication for testing. Moreover, they have not addressed the limitations of these assays and have provided conflicting information on test thresholds (2 thresholds were listed for each of these assays). A revision will be needed to better guide the andrologist/clinician regarding the utility of these important assays [12].

4. Reactive oxygen species (ROS) testing

The 6th edition recognizes the increasing clinical relevance of seminal oxidative stress by dedicating a section to the methods assigned for ROS testing. Possibly due to the limited availability of such testing, this assessment has been incorporated under "Advanced Examination" section, suggesting that this should still be considered a research tool. However, given the large number of recent papers on the reproductive impact of oxidative stress and the clinical utility of ROS testing, along with the description of Male Oxidative Stress Infertility (MOSI) as a clinical entity [13], one may consider ROS testing as part of the extended semen examination in clinical practice.

CONCLUSIONS

The 6th edition is a step forward in our understanding of the complex subject of male infertility through evaluation of the human ejaculate. Several objections

to the 5th edition have been addressed in the 6th edition, though areas of controversy remain. For the clinician, the most notable change is the introduction of "decision limits" rather than reference ranges, and the endorsement of SDF as an extended seminal test that can be ordered in certain clinical situations. For the laboratory personnel, the manual has been streamlined to facilitate a step-by-step examination with several old tests being abandoned, while several new tests have been introduced. The long-awaited 6th edition will be read and followed with great interest by everyone involved in the care of male reproductive health.

Conflict of Interest

The authors have nothing to disclose.

Author Contribution

Conceptualization: AA, HK. Writing – original draft: All the authors. Writing – review & editing: All the authors.

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