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INVITED OPINION

Semen Analysis

The new 6th edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen: is it a step toward better standard operating procedure?

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Semen analysis (SA) remains the cornerstone of male infertility evaluation and should ideally be performed by an accredited andrology laboratory or *in vitro* fertilization (IVF) clinics based on the standards defined by the World Health Organization (WHO) for the Examination and Processing of Human Semen and in accordance with the International Organization for Standardization's Basic Semen Examination on Specification and Test Methods.[1,2] The first edition of the WHO Laboratory Manual on Semen Analysis Processing was published in 1980, and since then, four modified and updated versions have been released to improve SA standard methods and incorporate new technologies to assess extended and advanced sperm parameters.[3] Early this year, an anticipated 6th edition draft has been sent out online for public reviewing and feedback before its forthcoming official publication.[4] The new 6th edition of the WHO manual aims to provide not only an update of the current methods and thresholds but also an insight in recent developments on semen examination, sperm preparation and cryopreservation, and quality control and assurance.

One of the most notable changes in the new 6th edition is the change in the definition of "abnormal ejaculates", While the reference ranges and limits on various sperm parameters have been revised to remove the existing dichotomy between "fertile" and "infertile" men and these values should be viewed as continua of normality, borderline or pathological semen parameters. Epidemiological data have repeatedly demonstrated that male fertility can change over the years[1,5] and fertility may vary depending on the regions of the world. The incorporation of a larger dataset of semen samples from across developing countries in Africa and Asia reduces potential bias related to sperm parameter data derived from Western countries and provides a more global representation of the sperm parameters. Various sperm parameters have been revised accordingly to afford more generalization and highlight the male fertility as continuum of semen parameters in the real life[6] and the fact that SA alone cannot predict fertility, pregnancy or its associated clinical outcomes taking the multifactorial nature of fertilization process into account. In the 6th edition WHO manual, the evaluation of sperm count has been revised to report lower concentrations ($<2 \times 10^6$ ml^{-1}), noting that errors associated with counting a small number of spermatozoa may be high. On the other hand, the categorization of sperm motility has reverted to fast progressive motile, slow progressive motile, nonprogressive motile and immotile (Grade a, b, c or d) based on older studies, despite rapid and slow progressive motility is well defined and can be taught effectively. In this new edition, more and better-quality micrographs of spermatozoa from unprocessed semen samples considered normal, borderline or abnormal are included, accompanied by explanations of why each spermatozoon has been classified the way it has, with description on morphological anomalies of the head, intermediate piece, and tail.

Significant advances in scientific knowledge and state-of-art technologies have improved the various aspects of functional assessment of male,[7] while advanced sperm selection methodologies have been updated or introduced, targeting especially the populations with poor reproductive prognosis in terms of natural conception and assisted reproductive technology (ART).[8] Dedicated chapters on sperm preparation extend beyond the ejaculate to include spermatozoa obtained from the testis and epididymis, while the previous reference to human cervical

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mucus tests has been removed from the new edition. On the other hand, the importance of genetic evaluation has been expanded in the 6th edition. The roles of sperm DNA fragmentation, seminal oxidative stress, and reactive oxygen species have been highlighted and the diverse forms of sperm chromosomal abnormalities and gene mutations are also emphasized. Already established and new techniques of sperm DNA fragmentation assessment such as sperm chromatin structure assay, sperm chromatin dispersion test, acridine orange flow cytometry, and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) assay have been added to assist sperm function evaluation, while the use of single cell gel electrophoresis (SCGE, also known as COMET) assay requires high level of expertise for interpretation given multiple methodological steps and inter-laboratory variation. Genetic testing for male infertility in this era is anticipated to extend beyond routine karyotype and azoospermia factor (AZF) Y-chromosome microdeletions analysis. While many genetic causes of male infertility remain unknown, it is recognized that the rates of genetic abnormalities are significantly higher among men, especially those requiring ART.[9] Epigenetic profiling of spermatozoa is an exciting and rapidly evolving field that will require the formulation of a consensus guideline by the WHO, given its potentially bioethical and medicolegal implications.

Although there have been significant advances in the understanding of male reproductive health, the translation from basic science to clinical practice on the management of male infertility is still limited. Various methodologies on sperm preparation techniques for clinical use or specialized sperm function assays and sperm cryopreservation need to be standardized. Contemporary and evidence-based reference ranges and reference limits are pivotal to male fertility regulation and clinical interventions. While the WHO 6th edition has addressed some of these limitations (or objections) from the last editions, many areas of controversy remain and will require further scientific validation.[10] The new WHO manual offers a framework of standardized and efficient semen processing protocols for laboratory scientists and allows clinicians the flexibility to interpret the reference value for various sperm parameters in a more dynamic manner. The introduction of new tests invariably provides greater opportunities for better patient counseling and therapeutic interventions. Nonetheless, it is important to understand that this WHO manual functions both as an aid and a foundation for human semen examination and processing but is not intended to replace actual clinical management of male infertility. Men with fertility risk factors or abnormal semen parameters should be referred to a male reproductive specialist for a full clinical evaluation, appropriate counseling, and evidence-based therapeutic interventions.

COMPETING INTERESTS

All authors declare no competing interests.

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