SPERM MOTILITY EVALUATION ACCORDING TO WHO VI EDITION: MOVING FORWARD TURNING BACK?

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The WHO Laboratory Manual

"WHO Laboratory Manual for the Examination and Processing of Human Semen" was first published in 1980 and was updated four times in the last thirty years (1987, 1992, 1999, 2010) [1]. Considerable efforts were made to translate the manual in numerous languages (in Italy it was edited by the Italian Society of Andrology and Sexual Medicine – SIAMS), allowing it to mark a level of standardization necessary for human semen analysis to improve both the overall quality and the comparability of results. This year the sixth edition of the WHO Manual is being published [2]. The 2021 version provides implementation of new topics of interest and several updates in the methodological descriptions. Briefly, this new edition covers semen analysis, spermatozoa preparation and cryopreservation and quality control. Procedures related to semen analysis include basic examinations (routine procedures for the evaluation of sperm parameters), second level analyses (extended semen examinations required in specific conditions), research analyses (advanced examinations, not yet considered for laboratory routine use).

Compared to previous versions, this new edition presents several changes in semen analyses procedures including simplifications of dilutions, a thorough descriptions of methods to manage very low sperm numbers. The diagnostic importance of total sperm number was confirmed since it accurately mirrors testicular production capability. On the other hand, its estimation requires a precise evaluation of semen volume and the weighting of the sample inside the collection container was confirmed. Moreover, the section dedicated to sperm morphology has been enriched with a number of high-quality pictures with morphological interpretation of presented cells. Regarding the reference values, those presented in the WHO 2010 have been reconfirmed, albeit with few minor differences **(Table I)**.

The sperm motility dilemma

A major change has involved sperm motility evaluation, returning to the motility classification of the 1999 edition of the manual [3]. The WHO experts board recommends distinguishing between rapidly and slowly progressive motility because the presence (or absence) of rapidly progressive spermatozoa is considered to be clinically relevant.

In particular, the WHO 2021 edition describes four categories:

- *Rapidly progressive motility:* it is a form of motility in which spermatozoa move actively in a linear or wide circle pattern. The distance from the starting to the ending point of the movement is covered with a speed of at least ≥25 μm/s (equivalent to approximately half of tail's length).

- *Slowly progressive motility:* this form of motility is similar to the previous but the speed of the movement is lower, between 5 to 25 μm/s (from approximately the length of the spermatozoa head to less than half of its tail's length)
- *Non-progressive motility:* this category includes all movements where no spatial progression can be detected. It could be the case of small circular movements where flagellar pulse moves the head less than 5 μm/s.
- *Immotile:* no sperm movement is present

The evaluation of spermatozoa velocity was eliminated in the previous edition of the manual since "*it is difficult for technicians to define linear progression so precisely without making mistakes*". As a matter of fact, the VI edition specifies that the recommended categories should have "*approximate speed limits*". Hence, it is legitimate to ask what sense does it make to indicate this parameter given that the categorization of the speed limits is an approximation prone to errors and misclassification. The evaluation of speed involves the analysis of two factors: space and time. Undoubtedly, an ocular grid, as the manual suggests, makes it easier to count motile and immotile spermatozoa and allows us to evaluate the displacement of the spermatozoon from a starting point to the final one. While we can easily account the space factor, what is missing is the evaluation of the second factor, the time of movement through a cell of the grid. In our opinion, a qualitative description of movement is more important for the purposes of assessing the individual's fertility, identifying linear and non-linear movements in progressively motile spermatozoa. In a previous publication, using Computer Aided Semen Analysis (CASA) [2], we could detect that these correspond to different speed and linearity classes [4]. CASA kinematic analysis software, through the use of several algorithms, provided a reliable and reproducible evaluation of several standardized variables such as curvilinear velocity (VCL μ/s), the velocity along the curvilinear path (μm/s), and linearity of the curvilinear path (LIN %) which were significantly different between linear and non-linear progressively motile semen samples (**Table II**). Thiswould make the analysis equally informative. Furthermore, why the indicated speed threshold has been set to 25 μm/s? The references indicated date back to the '80s, where spermatozoa velocity was evaluated with time-exposure photomicrography [5-7]. Recent technologies have allowed development of devices used in Computer-Aided Sperm Analysis with higher sensitivities to sperm kinetics, suggesting the possibility of a re-evaluation of this threshold. In fact, a following paper showed that the "poor fertilization outcome" group has higher kinetic parameters than indicated [8].

Anyone involved in the field of seminology knows how difficult it is to have common criteria for analysis. In fact, the evaluation of sperm motility and morphology mainly depends on the seminologist's experience. Adding the evaluation of speed would re-introduce an information that is

certainly less precise, increasing variability, possibility of error and, thus, reducing standardization among laboratories.

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Disclosures

The Authors declare no conflict of interest.

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