

ORIGINAL RESEARCH

The impact of the new 2021 reference limits of the World Health Organization criteria for semen analyses

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Abstract

The World Health Organization (WHO) published the first laboratory manual in the 1980s and this has been updated several times since. The sixth version of this manual, the latest to be issued, was published in 2021. In the sixth version, the distribution of semen parameters was given instead of the reference level and some parameter limits were decreased or increased. This study aimed to evaluate the impact of the new 2021 WHO semen analysis reference values by reclassifying previous semen analysis parameters. All semen analyses performed between 01 July 2021 and 01 September 2023 were collected retrospectively. Semen volume, sperm concentration ($\times 10^6/\text{mL}$), total sperm motility (%), and progressive sperm motility (%) were evaluated according to 2010 and 2021 WHO semen analysis reference values. Then, the percentile changes of the normal (at or above the reference limits) results of each parameter and overall semen parameters were evaluated. 2535 spermograms were included in the study. According to the 2010 WHO criteria, 42.99% of semen specimens were at or above the reference limits for all parameters whereas, samples with all parameter values at or above the 2021 WHO reference limits were 43.55%. It was found that the number of each semen parameter that were recategorized due to the new limits for volume, sperm concentration, progressive, and total motility were 0.9%, 1.1%, 3.2% and 2.8%, respectively. 29 semen results (1.1%) that had all parameter values at or above the new reference limits were reclassified as a normal spermogram based on the new 2021 criteria. The 2021 WHO semen reference limits do not influence the percentile of men who require infertility treatment.

Keywords

Semen analysis; WHO criteria; Male infertility

1. Introduction

The World Health Organization (WHO) published the first laboratory manual in the 1980s and this has been updated several times since. The main aim of the manual is to standardize semen analysis techniques used in andrology and research laboratories. The sixth version of this manual, the latest to be issued, was published in 2021 [1]. While normal [2, 3] and reference [4] values for the semen parameters are detailed in all previous versions of the manual, the lower fifth centile, which was used as the lower reference range obtained from presumed fertile men, was first included in the fifth version of the manual [5]. In the sixth version, the distribution of semen parameters was given instead of the reference level and some parameter limits were decreased or increased.

In this study, we aim to detect the change in the number of semen analyses that were classified as normal or abnormal according to the new WHO 2021 criteria.

2. Material and methods

After the ethical approval received from the local ethical committee; all semen analyses of men with unknown fertility status performed at Eskisehir City Hospital Andrology Laboratory, Eskisehir, Türkiye, between 01 July 2021 and 01 September 2023 were collected retrospectively. Semen specimens were collected by masturbation into clean collection cups and allowed to liquefy, and then the following variables were manually determined in accordance with the methods outlined in the WHO sixth edition. Results with azoospermia were excluded from the study. Semen volume, sperm concentration ($\times 10^6/\text{mL}$), total sperm motility (%), and progressive sperm motility (%) were evaluated. These parameters were reviewed individually in line with the 2010 WHO sperm reference values. The 2010 WHO sperm reference values for these parameters were as follows: a semen volume of 1.5 mL, sperm concentration of 15 million/mL, sperm total motility of 40%, and sperm progressive motility of 32%. All spermograms were then re-evaluated according to the 2021 WHO semen

parameters values. The 2021 WHO sperm reference values for these parameters are a semen volume of 1.4 mL, sperm concentration of 16 million/mL, sperm total motility of 42%, and sperm progressive motility of 30%. All semen parameters evaluated in this study were categorized as normal if the values were at or above the reference values of the 2010 or 2021 criteria. After this categorization, the percentile changes of the normal (at or above the reference limits) results of each parameter and overall semen parameters were evaluated.

3. Results

From 2692 semen analyses, 157 results with azoospermia were excluded from the study. The remaining 2535 spermograms were included in the study. The four semen parameters whose reference limits were changed in the WHO 2021 criteria were selected. Firstly, all these parameters were evaluated according to the WHO 2010 criteria. According to these criteria, the values at or above the reference limits for the volume, sperm concentration, progressive, and total motility were 86.55%, 81.74%, 68.09% and 80.51%, respectively (Table 1). The number of semens whose results of all parameter values were at or above the reference limits was 42.99%. The four parameters were then evaluated based on the WHO 2021 criteria. It was found that the values at or above the reference limits for the volume, sperm concentration, progressive, and total motility were 87.5%, 80.6%, 71.36% and 77.71%, respectively (Table 1). 43.55% of the samples had all parameter values at or above the new limits. It was found that the number of each semen parameter that were recategorized due to the new limits for volume, sperm concentration, progressive, and total motility were 0.9%, 1.1%, 3.2% and 2.8% respectively. 29 semen results (1.1%) that had all parameter values at or above the new reference limits were reclassified as a normal spermogram based on the new 2021 criteria.

4. Discussion

Semen analysis has been used for many years as a tool for assessing a man's fertility. If at least one parameter is below the reference limits, this man should be informed that WHO recommends that only 5% of men below these thresholds are in couples who had time to pregnancy <12 months and therefore they have no fertility problem. But this man could be mistakenly defined as an infertile man in andrological practice. Infertility is defined as the inability of a couple to conceive after one year of unprotected, frequent sexual intercourse. As seen, this definition of "infertility" is not related to the level of

semen parameters. However, the limits of semen parameters are still widely used in fertility clinics to classify the male as fertile or infertile. In addition, in some reports, semen quality has been defined as being at or above the reference limits of the WHO criteria [6–8], however, conventional sperm parameters could not detect the sperm quality. With improved technology, analysis of the bio-functional sperm parameters should be performed alongside conventional sperm parameters to detect semen quality.

The recent trend in andrology is that there has been a decline in conventional sperm parameters, however, there has been some disagreement on this topic [9]. While some reports found that there are no significant downward trends in sperm parameters [6, 8, 10]; other reports declared that significant declines in sperm counts have been detected [11–14]. The main reason for this is that there have been certainly methodological differences in the reports. The populations used in the studies have been selected non-randomly or from only a few geographical areas. It should be remembered that the sperm results of men who seek fertility treatment or who are sperm donors or living in different geographical areas do not give sufficient information about the trends of global sperm parameters. With the reports that found declining trends in the sperm parameters, the WHO 2021 manual launched new reference limits for the semen parameters [1]. In this new manual, the reference limits of the semen volume and progressive sperm motility were changed from 1.5 mL to 1.4 mL and 32% to 30%. As a result, popular media began to announce the uncontrollable news about the global trend in declining sperm parameters [15, 16]. The important point missed, however, was that the WHO renewed the reference limits due to the use of new methodology not because of decreasing sperm counts [17]. In the 6th edition, the reference limits of the 5th version were abandoned and the new 5th percentile of the distribution of semen variables was announced. The results of semen tests performed at validated laboratories in line with the fifth edition manual were collected from more fertile men from more countries across five continents [18]. With the improved validated methodology in the 6th version of the manual, while the new reference distribution of the semen volume and progressive sperm motility was lower than the reference limits of 5th version, the new reference distribution of the sperm concentration and total sperm motility were higher than the reference limits of 5th version [1]. As such, if there was a global declining trend in the sperm parameters, we would expect a decrease in all values. Actually, the 2010 WHO criteria reference limits were lower than the previous manual

TABLE 1. Evaluation of spermogram data according to 2010 and 2021 criteria.

Sperm parameter	At or above the reference limit of 2010 criteria	At or above the reference limit of 2021 criteria	Number of recategorized spermograms
Volume (mL)	2194 (86.55%)	2218 (87.5%)	24 (0.9%)
Concentration (million/mL)	2072 (81.74%)	2043 (80.6%)	29 (1.1%)
Progressive motility (%)	1726 (68.09%)	1809 (71.36%)	83 (3.2%)
Total motility (%)	2041 (80.51%)	1970 (77.71%)	71 (2.8%)
Normal	1380 (42.99%)	1409 (43.55%)	29 (1.1%)

reference limits due to the change of the methodology in the manual. Murray *et al.* [19] and Baker *et al.* [20] reported two studies separately after the 5th WHO manual to evaluate the effect of this decrease on the classification of semen results. They evaluated all spermograms according to the fourth and fifth WHO reference limits. 15.1% [19] and 17.7% [20] results were re-classified as at or above the 2010 WHO criteria respectively. They both concluded that fewer men would need infertility treatment with the 2010 WHO criteria.

In this study, we aimed to detect the effect of the WHO 2021 criteria on the classification of sperm parameters. All semen samples were evaluated in line with both the WHO 2010 and 2021 criteria. We found that while more men had at or above values for semen volume and progressive sperm motility; more men had below values for sperm concentration and total sperm motility as expected. When we analyzed the semen results overall as normal or abnormal according to the four sperm parameters affected by the change in the WHO criteria, only 1.1% were re-classified as normal according to the WHO 2021 criteria. With this result, we could not conclude that fewer or more men would need infertility treatment in contrast to the aforementioned studies.

The major limitation of this study is that the number of semen analyses should be larger to better evaluate the effect of the new reference limits. However, we especially chose the study period because we wanted to consider how many men would have been classified as having normal semen values by the new criteria.

5. Conclusions

Health professionals related to infertility treatment should know that the new reference limits were defined as a result of the new manual's methodology and the men should not be classified as fertile or infertile based on only semen analysis. The results of semen analysis should be recognized as a tool for determining fertility capacity of men.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

MEA and CK—designed the research study. ÖÖ and TÇ—performed the research. MS and AA—provided help and advice on searching data and writing manuscript. AA and MEA—analyzed the data. MS and CK—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Permission for this study was obtained from the Non-Interventional Clinical Research Ethics Committee of Eskişehir City Hospital on 27 December 2023 (Decision

number: ESH/GOEK 2023/61). Because the patients remained anonymous and the clinical data were deidentified, the requirement for informed consent was waived.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. Coşkun Kaya is serving as one of the Editorial Board members of this journal. We declare that Coşkun Kaya had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to AW.

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