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Interpretation of semen analysis using WHO 1999 and WHO 2010 reference values: Abnormal becoming normal

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Summary

Reference values of WHO 1999 manual were used for the interpretation of semen analysis until 2010 when new reference values were introduced which have lower cut-off compared to WHO 1999. Therefore, several men who previously were diagnosed abnormal based on their semen analysis have now become normal using new reference values. This study was conducted on semen analyses of 661 men from Middle East region and Pakistan. All semen analyses were reviewed using WHO 1999 and WHO 2010 criteria. Results showed that based on new criteria, 19% of the population changed classification from abnormal to normal when all normal semen parameters were considered. When at least one or more abnormal semen parameters were considered, of the total 661, 44% (288) of the population changed its classification from abnormal to normal with shift from WHO 1999 to 2010 criteria. These findings show that using new cut-off values, many more men are considered normal, but using old criteria (WHO 1999), the same men would be classified as abnormal. This warrants further discussion over the investigations and management plans for patients whose semen analyses fall below WHO 1999 but above WHO 2010 cut-offs.

1 | Introduction

Semen analysis is the primary test used in the evaluation of a couple's fertility status. The test is cost-effective and easy to perform. At the same time, it provides essential quantitative information of semen characteristics. The results of semen analysis are widely used to consider a male's fertility as normal or abnormal. Therefore, most practitioners refer the male partner to an infertility clinic for evaluation and possible treatment merely looking into the semen analysis (Murray et al., 2012). In the last decade, the decision to consider a semen analysis report as normal or abnormal was based on the criteria set by the World Health Organization (WHO) using the reference values reported in the 4th edition of the manual (WHO, 1999) until the 5th edition was introduced in 2010 (WHO, 2010). The significance of this manual is that it provides universal guidelines to help the practitioner in making decisions to evaluate a semen analysis as normal or abnormal. This manual also provides step-by-step procedure how to perform a routine semen analysis along with several other functional tests. However, the debate over the significance of the reference values which distinguish the fertile man from the infertile without reinforcing the importance of the clinical history of the patient is of great concern (Cooper et al., 2010; De Jonge, 2012). The reason for redefining the cut-off

values emerged due to lack of consensus over the suitability of the reference values set in the 4th edition of the WHO manual. Certain fertility centres considered the values set for sperm concentration, motility and morphology too high, whereas others believed that they were too low as reviewed and commented by the authors of WHO 2010 manual (Cooper et al., 2010).

The argument that the cut-off values are too high suggests that many fertile men would possibly be considered as subfertile or subnormal with regard to sperm concentration, motility and morphology results (Barratt, Dunphy, Thomas, & Cooke, 1988; Barratt, Naeeni, Clements, & Cooke, 1995; Chia, Tay, & Lim, 1998; Gao et al., 2007, 2008; Nallella, Sharma, Aziz, & Agarwal, 2006; Pasqualotto et al., 2006). Another major concern regarding such men who were considered infertile due to these high reference values was that these men would undergo unnecessary and expensive infertility examinations and treatments with assisted reproductive technologies (Cooper et al., 2010; Lemcke, Behre, & Nieschlag, 1997). On the other hand, the argument that these values are too low suggests that the pregnancy rate is directly proportionate in case the sperm concentration is between 40 and 50×10^6 sperm/ml (Bonde et al., 1998; Slama et al., 2002) considering that a sperm concentration of less than 20×10^6 sperm/ml would be too low to achieve pregnancy (WHO 1987, 1992, 1999). Further, sperm concentrations higher than the suggested cut-off of 20×10^6 sperm/ml were reported in infertile men (Nallella et al., 2006).

In order to address this controversy of too high or too low cut-off values, new reference values for semen characteristics were introduced in the 5th edition of the manual (WHO, 2010) which are lower compared to the 4th edition (WHO, 1999). Currently, almost all the organisations and practitioners follow the 2010 WHO reference values for semen parameters.

Except for very few studies with contradictory findings, the impact of the shift from the 1999 WHO guidelines to the 2010 guidelines on the patient referrals and the potential bias in counting an infertile man as fertile has not been reviewed yet. No change in the referral pattern was observed when the semen analyses were performed according to 2010 WHO guidelines (Baker, Li, & Sabanegh, 2015). However, they did report that 16% of the study population which was considered abnormal using WHO 1999 criteria became normal when the new criteria were applied. Murray et al., 2012 reported that 15% of the study population would be considered normal based on their semen parameters when shifting from WHO 1999 cut-off values to WHO 2010 reference values which may result in a lesser number of men referred for further infertility evaluation or treatment (Murray et al., 2012). Catanzariti, Cantoro, Lacetera, Muzzonigro, and Polito (2013) also reported that 15.8% of the study population would become normal based on their semen parameters when the new reference values were implemented.

This study is comprised of data from different countries from the Middle East and Pakistan and exhibit a heterogeneous representation of men. The objective was to classify the semen characteristics of 661 men according to WHO 1999 and 2010 reference values to analyse that percentage of the population that may change classification from abnormal to normal using the new 2010 WHO reference values. As the population included in this

study had no representation when the new reference values were defined, this study will add valuable knowledge about the impact of new reference values in the diagnostic value of semen analyses and possible changes in the referral pattern for subsequent male infertility assessment and treatment in this population.

2. | Materials and methods

Ethical approval was obtained from the concerned centres. Retrospective analysis of semen characteristics was performed from July 2011 to 2014 at four centres; Riyadh, Alkharj (Saudi Arabia), Cairo (Egypt) and Lahore (Pakistan). Irrespective of the female factor, semen results of men who had a history of more than 1 year of infertility were considered. Azoospermic subjects and men with more than 7 or less than 2 days of sexual abstinence were excluded. All men provided two semen samples within 2–3 weeks' time. The underlying aetiological factor of infertility or abnormal semen analysis was not taken into consideration. The goal of this article was to review the semen analyses of patients which were assessed according to WHO 2010 criteria at different centres during 2011 and 2014. The same semen analyses were interpreted using old criteria (WHO 1999) aiming to see how new reference values can impact the interpretation of a semen analysis as new criteria have lower cut-off values compared to the old. Only those semen analyses were included which were performed by manual method. The CASA results were not available for several patients and were not included. The core parameters on which the analysis were classified include semen volume, total sperm motility, concentration and normal sperm morphology.

3. | Results

After all exclusions, a total of 661 semen analyses from multiple centres were reviewed over a 3-year period. The WHO, 1999 and 2010 values along with per cent decline in these values with a shift to new criteria are given in Table 1. Overall, means ($\pm SD$) of semen volume, sperm concentration, motility and normal morphology of all samples were 3.1 ± 1.5 ml, 47.0 ± 51.3 million/ml, $40 \pm 20\%$ and $11 \pm 17\%$ respectively. A comparative classification of patient's semen analyses ($n = 661$) as normal or abnormal based on WHO 1999 and 2010 criteria is given in Table 2. This comparison shows that 4% of the subjects qualified as normal according to the WHO 1999 criteria, whereas 23% qualified as normal when the WHO 2010 reference values were applied. This indicates that when the overall semen parameters are taken into consideration, there was an increase in the number of subjects regarded as normal by 19%.

Semen analyses of the study population were also categorised as normal or abnormal considering the individual semen characteristics using both criteria. This analysis revealed that of the total 661 men, 8% changed their classification as normal for the semen volume, 7% for sperm concentration, 20% for sperm motility and 31% for normal sperm morphology with the application of 2010 WHO criteria, but were abnormal when WHO 1999 criteria were applied (Table 3).

TABLE 1 Cut-off and reference values and percentage decline in these values with shift from the WHO (1999) to WHO (2010) criteria

Parameter	WHO 1999	WHO 2010	% Decline
Semen volume (ml)	2	1.5	25
Concentration (10 ⁶ /ml)	20	15	25
Motility (%)	50	40	20
Normal Morphology (%)	14	4	71

TABLE 2 Classification of patient's (n = 661) semen analyses as normal or abnormal based on the WHO (1999, 2010) cut-off and reference values respectively

Category	WHO 1999 n (%)	WHO 2010 n (%)	% Shift
Normal	28 (4)	152 (23)	19
Abnormal (1 ≥ parameter)	633 (96)	509 (77)	
Total	661 (100)	661 (100)	

A subclassification based on the sperm parameter abnormalities (i.e., oligozoospermia, asthenozoospermia, teratozoospermia, oligoasthenozoospermia [OA], oligoteratozoospermia [OT], oligoasthenoteratozoospermia [OAT]) is depicted in Table 4. A clear shift in the respective total numbers of patients as well as the percentage of patients belonging to a specific group can be noticed from WHO 1999 to WHO 2010 criteria. Furthermore, the percentages of patients which are considered abnormal according to WHO 1999 but normal with regard to WHO 2010 were categorised. When sperm parameter abnormalities (concentration, motility, normal sperm morphology) were taken into consideration, either as single abnormal parameter or multiple abnormal parameters, of the 661 semen analyses, a total of 288 (44%) were below the fifth percentile of 1999 but above the fifth percentile of 2010 WHO criteria. This demonstrates that using 2010 criteria, 44% of the total semen analyses were regarded as normal, while these patients were abnormal according to 1999 WHO criteria. Of these 288 men, 84% had single sperm parameter abnormality (oligozoospermia, asthenozoospermia or teratozoospermia), 6% had abnormal concentration and motility together (OA), 9% had abnormal concentration and morphology (OT), 34% had abnormal motility and morphology (asthenoteratozoospermia) and 2% had abnormal concentration, motility and morphology (OAT).

4 | Discussion

Cut-off values for semen analysis defined in 4th edition of WHO manual (1999) were used to report semen parameters as normal or abnormal until 2010 when 5th edition of the WHO manual was introduced. The new edition describes the procedures for the routine semen analysis; sperm function tests and revised quality control sections in more detail. Yet, the most important feature of 2010 WHO manual is the inclusion of fertile men with known time taken to pregnancy from different countries which was lacking in the 1999 WHO manual. In order to understand these reference values and to be able to put them into context, it is important to note that these new reference values in the 2010 WHO manual were principally based on Northern European and American studies with 10%

representation from Australia and therefore show an over-representation of certain countries such as those from Northern Europe (Esteves et al., 2012). Other countries Africa, Eastern Europe, Central and South America and Asia were under-represented (Cooper et al., 2010; Esteves et al., 2012).

Previously, few studies have reported the impact of the new reference values on the evaluation of semen analysis and the percentage shift in the result thereof in men who were conceded abnormal according to the 1999 WHO guidelines, but changed their classification to normal when the 2010 WHO reference values were introduced (Baker et al., 2015; Catanzariti et al., 2013; Murray et al., 2012). The present study comprises a population from Middle Eastern and Indo-Pakistan subcontinent regions. Millions of fertile men are living in these countries, and this area has a high population growth rate. Yet, this region did not contribute any data when reference values for the 5th edition of the WHO manual were defined. Thus, the aim of the present study was to analyse the change in the diagnosis and interpretation of semen analysis as normal or abnormal, using the 2010 WHO reference values in comparison to the 1999 WHO cut-off values in this population. We also wanted to know the percentage population which was abnormal according to WHO 1999 guidelines, but changed the classification to normal when WHO 2010 criteria were applied.

Results showed that according to WHO 1999 criteria, 4% (28 of 661) of the patients were normal with respect to all parameters (volume, count, motility and normal sperm morphology). When the same study group was analysed against WHO 2010 reference values considering the same semen parameters, 23% (152/661) of the patients qualified as normal. These numbers show that by using the new criteria, 19% more patients are classified as normal who were abnormal according to the old criteria. On the other hand, when at least one abnormal parameter (volume, concentration, motility or normal sperm morphology) was considered, 44% (288 of 661) of the population fell below the fifth percentile of the 1999 WHO cut-off values but above the fifth percentile of the 2010 WHO reference values. This means that according to WHO 1999, at least one sperm parameter was abnormal in these men, and the semen analyses were declared abnormal. However, when the new reference values were implemented, their abnormal status was assessed as normal. For example, if a semen analysis had a volume of 1.5 ml, a sperm concentration of 18 million/ml, motility of 42% and 7% normal sperm morphology, and then according to WHO 1999, all semen parameters are below the cut-off values and the analysis was regarded as abnormal.

TABLE 3 Classification of patients (n = 661) below and above the WHO (1999) and WHO (2010) cut-off and reference values respectively

Parameter	> WHO 1999 criteria n (%)	> WHO 2010 but < WHO 1999 criteria n (%)	< WHO 2010 n (%)
Semen volume	555 (84)	51 (8)	55 (8)
Sperm concentration	399 (60)	47 (7)	215 (33)
Motility	208 (31)	133 (20)	320 (48)
Normal morphology	143 (22)	206 (31)	312 (47)

TABLE 4 Number and percentage of men with single and multiple abnormalities in standard semen analysis according to WHO 1999 and 2010 criteria

Category	WHO 1999		WHO 2010	
	n	%	n	%
Normal	27	4	152	23
Oligospermia	5	1	38	6
Asthenospermia	91	14	121	18
Teratospermia	145	22	111	17
Oligoasthenospermia	17	3	32	5
Oligoteratospermia	34	5	43	6
Asthenoteratospermia	141	21	60	9
Oligoasthenoteratospermia	201	30	104	16

However, according to the 2010 WHO manual, all these parameters are above the lower reference values, and therefore, the analysis was regarded as normal. Likewise, another semen analysis with a volume of 2 ml, a sperm concentration of 16 million/ml, a motility of 52% and 14% normal sperm morphology was declared oligozoospermic according to WHO 1999 because only the concentration was below the cut-off value. According to WHO 2010, however, the same concentration is above the lower reference value and the analysis was regarded as normal.

Most of the gynaecologist who also treat male infertility and those infertility experts who lack extensive training in male infertility usually ignore the total sperm count number and are merely looking at sperm concentration when reporting the results. However, it should be kept in mind that just sperm concentration should not be considered sufficient in declaring a semen report as normal or abnormal based on sperm number. The total sperm count is one of the key parameter which should also be considered. If a semen sample shows sperm concentration of 13 million/ml and volume of 5 ml; merely looking at concentration it will be regarded as oligospermia. However, based on total sperm count, it qualifies as normal according to both criteria (WHO 1999, 2010).

The same attention must be given when looking for sperm motility results. The infertility practitioners lacking advanced training in andrology especially in developing countries where much of infertility practice (males and females) is handled by gynaecologists, the main focus is given to total motility and progressive motility is ignored. Progressive motility is one of the key parameter of sperm motion, but it needs expert eye to be assessed correctly when using manual method. In single semen sample, the results of progressive motility assessed by two different observes are more variable compared to total motility. Further, the assessment of semen analysis by manual method is subject to observer's experience particularly in case of progressive motility. In the current study, data were collected from four different centres therefore, chances of inter-observers variations in case of progressive motility were relatively higher compared to total motility. Additionally, the criteria to assess progressive motility in WHO 1999 and WHO 2010 differ. According to WHO 1999, progressive motility refers to "grade a" only and should be $\geq 25\%$, whereas in WHO 2010, it refers to "grades a + b" and should be $\geq 32\%$. Therefore,

to minimise such bias, we have reported total motility which is the cumulative number of all grades assessed by either criterion (WHO 1999 or WHO 2010). Several recent key studies have also reported total motility instead of progressive motility (Baker et al., 2015; Catanzariti et al., 2013; Murray et al., 2012).

Almost all the andrology and clinical laboratories dealing with infertility have switched to the 2010 WHO reference values, and scientists and clinicians are reporting the semen analysis as normal or abnormal based on the new reference values. In this study, 44% of the subjects were regarded abnormal according to the old criteria but normal when the new criteria were applied. If the same study cohort was to be analysed before the implementation of WHO 2010 values, these men would have been subjected to further fertility evaluations such as detailed clinical and radiographic examinations of the male reproductive tract including the testes and sperm function tests, that is sperm DNA fragmentation. However, as these semen analyses are now regarded as normal, clinicians may not ask for further male evaluation and would rather turn towards assisted reproductive techniques such as intrauterine insemination which may cause extra financial and psychological burden.

Now the problem is if the semen analysis shows numbers which are abnormal according to WHO 1999 but normal according to WHO 2010; the person should be considered normal. Usually, the couples consult for fertility issues after one year or more of marriage or living together. The question is that after a defined period of infertility (1 year or more) if the couple is still unable to conceive and the semen numbers appear constantly normal what decision the provider will take? Will he or she go for female investigations and if found normal, will he or she return back towards intensive male evaluation, declare unexplained infertility, go for assisted reproductive treatment or do nothing? These are the questions which should be considered while dealing with men whom semen analyses fall in the grey zone. The couples with no conception after unprotected intercourse of longer than 12 months and those with advanced age even if no conception occurred during a period less than 12 months must be explored beyond semen analysis for sperm function tests (sperm DNA fragmentation, sperm chromatin condensation), varicocele or any other underlying aetiological factor.

There is well-established consensus that advanced age of women is negatively related to reproductive outcome. Therefore, such women are advised infertility treatment as soon as possible to avoid further delay. However, in couples where the male is at advanced age, the decision-making is not as firm as in females because of controversy in defining the threshold of advanced paternal age. One study (Zhu et al. (2011)) reported decline in motility and normal sperm morphology as from 30 years of age, while another study (Kidd, Eskenazi, & Wyrobek, 2001) reported decline in sperm concentration at the age of 34 years. In turn, others report declines in sperm motility and sperm functions such as DNA fragmentation beyond 40 years (Marcon & Boissonneault, 2004; Singh, Muller, & Berger, 2003; Stone, Alex, Werlin, & Marrs, 2013). Nonetheless, advanced paternal age has shown to affect the reproductive outcome not only after natural conceptions but also achieved through assisted reproduction (Sharma et al., 2015). Therefore, this may be of concern that normal semen numbers based on new reference values may cause delay in

procreation in couples where the male is at advanced age and possibility of unnecessary female examinations may increase.

Despite the fact that a semen analysis can provide important information on spermatozoon, motility concentration and normal morphology as predictive parameters for fertilisation, it does not predict sperm functional defects such as DNA fragmentation, oxidative stress and antisperm antibodies (Agarwal, Makker, & Sharma, 2008; Bungum, Bungum, & Giwereman, 2011). Around 30% of men with normal semen analyses diagnosed with unexplained infertility exhibit sperm function defects, and further investigations are warranted (Agarwal et al., 2008; Bungum et al., 2011).

The other facet of the new reference values is how to deal patients with varicocele. Around 15% of men from the general population suffer from varicocele, a number which ranges from 19% to 41% in primary male infertility and 45–81% in males with secondary infertility (Kibar, Seckin, & Erduran, 2002). In case of abnormal semen parameters, varicocele repair is recommended. However, there is lack of clear consensus with regard to the improvement in semen characteristics after varicocele surgery (Kim et al., 2016). Nonetheless, varicocelectomy has shown improvement in semen characteristics in young men (≤ 37 years) compared to older men (≥ 37) (Kimura, Nagao, Tai, Kobayashi, & Nakajima, 2016). Surgical repair of the varicocele improved the spontaneous conception rate by 2.87 times compared to those men who were not offered any treatment either surgical or medicinal (Marmar et al., 2007). However, the treatment becomes a challenge in cases where the semen analysis is categorised as normal according to new 2010 WHO reference values, particularly for those men who fall into the grey zone (below fifth percentile of WHO 1999, but above fifth percentile of WHO 2010). Several guidelines advocate varicocelectomy when semen parameters are abnormal, and the varicocele is palpable. The situation becomes more complex where reimbursement from the health insurance companies is involved. Further, the patient himself will be confused when the practitioner would explain that his semen analysis is normal, and the improvement in semen parameters after varicocelectomy is not guaranteed. On the other side, using the new reference values when the initial analysis shows normal values (grey zone population), the probability to refer men to clinicians for further fertility assessment will reduce. The good numbers of semen analysis may lead to either deferment or complete absence in referral pattern because a large majority of the clinicians make their decisions on the semen analysis results.

Previous studies (Baker et al., 2015; Catanzariti et al., 2013; Murray et al., 2012) which have compared the impact of the new reference values on interpretation of the semen analysis have reported a shift of not more than 16% with the implementation of new cut-off values, which is discernibly lower than the one of 44% in this report. Nonetheless, this current study has certain limitations such as the underlying aetiology for abnormal semen parameters which was not taken into consideration. Ideally, there should be three semen analyses from each man to obtain more precise results because of the variability of the parameters in seminal ejaculates from same individual.

5 | Conclusion

In conclusion, this is the first study reporting a comparison of the evaluation of human semen as being “normal” according to the 1999 and 2010 WHO laboratory manual from the Middle East and Indo-Pakistan region. Results suggest that a reasonable percentage of men examined for fertility problems may be considered “normal” using the 2010 new criteria and may not be given attention for further evaluation. However, it should be kept in mind that a standard semen analysis including seminal volume, sperm concentration, motility and normal sperm morphology is not sufficient to predict the male fertility potential as it cannot provide information about physiological sperm functions. Hence, the implementation of the new (WHO, 2010) criteria may result in lesser numbers of men referred for further evaluations, and more focus would shift towards female investigations.

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