Comparison between WHO (World Health Organization) 2010 and WHO 1999 parameters for semen analysis – interpretation of 529 consecutive samples

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Summary

Objective: To quantify how many men with normal semen according to WHO (WHO - World Health Organization) 1999 criteria, should be considered with abnormal semen according to 2010 criteria and vice versa; to study which parameter of volume, concentration, motility and morphology is the most responsible of this change.

Materials and methods: We studied, using WHO 1999 parameters, 529 consecutive semen samples from 427 men, collected in our Department from January 2008 to December 2009, then we re-evaluated those results using WHO 2010 parameters; we also studied each parameter to understand how changed the classification from normal (defined normal by all parameters) to abnormal (defined abnormal by at least one parameter) using the two WHO criteria.

Results: 3 men (0.56%) were azoospermic. Among the remaining 526 samples, 199 (37.83%) were considered normal and 246 (46.76%) abnormal both according to WHO 1999 and WHO 2010 criteria; we found that none of the samples classified normal according to the previous criteria was classified abnormal according the more recent criteria, while 82 (15.58%) evaluated as abnormal according 1999 criteria changed to normal according 2010 criteria. The concordance between 1999 and 2010 evaluation was 84.44%.

Conclusions: In this study we noted that the changes from WHO 1999 to WHO 2010 criteria did not modify the interpretation of semen quality, because comparing the two classifications we demonstrated that there is a substantial agreement, considering the three parameters (count, motility and morphology) all together, and also considering each single parameter. Anyhow, almost 16% of the patients considered infertile according to the old criteria, should be evaluated normal by the new classification and they should not need any treatment for infertility.

Key words: WHO 2010 parameters; Infertility; Semen analysis.

Submitted 5 June 2013, Accepted 30 June 2013

No conflict of interest declared

Introduction

Semen analysis is a crucial and irreplaceable tool for evaluating male infertility and precise thresholds are needed. Since 1951, the scientific community recognized this concept and McLoad et al. (1, 2) indicated the cut-off values for sperm counts (> 20 x 106 /mL, total sperm count > 100 x 106) for the first time, to distinguish fertility from subfertility. However the clinicians noted several times that some men with a sperm count below these ranges were able to conceive and this created an uncertainty in the clinical practice. In 1980 the World Health Organization (WHO) tried to clarify these doubts, by publishing the first (3) of 5 editions of guidelines for semen analysis. The weak point of that edition was that its criteria had never been prospectively validated by any study, because all the works present in the literature of the following years used either a case-control design, comparing fertile and subfertile couples, or a cohort design among the first pregnancy planners (4, 5).
The ultimate edition published in 2010 (6) used the concepts of “percentile” and “confidence intervals”, for the first time, allowing the clinicians to evaluate the individual semen analysis values in the context of measurement error and indicated reference values for semen parameters based on data from fertile men above the 5th percentile. Furthermore, the results were generated in multiple laboratories using standardized procedures and based on real world data.

However the last WHO guidelines for semen analysis radically changed the interpretation of semen analysis of the previous WHO 1999 (7) guidelines especially regarding the parameters of number (from 20 x 10^6/mL to 15 x 10^6/mL) and morphology (from 30% to 4%). This change mean that some of the patients, who were considered abnormal for the quality of their semen according to the old classification, would be considered normal, as a result of the new classification. Our study tried to quantify this change and to understand the percentage of concordance between the two classifications in the assessment of a sample, considering all three parameters at the same time (sperm count, motility and morphology) and analyzing each parameter individually to understand which of them is most responsible for the shift from normospermia to dyspermia.

**Materials and methods**

We studied 529 consecutive semen samples from 427 men collected in our Department from January 2008 to December 2009. Semen analysis was performed 2 or more times in 74 patients during this period. This group was composed of healthy men and men affected by different diseases (infertility, infections, varicocele, and other pathologies), Semen analysis was performed after at least 3 days of sexual abstinence. Semen samples were collected at the hospital by masturbation directly into a 120 mL sterile jar. Semen samples were analyzed within 1 hour of ejaculation. After liquefaction, semen volume was measured in a graded syringe with 0.1-mL accuracy. Sperm concentration was counted and motility assessed in a Makler counting chamber at a magnification of x 200. All semen parameters were classified first, according to the 1999 WHO criteria, then retrospectively using WHO 2010 parameters; we also studied each parameter to understand how the results changed from normal (defined as all parameters normal) to abnormal (defined with at least one parameter abnormal) using the two criteria.

**Results**

The mean age of participants was 30.26 (18-60). Sperm characteristics are summarized in Table 1. Between the 529 samples, we detected 3 (0.56%) cases of azoospermia. In analysing the specimens using the old criteria (Figure 1) we found 199 (37.62%) cases of normospermia, 140 (26.47%) cases of asthenozoospermia and 83 (15.69%) cases of oligoasthenozoospermia, while using WHO 2010 criteria (Figure 2) we found slightly different rates of prevalence: 283 (53.50%) cases of normospermia, 105 (19.85%) cases of asthenozoospermia and 85 (16.07%) cases of oligoasthenozoospermia.

![Figure 1. Number of patients in each category (WHO 1999).](image1)

![Figure 2. Number of patients in each category (WHO 2010).](image2)

When we considered only the number (Figures 3, 4) we observed that 355 (67.49%) patients had a normal number according to both WHO 1999 and WHO 2010 criteria, 138 (26.24%) patients were considered to have oligozoospermia by both classifications and 33 (6.27%) patients were considered to have a normal number according to WHO 2010 criteria and to have oligo-
zoospermia according to the WHO 1999 classification. We found that some patients who had an abnormal number according to old criteria were not considered abnormal according the new one. Therefore the concordance between the two classifications for number was 93.73%. Considering only the parameter of motility (Figures 5, 6) we found that 248 (47.15%) cases had normal motility both according to WHO 1999 and WHO 2010 criteria, 192 (36.50%) patients were considered to have asthenozoospermia according to both classifications and 86 (16.35%) patients were considered to have a low motility according to WHO 1999 parameters and to have a normal motility according to the new parameters. We found that some patients who had an abnormal motility by the old criteria were not considered abnormal according to the new one.

The concordance according to the two criteria about motility was 83.65%. Studying only morphology (Figures 7, 8) we observed that 469 (89.16%) cases presented normal morphology according to both classifications, 2 (0.38%) cases had teratozoospermia according to both WHO 1999 and WHO 2010 classification and 55 (10.46%) patients were considered to have abnormal morphology according to the old classification and to have normal morphology according to the new one. We found that patients who had an abnormal morphology by the old criteria were not considered abnormal according to the new one. Therefore the concordance between the two classifications of morphology was 89.54%.

Considering the three parameters together (Figures 9, 10) 199 (37.83%) patients were considered normal according
to both WHO 1999 and WHO 2010 classification, 246 (46.77%) cases were considered abnormal according to both classifications and 82 (15.59%) cases were evaluated abnormal according to the 1999 parameters and normal according to those of 2010. We found that patients who had all three parameters abnormal by the old criteria were not considered abnormal according to the new one, so that the concordance between 1999 and 2010 interpretation was 84.44%.

**Discussion**

In literature there are few studies that analyze the changes in the interpretation of semen analysis from WHO 1999 to WHO 2010 criteria. Murray et al. (8) recently did a multi-institutional retrospective study involving 387 infertile men, with the aim to understand how many semen samples of patients who were classified as infertile by timeline and previous semen analysis criteria would change classifications to be in the normal fertile range based on the 2010 WHO lower reference limits. They observed that overall, 43 (11.1%) patients who had one or more abnormal parameters in the original analysis would be converted to having all parameters within normal parameters and the most important changes in the interpretation of the data were in motility and morphology. These results are similar to our (11.1% vs 15.59%).

Another recent study by Zou et al. (9) indirectly analyzed this change of interpretation. In his work Zou examined
the determinants of semen quality in a large sample of military personnel from different geographical areas of the People’s Republic of China. Among 1194 patients he found that 88.3% had at least one semen parameter below the normal values according to World Health Organization (WHO) recommendations (1999), and 62.5% according to WHO recommendations (2010). Therefore, this study also demonstrated that the new classification created a little shift in the patients from infertile to fertile.

Metha et al. (10) instead, evaluated the improvement in semen parameters and serum testosterone (T) in their study, following varicocelectomy in those men considered abnormal according to the 1999 WHO criteria yet normal by the new 2010 criteria. They analysed 152 patients in total, that met the inclusion criteria (sperm concentration 15-20 million/mL, motility 40-50%, or morphology 4-14%): 111 patients (73%) underwent bilateral varicocelectomy, while 41 (27%) underwent a left side varicocelectomy. Overall, sperm concentration and serum testosterone (T) improved following surgery. Among men who met the inclusion criteria for sperm concentration, only sperm concentration was significantly increased (17.8 vs. 38.0 million/mL, p = 0.03). They concluded that microsurgical varicocelectomy in the subset of men considered to have normal semen parameters according to the 2010 WHO reference ranges, but abnormal according to the 1999 reference ranges lead to a significant improvement in serum T, sperm concentration, and, in some cases, sperm motility.

Metha and his colleagues underlined that even among patients considered normal for the new classification but abnormal for the old one, microsurgical varicocelectomy should be performed because of its improvement of fertility potential as well as T levels.

Our study evaluated if there were any changes in the evaluation of the semen quality according to WHO 1999 criteria and WHO 2010 criteria in order to understand if there are cases, which can change from abnormal to normal, and vice versa using the two classifications. We concluded that, considering each parameter by itself and all three parameters together, the concordance is very high. Concordance was high according to the number parameter (93.73%) that was slightly changed, from 20 x 10^6/mL (WHO 1999) to 15 x 10^6/mL (WHO 2010), but also according to morphology parameter (89.54%) that was radically changed from 30% (WHO 1999) to 4% (WHO 2010). Another interesting observation from our results is that there are not cases considered normal by the old classification that change to abnormal by the new one, while there are patients that resulted abnormal by the old criteria and normal according to new WHO 2010 parameters. This change is more consistent for motility (16.35%) than morphology (10.46%), as we expected. This implies that none of the patients that were previously considered normal changed to abnormal, according to the new classification, but some patients, about 15%, changed from abnormal to normal by the new classification. So thanks to the new criteria, the rate of patients that do not need any treatment for infertility is now reduced because they are no more considered infertile.

Acknowledgements
The Authors would like to thank all of the patients for their contribution to this study and all the Urologists of the Department of Urology of the Polytechnic University of Marche in Ancona, who contributed to this study, with their data collection and sharing.

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Archivio Italiano di Urologia e Andrologia 2013; 85, 3