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Distribution of semen examination results 2020 – A follow up of data collated for the WHO semen analysis manual 2010

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Abstract

Background: It is now 11 years since publication of the WHO 2010 guidelines for semen assessment values, and it is critical to determine whether they are still valid and/or whether they should be modified.

Objectives: To utilise data published since 2010 and combine these with data used in the 2010 assessment to provide an updated and more comprehensive representation of the fertile man. This may be utilised to present an updated distribution of values for use by WHO in 2021.

Materials and Methods: Two specific analyses were performed namely, (1) Analysis 1: Examination of published data following publication of WHO 2010 [termed 2010–2020 data]. (2) Analysis 2: Examination of the data used to help formulate the 2010 distribution of values combined with the data from Analysis (1) [termed WHO 2020].

Results: In total, data from more than 3500 subjects, from twelve countries and five continents were analysed. The 5th centile values for concentration, motility and morphology are: 16 × 10^6/ml, 30% progressive motility [42% total motility] and 4% normal forms.

Discussion: This study presents substantial additional information to establish more comprehensive and globally applicable lower reference values for semen parameters for fertile men although they do not represent distinct limits between fertile and subfertile men. There are still data missing from many countries and, some geographical regions are not represented. Moreover, the number of subjects although significant is still relatively low (<4000).

Conclusion: These distributions of values now include semen analysis providing a more global representation of the fertile man. Increasing the number of subjects provides robust information that is also more geographically representative.

KEYWORDS
semen analysis, WHO, reference values, fertile man

1 | INTRODUCTION

Although the World Health Organisation (WHO) published the first laboratory manual for the examination of human semen and semen-cervical mucus interaction in 1980 (WHO 1980), the 5th Edition[1] was the first to present distribution of semen parameter values that were based on significant supporting data.[2] These were defined from a population-based analysis of fertile men namely those with current or formerly pregnant partners with a known Time to Pregnancy (TTP) up to and including 12 months.[2] The 5th centiles were presented as the lower reference values[1,2] and, as expected, these have been ubiquitously used in the literature and clinical practice.

It is 11 years since publication, and it is critical to determine whether they are still valid and/or whether they should be modified. For example, there was a relatively limited range of subjects to formulate the WHO 2010 reference ranges – between 428 (for vitality) and 1941 (for semen volume). Moreover, a more global representation is required as there was no data from, for example, Africa or China.

To address this, we present substantial additional data obtained from published sources to potentially formulate more comprehensive and up to date lower reference values. These new data, combined with the previous information, comprises a database of semen analysis results of >3500 subjects, from twelve countries and five continents. This constitutes an updated and more comprehensive representation of the fertile man.

2 | MATERIALS AND METHODS

2.1 | Experimental design

This study presents two specific analyses. Analysis 1 presents a more recent reflection of semen parameters (post publication of WHO 2010), while Analysis 2 is a comprehensive presentation of values including data used for the WHO 2010 guidelines.

1. Analysis 1: Examination of published data following publication of the 5th Edition of the WHO laboratory manual for the examination and processing of human semen.[1,2] We term this Analysis 1, 2010–2020 data.
2. Analysis 2: Examination of the data used to help formulate the 2010[2] reference values combined with the data from Analysis 1 (2010–2020 data). We term this Analysis 2, WHO 2020.

2.2 | Analysis 1: 2010–2020 data. Review and assessment of the literature

A literature review of papers that contained data on semen analysis was performed and the search strategy is described and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Flow Diagram[3] (Appendix S1 and S2). In addition, Scopus (Elsevier's abstract and citation database) was used to search for papers that cited Cooper et al. 2010.[2] The publication dates examined were 1st January 2010 – 30th April 2020.

In this analysis we only assessed data from fertile men who have achieved a natural pregnancy with a known TTP ≤ 12 months and a sexual abstinence period of 2–7 days. Patients who were attending an infertility clinic and/or for fertility assessment were excluded.

2.3 | Data identification and processing Analysis 1: 2010–2020 data

Screening of the literature and analysis was performed by M. J. C. and C. L. R. B. Authors of the identified studies were contacted directly to obtain the raw semen analysis data related to their study (Table 1). All data examined was published in peer reviewed journals. The data was collated within Microsoft Excel spreadsheets and statistical analysis was carried out using SPSSv25. The new data set presents a total of up to 1789 subjects, and incorporates data from three continents Africa, Asia and Europe.

The methodologies as described in the 5th edition of the WHO[1] manual provided guidance on quality control and quality assurance. When semen analysis was performed according to 5th edition of WHO laboratory manual compliance to these procedures was assumed. PRISMA[3] Flow diagram is presented in Appendix S2.

2.4 | Analysis 2: WHO 2020

The dataset used to help formulate the WHO 2010 distribution of values was obtained from the editorial team of the sixth edition of the upcoming 'WHO laboratory manual for the examination and processing of human semen'. This data was examined and prepared to include only those semen analysis results with a confirmed TTP ≤ 12 months and a sexual abstinence range between 2 and 7 days. The data was then combined with 2010–2020 data (Analysis 1) to formulate WHO 2020 (Analysis 2). This combined data set presents a total of up to 3589 subjects, and incorporates data from five continents Africa, Oceania, Americas, Asia and Europe.

The data is freely available here https://doi.org/10.15132/10000163 and thus can be examined by investigators, added to and reanalysed, as and when appropriate.

3 | Results

Since 2010, seven published studies have been identified as potentially suitable for detailed assessment. Data was kindly supplied from five published studies[4–8] (Table 1). Five studies documented using WHO standards for assessment (WHO 2010) however the morphology assessment by Aboutorabi et al.[5] was not compliant with WHO 2010 and therefore has been excluded. Vitality assessments were only available from the study by Evgeni et al.[8] Lotti et al.[4], Tang et al.[7] and Zedan et al.[6] After accounting

TABLE 1 Study and number of subjects used for WHO 2020 reference values. Data includes published sources. The data is presented to indicate those studies included since publication of WHO 2010 and the data used for WHO 2010 reference values (Cooper et al., 2010[2])

Origin of study	Number of subjects	Reference to publication containing data	Data obtained
New subjects since WHO 2010			
Italy, Europe	105	Lotti et al. (2020)[4]	Yes
Iran, Asia[a]	168	Aboutorabi et al. (2018)[5]	Yes
Egypt, Africa	240	Zedan et al. (2017)[6]	Yes
China, Asia	1200	Tang et al. (2015)[7]	Yes
Greece, Europe	76	Evgeni et al. (2015)[8]	Yes
Subjects used in WHO 2010			
Australia, Oceania	206	Stewart et al. (2009)[19]	Yes[b]
Norway, Europe	82	Haugen et al. (2006)[20]	Yes[b]
United States of America, Americas	487	Swan et al. (2003)[21]	Yes[b]
France, Finland, Denmark, United Kingdom, Europe	826	Auger et al. (2001)[22], Jørgensen et al. (2001)[23], Slama et al. (2002)[24]	Yes[b]
Denmark, Europe	199	Bonde et al. (1998)[25], Jensen et al. (2001)[26]	Yes[b]

[a]Morphology results not utilised in this study. The samples used are only those subjects where there was a defined abstinence period of 2–7 days and TTP up to and including 12 months consequently the sample size can differ from that presented in Cooper et al. (2010)[2] and in some of the tables in the original data papers. In Lotti et al., the number of subjects reported (n = 105) refers only to men enrolled from the Florence spin-off of the "European Academy of Andrology (EAA) ultrasound study" with a TTP ≤ 12 months.

[b]The dataset used to help formulate the WHO 2010 reference values was obtained from the editorial group of the of the sixth edition of the upcoming 'WHO laboratory manual for the examination and processing of human semen'.

TABLE 2 Distribution of semen parameters for the fertile man and the 95% confidence intervals (CI) for the 5th centile, collated from published sources used in Analysis 1: 2010–2020 data

		Centiles									
	N	2.5th	5th	(95% CI)	10th	25th	50th	75th	90th	95th	97.5th
Semen volume (ml)	1789	1.0	1.2	(1.1-1.4)	1.5	2.0	2.8	3.8	5.0	5.5	6.0
Sperm concentration (10^6 per ml)	1789	14	18	(15-20)	22	31	60	104	157	203	243
Total sperm number (10^6 per ejaculate)	1789	30	36	(33-40)	50	88	168	288	467	573	679
Total motility (PR + NP, %)	1789	35	43	(40-45)	50	59	67	78	90	92	94
Progressive motility (PR,%)	1789	22	27	(26-30)	34	44	54	64	75	80	84
Non-progressive motility (NP, %)	1789	0	1	(1-1)	2	6	12	20	32	37	40
Immotile spermatozoa (IM, %)	1101	10	15	(12-17)	20	27	35	43	50	57	66
Vitality (%)	1337	45	54	(50-56)	60	69	78	88	95	97	98
Normal forms (%)	1621	3	4	(3.1-4.0)	5	7	12	19	30	32	34

The 5th centile is indicated above, of fertile men from published sources, from 01/01/2010 – 30/04/2020.

for an abstinence period of 2–7 days and a TTP of ≤12 months, up to 1789 subjects were analysed (Analysis 1: 2010–2020). Not all information was available for each subject. The key centiles are presented in Table 2.

The dataset used to help formulate the WHO 2010 values[1] was obtained from the editorial team of the sixth edition of the upcoming WHO laboratory manual. After accounting for an abstinence period of 2–7 days and confirming a TTP ≤ 12 months, up to 1800 subjects

were examined. Not all information was available for each subject and vitality was not available.

To formulate the WHO 2020 distribution values, we combined the data from 2010 with 2010–2020, which resulted in up to 3589 subjects for analysis (Analysis 2: WHO 2020). The data are presented in Table 3. The 5th centiles for concentration, motility and morphology are: 16 × 10^6/ml, 30% progressive motility [42% total motility] and 4% normal forms. For comparison the 5th centiles in WHO 2010[1] are 15 × 10^6/ml, 32% progressive motility [40% total motility] and 4% normal forms.

4 | DISCUSSION

This study presents substantial additional information to establish more comprehensive and globally applicable data for semen parameters. These new data, combined with the previous information, comprises more than 3500 subjects, from twelve countries and five continents. The 5th centiles are not noticeably different from those of WHO 2010[1] giving confidence that such values are relatively robust.

Recently, semen analysis has come under increasing scrutiny for its potential lack of reliability and poor reproducibility. With the introduction of the 2010 WHO manual[1] came a further call for improved quality control, internally and externally with clear methods that could be performed to achieve this. In this study (Analysis 1: 2010–2020), we only included studies that cited WHO 2010 methods and provided some detail (either in the study or by correspondence) of adherence to these methods. To provide further transparency regarding the data we utilised the matrix provided by Björndahl et al.[9] This checklist was produced, in part, for researchers performing semen analysis to provide important information to help the reader assess the quality of the analysis. We explored how this checklist could be incorporated (Appendix S3). Whilst there is no such thing as the ideal study, we note that assessment against the Björndahl template showed more information available for the post 2010 data (Appendix S3) compared to pre 2010 data (data not shown). For example, in the original data set[2] there was several different counting chambers used to assess concentration (including Neubauer, Makler, Burker-Turk, and Thoma chambers) with no clear information regarding the number of samples assessed using each chambers or comparable accuracy of the different methods. In this context, the inclusion of data from Tang et al.,[7] who used computer assisted sperm analysis (CASA) to assess sperm concentration and motility, requires comment. The 2010 WHO manual states 'provided that adequate care is taken in preparing specimens and using the instrument, CASA can now be used for some routine diagnostic applications'.[1] Tang et al.[7] utilised a SCA CASA 2000 to assess concentration and motility. The authors commented that, to reduce variation all samples were analysed by two well trained technicians who participated in a continuous external quality control system (based on WHO semen laboratory manual).[7]

The distribution of values in this data set for 2010, when analysed from the data provided, are not exactly the same as reported by Cooper et al.[2] We can only speculate as to why this is so. Firstly, the dataset used to formulate the WHO 2010 reference ranges is not publicly available. Moreover, we are unaware of any independent analysis of this specific data set. We obtained the data from the editorial team of the sixth edition of the upcoming, 'WHO laboratory manual for the examination and processing of human semen'. As such, the data used for the analysis presented by Cooper et al.[2] may have been subtly different from that which we obtained and there is no way of verifying this. Secondly, it's unclear from the information presented in the Cooper paper exactly which studies were used (and subjects) for construction of the reference values.[2] Thirdly, the sample size in the data sets are slightly different. Moreover, we edited the data, where appropriate, to include only those semen analysis results with a confirmed TTP ≤ 12 months and an abstinence range of 2 but no more than 7 days

TABLE 3 Distribution of semen parameters for the fertile man and the 95% CI for the 5th centile, Analysis 2: WHO 2020

		Centiles									
	N	2.5th	5th	(95% CI)	10th	25th	50th	75th	90th	95th	97.5th
Semen volume (ml)	3586	1.0	1.4	(1.3-1.5)	1.8	2.3	3.0	4.2	5.5	6.2	6.9
Sperm concentration (10^6 per ml)	3587	11	16	(15-18)	22	36	66	110	166	208	254
Total sperm number (10^6 per ejaculate)	3584	29	39	(35-40)	58	108	210	363	561	701	865
Total motility (PR + NP, %)	3488	35	42	(40-43)	47	55	64	73	83	90	92
Progressive motility (PR, %)	3389	24	30	(29-31)	36	45	55	63	71	77	81
Non-progressive motility (NP, %)	3387	1	1	(1-1)	2	4	8	15	26	32	38
Immotile spermatozoa (IM, %)	2800	15	20	(19-20)	23	30	37	45	53	58	65
Vitality (%)	1337	45	54	(50-56)	60	69	78	88	95	97	98
Normal forms (%)	3335	3	4	(3.9-4.0)	5	8	14	23	32	39	45

The 5th centile, is indicated above, and provides the lower reference values, of the fertile man.

as suggested in the WHO 2010 manual. Furthermore, there was no vitality information in the data provided by the editorial board.

Although the combined data (Analysis 2: WHO 2020, Table 3) present a more geographically representative global population, of 12 countries, in comparison to 7 countries previously presented,[2] including data from countries previously excluded such as China and Africa, there are limitations to our study. Firstly, it was surprising that relatively few studies were identified from 2010 onwards. Additionally, some were excluded as the methods used were not those recommended by WHO e.g. 'Semen Quality and Time-to-Pregnancy, the LIFE Study',[10] whereby semen samples were posted and analysed the following day. In 167 studies, we could not determine key details e.g. TTP of the fertile cohort, as this was outwith the study design. Moreover, even where data was originally indicated as potentially appropriate some data could unfortunately not be obtained e.g. data from Tang L-X et al.,[11] was not available as the main author was deceased, in the case of Punab et al.[12] data was not released by the author. Secondly, there are still data missing from many countries and, some geographical regions are not represented e.g. South America. What is more, data is only available for one African country (Egypt) which is unlikely to be representative of the whole continent. Lastly, the number of subjects, although significant, is still relatively low (<4000). More data is required from regionally diverse populations. If the WHO come to revise the manual (7th edition, ~2030) it is hoped that the above issues will be addressed. To aid in transparency and continual assessment, the data is available here https://doi.org/10.15132/10000163 and can be examined, added to and reanalysed as and when appropriate.

The WHO (2010) manual and the generation and use of reference values have been the subject of considerable debate e.g. Ford,[13] Boyd,[14] Björndahl.[15] It is not appropriate to repeat the minutia of these arguments here. However, it is important to emphasis key points. For example, as noted by MacLeod and Gold[16] there is substantial overlap between fertile and subfertile populations and thus reference values for a specified population (here fertile men) are not, and never will be, clear delineations of fertile and infertile men. For a reference range (or reference interval) to be meaningful it is necessary that the limits constitute true and distinct borders between "normal" and "abnormal", i.e. fertile and infertile. In case of indistinct boundaries between normal and pathology, the establishment of decision limits based on scientific evidence are much more helpful.[17] Furthermore, different decision limits should be established for different purposes e.g. for the choice of treatment with ICSI compared to other reproductive interventions.

It is necessary to see the distribution of semen assessment results in context. What is reassuring about the recent and combined analysis was that the 5th centiles were similar to WHO 2010.[1,2] Moreover, the sperm concentration of 16 million/ml (5th centile) is reasonably consistent with the original studies presented over 70 years ago from MacLeod and Gold 1951[16] where 5% of 1000 fertile men had sperm concentrations less than 20×10^6/ml. Other historical studies show a similar pattern. For example, in the analysis of Naghma-E-Rehan et al.,[18] 7% of 1300 fertile men had a sperm concentration less than 20 million/ml. Suffice it to say we can suggest that, using these studies as benchmarks over the last 70 years, the 5th centile for fertile males is realistically between 15–20 million/ml.

In summary, the distribution of semen assessment values now includes results from more than 3500 subjects, from twelve countries and five continents, providing a more global representation of the fertile male. Increasing the number of subjects provides robust information that is also more geographically representative. However, as emphasised, in the future, significantly larger studies encompassing data from regionally diverse populations will be needed to provide updated values.

DISCLAIMER

Some of the authors are present or former staff members of the World Health Organization. The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the views, decisions or policies of the World Health Organization.

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CONFLICT OF INTEREST

CLRB is on the editorial board for RBMO, has received lecturing fees from Merck and Ferring and was on the Scientific Advisory Panel for Ohana BioSciences (2018). None of the other authors declared a conflict of interest.

AUTHOR CONTRIBUTIONS

MJC and CLRB analysed the data and produced a first draft of the manuscript. Other authors assisted with interpretation of the data and further editing/comments/suggestions on further manuscript drafts. All authors approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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