LETTER

Reference values for human semen characteristics: A multicentre study in China

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Dear Editor,

The applicability of the current standard reference values to Chinese and Asian males is limited. Prior to this study, there has been no comprehensive evaluation of semen characteristics in reproductive age group nationwide in China. Therefore, it is urgent to supplement nationwide reference values for human semen characteristics in China, which could inform a more applicable evaluation for Chinese males and even provide data support for larger-scale research in Asia.

LIMITED APPLICABILITY OF THE CURRENT STANDARD REFERENCE VALUES

The WHO published the 5th edition of semen reference values in 2012 and then updated the latest version (termed WHO 2020 reference values) in 2021 by collating a follow up of published data.[1,2] The multi-ethnic applicability and broad representativeness of the WHO-5th semen reference are controversial due to lack of studies from Asia, Africa, the Middle East and Latin America,[3] while WHO 2020 reference values further added data from Asia and Africa. Nevertheless, the latest version was still estimated from a relatively small number of men (n = 3,589) with a known history of recent paternity, rather than from general men. Recent literature pointed out that the application of semen reference values from certain fertile men might not be applicable to general men.[3] In addition, the WHO 2020 reference values integrated the Chinese data that covered only a province (i.e., Guangdong). It's critical to determine whether they are appropriate and valid for the general male population in China.

To fill this evidence gap, we presented detailed analyses of relevant data from 16 human sperm banks covering 7 representative geographical regions in China, from April 20, 2013 to August 31, 2019. We aimed to supplement the reference values of semen parameters for general Chinese population.

DATA SOURCES FOR THIS STUDY

We used semen parameter data of 65,335 semen donors from 16 human sperm banks in China (i.e., including Jilin, Liaoning, Inner Mongolia, Beijing, Shanxi, Gansu, Shaanxi, Henan, Hubei, Jiangsu, Zhejiang, Sichuan, Chongqing, Yunan, Guangdong and Guangxi). The semen analysis methods are consistent with the WHO-5th semen reference values.[1] The study subjects aged 20-45 years (approximately covering the range of reproductive age) were from the general population, which is a mixed male population of unknown fertility and is assumed to be representative of the general men with reproductive age. After the sperm donors underwent routine semen screening tests, they were further required to have blood drawn to test for sexually transmitted diseases (hepatitis B, hepatitis C, syphilis, and AIDS), toxoplasma gondii, cytomegalovirus, herpes simplex virus, rubella virus, karyotype analysis, and thalassemia screening. In addition, pathogenic microorganisms such as gonococcus, chlamydia and mycoplasma were examined in semen samples. If the above test results are abnormal, the sperm donors were eliminated. For a male with multiple semen analyses, only the first test was adopted in data analysis, as the first sample was obtained under the "unscreened" condition to be more representative of the general population.[1]

STATISTICAL ANALYSIS

We applied both variance-weighted and non-weighted methods, referred to the latest WHO procedures,[1,4] to establish the reference values of six semen parameters. For variance-weighted analysis, we estimated the 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th and 97.5th centiles and corresponding variance of each sperm bank using quantile regression; we then calculated the pooled estimates (termed as C_{pooled}) of these centiles as equation 1:

$$C_{pooled} = \frac{\frac{b_1}{v_1} + \frac{b_2}{v_2} + \ldots + \frac{b_n}{v_n}}{\frac{1}{v_1} + \frac{1}{v_2} + \ldots + \frac{1}{v_n}} = w_1 b_1 + w_2 b_2 + \ldots + w_n b_n \quad (1)$$

where

$$w_i = \frac{\frac{1}{v_i}}{\frac{1}{v_1} + \frac{1}{v_2} + \ldots + \frac{1}{v_n}}, (i = 1, 2, \ldots, n) \quad (2)$$

In the above equations, b_i represents specific centile estimated from center i, and v_i represents the estimated variance of b_i obtained by quantile regression. The estimation of the standard error of C_{pooled} (termed as SE_{pooled}) is

A

Sperm parameter	Centiles (%)								
	2.5	5 (95% CI)	10	25	50	75	90	95	97.5
Semen volume (mL)	1.027	1.248 (1.248, 1.248)	1.637	2.089	2.894	3.336	4.767	5.494	6.572
Sperm concentration (10^6/mL)	7.372	10.776 (10.749, 10.804)	17.604	32.851	54.982	65.704	74.990	86.590	92.478
Total sperm count (10^6)	9.130	16.513 (16.304, 16.722)	30.058	75.284	139.593	215.199	301.305	377.775	445.940
Progressive motility (%)	16.609	24.430 (24.369, 24.492)	29.890	49.760	55.465	60.373	64.449	67.281	70.824
Total motility (%)	20.487	25.926 (25.861, 25.990)	35.802	48.094	58.456	64.976	71.120	77.128	81.183
Total motile sperm count (10^6)	2.796	5.829 (5.770, 5.887)	11.700	32.070	74.315	118.516	172.233	216.836	256.584

B

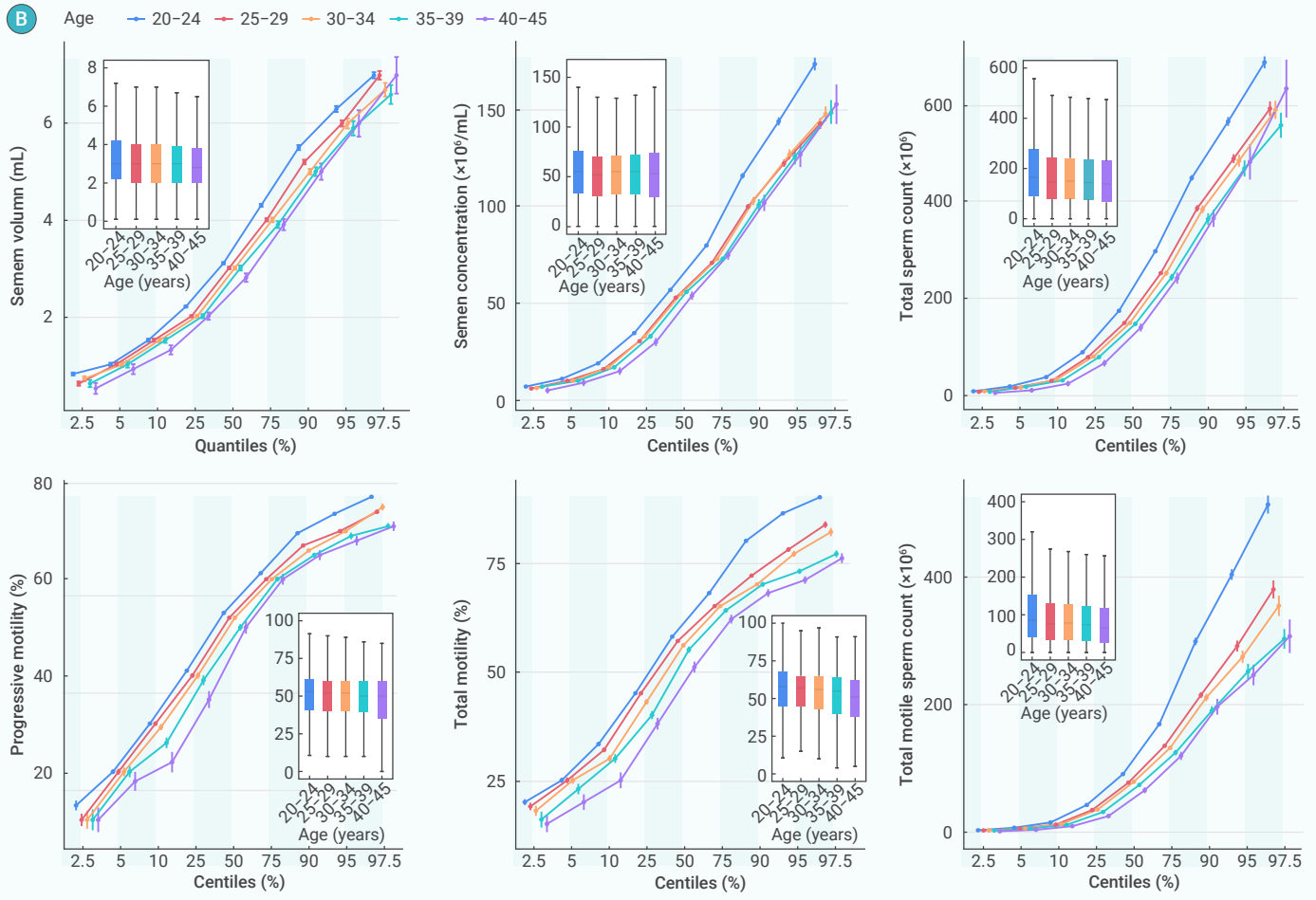

Figure 1. Reference values for semen parameters (A) Distribution of semen parameters for the general man and 95% confidence interval (CI) for the 5th centiles by variance-weighted analysis. (B) Distribution of values and corresponding 95% confidence intervals (CI) for semen parameters by non-weighted analysis, stratified by age. Each error bar represents the estimated centile and the corresponding 95% CI by quantile regression for semen volume (ml), sperm concentration (×10^6/ml), total sperm count (×10^6), progressive motility (%), total motility (%) and total motile sperm count (×10^6). For each semen parameter, the embedded mini-graph of box-and-whisker shows the distribution of raw data by age group.

$w_1^2v_1 + w_2^2v_2 + \ldots + w_n^2v_n$. The 95% confidence interval (CI) of C_{pooled} was calculated by the normal approximation, $C_{pooled} \pm (1.96 \times SE_{pooled})$. The non-weighted analysis was directly performed on the non-weighted pooled dataset. We performed statistical comparisons between results of variance-weighted and non-weighted to identify whether there was the existence of significant differences among centers.

For 62,231 men with accurate age at sample collection, we divided the data into five groups by age for calculation of reference values. Since center-specific sample size might not be enough for accurately estimating centiles in quantile regression when data was stratified by age groups, we only used non-weighted analysis for calculating age-dependent reference values.

THE ESTIMATED REFERENCE VALUES FOR SEMEN CHARACTERISTICS

The reference values of semen parameters by weighted analysis were fundamentally different from estimates in the non-weighted analysis (all *P*-values < 0.05) (Figure 1A), suggesting significant disparity among sperm banks. Thus, we adopted the weighted reference values of the semen parameters for general men in this study. The 5th centiles as lower reference values calculated by variance-weighted method was 1.2478 ml (95% CI: 1.2477, 1.2479) for semen volume, 10.776×10^6/ml (95% CI: 10.749, 10.804) for sperm concentration, 16.513×10^6/ejaculate (95% CI: 16.304, 16.722) for total sperm count, 24.430% (95% CI: 24.369, 24.492) for progressive motility, 25.926% (95% CI: (25.861, 25.990) for total motility, and 5.829×10^6/ejaculate (95% CI: 5.770, 5.887) for total motile sperm count. We also expressed semen parameters as a continuum by reporting the estimates of 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th and 97.5th centiles (except 5th centile, other centiles' 95% CIs were not listed due to the limitation of Figure layout), which might yield more

helpful information for distribution of semen parameters.

Figure 1B also presents age-specific reference values obtained by the non-weighted method. The trends in the distribution of reference values were largely similar across different age groups. At the same centile, a declining figure for older men was observed, which was more obvious at centiles higher than 50th. For all semen parameters, the highest reference value across different centiles was generally in those aged 20-24 years, whereas the lowest reference value was always observed in the oldest group aged 40-45 years. Overall, the semen volume, sperm concentration, total sperm count and total motile sperm count of investigated general men declined relatively rapidly from 20-24 years groups to 25-29 years groups, and then gradually decreased with age. The progressive motility and total motility gradually decreased from 20-24 years groups to 35-39 years groups, and then declined relatively rapidly from 35-39 years groups to 40-45 years groups.

CHOICE OF THE POPULATION FOR ESTABLISHING SEMEN REFERENCE VALUES

The WHO-5th semen reference values remained controversial in terms of its studied population and limited generality,[3] and such a debate might still exist in WHO 2020 reference values; some studies still adopt the older standards or other reference values.[3,5] For both WHO-5th and WHO 2022 semen reference, the semen data were from a group of fertile men that had a currently or formerly pregnant partner with time-to-pregnancy (TTP) ≤ 12 months. Chiles and his colleagues pointed that semen parameters established from a pool of men representing general population would be more applicable and generalized.[3] Besides, the WHO-5th semen reference combined data of 1,953 semen samples from five studies in eight countries (not including China), the WHO 2020 reference extended to 3,589 samples from twelve countries (only including a single-center data in China). Their external applicability to Chinese population with geographically and climatologically diversity is limited. Thus, a large-scale analysis of semen samples from general population in China helps to provide new insights into the reproductive status of men in China. Some previous studies tended to study on single-center and specific groups,[6-10] however, before the current study, there was no research devoted to addressing these gaps.

COMPARISON OF REFERENCE VALUES BETWEEN CURRENT STUDY AND WHO 2020 GUIDELINE

When compared with lower reference values (i.e., 5th centiles) of latest WHO 2020 guideline for semen characteristics, we observed that the percentage below to lower WHO reference values of semen volume, sperm concentration and total sperm count were less than 10% for most regions except for the central China, while the percentages of motility parameters seemed to be disproportionate among different areas. This suggests that the current WHO-5th semen reference values might be not very applicable to the general population with reproductive age in China.

In this study, the 5th centile estimated by variance-weighted method was considered as the lower reference values for the investigated Chinese general population with reproductive age: 1.2478 ml (95% CI: 1.2477, 1.2479) for semen volume, 10.776×10^6/ml (95% CI: 10.749, 10.804) for sperm concentration, 16.513×10^6/ejaculate (95% CI: 16.304, 16.722) for total sperm count, 24.430% (95% CI: 24.369, 24.492) for progressive motility, 25.926% (95% CI: (25.861, 25.990) for total motility, and 5.829×10^6/ejaculate (95% CI: 5.770, 5.887) for total motile sperm count. Compared with WHO 2020 lower reference values, our lower reference values are smaller for all semen parameters. The results of this study are reasonable, because our reference values were estimated based on the unselected general population, while the WHO 2020 reference values were estimated based on men with clear fertility. The developed reference values in this study could serve as a more applicable representation of the general men in China. A larger sample size yields more robust insights and enhances regional representativeness.

PERSPECTIVE

In this study, we established reference values for semen characteristics by standard methods, which potentially serve as a more applicable representation of the general men in China. Lower reference values have been established for sperm concentration, total sperm count, progressive motility, total motility, and total motile sperm count, differing from current standard reference values. These newly developed reference values could serve as a more applicable representation of the general male population in China, benefiting from a large number of subjects that provide robust and geographically representative information.

However, limitations exist in this study. Considering the sample source, the reference standard derived from our study may be more applicable to sperm donors rather than the broader demographic of all Chinese men. This nationwide study recruited semen donors from 16 human sperm banks aged 20-45 years from a mixed male population of unknown fertility. Although the large sample size of study population covered the seven representative geographic regions in China, semen samples obtained from sperm banks may predominantly represent younger, presumably healthier men with above-average semen quality. Therefore, it is necessary for future research to capture a more comprehensive understanding of sperm quality by encompassing a broader and more diverse sample of men across different demographics in China.

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AUTHOR CONTRIBUTIONS

P.G., X.Z.Z., J.Y.Z. and H.S.W. conceived and designed the study. J.Y.Z., H.S.W., Q.L.W, Y.S.L., T.Q.M., J.G.Y., Z.Z., X.W.L., H.Q.S., F.X.L., F.P.L., Z.Q.W., X.H.H., C.Y.S., Z.W.L., H.D., A.P.Z., G.H.G. and X.Z.Z performed the research. J.Y.Z., H.S.W., Q.L.W., T.T.D., L.X.W., X.Y.C., Y.C.Y. and L.N.X. carried out the data analyses. J.Y.Z, H.S.W., Q.L.W, H.B.N. and P.G. wrote the paper. J.Y.Z, H.S.W., T.X., Q.L.W., P.G. and X.Z.Z. edited the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

ETHICAL STATEMENT AND PATIENT CONSENT

The study has been reviewed and approved by the Ethics Committee of Guangdong Provincial Reproductive Science Institute (ID of the ethics approval: 2021-13). The study is in line with the Helsinki ethical guidelines. Data used in this work were anonymous, and no individual identifiable information was available in our study.

DATA AND CODE AVAILABILITY

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.