OPINION

Semen Analysis in African Laboratories: Is it Valid to Use the Reference Ranges from the WHO 5th Edition Manual for Clinical Interpretation?

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Abstract
Semen analysis is the core screening test for male fecundity, and the WHO has produced a series of manuals with recommended methodology to minimize technical variation together with reference ranges to help standardize clinical interpretation. Whilst numerous reports show semen characteristics vary between geographical locations and racial groups, African men were not included in the analyses undertaken to provide data for the reference ranges included in the WHO 5th Edition manual. Before the current global reference ranges published by WHO can be used by African laboratories, they must confirm their suitability for local African men. Futhermore, the standardized methods described in the WHO 5th Edition manual need to be used and validated in African Laboratories before verification studies for African subjects can be undertaken, and the inclusion of results from African men when the 6th Edition manual is written would seem warranted.

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Introduction

Infertility affects more than 45 million couples globally and that number is rising, with the highest prevalence seen in South Asia, Sub-Saharan Africa, North Africa/Middle East, and Central/Eastern Europe and Central Asia (Mascarenhas et al., 2012). Semen analysis is the core screening test for male infertility but has repeatedly been observed to show large technical variation due to difficulties in the control of accuracy and precision (Filimberti et al., 2013, Matson, 1995, Punjabi et al., 2016), leading to discussion regarding the requirements necessary for a laboratory to function optimally (Björndahl et al., 2004, Tomlinson, 2010). This current opinion paper discusses the challenges faced by African laboratories in using the reference ranges currently provided by the World Health Organisation (WHO, 2010), and ensuring widespread accuracy and precision using appropriate methodology.

Challenges for African laboratories

Racial and geographical differences in semen parameters

The measurement of semen quality as a surrogate measure of male fecundity in clinical andrology must consider racial and geographical differences that may exist in semen values. Four studies comparing Black and White men were identified and the details are listed in Table 1. A study investigating men from different racial groups within one country (the United States) that had recently fathered a child reported that Black men had a lower semen volume, sperm concentration and total motile count than White men (Redmon et al., 2013). Similary, a study using data for prospective semen donors showed a reduced total motile sperm concentration in specimens from African American men compared to White men (Chang et al., 2018). Taking a different approach by investigating geographical differences, a study comparing infertile men from the Middle East

Table 1. A summary of four publications showing differences in semen quality between different racial groups of men. The data is restricted to the findings relating to Black or Middle Eastern and North African (MENA) men, and White or non-MENA men. Studies were undertaken in the United States of America (US), Austria or Qatar

Authors	Setting	Period	Men	Findings
Redmon et al. (2013)	The Study for Future Families (SFF) in US	1999-2005	Partners of pregnant women, Black (n=57) and white (n=557) men, attending prenatal clinics in US.	Black men had significantly lower (geometric mean) sperm concentration (43 [34-56] vs 60 [56-64]) and total motile sperm concentration (57 [40-80] vs 108 [99-117]) than White men.
Feichtinger et al. (2016)	Fertility Clinic in Austria	2000-2011	Male partners from MENA (n=218) and non-MENA countries	After adjustment for age and smoking status of the male partner, MENA men were more likely to suffer from male factor infertility (OR 1.62, 95% CI 1.09–2.41, P = 0.017).
Elbardisi et al. (2018)	Fertility Clinic in Qatar	2012-2015	Infertile men from MENA (n=8799) and non-MENA (n=5093) countries.	Compared to non-MENA men, MENA men had significantly lower (mean ± SEM) sperm concentrations (29.8±0.3 vs 36.9±0.4)), total motility (45.0±0.3 vs 47.3±0.3) and progressive motility (24.6±0.3 vs 25.7±0.3), and higher proportion of abnormal forms (80.7±0.3 vs 78.5±0.3). Using WHO (2010) reference ranges, MENA men also showed a significantly higher prevalence of oligozoospermia (26.5% vs 17.6%), asthenozoospermia (27.6% vs 23.3%), and teratozoospermia (49.8% vs 46.9%).
Chang et al. (2018)	Donor sperm banks in USA	2007-2017	1929 semen donors, including White and African American men.	Decrease in total motile sperm concentration in specimens from African American men compared to White men ($p<0.03$). Data not provided

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and North Africa (MENA) with non-MENA infertile men showed MENA men had an increased semen volume but reduced sperm concentration, motility and morphology, and an increased prevalence of the classifications of oligozoospermia, asthenozoospermia and teratozoospermia (Elbardisi et al., 2018). Interestingly, methods and reference ranges of the WHO manual 5th Edition (WHO, 2010) were said to have been used. Elsewhere, a semen abnormality was reported as being present more frequently in MENA men attending a European clinic (Feichtinger et al., 2016).

Semen analysis in Africa and clinical interpretation

Reports on the frequency of different categories of semen quality have been made for male partners of infertile couples in Madagascar (Idrisa et al., 2001) and different parts of Nigeria (Akinola et al., 2010, Garba‐Alkali et al., 2018, Jimoh et al., 2012, Obiechina et al., 2002, Ugwuja et al., 2008) although there was no comparative aspect, only the epidemiological findings. Other reports from Nigeria have described the semen of fertile men (Akande et al., 2011) and both fertile and infertile men (Sobowale and Akiwumi, 1989). A meta-analysis has used publications from a number of African countries to investigate changes in semen quality over time (Sengupta et al., 2017) showing an overall 72.6% decrease in mean sperm concentration over the past 50 years, and is in stark contrast to the smaller overall 32.5% decrease in mean sperm concentration in European men over the same time frame (Sengupta et al., 2018). This difference in the rate of decline may also make the current use of the same reference ranges for both groups of men to be inappropriate. Similarly, two studies with increased proportions of Black men with semen abnormalities when compared with White men may be a true finding (Elbardisi et al., 2018, Feichtinger et al., 2016), but it could also simply be that the clinical interpretation may not be aligned with the current reference ranges.

Whilst one can understand how semen analysis techniques can be evaluated and reliable ones identified, it is less clear how reference ranges are derived and whether they apply to all racial groups. The current WHO manual (WHO, 2010) is the most transparent of the WHO series of manuals, using data collected from a number of sites (Cooper et al., 2010). Interestingly, there were no African sites included in the study and so there is a need to verify these global ranges for African men given the previous reports of racial differences. However, before this can be done or data generated for future revisions of the reference ranges, African laboratories should demonstrate that they are using the recommended methods.

Standardization of methods used, accuracy and precision

The results of semen analysis depend significantly on the methodology used and studies continue to confirm this (Peng et al., 2015), so the World Health Organization has worked hard to define standardized methods for semen analysis, producing five manuals from 1980 (Belsey et al., 1980) through to the current 5th Edition (WHO, 2010). Unfortunately, however, surveys in a range of countries such as the USA, Germany, UK and Poland consistently show that the recommended methods are not adopted widely (Keel et al., 2002, Nieschlag et al., 2018, Riddell et al., 2005, Walczak-Jedrzejowska et al., 2013). Whilst publications from African laboratories indicate that the WHO methodology of the day is being followed, as described above, the methodology used by routine laboratories performing semen analysis is yet to be defined.

Laboratories around the world responsible for the analysis of men's semen have consistently displayed a poor accuracy of results as reported by external quality assurance schemes (Alvarez et al., 2005, Keel et al., 2000, Matson, 1995, Punjabi et al., 2016). The real value of external quality assurance schemes is then as a surveillance tool so that laboratories can monitor their own accuracy and implement corrective action if there is a problem, and the utilization of such schemes by African laboratories would be valuable in identifying methodological bias.

Summary

There is a significant body of evidence to show differences in values obtained at semen analysis for Black and non-Black men. Whilst the current WHO reference ranges were derived using evidence-based methodology, the men studied were only from a relatively small number of WHO center in a small number of countries and there were no men from Africa. Before using

these global reference ranges, African laboratories must confirm the suitability of the reference ranges for local African men. However, before undertaking such verification studies on African men or generating data for future revisions of the reference ranges, laboratories need to use standardized methodology as described in the WHO 5th edition to maximize accuracy and precision, using methods matched to the reference ranges. Some form of audit of methods used routinely by African laboratories to confirm adherence to the recommended methods would appear necessary. In addition, the inclusion of results from African men in the data used to derive the reference ranges for the forthcoming WHO 6th Edition manual must be considered, and some form of African representation to the WHO would seem warranted.

Note Added at Proof

Thank you to Dr Franken for his insightful comments (Franken, D. Personal communication, 2019). Due to the large technical variability of semen analysis, as revealed repeatedly by EQA program, it is suggested any new data used in the calculation of reference ranges come from laboratories that use WHO methodology which has been fully verified to guarantee acceptable accuracy and precision.

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