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Evaluation of reference values of standard semen parameters in fertile Egyptian men

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Summary
The reference values of human semen, published in the WHO's latest edition in 2010, were lower than those previously reported. The objective of this study was to evaluate reference values of standard semen parameters in fertile Egyptian men. This cross-sectional study included 240 fertile men. Men were considered fertile when their wives had recent spontaneous pregnancies with time to pregnancy (TTP) ≤12 months. The mean age of fertile men was 33.8 ± 0.5 years (range 20–55 years). The 5th percentiles (95% confidence interval) of macroscopic semen parameters were 1.5 ml for volume and 7.2 for pH. The 5th percentiles of microscopic parameters were 15 million/ml for sperm concentration, 30 million per ejaculate for total sperm count, 50% for total motility, 40% for progressive motility, 62% for vitality, 4% for normal sperm forms and 0.1 million/ml for seminal leucocyte counts. In conclusion, fertile Egyptian men had higher reference values of sperm total motility, progressive motility and vitality, and lower reference values for total sperm counts as compared to those determined by the latest edition of the WHO laboratory manual in 2010. Other semen parameters were identical to those defined by the WHO 2010 manual.

1 | Introduction

Standard procedures of semen analysis are routinely used in most laboratories for initial evaluation of male fertility potential. These procedures include initial macroscopic examination of semen appearance, liquefaction, volume, viscosity and pH; and microscopic investigation of sperm concentration, motility and morphology; and assessment of seminal leucocytes and immature germ cells (WHO, 2010). Despite its weaknesses as a diagnostic tool, standard semen analysis allows for detection of remarkable cases of infertility such as azoospermia (Saleh et al., 2002). In addition, with repetitively abnormal semen analyses results, men can be diagnosed as infertile and an approximate prognosis can be given.

The methods of human semen evaluation are provided by the WHO, which periodically releases manuals including specific protocols and reference standards (Esteves, 2014). The WHO published its updated 5th edition of the laboratory manual for the examination of human semen in late 2010. This latest edition of the manual established reference values derived from data belonging to eight countries located in three continents from 1953 fertile men with a time to pregnancy (TTP) of <1 year (Cooper et al., 2010). The new reference values

for human semen characteristics were markedly lower than those previously reported (Esteves, Zini et al., 2012).

A series of reports has questioned the validity of these reference values because they categorised men who were previously considered infertile as fertile (Esteves, Hamada, Kondray, Pitchika, & Agarwal, 2012; Haidl, 2011; Murray et al., 2012; Yerram, Sandlow, & Brannigan, 2012). In addition, the new reference values were derived from data belonging to fertile men from Europe, Australia and North America, thus ignoring the vast majority of fertile men living in Africa, Asia, Middle East and South America. Recent controlled prospective studies confirmed regional differences between countries and continents as regards semen quality (Ellekilde Bonde, 2010; Vieira, 2013). Therefore, a major concern surrounded the ability of the new reference values for human semen to represent fertile men worldwide.

The objective of this study was to determine the reference values of standard semen parameters in a population of proven fertile Egyptian men. In order to avoid potential sources of bias and confounding factors, the following points were taken into consideration: (i) men's natural fertility was defined as the ability to initiate a recent spontaneous pregnancy with a TTP ≤12 months; (ii) semen analysis was performed according to the guidelines of the 5th edition of the WHO laboratory manual for examination of human semen (WHO, 2010); and (iii) the 5th percentile was used as the lower reference limit for semen parameters, thus allowing to cross-match the results of this study with the reference values reported in the latest edition of the WHO in 2010.

2 | Materials and methods

2.1 | Population and sample

This cross-sectional study was conducted in Assiut University Hospital, a central referral hospital located in Upper Egypt, between March 2014 and September 2016. The study was approved by the Institutional Research and Ethical Committees. Informed written consents were obtained from all participants. The study included fertile men whose wives were pregnant, at the time of recruitment, with TTP ≤12 months. Time to pregnancy was defined as the number of months (or cycles) from starting regular unprotected sexual intercourse to achieving pregnancy.

For sample size calculation, the equation of $N=(z_{1-\alpha/2})^2P(1-P)/D^2$ was used as previously described (Kasiulevicius, Sapoka, & Filipaviciute, 2006). In that equation, N, the minimum sample size required; $Z_{\frac{1-\alpha}{2}}$, the number of standard errors from the mean; P, the proportion of the best guess about the value of the proportion of interest; and D, the absolute precision required on either side of the proportion, or the distance; how close to the proportion of interest the estimate is desired to be. We considered confidence level at 95%, P = 0.85, D = 0.05, $(1 - P)$ = 0.15, $Z_{\frac{1-\alpha}{2}}=1.96.$ The minimum number required to achieve the objective of the study was 196 participants.

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A total of 390 men were recruited to increase the statistical reliability of the results. Men with history of primary or secondary infertility; general or local medical conditions affecting fertility, for example recent febrile illness or epididymo-orchitis; or drugs reducing fertility such as chemotherapy or anti-androgens were excluded. Genital examination was carried out by a single andrologist.

2.2 | Semen analysis

Standard semen analysis was performed according to the guidelines of the 5th edition of the WHO laboratory manual for examination of human semen (WHO, 2010).

Participants were asked to deliver a semen sample into a sterile plastic container after a period of sexual abstinence of 2–7 days. The samples were left to liquefy in a 37°C incubator and were analysed within one hour of delivery. Standard procedures included macroscopic features (semen volume, viscosity and pH); and microscopic parameters (sperm concentration, motility, vitality, morphology and seminal leucocyte counts). Hypo-osmotic swelling test was used for assessment of sperm vitality, and peroxidase-staining test was used for seminal leucocyte quantification. Leukocytospermia was considered at seminal leucocyte counts greater than one million peroxidase positive leucocytes per ml of semen (WHO, 2010).

To avoid inter-observer variability, all samples were examined by the same investigator and results were verified by a second observer. Two aliquots of thoroughly mixed semen samples were taken for replicate analysis. For each sample, replicate values were compared to check if they were acceptably close (<10% difference). If the difference was higher, two new aliquots were taken from the semen sample, two new preparations were made, and assessment was repeated.

2.3 | Statistical analysis

Statistical analysis of the data was performed using SPSS version 21 program (SPSS Inc., Chicago, IL, USA). Quantitative data were presented as mean ± standard deviations (*SD*), and median (5th and 95th percentiles). The lower reference values were considered at the 5th percentiles. Spearman rank correlation test was applied to determine relationship between quantitative variables. p value <.05 was set as statistically significant.

3 | Results

Out of 390 recruited fertile men, 240 (61.5%) fulfilled the study's inclusion criteria, agreed to participate and were, therefore, enrolled in the study. The mean ± *SD* of age of participants was 33.8 ± 0.5 years (range 20–55 years). The mean ± *SD* of duration of marriage was 5.98 ± 5.44 years. Clinically palpable varicocele was detected in 32 of 240 (13.3%) of fertile men in this study (15 bilateral and 17 left side). The remaining 208 of 240 (86.7%) of fertile men had no remarkable abnormality in their genital examination.

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All samples had normal viscosity (threading less than 2 cm). Minimum and maximum values, mean ± *SD* and 5th, and 95th percentiles of seminal macroscopic (volume and pH) and microscopic parameters (sperm concentration, motility, vitality, morphology and seminal leucocyte counts) in fertile Egyptian men are shown in Table 1. The 5th percentiles (95% confidence interval) of semen parameters among fertile Egyptian men and those reported in the latest edition of the WHO laboratory manual for examination of human semen (WHO, 2010) are shown in Table 2. A demonstration of the mean values and the 5th percentiles of semen parameters in the present, and in previous studies, is shown in Table 3.

When latest WHO (2010) lower reference values of normal semen parameters were applied to the 240 fertile men included in this study, 4% of the subjects had sperm concentrations lower than 15 million/ml, 2% had progressive motility less than 32% and 4.6% had less than 4% normal sperm forms. On the other hand, 101 of 240 (42%) of fertile men, in this study, who were normozoospermic according to the WHO (2010), had at least one parameter below the reference values of 1999 WHO criteria. The differences in semen parameters of fertile men with and without clinically palpable varicoceles were not significant ($p > .05$).

TABLE 1 Minimum and maximum values (range), mean ± *SD* and 5th, and 95th percentiles of seminal macroscopic (volume and pH) and microscopic parameters (sperm concentration, motility, viability, morphology and seminal leucocyte counts) in fertile Egyptian men ($n = 240$)

Semen parameters	Range (minimum to maximum)	Mean ± *SD*	5th and 95th percentiles
Semen volume (ml)	0.4–7	2.87 ± 1.38	1.5–5.5
pH	7–8	7.3 ± 0.22	7.2–7.8
Sperm concentration ($\times 10^6$/ml)	4–84	37.26 ± 19.38	15–83
Total sperm count ($\times 10^6$/ ejaculate)	16–336	100.1 ± 64.4	30–216
Total sperm motility (%)	30–80	62.17 ± 8.60	50–75
Progressive sperm motility (%)	15–70	49.23 ± 8.32	40–60
Sperm vitality (%)	30–85	69.94 ± 9.68	62–80
Sperm normal forms (%)	2–31	10.34 ± 7.04	4–25
Seminal leucocytes ($\times 10^6$/ ml)	0.1–3	0.44 ± 0.44	0.1–0.8

SD, standard deviation.

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TABLE 2 Lower reference limits (5th percentile, 95% confidence interval) of semen parameters among fertile Egyptian men (n = 240) and those reported in the latest edition of the WHO laboratory manual for examination of human semen (WHO, 2010)

Semen parameter	WHO (2010) (5th percentile)	Fertile Egyptian men (n = 240) (5th percentile)
Volume (ml)	1.5	1.5
pH	7.2[a]	7.2
Sperm concentration ($\times 10^6$/ml)	15	15
Total sperm count ($\times 10^6$/ejaculate)	39	30
Total sperm motility (%)	40	50
Progressive sperm motility (%)	32	40
Sperm vitality (%)	58	62
Sperm normal forms (%)	4	4
Seminal leucocyte count ($\times 10^6$/ml)	<1	0.1

[a]The WHO retained the value of 7.2 as a consensus lower reference limit for semen volume as per previous edition (WHO, 1999) due to lack of data in the literature (WHO, 2010).

4 | Discussion

The WHO considered men fertile when their partners had spontaneous pregnancy with TTP ≤12 months (or cycles) following regular unprotected sexual intercourse (WHO, 2010). Time to pregnancy has been generally accepted to reflect the fertility of a couple because it correlates well with sperm quality and quantity as well as sexual activity (Olsen & Ramlau-Hansen, 2014). In the majority of cases involving male factor infertility, the diagnosis was based on abnormalities of semen quality with varying severity and poorly understood aetiology (Tomlinson, Kessopoulou, & Barratt, 1999). Until the causes of male infertility are better understood, it is unlikely that any given descriptive test of sperm quality or sperm function will predict with absolute certainty that a man will be fertile or infertile in a given time period (Evenson et al., 1999).

Establishing reference values for semen parameters in fertile men is essential for accurate evaluation, counselling and treatment of men with male infertility (Redmon et al., 2013). In general, one-sided lower reference limits of semen parameters were used for discrimination between fertile and infertile men (WHO, 2010). However, there is controversy as to the cut-off values below which semen parameters are described as abnormal, and a diagnosis of male infertility could be given. Previous studies have proposed the 2.5th percentile (Cooper, Jockenhoevel, & Nieschlag, 1991), 5th percentile (Andersen et al., 2002; Gao et al., 2007) or 10th percentile (Menkveld et al., 2001; van der Merwe, Kruger, Oehninger, & Lombard, 2005) as lower reference values. The latest edition of the WHO laboratory manual for examination of human semen has determined the 5th percentile as a lower reference limit that discriminates between fertile and infertile men based on their semen analyses results (WHO, 2010).

In the present study, the 5th percentile of semen volume (1.5 ml) was similar to that determined by the WHO 2010 and to the value reported in a recent study of semen

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parameters among American fertile men (Redmon et al., 2013). Lower cut-off values of semen volume were reported in recent studies on 1,213 fertile men in Guangdong Province, China (1.3 ml) (Tang et al., 2015); and 792 fertile men from four large cities in Japan (1 ml) (Iwamoto et al., 2013). The 5th percentile of semen viscosity (threading <2 cm) and pH (7.2), in the present study, were similar to those reported in the WHO laboratory manual in 2010. The latter has retained the cut-off values of semen viscosity and pH that were determined in the previous 4th edition of the WHO manual (WHO, 1999) due to lack of sufficient data to provide new reference cut-off values (WHO, 2010).

TABLE 3 Mean values and 5th percentiles of semen parameters in the present study and in previous studies

Study	Method	Volume (ml)	Sperm concentration ($\times 10^6$/ml)	Total sperm count ($\times 10^6$/ejaculate	Progressive sperm motility %	Total sperm motility %	Sperm vitality %	Normal sperm forms %
Chia et al. (1998)	5th Centile	ND	ND	ND	ND	ND	ND	ND
	Mean	2.3	44.7	ND	ND	54.8	73.6	20
Gunalp et al. (2001)	5th Centile	ND	ND	ND	ND	ND	ND	ND
	Mean	4	50	ND	49	59	ND	14.9
Haugen et al. (2006)	5th Centile	1.7	10.6	22.3	33	ND	ND	3
	Mean	4.2	93	366	53	ND	ND	13.6
Crazzolara et al. (2007)	5th Centile	ND	ND	ND	ND	ND	ND	ND
	Mean	2.6	60	160	36		47	8
Redmon et al. (2013)	5th Centile	1.5	12	32	ND	28	ND	3
	Mean	3.9	60	209	ND	51	ND	11
Iwamoto et al. (2013)	5th Centile	1	18	38	ND	31	ND	1.5
	Mean	3	110	317	ND	67	ND	9.8
Tang et al. (2015)	5th Centile	1.3	20	40	25	39	48	5
	Mean	3.0	80	238	55	68	78	16
Present study	5th Centile	1.5	15	30	40	50	62	4
	Mean	2.867	37.26	100.1	49.23	62.167	69.94	10.34

ND, not done.

The 5th percentile for sperm concentration among fertile men, in the current study (15 million/ml), was similar to the reference value determined by the WHO in 2010. A lower cut-off value of sperm concentration (12 million/ml) was reported among American fertile men (Redmon et al., 2013). Higher cut-off values of sperm concentrations were reported among Japanese fertile men (18 million/ml) (Iwamoto et al., 2013) and Chinese fertile men (20 million/ml) (Tang et al., 2015). The latest WHO manual has recommended to calculate and report the total sperm count as it provides a measure for the capability of the testis to produce spermatozoa as well as a test for the patency of the male genital tract (WHO, 2010). In the present study, the 5th percentile of the total sperm count (30 million per ejaculate) did not match the value of 39 million per ejaculate, recorded by the 5th edition (WHO, 2010), despite having similar cut-off values for semen volume and sperm concentration.

According to the WHO manual of 2010, the lower reference limits, for semen volume (1.5 ml) and sperm concentration (15 million/ml), were 25% less than those reported in the previous 4th edition (WHO, 1999) (2 ml and 20 million/ml respectively). However, the reference value for total sperm count (sperm concentration multiplied by volume) did not show a similar reduction (39 million per ejaculate in the WHO 2010 manual versus 40 million per ejaculate in 1999). The lower reference value for the total sperm count was 32 million spermatozoa/ejaculate in fertile American men (Redmon et al., 2013), 38 million spermatozoa/ejaculate in fertile Japanese men (Iwamoto et al., 2013) and 40 million

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spermatozoa/ejaculate in fertile Chinese men (Tang et al., 2015) respectively. Taken together, the lower reference value for total sperm count in fertile men from different geographical regions and ethnic backgrounds is a range between 30 and 40 million spermatozoa per ejaculate (mean ± *SD* = 35.8 ± 4.5). The wide range of lower reference limits for total sperm count in fertile men may be attributed to many factors including methodological differences.

The 5th percentile for progressive sperm motility, in the present study (40%), was higher than the value of 32% reported by the 5th edition of the WHO manual (WHO, 2010) and 25% reported among fertile Chinese men (Tang et al., 2015). The 5th percentile of total sperm motility, in the present study (50%), was higher than the value of 40% reported by the 5th edition of the WHO manual (WHO, 2010), 39% in the Chinese study (Tang et al., 2015) and 31% in the Japanese study (Iwamoto et al., 2013). The Chinese study used computer-assisted semen analysis (CASA) for sperm motility evaluation (Tang et al., 2015), and the Japanese study included 1.5% with a history of infertility and 12.6% with TTP greater than 12 months (Iwamoto et al., 2013).

The current WHO laboratory manual (WHO, 2010) determined the lower reference value for normal sperm forms as 4% based on the Tygerberg classification (strict criteria) that considers minimal morphological deviations as abnormal. Early reports found men with normal sperm forms between 5 and 14% to have better fertilisation rates than those with 4% or less (Coetzee, Kruger, & Lombard, 1998). Strict criteria for assessment of sperm morphology were also correlated with the in vivo fertility potential (Eggert-Kruse et al., 1996). The 4% lower reference limit (5th percentile) of normal sperm forms, in our study, matched the cut-off value of the WHO 2010 manual and was close to the 3% value among fertile American men (Redmon et al., 2013) and 5% among fertile Chinese men (Tang et al., 2015).

Interestingly, 42% of normozoospermic fertile men, in this study, would be considered infertile according to the 4th edition in 1999 (WHO, 1999), with at least one parameter below the reference values of the later. It has been reported that 15.1% of men, who, on a previous analysis (according to the WHO manual of 1999), were deemed in the infertile range, would be classified at the fertile range using the WHO (2010) reference values (Murray et al., 2012).

5 | Conclusions

Proven fertile Egyptian men had higher reference values (5th percentiles) of sperm total motility, progressive motility and vitality, and lower reference values for total sperm counts as compared to those determined by the latest (2010) edition of WHO laboratory manual. Other semen parameters were identical to those defined by the WHO 2010 manual. Despite the cross-sectional nature of the study, it provided basic data of proven fertile men in Egypt, and the findings are interesting in many aspects.

First, these findings are in agreement with recent reports of a general trend towards lower reference values of sperm parameters. This trend was reflected in the latest edition of the WHO laboratory manual in 2010 and may indicate a decline in men's fertility potential in

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recent years. Second, differences in sperm parameters among fertile men, in different studies including the current one, may be related to genetic, ethnic, geographical and environmental factors. It may also reflect, at least in part, a degree of methodological and inter-observer variability. Future studies adjusting for potential sources of bias and variability may help establish new cut-off values for sperm parameters that can accurately discriminate between fertile and infertile men. Adherence to TTP of less than 12 months as a time limit for fertility potential may help define the fertile population more precisely. Also, a longitudinal study of semen quality over time would be the ideal design to address the issue of establishing reference values of semen parameters of fertile men in a certain population.

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