Original Article

Clinical Presentations and Semen Characteristics of Men Attending the Secondary Referral Infertility Clinic at Mumbai, India

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Abstract

Background: Limited information is available on the aetiology and semen profiles of male infertility in Indian population. **Aim:** The aim of this study is to study the clinical and semen characteristics of men attending the infertility clinic and also to understand the impact of World Health Organization (WHO) 2010 reference values on the diagnosis of male infertility. **Setting and Design:** A retrospective study evaluating the medical case records (January 2005 to December 2015, [n = 1906]) of men attending infertility clinic in Mumbai, India. **Materials and Methods:** The aetiology was classified based on the andrology evaluation and other investigations. Semen profiles were compared during the years 2005–2010 and 2011–2015 using WHO 1999 and WHO 2010 criteria, respectively. **Statistical Analysis:** The Chi-square and Mann–Whitney U tests were performed using Open Source Epidemiological software and Social science calculators. **Results:** The aetiology of male infertility was determined in 62% of the men; while the cause remained undetermined in 38%. Varicocele (25%), urogenital infections (10%), sexual dysfunctions (8%) and vas aplasia (8%) were identified as major aetiologies in our cohort. Men with sexual dysfunctions and vas aplasia were significantly higher during the years 2011–2015 as compared to 2005–2010. Men having normozoospermia (10%) and azoospermia (3%) were increased, whereas those having oligoasthenozoospermia (17%) were reduced in 2011–2015 as compared to 2005–2010. According to WHO 1999 criteria , 12-15% of men showed abnormal semen profiles. The semen parameters of these men became normal on using WHO 2010 reference values. **Conclusions:** Varicocele is the most common aetiology in infertile men. Idiopathic infertility was seen in a higher proportion among the infertile men.

Keywords: *Aetiology of male infertility, semen, sperm, varicocele, World Health Organization reference value*

Introduction

Globally, around 15% of the couples are unable to conceive after trying 1 year of unprotected intercourse.[1] Male factor is observed in 50% of couples experiencing infertility.[2,3] In addition, geographical and ethnic variations in the incidence of male infertility in North America (4.5%–6%), Australia (8%–9%), Europe (7.5%) and sub-Saharan Africa (2.5%–4.8%) have been observed.[4] There are geographical variations in the estimated incidence of infertility among different Indian states ranging from 3.7% (Uttar Pradesh, Himachal Pradesh, and Maharashtra)[5]

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to 15% Kashmir.[6] The prevalence of primary infertility was reported to be different amongst different tribes and communities within the same region in India[5,7] suggesting the heterogeneity of Indian population.

The aetiology of male infertility has been sub-categorised as pre-testicular, testicular and post-testicular.[8,9] Globally, there is a variation in reporting the aetiology of male infertility in different populations. Varicocele has been reported in 10%–22%, followed by urogenital infections (5%–12%), immunological cause (5%–11%), cryptorchidism (3%–17%) and sexual dysfunctions (2%–5%).[10-12] Idiopathic male infertility was reported in 40%–45% of the cases[11] while genetic causes contributed around 15%.[13] The majority of these studies are conducted in the Caucasian population considering the paucity of male infertility-related data and the heterogeneity of the Indian population, studies need to be conducted for understanding the causes of male infertility in Indian men.

Semen analysis is a crucial investigation for the evaluation of infertility and it helps to classify the severity of male factor infertility.[14,15] The World Health Organization (WHO) periodically provides guidelines for the evaluation of semen and also provides reference values that are considered for the diagnosis of male infertility. The WHO manuals were published in 1980, 1987, 1992 and 1999 to standardise the semen analysis procedures in andrology laboratories worldwide.[16] The fifth manual of the WHO released in 2010[17] provided new reference values which were based on the semen profiles from men whose partners had a time to pregnancy of 12 months or less, the cutoffs for abnormal seminogram were then proposed based on the new cut off values.[18] The reference values for semen analysis as per the WHO 2010 guidelines[17] are lower than those in WHO 1999 manual[19] and hence sparked several debates among the scientific community.[20,21] Studies from the different parts of the world showed that 15%–19% of men having abnormal semen analysis according to the WHO 1999 criteria were reclassified as normal as per WHO 2010 reference values.[22-25] Such studies have not been reported in the Indian population.

The implementation of WHO 2010 reference values in men with varicocele is likely to delay referrals for further evaluation and treatment.[26] In addition, the impact of WHO 2010 reference values on the interpretation of semen parameters among men having varicocele has not been studied in Indian men. Although Indian population contributes to 1/6th of the global population, the normal values for semen generated by the WHO are largely based on the Caucasian data and have no representation from China, Africa, the Middle East, South America as well as India.[20] This is probably due to a lack of data on sociodemographics, clinical characteristics and the aetiology of male infertility from these populations. To address these unmet needs, a large scale retrospective study was undertaken with the objectives: (i) To classify the aetiology of male infertility; (ii) understand the semen characteristics of the men with infertility and (iii) to analyse the impact of WHO 2010 reference values on the interpretation of semen parameters among Indian infertile men.

Materials and Methods

The present study was approved by the Institutional Ethics Committee for Clinical Studies at Indian Council of Medical Research-National Institute for Research in Reproductive Health (ICMR-NIRRH), Mumbai, India (IEC approval: D/ICEC/Sci-80/114/2016). The study was focussed on retrospective data collection from medical case records of men attending the infertility clinic during the period 1st January 2005–31st December 2015. The waiver of consent was granted by the Institutional Ethics Committee for Clinical Studies as the data were anonymised, and due precautions were taken to maintain the patient's confidentiality. The study was adequately powered to address the outcome measures. The power of the study was judged by the width and magnitude of the 95% confidence interval and the *post hoc* power calculator (https://clincalc.com/). The flow chart represents men attending the infertility clinic, undergoing andrology evaluation, the process of data entry, quality control and analysis [Figure 1].

Data entry and quality control

The infertility clinic of ICMR-NIRRH is a secondary referral centre situated at Mumbai, Western India. A dedicated team of clinicians and andrologists provided clinical and laboratory services to the men attending the clinic. The detailed examination of penis, position, and size of testicles, scrotal examination for detecting the pathologies of the genital ducts (epididymis and ductus deferens), the presence of a varicocele, secondary sexual characteristics was performed by the team of andrologists. The details of the clinical and laboratory parameters recorded in the male infertility case record forms are presented in Supplementary Table 1. Grading of the varicocele was carried out on Doppler for 214 men (Grade I [n = 101], Grade II [n = 56] and Grade III [n = 57]). Semen analysis was performed by a single trained operator for the entire period 2005–2010 and 2011–2015 as per the WHO 1999 and 2010 manual, respectively. Asthenozoospermia is classified as those having spermatozoa with total motility <50% as per WHO 1999 criteria and <40% as per WHO 2010. Oligozoospermia is defined as those with sperm concentration <20 million/ml as per WHO

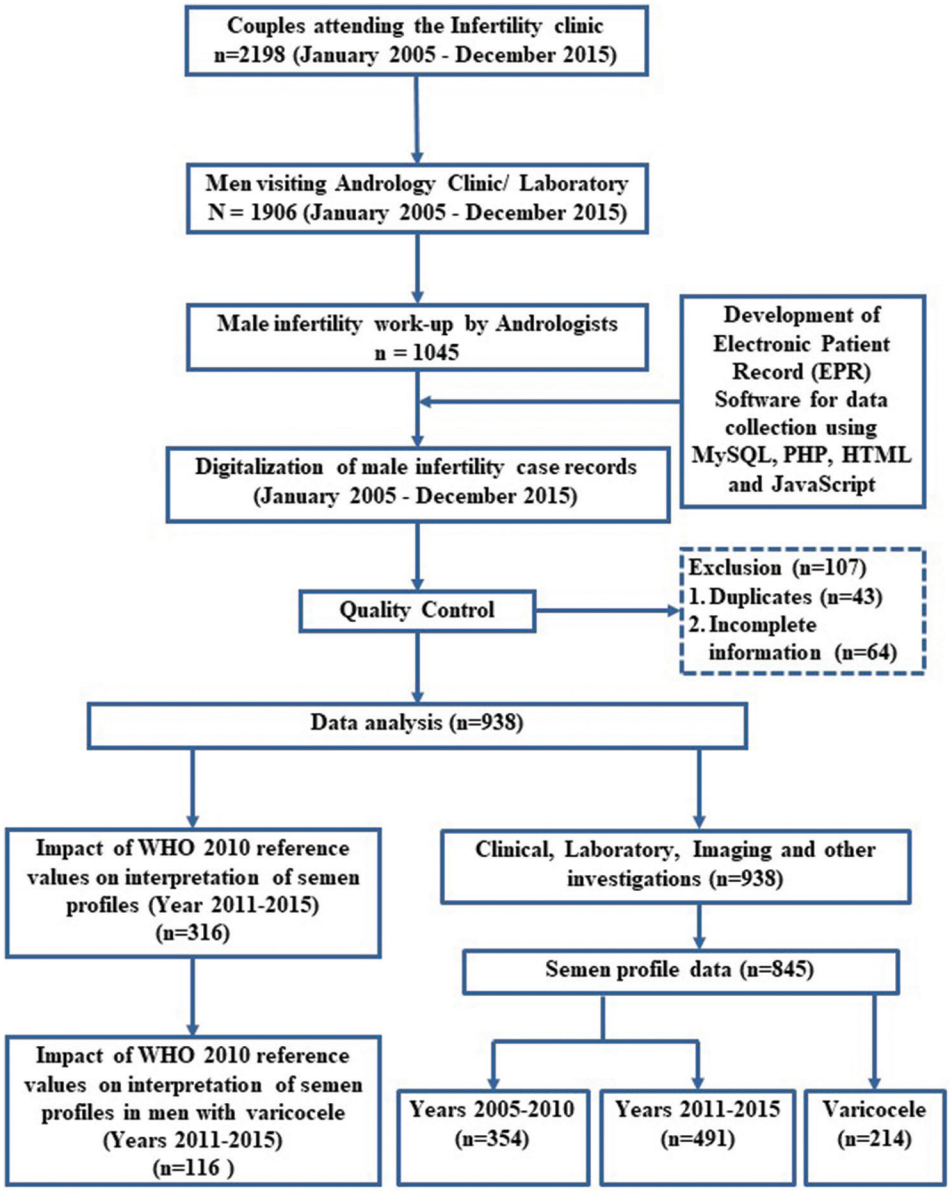

Figure 1: Flow chart showing men attending the infertility clinic for andrology evaluation, methods employed for software development, data collection, quality control and analysis

1999 criteria and <15 million/ml as per the WHO 2010. Oligoasthenozoospermia is the combination of both oligozoospermia and asthenozoospermia.[17,19]

Electronic patient record software for data entry was developed to capture the information from the male infertility case records. Each case was assigned with a single aetiology. In men who had been diagnosed with two aetiologies, the primary cause with a higher severity was considered for reporting.

To study the impact of WHO 2010 reference values on the diagnosis of male infertility, we analysed the semen analysis records of 316 men attending the clinic during the period 2011–2015. The semen analysis data were interpreted as per the WHO 1999[19] and WHO 2010[17] criteria. Azoospermia cases (n = 175) were excluded from this analysis. We also conducted the analysis of semen parameters in men with varicocele (n = 116) during the period 2011–2015.

Statistical analysis

Statistical analysis was carried out using Open Source Epidemiologic Statistics for Public Health version 3.01 and Social Science Statistics online calculator. The categorical data were recorded as proportions. The continuous variables were not normally distributed and expressed as median and inter-quartile range (IQR). The comparisons between the groups were done using the Chi-square test and Mann–Whitney U test.

A two-sided $P<0.05$ was considered statistically significant.

Results

Sociodemographic and clinical characteristics

The sociodemographic and clinical characteristics of men attending the infertility clinic for andrology work-up are represented in Table 1 and Supplementary Table 2, respectively. The median age of men attending the andrology clinic was 33 years (range 14–61). The majority of men (61%) were between the age group of 30 and 39 years. Around 40% of the men had a duration of infertility of more than 5 years. Around 46% of men were employed either in the public or private sector while 20% were laborers. The majority of men had primary infertility (91%). Primary infertility varied significantly in the period 2005–2010 (54%) as compared to 2011–2015 (46%) *(P* = 0.008).

Table 1: Clinical characteristics of men attending the infertility clinic

Variables	*n*=938, *n* (%)
Duration of infertility (years)	783
1-2	178 (22.7)
2-5	292 (37.3)
5-10	225 (28.8)
Above 0	88 (11.2)
Type of infertility	923
Primary	837 (90.7)
Secondary	86 (9.3)
Past medical history/systemic illness	
Tuberculosis	49 (5.2)
Jaundice	40 (4.3)
Typhoid	23 (2.5)
Diabetes	18 (1.9)
Mumps	11 (1.2)
Thyroid	6 (0.6)
Hypertension	5 (0.5)
Measles	4 (0.4)
Anxiety	3 (0.3)
History of surgery	93
Testicular biopsy	26 (2.8)
Varicocelectomy	20 (2.2)
Hernia repair	16 (1.7)
Hydrocele surgery	15 (1.6)
Orchiopexy	7 (0.8)
Appendectomy	4 (0.4)
Secondary characteristics	
Gynaecomastia	25 (2.7)
Reduced masculine traits	19 (2.0)
Sexual history	615
Coital frequency	
1-3 times/week	455 (76.0)
4-5 times/week	149 (24.0)
Premature ejaculation	55 (5.9)
Poor libido	44 (4.7)

Aetiology of male infertility

The aetiology of male infertility was determined in 62% of men; while in 38% of cases, it remained undetermined [Table 2]. Varicocele (25%) was the most common aetiology followed by urogenital infections (10%), sexual dysfunction (8%), vas aplasia (8%) and endocrine causes (6%). Among men with varicocele, 90% had primary infertility while 10% had secondary infertility. Left side varicocele (55.6%, *n* = 119/214) was most commonly reported followed by bilateral (43.5%, *n* = 93/214) and right varicocele (0.9%, *n* = 02/214). Grade I (60%, *n* = 71/119) varicocele was common than Grade II − III (40%, *n* = 48/119) varicocele on the left side. On the contrary, Grade II − III (69%, *n* = 64/93) was common

Table 2: Aetiology of male infertility in men attending the infertility clinic

Aetiology	*n*=938, *n* (%)
Undetermined	361 (38.0)
Varicocele	232 (25.0)
Urogenital infections	95 (10.0)
Epididymo-orchitis	70 (7.4)
Epididymitis	11 (1.1)
Funiculitis	7 (0.8)
Genital tuberculosis	6 (0.6)
Vasitis	1 (0.1)
Sexual dysfunction	75 (8)
UCM	29 (3.1)
Erectile dysfunction	24 (2.6)
Premature ejaculation	10 (1.1)
Anorgasmic ejaculation	8 (0.8)
Delayed ejaculation	2 (0.2)
Situation anejaculation	2 (0.2)
Vas aplasia	71 (7.6)
CBAVD	59 (6.3)
CUAVD	7 (0.8)
EDO	5 (0.5)
Endocrine causes	52 (5.5)
Hypergonadotropic hypogonadism	19 (2.0)
Diabetes mellitus	15 (1.6)
Sertoli only syndrome	6 (0.64)
Klinefelter syndrome	6 (0.64)
Thyroid disorders	5 (0.53)
Kallmannn's syndrome	1 (0.11)
Undescended testis	22 (2.4)
Occupational	20 (2.1)
Testicular trauma, torsion	8 (0.9)
Testicular cancer	2 (0.2)

UCM=Unconsummated marriage, CBAVD=Congenital absence of vas deferens, CUAVD=Congenital unilateral absence of vas deferens, EDO=Ejaculatory duct obstruction

than Grade I (31%, 29/93) in men with bilateral varicocele. Among men with urogenital infections, epididymo-orchitis was most frequently observed (8%). Unconsummated marriage (UCM) (3%) was observed in men having sexual dysfunction. Men with vas aplasia showed a high frequency of congenital bilateral absence of vas deferens (6%) compared to congenital unilateral absence of vas deferens (0.8%) and ejaculatory duct obstruction (0.5%). There was a significant increase in the sexual dysfunction ($P \leq 0.001$), vas aplasia cases ($P \leq 0.001$) during the period 2011–2015 as compared to 2005–2010 [Supplementary Table 3].

Semen characteristics

Excluding data of men with azoospermia (n = 289), the sperm concentration, percentage of motile, and progressively motile sperm were analysed for 556 men attending the clinic from 2005 to 2015. The sperm concentration (median, IQR) was 11, (3–21) × 10^6/ml and the total sperm count was 22, (6–54.4) × 10^6/ejaculate. The total motility (median, IQR) was 35, (20–47) % and progressive motility was 10, (10–33)%. A significant increase was observed in the sperm parameters (volume, sperm concentration, total sperm count, and progressive motility) during 2011–2015 as compared to 2005–2010 [Supplementary Table 4]. Amongst the men with normozoospermia the median sperm concentration was 28, (18–53) ×10^6 per ml.

In men with Grade II-III varicocele, sperm concentration (P=0.0114) and total sperm count (P=0.0054) were significantly lower as compared to those with Grade I varicocele [Supplementary Table 4]. Majority of men (89%) with Grade I varicocele had abnormal sperm concentration and motility.

Comparison of semen profiles categorized

Categorization of semen profiles based on sperm parameters compared during 2005–2010 and 2011–2015 is depicted in Table 3. Overall, Azoospermia (34%) and oligoasthenozoospermia (34%) were reported during 2005–2015. Amongst the men with azoospermia (n=289), 61% were classified as non-obstructive azoospermia while obstructive azoospermia was reported in 39% of men. Amongst the men with oligozoospermia, 47% (41/89) men with counts <5 million/ml were classified as severe oligozoospermia. There was a significant decrease in number of men with oligoasthenozoospermia ($P \leq 0.001$) and an increase in normozoospermia ($P \leq 0.001$) in 2011–2015 as compared to 2005–2010 [Table 3].

Irrespective of the grade of varicocele, almost 50% had oligoasthenozoospermia, while 8% had azoospermia. Amongst the men with varicocele, there was a significant increase in a number of cases with normozoospermia (P=0.017) and a decrease in oligoasthenozoospermia (P=0.037) in 2011–2015 as compared to 2005–2010 [Table 3].

Impact of World Health Organization 2010 reference values

Among 316 men attending infertility clinic during 2011–2015, the use of WHO 1999 reference values, classified 10% of men to have normal semen profile, whereas when the WHO 2010 reference values were used, 24% of men had semen categorized as normal [Table 4]. Thus, there was an increase of 14% semen profiles being classified as normal due to revision of reference values as per WHO 2010 guidelines. A significant shift in the percentage of semen parameter abnormalities were reported due to the change in reference values [Table 4].

The impact was also seen in men with varicocele, where 3% showed normozoospermia using WHO 1999 reference values. However using WHO 2010 reference values, 15% of men with varicocle were classified as

Table 3: Comparison of sperm parameters in infertile men

Sperm parameters	2005-2015	2005-2010††	2011-2015‡‡	Percent variation	*P*
Overall	*n*=845	*n*=354	*n*=491		
Normozoospermia	98 (11.6)	21 (5.9)	77 (15.7)	+10	<0.001
Asthenozoospermia	86 (10.5)	39 (11.0)	47 (9.6)	−1	0.492
Oligozoospermia	89 (10.5)	25 (7.1)	64 (13.0)	+6	0.006
Oligoasthenozoospermia	283 (33.5)	155 (43.8)	128 (26.1)	−17.7	<0.001
Azoospermia	289 (34.2)	114 (32.2)	175 (35.6)	+3	0.305
Varicocele	*n*=214	*n*=85	*n*=129		
Normozoospermia	20 (9.3)	3 (3.5)	17 (13.2)	+9.6	0.017
Asthenozoospermia	20 (9.3)	11 (12.9)	9 (6.9)	−6	0.156
Oligozoospermia	52 (24.3)	17 (20.0)	35 (27.1)	+6.9	0.258
Oligoasthenozoospermia	104 (48.6)	49 (57.6)	55 (42.6)	−15.3	0.037
Azoospermia	18 (8.4)	5 (5.9)	13 (10.1)	+4.9	0.324

Values are presented as *n* (%). ††WHO 1999 criteria, ‡‡WHO 2010 criteria. WHO=World Health Organization

normozoospermia [Table 4]. There was a 12% shift in men with varicocele being classified as having normal semen parameters using revised reference values of WHO 2010. In men with Grade I varicocele (n = 60), using WHO 2010 reference values a 15% shift in abnormal semen being classified as normal was observed.

Discussion

We report varicocele as the most common cause of male infertility and undetermined (idiopathic) cases in 38%. The incidence of idiopathic male infertility in our population is lower than study reported in Dutch and Estonian population as 44% and 60% respectively.[11,27] More than 500 target genes were postulated to be associated with idiopathic male infertility through various genomic studies.[28]

The incidence of varicocele (25%) reported in our study is similar to Caucasian[10,11] and southern Indian populations (24%),[29] but higher than north Indian population (16.6%),[30] thus indicating the population specific differences. Among men diagnosed with varicocele, 90% had primary infertility while 10% had secondary infertility. In contrast, others have reported a higher prevalence of varicocele in secondary infertility than primary infertility.[31,32] In our study, there was significant difference in men presenting with left varicocele and bilateral varicocele, which is similar to other studies.[33-35]

Urogenital infections were noted in 10% of infertile men, which corroborates with studies reported.[12,36] Since, infections are treatable conditions, timely diagnosis of this group would aid in early management of infertility with high success rate.

The prevalence of sexual dysfunction as a primary cause of infertility varies from 0.4% to 4.6%.[37] We report 8% infertile men diagnosed with sexual dysfunction, with significant increase in 2011–2015 as compared to 2005–2010. The reasons for higher incidence of sexual dysfunction in our study population could be due to the fact that limited andrologists are available in western region, RS and VK being the leading Andrologists in India, large number of cases are referred to our center for evaluation. Another possibility could be due to altered lifestyle, stress, chronic diseases, environmental pollutants and side effects of drugs.[38] A prospective study in 120 Indian men reported 30% with erectile dysfunction.[30] However, further large scale case-control studies are required to identify the risk factors for sexual dysfunction.

Among the sexual dysfunction, UCM was observed in 29 of the 938 men (3.1%) where the female factor of UCM was ruled out. Generally, UCM is mainly reported from non-Western societies with 8%–17% incidence in different geographical regions.[39-41] In the Indian context, UCM is hard to diagnose owing to the socio-cultural barriers and infertility specialists need to pay close attention to this condition.

The genetic causes of male infertility were identified in our study population. A high incidence of Vas aplasia was referred to our clinic in 2011–2015 as compared to 2005–2010. Previously, we reported known mutations and novel variants in the CFTR gene in vas aplasia in a study undertaken from 2013 onwards.[42-46] Mutations were reported in 66% men, while 34% were negative for CFTR mutations.[45] The possibility of other genes responsible for vas aplasia in CFTR negative men is being explored using exome sequencing study. Men with CFTR mutations have the possibility of transmitting the mutant to their progeny and hence the chance of having a child with cystic fibrosis. Hence, we screened their female partners and nine were found to harbour CFTR gene variants.[45] We therefore provided genetic counselling to these couples prior to undergoing ICSI. Karyotyping and Yq microdeletions were studied in limited infertile men,[47,48] and hence not reported in present study.

Semen analysis is the cornerstone for investigating male infertility and the WHO references are used worldwide as cut-offs to interpret seminograms and provide a diagnosis of male infertility. A significant increase in normozoospermia, oligozoospermia and azoospermia cases, and a reduction in Oligoasthenozoospermia in 2011–2015 than 2005–2010 were observed. Similar reports in Bangladeshi infertile men, indicated 18% increase in azoospermia and 7.2% in normozoospermia by while severe oligozoospermia decreased by 3.1% between 2000–2010 and 2011–2016.[49] Increase in normozoospermia is due to change in cut-offs defined

Table 4: Impact of World Health Organization 2010 reference values on semen parameters categorized in men attending the infertility clinic from 2011 to 2015

Sperm parameters	WHO 1999	WHO 2010	Percent shift	*P*
Overall (n=316)				
Normozoospermia	33 (10)	77 (24)	+14	0.008
Asthenozoospermia	56 (18)	47 (15)	−3	0.567
Oligozoospermia	52 (17)	64 (20)	+3	0.585
Oligoasthenozoospermia	175 (55)	128 (41)	−14	0.047
Varicocele (n=116)				
Normozoospermia	3 (3)	17 (15)	+12	0.003
Asthenozoospermia	15 (13)	9 (8)	−6	0.249
Oligozoospermia	22 (19)	35 (30)	+11	0.071
Oligoasthenozoospermia	76 (66)	55 (47)	−19	0.010

Values are presented as n (%). WHO=World Health Organization

for sperm count and motility in the WHO 2010 manual for semen analysis. Another study in South Indian men, showed a significant increase in severe oligozoospermia, and a slight increase in azoospermia between the years 2002–2005 than in 1993–1997.[50]

Using the WHO 1999 criteria, we observed 10% of men were categorized as having normal semen parameters. When the 2010 criteria were applied to the same study population 24% men had normal semen parameters, causing a shift of 14%, in abnormal semen being classified as normal. Similar observations (around 15%–19% shift) were made in studies from USA,[22] Italy,[23] Egypt,[24] Middle East and Pakistan.[25] To the best of our knowledge this is the first report to assess the change in the interpretation of semen analysis in Indian infertile men using the cut off values based on WHO 1999 and 2010 manual for semen analysis.

Clinically, these observations would have significant impact in decision making in the borderline cases who are likely to undergo further evaluation by specialists thereby delaying definitive diagnosis and management.[20,26] On the other hand, a study found a 16% shift but indicated no change in the referral pattern when using both WHO 1999 and 2010 criteria.[24]

We observed that amongst men with varicocele, nearly 50% had oligozoospermia and 8% had azoospermia. We observed that about 89% men with Grade I varicocele had abnormal sperm parameters suggesting effect of Grade I varicocele on spermatogenesis. We specifically looked at the impact of reference values of WHO 2010 in men with varicocele and observed a 12% shift in the classification of semen as normal which were in the abnormal category as per the WHO 1999 criteria. This shift was 15% among those with Grade I varicocele. Conversely, a meta-analysis indicated that WHO 2010 reference values for human semen analysis had no impact on the association between varicocele and semen parameters compared to previous WHO reference values.[51] According to the WHO guidelines, treatment has to be given to men with palpable varicocele and abnormal semen analyses.[52] It is known that varicocelectomy in men with mild oligozoospermia improves the spontaneous conception rate as compared to those men who do not undergo any treatment.[53-55] With the implementation of WHO 2010 reference values, men with varicocele and mild oligozoospermia who were previously considered as candidates for varicocele repair would be denied surgery.

Limitations of the study

The data was incomplete for sperm morphology so we could not analyse findings on sperm morphology which is an important parameter. The present study is a hospital-based study with a referral population and hence the findings cannot be generalised. We did not compare the records of proven fertile men which could have added value to the analysis.

Conclusions

In conclusion, the cause of male infertility was determined for 62% and remained undetermined in 38% of men in our study cohort. Based on this observation, we recommend genetic and genomic studies to be planned to delineate the genetic aetiology of the subgroup of male infertility where the cause is undetermined. This is the first report from India on comparison of the semen analysis data of male infertility clinic as per 1999 and 2010 WHO laboratory manual. Our study reports 12%–15% of men evaluated in our clinic for infertility may be considered "normal" using 2010 WHO reference criteria and could have been missed out from further evaluation especially those with varicocele who could have been benefited from treatment. Therefore, the implementation of the WHO, 2010 criteria would restrict the reasonable numbers of men being referred for further evaluations, and shift the focus of fertility evaluation more towards the female partner. The evaluation of male infertility should not be solely dependent upon semen analysis and physicians should consider a proper physical examination, detailed history taking, and appropriate endocrine, genetic, and other investigations. Based on the results of this study, we recommend the inclusion of the Indian population while considering the global reference values for WHO manual on semen analysis.

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Data availability statement

The data set used in the current study is available with the corresponding author and will share upon reasonable request.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary Table 1: Details of sociodemographic, clinical and laboratory parameters as per the male infertility case record form

Sociodemographic	History	Sexual history	General examination	Genital examination	Investigations
Age	Type of infertility	Coital frequency	Masculine traits	Testicular size	Semen analysis
Occupation	Duration of infertility	Libido	Gynaecomastia	Epididymis	Hormonal profile
Marriage duration	Childhood illness	Erection	Scrotum	Vas deferens	Scrotal ultrasound colour doppler
Smoking/chewing tobacco/recreational drugs	Systemic illness medication	Potency Ejaculation	Penis	Ejaculatory duct	TRUS
Alcohol consumption	History of surgery	Night emission	Urethra	Prostate	Karyotyping
Rest and sleep	Family history of infertility			Seminal vesicles	
Diet and appetite					
Types of bath					
Exposure to heat					

TRUS=Trans-rectal ultrasound

Supplementary Table 2: Sociodemographic characteristics of men attending infertility clinic

Variables	*n*=938, *n* (%)
Age (years), median (IQR)	33 (7.0)
Below 19	5 (0.5)
20-29	205 (21.9)
30-39	574 (61.2)
Above 40	154 (16.4)
Occupation	
Public/private sector job	427 (45.5)
Labor	205 (21.9)
Self-employed/business	84 (9.0)
Industrial worker	44 (4.7)
Driver	43 (4.6)
Professional cook	5 (0.5)
Unemployed	5 (0.5)
Student	4 (0.4)
Data not available	121 (13.0)
Types of bath	
Normal	864 (92.1)
Hot water	58 (6.2)
Sauna/steam	16 (1.7)
Addiction	
Alcohol	180 (19.2)
Tobacco	171 (18.2)
Both tobacco and alcohol	76 (8.1)

Supplementary Table 3: Aetiology of male infertility among men attending the infertility clinic

Aetiology	2005-2015 (*n*=938), *n* (%)	2005-2010 (*n*=411), *n* (%)	2011-2015 (*n*=527), *n* (%)	*P*
Undetermined	361 (38.5)	171 (41.6)	190 (34.1)	0.008
Varicocele	232 (24.7)	100 (24.3)	132 (25.1)	0.8
Urogenital infections	95 (10.1)	41 (9.9)	54 (10.2)	0.89
Sexual dysfunction	75 (8.0)	23 (5.6)	52 (9.9)	<0.001
Vas aplasia	71 (7.6)	11 (2.7)	60 (11.4)	<0.001
Endocrine cause	52 (5.5)	27 (6.6)	25 (4.7)	0.22
Undescended testis	22 (2.4)	13 (3.2)	9 (1.7)	0.07
Occupational	20 (2.1)	11 (2.7)	9 (1.7)	0.197
Testicular trauma and torsion	8 (0.9)	3 (0.7)	5 (0.9)	0.717
Testicular cancer	2 (0.2)	1 (0.2)	1 (0.2)	0.86

Supplementary Table 4: Semen parameters among infertile men

	n	Semen volume (ml)	Sperm concentration (106/ml)	Total sperm count (106/per ejaculate)	Total motility (%)	Progressive motility (%)
Years						
2005-2015	556	2.4 (1.9-3.3)	11 (3-21)	22 (6-54.4)	35 (20-47)	10 (10-33)
2005-2010††	240	2.3 (1.8-3)	8.5 (2-20)	18 (4.2-45.5)	35 (20-44)	0 (0-8)
2011-2015‡‡	316	2.5 (1.9-3.7)	11.5 (3-23.3)	27.8 (7.7-61.7)	36 (20-50)	30 (10-41)
P^a		0.0394	0.0209	0.0056	0.204	<0.001
Varicocele						
Grade I	96	2.5 (2-3.7)	9.3 (3-16)	23.5 (7.7-51)	33.5 (19.8-47.3)	11 (0-33)
Grade II + III	100	2.5 (2-3.2)	5 (1.2-12)	12 (3-27.5)	35 (20-46)	13 (0-32)
P^b		0.435	0.0114	0.0054	0.842	0.810

††WHO 1999 criteria, ‡‡WHO 2010 criteria, Data represented as median (IQR). Mann–Whitney test used for analysis, P^a=Comparison between 2005 and 2010 and 2011-2015, P^b=Comparison between Grade I and Grade II + III. Azoospermia cases were excluded from the analysis. IQR=Interquartile range, WHO=World Health Organization