

# Significance of sperm characteristics in the evaluation of male infertility

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**Objective:** To compare sperm characteristics among: patients undergoing infertility evaluation, patients with male factor infertility (MFI), healthy sperm donors, and men with proven fertility; to examine the overlap of sperm characteristics in all four of these groups; and to identify good discriminators of fertility versus infertility among sperm characteristics.

**Design:** Retrospective study.

**Setting:** Male infertility clinic at a tertiary care hospital.

**Patient(s):** Proven fertile men (n = 56), normal donors (n = 91), men presenting for infertility evaluation (n = 406), and MFI patients (n = 166).

**Intervention(s):** None.

**Main Outcome Measure(s):** Routine semen analysis.

**Result(s):** Using current World Health Organization (WHO) reference values, a large group of MFI patients presented with higher sperm concentration ( $27.5 \times 10^6$  to  $99.2 \times 10^6$ ), resulting in broader overlap with fertile men and poor sensitivity (0.48). Similarly, percentage normal morphology (%) using Tygerberg's strict criteria was low in most of the MFI patients (sensitivity 0.83), almost half of the fertile men also presented with abnormal morphology (specificity 0.51). Of all the variables examined, sperm motility (%) was superior, having minimum overlap range (lower and upper cut-off values 46% and 75%) and high sensitivity (0.74) and specificity (0.90). Areas under curve were higher for motility (0.90) and concentration (0.84) compared with morphology (WHO 0.65 and Tygerberg's strict criteria 0.74).

**Conclusion(s):** Sperm motility and concentration provide more accurate information than morphology (WHO and Tygerberg's criteria) during infertility evaluation. Redefining the reference values for concentration and morphology may significantly increase the importance of routine semen analysis. (*Fertil Steril*® 2006;85:629–34. © 2006 by American Society for Reproductive Medicine.)

**Key Words:** Spermatozoa, male factor infertility, sperm parameters, sperm characteristics, infertility evaluation

Infertility has major public health, economic, and psychosocial consequences in the United States. It affects approximately 15% of couples of reproductive age (1). A male-related factor is solely responsible in about 20% of cases of infertility and is a contributory factor in another 30%–40% (2). Infertility evaluation plays a major role in identifying the underlying cause, directing treatment of reversible conditions to allow natural conception (3), and giving couples with irreversible conditions the opportunity to pursue assisted reproductive options without delay.

Semen analysis is the initial and most essential step of the infertility evaluation, which also includes a physical exam, hormonal evaluation, sperm function testing, and genetic

Received May 10, 2005; revised and accepted August 8, 2005.  
Supported by the Glickman Urological Institute, Cleveland Clinic Foundation.

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analysis. It is also considered a cornerstone of the laboratory evaluation of the infertile male and helps define the severity of male factor infertility (MFI) (4). The diagnosis of MFI is made when the results of semen analysis are repeatedly abnormal according to World Health Organization (WHO) criteria. Therefore, the cause of MFI in most instances is linked to an abnormality in one or more of the semen characteristics (5).

Human spermatozoa display marked heterogeneity, and therefore a variety of sperm abnormalities may be found in the semen samples—even those from fertile men (6). Moreover, a normal spermiogram does not necessarily indicate satisfactory fertility potential. Owing to these inherent limitations in the methods of assessment, an accurate diagnosis of MFI can be made in only 40% of affected males seeking assistance (7).

WHO published manuals in 1980, 1987, 1992, and 1999 with the objective of standardizing semen analysis procedures in andrology laboratories worldwide. Most human semen analysis laboratories have adopted the WHO-recommended reference values for assessing semen characteristics. However,

there is a prevailing concern among clinicians that the current WHO reference values are too stringent.

Some recent studies have called for these reference values to be reconsidered, and others have proclaimed that these values fail to satisfy vigorous clinical, technical, and statistical standards (8–10). The repercussive implications of these criticisms can be readily evident from the changes in WHO nomenclature reported in the last four manuals.

There are no clear-cut guidelines for discriminating fertile men from infertile men based on the reference values put forth by WHO. This is partly attributed to the fact that these criteria do not strictly fit with the true fertility potential of subjects. Because there are no standard reference normal values, there is a risk of misclassifying patients with normal sperm parameters as having abnormal sperm function (8). This error could cause unnecessary anxiety on the part of the affected patients. It might also lead to unwarranted investigations and interventions for the patients and perhaps their spouses.

The purpose of our study was to: 1) evaluate sperm characteristics in patients undergoing infertility evaluation, in MFI patients with normal and abnormal semen characteristics, and in normal healthy volunteers and donors with proven fertility; 2) examine the overlap of sperm characteristics in all groups of the study population; 3) identify sperm characteristics that are good discriminators between proven fertile and infertile patients; and 4) identify improved cut-off values of sperm characteristics that better discriminate infertile from fertile men.

## MATERIALS AND METHODS

The Institutional Review Board of the Cleveland Clinic Foundation approved the study. Medical charts of the patients attending our infertility clinic for infertility evaluation during the years 1999–2000 were reviewed. These patients had attended our male infertility clinic for infertility evaluation and were asked to provide a semen sample for routine semen analysis as the first step in the evaluation of their infertility.

### Study Population

The study population consisted of 572 men presenting for infertility evaluation; of these, 406 patients presented for infertility with or without known female factor infertility and 166 with MFI only with no female factor infertility ( $n = 166$ ). Male factor infertility is defined as the inability of a couple to conceive a child after one year of unprotected sexual intercourse with a normal female partner or spouse, i.e., normal reproductive history, normal ovulation (by follicular ultrasound scan, luteal phase progesterone levels, and endometrial biopsy), and tubal patency (hysterosalpingogram).

The control population included two groups: 1) normal healthy donors with unproven fertility and those who estab-

lished a pregnancy more than 2 years before ( $n = 91$ ); and 2) men with proven fertility, i.e., established a pregnancy in the last 2 years ( $n = 56$ ).

### Semen Analysis

Semen was collected by masturbation after 2–3 days of sexual abstinence. After liquefaction, both manual and computer-assisted semen analysis (CASA) (IVOS 10.7s; Hamilton Thorne Research, Beverly, MA) were performed. For each measurement, a 5- $\mu\text{L}$  sample from either a control or an infertile patient sample was loaded on a MicroCell slide (Conception Technologies, San Diego, CA). A minimum of 200 cells were counted per sample. Sperm motion kinetics measured by CASA included the following: sperm concentration ( $\times 10^6/\text{mL}$ ), percentage motility, curvilinear velocity (VCL,  $\mu\text{m}/\text{sec}$ ), straight-line velocity (VSL,  $\mu\text{m}/\text{sec}$ ), average path velocity (VAP,  $\mu\text{m}/\text{sec}$ ), linearity (LIN, %), and amplitude of lateral head displacement (ALH,  $\mu\text{m}$ ).

In addition to the computerized results, manual results were calculated for sperm concentration and motility. All manual and CASA measurements were performed by trained and licensed medical technologists. CASA results were used when the difference between the manual and CASA values was less than 20%. In cases where the difference was greater than 20%, the manual results were reported. For morphologic evaluation, seminal smears were stained with Giemsa stain (Diff-Quick; Baxter Healthcare, McGraw Park, IL) and the percentage of sperm with normal morphology was assessed by WHO guidelines (3) and Tygerberg's strict criteria (11).

### Statistical Analysis

Basic descriptive statistics such as the mean  $\pm$  standard deviation and the median (25%, 75% interquartile ranges) were calculated for all study groups and compared using unpaired  $t$  test. Statistical significance was assessed at  $P < .05$ . The range (scale) was determined in order to observe the overlap of sperm characteristics between two groups (i.e., fertile men and MFI patients). The lower cut-off point of the range was selected where 95% (5th percentile or two standard deviations) of fertile donors were above this value. The upper cut-off point of range was selected where 95% (95th percentile or two standard deviations) of MFI patients were below this value. The percentages of the two populations falling within this range were calculated.

The diagnostic ability of the individual semen characteristics in differentiating infertile from fertile men was analyzed using Receiver Operating Characteristic (ROC) curves (JMP IN, Version 5.1, SAS Institute, Cary, NC). The sensitivity of a test was defined as the percentage of individuals with infertility (disease) that is classified as having infertility. The specificity of a test was defined as the percentage of proven fertile individuals (without the disease).

## RESULTS

The various semen characteristics of the study population (mean  $\pm$  standard deviation or median (upper, lower CI)) are shown in Table 1. Sperm concentration ( $\times 10^6/\text{mL}$ ), motility (%), and morphology (%) (WHO and Tygerberg's strict criteria) were significantly higher in the men with proven fertility and in the normal donors than in the patients undergoing infertility evaluation and in those with MFI ( $P < .05$ ). However, the values of sperm characteristics were comparable between the fertile men and normal donors.

### Overlap of Sperm Characteristics

The overlap and its ranges in the study population for various sperm characteristics are shown in the Table 2. The reason for selecting these two groups was that we wanted to compare semen characteristics between proven fertile men and proven MFI patients. Although the fertile men had significantly higher values for many of the sperm characteristics than the men with MFI, a significant proportion of overlap was seen in the sperm characteristics between these two groups (Table 2).

Though most of the fertile men (95% or 5th percentile lower cut-off) demonstrated higher sperm concentration and motility values ( $> 27.5 \times 10^6/\text{mL}$  and 46%), a subgroup of MFI patients also presented with high sperm concentration ( $> 36 \times 10^6/\text{mL}$ ) and motility (28%). This resulted in a broader range of overlap, particularly for sperm concentration ( $27.5 \times 10^6$  to  $99.2 \times 10^6$ ).

Furthermore, in comparison to concentration and morphology, a smaller proportion of the MFI patients had higher motility (greater than the lower cut-off value, i.e., 5th percentile), resulting in a narrow range of overlap (lower and upper cut-off values 46% and 75%); the smallest percentage of the study population fell within this range (proven fertile: 47%; MFI: 29%).

When the distribution of normal sperm morphology in both the fertile donors and the MFI population was analyzed, we found that the lower cut-off value of the overlap range was far below the WHO cut-off value of 30% and Tygerberg's strict criteria of 14% (18% for WHO morphology, 4% for Tygerberg's morphology). This indicates that most of the fertile population had significantly poor sperm morphology and that the results (WHO and Tygerberg's strict criteria) were far below the current reference values. Furthermore, in our study population, almost all of the fertile donors and MFI patients were within this range of overlap (Table 2).

### Receiver Operating Characteristic (ROC) Curves

The areas under the curve (AUC) for concentration (0.84) and motility (0.90) were significantly higher than those for morphology (WHO morphology: 0.63; Tygerberg's strict criteria: 0.74). At current WHO cut-off values, the specificities of sperm characteristics were higher for concentration (0.98) and motility (0.90) but not for morphology (WHO

**TABLE 1**

**Comparison of sperm characteristics between proven fertile men (PF), normal healthy donors (NHD), patients undergoing infertility evaluation, and patients with male factor infertility (MFI).**

Sperm characteristic	PF (n = 56)	NHD (n = 91)	Infertility evaluation (n = 406)	MFI patients (n = 166)	PF vs. NHD	PF vs. infertility evaluation	Infertility evaluation vs. NHD	MFI vs. NHD	MFI vs. infertility evaluation	MFI vs. PF
Concentration ( $10^6/\text{mL}$ ) <sup>a</sup>	69.9 (48.3, 120.0)	65.0 (40.7, 95.7)	32.9 (5.0, 75.4)	21.3 (7.95, 44.21)	NS	$P < .001$	$P < .001$	$P < .001$	$P < .05$	$P < .001$
Motility (%)	72.5 $\pm$ 16.6	72.9 $\pm$ 15.7	49.3 $\pm$ 30.4	37.0 $\pm$ 20.6	NS	$P < .001$	$P < .001$	$P < .001$	$P < .001$	$P < .001$
Morphology (%)										
WHO	37.7 $\pm$ 16.1	35.7 $\pm$ 12.8	17.8 $\pm$ 12.8	29.6 $\pm$ 11.5	NS	$P < .001$	$P < .001$	$P < .001$	$P < .01$	$P < .01$
Kruger's strict criteria	13.8 $\pm$ 7.1	10.9 $\pm$ 5.06	5.65 $\pm$ 4.6	8.1 $\pm$ 4.5	NS	$P < .05$	$P < .001$	$P < .001$	$P < .01$	$P < .01$

Note: Comparisons between groups were made using unpaired *t* test. Statistical significance was assessed at  $P < .05$ , and the summary statistics are presented as mean  $\pm$  standard deviation. NS = Not significant; WHO = World Health Organization.

<sup>a</sup> Values are median (25%, 75% interquartile ranges).

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**TABLE 2****Overlap ranges of sperm characteristics in fertile men and patients with male factor infertility (MFI).**

	Overlap range (cut-off values)		Percentage of overlap	
	Lower	Higher	Proven fertile (%)	MFI (%)
Concentration (10 <sup>6</sup> /mL)	27.5	99.2	61.8 (34/55)	36.7 (61/166)
Motility (%)	46	75	47.2 (26/55)	28.9 (48/166)
Morphology (%)				
WHO	18	51	81.0 (30/37)	81.3 (135/166)
Tygerberg's strict criteria	4	15	61.1 (33/54)	84.3 (140/166)

Note: WHO = World Health Organization.

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morphology: 0.68; Tygerberg's strict criteria: 0.51). These results also indicate that in most of the fertile men, sperm concentration and motility were higher than the current WHO cut-off values but the morphology was poorer.

We next examined the cut-off points for all the sperm characteristics provided by the statistical program by assigning equal weight to sensitivity and specificity (Table 3). Surprisingly, these values were different from the established WHO cut-off values, especially for sperm concentration and morphology (WHO and Tygerberg's strict criteria). Sperm motility had higher accuracy of correctly identifying the patient population among all the semen characteristics, even at the current WHO cut-off point.

## DISCUSSION

Our findings and recommendations are based on 166 MFI patients with no female factor infertility and 56 men with

proven fertility (control), although data from 572 men was included in the study. The results of this study suggest that the average values for sperm characteristics were significantly higher in fertile men compared with men with MFI. Moreover, these values were significantly higher than the cut-off values established by WHO. Patients undergoing infertility evaluation (not confirmed "infertile") also presented with sperm characteristics that were lower than those of the fertile men at current WHO cut-off values. However, they all had significantly higher values for sperm characteristic than the MFI patients ( $P < .05$ ). This may be due to the fact that in a group of patients attending infertility evaluation, the cause of infertility may be due to female factors.

A significant proportion of the fertile men had lower sperm morphology values than the WHO cut-off values despite the fact that the average values were generally higher. Similarly, the MFI patients also demonstrated higher

**TABLE 3****Receiver operating characteristic curve analysis of sperm concentration, motility and sperm morphology for established and proposed cutoff values.**

	Cut-off values ( $\geq$ )	Area under curve	Sensitivity	Specificity
Concentration (10 <sup>6</sup> /mL)				
WHO	20	0.84	0.48	0.98
Proposed	31.2	0.84	0.64	0.90
Motility (%)				
WHO	50	0.90	0.74	0.90
Proposed	57.8	0.90	0.83	0.88
Morphology (%)				
WHO	30.00	0.63	0.52	0.68
Proposed	33.00	0.63	0.67	0.58
Tygerberg's strict criteria				
Proposed	11	0.74	0.76	0.65

Note: WHO = World Health Organization; Proposed = cut-off point provided by statistical program weighing sensitivity and specificity equally.

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values for sperm concentration and motility than the established WHO cut-off values. This resulted in significant overlap of sperm characteristics (concentration and morphology) between the fertile men and the MFI patients.

To provide a clear picture of this overlap, we calculated the lower and upper cut-off values of overlap and determined the percentage of fertile men and MFI patients falling within this range. If sperm concentrations are below this cut-off value ( $<27.5 \times 10^6$  sperm/mL) it denotes significantly reduced occurrence of in vivo pregnancy (12). Similarly, concentrations above this cut-off value ( $>99.2 \times 10^6$  sperm/mL) indicate significantly higher occurrence of pregnancy.

Our study results clearly show that motility and concentration are better predictors of fertility potential than sperm morphology assessed both by WHO guidelines as well as by Tygerberg's strict criteria. Not only was the mean and standard deviation of motility higher in the fertile population and significantly lower in the infertile population, the range of overlap was shorter and the percent of MFI patients with high motility was smaller compared to both sperm concentration and morphology (Table 2). Comparing these values to both concentration and morphology (WHO and Tygerberg's strict criteria) shows that concentration has the broader range of overlap, with a large proportion of MFI patients having higher sperm concentration when compared with the current WHO cut-off values.

The AUC indicates the accuracy of characteristics in discriminating fertile men from infertile patients (13). Our results clearly show that the AUC was higher for motility (0.90) when compared with concentration (0.84), WHO morphology (0.63), and Tygerberg's strict criteria (0.74) (Table 3). This further strengthens the observation that among all the sperm characteristics in our study population, motility is superior in discriminating fertile from infertile men.

Several studies have recommended reconsidering the current WHO cut-off values in an attempt to improve the current accuracy of sperm characteristics because of either poor sensitivity or poor specificity (8, 14). The optimum cut-off value provided by the statistical software program that gives equal weight to the sensitivity and specificity (better accuracy) showed that this value was comparable with high sensitivity and specificity to the current WHO cut-off value only for motility. However, these values were much different for concentration and morphology (WHO and Tygerberg's strict criteria) (Table 3).

Several studies have demonstrated the correlation of motility with the fertilization rate in vivo and in vitro (15–17). Krause (18) also found sperm concentration and percentage of motile spermatozoa to be predictors of fertility outcome in vivo. Normal motility is indicative of normal development of spermatozoal axoneme during spermatogenesis in the testis, a normal maturation process in the epididymis, and normal seminal plasma constituents (19, 20). There are several proven and unproven causes for isolated and combined as-

thenozoospermia, which include defective functioning involving any of these processes.

Sperm motility is a critical indicator of semen quality and fertility potential, because it is required for penetration of cervical mucus, transport through the female genital tract, and penetration through the corona radiata and zona pellucida before oocyte fertilization (21). Isolated asthenozoospermia has been reported in as many as 24% of patients undergoing infertility evaluation and contributed to another 55% patients with other sperm defects such as oligozoospermia and teratozoospermia (20). In our study population, isolated asthenozoospermia was observed alone in 16% of MFI patients and in another 61% of MFI patients along with other defective sperm characteristics.

In our study, almost all of the fertile men had higher motility than the current WHO cut-off value (specificity 0.92). However, a group of MFI patients had higher motility (sensitivity 0.74). The results in this MFI group indicate that several other mechanisms and characteristics play an important role during fertilization, even though motility significantly correlates with fertility status ( $X^2 = 45.26$ ;  $P < .0001$ ).

Several studies in the literature have reported that percentage normal morphology is an essential characteristic for in vivo fecundity and in vitro fertilization (22). There is a continuing debate over the role of normal morphology in male infertility and its value in the evaluation and management of infertile men (9, 22). Most of these studies indicate that morphology is the best predictor among all of the sperm characteristics and that the current cut-off values of both morphologies (WHO and Tygerberg's strict criteria) need to be further reduced.

Our results are in accordance with these studies in that most of our fertile population had Tygerberg's morphology that was poorer than current cut-off values (specificities: WHO morphology: 0.68; Tygerberg's strict criteria: 0.51). Furthermore, almost all of the fertile men and MFI patients fell within the defined range of overlap, resulting in poor sensitivity or specificity at a given cut-off value and therefore in poor discriminating characteristics of fertility (AUC: WHO morphology: 0.65; Tygerberg's strict criteria: 0.72) (Tables 2 and 3).

Our results also strongly favor lowering of the cut-off values of sperm morphology to further improve their ability to discriminate MFI patients from fertile men (Table 3). Bartoov et al. (23) demonstrated that a diagnostic semen profile based on the number of deviations from the WHO normal standard values is not accurate. Even though their study included up to six semen characteristics, none of them, including normal sperm morphology, was useful as a sole predictor in the evaluation of male fertility potential. They concluded that optimal evaluation of male fertility potential can be achieved only by proportionally combining semen analysis characteristics in which fertile and infertile males differ significantly.

Multivariate discriminant analysis demonstrates that a combination of sperm characteristics, such as semen volume, sperm count, and percentage of motile and normal forms, considered in correct proportions, provides the best diagnostic profile that could discriminate the fertile from the suspected infertile male groups. Each semen characteristic contributes a different aspect to fertility potential, which in conjunction with the others yields maximum potency. This was elegantly demonstrated in our earlier studies (24, 25), where we derived novel semen quality scores based on principal component analysis. Each variable was assigned a weight to provide an overall semen quality score capable of discriminating the two populations.

In the current study, we examined and analyzed routine semen analysis reports of all possible study groups (i.e., fertile men, normal healthy donors as controls, patients undergoing infertility evaluation, and patients with MFI). The major limitation was the retrospective nature of the study. In conclusion, a significant proportion of patients with MFI present with normal sperm concentration, and a significant proportion of the fertile population present with poor sperm morphology. This is reflected in the fact that we found a significant overlap of sperm characteristics among fertile and infertile men. However, among the sperm characteristics, sperm motility and concentration are more accurate than sperm morphology (both WHO morphology and Tygerberg's strict criteria). There is a need to reevaluate the cut-off values established by WHO to further improve the ability of these characteristics to differentiate patients with MFI from fertile men. Finally, additional studies in larger clinical setting are needed to identify the exact reference range that can differentiate infertile men from fertile ones.

*Acknowledgments:* The authors thank Karen Seifarth, M.T. (A.S.C.P.), Cheryl Ackerman, M.T. (A.S.C.P.), and Lora Cordek, M.T. (A.S.C.P.), from the Clinical Andrology Laboratory for their technical assistance and Robin Verdi for secretarial help.

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