

Capacity of objectively assessed sperm motility characteristics in differentiating between semen of fertile and subfertile men

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A simple and inexpensive computer-assisted method for the objective assessment of sperm motility characteristics was employed to evaluate semen samples of 42 fertile men and 70 subfertile patients. The capacity of each motility parameter to discriminate between semen of the two groups was evaluated by receiver operating characteristic curve analysis. Velocity, linear velocity, and "angular" velocity are reasonably accurate, whereas linearity and angularity index had a very poor discriminating power. The best discrimination between the two groups was the proportion and concentration of sperm with rapid linear progressive motility, based on the cut-off value of linear velocity ≥ 22 $\mu\text{m}/\text{sec}$. This parameter was 90% accurate in discriminating semen of fertile men from that of subfertile patients. *Fertil Steril* 50:635, 1988

Several studies have shown that motility characteristics of spermatozoa are of the utmost importance for men's fertility.¹⁻³ Velocity has emerged as the most important parameter to assess the fertilizing ability of spermatozoa *in vivo*⁴ and perhaps *in vitro*.⁵ However, the technology for the assessment of sperm motility characteristics has been insufficiently standardized and validated.⁶

Recently, we have developed a simple and inexpensive computer-assisted method for the objective assessment of sperm motility characteristics. This method has been validated as to its accuracy and reproducibility.⁷ With this method, a study of sperm motility characteristics was carried out in a group of subfertile and fertile men. The diagnostic performance of different parameters of motility in

differentiating the two groups was analyzed with receiver operating characteristics (ROC) curves.

MATERIALS AND METHODS

Subjects

The subfertile group consisted of 70 men who were the male partners of infertile couples of at least 12 months' duration, and whose wives had no demonstrable cause of infertility by standard World Health Organization criteria.⁸ Because sperm motility could only be assessed in semen samples with sperm present, men with azoospermia were excluded. No consideration was given to the alleged cause of the male subfertility for including the patients into the study.

The fertile group consisted of 42 men who had fathered a pregnancy between 3 months and 2 years before the present examination.

Assessment of Sperm Motility Characteristics

Sperm motility characteristics were assessed by means of a simple computer-assisted method as de-

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Figure 1 Diagram of the microscope equipped with a drawing tube, digitizing tablet with cursor, and microcomputer.

scribed by Hinting et al.⁷ The method is based on the use of a digitizing tablet with cursor equipped with a light point (Numonics digitizing tablet 2210, Numonics Europe, Leuven, Belgium) and a microcomputer (IBM XT, IBM Corp., Armonk, NY).

A zoom drawing tube (Carl Zeiss, Oberkochen, West Germany) is introduced between the objective and the oculars of a phase-contrast or dark-field microscope. The drawing tube permits simultaneous observation of the microscopic field and the digitizing tablet, which is placed beside the microscope (Fig. 1). A square of 7×7 cm with a grid is drawn on the digitizing tablet, and this is superimposed at the center of the microscopic field. The zoom of the drawing tube is adjusted so that the superimposition of the square corresponds exactly to $100 \times 100 \mu\text{m}$ on a micrometer slide.

An aliquot of $11.5 \mu\text{l}$ of thoroughly mixed undiluted fresh semen is placed on a microscope slide and covered with a standard coverslip of 24×24 mm, giving a uniform specimen depth of $20 \mu\text{m}$. Analysis is performed at magnification $\times 500$ and at room temperature. The movement of each spermatozoon encountered is tracked during 3.2 seconds by the cursor on the digitizing tablet.⁷

Parameters of Sperm Motility Characteristics

Five parameters concerning the sperm motility were calculated using the Autosperm software package (Amsaten Corp., De Pinte, Belgium).

1. Velocity, also called average curvilinear velocity (expressed in $\mu\text{m}/\text{sec}$), which is the total distance covered by the spermatozoa per unit of time,
2. Linear velocity, also called straight line velocity (expressed in $\mu\text{m}/\text{sec}$), which is the shortest distance between the start and end point, calculated per unit of time,
3. Linearity index, also called linearity of forward progression (expressed in %), which is

the quotient of linear velocity divided by velocity, multiplied by 100,

4. Angularity index (expressed in %), which is the deviation of linearity calculated as the mean of the cosine of angles, transformed to a percentage. A straight track has an angularity index of 100%; and
5. Angular velocity (expressed in $\mu\text{m}/\text{sec}$), which is calculated by multiplying angularity index with velocity, divided by 100.

Furthermore, spermatozoa were classified according to their motility characteristics as grade a, b, c, and d in agreement with the World Health Organization Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction.⁹ In a previous study, we have established the criteria for classification⁷ as follows: grade a: linear velocity $\geq 22 \mu\text{m}/\text{sec}$; grade b: linear velocity $< 22 \mu\text{m}/\text{sec}$ and velocity $\geq 5 \mu\text{m}/\text{sec}$; grade c: velocity $< 5 \mu\text{m}/\text{sec}$; and grade d: immotile spermatozoa.

In addition, the proportion of grade a spermatozoa was calculated at different cut-off values of linear velocity, namely $\geq 20 \mu\text{m}/\text{sec}$, $\geq 22 \mu\text{m}/\text{sec}$, $\geq 25 \mu\text{m}/\text{sec}$, $\geq 30 \mu\text{m}/\text{sec}$ and $\geq 35 \mu\text{m}/\text{sec}$.

Statistical Analysis

The main objective of the present study was to assess the ability of each parameter of sperm motility to discriminate between semen of the subfertile and fertile groups. The ROC curves^{3,10} were used since they provide both visual and numeric evidence of the quality of a particular test and guide the selection of a normal threshold.¹¹ In brief, the curves were constructed from the cumulative frequency distribution of the result of the different motility characteristics in the two separate groups, plotting the proportion of subjects in the first group against the proportion in the second group. If the distribution of the result of a particular test is identical in both groups, the ROC curves will coincide with the diagonal. The greater the difference in the distribution of the characteristic in the two groups, the further the curve will shift from the diagonal to the upper left hand corner. The ROC curve for an ideal diagnostic test reaches the upper left hand corner. The point on the curve which is the farthest away from the diagonal is the "best" criterion value with respect to making the fewest classification errors. With the use of this criterion value, sensitivity and specificity were calculated.

In addition, the percentiles were calculated

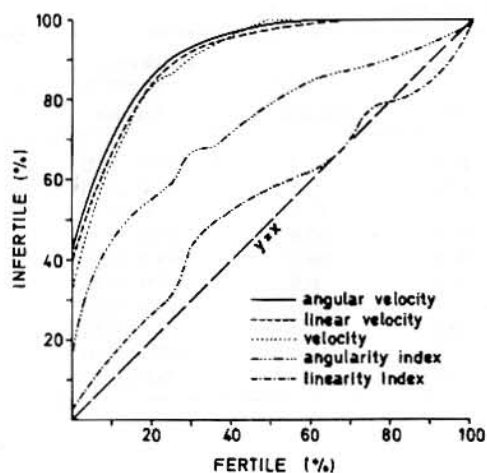


Figure 2 ROC curves of sperm motility parameters of the fertile compared to the subfertile men.

based on the frequency distribution characteristics in semen of subfertile and infertile men.

RESULTS

The median sperm concentration in the subfertile group was $28.6 \times 10^6/\text{ml}$ (5th percentile: $5.1 \times 10^6/\text{ml}$, 95th percentile: $113.3 \times 10^6/\text{ml}$). The median sperm concentration in the fertile group was $95.9 \times 10^6/\text{ml}$ (5th percentile: $37.8 \times 10^6/\text{ml}$, 95th percentile: $284.4 \times 10^6/\text{ml}$).

The ROC curves for the sperm motility parameters in the fertile and subfertile group are shown in Figure 2, and accuracy parameters are listed in Table 1.

Angular and linear velocities have the strongest power to discriminate between semen from the two

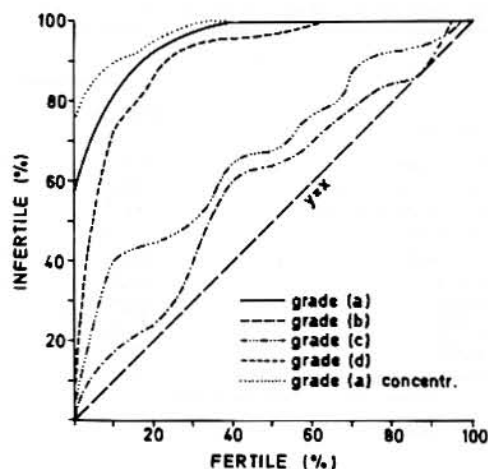


Figure 3 ROC curves of the proportion of different groups of spermatozoa (classified as grade a, b, c, and d) and grade a sperm concentration of the fertile compared to the subfertile men.

groups, followed by velocity. Angularity index has less discriminating power and the linearity index seems to be nondiscriminant.

The ROC curves for the proportion of different categories of sperm in the fertile and subfertile groups are shown in Figure 3, and accuracy parameters are listed in Table 1. The concentration and percentage of spermatozoa with rapid linear progression (grade a spermatozoa) are the best discriminants between semen of the two groups.

The accuracy at different cut-off values of linear velocity in classifying grade a spermatozoa shows that the value of $22 \mu\text{m}/\text{sec}$ is the best in dividing between the two groups. Less accuracy is attained when either higher cut-off values of $25 \mu\text{m}/\text{sec}$ (15% error rate), $30 \mu\text{m}/\text{sec}$ (18% error rate), 35

Table 1 Accuracy of Sperm Motility Parameters and of the Proportion of Different Groups of Spermatozoa and the Concentration of Grade a Sperm in Differentiating Between Semen of Fertile and Subfertile Men

	Criterion value	Sensitivity ^a (%)	Specificity ^a (%)	Error ^b rate (%)
Velocity ($\mu\text{m}/\text{sec}$)	22.7	83	82	17.5
Linear velocity ($\mu\text{m}/\text{sec}$)	20.9	84	81	17.5
Angular velocity ($\mu\text{m}/\text{sec}$)	21.0	85	81	17.0
Linearity index (%)	90.1	45	69	43.0
Angularity index (%)	90.4	66	71	31.5
Grade a (%)	21	89	86	12.5
Grade b (%)	24	61	60	39.5
Grade c (%)	3	41	90	34.5
Grade d (%)	47	88	79	16.5
Grade a concentration ($10^6/\text{ml}$)	9.5	86	95	9.5

^a Sensitivity is the percentage of true fertile classifications; specificity is the percentage of true infertile classifications.

^b Error rate is the percentage false fertile plus the percentage false infertile classifications divided by 2.

Table 2 Description of Sperm Motility Characteristics of Fertile and Subfertile Men

	Fertile			Subfertile		
	Median	(Range)	5P ^a	Median	(Range)	95P ^a
Velocity ($\mu\text{m}/\text{sec}$)	27.2	(18.1–41.9)	18.5	18.8	(10.0–26.2)	25.3
Linear velocity ($\mu\text{m}/\text{sec}$)	24.2	(16.1–37.3)	17.1	16.9	(8.7–26.0)	23.5
Angular velocity ($\mu\text{m}/\text{sec}$)	24.7	(16.4–40.0)	17.0	17.0	(7.6–26.1)	23.7
Linearity index (%)	92.0	(68.4–97.7)	84.4	90.6	(67.6–99.1)	97.3
Angularity index (%)	92.0	(82.1–97.5)	86.5	88.7	(65.2–99.5)	95.4
Grade a (%)	38.0	(13.0–81.0)	15.0	6.5	(0.0–33.0)	27.0
Grade b (%)	22.5	(4.0–49.0)	4.6	25.0	(4.0–72.0)	50.0
Grade c (%)	4.0	(0.0–17.0)	0.0	6.0	(0.0–21.0)	16.0
Grade d (%)	29.0	(10.0–61.0)	15.0	55.0	(18.0–92.0)	79.0
Grade a concentration ($10^6/\text{ml}$)	37.1	(8.9–226.7)	9.4	1.7	(0.0–28.9)	15.0

^a P, percentile.

$\mu\text{m}/\text{sec}$ (21.5% error rate) or a lower cut-off value of 20 $\mu\text{m}/\text{sec}$ (15% error rate) is used. It appears that the cut-off value of linear velocity of 22 $\mu\text{m}/\text{sec}$ is the best in dividing the two groups.

In Table 2, the values of median, range, and 95th percentile of the semen characteristics in the two groups are given. Velocity parameters present a rather narrow range within the groups.

DISCUSSION

In this study, a simple computer-assisted method for objective assessment of sperm motility characteristics was used. Sperm movement is tracked by hand, and data are immediately analyzed without the intermediate step of video or photographic recording. Thanks to this simplification, the system is inexpensive and the time needed to completely evaluate a semen sample is no more than 5 minutes. In comparison with conventional methods, the Autosperm method gives more accurate results. Indeed, with a similar study population but semisubjective sperm motility evaluation,⁹ Comhaire et al.³ recorded a minimal misclassification rate of 17.5% in discriminating between semen of fertile and subfertile men, whereas with the present technology a misclassification rate of only 9.5% is reached.

Until now, none of the reports on objective sperm motility analysis have critically evaluated the diagnostic performance of each parameter as to assessing men's fertility *in vivo*. The ROC curve and associated calculations provide a model for incorporating information from new tests of male gamete function into the diagnostic assessment of infertility. ROC curves analysis may be superior to correlation methods particularly to determine

whether the test results provide clearly dichotomous information, which is required for clinical decision.¹¹

Among the motility characteristics, velocity, linear velocity, and angular velocity are almost as accurate. The indexes have poor or no discriminating ability. This finding is in agreement with results of Mathur et al.¹² showing no significant differences in linearity between the sperm of infertile and fertile men.

The criterion value for discriminating the two groups is similar for linear and angular velocity at 21 $\mu\text{m}/\text{sec}$. This value is identical to that reported in our previous study where we found that grade a and grade b spermatozoa could accurately be discriminated on the basis of a linear velocity ≥ 22 $\mu\text{m}/\text{sec}$.

The present study provides evidence that the proportion and concentration of spermatozoa with rapid linear progression is the best discriminant between semen of subfertile and fertile men. Similar findings have been reported by Aitken et al.,¹³ Holt et al.,⁵ and Mathur et al.,¹² with cut-off values of velocity of 25, 20, and 30 $\mu\text{m}/\text{sec}$, respectively. With our method, it appears that cut-off value of linear velocity of 22 $\mu\text{m}/\text{sec}$ provides the best discrimination. However, this value may not be entirely comparable with the results of others since the method of objective semen analysis may yield different results unless identical parameter settings are used.⁶

The criterion value of the proportion of grade a motile spermatozoa is 21%. This value is close to the World Health Organization criteria for diagnosis of asthenozoospermia, which is suggested 25% of grade a motility.⁹ The concentration of grade a sperm provides 90% accuracy in differentiating be-

tween semen of the two groups. However, this parameter has a relatively wide range contrasting with the swimming speed parameters, which have a more narrow range within each group of subjects. Holt et al.⁵ reported that the mean swimming speeds of spermatozoa in different samples of the same person remain mostly within one standard deviation of each other. Similarly, Katz and Overstreet¹⁴ noted that sperm swimming speed was a relatively stable parameter in contrast to sperm concentration. Hence, combining concentration and motility characteristics may result in less stable values due to fluctuations of sperm concentration. In practice, this could mean that the proportion of grade a spermatozoa and linear or angular velocity might have greater value for distinguishing between fertile and subfertile men.

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