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Diagnostic Value of Sperm Function Tests and Routine Semen Analyses in Fertile and Infertile Men

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The results of routine semen analyses, the zona-free hamster oocyte penetration test, the hypoosmotic swelling test, and semen adenosine triphosphate levels were studied in 66 fertile and 130 infertile men. Multivariate discriminant analysis demonstrated that routine semen parameters including semen volume, sperm count, percent sperm motility, and percent normal spermatozoa in combination could predict the fertility of these patients with 70.4% accuracy. Of the three sperm function tests evaluated, the zona-free hamster oocyte penetration test and the hypoosmotic swelling test were selected by the multivariate discriminant analysis as variables capable of providing significant information on the fertility status of the patients. However, the addition of the results of these two tests to the routine semen analysis did not significantly improve the predictability of fertility. The overall correct prediction rate was 77.6% after incorporation of the results of these two sperm function tests. In this group of subjects, the presently available sperm function tests did not predict the fertility status of a patient with a high degree of accuracy.

Key words: routine semen parameters, sperm function tests, male fertility and infertility, multivariate discriminant analysis.

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Clinicians usually rely on the results of semen analysis for the assessment of male fertility. The parameters monitored in most laboratories, including sperm count, motility, and morphologic features, cannot clearly discriminate between fertile and infertile men. However, multivariate discriminant analyses using these conventional semen parameters showed that sperm concentration and percent normal forms of the sperm head were the variables providing the most significant information discriminating fertile and infertile men (Sherins et al, 1977; Wickings et al, 1983). In recent years, much effort had been directed towards the development of sperm function tests that can assess the functional capacity of human spermatozoa (Blasco, 1984). These sperm function tests included the evaluation of sperm motility (Overstreet et al, 1979), the zona-free hamster oocyte penetration test (Yanagimachi, 1984; Rogers, 1985), semen adenosine triphosphate (ATP) determination (Comhaire et al, 1983), and the hypoosmotic swelling test (Jeyendran et al, 1984). Wickings et al (1983) reported that assessment of sperm fertilizing capacity by the zona-free hamster oocyte penetration test did not contribute additional information

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TABLE 1. Clinical Diagnosis of the Patients

Group	N
Fertile	66
Infertile (total)	171
1. Azoospermia	41
Hypogonadotropic hypogonadism	4
Klinefelter's syndrome	2
Germinal epithelium damage	20
Congenital absence of vas	6
Obstructive azoospermia	9
2. Idiopathic oligoasthenozoospermia	79
3. Sperm autoimmunity	4
4. Varicocele	24
5. Unexplained infertility	23

TABLE 2. Diagnoses of the Female Partners of the Patients

Group	Fertile (N = 66)		Infertile (N = 130)	
Normal	26	(39.4%)	89	(68.5%)
Ovulatory problems	24	(36.4%)	22	(16.9%)
Tubal problems*	9	(13.6%)	8	(6.2%)
Endometriosis	3	(4.5%)	10	(7.7%)
Cervical mucus problems	2	(3.0%)	1	(0.8%)
Corpus luteal defect	2	(3.0%)	0	(0%)

*Patients with bilateral tubal blockage and dysfunction were excluded from the study.

to the diagnosis of infertility. In another study, both quantitative measurement of sperm motility by multiple exposure photomicrography and the zona-free hamster oocyte penetration test were found to be superior to routine semen analysis in identifying infertile patients (Albertsen et al, 1983). Studies by Aitken et al (1984, 1985) showed that, in addition to sperm density and morphology, sperm movement parameters and the results of the zona-free hamster oocyte penetration tests were important factors in predicting the fertility potential of patients with unexplained infertility or oligozoospermia. Although the percentage of swollen spermatozoa after hypoosmotic treatment was higher in fertile than in infertile men (Chan et al, 1985; Van der Ven et al, 1986), the hypoosmotic swelling test cannot clearly distinguish between the two groups because of significant overlapping of values (Chan et al, 1985).

In the present study, we investigated whether these newer sperm function tests would add significant information to traditional semen parameters in assessing the fertility status of patients presenting with infertility.

Materials and Methods

Subjects

We investigated a total of 171 patients presenting with a possible male factor attending the Infertility Clinic of Queen Mary Hospital, University of Hong Kong, from 1984 to 1986. Based on clinical history and examination, serum FSH, LH, prolactin, and testosterone levels and the results of routine semen analysis, the causes of infertility for these patients are listed in Table 1. Forty-one patients had azoospermia due to various causes (Table 1) and were subsequently excluded from the data analyses. Of the remaining 130 infertile patients, a large proportion (n = 79) had idiopathic oligoasthenozoospermia. At the same time, another 66 men defined as fertile were recruited from those infertility clinic patients who had impregnated their wives during or following evaluation of their fertility status. The pregnancies were spontaneous or as a consequence of treatment of the female partners. The diagnoses of the female partners before treatment in the fertile and infertile groups of male patients are detailed in Table 2. The female partners of both groups of patients were seen and assessed by our gynecologists and treated appropriately with correction of all treatable conditions. Patients whose female partners had persistent abnormalities leading to infertility were excluded from the study.

Semen Analyses

Semen samples were collected by masturbation after 2 to 5 days abstinence. Of the 196 subjects (66 fertile and 130 infertile) with spermatozoa in the ejaculate, 123 had more than one sample (two to 11 samples per patient) analyzed. Semen analyses were performed within 2 hours after collection according to standardized methods defined by the World Health Organization (1980). *In vitro* sperm fertilizing capacity was assessed by the standard zona-free hamster oocyte penetration assay as previously described (Chan et al, 1983). Semen ATP concentration was quantitated by the bioluminescent assay (Comhaire et al, 1983; Chan and Wang, 1987). The functional integrity of the sperm membrane was studied by the hypoosmotic swelling technique (Jeyendran et al, 1984; Chan et al, 1985).

Data Analyses

A total of 577 semen samples were collected from the 196 subjects for routine semen analyses and sperm function tests. If a patient provided more than one semen sample, then the mean results for the different tests were used for statistical analyses. Logarithmic transformations of sperm count were used to reduce the skewness of the frequency distribution. Statistical evaluation was performed using the Statistical Package for the Social Sciences (Nie et al, 1979). Comparisons between group means were performed using the Mann–Whitney test. Simple and partial correlation coefficients were calculated for all variables of semen quality. To determine whether any given variable or set of variables could contribute significant information to the prediction of fertility or infertility, the multivariate discriminant analysis using the Rao's method was performed (Nie et al, 1979).

Results

Routine semen analyses were performed in all 196 patients in the present study. However, complete data for all the sperm function tests (semen ATP, zona-free hamster oocyte penetration test, and hypoosmotic swelling test) were available for only 107 (34 fertile and 73 infertile) patients. The initial statistical analyses were based on data obtained from the 107 patients who had complete data for all parameters of semen quality. For both fertile and infertile groups, the mean values for the routine semen parameters and the results of various sperm function tests are shown in Table 3. The mean age and duration of infertility were not significantly different between the two groups. Mean values for sperm concentration, motility, percent normal sperm forms, the penetration rate of the zona-free hamster oocyte penetration test, the percentage swollen spermatozoa after hypoosmotic treatment, and semen ATP concentration were significantly lower in the infertile group.

TABLE 3. Characteristics of the Fertile and Infertile Patients*

Parameter	Fertile (N = 34)	Infertile (N = 73)	Significance
Age (years)	34.3 ± 1.0	34.2 ± 0.6	NS
Duration of infertility (years)	3.0 ± 0.6	3.9 ± 0.3	NS
Semen volume (ml)	2.5 ± 0.2	3.0 ± 0.2	NS
Log_{10} sperm count (10^6/ml)	1.6 ± 0.1	1.3 ± 0.1	$P < 0.001$
% sperm motility (2 hours)	50.6 ± 1.6	36.7 ± 1.7	$P < 0.001$
% normal forms	68.5 ± 1.7	59.8 ± 1.2	$P < 0.001$
Zona-free hamster oocyte penetration rate (%)	19.2 ± 3.6	5.4 ± 1.0	$P < 0.001$
% swollen cells after hypoosmotic treatment	72.4 ± 2.3	56.8 ± 1.8	$P < 0.001$
Semen ATP concentration (10^{-10} mol/ml)	135.0 ± 17.0	83.4 ± 8.1	$P < 0.005$

*Mean ± SEM; NS, not significant ($P > 0.05$).

Linear correlation analysis on all semen parameters in the 107 patients (Table 4) showed significant correlations among sperm concentration, motility, and morphology. Moreover, the results of the zona-free hamster oocyte penetration test and the hypoosmotic swelling test were interrelated and both were correlated with sperm count, motility, and morphology. Semen ATP concentration was also significantly correlated with sperm count, motility, and morphology. There was a positive correlation between semen ATP concentration and the result of the hypoosmotic swelling test, but there was no significant correlation between semen ATP concentration and the result of zona-free hamster oocyte penetration test.

Because the results of the sperm function tests were dependent on the results of conventional semen parameters, the correlation coefficients between the results of these sperm function tests were recalculated after removing the combined effects of the routine semen parameters by partial correlation

TABLE 4. Linear Correlation Coefficients for Different Semen Parameters

Parameter	Semen Volume	Log_{10} Sperm Count (10^6/ml)	% Sperm Motility (2 hours)	% Normal Sperm Forms	% Zona-free Hamster Oocyte Penetration	% Swollen Spermatozoa after Hypoosmotic Treatment
Log_{10} sperm count (10^6/ml)	−0.0247	—	—	—	—	—
% sperm motility (2 hours)	0.0536	0.3286*	—	—	—	—
% normal sperm forms	−0.1334	0.4110*	0.5293*	—	—	—
% zona-free hamster oocyte penetration	−0.2390‡	0.3940*	0.3650*	0.4962*	—	—
% swollen sperm after hypoosmotic treatment	−0.0609	0.1865	0.6020*	0.4057*	0.4121*	—
Semen ATP concentration	−0.0295	0.5410*	0.3320†	0.2042‡	0.1519	0.3696*

*$P < 0.0001$.
†$P < 0.01$.
‡$P < 0.05$.

TABLE 5. Standardized Discriminant Function Coefficients Assigned to the Variables of Semen Quality Selected for their Combined Capacity to Differentiate Between Fertile and Infertile Patients*

Discriminating Variable	Standardized Discriminant Function Coefficient	
	Analysis 1†	Analysis 2‡
Semen volume (ml)	−0.22	−0.39
% sperm motility (2 hours)	0.36	0.79
Log_{10} sperm count (10^6/ml)	0.30	10.67
% zona-free hamster oocyte penetration	0.35	—
% swollen spermatozoa after hypoosmotic treatment	0.44	—

*N = 107.
†Analysis 1: all semen parameters.
‡Analysis 2: routine semen parameters only.

analysis. There was now no significant correlation between semen ATP concentration and the results of the zona-free hamster oocyte penetration test ($r = -0.105$, $P > 0.05$). Significant but weak correlations were observed between the results of the hypoosmotic swelling test and both the semen concentration of ATP ($r = 0.289$, $P < 0.01$) and the zona-free hamster oocyte penetration test ($r = 0.248$), $P < 0.05$).

Multivariate discriminant analysis was used to determine which semen parameters were capable of predicting the fertility status of the 107 patients. This analysis selected five discriminating variables, including semen volume, sperm motility, the log of sperm count, the percent penetration rate in the zona-free hamster oocyte penetration test and the percent swollen cells after hypoosmotic treatment (Table 5; Analysis 1). The percent normal sperm morphology and semen ATP concentration were not selected as significant discriminating factors for predicting fertility status. These five discriminating variables in combination could predict accurately the fertility status of 77.6% of the patients (Table 6; Analysis 1).

TABLE 6. Correct Classification of the Fertility Status of Patients According to the Discriminating Variables Listed in Table 5*

Actual Group Membership	Predicted Group Membership			
	Analysis 1†		Analysis 2‡	
Fertile (n = 34)	25	(73.5%)	26	(76.5%)
Infertile (n = 73)	58	(79.5%)	52	(71.2%)
Overall correctly classified	83	(77.6%)	78	(72.9%)

*N = 107.
†Analysis 1: all semen parameters.
‡Analysis 2: routine semen parameters only.

TABLE 7. Standardized Discriminant Function Coefficients Assigned to the Routine Semen Parameters Selected for Their Combined Capacity to Differentiate Between Fertile and Infertile Patients*

Discriminating Variable	Standardized Discriminant Function Coefficient
Semen volume	−0.18
% sperm motility (2 hours)	0.64
% normal sperm forms	0.22
Log_{10} sperm count (10^6/ml)	0.41

*N = 196.

The significance of using routine semen parameters alone to predict the fertility status of the 107 patients was then retested by the multivariate discriminant analysis. Semen volume, percent sperm motility, and the log of sperm count were selected as capable of discriminating fertile from infertile patients (Table 5; Analysis 2). These variables in combination could accurately predict the fertility status in 72.9% of the patients (Table 6; Analysis 2).

Multivariate discriminant analyses were then used to study the whole group of 196 patients to determine which routine semen parameters were of discriminating value in predicting their fertility status. All four routine semen parameters had significant discriminating value (Table 7). The most powerful discriminating factor was sperm motility and the least powerful was semen volume. These four discriminating factors in combination could accurately predict the fertility status of 70.4% of the patients (Table 8).

TABLE 8. Classification of the Fertility Status of Patients According to the Discriminating Variables Listed in Table 7*

Actual Group Membership (N = 196)	Predicted Group Membership			
	Fertile		Infertile	
Fertile (N = 66)	51	(77.3%)	15	(22.7%)
Infertile (N = 130)	43	(33.1%)	87	(66.9%)

*Overall percentage of grouped cases correctly classified: 138/196 × 100% = 70.4%.

Chi-square analysis demonstrated that the rates for correctly predicting the fertility status of a patient for the discriminating variables listed in Table 5 and those listed in Table 7 were not significantly different from each other ($P > 0.05$).

Discussion

The results of the present study of 66 fertile and 130 infertile patients indicate that when only routine semen parameters were evaluated by multivariate discriminant analysis, semen volume, sperm count, motility, and morphology were useful in predicting the fertility status correctly in 70.4% of the subjects (Tables 7, 8). The importance of sperm concentration and morphology have been previously reported (Sherins et al, 1977; Wickings et al, 1983). Furthermore, as in our present study, Wickings et al (1983) found significant correlations between sperm count and motility and the results of the zona-free hamster oocyte penetration test in oligozoospermic men. However, Aitken et al (1984) and Irvine and Aitken (1985, 1986) observed that the conventional semen parameters *per se* were not of significant value in discriminating the fertility potential of patient populations characterized by normal semen profiles, such as in couples with unexplained infertility and in semen donors from an artificial insemination program. The high proportion of study subjects with oligoasthenozoospermia (60.8%; Table 1) in our analysis would favor multivariate discriminant analysis for selecting the routine semen parameters having discriminatory value.

The differences between the present and some previous studies could be due to the selection of patients and also the variations in research methodology. In the present study, 123 of the total 196 patients (62.7%) had provided more than one semen sample for analysis, and the mean values for various parameters of semen quality, including sperm function tests, were used for the multivariate discriminant analysis. This could have reduced the random error in the analysis caused by variations in semen quality (Poland et al, 1985; Bostofte et al, 1987).

Another problem of this study was that a large number of the female partners (60.6%) in the fertile group presented with treatable gynecologic problems, whereas a smaller proportion (31.5%) had such problems in the infertile group (Table 2). The variation in the pattern and the disease of the female partner and its effect on the diagnosis of infertility have been well recognized (Silber and Cohen, 1983; Steinberger and Rodriguez-Rigau, 1983). Furthermore, although all treatable causes in the female partners had been corrected clinically and patients with female partners with persistent abnormalities were excluded from the study, the effect of variations in the fertility status of the female partner cannot be adequately controlled in retrospective studies of this nature.

It was determined by a partial correlation analysis that semen ATP concentration was significantly correlated with sperm count, motility, morphology, and sperm swelling after hypoosmotic treatment. These findings are comparable to those of our previous report (Chan and Wang, 1987). In the present study, similar to the findings of Irvine and Aitken, (1985, 1986), semen ATP measurement was not selected by the multivariate discriminant analysis as a factor useful in predicting the fertility status of patients.

The diagnostic value of the zona-free hamster oocyte penetration test in discriminating fertile and infertile men in cases of unexplained infertility and in oligozoospermia has been recognized (Aitken et al, 1984; Aitken, 1985). Recently, Van der Ven et al (1986) reported a strong relationship between the results of the hypoosmotic swelling test and the performance of spermatozoa in a human *in vitro* fertilization (IVF) program, but no multivariate discriminant analysis was performed to determine whether the sperm swelling would significantly predict the success of IVF. In the present study, both these sperm function tests were selected by the multivariate discriminant analysis as variables capable of providing significant information for determining the fertility status of the patients. However, incorporation of the results of these two tests into the multivariate discriminant analysis did not significantly improve the prediction of fertility when compared with using only the mean results of multiple routine semen analyses. This was a consequence of the dependence of these two sperm function tests on the routine semen parameters (e.g., sperm concentration, motility, and morphology) demonstrated in the present study. The inability to improve significantly the predictability of fertility status by the sperm function tests performed in the present study may indicate that there are other sperm factors that should be assessed. The highest discrimination rate for male fertility status ever reported in the literature was that of Irvine and Aitken (1986), who achieved an overall correct prediction rate of 81.3% for the *in vivo* fertility potentials of cryopreserved ejaculates used in an artificial insemination program. The discrimi-

nating variables selected by the multivariate discriminant analysis in their study included not only the concentration of seminal motile spermatozoa and the result of the zona-free hamster egg penetration test by human spermatozoa after calcium ionophore treatment, but also the sperm movement parameters of capacitated spermatozoa.

In conclusion, the results of the present study indicate that the zona-free hamster egg penetration test and the hypoosmotic swelling test in our study population do not add significant information in discriminating the fertility status of infertility patients in addition to the averaged results of multiple routine semen analyses. It remains to be investigated whether the results of these sperm function tests could be useful for this purpose if further improvements are made in their quality and sensitivity. Our results support the notion of Irvine (1986) that the present generation of sperm tests may not accurately predict the fertility status of a patient presenting to the infertility clinic. The development of another generation of tests may provide diagnostic information with greater precision and accuracy.

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