Prediction of the in-vitro fertilization (IVF) potential of human spermatozoa using sperm function tests: the effect of the delay between testing and IVF

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To examine the diagnostic significance of several criteria of semen quality and to determine whether their prognostic value is eroded by the time interval between assessment and the attempt at in-vitro fertilization (IVF) with embryo transfer, 73 couples undergoing IVF and embryo transfer therapy were studied. The ability of human spermatozoa to achieve fertilization in vitro was examined in relation to the conventional semen profile, sperm morphology, the computer-aided assessment of sperm movement, ionophore-induced acrosome reaction, acridine orange staining, and chemiluminescent signals induced by phorbol ester and N-formyl-methionyl-leucyl-phenylalanine (FMLP). Spermatozoa were examined both in semen and after preparation on Percoll, some weeks prior to IVF. Fertilization rates were noted to be significantly correlated with elements of sperm movement characteristics, sperm morphology, and reactive oxygen species generation. Prediction of fertilization rates in a stepwise multiple regression analysis was obtained using four variables: sperm morphology, FMLP-induced chemi-luminescence and sperm movement characteristics (beat cross frequency and straightness) (r = -0.5). When multiple logistic regression analysis was used to predict which samples would achieve fertilization rates above and below a 50% threshold, three variables of predictive value including linearity, average path velocity and FMLP-induced chemiluminescence were selected. Combination of these variables classified the samples achieving good or poor fertilization with an overall accuracy of 83.6%. The time interval between semen assessment and IVF had little effect on the predictive value of these tests. In conclusion, the fertilizing ability of human spermatozoa is related to sperm morphology, attributes of sperm movement and reactive oxygen species production. The time delay between testing and IVF did not appear to affect predictive accuracy.

Key words: acrosome reaction/in-vitro fertilization/movement characteristics/reactive oxygen species/sperm morphology

Introduction

Techniques of assisted conception have come to play a major role in the treatment of couples with male factor infertility. In the most recent report of the UK's Human Fertilization and Embryology Authority, it was notable that there was a male factor present in 9890 of 14 606 (67.7%) in-vitro fertilization (IVF) cycles undertaken in the UK in 1992. Since poor semen quality is almost inevitably associated with lower fertilization rates (Balmaceda et al., 1993; Irvine, 1994), the prediction of successful fertilization in vitro remains one of the major challenges to the andrology laboratory. The diagnosis of defective sperm function is difficult because the spermatozoon is a highly-specialized cell that must express a diverse array of biological properties, including elements of sperm movement, zona recognition, signal transduction, exocytosis and cell fusion, in order to achieve its ultimate goal of fertilizing the oocyte. Thus, failure of fertilization could be due to defects in any one of a number of different aspects of sperm physiology. Characterization of the nature of the abnormalities present in dysfunctional human spermatozoa therefore necessitates the use of an integrated battery of techniques, each component of which focuses on a different aspect of sperm cell biology. The diagnosis of defective sperm function has been facilitated by the recent development of improved diagnostic tests including assessments of sperm morphology (Kruger et al., 1988), sperm movement including hyperactivation (Burkman, 1991), the acrosome reaction (Aitken and Brindle, 1993), sperm-oocyte fusion (Aitken, 1994), sperm-zona binding and penetration (Liu and Baker, 1994), nuclear DNA normality (Tejada et al., 1984) and biochemical assays for monitoring the generation of reactive oxygen species by human spermatozoa and contaminating leukocytes (Aitken et al., 1992a).

By using combinations of such assays, several investigators have attempted to predict the outcome of IVF with varying success (Kruger et al., 1988; Liu et al., 1988; Liu and Baker, 1994; Sukcharoen et al., 1995). Many such studies have attempted to address the relationship between tests of semen quality and fertilizing potential in vitro by focusing on the same semen sample used for the IVF and embryo transfer attempt (De Geyter et al., 1992; Franken et al., 1993; Sukcharoen et al., 1995). This approach minimizes the effects of inter-ejaculate variability (Schwartz et al., 1979) and optimizes the quality of the predictive data with respect to the functional tests under consideration. However, in clinical practice, there is a need to provide patients with advice on the likely outcome of fertilization in IVF to be undertaken at some time in the future. Comparatively few studies have examined the predictive value of functional tests in this context (Aitken et al., 1987;
Soffer et al., 1992). The purposes of this study were therefore to: (i) examine a combination of sperm function tests assessed prior to IVF treatment in predicting the subsequent fertilizing capacity of human spermatozoa in vitro; and (ii) examine whether the time gap between functional testing and subsequent IVF eroded the predictive value of the tests.

Materials and methods

Study population

The study population consisted of a cohort of 73 unselected patients undergoing semen analysis and sperm function testing at the time of an initial consultation with the assisted conception programme at the Simpson Memorial Maternity Pavilion, Edinburgh, UK, between November 1993 and May 1994. The indications for IVF were unexplained infertility (n = 23, 31.5%), bilateral tubal occlusion (n = 23, 31.5%), male infertility (n = 19, 26.0%), and endometriosis (n = 8, 11%). Semen samples were collected by masturbation into sterile plastic containers following a 2 day period of sexual abstinence. The semen was then assessed according to the World Health Organization guidelines (WHO, 1992) with regard to the sperm concentration, motility and morphology. For this cohort of patients, the constituents of the conventional semen profile, expressed as mean ± SEM were: volume 3.0 ± 0.1 ml; sperm concentration 68.8 ± 9.5×10⁶ spermatozoa/ml; normal morphology, 45.4 ± 1.9% and motility, 53.4 ± 2.4%.

Sperm preparation

The spermatozoa were then separated from seminal plasma by centrifugation on a discontinuous Percoll gradient (Pharmacia, Uppsal, Sweden), using a two-step gradient comprising 3 ml layers of 40 and 80% Percoll respectively (Aitken et al., 1993). Isotonic Percoll was produced by supplementing 10 ml of 10× concentrated medium 199 (Flow Laboratories, Irvine, UK) with 300 mg human or bovine serum albumin (BSA; Armour Pharmaceutical Co., Eastbourne, UK), 3 mg sodium pyruvate and 0.37 ml of a sodium lactate syrup followed by the addition of 90 ml of Percoll. This preparation was designated 100% Percoll (Aitken et al., 1993) and was subsequently diluted with HEPES-buffered Biggers-Whitten-Whittingham (BWW) medium (Biggers et al., 1971). Semen was placed on the top of the gradient and centrifuged at 500 g for 20 min. The spermatozoa at the base of the 80% fraction were collected and washed with a 5 ml volume of BWW medium and finally resuspended in BWW at a concentration of 20×10⁶/ml and used for the following assays of sperm function.

Tests of sperm function

Sperm morphology

Sperm morphology was assessed in both semen and in the 80% Percoll fraction using high quality phase-contrast optics on wet parameters at ×500 magnification and the scoring criteria laid down by the World Health Organization (WHO, 1992)

Acrosome reaction

The isolated spermatozoa in the 80% Percoll fraction were diluted 1:1 with the ionophore A23187, formulated as the free acid and prepared as a 100 mM stock solution in dimethylsulphoxide that was diluted to the working concentration (5 μM to give a final concentration of 2.5 μM) on 1:1 dilution with spermatozoa) immediately before the initiation of each experiment. After 3 h incubation with A23187 at 37°C in an atmosphere of 5% CO₂ in air, the acrosome reaction was assessed. The protocol developed for assessing the acrosome reaction involved the use of a detection reagent targeting the acrosomal region of the sperm head, in conjunction with the hypo-osmotic swelling test to monitor sperm viability (Aitken et al., 1993).

Sperm movement characteristics

The percentage of motile spermatozoa was assessed manually in both semen and the 80% Percoll fraction at ×100 magnification with the aid of a grid on an eye-piece graticule. The percentage of motile spermatozoa and the attributes of sperm movement were also assessed in both semen and the 80% Percoll fraction 1 h after the spermatozoa had been prepared using the European (25 Hz) version of the Hamilton-Thorn motility analyser (HTMA model 2030, Version 7; Hamilton-Thorn Research, Denver, MA, USA) at a temperature of 37°C using the following settings: minimum contrast, 12; minimum size, 3; low and high size gates, 0.4 and 1.6 respectively; low and high intensity gates, 0.5 and 2.0 respectively; non-motile head size, 8; non-motile head intensity, 201. The measurements were conducted in 200 μm deep flat capillary tubes (Camlab, Cambridge, UK) and at least 100 motile cells were assessed for each determination. These determinations were carried out in duplicate and the results were averaged. The criteria of movement assessed in this study were: curvilinear velocity (VCL); straight line velocity (VSL); average path velocity (VAP); percentage motile (the percentage of cells exhibiting a VAP of ≥10 μm/s); percentage rapid (the percentage of cell exhibiting a VAP of ≥25 μm/s); the beat cross frequency (BCF) in Hz and the amplitude of lateral sperm head displacement (ALH) in μm. Linearity (LIN) was defined as VSL/VCL×100, while straightness (STR) was VSL/VAP×100. Percentage progressive motility equated with a STR of >75%. The playback function of the HTMA was used to verify the validity of the cell identification process and minor adjustments were made to the analyser settings when necessary.

Acridine Orange staining

Sperm nuclear normality was assessed in the 80% Percoll fraction by the Acridine Orange fluorescence method (Tejada et al., 1984). The spermatozoa were smeared onto precleaned microscope slides and allowed to air-dry for 20 min. The smears were then fixed overnight in Carnoy's solution (3 parts of methanol and 1 part glacial acetic acid) which was prepared daily. Following fixation, the slides were allowed to dry for a few minutes before staining with Acridine Orange (Sigma, St Louis, MO,- USA). The Acridine Orange staining solution was prepared daily as follows: 10 ml of 1% Acridine Orange in distilled water was added to a mixture comprising 40 ml of 0.1 M citric acid and 2.5 ml of 0.3 M Na₂HPO₄·7H₂O, pH 2.5. The sperm smears were stained for 5 min, gently rinsed and mounted with distilled water. The slides were evaluated on the same day using a fluorescence microscope (Leitz, Oberkochen, Germany) equipped with a 490 nm excitation filter and 530 nm barrier filter. A total of 300 cells was counted on each slide and the duration of microscopic evaluation did not exceed 40 s per field. Spermatozoa with a normal DNA content exhibited green fluorescence over the head region while abnormalities of the DNA content were indicated by a spectrum of fluorescence varying from yellow-green to red.

Reactive oxygen species

The measurement of reactive oxygen species was carried out on a Berhold LB 9505 luminometer (Berhold, Wildbad, Germany) at a chamber temperature of 37°C over a total time period of 30 min. A 400 μl aliquot of the sperm suspension at 10×10⁶/ml was incubated with 25 μM luminol (5-amino-2,3 dehydro-1,4 phthalazine-dione; Sigma) supplemented with 12.4 IU horseradish peroxidase (Type VI, 310 IU/mg; Sigma) for 6 months to sensitize the assay for the
generation of extracellular hydrogen peroxide (Aitken et al., 1992b). After allowing 6 min to capture this basal luminal-dependent signal, the cells were stimulated with N-formyl-methionyl-leucyl-phenylalanine (FMLP) and monitored for an additional 7 min to determine the magnitude of the peak chemiluminescent response and allow the system to return to baseline. The cell suspensions were then stimulated with 100 nM 12-myristate, 13-acetate phorbol ester (PMA) and monitored for 7 min to assess the residual capacity of the cell population for reactive oxygen species generation (Aitken et al., 1992b). The basal response and the response to FMLP and PMA were recorded as the integration of chemiluminescence counts over a 5 min observation period.

**In-vitro fertilization**

Ovarian stimulation consisted of the following regimen: pituitary down-regulation was achieved with a GnRH agonist (Suprefact, buserelin; Hoechst, Middlesex, UK) starting on day 1 of menstrual cycle. When ovarian suppression was documented by the absence of ovarian follicles and attenuation of the endometrial lining by ultrasound evaluation, ovulation was initiated with human menopausal gonadotrophin (HMG, Pergonal; Serono Laboratories, Herts, UK or Humegon; Organon Laboratories, Cambridge, UK). Changes in gonadotrophin dosage were based on follicular development as reflected by changes in follicular diameter and number assessed by serial transvaginal ultrasonography. Human chorionic gonadotrophin (HCG, Profasi; Serono Laboratories), 5000 IU, was administered when at least three leading follicles reached a mean diameter of at least 16 mm. Oocyte retrieval was performed 34 h after the HCG injection, using transvaginal ultrasound. The oocytes were assessed according to the criteria described by Veeck (1988). Grade I oocytes were excluded from the study. To provide a reliable assessment of sperm fertilizing potential, only female patients who had four or more grade II–III oocytes collected were included in this study.

**Semen samples and semen preparation for IVF therapy**

At 1 h before the scheduled time of oocyte retrieval, a semen sample was collected by the male partner by masturbation into sterile containers. After liquefaction, routine semen analyses were performed (WHO, 1992). The semen samples were then prepared by two-layer (40 and 80%) discontinuous Percoll separation, after which sperm pellet was gently overlaid with Earle’s medium (Flow Laboratories) supplemented with 10% human serum albumin (4.5% human albumin solution; Immuno AG, Vienna, Austria). After 1 h incubation, the upper layer of culture medium containing the motile spermatozoa was used for oocyte insemination; 1–3 h after oocyte retrieval, 100 µl of sperm suspension containing ∼100,000 motile spermatozoa was added to 750 µl of medium containing up to four ova to give a final sperm concentration of ∼120,000 spermatozoa/ml. This insemination concentration was uniform for all patients and was not affected by the outcome of the sperm function tests. Fertilization of the oocytes was assessed after 20–22 h incubation at 37°C in an atmosphere of 5% CO₂ in air.

**Statistical analysis**

Five cases were excluded from the analysis because fewer than four oocytes were collected. Data are generally presented as mean ± SEM. The data were first examined by inspection of frequency distribution plots and calculations of kurtosis and was normalized, where appropriate, by log transformation (reactive oxygen species chemiluminescence data). The data were weighted by the number of eggs inseminated and analysed using linear and stepwise multiple regression analysis (SPSS for Windows v6.0; SPSS Inc., Chicago, IL, USA). Stepwise regression analysis identified the optimum combination of independent variables (sperm function tests) that can be used to predict the dependent variable (fertilization rate). Cluster analysis was then used to subdivide the fertilization rate data into 'poor' and 'good' fertilization groups, and multiple logistic regression analysis used to examine which variables could distinguish between samples achieving poor or good fertilization. P < 0.05 was considered to be significant.

**Ethics**

This study was approved by the Paediatrics/Reproductive Medicine Research Ethics Subcommittee of Lothian Health, UK.

**Results**

**Outcome of IVF**

From the 73 couples studied, the mean number of grade II–III oocytes retrieved and inseminated was 9.7 ± 0.7 (range 1–23) while the mean value of the fertilization rate was 61.8 ± 3.5% (0–100%). The mean time-lapse from semen assessment to oocyte retrieval was 137.3 ± 11.0 days (30–372 days). In all subsequent analyses, the five cases in which fewer than four oocytes were collected were omitted.

**Sperm function test results**

**Simple linear regression analysis**

When the relationship between the observed fertilization rate and the conventional semen profile was examined, significant correlations were observed with several parameters, including sperm concentration in semen (r = 0.215; P < 0.001), and the percentage of morphologically normal spermatozoa in semen (r = 0.335; P = 0.001). Several attributes of semen quality measured on the 80% Percoll fraction were also found to be significantly positively correlated with the fertilizing ability of the spermatozoa in vitro, including the percentage of morphologically normal spermatozoa (r = 0.333; P = 0.001), the hypo-osmotic swelling test (r = 0.203; P < 0.001), ALH (r = 0.244; P < 0.001) and the percentage rapid cells (r = 0.198, P < 0.001); negative correlations were observed with STR (r = -0.276; P < 0.001), and LIN (r = -0.257; P < 0.001). Of the chemiluminescence measurements recorded in this study, significant negative correlations were observed with the basal levels of reactive oxygen species generation following stimulation with both FMLP and PMA (r = -0.304; P < 0.001) and phorbol ester (r = -0.276; P < 0.001).

**Stepwise multiple regression analysis**

In order to determine whether a combination of variables describing different attributes of the semen and 80% Percoll fraction could account for the observed variation in fertilization rates, a stepwise multiple regression analysis, weighted by oocyte number, was performed. A multiple regression equation was generated that gave a multiple regression coefficient of r = 0.51 (r² = 0.261) for the relationship between gamete quality and fertilization rate in vitro. This analysis was based on four variables (Table I) comprising (in the order in which they were entered into the equation) the percentage of morphologically normal spermatozoa in semen, STR in 80% Percoll
function and the fertilizing ability of spermatozoa in human IVF (De Geyter et al., 1992; Franken et al., 1993; Sukcharoen et al., 1995). Most often, these studies have examined spermatozoa at the time of the IVF attempt, maximizing the chance of revealing meaningful relationships between gamete quality and IVF outcome. Whilst this is important in examining relationships between in-vitro bioassays and function, in clinical practice there is a need to predict the outcome of a treatment in advance of that treatment being undertaken. In the present study, spermatozoa in semen and following sperm preparation by discontinuous Percoll separation were assessed some time prior to the IVF attempt to determine which attributes of sperm function were related to outcome, and to examine whether the time lapse between semen assessment and subsequent treatment was related to the predictive value of the tests in question. In previous studies involving the zona-free hamster oocyte penetration test, we have observed that the relationship between the functional bioassay and IVF outcome is excellent when performed on the same sample as is used for IVF, but less good when performed on a previous semen sample from the same patient (Aitken et al., 1987).

It was observed that several different attributes of gamete quality were significantly correlated with the fertilizing potential of a subsequent ejaculate. In particular, the percentage of normal morphology, both in semen and after separation on 80% Percoll, was correlated with subsequent fertilization, in support of several previous studies affirming the diagnostic significance of this criterion (Jeulin et al., 1986; Kruger et al., 1986, 1988; Liu et al., 1988; Sukcharoen et al., 1995). Furthermore, several sperm movement characteristics, particularly in the 80% Percoll fraction, including STR, ALH and LIN were negatively correlated (Liu et al., 1986), while STR and LIN were negatively correlated (Liu et al., 1991) with the fertilization rates. Importantly, there were significant negative correlations between fertilization rates and the generation of reactive oxygen species, both in the basal state, and following stimulation with FMLP and PMA, confirming previous findings (Sukcharoen et al., 1995).

When stepwise multiple regression analysis was used, some 26% of the observed variation in fertilization rates could be explained by the combination of sperm morphology in semen, BCF in semen, STR in the 80% Percoll fraction and FMLP-induced chemiluminescence (Table I). The most important variable in the group was STR in 80% Percoll fraction, FMLP-induced chemiluminescence and BCF in semen (Table I). In order to examine the impact of the time delay between the assessment of semen quality and subsequent IVF with embryo transfer, the residual values (the difference between observed and predicted fertilization rates in the above analysis) were related to the time delay. No significant relationship was observed ($r = 0.147, P = 0.234$), suggesting that the time delay between sperm assessment and IVF treatment is not of major importance.

**Multiple logistic regression analysis**

Fertilization rate data were classified into two groups by using K-means cluster analysis. This analysis resulted in a threshold of 50% fertilization rate being selected as a division between ‘poor’ and ‘good’ fertilization rates. Stepwise multiple logistic regression analysis was then used to examine which variables were of value in distinguishing between samples achieving more or less than 50% fertilization of oocytes. Using this approach, LIN in the 80% Percoll fraction, VAP in semen and levels of reactive oxygen species generation observed following stimulation with FMLP were selected as being of value in predicting samples with good or poor fertilizing capacity (Table II). These three parameters correctly classified 76% of samples with poor fertilization and 88.1% of samples with good fertilization; the overall predictive accuracy was 83.6% ($\chi^2 = 27.774, P < 0.0001$) (Table III).

**Discussion**

Several previous studies have examined the relationships that exist between various attributes of semen quality or sperm fraction, FMLP-induced chemiluminescence and BCF in semen (Table I). In order to examine the impact of the time delay between the assessment of semen quality and subsequent IVF with embryo transfer, the residual values (the difference between observed and predicted fertilization rates in the above analysis) were related to the time delay. No significant relationship was observed ($r = 0.147, P = 0.234$), suggesting that the time delay between sperm assessment and IVF treatment is not of major importance.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Regression coefficient</th>
<th>Standard coefficient</th>
</tr>
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<tbody>
<tr>
<td>Intercept</td>
<td>130.306</td>
<td>0.251</td>
</tr>
<tr>
<td>Normal sperm morphology in semen</td>
<td>0.417</td>
<td>0.215</td>
</tr>
<tr>
<td>BCF in semen</td>
<td>1.590</td>
<td>0.174</td>
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<tr>
<td>STR in 80% Percoll fraction</td>
<td>-0.634</td>
<td>-0.285</td>
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<tr>
<td>Chemiluminescence induced by FMLP in 80% Percoll fraction</td>
<td>-0.58</td>
<td>-0.219</td>
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BCF = beat cross frequency; STR = straightness; FMLP = N-formyl-methionyl-leucyl-phenylalanine.

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<tr>
<td>Intercept</td>
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<td>-0.321</td>
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<tr>
<td>LIN in 80% Percoll fraction</td>
<td>-0.92</td>
<td>-0.181</td>
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<tr>
<td>Chemiluminescence induced by FMLP in 80% Percoll fraction</td>
<td>-1.176</td>
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<tr>
<td>VAP in semen</td>
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LIN = linearity; VAP = average path velocity; FMLP = N-formyl-methionyl-leucyl-phenylalanine.

It was observed that several different attributes of gamete quality were significantly correlated with the fertilizing potential of a subsequent ejaculate. In particular, the percentage of normal morphology, both in semen and after separation on 80% Percoll, was correlated with subsequent fertilization, in support of several previous studies affirming the diagnostic significance of this criterion (Jeulin et al., 1986; Kruger et al., 1986, 1988; Liu et al., 1988; Sukcharoen et al., 1995). Furthermore, several sperm movement characteristics, particularly in the 80% Percoll fraction, including STR, ALH and LIN were significantly related to the fertilization rate in vitro. In agreement with previous studies, ALH was significantly positively correlated with fertilization rates (Jeulin et al., 1986), while STR and LIN were negatively correlated (Liu et al., 1991) with the fertilization rates. Importantly, there were significant negative correlations between fertilization rates and the generation of reactive oxygen species, both in the basal state, and following stimulation with FMLP and PMA, confirming previous findings (Sukcharoen et al., 1995).

When stepwise multiple regression analysis was used, some 26% of the observed variation in fertilization rates could be explained by the combination of sperm morphology in semen, BCF in semen, STR in the 80% Percoll fraction and FMLP-induced chemiluminescence (Table I). The most important variable in the group was STR in 80% Percoll fraction, FMLP-induced chemiluminescence and BCF in semen (Table I). In order to examine the impact of the time delay between the assessment of semen quality and subsequent IVF with embryo transfer, the residual values (the difference between observed and predicted fertilization rates in the above analysis) were related to the time delay. No significant relationship was observed ($r = 0.147, P = 0.234$), suggesting that the time delay between sperm assessment and IVF treatment is not of major importance.

**Multiple logistic regression analysis**

Fertilization rate data were classified into two groups by using K-means cluster analysis. This analysis resulted in a threshold of 50% fertilization rate being selected as a division between ‘poor’ and ‘good’ fertilization rates. Stepwise multiple logistic regression analysis was then used to examine which variables were of value in distinguishing between samples achieving more or less than 50% fertilization of oocytes. Using this approach, LIN in the 80% Percoll fraction, VAP in semen and levels of reactive oxygen species generation observed following stimulation with FMLP were selected as being of value in predicting samples with good or poor fertilizing capacity (Table II). These three parameters correctly classified 76% of samples with poor fertilization and 88.1% of samples with good fertilization; the overall predictive accuracy was 83.6% ($\chi^2 = 27.774, P < 0.0001$) (Table III).

| Table I. Multiple regression analysis of the relationship between the quality of semen and the 80% Percoll fraction with subsequent fertilization rates in vitro |
|----------------|-----------------|-------------------|
| Variables       | Regression coefficient | Standard coefficient |
| Intercept       | 130.306          | 0.251             |
| Normal sperm morphology in semen | 0.417 | 0.215 |
| BCF in semen    | 1.590            | 0.174             |
| STR in 80% Percoll fraction | -0.634 | -0.285 |
| Chemiluminescence induced by FMLP in 80% Percoll fraction | -0.58 | -0.219 |

BCF = beat cross frequency; STR = straightness; FMLP = N-formyl-methionyl-leucyl-phenylalanine.

| Table II. Multiple logistic regression analysis of the quality of human semen and the 80% Percoll fraction in prediction of ‘good’ (≥50%) and ‘poor’ (<50%) fertilization in vitro |
|----------------|-----------------|-------------------|
| Variables       | Regression coefficient | Standard coefficient |
| Intercept       | 10.703           | -0.321            |
| LIN in 80% Percoll fraction | -0.92 | -0.181 |
| Chemiluminescence induced by FMLP in 80% Percoll fraction | -1.176 | -0.181 |
| VAP in semen    | 0.092            | 0.135             |

LIN = linearity; VAP = average path velocity; FMLP = N-formyl-methionyl-leucyl-phenylalanine.

| Table III. Classification of semen samples into ‘good’ and ‘poor’ fertilization rates based on variables listed in Table II. Values in parentheses are percentages |
|----------------|-----------------|-------------------|
| Actual fertilization group | Predicted fertilization group |
| Poor (n = 25) | 19 (76.0) | 6 (24.0) |
| Good (n = 42) | 5 (11.9) | 37 (88.1) |

*Good ≥50%; poor <50%.

bOverall correct prediction rate = 83.6%.

References

De Geyter et al., 1992
Franken et al., 1993
Sukcharoen et al., 1995

followed by the percentage normal morphology and FMLP-induced chemiluminescence. In a previous study, a number of variables measured on the inseminated sperm suspension were found to be significantly related to the fertilization rate in vitro obtained with the same sample, particularly sperm morphology, leukocyte contamination, acrosome reaction, and sperm movement characteristics (Sukcharoen et al., 1995). The significance of these attributes of semen quality were confirmed in the present study with the exception of acrosomal status which was not found to be related to outcome. Consistently, the percentage of normal forms and FMLP-induced chemiluminescence have been found to be significant predictors of sperm function, whether measured on the same sample as used for IVF (Sukcharoen et al., 1995) or on a previous ejaculate from the same patient. Given the problems of intra-observer, inter-observer and inter-laboratory variations known to occur in the assessment of sperm morphology (Newminger et al., 1990), the determination of leukocyte contamination with the FMLP provocation test and the objective assessment of sperm movement may represent more robust means by which to predict the fertilizing potential of human spermatozoa in vitro.

In summary, we have presented data showing that the outcome of subsequent IVF can be predicted by semen analysis and sperm function tests undertaken prior to IVF therapy. The model for this prediction of poor fertilization outcome had a sensitivity of 76% and thus might be useful in identifying those couples who are likely to require micro-assisted fertilization techniques. Therefore the application of these tests may permit counselling and selection of appropriate therapeutic options prior to the start of the first IVF cycle. However, such models should be tested in a prospective manner to properly assess their applicability.

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References


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