Sperm Movement Characteristic in Prediction of in Vitro Fertilization

Nares Sukcharoen MD,*
John Keith PhD.**

* *Department of Obstetrics and Gynaecology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.* ** *Assisted Conception Unit, Simpson Memorial Maternity Pavilion, Edinburgh, U.K.*

Abstract : *To determine the relationship between the in vitro fertilization rates and sperm movement characteristics, inseminated sperm suspensions used for in vitro fertilization in 125 patients were assessed using the Hamilton Thorn Motility Analyzer version 10 (HTMA-IVOS). Several sperm movement characteristics of the inseminated sperm suspensions were found to be weakly significantly correlated with the fertilization rate in vitro, such as, straightness (STR) ($r = -0.1285$; $P < 0.0001$), linearity (LIN) ($r = -0.1188$; $P < 0.0001$), amplitude of lateral head displacement (ALH) ($r = 0.0938$; $P < 0.005$), curvilinear velocity (VCL) ($r = 0.0607$; $P < 0.05$) and percentage of rapid motility ($r = 0.0601$; $P < 0.05$). With stepwise regression analysis of all data, poor prediction of fertilization rates ($R = 0.217$) was obtained using a multiple regression equation incorporating 2 variables, including percentage of sperm motility and STR in inseminated sperm suspension. Comparison of sperm preparations giving poor with good in vitro fertilization rates revealed only a significant difference of STR in the inseminated sperm suspensions between both groups ($p = 0.0339$). The STR in the inseminated sperm suspension was the most significant factor negatively related to fertilization rate in vitro. However, the overall predictivity of sperm movement characteristics for in vitro fertilization was still poor. In conclusion, the objective measurement of the sperm movement characteristics by HTMA has no practical value in predicting fertilization rate in vitro. (Thai J Obstet Gynaecol 1995;7:113-122)*

Short title : Sperm movement characteristic and in vitro fertilization

Key words : sperm movement characteristic, conventional semen analysis, in vitro fertilization

The sperm motility has long been recognized as an important functional characteristic for evaluating the fertility potential of spermatozoa because it is necessary not only for sperm penetration through cumulus

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and zona pellucida but also in sperm-egg interaction. The measurement and analysis of sperm motion are technologically challenging. Sperm cell motions are kinematically complex, and are optically difficult to image with high resolution using the light microscope. Historically, the clinical assessment of sperm motility was based on subjective visual impressions with limited accuracy and precision. The limitations themselves contributed to the paucity of direct evidence of positive relationships between measures of conventional seminal sperm motility and fertilization in vitro.[1,2] In recent years, the evolution of computer vision technology has given rise to a new generation of instruments that have overcome these historical limitations and are becoming amenable to practical clinical utilization. Computer-aided sperm analysis (CASA) technology has become commercialized and is now used in several laboratories worldwide. It has been demonstrated clearly that they provide objective assessment of sperm motility and details of movement characteristics not obtainable by subjective assessment of motility. At least six CASA systems are currently available. Hamilton Thorn Motility Analyzer (HTMA) used in this study is one of the most popular CASA systems.

Several sperm motility parameters were evaluated to allow the clinician to predict the relative probability of fertilization in vitro.[3-5] Among those parameters, curvilinear velocity (VCL),[4] amplitude of lateral head displacement (ALH),[3] linearity (LIN) and straight line velocity (VSL)[5] have been reported to be a useful predictor of fertilization rate in vitro. However, very few studies have analyzed the relationship between sperm movement characteristics and fertilization rate in vitro and available data are still controversial.

The aim of this study was to examine the clinical value of the sperm movement characteristics obtained by HTMA together with data from standard semen analysis. In this prospective study, inseminated sperm suspensions were assessed with the Hamilton-Thorn Motility Analyzer (HTMA) to determine which sperm movement characteristics are related to IVF rates and assess whether these sperm movement characteristics have any relevance in predicting fertilization rate in vitro.

Materials and Methods

Study population

The study population consisted of a cohort of 125 unselected patients undergoing IVF therapy at the Simpson Maternity Memorial Pavilion, Edinburgh, between February and May 1994, without the use of donor gametes.

In vitro fertilization

Controlled ovarian hyperstimulation 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 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 (Profasi® ; Serono Laboatories), 5,000 IU, was administered when at least three leading follicles reached a mean diameter of at least 16 mm. Oocyte retrieval was performed 34 hours after the hCG injection, using transvaginal ultrasound.

Semen samples and semen preparation

One hour 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 according to WHO guidelines[6] and sperm movement characteristics assessment as described below were performed. The semen samples were then prepared by two-layer (40% and 80%) discontinuous Percoll® (Pharmacia, Uppsala, Sweden) centrifugation, after which the sperm pellet was gently overlaid with Earle's medium (Flow Laboratories, Irvine, UK.) supplemented with 10% human serum albumin (Human Albumin Solution, 4.5%, Immuno AG, Vienna, Austria.). After one hour of incubation, the upper layer of culture medium containing the motile sperm was used for oocyte insemination. The oocytes were inseminated with 100 µl of sperm suspension containing approximately 100,000 motile spermatozoa 1 to 3 hours after oocyte retrieval. Fertilization of the oocytes was assessed after 20 to 22 hours incubation at 37 °C in an atmosphere of 5% CO_2 in air. The remainder of the swim-up sperm suspension was centrifuged (500g for 5 minutes) and resuspended in BWW medium[7] at a concentration of 20 x 10^6 sperm/mL for the following sperm movement characteristics assessment.

Sperm movement characteristics

The sperm movement characteristics were assessed using the European (25 Hz) version of the Hamilton Thorn Motility Analyzer (HTMA-IVOS Version 10 : Hamilton Thorn Research, Danvers, MA, USA) at a temperature of 37 °C using the following settings : minimum contrast, 10 ; minimum size, 5 ; low and high head size gates, 0.18 and 2.18, respectively ; low and high head intensity gates, 0.4 and 1.67, respectively ; nonmotile head size, 5 ; nonmotile head intensity, 80 ; magnification factor, 2.53. 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 deter-

minations 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 rapid" (the percentage of cells exhibiting a VAP of ≥ 25 μm/s) and ALH (the amplitude of lateral sperm head displacement in μm). Linearity (LIN) was defined as VSL/VCL x 100, while straightness (STR) was VSL/VAP x 100. "Percent progressive motility" equated with an STR of >75%. The playback function of the HTMA-IVOS was used to verify the validity of the cell identification process and minor adjustments made to the analyzer set up when necessary.

Statistical Analysis

All data are presented as means ± SEM. The paired *t* test was used to assess the statistical significance of differences between the sperm movement characteristics in semen and inseminated sperm suspension. Ten cases were excluded from statistical analysis because the number of oocytes collected were less than four oocytes to avoid misinterpretation of the percentage of fertilization rates and increase statistical significance. The data were weighted with the number of eggs and then analysed by linear and stepwise regression analysis. Stepwise regression analysis identifies the optimum combination of independent variables (conventional semen analysis parameters and sperm movement characteristics) that can be used to predict the dependent variable (fertilization rate). The unpaired *t* test was used to assess the statistical significance of differences between the sperm movement characteristics in samples exhibiting good and poor fertilization. All data were analysed using Statistical Package for the Social Sciences (SPSS for MS Windows Release 6, Microsoft Ltd., Wokingham). Probability values (p) < 0.05 were considered significant.

Ethics

This study was approved by the Pediatrics/ Reproductive Medicine Research Ethics Sub-committee of Lothian Health.

Results

Study population

For the 125 couples studied, the indication for IVF was unexplained infertility in 49 (39.2%), bilateral tubal occlusion in 50 (40%), male infertility in 19 (15.2%), and endometriosis in 8 (6.4%).

The semen was assessed according to the World Health Organization guidelines[6] with regard to the sperm concentration, motility and morphology. For this cohort of patients, the constituents of the conventional semen profile, expressed as mean ± standard error of means (SEM), were : volume, 3.2 ± 0.2 mL ; sperm concentration, 55.8 ± 3.1 x 10^6 spermatozoa/mL ; normal morphology, 51.4 ± 1.1% and

motility, 49.0 ± 1.1%.

Outcome of IVF

For the 125 couples studied, the mean value of the number of oocytes retrieved and inseminated was 9.6 ± 0.5 (1 - 24) while the mean value of fertilization rate was 69.7 ± 2.5% (0% - 100%).

Sperm movement characteristics results

Sperm movement characteristics in semen and inseminated sperm suspension

Table 1 shows the mean ± SEM of all sperm movement characteristics obtained by HTMA-IVOS from 125 patients. Most of the sperm movement characteristics (Percentage of rapid motility, percentage of progressive motility, VAP, VCL, VSL, ALH and BCF) were significantly increased after selection of motile spermatozoa by discontinuous Percoll centrifugation and swim-up techniques.

Simple linear regression analysis

Linear regression analysis of fertilization rate weighted by number of eggs against the parameters of the conventional semen profile for this cohort of patients revealed that weakly significant correlations with fertilization rate were observed for the sperm concentration (r = 0.0893; $P < 0.005$) and the percentage of sperm motility (r = 0.1156; $P < 0.0001$). Several sperm movement characteristics of the inseminated sperm suspensions were also found to be weakly significantly correlated with the fertilizing potential of the spermatozoa in vitro, such as, STR (r = -0.1285; $P < 0.0001$), LIN (r = -0.1188; $P < 0.0001$), ALH (r = 0.0938; $P < 0.005$), VCL (r = 0.0607; $P < 0.05$) and percentage of rapid motility (r = 0.0601; $P < 0.05$).

Stepwise regression analysis

In order to determine whether a combination of variables describing different sperm movement characteristics of the inseminated sperm suspensions and conventional semen analysis could adequately explain the variance in in vitro fertilization rates, a stepwise multiple regression analysis was performed. In this model, independent variables are added to the regression analysis in order of their ability to predict the dependent variable. With this data set a regression equation was generated that gave a multiple regression coefficient of R = 0.217 (R^2 = 0.0473) for the relationship between the conventional semen analysis and sperm movement characteristics of the inseminated sperm suspension and in vitro fertilization rate. This analysis was based on 2 variables, comprising, the percentage of sperm motility and STR in inseminated sperm suspension (Table 2). Included in this table are the standardized β coefficients, which give an indication of the relative importance of each of the selected independent variables in predicting the dependent variable, fertilization

rate. Examination of these coefficients reveals that the most important variables were STR in inseminated sperm suspensions.

(Comparison of samples exhib-iting good or poor fertilization)

The other approach towards assessing the relationship between sperm movement characteristics and in vitro fertilization success was to compare those samples exhibiting an impaired capacity for fertilization with the rest of the study population. For this purpose, a threshold value of less than 60% fertilization was selected (n = 29) since it captured those samples in the lowest quartile of the data distribution. Comparison of sperm preparations giving in vitro fertilization rates of less than 60% (n = 29) with the remainder of the study population (n = 86) revealed only a significant difference of STR in the inseminated sperm suspensions between both groups (p = 0.0339) (Table 3).

Discussion

Recently, it has become possible to assess the fertilization potential directly by observing fertilization in IVF treatment. The advent of in vitro fertilization has contributed in eliminating various male and female factors by bringing human spermatozoa and oocytes into direct physical contact. Therefore, clinical IVF provides a useful approach for evaluation tests of human sperm function[8]. In this study, inseminated sperm suspensions were assessed with the Hamilton-Thorn Motility Analyzer (HTMA) to determine which sperm movement characteristics are related to fertilization rate in vitro and assess whether these sperm movement characteristics have any relevance in predicting fertilization rate in vitro. Great care was taken to standardize the fertilizing environment and to use the proper statistics that can evaluate the effect of

Table 1 ***The sperm movement characteristics assessed by HTMA-IVOS in semen and inseminated sperm suspension.***

Sperm movement characteristics	Semen	Inseminated sperm suspension
Percentage of rapid motility (%)	47.7 ± 3.0	76.8 ± 2.8†
Percentage of progressive motility (%)	26.3 ± 1.3	40.9 ± 1.5†
Average path velocity (VAP : μm/s)	40.2 ± 1.3	55.2 ± 1.7†
Straight line velocity (VSL : μm/s)	26.2 ± 0.8	36.0 ± 1.1†
Curvilinear velocity (VCL : μm/s)	56.3 ± 1.9	78.6 ± 2.3†
Straightness (STR : %)	66.7 ± 0.7	66.8 ± 0.8
Amplitude of lateral head displacement (ALH : μm)	3.8 ± 0.1	4.7 ± 0.1†
Linearity (LIN : %)	48.4 ± 0.6	48.0 ± 0.7
Beat cross frequency (BCF : Hz)	9.1 ± 0.2	9.9 ± 0.3*

* $p < 0.05$, † $p < 0.0001$

sperm movement characteristics on fertilization rate in vitro.

After sperm preparation by discontinuous Percoll centrifugation and swim-up techniques, most of the sperm movement characteristics (Percentage of rapid motility, percentage of progressive motility, VAP, VCL, VSL, ALH and BCF) were significantly increased (Table 1) which confirmed the previous study.[9]

Many attempts have been made to correlate standard semen analysis characteristics and fertilization outcome, no single parameter appears to account for enough of the total variation to allow for high predictive value.[10,11] In agreement with these studies our study confirmed that conventional semen analysis had limited clinical value for predicting fertilization rate in vitro.

In this study, we used the computer assisted image analyzer to evaluate the movement characteristics of semen and inseminated semen suspension. One advantage of such analysis is that it can provide objective measurements of sperm quality. Our studies demonstrated that several sperm movement characteristics were weakly significantly related to the fertilization rates in vitro, including VCL, ALH, LIN, STR, and the percentage of rapid motility. In previous studies, curvilinear velocity (VCL),[4] amplitude of lateral head displacement (ALH),[3] linearity (LIN) and straight line velocity (VSL)[5] have been reported to be a useful predictor of fertilization rate in vitro. In agreement with these studies, the significant correlation between fertilization rate and some sperm movement characteristics, such as, VCL, ALH, and LIN in inseminated sperm suspension were demonstrated. However, we were unable to confirm these studies, which reported a moderate correlation between these sperm movement characteristics and fertilization rate in vitro. On the basis of our data, it seems that relationship between the sperm movement characteristics and the fertilization rate is very poor. Apart from VCL, ALH and LIN, STR had negatively significant correlation with fertilization rates in vitro.

To determine whether a combination of variables describing different sperm movement characteristics of the inseminated sperm suspensions and conventional semen analysis could adequately explain the variance in in vitro fertilization rates, a stepwise multiple regression analysis was performed. Stepwise regression analysis is the most appropriate method for examining the relationship between the probability to response and multiple explanatory variables. In this study, this approach was used to examine how several sperm movement characteristics and standard semen analysis parameters are related to the fertilization rate in vitro weighted by number of the eggs. With stepwise regression analysis of all data, poor prediction of fertilization rates ($R = 0.217$) was obtained using a multiple regression equation incorporating 2 variables,

Table 2 ***Stepwise regression analysis of the relationship between the combination of conventional semen analysis and sperm movement characteristics of inseminated sperm suspension with subsequent fertilization rates in vitro.***

Variables	Coefficient	Standard coefficient
Intercept	86.1663	
STR in sperm suspension	- 0.4798	- 0.1777
Percentage of sperm motility	0.3084	0.1261

Table 3 ***Comparison of the sperm movement characteristics in samples exhibiting good and poor fertilization.***

Sperm movement characteristics	Good fertilization (≥ 60%)	Poor fertilization (< 60%)
Percentage of rapid motility (%)	69.9 ± 3.0	62.5 ± 5.4
Percentage of progressive motility (%)	40.0 ± 1.5	42.4 ± 2.8
Average path velocity (VAP : μm/s)	55.5 ± 2.6	52.5 ± 2.6
Straight line velocity (VSL : μm/s)	38.8 ± 1.4	38.8 ± 2.5
Curvilinear velocity (VCL : μm/s)	75.5 ± 2.4	69.6 ± 3.5
Straightness (STR : %)	69.8 ± 1.0	74.2 ± 1.9*
Amplitude of lateral head displacement (ALH : um)	4.3 ± 0.2	3.8 ± 0.3
Linearity (LIN : %)	53.3 ± 1.3	58.2 ± 2.7
Beat cross frequency (BCF : Hz)	9.3 ± 0.3	9.4 ± 0.4

* $p < 0.05$

including percentage of sperm motility and straightness (STR) in inseminated sperm suspension (Table 2). Our results illustrate that although sperm movement characteristics have been shown to be correlated with in vitro fertilization, this parameter by itself does not process a sufficiently high discriminating power to be practical for use in predicting the fertilizing potential of semen samples for IVF.

In this study, the STR in inseminated sperm suspensions seems to be one of the most significant factors related to IVF rates because the STR had negatively significant correlation with fertilization rates in vitro and it was selected by stepwise regression analysis to incorporate in the multiple regression equation. Furthermore, there was a significant difference of STR in the inseminated sperm suspensions between the good and poor fertilization group. However, the overall predictivity of sperm movement characteristics for in vitro fertilization was still poor.

The predictivity of sperm movement characteristics for in vitro fertilization is not consistent with several previous studies because of many reasons. First, CASA system

reports mean values of each movement characteristics of the whole sperm populations. These values may not reflect the sperm movement characteristics of the spermatozoa with fertilizing potential. Therefore, several sperm movement characteristics are weakly significantly related to the fertilization rates in vitro. The reliance on mean values of sperm movement characteristics in the prediction of fertilization rate in vitro may be inappropriate. This could explain some of the inconsistent results of earlier studies. Identification of the subpopulations of spermatozoa with fertilizing potential, such as, hyperactivated motility is much more logical strategy for finding predictors of fertilization in vitro. Second, in vitro fertilization is influenced by many interactive factors, such as, oocyte quality, culture system, etc.

Although a number of statistically significant correlation were found in this type of study, often none of them are useful in clinical practice for predicting the likelihood of successful IVF. Some of the reasons are the variation of the oocyte quality, the variation of the culture system. However, this study is useful for indicating which measurements are worth further investigation to develop a set of prognostic factors for clinical IVF.

There are some studies about the usefulness of the sperm movement characteristics as a method to predict oocyte fertilization in culture.[3-5] However, the results of the present study could not confirm the above observations. Therefore, we conclude that the sperm movement characteristics have no practical value in predicting fertilization rate in vitro. However, further study is required to identify the appropriate mathematical approach to define the sperm movement characteristics that will be useful clinically. Moreover, further studies on other aspects of human sperm movement characteristics are necessary to identify the other sperm parameters, such as, hyperactivated sperm motility, which is important for the success of IVF and to improve the prediction accuracy.

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