Prognostic Value of Objective Semen Parameters in an In Vitro Fertilization Program

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Submitted: February 26, 1997
Accepted: March 24, 1997

Purpose: *The basic semen parameters seem to have a limited predictive value in male fertility. Could other objective sperm analyses be helpful in the choice of the most adapted assisted procreation technique?*
Methods: *This study concerns 78 infertile couples with insemination failures. For each semen, 21 objective parameters are analyzed in fresh semen and after sperm selection procedure. The 78 couples are then included in an IVF protocol and classified into two groups: fertile (at least one cleaved embryo is obtained) and infertile.*
Results: *Using multiple variant discriminant factorial analysis, we have found nine nonconventional parameters which induce us to define two classes of semen. These two classes fit with the classification into fertile and infertile groups in 74.4% of the cases.*
Conclusions: *So these parameters allow us to predict the chance of obtaining embryos during an IVF trial and to choose for each couple the most appropriate technique: IVF or ICSI.*

KEY WORDS: acrosome reaction; hyperactivated motility; in vitro fertilization; intracytoplasmic sperm injection; sperm kinetics.

INTRODUCTION

Male-related problems could account for 43% of infertile couples (1) and male-factor infertility constitutes an important part of the assisted reproduction technology (ART) population. The advent of assisted reproductive techniques [in vitro fertilization (IVF) and, more recently, intracytoplasmic sperm injection (ICSI)] appears to be real progress in the treatment of male infertility.

The fundamental problem for clinicians remains the fact that the conventional criteria of semen quality (sperm density, motility and sperm morphology) are of limited prognostic value in predicting the success of the IVF attempt (2). As a consequence, additional tests of sperm functions have evolved in order to characterize semen quality better.

Today there is no single test of sperm function that will accurately predict fertility, except when there is a specific disorder affecting all sperm (3).

Objective studies of human sperm movement have shown the real value of such parameters in predicting the ability for a couple to achieve pregnancy (4). Sperm movement is related to specific aspects of sperm function including capacitation and interaction with zona pellucida (5).

Recently attention has been focused on the assessment of acrosomal status of human spermatozoa, since acrosome discharge is prerequisite to fertilization (6). Acrosome discharge seems to be impaired among many men suspected of being infertile (7).

The aim of this study is to examine the predictive value for success in IVF of three standard data of fresh semen (spermatozoa, round cells, and polynuclear density) and of objective parameters which test different functions: motility in fresh semen and after treatment and the acrosomal reaction (AR). The purpose is to target the most effective method of ART for couples with intrauterine insemination failures: IVF or ICSI. Female parameters, except oocyte quality, are not included in this analysis, although morphologically oocyte quality is not a guarantee of metabolic quality. We took into account only the fertilization results, and not the occurrence of pregnancy, which introduces various female factors. To perform our study we selected couples with at least three intrauterine insemination (IUI) failures and scheduled in an IVF program.

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1058-0468/97/0700-0321$12.50/0

Using multiple variant discriminant factorial analysis, we have found nine nonconventional variable parameters which allowed us to define a good prognostic value of fertility potential for the studied group.

MATERIALS AND METHODS

This study was conducted between March 1994 and December 1995. Semen samples were obtained from 112 male partners of couples scheduled in an IVF program after having undergone failures in at least three intrauterine inseminations (from three to six) after controlled ovarian hyperstimulation. For a defined couple, IVF attempt was performed from 3 to 8 months after the detailed semen analysis. The age of male patients was between 29 and 45 (average, 35.3), and that of the female partner between 26 and 42 (average, 33.3).

Twenty-one parameters were studied and are described in Table I.

Fresh Semen Analysis. Semen was collected by masturbation after 48 to 72 hr of abstinence. The semen sample was allowed to liquefy for 1 hr after collection at room temperature. Sperm and round-cell density and percentage of polynuclear cells were then determined according to the WHO guidelines (8).

Motility Analysis. Samples were evaluated with CASA (Hamilton–Thorn HTM-S 2030) in microcells of 20-mm depth at 37°C. Analysis was based upon counting a minimum of 100 motile cells (minimum, 5 fields) from a semen with a sperm density of less than 80 million/ml. The machine parameters were set at a frame rate of 20 to 25/sec, a minimum contrast of 7, a minimum size of 6, low and high size gates of 0.4 to 1.2, low and high intensity gates of 0.4 to 1.2, a nonmotile head size of 11, a nonmotile intensity of 241, and a path velocity of more than 10 mm/sec to be counted as motile. To check the accuracy of these settings, the playback function of the HTM-S was used throughout the study, confirming that nonmotile and motile cells were correctly identified. The measured parameters were curvilinear motility (VCL), straight-line velocity (VSL), linearity (LIN), and amplitude of lateral head displacement (ALH).

Sperm Preparation. Semen samples were layered on a 40–80% Percoll gradient. Following centrifugation for 30 min at 300*g* the 80% Percoll fraction was recovered and washed twice with tyrode medium (GIBCO BRL). The final pellet was resuspended in 2 ml of Ménézo-B2 medium (BioMéreieux, Marcy l'Etoile, France) and incubated for 5 hr at 37°C with 5% CO_2 in air. Sperm with hyperactivated motility were determined using the parameters defined by Pilikian (9): VCL, >80 μm/sec; LIN, <65% and ALH, >5.5 μm. They were counted on fresh semen and after capacitated treatment. The hyperactivity motility is expressed as the percentage of motile sperm.

Assessment of Acrosomal Status. The human acrosomal reaction was assessed by the method described by Mendoza *et al.* (10): capacitated sperm were exposed to ionophore calcium (A23187, Sigma) at a concentration of 10 mM in B2 medium for 30 min. The acrosome reaction (AR) was quantified by the binding of fluorescein isothiocyanate (FITC)-labeled *Pisum sativum* lectin (which binds specifically to acrosin). At least 100 sperm were counted, assessed, and classified according to Mendoza *et al.* into three classes: unreacted, when fluorescence was distributed over the entire acrosome; equatorial, when fluorescence was located around the equatorial acrosomal segment; and reacted, when the sperm was not fluorescent at all. The AR results are expressed as the percentage of 100 analyzed cells before and after incubation in ionophore calcium. Cells were double-stained with vital stain Hoechst 23356 in order to see if the sperm were dead.

Table I. The Parameters Studied[a]

Fresh semen	Capacitated treatment	Acrosomal status
Sperm density	Motile sperm density	Unreacted sperm before induction
Round cells density	VCL	Unreacted sperm after induction
Polynuclear/total round cells	VSL	Equatorial reacted sperm before induction
VCL	LIN	Equatorial reacted sperm after induction
VSL	ALH	Delta equatorial sperm
LIN	Hyperactivated sperm	Reacted sperm before induction
ALH		Reacted sperm after induction
Hyperactivated sperm		

[a] VCL, curvilinear velocity; VSL, straight-line velocity; LIN, linearity; ALH, lateral head displacement; delta, difference between before and after treatment.

IVF Procedure. Subsequently, from 15 days to 8 months, the couples underwent an IVF trial. The women underwent desensitization with LH-RH analogue (Buserilline; Hoechst, Puteaux, France) administered subcutaneously for 12 days. During the early follicular phase (days 1 to 5) ovarian stimulation was obtained with combined FSH (Metrodine HP; Serono)–hMG (Humegon; Organon) (225 IU FSH and 75 IU LH) (ratio ≥3). On day 6, stimulation was continued with doses adapted to changes in 17 β-estradiol (E_2) level. When three or more follicles showed a mean diameter of approximately 18 mm, the stimulation was discontinued and 5000 IU of hCG was administered. Follicles were aspirated 36 hr later by ultrasound-guided transvaginal puncture. After retrieval, cumulus–corona–oocyte complexes were scored according to Veeck's classification (11). Only metaphase II oocytes were included in this study. For each couple, up to 10 oocytes were transferred into a culture dish containing 2 ml of Ménézo-B2 medium, inseminated with prepared spermatozoa at a concentration of 200,000 motile sperm per ml, and cultured for 18–20 hr at 37°C with 5% CO_2 in air.

At this time, oocytes were examined for fertilization as determined by the presence of two pronuclei and transferred into fresh Ménézo-B2 medium. Twenty-four hours later, embryo quality was assessed according to morphological appearance and cleavage stage and up to three embryos were transferred into the uterine cavity. At least three mature oocytes were necessary to consider the attempt and at least one cleaved embryo was required to have a positive fertilization result.

Statistical Analysis

Two methods of statistical analysis were used. (i) The results were evaluated by a nonparametric test: the Mann–Witney *U* test used for the comparison of the two groups of semen—with or without fertilization; and (ii) the relative value of the measured variables in discriminating between semen samples which induce fertilization or not was examined using a statistical method—multivariate discriminant factorial analysis (software STATITCF, INRA, France).

RESULTS

The study recruited 112 couples; 34 couples were excluded for different reasons: bacterial infection, ovarian stimulation failure, unavailability of follow-up information, or technical reasons [the analysis does not allow an accurate measurement of kinetic parameters using a CASA instrument when there are more than 80 million sperm/ml (5)]. The 78 remaining couples were studied. Forty-eight couples did not fertilize during their IVF trial; the mean number of recovered oocytes in these cases was 8.2 (from 3 to 14).

Thirty couples obtained at least one embryo during their first IVF attempt: the mean number of recovered oocytes per couple was 9.3 (from 3 to 13), and the mean number of cleaved embryos per couple was 3.2 (from 1 to 10).

Differences between couples with (30 cases) and couples without (48 cases) fertilization using a nonparametric test (Mann–Whitney *U* test) were significant for two variables studied: VCL in fresh semen ($P = 0.05$) and delta equatorial sperm in acrosomal status ($P = 0.030$). Differences were nonsignificant for the other 19 variables, although for VSL in fresh semen $P = 0.085$ and for VSL after capacitated treatment $P = 0.095$. We excluded from the studied semen parameters sperm morphology, for it is not a real objective parameter. The ability of sperm morphology to predict fertilization is an area of debate (12).

Sperm density is higher in unsuccessful ejaculates than in successful ones, but this difference is not statistically significant by the Mann–Whitney *U* test. No differences between the concentration of round cells are found in the two populations. The other parameters compared between the two populations have an average value which is different in the expected direction but not statistically significant (Mann–Whitney *U* test).

Table II presents the mean values, the standard deviations, and the significant differences (determined by the Mann–Whitney *U* test) of the analyzed parameters in the fertile and nonfertile group.

Using a multiple-variate discriminant factorial analysis on these 21 parameters, 9 variables were revealed to have the best predictive value for fertilization. These variables are described in Table III.

In fresh semen four variables were important: VCL, VSL, ALH, and hyperactivated sperm. After capacitated treatment three variables were important: VCL, VSL, and LIN. Concerning the acrosomal reaction, the number of unreacted sperm before induction and the difference between the equatorial acrosomal reaction before and that after induction are important.

According to these nine discriminant parameters the couples were classified into two groups; and according to the IVF results, two classes were distinguished: with and without fertilization.

Table II. Mean Values, Standard Deviations, and Significant Differences of the Studied Parameters in the Fertile and Nonfertile Groups

Parameters	Units	Fertile semen		Nonfertile semen		P^a
		Mean	SD	Mean	SD	
Fresh semen						
Sperm density	Million/ml	27.15	19.02	36.96	48.14	0.861
Round cell density	Million/ml	1.81	1.34	2.49	4.24	0.429
Polynuclears	% of round cells	13.96	25.03	13.38	25.21	0.877
VCL	µm/sec	52.50	9.54	44.89	13.19	**0.035**
VSL	µm/sec	36.36	8.45	30.43	9.33	0.085
LIN	Percentage	69.03	7.33	66.56	12.15	0.572
ALH	µm	2.35	1.49	1.78	0.94	0.137
Hyperactivated sperm	Percentage	3.34	4.60	2.60	3.47	0.517
Capacitated treatment						
Motile sperm density	Million/ml	6.69	7.59	5.33	7.11	0.144
VCL	µm/sec	87.63	17.17	77.06	20.25	0.238
VSL	µm/sec	65.93	15.36	55.64	16.59	0.095
LIN	Percentage	74.80	7.64	71.97	8.54	0.157
ALH	µm	3.85	1.40	3.50	1.39	0.295
Hyperactivated sperm	Percentage	9.83	7.00	8.98	7.90	0.441
Acrosomal status						
Unreacted sperm before induction	Percentage	61.76	24.84	58.00	21.21	0.162
Unreacted sperm after induction	Percentage	39.76	19.97	45.97	21.20	0.292
Equatorial reacted sperm before induction	Percentage	3.66	3.68	4.85	4.34	0.252
Equatorial reacted sperm after induction	Percentage	21.56	15.12	13.79	12.38	0.139
Delta equatorial sperm	Percentage	17.90	14.48	8.79	10.84	**0.030**
Reacted sperm before induction	Percentage	12.40	11.47	13.72	9.63	0.393
Reacted sperm after induction	Percentage	16.43	10.15	16.75	10.30	0.954

[a] Comparison by Mann–Whitney *U* test.

Table III. Discriminant Parameters

Fresh semen	Capacitated treatment	Acrosomal status
VCL	VCL	Unreacted sperm before induction
VSL	VSL	Delta equatorial sperm
ALH	LIN	
Hyperactivated sperm		

Table IV. Multiple Variant Discriminant Factorial Analysis Comparing Fertile and Nonfertile Couples

	Fertile couples	Nonfertile couples	Overall
Number correctly classified	16	42	58
Number incorrectly classified	14	6	22
% correct classification	53.3	87.5	74.4

As shown on Table IV, 74.4% of the couples were classified as "fertile" according to the discriminant parameters and did fertilize during their IVF trial.

DISCUSSION

According to some authors conventional semen analysis is enough to evaluate the fertility potential of the semen (13). However, new technologies such as CASA and the study of the acrosomal status increase the objectivity of the measurement and the appreciation of the functional status of the spermatozoa. It is now well accepted that conventional analysis is not an accurate predictor of the true fertility potential (14). The aim of our study was to determine, for a population with IUI failures, which semen parameters (standard and/or more specific criteria: computer-aided sperm analysis and acrosomal reaction) allow discrimination between fertile and nonfertile men during IVF, in order to target the most adapted procreation technique: IVF or ICSI. We found nine discriminant parameters which allowed good classification of the couples, according to the IVF results, in 74.4% of the cases.

Since in this study the analysis of the semen was made from 15 days to 8 months before the day of the IVF attempt, the problem of intervariability of the ejaculate is posed. According to Calvo *et al.* (7), for a majority of men the acrosomal reaction is a stable parameter of sperm function. In a preliminary study, 100 different semens were analyzed several times

every 3 months by CASA. Kinetics parameters appeared to be reproductive in the absence of intercurrent problems (15,16).

Most of the parameters studied here are subject to debate.

From a traditional view, a high concentration of leukocytes is detrimental to sperm function (8). But other authors (17) suggest that such cells may enhance fertility by removing abnormal and degenerated spermatozoa and generating hydrogen peroxide, which is believed to be an essential part of sperm capacitation. In our study no statistically significant difference could be found between the "fertile" and the "nonfertile" group.

Early works on the study of human sperm movement using time-exposure photomicrography established that these measurements were of clinical value in predicting the ability of spermatozoa to fertilize human oocytes in vitro (4). The parameters obtained by CASA have been shown to correlate with the achievement of pregnancy (1), the outcome after IUI (14), and the hamster test assay (18). In fresh semen, the relative values of CASA parameters are variable according to different studies. The most correlated parameters with IVF results are VCL, VSL, ALH, and LIN for Pilikian *et al.* (9) and VCL and VAP (average path velocity) for Barrat *et al.* (19). For Liu & Baker (3), only linearity is significantly related to fertilization rate, and not ALH. According to MacLeod *et al.* (2) VSL, LIN, and VCL are the most important, and for Montagut *et al.* (20) the useful parameters are VCL and ALH.

We have shown here that CASA values on fresh semen that seem to be the most significant are the VCL, VSL, ALH, and percentage of hyperactivated sperm.

A low level of hyperactivation is associated with a poor fertilization rate (9,21,22). For Sukchaoren (23) the most important movement for IVF success is the incidence of hyperactivation (HA) after 3 hr.

According to Aitken *et al.* (18), ALH is strongly correlated with sperm–oocyte fusion. For Wang *et al.* (24) the percentage of spermatozoa with transitional HA after 6 hr of incubation correlated significantly with the fertilization rate of human oocytes. On the other side, Ginburgh (25), Hinney *et al.* (26), and Mak *et al.* (27) found no correlations between the percentage of hyperactivated sperm and the fertilization rate or sperm penetration assay rate. We conclude from our study that no significant differences are found between the percentage of HA sperm and the success of IVF attempts, although the percentage of hyperactivated sperm is one of the nine discriminant parameters.

Concerning the acrosomal reaction the results of different studies are more in agreement: a low percentage of reacted sperm after induction is associated with a low fertilization rate (7,21,28–30).

In our study the results of fertilization are well related to two parameters of the acrosomal reaction: the number of unreacted sperm before induction and the difference between the equatorial acrosomal reaction before and that after induction.

Some authors have tried to determine the most predictive associated parameters for IVF success or pregnancy. Wang *et al.* (24) found that the percentage of spermatozoa with HA transitional motility and the acrosome variables classified the samples achieving a good (>70%) or poor fertilization rate with 80% accuracy. Paston *et al.* (14) used eight variables (one clinical factor, three conventional, and four nonconventional parameters of sperm analysis) to predict with an accuracy of 74.4% the fertility potential of an infertile group. According to MacLeod *et al.* (2) the association of the percentage of sperm with progressive motility, mean VAP, mean VSL, sperm concentration, and morphometry correctly classified women who became pregnant in 62.3% of his studied population. MacLeod and Irvine (31), studying human donor semen, were able to discriminate significantly between successful and unsuccessful ejaculates with an overall accuracy of 87%. Parinaud *et al.* (32) studied sperm morphology, vitality, motility, and acrosome reaction and obtained an accuracy of 83%. In our study we obtained a good classification in 74.4% of the semen without studying sperm morphology. The 25% chance of no fertilization despite a favorable assessment could lead to the use of a proportion of the oocytes obtained for ICSI. For ethical reasons, it seems difficult to transfer embryos which would be obtained by different methods (IVF and ICSI).

As noted in the literature, it is often difficult to compare the different studies. Some authors indeed compare the results with IVF; others compare the results with pregnancy. The CASA instruments are not always the same, and in addition, the samples do not have the same number of patients. Another difficulty is the statistical method which was used. In general if the variable lacks statistical significance, the variable either is not related or could be related but the relationship is not detected as statistically significant in the analysis. There may be statistically insufficient power, the sample size being too small with regard to the magnitude of the effect and its variability.

According to Krause (13) the statistical significance of the results is not the same, depending on the statisti-

cal method used. Multivariate statistical analysis is powerful and available to examine the impact of groups of predictor variables of any form of pregnancy or fertilization rate, as recommended by Aitken *et al.* (12). Using this approach, it should be possible to define optimized sets of prognostic criteria for assessing fertility.

Our population is peculiar because it consists of couples with at least three IUI failures and with subnormal classical semen parameters (sperm density, motility, and morphology). These couples fertilize in the first attempt at IVF or do not fertilize sometimes even after two IVF trials. In this population it was interesting to see if there was a difference in the semen characteristics of the male partner which would suggest carrying on with IVF or directly scheduling ICSI. In our study we found nine parameters which were the most discriminative in predicting, in 74.4% of the cases, IVF success. These parameters are as follows: in fresh semen, VCL, VSL, ALH, and percentage of hyperactivated sperm; after capacitated treatment, VCL, VSL, and LIN; and for the acrosomal reaction, the number of unreacted sperm before induction and the difference between the equatorial acrosomal reaction before and that after induction.

Prospective studies will be needed to confirm that this diagnostic test has value in separating fertile from infertile males based on semen analysis.

REFERENCES

1. Irvine DS, Mac Leod IC, Templeton AA, Masterton A, Taylor A: A prospective clinical study of the relationship between the computer assisted assessment of human semen quality and the achievement of pregnancy in vivo. Hum Reprod 1994;9:2324–2334
2. Mac Leod IC, Irvine DS, Masterton A, Taylor A, Templeton AA: Assessment of the conventional criteria of semen quality by computer-assisted image analysis: Evaluation of the Hamilton-Thorn motility analyser in the context of a service andrology laboratory. Hum Reprod 1994;9:310–319
3. Liu DY, Baker HWG: Tests of human sperm function and fertilization in vitro. Fertil Steril 1992;58:465–482
4. Aitken RJ: Assessment of sperm function for IVF. Hum Reprod 1988;3:89–95
5. Davis RO, Katz DF: Operational standards for CASA instruments. J Androl 1993;14:385–394
6. Calvo L, Dennison-Lagos L, Banks S, Dorfmann A, Thorsell L, Bustillo M, Schulmann J, Sherins RJ: Acrosome reaction inducibility predicts fertilization success at in vitro fertilization. Hum Reprod 1994;9:1880–1886
7. Calvo L, Dennison-Lagos L, Banks S, Sherins RJ: Characterization and frequency distribution of sperm acrosome reaction among normal and infertile men. Hum Reprod 1994;9:1875–1879
8. WHO (World Health Organisation): Laboratory Manual for the Examination of Human Semen and Sperm Cervical Mucus Interaction, 3rd ed. Cambridge, Cambridge University Press, 1992
9. Pilikian S, Czyba JC, Guerin JF, Pinatel MC, Adeleine P, Ecochard R: Analyse fonctionnelle des spermes inféconds présentant une asthénozoospermie isolée. 10th Congress of Société d'Andrologie de Langue Française, Oct. 29–31, 1992
10. Mendoza C, Carreras A, Moos J, Tesarik J: Distinction between true acrosome reaction and degenerative acrosome loss by a one step staining method using Pisum sativum agglutinin. J Reprod Fertil 1989;95:755–763
11. Veeck L: Typical morphology of the human oocyte and conceptus. *In* Atlas of the Human Oocyte and Early Conceptus, Vol 2. Baltimore, Williams and Wilkins, 1991, pp 1–99
12. Aitken RJ, Baker HWG, Irvine DS: On the nature of semen quality and infertility. Hum Reprod 1995;10:248–249
13. Krause W: Computer assisted semen analysis systems: Comparison with routine evaluation and prognostic value in male fertility and assisted reproduction. Hum Reprod 1995;10(Suppl 1):60–66
14. Paston MJ, Sarkar S, Oates RP, Badawy SZA: Computer aided semen analysis variables as predictors of male fertility potential. Arch Androl 1994;33:93–99
15. Fillion C, Kraemer M, Martin-Pont B, Gonzales J: Analyse automatisée du mouvement des spermatozoïdes: Reproductibilité et intérêt pour le choix d'une technique d'AMP. 10th Congress of Société d'Andrologie de Langue Française, Oct. 29–31, 1992
16. Sukcharoen N, Keith J, Irvine S, Aitken RJ: Prediction of the in vitro fertilization potential of human spermatozoa using sperm function test: The effect of delay between testing and IVF. Hum Reprod 1996;11:1030–1034
17. Aitken RJ, Baker HWG: Seminal leucocytes: Passengers, terrorists or good samaritans? Hum Reprod 1995;10:1736–1739
18. Aitken RJ, Buckingham D, Harkiss D: Analysis of the extent to which sperm movement can predict the results of ionophore-enhanced functional assays of the acrosome reaction and sperm-oocyte fusion. Hum Reprod 1994;9:1867–1874
19. Barratt CLR, Tomlinson MJ, Cooke ID: Prognostic significants of computerized motility analysis for in vivo fertility. Fertil Steril 1993;60:520–525
20. Montagut J, Regnier-Vigouroux G, Leprêtre S, Artigues MJ, Manzano M, Degoy J: Analyse cinétique des spermatozoïdes: Effets des conditions expérimentales sur la fonction flagellaire et détermination de seuils prédictifs de non-fécondation en fécondation in vitro. Réf Gyn Obs 1995;3:275–284
21. Mortimer D: The essential partnership between diagnostic andrology and modern assisted reproductive technologies. Hum Reprod 1994;9:1209–1213
22. Wang C, Leung A, Tsoi WL, Leung J, Ng V, Lee KF, Chang SYW: Evaluation of human sperm hyperactivated motility and its relationship with the zona-free hamster oocyte sperm penetration assay. J Androl 1991;12:253–257
23. Sukcharoen N, Keith J, Irvine DS, Aitken RJ: Definition of the optimal criteria for identifying hyperactivated human spermatozoa at 25Hz using in vitro fertilization as a functional end point. Hum Reprod 1995;10:2928–2937
24. Wang C, Lee GS, Leung A, Surrey ES, Chang SYW: Human sperm hyperactivation and acrosome reaction and their relationships to human in vitro fertilization. Fertil Steril 1993;59:1221–1227

25. Ginburgh KA, Sacco AG, Ager JW, Moghissi KS: Variation of movement characteristics with washing and capacitation of spermatozoa. II. Multivariate statistical analysis and prediction of sperm penetration ability. Fertil Steril 1990;53:704–708
26. Hinney B, Wilke G, Michelmann HW: Prognostic value of an automated sperm analysis in IVF or insemination therapy. Andrology 1993;25:195–202
27. Mak C, Van Kooij RJ, Eimers JM, Te Velde ER: Human sperm movement assessed with the Hamilton-Thorn motility analyser and in vitro fertilization. Andrology 1994;26:323–329
28. Henkel ER, Müller C, Miska W, Gips H, Schill WB: Determination of the acrosome reaction in human spermatozoa is predictive of fertilization in vitro. Hum Reprod 1993;8:2128–2132
29. Bielsa MA, Andolz P, Gris JM, Martinez P, Egozcue J: Which semen parameters have a predictive value for pregnancy in infertile couples? Hum Reprod 1994;9:1887–1890
30. Parinaud J, Vieitez G, Moutaffian H, Richoilley G, Labal B: Variations in spontaneous and induced acrosome reaction: Correlations with semen parameters and in vitro fertilization results. Hum Reprod 1995;10:2085–2089
31. MacLeod IC, Irvine DS: The predictive value of computer assisted semen analysis in the context of a donor insemination program. Hum Reprod 1995;10:580–586
32. Parinaud J, Richoilley G, Milhet P, Moutaffian H, Vieitez G: Validation of a scoring method predicting the in-vitro fertilizing ability of human spermatozoa. Int J Androl 1996;19:18–22