

Predictive value of sperm motility characteristics assessed by computer-assisted sperm analysis in intrauterine insemination with superovulation in couples with unexplained infertility

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Objective: To determine whether characteristics of sperm motility obtained by computer-assisted sperm analysis (CASA) could predict pregnancy after intrauterine insemination (IUI) in couples with unexplained infertility.

Methods: Three hundred eighty-three cycles of intrauterine insemination with superovulation were retrospectively analyzed. Semen analysis was performed with CASA before and after swim-up and the parameters were compared between pregnant and non-pregnant women.

Results: The pregnancy rate per cycle was 14.1%. Pregnant and non-pregnant women were comparable in terms of age, infertility duration, the number of dominant follicles. While sperm concentration, motility, and parameters such as average path velocity (VAP) and percentage rapid (RAPID) before semen preparation were significantly different between the pregnancy and non-pregnancy groups, there were no differences in sperm parameters when comparing the two groups after preparation. Using a receiver operating characteristic curve to measure sensitivity and specificity, the optimal threshold value for the predictors of pregnancy was revealed to be a concentration of $\geq 111 \times 10^6/\text{mL}$, a motility of $\geq 51.4\%$, and RAPID $\geq 30.1\%$ before preparation for IUI.

Conclusion: Sperm parameters including concentration, motility, and RAPID before sperm preparation could have predictive value for pregnancy outcome after intrauterine insemination with superovulation in couples with unexplained infertility, and would be helpful when counseling patients before they make the decision to proceed with IVF/ICSI-ET.

Keywords: Insemination, Artificial; Infertility; Semen Analysis; Image Processing, Computer-assisted; Sperm Motility; Human

Introduction

Although assisted reproductive technologies (ARTs) including *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) have

been developed, intrauterine insemination (IUI) still remains a reliable method that can be applied to many infertile couples. Compared to IVF or ICSI, IUI is less invasive and associated with lower costs and a low incidence of complications, making it a relatively cost-effective treatment for many forms of infertility [1]. Indications for IUI have included mild male factor infertility (oligozoospermia, asthenozoospermia, pyospermia), cervical factor infertility, and cases of unexplained infertility.

In cases of unexplained infertility, the pregnancy rate with IUI has been reported to be 9-20% [2-5]. Moreover, a meta-analysis revealed that the live birth rate of IUI increased twofold when it was under-

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gone with ovarian stimulation rather than the natural cycle [6]. The success rates of IUI with superovulation depend on the woman's age, duration of infertility, sperm quality, and tubal patency on either side [3,7]. Among sperm parameters, total motile sperm count and morphology are known to be closely associated with the success of IUI [2, 8-10].

Standard semen quality assessment describes the volume, viscosity, concentration, normal and abnormal morphology, and motility of spermatozoa when conducted according to guidelines by the World Health Organization (WHO) [11]. However, it is a rather subjective method which has been associated with large inter-laboratory variation in estimating the respective percentages of progressive and non-progressive motile and immotile spermatozoa, which makes it difficult to predict fertility [12,13]. In an attempt to make assessments of sperm movements more objective and detailed, tools for computer-assisted sperm analysis (CASA) have been developed. CASA is an automated method used to visualize sperm that can assess several specific motility parameters describing the movements of spermatozoa. Several studies have addressed CASA parameters as predictors of male fertility. Larsen et al. [14] reported that CASA parameters were useful in predicting the possibility of natural pregnancy in the general male population. In studies of male infertility, some CASA parameters were reported to be useful in predicting pregnancy after IUI with partner or donor sperm [15-18], and in the assessment of sperm fertilizing ability in IVF [19].

On the other hand, some studies have failed to show the association of CASA parameters with the success of IUI [20,21], and there have been few studies about its usefulness in predicting pregnancy after IUI in cases of unexplained infertility.

The aim of this study was to determine whether assessing characteristics of sperm motility using CASA could predict pregnancy after IUI with superovulation in couples with unexplained infertility.

Methods

1. Subjects

This is a retrospective case-control study that was performed in our infertility clinic between October 2006 and September 2008. Three hundred and eighty-three patients with unexplained infertility that underwent IUI with superovulation were included in this study. Patients were divided into two groups, the pregnant group and non-pregnant group.

The inclusion criteria were a follicular stimulating hormone (FSH) level no greater than 12 mIU/mL, at least unilateral tubal patency demonstrated in hysterosalpingogram, and semen analysis resulting in normal sperm counts, morphology, and motility according to the WHO classification. Exclusion criteria were a woman's age of more

than 40 years, ovarian cyst detected in the ultrasound examination, uterine lesions such as submucosal leiomyoma, and a previous diagnosis of moderate to severe pelvic endometriosis.

Successful pregnancy after intrauterine insemination was confirmed by ultrasound as one or more visible gestational sacs in the uterine cavity.

2. Superovulation and intrauterine insemination

Superovulation was conducted with either 150 IU of intramuscularly injected uHMG (IVF-M; LG Life Science, Seoul, Korea) or 100 mg of oral clomiphene citrate (Clomifene; Young Poong Pharmaceutical, Incheon, Korea) for five days starting on day 3 of the menstrual cycle, followed by daily injection of 150 IU of hMG. When more than two dominant follicles reached a diameter of 17 mm or the urinary specimen showed positive results for LH surge, 5,000 IU of hCG (Pregnyl; Organon, Oss, the Netherlands) was injected intramuscularly, and after 24 to 36 hours, IUI was performed only once.

Semen was collected from subjects via ejaculation after at least 48 hours of abstinence. The semen was then analyzed after at least 30 minutes of liquefaction. For semen analysis, 10 μ L of semen was analyzed by computer assisted sperm analysis (CASA; Sperm Analysis Imaging System [SAIS] ver. 10.1, Medical Supply, Wonju, Korea), and concentration, sperm motility, total motile sperm count, hyperactivity percent (HYP), curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), linearity (LIN), straightness (STR), beat cross frequency (BCF), and amplitude of lateral head displacement (ALH), percentage rapid (RAPID) were reported. Semen preparation was performed using the conventional swim-up method, and the same parameters were reanalyzed via a computerized process.

3. Statistical analysis

Statistical analysis was carried out with the SPSS ver. 16.0 (SPSS Inc., Chicago, IL, USA). Calculations of statistical differences between the groups were carried out by a Student's *t*-test or a chi-square test. Receiver operating characteristic (ROC) curves were constructed for the calculation of the clinically acceptable cut-off value of each individual parameter in predicting pregnancy by IUI. A *p*-value of < 0.05 was defined as representing a significant difference.

Results

A total of 383 cycles of intrauterine insemination with superovulation were conducted. There were 54 cases of successful intrauterine pregnancy and the pregnancy rate was estimated to be 14.1%. There were no significant differences between the pregnant group and the non-pregnant group in terms of female age, duration of infertility, basal hormone levels, number of dominant follicles, or endometrial

thickness at the time of hCG administration (Table 1).

Statistically significant differences were found in some variables associated with semen analysis before swim-up: sperm concentration ($152.9 \pm 13.7 \times 10^6/\text{mL}$ vs. $118.8 \pm 4.3 \times 10^6/\text{mL}$ in the pregnant and non-pregnant group, respectively; $p=0.021$); motility ($56.8 \pm 1.8\%$ vs. $51.2 \pm 0.9\%$; $p=0.007$); VAP ($40.8 \pm 1.05 \mu\text{m}/\text{sec}$ vs. $38.4 \pm 0.4 \mu\text{m}/\text{sec}$; $p=0.033$); and RAPID ($28.1 \pm 2.16\%$ vs. $24.5 \pm 0.87\%$; $p=0.022$). VCL ($67.2 \pm 1.85 \mu\text{m}/\text{sec}$ vs. $63.3 \pm 0.74 \mu\text{m}/\text{sec}$; $p=0.050$) and VSL ($32.8 \pm 1.01 \mu\text{m}/\text{sec}$ vs. $30.7 \pm 0.37 \mu\text{m}/\text{sec}$; $p=0.050$), parameters of sperm motility, showed some degree of difference that was not statistically significant. Differences in LIN, STR, BCF, and ALH were not statistically significant. After swim-up, semen analysis parameters were not significantly different between the two groups (Table 2).

According to the ROC curve, RAPID, motility, and concentration parameters in the unprocessed semen were predictive of pregnancy

and the cut-off values were 30.1% for RAPID, 51.4% for motility, and $111 \times 10^6/\text{mL}$ for concentration. Further analysis was performed according to the number of parameters that were consistent with these criteria. The pregnancy rate per cycle was 6.7% (7/104) if no criteria were met, 11.4% (13/114) for one criterion, 15.7% (11/70) for two criteria, and 24.2% (23/95) if all three criteria were met (Tables 3, 4, Figure 1).

Discussion

Intrauterine insemination with or without superovulation is usually the first-line treatment choice for patients with unexplained infertility who failed to conceive by conventional ovulation induction with timed coitus. Although the IUI procedure is less invasive and less expensive than IVF or ICSI, both infertile couples and doctors bear a

Table 1. Patient characteristics of pregnancy and non-pregnancy groups

	Pregnant group	Non-pregnant group	p-value
No. of cycles	54	329	
Age of female (yr)	32.7 ± 2.8	32.5 ± 2.9	NS
Infertility duration (yr)	2.1 ± 1.3	2.0 ± 1.8	NS
Basal FSH (mIU/mL)	7.7 ± 2.1	7.5 ± 2.1	NS
Basal LH (mIU/mL)	3.3 ± 2.3	2.9 ± 2.0	NS
Basal E ₂ (pg/mL)	16.3 ± 10.0	17.6 ± 16.1	NS
Number of follicles ≥ 17 mm	3.1 ± 1.2	3.5 ± 2.1	NS
EMT on hCG day (mm)	7.3 ± 0.7	7.9 ± 0.9	NS

The values are expressed as means ± SD.

NS, not significant; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E₂, estradiol; EMT, endometrial thickness. hCG, human chorionic gonadotropin.

Table 3. Comparison of CASA parameters as predictors of pregnancy using ROC curves

Test result variable	Area (mean ± SE)		Asymptotic sig.	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
Concent	0.599	0.043	0.019	0.515	0.684
MOT	0.598	0.038	0.021	0.522	0.673
VCL	0.585	0.043	0.045	0.500	0.670
VSL	0.586	0.043	0.043	0.501	0.671
VAP	0.594	0.044	0.026	0.509	0.680
HYP	0.556	0.044	0.186	0.469	0.643
RAPID	0.630	0.042	0.002	0.548	0.712

CASA, computer-assisted sperm analysis; ROC, receiver operating characteristic; Concent, concentration; MOT, motility; VCL, curvilinear velocity; VSL, straight line velocity; VAP, average pathway velocity; HYP, hyperactivity percent; RAPID, percentage rapid.

Table 2. Semen parameters before and after swim-up

	Before swim-up			After swim-up		
	Pregnant group	Non-pregnant group	p-value	Pregnant group	Non-pregnant group	p-value
Volume (mL)	2.6 ± 0.15	2.8 ± 0.07	NS	1.0 ± 0.09	1.0 ± 0.20	NS
Concentration ($\times 10^6/\text{mL}$)	152.9 ± 13.7	118.8 ± 4.4	0.021	32.6 ± 1.24	30.2 ± 0.58	NS
Motility (%)	56.8 ± 1.8	51.2 ± 0.9	0.007	89.8 ± 1.2	89.3 ± 0.5	NS
VCL ($\mu\text{m}/\text{sec}$)	67.2 ± 1.8	63.3 ± 0.7	0.050	109.9 ± 3.0	109.9 ± 1.3	NS
VSL ($\mu\text{m}/\text{sec}$)	32.7 ± 1.0	30.7 ± 0.3	0.050	53.2 ± 2.1	52.1 ± 0.9	NS
VAP ($\mu\text{m}/\text{sec}$)	40.8 ± 1.0	38.4 ± 0.4	0.033	59.5 ± 1.8	58.4 ± 0.8	NS
LIN	48.8 ± 0.87	48.9 ± 0.35	NS	48.5 ± 1.48	47.4 ± 0.54	NS
STR	79.9 ± 0.94	79.7 ± 0.38	NS	88.2 ± 1.20	87.9 ± 0.45	NS
BCF	18.4 ± 0.44	18.8 ± 0.17	NS	17.1 ± 0.39	17.0 ± 0.16	NS
ALH	3.42 ± 0.09	3.25 ± 0.03	NS	4.89 ± 0.11	4.86 ± 0.05	NS
RAPID (%)	28.1 ± 2.16	24.5 ± 0.87	0.022	64.3 ± 2.75	62.7 ± 1.08	NS

The values are expressed as means ± SE.

NS, not significant; VCL, curvilinear velocity; VSL, straight line velocity; VAP, average pathway velocity; LIN, linearity of movement; STR, straightness; BCF, beat cross frequency; ALH, amplitude of lateral head displacement; RAPID, percentage rapid.

Table 4. ROC curve analysis: AUC and cut-off with best sensitivity and specificity combination

	AUC	Cut-off	Sensitivity	Specificity
Motility (%)	0.598	51.4	74	49
Concentration ($\times 10^6$ /mL)	0.599	111	57.4	56
RAPID (%)	0.630	30.1	57.4	70

ROC, receiver operating characteristic; AUC, area under curve; RAPID, percentage rapid.

psychological burden because of the relatively low success rate per cycle, leading to a repetition of the procedure and a risk of higher-order multiple pregnancy associated with superovulation [1,2,4,5].

Many investigators have attempted to determine the optimal predictor of success to help counsel patients before they make the decision to proceed with IVF/ICSI-ET. It is known that female age, duration of infertility, tubal patency, the number of induced follicles, the insemination technique, timing, and several semen parameters may affect the outcome of the IUI procedure [3,7]. Among semen parameters, sperm motility is believed to be one of the most important characteristics correlated with fertility [22,23].

Prospective studies have shown that the conventional semen profile of the WHO is unable to discriminate between fertile and infertile men, especially in idiopathic infertility [22,24-26]. Accordingly, additional functional tests, such as the zona-free hamster egg penetration test [27] and evaluation of acrosome reaction combined with assessments of cell viability [28], have been suggested to provide more information on the fertilizing potential of human spermatozoa in addition their number or appearance. However, many of the assays involved are technically complex.

CASA is an automated method that can measure several specific motility parameters that describe the movements of spermatozoa with the advantage of providing more objective, reproducible, and detailed data on sperm motility. Many studies have indicated that the direct quantitative assessment of sperm movement by CASA reflects the fertilizing ability of human spermatozoa *in vitro* under conditions where the conventional semen profile is of limited diagnostic value. A prospective follow-up study reported that the concentration of motile spermatozoa, defined as spermatozoa with a VCL $> 25 \mu\text{m}/\text{sec}$, was the most significant and independent CASA parameter in predicting the chance of natural conception in the general male population [14]. Barratt et al. [29] found the total number of spermatozoa and VAP to be predictors of pregnancy. Other parameters such as ALH, VSL, VCL, and LIN have also been reported to be correlated with fertility [28,30].

The relative influence of semen parameters on the likelihood of a successful outcome in IUI remains controversial. Shibahara et al. identified three semen parameters including normal morphology $\geq 15.5\%$

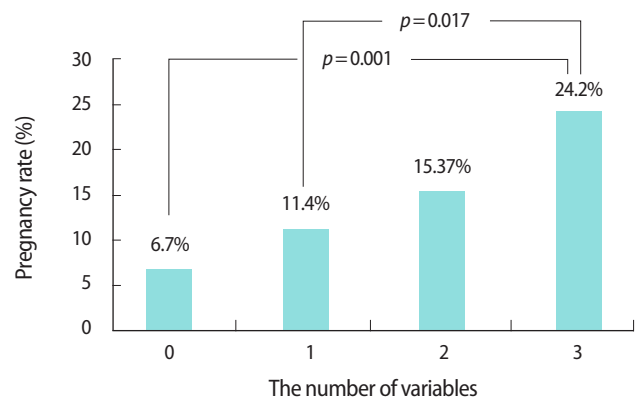


Figure 1. Association between pregnancy rate and number of variables exceeding the threshold for the three independent predictive parameters.

before sperm separation, rapid motility $> 25.5\%$, and VCL $> 102.6 \mu\text{m}/\text{sec}$ after sperm preparation as predictors of pregnancy by IUI in male infertility, and reported that no pregnancy was achieved if none of these three parameters were satisfied [15]. Freour et al. [20] found that the concentration and proportion of progressive motile spermatozoa, defined as cells with VAP $> 25 \mu\text{m}/\text{sec}$ and VSL $> 100 \mu\text{m}/\text{sec}$, were higher in the pregnancy group than in the failure group, while respective sperm motility parameters measured by CASA failed to predict pregnancy in IUI cycles conducted with donor sperm. They also reported that ALH improvement during the sperm preparation is necessary for pregnancy in an IUI with frozen donor semen and speculated that it could help in improving pregnancy rates in IUI with donor semen programs, as ALH evolution before and after preparation can be easily calculated [21].

In a retrospective analysis of the relationship between the CASA estimates and fertilization rates in IVF-ET cycles, two parameters, including VCL and rapid, provide reliable estimations of the fertilizing ability of human sperm [19].

Most of the studies on CASA parameters and fertility outcome in IUI have been conducted in couples with male infertility, whereas few studies were conducted in couples with unexplained infertility. In this study, we assessed the sperm motility parameters by CASA in 383 IUI cycles with superovulation indicated in couples with unexplained infertility. Specific CASA parameters such as VAP, RAPID, VSL, VCL, as well as sperm concentration and motility were significantly different between the pregnant group and the non-pregnant group. We also found that the combination of parameters such as RAPID $\geq 30.1\%$, motility $\geq 51.4\%$, and concentration $\geq 111 \times 10^6/\text{mL}$ before sperm preparation could be useful in predicting pregnancy in IUI. For instance, the pregnancy rate was highest if all three parameters were satisfied, while it was lowest if no parameters were satisfied.

Many studies reported that the improvement of sperm motility pa-

rameters during the preparation process or their value after preparation were correlated with their pregnancy outcome of IUI [15-21], whereas we did not find any difference between CASA parameters after sperm preparation according to cycle outcome. This may have been due to our use of fresh semen of normal values by WHO criteria, in contrast to previous studies that utilized semen with male factor infertility or frozen-thawed donor sperm.

In general, one of the most important movements associated with sperm fertilizing ability is known to be *hyperactivated* motility [31, 32]. Hyperactivation is characterized by the development of high velocity, large amplitude, asymmetrical flagellar waves, and is thought to facilitate the detachment of spermatozoa from the oviductal epithelium and penetration of the matrix of the cumulus oophorus and the zona pellucida [32]. CASA has been used to detect hyperactivation, as it generates a characteristic non-progressive star-spin pattern of movement, and to identify the percentage of sperm in a sample that are hyperactivated. As for hyperactivation, we did not find any difference between the pregnant and the non-pregnant groups. Also, there was no difference in the pregnancy rate according to hyperactivation. It can be postulated that hyperactivation occurred when the spermatozoa reached the oocyte vicinity and CASA could only be of limited value to the assessment of hyperactivation *in vitro* before the IUI procedure. Unfortunately, factors triggering hyperactivation *in vivo* are poorly understood, and it has been suggested that the trigger may be hormones, ions, secretions of the oviductal epithelium, or a change in the pH of oviduct fluid or follicular fluid introduced into the oviduct by the oocyte-cumulus mass [33,34].

In conclusion, concentration and motility assessment of semen by CASA is a fairly useful method for predicting pregnancy outcome after IUI with superovulation, which could be helpful in planning strategies for the further treatment of unexplained infertile couples by proceeding with IVF/ICSI-ET.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

References

1. ESHRE Capri Workshop Group. Intrauterine insemination. Hum Reprod Update 2009;15:265-77.
2. Stone BA, Vargyas JM, Ringler GE, Stein AL, Marrs RP. Determinants of the outcome of intrauterine insemination: analysis of outcomes of 9963 consecutive cycles. Am J Obstet Gynecol 1999;180:1522-34.
3. van der Westerlaken LA, Naaktgeboren N, Helmerhorst FM. Evaluation of pregnancy rates after intrauterine insemination according to indication, age, and sperm parameters. J Assist Reprod Genet 1998;15:359-64.
4. Ransom MX, Blotner MB, Bohrer M, Corsan G, Kemmann E. Does increasing frequency of intrauterine insemination improve pregnancy rates significantly during superovulation cycles? Fertil Steril 1994;61:303-7.
5. Dodson WC, Haney AF. Controlled ovarian hyperstimulation and intrauterine insemination for treatment of infertility. Fertil Steril 1991;55:457-67.
6. Verhulst SM, Cohlen BJ, Hughes E, Te Velde E, Heineman MJ. Intra-uterine insemination for unexplained subfertility. Cochrane Database Syst Rev 2006;(4):CD001838.
7. Steures P, van der Steeg JW, Mol BW, Eijkemans MJ, van der Veen F, Habbema JD, et al. Prediction of an ongoing pregnancy after intrauterine insemination. Fertil Steril 2004;82:45-51.
8. Shulman A, Hauser R, Lipitz S, Frenkel Y, Dor J, Bider D, et al. Sperm motility is a major determinant of pregnancy outcome following intrauterine insemination. J Assist Reprod Genet 1998; 15:381-5.
9. Hauser R, Yogev L, Botchan A, Lessing JB, Paz G, Yavetz H. Intrauterine insemination in male factor subfertility: significance of sperm motility and morphology assessed by strict criteria. Andrologia 2001;33:13-7.
10. van Voorhis BJ, Barnett M, Sparks AE, Syrop CH, Rosenthal G, Dawson J. Effect of the total motile sperm count on the efficacy and cost-effectiveness of intrauterine insemination and in vitro fertilization. Fertil Steril 2001;75:661-8.
11. World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. Cambridge: Cambridge University Press; 1999.
12. Jørgensen N, Auger J, Giwercman A, Irvine DS, Jensen TK, Jouannet P, et al. Semen analysis performed by different laboratory teams: an intervariation study. Int J Androl 1997;20:201-8.
13. Brazil C, Swan SH, Drobnis EZ, Liu F, Wang C, Redmon JB, et al. Standardized methods for semen evaluation in a multicenter research study. J Androl 2004;25:635-44.
14. Larsen L, Scheike T, Jensen TK, Bonde JP, Ernst E, Hjøllund NH, et al. Computer-assisted semen analysis parameters as predictors for fertility of men from the general population. The Danish First Pregnancy Planner Study Team. Hum Reprod 2000;15:1562-7.
15. Shibahara H, Obara H, Ayustawati, Hirano Y, Suzuki T, Ohno A, et al. Prediction of pregnancy by intrauterine insemination using CASA estimates and strict criteria in patients with male factor infertility. Int J Androl 2004;27:63-8.
16. Macleod IC, Irvine DS. The predictive value of computer-assisted semen analysis in the context of a donor insemination programme. Hum Reprod 1995;10:580-6.

17. Marshburn PB, McIntire D, Carr BR, Byrd W. Spermatozoal characteristics from fresh and frozen donor semen and their correlation with fertility outcome after intrauterine insemination. *Fertil Steril* 1992;58:179-86.
18. Sidhu RS, Sharma RK, Agarwal A. Effects of cryopreserved semen quality and timed intrauterine insemination on pregnancy rate and gender of offspring in a donor insemination program. *J Assist Reprod Genet* 1997;14:531-7.
19. Hirano Y, Shibahara H, Obara H, Suzuki T, Takamizawa S, Yamaguchi C, et al. Relationships between sperm motility characteristics assessed by the computer-aided sperm analysis (CASA) and fertilization rates in vitro. *J Assist Reprod Genet* 2001;18:213-8.
20. Freour T, Jean M, Mirallie S, Langlois ML, Dubourdiou S, Barriere P. Predictive value of CASA parameters in IUI with frozen donor sperm. *Int J Androl* 2009;32:498-504.
21. Fréour T, Jean M, Mirallié S, Dubourdiou S, Barrière P. Computer-Assisted Sperm Analysis (CASA) parameters and their evolution during preparation as predictors of pregnancy in intrauterine insemination with frozen-thawed donor semen cycles. *Eur J Obstet Gynecol Reprod Biol* 2010;149:186-9.
22. Bostofte E, Bagger P, Michael A, Stakemann G. Fertility prognosis for infertile men: results of follow-up study of semen analysis in infertile men from two different populations evaluated by the Cox regression model. *Fertil Steril* 1990;54:1100-6.
23. Eimers JM, te Velde ER, Gerritse R, Vogelzang ET, Looman CW, Habbema JD. The prediction of the chance to conceive in subfertile couples. *Fertil Steril* 1994;61:44-52.
24. Aitken RJ, Best FS, Warner P, Templeton A. A prospective study of the relationship between semen quality and fertility in cases of unexplained infertility. *J Androl* 1984;5:297-303.
25. Irvine DS, Aitken RJ. Predictive value of in-vitro sperm function tests in the context of an AID service. *Hum Reprod* 1986;1:539-45.
26. Ducot B, Spira A, Feneux D, Jouannet P. Male factors and the likelihood of pregnancy in infertile couples. II. Study of clinical characteristics—practical consequences. *Int J Androl* 1988;11:395-404.
27. Shibahara H, Mitsuo M, Inoue M, Hasegawa A, Shigeta M, Koyama K. Relationship between human in-vitro fertilization and intracytoplasmic sperm injection and the zona-free hamster egg penetration test. *Hum Reprod* 1998;13:1928-32.
28. Aitken RJ. Sperm function tests and fertility. *Int J Androl* 2006;29:69-75.
29. Barratt CL, Tomlinson MJ, Cooke ID. Prognostic significance of computerized motility analysis for in vivo fertility. *Fertil Steril* 1993;60:520-5.
30. Paston MJ, Sarkar S, Oates RP, Badawy SZ. Computer-aided semen analysis variables as predictors of male fertility potential. *Arch Androl* 1994;33:93-9.
31. Sukcharoen N, Keith J, Irvine DS, Aitken RJ. Definition of the optimal criteria for identifying hyperactivated human spermatozoa at 25 Hz using in-vitro fertilization as a functional end-point. *Hum Reprod* 1995;10:2928-37.
32. Suarez SS. Control of hyperactivation in sperm. *Hum Reprod Update* 2008;14:647-57.
33. Nichol R, Hunter RH, Gardner DK, Leese HJ, Cooke GM. Concentrations of energy substrates in oviductal fluid and blood plasma of pigs during the peri-ovulatory period. *J Reprod Fertil* 1992;96:699-707.
34. Yao Y, Ho P, Yeung WS. Effects of human follicular fluid on the capacitation and motility of human spermatozoa. *Fertil Steril* 2000;73:680-6.