*Original Article*

# Accuracy of sperm velocity assessment using the Sperm Quality Analyzer V

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*Aim*: The correct diagnosis of the functional capacity of human sperm is limited. The Sperm Quality Analyzer (SQA) with the visualization system (SQA V, Medical Electronic System, Hatavorzo, Israel), an upgraded version of SQA, was recently developed to provide a rapid and low-cost quantitative evaluation of sperm quality as well as sperm velocity assessment. The aim of the present study was to evaluate whether the SQA V's new parameters correlate with computer-aided sperm analysis (CASA) estimates.

*Methods*: Semen quality analysis of 66 fresh samples was determined using SQA V and CASA.

*Results*: There were significant correlations of total sperm concentration ($P < 0.001$), sperm motility ($P = 0.145$), and percentage of progressive motile sperm ($P = 0.001$), between the SQA V variables and the CASA estimates. The sperm velocity assessed by SQA V was significantly correlated with

some of the CASA estimates, including sperm motility ($P = 0.001$), the percentage of progressively motile sperm ($P < 0.001$), straight-line velocity ($P < 0.001$), curvilinear velocity ($P < 0.001$) and average path velocity ($P < 0.001$). However, it did not correlate with amplitude of lateral head displacement, beat cross frequency, straightness, or linearity, assessed by CASA.

*Conclusion*: Assessment of sperm motility has been shown as one of the important factors to predict the functional capacity of human sperm. On the basis of the present study, SQA V is considered useful for screening sperm quality in the management of male infertility. (Reprod Med Biol 2003; **2**: 151–157)

**Key words:** computer-aided sperm analysis, male infertility, semen analysis, Sperm Quality Analyzer visualization system, velocity.

## INTRODUCTION

THE SEMEN PARAMETERS are one of the most important predictive values in fertilization and pregnancy rates in *in-vitro* fertilization and embryo transfer (IVF-ET).[1] However, the traditional microscopic measurement of semen parameters has been limited.[2–4] The most frequently used method is the Makler chamber for measuring sperm concentration and sperm motility. However, this technique is subject to high variability. Sperm motility is influenced by temperature, the depth and nature of the chamber, and the subjective component when differentiating the grades of sperm motility.[5] However, assessment of morphology

is influenced by fixation and staining skills, quality of the microscope, and the observer's subjectivity. As the methods are highly variable, several programs for objective analyzing have been developed.[6–11]

In recent years, some automated systems, known as computer-aided sperm analysis (CASA), have been available.[12] The automated system of CASA was developed to provide two advantages over manual methods if appropriately followed.[13] One advantage is high precision and the other is provision of quantitative data on sperm kinetics. There are several reports demonstrating good correlations of sperm motions estimated by CASA with fertilization rates.[14,15] However, CASA instruments are not easy to master and their reliability depends on training, ability, and the experience of the user. Additionally, the equipment is expensive and therefore it is inaccessible for most laboratories.

In contrast, Sperm Quality Analyzer (SQA; Medical Electronic System, Hatavorzo, Israel), a unique compact desktop instrument that combines optical detection with

an internal computer, was also developed to provide a rapid, easy and low-cost quantitative evaluation of semen quality. Although the clinical usefulness of SQA has previously been demonstrated,[16–18] we have shown that the sperm motility index (SMI), assessed by SQA, provides a reliable estimation of human sperm quality,[19] and correlates well with CASA estimates.[20] Recently, SQA with the visualization system (SQA V), an upgraded version of SQA, was developed to provide sperm velocity assessment. The aim of the present study was to evaluate whether the sperm velocity assessment by SQA V correlates with CASA estimates, especially sperm motion variables.

## MATERIALS AND METHODS

### Semen specimens

SIXTY-SIX FRESH SEMEN SAMPLES were collected from 66 men (collected via masturbation), who visited the Department of Obstetrics and Gynecology at the Hospital of Jichi Medical School or the Central Clinic, between March 2002 and June 2002. After obtaining informed consents, the semen samples were analyzed by CASA and SQA V, simultaneously.

### Routine semen analysis

After liquefaction, routine semen analysis was carried out by the CASA system (Hamilton Thorne Research, Beverly, MA, USA) at room temperature, as we previously described.[15] In brief, a 5 μL aliquot of semen sample was placed in the Makler chamber. A total of 200 sperm were counted with CASA to examine the sperm concentration, sperm motility, and sperm motion variables, including amplitude of lateral head displacement (ALH), beat cross frequency (BCF), curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), linearity (LIN = VSL/VCL), and straightness (STR = VSL/VAP). The CASA settings were followed according to the manufacturer's instructions.

### Semen analysis using Sperm Quality Analyzer visualization system

The SQA V device is shown in Fig. 1. A 0.6 mL of semen sample was aspirated into the capillary and inserted into the electro-optical chamber of the instrument. Immediately, light beams translated their characteristics into electrical signals. The computer analyzed the data applying special algorithms and reported the results according to the World Health Organization (WHO)
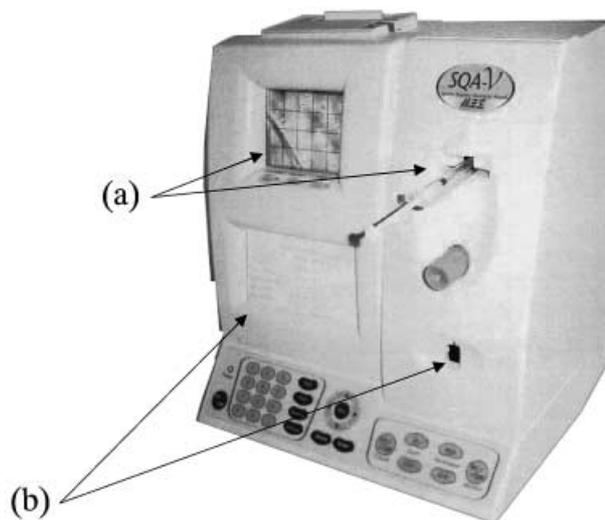


**Figure 1** The Sperm Quality Analyzer visualization system (SQA V) device. (a) The SQA V composite capillary is filled with a sperm sample according to the attached instructions, and tested in SQA V according to the on-screen instructions. (b) The same sample is used for the phase-contrast microscopic examination in a Makler counting chamber.

manuals and other parameters. After a 75 s period, the SQA V digitally displayed the following data: total sperm concentration (TSC), motility percentage, progressive motility percentage, normal morphology percentage, motile sperm concentration (MSC), progressively motile sperm concentration (PMSC), functional sperm concentration (FSC), and average velocity of the progressively motile sperm (velocity).

### Statistical analysis

Statistical analysis of the data was carried out using the Statistical Package for Social Science (SPSS) for personal computers, version 10.0 for Windows (SPSS Institute, Chicago, IL, USA). Results were presented as mean ± SD. The statistical analysis consisted of analysis of variance. A value of $P < 0.05$ was defined as representing a significant difference. Correlations were measured with Pearson's correlation coefficient. Correlations were estimated through linear regression analysis.

## RESULTS

### Semen characteristics of 66 men

SEMEN CHARACTERISTICS WERE measured by CASA performed on 66 patients. The mean ± SD of the sperm

**Table 1** Semen characteristics using computer-aided sperm analysis for 66 samples

| Characteristics | Mean ± SD | Range |
|---|---|---|
| CASA estimates | | |
|   Concentration ($10^6$/mL) | 144.5 ± 158.4 | 1.4–1135.8 |
|   Motility (%) | 45.3 ± 22.0 | 3.0–88.0 |
|   Rapid (%) | 20.2 ± 15.0 | 0–52.0 |
| Sperm motion variables | | |
|   VCL (μm/s) | 82.5 ± 15.7 | 40.5–133.0 |
|   VSL (μm/s) | 46.1 ± 9.7 | 21.4–73.7 |
|   VAP (μm/s) | 56.1 ± 10.1 | 27.3–86.1 |
|   ALH (μm) | 4.0 ± 4.4 | 0–37.4 |
|   BCF (Hz) | 23.6 ± 5.0 | 9.7–32.3 |
|   STR (VSL/VAP) | 80.5 ± 6.1 | 68.0–91.0 |
|   LIN (VSL/VCL) | 57.4 ± 8.9 | 38.0–76.0 |

ALH, amplitude of lateral head displacement; BCF, beat cross frequency; LIN, linearity; STR, straightness; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight-line velocity.

concentration, sperm motility, rapid motility, VCL, VSL, VAP, ALH, BCF, STR, and LIN were 144.5 ± 158.4 × $10^6$/ mL (range: 1.4–1135.8), 45.3 ± 22.0% (range: 3.0–88.0), 20.2 ± 15.0% (range: 0–52.0), 82.5 ± 15.7 μm/s (range: 40.5–133.0), 46.1 ± 9.7 μm/s (range: 21.4–73.7), 56.1 ± 10.1 μm/s (range: 27.3–86.1), 4.0 ± 4.4 μm (range: 0– 37.4), 23.6 ± 5.0 Hz (range: 9.7–32.3), 80.5 ± 6.1 (range: 68.0–91.0) and 57.4 ± 8.9 (range: 38.0–76.0), respectively (Table 1).

## Assessment of Sperm Quality Analyzer visualization system variables

Assessment of SQA V was simultaneously carried out on 66 men. The mean ± SD of the SQA variables, including TSC, motility percentage, progressive motility percentage, normal morphology percentage, MSC, PMSC, FSC, and sperm velocity were 74.3 ± 48.2 × $10^6$/mL (range: 8.1– 227.8), 36.4 ± 21.1% (range: 0–90.3), 29.6 ± 17.7% (range: 0–82.7), 7.0 ± 6.7% (range: 0–41.6), 22.4 ± 17.0 × $10^6$/mL (range: 1.2–87.5), 19.3 ± 15.5 × $10^6$/mL (range: 0.5–79.2), 2.8 ± 3.2 × $10^6$/mL (range: 0–15.1) and 14.1 ± 4.2 μm/s (range: 1.0–20.0), respectively (Table 2).

## Comparison of Sperm Quality Analyzer visualization system variables and computer-aided sperm analysis estimates

The relationships between the SQA variables and the CASA estimates were compared. Regarding the basic semen characteristics, there were significant correlations of total sperm concentration ($P < 0.001$, $r = 0.693$; Fig. 2a), and percentage of progressively motile sperm ($P = 0.001$, $r = 0.397$; Fig. 2c), between the SQA V variables and the CASA estimates. However, there was no significant relationship for sperm motility ($P = 0.145$, $r = 0.186$; Fig. 2b) between the two devices.

Regarding the sperm velocity assessment by SQA V, it was compared with the sperm motion variables by CASA. There were significant correlations with the CASA estimates, including sperm motility ($P = 0.001$, $r = 0.514$; Fig. 3a), percentage of progressively motile sperm ($P < 0.001$, $r = 0.472$; Fig. 3b), VCL ($P < 0.001$, $r = 0.459$, Fig. 3c), VSL ($P < 0.001$, $r = 0.508$; Fig. 3d), and VAP ($P < 0.001$, $r = 0.522$; Fig. 3e). However, it did not correlate with ALH, BCF, LIN, or STR assessed by CASA (data not shown).

## DISCUSSION

SEMEN ANALYSIS IS one of the most important predictive values of male infertility, as WHO has suggested that a male problem may be present in as many as 43% of couples.[20] The assessment of male infertility is still

**Table 2** Semen characteristics using Sperm Quality Analyzer visualization system of 66 samples

| Characteristics | Mean ± SD | Range |
|---|---|---|
| TSC ($10^6$/mL) | 74.3 ± 48.2 | 8.1–227.8 |
| Percentage of motile sperm (%) | 36.4 ± 21.1 | 0–90.3 |
| Percentage of progressive motile sperm (%) | 29.6 ± 17.7 | 0–82.7 |
| Normal morphology (%) | 7.0 ± 6.7 | 0–41.6 |
| MSC ($10^6$/mL) | 22.4 ± 17.0 | 1.2–87.5 |
| PMSC ($10^6$/mL) | 19.3 ± 15.5 | 0.5–79.2 |
| FSC ($10^6$/mL) | 2.8 ± 3.2 | 0–15.1 |
| Sperm velocity (μm/s) | 14.1 ± 4.2 | 1.0–20.0 |

FSC, functional sperm concentration; MSC, motile sperm concentration; PMSC, progressively motile sperm concentration; TSC, total sperm concentration.
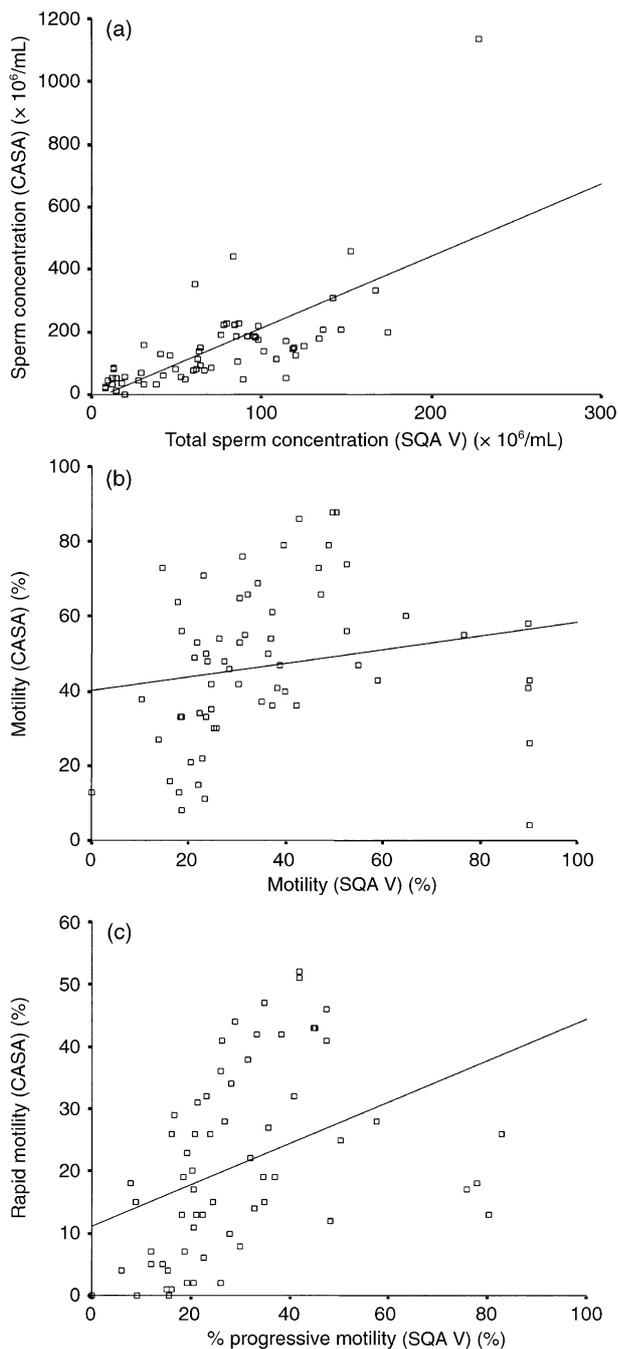
**Figure 2** Comparison of basic semen characteristics between the Sperm Quality Analyzer visualization system (SQA V) variables and the computer-aided sperm analysis (CASA) estimates. Regarding the basic semen characteristics, there were significant correlations of (a) total sperm concentration ($P < 0.001$, $r = 0.693$) and (c) the percentage of progressively motile sperm ($P = 0.001$, $r = 0.397$), between the SQA V variables and the CASA estimates. However, there was no significant relationship of (b) sperm motility ($P = 0.145$, $r = 0.186$) between the two devices.

based on conventional criteria of semen quality, principally semen volume, sperm concentration, percentage motility, and percentage normal morphology. Although WHO standardized the laboratory procedures involved in assessing human semen,[21] the fundamental problem remains that the conventional criteria of semen quality are of very limited prognostic value in predicting the achievement of pregnancy.[22–24] There are some widely used sperm function tests, such as the zona-free hamster egg penetration test (HEPT) and the zona-binding test. Although the diagnostic usefulness of HEPT has been shown,[25] it is recommended that only laboratories with a proven record of assay repeatability should perform the technique.[26] The zona-binding assays may have limited availability of sufficient quantities of human zona-pellucida.[27] As a consequence, several tests of sperm function have evolved, initially using photographic or videomicrographic techniques.[28,29] More recently, CASA was developed, and it has been revealed that CASA provides a rapid and accurate assessment of the attributes of sperm motion.[30] It has also been revealed that CASA provides reliable estimations of the fertilization ability of human sperm.[15] Therefore, sperm velocity, one of the CASA estimates, is a sensitive indicator of sperm functional ability in a postcoital test, artificial insemination with the husband's semen[31] and fertilization rates in IVF.[14,32] We have previously shown that CASA estimates, including VCL and rapid (before after swim-up), have a significant relationship with human sperm fertilizing ability.[15]

We have also shown that, CASA and SQA are likely to be useful in predicting the fertilization rate in IVF-ET.[33] Despite the advantages of CASA, the systems are currently still expensive. The CASA techniques depend on training, ability, and experience of the user. It has also been suggested that there is a problem associated with the distinction between the debris and the sperm.

Sperm Quality Analyzer visualization system is a simple and inexpensive device providing a quantitative estimation of sperm motility and morphology. Moreover, by using this up-graded version, we can directly visualize sperm movement on a screen (Fig. 1). Additionally, SQA V has good reproducibility, particularly in comparison with conventional semen analysis, which is highly subjective. Sperm Quality Analyzer uses light passed through a small sample of the semen to detect variations in optical density (OD) that result from moving particles. Fluctuations in OD are registered by a photometric cell, and the frequency of the analog wave-like electrical signal is converted into a digital one to provide a numerical
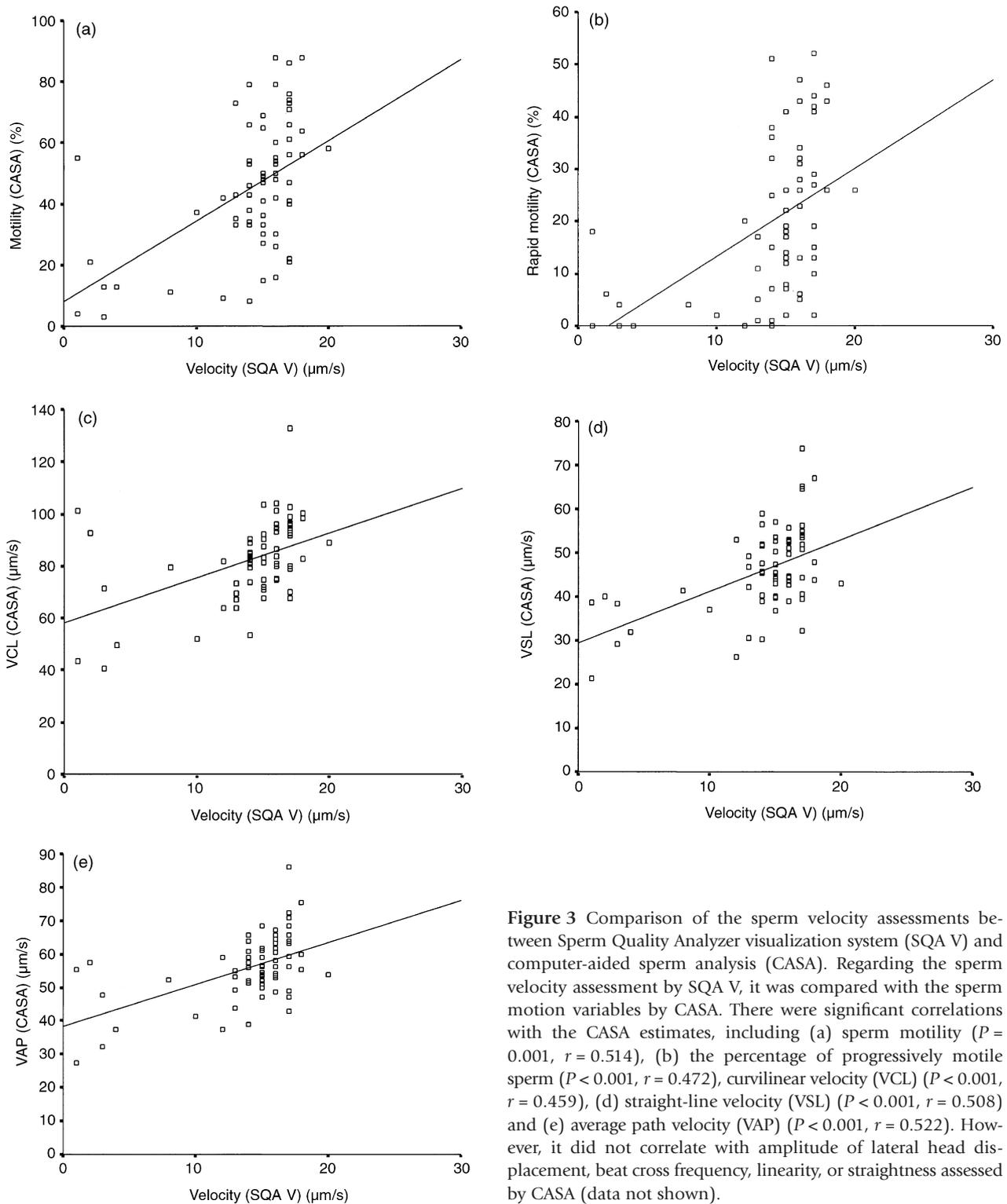
**Figure 3** Comparison of the sperm velocity assessments between Sperm Quality Analyzer visualization system (SQA V) and computer-aided sperm analysis (CASA). Regarding the sperm velocity assessment by SQA V, it was compared with the sperm motion variables by CASA. There were significant correlations with the CASA estimates, including (a) sperm motility ($P = 0.001$, $r = 0.514$), (b) the percentage of progressively motile sperm ($P < 0.001$, $r = 0.472$), curvilinear velocity (VCL) ($P < 0.001$, $r = 0.459$), (d) straight-line velocity (VSL) ($P < 0.001$, $r = 0.508$) and (e) average path velocity (VAP) ($P < 0.001$, $r = 0.522$). However, it did not correlate with amplitude of lateral head displacement, beat cross frequency, linearity, or straightness assessed by CASA (data not shown).

output, and the various parameters are expressed. As SQA V recognizes only motile particles, problems with debris, which may have influenced the difference of the sperm concentration between CASA and SQA V, have been excluded.

Assessment of sperm motility has been shown as one of the important factors to predict the functional capacity of human sperm. In the present study, sperm velocity assessed by SQA V showed significant correlations with some of the CASA estimates (Fig. 3a–e). In particular, sperm velocity assessed by SQA V correlated well with VCL (Fig. 3c) and rapid (Fig. 2c), which we have shown to be the most important CASA parameters to predict fertilization rates in IVF. These results indicate that SQA V could be used as a routine semen examination to select the appropriate treatment for patients with male factor infertility.

On the basis of the results from the present study, SQA V is considered useful for the screening of semen quality in the management of male infertility. Further studies are required to investigate the relationship between the SQA V parameters and fertilization rates in IVF.

## REFERENCES

1 Donnelly ET, Lewis SE, McNally JA, Thompson W. In vitro fertilization and pregnancy rates: the influence of sperm motility and morphology on IVF outcome. *Fertil Steril* 1998; **70**: 305–314.

2 Mahadevan MM, Trounson AO. The influence of seminal characteristics on the success rate of human in vitro fertilization. *Fertil Steril* 1984; **42**: 400–405.

3 Dunphy BC, Neal LM, Cooke ID. The clinical value of conventional semen analysis. *Fertil Steril* 1989; **51**: 324–329.

4 Silber SJ. The relationship of abnormal semen parameters to male fertility. *Hum Reprod* 1989; **4**: 947–953.

5 Yeung CH, Cooper TG, Nieschlag E. A technique for standardization and quality control of subjective sperm motility assessments in semen analysis. *Fertil Steril* 1997; **67**: 1156–1158.

6 Chong AP, Walters CA, Weinrieb SA. The neglected laboratory test. The semen analysis. *J Androl* 1983; **4**: 280–282.

7 Jequier AM, Ukombe EB. Errors inherent in the performance of a routine semen analysis. *Br J Urol* 1983; **55**: 434–436.

8 Mortimer D, Shu MA, Tan R. Standardization and quality control of sperm concentration and sperm motility counts in semen analysis. *Hum Reprod* 1986; **1**: 299–303.

9 Dunphy BC, Kay R, Barratt CL, Cooke ID. Quality control during the conventional analysis of semen, an essential exercise. *J Androl* 1989; **10**: 378–385.

10 Neuwinger J, Behre HM, Nieschlag E. External quality control in the andrology laboratory: an experimental multicenter trial. *Fertil Steril* 1990; **54**: 308–314.

11 Cooper TG, Atkinson AD, Nieschlag E. Experience with external quality control in spermatology. *Hum Reprod* 1999; **14**: 765–769.

12 Holt W, Watson P, Curry M, Holt C. Reproducibility of computer-aided semen analysis: comparison of five different systems used in a practical workshop. *Fertil Steril* 1994; **62**: 1277–1282.

13 Davis RO, Katz DF. Standardization and comparability of CASA instruments. *J Androl* 1992; **13**: 81–86.

14 Parinaud J, Richoilley G, Moutaffian H *et al.* Are the characteristics of spermatozoa in the insemination medium useful for predicting in-vitro fertilization results? *Int J Androl* 1996; **19**: 103–108.

15 Hirano Y, Shibahara H, Obara H *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**: 213–218.

16 Bartoov B, Ben-Barak J, Mayevsky A, Sneider M, Yogev L, Lightman A. Sperm motility index: a new parameter for human sperm evaluation. *Fertil Steril* 1991; **56**: 108–112.

17 Martinez C, Mar C, Azcarate M, Pascual P, Aritzeta JM, Lopez-Urrutia A. Sperm motility index: a quick screening parameter from sperm quality analyser-IIB to rule out oligo- and asthenozoospermia in male fertility study. *Hum Reprod* 2000; **15**: 1727–1733.

18 Shibahara H, Suzuki T, Obara H *et al.* Accuracy of the normal sperm morphology value by Sperm Quality Analyzer IIC: comparison with the strict criteria. *Int J Androl* 2002; **25**: 45–48.

19 Shibahara H, Naito S, Hasegawa A, Mitsuo M, Shigata M, Koyama K. Evaluation of sperm fertilizing ability using the Sperm Quality Analyzer. *Int J Androl* 1997; **20**: 112–117.

20 Cates W, Farley TM, Rowe PJ. Worldwide patterns of infertility: is Africa different? *Lancet* 1985; **14**: 596–598.

21 World Health Organization. *WHO Laboratory Manual for the Examination of Human Sperm and Semen–Cervical Mucus Interaction*, 4th edn. Cambridge: Cambridge University Press, 1999.

22 Aitken RJ, Sutton M, Warner P, Richardson DW. Relationship between the movement characteristics of human spermatozoa and their ability to penetrate cervical mucus and zona-free hamster oocytes. *J Reprod Fertil* 1985; **73**: 441–449.

23 Irvine DS, Aitken RJ. Predictive value of in-vitro sperm function tests in the context of an AID service. *Hum Reprod* 1986; **1**: 539–545.

24 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**: 1100–1106.

25 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**: 1928–1932.

26 ESHRE Andrology Special Interest Group. Consensus workshop on advanced diagnostic andrology techniques. *Hum Reprod* 1996; **11**: 1463–1479.

27 Burkman LJ, Coddington CC, Franken DR *et al*. The hemizona assay (HZA): development of a diagnostic test for the binding of human spermatozoa to the human hemizona pellucida to predict fertilization potential. *Fertil Steril* 1988; **49**: 688–697.

28 Overstreet JW, Katz DF, Hanson FW, Fonseca JR. A simple inexpensive method for objective assessment of human sperm movement characteristics. *Fertil Steril* 1979; **31**: 162–172.

29 Katz DF, Overstreet JW. Sperm motility assessment by videomicrography. *Fertil Steril* 1981; **35**: 188–193.

30 Mathur S, Carlton M, Ziegler J, Rust FP, Williamson HO. A computerized sperm motion analysis. *Fertil Steril* 1986; **46**: 484–488.

31 Beauchamp PJ, Galle PC, Blasco L. Human sperm velocity and postinsemination cervical mucus test in the evaluation of the infertile couple. *Arch Androl* 1984; **13**: 107–112.

32 Fetterolf PM, Rogers BJ. Prediction of human sperm penetrating ability using computerized motion parameters. *Mol Reprod Dev* 1990; **27**: 326–331.

33 Suzuki T, Shibahara H, Tsunoda H *et al*. Comparison of the Sperm Quality Analyzer IIC variables with the computer-aided sperm analysis estimates. *Int J Androl* 2002; **2**: 49–54.