*Journal of Assisted Reproduction and Genetics, Vol. 18, No. 4, 2001*

# ANDROLOGY

## Relationships Between Sperm Motility Characteristics Assessed by the Computer-Aided Sperm Analysis (CASA) and Fertilization Rates In Vitro

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*Submitted: September 27, 2000*
*Accepted: November 8, 2000*

***Purpose:*** *Some studies have suggested that computer-aided sperm analysis (CASA) estimates of concentration and movement characteristics of progressively motile spermatozoa are related to fertilization rates in vitro. However, it has also been suggested that the greater number of motility parameters assessed by CASA does not imply more precision in predicting fertility. This study was carried out to investigate the relationships between the CASA estimates and fertilization rates in vitro.*

***Methods:*** *Semen quality analysis was performed using CASA in 136 in vitro fertilization-embryo transfer (IVF-ET) cycles with at least 3 oocytes collected. The CASA estimates before and after swim-up were compared between 108 cycles with fertilization rate >50% ("good" group) and 28 cycles with fertilization rate ≤50% ("poor" group).*

***Results:*** *Before swim-up, there were significant correlations between fertilization rates and CASA estimates, including amplitude of lateral head displacement (ALH) (*$r = .269$*), curvilinear velocity (VCL) (*$r = .297$*), straight line velocity (VSL) (*$r = .266$*), and rapid sprm movement (Rapid) (*$r = .243$*). There was also a significant correlation between the fertilization rates and straightness (STR) after swim-up (*$r = -0.178$*). As for sperm movement characteristics, there were significant differences of ALH (*$p < .005$*), VCL (*$p < .001$*), VSL (*$p < .005$*), and Rapid (*$p < .01$*) between "good" and "poor" groups before swim-up. After swim-up, there were significant differences of VCL (*$p < .005$*), average path velocity (VAP) (*$p < .005$*), and Rapid (*$p < .05$*) between the two groups.*

***Conclusions:*** *These results indicate that some of the CASA estimates provide reliable estimation of the fertilizing ability of human sperm. There were significant differences of the two sperm movement characteristics, including VCL and Rapid (before and after swim-up), indicating that the total distance traveled by rapid sperm movement might be important in human sperm fertilizing abilities.*

**KEY WORDS:** Computer-aided sperm analysis; fertilization; sperm motility.

### INTRODUCTION

Examination of the functional capacity of human spermatozoa in vitro is likely to be of greater value in predicting fertility than the routine semen examinations. Such information would be helpful when counseling couples before they make the decision to proceed with in vitro fertilization-embryo transfer (IVF-ET). This information could aid the laboratory in planning its strategy at the time of insemination. Although IVF provides the best means of investigating sperm–egg interaction and estimating sperm fertilizing ability for diagnostic purposes, it cannot obviously be used as a routine screening test. Because the absolute predictive value of the so-called "basic"

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1058-0468/01/0400-0213$19.50/0 

semen analysis is relatively poor in relation to fertility potential through either spontaneous conception or following assisted conception treatment (1–5), several discriminatory tests that can foretell the fertilizing potential of spermatozoa have been identified. These include the zona-free hamster egg penetration test (6), Sperm Quality Analyzer (7,8), and the "strict criteria" for sperm morphology (9) to determine an indication of intracytoplasmic sperm injection (ICSI).

We used the computer-aided sperm analysis (CASA) system to investigate the sperm motility characteristics in semen samples from infertile patients treated by IVF-ET. The development of CASA systems that can identify and track human sperm has revolutionized the research of the movement of human sperm (10–12). The CASA has the advantage of providing objective semen analysis data. It has increased the accuracy and reproducibility of sperm count and motility. However, it has also been suggested that the greater number of motility parameters assessed by CASA does not imply more precision in predicting fertility. The aim of this study was to investigate the usefulness of the CASA as a sperm function test, by a retrospective analysis of the relationship between the CASA estimates and fertilization rates in vitro.

## MATERIALS AND METHODS

### Semen Samples

One hundred and thirty-six fresh semen samples from 99 men were obtained by masturbation, and at least three oocytes were collected in conventional IVF-ET cycles between May 1995 and December 1999. All IVF-ET cycles were performed according to the procedure as we previously described (6,7). A total of 99 women with a mean age of 33.5 years were treated in 136 cycles. The medical indications for conventional IVF-ET treatment were as follows: a tubal factor in 46 cases, a female immunological factor (such as sperm-immobilizing antibodies) in 2 cases, unexplained in 42 cases, and a male factor in 9 cases.

### Routine Semen Analysis Using CASA System

After liquefaction, semen quality analysis was performed using the CASA system (Hamilton Thorne Research, Beverly MA, USA) in the 136 IVF-ET cycles. Briefly, a 5-$\mu$L aliquot of semen sample was placed in the Makler chamber. At least 200 sperm were counted with CASA to evaluate 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. Sperm morphology was assessed following the method by Kruger *et al.* (13).

### Swim-Up Method

The swim-up procedure was followed as we previously described (9). Briefly, semen samples were mixed with 7 mL of Sydney IVF Sperm Buffer (COOK IVF, Australia) containing human serum albumin (HSA) and centrifuged at 569 $\times$ *g* (1800 rpm) for 5 min. The pellet was resuspended with 0.5 mL of the same medium. Swim-up was performed using a combined migration–sedimentation method (14) in a BIO-LABO tube (Jyuji Field, Tokyo). Spermatozoa migrate from semen contained in a ring-shaped well that is completely overlaid with a layer of culture medium. The central hole of the ring constitutes the collection well into which motile spermatozoa settle within 1–2 h at 37°C. The upper two-thirds of the supernatant was collected and mixed with Sydney IVF Sperm Medium (COOK IVF, Australia), followed by centrifugation at 569 $\times$ *g* for 5 min. The pellet was resuspended with 0.5 mL of the same medium and the sperm motility characteristics were assessed using CASA.

### IVF Protocol

The patients were stimulated using a combination of gonadotropin releasing hormone (GnRH) agonist started in the luteal phase (suppression protocol) followed by gonadotropins, as we reported previously (6–8). On the second or third day after oocyte retrieval, the morphological assessment of embryos was performed under the inverted microscope, and the maximum number of embryos with good quality transferred was three. Clinical pregnancy was diagnosed when the gestational sac was detected by transvaginal ultrasonography.

The IVF treatment cycles were classified into two categories according to the fertilization rate: "good" (fertilization rate >50%), and "poor" (fertilization rate $\leq$50%). These categories were used to determine which CASA estimates were important to predict better fertilization rates in both fresh ejaculates and post–swim-up sperm.

### Statistical Analysis

Statistical analysis of the data was performed by Student's $t$-test, using Statview 4.5 (Abacus Concepts, Berkeley, CA) for Macintosh, and $p < .05$ was defined as representing a significant difference. Pearson's correlation coefficient was used to analyze the predictability of the fertilization rate by the semen characteristics.

## RESULTS

### Results of IVF-ET

The average number of oocytes collected was $10.9 \pm 6.7$ (mean $\pm$ SD) in the 136 IVF-ET treatment cycles. The overall fertilization rate was $75.6 \pm 29.3\%$. No embryo was available in seven cycles. ET was intentionally canceled to avoid developing severe ovarian hyperstimulation syndrome (OHSS) in seven cycles. Clinical pregnancies, including 29 single, 5 twin, and 3 triplet, out of 123 fresh ET were established, giving a pregnancy rate of 30.1%. The implantation rate was 11.6% (48/415).

### Semen Characteristics and Sperm Morphology in 136 Fresh Samples

The semen characteristics and sperm morphology for a total of 136 fresh semen samples from 99 men were evaluated. Table I shows the semen characteristics. The mean $\pm$ SD for semen volume, sperm concentration, sperm motility, motile sperm concentration, and normal sperm morphology were $4.1 \pm 1.5$ mL, $(183.3 \pm 110.1) \times 10^6$/mL, $57.6 \pm 18.3\%$, $(113.7 \pm 83.2) \times 10^6$/ml, and $23.1 \pm 9.2\%$ respectively. As for the sperm motion variables, ALH, BCF, VCL, VSL, VAP, LIN, STR, and Rapid were $3.4 \pm 0.9$ $\mu$m, $25.4 \pm 3.6$ Hz, $83.7 \pm 16.5$ $\mu$m/s, $47.8 \pm 9.7$ $\mu$m/s, $61.4 \pm 45.2$ $\mu$m/s, $58.8 \pm 7.2\%$, $81.4 \pm 5.3\%$, and $36.7 \pm 18.5\%$ respectively.

**Table I.** Semen Characteristics and Sperm Morphology in 136 Fresh Samples from 99 Infertile Subjects Treated with IVF-ET

| Characteristics | Mean $\pm$ SD | Range |
|---|---|---|
| Volume (mL) | $4.1 \pm 1.5$ | 0.5–8.0 |
| Concentration ($10^6$/mL) | $183.3 \pm 110.1$ | 2.2–521.6 |
| Motility (%) | $57.6 \pm 18.3$ | 12.0–92.0 |
| Motile sperm conc. ($10^6$/mL) | $113.7 \pm 83.2$ | 0.6–397.1 |
| Normal morphology (%) | $23.1 \pm 9.2$ | 5.0–58.0 |
| Sperm motion variables | | |
| ALH ($\mu$m) | $3.4 \pm 0.9$ | 0.0–7.20 |
| BCF (Hz) | $25.4 \pm 3.6$ | 14.1–36.0 |
| VCL ($\mu$m/s) | $83.7 \pm 16.5$ | 44.0–131.4 |
| VSL ($\mu$m/s) | $47.8 \pm 9.7$ | 21.2–75.4 |
| VAP ($\mu$m/s) | $61.4 \pm 45.2$ | 31.6–570.7 |
| Linearity (VSL/VCL) | $58.8 \pm 7.2$ | 39.0–77.0 |
| Straightness (VSL/VAP) | $81.4 \pm 5.3$ | 60.0–94.0 |
| Rapid (%) | $36.7 \pm 18.5$ | 0.0–79.0 |

**Table II.** Predictability of the IVF Fertilization Outcome by the Semen Characteristics Before and After Swim-Up

| Characteristics | Correlation | |
|---|---|---|
| | Before swim-up | After swim-up |
| Volume (mL) | −0.043 | NT |
| Concentration ($10^6$/mL) | 0.265*** | 0.176* |
| Motility (%) | 0.298**** | 0.190* |
| Total motile count ($10^6$) | 0.226** | NT |
| Normal morphology (%) | 0.278*** | 0.227** |
| Sperm motion variables | | |
| ALH ($\mu$m) | 0.269*** | 0.058 |
| BCF (Hz) | 0.042 | −0.120 |
| VCL ($\mu$m/s) | 0.297**** | 0.153 |
| VSL ($\mu$m/s) | 0.266*** | 0.065 |
| VAP ($\mu$m/s) | 0.053 | 0.167 |
| Linearity (VSL/VCL) | −0.046 | −0.126 |
| Straightness (VSL/VAP) | 0.015 | −0.178* |
| Rapid (%) | 0.243*** | 0.110 |

*Note.* NT: not tested.
* $p < .05$; ** $p < .01$; *** $p < .005$; **** $p < .001$.

### Predictability of the IVF Fertilization Outcome by the Semen Characteristics

The relationships between the fertilization rates in vitro and semen characteristics were investigated (Table II). There were significant correlations between fertilization rates and semen characteristics, including sperm concentration ($r = .265$, $p < .005$), sperm motility ($r = .298$, $p < .001$), total motile count ($r = .226$, $p < .01$), normal morphology ($r = .278$, $p < .005$), ALH ($r = .269$, $p < .005$), VCL ($r = .297$, $p < .001$), VSL ($r = .266$, $p < .005$), and Rapid ($r = .243$, $p < .005$). However, there were no significant correlations between fertilization rates and semen characteristics such as semen volume, BCF, VAP, LIN, and STR.

### Predictability of the IVF Fertilization Outcome by the Post Swim-Up Semen Characteristics

The relationships between the fertilization rates in vitro and the post swim-up semen characteristics were investigated (Table II). There were significant correlations between fertilization rates and the post swim-up semen characteristics including sperm concentration

($r = .176$, $p < .05$), sperm motility ($r = .190$, $p < .05$), normal morphology ($r = .227$, $p < .01$), and STR ($r = -.178$, $p < .05$). However, there were no significant correlations between fertilization rates and the post swim-up semen characteristics such as ALH, BCF, VCL, VSL, VAP, LIN, and Rapid.

### Comparison of the CASA Estimates in Fresh Ejaculates Between "Good" and "Poor" Fertilization Groups

The CASA estimates in fresh ejaculates were compared between 108 cycles with "good" fertilization rate group and 28 cycles with "poor" fertilization rate group (Table III). There were significant differences of sperm concentration ($p < .005$), sperm motility ($p < .005$), and motile sperm concentration ($p < .01$) between the "good" and "poor" groups. As for sperm movement characteristics, there also were significant differences of ALH ($p < .005$), VCL ($p < .001$), VSL ($p < .005$), and Rapid ($p < .01$) between the groups. However, there were no significant differences of BCF, VAP, LIN, and STR between the groups.

### Comparison of the CASA Estimates in Post Swim-Up Sperm Between "Good" and "Poor" Fertilization Groups

The CASA estimates in post swim-up sperm were compared between the "good" and "poor" fertilization groups (Table IV). There were significant differences of sperm concentration ($p < .05$), sperm motility ($p < .01$), motile sperm concentration ($p < .05$), VCL ($p < .005$), VAP ($p < .005$), and Rapid ($p < .05$) between the groups. However, there were no significant differences of ALH, BCF, VSL, LIN, and STR between the groups.

**Table III.** Comparison of the CASA Estimates in Fresh Ejaculates Between "Good" and "Poor" Fertilization Groups

| Category | Good | Poor |
|---|---|---|
| Fertilization rate (%) | >50 | ≤50 |
| No. of cycles treated | 108 | 28 |
| Concentration ($10^6$/mL) | 198.3 ± 108.8** | 125.5 ± 96.3** |
| Motility (%) | 59.9 ± 16.5** | 48.9 ± 22.1** |
| Motile sperm conc. ($10^6$/mL) | 123.5 ± 81.2* | 76.3 ± 81.6* |
| Sperm motion variables | | |
| ALH ($\mu$m) | 3.5 ± 0.8** | 2.9 ± 1.0** |
| BCF (Hz) | 25.4 ± 3.4 | 25.5 ± 4.5 |
| VCL ($\mu$m/s) | 86.2 ± 16.0*** | 74.3 ± 15.2*** |
| VSL ($\mu$m/s) | 49.1 ± 9.2** | 42.7 ± 9.8** |
| VAP ($\mu$m/s) | 64.0 ± 50.2 | 51.5 ± 9.9 |
| Linearity (VSL/VCL) | 58.7 ± 6.7 | 59.4 ± 8.9 |
| Straightness (VSL/VAP) | 81.4 ± 4.8 | 81.3 ± 6.9 |
| Rapid (%) | 38.8 ± 17.8* | 28.6 ± 19.0* |

*Note.* Values are mean ± SD.
* $p < .01$; ** $p < .005$; *** $p < .001$.

**Table IV.** Comparison of the CASA Estimates in Post–Swim-Up Sperm Between "Good" and "Poor" Fertilization Groups

| Category | Good | Poor |
|---|---|---|
| Fertilization rate (%) | >50 | ≤50 |
| No. of cycles treated | 108 | 28 |
| Concentration ($10^6$/mL) | 62.5 ± 42.5* | 43.1 ± 47.8* |
| Motility (%) | 92.2 ± 7.2** | 85.7 ± 19.3** |
| Motile sperm conc. ($10^6$/mL) | 57.7 ± 39.8* | 40.3 ± 46.4* |
| Sperm motion variables | | |
| ALH ($\mu$m) | 5.7 ± 1.2 | 5.2 ± 1.7 |
| BCF (Hz) | 30.7 ± 3.7 | 30.7 ± 6.7 |
| VCL ($\mu$m/s) | 158.6 ± 24.7*** | 140.0 ± 40.3*** |
| VSL ($\mu$m/s) | 81.3 ± 10.1 | 76.9 ± 17.5 |
| VAP ($\mu$m/s) | 97.5 ± 10.7*** | 88.8 ± 21.3*** |
| Linearity (VSL/VCL) | 54.9 ± 8.1 | 56.9 ± 14.2 |
| Straightness (VSL/VAP) | 83.4 ± 5.8 | 83.7 ± 17.4 |
| Rapid (%) | 65.0 ± 19.9* | 55.3 ± 22.0* |

*Note.* Values are mean ± SD.
* $p < .05$; ** $p < .01$; *** $p < .005$.

## DISCUSSION

Male infertile patients have been assessed on the basis of a semen profile including sperm concentration, sperm motility, and sperm morphology, incorporating descriptive criteria by the WHO (15). Such descriptive criteria are adequate to identify the most severe cases of male factor, however, prospective studies have shown that the conventional semen profile is incapable of discriminating between fertile and infertile men, especially in idiopathic infertility (1–5). Therefore, in vitro tests have been developed to assess the functional capacity of human sperm to predict fertility. While various attributes of sperm function have been studied in some detail, many of the assays involved are technically complex. Sperm motility is commonly believed to be one of the most important characteristics correlated with fertility (5,16). Recent studies have indicated that sperm motility data obtained by CASA also may be predictive of fertility (10–12,17–21).

The CASA instruments were initially available in the mid-1980s (10–12), and there has now been a positive and concerted action to define the role of CASA in both the clinical andrology laboratory as well as the research laboratory. However, it was suggested that too few studies related to the use of sperm motion analysis and prediction of IVF outcome to reach general conclusions (22).

We assessed the sperm motility characteristics by CASA and compared them with the fertilization rates in vitro in 136 IVF-ET cycles. As for the CASA estimates before swim-up, there were significant correlations between fertilization rates and the sperm motility characteristics, including ALH ($r = .269$, $p < .005$), VCL ($r = .297$, $p < .001$), VSL ($r = .266$, $p < .005$), and Rapid ($r = .243$, $p < .005$) (Table II). Only STR ($r = -.178$, $p < .05$) was significantly correlated with fertilization rates in the post swim-up sperm (Table II). ALH, velocity, and STR were found to be better in predicting the achievement of pregnancy than the conventional criteria of semen quality (4,15). Our study demonstrated that ALH and two velocity parameters (VCL and VSL) before swim-up correlated with fertilization rates, indicating ALH and velocity before swim-up can be one of the good predictors of the fertilization outcome, which supported the previous studies. It also showed that STR post swim-up correlated with fertilization rate, suggesting STR post swim-up can be another good predictor of the fertilization outcome.

The CASA estimates in fresh ejaculates were compared between 108 "good" patients and 28 "poor" patients (Table III). As for the sperm movement characteristics, there were significant differences of ALH ($p < .005$), VCL ($p < .001$), VSL ($p < .005$), and Rapid ($p < .01$) between the two groups. It was shown that there were significant differences of VCL ($p < .005$), VAP ($p < .005$), and Rapid ($p < .05$) in post swim-up sperm between the "good" and "poor" fertilization groups (Table IV). VCL represents total distance traveled by the sperm head, while Rapid indicates average path velocity $>25$ $\mu$m/s. These two CASA estimates were significantly better in the "good" fertilization group, both in fresh ejaculates and post swim-up sperm. It may suggest that the total distance traveled by rapid sperm reflect the fertilizing potential of human sperm.

In conclusion, some of the CASA estimates provide reliable estimation of the fertilizing ability of human sperm. There were significant differences of the two sperm movement characteristics, including VCL and Rapid (before and after swim-up), indicating that the total distance traveled by the rapid sperm movement might be important in human sperm fertilizing abilities. Such information would be useful when counseling the couples before they make the decision to proceed with IVF-ET. Further studies are required to determine the cut-off values of the CASA estimates that could aid the laboratory in planning its strategy at the time of insemination.

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