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ment options to patients undergoing infertility evaluation. CASA results are significantly affected by threshold settings, video frame rates, parameter settings, evaluation algorithms, and other variables, including sperm count.[5,12] To ensure accuracy, CASA analyzers should be calibrated to obtain results that are comparable to a manual method.[13] A manual sperm count, when performed correctly, is less subject to error and is therefore more accurate than an automated count.[14]

The quality of the counting chamber (eg, the hemacytometer and the Makler chamber) is one of the sources of error in CASA and manual semen analysis.[12] Potential sources of error arise from repeated handling, cleaning, and reassembly. The MicroCell counting chamber used in this study has been reported to be more accurate and precise for determining the concentration and motility of semen specimens than are other counting chambers.[15,16]

In prefreeze semen samples, the overestimation of sperm count by CASA was concentration dependent: the percent disagreement between CASA and manual sperm concentration was inversely related to the manual sperm count. Overestimation was higher in low sperm-count specimens, and similar findings have been reported by others.[5,17] Overestimation of sperm concentration at the lower end of the range (less than 20×10^6/mL; here, in patients with cancer) is much higher and is probably the result of including refractile, nonsperm particles (debris) in the estimate. In these low-count specimens, manual semen analysis with a MicroCell chamber provides more reliable results.[4] A new approach using a deoxyribonucleic acid (DNA)-specific stain can also be used.[15] The overestimation of sperm count by CASA that we found was lower than values that have been reported earlier (from 16% to 30%).[2] This discrepancy may be the result of differences in the analyzers used in these two studies. The overestimation of sperm count also reduces the percent motility calculated by CASA. Another reason for the lower sperm motility recorded with CASA may be the slow movement of the sperm tail, which can be observed in the manual methods but which may be below the threshold to which CASA is sensitive. However, a few investigators have reported higher sperm motility values with CellSoft CASA than with manual methods.[18]

Sperm concentration and motility can be evaluated over a wider range (greater than 20×10^6/mL) with fair accuracy (acceptable difference less than 15% from manual results) when the MicroCell chamber is used with CASA.[12] Our results indicate that CASA can be used to estimate sperm concentration and motility in the sperm concentration range of 20 to 300 million/mL for most specimens, but these estimates need to be verified manually. These results are comparable to those reported earlier (20 to 149 million/mL and 20 to 340 million/mL).[3,4] However, this range is much wider than that reported by other investigators.[19]

The sperm concentrations measured by two analyzers were similar, as reported earlier.[20] Both analyzers overestimated the prefreeze sperm count and underestimated the post-thaw sperm count. The post-thaw percent disagreement in sperm concentration between automated and manual methods was significant in both normal donors and patients with cancer, indicating that all post-thaw sperm concentrations should be verified manually. One reason for this difference may be the variable clumping to which post-thaw immotile cells are prone. The present settings of the analyzer's parameters may not be optimum for cryopreserved sperm counts where a freezing medium containing 25% egg yolk is added to semen. Washed semen specimens require different CASA settings for semen analysis; similarly, post-thaw specimens with freezing medium may require different settings when the viscosity of the freezing medium is different from that of the ejaculate.

Differences in the post-thaw motility results of the two analyzers may be the result of different algorithms used for deriving identical motion parameters and of differences in image capturing and hardware processing between systems. The post-thaw motility values obtained with the MA analyzer in both patients with cancer and normal donors were close to the manual values, whereas those obtained with the HT-IVOS analyzer differed significantly from the manual values. This differences between the two analyzers may be the result of different parameter settings and perhaps different sample sizes (HT-IVOS, n = 217; MA, n = 29).

Automated sperm concentration results (beyond the range of sperm concentrations recommended by the manufacturer) need to be verified by manual methods until the technology can accurately determine the entire range of sperm concentrations in an ejaculate.[21] At low sperm counts (less than 20×10^6/mL), CASA values for sperm count and percent motility are likely to be unreliable, and values for post-thaw sperm motility are likely to be underestimated with wide variations. Oligospermic patients (true or borderline) may be categorized by CASA as normal on the basis of fresh ejaculates and abnormal on the basis of post-thaw specimens. This misclassification has clinical implications in the diagnosis and treatment of subfertile men. Therefore, we recommend that assisted reproductive procedures be advocated with caution in such cases.

TABLE II. ***Percent disagreement in CASA sperm motility and count compared to manual results***

	Sperm Motility			Sperm Count		
Factors	Mean (SE)*	P†	P‡	Mean (SE)*	P†	P‡
Prefreeze (n = 246)						
Analyzers						
HTM (n = 217)	−2.1 (3.6)	0.56	0.15	13.8 (4.7)	<0.001	0.94
MA (n = 29)	4.2 (4.9)	0.39		14.2 (5.8)	0.016	
Diagnostic groups						
Donors (n = 201)	−0.58 (5.9)	0.92	0.65	2.1 (7.7)	0.78	0.011
Cancer patients (n = 29)	2.7 (4.4)	0.54		25.8 (5.2)	<0.001	
Interaction between analyzer and diagnostic group	—	—	0.27	—	—	0.23
Post-thaw sperm motility (n = 230)						
Analyzers						
HTM (n = 195)	−16.8 (4.9)	<0.001	0.019	−11.0 (2.5)	<0.001	0.65
MA (n = 35)	−0.98 (6.6)	0.88		−0.98 (3.9)	0.012	
Diagnostic groups						
Donors (n = 200)	−11.5 (6.7)	0.089	0.58	−12.4 (2.5)	<0.001	0.51
Cancer patients (n = 30)	−6.3 (6.7)	0.34		−9.3 (4.0)	0.021	
Interaction between analyzer and diagnostic group	—	—	0.13	—	—	0.052

* *Values are expressed as least square mean along with standard error in parentheses.*
† *Probability of least square mean different from zero.*
‡ *Value for any effect from a given factor.*

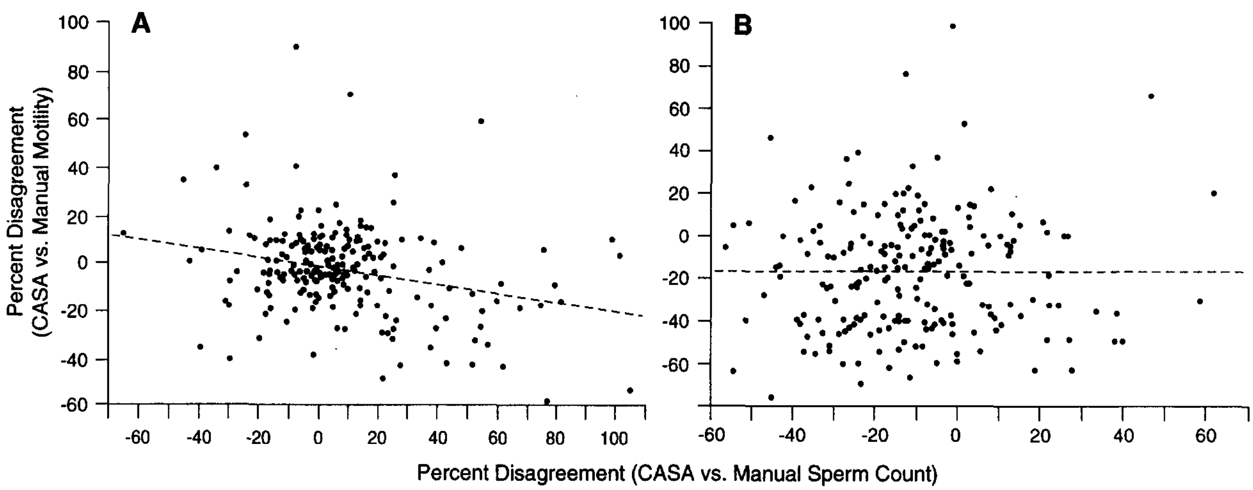

FIGURE 3. *Plots of correlation between percent disagreement in motility and percent disagreement in sperm count: (A) prefreeze condition, (B) post-thaw condition.*

<0.01). The prefreeze CASA results were significantly higher than were the manual results for the cancer group ($P < 0.001$) and were significantly lower than the manual results for post-thaw sperm counts (HT-IVOS, $P < 0.001$; MA, $P = 0.012$).

Post-thaw Versus Prefreeze Differences in Count and Motility

The percent disagreement between CASA and manual results for prefreeze and post-thaw specimens did not differ significantly between diagnostic groups or between analyzers.

Relationship of Percentage Differences Between CASA and Manual Count and Motility

The percent disagreement between CASA and manual sperm counts in prefreeze specimens was inversely related to the percent disagreement between CASA and manual motility results (regression coefficient from the mixed effect model −0.155; $P < 0.0007$; Fig. 3A). No such relationship was seen in the post-thaw specimens (Fig. 3B).

COMMENT

Accurate semen analysis results are important for interpreting and recommending various treat-

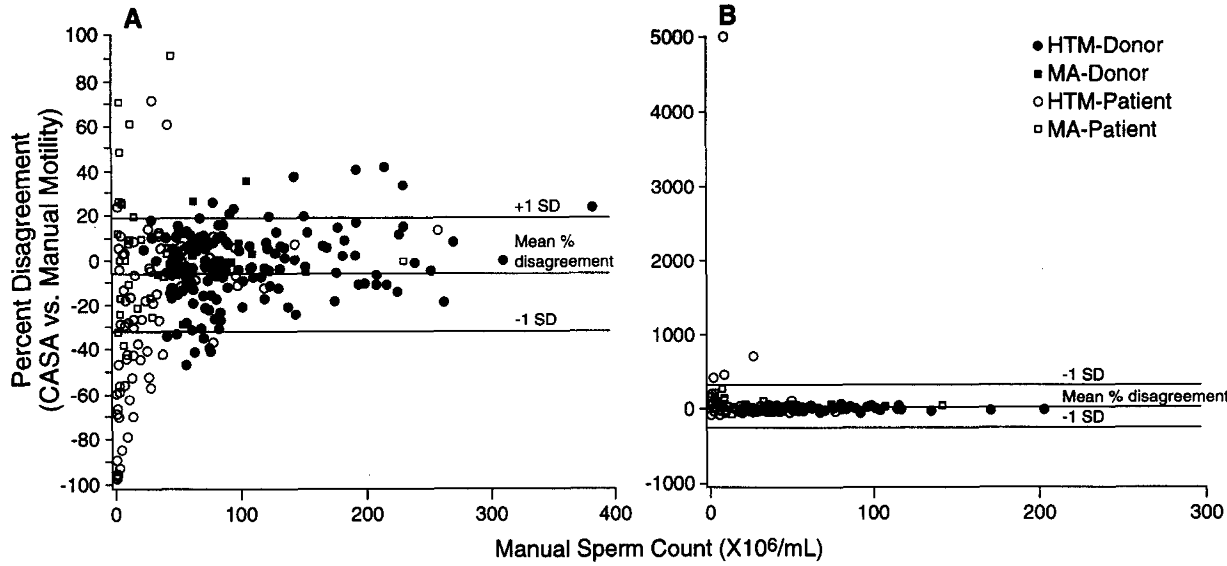

FIGURE 1. *Modified Bland-Altman agreement plot for prefreeze motility (mean ± standard deviation reference lines) (A); and post-thaw motility (mean ± standard deviation reference lines) (B).*

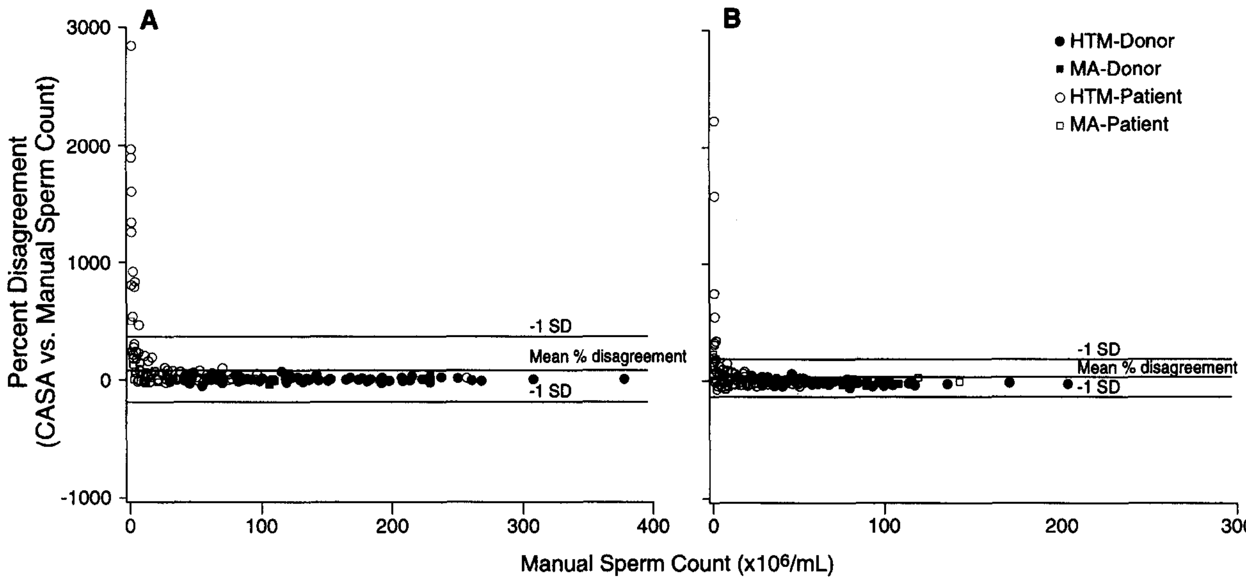

FIGURE 2. *Modified Bland-Altman agreement plot for prefreeze count (mean ± standard deviation reference lines) (A); and post-thaw count (mean ± standard deviation reference lines) (B).*

10^6/mL were analyzed further in the mixed regression analyses.

Prefreeze and Post-thaw Percent Disagreement Between CASA and Manual Methods in Sperm Motility

Manual and CASA sperm motility counts did not differ significantly between the analyzers or between donors and patients with cancer in prefreeze specimens (Table II). The MA analyzer motility results did not differ from the manual results in post-thaw specimens, but lower motility results were obtained with the HT-IVOS (P <0.001, Table II). The percent disagreement between the HT-IVOS analyzer and manual counts was significantly different from that of the MA analyzer and manual counts ($P = 0.019$). Percent disagreement between the CASA and manual motility results did not differ significantly by diagnostic group.

Prefreeze and Post-thaw Percent Disagreement Between CASA and Manual Methods in Sperm Count

The CASA values of sperm counts were significantly higher than were manual values in prefreeze specimens (Table II), but the differences between the analyzers were not significant. However, CASA and manual sperm count methods differed significantly by diagnostic group (P

TABLE I. ***Differences in sperm count and percent motility between CASA and manual measurements, before and after cryopreservation***

Group by Sperm Count	Number of Specimens	Cancer Patients (%)	% Count Difference Between CASA & Manual*	% Count Difference Between CASA & Manual by CASA Machine*	% Motility Difference Between CASA & Manual*	% Motility Difference Between CASA & Manual by CASA Machine*
Prefreeze						
0–10	41	100.0	471.8 (644.8)	HTM: 637.3 (740.6) MA: 152.0 (122.7)	−29.7 (43.7)	HTM: 46.6 (37.2) MA: 7.9 (33.2)
11–20	22	100.0	40.4 (67.7)	HTM: 58.0 (78.5) MA: 15.9 (39.4)	−19.9 (30.0)	HTM: −33.7 (21.6) MA: 2.6 (28.8)
21–50	65	46.2	22.9 (32.7)	HTM: 22.5 (32.8) MA: −5.9 (23.4)	−4.1 ± 24.6	HTM: 23.8 (33.6) MA: 3.1 (29.3)
51–80	96	12.5	10.4 (24.2)	HTM: 11.2 (24.9) MA: −4.6 (16.3)	−4.2 ± 16.1	HTM: 4.0 (16.2) MA: −0.45 (14.4)
>80	90	7.8	0.06 (16.7)	HTM: 0.51 (16.5) MA: −4.5 (19.3)	1.2 (15.0)	HTM: 0.90 (15.2) MA: 4.4 (13.1)
Post-thaw						
0–10	65	98.5	143.2 (352.4)	HTM: 196.1 (432.1) MA: 52.9 (85.9)	85.0 (633.0)	HTM: 118.3 (790.0) MA: 25.3 (82.6)
11–20	24	87.5	11.9 (35.3)	HTM: 12.0 MA: 11.0 (36.6)	−19.2 (31.7)	HTM: −18.3 (31.6) MA: −24.1 (36.5)
21–50	165	16.4	−9.7 (22.4)	HTM: −8.8 (21.7) MA: −14.0 (25.5)	−13.9 (63.2)	HTM: −14.4 (67.9) MA: −11.4 (30.3)
51–80	42	4.8	−12.4 (14.3)	HTM: −12.2 (15.0) MA: −13.7 (10.1)	−12.3 (27.2)	HTM: −15.0 (27.7) MA: 4.5 (18.0)
>80	26	7.7	−15.4 (18.1)	HTM: −16.1 (17.6) MA: −10.4 (25.4)	−15.9 (27.4)	HTM: −18.4 (27.1) MA: 12.5 (10.6)

Key: CASA = computer-assisted sperm analysis; HTM = Hamilton-Thorn Motion analyzer; MA = Motion Analysis analyzer; SD = standard deviation.
* *Mean (SD).*

were underestimated. Motility (prefreeze and post-thaw) was underestimated by CASA.

Prefreeze and Post-thaw Sperm Count and Motility Agreement Plots

A plot of the percentage differences in manual and CASA results for prefreeze motility indicated large variations at low sperm counts (Fig. 1A). The HT-IVOS analyzer gave lower motility values than did manual counting, whereas the MA analyzer gave higher values. Patients with cancer showed more variation than did normal donors. In post-thaw motility, the CASA values were higher than were manual values for low sperm counts. The variation was higher among patients with cancer than among normal controls (Fig. 1B). Similar variations were seen when percentage differences in sperm count were plotted against prefreeze sperm counts (Fig. 2A) and post-thaw sperm counts (Fig. 2B).

The differences in sperm count and percent motility between the CASA and the manual methods were higher and skewed at low sperm counts; therefore, data for sperm counts greater than 20 ×

Semen from patients with cancer is generally of poor quality (low sperm counts and motility), so it is important to determine whether CASA evaluation of these specimens before and after freezing differs significantly from manual evaluation. We compared the concentration and motility results from CASA with standardized, quality-controlled manual methods based on World Health Organization recommendations.[7] These methods have high precision, accuracy, and reliability.[8]

Our study had three aims: (1) to measure and compare the accuracy and/or precision of two CASA instruments in measuring sperm concentration and motility in prefreeze and post-thaw semen specimens; (2) to measure and compare the accuracy and/or precision of automated versus manual semen analysis; and (3) to determine whether results differed by the type of semen specimen analyzed (ie, between normal volunteers and patients with cancer).

MATERIAL AND METHODS

We analyzed semen from normal donors and patients with cancer who were referred to the Andrology Laboratory from 1993 to 1996. Prefreeze or post-thaw semen specimens were analyzed for those normal donor and cancer patients whose semen analysis results by both CASA and manual analysis were available. The healthy donors were selected on the basis of a normal semen analysis.[7]

Assessment of Semen Characteristics

All specimens were collected on site by masturbation into sterile cups after 48 to 72 hours of sexual abstinence. Specimens were allowed to liquefy at 37°C for 30 minutes before evaluation. All specimens were analyzed on one of the two computer-assisted motion analyzers: Hamilton-Thorne Research IVOS (Beverly, Mass) or the Motion Analysis Cell-Trak, Model VP 110, Version 4.22B (Santa Rosa, Calif). Sperm concentration and motility were also verified manually. For each measurement, a 5-μL sample aliquot was loaded onto a 20-μm counting chamber (MicroCell, Conception Technologies, La Jolla, Calif) and analyzed for sperm count and motility. Sperm count and motility were also evaluated manually under an Olympus BH2-S microscope (Olympus, New York, NY) with a ×20 positive phase-contrast objective.[9] For CASA analysis, representative fields containing at least 200 motile spermatozoa were examined. Raw samples were analyzed at a frame rate of 30 frames. Samples were analyzed for sperm concentration, percent motility, and other motion characteristics,[10] although only sperm count and motility are considered in this study.

The Motion Analysis Cell-Trak was set as follows: 2-well, 20 μm, duration of data capture, 15 frames; frame rate, 30; minimum motile speed, 2 μm/s; maximum burst speed, 600 μm/s; distance scale factor, 0.9457 μm/pixel; centroid cell size maximum, 8 pixels; number of cells to find per well, 200; and minimum number of fields per sample, 3. The reproducibility of the CASA results was determined with a manual count using a calibrated videotape.[2] The intra-assay variation was less than 10% for sperm counts and motility.

The parameter settings used with the Hamilton-Thorne IVOS were as follows: frames acquired, 30; frame rate, 30 Hz; minimum contrast, 90; minimum cell size, 5 pixels; nonmotile head size, 5 pixels; nonmotile brightness (head intensity), 90; low path velocity (VAP), 5 μm/s; medium path velocity (VAP), 25μm/s; slow cells motile, yes; and threshold straightness (STR), 80. Reproducibility of the analyzer was determined by using a fixed concentration of latex beads ("Accubeads," from Hamilton-Thorne Research). Inter- and intraobserver variability were determined with standard latex bead concentrations.

Cryopreservation

For cryopreservation, semen was slowly mixed with an equal volume of TEST-yolk buffer with glycerol (Irvine Scientific, Santa Ana, Calif) in four equal aliquots at 5-minute intervals so as to have a final semen-to-freezing medium ratio of 1:1. Mixed aliquots were frozen in cryogenic vials using a three-stage freezing procedure: exposure to −20°C for 10 minutes, exposure to −100°C nitrogen vapors for 2 hours, and immersion in liquid nitrogen at −179°C.[11] For post-thaw semen analysis, an aliquot of each specimen was thawed after 24 hours and examined by both manual and CASA methods, as described.

Statistical Methods

Using the modified Bland-Altmann method, we plotted the data for prefreeze and post-thaw motility and sperm counts. The modified Bland-Altmann method plots the difference between two values on the y-axis and the average of the two values on the x-axis. The closer the data points are to the zero point on the y-axis, the more agreement there is among them.

The percent disagreement between the automated and manual methods was calculated by subtracting the manual readings from the CASA readings: [(CASA reading − manual reading) × 100]/manual reading. The manual method was used as the gold standard in the study. A mixed-effects linear regression model was used to evaluate how the type of analyzer and type of specimen examined affected the accuracy of CASA results. To assess the relationship between sperm count and motility, a separate mixed-effects regression model was used to analyze both prefreeze and post-thaw conditions. Alpha was set at 0.05, and all tests were two-tailed. Multiple comparisons were controlled with Bonferroni's correction. Analyses were performed with the SAS statistical software package (SAS Institute, Cary, NC).

RESULTS

We analyzed the prefreeze and post-thaw semen results of 220 specimens from 8 normal donors and 131 specimens from 57 patients with cancer who participated in our sperm banking program. The percent disagreement between CASA and manual readings in both sperm count and percent motility under prefreeze and post-thaw conditions were large when the manual sperm count was less than 20×10^6/mL and especially if it was less than 10×10^6/mL (Table I). The percent disagreement between prefreeze CASA and manual sperm counts and motility measurements decreased when the manual count increased. The lowest percent disagreement between CASA and manual sperm counts was seen at sperm concentrations of greater than 80×10^6/mL for prefreeze specimens and at concentrations of 20 to 50×10^6/mL for post-thaw specimens. Prefreeze sperm counts were thus overestimated by CASA, whereas post-thaw counts

ACCURACY OF COMPUTER-ASSISTED SEMEN ANALYSIS IN PREFREEZE AND POST-THAW SPECIMENS WITH HIGH AND LOW SPERM COUNTS AND MOTILITY

RAJINDER S. SIDHU, RAKESH K. SHARMA, JAR-CHI LEE, AND ASHOK AGARWAL

ABSTRACT

Objectives. We evaluated the accuracy and precision of computer-assisted semen analysis (CASAs) to determine whether variations in the CASA results were related to the type of analyzer or to the type of specimens analyzed.

Methods. Semen specimens were analyzed manually and by CASA before freezing and after thawing. Multiple ejaculates (220 semen specimens) from normal healthy donors (n = 8) and 131 semen specimens from 57 patients with cancer were assessed.

Results. The differences between CASA and manual sperm counts and percent motility were higher in prefreeze and post-thaw specimens at sperm concentrations of less than 20×10^6/mL. The differences between CASA and manual results were significant for cancer patients, compared with normal donors ($P < 0.01$).

Conclusions. CASA results are unreliable at sperm counts of less than 20×10^6/mL and post-thaw motility is generally underestimated by CASAs. UROLOGY **51:** 306–312, 1998. © 1998, Elsevier Science Inc. All rights reserved.

The results of semen analysis are used to evaluate male fertility potential and to predict the outcome of assisted reproductive procedures, such as intrauterine insemination or in vitro fertilization. Sperm concentration and motility have clinical value as an adjunct to sperm function tests in predicting successful in vivo and in vitro fertilization.[1] Whereas sperm concentration can be determined manually with only small inter- and intraobserver differences,[2] evaluation of sperm motility by visual assessment shows large inter- and intraobserver variations. Computer-assisted semen analysis (CASA) was introduced during the 1980s to analyze additional sperm motion characteristics such as velocity, linearity, and lateral head displacement, which cannot be evaluated visually. CASA was developed to reduce the amount of time a technician spent in sperm observation, reduce intraobserver differences, and improve the accuracy of final results. CASA equipment has been evaluated for accuracy, precision, and technician error,[2,3] and has improved from being semi-automated to nearly fully automated.

CASA is objective and rapid. However, measurements of sperm concentration with CASA do not correlate well with those of manual methods at low sperm concentrations.[4] Abnormal semen quality (characterized by low sperm count) is common in extreme male-factor infertility, such as oligospermia or teratospermia, and in patients with cancer who bank their semen specimens before undergoing treatment for cancer. CASA results are objective, but depend on the parameter settings,[5] which are at the discretion of the user. Measuring sperm concentration and motion characteristics requires different parameter settings for raw and washed (after removal of seminal plasma) semen specimens. In cryopreserved specimens, post-thaw semen samples may require different parameter settings because a freezing medium has been added to the ejaculate. As a consequence, sperm motion characteristics should not be viewed as absolute values, but instead should be interpreted in light of parameter settings.[6]

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