Research Article

Performance comparison of two computer-aided sperm analysis systems

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Article Info

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Keywords

Computer assisted semen analyzer, Male infertility, Semen analysis

Abstract

Background: Automated systems are promising tools for the standardization of laboratory semen analysis, but the assessments on these systems are still insufficient to support their use in daily practice. We aimed to evaluate the analytic performance of two computer-aided sperm analysis (CASA) systems, having manual evaluation as a reference.

Methods: Fifty-two seminal fluid samples were analyzed in accordance with the WHO 6th Edition guidelines at the Clinical Pathology Laboratory of Trento. A LensHooke X1 PRO instrument and a SCA SCOPE system were used for the automated analysis of the following parameters: sperm concentration count, total sperm motility count, spermatozoa with progressive motility count, spermatozoa with rapid progressive motility count, and spermatozoa morphology.

Results: Correlation and consistency between manual method and both the two CASA systems were good-to-moderate for all the parameters, with the exception of morphology (no correlation). However, the agreement was widely variable, with slight to moderate, or even considerable differences, and proportional biases towards overestimations.

Conclusions: Our data suggest that the two here studied CASA systems cannot be an alternative to expert manual semen analysis at present but might assist and facilitate the analysis.

Introduction

Human semen examination is the most widely used test for the diagnosis of male infertility, recommended as first laboratory analysis in couple infertility work-up, and necessary before storing, or donating semen samples [1]. Misdiagnosis, and delay in infertility treatments can follow inaccurate laboratory reports, mainly due to analytical issues [1].

Seminal fluid analysis usually relies on experienced operators' subjectivity for appearance, viscosity, and fluidization, as well as, on microscope, sperm concentration, total number, viability, and morphology [2, 3]. The lack of between-laboratory standardization, assay heterogeneity, and difficulty in identifying pathologic cut-offs still affect expert manual analysis [4].

Therefore, computer-aided sperm analysis (CASA) systems have been developed, igniting debates on their current performance and potentialities [3, 4]. Main suitable tasks include basic sperm function and motility assessment. Notwithstanding promising results [5-8], these systems are still insufficiently performing to enter routine laboratory practice [4]. Their main weakness are the lack of standardized algorithms and of extensive quality control procedures, and small volumes required for testing, which are scarcely representative of the whole content sample [4].

Having microscope analysis as the referral technique, we aimed to evaluate the analytic performance of two CASA systems in assessing semen count, mobility, and morphology.

Materials and methods

Fifty-two seminal fluid samples were from persons older than 18 years, who underwent sperm analysis at the APSS of Trento, between July and August 2024, for the following purposes: infertility issues in couples, medically assisted procreation procedures, post-vasectomy control, or sperm cryopreservation. Samples with sperm concentration counts lower than 2 million/mL were excluded from the study. The samples were collected and analyzed in accordance with the WHO Laboratory Manual for the Examination and Treatment of Seminal Fluid criteria [3]. Compliance with the pre-analytical phase rules of the WHO Manual was verified, particularly those regarding ejaculatory abstinence for 2-7 days before seminal fluid collection. The study complied with the ethical principles outlined in the 2008 revised Helsinki Declaration.

The following four key parameters were considered: a) sperm concentration count (SCC), b) total sperm motility count (TSMC), c) spermatozoa with progressive motility count (SPMC), d) spermatozoa with rapid progressive motility count (SRPMC). In addition, spermatozoa morphology was evaluated, subdivided into the following categories: normal, head defects, intermediate trait defects, tail defects.

Seminal fluids were analyzed with: a) light microscopy (manual), b) LensHooke X1 PRO instrument (Bonraybio, Taichung City, Taiwan), c) SCA SCOPE system, version 6.6 (Microptic, Barcelona, Spain).

LensHooke X1 PRO technology exploits integrated high-resolution, autofocusing optical lens in combination with an automatic computational system. Seminal fluid samples are applied into two special containment wells and then analyzed. SCA SCOPE is equipped with an optical microscope with high-resolution lenses, and with an integrated high-resolution digital camera with a Complementary Metal Oxide Semiconductor sensor. Four slides are set up for each sample for microscopy evaluation. Images, obtained from different areas of the slide, are displayed on a monitor along with the test results.

Automated semen analysis was performed in accordance with the manufacturers' protocols. Seminal fluid samples were simultaneously analyzed with both the systems, and by experienced operators (E.B. and R.C.) under a microscope.

Statistical analysis

Bland-Altman analysis was used to assess the agreement between manual sperm analysis and the two automated systems. Mean difference (bias) and 95% limits of agreement (LoA) were calculated to determine the range within which most differences fell. Using a two-way random-effect model for absolute agreement, we assessed the between-measurement degree of agreement through intraclass correlation coefficients (ICC; 95% confidence interval), which were interpreted as follows: <0.50, poor; 0.50-0.74, moderate; 0.75-0.90, good; >0.90, excellent. Statistical analysis was performed with "Software R" (4.3.3 version; R foundation, Vienna, Austria) [9].

Results

Table 1 shows the data on seminal fluid analysis obtained with the two CASA technologies and with microscope evaluation.

Table 1: Data of the seminal fluid analysis obtained with two CASA technologies and with microscope evaluation.

	Microscopy evaluation	LensHooke	SCA SCOPE
SCC, 10^6/mL, median (IQR)	19 (4, 44)	23 (6, 65)	25 (11, 51)
TSMC, %, median (IQR)	51 (37, 64)	55 (25, 68)	37 (20, 54)
SPMC, %, median (IQR)	36 (21, 50)	38 (19, 58)	25 (13, 36)
SRPMC, %, median (IQR)	21 (14, 36)	16 (5, 22)	10 (5, 21)
Morphology			
Normal, %, median (IQR)	4.0 (2.0, 6.0)	4.0 (3.0, 5.0)	1.5 (0.9, 3.0)
Head defects, %, median (IQR)	85 (80, 91)	80 (76, 85)	93 (83, 97)
Intermediate trait defects, %, median (IQR)	31 (30, 40)	50 (44, 57)	85 (78, 90)
Tail defects, %, median (IQR)	17 (13, 25)	25 (23, 26)	0 (0, 0)

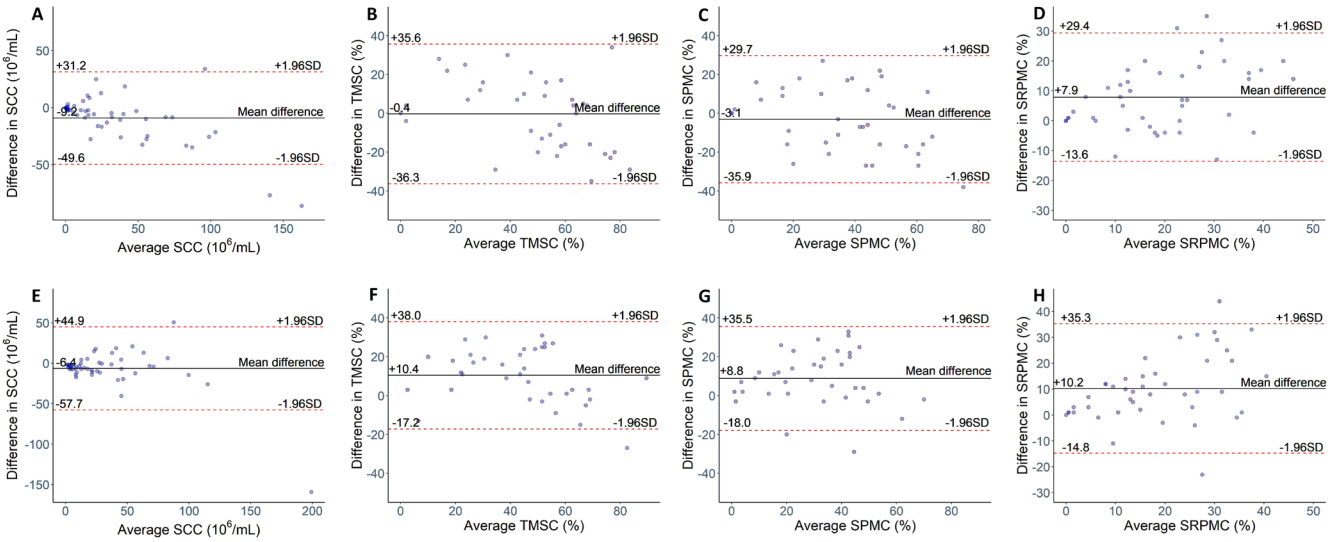
CASA, computer-aided sperm analysis; SCC, sperm concentration count; TSMC, total sperm motility count; SPMC, spermatozoa with progressive motility count; SRPMC, spermatozoa with rapid progressive motility count; IQR, interquartile ranges

Correlations between microscope measurements vs CASA results were classifiable as: a) "good" for SSC [ICC, 0.842 (IQR, 0.701-0.914; p<0.001) vs LensHooke, and 0.767 (0.621-0.862; p<0.001) vs SCA SCOPE]; b) "moderate" for TSMC [0.721 (0.520-0.846; p<0.001) vs LensHooke, and 0.712 (0.311-0.870; p<0.001) vs SCA SCOPE]; c) "moderate" for SPMC [0.694 (0.483-0.829; p<0.001) vs LensHooke, and 0.66 (0.311-0.832; p<0.001) vs SCA SCOPE]; d) "moderate" for SRPMC [0.58 (0.189-0.783; p=0.003] vs LensHooke, and "poor" for SRPMC [ICC, 0.406 (0.025-0.662; p=0.018) vs SCA SCOPE]. CASA technologies and microscope evaluation did not correlate for spermatozoa morphology.

LensHooke X1 PRO vs microscope evaluation

For SCC, between-method mean difference was -9.2 × 10^6/mL [95% LoA range, -49.6×10^6/mL to +31.2×10^6/mL] (Figure 1 panel A), indicating LensHooke X1 PRO underestimations. Most data points cluster around the mean difference value, and dispersion increases at the highest SCC, with few outliers. This suggests that between-method discrepancies became more pronounced as SCC increased, potentially indicating a proportional bias. For TMSC, the overall agreement was good, with minimal systematic bias (-0.4%). However, the wide variability (95% LoA range, -36.3% to +35.6%) suggests substantial differences in outliers (Figure 1 panel B). A slight proportional bias could be present, as larger deviations tended to associate with higher TMSC values. Agreement for SPMC was strong, with a small systematic bias (-3.1%). However, variability was considerable, with 95% LoA ranging from -35.9% to +29.7% (Figure, 1 panel C). For SRPMC, LensHooke X1 PRO overestimated the counts (Figure 1 panel D). Moderate variability was observed (95% LoA: -13.6% to +29.4%), with a possible proportional bias, since the discrepancies increased proportionally to higher SRPMC values.

Figure 1: Difference against average data between manual vs LensHooke X1 PRO (A-D), and manual vs SCA SCOPE (E-H) for the following parameters: sperm concentration count (SCC), total sperm motility count (TSMC), spermatozoa with progressive motility count (SPMC), spermatozoa with rapid progressive motility (SRPMC); 95% limits of agreement (broken lines).



SCA SCOPE vs microscope evaluation

A mean difference of -6.4×10⁶/mL was found for SCC (Figure, panel E), indicating that SCA SCOPE slightly underestimated sperm concentration. LoA extended from +44.9×10⁶/mL to -57.7×10⁶/mL, highlighting considerable variability. For TMSC, mean difference was +10.4% (95% LoA range, -17.2% to +38.0%; Figure, panel F), suggesting a general overestimation and moderate variability of the parameter. A systematic bias of +8.8% affected SPMC, along with wide variability (95% LoA range, -18.0% to +35.5%; Figure, panel G). For SRPMC, SCA SCOPE overestimated the values by 10.2% (Figure, panel H), with moderate variability (95% LoA range, -14.6% to +35.3%). The data distribution suggested a possible bias proportional to higher SRPMC values.

SCA SCOPE vs LensHooke X1 PRO evaluation

Overall, the performance of the two CASA systems did not differ significantly. Correlations between SCA SCOPE vs LensHooke results were classifiable as: a) "good" for SSC [ICC, 0.895 (IQR, 0.822-0.939; p<0.001)]; b) "moderate" for TSMC [0.509 (0.270-0.690; p<0.001); c) "moderate" for SPMC [0.528 (0.247-0.717; p<0.001); d) "moderate" for SRPMC [0.712 (0.543-0.827; p<0.001].

Discussion

According to the WHO, seminal fluid analysis is the reference test for defining the role of male infertility in couple infertility. Instruments that can ensure greater standardization with good performance in this setting could improve laboratory practice, especially in non-specialist laboratories. CASA systems are expected to perform in the basic sperm analysis [4], which is why we selected sperm concentration, motility, and morphology for this study.

For sperm concentration, we found good correlation and consistency between manual method and the two CASA systems. However, it is well-known that at low concentrations only the human eye can discriminate all the components that make spermatozoa identifiable as such [3]. This is a difficult to overcome technological limit at present. However, both CASA systems extend the 'validated' concentration ranges below the threshold of 2 million spermatozoa/ml, which we, on the contrary, have identified as the lower limit of accuracy. Correlation and consistency were only moderate when the motility parameters were compared between microscopy visualization and the CASA systems.

Our findings on agreement between manual method and the CASA systems showed wide variability, ranging from slight to, more frequently, moderate-to-considerable differences for all the considered parameters. The biases were often towards overestimations, and affected both the systems, with slight differences.

Efficient use of CASA technologies in seminal fluid analysis is still a matter of debate [2, 5-8, 10-12]. Agarwal and colleagues, who developed the LensHooke X1 Pro-based system, found good correlation data on sperm concentrations and motility in comparison with manual microscopy [2]. Analogously, these parameters resulted well correlated in a comparison study between an earlier version of the SCA SCOPE and manual evaluation [5]. Both these studies, however, did not take into account rapid and slow progressive motility, namely, the parameters reintroduced in the sixth edition of the WHO3 manual [3].

In a more recent study, Jani and colleagues compared microscopy visualization and LensHooke X1 Pro system, showing good correlation for sperm concentration and for total motility [8]. As for the agreement, they judged it acceptable

and concluded that LensHooke X1 Pro system could help standardize reports in non-specialist laboratories [8].
Overall, both our and literature data suggest that evidence is still insufficient to make CASA completely substitute semen manual analysis in routine laboratory practice [4]. In particular, our results cannot confirm the high degree of agreement with the manual method regarding sperm concentration and motility, which the SCA SCOPE and LensHooke X1 Pro systems are supposed to guarantee, in accordance with the manufacturers' claims. These technologies could help assess sperm motility, especially fast and slow progressive motility, since the human eye does not have standard parameters to define these features [2, 5, 11-12]. However, they are at risk of mistaking debris for sperm at low concentrations, thus falsely lowering the motility values, and of failing in recognizing spermatozoa at high concentrations [4]. Practically, the use of microscopy should be mandatory to assign motility around and below the decision-making limits [3].
At present, CASA technologies could thus be useful in the initial evaluation of seminal fluids, necessitating expert human eye confirmation, especially when sperm concentration and motility are low. As for morphology, artificial intelligence is expected to improve CASA performance.
In conclusion, the analytic performance of LensHooke x1 Pro and SCA SCOPE was substantially comparable. LensHooke X1 Pro instrument has the advantage of 'all-in-one' analysis, which takes few seconds and less staff time. SCA SCOPE is more complex, since slide preparation needs time and dexterity, and morphological analysis time is longer. These features make SCA SCOPE more suitable for research and second-level examination, whereas LensHooke x1 Pro could better fit for laboratories dealing with large number of samples to be processed as soon as possible.

Author contributions
Conceptualization: Efrem Bonelli, Adriano Anesi; Material preparation, data collection: Efrem Bonelli, Roberta Cont; Statistical analysis: Patrizia Bartolotta; Original draft preparation: Efrem Bonelli, Diego Franciotta; Review, editing, and final approval: Efrem Bonelli, Roberta Cont, Patrizia Bartolotta, Luca Pio Dicembre, Diego Franciotta, Adriano Anesi

Data availability
The data supporting the findings of this study are available on request from the corresponding author.

Declaration of conflict of interest
The authors declare no conflict of interest.

Ethical Approval
The local Institutional Review Board deemed the study exempt from review.

Funding Statements
This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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