Manual versus computer-automated semen analysis

M. Köse[1], L.Karakoc Sokmensuer[2], A. Demir[3], G. Bozdag[2], S. Gunalp[2]

[1]Afyon State Hospital, Department of Obstetrics and Gynecology, Afyon; [2]Hacettepe University, Department of Obstetrics and Gynecology, Ankara; [3] Elazığ Training and Research Hospital, Department of Obstetrics and Gynecology, Elazığ (Turkey)

Summary

Objective: To evaluate agreement of conventional sperm analysis with computer-aided semen analysis (CASA) regarding concentration, motility, and morphology using samples from infertile men. *Materials and Methods:* In this study a total of 195 male partners of couples who underwent evaluation of infertility were included. All semen samples were examined by conventional method and CASA in terms of morphology, motility, and concentration. Pearson correlation analysis and the Bland-Altman method were used to assess correlation and agreement between conventional semen analysis and CASA measurements. *Results:* When the two methods were compared in terms of concentration, motility, and morphology, there was a statistically significant correlation in all variables. The best correlation was obtained for sperm concentration. However, there was a poor correlation for sperm morphology between conventional method and CASA. Sperm concentration and morphology obtained by CASA were 14% and 87% lower, respectively; motility was 21% higher than the conventional method. *Conclusion:* Although CASA systems are objective and rapid, they should be evaluated in terms of cost-effectiveness, however they may be useful in over-loaded assisted reproductive technique (ART) clinics.

Key words: Sperm; CASA; Infertility; Assisted reproductive techniques.

Introduction

Abnormalities in sperm production or function, alone or in combination with other factors, account for 20-40% of all infertile couples [1]. After a detailed investigation with history and examination, semen analysis is generally demanded from male partners seeking fertility [2]. The findings of sperm analysis do not only clarify the etiology of infertility but also assist the management of treatment. Nevertheless, despite simplicity of semen analysis, careful analysis and a great deal of technical expertise is crucial for confidential results.

The method for conventional semen analysis is a somewhat subjective and time-consuming technique and inter-observer variability should not be disregarded [3]. Alternatively, computer-aided semen analysis (CASA) is developed to improve accuracy of sperm count and to establish a standardized method of test. However, the success of the technique is based on the advancement of technology, analytic conditions, and skills of the technicians. Additionally, the current data about their role for prediction of pregnancy either in natural conception or assisted reproduction cycles is still inconclusive [4].

The aim of current study is to evaluate agreement of conventional sperm analysis with CASA regarding concentration, motility, and morphology using samples from infertile men.

Materials and Methods

A total of 195 male partners of couples who underwent evaluation of infertility were included in this study. All semen samples were obtained in Hacettepe University, School of Medicine, Andrology Laboratory according to the local and World Health Organization (WHO) Laboratory Manual for the Examination of Human Semen and Cervical Mucus Interaction guidelines [5]. Specimens were obtained by masturbation after three to five days of sexual abstinence into sterile polypropylene container and allowed to liquefy for a minimum of 30 minutes at 37°C before semen analysis.

Conventional manual analysis was performed according to the methods described by the WHO guidelines [3]. Briefly, the concentration of spermatozoa was determined by using Makler counting chamber by single observer. Regarding the motility ratio, minimum of 200 spermatozoa were evaluated. Motility of each spermatozoon was classified as progressive motile, non-progressive motile, and immotile. Sperm morphology was assessed by Kruger's strict criteria after observing 100 spermatozoon per slide [9].

Aliquots of the same semen samples were also examined with CASA for sperm concentration, motility, and morphology.

Statistical methods

SPSS 16.0 statistical analysis software was used to evaluate variables. Pearson correlation analysis was used to assess correlation between conventional semen analysis and CASA measurements. The Bland-Altman method was used to assess agreement between conventional semen analysis and CASA measurements. The p values less than 0.05 were considered as statistically significant.

Results

The mean male age was 31.6 ± 6.2 years and mean abstinence duration was four ± 1.4 days. The mean sperm volume was 3.7 ± 1.7 ml. The descriptive statistics of semen parameters assessed by conventional method and CASA are given in Table 1. When the two methods were compared in terms of concentration, motility and mor-

Revised manuscript accepted for publication November 20, 2013

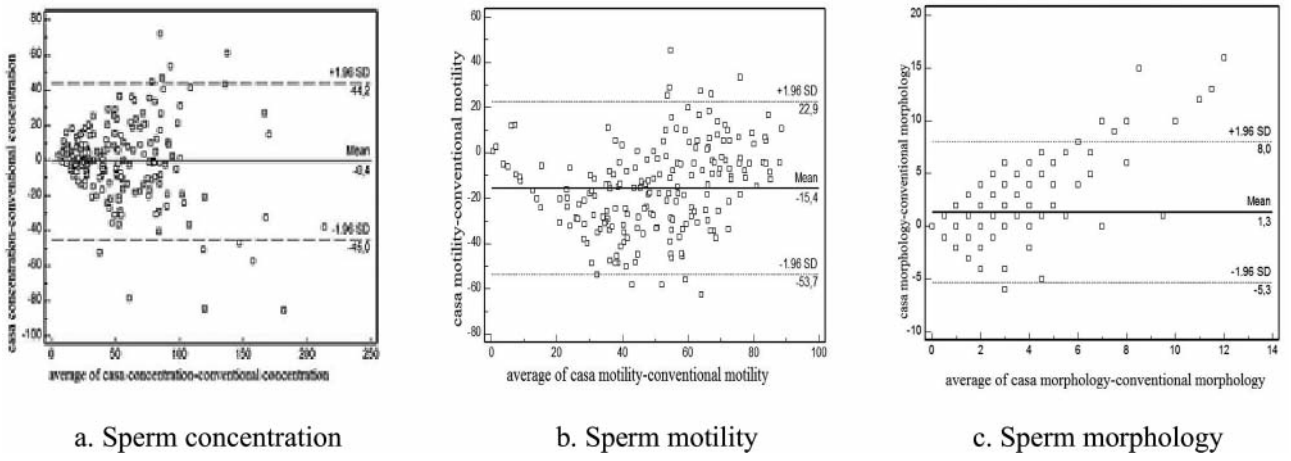

Figure 1. — Bland-Altman plots showing methods differences (conventional vs CASA). Solid lines represent mean differences and dotted lines indicate limits of agreement.

Table 1. — *The descriptive statistics of semen parameters assessed by conventional semen analysis and CASA.*

	Concentration (x10^6/ml)		Motility (%)		Morphology (%)	
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Conventional	55.4 (40.5)	4 - 232	57.8 (19.7)	0 - 95	1.5 (1.6)	0 - 9
CASA	54.4 (36.6)	7.3 - 193.9	42.7 (24.2)	0.9 - 93.9	2.8 (3.7)	0 - 20
p value	< 0.001		< 0.001		< 0.001	

SD: Standard deviation.

phology, there was a statistically correlation between the two method's measurements in all variables ($p < 0.001$) (Table 1).

The best correlation was obtained for sperm concentration. However, there was a poor correlation for sperm morphology between conventional method and CASA (Table 2).

Other than correlation analysis, when agreement was tested between two methods by Bland Altman, the mean biases (upper and lower limits of agreement) between conventional method and CASA according to sperm concentration, sperm motility, and sperm morphology are given in Table 2 and depicted in Figure 1. While sperm concentration and morphology obtained by CASA were 14% and 87% lower, respectively, motility was 21% higher than the conventional method.

Table 2. — *Correlation coefficient and mean differences of conventional method and CASA.*

Sperm parameter	Correlation coefficient	Mean bias (upper and lower limits of agreement)
Concentration (x10^6 cells ml$^-$)	0.84	-0.4 (-45.0 - 44.2)
Motility %	0.62	-15.4 (-53.7 - 22.9)
Morphology %	0.44	1.3 (-5.3 - 8.0)

Discussion

Conventional semen analysis with Makler chamber or hemocytometer is simple and routinely used test for the most of the andrology laboratories. Notwithstanding its simplicity, it shows intra- and interlaboratory variations. Therefore, there is a need to develop new methods to assess semen quality objectively.

Initially, CASA was developed to perform more accurate semen analysis. Its aim is to achieve standardized, objective, and reproducible test for sperm concentration, morphology, and motility. Use of CASA system in assisted reproductive techniques (ART) laboratories assumed to help in improvement of laboratory standardization and quality control studies. Besides these advantages, there are some disadvantages of CASA system such as the cost of the equipment and extreme need of validation.

There are some reports about CASA in andrology research and veterinary practise. Authors concluded CASA instruments were precise, efficient and reliable tool to evaluate fertility objectively and improve artificial insemination techniques [6, 7].

There are also a few studies in literature which compare these two semen analysis methods in human sperm. In one of them Cooper and Yeung compared two methods according to percentage of rapidly progressive (grade a) spermatozoa [8]. They reported that percentage of grade

a spermatozoa from both methods were similar but did not agree well with each other. Vested *et al.* also compared the two methods in terms of sperm motility and concentration. They found the interclass correlation coefficients (ICE) for sperm concentration assessments was high (0.92), whereas there was no correlation for rapidly progressive and slowly progressive spermatozoa (ICC = 0) between the two methods. They also reported low correlation (ICC = 0.54) for non-progressive sperm between conventional and CASA assessments [9]. In another study, authors concluded that CASA system was able to provide sperm concentration and motility measurements which were at least as reliable as current manual methods [10].

In a recently published study, Lammers *et al.* reported that correlation coefficients of both automated systems with manual analysis were very high for sperm concentration, total sperm number, motile sperm concentration, and progressively motile sperm concentration. In same study concerning morphology, specificity (Sp), and negative predictive values (NPV) of CASA versus manual assessment were: SpCASA – 84%, NPVCASA – 96%, respectively. As a result; they concluded that automated sperm analysis systems can be considered as accurate tools for routine sperm analysis, providing high quality results and allowing better standardization than manual analysis [11].

In the present study, sperm concentration, motility, and morphology results were markedly similar between conventional and manual methods ($p < 0.001$). However, ICC was found high (0.89) for sperm concentration and low for sperm motility and morphology, 0.62 and 0.44, respectively. The accuracy of sperm concentration appears to be diminished in the presence of severe oligospermia or excessive numbers of sperm [12, 13]. The high correlation found in the present study in terms of sperm concentration might be due to the small sample size or to the presence of phenomenal severe oligopolyspermia in the cohort.

Despite the similarity between two methods results, measurements of sperm concentration, motility, and morphology with CASA do not correlate very well with sperm motility and morphology measurements of manual methods.

In conclusion, although CASA systems are objective and rapid, they should be evaluated in terms of cost-effectiveness, however they may be useful in over-loaded ART clinics.

Acknowledgements

This project was supported by the Hacettepe University Research Foundation, Ankara, Turkey. Grant number: 06 A 101 005.

References

[1] Thonneau P., Marchand S., Tallec A., Ferial M.L., Ducot B., Lansac J., *et al.*: "Incidence and main causes of infertility in a resident population (1,850,000) of three French regions (1988–1989)". *Hum. Reprod.*, 1991, *6*, 811.

[2] Fréour T., Jean M., Mirallie S., Barriere P.: "Computer-assisted sperm analysis parameters in young fertile sperm donors and relationship with age". *Syst. Biol. Reprod. Med.*, 2012, *58*, 102.

[3] World Health Organization: "*WHO Laboratory Manual for the Examination and Processing of Human Semen",* 5th ed., 2010. Available at: http://whqlibdoc.who.int/publications/2010/9789241547789_ eng.pdf

[4] Check J.H., Shanis B., Bollendorf A.: "Computer assisted semen analysis not superior to routine analysis in distinguishing fertile from subfertile men". *Arch Androl.*, 1990, *25*, 13.

[5] World Health Organization: " *Laboratory manual fort the examination of human semen and sperm-cervical mucus interaction.* 4th ed. Cambridge: Cambridge University Press,, 1999, 9.

[6] Verstegen J, Iguer-Ouada M, Onclin K.: "Computer assisted semen analyzers in andrology research and veterinary practice". Theriogenology, 2002, *57*, 149.

[7] Broekhuijse M.L., Soštarić E., Feitsma H., Gadella B.M.: "Additional value of computer assisted semen analysis (CASA) compared to conventional motility assessments in pig artificial insemination". *Theriogenology*, 2011, *76*, 1473.

[8] Cooper T.G., Yeung C.H.: "Computer-aided evaluation of assessment of "grade a" spermatozoa by experienced technicians". *Fertil. Steril.*, 2006, *85*, 220.

[9] Vested A., Ramlau-Hansen C.H., Bonde J.P., Thulstrup A.M., Kristensen S.L., Toft G.: "A comparison of conventional and computer-assisted semen analysis (CRISMAS software) using samples from 166 young Danish men". *Asian J. Androl.*, 2011, *13*, 453.

[10] Tomlinson M.J., Pooley K., Simpson T., Newton T., Hopkisson J., Jayaprakasan K.: "Validation of a novel computer-assisted sperm analysis (CASA) system using multitarget-tracking algorithms". *Fertil Steril.*, 2010, *93*, 1911.

[11] Lammers J., Mansour W., Lattes S., Jean M., Barriere P., Freour T.: "Automated semen analysis…the new gold standard? a comprehensive study comparing manual and automated semen analysis". *Fertil Steril.*, 2012, *98*, S150.

[12] Irvine D.: "The computer assisted semen analysis systems:sperm motility assessment". *Hum. Reprod.*, 1995, *10*, 53.

[13] Krause W.: "Computer assisted semen analysis systems: comparison with routine evaluation and prognostic value in male fertility and assisted reproduction". *Hum. Reprod.*, 1995, *10*, 60.

Address reprint requests to:
L. KARAKOC SOKMENSUER, M.D.
Hacettepe University Hospital
Department of Obstetrics and Gynecology, IVF Unit
Sihhiye, Ankara (Turkey)
e-mail: lalekarakoc@hacettepe.edu.tr