

RESEARCH ARTICLE

Comparison of manual sperm analysis with computer-assisted sperm analysis: A comparative cross-sectional study

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ABSTRACT


Background: Conventional manual semen analysis is done routinely in most reproductive laboratories. Computer-assisted semen analysis (CASA), on the other hand, is more objective and timesaving counterpart to the conventional method. Different CASA software systems are available, some are fully automated. Thus, it is essential to analyze its performance and compare it with the conventional semen analysis method. **Aims and Objectives:** Our objective was to analyze the same semen sample by manual method using Makler chamber and using sophisticated CASA software. Our aim was to find the level agreement between the two methods. **Materials and Methods:** Ethical clearance was taken before the study. All the subjects were given proper instructions on how to collect complete semen sample. $n = 60$ diagnosed primary infertile male patients referred from the obstetrics and gynecology department. Semen of the same patient was analyzed by manual method and CASA software in accordance with the WHO guidelines. The results obtained were analyzed using Pearson's correlation and Bland and Altman plot. **Results:** On statistical analysis, "r" value obtained is 0.99 which is highly statistically significant. CASA and manual methods both agree with each other as proved by Bland and Altman plot. **Conclusion:** Both methods can be used to screen semen samples in reproductive laboratories. Manual method is easy, feasible, requires expert analysis, and has subjective variability. CASA, on the other hand, is faster newer method with better reproducibility.

KEY WORDS: Manual Sperm Analysis; Computer-assisted Semen Analysis; Bland and Altman Plot

INTRODUCTION

Semen analysis is the first diagnostic tool to evaluate the male infertility. Before the arrival of sophisticated softwares, semen analysis was done on counting chamber that required pipetting the semen sample. Later on, improvements in the counting chamber led to the invent of Makler chamber

which made semen analysis much easier and acceptable too. Even though the recently published WHO 5th edition manual recommendations (2010) should improve the analytical performance of manual semen analysis, existing high-performance automated analysis systems can provide an accurate technical alternative.^[1] Conventional manual semen analysis is the routine method in most assisted reproductive technology laboratories, but this method suffers from subjectivity and lack of standardization.^[2] No established method for counting sperm has been fully validated as the gold standard by the WHO. Manual method is still used because it is very cheap and easy method. It is rational to compare a new, more appropriate or additional method to a conventional one.

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Aims and Objectives

Our objective is to analyze the same semen sample by manual method using Makler chamber and using sophisticated computer-assisted semen analysis (CASA) software. Our aim was to see the level agreement between the two methods and thus conclude that both the methods can be incorporated at various fertility clinics.

MATERIALS AND METHODS

Ethical clearance was taken before the study and subjects were explained the procedure in detail. Written informed consent was taken from all the subjects. All the subjects were given proper instructions on how to collect complete semen sample. $n = 60$ diagnosed primary infertile male patients referred from the obstetrics and gynecology (OBGY) department. Semen of the same patient was analyzed by manual method and CASA software in accordance with the WHO guidelines. The results obtained were analyzed using Pearson's correlation and Bland and Altman plot.

Procedure

$n = 60$ diagnosed primary infertile male patients referred from the OBGY department. Semen analysis was done by manual method using Makler chamber and by CASA.

1. Semen analysis by manual method: Complete semen sample of the patient was collected in a non-toxic labeled glass bottle. The semen sample was left to liquefy for 30 min. A drop of a semen sample, using a sterile dropper was placed at the center of the lower surface of coverslip. There is a $1 \text{ mm} \times 1 \text{ mm}$ grid on the coverslip of Makler chamber, subdivided into 100 squares, each square of $0.1 \times 0.1 \text{ mm}$. The number of sperm heads in 10 squares was counted which indicated their concentration in million/ml.
2. CASA: The same sample was analyzed by CASA software (BIOVIS 2000 CASA Expert Vision Labs Pvt. Limited, India). CASA is a computer system which has a high-resolution camera connected to a phase-contrast microscope. The analysis of a single view field takes 1 s. Sperm concentration and motility were examined with the CASA system. Each analyzed slide may be saved in system memory and the examination may be repeated. Semen analysis was conducted with strict adherence to the program settings provided by the manufacturer. The results obtained were compared using Pearson's correlation and Bland and Altman plot. Statistical evaluation was performed (MedCal software) to analyze data using Pearson's correlation and Bland and Altman plot.

RESULTS

Using Pearson's correlation, we got (correlation coefficient) $r = 0.9916$ with $P < 0.0001$ which is highly statistically significant. Bland and Altman plot shows a decent level of agreement between the two methods [Figures 1 and 2].

DISCUSSION

Using Pearson's correlation, we got $r = 0.9916$ with $P < 0.0001$ which is highly statistically significant. Sperm counts obtained by CASA and Makler chamber show linear association. A scatter diagram depicting the same is also shown. Difference of the two paired measurements is plotted against the mean of the two measurements using the Bland and Altman plot. It shows acceptable decent level of agreement between sperm counts obtained by CASA and manual method.

The sperm count is a basic test for assessing male fertility, and there have been calls for global standardization of this test.^[3] The importance of semen analysis in the diagnostic process and treatment of male infertility remains indisputable.^[4] Manual semen analysis is very cost effective and simple procedure yet it requires expert analysis. No procedure till date is validated as a gold standard by the WHO for semen analysis.

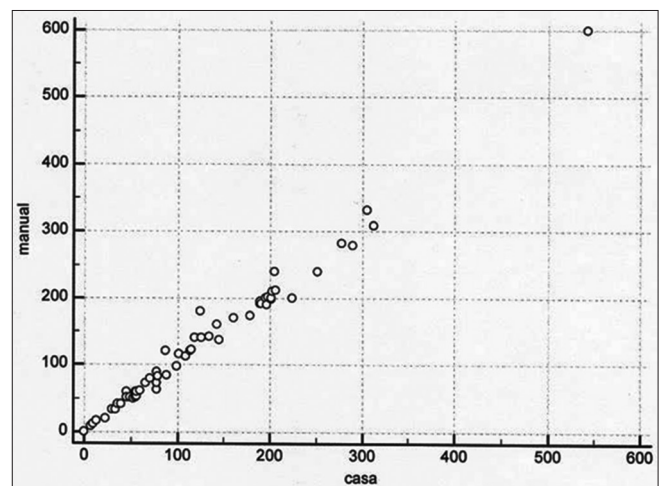


Figure 1: Scatter diagram showing sperm count in million using computer-assisted semen analysis and manual method

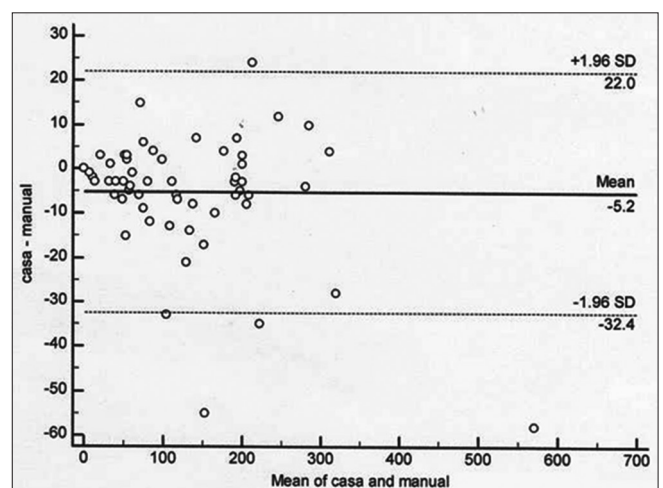


Figure 2: Bland and Altman plot showing agreement between computer-assisted semen analysis and manual method

CASA assessment is more objective and reproducible than technician-based manual motility assessment. CASA being a semi-automated computer analysis is less time consuming and accurate. In addition to sperm count, CASA also gives information about sperm kinematics which are predictors of *in vitro* fertilization.^[5] Furthermore, CASA is superior to manual method in that; it can accurately count sperms based on their progression whether rapid progressive, medium, slow, or static which is accurately not possible by manual method.

Lammer *et al.*, in 2013, did a double-blind prospective study comparing two automated sperm analyzers versus manual semen assessment. Statistical analysis of the test results from the automated systems and the manual method demonstrated no significant differences for most of the semen parameters measured.^[6] The study done by Komori *et al.*, in 2006, showed that sperm motility analysis system and manual microscopic sperm analysis show strong agreement in estimating sperm counts.^[7]

Fuse *et al.*, in 2005, showed that measurement of total sperm concentration and percent progressive motility by sperm quality analyzer IIB showed high correlations with those of conventional manual method.^[8] On the contrary, the study by Vested *et al.*, in 2011, showed that CRISMAS CASA results and results from the conventional method were not comparable with respect to sperm concentration and motility analysis.^[2]

Strength of our study is that we have taken a large sample size, $n = 60$. We have analyzed the data using both Pearson's correlation and also by Bland and Altman plot. Limitation of our study is that we could have compared more than two methods.

CONCLUSION

Both CASA and manual methods demonstrated acceptable agreement. However, to avoid subjective variations and for better standardization, CASA is a better tool. Furthermore,

CASA is no doubt superior to manual method in terms of giving sperm kinematics alongside with sperm counts.

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