

Comparison of semen analysis between the two Hamilton-Thorn semen analysers

A. Agarwal, E. Ozturk and K. R. Loughlin

Key words. Semen analysis -Hamilton -Thorn motility analyser- human

Summary. This study was designed to assess the reproducibility of the computer-assisted semen analysis (CASA) system. Semen specimens from 41 suspected subfertile patients were analysed on two identical Hamilton-Thorn Motility analysers (HTM, model 2030, version 7). Specimens were analysed after liquefaction at 37 °C for 30 min on two HTMs adjusted to the same gate settings and by using the same semen droplet loaded on a Makler counting chamber pre-warmed at 37 °C. Significant differences were seen in the total sperm count ($P < 0.0004$), motile sperm count ($P < 0.004$), amplitude of lateral head displacement ($P < 0.0001$), linearity ($P < 0.01$), and beat cross frequency ($P < 0.0001$) between the two HTMs. No significant differences between the two machines was seen in the case of other sperm motion parameters: percentage motility, average path velocity, straightline velocity, curvilinear velocity, straightness, and progressive motility. Our results indicate that the reproducibility of several semen parameters analysed by HTM is poor, and steps to remedy this problem are necessary.

Introduction

The commercial introduction of various computer-assisted sperm analysis (CASA) systems has greatly facilitated the analysis of semen in

many andrology laboratories. The use of an automated CASA system eliminates subjective human error and provides the potential for accurate quantitative evaluation of semen.

Previous reports in the literature have primarily focused on comparing the accuracy of results (count, percentage motility) generated by various CASA systems (Cell Soft [Cryo Resources Ltd, NY], Hamilton Thorn [Hamilton Thorn Research Inc., Danvers, MA], Cell Trak [Motion Analysis Corp., Santa Rosa, CA] etc.) to those of manual methods and on comparing sperm motion parameters among different CASA systems under various conditions (Knuth et al., 1987; Gill et al., 1988; Ginsburg et al., 1988; Knuth & Nieschlag, 1988; Mahony et al., 1988; Mortimer et al., 1988; Pedigo et al., 1989; Yantman et al., 1988). The goal of this study was to test the accuracy and reproducibility of semen analysis results between two identical Hamilton-Thorn Motility analysers under similar experimental and laboratory conditions. The following semen parameters were compared: total sperm count (TSC), motile sperm count (MSC), percentage motility (% MOT), average path velocity (YAP), straightline velocity (YSL), straightness (STR), amplitude of lateral head displacement (ALH), progressive motility (PM), curvilinear velocity (YCL), linearity (LIN), and beat cross frequency (BCF).

Division of Urology and Reproductive-Endocrinology, Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA.

Correspondence: Ashok Agarwal, Ph.D., Urology Research Laboratory, MRB 414, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA.

Material and methods

Semen collection and analysis

Semen samples were obtained by masturbation from 41 suspectedly subfertile patients. Specimens were produced into sterile widemouthed plastic containers after 48- 72 h of sexual abstinence. The specimens were allowed to liquefy at 37 °C for 30 min. Semen analyses were performed on two HTMs adjusted to the same gate settings and using the same semen droplet (5 µl) placed on a Makler counting chamber (Sefi Medical, Haifa, Israel) prewarmed at 37 °C. The same technician used the same micropipette to fill the Makler chamber. A total of 200 cells or more were analysed in each specimen by a single trained technician by scanning at least four fields. To control for specimen drying effect, half of the specimens were analysed first on HTM 1 and then on HTM 2; the other half of specimens were analysed first on HTM 2 and then on HTM 1.

Manual vs. HTM analysis

Optimization of acquisition conditions for sperm concentration provides a reliable starting point for assessing the precision of HTM. Sperm concentration readings from both HTMs were therefore compared to manual readings in a random group of subfertile patients (n= 103).

Hamilton-Thorn analyser

The HTM uses a combination of components to automatically determine sperm motility and concentration. An infrared beam is used to acquire images of the sample. After conversion into a digital format, the images are analysed by processing algorithms that determine the properties of the sperm motion (Gill et al., 1988). The measurement parameters in the two HTMs were as follows: number of frames = 20, frame rate = 30 frames per second, minimum contrast = 7, minimum size = 5, Lo/Hi size gates = 0.5-1.8, Lo/ Hi intensity gates = 0.5-1.8, non-motile head size = 10, non-motile intensity = 200, medium VAP value = 25, low VAP value = 10, slow

cells motile = no; threshold STR=80, VAP = 10-500, VSL = 0-500 VCL = 0-500 STR = 0-100, LIN = 0-100, ALH = 0-100, BCF = 0-0.

Statistical analysis

Statistical analysis was done by paired Student's t-test, with use of the Statview II program (Abacus Concepts, Inc., Berkeley, CA, USA) on a Macintosh SE-30 personal computer.

Results and discussion

Sperm concentration measurement by HTM and Manual Method

A comparison of sperm concentration results between both HTMs (mean: 65.6±8.3) and the manual method (76.3±9.7) showed a strong correlation: 0.989, r= 0.979 (Fig. 1) .

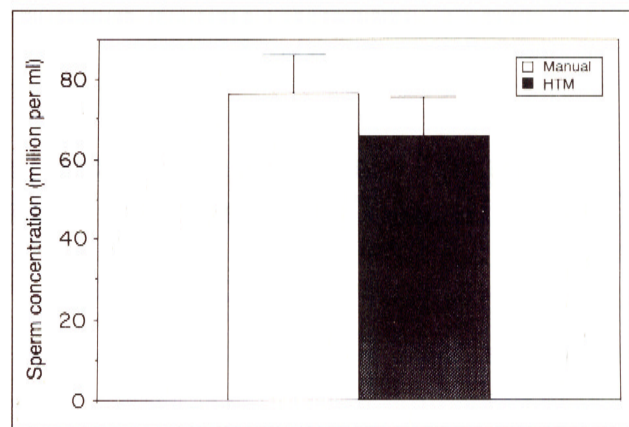


Figure 1. Comparison of sperm concentration between manual readings and HTM results show a strong correlation (r=0.979).

Comparison of semen analysis results between the two HTMs

Total and Motile sperm Count: There was a significant difference in total sperm count between HTM 1 (mean: 65.5±9.8) and HTM 2 (mean: 78.1 ±12.3) (P<0.0004) (Fig. 2). Motile sperm count also differed between HTM 1 (mean: 33.9±8.0) and HTM2 (mean: 43.8±10.8) (P<0.0041) (Fig. 2).

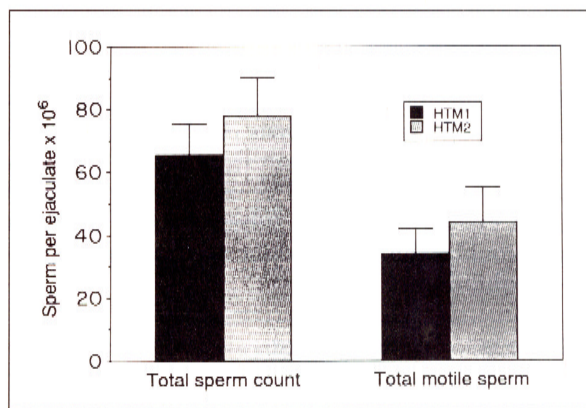


Figure 2. Comparison of total ($P < 0.0004$) and motile sperm count ($P < 0.004$) between the two HTM's show significant differences.

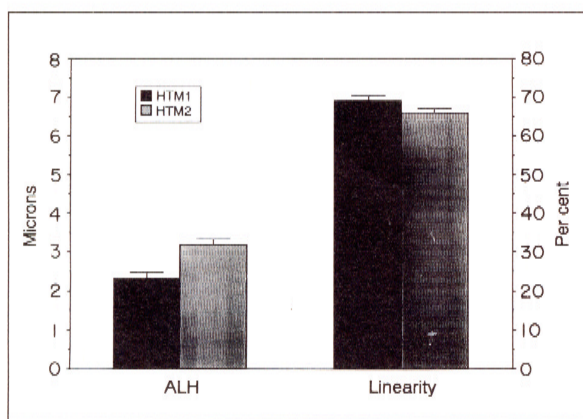


Figure 3. Comparison of sperm motion parameters, ALH ($P < 0.0004$) and linearity ($P < 0.004$) between the two HTM's show significant differences.

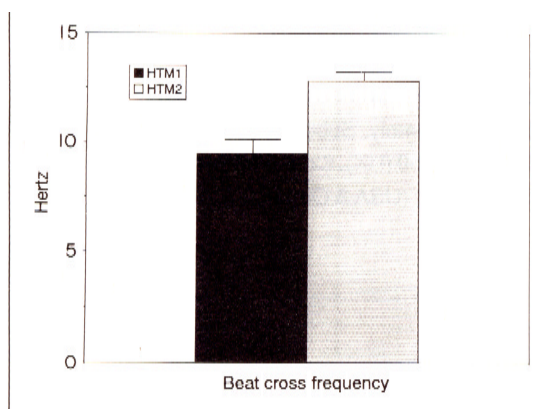


Figure 4. Comparison of beat cross frequency between the two HTM's show significant difference ($P < 0.0001$).

Sperm motion parameters: Significant differences between the two HTMs were seen in the following motion parameters: ALH (HTM 1 = mean 2.3 ± 0.17) vs. (HTM 2 = mean 3.18 ± 0.15) ($P < 0.0001$) (Fig.3), LIN (HTM 1 = mean 69.3 ± 1.2) vs. (HTM 2 = mean 66.2 ± 1.1) ($P < 0.0011$) (Fig. 3), and BCF (HTM 1 = mean 9.5 ± 0.6) vs. (HTM 2 = 12.8 ± 0.4) ($P < 0.0001$) (Fig.4). No significant differences between the two HTMs was seen for other motion parameters (% MOT, YAP, YSL, YCL, STR and PM).

The accuracy of semen analysis is essential in the investigation of the infertile male so that ensuing treatment is of maximal benefit to the patient. The use of an automated, computerized semen analyser eliminates subjective human error and provides the potential for accurate quantitative estimation of semen. In the present study, good correlation between sperm concentration measurements from both HTMs with the manual method validates the accuracy of HTM results. Analysis of results between the two HTMs showed significant differences in the absolute values of some important sperm parameters, despite identical conditions of the illumination system, magnification of the optical system, temperature of the sampling chamber, specimen volume, intensity and size of the sperm identified, number of frames, and frame rate used for acquisition.

In summary, the usefulness of data generated by HTM can be affected by lack of reproducibility among various semen parameters measured on two identical machines operating under similar experimental and clinical conditions. The use of Makler counting chamber may have attributed to lack of reproducibility in this study. Previous studies have found that Makler chamber gives inaccurate results compared to haemocytometer and Micro-Cell (Ginsburg & Armant, 1990). Steps to correct this problem are necessary.

References

- Gill HS, Van Arsdalen K, Hypolite J, Levin RM, Ruzich JV (1988) Comparative study of two computerized semen motility analysers. *Andrologia* 20:433-440.
- Ginsburg KA, Armant DR (1990) The influence of chamber characteristics on the reliability of sperm concentration and movement measurements obtained by manual and videomicrographic analysis. *Fertil Steril* 53:882-887.
- Ginsburg KA, Moghissi KS, Abel EL (1988) Computer-assisted human semen analysis sampling errors and reproducibility. *J Androl.* 9:82-90.
- Knuth UA, Nieschlag E (1988) Comparison of computerized semen analysis with the conventional procedure in 322 patients. *Fertil Steril* 49:881-885.
- Knuth UA, Yeung C-H, Nieschlag E (1987) Computerized semen analysis: Objective measurement of semen characteristics is biased by subjective parameter setting. *Fertil Steril* 48:118-124.
- Mahony MC, Alexander NJ, Swanson RJ (1988) Evaluation of semen parameters by means of automated sperm motion analysers. *Fertil Steril* 49:876-880.
- Mortimer D, Goel N, Shu MA (1988) Evaluation of the CellSoft automated semen analysis system in a routine laboratory setting. *Fertil Steril* 50:960-968.
- Pedigo NG, Vernon MW, Curry TE (1989) Characterization of a computerized semen analysis system. *Fertil Steril* 52:659-666.
- Vantman D, Koukoulis G, Dennison L, Zinaman M, Sherins RJ (1988) Computer-assisted semen analysis: evaluation of method and assessment of the influence of sperm concentration on linear velocity determination. *Fertil Steril* 49:510-515.