

# Defining the fertile man with Clear CASA: Capacitation (CAP) and Hyperactivation (HA)

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**ABSTRACT: Objective:** Is there a proven alternative to the basic semen analysis (SA)? HA and CAP are the true measure of fertilizing function. We should resolve two nagging questions: 1) Any CAP/ HA test must be wedded to pregnancy. 2) How do CASA definitions for sperm HA compare with one another?

**Design:** IVOS instruments and deep chambers were used. HA studies compared the 3 most cited HA systems: Burkman (1990, 1991), Mortimer and Mortimer 1990, and also Mortimer & Mortimer (M&M, 1995). The IVOS Sort program defined HA swimming patterns: thrash, star, helical, & circling, or all four together. HDATA pulled data from each analysis into Excel files for later HA sorting according to 12 equations.

**Materials and Methods:** Over 50,000 sperm were evaluated, from 9 different donors. Sperm were sampled at all stages of CAP. 1) % of sperm with the individual HA patterns, or ALL HA, when Burkman HA criteria were put into SORT ; 2) and when the sperm were reassessed using M&M 1990 criteria; 3) The same comparison was performed, but using the 1995 criteria; 4) Modifications to the 1995 criteria were tested, especially amplitude. Statistics: regression, ANOVA, chi-squared.

**Results:** Readings of human sperm using the Burkman 1990 criteria were compared to the M&M 1990 definitions: no statistical difference (P equal to 0.47). Comparison of Burkman criteria for HA to M&M 1995 values gave a P value less than 0.001. Parallel comparisons were made for individual patterns. Burkman values for Helical, Thrash and Star, added together, were virtually equal to the 1995 result. Inclusion of Circling dropped dramatically with M&M 1995.

**Conclusion:** Pregnancy, as an endpoint, is our gold standard (see other abstract). We find no Pregnancy data as validation for the 1995 paper. LifeCell Dx can provide guidelines for initiating Clear CASA in any lab. All labs must validate their own specific protocols for Clear CASA (medium, temperature, sperm handling, etc.). We emphasize that Circling HA adheres to the classical drawings of HA. **Support: N/A**

## INTRODUCTION: The Challenge

The true role of a semen analysis should be to answer this question: *is he fertile?*

Is there a proven alternative to the basic semen analysis (SA)?

HA (Hyperactivation) and CAP (Capacitation) are true measures of fertilizing function.

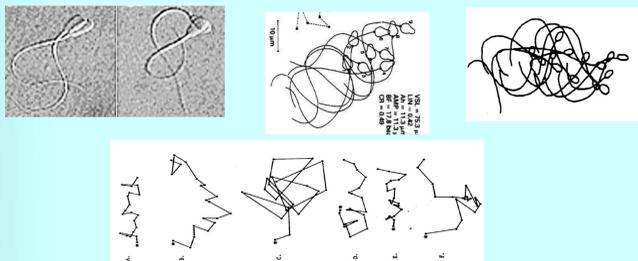
Isn't pregnancy prediction ultimately on a higher scale than a mathematical equation?

Less than 10% of all infertile couples in the U.S. resolve their fertility questions through use of IVF/ ICSI. Urologists, most Gynecologists, and primary care providers are not connected to IVF. Most couples, and their physician, will look to a semen analysis for guiding the first treatment step, or their second/third option: surgery, number of IUI cycles attempted, use of donor sperm, adoption, surrogacy, etc., or stop. From the male side, they need an answer to the question: *is he fertile?* Many of them realize that the basic, manual semen analysis has poor predictive capacity.

In these 3 posters, we present the role of Clear CASA in semen analysis and predicting the male's fertility. This approach is termed Advanced Semen Analysis, and we have used the IVOS instrument. PLEASE SEE Poster 1007 & Poster 1008, for the complete picture.

Sperm that can fertilize an egg/oocyte must achieve Capacitation (1), yet many functional markers of Capacitation require complex or lengthy assays (acrosome, biochemical changes, elevation of biomarkers, zona binding, etc.). In contrast, computerized tracking of vigorous swimming patterns (CASA) during Capacitation is rapid and reproducible. Since 1969, these capacitative, vigorous patterns have been called Hyperactivation (HA). See Figure 1 for examples of classical HA swimming patterns. Sperm that lack Hyperactivation cannot fertilize (2, 3).

## Fig. 1: Classical Images of Hyperactivation



[Yanagimachi, Morales, Burkman ]

Validation of the Clear CASA approach comes from prediction of pregnancy (see Poster 1007). We have defined Clear CASA based on careful studies of variables which significantly alter CASA results. Hyperactivation (HA) is a key component of Capacitation. For many years, we have utilized Hyperactivation (HA) as the critical parameter for predicting his "pregnancy potential." (See Poster 1007 for data on "90% accurate in predicting a pregnancy").

**Objective 1 for Clear CASA: How do competing CASA definitions for sperm HA compare with one another? (this Poster 993)**

[ Poster 1008: Objective 2 for Clear CASA : How will variations in CASA usage change semen analysis data? Poster 1007: Objective 3: Predicting his fertilizing potential using Clear CASA.

**METHODS and DESIGN:** All studies were performed at the prior Burkman lab in the School of Medicine, Dept. of Gyn/Ob, as well as the current private Burkman lab (LifeCell Dx, e-fertility diagnostics). In every study, Hamilton Thorne IVOS instruments were utilized. For this poster, fresh semen samples from screened research donors were studied at 37°C, after a simple swim-up (Ham's F10 with 0.3% HSA), and within deep chambers. Some men were "low HA donors" and others were "high HA donors." In total, the studies evaluated 12 semen samples from 9 men, representing more than 50,000 sperm analyzed (two posters). Sperm were sampled at early and late stages of Capacitation (2, 4, 6, and 8 hours). For Poster 993, all studies utilized 0.5 sec tracking with CASA.

Study 1: Compare the 3 most cited HA sorting systems. First, compare the HA values obtained using the Burkman criteria (1990, 1991) versus the 1990 Mortimer and Mortimer definitions for HA.

**The questions were:** How will HA results differ for a given sample, as the several HA criteria are applied? Is there a clinical rationale for choosing the HA criteria? How might this make a difference in predicting a man's fertilizing potential?

Study 2: Compare Burkman criteria against revised HA definitions by Mortimer & Swan in 1995 (Mortimer 1995).

Study 3: Evaluate modifications to the Mortimer 1995 criteria. Which HA vigorous patterns are affected?

Table 1: Burkman Criteria for Hyperactivation (All HA is equivalent to all four patterns put together; 1990, 1991)

All HA	Circling	Helical	Thrash	Star
VCL ≥ 100	VCL ≥ 100	VCL ≥ 80	VCL ≥ 100	VCL ≥ 80
ALH ≥ 7.5	VSL ≥ 60	VSL 40 to 59	VSL 16 to 39	VSL ≤ 15
LIN ≤ 65	ALH ≥ 7.5	ALH ≥ 8.0	ALH ≥ 8.0	ALH ≥ 8.0
	LIN = 36 to 65	LIN = 20 to 60	LIN ≤ 35	LIN < 20

Table 2: Mortimer Published Criteria for Hyperactivation (1990 vs 1995)

1990 Mortimer without VSL	1990 Mortimer with VSL	Mortimer & Swan 1995
VCL ≥ 100	VCL ≥ 100	VCL ≥ 180
ALH ≥ 5.0	ALH ≥ 5.0	ALH ≥ 6.0
LIN ≤ 60	LIN ≤ 60	LIN ≤ 45
	VSL < 30	

Table 3: We tested modifications of the Mortimer 1995 Hyperactivation criteria

Mortimer 1995 criteria	Modify the VCL (velocity)	Modify the LIN (linearity)
VCL ≥ 180	VCL ≥ <b>140</b>	VCL ≥ 180
ALH ≥ 6.0	ALH ≥ 6.0	ALH ≥ 6.0
LIN ≤ 45	LIN ≤ 45	LIN ≤ <b>60</b>

We evaluated the change in final % Hyperactivation, and the type of HA pattern, when the Hamilton Thorne IVOS "Sort" pages were altered as detailed in Tables 1-3. In the software "Sort" page, one enters the desired minimum and maximum values for up to 10 specific movement characteristics (VCL, VSL, LIN, ALH, etc.).

Secondly, we utilized the Hamilton Thorne program to dump the data for many of the analyzed sperm into Excel files (HDATA program). [The power of the program was quite useful: after a single 5-minute analysis, we could download all of the motility parameters for 300 to 1000 motile sperm, delete all sperm that had less than 15 data points, type in the Excel equation which represented one of the 12 HA definitions, and perform an Excel "sort" against each of the 12 equations. We used "if, then" equations].

**STATISTICS:** In various computations, logistic regression, ANOVA, and chi-squared analysis were used.

## RESULTS Figure 2: Comparing 7 different HA definitions with CASA (Mean ± SEM; 60 hz, 0.5 sec). One typical donor.

Circling is the major contributor to All HA. The matching Mortimer 1995 data are nearly equivalent to the calculated Helical + Thrash + Star value (p > 0.05). There is a significant difference between All HA and Mortimer 1995 (\* ; p < 0.001).

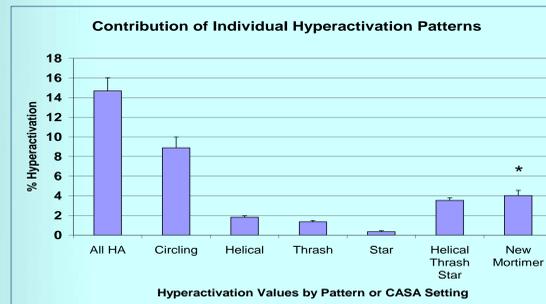


Figure 3: Mortimer data on the right. The 1990 Mortimer criteria (without the VSL restriction) matches ALL HA. By dropping VSL, the Circling HA sperm are recognized. The "new" 1995 criteria eliminated most of Circling and part of Helical.

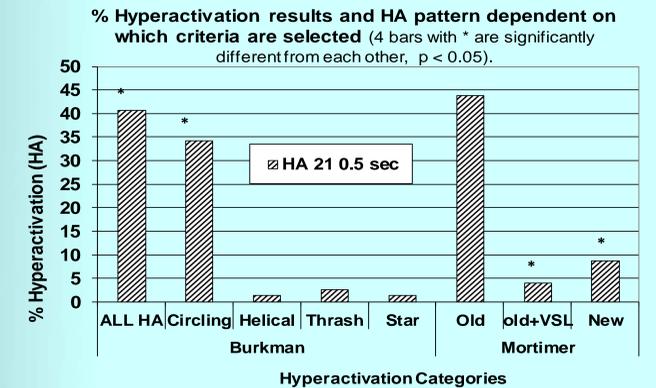


Figure 4: Significant change in %Circling HA, when using Mortimer 1990 criteria, compared to Mortimer 1995. Not the same donor as Figure 3.

Type of HA swimming pattern	Mortimer 1990	Mortimer 1995	Difference	P-value
Circling HA	20%	0.9%	19.1%	0.000
Helical	5.2%	0.5%	4.7%	0.05
Thrash	6.3%	8.2%	+1.9%	0.579
Star	4.2%	2.3%	1.9%	0.406

## DISCUSSION

Numerous papers have emphasized the inability of a basic semen analysis to predict a given man's fertilizing potential (ability to produce a pregnancy, excluding ICSI). A basic sperm count will not predict his potential. With coitus, IUI, and standard IVF, the fertilizing sperm must have Hyperactivated motility. Validation of CASA criteria when identifying HA for human sperm must be directly associated with the ability to produce a pregnancy.

Classic papers on HA report that sperm will have high-amplitude, non-linear and vigorous movement patterns. As shown in Poster 1007, reliance on the Burkman criteria for HA, using CASA, yields excellent prediction of those men who go on to produce a pregnancy.

We have defined Clear CASA to include identification of HA sperm that show the Thrashing, or Helical, or Star patterns, and including vigorous Circling patterns. Other popular definitions for HA use a Velocity cut-off that is too high, or a Linearity cut-off that is too low. Our pregnancy data (Poster 1007) give strong corroboration for defining Hyperactivated patterns in this manner.

**Excellent CASA is a very powerful clinical tool within Andrology.** The large majority of infertile couples in the U.S. will not seek in vitro fertilization. Within urology offices, and gynecology services, the non-ICSI patient deserves to have more than a basic semen analysis. Whether using CASA or a trained eye, assessment of Hyperactivated motility will guide the physician in making treatment decisions.

## References:

- Yanagimachi, R (1994): Mammalian fertilization. In: Knobil E & JD Neil, editors. The physiology of reproduction. New York, NY: Raven Press, Vol 1:189-317.
- Fleming AD, Yanagimachi R (1982). Fertile life of acrosome-reacted guinea pig spermatozoa. J Exp Zool 220:109-15.
- Qi, H, et al., (2007). All four CatSper ion channel proteins are required for male fertility and sperm cell hyperactivated motility. Proc Natl Acad Sci, 104:1219-23.
- Burkman, LJ (1991): Discrimination between nonhyperactivated and classical hyperactivated motility patterns in human spermatozoa using computerized analysis, Fertil and Steril, 55:363-371.
- Burkman LJ (1990): Hyperactivated motility of human spermatozoa during in vitro capacitation. In Gagnon (ed): "Controls of Sperm Motility". Boca Raton: CRC Press, pp 304-329.
- Mortimer ST, Mortimer D (1990) Kinematics of human spermatozoa incubated under capacitating conditions. J Androl., 11: 195-203.
- Burkman LJ (1995). Motility of human spermatozoa during capacitation. IN: Cambridge Reviews in Human Reproduction, volume on Gametes - the Spermatozoon, ed. Grudzinski JG.
- Mortimer ST, Swan MA (1995) Kinematics of capacitating human spermatozoa analysed at 60 Hz. Hum. Reprod., 10: 873-879.
- Mortimer ST (2000) CASA - Practical aspects. J. Androl., 21: 515-524.
- Mortimer D, Mortimer ST (2013). Computer-aided sperm analysis (CASA) of sperm motility and hyperactivation. Methods Mol Biol. 2013;927:77-87.
- Lamb, D. J. (2010). Semen analysis in 21st century medicine: the need for sperm function testing. Asian Journal of Andrology, 12:64-70.