

# P-1007 Consistent Predictor of Pregnancy (PREG) and sperm fertilizing potential : Advanced Semen Analysis (ASA) and Hyperactivation (HA) using Clear CASA for local or remote patients

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**ABSTRACT. Objective :** Many couples despair of knowing the male partner's true fertilizing potential. Why is the basic SA still heavily utilized? Can ASA with Clear CASA predict a man's ability to produce a pregnancy? Classically, a capacitated sperm (CAP) reflects the functions needed to fertilize an egg. Vigorous swimming/HA is the easiest CAP function to evaluate. (See Clear CASA Posters 993, 1008). In 1996, we reported on regression & Sensitivity for Hyperactivation vs. PREGNANCY. Here, we have studied 4 new groups.

**DESIGN:** Four random groups of new couples were seen (1998-2012). These represent > 300 ASA at the Burkman Gyn/Ob Andrology Lab, or in Dr. Burkman's private lab, LifeCell Dx. At 9-36 months later, all possible patients were called, in a blind fashion, to learn whether a PREGNANCY had occurred (natural, IUI, IVF, no ICSI). His ASA report was then read, showing the highest HA value.

**METHODS:** Samples were collected into F10 medium (diluted), and the lab utilized Clear CASA methods with CAP/HA analysis (IVOS). We adopted an HA cut-off of  $\geq 5\%$  (Burkman criteria). We studied those cases which had PREG and HA  $\geq 5\%$ .

**RESULTS:** See data table for each new group [1998; 2004; 2009; 2012]. Original data (1996) showed a mean HA of  $10.1 \pm 1.0$  (PREG) vs.  $4.4 \pm 0.9$  (Non-PREG;  $P < 0.001$ ); 49 had a PREG, and 43 of these had HA  $\geq 5\%$ . Thus, 88% PASSED. In Groups 2, 3, 4, 5, the PASS rates were 88%, 100%, 91%, & 91%. In total, 90.3% received a PASS, leaving 9.7% who failed our testing ( $P < 0.01$ , chi-squared). Over 17 years, the ASA methods & results were robust, with no drift.

**CONCLUSIONS:** Rigorous HA predicts a given man's ability to produce a pregnancy—this is not a correlation. Collection into medium initiates CAP. PREG indicates that the female issues are okay AND that the sperm were fertile. HA motility is very visual, if CASA is not available. Prediction of PREG by HA & Clear CASA has now reached the next level via remote semen analysis in Japan, and in cities far from Buffalo. Excellent CASA is a very powerful tool within Andrology.

## INTRODUCTION: The Challenge

Can Advanced Semen Analysis (with Clear CASA) predict each man's "pregnancy potential" ?

Can we achieve 90% accuracy in predicting? PREG is our gold standard.

The ability to predict serves as validation for our ASA and Clear CASA.

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

Many couples/physicians despair of knowing the male partner's true fertilizing potential. This gap cripples their ability to make informed decisions about treatment. Leading clinics state that the basic semen analysis has poor prediction (ref). Human sperm Capacitation (CAP) is a true marker for the man's fertilizing potential.



Less than 10% of all infertile couples in the U.S. resolve their fertility questions through use of IVF/ICSI. Yet most Urologists, 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 first need an answer to the question: *is he fertile?*

Since most offices/labs do not use CASA today (computer-assisted semen analysis), we challenge the discerning provider to consider: 1) visual training for identification of HA in a diluted semen sample (1); or 2) to resurrect their dusty CASA; or 3) to purchase a 60 hz CASA instrument with SORT; or 4) to learn about our remote semen analysis services (all digital).

Sperm that can fertilize an egg/oocyte must achieve Capacitation (2); however, many functional markers of Capacitation require complex or lengthy assays (acrosome, biochemical changes, elevation of biomarkers, zona binding, etc.). Many functional markers show a correlation, but are not validated to predict fertilizing potential for the individual man. Clear CASA methods (P-993, P-1008) permit a Capacitation analysis that is rapid and reproducible (3). Vigorous Hyperactivated swimming (HA) is a robust marker of overall Capacitation function. Sperm that lack HA cannot fertilize (4,5). Since 1969, HA patterns have been described as high-amplitude and non-progressive. Our SORT criteria include the thrashing, circling, helical and star patterns (see Figure above and P-993).

Our 1996 studies (6) presented the earliest data shown in Table 1 below. For 17 years, our Andrology Laboratories have relied on %HA as the critical parameter for predicting his "pregnancy potential."

## METHODS and DESIGN:

Four groups of new infertile couples were evaluated during 1998-2012. These followed on the group presented in the 1996 abstract (6; shown as Original in Table 1). Couples were seen at the earlier Burkman Gyn/Ob Andrology and research lab, or in Burkman's current private lab, LifeCell Dx. Semen evaluation and sperm function were carried out with our Advanced Semen Analysis (ASA) protocols and Clear CASA (Hamilton Thorne IVOS instruments).

At periods of 9 months to 36 months later, all possible patients were contacted to record whether a PREGNANCY had occurred since they visited the lab (Yes/No; natural, IUI, standard IVF; with IRB approval). This was also carried out 4 times later (4 new patient groups: 2, 3, 4, and 5). The person calling was blind to male and female history. Donor cases and ICSI cases were excluded. If the call was successfully completed, that person reviewed the male partner's ASA report(s) to record the highest Hyperactivation (HA) value.

Since lack of PREGNANCY does not reveal whether the female had a problem, or the sperm had a problem, data analysis focused on the cases that had a PREGNANCY (miscarriage was counted as a pregnancy). Among the PREG cases, those with HA  $\geq 5\%$  were marked as PASS for HA/Capacitation. Cases with HA  $< 5\%$  were marked as FAIL for HA/CAP.

Fig 2. PREGNANT Cases by % Hyperactivation

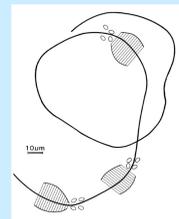
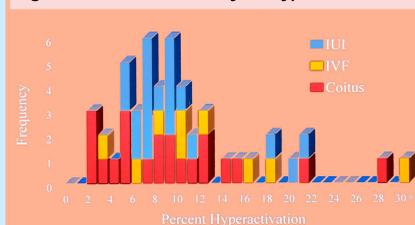


Fig 3. High-amplitude, non-progressive Circling HA

See Poster 993 and P-1008 for discussion of the Burkman SORT criteria to identify HA with Clear CASA. Sorting of HA sperm was carried out according to Burkman criteria (7 ; VCL  $\geq 100 \mu\text{m}/\text{sec}$  and ALH  $\geq 7.5 \mu\text{m}/\text{sec}$  and LIN  $\leq 65$ ; curvilinear velocity, amplitude, linearity). See other posters for limits on tracking duration, multi-phasic vior, etc. Here, %HA is essentially the sum of all 4 individual patterns among the sperm (ALL HA). In neat semen, % HA usually ranges from 0-4 (8). 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, with rigorous external and internal quality control.

For this poster, fresh semen samples were received from patients. The semen was collected into one or two vials, containing Ham's F10 medium (GIBCO). Hence, by the time analysis was performed at 37°C, Capacitation was initiated. Some patients also had a mock IUI washing procedure. Clear CASA data were obtained on the diluted semen within deep chambers, and at times also on the washed sperm sample ( $\geq 200$  motile sperm). The final ASA reports included count, % motility, VCL, ALH, % Hyperactivation, strict morphology, and notations on proprietary endpoints. If the patient provided multiple samples, the highest HA value was identified for these Pregnancy Potential studies. Statistics in this study included logistic regression, chi-squared, and student's "t" test.

Fig 4. Non-Pregnant cases from Original Group

## RESULTS:

Review of the first data set presented in 1996 (6) with additional cases from 1997. Among 200 initial cases, 49 were accepted as having a pregnancy (PREG) with no missing data (also no ICSI and no donor). Figure 2 illustrates the HA values for these 49 men who produced a pregnancy after we performed ASA (Advanced Semen Analysis).

The mean HA was  $10.1 \pm 1.0$ , compared to a mean of 4.4% for the Non-Pregnant group in Fig. 4 ( $P < 0.01$ ; mean  $\pm$  sem). For the PREGNANT group, there are no cases with 0% HA, or 1% HA. For HA of 2-4%, 5 out of 6 pregnancies occurred with natural intercourse. Standard IVF is a more hostile environment for borderline functioning sperm, compared to natural. Figure 4 (Non-Pregnant) shows that 71% of the data points ( $n = 30$ ) occur at the lowest end of the range, that is, 0-4 % Hyperactivation. One-third of the sperm samples have 0-1% Hyperactivation. Mean sperm concentrations were not significantly different between the 2 groups (data not shown;  $P > 0.05$ ).

HA of 5% was selected as the cut-off --- that is, samples with  $\geq 5\%$  HA, using our methods, are marked as PASS for our HA/Capacitation test. HA  $< 5\%$  is taken as a negative indicator for achieving pregnancy. As shown in the next section, relatively few pregnancies occurred in the new groups if HA was  $< 5\%$ .

**As shown in Table 1, 43 of the 49 cases had an ASA report stating vigorous Hyperactivated swimming of  $\geq 5\%$ . Therefore, the rate of correctly predicting these pregnancies was 88%.**

We might say that Sensitivity is 88%, but we cannot effectively state Specificity: as a couple, failure to get pregnant cannot be strictly assigned to the sperm, in most cases. However, if the male partner's sperm exhibit Hyperactivation (Capacitation) of  $< 3\%$ , their chances of pregnancy are much lower.

## RESULTS for Groups 2, 3, 4, and 5: HA and Predicting Pregnancy See Table 1.

Every three years or so, a fresh group of infertile patients was analyzed. These were referred to the lab for Advanced Semen Analysis. Samples were collected in the same manner as above, and digital analysis was carried out in the same way using our Clear CASA methods. Patients were called long after the report was sent out (9-36 months) and data were analyzed for those couples who reported a Pregnancy (no ICSI, no donor).

The data for each new group [1998; 2004; 2009; 2012] are presented in Table 1, along with the Original data set (1996-7). Table 1 represents 17 years of utilizing the HA cut-off of 5% in order to predict his "pregnancy potential."

The HA pass rate relative to Pregnancy has remained very stable over the years: 88%, 88%, 100%, 91%, 91% (Table 1.)

[Note: the % of cases with a pregnancy has declined since the local IVF group moved away from the University, and more "general infertility" patients visit the lab ]

Table 1	Number of Couples With Pregnancy	How many Passed our Test	% of Pregnancy couples who passed our test
Original group 1996-7	49	43 of 49	88%
New group 2	17	15 of 17	88%
New group 3	14	14 of 14	100%
New group 4	11	10 of 11	91%
New group 5	12	11 of 12	91.7%
<b>All 5 groups</b>	<b>103</b>	<b>93</b>	<b>90.3%</b>

## CONCLUSIONS:

- Covering approximately 400 Advanced Semen Analyses, with 103 pregnancies, 90% of the pregnancies were correctly predicted. The residual 9.7% were false negatives (see Figure 2).
- Rigorous and consistent protocols allow Clear CASA to predict that "this man can produce a pregnancy" [if the female does not have a serious problem].
- A rigorous, consistent Hyperactivation test, as part of our routine ASA, reflects Capacitation in each sample and the ability to produce a pregnancy.
- Step-wise logistic regression revealed that HA alone predicted pregnancy for 90.7% of the Original data set. Together, % Motility, ALH, & VCL added another 4% to the model.
- These data come from the hands of 8 lab staff members who were trained in the same protocols for sperm handling, and the same CASA steps.
- Over this time, we have used 3 different IVOS and CEROS instruments.
- We recommend this approach to other laboratories in validating a plausible alternative to the basic semen analysis.
- Our new patented methods for Remote Semen Analysis now support a platform for multiple laboratories to collaborate in confirming whether this approach can replace the basic semen analysis in private clinics, hospitals, and research settings.

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