

# CORRELATION OF COMPUTERIZED SEMEN ANALYSIS WITH SUCCESSFUL FERTILIZATION OF OOCYTES IN AN IN VITRO FERTILIZATION PROGRAM

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Sixty-nine couples enrolled in 123 in vitro fertilization-embryo transfer cycles were categorized by percentage fertilization; the results of categorization were compared with those of computerized semen analysis carried out with the CellSoft semen analyzer. Four groups were established: group 1 had 75% fertilization or greater; group 2 had 34% to 74% fertilization; group 3 had 1% to 33% fertilization; and group 4 had 0% fertilization. Statistical differences in certain semen parameters (motility, linearity, and straight-line velocity) were found comparing groups 1 and 3 using the initial ejaculate. A significant number of patients in group 1 had all normal semen parameters, but no statistical difference could be found in group 3 or 4 because of variations in specific abnormal parameters in the groups. When the straight-line velocity-motile density (SLVMD) calculation was used, a significant difference was seen between group 1 and group 3 and between group 1 and group 4 ( $p < 0.01$ ); 65% of group 3 and 76% of group 4 had an abnormal SLVMD. SLVMD is a useful calculation to predict fertilization rates in vitro from the initial ejaculate.

**Key Words:** Computerized semen analysis; In vitro fertilization; Straight-line velocity; Motile density.

## INTRODUCTION

In vitro fertilization-embryo transfer (IVF-ET), originally developed to treat tubal or idiopathic infertility [12], is also an alternative fertilization method in male infertility. Improved methods of IVF make it possible to use lower concentrations of motile spermatozoa than those originally reported to achieve fertilization [15]. The correlation between male factor infertility

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and success of IVF has been the topic of several investigations [16]. Decreased fertilization rates have been observed with tubal factor infertility [2, 5, 8]. A lower fertilization rate has also been reported in couples for whom results are subnormal in the hamster egg penetration assay [1, 9]. A study by Corson et al. [3], however, did not show the same correlation. The present study evaluates the parameters of computer-assisted semen analysis (CSA) carried out with the CellSoft semen analyzer (CRYO Resources) and their correlation with percentage fertilization of oocytes in vitro.

## MATERIALS AND METHODS

*Semen Analysis.* Sixty-nine couples involved in 123 IVF-ET cycles were enrolled prospectively in this study. For each couple, two baseline semen samples were evaluated by CSA and averaged before IVF-ET. Semen samples (7  $\mu$ l) were placed in a Makler chamber on a heated (37°C) stage and assayed directly. All samples with a sperm concentration in excess of  $40 \times 10^6$ /ml were diluted with the patient's sperm-free seminal plasma to a concentration of (25 to 35)  $\times 10^6$ /ml to ensure accurate velocity measurements [7, 13]. All samples with a sperm concentration of less than  $20 \times 10^6$ /ml were counted manually in the Makler chamber; measurements were compared with CSA results to ensure accurate count and motility results [10, 13]. With the Makler chamber, manual semen analysis was performed on the day of egg retrieval. There were no significant differences among count, motility, or between previous CSA and manual semen analysis on the day of the egg retrieval.

The normal values established in our laboratory from the minimum values achieved by fertile donor semen samples were in accordance with suggested reference values from CRYO Resources to be used with the CellSoft system. These values are as follows: count (CT),  $\geq 20 \times 10^6$ /ml; motility (%M),  $\geq 50\%$ ; curvilinear velocity (CVL),  $\geq 40 \mu$ m/sec; linearity (LIN),  $\geq 5.5$ (CLV/straight-line velocity  $\times 10$ ); mean amplitude lateral head displacement (ALHmn),  $\geq 1.80$ ; ALH maximum (ALHmax),  $\geq 2.30$ ; and beat/cross frequency (BCF),  $\geq 13.00$  Hz. Calculations were as follows: motile density (MD), (CT  $\times 10^6$ /ml  $\times$  %M)/100; and straight-line velocity (SLV), (velocity  $\times$  LIN)/10 (22  $\mu$ m/sec). SLV is a direct measurement but is not part of the routine CellSoft report. A new parameter, straight-line velocity-motile density (SLVMD;  $\geq 10$ [SLV  $\times$  MD]/100), was also evaluated in this study.

*Follicular Stimulation and Embryo Retrieval.* Oocyte fertilization during each IVF-ET cycle was characterized as good (group 1, 75% to 100%), adequate (group 2, 34% to 74%), poor (group 3, 1% to 33%), or none (group 4, 0%). Follicular stimulation was accomplished by first suppressing endogenous gonadotropins with administration of 1 mg leuprolide acetate subcutaneously 7 days after ovulation for a period of 10 days. Human menopausal gonadotropin (hMG) was administered intramuscularly at a dosage of 150 IU two times daily starting on day 11 of the cycle for 3 days. The hMG was then reduced to 225 IU per day for 1 day and then to 150 IU daily thereafter. Patients were monitored daily by vaginal ultrasonography and serum estradiol (E<sub>2</sub>) measurement. At the point when a serum E<sub>2</sub> level of at least 600 pg/ml and when at least three follicles larger than 16 mm in diameter were observed, human chorionic gonadotropin (hCG) was administered at a dose of 10,000 IU intramuscularly. Oocyte retrieval was performed by means of vaginal ultrasonography 30 to 32 hours after administration of hCG.

The retrieved oocytes were classified into four categories: immature, mature, postmature, or atretic [11, 14]. All preovulatory oocytes were incubated in Ham's F-10 medium (Gibco) supplemented with 5% bovine serum albumin (BSA; Sigma), pH 7.4, at 275 to 280 mOsm/kg H<sub>2</sub>O. Motile sperm concentrations of (5 to 10)  $\times 10^4$ /ml were used for inseminations. Higher sperm concentrations of (1.0 to 2.5)  $\times 10^5$ /ml were used for oligospermic patients. Semen was subjected to centrifugation at 400g for 8 min in a double volume of F-10 plus BSA, and the centrifugate was overlaid with 0.2 to 0.5 ml of modified F-10. Motile sperm were allowed to swim up into the overlying medium for 30 to 60 min. After the motile fraction was removed, semen parameters were measured and used for insemination. One

to four embryos were transferred on the second day after retrieval. When there were more than four embryos, the remaining ones were cryopreserved for subsequent transfer by means of a modification of the one-step method [6]. All patients who underwent ET received supplemental progesterone (25 mg in oil intramuscularly) daily starting on the day of retrieval and continuing until a negative  $\beta$ -hCG test result was obtained.

*Statistics.* Differences in CellSoft semen variables among groups were analyzed by analysis of variance (ANOVA). Pairwise comparisons among the three fertilization groups were made by means of the protected Fisher's least significant difference test. Differences among fertilization group frequency distributions sorted by CellSoft semen variables were analyzed by the  $\chi^2$  test. A  $p$  value of 0.05 or less was considered statistically significant. Multivariate regression and ANOVA were also performed with the SYSTAT statistical package (SYSTAT, Inc.). Numerical data are presented as the mean  $\pm$  standard deviation.

## RESULTS

In the 123 IVF-ET cycles studied, 20% resulted in 0% fertilization. All patients had at least 50% of their oocytes judged as mature. All retrieved mature, immature, and postmature oocytes were used for insemination 3 to 5 hours after the first oocyte retrieval time. Count and motility measured on the day of oocyte retrieval were not significantly different from the initial CSA results. When all semen parameters were normal, 81% (22/27) had a percentage fertilization of 75% or more (group 1). In group 3, with 1% to 33% fertilization, abnormal results varied among the parameters. The values of CellSoft semen variables and the derived quantity SLVMD in the four fertilization groups are presented in Table 1. There were statistically significant differences among the groups for SLV ( $p < 0.013$ ), MD ( $p = 0.015$ ), linearity ( $p = 0.001$ ), and SLVMD ( $p = 0.001$ ).

Count and curvilinear velocity were not statistically different among the groups, although there was a trend for low values in the low percentage fertilization groups. Motility, MD, SLV, and SLVMD were all statistically higher in group 1 compared to group 3 and in group 1 compared to group 4, as were these variables (with the exception of SLV) in group 1 compared to group 2. These differences were found primarily between groups 1 and 2, groups 1 and 3, and groups 1 and 4. There were no statistically significant differences among any of the variables between group 2 and group 3.

TABLE 1 CellSoft Parameters in Different Oocyte Fertilization Groups

Group	1	2	3	4
Oocytes fertilized (%)	$\geq 75$	34-74	1-33	0
N	49	33	16	25
CT	$119.3 \pm 75.4$	$84.6 \pm 57.1$	$91.5 \pm 0.9$	$71.37 \pm 65.4$
%M	$66.8 \pm 20.9^{a,b}$	$53.2 \pm 24.5$	$53.0 \pm 23.4$	$37.4 \pm 21.4$
MD	$81.3 \pm 69.8^{a,b}$	$51.3 \pm 53.1$	$61.5 \pm 67.7$	$36.07 \pm 37.58$
SLV	$27.9 \pm 8.1^b$	$26.3 \pm 1.3$	$23.4 \pm 6.82$	$23.37 \pm 7.9$
CLV	$44.3 \pm 8.4$	$42.1 \pm 9.8$	$41.8 \pm 7.13$	$39.2 \pm 13.0$
LIN	$6.2 \pm 1.0$	$6.1 \pm 1.1$	$5.53 \pm 0.97$	$5.64 \pm 2.5$
SLVMD	$23.4 \pm 22.7^{a,b}$	$12.6 \pm 12.3$	$14.48 \pm 15.4$	$8.61 \pm 9.9$

<sup>a</sup> $p < 0.01$  compared to group 2.

<sup>b</sup> $p < 0.01$  compared to group 3.

TABLE 2 Oocyte Fertilization by CellSoft Semen Parameters

CellSoft parameter	Value in normal sperm	Oocytes fertilized with normal parameter (%)	Oocytes fertilized with abnormal parameter (%)
CT	$\geq 20 \times 10^6/\text{ml}$	57.3 $\pm$ 37.9	34.5 $\pm$ 36.7*
%M	$\geq 30$	59.7 $\pm$ 36.9	40.7 $\pm$ 38.9*
LIN	$\geq 5.5$	65.4 $\pm$ 35.0	42.7 $\pm$ 38.6**
CLV	$\geq 40 \mu\text{m}/\text{sec}$	60.3 $\pm$ 37.1	40.6 $\pm$ 38.2*
ALHmn	$\geq 1.8 \mu\text{m}$	58.6 $\pm$ 38.5	52.0 $\pm$ 37.0
BCF	$\geq 13 \text{ Hz}$	58.2 $\pm$ 38.0	32.1 $\pm$ 29.8
MD	$\geq 8$	57.7 $\pm$ 37.7	36.2 $\pm$ 37.9*
SLV	$\geq 22 \mu\text{m}/\text{sec}$	62.1 $\pm$ 36.6	41.9 $\pm$ 38.4**
SLVMD	$\geq 10$	70.5 $\pm$ 34.6	35.0 $\pm$ 34.2***

\* $p < 0.05$  compared to normal.

\*\* $p < 0.01$  compared to normal.

\*\*\* $p < 0.001$  compared to normal.

There was considerable overlap in the values of all variables, as indicated by the large standard deviations, and individual variables correlated poorly with percentage fertilization. Multiple regression with a stepwise approach, performed with the variables with significant  $p$  values in the ANOVA, yielded a model for percentage fertilization incorporating SLV and MD. The correlation coefficient for this model was only 0.418. The addition of CellSoft variables did not improve the correlation coefficient.

Because of the lack of a good quantitative relationship among any of the CellSoft semen variables examined and percentage oocyte fertilization, the data were analyzed categorically for each of these variables. Semen parameters were divided into two groups according to whether or not a particular variable was greater or less than a normal threshold (Table 2). When so divided, there were significant differences in percentage oocyte fertilization for count, linearity, and velocity. Motility was not significant unless a normal of 30% or more was used. Fertilization was not significantly different when semen parameters were divided into groups on the basis of ALH, morphology, or BCF.

In examining these relationships further, the frequency distribution of oocyte fertilization groups was evaluated for normal and abnormal semen parameters divided according to the previously mentioned criteria. A significantly greater frequency of good and adequate oocyte fertilization occurred in groups 1 and 2 compared to groups 3 and 4. These differences were found in motility (73/101 compared to 9/22), linearity (60/75 compared to 22/48), SLV (59/77 compared to 23/46), and SLVMD (53/65 compared to 29/58) (Table 3). The frequency distribution of oocyte fertilization was not significantly different when semen parameters were sorted by count or velocity. There was a trend toward a difference in MD, but the low threshold was set at a minimum of 8.

Of the 69 couples involved in this study, 15 achieved a pregnancy. In group 1, six pregnancies occurred. Four of the six were normal pregnancies, and all four had a SLVMD value of more than 10. In group 2 six pregnancies were achieved, four of which were normal and two of which spontaneously terminated. In group 3 three pregnancies were achieved; one was normal, and two spontaneously terminated. The two cases of lost pregnancies each had a SLVMD value less than the normal range of 10.

## DISCUSSION

In this study, the largest differences in semen parameters were seen between those patients with 33% oocyte fertilization or less and those having 75% fertilization or more (groups 3 and 1, respectively). The parameters with the most striking difference between these two groups were linearity and SLV as well as SLVMD. SLV has been reported to distinguish between fertile and infertile men [4]. SLVMD was highly associated with an increased frequency of good and adequate oocyte fertilization. These parameters reinforce the importance of having sufficient numbers of motile sperm with linear SLV to achieve fertilization. These are the cells that are most likely to traverse the cumulus-corona complex to interact with the zona pellucida of the oocyte and to initiate the fertilization process.

The SLVMD calculation links linearity, velocity, count and motility into one accurate measurement with significant consequences. This parameter is an important new factor to be considered in the CSA male andrologic analysis. SLVMD is another measurement to add to the battery of semen analysis tests that can help predict the fertilizing potential of an individual's ejaculate. SLVMD is measured in a sample of the original ejaculate, not a washed sample. This makes the measurement easy to perform because the sample requires little additional manipulation. It also allows the clinic to predict with CSA the potential outcome of an IVF cycle.

TABLE 3 Distribution of Oocyte Fertilization by CellSoft Semen Parameter

Parameter	Oocyte fertilization (%)				<i>p</i>
	≥ 75	34-74	1-33	0	
CT					
< 20 × 10 <sup>6</sup> /ml	3	5	1	6	NS <sup>a</sup>
≥ 20 × 10 <sup>6</sup> /ml	46	28	15	19	
%M					
< 30	4	5	5	8	<0.05
≥ 30	45	28	11	17	
LIN					
< 5.5	12	10	11	15	<0.01
≥ 5.5	37	23	5	10	
CLV					
> 40 μm/sec	4	5	5	12	NS
≥ 40 μm/sec	45	28	11	13	
MD					
< 8	4	5	2	8	NS
≥ 8	45	28	15	17	
SLV					
< 22 μm/sec	12	11	10	13	<0.01
≥ 22 μm/sec	37	22	6	12	
SLVMD					
< 10	12	17	11	19	<0.001
≥ 10	37	16	5	6	

<sup>a</sup>NS, not significant.

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