Journal of Andrology, Vol. 21, No. 1, January/February 2000

Semen Quality and Human Fertility: A Prospective Study With Healthy Couples

MICHAEL J. ZINAMAN,* CHARLES C. BROWN,†‡ SHERRY G. SELEVAN,§ AND ERIC D. CLEGG§

*From the *Department of Obstetrics and Gynecology, Georgetown University Medical Center, Washington, DC; the †Division of Cancer Prevention and Control, National Cancer Institute, Bethesda, Maryland; and the §National Center for Environmental Assessment, US Environmental Protection Agency, Washington, DC.*

ABSTRACT: Measures of semen quality are used as surrogate measures of male fertility in clinical andrology, reproductive toxicology, epidemiology, and risk assessment. However, only limited data are available to relate those measures to fertility. This prospective study with 210 reproductive-age couples was conducted to provide information on the value of semen quality measures for predicting human male fertility potential and for development of models to estimate the effects of changes in semen quality on fertility in a given population for risk assessment. Couples without known risk factors for infertility and who had discontinued contraception to have a child were accepted. The study followed each couple for up to 12 menstrual cycles while they attempted to conceive and evaluated semen quality measures from multiple ejaculates per man with known abstinence intervals. For each cycle, the day of ovulation was predicted, and the couple was advised to have intercourse multiple times on that day and on the days around it. Among the demographic variables assessed, parity, contraception status prior to entering the study, male education level, and male smoking were associated significantly with 12-cycle pregnancy rate. Several semen quality measures also were associated significantly with pregnancy rate, with percentage morphologically normal sperm by strict criteria and measures involving total number of sperm showing particularly strong associations. Localized regression-smoothing plots of semen quality data against proportion of couples pregnant suggested levels below which fertility declines for several semen quality measures. These results have applications in both clinical andrology and in assessment of risk to male fecundity from environmental or pharmaceutical exposures. In particular, they contribute information on behavior of fertility with varying semen quality and can allow development of models to predict effects on fertility in populations from decrements in semen quality.

Key words: Male fertility, sperm parameters.

J Androl 2000;21:145–153

Measures of semen quality are used as surrogate measures of male fecundity in clinical andrology, reproductive toxicology, epidemiology, and risk assessment. In clinical settings, semen quality is examined routinely, and is considered an important component of the evaluation of couples presenting for fertility evaluation (Rowe et al, 1993). However, the implications of even moderate alterations in semen quality are poorly understood, and only limited data are available for relating these measures to the likelihood of achieving pregnancy (Meistrich and Brown, 1983). Risks to men's fertility from decrements in semen quality induced by environmental or therapeutic agents need to be assessed for reproductive toxicity. Adequate protection of human reproductive health requires that potential reductions in fertility, and not just infertility, be discernible (Zenick et al, 1994; US EPA, 1996).

At present, methods used for human semen evaluations vary substantially, ranging from those recommended by the World Health Organization (WHO, 1992) to detailed computer-assisted analysis of sperm motility, morphometric evaluation of sperm shape, and various physical and biochemical analyses (Boyle et al, 1992; Barratt et al, 1993; Comhaire, 1993; MacLeod and Irvine, 1995). These measures lack sufficient corresponding information for accurate characterization of their relationships with fertility. Most existing data have been derived primarily from infertile or subfertile populations, from semen donors, or from men undergoing vasectomy (MacLeod and Gold, 1951; Nelson and Bunge, 1974; Smith and Steinberger, 1977; Zukerman et al, 1977). Recently, Bonde et al (1998) investigated associations between semen quality measures and pregnancy within 6 months for 430 men without children who were in a relationship in which contraception was discontinued. Abstinence interval was known and controlled in the analyses. Semen quality measures included sperm concentration, total number of

Supported by a cooperative agreement from the US Environmental Protection Agency, Washington, DC.

The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the US Environmental Protection Agency.

Correspondence to: Michael J. Zinaman, MD, Department of Obstetrics and Gynecology, Loyola University Medical Center, 2160 South First Ave, Maywood, IL 60153.

‡Present address: 14017 Castaway Dr, Rockville, MD 20853.

Received for publication March 4, 1999; accepted for publication March 4, 1999.

Table 1. *Description of the study group*

Characteristic		Total Group (n = 200)	Pregnant (n = 156)	Not Pregnant (n = 44)	*P**
Age (y)					
Female	Mean (SD)	30.6 (3.3)	30.6 (3.3)	30.4 (3.1)	.52
	Median	30	31	30	
	Range	23–37	23–37	23–37	
Male	Mean (SD)	32.3 (4.8)	32.3 (4.4)	32.2 (5.9)	1.00
	Median	32	32	32.5	
	Range	20–51	24–47	20–51	
Educational attainment†					
Female	HS graduate	5%	4.5%	6.8%	.39
	Some college	14%	16.0%	6.8%	
	College degree	46%	46.2%	45.5%	
	Advanced training	35%	33.3%	30.9%	
Male	Some HS	0.5%	0.6%	—	.02
	HS graduate	3.5%	1.9%	9.1%	
	Some college	12.5%	13.5%	9.0%	
	College degree	37.5%	41.7%	22.7%	
	Advanced training	46.0%	42.3%	59.1%	
Race					
Female	White	93.5%	93.6%	93.2%	.92
	African American	4.0%	3.2%	6.8%	
	Asian	2.0%	2.6%	—	
	Other	0.5%	0.6%	—	
Male	White	95.5%	95.5%	95.5%	.99
	African American	3.0%	3.2%	2.3%	
	Asian	—	—	—	
	Other	1.5%	1.3%	2.3%	
Contraception practiced at enrollment‡		41.5%	45.5%	27.3%	.03
Number of cycles since contraception stopped‡§	Mean (SD)	2.2 (1.1)	2.2 (1.0)	2.3 (1.2)	.89
	Median	2	2	2	
	Range	1–5	1–5	1–5	
% With prior pregnancy, this partner (women's reports)					
Female		31.5%	37.2%	11.4%	.001
No. pregnancies§	Mean (SD)	1.4 (0.8)	1.5 (0.8)	1.2 (0.4)	.22
	Median	1	1	1	
	Range	1–4	1–4	1–2	
% With prior pregnancy, all partners (women's reports)					
Female		47.5%	51.9%	31.8%	.02
No. pregnancies§	Mean (SD)	1.6 (0.8)	1.6 (0.9)	1.3 (0.5)	.15
	Median	1	1	1	
	Range	1–4	1–4	1–2	
Male		42%	45.5%	29.6%	.06
Cigarette smoking status at enrollment					
Female	% Smoked	3.5%	3.8%	2.3%	.62
No. cigarettes/d in smokers§	Mean (SD)	7.3 (6.3)	7.8 (6.7)	n = 1	.80
	Median	6	6.5	4	
	Range	1–20	1–20		
Male	% Smoked	6.0%	3.8%	13.6%	.02
No. cigarettes/d in smokers§	Mean (SD)	10.8 (7.1)	6.8 (6.2)	14.2 (6.3)	.12
	Median	12	5	15	
	Range	1–20	1–16	3–20	

Table 1. *Continued*

Characteristic		Total Group (n = 200)	Pregnant (n = 156)	Not Pregnant (n = 44)	*P**
Drank alcohol in preceding month					
Female	% Drank	77.9%	78.1%	77.3%	.97
Drinks/mo§	Mean (SD)	7.5 (7.5)	7.3 (6.9)	8.4 (9.6)	.51
	Median	5	5	5	
	Range	1–48	1–32	1–48	
Male	% Drank	81.4%	81.9%	79.5%	.78
Drinks/mo§	Mean (SD)	16.8 (6.3)	15.8 (14.6)	20.4 (21.0)	.39
	Median	12	12	12	
	Range	1–80	1–80	1–80	

* Tests of differences between those with clinical pregnancies within 12 cycles and those not pregnant using chi-square test or trend analysis (proc freq, SAS 6.12) for categoric data or Wilcoxon test (proc npar1way, SAS 6.12) for continuous data.

† HS indicates high school.

‡ Proportion of couples practicing contraception at enrollment followed by the number of cycles since terminating contraception for the remainder. Data are based on women's responses.

§ Means and other values are calculated for those with >0 responses.

sperm, percentage motile sperm, and sperm morphology using the traditional WHO criteria (WHO, 1992). Sperm concentration and sperm morphology were most strongly associated with pregnancy.

In this report, a prospective study provides additional information to characterize relationships between semen parameters and male fertility. These data provide information on the value of semen quality measures to predict fertility in populations, and can be used to estimate decrements in fertility for risk assessment.

Methods

Study Description

This longitudinal study was conducted with volunteers in the greater Washington, DC, metropolitan area. Many of the study design details have been reported previously (Beltsos et al, 1996; Zinaman et al, 1996). Briefly, the study cohort consisted of 210 couples of reproductive age who were discontinuing contraception to achieve pregnancy. Recruitment was principally by radio and newspaper advertising and local physician referral. Initial screening, conducted by telephone, required the women to be between the ages of 21 and 37 and the men to be between 21 and 60. The women were required to have a history of regular menstrual cycles (intervals between 25 and 33 days) and no other identified risk factors for infertility at study entry. The men were required not to have had a history of evaluation or treatment for infertility. All couples who fit those requirements went on to the next stage of evaluation, in which each partner was interviewed separately, using a detailed questionnaire on reproductive and health histories, lifestyle, and occupational and environmental exposures. All screening and intake interviews were administered by the same person, a certified infertility nurse, who served as the study coordinator.

Each couple was followed for up to 12 complete menstrual cycles while they attempted to conceive. During the first 3 cycles (Phase 1), the women collected daily early-morning urine samples and maintained menstrual diaries recording intercourse and medications. Postcoital tests were done to confirm sexual activity at midcycle. If a woman was >1 day late for her anticipated menses, clinical pregnancy was confirmed by routine serum hCG pregnancy testing at the clinic. The men provided a semen sample on site early in each menstrual cycle after a requested 36 to 48 hours of abstinence. Actual abstinence was reported. If women tested positive for pregnancy in either of the first 2 cycles, another semen sample was requested shortly thereafter. Azoospermic men were informed of their condition, and all chose to leave the study to seek treatment. For each cycle, the day of ovulation was predicted by the infertility nurse on the basis of the preceding cycle length, and the couple was advised to have intercourse at least 3 times in the ovulatory period, including on the predicted day of ovulation.

In Phase 2, the couples who had not achieved pregnancy earlier continued to attempt pregnancy for up to 9 additional menstrual cycles. Completion of menstrual and intercourse diaries and of monthly phone contacts were continued. Similarly, if menstruation was delayed, women were given routine serum hCG testing for confirmation of pregnancy.

Semen Analysis

Semen specimens were collected at the study site and transferred immediately to the laboratory, where they were kept at 37°C until liquefied. Sperm concentration was determined by counting 2 sides of a hemacytometer. If a difference of >10% between the 2 sides was noted, a second aliquot was counted and all 4 sides were averaged. Semen volume was measured in a graduated pipette. Motility was defined as the proportion of sperm that were progressively motile at 37°C, and it was measured with a Makler chamber. Men were considered azoospermic if no sperm were seen or if sperm were seen only after centrifugation of the semen sample.

Dried semen smears were treated with a modified Papanicolaou stain (MacLeod and Irvine, 1995). Sperm morphology was evaluated by a single examiner using strict criteria (Menkveld et

al, 1990). Sperm were classified into 1 of 3 groups: normal, slightly amorphic, and amorphic. Twenty slides repeatedly were examined blind during the study as internal controls, giving a coefficient of variation of 7% for the repeated measures.

Statistical Methods

Two types of measures, potentially of prognostic utility, were considered: 1) demographic and other personal characteristics including age of the male, age of the female, smoking, alcohol consumption, number of previous pregnancies, and whether the couple had been practicing contraception just before entering the study and 2) semen quality measures including volume, sperm concentration, number of sperm (sperm count), percentage and number of motile sperm, percentage and number of morphologically normal sperm, and percentage amorphic sperm. Because a preliminary categorical analysis showed a leveling-off in pregnancy rates at higher semen levels, each semen measure was examined both as a linear and as a $\log_e$-transformed predictor. The above measures were compared in couples with and without pregnancies in the 12-month period using a chi-square or Wilcoxon test (Snedecor and Cochran, 1969), depending on the scaling of the measure. Statistical significance was defined as $P < .05$.

Univariate and multivariate relationships between these potential prognostic variables and the number of cycles required to achieve pregnancy (up to 12 cycles) were evaluated by Cox discrete time period regression (Cox, 1958).

Graphs demonstrating the relationship of the proportion of couples achieving pregnancy within 12 cycles versus changes in semen quality measures were produced with a univariate logistic regression model using a least squared local regression method (Cleveland, 1979; Harrell et al, 1996) to estimate the true predictor-response relationship (S-Plus Version 3.2, Mathsoft Inc., Seattle, Wash).

Results

Demographic Characteristics

Two hundred ten couples volunteered for the study. Ten couples were excluded from the analysis: 6 for azoospermia (all left the study to seek treatment), 2 for noncompliance with the study protocol, 1 for bilateral uterine tubal occlusion detected shortly after acceptance, and 1 because they dropped out. The remaining 200 couples represented here (Table 1) tended to be white/non-Hispanic (>90%) and to be of higher socioeconomic status, as demonstrated by education and income levels (Zinaman et al, 1996). At enrollment, the males' ages ranged from 20 to 51 (mean = 32.3), whereas the females' ages ranged from 23 to 37 (mean = 30.6). Some differences were observed between those couples achieving a clinical pregnancy within 12 cycles and those who did not. Statistically significant differences were found for male education (42.3% of couples who achieved pregnancy had advanced training, compared with 59.1% of couples who did not achieve pregnancy), use of contraception at enrollment (45.5% versus 27.3%), and percentage of women with any prior pregnancies with current partner and with all partners (37.2% versus 11.4% for current partner and 51.9% versus 31.8% with all partners).

No relationships were observed for either partner's age with time to pregnancy, while contraception status (practicing versus not practicing contraception at enrollment) and number of prior pregnancies were strongly related to time to pregnancy. Only 3.5% of the females and 6.0% of the males smoked at enrollment. The proportion of men who smoked was significantly higher in the couples without clinical pregnancies at the end of the study (3.8% in those with pregnancies versus 13.6% in those without). Most men (81.4%) and women (77.9%) reported some alcohol consumption at enrollment. Drinking patterns (proportion of drinkers and number of drinks per month for each drinker) were not significantly different between the pregnant and not-pregnant groups.

Semen Quality Measures and Pregnancy

Semen characteristics for couples with a clinical pregnancy within 12 cycles were compared with those without (Table 2; Figure 1). The values for each man were the average of his semen samples (mean = 2.5, range = 2–4). Overall, semen volumes ranged from 0.5 to 6.5 mL (mean, 2.8 mL). Sperm concentrations ranged from 3×10^6 to 261×10^6 (mean, 65.5×10^6), and sperm motility values ranged from 17 to 78% (mean, 56.3%). Proportion of sperm with normal morphology ranged from 0–20.5% (mean, 5.8%) and proportion of amorphic sperm ranged from 65.1–99.5% (mean, 89.0%). Total numbers of motile sperm and morphologically normal sperm ranged from 1.5–436×10^6 (mean, 99.6×10^6) and 0–88.6×10^6 (mean, 10.9×10^6), respectively.

The cumulative percentage graphs (Figure 1) show differences in distributions of the semen quality measures for men whose partners had clinically-identified pregnancies within 12 cycles and for men whose partners did not. For 7 of the semen quality measures, the distributions for men with partner pregnancies were shifted to the right of those without, showing consistently higher total numbers of sperm, sperm concentration, number and percentage motile, number and percentage morphologically normal, and volume. The distribution of percentage amorphic sperm for men with partner pregnancies was shifted to the left (lower percentages of amorphic sperm). Of these measures, percentage motile (57.0% for those with pregnancies versus 52.6% for those without), percentage morphologically normal (6.2% versus 4.1%), percentage amorphic sperm (88.3% versus 91.3%), average total numbers of sperm (178 million versus 147 million), motile sperm (104 million versus 83.6 million), and morphologically normal sperm (12.4 million versus 5.7 million) were significantly different in the two groups (Table

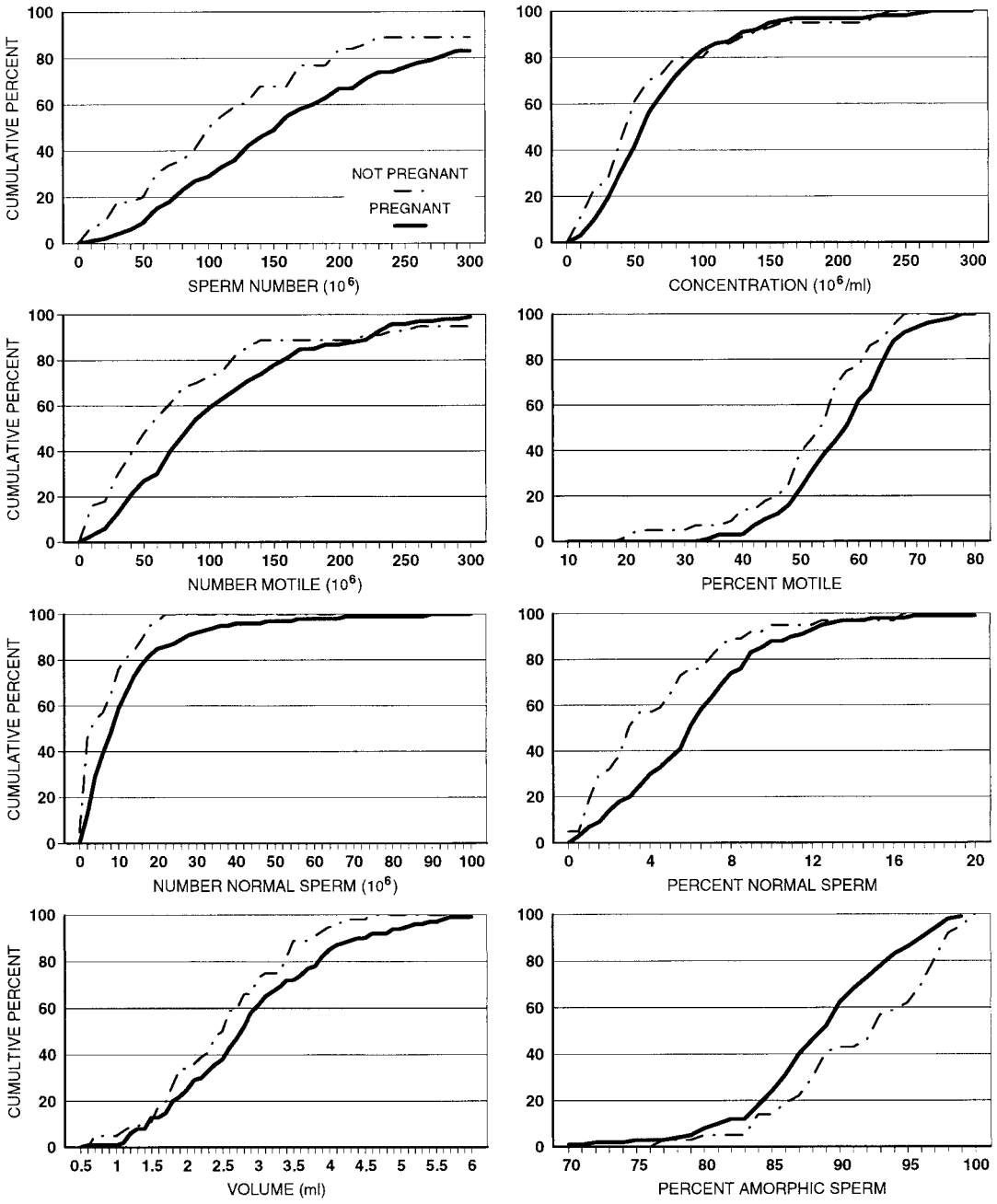

Figure 1. Distributions of averages across ejaculates of number of sperm, sperm concentration, number of motile sperm, percentage motile sperm, number of morphologically normal sperm, percentage morphologically normal sperm, semen volume, and percentage amorphic sperm.

2). Semen volume (P = .10) and sperm concentration (P = .08) were not significantly different.

Cox discrete time period regression analyses examined the relationships of the semen quality measures with time to clinically recognized pregnancy over the 12 cycles of data collection. Age, parity, contraception status, and abstinence interval were first considered for inclusion in these analyses. Of those, only parity and contraception status required control in the final analyses (Table 3). Because 86% of the abstinence intervals were clustered tightly between 30 and 50 hours, the relationship of abstinence interval with time to pregnancy was not significant. With the exceptions of semen volume and percentage amorphic sperm, the relationships were stronger when modeled as $\log_e$-transformed variables. Sperm concentration, number of sperm, number of motile sperm, percentage morphologically normal, number morphologically normal, and percentage amorphic were all significantly associated with 12-cycle time to pregnancy when modeled as $\log_e$-transformed variables.

Table 2. *Semen analysis summary statistics*

Variable	Overall*	Pregnant	Not pregnant	*P*†
Number of men	200	156	44	
Volume (mL)	2.8 (1.1) 2.7 0.5–6.5	2.9 (1.1) 2.8 0.5–6.5	2.5 (0.9) 2.5 0.7–4.6	.10
Sperm concentration (10^6/mL)	65.5 (49.1) 53.9 3.0–261	67.2 (47.8) 56.1 3.2–261	59.5 (53.7) 43.1 3.0–234	.08
Number of sperm (10^6)	172 (131) 139 7.9–732	178 (122) 151 9.0–684	147 (158) 105 7.9–732	.01
% Motile sperm	56.0 (9.7) 56.3 17.4–77.6	57.0 (9.0) 57.9 35.0–77.6	52.6 (11.2) 53.7 17.4–70.7	.02
Number of motile sperm (10^6)	99.6 (79.5) 79.4 1.5–436	104 (74.5) 86.0 4.3–424	83.6 (94.1) 53.6 1.5–436	.006
% Normal sperm[3]	5.8 (3.8) 5.6 0.0–20.5	6.2 (3.7) 5.9 0.2–20.5	4.1 (3.5) 2.9 0.0–16.4	.0002
Number of normal sperm[3] (10^6)	10.9 (13.0) 7.7 0.0–88.6	12.4 (14.1) 8.6 8.8–88.6	5.7 (6.0) 3.0 0.0–20.9	.0002
% Amorphic sperm[3]	89.0 (6.0) 89.1 65.1–99.5	88.3 (5.9) 88.8 65.1–99.1	91.3 (5.9) 92.3 76.9–99.5	.004

* Values in columns represent mean (SD); median; and range for each semen variable.
† Tests of differences in those with clinical pregnancies within 12 cycles and those not pregnant using Wilcoxon test for continuous data.
‡ n = 198, 154, and 44 for overall, pregnant, and not pregnant categories.

When all of the demographic and semen quality variables were evaluated in 1 multivariable Cox regression analysis, 4 variables were significant (Table 4): 1) number of previous pregnancies, 2) contraception status prior to study entry, 3) $\log_e$ percentage morphologically normal sperm, and 4) $\log_e$ number of sperm. The other variables measured during this study did not add substantially to those factors. The contribution of number of motile sperm was a reasonable replacement for total number of sperm (regression coefficient = 0.24, $P = .03$).

A LOESS local-smoothing method was applied to logistic regression to nonparametrically visualize the relations of the semen quality measures to the probability of pregnancy within 12 cycles (Figure 2a through f). To achieve acceptably smooth estimated curves, a smoothness parameter of 3/4 was used. A decline in pregnancy

Table 3. *Cox analysis results relating semen variables with time to pregnancy**

	Untransformed					
Semen Variable	Estimated Regression Coefficient	Standard Error	*P* Value	Estimated Regression Coefficient	Standard Error	*P* Value
Volume (mL)	0.114	0.107	.29	0.237	0.288	.41
Sperm concentration (10^6/mL)	0.0037	0.0018	.04	0.299	0.107	.005
Number total sperm (10^6)	0.0016	0.0007	.02	0.348	0.113	.002
% Motile sperm	1.35	0.98	.17	0.791	0.452	.08
Number motile sperm (10^6)	0.0023	0.0011	.05	0.308	0.099	.002
% Morphologically normal sperm	7.61	2.56	.003	0.341	0.104	.001
Number morphologically normal sperm (10^6)	0.0201	0.0076	.009	0.304	0.072	<.0001
% Amorphic sperm	−3.70	1.59	.02	−3.03	1.30	.02

* Controlling for parity and contraception status.

Table 4. *Final multivariate Cox regression model*

Variable	Estimated Regression Coefficient	Standard Error	*P*-value
Number of previous pregnancies	0.48	0.11	<.0001
Contraception status (yes)	0.58	0.19	.002
Log_e % normal sperm	0.29	0.11	.006
Log_e number of sperm (10^6)	0.30	0.12	.01

rate was observed below approximately 30×10^6 sperm/mL for sperm concentration. For total number of sperm, a decline was suggested below approximately 80×10^6 sperm. Number of motile sperm paralleled closely the pattern of total number of sperm. Percentage morphologically normal sperm suggested a decline below 8%, whereas pregnancy rate appeared to decline when percentage amorphic sperm exceeded 90%. Number of morphologically normal sperm suggested a decline in pregnancy rate below 4×10^6.

Discussion

This prospective longitudinal study provides new data for quantifying the relationships between semen quality measures and male fertility. The study population consisted of volunteers and was predominantly white and of high socioeconomic status (SES). At this time, there are no data to suggest that the relationships between semen quality parameters and fertility would vary substantially depending on race or SES.

This study incorporated experimental design factors that collectively have not previously been brought to bear. Included in this study were the use of couples who had discontinued contraception to actively attempt pregnancy and strict control of factors affecting conception. Factors were controlled that could contribute to reduced fertility of the female partners, including age, prior medical history, and menstrual cycle characteristics. In addition, the study design controlled factors such as timing of intercourse around ovulation, length of abstinence prior to semen sample collection and intercourse, collection of semen samples close to the time of conception for the large majority of couples, and clinical identification of pregnancy at about the same early gestational age. Combined, those factors served to maximize the chance for conception in each cycle and to allow comparisons of consistent information. Those considerations, plus the ability to use Cox time-to-event analyses and adjust for other contributing variables, have provided information not available previously.

Four demographic characteristics influenced fertility as measured by 12-cycle time to pregnancy. Those included parity, contraception at entrance to the study, male education, and smoking status. Of those, parity was by far the strongest. The influence of prior contraception in this study has been discussed previously (Zinaman et al, 1996), with number of cycles of no contraception use prior to study entry (1 to 5 cycles) being less predictive than whether contraception was practiced right up to study entry. As this study group contained few smokers, that factor did not contribute to the overall analysis. No differences were observed with alcohol consumption. Age of either partner was not related to fertility, probably because of the rigorous screening for factors indicative of reduced female fecundity. Women above age 37 were not accepted, and few men were above age 40.

The distributions of the semen quality measures had wide ranges even with tight control of abstinence intervals. However, the availability of multiple ejaculates from each man and the use of adjusted time-to-conception regression approaches allowed the identification of significant associations between many of those measures and fertility. When the semen quality measures were tested in multivariable models with parity and contraception status at entry, percentage normal sperm by strict criteria and total number of sperm per ejaculate combined to provide predictive capability that could not be improved by adding any of the other semen measures. However, in the absence of data on percentage normal sperm by strict criteria, other measures could contribute meaningfully to the regression model. The utility of the various permutations of these measures in multivariate models is being examined and will be published separately. These data will allow development of models that improve prediction of fertility and estimate decrements in fertility from effects on semen quality (Meistrich and Brown, 1983; Zinaman and Katz, 1997). The results potentially have applications in both clinical andrology and in assessment of risk to male fecundity in populations from environmental or pharmaceutical exposures.

A recent report by Bonde et al (1998) has also found significant relationships between certain measures of semen quality and fertility with men aged 20 to 35 who lived with a partner and had no children. Significant relationships with pregnancy were found for sperm concentration, number of sperm, and sperm morphology using traditional WHO evaluation. Sperm morphology was significantly associated only when sperm concentration was greater than 40×10^6 per mL.

Currently, the lower limits for WHO normal values for human semen variables (WHO, 1992) are 2.0 mL for volume, 20×10^6 sperm per mL for concentration, 40×10^6 for number of sperm per ejaculate, 50% with progressive motility, and 30% with normal morphology

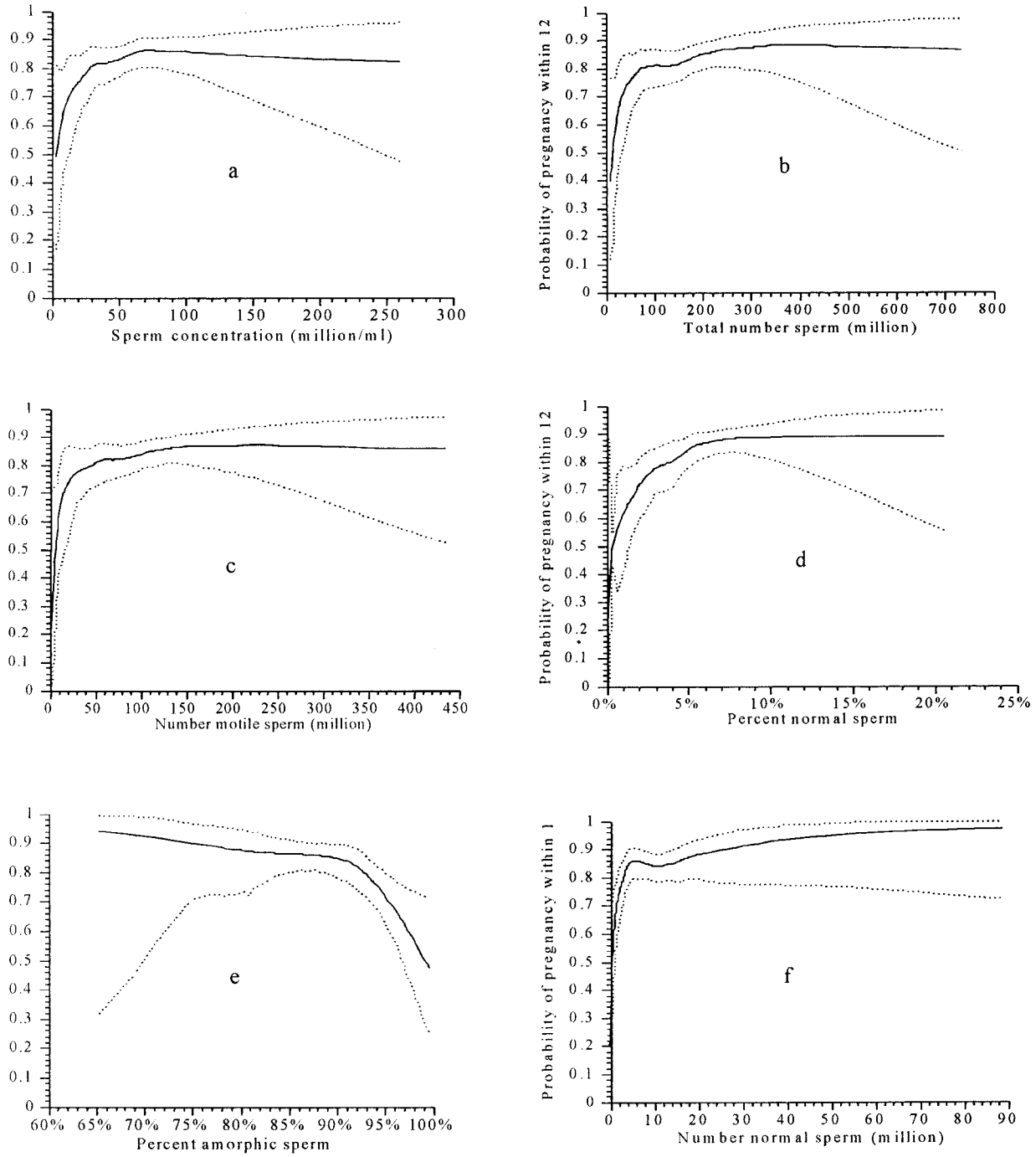

Figure 2. Smoothed patterns showing behavior of pregnancy rates with increasing levels in semen of sperm concentration (a), total number of sperm (b), number of motile sperm (c), percentage morphologically normal sperm (d), percentage amorphic sperm (e), and number of morphologically normal sperm (f). Solid line represents smoothed values using LOESS local regression approach. Dotted lines represent 95% confidence intervals of the estimates.

(nonstrict). No limit is given for percentage normal morphology by strict criteria. The results from this study, visually examined using LOESS local regression smoothing, suggest that the 12-cycle fertility rate began to decline at higher levels for equivalent measures. Finally, the results from this study add information to the existing data for selecting the limits used in the evaluation of fertility. Reexamination of the criteria to be used is suggested, with emphasis placed on sperm morphology by strict criteria.

References

Barratt CLR, Tomlinson MJ, Cooke ID. Prognostic significance of computerized motility analysis for in vivo fertility. *Fertil Steril.* 1993;60: 520–525.

Beltsos AN, Fisher SG, Uhler ML, Clegg ED, Zinaman MJ. The relationship of the postcoital test and semen characteristics to pregnancy rates in 200 presumed fertile couples. *Int J Fertil.* 1996;41:405–411.

Bonde JPE, Ernst E, Jensen TK, Hjollund NHI, Kolstad H, Henriksen TB, Scheike T, Giwercman A, Olsen J, Skakkebæk NE. Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. *Lancet.* 1998;352:1172–1177.

Boyle CA, Khoury MJ, Katz DF, Annest JL, Kresnow M, DeStefano F, Schrader S. The relation of computer-based measures of sperm morphology and motility to male fertility. *Epidemiology.* 1992;3:239–246.

Cleveland WS. Robust locally weighted regression and smoothing scatterplots. *J Am Stat Assoc.* 1979;74:829–836.

Comhaire FH. Methods to evaluate reproductive health of the human male. *Reprod Toxicol.* 1993;7:39–46.

Cox DR. The regression analysis of binary sequences (with discussion). *J R Stat Soc B.* 1958;20:215–242.

Harrell FE, Lee KL, Mark DB. Tutorial in biostatistics multivariate prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med.* 1996; 15:361–387.

MacLeod IC, Irvine DS. The predictive value of computer assisted semen analysis in the context of donor insemination programme. *Hum Reprod.* 1995;10:580–586.

MacLeod J, Gold RZ. The male factor in fertility and infertility. II. Spermatozoon counts in 1000 men of known fertility and 1000 cases of infertility marriage. *J Urol.* 1951;66:436–449.

Meistrich ML, Brown CC. Estimation of the increased risk of human infertility from alterations in semen characteristics. *Fertil Steril.* 1983; 40:220–230.

Menkveld R, Stander FSH, Kotze TJ, Kruger TF, van Zyl JA. The evaluation of morphological characteristics of human spermatozoa according to stricter criteria. *Hum Reprod.* 1990;5:586–592.

Nelson CM, Bunge R. Semen analysis: evidence for changing parameters of male fertility potential. *Fertil Steril.* 1974;25:503–507.

Rowe PJ, Comhaire FH, Hargreave TB, Mellows HJ. *WHO Manual for the Standardized Investigation and Diagnosis of the Infertile Couple.* Cambridge, United Kingdom: Cambridge University Press; 1993.

Smith KD, Steinberger E. What is oligospermia? In: Troen P, Nankin HR, eds. *The Testis in Normal and Infertile Men.* New York: Raven Press; 1977:489–503.

Snedecor GW, Cochran WG. *Statistical Methods.* Ames: Iowa State University Press; 1969.

US Environmental Protection Agency. Guidelines for reproductive toxicity risk assessment. *Fed Reg.* 1996;61(212):56;274–322.

World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interactions.* 3rd ed. Cambridge, United Kingdom: Cambridge University Press; 1992.

Zenick H, Clegg ED, Perreault SD, Klinefelter GR, Gray LE. Assessment of male reproductive toxicity: a risk assessment approach. In: Hayes AW, ed. *Principles and Methods of Toxicology.* 3rd ed. New York: Raven Press; 1994:937–988.

Zinaman MJ, Clegg ED, Brown CC, O'Connor J, Selevan SG. Estimates of human fertility and pregnancy loss. *Fertil Steril.* 1996;65:503–509.

Zinaman MJ, Katz DF. Incidence and implications of altered semen quality on family planning. *Adv Contracept.* 1997;13:123–128.

Zukerman Z, Rodriguez-Rigau LJ, Smith KD, Steinberger E. Frequency distribution of sperm counts in fertile and infertile males. *Fertil Steril.* 1977;28:1310–1313.