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Semen Quality and Time-to-Pregnancy, the LIFE Study

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Abstract

Objective—To assess semen parameters and couple fecundity as measured by time-to-pregnancy (TTP).

Design—Observational prospective cohort with longitudinal measurement of TTP.

Setting—16 Michigan/Texas counties

Participants—501 couples discontinuing contraception were followed for one year while trying to conceive; 473 (94%) men provided one semen sample and 80% provided two samples.

Interventions—None

Main Outcome Measures—Using prospectively measured TTP, fecundability odds ratios (FORs) and 95% confidence intervals (CIs) were estimated for 36 individual semen quality parameters accounting for repeated semen samples, time off contraception, abstinence, enrollment site, and couples' ages, body mass indices (BMI), and serum cotinine concentrations.

Results—In adjusted models, semen quality parameters were associated with significantly shorter TTP as measured by FORs >1: percent motility, strict and traditional morphology, sperm head width, elongation factor, and acrosome area. Significantly longer TTPs or FORs <1 were observed for morphologic categories amorphous and round sperm heads neck/midpiece abnormalities. No semen quality parameters achieved significance when simultaneously modeling all other significant semen parameters and covariates, except for percent coiled tail when adjusting for sperm concentration (FOR=0.99; 95% CI 0.99, 1.00). Male age was consistently associated with reduced couple fecundity (FOR=0.96; 95% CI 0.93–0.99) reflecting a longer TTP across all

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combined models. Female but not male BMI also conferred a longer TTP (FOR=0.98; 95% CI 0.96–0.99).

Conclusions—Several semen measures were significantly associated with TTP when modeled individually, but not jointly and in the context of relevant couple based covariates.

Keywords

epidemiology; fecundity; semen; sperm; time-to-pregnancy

Introduction

Semen quality is believed to be informative about male fecundity, which is defined as men's biologic capacity for reproduction irrespective of pregnancy intentions (1). Semen analysis remains the clinical standard for assessing male fecundity and related impairments including hormone production (2), and key components such as sperm concentration, motility and morphology are reported to be capable of classifying men by fertility potential (3). The World Health Organization publishes reference values for semen parameters as derived from a compilation of largely retrospective research that represents men from various countries (4, 5). However, the predictive value of these reference value parameters has long been debated with no single or set of semen parameters being highly predictive of male fertility (6–8). To this end, authors have noted the need for inclusion of the female partner for etiologic and prediction models (9), along with the development of new biomolecular or methodologic (e.g., sperm energy index, omics) approaches beyond functional tests for assessing and predicting male fecundity (10, 11).

A valuable literature suggests that semen quality is important for pregnancy, though most research relies upon samples of couples seeking infertility treatment or pregnant women (12–14). Noticeably absent are prospective cohorts with the preconception recruitment of couples of unknown fertility status (15). Only two previous studies utilized prospective cohort designs with preconception enrollment of couples in which semen quality was assessed in relation to TTP (16, 17). Unique strengths of this design are the inclusion of all couples trying for pregnancy and not just those achieving a recognized pregnancy, and the ability to assess semen quality in the context of couples' demographics and lifestyle consistent with the couple dependent nature of reproduction. Bonde and colleagues (1998) first assessed the association between semen quality and the probability of pregnancy within six months of observation for 430 Danish couples planning their first pregnancies who were recruited from trade-unions (16). While no significant associations were observed between semen volume and motility, sperm concentration up to 40×10^6/mL and percent normal morphology (10% to 60%) were independently associated with the probability of pregnancy. The findings were corroborated using computer-assisted semen analysis (CASA) techniques (18). Zinaman and colleagues (2000) recruited a convenience sample of 210 U.S. couples who were either discontinuing or off conception for <3 months for purposes of becoming pregnant. Using prospectively measured TTP for up to 12 months, both sperm count and percentage of normal sperm were associated with couple fecundity (17). Statistical analyses for both studies included attention to couples' ages, body mass indices and cigarette smoking histories. At least one study reported no association between semen quality parameters and TTP in either fresh samples or after density gradient separation among fertile men (19). We designed the Longitudinal Investigation of Fertility and the Environment (LIFE) Study to fully explore a spectrum of environmental and lifestyle factors and couple fecundity.

Materials and Methods

Design and Study Population

The LIFE Study utilized a prospective cohort design to enroll 501 couples discontinuing contraception for purposes of becoming pregnant from 16-targeted counties in Michigan and Texas. State-specific sampling frameworks were needed for recruiting purposes, given the absence of uniform registries for identifying couples planning pregnancy. Specifically, we utilized the Texas fishing/hunting license registry and a commercial marketing database for Michigan. Introductory letters were sent to the target population followed by telephone screening with each partner within two weeks. Few differences were observed with regard to socio-demographic or reproductive characteristics by site (20). Given the limited empirical evidence regarding the determinants of couple fecundity from a population perspective, the cohort was designed to be inclusive and only excluded couples with clinically diagnosed infertility. Inclusion criteria were: 1) females aged 18–40 and males aged ≥18 years; 2) in a committed relationship; 3) females' menstrual cycles between 21–42 days; 4) no injectable contraceptives within past year; 5) planning a pregnancy and off contraception for <2 months; and an 6) ability to communicate in English or Spanish.

Data Collection and Operational Definitions

Research assistants traveled to couples' homes and completed baseline in-person interviews that were conducted separately with each partner of the couple, followed by anthropometric assessments to measure height (cm), weight (kg) and hip and waist circumferences (cm) (21). Baseline urine samples were tested to ensure women were not pregnant. Women recorded menstruation and sexual intercourse in daily journals and used the Clearblue® Easy home urinary-based fertility monitor (Swiss Precision Diagnostics, formerly Unipath). This monitor tracks the rise in estrone-3-glucuronide (E_3G), a metabolite of estrogen, and luteinizing hormone (LH), and displays a low, high or peak fertility prompt for timing intercourse relative to ovulation. The monitor is reported to be 99% accurate in detecting the LH surge compared with vaginal ultrasonology (22). We used the monitor date for menses along with daily journal information to establish menstrual cycles and TTP. Women also were trained in the accurate use of the Clearblue® Easy home pregnancy test, which is sensitive for detecting 25 mIU/L of human chorionic gonadotropin (hCG). Each partner of the couple was remunerated $75 for complete participation. Human subjects' approval was received from all collaborating institutions, and all study participants gave informed consent before data collection.

Semen Collection

Male partners were asked to collect a baseline sample and another the following month irrespective of pregnancy status. Men collected samples via masturbation without the use of any lubricant following two days of abstinence using home collection kits that comprised an insulated shipping container (Hamilton Research, Beverly, MA) for maintaining sperm integrity (23), a glass specimen jar with an attached temperature data logger (I-Button, Maxim Integrated, Jan Jose, CA), a sperm migration straw filled with hyaluronic acid and plugged at one end, and packing materials (Vitrotubes #3520, VitroCom Inc., Mt. Lakes, NJ) (24). Couples were instructed to freeze insulation packs, refrigerate migration straws and to keep the remainder of the kit at room temperature. After collection, the male placed the open end of the migration straw into the semen as a global marker of motility at specimen collection, and recorded the date of last ejaculation and any spillage on labels. Semen was shipped via prepaid overnight service, and analyses were conducted the next day consistent with the survival of some sperm past 24 hours and the integrity of chromatin structure (25, 26).

Andrology Analysis

All semen samples were received at the National Institute for Occupational Safety and Health's andrology laboratory. An aliquot was placed in a 20μm deep chamber slide (Leja, Luzemestraat, Netherlands), and sperm motility was assessed using the HTM-IVOS (Hamilton Thorne, Beverly, MA) computer assisted semen analysis system (CASA). Sperm concentration was also measured using the IVOS system and the IDENT™ stain. Microscope slides were prepared for sperm morphometry and morphology assessment. An aliquot of the whole semen was diluted in TNE buffer with glycerol and frozen for the sperm chromatin stability assay (SCSA®) analysis (27). Sperm viability was determined by hypo-osmotic swelling (HOS assay). The sperm migration straw was removed from the semen and viewed under the microscope. The distance traveled by the vanguard sperm was measured to the nearest mm. Sperm morphometry was conducted using the IVOS METRIX system. A contract laboratory (Fertility Solutions Inc, Cleveland, OH) assessed sperm morphology on the prepared slides from the first specimen. Both the traditional with differential classification and strict morphology assessments were performed on each slide (28, 29). SCSA® was measured on the first specimen according to the methods of Evenson (27). 100 μl of whole semen was diluted into 900 μl TNE buffer and kept frozen at −70°C until analysis.

The SCSA® procedure was conducted on a Coulter Epics Elite Flow Cytometer using SCSA® (SCSA diagnostics, Brookings, SD) software. For budgetary reasons, an abbreviated semen analysis was conducted on the second sample, viz., volume, concentration and motility. We assessed 36 parameters including 5 general characteristics (volume, straw distance, sperm concentration, total count, hypo-osmotic swollen), 8 motility measures, 6 sperm head measures, 15 morphology measures, and 2 sperm chromatin stability assay measures. An established laboratory quality assurance plan and quality control was in place (30).

Statistical Analysis

We explored and quantified the distributions for the 36 semen quality parameters as medians and interquartile ranges (IQRs). Semen parameters were empirically assessed for skewedness and all were left in their original scale, given their use as covariates not outcomes requiring normality assumptions, and recognizing that some parameters were either counts or percent measurements. Socio-demographic comparisons of male participants and semen collection and appearance characteristics by site were made to ensure no systematic differences. We also assessed the comparability of the two semen samples to identify any significant differences using the nonparametric Wilcoxon rank sum test.

The relation between semen parameters and couple fecundity as measured by TTP was extensively assessed. Couples achieving pregnancy within the first few weeks following enrollment or before a fully observed menstrual cycle were defined as having a TTP=0 to differentiate them from couples with at least one fully observed menstrual cycle. Couples not achieving pregnancy within 12 months were censored as a TTP >12 consistent with the clinical definition of infertility (31). We have no information on medical care seeking behavior or outcome for couples not achieving pregnancy within 12 months. TTP was defined as the total number of menstrual cycles required for pregnancy, with cycles defined as day one to day one followed by at least two days of bleeding using the combined information from the daily journals and fertility monitor (32). *A priori*, we included covariates reported to be determinants of couple fecundity or TTP: male age (years), the difference of the male and female's age given the correlation between partners' ages, male and female body mass indices (weight in kg/ height in m^2), abstinence time (# days), male and female smoking as quantified by serum cotinine concentrations, and enrollment site

(Texas as referent) as a proxy for any residual confounding (33–44). We did not adjust for sexual intercourse, which may be an intermediate on the pathway or an independent predictor but not a confounder, and given the lack of any significant associations between frequency of either intercourse or ejaculation by the WHO semen quality parameters, i.e., volume, count, concentration, percent motility or normal morphology (data not shown). We also did not adjust for caffeine, given the limited available data suggesting an adverse relation as recently reviewed to preserve precision (45) coupled with the number of covariates already in models. Serum cotinine concentrations were quantified using an established protocol (46), and were log transformed and standardized by their standard deviations and reported as ng/g serum.

Cox proportional hazards models for discrete survival time were implemented to assess semen parameters and fecundability adjusting for left truncation or time off contraception and accounting for the use of both semen samples in the models. Missing data for each semen sample were imputed using the Markov Chain Monte Carlo methods under the missing at random assumption to minimize any bias associated with missing data (47). Ten imputations were run (PROC MI) and results were combined (PROC MIANALYZE) using SAS (version 9.2, SAS Institute, Inc., Cary, North Carolina). Summary statistics included fecundability odds ratios (FORs) that estimate the odds of becoming pregnant each cycle conditional on not becoming pregnant in the previous cycle and 95% confidence intervals (CIs) (48). FORs <1.0 denote a reduction in fecundity or a longer TTP, while FORs >1.0 denote enhanced fecundity or a shorter TTP. Separate models were run for each semen parameter consistent with our aim to fully explore the many facets of semen quality relative to TTP. Finally, we utilized multivariable models to assess the combined effects of all significant semen parameters identified from individual adjusted FORs and relevant covariates to more closely model couples' trying attempts. Specifically, we ran six multivariable models because some semen parameters are derived from others (i.e., volume, concentration and total count) or from varying methods (i.e., WHO normal or Strict) to avoid collinearity. To facilitate clinical interpretation, we also ran models for sperm concentration and total count where we rescaled the parameters by their standard deviation (SD) rather than keeping in the original scale (x106/mL). The linearity and proportional hazards assumptions were verified in all models.

Results

A semen sample was returned from 473 (94%) participating men, of which 378 (80%) provided a second sample. No significant differences were observed between the two samples. Five (1%) men were determined to be azoospermic for both samples. A description of the cohort by research site is presented in Table 1, and reflects that overall approximately 12% of participants were non-white, 11% were without a college education, 11% had no health insurance, and 42% of men had never fathered pregnancies. On average, male partners were 31.8±4.9 years of age with a BMI of 29.8±5.6. Female BMI was the only variate that differed significantly by enrollment site, with a higher mean BMI for Michigan than Texas women (29.3 ±8.6 and 27.2 ±6.8, respectively).

The distributions for semen quality endpoints for the LIFE Cohort are presented in Table 2. Semen characteristics quantified in both samples per individual (volume, concentration, total count and strict morphology) and were similar. Distributions for volume, concentration, total count, and morphology (strict) were generally similar to those reported by WHO (4). Sperm motility and viability measures (HOS) were next day measurements and are not directly comparable with WHO values, nor were any of our remaining parameters that are not reported by WHO.

Table 3 presents the unadjusted and adjusted FORs modeled separately for each semen quality parameter excluding the five azoospermic men from the analysis consistent with our exploratory approach. Of note is the identification of 12 semen parameters that were associated with enhanced (FORs >1.0; shorter TTP) and 6 with diminished (FORs <1.0; longer TTP) couple fecundity, when adjusting for potential confounders. Many FORs and CIs were close to one reflecting our study's statistical power. Semen parameters associated with a shorter TTP included: sperm head width (FOR 2.54; 95% CI 1.42, 4.57); acrosome head area (FOR 1.03; 95% CI 1.01, 1.05; elongation factor (FOR 1.03; 95% CI 1.01, 1.06); % normal morphology - strict criteria (FOR 1.03; 95% CI 1.01, 1.04); % normal morphology – traditional criteria (FOR 1.02 95% CI 1.01, 1.04); % motility on day after collection (FOR 1.02; 95% CI 1.01, 1.02); straight line velocity (FOR 1.01; 95% CI 1.00, 1.02); % hypo-osmotic (FOR 1.01; 95% CI 1.00, 1.02); sperm path linearity (FOR 1.01; 95% CI 1.00, 1.02); straightness (FOR 1.01; 95% CI 1.00, 1.01); sperm concentration (FOR 1.00; 95% CI 1.00, 1.01); and total sperm count (FOR 1.00; 95% CI 1.00, 1.00). FORs for the latter two rescaled parameters were 1.19 (1.06, 1.34) and 1.20 (1.06, 1.36), respectively. Semen quality parameters associated with diminished fecundity or a longer TTP included (in descending order of magnitude): % round head (FOR 0.89; 95% CI 0.81, 0.98); % amorphous head (FOR 0.98; 95% CI 0.97, 0.99); % neck/midpiece abnormalities (FOR 0.99; 95% CI 0.97, 1.00), % coiled tails (FOR 0.98; 95% CI 0.97, 1.00); % high DNA stainability (FOR 0.97; 95% CI 0.95, 1.00); and % DNA fragmentation index (FOR 0.98; 95% CI 0.97, 1.00).

Table 4 presents the FORs when modeling semen parameters significant in adjusted models of individual endpoints except when a parameter is a composite of others (i.e., elongation factor) or a relatively similar measure (i.e., sperm concentration and total sperm count; strict or traditional criteria). Percent coiled tail was the only semen parameter to be significantly associated with fecundity before rounding (FOR 0.99; 95% CI 0.98, 1.00). Moreover, older male age was associated with reduced fecundity (FORs <1.0) across all models, and female but not male BMI reflected a similar negative pattern in 4/6 models.

Discussion

In the most comprehensive analysis of semen quality parameters and couple fecundity analyzed to date, we found that semen quality parameters were both positively and negatively associated with couple fecundity in expected directions when modeled separately resulting in shorter and longer TTP, respectively. Our findings reflected that select parameters characterizing sperm motility, head and morphology (i.e., strict and traditional criteria) were associated with a shorter time to pregnancy, though all FORs were small in magnitude even when rescaling two key parameters - sperm concentration and total count by their SDs. One notable exception was the strong positive association between sperm head width (μm) and TTP, conferring an approximately 2.5-fold reduction in TTP per unit increase in sperm head width. To our knowledge, sperm head width has received little investigation in relation to couple fecundity or TTP. Also of note is that among four other morphologic sperm characteristics associated with a longer TTP, the largest effect was observed between percent round headed sperm and TTP, reflecting an 11% increase in TTP per every percent increase reflecting diminished fecundity. Both the DNA fragmentation index and high DNA stainability were negatively associated with TTP, though the point and interval estimates were close to one. However, in combined models accounting for the simultaneous effect of several semen parameters and covariates, only percent coiled tail was significantly associated with diminished fecundability before rounding (FOR 0.99; 95% CI 0.98, 1.00). The absence of strong semen parameter effects in our multivariate models may suggest the biologically inherent programming of male fecundity to compensate for deficiencies in one or more semen parameters relative to couple fecundity or our inability to

empirically characterize the fertilizing capabilities of semen endpoints. Conversely, male age was consistently associated with an approximately 4% reduction in fecundability per increasing year and female BMI about a 2% reduction per increasing unit in most models.

Comparison of our findings with the two previous prospective cohort studies with preconception enrollment of couples requires caution for a number of reasons. While we corroborated a positive relation between morphology (strict and traditional) and fecundity similar to the earlier studies (16, 17), these parameters were not retained in models inclusive of other semen parameters. Similarly, total sperm count also was associated, though the point and interval estimates were essentially one after rounding suggesting little impact on fecundity after accounting for couple factors. Our findings are based upon all semen samples per participant, whereas one earlier study had one sample (16) while the other study averaged semen values across samples (17). Another key difference is the 12-month follow-up period in the LIFE Study, which is comparable to only one (17) previous study. Another important consideration is the low sperm concentration reported for the Danish population (49, 50).

Findings from the LIFE Study are strengthened by several unique features including the largest number of semen quality parameters explored to date in relation to TTP, the enrollment of couples prior to conception with prospective daily follow-up through 12 months of trying, and the measurement of BMI and cotinine for both partners. Use of the fertility monitor provided all couples with a standardized prompt for timing intercourse, while capturing longitudinal menses data for measuring TTP. Use of digital pregnancy kits permitted the sensitive detection of hCG pregnancies. In addition, we modeled data from all semen samples while addressing the inherent correlation of repeated samples.

Our findings support past research focusing on age, BMI and smoking when assessing couple fecundity. For example, our finding that older paternal age is significantly and negatively associated with TTP underscores the importance of biology for couple fecundity, and corroborates previous research reporting a decline in fecundity with age (51, 52) including male age beginning at approximately 30 years of age and a 50% lower probability of pregnancy for men >35 years of age even after controlling for female age (35, 38). Evidence suggests that male age more so than BMI may be associated with semen quality (41, 53), a finding we corroborated in our combined models. Age related effects on male fecundity are diverse and may reflect genetic (54), hormonal (55) or sexual dysfunction (56) aberrations. In research that assessed couples' BMIs, female rather than male BMI appeared to exert more of an effect on TTP (36), a finding we corroborate. A recent systematic review concluded that there was no evidence for an association between increased BMI and semen parameters (57). A clearer understanding of couples' characteristics including lifestyle and fecundity is lacking despite the diagnostic importance of semen analysis, and most likely accounts for the absence of evidence-based guidance on lifestyle and male fertility (58, 59).

Careful interpretation of the LIFE Study's findings is needed in light of important considerations including its *a priori* exploratory approach for assessing the spectrum of semen parameters relative to TTP. Given the reported limited predictability of traditional semen parameters, sperm function or DNA tests for fertility (7, 8, 60), we assessed a spectrum of parameters without controlling for multiple comparisons to more completely assess semen quality and couple fecundity. As such, we cannot eliminate false positive findings and further recognize the need for development of novel models suitable for the analysis of mixtures including semen quality biomarkers. While our findings are believed to be internally valid, the external validity or generalizability of our findings to other populations is limited and await corroboration. While the aim of the LIFE Study was to assess persistent environmental chemicals in the context of lifestyle and human fecundity

including semen quality, it is important to note that such chemical exposure is widespread with ubiquitous exposure through diet, inhalation and other routes for contemporary populations (61, 62). Moreover, the geometric mean chemical concentrations in the Life Study were usually lower than those quantified for U.S. men participating in the nationally representative NHANES biomonitoring survey (63). Another study limitation is the use of 24-hour semen quality analysis, which is not ideal for time sensitive endpoints such as motility and viability. To globally assess motility at the time of collection, men placed a glass straw into their specimen as described above. This step allowed us to assess the presence of motile sperm at collection. We recognize that 24-hour analysis may account for the lack of any significant motility associations in final analyses. Still, it is important to keep in mind that while efficiency is reduced by use of the 24-hour semen analysis, we have no evidence supporting the introduction of bias. This reflects not only the blinding of laboratory staff to fecundity status, but the absence of empirical evidence supporting a systematic difference in the integrity of semen samples collected in the home by couples' TTP. Still, the 24-hour analysis is suitable for research but not clinical purposes, and the findings should be interpreted as such. Of note is the successful use of at home semen collection in another study, which formally assessed the impact of overnight semen analysis relative to analysis within 1.5 hours with no observed differences for (non-motility) various semen endpoints (64, 65). Lastly, the success of the LIFE Study underscores the willingness of men from disparate geographic areas to collect semen for research purposes underscoring the utility and feasibility of population based research focusing on male and couple fecundity.

Given that human semen quality is reported to be poorer in relation to other species (66), it is essential to identify not only etiologic determinants but also biomarkers predictive of male fecundity. Overcoming these critical data gaps is important for determining whether male fecundity is declining as reported for contemporary birth cohorts (67, 68) and in understanding the reported geographical differences in both semen quality (49, 69–74) and TTP (75–77), and its ultimate impact on population fecundity. Novel research paradigms for untargeted and targeted investigation of environmental influences on human fecundity and fertility, such as the exposome paradigm (78, 79), may offer promise for elucidating the biomarker of male fecundity and its role in couple fecundity.

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Table 1

Comparison of male partners by enrollment site, LIFE Study (n=473).

Characteristic	Michigan (n=104)	Texas (n=397)
	n (%)	n (%)
Nonwhite race	11 (10.8)	49 (12.5)
≤High school education	10 (9.7)	34 (8.6)
No health insurance	10 (9.6)	33 (8.3)
Female partner's parity:		
Never pregnant	39 (37.5)	171 (43.4)
Pregnant, no previous births	13 (12.5)	40 (10.2)
Pregnant, previous births	52 (50.0)	183 (46.5)
Never fathered pregnancy prior to study entry	42 (40.4)	173 (43.7)
Current smoker	19 (18.6)	65 (16.6)
	Mean (±SD)	Mean (±SD)
Male age (in years)	31.9 (4.7)	31.7 (4.9)
Female age (in years)	30.3 (4.3)	29.9 (4.1)
Male body mass index (kg/m^2)	29.7 (5.3)	29.8 (5.6)
Female body mass index (kg/m^2)*	29.3 (8.6)	27.2 (6.8)

* $p < 0.01$

Table 2

Distribution of semen quality endpoints, LIFE Study.

Semen Endpoints	Total (n=473)	
	Mean (SD)	Centiles (5th, 95th)
General Semen Characteristics:		
Volume (mL)		
1st sample	3.4 (1.7)	(1.0, 6.8)
2nd sample	3.4 (1.5)	(1.3, 6.1)
Motility (%)[a]		
1st sample	12.2 (12.5)	(0.0, 40.0)
2nd sample	12.5 (12.5)	(0.0, 41.0)
Straw (mm distance sperm traveled)		
1st sample	10.5 (6.2)	(3.3, 22.6)
Sperm concentration (x10^6/mL)		
1st sample	73.8 (56.4)	(9.6, 189.0)
2nd sample	74.9 (58.4)	(10.9, 194.6)
Total sperm count (x 10^6/mL)		
1st sample	231.2 (187.7)	(23.9, 596.5)
2nd sample	238.1 (205.0)	(29.1, 691.9)
Hypo-osmotic swollen (%)[a]		
1st sample	67.1 (10.1)	(49.1, 80.8)
2nd sample	65.4 (11.1)	(45.4, 80.3)
Sperm Motility:[a]		
Average path velocity (μm/sec)		
1st sample	67.1 (10.1)	(49.1, 80.8)
2nd sample	65.4 (11.1)	(45.4, 80.3)
Straight line velocity (μm/sec)		
1st sample	27.1 (11.0)	(0, 42.8)
2nd sample	27.8 (10.7)	(0, 42.8)
Curvilinear velocity (μm/sec)		
1st sample	62.2 (23.1)	(0, 90.3)
2nd sample	63.9 (22.2)	(0, 93.7)
Amplitude head displacement (μm)		
1st sample	3.1 (1.5)	(0.0, 5.0)
2nd sample	3.2 (1.5)	(0.0, 5.2)
Beat cross frequency (Hz)		
1st sample	19.7 (7.7)	(0.0, 28.7)
2nd sample	20.2 (7.6)	(0.0, 28.7)

Semen Endpoints	**Total (n=473)**	
	Mean (SD)	**Centiles (5th, 95th)**
Straightness (%)		
1st sample	67.5 (21.6)	(0.0, 84)
2nd sample	68.4 (19.7)	(0.0, 84)
Linearity (%)		
1st sample	40.8 (14.2)	(0.0, 55)
2nd sample	41.6 (13.3)	(0.0, 56)
Percent Motility (%)		
1st sample	12.2 (12.5)	(0.0, 40)
2nd sample	12.5 (12.5)	(0.0, 41)
Sperm Head Measurements:		
Length (μm)		
1st sample	4.9 (0.3)	(4.4, 5.4)
2nd sample	4.9 (0.3)	(4.4, 5.4)
Area (μm^2)		
1st sample	12.2 (0.9)	(10.6, 13.7)
2nd sample	12.2 (0.9)	(10.8, 13.8)
Width (μm)		
1st sample	3.2 (0.2)	(2.9, 3.4)
2nd sample	3.2 (0.2)	(2.9, 3.4)
Perimeter (μm)		
1st sample	13.2 (0.5)	(12.4, 14.1)
2nd sample	13.2 (0.5)	(12.4, 14.1)
Elongation factor (%)		
1st sample	65.8 (5.4)	(56.8, 74.5)
2nd sample	66 (5.4)	(57.3, 75.3)
Acrosome area of head (%)		
1st sample	25.6 (5.3)	(17.1, 34.5)
2nd sample	25.9 (4.8)	(18.0, 33.9)
Morphology[b]:		
Strict criteria (%)	20.1 (10)	(4.0, 38.0)
WHO Normal (%)	30.4 (12.5)	(10.0, 51.0)
Amorphous (%)	30.6 (11.1)	(14.5, 49.5)
Round (%)	1.2 (1.6)	(0.0, 4.0)
Pyriform (%)	6.3 (6.2)	(0.5, 19.5)
Bicephalic (%)	1.2 (1.7)	(0.0, 4.5)
Taper (%)	2.8 (2.7)	(0.0, 8.0)
Megalo head (%)	2.4 (1.8)	(0.5, 6.0)

Semen Endpoints	**Total (n=473)**	
	Mean (SD)	**Centiles (5th, 95th)**
Micro head (%)	1.5 (1.2)	(0.0, 3.5)
Neck & midpiece abnormalities (%)	26.6 (10)	(13.0, 45.5)
Coiled tail (%)	24.2 (11.2)	(10.0, 46.5)
Other tail abnormalities (%)	5.2 (4.2)	(1.0, 11.5)
Cytoplasmic droplet (%)	10.2 (5.3)	(3.0, 19.5)
White blood cell count (# wbc)	1.1 (14)	(0.0, 2.0)
Immature sperm (# immature sperm)	6.2 (16.7)	(0.0, 18.0)
Sperm Chromatin Stability Assay:		
DNA fragmentation index (%)	15.3 (10.4)	(5.1, 33.8)
High DNA stainability (%)	7.4 (5.2)	(2.0, 19.3)

NOTE: Five men were azoospermic for both samples and were excluded from analysis. Morphology and CASA was not performed for the second sample.

[a] Denotes next day analysis

[b] Traditional and strict criteria

Table 3

Unadjusted and adjusted fecundability odds ratios (FORs) for individual semen quality endpoints, LIFE Study (n=468).

Semen Endpoint	Unadjusted FOR (95% CI)	Adjusted FOR (95% CI)[a]
General Characteristics		
Volume (mL)	1.06 (0.99, 1.13)	1.03 (0.96,1.11)
Straw distance (mm)	1.01 (0.99, 1.03)	1.01 (0.99, 1.03)
Sperm concentration (x10^6/mL) Sperm concentration (per 1 SD)	1.0 (1.00, 1.01)** 1.16 (1.05, 1.28)**	1.00 (1.00, 1.01)** 1.19 (1.06, 1.34)**
Total sperm count (x10^6/mL) Total sperm count (per 1 SD)	1.0 (1.00, 1.00)** 1.20 (1.06, 1.36)**	1.00 (1.00, 1.00)** 1.20 (1.06, 1.36)**
Hypo-osmotic swollen (%)	1.02 (1.01, 1.03)**	1.01 (1.00, 1.02)*
Sperm Motility[b]		
Average path velocity (μm/sec)	1.01 (1.00, 1.02)*	1.01 (1.00, 1.02)
Straight line velocity (μm/sec)	1.01 (1.00, 1.02)*	1.01 (1.00, 1.02)*
Curvilinear velocity (μm/sec)	1.00 (1.00, 1.02)*	1.00 (1.00, 1.01)
Amplitude head displacement (μm)	1.09 (1.03, 1.16)**	1.07 (1.00, 1.14)
Beat cross frequency (Hz)	1.01 (1.00, 1.02)	1.01 (1.00, 1.02)
Straightness (%)	1.01 (1.00, 1.01)**	1.01 (1.00, 1.01)*
Linearity (%)	1.01 (1.00, 1.01)*	1.01 (1.00, 1.02)*
Percent Motility (%)	1.02 (1.01, 1.02)**	1.02 (1.01, 1.02)**
Sperm Head Measurements		
Length (μm)	0.84 (0.63, 1.12)	0.74 (0.51, 1.07)
Area (μm^2)	1.15 (1.05, 1.26)**	1.11 (0.99, 1.24)
Width (μm)	2.73 (1.70, 4.39)**	2.54 (1.42, 4.57)**
Perimeter (μm)	1.07 (0.91, 1.26)	0.99 (0.81, 1.22)
Elongation factor (%)	1.03 (1.01, 1.05)**	1.03 (1.01, 1.06)**
Acrosome area of head (%)	1.03 (1.01, 1.04)**	1.03 (1.01, 1.05)**
Morphology[c]		
Strict criteria (%)	1.03 (1.01, 1.04)**	1.03 (1.01, 1.04)**
Traditional normal (%)	1.02 (1.01, 1.03)**	1.02 (1.01, 1.03)**
Amorphous (%)	0.98 (0.97, 0.99)**	0.98 (0.97, 0.99)**
Round (%)	0.90 (0.82, 0.98)*	0.89 (0.81, 0.98)*
Pyriform (%)	0.98 (0.96, 1.00)*	0.98 (0.96, 1.00)
Bicephalic (%)	0.93 (0.85, 1.01)	0.93 (0.86, 1.01)
Taper (%)	0.96 (0.91, 1.00)	0.97 (0.93, 1.01)
Megalo head (%)	0.96 (0.90, 1.04)	0.96 (0.89, 1.04)

Semen Endpoint	Unadjusted FOR (95% CI)	Adjusted FOR (95% CI)[a]
Micro head (%)	0.96 (0.86, 1.07)	0.99 (0.89, 1.11)
Neck & midpiece abnormalities (%)	0.99 (0.98, 1.00)*	0.99 (0.97, 1.00)*
Coiled tail (%)	0.98 (0.97, 0.99)**	0.98 (0.97, 1.00)**
Other tail abnormalities (%)	0.98 (0.95, 1.01)	0.98 (0.95, 1.02)
Cytoplasmic droplet (%)	0.99 (0.96, 1.01)	0.98 (0.96, 1.00)
White blood cell count (# wbc)	0.99 (0.97, 1.02)	0.99 (0.96, 1.02)
Immature sperm (# immature sperm)	0.99 (0.97, 1.00)	0.98 (0.97, 1.00)
Sperm Chromatin Stability Assay		
DNA fragmentation index (%)	0.98 (0.97, 1.00)**	0.98 (0.97, 1.00)*
High DNA stainability (%)	0.98 (0.95, 1.00)	0.97 (0.95, 1.00)*

NOTE: Excludes 5 azoospermic men from analysis. Semen samples were imputed using the MCMC method. All point and interval estimates were rounded to two decimal places.

[a] FORs estimated using repeated measures and adjusted for left truncation to account for time off contraception before enrollment, male age, difference of couples' ages, male and female BMI, male and female serum cotinine concentrations, abstinence, and site.

[b] Denotes 24-hour motility

[c] Traditional and strict criteria – differentials were conducted using the traditional morphology.

* $p \leq 0.05$;

** $p \leq 0.01$ before rounding to two decimal places.

SD, denotes standard deviation. The SDs for sperm concentration and total sperm count are 57.44 and 196.13, respectively.

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Table 4

Multivariable regression model based fecundability ratios for significant semen quality endpoints, LIFE Study (n=468).

Semen Quality Parameter	Model 1 Sperm Concentration FOR (95% CI)	Model 2 Total Sperm Count FOR (95% CI)	Model 3 Sperm Concentration + Strict Criteria FOR (95% CI)	Model 4 Sperm Concentration + Traditional Normal FOR (95% CI)	Model 5 Total Sperm Count + Strict Criteria FOR (95% CI)	Model 6 Total Sperm Count + Traditional Normal FOR (95% CI)
Strict Criteria	--	--	1.02 (1.00, 1.04)	--	1.02 (0.99, 1.04)	--
% Traditional normal	--	--	--	1.01 (0.99, 1.03)	--	1.01 (0.99, 1.03)
Sperm concentration (x10^6/mL)	1.00 (1.00, 1.00)	--	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	--	--
Sperm concentration (per 1 SD)	1.07 (0.94, 1.21)	--	0.97 (0.94, 1.22)	0.97 (0.75, 1.26)	--	--
Total sperm count (x10^6/mL)	--	1.00 (1.00, 1.00)	--	--	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)
Total sperm count (per 1 SD)	--	1.11 (0.97, 1.27)	--	--	0.96 (0.77, 1.21)	0.97 (0.75, 1.25)
Hypo-osmotic swollen	1.01 (0.99, 1.02)	1.01 (0.99, 1.02)	1.01 (0.99, 1.03)	1.01 (0.99, 1.02)	1.01 (0.99, 1.02)	1.01 (0.99, 1.02)
Straight line velocity	1.00 (0.98, 1.03)	1.00 (0.97, 1.02)	1.00 (0.98, 1.03)	1.00 (0.98, 1.03)	1.00 (0.98, 1.03)	1.00 (0.98, 1.03)
Straightness	1.00 (0.98, 1.03)	1.01 (0.99, 1.03)	1.00 (0.98, 1.02)	1.00 (0.98, 1.02)	1.00 (0.98, 1.03)	1.00 (0.98, 1.03)
Linearity	0.99 (0.97, 1.02)	0.99 (0.97, 1.02)	0.99 (0.97, 1.02)	0.99 (0.97, 1.02)	0.99 (0.97, 1.02)	0.99 (0.97, 1.02)
Percent motility	1.00 (0.99, 1.02)	1.00 (0.99, 1.02)	1.00 (0.99, 1.02)	1.00 (0.99, 1.02)	1.00 (0.99, 1.02)	1.00 (0.99, 1.02)
Sperm head width	1.64 (0.79, 3.38)	1.58 (0.76, 3.29)	1.54 (0.75, 3.20)	1.49 (0.71, 3.10)	1.50 (0.72, 3.13)	1.44 (0.68, 3.03)
Acrosome head area	0.99 (0.96, 1.02)	0.99 (0.96, 1.02)	0.99 (0.97, 1.02)	0.99 (0.96, 1.02)	0.99 (0.97, 1.02)	0.99 (0.96, 1.02)
Amorphous	0.99 (0.98, 1.00)	0.99 (0.98, 1.00)	1.00 (0.98, 1.01)	1.00 (0.98, 1.01)	1.00 (0.98, 1.01)	1.00 (0.98, 1.01)
Round	0.95 (0.86, 1.06)	0.95 (0.85, 1.05)	0.95 (0.85, 1.05)	0.95 (0.85, 1.06)	0.94 (0.85, 1.05)	0.95 (0.85, 1.05)
Neck/midpiece abnormalities	1.01 (0.99, 1.02)	1.01 (0.99, 1.02)	1.01 (0.99, 1.03)	1.01 (0.99, 1.03)	1.01 (0.99, 1.03)	1.01 (0.99, 1.03)
Coiled tail	0.99 (0.98, 1.00)*	0.99 (0.98, 1.00)	0.99 (0.98, 1.00)	0.99 (0.98, 1.01)	0.99 (0.98, 1.01)	0.99 (0.98, 1.01)
DNA fragmentation index	0.99 (0.98, 1.01)	0.99 (0.98, 1.01)	0.99 (0.98, 1.01)	0.99 (0.98, 1.01)	0.99 (0.98, 1.01)	0.99 (0.98, 1.01)
High DNA stainability	0.99 (0.96, 1.02)	0.99 (0.96, 1.02)	0.99 (0.96, 1.02)	0.99 (0.96, 1.02)	0.99 (0.96, 1.02)	0.99 (0.96, 1.02)
Male age	0.96 (0.93, 0.99)**	0.96 (0.93, 0.99)**	0.96 (0.93, 0.99)**	0.96 (0.93, 0.99)**	0.96 (0.93, 0.99)**	0.96 (0.93, 0.99)**
Difference in couples' ages	0.97 (0.93, 1.01)	0.96 (0.92, 1.01)	0.97 (0.93, 1.01)	0.97 (0.93, 1.01)	0.97 (0.93, 1.01)	0.97 (0.93, 1.01)
Male BMI	1.01 (0.98, 1.04)	1.01 (0.99, 1.04)	1.01 (0.99, 1.04)	1.01 (0.99, 1.04)	1.01 (0.99, 1.04)	1.01 (0.99, 1.04)
Female BMI	0.98 (0.96, 1.00)*	0.98 (0.96, 1.00)	0.98 (0.96, 0.99)*	0.98 (0.96, 0.99)*	0.98 (0.96, 0.99)*	0.98 (0.96, 0.99)*
Male serum cotinine	0.88 (0.76, 1.01)	0.88 (0.77, 1.02)	0.88 (0.77, 1.02)	0.88 (0.77, 1.02)	0.89 (0.77, 1.02)	0.89 (0.77, 1.02)
Female serum cotinine	0.88 (0.75, 1.04)	0.89 (0.75, 1.05)	0.89 (0.75, 1.05)	0.89 (0.75, 1.05)	0.89 (0.75, 1.06)	0.89 (0.76, 1.06)

Semen Quality Parameter	Model 1 Sperm Concentration FOR (95% CI)	Model 2 Total Sperm Count FOR (95% CI)	Model 3 Sperm Concentration + Strict Criteria FOR (95% CI)	Model 4 Sperm Concentration + Traditional Normal FOR (95% CI)	Model 5 Total Sperm Count + Strict Criteria FOR (95% CI)	Model 6 Total Sperm Count + Traditional Normal FOR (95% CI)
Abstinence	1.01 (0.97, 1.05)	1.01 (0.97, 1.05)	1.01 (0.97, 1.05)	1.01 (0.97, 1.05)	1.01 (0.97, 1.05)	1.01 (0.97, 1.05)
Enrollment site	0.89 (0.64, 1.22)	0.90 (0.66, 1.25)	0.89 (0.64, 1.22)	0.89 (0.64, 1.22)	0.90 (0.66, 1.25)	0.90 (0.66, 1.25)

NOTE: All units for semen parameters are the same as those reported in Table 3. Semen samples were imputed with ten imputations using the MCMC method. FORs adjusted for left truncation to account for time off contraception before enrollment, male age, difference of couples' ages, male and female BMI, abstinence, and site.

* $p < 0.05$;

** $p < 0.01$ before rounding to two decimal places.

SD, denotes standard deviation. The SDs for sperm concentration and total sperm count are 57.44 and 196.13, respectively.