ORIGINAL ARTICLE: ANDROLOGY

Semen quality and pregnancy loss in a contemporary cohort of couples recruited before conception: data from the Longitudinal Investigation of Fertility and the Environment (LIFE) Study

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Objective: To study the relationship between semen quality and pregnancy loss in a cohort of couples attempting to conceive.
Design: Observational prospective cohort.
Setting: Not applicable.
Patient(s): Three hundred and forty-four couples with a singleton pregnancy observed daily through 7 postconception weeks of gestation.
Intervention(s): None.
Main Outcome Measure(s): Association between semen quality and pregnancy loss.
Result(s): Ninety-eight (28%) of the couples experienced a pregnancy loss after singleton pregnancy. No differences were observed in semen volume, sperm concentration, total sperm count, sperm viability, or sperm morphology (World Health Organization [WHO] and strict criteria) by couple's pregnancy loss status irrespective of whether they were analyzed continuously or as dichotomous variables per the WHO 5th edition semen criteria. A dichotomous DNA fragmentation measure of $\geq$30% was statistically significantly associated with pregnancy loss. No association was identified with other sperm morphometric or movement measures. Of the 70 couples who re-enrolled after a pregnancy loss, 14 experienced a second loss. Similar findings were identified when examining semen quality from couples with recurrent pregnancy loss.
Conclusion(s): Although a few trends were identified (e.g., DNA fragmentation), general semen parameters seemed to have little relation with risk of pregnancy loss or recurrent pregnancy loss at the population level. However, given that 30% of pregnancies end in miscarriage and half the fetal genome is paternal in origin, the findings await corroboration. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: DNA fragmentation, fertility, male infertility, semen analysis, spontaneous abortion

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Pregnancy loss affects 30% of pregnancies (1–3). Although most pregnancy losses are multifactorial in nature, most identified etiologies center around the woman. However, as a man contributes 50% of the genome to an embryo, it is reasonable to assume that male factors may also contribute to pregnancy loss. Indeed, up to 50% of all cases of infertility are due to a male factor (4, 5). To date, there are relatively limited data

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on male factors contributing to pregnancy loss, especially research that captures loss during the peak weeks in early gestation. However, a recent study by our group using the same cohort for the present analysis did identify an association between paternal lifestyle factors (i.e., caffeine consumption) and pregnancy loss in a prospective cohort study (6).

Recurrent pregnancy loss (RPL), defined as two or more consecutive losses for a couple, affects 1% to 5% of women (1, 3). As with pregnancy loss, the evaluation for RPL centers around the woman. Yet even after uterine, oocyte, and chromosomal factors are excluded, an idiopathic etiology is left approximately 50% of the time (3). Investigators have also attempted to determine the male factors associated with RPL. Zidi-Draj et al. (7) reported higher levels of sperm immotility, abnormal morphology, and elevated sperm DNA fragmentation in the male partners in couples with RPL. Elevated levels of sperm aneuploidy have also been reported among men from couples with RPL (8). However, as these studies relied on case control designs with fertile couples serving as the control groups, prospective studies are required to confirm the reported associations.

Surprisingly, few studies have attempted to assess semen quality and the risk of incident pregnancy loss. This may reflect the very few couple-based preconception cohort studies conducted worldwide, with even fewer collecting semen samples (9). Preconception cohort studies are needed to address this question, given the marked concentration of losses early in pregnancy or before seeking prenatal care. Using data from the Longitudinal Investigation of Fertility and the Environment (LIFE) Study, we examined the association between semen quality and pregnancy loss in a prospective study. Given that RPL represents a unique group, we also performed a subanalysis on couples with two or more losses.

MATERIALS AND METHODS

Study Design and Population

The study cohort comprised 347 couples (69%) whose female partners had an observed pregnancy (denoted by a positive urine pregnancy test) while participating in the LIFE Study, which was designed to examine the association between environmental and lifestyle factors and fecundity end points, including pregnancy loss. Three couples with twin pregnancies were excluded, resulting in a cohort comprising 344 couples with singleton pregnancies.

The LIFE Study used population-based sampling frameworks to recruit couples discontinuing contraception for purposes of becoming pregnant from 16 counties in Michigan and Texas. By design, the eligibility criteria were minimal and included [1] couples in a committed relationship; [2] ability to communicate in English or Spanish; [3] women aged 18 to 40 and men aged $\geq$ 18 years; [4] women with menstrual cycles between 21 and 42 days, as required by the fertility monitors; [5] no history of injectable hormone contraception in the past year; [6] no clinically diagnosed infertility in either partner; and [7] off contraception $<$2 months. Before enrollment, the women's urines were tested to ensure they were not already pregnant.

Human subjects approval was obtained from the participating institutions, and all men and women gave written informed consent before data collection. Complete details about the study design of LIFE have been previously published elsewhere (10).

Data Collection and Follow-up

Couples were interviewed individually upon enrollment to ascertain their sociodemographic, lifestyle, and medical history information, followed by measurement of height and weight to calculate body mass index (BMI). The couple was then instructed in the completion of daily journals to record their lifestyle in a manner consistent with how people think about such exposures (e.g., number of cigarettes smoked per day, number of alcoholic and caffeinated beverages consumed per day, number of daily multivitamins). Pregnant women completed their journals daily through 7 postconception weeks' gestation then continued as monthly journals until a loss or delivery. Couples experiencing a loss had the option of re-entering the study.

Biospecimen Collection and Analysis

Semen samples were collected via masturbation without the use of any lubricant after 2 days of abstinence using home collection kits, which included an insulated shipping container (Hamilton Research) for maintaining sperm integrity at the time of enrollment. Other studies have used similar approaches (11, 12). All semen samples were received at the study's andrology laboratory.

The complete laboratory methodology has been previously reported (10). Briefly, an aliquot of semen was placed in a 20-μm-deep chamber slide (Leja), and sperm motility was assessed using the HTM-IVOS (Hamilton Thorne) 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 assessments. An aliquot of whole semen was diluted in TNE (Tris, NaCl, and EDTA) buffer with glycerol and frozen for the sperm chromatin stability assay (SCSA) analysis (13). Sperm viability was determined by hypo-osmotic swelling (HOS assay).

To ensure integrity of the 24-hour analysis, steps were taken to ensure the quality of the semen parameters. A thermometer was attached to all collection jars to ensure the temperature of the sample was within acceptable limits (all were). Upon receipt, the andrology laboratory assessed the integrity of the samples, and all were found to be acceptable.

Home Fertility and Pregnancy Testing

Women were trained in the use of the Clearblue digital fertility monitor (SPD Swiss Precision Diagnostics GmbH), which has been demonstrated to be accurate in detecting ovulation relative to the gold-standard, ultrasound visualization (14). The monitor records the ratios of estrone-3-glucuronide (E3G) and luteinizing hormone (LH) and stores data for up to 6 months. Study personnel downloaded the data every 45 days. Day of ovulation in the study was approximated by the day of peak LH as indicated by the fertility monitor.

Women were also trained in the use of the Clearblue digital pregnancy test (with readouts of "pregnant" or "not pregnant"), which has a demonstrated sensitivity and reliability for detecting 25 mIU/mL of human chorionic gonadotropin, and demonstrated accuracy by women (15). Women tested their urine for pregnancy on the day they expected menstruation consistent with manufacturer's guidance.

Pregnancy Loss Ascertainment

Pregnancy loss was defined as conversion to a negative pregnancy test as subsequently recorded in the woman's journal, clinical confirmation of loss recorded on a separate pregnancy loss card, or onset of menstruation recorded in the journal, depending upon gestational age at loss (17). Gestational age at loss was measured in days after conception, which was approximated by the day of ovulation (LH peak) as recorded by the fertility monitor.

Statistical Analysis

We summarized the distributions as mean ± standard deviation (SD) or median and interquartile range (IQR) for continuous variables, and frequency and percentage for categorical variables. Table 1 includes age (years, continuous), body mass index (BMI in kg/m^2), race (white, non-white), education level (high school or below, some college or above), prior paternity (yes/no), self-reported smoking (yes/no), and self-reported alcohol consumption (yes/no). We dichotomized semen quality parameters (semen volume, sperm concentration, total sperm count, sperm viability, or sperm morphology by World Health Organization (WHO) and strict criteria using clinical cut points of male participants in the LIFE Study, based on WHO standards (16). The DNA fragmentation index (DFI) was also dichotomized (DFI $\geq$30) based on a previously reported cut point (18–20).

We assessed differences in semen parameters by couple's pregnancy loss status using the nonparametric (Kruskal-Wallis) test for continuous variables and chi-square test for categorical variables. The findings are reported in Table 2.

We used discrete time survival models to assess how semen quality parameters are related to pregnancy loss, with time-to-loss as an outcome and semen quality parameters as separate independent variables. These models were run unadjusted as well as with adjustment for covariates, including self-reported smoking (yes/no) and self-reported alcohol consumption (yes/no). We performed several sensitivity analyses for the recurrent pregnancy loss (RPL) group by varying the comparison groups, but the conclusions remained unchanged. $P<.05$ was considered statistically significant without adjusting for multiple comparisons. All statistical analyses were performed by the SAS version 9.3 (SAS Institute).

RESULTS

In all, 98 (28%) couples who became pregnant during the study experienced an incident pregnancy loss. Demographic characteristics of the entire cohort are presented in Table 1.

TABLE 1

Baseline characteristics of men who achieved a pregnancy, LIFE Study (n = 344).

Characteristic	Male (N = 344)
Demographic	
Age (y), mean ± SD	31.6 ± 4.59
BMI (kg/m^2), mean ± SD	29.39 ± 4.98
White	285 (83.33)
College educated	323 (94.72)
Prior paternity	207 (60.17)
Smoker	37 (10.76)
Alcohol	298 (86.63)
Semen parameter	
Volume (mL)	
<1.5	32 (9.64)
Median (IQR)	3.4 (2.3, 4.4)
Concentration (million/mL)	
<15	19 (5.72)
Median (IQR)	66.7 (37.65, 97.05)
Total sperm count (million)	
<39	21 (6.33)
Median (IQR)	202.9 (107.3, 338.1)
Morphology (% WHO normal)	
<30	139 (43.85)
Median (IQR)	31.5 (23, 40)
Morphology (% strict criteria)	
<4	9 (2.84)
Median (IQR)	21 (14.0, 27.5)
DNA (% fragmentation index)	
≥30	20 (6.13)
Median (IQR)	11.89 (8.4,18.04)

Note: Data presented as n (%), unless otherwise specified otherwise. BMI = body mass index; IQR = interquartile range; LIFE = Longitudinal Investigation of Fertility and the Environment; SD = standard deviation; WHO = World Health Organization.

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No differences were observed in semen volume, sperm concentration, total sperm count, sperm viability, or sperm morphology (WHO and strict, Table 2) by couple's pregnancy loss status, irrespective of whether they were analyzed continuously or as dichotomous outcomes per the WHO 5th edition criteria (16). Sperm motility end points as measured by average path velocity, straight line velocity, curvilinear velocity, amplitude head displacement, beat cross frequency, percentage with a straight trajectory, and percentage with a linear trajectory were also similar among the groups. Several measures of sperm head characteristics including head length, width, area, perimeter, percentage of elongation, and percentage occupation by the acrosome were also similar relative to pregnancy loss status. Other measures of morphology such as percentage of amorphous, round, pyriform, bicephalic, tapered, megalo-headed, and micro-headed were also similar between male partners of couples with and without a pregnancy loss. The percentage distributions of DFI and high DNA stainability were similar irrespective of pregnancy loss status, but dichotomizing DFI (i.e., DFI $\geq$30) was positively associated with pregnancy loss (11% and 4% of men with and without loss; $P=.03$). This difference remained after adjusting for BMI, smoking, and alcohol consumption.

As there was little evidence for any statistically significant differences in semen quality between men with and without a pregnancy loss, we also graphically compared semen quality patterns by couples' pregnancy loss status.

TABLE 2

Semen characteristics of men from couples with no pregnancy loss, one pregnancy loss, or two pregnancy losses.

Characteristic	No pregnancy loss (n = 246)	Pregnancy loss (n = 98)	Recurrent pregnancy loss (n = 14)	*P* value[a]
General				
Volume (mL)	3.58 ± 1.75	3.28 ± 1.51	2.71 ± 0.78	.19
Sperm concentration ($\times 10^6$/mL)	79.00 ± 59.18	77.18 ± 57.38	63.22 ± 48.10	.61
Total sperm count ($\times 10^6$/ejaculate)	253.88 ± 189.83	242.15 ± 213.57	168.54 ± 135.88	.54
Hypo-osmotic swelling (%)	68.24 ± 8.72	68.18 ± 10.65	66.69 ± 12.35	.83
Straw (mm distance sperm traveled)	10.64 ± 6.45	10.91 ± 6.62	9.75 ± 3.93	.70
WHO parameter, n (%)				
Volume <1.5 mL	21 ± 8.90	11 ± 11.46	0.00 ± 0.00	.79
Concentration <15 M/mL	12 ± 5.08	7 ± 7.29	2 ± 14.29	.27
Total count <39 M	13 ± 5.51	8 ± 8.33	3 ± 21.43[b]	.51
WHO morphology <30%	103 ± 45.98	36 ± 38.71	6 ± 42.86	.19
Strict morphology <4%	8 ± 3.57	1 ± 1.08	0 ± 0.00	.30
DFI (≥30%)	10 ± 4.29	10 ± 10.75[b]	1 ± 7.14	.04
Motility				
Average path velocity (μm/s)	37.04 ± 12.54	36.58 ± 13.54	32.69 ± 19.46	.70
Straight line velocity (μm/s)	27.62 ± 10.26	27.54 ± 11.30	24.34 ± 15.63	.89
Curvilinear velocity (μm/s)	63.95 ± 21.59	61.94 ± 22.52	56.21 ± 32.12	.39
Amplitude head displacement (μm)	3.21 ± 1.37	3.14 ± 1.38	2.65 ± 1.56	.63
Beat cross frequency (Hz)	20.23 ± 7.23	19.52 ± 7.85	17.66 ± 10.37	.38
Straightness (%)	68.70 ± 19.40	67.90 ± 21.56	56.86 ± 31.42	.67
Linearity (%)	41.40 ± 13.05	41.27 ± 14.27	34.00 ± 19.52	.88
Percent motility (%)	12.97 ± 12.87	14.08 ± 12.61	16.57 ± 16.02	.58
Head measurement				
Length (μm)	4.88 ± 0.26	4.88 ± 0.32	4.98 ± 0.38	.98
Area (μm)	12.25 ± 0.84	12.22 ± 1.04	12.45 ± 0.91	.95
Width (μm)	3.18 ± 0.18	3.18 ± 0.18	3.19 ± 0.11	.97
Perimeter (μm)	13.25 ± 0.48	13.25 ± 0.62	13.45 ± 0.63	.89
Elongation factor (%)	66.14 ± 5.18	65.95 ± 5.20	64.90 ± 5.07	.99
Acrosome area of head (%)	25.78 ± 5.06	26.67 ± 5.30	27.31 ± 3.47	.26
Morphology (%)				
Strict criteria	20.95 ± 9.59	22.01 ± 10.56	21.18 ± 9.45	.35
WHO normal	31.24 ± 11.99	33.38 ± 12.40	33.39 ± 12.18	.12
Amorphous	29.62 ± 10.80	28.60 ± 10.09	25.79 ± 8.59	.49
Round	1.14 ± 1.67	0.90 ± 1.09	0.79 ± 0.93	.29
Pyriform	6.04 ± 5.46	6.49 ± 7.22	8.50 ± 6.57	.68
Bicephalic	1.12 ± 1.70	1.04 ± 1.48	1.04 ± 1.43	.69
Tapered	2.80 ± 2.76	2.54 ± 2.30	2.39 ± 1.69	.27
Megalo-head	2.32 ± 1.72	2.47 ± 2.22	3.25 ± 1.77[b]	.48
Micro-head	1.44 ± 1.32	1.49 ± 1.00	1.75 ± 1.40	.58
Neck and midpiece abnormalities	26.02 ± 9.80	26.41 ± 8.42	26.79 ± 9.58	.68
Coiled tail	23.90 ± 10.70	21.68 ± 9.38	21.57 ± 9.67	.07
Other tail abnormalities	5.17 ± 4.80	5.17 ± 3.29	5.43 ± 2.20	.64
Cytoplasmic droplet	10.15 ± 5.31	9.69 ± 4.64	10.04 ± 4.31	.47
Immature germ cell count	4.81 ± 5.58	5.51 ± 10.25	5.93 ± 5.28	.13
SCSA (%)				
DNA fragmentation index	14.33 ± 9.72	15.84 ± 11.29	15.83 ± 17.20	.10
High DNA stainability	7.21 ± 5.15	6.77 ± 5.10	6.99 ± 5.36	.69

Note: Data presented as mean ± standard deviation, unless specified otherwise. DFI = DNA fragmentation index; SCSA = sperm chromatin stability assay; SD = standard deviation; WHO = World Health Organization.

[a] Multivariable models adjusted for smoking and alcohol use comparing men from couples with pregnancy loss versus no pregnancy loss.

[b] $P < .05$.

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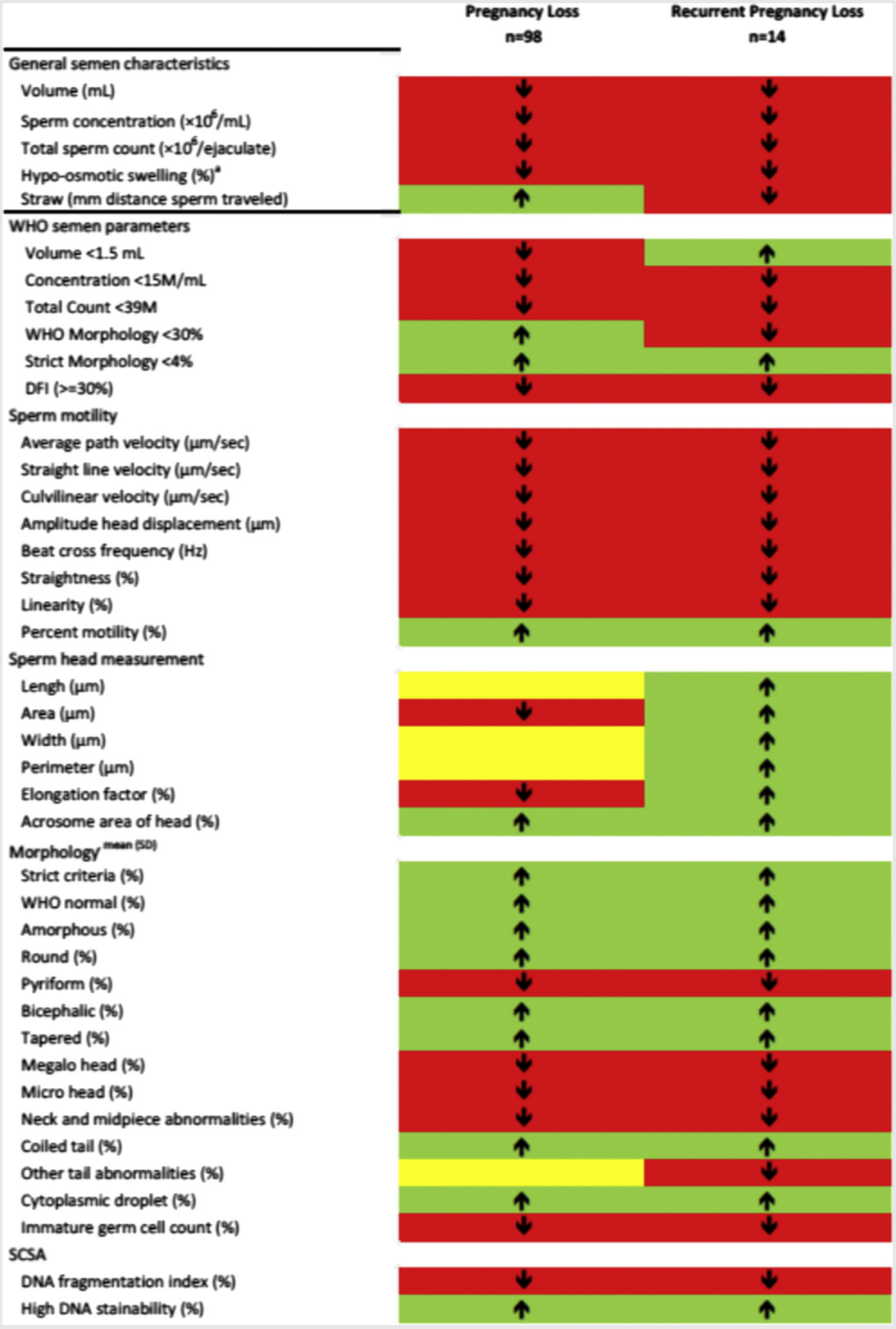
For most parameters, we found lower distributions in semen quality for couples experiencing a pregnancy loss. Figure 1 illustrates a summary of the comparison between men without a pregnancy loss, with one pregnancy loss, and with two or more pregnancy losses. Of the 35 semen and sperm end points assessed, 19 (54%) were worse among couples experiencing a pregnancy loss in comparison with the couples without a loss; 12 (34%) end points were better in the former group. Among the measures of motility, 7 (78%) of 9 were lower among couples with than without a loss, as were 7 (35%) morphometric end points.

Of the 98 couples with pregnancy loss, 70 re-entered the study. Fourteen couples experienced more than one loss while being followed in the study. Similar to men in couples experiencing one loss, few associations between semen quality and RPL were identified. A higher percentage of men whose female partner experienced RPL were found to have lower total sperm counts (<39 M) in comparison with the male partners in couples without losses (21.4% and 5.5%, respectively; Table 2). Overall, 20 (57%) measured semen end points were worse in couples with than without RPL (Fig. 1). Eight (89%) motility parameters were worse

FIGURE 1



Semen parameters for men with pregnancy loss or recurrent pregnancy loss compared with men without pregnancy loss. *Red* denotes a lower semen parameter value, *green* denotes a higher value, and *yellow* denotes an equal value compared with men without pregnancy loss.

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in men from RPL couples, as were 6 (30%) morphologic measures.

DISCUSSION

To our knowledge, our study is the first to evaluate a range of semen end points beyond traditional clinical outcomes such as count, motility, and morphology in relation to risk of incident pregnancy loss. Moreover, our findings are strengthened by the preconception enrollment of couples from the general population, along with daily follow-up observation through 7 postconception weeks and monthly thereafter. We found no evidence that semen quality in men recruited from the general population was associated with incident pregnancy loss. These findings were consistent when restricting the analysis to couples with observed RPL. However, couples experiencing a pregnancy loss were more likely to have male partners with abnormal sperm DNA fragmentation. In addition, male partners in couples with RPL were more likely to have lower total sperm counts than the couples without pregnancy loss. In addition when examining all measured semen end points, male partners of couples experiencing pregnancy losses tended to have worse semen quality in comparison with the male partners in couples without losses.

Most prior research on pregnancy loss has focused on maternal factors (21), though there are some limited data suggesting possible male factors (6–8,22–26), especially for RPL. Studies of RPL have reported associations with DNA fragmentation, sperm aneuploidy, sperm morphology, and motility (7, 8, 26). However, these prior studies have relied upon a case-control design. A key limitation of the case control design is that the control group is not at risk for loss, as by definition their pregnancy went to term. Although these studies make important contributions to our understanding of the association between semen quality and pregnancy loss, methodologic limitations from such retrospective designs make definitive conclusions challenging. To our knowledge, our study represents the first prospective examination of semen quality and risk of pregnancy loss among couples attempting to conceive.

Similar to prior reports, we did find an association between DNA fragmentation and pregnancy loss. Importantly, the only statistically significant result was identified after dichotomizing the DFI based on the defined abnormal cut point for DNA fragmentation but not when examined on continuous scale. Given the variability in semen quality, it may be that defining abnormal based on strict criteria (e.g., a defined and validated cut point) is more useful than a continuous scale, especially as our results also support a threshold effect of DFI. However, given the number of parameters examined, it is also conceivable that our association was due to chance alone. Indeed, for 35 tests performed, there is an 83% chance for finding at least one statistically significant association.

When examining RPL, there was a trend identified for several semen parameters. Semen volume and total sperm count were both lower in the male partners of couples with RPL than those without. However, on a continuous scale, neither reached statistical significance. It is important to know that RPL is a rare outcome, affecting less than 5% of pregnant couples (1). In our cohort, only 14 (4%) couples were observed to experience RPL, which is consistent with other population estimates (1, 27). The current report represents the first data on prospectively recruited men or men from the general population regarding semen quality and RPL.

Given the few associations identified in our primary analysis, we performed a visual analysis to examine the collective findings. Overall, we did observe that male partners of couples with (recurrent) pregnancy loss tended to have lower semen quality in comparison with the unaffected couples. Although many examined parameters and morphometrics are not part of routine patient care, our in-depth analysis still did not identify possible signals.

Several other important limitations warrant mention. Given the close monitoring of couples, very early pregnancies and losses may be more common in this cohort than would be observed in clinical practice. Indeed, all losses occurred before 22 weeks' gestation (17). It is conceivable that an analysis of later pregnancies would give different results. However, given the contribution of the sperm to early embryonic development, we would expect a larger effect earlier in gestation rather than later. As genetic abnormalities are thought to contribute to early pregnancy losses, our findings of abnormal DFI in men from couples with pregnancy loss is consistent with a paternally derived genetic origin of fetal loss. We analyzed nearly 100 pregnancy losses, but it is possible that we were underpowered to identify some associations with semen quality, particularly if they are reflected in small difference that would require larger cohorts.

Nevertheless, the current report represents the first prospective examination of the association between semen quality and pregnancy loss. A few trends were identified (e.g., DNA fragmentation), but general semen parameters seemed to have little relation to the risk of incident pregnancy loss at the population level. However, given that 30% of pregnancies end in miscarriage and half of the fetal genome is paternal in origin, these findings await corroboration.

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REFERENCES

1. Stirrat GM. Recurrent miscarriage. Lancet 1990;336:673–5.
2. Regan L, Rai R. Epidemiology and the medical causes of miscarriage. Baillieres Best Pract Res Clin Obstet Gynaecol 2000;14:839–54.
3. Practice Committee of the American Society for Reproductive Medicine. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. Fertil Steril 2012;98:1103–11.
4. Thonneau P, Marchand S, Tallec A, Ferial ML, Ducot B, Lansac J, et al. Incidence and main causes of infertility in a resident population (1,850,000) of three French regions (1988–1989). Hum Reprod 1991;6:811–6.
5. Eisenberg ML, Li S, Behr B, Pera RR, Cullen MR. Relationship between semen production and medical comorbidity. Fertil Steril 2015;103:66–71.

6. Buck Louis GM, Sapra KJ, Schisterman EF, Lynch CD, Maisog JM, Grantz KL, et al. Lifestyle and pregnancy loss in a contemporary cohort of women recruited before conception: the LIFE Study. Fertil Steril 2016;106:180–8.
7. Zidi-Jrah I, Hajlaoui A, Mougou-Zrelli S, Kammoun M, Meniaoui I, Sallem A, et al. Relationship between sperm aneuploidy, sperm DNA integrity, chromatin packaging, traditional semen parameters, and recurrent pregnancy loss. Fertil Steril 2016;105:58–64.
8. Ramasamy R, Scovell JM, Kovac JR, Cook PJ, Lamb DJ, Lipshultz LI. Fluorescence in situ hybridization detects increased sperm aneuploidy in men with recurrent pregnancy loss. Fertil Steril 2015;103:906–9.e1.
9. Buck GM, Lynch CD, Stanford JB, Sweeney AM, Schieve LA, Rockett JC, et al. Prospective pregnancy study designs for assessing reproductive and developmental toxicants. Environ Health Perspect 2004;112:79–86.
10. Buck Louis GM, Schisterman EF, Sweeney AM, Wilcosky TC, Gore-Langton RE, Lynch CD, et al. Designing prospective cohort studies for assessing reproductive and developmental toxicity during sensitive windows of human reproduction and development—the LIFE Study. Paediatr Perinat Epidemiol 2011;25:413–24.
11. Luben TJ, Olshan AF, Herring AH, Jeffay S, Strader L, Buus RM, et al. The healthy men study: an evaluation of exposure to disinfection by-products in tap water and sperm quality. Environ Health Perspect 2007;115:1169–76.
12. Olshan AF, Perreault SD, Bradley L, Buus RM, Strader LF, Jeffay SC, et al. The healthy men study: design and recruitment considerations for environmental epidemiologic studies in male reproductive health. Fertil Steril 2007;87:554–64.
13. Evenson DP, Larson KL, Jost LK. Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl 2002;23:25–43.
14. Behre HM, Kuhlage J, Gassner C, Sonntag B, Schem C, Schneider HP, et al. Prediction of ovulation by urinary hormone measurements with the home use ClearPlan Fertility Monitor: comparison with transvaginal ultrasound scans and serum hormone measurements. Hum Reprod 2000;15:2478–82.
15. Johnson S, Cushion M, Bond S, Godbert S, Pike J. Comparison of analytical sensitivity and women's interpretation of home pregnancy tests. Clin Chem Lab Med 2015;53:391–402.
16. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update 2010;16:231–45.
17. Sapra KJ, Buck Louis GM, Sundaram R, Joseph KS, Bates LM, Galea S, et al. Signs and symptoms associated with early pregnancy loss: findings from a population-based preconception cohort. Hum Reprod 2016;31:887–96.
18. Practice Committee of the American Society for Reproductive Medicine. The clinical utility of sperm DNA integrity testing: a guideline. Fertil Steril 2013;99:673–7.
19. Agarwal A, Majzoub A, Esteves SC, Ko E, Ramasamy R, Zini A. Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios. Transl Androl Urol 2016;5:935–50.
20. Evenson DP, Jost LK, Marshall D, Zinaman MJ, Clegg E, Purvis K, et al. Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod 1999;14:1039–49.
21. Brown S. Miscarriage and its associations. Semin Reprod Med 2008;26:391–400.
22. Gil-Villa AM, Cardona-Maya W, Agarwal A, Sharma R, Cadavid A. Assessment of sperm factors possibly involved in early recurrent pregnancy loss. Fertil Steril 2010;94:1465–72.
23. Bareh GM, Jacoby E, Binkley P, Chang TC, Schenken RS, Robinson RD. Sperm deoxyribonucleic acid fragmentation assessment in normozoospermic male partners of couples with unexplained recurrent pregnancy loss: a prospective study. Fertil Steril 2016;105:329–36.e1.
24. P K, Malini SS. Positive association of sperm dysfunction in the pathogenesis of recurrent pregnancy loss. J Clin Diagn Res 2014;8:OC07–10.
25. Ruixue W, Hongli Z, Zhihong Z, Rulin D, Dongfeng G, Ruizhi L. The impact of semen quality, occupational exposure to environmental factors and lifestyle on recurrent pregnancy loss. J Assist Reprod Genet 2013;30:1513–8.
26. Tomlinson M, Lewis S, Morroll D, British Fertility S. Sperm quality and its relationship to natural and assisted conception: British Fertility Society guidelines for practice. Hum Fertil (Camb) 2013;16:175–93.
27. Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, et al. Incidence of early loss of pregnancy. N Engl J Med 1988;319:189–94.