Association between semen quality among men with different occupational exposures and risk of recurrent spontaneous abortion in island residents

Wenli Cao¹, Xinyao Pan^{2,3,4}, Feijun Ye¹, Jing Zhou^{2,3,4}, Zengshu Huang^{2,3,4},
 Chuyu Li^{2,3,4}, Yanpu Zhang¹, Jianwei Fang¹, Yan Jiang¹, Hongyu Lian¹,
 Zhongxing Fu⁵, Yan Du^{2,3,*‡} and Ling Wang^{2,3,4,†‡}

¹*Reproductive Medicine Center of Zhoushan Maternal and
 Child Health Care Hospital
 Zhoushan, Zhejiang, P. R. China*

²*Laboratory for Reproductive Immunology
 Obstetrics and Gynecology Hospital of Fudan University
 Shanghai 200011, P. R. China*

³*The Academy of Integrative Medicine of Fudan University
 Shanghai, P. R. China*

⁴*Shanghai Key Laboratory of Female Reproductive
 Endocrine-Related Diseases
 Shanghai, P. R. China*

⁵*Ningguo Bio-Leader Biotechnology Co., Ltd.
 Anhui, P. R. China*

*sophiedu_61@163.com

†dr.wangling@fudan.edu.cn

Received 4 May 2019; Accepted 27 May 2019; Published 10 July 2019

Objective: This study aimed to evaluate the semen quality of male adults whose spouses suffer from recurrent spontaneous abortion (RSA) in the island area, and to explore the association between RSA and occupational exposures.

Methods: A total of 131 male patients were recruited and divided into two groups: spouse with recurrent spontaneous abortion group (RSA group, $n = 57$) and the normal fertility group (control group, $n = 74$). Information such as height, weight and occupational exposure history of 131 men were obtained. Semen samples were collected and analyzed. Differences in semen parameters and DNA fragmentation index (DFI) between the two groups were compared. Odds ratios (ORs) and their corresponding 95% confidence intervals were calculated to evaluate the association between occupational exposures and RSA status.

Results: Overall, no significant difference was found in sperm concentration, progressive motility and normal morphology rate between RSA and control groups. Only DFI was observed to be significantly higher in the RSA group. Evaluation of the receiver operating characteristic (ROC) curve showed DFI (AUC: 0.623, $p < 0.05$) could discriminate between males from the couples with RSA and without RSA.

Conclusion: The analysis of conventional semen parameters could not directly reflect their influence on embryonic development, and test of integrity of the sperm DNA is of paramount importance to fully understand male fertility. It is crucial to conduct studies regarding occupational exposures and pregnancy loss and/or RSA risk, since it will

‡Corresponding authors.

provide population-level data to aid in the identification of important risk factors that warrant further mechanistic investigation, and eventually lead to effective prevention and treatment strategies.

Keywords: Recurrent spontaneous abortion (RSA); semen quality; DNA fragmentation index (DFI); occupational exposure.

Introduction

Recurrent spontaneous abortion (RSA) refers to two or more consecutive spontaneous miscarriages with the same partner.¹ It has been recognized as one of the most complicated and challenging issues in reproductive medicine, with an estimated prevalence of 26.6% in Chinese couples seeking for medical assistance in the reproductive center according to a recent epidemiological study.² RSA is a heterogeneous disease which involves multiple risk factors. In spite of continuing efforts to identify the causes, its pathogenesis remains unclear. Given that 30% of pregnancies end in miscarriage,³ elucidating the etiology of RSA will aid in providing comprehensive counseling and adapted therapies to the couples.

Due to the intimate maternal relationship with the developing embryo, most identified causes of RSA center around the woman. However, half of the fetal genome is paternal in origin, therefore it is expected that sperm genetic alterations contribute to RSA. Since it is still controversial to use the routine semen analysis to diagnose RSA,^{4–8} the innovation of sperm DNA fragmentation index (DFI) has started a new era for evaluating semen quality. Studies have documented that sperm is not a mere vector of paternal DNA but also plays a critical role in embryogenesis.⁹ Sperm DNA damage is considered to be associated with decreased pregnancy rate in IVF,⁵ increased aneuploidy rate,⁶ gene mutation-related autosomal dominant disorders and childhood cancer.¹⁰

Although semen quality differs by region and ethnicity,^{11–13} a major risk for male reproductive health comes from the contamination of water, air, soil, food and beverages as well as numerous environmental toxicants.¹⁴ The pronounced effects of environmental exposures on male infertility have been suggested by studies conducted on animals as well as humans.^{15–18} Surprisingly, few studies have attempted to assess the influence of male occupational exposures on semen quality and its association with RSA in island residents. The current study aimed to explore the effect of male occupational exposure on semen quality, and its association with RSA in Zhoushan City, an island city which is located to the southeast of Shanghai in Eastern China Coast.

Materials and Methods

Clinical data

From January 2017 to December 2017, male patients who sought treatment for RSA at the Reproductive Center and Department of Embryology and Andrology of Zhoushan

Maternal and Child Health Care Hospital in Zhoushan City were recruited as the case group. Couples in the RSA group were confirmed as having normal chromosomal karyotypes and no reported exposure to toxic or severe radiation in living or working environment. Male patients were excluded if their spouses met the following exclusion criteria: (1) abnormality in immunological examination; (2) endocrine abnormalities such as abnormal thyroid function, luteal dysfunction, polycystic ovary syndrome (PCOS), hyperprolactinemia (PRL) and diabetes; (3) reproductive organ abnormalities including uterine malformation, adenomyosis, hysteromyoma, endometriosis, intrauterine adhesions, cervical incompetence and genital tract infections; (4) history of systemic diseases or malignant tumors such as TORCH positive, severe infection with high fever, severe anemia or heart failure, chronic consumptive disease, liver and kidney diseases and hypertension; (5) tobacco and alcohol addiction. Healthy males who came to the hospital for pregnancy-related examination during the same period, and whose spouses had at least one normal birth and no spontaneous abortion histories, were recruited as controls.

The study protocol conformed to the ethical guidelines of the 2000 Declaration of Helsinki and was approved by the Research Ethics Board at the Reproductive Medicine Center of Zhoushan Maternal and Child Health Care Hospital. Informed consent was obtained from all participants.

Occupational exposure survey

A survey questionnaire was designed by a group of reproductive medicine experts and epidemiologists to assess the occupational exposures. Five main occupational exposure categories were established based on the economic and natural environment characteristics of the islands: (1) seawater contacts (referring to fishermen or sailors engaged in marine fishing); (2) IT workers (mainly specializing in certain IT fields such as computer hardware, software and networking); (3) decorators (including electricians, carpenters and painters engaged in construction); (4) office workers (referring to accountants, secretaries and school teachers); (5) other occupations (including skilled labor, self-employed, security guards and entertainment workers). Occupational exposure history was defined as having continuously engaged in a specific type of occupation over the past one year. A committed research nurse conducted the one-on-one interviews after three standard training sessions. The presence of both husband and wife was required during the entire interview to improve the reliability of the information.

Routine semen analysis and sperm DNA fragmentation detection

Participants were abstinent for 2–7 days before semen collection, which was performed at the laboratory of the Reproductive Center. Whole semen was collected using the masturbation method into a sterile cup and placed in a constant-temperature water bath at 37°C until the semen was completely liquefied. The liquefaction time was recorded, and parameters including concentration, motility and morphology of semen were analyzed by a computer-assisted sperm analysis (CASA) system [Sperm Class Analyzer (SCA); Microptic, Barcelona, Spain]. Sperm freezing agent was added into 0.5–1-mL liquefied semen, and the mixture was frozen for sperm DNA fragmentation detection. Indexes in the fifth edition of the “WHO Laboratory Manual for Human Semen and Sperm–Cervical Mucus Interaction Test” was used as the reference to determine normal semen quality: (1) volume ≥ 1.5 mL; (2) concentration $\geq 15 \times 10^6$ /mL; (3) pH ≥ 7.2 ; (4) progressive motility (a) $> 25\%$ or ($a + b$) $\geq 32\%$; (5) liquefaction time ≤ 60 min; (6) normal morphology rate $\geq 4\%$.¹⁹ DFI was detected using modified sperm chromatin dispersion (SCD) test method, and the sperm DFI test kit (Anke Biological, Anhui, China) was utilized following the manufacturer’s instructions:

DFI = sperms with fragmented DNA count/total sperm count $\times 100\%$.

Based on the ratio of halo diameter and transverse diameter of the sperm head, the halo could be divided into four grades: large, medium, small and no halo. Large and/or medium halo represented complete sperm DNA and no fragment, whereas the small and/or no halo referred to sperm with fragmented DNA. A total of 500 sperms were counted each time, DFI $< 25\%$ was considered as normal.

Statistical analyses

Continuous variables were presented as mean $\pm$ standard deviation (SD), and the differences were compared by Student’s *t*-test or one-way ANOVA if normally distributed. Variables that did not assume Gaussian distribution were analyzed by Mann–Whitney test or Kruskal–Wallis test. Categorical variables were presented as number and percentage (%), and were compared using chi-square tests. Correlation between DFI and other parameters were determined with Pearson correlation coefficient. Logistic regression was performed to investigate the association between RSA and various sperm parameters. The predictive power of semen parameters on RSA was determined by the receiver operating characteristic (ROC) analysis. All above analyses were two-sided and a *p*-value of less than 0.05 was deemed statistically significant. All statistical analyses were

conducted using SPSS 22.0 for Windows (IBM SPSS, IBM Information Management, Armonk, NY, USA).

Results

Population characteristics of RSA cases and controls concerning different occupations

A total of 57 RSA cases and 74 controls were included in the analyses. There were no significant differences in age and BMI between the two groups ($p > 0.05$). The number of pregnancy losses from couples of RSA group ranged from 2 to 5 with a mean of 2.37 losses. There was no significant difference in sperm concentration, progressive motility and normal morphology rate between RSA group and the control group ($p > 0.05$). DFI in the RSA group was 1.2 times higher compared to that of the controls ($16.35 \pm 8.14\%$ versus $13.20 \pm 7.60\%$, $p < 0.05$; see Table 1).

When all subjects were classified by different occupations, a general description about population characteristics is shown in Table S1. Age and BMI are comparable among the five occupational groups ($p > 0.05$). None of the sperm parameters tested showed any significant difference among the five groups ($p > 0.05$).

Stratified analyses were then performed according to the occupational exposure status and reproductive history of the patients (Fig. S1). DFI of the RSA group was found to be significantly higher than that of the control group within the “other” occupational category [$p < 0.05$, Fig. S1(d)], while no other difference was observed.

Risk estimates of RSA cases and controls concerning different occupations

Risk estimates of different occupational exposures are shown in Table 2. There was a higher fraction of RSA in males working as seawater contact and IT worker than in males working as decorator, office worker or others. However, the

Table 1. Population characteristics of the RSA group and the control group.

Variable	RSA ($n = 57$)	Control ($n = 74$)	<i>p</i> -Value
Age (years)	32.89 ± 4.54	34.92 ± 5.86	0.06
BMI (kg/m^2)	23.51 ± 0.74	23.63 ± 0.75	0.35
Pregnancy loss (n , %)			—
2	43 (75.4%)	—	
3	9 (15.8%)	—	
4	3 (7.0%)	—	
5	2 (4.5%)	—	
Sperm concentration ($\times 10^6/\text{mL}$)*	107.17 ± 97.15	107.14 ± 95.15	0.71
Progressive motility (%)	50.81 ± 18.41	47.30 ± 20.49	0.31
Normal morphology rate (%)*	7.12 ± 6.11	5.86 ± 5.05	0.30
DFI (%)	16.35 ± 8.14	13.20 ± 7.60	0.02

Note: *Rank sum test.

Table 2. Risk estimates of RSA among males with different occupations.

Occupation (n, %)	RSA (n = 57)	Control (n = 74)	OR	95% CI	p-Value
Seawater contact	18 (31.6%)	15 (20.3%)	1.82	0.82–4.02	0.14
IT worker	6 (10.5%)	0 (0%)	—	—	0.02
Decorator	13 (22.8%)	18 (24.3%)	0.92	0.41–2.08	0.84
Office worker	11 (19.3%)	19 (25.7%)	0.69	0.30–1.60	0.39
Other	9 (15.8%)	22 (29.7%)	0.44	0.19–1.06	0.06

Note: OR: Odds ratio; 95% CI: 95% confidence interval.

Table 3. Correlation between analyzed sperm parameters and the DFI.

Variable	Correlation coefficient (r)	p-Value
Sperm concentration	−0.08	0.37
Progressive motility	−0.04	0.61
Normal morphology rate	0.07	0.44
Number of miscarriages	0.22	0.01

odds ratios (ORs) and their corresponding 95% confidence intervals (95% CIs) did not show that men having a history of certain occupational exposures were associated with increased risk of RSA.

Correlation analysis of DFI and other semen parameters

Table 3 summarizes the results of correlation analysis between DFI and other sperm measurements. In this population, no significant correlation was detected between DFI and any of the sperm parameters ($p > 0.05$ for all). However, increased DFI was significantly associated with an increase in the number of spontaneous abortions ($p < 0.05$).

Association between RSA, DFI and other semen parameters

Association between semen quality as a dichotomous outcome according to the WHO 5th edition criteria and RSA risk was further analyzed (Table S2). The percentage of males with normal sperm concentration ($\geq 15 \times 10^6/\text{mL}$), progressive motility $\geq 32\%$, normal morphology rate $\geq 4\%$ and

DFI $< 25\%$ was similar between the two groups ($p > 0.05$ for all).

Next, using the above-mentioned dichotomous definition of normal semen quality, we further categorized the population into four subgroups: control with normal semen quality, control with abnormal semen quality, RSA with normal semen quality and RSA with abnormal semen quality. These four groups were balanced in terms of baseline characteristics such as age and BMI. An increasing trend is observed as shown in Fig. S2.

Logistic regression analysis was performed to further investigate the association between RSA and various factors. Our results indicated that DFI was positively associated with the occurrence of RSA, whereas all other variables were not significantly associated with RSA (Table 4).

Predictive values of DFI and other semen parameters in RSA

ROC curve was constructed to evaluate the predictive values of DFI and other sperm measurements in RSA (Fig. 1). The low values of areas under the curve (AUCs) indicated the absence of a predictive power of sperm concentration, progressive motility and normal morphology with respect to RSA. Only DFI (AUC: 0.623, $p < 0.05$) presented a mild capacity to adequately discriminate between males from the couples with RSA and without RSA. The combination of all four parameters had a higher AUC of 0.653 ($p < 0.05$), which is an optimal predictor of RSA.

Discussion

Sperm provides about half of the genetic information for the fertilized egg and plays an important role in embryonic development. Sperm concentration, motility, morphology and DFI are widely used as indicators for clinical evaluation of male infertility. The current study indicated that DFI may be associated with RSA risk, but not the parameters from routine semen analysis (sperm concentration, motility and normal morphology rate). Further subgroup analysis revealed that DFI value was the highest among the RSA group with

Table 4. Logistic regression analysis results between RSA risk and sperm parameters.

Variable	Crude OR (95% CI)	Adjusted OR (95% CI)*	Fully adjusted OR (95% CI)†
Sperm concentration	1.00 (0.996–1.004)	0.999 (0.995–1.003)	0.999 (0.995–1.003)
Progressive motility	1.01 (0.99–1.03)	1.01 (0.99–1.02)	1.01 (0.99–1.02)
Normal morphology rate	1.04 (0.97–1.11)	1.03 (0.97–1.10)	1.03 (0.96–1.10)
DFI	1.05 (1.01–1.10)	1.06 (1.01–1.11)	1.06 (1.01–1.11)

Notes: OR: Odds ratio; 95% CI: 95% confidence interval;

*adjusted for age and BMI;

†adjusted for age, BMI, sperm concentration, progressive motility, normal morphology rate and DFI.

Diagnostic value of various sperm parameters

Variable	AUC (95%CI)	P
Sperm concentration	0.519 (0.420-0.619)	0.71
Progressive motility	0.550 (0.450-0.649)	0.33
Normal morphology rate	0.553 (0.453-0.652)	0.30
DFI	0.623 (0.526-0.720)	0.02
Combination	0.653 (0.558-0.748)	0.003

Figure 1. ROC curve analysis of the predictive values of sperm parameters to forecast RSA.

abnormal semen quality. Evaluation of the receiver operating characteristic curve showed that DFI, combined with routine semen analysis, is somewhat of clinical predictive value for the diagnosis of RSA. When considering the contribution of occupational exposure, although significant quantitative differences could be seen, there was no convincing evidence that occupation correlated with sperm quality in RSA patients. To the best of our knowledge, it is the first study evaluating the effect of occupational exposure on male semen quality of RSA couples in the island environment.

Studies on assisted reproductive technology (ART) have proposed that the risk factors for RSA in males include age, chromosomal abnormalities, low percentage of morphologically normal sperm, anti-sperm antibody positive, sperm nuclear abnormalities, sperm DFI and other factors that may affect the survival rate and motility of the sperm.²⁰ During the search of pathogenic factors of RSA in males, it is

difficult if not impossible to gain a thorough understanding of male fertility if only focusing on the routine semen analysis. Therefore, our study analyzed sperm concentration, motility, morphology as well as DFI in male participants, and observed different roles those indexes play in RSA. Our study results are similar to previous studies. In a prospective cohort of 344 couples attempting to conceive, general semen parameters were not related with the risk of pregnancy loss or recurrent pregnancy loss, whereas DNA fragmentation was significantly associated with pregnancy loss.⁴ In a study conducted among Italian couples, the conventional seminal profile of patients with RSA was similar to that of fertile patients. Despite good spermatogenesis, however, sperm DNA integrity was impaired in the RSA group, with DFI being significantly higher than that of the fertile controls.⁵ Recent meta-analyses demonstrated that patients with high DNA damage experienced significantly increased

miscarriage compared with those who had low DNA damage, regardless of IVF/ICSI cycles.^{21–23}

Studies regarding parameters from the routine sperm analysis in relation to RSA seemed contradictory. A study on 22 Tunisian men from RSA couples showed a significantly lower sperm progressive motility, significantly higher morphological abnormalities and DFI.⁶ Similar results of lower sperm motility were also reported by another two studies. While one study from Iran reported a higher DFI but not abnormal morphology in the RSA group,⁷ another study in France suggested a higher abnormal morphology in the RSA group, but no difference in DFI.⁸ Our study results suggested that routine semen analysis, which is traditionally recommended as the first step in the guideline,²⁴ is inadequate to determine the reproductive outcome. It is likely that the anomalies can be detected by sperm functionality in DFI, but not in morphologic examination using the conventional methods. Some RSA females whose husbands have normal routine semen parameters could also conceive, however, the problem remains as to why those fertilized embryos fail to produce term pregnancy. Both animal and human studies have demonstrated that sperm DNA integrity is crucial for paternal transmission of information to the embryo.²⁵ Changes of metabolic behavior in embryos may stem from DNA lesions which compromise embryonic development. A positive correlation between the value of DFI and frequency of miscarriage loss in the current study suggested that sperm DNA damage could be associated with loss of genes involved in normal embryonic development. Tesarik *et al.*²⁶ first proposed the concept of “late paternal effect” as a non-perceptible deterioration of zygote and cleaving embryo morphology along at the early stage of embryo development, which was associated with an increased percentage of sperm DNA fragmentation that leads to repeated ART failures. The notion was supported by Simon *et al.* as they showed that the progression of development from good-quality day-3 embryos to blastocysts is highly influenced by the degree of sperm DNA damage.²⁷ The probability of eliminating an embryo fertilized by a sperm with fragmented DNA is higher at the blastocyst stage than at the cleavage stage. Although human oocytes are well equipped to handle DNA lesions in multiple ways including direct reversal of damage, single-strand damage repair, base excision repair and nucleotide excision,²⁸ there is a threshold beyond which the lesion may not be repaired.⁹ Thus, the incomplete repair of sperm DNA damage by the oocyte produced by RSA females may lead to miscarriages.

Many types of insults have been considered to provoke DNA damage mainly through two pathways: an apoptotic process causing activation of endonucleases and a direct attack on DNA by free radicals that produce both base oxidation and strand breaks.²⁹ The finding that reduced fecundity in men correlates to an increase in reactive oxygen

species (ROS) generation suggested that an oxidative attack on the DNA might be a reason for defective human spermatozoa.³⁰ An increased semen oxidative stress and DNA fragmentation, combined with reduced testicular steroidogenesis, was found in male partners of women with RSA.³¹ Furthermore, defects in the telomere structure are prone to make sperm chromosome ends more accessible to oxidative radicals,^{32,33} which, in turn, might contribute to an increase in the percentage of sperm DNA lesions.³⁴ Interestingly, in the examined normal morphology rate of spermatozoa in this study, the frequencies of morphological sperm abnormalities in the two groups were only 66.7% and 60%, respectively, suggesting that other factors such as island environment may be responsible.

As a possible extrinsic environment-derived risk factor of miscarriage, the role of environmental exposure has been continuously discussed. It is well documented that outdoor air pollutants (PM_{2.5}, PM₁₀, SO₂, NO_x, O₃, PAHs) could affect at least one of the semen parameters (DNA fragmentation, morphology and motility).³⁵ It is well established that smoking is associated with impaired male fertility, increased DNA damage, aneuploidies and mutations in sperms. These effects could be heritable if smoking-induced *de novo* mutations occur in the sperms and thus can pass on from fathers to offspring.³⁶ Other studies have shown an association between sperm quality and endocrine disruptors ranging from phthalates (PCBs), bisphenol A (BPA), organophosphate pesticides, perfluoroalkyl compounds (PFCs) to metal cadmium.^{37,38}

Despite numerous researches providing evidences that chemical toxicants are adversely correlated to male reproduction, studies on island environmental factors are sparse. A European study on fertility (INUENDO) revealed a much higher concentration of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153), an organochlorine pollutant (POP), in Inuits and Swedish fishermen compared to men from Eastern European countries. What is more, the concentration of CB-153 was inversely correlated with sperm quality.^{39,40} The effects of chemical-induced alterations on general sperm characteristics and DNA integrity among different populations are contradictory.^{40–44} Dietary seafood and area of residence are important for the POP biomarker concentrations since POP accumulates in seafood from the combination of environmental conditions and biomagnification in the aquatic food chains.⁴⁵ An assessment conducted on the Seine Estuary showed significant abnormal sperm DNA integrity within the native population of *Palaemon longirostris*, which may be caused by the accumulation of micro-pollutants such as polycyclic aromatic hydrocarbons (PAHs), trace metals and PCB.⁴⁶ More importantly, sperm DNA damages persist even 20 days after toxic stress cessation.

The present study was conducted in Zhoushan, a port city located at the juncture of China eastern coastline and Yangtze waterway. With more than 1,390 islands and over 270 miles

deep-water shoreline, it is the largest archipelago in China. Fieldworks^{47–49} carried out by local researchers revealed a pronounced PCB accumulation in surface sediments, and organochlorine pesticides (OCPs) especially hexachlorocyclohexane (HCH) and dichloro-diphenyl-trichloroethane (DDT) in seafood from the area.⁴⁷ However, we did not detect significant impairment of semen quality in association with various vocations, only a declining trend of morphologically normal sperm in both RSA and control groups. It is noteworthy to mention that other environmental exposures also existed as well as the occupational exposure in our study population. The men with seawater contact, for instance, could be exposed to all kinds of ocean contaminants, such as natural or synthetic organics or minerals. The same population is also vulnerable to occupational noise exposure and solar radiation exposure.^{50,51} As a result, verification of potential determinants specific to one particular occupation can be challenging. Additionally, it is not yet clear the threshold of certain exposure in semen to alter the DNA structure, or how much DNA fragmentation would have serious implications on miscarriage. Our study has several limitations. First, as a cross-sectional study, it is difficult to infer causality. Then, our sample size is not adequate for multiple comparisons, particularly in the subgroup analyses. Future studies need to be carried out prospectively with a large sample size. Last, our questionnaire is not designed to quantitatively assess occupational exposure, but to merely classify participants into a general occupational category.

To summarize, to the best of our knowledge, this is the first study of the relationship among sperm quality, RSA and occupational exposure in the island environment. Our results showed that functional test of sperm DNA fragmentation was related to RSA risk, whereas the conventional semen analysis had no predictive value of RSA, suggesting DNA fragmentation assay may be a preferred diagnostic method in clinical application. It is crucial to conduct studies regarding occupational exposures and pregnancy loss and/or RSA risk, since it will provide population-level data to aid in the identification of important risk factors that warrant further mechanistic investigation, and eventually lead to effective prevention and treatment strategies.

Conflict of Interest

There is no conflict of interest to disclose.

Acknowledgments

This work was supported by the Science and Technology Commission of Shanghai Municipality (2015 YIXUEYINGDAO Project No. 15401932200 and 2018 YIXUEYINGDAO Project No. 18401902200; to Ling Wang), the

Shanghai Committee of the China Democratic League (No. 02054; to Ling Wang), the National Natural Science Foundation of China (Nos. 30801502 and 31571196; to Ling Wang), the FY2008 JSPS Postdoctoral Fellowship for Foreign Researchers (P08471; to Ling Wang), the Shanghai Pujiang Program (No. 11PJ1401900; to Ling Wang) and the Development Project of Shanghai Peak Disciplines-Integrative Medicine (No. 20150407). Wenli Cao and Xinyao Pan contributed equally to this work.

References

1. Practice Committee of American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss: A committee opinion. *Fertil Steril* 2013;99:63.
2. Wang HY, Qiao J, Sun XX, Wang SY, Liang XY, Sun Y, Liu FH. Epidemiological survey and risk factor analysis of recurrent spontaneous miscarriages in infertile women at large infertility centers. *Chin Med J (Engl)* 2017;130:2056–62.
3. Practice Committee of American Society for Reproductive Medicine. Evaluation and treatment of recurrent pregnancy loss: A committee opinion. *Fertil Steril* 2012;98:1103–11.
4. Eisenberg ML, Sapra KJ, Kim SD, Chen Z, Louis GMB. 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. *Fertil Steril* 2017;108:613–9.
5. Carlini T, Paoli D, Pelloni M, Faja F, Dal Lago A, Lombardo F, Lenzi A, Gandini L. Sperm DNA fragmentation in Italian couples with recurrent pregnancy loss. *Reprod Biomed Online* 2017;34:58–65.
6. Zidi-Jrah I, Hajlaoui A, Mougou-Zerelli S, Kammoun M, Meniaoui I, Sallem A, Brahem S, Fekih M, Bibi M, Saad A, Ibala-Romdhane S. Relationship between sperm aneuploidy, sperm DNA integrity, chromatin packaging, traditional semen parameters, and recurrent pregnancy loss. *Fertil Steril* 2016;105:58–64.
7. Kamkar N, Ramezanali F, Sabbaghian M. The relationship between sperm DNA fragmentation, free radicals and antioxidant capacity with idiopathic repeated pregnancy loss. *Reprod Biol* 2018;18:330–5.
8. Esquerre-Lamare C, Walschaerts M, Chansel Debordeaux L, Moreau J, Bretelle F, Isus F, Karsenty G, Monteil L, Perrin J, Papaxanthos-Roche A, Bujan L. Sperm aneuploidy and DNA fragmentation in unexplained recurrent pregnancy loss: A multicenter case-control study. *Basic Clin Androl* 2018;28:4.
9. Kumar K, Deka D, Singh A, Mitra DK, Vanitha BR, Dada R. Predictive value of DNA integrity analysis in idiopathic recurrent pregnancy loss following spontaneous conception. *J Assist Reprod Genet* 2012;29:861–7.
10. Conti SL, Eisenberg ML. Paternal aging and increased risk of congenital disease, psychiatric disorders, and cancer. *Asian J Androl* 2016;18:420–4.
11. Elbardisi H, Majzoub A, Al Said S, Al Rumaihi K, El Ansari W, Alattar A, Arafa M. Geographical differences in semen characteristics of 13 892 infertile men. *Arab J Urol* 2018;16:3–9.
12. Consales C, Leter G, Bonde JP, Toft G, Eleuteri P, Moccia T, Budillon A, Jonsson BA, Giwercman A, Pedersen HS,

- Ludwicki JK, Zvezdai V, Heederik D, Spano M. Indices of methylation in sperm DNA from fertile men differ between distinct geographical regions. *Hum Reprod* 2014;29:2065–72.
13. Khandwala YS, Zhang CA, Li S, Behr B, Guo D, Eisenberg ML. Racial variation in semen quality at fertility evaluation. *Urology* 2017;106:96–102.
14. Muratori M, De Geyter C. Chromatin condensation, fragmentation of DNA and differences in the epigenetic signature of infertile men. *Best Pract Res Clin Endocrinol Metab* 2018; doi: 10.1016/j.beem.2018.10.004.
15. Minguez-Alarcon L, Afeiche MC, Williams PL, Arvizu M, Tanrikut C, Amarasiwardena CJ, Ford JB, Hauser R, Chavarro JE. Hair mercury (Hg) levels, fish consumption and semen parameters among men attending a fertility center. *Int J Hyg Environ Health* 2018;221:174–82.
16. Singh S, Singh SK. Chronic exposure to perfluorononanoic acid impairs spermatogenesis, steroidogenesis and fertility in male mice. *J Appl Toxicol* 2018;39(3):420–31.
17. Zhai J, Geng X, Ding T, Li J, Tang J, Chen D, Cui L, Wang Q. An increase of estrogen receptor alpha protein level regulates BDE-209-mediated blood-testis barrier disruption during spermatogenesis in F1 mice. *Environ Sci Pollut Res Int* 2018;26(5):4801–20.
18. Tian M, Liu L, Zhang J, Huang Q, Shen H. Positive association of low-level environmental phthalate exposure with sperm motility was mediated by DNA methylation: A pilot study. *Chemosphere* 2018;220:459–67.
19. World Health Organization. *WHO Laboratory Manual for the Examination and Processing of Human Semen*. 5th ed. Geneva: World Health Organization, 2010:13,16,26,44,100.
20. 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.
21. Robinson L, Gallos ID, Conner SJ, Rajkhowa M, Miller D, Lewis S, Kirkman-Brown J, Coomarasamy A. The effect of sperm DNA fragmentation on miscarriage rates: A systematic review and meta-analysis. *Hum Reprod* 2012;27:2908–17.
22. Zhao J, Zhang Q, Wang Y, Li Y. Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after *in vitro* fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis. *Fertil Steril* 2014;102:998–1005.e8.
23. Tan J, Taskin O, Albert A, Bedaiwy M. Association between sperm DNA fragmentation and idiopathic recurrent pregnancy loss: A systematic review and meta-analysis. *Reprod Biomed Online* 2018;38(6):951–60.
24. Jungwirth A, Diemer T, Kopa Z, Krausz C, Tournaye H, Kelly B, Pal R. EAU Guidelines on Male Infertility 2017. Arnhem, The Netherlands: European Association of Urology, 2017:223.
25. Uppangala S, Pudukalakatti S, D'Souza F, Salian SR, Kalthur G, Kumar P, Atreya H, Adiga SK. Influence of sperm DNA damage on human preimplantation embryo metabolism. *Reprod Biol* 2016;16:234–41.
26. Tesarik J, Greco E, Mendoza C. Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. *Hum Reprod* 2004;19:611–5.
27. Simon L, Murphy K, Shamsi MB, Liu L, Emery B, Aston KI, Hotaling J, Carrell DT. Paternal influence of sperm DNA integrity on early embryonic development. *Hum Reprod* 2014;29:2402–12.
28. Menezo Y, Dale B, Cohen M. DNA damage and repair in human oocytes and embryos: A review. *Zygote* 2010;18:357–65.
29. Rex AS, Aagaard J, Fedder J. DNA fragmentation in spermatozoa: A historical review. *Andrology* 2017;5:622–30.
30. Aitken RJ, Bronson R, Smith TB, De Iuliis GN. The source and significance of DNA damage in human spermatozoa; a commentary on diagnostic strategies and straw man fallacies. *Mol Hum Reprod* 2013;19:475–85.
31. Jayasena CN, Radia UK, Figueiredo M, Revill LF, Dimakopoulou A, Osagie M, Vessey W, Regan L, Rai R, Dhillon WS. Reduced testicular steroidogenesis and increased semen oxidative stress in male partners as novel markers of recurrent miscarriage. *Clin Chem* 2019;65:161–9.
32. Raha S, Robinson BH. Mitochondria, oxygen free radicals, disease and ageing. *Trends Biochem Sci* 2000;25:502–8.
33. Liu L, Trimarchi JR, Smith PJ, Keefe DL. Mitochondrial dysfunction leads to telomere attrition and genomic instability. *Aging Cell* 2002;1:40–6.
34. Rocca MS, Speltra E, Menegazzo M, Garolla A, Foresta C, Ferlin A. Sperm telomere length as a parameter of sperm quality in normozoospermic men. *Hum Reprod* 2016;31:1158–63.
35. Jurewicz J, Dziewirska E, Radwan M, Hanke W. Air pollution from natural and anthropic sources and male fertility. *Reprod Biol Endocrinol* 2018;16:109.
36. Beal MA, Yauk CL, Marchetti F. From sperm to offspring: Assessing the heritable genetic consequences of paternal smoking and potential public health impacts. *Mutat Res* 2017;773:26–50.
37. Mima M, Greenwald D, Ohlander S. Environmental toxins and male fertility. *Curr Urol Rep* 2018;19(7):50.
38. Di Nisio A, Foresta C. Water and soil pollution as determinant of water and food quality/contamination and its impact on male fertility. *Reprod Biol Endocrinol* 2019;17(1):4.
39. Jonsson BA, Rylander L, Lindh C, et al. Inter-population variations in concentrations, determinants of and correlations between 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p'-DDE): A cross-sectional study of 3161 men and women from Inuit and European populations. *Environ Health* 2005;4:27.
40. Toft G, Rignell-Hydbom A, Tyrkiel E, et al. Semen quality and exposure to persistent organochlorine pollutants. *Epidemiology* 2006;17:450–8.
41. Toft G. Persistent organochlorine pollutants and human reproductive health. *Dan Med J* 2014;61:B4967.
42. Rignell-Hydbom A, Rylander L, Giwercman A, Jonsson BA, Lindh C, Eleuteri P, Rescia M, Leter G, Cordelli E, Spano M, Hagmar L. Exposure to PCBs and p,p'-DDE and human sperm chromatin integrity. *Environ Health Perspect* 2005;113:175–9.
43. Rignell-Hydbom A, Rylander L, Giwercman A, Jonsson BA, Nilsson-Ehle P, Hagmar L. Exposure to CB-153 and p,p'-DDE and male reproductive function. *Hum Reprod* 2004;19:2066–75.
44. Toft G, Axmon A, Giwercman A, Thulstrup AM, Rignell-Hydbom A, Pedersen HS, Ludwicki JK, Zvezdai V, Zinchuk A, Spano M, Manicardi GC, Bonefeld-Jorgensen EC, Hagmar L, Bonde JP. Fertility in four regions spanning large contrasts in serum levels of widespread persistent organochlorines: A cross-sectional study. *Environ Health* 2005;4:26.
45. Artic Monitoring and Assessment Programme. Human Health in the Arctic. Oslo: AMAP, 2003:104.

46. Erraud A, Bonnard M, Geffard O, Chaumot A, Duflot A, Geffard A, Forget-Leray J, Xuereb B. Assessment of sperm DNA integrity within the *Palaemon longirostris* (H. Milne-Edwards, 1837) population of the Seine estuary. *Environ Pollut* 2018;245:485–93.
47. Zhou S, Pan Y, Zhang L, Xue B, Zhang A, Jin M. Bio-magnification and enantiomeric profiles of organochlorine pesticides in food web components from Zhoushan Fishing Ground, China. *Mar Pollut Bull* 2018;131:602–10.
48. Wang JY, Yu XW, Fang L. Organochlorine pesticide content and distribution in coastal seafoods in Zhoushan, Zhejiang Province. *Mar Pollut Bull* 2014;80:288–92.
49. Wang X, Xu H, Zhou Y, Wu C, Kanchanopas-Barnette P. Spatial distribution and sources of polychlorinated biphenyls in surface sediments from the Zhoushan Archipelago and Xiangshan Harbor, East China Sea. *Mar Pollut Bull* 2016;105:385–92.
50. Levin JL, Curry, 3rd WF, Shepherd S, Nalbone JT, Nonnenmann MW. Hearing loss and noise exposure among commercial fishermen in the Gulf Coast. *J Occup Environ Med* 2016;58:306–13.
51. Lee DH, Park KS, Cho MC. Laboratory confirmation of the effect of occupational sun exposure on serum 25-hydroxyvitamin D concentration. *Medicine (Baltimore)* 2018;97:e11419.