Original Article

SARS-CoV-2 vaccination and semen quality: a study based on sperm donor candidate data in southwest China

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Background: The coronavirus disease 2019 (COVID-19) pandemic has been a global health crisis and continues to pose risk to population health at the present. Vaccination against this disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus has become a public health priority worldwide. Yet, limited information is available on the potential impact of such vaccines on human fertility.

Methods: To examine the relationship between COVID-19 vaccination and male fertility, we conducted an observational study on sperm donor candidates in China who received Chinese COVID-19 vaccines between January 1, 2020 to December 31, 2021.

Results: A total of 2,955 semen samples from 564 individuals were assessed along with vaccination information. Statistical analyses were conducted on both the entire study population and the subgroup of individuals who provided repeated semen samples before and after vaccination. While motility related parameters [progressive rate, curvilinear velocity (VCL), average path velocity (VAP), straight-line velocity (VSL), wobble (WOB), straightness (STR), linearity (LIN), amplitude of lateral head displacement (ALH), beat-cross frequency (BCF)] exhibited statistically significant difference before and after vaccination based on Welch two-sample test, mixed effects regression results based on repeated measures from the same individuals indicated that vaccination was not statistically associated with sperm quality parameters except for VCL, VAP, and VSL. Individual variability was the key determinant of sperm quality variance, with contribution ranging from 19% to 82%.

Conclusions: Findings from our study could help to enhance current understanding of male reproductive health in the context of the global pandemic.

Keywords: Sperm quality; severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); coronavirus disease 2019 vaccination (COVID-19 vaccination); male fertility; mixed effects model

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Introduction

Numerous reports have indicated that sperm quality in men is declining at the population level (1-3). Non-genetic factors, such as environmental exposure and lifestyle, may be the most important factors contributing to the observed trends (4). Since December 2019, the global coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in high morbidity and mortality worldwide. It has affected social interaction, public health, and economics across the world (5). Vaccination against COVID-19 has become the most important method to reduce the COVID-19 morbidity and mortality burden (6). Consequently, the potential side effects from COVID-19 vaccines are of intense clinical, public health, and societal interests. Since the global sperm quality decline is of concern and male fertility plays a key role in human reproduction, the potential impact of SARS-CoV-2 vaccination on the male fertility is of particular interest.

Early studies have established that SARS-CoV-2 can impact on male reproductive system. Some scientific evidence has shown that COVID-19 infection can adversely affect testes and sperm quality (7-9), but other studies suggest that SARS-CoV-2 genetic material was rarely found in semen samples, with no observed viral transmission during sexual contact or assisted reproductive techniques (10-12). Recent bioinformatics evidence shows that the testis is a suspected target organ for SARS-CoV-2, and it is potentially vulnerable towards SARS-CoV-2 infection. Angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) have been shown to be highly expressed in spermatogonia and Sertoli and Leydig cells (13,14). Yet, SARS-CoV-2 infects host cells through ACE2 receptors, and TMPRSS2 plays a major role in SARS-CoV-2's entry into cell (14). Furthermore, COVID-19 infection may cause testicular structure damage (7,13,15-17). According to histopathology and ultrastructural findings from COVID-19 fatalities, the SARS virus is prevalent in testicular tissue and causes testicular lesions by orchitis, vascular changes, basal membrane thickening, Leydig and Sertoli cell scarcity (13,16).

Because COVID-19 infection can result in serious illness and injury of multiple organs, and even deaths, developing vaccines against SARS-CoV-2 has been of utmost priority (18). The safety, efficacy, tolerability, and immunogenicity of these vaccines are emphasized in a number of clinical trials. To date, the majority of COVID-19 vaccines demonstrate relatively excellent efficacy based on published data of phase III clinical trials, and the risk of adverse events is acceptable (19,20). Nevertheless, although mass vaccination against SARS-CoV-2 has been authorized and implemented worldwide since 2020, their epidemiological impact on male fertility is not well understood, especially at the population level. A joint statement from the Society for Male Reproduction and Urology and the Society for the Study of Male Reproduction advises that COVID-19 vaccination should be offered to fertile men of reproductive age if they meet the vaccination criteria (https://connect.asrm.org/smru/forprofessionals/covid/new-page?ssopc=1). However, results from surveys conducted on couples preparing for pregnancy indicate that attitudes towards COVID-19 vaccination range from hesitant to refusal (21-25).

Vaccination has evolved into a routine and highly effective method for illness prevention during the COVID-19 pandemic. In China, at least three types of COVID-19 vaccines, which are inactivated SARS-CoV-2 vaccine, mRNA vaccine, and recombinant protein vaccines respectively, have received emergency-use approval (https://www.chinacdc.cn/jkzt/crb/zl/

Highlight box

Key findings

- An observational study was conducted on 2,955 semen samples from 564 sperm donor candidates in China who received the Chinese coronavirus disease 2019 (COVID-19) vaccines between January 1, 2020 to December 2021.
- Statistical analyses were conducted on both the entire study population and a subgroup of individuals who provided semen samples pre- and post-vaccination. Mixed effects regression model was constructed to analyze the potential association between semen quality and vaccination.
- Semen quality parameters were not statistically associated with vaccination, and individual variability is the key determinant of sperm quality variance.

What is known and what is new?

- Vaccination has evolved into a routine and highly effective method for illness prevention during the COVID-19 pandemic.
- The potential side effects of male reproductive health from COVID-19 vaccines are of intense clinical, public health, and societal interests.

What is the implication, and what should change now?

- Current finding is in line with previous reported results and could provide more evidence to the understanding of male reproductive health in the context of global epidemic

szkb_11803/jszl_12208/202103/t20210329_225214.html) based on their high efficacies and safety levels (26-31). The first type is an inactivated SARS-CoV-2 vaccine manufactured by Sinovac (PiCoVacc), the Beijing Institute of Biological Products (BBIBP-CorV), and the Wuhan Institute of Biological Products (Sinopharm-Wuhan inactivated vaccine). The second type is an mRNA vaccine manufactured by CanSinoBIO based on an adenovirus in partnership with the Chinese Academy of Military Medical Sciences. The third type, a recombinant new coronavirus vaccine [Chinese hamster ovary (CHO) cells, which are stably expressing SARS-CoV-2 spike protein], is produced by Zifivax using a protein to trigger an immune response (18). PiCoVacc9 and BBIBP-CorV10 are widely used, eliciting neutralizing antibodies (NAbs) in mice, rats, and non-human primates, with the nonhuman primates in the high-dose group being fully protected from infection by SARS-CoV-2 with no antibody-dependent enhancement (ADE) (18,32), while ADE has been observed in clinical cases (33,34). Evaluations of COVID-19 vaccine safety and efficacy have shown that vaccination can yield excellent efficacy against the original strain and some variants, while the risk of adverse events is acceptable (19,35-37). As of November 28, 2022, more than 3.44 billion vaccine doses had been provided freely for all people in China. China's State Council stated that more than 1.34 billion people, or 92.54% of national population, had received two vaccine doses (http://www.gov.cn/xinwen/gwylflkjz216/index.htm).

In light of the long-term decreasing trend in human fertility and the ongoing COVID-19 pandemic, we examined the relationship between sperm quality and COVID-19 vaccination in China by analyzing data from a provincial sperm bank. To conduct this observational study, we recruited young adult men aged 20 to 45 years who applied to be sperm donors at Sichuan Human Sperm Bank of China, conducted survey to collect habitual information, and assessed sperm quality parameters before and after the administration of COVID-19 vaccination to understand the association between vaccination and sperm quality. This assessment is one of the first attempts to investigate the vaccination effect on male reproductive health using an epidemiological approach in China. We hope the findings could contribute to scientific knowledge about male reproductive health in the global pandemic era. We present this article in accordance with the STROBE reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-23-395/rc).

Methods

Study population

To investigate the potential effect of COVID-19 vaccination on sperm quality in China, young adult men of age 20 to 45 years old who were sperm donor candidates at the Sichuan Human Sperm Bank of China were recruited between January 1, 2020 to December 31, 2021. And the sperm donor candidates, who have been ever infected with COVID-19, were excluded in the study. More information about Sichuan Human Sperm Bank of China and sperm donor recruitment could be obtained from previously published studies (38,39).

At the time of semen sample collection, the following demographic information was obtained through in-take survey: weight, height, age, education level, ethnicity, abstinence time, smoking and drinking history, residential address at the time of sample collection, and working address during study period. Information on SARS-CoV-2 vaccination (vaccination date, vaccination type, and dose number) and residential address at the time of sample collection was collected using self-reported electronic survey retrospectively.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Ethics approval for this study was obtained from the Institutional Review Board of West China Second University Hospital of Sichuan University (No. WCSUH-SCU IRB 2021-197), and individual consent for this retrospective analysis was waived.

Semen analysis

Semen collection and analysis followed the guidelines of World Health Organization (WHO) [2010]. In brief, semen samples were collected into a sterile container by masturbation. After collection, the sample was immediately incubated at 37 ℃ and evaluated directly. The volume of semen was obtained by weighing. Sperm concentration, motility, movement parameters were determined according to the WHO 2010 recommendations, measured by the Makler chamber and Computer Assisted Sperm Analysis (CASA) system [including sperm concentration (millions/mL), progressive sperm rate (%), average path velocity (VAP, μm/s), curvilinear velocity (VCL, μm/s), amplitude of lateral head displacement (ALH, μm/s), linearity (LIN, %), straight-line velocity (VSL, μm/s), beat-cross frequency

(BCF, times/s), straightness (STR, %), and wobble (WOB, %)]. More information on semen analysis could be found in our earlier study (39).

Air pollution data and exposure assessment

To estimate person-specific exposure, we extracted each study individual's residential address and converted the longitude and latitude to grid cells code at 0.01° resolution, then we matched the environmental data with the individual's address grid. We created a grid with 0.01° resolution and then aligned the environmental data and the individuals' addresses to the predefined grid. The daily full-coverage particulate matter $(PM)_{2.5}$ (μg/m^3), PM_{10} (μg/m^3), O_3 (μg/m^3), NO_2 (μg/m^3), SO_2 (μg/m^3), and CO (mg/m^3) concentrations, as well as daily temperature (℃) and relative humidity (%) were estimated to match the address grids. Hourly raw meteorological data with a spatial resolution of 0.25° were obtained from the National Oceanic and Atmospheric Administration (NOAA, https://nomads.ncep.noaa.gov/). We resampled the temperature and relative humidity into 0.01° grid cells by using bilinear interpolation. We collected the hourly concentrations of six air pollutants from the China National Environmental Monitoring Centre (CNEMC, http://www.cnemc.cn/), including $PM_{2.5}$, PM_{10}, O_3, NO_2, SO_2, and CO. For satellite retrievals, we obtained the aerosol optical depth (AOD) data from the Himawari-8 and the Multi-Angle Implementation of Atmospheric Correction (MAIAC) products for estimating $PM_{2.5}$ and PM_{10} (40,41). We also collected the tropospheric NO_2 columns and the total SO_2 (and O_3, CO) columns from the Sentinel-5P TROPOspheric Monitoring Instrument (TROPOMI) products to estimate the surface concentrations of gaseous pollutants (NO_2, SO_2, O_3, and CO) (40-42). Based on the air quality measurements, satellite retrievals, and various auxiliary variables such as meteorological conditions, population density, and land use types, we developed six separate machine learning models (i.e., extreme gradient boosting) to estimate the gridded concentrations of each air pollutant. The hourly estimations of six air pollutants, temperature, and relative humidity were then averaged to daily mean values. For details of the modeling process and prediction performance, please refer to the previous studies (42-44).

Statistical analysis

Two statistical models were constructed to analyze the potential association between vaccination and sperm quality. First, a generalized linear model examined whether vaccination (before or after vaccination) has significant association with each sperm quality parameter separately. The sperm quality parameters included indicators for semen characteristics (such as volume) and sperm motility (such as progressive rate). The model was adjusted for time between vaccination and semen sample collection, age, body mass index (BMI), education level, ethnicity, abstinence duration (days), drinking status (yes/no), smoking status (yes/no), 90-day average concentrations of air pollutants ($PM_{2.5}$, PM_{10}, O_3, SO_2, NO_2 and CO), 90-day average weather parameters (temperature and relative humidity) and vaccine manufacturer. Previous studies found statistically significant associations between the 90-day average pollutant concentrations and weather parameters with sperm quality parameters (38,39). Second, a mixed-effects model used the same aforementioned dependent, independent, and covariate variables as fixed affects, with individual identity document (ID) included as the random effect.

A subgroup analysis was conducted for semen samples that were collected between 60 and 90 days (inclusive) after first vaccination shot. The same two models (generalized linear and mixed effects) were constructed for this subgroup analysis, using the same model structure and variables. Sperm quality before and after vaccination was analyzed using two sample or pairwise *t*-test with significance level at 0.05.

Results

Based on sample selection criteria, a total of 2,955 semen samples collected from 564 individuals between January 1, 2020 to December 31, 2021 were included in our study. All study participants received at least one COVID-19 vaccine dose, and most (94.11%) received two doses. Out of the 564 individuals, 506 (89.72%) were vaccinated with an inactivated COVID-19 vaccine, while 10 (1.77%) and 7 (1.24%) were vaccinated with mRNA vaccine and recombinant new coronavirus vaccine (CHO cells), respectively (*Table 1*).

The study population was concentrated in metropolitan

Table 1 Summary statistics of study population

Population characteristics	Values (N=564, n=2,955)
Age (years), mean (SD)	25.11 (5.22)
Height (cm), mean (SD)	173.8 (5.19)
Weight (kg), mean (SD)	67.91 (8.94)
BMI (kg/m^2), mean (SD)	22.56 (5.13)
Ethnicity, N (%)	
Han	533 (94.50)
Yi	7 (1.24)
Manchu	6 (1.06)
Qiang	4 (0.71)
Others	14 (2.48)
Education level, N (%)	
High school and below	50 (8.87)
College	423 (75.00)
Graduate school	82 (14.54)
NA	9 (1.60)
Marriage status, N (%)	
Single, never married	490 (86.88)
Married	73 (12.94)
Divorced	1 (0.18)
Vaccination information, N (%)	
Received one shot	2,955 (100.00)
Received two shots	2,781 (94.11)
Received three shots	175 (5.92)
Drinking and smoking habits, N (%)	
Smoking frequency	
1–3 times/week	54 (9.57)
4–6 times/week	21 (3.72)
7 times or more/week	30 (5.32)
Never	459 (81.38)
Smoking amount, N (%)	
1–5/day	82 (14.54)
6–12/day	23 (4.08)
0/day	459 (81.38)
Drinking frequency, N (%)	
1–3 times/week	216 (38.30)
4–6 times/week	1 (0.18)
7 times or more/week	2 (0.35)
Never	345 (61.17)
Drinking amount, N (%)	
50–150 mL/time	204 (36.17)
151–250 mL/time	12 (2.13)
>250 mL/time	3 (0.53)
0 mL/time	345 (61.17)
Vaccine types, N (%)	
Inactivated vaccine	506 (89.72)
mRNA vaccine	10 (1.77)
Recombinant new coronavirus vaccine (CHO cells)	7 (1.24)
Unknown or forget	41 (7.27)

N, number of unique individuals; n, number of samples; SD, standard deviation; BMI, body mass index; NA, not available; CHO, Chinese hamster ovary.

Chengdu, capital city of Sichuan Province, where there was higher pollution from particulates ($PM_{2.5}$ and PM_{10}), NO_2 CO, and O_3 during the study period (January 1, 2020 to December 31, 2021) compared to the surrounding regions (*Figure 1*). The study population was young, healthy (81.38% did not smoke and 61.17% did not drink) and with normal BMI (*Table 1*). They were overall highly-educated with 75.0% of individuals having a college education and approximately 14.54% having received graduate school education. Most of the study population was single and never married (86.88%). The sperm quality parameters from all sperm donor candidates before and after vaccination are summarized in *Table 2* and Table S1. Based on analysis using all 2,955 semen samples, sperm motility parameters appeared to show statistical difference before and after the first vaccination (*Table 2*). And it was same to the results of before and after the second vaccination (Table S1). However, pair-wise comparison based on the 305 samples from the 71 individuals who donated before and after vaccination showed no statistical difference for any sperm quality parameters (*Table 2*, right column and Table S2).

Similarly, based on generalized linear model, semen samples collected before and after vaccination appeared to show statistically different concentration, progressive rate, total forward sperm, and motility (VCL, VAP, VSL, ALH) (Table S3). However, once individual ID was included in the mixed effects model, only VCL, VAP and VSL exhibited

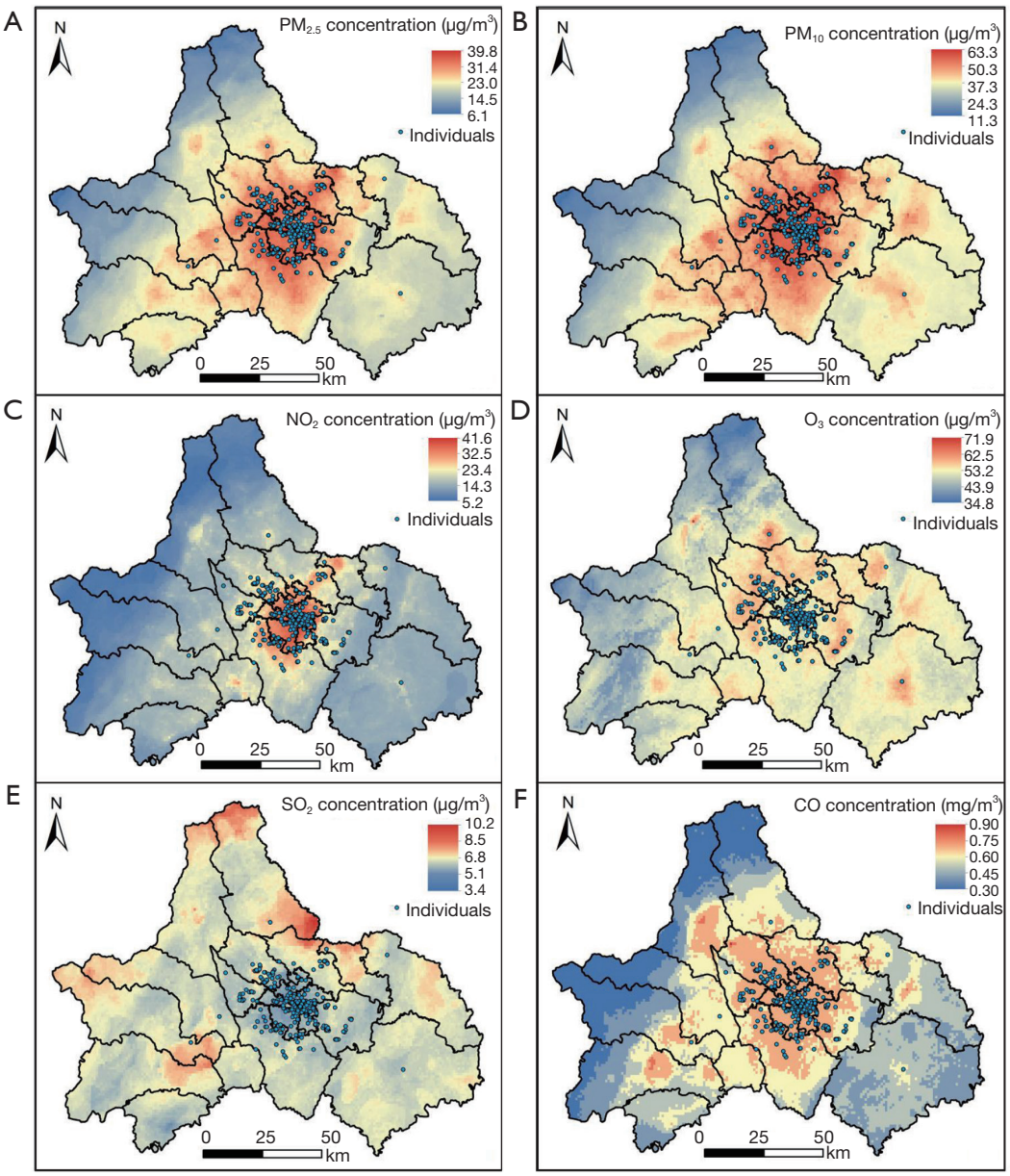

Figure 1 Average pollutant concentrations across the study area and distribution of primary residence of study participants (January 1, 2020 to December 31, 2021).

significant association (P values are 0.04, 0.02 and 0.009, respectively) with vaccination (*Table 3*). ID (or individual) played the dominant role in variance contribution for semen volume (77%), sperm concentration (57%), progressive rate (58%), and for motility related parameters such as VCL (60%), VAP (56%), VSL (56%), STR (61%), ALH (53%) and BCF (56%). Interestingly, the time duration between semen sample collection and vaccination did not appear to have statistical association with sperm quality parameters (results not shown).

The characteristics of a sub-group of individuals who provided semen samples between 60 to 90 days post-vaccination were similar to the larger study population (Table S4) and the overall sperm quality was similar to that of the larger sample (Table S5). The lack of association between vaccination time and sperm quality parameters from mixed

Table 2 Summary of semen quality parameters before and after first vaccine dose

Semen quality parameters	Total (N=564, n=2,955), mean (SD)	Pre-vaccination (N=347, n=869), mean (SD)	Post-vaccination (N=288, n=969), mean (SD)	P value†	P value‡
Volume (mL)	4.06 (1.47)	4.05 (1.40)	4.06 (1.60)	0.9007	0.676
Sperm concentration (×10^6/mL)	127.76 (70.61)	128 (71.4)	127 (69.0)	0.8454	0.940
Total sperm count (×10^6)	493.29 (277.64)	492 (267)	496 (298)	0.6967	0.676
Total forward sperm (×10^6)	344.43 (206.56)	347 (203)	338 (214)	0.2671	0.267
Progressive rate (%)	68.95 (9.93)	69.70 (9.88)	67.40 (9.85)	<0.001	0.617
VCL (μm/s)	49.29 (11.11)	47.4 (10.3)	53.2 (11.8)	<0.001	0.342
VAP (μm/s)	34.67 (8.13)	33.4 (7.67)	37.4 (8.37)	<0.001	0.970
VSL (μm/s)	26.18 (7.30)	24.9 (6.84)	28.8 (7.48)	<0.001	0.772
WOB (%)	0.69 (0.07)	0.68 (0.07)	0.70 (0.06)	<0.001	0.032
STR (%)	0.60 (0.08)	0.60 (0.08)	0.59 (0.09)	<0.001	0.351
LIN (%)	0.51 (0.09)	0.49 (0.09)	0.54 (0.08)	<0.001	0.109
ALH (μm/s)	4.36 (1.00)	4.59 (1.00)	3.90 (0.81)	<0.001	0.870
BCF (times/s)	11.64 (2.16)	11.3 (2.03)	12.3 (2.28)	<0.001	0.190

†, P values obtained from Welch two sample *t*-test; ‡, P values obtained from pair-wise *t*-test based on 305 semen samples from 71 individuals who provided semen samples before and after receiving COVID-19 vaccine. N, number of unique individuals; n, number of samples; SD, standard deviation; VCL, curvilinear velocity; VAP, average path velocity; VSL, straight-line velocity; WOB, wobble; STR, straightness; LIN, linearity; ALH, amplitude of lateral head displacement; BCF, beat-cross frequency.

Table 3 Summary of mixed effects model

Variables	Estimate (SE)	Variance by ID (%)
Semen volume (mL)	−0.16 (0.09)	77
Sperm concentration (×10^6/mL)	2.67 (5.36)	57
Progressive rate (%)	1.04 (0.81)	58
Total sperm count (×10^6)	4.89 (21.81)	51
Total forward sperm (×10^6)	13.72 (16.24)	50
VCL (μm/s)	−1.70 (0.84)*	60
VAP (μm/s)	−1.35 (0.62)*	56
VSL (μm/s)	−1.44 (0.55)*	56
WOB (%)	0.0087 (0.0051)	46
STR (%)	0.00055 (0.0064)	61
LIN (%)	0.0019 (0.0067)	49
ALH (μm/s)	0.13 (0.073)	53
BCF (times/s)	−0.17 (0.17)	56

The primary dependent variable of interest was whether the sample was collected before or after vaccination. Model adjusted for time between sample collection and vaccination, vaccine manufacturer, age, BMI, education level, ethnicity, abstinence duration (days), drinking status, smoking status, relative humidity and temperature, 90-day average concentrations of $PM_{2.5}$, PM_{10}, CO, O_2, NO_2, and O_3. Individual ID was the random variable. Significance level: *, P<0.05. VCL, curvilinear velocity; VAP, average path velocity; VSL, straight-line velocity; WOB, wobble; STR, straightness; LIN, linearity; ALH, amplitude of lateral head displacement; BCF, beat-cross frequency; SE, standard error; ID, identity document; BMI, body mass index; PM, particulate matter.

effects regression analysis on this subgroup with samples obtained 60–90 days (inclusive) after vaccination resembled that of the larger study population (Table S6).

Discussion

Our study examined the potential relationship between COVID-19 vaccination and sperm quality parameters. In this study, our results suggest that the Chinese COVID-19 vaccination is not statistically associated with changes in most sperm quality parameters except VCL, VSL and VAP, but rather variability between individuals appears to play the dominate role in contributing variance for these sperm quality parameters. The subgroup analysis on semen samples collected 60–90 days (inclusive) after vaccination showed no statistically significance at all.

To date, there are only a few studies reporting the potential relationship between COVID-19 vaccination and sperm quality. These study results indicated no significant decrease in sperm parameters after receiving the mRNA vaccine against SARS-CoV-2, but the sample sizes in those studies were relatively limited (14,45-47). A recent study from Gat *et al.* [2022] on 37 sperm donors found a temporary decline in sperm concentration and total motile count occurred three months post-vaccination with the BNT162b2 vaccine, and this decline was followed by subsequent recovery (48). While there seems to be limited to no negative impact on sperm quality from COVID-19 vaccination, the damage on sperm quality caused by COVID infection is evident. Previous studies found that SARS-CoV-2 mRNA could be detected in the semen, testis, and prostatic fluid of infected males (7,10). Additionally, patients with moderate COVID symptoms had worse sperm quality parameters after infection compared to the baseline or to patients with mild symptoms and healthy controls (7,49). The impaired sperm quality could be the result of fever or inflammation due to infection.

Moreover, mounting evidence suggests that the COVID-19 vaccination seemingly does not have significant effect on female reproductive health neither. For example, a recent study found that mRNA SARS-CoV-2 vaccination did not significantly change plasma anti-Müllerianhormone (AMH) levels and was not associated with a decrease in ovarian reserve after three months (50). Another assessment on ovarian follicle function reported no measurable difference between vaccinated and unvaccinated women (51). Similarly, recent studies showed the SARS-CoV-2 vaccination had no detrimental effect on sperm numbers and motility among men (14,52-54), whose results are consistent with our outcome.

Individuals contemplating pregnancy face uncertainty surrounding reproductive decision making and provision of care. The absence of data regarding the effect of the pandemic and vaccination on reproductive health accentuated the hurdles faced by those considering pregnancy. Further studies are needed to determine the effects of COVID-19 vaccination on male reproductive health and conception, because results from such studies could help to optimize the decision-making process and management for individuals of reproductive age.

Strengths and limitations

This study utilized a relatively large study population and sample size compared with previous studies. This larger sample size enhances the robustness of our study results. Moreover, our statistical analysis involved the control for potential confounders for sperm quality, including air pollution, lifestyle, habits, and social-demographic characteristics (such as age, education, and BMI). However, since our study population included sperm donor candidates and participation in the study was voluntary, the results must be interpreted cautiously and may have limited generalizability to the broader public. Moreover, not of all subjects who donated sperm before and after vaccination, which is another limitation. And because of the limitation of the cost and conditions, more sperm parameters of the all samples, such as the sperm DNA damage, are not analysed in the study.

Conclusions

In this study, we examined the association between Chinese COVID-19 vaccines and sperm quality parameters. Results from our assessment of repeated semen samples provided by sperm donor candidates suggest that receiving the COVID-19 vaccine is not significantly associated with sperm quality parameters. Individual variability plays the dominant role in explaining the sperm quality variance. Our findings agree with other recent studies and provide additional scientific evidence to guide vaccination policies for reproductive age males.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Institutional Review Board of West China Second University Hospital of Sichuan University (No. WCSUH-SCU IRB 2021-197), and individual consent for this retrospective analysis was waived.

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Supplementary

Table S1 Summary of semen quality parameters before and after the second vaccine dose

Semen quality parameters	Total (N=564, n=2,955), mean (SD)	Pre-vaccination (N=347, n=869), mean (SD)	Post-vaccination (N=277, n=942), mean (SD)	P value[†]
Volume (mL)	4.06 (1.47)	4.05 (1.40)	4.07 (1.60)	0.8847
Sperm concentration (×10^6/mL)	127.76 (70.61)	128 (72.4)	128 (69.4)	0.9873
Total sperm count (×10^6)	493.29 (277.64)	492 (267)	500 (300)	0.6999
Total forward sperm (×10^6)	344.43 (206.56)	347 (203)	341 (216)	0.2326
Progressive rate (%)	68.95 (9.93)	69.70 (9.88)	67.40 (9.89)	<0.001
VCL (μm/s)	49.29 (11.11)	47.4 (10.3)	53.2 (11.8)	<0.001
VAP (μm/s)	34.67 (8.13)	33.4 (7.67)	37.4 (8.39)	<0.001
VSL (μm/s)	26.18 (7.30)	24.9 (6.84)	28.8 (7.50)	<0.001
WOB (%)	0.69 (0.07)	0.68 (0.07)	0.70 (0.06)	<0.001
STR (%)	0.60 (0.08)	0.60 (0.08)	0.59 (0.09)	<0.001
LIN (%)	0.51 (0.09)	0.49 (0.09)	0.54 (0.08)	<0.001
ALH (μm/s)	4.36 (1.00)	4.59 (1.00)	3.90 (0.81)	<0.001
BCF (times/s)	11.64 (2.16)	11.3 (2.03)	12.3 (2.28)	<0.001

[†], P values obtained from Welch two-sample *t*-test. N, number of unique individuals; n, number of samples; SD, standard deviation; VSL, straight-line velocity; WOB, wobble; STR, straightness; LIN, linearity; ALH, amplitude of lateral head displacement; BCF, beat-cross frequency.

Table S2 Summary of semen quality parameters of 305 semen samples from 71 individuals who provided semen samples before and after receiving COVID-19

Semen quality parameters	Total (N=71, n=305), mean (SD)	Pre-vaccination (N=71, n=71), mean (SD)	Post-vaccination (N=71, n=234), mean (SD)	P value
Volume (mL)	4.44 (1.31)	4.46 (1.31)	4.40 (1.32)	0.676
Sperm concentration (×10^6/mL)	113 (50.61)	120 (49.7)	121 (54.8)	0.940
Total sperm count (×10^6)	509 (267.64)	502 (260)	514 (278)	0.676
Total forward sperm (×10^6)	346 (206.56)	348 (201)	336 (216)	0.267
Progressive rate (%)	67.89 (8.93)	68.14 (7.90)	67.49 (9.52)	0.617
VCL (μm/s)	53.87 (11.8)	53.5 (11.9)	55.2 (11.8)	0.342
VAP (μm/s)	38.67 (8.80)	39.1 (8.81)	39.0 (8.79)	0.970
VSL (μm/s)	30.13 (8.10)	30.5 (7.88)	30.2 (8.15)	0.772
WOB (%)	0.72 (0.04)	0.72 (0.05)	0.71 (0.04)	0.032
STR (%)	0.59 (0.08)	0.59 (0.07)	0.58 (0.09)	0.351
LIN (%)	0.56 (0.07)	0.56 (0.07)	0.55 (0.08)	0.109
ALH (μm/s)	3.97 (0.75)	3.99 (0.79)	3.97 (0.72)	0.870
BCF (%)	12.41 (2.08)	12.66 (1.98)	12.3 (2.25)	0.190

COVID-19, coronavirus disease 2019; N, number of unique individuals; n, number of samples; SD, standard deviation; CL, curvilinear velocity; VAP, average path velocity; VSL, straight-line velocity; WOB, wobble; STR, straightness; LIN, linearity; ALH, amplitude of lateral head displacement; BCF, beat-cross frequency.

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Table S3 Summary of generalized linear model

Variables	Estimate (SE)
Semen volume (mL)	−0.0069 (0.099)
Sperm concentration (×10^6/mL)	9.54 (4.78)*
Progressive motility PR (%)	1.91 (0.67)**
Total sperm count (×10^6)	35.74 (18.80)
Total forward sperm (×10^6)	36.61 (14.00)*
VCL (μm/s)	−2.07 (0.73)**
VAP (μm/s)	−1.86 (0.53)***
VSL (μm/s)	−2.16 (0.47)***
WOB (%)	0.0054 (0.0043)
STR (%)	0.0044 (0.0055)
LIN (%)	−0.0062 (0.0057)
ALH (μm/s)	0.28 (0.06)***
BCF (times/s)	−0.24 (0.14)

The primary dependent variable of interest was whether the sample was collected before or after vaccination. Model adjusted for time between sample collection and vaccination, vaccine manufacturer, age, BMI, education level, ethnicity, abstinence duration (days), drinking status, smoking status, relative humidity and temperature, 90-day average concentrations of $PM_{2.5}$, PM_{10}, CO, O_2, NO_2, and O_3. Significance level: *, P<0.05; **, P<0.005; ***, P<0.001. PR, progressive rate; VCL, curvilinear velocity; VAP, average path velocity; VSL, straight-line velocity; WOB, wobble; STR, straightness; LIN, linearity; ALH, amplitude of lateral head displacement; BCF, beat-cross frequency; SE, standard error; BMI, body mass index; PM, particulate matter.

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Table S4 Summary characteristics for subgroup with semen samples 60–90 days after vaccination

Variables	Values (N=71, n=133)
Population characteristics, mean (SD)	
Age (years)	23.76 (4.65)
Height (cm)	174.78 (4.49)
Weight (kg)	68.13 (9.37)
BMI (kg/m^2)	22.31 (3.05)
Ethnicity, N (%)	
Han	69 (97.18)
Other[†]	2 (2.82)
Education level, N (%)	
High school and below	9 (12.68)
College	46 (64.79)
Graduate school	12 (16.90)
NA	4 (5.63)
Marriage status, N (%)	
Single, never married	65 (91.55)
Married	6 (8.45)
Duration between semen sample collection and first vaccination shot (days), mean (SD)	74.07 (8.21)
Drinking and smoking habits, N (%)	
Smoking frequency	
1–3 times/week	9 (12.68)
4–6 times/week	2 (2.82)
7 times or more/week	6 (8.45)
Never	54 (76.06)
Smoking amount	
1–5/day	13 (18.31)
6–12/day	4 (5.63)
0/day	54 (76.06)
Drinking frequency	
1–3 times/week	26 (36.62)
4–6 times/week	0
7 times or more/week	0
Never	45 (63.38)
Drinking amount	
50–150 mL/time	22 (31.00)
151–250 mL/time	1 (1.41)
More than 250 mL/time	1 (1.41)
0 mL/time	47 (66.20)

[†], other ethnicities are Tujia (1, or 1.41%) and Yi (1, or 1.41%). N, number of unique individuals; n, number of samples; SD, standard deviation; BMI, body mass index; NA, not available.

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Table S5 Summary of semen quality parameters for subgroup of samples analyzed 60–90 days after vaccination

Parameters	Mean (SD) (N=71, n=133)
Abstinence (days)	4.35 (1.07)
Total sperm count (×10^6)	467.72 (281.29)
Total forward sperm (×10^6)	316.88 (215.11)
Sperm concentration (×10^6/mL)	119.55 (63.02)
Volume (mL)	4.03 (1.58)
Progressive rate (%)	65.61 (12.46)
Round cells (×10^6/mL)	0.21 (0.20)
VCL (μm/s)§	49.97 (12.69)
VAP (μm/s)§	35.73 (9.12)
VSL (μm/s)§	27.71 (7.95)
WOB (%)§	0.71 (0.06)
STR (%)§	0.59 (0.096)
LIN (%)§	0.56 (0.088)
ALH (μm/s)§	3.78 (0.93)
BCF (times/s)	12.19 (2.57)

§, quality of sperm motion analyzed by CASA, only for samples with at least 25 sperm tracks, including VCL (μm/s), VAP (μm/s), VSL (μm/s), WOB (%), STR (%), LIN (%), ALH (μm/s), and BCF (times/s). N, number of unique individuals; n, number of samples; SD, standard deviation; VCL, curvilinear velocity; VAP, average path velocity; VSL, straight-line velocity; WOB, wobble; STR, straightness; LIN, linearity; ALH, amplitude of lateral head displacement; BCF, beat-cross frequency.

Table S6 Summary of mixed effects model for subgroup of samples analyzed 60–90 days after first dose of vaccination (N=71, n=133)

Variables	Estimate (SE)	Variance by ID (%)
Semen volume (mL)	0.0015 (0.012)	82
Sperm concentration (×10^6/mL)	−0.89 (0.69)	66
Progressive motility PR (%)	0.0081 (0.14)	29
Total sperm count (×10^6)	−3.21 (2.72)	73
Total forward sperm (×10^6)	−2.15 (2.03)	77
VCL (μm/s)	0.12 (0.13)	37
VAP (μm/s)	0.078 (0.10)	30
VSL (μm/s)	0.056 (0.088)	27
WOB (%)	−0.00031 (0.00073)	19
STR (%)	2.126e−03 (1.016e−03)	44
LIN (%)	−0.00041 (0.0010)	22
ALH (μm/s)	0.0049 (0.011)	35
BCF (times/s)	0.054 (0.028)	34

Primary dependent variable of interest was sample collection time since vaccination. Mixed effects model adjusted for age, BMI, education level, ethnicity, abstinence duration (days), drinking status, smoking status, relative humidity and temperature, 90-day average concentrations of $PM_{2.5}$, PM_{10}, CO, SO_2, NO_2, and O_3. Individual ID was the random variable. PR, progressive rate; VCL, curvilinear velocity; VAP, average path velocity; VSL, straight-line velocity; WOB, wobble; STR, straightness; LIN, linearity; ALH, amplitude of lateral head displacement; BCF, beat-cross frequency; SE, standard error; ID, identity document; BMI, body mass index; PM, particulate matter.

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