

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 ($\times 10^6$ /mL)	127.76 (70.61)	128 (72.4)	128 (69.4)	0.9873
Total sperm count ($\times 10^6$)	493.29 (277.64)	492 (267)	500 (300)	0.6999
Total forward sperm ($\times 10^6$)	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 (μ m/s)	49.29 (11.11)	47.4 (10.3)	53.2 (11.8)	<0.001
VAP (μ m/s)	34.67 (8.13)	33.4 (7.67)	37.4 (8.39)	<0.001
VSL (μ m/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 (μ m/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 ($\times 10^6$ /mL)	113 (50.61)	120 (49.7)	121 (54.8)	0.940
Total sperm count ($\times 10^6$)	509 (267.64)	502 (260)	514 (278)	0.676
Total forward sperm ($\times 10^6$)	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 (μ m/s)	53.87 (11.8)	53.5 (11.9)	55.2 (11.8)	0.342
VAP (μ m/s)	38.67 (8.80)	39.1 (8.81)	39.0 (8.79)	0.970
VSL (μ m/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 (μ m/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.

Table S3 Summary of generalized linear model

Variables	Estimate (SE)
Semen volume (mL)	-0.0069 (0.099)
Sperm concentration ($\times 10^6/\text{mL}$)	9.54 (4.78)*
Progressive motility PR (%)	1.91 (0.67)**
Total sperm count ($\times 10^6$)	35.74 (18.80)
Total forward sperm ($\times 10^6$)	36.61 (14.00)*
VCL ($\mu\text{m/s}$)	-2.07 (0.73)**
VAP ($\mu\text{m/s}$)	-1.86 (0.53)***
VSL ($\mu\text{m/s}$)	-2.16 (0.47)***
WOB (%)	0.0054 (0.0043)
STR (%)	0.0044 (0.0055)
LIN (%)	-0.0062 (0.0057)
ALH ($\mu\text{m/s}$)	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 $\text{PM}_{2.5}$, 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.

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.

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 ($\times 10^6$)	467.72 (281.29)
Total forward sperm ($\times 10^6$)	316.88 (215.11)
Sperm concentration ($\times 10^6/\text{mL}$)	119.55 (63.02)
Volume (mL)	4.03 (1.58)
Progressive rate (%)	65.61 (12.46)
Round cells ($\times 10^6/\text{mL}$)	0.21 (0.20)
VCL ($\mu\text{m/s}$) [§]	49.97 (12.69)
VAP ($\mu\text{m/s}$) [§]	35.73 (9.12)
VSL ($\mu\text{m/s}$) [§]	27.71 (7.95)
WOB (%) [§]	0.71 (0.06)
STR (%) [§]	0.59 (0.096)
LIN (%) [§]	0.56 (0.088)
ALH ($\mu\text{m/s}$) [§]	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 ($\mu\text{m/s}$), VAP ($\mu\text{m/s}$), VSL ($\mu\text{m/s}$), WOB (%), STR (%), LIN (%), ALH ($\mu\text{m/s}$), 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 ($\times 10^6/\text{mL}$)	-0.89 (0.69)	66
Progressive motility PR (%)	0.0081 (0.14)	29
Total sperm count ($\times 10^6$)	-3.21 (2.72)	73
Total forward sperm ($\times 10^6$)	-2.15 (2.03)	77
VCL ($\mu\text{m/s}$)	0.12 (0.13)	37
VAP ($\mu\text{m/s}$)	0.078 (0.10)	30
VSL ($\mu\text{m/s}$)	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 ($\mu\text{m/s}$)	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}, PM₁₀, CO, SO₂, NO₂, and O₃. 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.