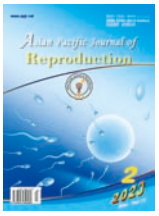

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Effect of BNT162b2 mRNA COVID-19 vaccine on sperm morphokinetics and DNA integrity: A prospective observational study in Japan

Yasuhiro Ohara[1,2✉], Shimpei Mizuta[1,3], Hidehiko Matsubayashi[1,3], Tomomoto Ishikawa[1,3], Tsuyoshi Takiuchi[4], Tadashi Kimura[2]

[1]*Department of Reproductive Medicine, Reproduction Clinic Osaka, 4-20, Ofuka-cho, Kita-ku, Osaka, Japan*

[2]*Department of Obstetrics and Gynecology, Graduate School of Medicine, Osaka University, 2-15, Yamadaoka, Suita City, Osaka, Japan*

[3]*Department of Reproductive Medicine, Reproduction Clinic Tokyo, 1-5-2, Higashi-shinbashi, Minato-ku, Tokyo, Japan*

[4]*Department of Clinical Genomics, Graduate School of Medicine, Osaka University, 2-15, Yamadaoka, Suita City, Osaka, Japan*

ABSTRACT

Objective: To assess whether the coronavirus disease 2019 (COVID-19) mRNA vaccine affects sperm morphokinetics using a computer-assisted semen analyzer and other semen parameters using a sperm chromatin structure assay.

Methods: Healthy male volunteers in two Japanese clinics between May 2021 and December 2021 were prospectively analyzed. Participants donated sperm twice, two days apart, in the following phases: before vaccination, 2 weeks after the first vaccine dose, and 2, 4, and 12 weeks after the second dose. Basic sperm parameters, sperm motility characteristics, and the percentage of DNA-damaged sperm were compared among the different phases.

Results: Ninety-six semen samples from ten volunteers, who were vaccinated with the BNT162b2 mRNA vaccine, were evaluated. There were no significant differences between any phases in basic semen findings and parameters of the sperm chromatin structure assays. Regarding sperm motion characteristics, the average linear velocity, beat-cross frequency, and sperm motility index significantly decreased after the second vaccine dose (P=0.018, P=0.003, and P=0.027, respectively), with no significant differences between any two phases by *post-hoc* pairwise comparisons.

Conclusions: After COVID-19 mRNA vaccination, while sperm motion characteristics might fluctuate, no apparent deterioration of basic sperm parameters or sperm DNA integrity was observed. Given the adverse effects of COVID-19 on sperm, our findings suggest that there might be no reason to refrain from vaccination for healthy individuals.

KEYWORDS: Computer-assisted semen analyzer; COVID-19 vaccine; Flow cytometry; Male fertility; Sperm chromatin structure assay

1. Introduction

The 2019–2022 coronavirus disease 2019 (COVID-19) pandemic triggered pregnancy hesitancy due to its unknown reproductive consequences. In 2020, the birth number in Japan dropped to 840 832, the lowest since 1899[1]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA has been detected in the semen of symptomatic patients with COVID-19 and has been reported to worsen basic semen findings[2]. To control this infection, BNT162b2 mRNA COVID-19 vaccine was developed with inoculation in Japan in February 2021. Findings on impaired

Significance

Although the impact of COVID-19 vaccines on basic sperm parameters has been reported, their effects on sperm functional parameters, including morphokinetics and DNA fragmentation, are unknown. Thus, evaluating sperm function is important for assessing fecundity after vaccination. This study highlighted the influence of the BNT162b2 mRNA COVID-19 vaccine on sperm function and revealed its safety for conventional semen parameters and DNA integrity, despite fluctuations in several sperm motion characteristics.

✉To whom correspondance may be addressed. E-mail: yasumots@yahoo.co.jp

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spermatogenesis in COVID-19 patients initially prompted concerns about the vaccine's impact on male fertility[3–5]. While COVID-19 mRNA vaccination showed no detrimental effects on basic sperm parameters[6–11], the vaccine's impact on sperm morphology and function remains incompletely understood. Since elevated sperm DNA fragmentation has been found to adversely affect many reproductive outcomes and sperm kinetics can be associated with sperm DNA damage, evaluating sperm function is important in assessing fecundity after vaccination[12–15]. Therefore, this study aimed to characterize the BNT162b2 mRNA vaccine's impact on male reproductive function.

2. Materials and methods

2.1. Study population

This prospective cohort study was performed in two private fertility clinics in Japan. Male volunteers, including medical staff, were recruited from the clinics. The inclusion criteria were as follows: a) healthy men; b) aged 20-50 years; c) those who received the first BNT162b2 mRNA vaccine (BioNTech SE, Mainz, Germany; and Pfizer Inc., New York, NY, USA) between May 2021, when vaccination became publicly available in Japan, and December 2021; and d) those who were adequately informed about this study and provided voluntary written consent. The exclusion criteria were as follows: a) those using drugs, such as supplemental testosterone or anabolic steroids, which might affect semen findings; b) those with a history of coronavirus infection at the time of study entry; and c) those who discontinued vaccination after the first dose.

Participants who met the criteria underwent flow cytometry (FCM)-based semen evaluation with a computer-assisted sperm analyzer (CASA) and a sperm chromatin structure assay (SCSA), which are widely used in assisted reproductive technology, at five different phases: before the first mRNA vaccination (T0), 2 weeks after the first dose (T1), and 2, 4, and 12 weeks after the second dose (T2-4, respectively)[16,17]. Volunteers donated semen twice, two days apart, at each phase. The vaccine was administered in two intramuscular injections, 21 days apart (Figure 1). Participants were instructed to provide semen samples after 2 days of abstinence and were asked to complete a short questionnaire about their vaccination dates and side effects after each vaccine.

2.2. Outcome measures

Trained embryologists performed the analysis in the andrology laboratory, located near the sperm collection room. Semen samples were collected by masturbation into sterile plastic containers during the day. Each specimen was allowed to liquefy for at least 30 min at ambient temperature (from 15 ℃ to 25 ℃). All semen parameters were interpreted based on guidelines of the 2021 World Health Organization (WHO) Laboratory Manual for examining and processing of human semen[18].

2.3. Analysis of motility and kinematic parameters by CASA

CASA analyses on fresh donated semen samples were performed with a sperm motility analysis system (SMAS) (version 3.6.19.585, DITECT, Tokyo, Japan)[19]. Sperm from the conditioned medium was spotted onto a MAKLER counting chamber to analyze sperm motility. SMAS contains a high-resolution digital scanning camera, a personal computer with a digital frame grabber and image-processing software, and a computer monitor. The system records images at the rate of 60 frames per second and can analyze up to 5000 motile sperms at once. SMAS findings have been shown to significantly correlate with those obtained from manual microscopic sperm analysis based on the WHO Laboratory Manual[20]. Semen volume (mL), sperm concentration (number/mL), sperm motility (%), total sperm count, total motile sperm count, linear velocity (μm/min), curvilinear velocity (μm/min), the amplitude of lateral head displacement (μm), beat-cross frequency (Hz), and sperm motility index were all evaluated in this study.

2.4. Sperm chromatin analysis by FCM

FCM provides a high-throughput evaluation of thousands of spermatozoa within seconds, with the characterization of multiple

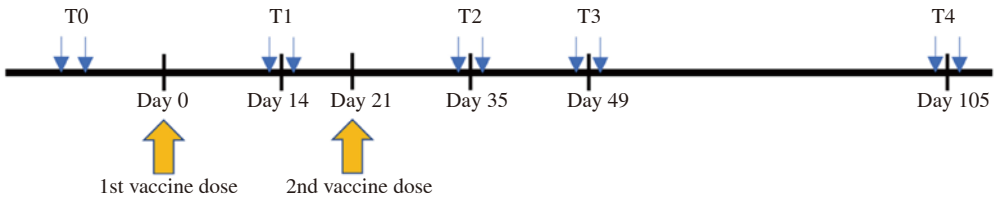

Figure 1. Study protocol of timing of semen analysis and administration of the vaccine (BNT162b2). Day 0, first mRNA vaccine dose; T0, before the first mRNA vaccine; T1, 2 weeks after the first dose; T2, 2 weeks after the second dose; T3, 4 weeks after the second dose; T4, 12 weeks after the second dose.

physiological features of each spermatozoon and the analysis of sperm viability, immaturity, acrosome integrity, apoptotic markers, DNA damage, *etc*. In the current study, FCM was used to evaluate the sperm DNA damage rate, sperm advanced DNA damage rate, and immature sperm rate.

2.5. Acridine orange (AO) staining of human sperm

All FCM samples were evaluated for semen quality after thawing. As the BNT162b2 vaccine reportedly did not alter semen quality after freezing[21], we analyzed frozen-thawed semen samples for FCM. The collected raw semen (100 μL) was placed in a 1.5-mL tube and frozen in liquid nitrogen (-196℃). At the measurement time, the frozen sample was placed in a refrigerator (4℃) for 40 min and subsequently mixed with 1 mL of a culture solution (heated to 35℃, GxIVFTM, Vitrolife, Gothenburg, Sweden). This diluted solution was used for the measurement. Additionally, 200 μL of acid detergent solution (80 mM HCl, 15 mM NaCl, 0.1% Triton X-100; pH 1.2) was added, and the mixture was allowed to acidify for 30 s. Subsequently, 600 μL of AO solution (6 mg/mL AO, 37 mM citrate acid, 126 mM Na_2HPO_4, 1.1 mM EDTA, and 150 mM NaCl) were consecutively pipetted to the mixture.

2.6. AO fluorescence detection by FCM

After AO staining, the human sperm suspension was transferred to a tube for FCM analysis. AO green and red were detected with fluorescein isothiocyanate (FITC, 525 nm) and peridinin-chlorophyll protein-cyanine 5.5 (PC5.5, 610 nm) filters, respectively. The fluorescence signals of FITC and PC5.5 were displayed on the vertical and horizontal axes, and the degree of fragmentation (DFI) of sperm DNA was measured using the fluorescence signal of AO red as an index (Figure 2).

2.7. Statistical analysis

The EZR software (version 4.0.2, Saitama Medical Center, Jichi Medical University, Saitama, Japan) was used for statistical analyses. This study aimed to include at least 16 samples in each group, which was expected to yield a power of ≥0.80, based on an α-value of ≤0.05 and assumed effect size of 0.40[22]. Statistical analyses were performed using a parametric one-way repeated measures analysis of variance or the non-parametric Friedman's test, according to the distribution of variables, followed by *post-hoc* pairwise comparisons with the Holm's correction. All statistical analyses were two-sided. Data were presented as number (%), mean±standard deviation, or median [interquartile range (IQR)]. $P<0.05$ was considered statistically significant.

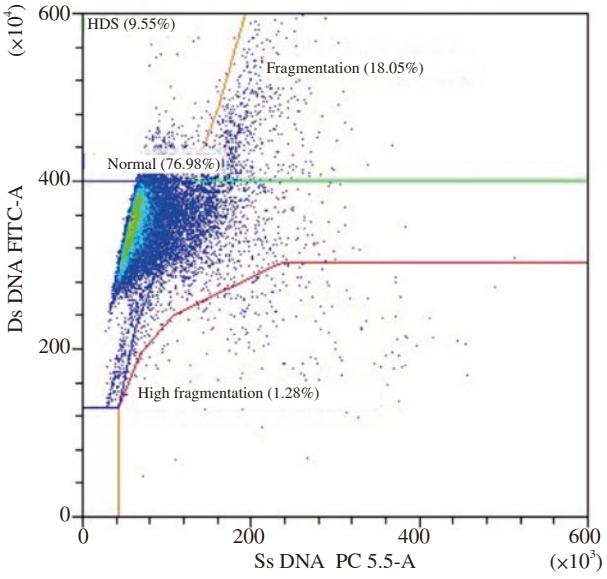

Figure 2. Scattergram of sperm DNA fragmentation and maturity by flow cytometry. A scattergram of green fluorescence intensity by double-stranded (ds) DNA FITC (525 nm) *versus* red fluorescence intensity by single-stranded PC5.5 (610 nm), processed according to the sperm chromatin structure assay.

2.8. Ethics approval statement

This study was approved by the Review Board of our institution on May 7, 2021 (approval number: 2021-O-D-01). Written informed consent was obtained from all participants. The study conformed to the principles of the Helsinki's Declaration. This trial was registered in the University Hospital Medical Information Network Clinical Trial Registry (UMIN-CTR) on April 26, 2021 and started on May 15, 2021.

3. Results

3.1. Participants' characteristics

A total of 96 semen samples from ten male volunteers, who were vaccinated with the BNT162b2 mRNA vaccine, were collected between May 2021 and December 2021 and analyzed. Twenty samples were collected at T0, T1, and T2, and 18 samples were collected at T3 and T4. The median (IQR) age of patients was 32 (22-38) years. One participant did not give samples after T2 for personal reasons. No participants had COVID-19 symptoms or a positive test result during the study period. No participants received any infertility treatment within the study period. Regarding side effects, five participants reported fever after the second mRNA vaccination. All sperm donors in the present study did not have any severe side effects from the vaccine.

3.2. SMAS results

Table 1 summarizes the average semen parameters determined by SMAS in each phase. There were no differences in the basic semen parameters among all phases. No participants had azoospermia after vaccination, and no semen parameters deteriorated significantly. Among sperm morphokinetic parameters, the average linear velocity, beat-cross frequency, and sperm motility index significantly decreased after vaccination (P=0.018, P=0.003, and P=0.027, respectively), with no significant differences between any two phases. Five of the ten donors had a fever of 37 ℃ or higher after the second mRNA vaccination. Additionally, we compared participants with and without fever in terms of changes in linear velocity, beat-cross frequency, and sperm motility index. In this subgroup analysis, no significant differences were observed in these parameters between the fever-positive and fever-negative groups during the observation period (Table 2).

3.3. FCM results

Table 3 summarizes the average semen parameters obtained by FCM at each phase. No significant differences were observed in SCSA parameters (sperm DNA damage rate, sperm advanced DNA damage rate, and immature sperm rate).

Table 1. Semen parameters of 96 sperm samples before the first vaccination dose (T0), 2 weeks after the first dose (T1), and 2 (T2), 4 (T3), and 12 (T4) weeks after the second dose.

Parameters	T0 (N=20)	T1 (N=20)	T2 (N=20)	T3 (N=18)	T4 (N=18)	P-value	*Post hoc* test
Semen volume, mL	3.39±1.10	3.72±1.10	3.59±1.50	3.64±0.80	3.40±1.10	0.75	-
Sperm concentration, million/mL	58.3 (38-69)	57.9 (44-70)	59.6 (51-107)	60.2 (48-68)	39.0 (36-61)	0.64	-
Motility, %	65.30±18.0	62.50±22.00	64.10±19.00	58.30±18.00	57.30±21.00	0.40	-
Total sperm count, million	161.5 (120-271)	182.3 (144-345)	286 (208-347)	190 (171-295)	119.0 (100-196	0.63	-
Total motile sperm count, million	116 (66-223)	149 (68-215)	212.0 (103-262)	128.6 (82-169)	79.4 (46-138)	0.73	-
Linear velocity, μm/s	19.90±2.50	20.20±3.70	18.90±3.10	18.90±2.10	17.50±3.60	0.018	NS
Curvilinear velocity, μm/s	51.60±9.90	52.70±12.00	49.90±10.00	51.30±11.00	47.40±11.00	0.38	-
Amplitude of lateral head displacement, μm	1.13±0.26	1.16±0.31	1.11±0.29	1.11±0.31	1.05±0.24	0.84	-
Beat-cross frequency, hz	8.26±0.59	8.24±0.65	8.07±0.62	7.96±0.57	7.54±1.00	0.003	NS
Sperm motility index	238.6 (151-315)	285.1 (172-348)	274.1 (161-310)	194.7 (128-249)	199.6 (91-242)	0.027	NS

Data are expressed as mean±standard deviation or median (IQR). P value is calculated by parametric one-way repeated measures analysis of variance or the non-parametric Friedman's test. NS indicates no statistically significant difference between any two phases by *post-hoc* pairwise comparisons with the Holm's correction (P>0.05). N=semen sample numbers.

Table 2. Semen parameters in participants with (n=5) or without (n=5) fever after the second vaccination at the following phases: before the first dose (T0), and 2 (T2), 4 (T3), and 12 (T4) weeks after the second dose.

Parameters	T0 (N=10)	T2 (N=10)	T3 (N=8)	T4 (N=8)	P-value
Linear velocity, μm/s					
Fever positive	20.30±1.50	17.60±2.20	19.00±1.00	16.50±2.80	0.06
Fever negative	19.40±3.10	20.20±3.30	18.80±2.70	18.30±4.00	
Beat-cross frequency, hz					
Fever positive	8.50±0.70	8.14±0.70	8.01±0.50	7.60±1.40	0.40
Fever negative	8.02±0.3	8.01±0.50	7.65±0.40	7.49±0.70	
Sperm motility index					
Fever positive	218.2 (179-280)	254.4 (167-284)	169.3 (127-212)	105.5 (91-120)	0.86
Fever negative	258.9 (146-321)	300.8 (231-381)	194.7 (142-345)	228.5 (199-242)	

Data are expressed as mean±standard deviation or median (IQR). N=semen sample numbers.

Table 3. Sperm DNA fragmentation and maturation of 96 sperm samples before the first vaccination dose (T0), 2 weeks after the first dose (T1), and 2 (T2), 4 (T3), and 12 (T4) weeks after the second dose.

Parameters	T0 (N=20)	T1 (N=20)	T2 (N=20)	T3 (N=18)	T4 (N=18)	P-value
DNA fragmentation index, %	6.24 (4.1-12.0)	6.72 (4.4-9.5)	6.03 (4.2-10)	6.78 (4.1-10.0)	8.16 (5.0-12.0)	0.95
High DNA fragmentation index, %	0.79 (0.4-1.3)	0.85 (0.6-1.0)	0.85 (0.5-1.5)	0.69 (0.5-0.9)	0.69 (0.6-1.3)	0.77
High stainable DNA, %	12.80 (8.6-12.0)	15.20 (7.3-19.0)	15.10 (7.5-18.0)	15.00 (5.3-20.0)	15.40 (13.0-24.0)	0.16

Data are expressed as median and interquartile range (IQR). High DNA fragmentation index is defined as over 30%. N=semen sample numbers.

4. Discussion

This study showed that COVID-19 vaccination had no influence on basic semen parameters or sperm DNA integrity, despite fluctuations in sperm morphokinematic parameters, such as linear velocity, beat-cross frequency, and sperm motility index after vaccination. These findings were consistent with previous reports, which showed no significant differences in semen parameters and fertilization rates[8,23]. In support of these findings, the COVID-19 mRNA vaccine, an inactivated vaccine containing only antigenic components without a live virus, induces a mild immune response without genome transcription or viral DNA replication[16]. Furthermore, vaccine-induced systemic inflammation is expectedly mild, and may have little influence on semen findings.

Increases in semen parameters, including sperm volume, concentration, motility, total sperm count, and total motile sperm count, have also been reported for the COVID-19 vaccines[6,21,24,25]. Our results showed a similar trend of higher basic semen parameters (semen volume, concentration, total sperm count, and total motile sperm count) at T1, T2, and T3 compared to T0, with no significant differences. These discrepancies might result from the high variability in samples taken from the same individual, a common characteristic of semen analysis.

Furthermore, our study showed that COVID-19 vaccination affected three sperm morphokinematic parameters by SMAS: linear velocity, beat-cross frequency, and sperm motility index. Recent studies showed that mRNA vaccines had negative effects on sperm concentration, total motile sperm count, and total/progressive sperm motility[26,27]. In this study, the average linear velocity, beat-cross frequency, and sperm motility index significantly decreased after the second vaccination, with no significant differences among any of the phases. This discrepancy may be explained by our relatively large number of measurement time points.

Since fever due to SARS-CoV-2 infection could induce a reversible negative effect on semen parameters[28-30], we compared the changes in linear velocity, beat-cross frequency, and sperm motility index between participants with and without fever, which showed no significant between-group differences. However, in the fever-positive group, the linear velocity tended to decrease from T0 to T4 compared to that in the fever-negative group. Therefore, the effect of fever on sperm motility cannot be denied. Based on the above findings, we considered that a systemic immune response, fever, or both might be responsible for the fluctuating sperm results after COVID-19 vaccination.

Apparent deleterious effects of COVID-19 vaccination on sperm parameters have not been shown. However, previous studies only assessed basic semen parameters and did not investigated the effects of COVID-19 vaccines on sperm functions, such as DNA fragmentation, which has been associated with diminished fertilization rates, embryo quality, pregnancy rates, and higher rates of spontaneous miscarriage[31-36]. To our best knowledge, there are no reports on sperm function, such as CASA analysis or sperm DFI, after COVID-19 mRNA vaccination. So far, only one prospective study on sperm functions included the analysis of reactive oxygen metabolites, electrolytes, and interleukin 6 (IL-6, a marker of inflammation), and showed no significant differences in these parameters before and after vaccination[37]. These findings were consistent with our work. Moreover, we found that COVID-19 vaccination did not impair sperm DNA integrity. As such, the BNT162b2 vaccine is considered fundamentally safe.

Our study had several strengths. First, this study was specifically designed to investigate the differences in semen parameters before and after the first and second COVID-19 vaccinations. We established five phases to evaluate both acute and chronic sperm reactions to the vaccine. As sperm quality may differ among participants, average data of all sperm samples (donated twice, 2 days apart at each phase) were assessed. Second, unlike previous studies, we evaluated semen functions with FCM.

The study's limitations included the small number of participants with relatively good semen findings, unknown long-term prognosis, and the lack of a control group. While COVID-19 vaccine and booster reportedly did not affect long-term sperm parameters[2, 25,26], further long-term follow-up on the impact of COVID-19 vaccination on sperm function is needed.

In conclusion, while there may be fluctuations in sperm motion characteristics after COVID-19 mRNA vaccination, we did not find any deterioration of sperm DNA integrity after such vaccinations. Our findings suggest that there may be no reason to refrain from vaccination for healthy individuals.

Conflict of interest statement

The authors declare no conflicts of interest.

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This study received no extramural funding.

Authors' contributions

Yasuhiro Ohara was the principal investigator in this study, designed the study, collected the data, performed statistical analyses, analyzed the data, and drafted the manuscript. Dr Shimpei Mizuta designed the study, collected the data, performed statistical analyses, analyzed the data, and reviewed the manuscript. Dr Hidehiko Matsubayashi, and Dr Tomomoto Ishikawa, supported the collection of data, helped in review of literature, reviewed the manuscript. Dr Tsuyoshi Takiuchi and Dr Tadashi Kimura designed the study and revised the manuscript. All the authors approved the manuscript for publication.

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