

Exhibit 149

Covid-19 vaccination BNT162b2 temporarily impairs semen concentration and total motile count among semen donors

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Abstract

Background: The development of covid-19 vaccinations represents a notable scientific achievement. Nevertheless, concerns have been raised regarding their possible detrimental impact on male fertility

Objective: To investigate the effect of covid-19 BNT162b2 (Pfizer) vaccine on semen parameters among semen donors (SD).

Methods: Thirty-seven SD from three sperm banks that provided 216 samples were included in that retrospective longitudinal multicenter cohort study. BNT162b2 vaccination included two doses, and vaccination completion was scheduled 7 days after the second dose. The study included four phases: T0 – pre-vaccination baseline control, which encompassed 1–2 initial samples per SD; T1, T2 and T3 – short, intermediate, and long terms evaluations, respectively. Each included 1–3 semen samples per donor provided 15–45, 75–125 and over 145 days after vaccination completion, respectively. The primary endpoints were semen parameters. Three statistical analyses were conducted: (1) generalized estimated equation model; (2) first sample and (3) samples' mean of each donor per period were compared to T0.

Results: Repetitive measurements revealed –15.4% sperm concentration decrease on T2 (CI –25.5%–3.9%, $p = 0.01$) leading to total motile count 22.1% reduction (CI –35% – –6.6%, $p = 0.007$) compared to T0. Similarly, analysis of first semen sample only and samples' mean per donor resulted in concentration and total motile count (TMC) reductions on T2 compared to T0 - median decline of 12 million/ml and 31.2 million motile spermatozoa, respectively ($p = 0.02$ and 0.002 respectively) on first sample evaluation and median decline of 9.5×10^6 and 27.3 million motile spermatozoa ($p = 0.004$ and 0.003 , respectively) on samples' mean examination. T3 evaluation demonstrated overall recovery without. Semen volume and sperm motility were not impaired.

Discussion: This longitudinal study focused on SD demonstrates selective temporary sperm concentration and TMC deterioration 3 months after vaccination followed by later recovery verified by diverse statistical analyses.

Conclusions: Systemic immune response after BNT162b2 vaccine is a reasonable cause for transient semen concentration and TMC decline. Long-term prognosis remains good.

KEYWORDS

BNT162b2, Covid-19, male infertility, semen donors

1 | INTRODUCTION

In December 2019, an initial local pneumonia outbreak in Wuhan City of China has quickly developed into the worst global health crisis over a century, as humanity faced a dramatic challenge, which affected daily lives worldwide. On 30 January 2020, World Health Organization (WHO) officially declared the COVID-19 epidemic as a public health emergency of international concern.¹ Full genome sequencing, published shortly after initiation global spread, resulted with identification of new coronavirus initially named 2019-nCoV later turned to Covid-19 or SARS-CoV-2. The new virus genome shared 77.6% sequence identity to SARS-CoV and 96% with bat coronavirus.² On September 7th, 2021, over 221 million people have been diagnosed and more than 4.5 million died from Covid-19 pandemic (<https://www.who.int/emergencies/diseases/novel-coronavirus-2019> accessed on September 7th, 2021).

Over the first pandemic months, there was insufficient data regarding the possible impact of Covid-19 on human reproduction. Yet, it was clear it employs the Angiotensin-Converting Enzyme 2 (ACE2) receptor for cellular entry.^{3,4} Various testicular cells including Leydig, Sertoli, spermatogonia and spermatozoa express ACE2 and related proteases resulting with viral fusion.^{5,6} Cytokine storm-induced dysfunction, autophagy regulation and damaged blood-testis barrier were also suggested as possible pathogenic mechanism for testicular damage.⁷ Clinical reports of orchitis, supported by histological findings, further emphasized testicular involvement.^{8,9} Therefore, detrimental impact on both spermatogenesis and testosterone production¹⁰ seem an obvious outcome. However, studies focused on Covid-19 detection on semen and testis resulted with conflicting results.⁷

Since the identification of the SARS-CoV-2 virus and its genome, an exceptional effort by the scientific community has led to the development of over 300 vaccine projects.¹¹ The rapid and successful development of the BNT162b2 vaccine, providing 95% protection 7 days after second dose,¹² is a notable scientific achievement. Israel was the first country to establish nationwide vaccination campaign. While initial candidates for vaccination were health care workers and citizens older than 65 years, gradual growing availability of the vaccinations enabled expansion of the campaign to all citizens older than 16 years.¹³ Unfortunately, vaccine hesitancy due to various reasons, including fears of “potential damage” to fertility is a major threat to vaccination programs’ success.¹⁴ Consequently, scientific answers are required based on objective methodological standards. There have been few

studies regarding the impact of COVID-19 vaccine of semen parameters, resulting with overall reassuring results, some even reported parameters improvements post-vaccination.^{15–17} However, careful examination raises two questions. First, is there a biological rationale for semen parameters improvement post-vaccination? Second, since semen analysis (SA) may vary significantly over time, what is the reliability of studies that include minimal samples per patient before and after vaccination? In order to answer these concerns, we applied for a methodology, which will answer two requirements: (1) long term follow up over time with repetitive samples per patient and (2) several statistical analysis approaches, which will enable detailed and comprehensive evaluation from different directions. Semen donors (SD) seem suitable for these methodological requirements to investigate vaccinations’ impact due to repetitive supply of semen samples over time. The aim of the research to compare SA parameters of fresh semen samples supplied by SD before and after first two doses of BNT162b2 vaccination. Post vaccination doses were divided to three-time frames (T1, T2 and T3) to supply continuous long-term follow-up.

2 | METHODS

2.1 | Sperm banks and semen donors

This retrospective multicenter study included SD from Shamir (#1), Sheba (#2) and Herzlyia (#3) Medical Centers’ sperm banks (SB), Israel. All SB act according to Israeli Minister of Health regulations and authorizations and all semen laboratories undergo routine annual internal and external supervision tests. Medical evaluation for candidates who apply to become SD are similar between all three SB including two initial semen samples examined both as fresh and freezing-thawing SA. Only those with appropriate scores continue further medical and genetic evaluations as well as general laboratory work.

Semen samples handling, processing and examinations were performed according to WHO guidelines.¹⁸ Briefly, upon sample’s acceptance and after 30–60 min liquification, semen volume (ml) was measured by syringe. Then, a drop of native sperm sample was delivered to Mackler Chamber for evaluation of sperm concentration ($\times 10^6$ per ml), motility (both progressive and non-progressive, measured by % of total sperm cells) and total motile count (TMC, millions).

2.2 | Semen samples prior and after vaccination

The current research included SD who completed both BNT162b2 vaccine doses. Due to previously concerns regarding possible covid-19 semen transmission, every donor had PCR test prior to sample providing. However, further PCR tests were performed only on cases of clinical suspicion, and no positive cases were documented. All SD had negative PCR/serological results with no Covid-19 symptoms. Once vaccinated, no further tests were applied.

Vaccination policy was applied according to general population and not as post-recovery. Donors were regarded as vaccinated a week after second dose¹² between February 1st ad April 16th, 2021 ("vaccinated date"). The study included four phases: T0 - pre vaccination baseline control, which included 1–2 initial samples. Each of the three post-vaccination time frames - T1, T2 and T3 (short, intermediate and long term evaluations, respectively) - included 1–3 samples per donor supplied 15–45, 75–125 and over 145 days after vaccinated date, respectively. Samples produced after third (buster) vaccination dose were excluded from the study.

Each donor supplied at least single pre-vaccination (T0) and single post-vaccination (either T1, T2 or T3) samples by masturbation after 3–5 days of abstinence.

2.3 | Statistical analysis

The primary endpoints were semen volume, sperm concentration, overall sperm motility (progressive and non-progressive) and TMC comparison between T0 versus T1, T2 and T3. Continuous parameters were evaluated for normal distribution using histogram and Q-Q plot. Since volume, concentration and TMC were squawked, they were transformed using the natural logarithm function.

The current research included three statistical analyses: (1) generalized estimated equation model was used for repeated measures analysis, (2) median difference between T1, T2 and T3 versus T0 comparing the first sample per period for each donor and (3) samples' mean of each donor per period using paired samples *t*-test or Wilcoxon test. Median differences analysis included 28, 29 and 22 SD who supplied samples on T0 and T1, T2 or T3, respectively.

All statistical tests were 2 sided and $p < 0.05$ was considered as statistically significant. SPSS software was used for all statistical analysis (IBM SPSS statistics for windows, version 25, IBM corporation, armnok, NY, USA, 2017)

IRB approval was obtained in all included institutions.

3 | RESULTS

The research included 37 SD who supplied at least single semen sample prior (T0) and post- (either T1, T2 or T3) vaccination. SB #1 included 9 SD who supplied total 60 samples while SB # 2 and # 3 included

TABLE 1 Semen donors and samples

	SB ¹ #1	SB #2	SB #3	Total
Sperm donors (<i>n</i>)	9	12	16	37
Age (years)	25.9 ± 4.3	25.8 ± 3.7	26.5 ± 4.7	26.1 ± 4.2 ^{2,3}
T0 samples	17	24	25	66
T1 samples	16	16	20	52
T2 samples	15	29	17	61
T3 samples	12	9	16	37
Total samples	60	78	78	216

¹SB - Sperm bank.

²Mean age, $p = 0.887$.

³± implies for standard deviation.

12 and 16 SD providing 78 samples each, resulting with total 216 samples. SD average age was 26.1 ± 4.2 years without significant difference between SBs (Table 1). While T0 samples were collected before vaccination (up to 2 samples per donor, total 51 samples), average collection intervals post-vaccination were 26.7 ± 10 , 92.5 ± 13.4 and 174.8 ± 26.8 days post-vaccination date for T1, T2 and T3 ($p < 0.0001$, respectively, up to 3 samples per donor on each time frame).

3.1 | Semen parameters' evaluations over time

The wide heterogeneity and variations between semen samples over time necessitates repetitive measurements per donor and diverse statistical approaches. The first analysis included repetitive measurements to evaluate the post-vaccination change compared to T0 as reference. No significant change was demonstrated between T1 and T0. However, while volume and motility changes on T2 were not significant, sperm concentration was significantly lower due to decrease of -15.4% (confidence interval -25.5% – -3.9%) compared to T0 ($p = 0.01$). Moreover, TMC percentage change reduction of 22.1% was significantly lower compared to T0 (confidence interval -35% – -6.6% , $p = 0.007$) as well. Although concentration and TMC were reduced also on T3, these values did not reach statistical significance (Table 2).

The second analysis focused on median differences between T1, T2 and T3 versus T0 according to the first semen sample of each donor on each time frame. The only significant changes were found for sperm concentration and TMC with median decline of 12 million/ml and 31.2 million motile spermatozoa, respectively ($p = 0.02$ and 0.002 , respectively) during T2 followed by later recovery during T3 (Table 3).

Last and similarly, median differences between T1, T2 and T3 versus T0 according to sample's mean of each donor were investigated. Again, the only significant differences were found specifically on sperm concentration and TMC on T2 - median decline of 9.5 million/ml and 27.3 million motile spermatozoa, respectively ($p = 0.004$ and 0.003 , respectively) followed by recovery on T3 (Table 4).

TABLE 2 Percentage and absolute change¹ compared to T0 as reference measured by repeated measures analysis (total samples)

		Change ¹	95% CI		p-Value
Semen volume	T0 ²	Ref			
	T1	10%	−3.9% 25.8%		0.214
	T2	−4.5%	−14.7% 7%		
	T3	9%	−6.3% 26.8%		
Sperm concentration	T0	Ref			
	T1	−14.5%	−27.9% 1.4%		0.044
	T2	−15.4%	−25.5% −3.9%		
	T3	−15.9%	−30.3% 1.7%		
Sperm motility	T0	Ref			
	T1	2.7	−1 6.6		0.058
	T2	−1.9	−4.9 1.7		
	T3	−4.1	−8.2 0.1		
Total motile count	T0	Ref			
	T1	−2%	−19.9% 20.1%		0.027
	T2	−22.1%	−35% −6.6%		
	T3	−19.4%	−35.4% 0.6%		

¹Volume, concentration and TMC are presented as *percentage* change compared to T0 while motility change is presented as *absolute* change.

²T0 – pre-vaccination baseline control; T1, T2 and T3 – short, intermediate and long-term evaluations after 15–45, 75–125 and over 145 days after vaccination date, respectively.

4 | DISCUSSION

Following rapid and successful pre-clinical and human trials, several vaccines have been developed by international partnerships including Astra Zeneca/Oxford University, Pfizer/BioNTech, Moderna.¹⁹ Over the past year, various studies supplied convincing data supporting vaccinations' efficiency not only be reducing mortality rate but also in lessening in illness severity, hospital admissions resulting with overall improved outcome and prognosis.^{13,20,21} These results demonstrate historic scientific medical achievement. Opposed to that magnitude success, a parallel dramatic phenomenon of the fake news is spread over societies and countries. Content analysis determined that fake news could be divided into Health- and non-health-related types such as religious beliefs, politics, economy, prevention of the infection, the origin of the disease, conspiracy theories etc.²² World Health Organization's Director-General declared the global 'over-abundance' of Covid-19 information an 'infodemic'.²³

One of the most concerning issues is the possible impact of vaccine on human reproduction.¹⁴ Previous reassuring publications were mainly based on single pre- and single-post-vaccination samples per participant.^{16,17,24,25} Safrai et al. investigated pre and post-vaccination semen samples of 72 patients undergoing IVF treatments. Only two samples were included with average time of 71 days between first vaccination dose and post-vaccination sample.²⁵ Lifshitz et al. conducted prospective study among fertile men with similar design including only 2 samples - single pre- and single post-vaccination – the later supplied on average of 37 days post-second vaccination dose.²⁴ Therefore, both

TABLE 3 Median differences between T1, T2 and T3 versus T0¹—first sample per donor in each time frame²

		Median	25 quadrantile	75th percentile	p- Value
Semen volume (ml)	T0-T1	0	−0.94	−0.45	0.29
	T0-T2	0.2	−0.4	−0.8	0.16
	T0-T3	−0.05	−0.5	−0.52	0.63
Sperm concentration (X 10 ⁶ /ml)	T0-T1	12.5	−10	27.25	0.09
	T0-T2	12	−8	31	0.02
	T0-T3	3.5	−15.5	27.5	0.4
Sperm motility (%) ^{3,4}	T0-T1	−5	−9.25	5	0.62
	T0-T2	5	−6	10	0.59
	T0-T3	0	−5	11	0.44
Total motile count (X 10 ⁶)	T0-T1	9.8	−23.2	−24.1	0.36
	T0-T2	31.2	2.5	57.8	0.002
	T0-T3	4.48	−18.3	48.6	0.39

¹T0 – pre-vaccination baseline control; T1, T2 and T3 – short, intermediate and long-term evaluations after 15–45, 75–125 and over 145 days after the vaccination date, respectively.

²Samples sizes: 28, 29 and 22 SD for T1, T2 and T3 comparisons, respectively.

³Progressive and non-progressive.

⁴Wilcoxon for all variables except motility, which was compared by *t*-test.

TABLE 4 Median differences between T1, T2 and T3 versus T0¹—samples' mean per donor in each time frame²

		Median	25 pe	75 percentile	p-Value
Semen volume (ml)	T0-T1	0	-0.95	0.45	0.54
	T0-T2	0.2	-0.4	0.8	0.058
	T0-T3	0	-0.5	0.43	0.66
Sperm concentration (X 10 ⁶ /ml)	T0-T1	6.3	-9.46	27.5	0.15
	T0-T2	9.5	2.75	21.25	0.004
	T0-T3	2.25	-11.1	37.3	0.34
Sperm motility (%) ^{3,4}	T0-T1	-2.1	-9.4	4.7	0.28
	T0-T2	5	-4.4	8.25	0.29
	T0-T3	-2.5	-5	6	0.91
Total motile count TMC (X 10 ⁶)	T0-T1	3.3	-22.8	24.9	0.72
	T0-T2	27.3	1.9	46.1	0.003
	T0-T3	-6.7	-23.5	28.4	0.99

¹T0 – pre-vaccination baseline control; T1, T2 and T3 – short, intermediate and long-term evaluations after 15–45, 75–125 and over 145 days after the vaccination date, respectively.

²Samples sizes: 28, 29 and 22 SD for T1, T2 and T3 comparisons, respectively.

³Progressive and non-progressive.

⁴Wilcoxon for all variables except motility, which was compared by *t*-test.

studies included only two semen samples with follow up equivalent to T1 in the current research yielding similar results but not relevant for the current concentration and TMC decline 3 months post-vaccination completion. Furthermore, Gonzales et al. and Barda et al. reported semen improvement post-vaccination^{16,17} without convincing scientific rationale for their observations. The current study, composed of 37 SD and 216 semen samples over four time points, demonstrates selective temporary deterioration of sperm concentration 3 months after vaccination resulting with impaired TMC without alternations in volume and motility, followed by later recovery. We insisted on verifying our findings by diverse statistical analyses since semen samples are characterized by high within- and between-subjects variations.²⁶ Hence, these results were not solely observed by repetitive analysis but also by using a single sample as well as samples' mean per donor for each time frame. Therefore, the long-term impact of BNT162b2 vaccine seems safe. To the best of our knowledge, this is the first longitudinal research that continuously examined semen analysis after vaccination over 6 months – beyond the spermatogenesis period in human.

Almost 2 decades ago, Carlsen et al. characterized the detrimental impact of febrile illness on various stages of spermatogenesis.²⁷ The COVID-19 vaccines can cause mild adverse effects after the first or second doses, including pain, redness or swelling at the site of vaccine shots, fever, fatigue, headache, etc.²⁸ Therefore, rather than a direct effect on testicular cells (ex. via ACE receptor), we believe that systemic immune response is a more reasonable explanation for the temporary concentration decline. Interestingly, Mohamed Abdelhamid et al. have recently suggested that fever from SARS-CoV-2 virus infection induces a reversible negative effect on the semen parameters

until one cycle (74 days) of spermatogenesis.²⁹ The current study supports that notion not only regarding the febrile systemic response, which impairs spermatogenesis but also on the timing and duration of these alternations. Focusing on long-term follow up, Abdelhamid et al. emphasized *illness*-related testicular damage, which extends beyond patient's recovery. Consequently, they suggested to add that adverse effect to the list of long-term post-COVID-19 syndromes.^{29,30} On the contrary, our findings demonstrate long term recovery after vaccination.

The current study has several limitations. The most important is the focus on SD rather than the general population of patients with subfertility. However, since SD supplies semen on a regular base it enabled a longitudinal design over two post-vaccination time frames versus pre-vaccination baseline. Guo et al. have recently reported temporary decreased semen parameters (sperm concentration, sperm motility etc.) among 41 patients who recovered from Covid-19 compared to healthy controls 75 days after symptoms' appearance. However, significant improvement was noted among 21 patients who supplied a second sample a month later,³⁰ demonstrating the importance of continuous follow-up as performed in the current research. Another limitation is the retrospective design, although we assume its impact on our results and conclusions is small due to high overall similarity among all examined parameters.

In conclusion, in this longitudinal multicenter study, we found a selective temporary decline of sperm concentration and total motile count 3 months post-vaccination followed by recovery among SD. While on first look, these results may seem concerning, from a clinical perspective they confirm previous reports regarding

vaccines' overall safety and reliability despite minor short-term side effects. Since misinformation about health-related subjects represents a public health threat,²³ our findings should support vaccinations programs. Further studies concentrating on different vaccines and populations (ex. subfertile patients) are urgently required.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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The authors received no specific funding for this work.

AUTHOR CONTRIBUTIONS

I G participated in study design, data collection, data analysis and manuscript writing. A K participated in study design and data collection. M D, A U and M L participated in data collection. A H participated in study design data analysis and manuscript editing. M B participated in study design and data collection.

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