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SARS-CoV-2 infection reduces quality of sperm parameters: prospective one year follow-up study in 93 patients

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Summary

Background Short- and long-term implications of SARS-CoV-2 on the quality of the sperm and the results of this on fertility remain largely unknown due to lack of longitudinal studies. In this longitudinal observational cohort study, we aimed to analyse the differential effect and the impact of SARS-CoV-2 infection on different semen quality parameters.

Methods Sperm quality was assessed using the World Health Organization criteria, DNA damage to sperm cells by quantifying the DNA fragmentation index (DFI) and the high-density stainability (HDS), IgA- and IgG-anti-sperm antibodies (ASA) were assessed with light microscopy.

Findings SARS-CoV-2 infection was associated with sperm parameters that were independent of spermatogenic cycle like progressive motility, morphology, DFI and HDS, as well as spermatogenic cycle dependent parameters such as sperm concentration. Detection of IgA- and IgG-ASA allowed classification of patients in three different groups according to its sequence of appearance in sperm during post-COVID-19 follow-up. The maximum progressive motility was lowest during follow-up in patients without ASA (41.9%), intermediate in patients with only IgA-ASA (46.2%) and highest inpatients who had both IgA- and IgG-ASA (54.9%).

Interpretation SARS-CoV-2 infection was associated with changes of all analysed sperm parameters to a different degree which is also observed in their return to normality and is suggestive of individual variations in the patient's immune system performance. Firstly, sperm production is decreased through temporal immune mediated arrest of active meiosis, and secondly immune induced sperm DNA damage prevents fertilization if transferred to the oocyte. Both mechanisms are temporal, and most sperm parameters return to baseline after infection.

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Keywords: COVID-19; Fertility; Anti-sperm antibodies; Sperm DNA damage; DNA fragmentation Index (DFI); Sperm chromatin structure assay (SCSA)

Introduction

Although some studies have shown the presence of SARS-CoV-2 virus in the testis of deceased COVID-19 patients[1–3] and during the acute phase of the infection,[4,5] many studies[6–9] reported the absence of the virus in semen shortly after COVID-19. Our data showed that semen is not infectious at a median of 21 days after SARS-CoV-2 infection.[9] Despite the potential risk for sexual transmission of the SARS-CoV-2 virus therefore being low, short- and long-term effects on the quality of the sperm and the impact of this on fertility remain largely unknown due to lack of longitudinal studies. There are increasing amount of data showing that SARS-CoV-2 RNA is only sporadically present in the

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Research in context

Evidence before this study

Although some studies have shown the presence of SARS-CoV-2 virus in the testis of deceased COVID-19 patients and during the acute phase of the infection, many studies reported the absence of the virus in semen shortly after COVID-19. Despite the potential risk for sexual transmission of the SARS-CoV-2 virus therefore being low, short- and long-term effects on the quality of the sperm and the impact of this on fertility remain largely unknown due to lack of longitudinal studies.

Added value of this study

This large follow-up study of post COVID-19 patients demonstrated a harmful effect of SARS-CoV-2 infection on different sperm parameters through immune response. Recovery of the different sperm parameters varied between patients, could take more than one-year post infection, and depended on the initial immune response of the patient. Our data show for the first time that peak progressive sperm motility post-COVID-19 is dependent on the patient's antibody response. Patients producing both IgA/IgG-ASA had the fastest and most complete recovery, while patients without IgA/IgG-ASA experienced slow and incomplete recovery. Conversely, harmful sIgG-N in blood only significantly correlated with higher sperm DNA damage (DFI). Our longitudinal data show that the reduction in sperm concentration after SARS-CoV-2 infection is not caused by fever but rather by the immunological response that follows afterwards.

The main goal of the immune response after a SARS-CoV-2 infection is to protect cell populations amplified through meiosis. Firstly, sperm production is decreased through temporal arrest of active meiosis, and secondly the sperm DNA is damaged preventing fertilization if transferred to the oocyte. Both mechanisms are temporal, and most sperm parameters return to baseline after infection.

Implications of all the available evidence

Detection of IgA and IgG ASA that bind the tail of spermatozoa allowed a novel classification of patients in 3 different groups according to the detection and sequence of appearance in sperm during post COVID-19 follow-up. Detection of harmful (sIgG-N) and protective (RBD-IgG) antibodies in both serum and sperm (IgA/IgG-ASA) as well as the measurement of sperm DNA damage in sperm should be promoted in fertility assessment.

While we showed that the humoral immunity plays a key role in modulating sperm parameters after SARS-CoV-2 infection, future research will focus on how the cellular immunity and t-cell mediated response can also impact fertility.

reproductive tract, but the long-term effects of the virally induced immune responses on reproductive tissues remain to be uncovered. One of the most frequently reported epidemiological data is gender related COVID-19 mortality, men being more affected than women. In Belgium male patients accounted for 52.1% of deaths due to COVID-19 (https://epistat.wiv-isp.be/covid/). While women during their reproductive years in general only have one reduction division per month, their reproductive system is less susceptible to viral infections than men.[10] Furthermore, women are known to mount stronger humoral as well as cell-mediated immunity in response to viral infections.[11] Men, on the other hand, starting from puberty until they die, have millions of reduction divisions everyday producing gametes in an environment devoid of immune protection. Therefore, viral infections pose a greater threat for male than for female gametes. In men, not only during the production but also after finalizing the production of the spermatozoa, some viruses can gain access to the spermatozoa, get inside them, and get piggybacked into the oocyte, compromising the embryo (Zika).[12] Even worse, viruses, such as SARS-CoV-2, can alter protein-coding genes in spermatozoa, and transfer them to the offspring as shown in a study on sperm collected from patients after recovery from COVID-19.[13]

The purpose of this longitudinal observational cohort study was to analyse the differential effect and the impact of SARS-CoV-2 infection on different semen quality parameters. We present the sperm quality and the immunological data of a longitudinal follow-up of 120 unvaccinated patients after the first/second COVID-19 wave.

Methods

Study design and participants

In this observational prospective cohort study, patients (18–70 years old) with a proven SARS-CoV-2 infection during the first (March 2020 and June 2020) and second wave (August 2020 and February 2021) in Belgium, were followed up for up to six control visits in outpatient health care facilities in Antwerpen, Tienen or Genk.[9] Because at the start of the initial study in May 2020 the proportion of SARS-CoV-2 PCR positivity in semen was expected to be low but was unknown at that moment, we decided to include semen samples from 100 participants. With an expected dropout rate of 20%, we aimed for 120 inclusions. Of 120 included patients recruited from the general population, two vasectomized patients were excluded from follow-up, 93 patients had two or more control visits, 42 had three visits, nine had four visits, three had five visits and two had six visits (242 post-COVID-19 visits). For the follow-up part of the study, we did not expect that SARS-CoV-2 infection would have such an influence on sperm parameters

Characteristics	
Number of participants included in follow-up	93
Number of days post infection, mean ± SD [range]	169.5 ± 75.6 [38.0–401.0]
Age at inclusion, mean ± SD [range]	34.7 ± 8.7 [18.0–69.0]
Body mass index (kg/m^2), mean ± SD [range]	24.4 ± 4.4 [18.0–49.1]
Having children	40/93 (43.0%)
Reported infertility	6/93 (6.5%)
Current smoking	7/93 (7.5%)
Positive SARS-CoV-2 PCR nasopharyngeal swab	93/93 (100%)
Underlying conditions	11/93 (11.8%)
Total symptom score (0–15)	4.0 ± 1.6
Fever (>38 °C)	35/93 (37.6%)
Home recovery	90/93 (96.8%)
Hospitalization	3/93 (3.2%)

Data are shown as number (percentage of total) or mean ± SD.

Table 1: **Epidemiologic characteristics of patients with two or more follow-up visits included in the post COVID-19 follow-up trial.**

and that so many participants would come back several times for follow-up. For the follow-up part of the study, we did not conduct any sample size calculations that could have provided an assessment of study power. The epidemiologic characteristics of the 93 patients with two or more control visits is shown in Table 1. During each follow-up a fresh sperm sample produced by masturbation was provided and a blood sample was taken. The protocol and methods can be found in a former paper. The same laboratory procedures and tests were performed on all samples in follow-up.[9] Each subject served as its own control. The study was reviewed and approved by the ethical committee of University Hospital Antwerp on May 11, 2020 (B3002020000078).

SARS-CoV-2 IgG antibodies in serum and SARS-CoV-2 RNA in sperm

The semiquantitative detection of IgG antibodies to the receptor binding domain (RBD) of the subunit of the spike protein of SARS-CoV-2 (sIgG-RBD) and of IgG antibodies to the nucleocapsid protein (N) of SARS-CoV-2 (sIgG-N) were performed as previously described (ARCHITECT System, Abbott Laboratories, reference 6S60-30, 6R8620).[9] All post-COVID-19 sperm samples were tested for the presence of SARS-CoV-2 RNA with the SpermCOVID test.[9] In the SpermCOVID test we used the Chemagic Viral DNA/RNA 300 Kit H96 (Chemagen, PerkinElmer; cat no. CMG-1033-S, Zaventem, Belgium) on the Chemagic 360 instrument for automated isolation from 300 mL of semen. After isolation of viral RNA using the short protocol, the eluted viral RNA was subsequently converted to cDNA with the TaqPath COVID-19 CE-IVD RT-PCR Kit (ThermoFisher Scientific, Waltham, MA). The PerkinElmer SARS-CoV-2 Real-time RT-PCR assay was used to simultaneously detect two SARS-CoV-2 target genes (N-gene and ORF1ab-gene), an internal control gene (MS2-phage), and a human RNA control using Quantstudio 7 flex (Applied Biosystems, Waltham, MA). The detection limit of the SpermCOVID test is two SARS-CoV-2 copies/mL semen.

Analysis of semen quality parameters

Sperm quality was assessed using the World Health Organization (WHO) guidelines for semen analysis, measuring sperm concentration, motility and morphology.[14] Microscopic assessment of sperm motility was done at 37 °C. The motility of each spermatozoon was graded in three categories. Grade A progressive motility (progressive motility): spermatozoa moving actively, either linearly or in a large circle, regardless of speed. Non-progressive motility (grade B motility): all other patterns of motility with an absence of progression, e.g. swimming in small circles, the flagellar force hardly displacing the head, or when only a flagellar beat can be observed. Immotile spermatozoa (grade C): no movement. Percentages of DNA fragmentation index (DFI), high-density stainability (HDS), presence of IgA- and IgG-anti-sperm antibodies (ASA) were measured as previously described.[9] Briefly, when moving spermatozoa were present, direct IgA and IgG mixed antiglobulin reaction (MAR) tests were performed using the sperMar test kit for IgA and IgG (Fertipro, Beernem, Belgium). Light microscopy was used to determine the percentage of motile spermatozoa with attached latex particles. The location on the spermatozoan (head, midpiece, tail) where the latex particles attached was also recorded.

Effect, impact, and recovery of sperm parameters

As we did not have a baseline sample before COVID-19 occurred, we used the difference (increase or decrease) between measured peak and bottom values of sperm concentration, progressive motility, morphology and

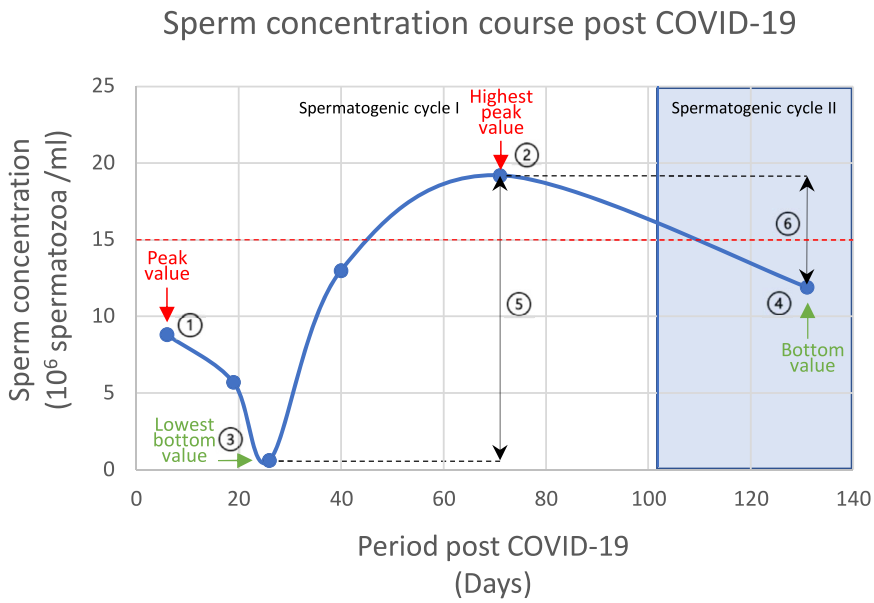

Fig. 1: Sperm concentration course post-COVID-19 (participant 023). ① peak value in first half of spermatogenic cycle I, 8.8 × 10^6 spermatozoa/mL at day six post-COVID-19, ② highest peak value, second half of spermatogenic cycle I, 19.2 × 10^6 spermatozoa/mL at day 71 post-COVID-19, sperm concentration maximum (baseline); ③ bottom value in spermatogenic cycle I, 0.6 × 10^6 spermatozoa/mL at day 26 post-COVID-19, lowest bottom concentration (minimum); ④ bottom value in spermatogenic cycle II, 11.9 × 10^6 spermatozoa/mL at day 131 post-COVID-19; ⑤ sperm concentration decrease post-COVID-19, the highest peak value or maximum and lowest bottom value or minimum was used to calculate the effect of SARS-CoV-2 on sperm concentration, decrease of 96.9% (high effect); ⑥ sperm concentration decrease in spermatogenic cycle II, decrease of 38.0%; The participant was classified as having a normal sperm concentration (no impact). At the last measurement (end of follow-up) the sperm concentration was abnormal and was classified as not recovered. Red dashed line: WHO cut-off (15 × 10^6 spermatozoa/mL).

sperm DNA damage (DFI/HDS) as a proxy to assess the effect of the SARS-CoV-2 infection on each of the sperm parameters. We assessed the extent of the impact by calculating how many patients fitted the diagnoses of oligozoospermia, asthenozoospermia, teratozoospermia and elevated DFI and HDS during follow-up (Fig. 1). Following WHO cut-offs values were used[14]: sperm concentration below 15 × 10^6 spermatozoa/mL (oligozoospermia), progressive motility below 32% (asthenozoospermia), and normal morphology <4% (teratozoospermia). In the last 20 years a limited number of studies determined the DFI threshold for both natural or intra-uterine insemination (IUI) fertilization using the Sperm Chromatin Structure Assay (SCSA),[15–19] all finding a consistent and narrow interval for the DFI threshold between 25% and 27%. A DFI of ≥25% was used as cut-off for an abnormal DFI. A value ≥ 15% was used as cut-off for an abnormal HDS.[17] Finally, we calculated how many of the sperm parameter diagnoses recovered to normal in function of the time lapse after infection and correlated these findings with the calculated phase in the spermiogenesis cycle.

ASA IgA and IgG classification

An immunologic cause of infertility was considered when ≥40% of the moving spermatozoa had latex particles attached.[14] During the microscopic detection of ASA, we recorded where the attachment of latex particles coated with anti-IgA or anti-IgG antibodies occurred on the spermatozoa. Three different attachment sites of ASA on spermatozoa were studied, ASA binding to the tail, the head, or covering the whole spermatozoa. For location specific attachment of ASA IgA/IgG on sperm, ≥2% was considered positive (see video 1–3). The spatial attachment of both IgA/IgG ASA to the same part on the spermatozoa could point to a common epitope for both antibody types against SARS-CoV-2 virus or its receptor. We classified our patients in three different groups according to the detection and sequence of IgA- and IgG-ASA appearance in semen post-COVID-19: 1) no IgA-/IgG-ASA, 2) IgA-ASA but no IgG-ASA, and 3) both IgA- and IgG-ASA present.

Sperm concentration

In the cycle of the seminiferous epithelium, 16 days are needed to prepare A_{pale}-spermatogonia to become committed to meiosis and enter active spermatogenesis. Thereafter a spermatogenic wave lasting 74 days ensues, followed by the epididymal transit lasting 12 days. The cycle releases spermatozoa constantly.[20,21] In normal healthy men this would result in a constant sperm concentration over time during follow-up. If

SARS-CoV-2 infection would influence this production process by impacting the seminiferous epithelium cycle or the spermatogenic wave, a reduced output should become visible as a proportional difference between measured peak and bottom sperm concentration (sperm concentration difference %) in relation to the number of days post-COVID-19.

We first calculated the number of days between the date of the SARS-CoV-2 RT-PCR positive nasal swab (day zero) and the date of ejaculation for each of the 242 visits. Secondly, we determined the number of days post infection when the sperm concentration was lowest (bottom concentration) and highest (peak concentration). Finally, we calculated the percentual difference between bottom and peak sperm concentration for each of the 93 patients who had attended at least two follow-up visits.

To analyse the effect of the viral infection across different rounds of spermatogenesis, we divided the follow-up in 102-day periods reflecting the duration of time needed for A_{pale}-spermatogonia to divide (16-days), complete spermatogenesis (74-days), go through epididymal transit (12-days) and be ejaculated.

To pinpoint the moments when SARS-CoV-2 infection was associated with the different phases of spermatogenesis, we calculated in which phase of the spermatogenesis time interval, the days post infection of the bottom and peak sperm concentrations occurred for each patient in relation to the time of ejaculation. We used following time intervals in relation to the time of ejaculation for our calculations: mitotic proliferation 86–58 days before; meiotic division 58–34 days before; spermiogenesis 34–12 days before and epididymal transit 12–0 days before ejaculation.[20–22]

To approximate the sperm concentration before infection (baseline), we used the peak sperm concentration measured during follow-up as a proxy, assuming asynchronous meiosis and constant release of spermatozoa over time.

To calculate the average number of days post-COVID-19 at which the sperm concentration bottom occurred, the whole spermatogenic cycle period was used (0–102; 103–204; 205–306 and 307–408 days). As there were two sperm concentration peaks during the first spermatogenic cycle, each 102-day cycle was split into a first and second half (0–51 and 52–102 days), to calculate the average number of days post-COVID-19 at which the sperm concentration peaks occurred (Fig. 1). For each patient the lowest sperm concentration bottom value was considered as sperm concentration minimum, and the highest sperm concentration peak value as sperm concentration maximum.

Statistical analysis

Excel 2017 was used for data collection and management, and statistical analysis was performed with MedCalc Software version 17.2–64 bit.[23] The Shapiro–Wilks test was performed to assess if variables were normally distributed. For non-normal distributed variables logarithmic transformation was applied and when transformed variables were normally distributed geometric means was reported. The serial measurements module was used in a first stage to calculate a suitable summary measure within the duration of follow-up for each subject (e.g. time to reach bottom, time to reach peak), and in a second stage these summary measures are analysed by simple statistical techniques as though they were raw data.[24] For normally distributed data, ANOVA was used to compare means of groups. When the distribution of the summary measures was not normal, we used the Kruskal Wallis tests, and for not normally distributed paired samples, the Wilcoxon test was used. The Mann–Whitney test was used to test the significance of the difference between two independent samples. Linear multiple regression analysis was performed after testing for collinearity of the variables used in the model. Collinearity was tested by calculating the variance of inflation factor (VIF); VIF <5.0 indicates absence of collinearity. Oldham's method to correct for the bias in the relation between change and initial value was used.[25] Repeated-measures ANOVA was performed to test for differences in continuous variables during the study at six time points. Fever was included as a covariate and post-hoc analysis with Bonferroni-Holm correction for multiple comparisons was performed to test differences between visits. The Levene's test was used to test the homogeneity of variance among groups. If homogeneity of variance assumption was violated, Welch test was performed, and the respective p value was reported. Probability (p) values of <0.05 were considered statistically significant.

Role of the funding source

Supported in part by Femicare Vereniging zonder Winstoogmerk (VZW) and AML (R20-014). AML and Femicare financed the testing and patient expenses. None of the investigators received any remuneration. The funding source had no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Results

Follow-up of COVID-19 patients

The mean post-COVID-19 follow-up period per patient was 169.5 ± 75.6 days. We did not detect SARS-CoV-2 RNA in any of the 242 follow-up semen samples.

Sperm concentration minima (lowest bottom values) in follow-up as a proxy of SARS-CoV-2 infection effect on spermatogenesis

For each of the 93 patients their minimum and maximum sperm concentration was identified (Tables 2 and 3). We

calculated that the diminished sperm concentration measured at 40.0 days (IQR 25.8–58.3 days) during the first spermatogenic cycle post-COVID-19 occurred during meiotic division. The following sperm concentration bottom values in spermatogenic cycles 2, 3 and 4 also occurred during meiosis (Fig. 2). There was no difference in sperm concentration minima between the subsequent spermatogenic cycles (Kruskal Wallis tests, Table 2).

There was a significant inverse correlation between the change in sperm concentration (bottom values—peak values) and the average sperm concentration ((bottom value + peak value)/2) of the same patient (Fig. 3A). In patients with the highest sperm concentrations SARS-CoV-2 infection was associated with the greatest sperm concentration difference between bottom and peak values. The sperm concentration minima correlated with the time to reach the bottom value post-COVID-19 (Fig. 3B, $R^2 = 0.0426$, $p = 0.0473$; Regression). The sperm concentration minima were lowest in the first spermatogenic cycle post infection, increased during the subsequent cycles and were highest in the fourth spermatogenic cycle post-COVID-19.

In 11 patients two separate sperm concentration bottom values occurred during follow-up. The median reduction in the first sperm concentration bottom value (69.6%) was higher than in the second sperm concentration bottom value (40.5%; $p = 0.0488$; Wilcoxon test).

Of 65 patients with a sperm concentration bottom value during the first spermatogenic cycle 23 (35.4%) reported fever (≥38.0 °C for at least 1 day) and 42 (64.6%) did not. None of the patients with sperm concentration bottom values in spermatogenic cycles 2–4 reported having fever. There was no difference between patients who experienced fever and who did not in time to reach bottom sperm concentration, sperm concentration bottom value or in % sperm concentration reduction (data not shown).

Sperm concentration peak value as proxy of asynchronous meiosis and constant release of spermatozoa in time (baseline)

The 93 sperm concentration maxima from each patient during follow-up clustered in six different time periods since infection (Table 3). There was no difference between the measured sperm concentration maxima across all patients in the six different time periods post-COVID-19 (Kruskal Wallis tests). The median maximum sperm concentration of all patients across all time periods was 56.110^6/mL (IQR 36.9–76.3). The sperm concentration maxima did not correlate with the time to reach the peak value post-COVID-19 ($R^2 = 0.0393$, $p = 0.6$, Regression).

Effect, impact, and recovery of sperm concentration

Table 4 provides an overview of the difference between sperm concentration bottom and peak values within

Spermatogenic cycle	Number of sperm concentration minima	Median number of days post COVID-19	IQR	Cycle of the seminiferous epithelium	Correction number of days	Days into spermatogenesis per spermatogenic cycle	Process	Median sperm concentration at minimum (10^6/mL)	IQR	Median sperm concentration decrease (%)	IQR	Percentage below WHO cutoff (<15 10^6/mL) (n/N)
1	65	40.0	25.8–58.3	0–1	0	40.0	Meiotic division	20.7	8.7–39.3	55.7	27.1–81.4	33.8 (26/65)
2	22	143.5	126.0–166.0	6	96	47.5	Meiotic division	29.3	13.5–56.3	54.5	34.4–78.9	31.8 (7/22)
3	5	222.0	214.5–235.0	11	176	46.0	Meiotic division	20.7	15.7–34.0	44.2	41.5–61.4	20.0 (1/5)
4	1	361.0	336.0–361.0	20	320	41.0	Meiotic division	28.0	28.0	50.6	50.6	0 (0/1)
All	93	NA	NA	NA	NA	43.0	Meiotic division	21.5	11.5–41.1	53.1	33.1–77.6	36.6 (34/93)

Sperm concentration minimum: lowest sperm concentration bottom value during follow-up, IQR: inter quartile range, NA: not applicable, Sperm concentration decrease (%) was calculated compared to sperm concentration maximum for the same patient, meiotic division: 58-34 days before ejaculation.

Table 2: **Characteristics of sperm concentration minima during consecutive spermatogenic cycles in follow-up post-COVID-19.**

Spermatogenic cycle	Half	Number of sperm concentration maxima	Median number of days post COVID-19	IQR	Cycle of the seminiferous epithelium	Correction number of days	Days into spermatogenesis per spermatogenic cycle	Process	Median sperm concentration maximum (10^6/mL)	IQR	% Below WHO cutoff (<15 10^6/mL)
1	First	10	35.0	30.0–38.0	0–1	0	35.0	Meiotic division	59.2	36.9–92.4	0 (0/10)
	Second	39	84.0	66.3–93.8	0–1	0	84.0	Mitotic proliferation	43.7	27.4–72.8	7.7 (3/39)
2	First	20	123.5	114.0–131.5	6	96	27.5	Spermiogenesis	64.7	43.7–76.0	0 (0/20)
	Second	10	178.5	173.0–184.0	6	96	82.5	Mitotic proliferation	47.0	32.6–68.4	10.0 (1/10)
3	Second	13	234.0	227.8–247.0	11	176	58.0	Meiotic division	58.3	44.6–106.5	0 (0/13)
4	Second	1	376.0	376.0	20	320	56.0	Meiotic division	85.3	85.3	0 (0/1)
All	NA	93	NA	NA	NA	NA	NA	NA	56.1	36.9–76.3	4.3 (4/93)

Sperm concentration maximum: highest sperm concentration peak value during follow-up, IQR: Inter quartile range, SD: Standard deviation, NA: Not applicable.

Table 3: **Characteristics of sperm concentration maxima during consecutive spermatogenic cycles in follow-up post-COVID-19.**

each patient follow-up, according to the type of effect (low (<40%), medium (≥40% and <70%), high (≥70%)) the SARS-CoV-2 infection has on reducing the %sperm concentration during follow-up. The sperm concentration maximum was used to determine if the patients had a sperm concentration above the WHO cut-off of 15×10^6 spermatozoa/mL, and if the SARS-CoV-2 infection had an impact. For sperm concentration the median decrease during follow-up was 53.1%, only four patients (4.3%) were affected and in 62 patients the sperm concentration recovered.

Effect, impact, and recovery of SARS-CoV-2 infection on progressive motility

The progressive sperm motility bottom values did not correlate with the time to reach bottom value (days post-COVID-19) and were therefore considered independent of the spermatogenic cycle (Fig. 3C). There was a correlation between the change in progressive sperm motility (bottom values—peak values) and the average progressive sperm motility ((bottom value + peak value)/2) of the same patient (Fig. 3D). The effect was lower in samples with higher progressive motility.

Table 4 gives an overview of the difference between progressive sperm motility bottom and peak values within each patient follow-up, according to the type of effect the SARS-CoV-2 infection has on reducing the percentage progressive motility.

In asthenozoospermic samples, SARS-CoV-2 infection always resulted in progressive motility below the 32% WHO cut-off. Whereas in samples with normal progressive sperm motility only a high reduction effect (≥50%) could reduce the progressive motility below the 32% WHO cut-off (34/82; 41.5%). For progressive sperm motility, the median decrease during follow-up was 42.9%, 11 patients (11.8%) were affected and in 61 patients the progressive sperm motility recovered.

Effect, impact, and recovery of SARS-CoV-2 infection on sperm morphology

In 28.0% of patients (26/93) there was no difference in sperm morphology during follow-up. There was a correlation between the difference in sperm morphology (bottom value-peak value) and the average sperm morphology ((bottom morphology + peak morphology)/2) from the same patient ($r = -0.2700$, $p = 0.0088$, 95% CI –0.4490 to –0.0702; Spearman's Rho). The sperm morphology bottom values did not correlate with the time to reach the bottom value (days post-COVID-19) and were therefore considered independent of the spermatogenic cycle (data not shown).

Table 4 gives an overview of the difference between normal sperm morphology bottom and peak values within each patient follow-up. In 20 patients (21.5%) the morphology worsened over time during follow-up, while in 47 patients (50.5%) the sperm morphology recovered in average 159 days post infection. In 20 patients the

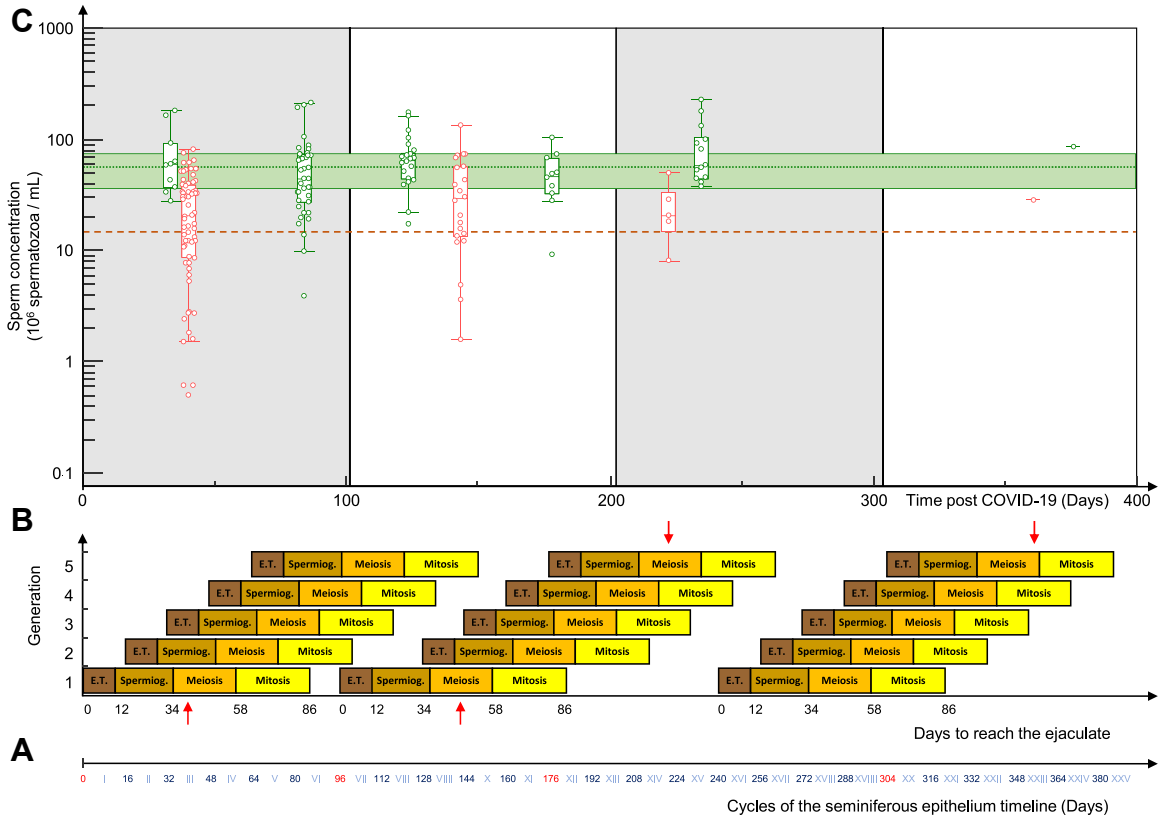

Fig. 2: A: Time line of cycles of the seminiferous epithelium (Roman numbers I to XV), during each 16-day cycle an A_{pale}-spermatogonium progenitor proliferates to become a committed A_{pale}-spermatogonium (designated start day 0) B: 4.6 cycles of the seminiferous epithelium are required for a committed A_{pale}-spermatogonium (designated start day 0) to produce a group of spermatozoa completing spermiation 74 days later and reaches the ejaculate after a 12 day epididymal transit time (E.T.); Red arrows indicate the sperm concentration bottom value in each spermatogenic cycle occurred during meiosis C: Sperm concentration in function of time post-COVID 19 infection. Green rectangle: calculated median sperm concentration maximum and inter quartile range in follow-up as proxy of asynchronous meiosis and constant release of spermatozoa in time. Box-and-Whisker plots: measured maximum sperm concentration (green) and sperm concentration minima (red). Dashed line: WHO cut-off (15 × 10^6 spermatozoa/mL).

recovery stayed below the 4% WHO cut-off in average after 140.7 days (95% CI 100.4–180.9 days) and in 27 patients the sperm morphology improved above the 4% WHO cut-off in average after 173.0 days (95% CI 145.4–200.7 days). In two of these 27 patients, recovery to normal morphology was only reached after 376- and 401-days post-COVID-19.

Effect, impact, and recovery of SARS-CoV-2 infection on sperm DNA damage

Overview of the effect, impact, and recovery of DFI and HDS within each patient follow-up upon infection with SARS-CoV-2 is shown in Table 5.

There was a strong inverse correlation between the difference in sperm DNA damage (bottom values—peak values) and the average sperm DNA damage ((bottom value + peak value)/2) of the same patient for both DFI (Fig. 3E) and HDS (Fig. 3I). The DFI and HDS peak values did not correlate with the time to reach the peak value and were therefore also considered independent of the spermatogenic cycle.

Only during the first spermatogenic cycle did sperm DNA peak values correlated with the time to reach the peak value for DFI (Fig. 3F) and HDS (Fig. 3J). In average the DFI peak was reached 45.6 days post-COVID-19 and the HDS peak after 51.3 days.

Having fever did not correlate with peak DFI or HDS values.

After SARS-CoV-2 infection sperm DNA damage recovered below cut-off for DFI in 76 patients, and for HDS in 63 patients. For both DFI (Fig. 3G) and HDS (Fig. 3K) there was a correlation between the effect of SARS-CoV-2 infection on sperm DNA increase and recovery back to baseline (bottom value). The higher the initial effect (increase of sperm DNA damage) the longer it took to recover to baseline.

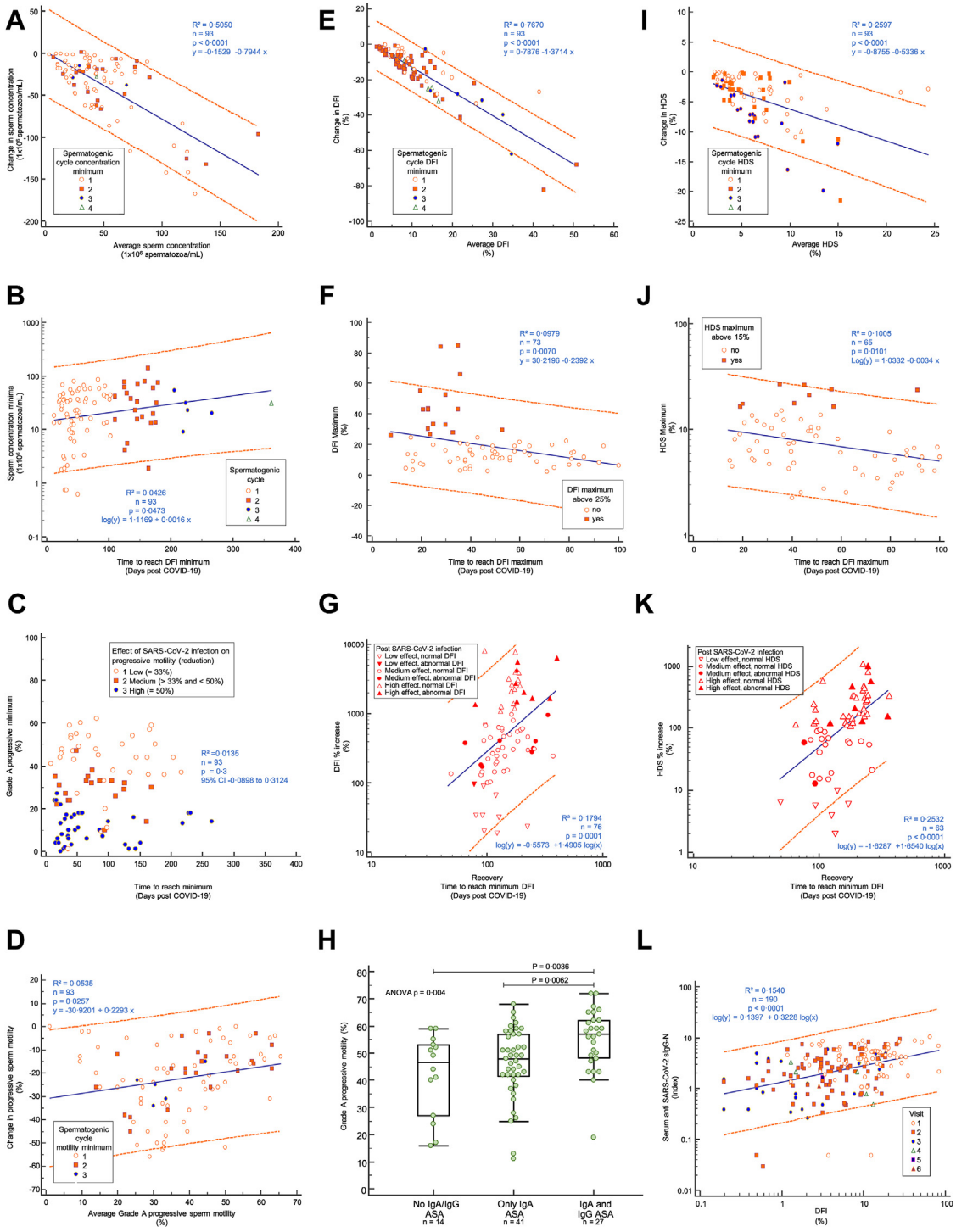

Fig. 3: A: Effect of SARS-CoV-2 on sperm concentration. Correlation between the change in sperm concentration (bottom values—peak values) and the average sperm concentration ((bottom value + peak value)/2) of the same patient. Orange dashed lines: 95% prediction. B: Correlation between sperm concentration bottom (10^6 spermatozoa/mL) and time to reach the sperm concentration bottom values post-COVID-19 (Days). C: Correlation between progressive sperm motility bottom (%) and time to reach progressive sperm motility bottom post-COVID-19 (Days). The progressive sperm motility bottom values did not correlate with the time to reach bottom (days post-COVID-19) and were considered independent of the spermatogenic cycle. D: Effect of SARS-CoV-2 infection on the bottom progressive sperm motility (%). Correlation between the change in progressive sperm motility (bottom values—peak values) and the average progressive sperm motility ((bottom value + peak

SARS-CoV-2 antibodies in serum

Serum anti SARS-CoV-2 sIgG-N levels declined during follow-up (n = 237, r = –0.5040, p < 0.0001, 95% CI –0.5934 to –0.4025; Spearman's Rho) and correlated with DFI between day 23 and day 218 post-COVID-19 (Fig. 3L; n = 190, R^2 = 0.1540, p < 0.0001; Regression), but not with HDS. Serum SARS-CoV-2 sIgG-RBD levels did not correlate with either DFI or HDS.

ASA in semen

In 19.4% (18/93) patients neither IgA nor IgG-ASA were detected post infection. In four of them IgA-ASA measurement failed, so these four were not considered for further analysis. In the remaining 75 patients IgA- and IgG-ASA binding to different locations on spermatozoa was detected. In five patients IgA/IgG-ASA were attached to the whole spermatozoa (5.4%), while in 70/75 patients IgA/IgG-ASA attached only to the tail of the spermatozoa (75.3%). In two patients of the latter group, only IgG-ASA were detected during follow-up. Because first measurement of these two patients were at day 36 and at 81 post infection, respectively, the window of detection of ASA IgA may have been missed, leading to exclusion of these two cases from the ASA classification.

As a result, concerning binding of ASA to the tail of spermatozoa, 14/82 samples showed no IgA/IgG binding at all (17.1%), 41/82 showed IgA binding only (50.0%) and 27/82 showed binding of both IgA and IgG (32.9%).

ASA IgA/IgG sperm tail binding did not correlate with sperm concentration, morphology, DFI nor HDS.

However, concerning mobility, we noted a significant increase in peak progressive motility from patients without ASA (41.9%) to patients with only IgA-ASA (46.2%) to patients who had both IgA/IgG-ASA binding to sperm tails (54.9%; ANOVA; p = 0.004; Fig. 3H). There was no difference between these three groups for %reduction of motility, for the period between progressive motility bottom and peak values or for progressive motility bottom value during follow-up.

Discussion

Our prospective follow-up study clearly shows that a SARS-CoV-2 infection has a profound adverse effect on all studied sperm parameters like concentration, progressive motility, morphology, DFI and HDS.

Strengths of the study are the large number of patients, the confirmation of SARS-CoV-2 infection by PCR, the knowledge of exact timing of the infection, and the long-term follow-up. Follow-up was organized if not all analysed sperm parameters were normal and stopped when all sperm parameters had normalized. The major disadvantage, not to have a control sample before infection, was addressed by comparing peak and bottom values of each parameter of sperm quality and using the peak/bottom value of any parameter during follow-up as a proxy of pre-infection base values. By calculating the time lapse to reach bottom values and assessing the percentage of decrease compared to peak values, this allowed us to obtain a unique insight in the pathogenesis of SARS-CoV-2 infection on sperm function. Also, the simultaneous testing of both serum and sperm for antibody production offered an ideal possibility to study the effect on the host antibody response.

One type of effect of SARS-CoV-2 infection on sperm function was clearly dependent of the spermatogenic cycle: sperm concentration. Other rather affected the finished spermatozoa and were not dependent of the spermatogenic cycle: progressive motility, proportion of normal morphology, and indicators of DNA damage, like DFI and HDS.

Recovery of the different sperm parameters varied between patients, could take more than one-year post infection, and could depend on the initial immune response of the patient. While for sperm morphology and sperm concentration, only part of the patients was affected, this could suggest that at least some patients would have had a t-cell mediated response. There could be several other mechanisms that impact metrics over the study period and could have influenced spermatogenesis such as the combination of SARS-CoV-2 and ACE2 expression levels in reproductive tissue, and the level of hormone production. We did however not detect a decline in sperm concentration maxima in time post COVID-19. This further indicates that it is the reduction division process that is impacted. Every participant has his own sperm production maximum, which reflects the efficiency to produce spermatocytes out of

value)/2) of the same patient. Orange dashed lines: 95% prediction. E: Effect of SARS-CoV-2 infection on DFI bottom value post-COVID-19. Correlation between the DFI increase (%) and DFI bottom values (%) post-COVID-19. The effect was measured as % increase in DFI. F: Effect of SARS-CoV-2 infection on DFI peak value post-COVID-19 during the first spermatogenic cycle. G: Correlation between the effect of SARS-CoV-2 infection on DFI increase and recovery of DFI to bottom during post-COVID-19 follow-up. H: Sperm motility peak value (%) during post-COVID-19 follow-up according to ASA IgA/IgG sperm tail binding classification. No IgA or IgG ASA sperm tail binding n = 14; only IgA ASA sperm tail binding n = 41 and both IgA and IgG ASA sperm tail binding n = 27, ANOVA p = 0.004. I: Effect of SARS-CoV-2 infection on HDS bottom value post-COVID-19. Correlation between the HDS increase (%) and HDS bottom (%) post-COVID-19. The effect was measured as % increase in HDS. J: Effect of SARS-CoV-2 infection on HDS peak value post-COVID-19 during the first spermatogenic cycle. K: Correlation between the effect of SARS-CoV-2 infection on HDS increase and recovery of HDS to bottom value during post-COVID-19 follow-up. L: SARS-CoV-2 sIgG-N antibodies in serum and sperm DNA damage (DFI). Correlation between sIgG-antibodies (Index) versus DFI (%) between day 23 and day 218 post-COVID-19 (n = 190, R^2 = 0.1540, p < 0.0001; Regression).

Sperm parameter	Effect					Impact			Recovery				
	Min/max difference (%)	Median decrease during follow-up (%)	IQR	Median bottom value[a]	Median peak value[a]	Cutoff at maximum	n	From total (%)	Diagnose at end: Normal (n)	Diagnose at end: Abnormal (n)	Recovered (n)	Mean[b] days to reach maximum	95% CI
Concentration	Low (<40%)	17.4	13.7–22.2[c]	9.9	11.9	Oligo	2	2.2	0	2	0	–	–
		20.5	13.6–29.4	39.1	53.8	Normal	29	0	29	0	19	120	94–154
	Medium (≥40% and <70%)	61.2	57.5–65.2[c]	2.6	6.6	Oligo	2	2.2	0	2	0	–	–
		53.0	49.0–58.9	29.3	61.0	Normal	28	0	27	1	18	139	110–176
	High (≥70%)	–	–	–	–	Oligo	–	–	–	–	–	–	–
		84.3	80.8–89.0	8.3	58.4	Normal	32	0	26	6	25	146	123–173
	All	53.1	46.4–65.4	21.5	56.1		93	4.3	82	11	62	136	121–153
Progressive motility	Low (≤33%)	16.8	7.7–19.1[c]	10.0	12.0	Astheno	4	4.3	0	4	0	–	–
		18.2	10.5–23.0	47.0	59.0	Normal	31	0	31	–	17	143	110–185
	Medium (>33% and <50%)	46.8	46.2–47.4[c]	12.0	22.5	Astheno	2	2.2	0	2	1	250	250
		40.9	38.6–44.0	32.0	52.0	Normal	17	0	15	2	13	134	100–179
	High (≥50%)	88.2	81.3–96.3[c]	2.0	24.0	Astheno	5	5.4	0	5	5	174	96–317
		72.5	67.2–83.1	13.0	50.0	Normal	34		27	7	25	122	97–155
	All	42.9	37.5–56.7	24.0[a]	51.0[a]		93	11.8	73	20	61	136	119–155
Morphology	No effect (0%)	0	0–0	1.4	1.4	Terato	19	20.4	0	19	0	–	–
		0	0–0	5.1	5.1	Normal	7	0	7	0	0	–	–
	Medium (<50%)	33.3	33.3–33.3	2.0	3.0	Terato	6	6.5	0	6	5	137	86–217
		25.0	16.7–27.1	4.7	6.1	Normal	29	0	26	3	17	144	119–174
	High (≥50%)	50.0	50.0–83.4	0.8	2.2	Terato	20	21.5	0	20	15	115	82–163
		50.0	50.0–75.0	1.9	5.1	Normal	12	0	10	2	10	189	135–265
	All	25.0	0–50.0	2.7	3.9		93	41.9	43	50	47	141	122–164

IQR: Interquartile range. [a]Bottom and peak sperm concentration in 10^6 spermatozoa/ml, progressive motility, and morphology in percent. [b]Geometric mean; 95% CI: 95% Confidence Interval. [c]Range, Oligo: Oligozoospermic, Astheno: Asthenozoospermic, Terato: Teratozoospermic.

Table 4: **Overview of the effect, impact, and recovery on sperm parameters concentration, progressive motility, and morphology within each patient follow-up upon infection with SARS-CoV-2.**

spermatogonia. Once the external modulation diminishes, sperm concentrations return to maximum (base value). Because all participants tested positive for SARS-CoV-2, and the date of testing is known, we can align them for recovery from viral infection. In some patients, the first measurement of sperm concentration represents pre-COVID values, because it takes another 86 days after meiosis to complete the sperm, and the measurements are made before meiosis is affected (before 43 days post COVID-19; see Fig. 2). Our data clearly show that peak progressive sperm motility post-COVID-19 is dependent on the patient's antibody response. Patients producing both IgA/IgG-ASA had the fastest and most complete recovery, while patients without IgA/IgG-ASA experienced slow and incomplete recovery. If IgA and IgG recognize the SARS-CoV-2 receptor Angiotensin-converting enzyme-2, this can explain the attachment of IgA/IgG-ASA to the tail of spermatozoa, because the receptor is also located on the sperm tail.[26,27] Conversely, harmful sIgG-N in blood only significantly correlated with higher DFI. Similarly, early in the pandemic it became clear that our immune system can produce antibodies that are protective or harmful.[28]

While we could measure a surge in sperm DNA damage during the first spermatogenic cycle post infection, the recovery to minimum baseline values only occurred during the second and third cycle. This could be due to a diminishing immunological response and its waning effect on finished spermatozoa. This is comparable to the effect seen after infection with Influenza in sperm in case studies.[22,29] Furthermore, our data show that the reduction in sperm concentration after SARS-CoV-2 infection is not caused by fever but rather by the immunological response that follows afterwards.

In healthy men the normal response upon SARS-CoV-2 infection (symptomatic) is the up and down regulation of cytokines by the host immune system to prevent harmful effects of the virus. In symptomatic patients infected with SARS-CoV-2 and recover, Hepatocyte Growth Factor (HGF) serum levels are significantly lower than in asymptomatic individuals and healthy uninfected controls.[30] In symptomatic COVID-19 patients who recovered, lowered HGF levels could

Sperm DNA damage	Effect					Impact			Recovery				
	Increase (%)	Mean[a] increase (%)	95% CI	Mean bottom value (%)	Mean peak value (%)	Cutoff at maximum	n	From total (%)	Diagnose at end		Recovered (n)	Mean[a] days to reach minimum	95% CI
									Normal (n)	Abnormal (n)			
DFI	Low (<100%)	34.0	21.2–54.8	7.0	9.8	Normal	12		12	0	8	118.8[c]	80.2–157.3
		95.7	–	28.1	55.0	Abnormal	1	1.1	0	1	1	77.0	77.0
	Medium (≥100% and <1000%)	301.5	248.7–365.5	2.9	11.6	Normal	45		45	0	36	146.6	126.5–166.8
		306.4	198.4–473.3	10.4	41.9	Abnormal	9	9.7	8	1	8	184.1	98.9–269.4
	High (≥1000%)	2490.1	1789.3–3465.6	0.6	14.8	Normal	16		16	0	14	162.4	147.0–177.8
		2517.3	1686.1–3758.3	1.7	41.6	Abnormal	10	10.8	9	1	9	223.2[c]	148.0–299.0
	All	406.6	299.7–551.7	3.9	18.5		93	21.5	90	3	76	158.7	142.7–174.7
HDS	Low (<10%)	4.2	2.1–6.4	5.1	5.3	Normal	10	0	10	0	6	119.0	74.8–163.2
		–	–	–	–	Abnormal	0	–	–	–	–	–	–
	Medium (≥10% and <100%)	47.7	40.2–55.1	4.4	6.4	Normal	37	0	37	0	17	138.2[d]	108.9–167.4
		29.6	12.7–58.9[b]	17.8	22.0	Abnormal	3	3.2	1	2	2	85.5	77.0–94.0
	High (≥100%)	283.1	210.7–355.5	2.5	8.2	Normal	35	0	35	0	31	190.4	167.5–213.3
		363.9	101.1–626.7	5.6	19.8	Abnormal	8	8.6	7	1	7	224.6[d]	158.1–291.0
	All	158.2	116.1–200.3	4.3	8.6		93	11.8	90	3	63	170.0	152.8–187.2

[a]Geometric mean; 95% CI: 95% Confidence Interval. [b]Range. [c]ANOVA $p = 0.012$. [d]ANOVA $p < 0.05$.

Table 5: **Overview of the effect, impact, and recovery of DFI and HDS within each patient follow-up upon infection with SARS-CoV-2.**

inhibit A_{pale}-spermatogonia to divide, cross the blood testis-barrier and become spermatocytes, resulting in a lower sperm concentration.[31,32] The meiotic reduction division is paramount in gametogenesis and its disturbance by a viral infection could cause a lot of harm to progeny. When the immune response to a viral infection blocks meiosis, the production of spermatozoa will momentarily stop, resulting in a sperm concentration minimum in average 43 days post infection. This however does not solve the problem for spermatozoa that can be ejaculated between the moment of infection and the sperm concentration minimum 43 days later. Because these spermatozoa have already been built the moment the infection begins, another strategy is needed to safeguard or inactivate these gametes. For ejaculated spermatozoa between days 0 and 43 post infection, elevated HDS and DFI render the spermatozoa inactive. While sperm DNA damage primarily leads to fragmentation of the paternal chromosomes followed by random distribution of the chromosomal fragments over the two sister cells in the first cell division.[33] Middelkamp and co-workers showed that an unexpected secondary effect of sperm DNA damage is the induction of direct unequal cleavages.[33] As a result, chaotic mosaicism is common in embryos derived from fertilizations with damaged sperm.[34]

In conclusion, in this follow-up study of post-COVID-19 patients, we demonstrated a harmful effect of SARS-CoV-2 infection on different sperm parameters. Besides the direct deleterious effects of the viral infection on the production of male gametes, we also demonstrated that deactivation of the finished spermatozoa occurs, by blocking their mobility and damaging the sperm DNA. We hypothesize both immune driven mechanisms are meant to prevent transfer of damaged DNA to our progeny.

Contributors

CD, EB, JJ, WO, and GD contributed to conceptualisation of the study. CD, EB, JJ, FD, WO, AC, and GD contributed to data collection and performance of laboratory tests for the study. EB, JJ, WO, AC, and GD supervised these investigations. CD, EB, and GD accessed the original data and verified the underlying data. CD, EB, and GD developed the statistical analysis plan. All authors had full access to the data. CD created the figures and wrote the first draft of the manuscript. All authors critically revised and edited the manuscript and approved the final version for submission. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Data sharing statement

The datasets generated for this study are available on request from the corresponding author.

Declaration of interests

We declare no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2023.104640.

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