



ARTICLE

Study of SARS-CoV-2 in semen from asymptomatic donors with the presence of virus in nasopharyngeal swabs



BIOGRAPHY

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KEY MESSAGE

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was not detected in semen samples from asymptomatic individuals infected with SARS-CoV-2. This result supports the safety of assisted human reproduction treatments using this type of sample.

ABSTRACT

Research question: Is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) present in semen samples from asymptomatic donors who have positive virus results from nasopharyngeal swabs?

Design: Nasopharyngeal PCR was performed on 1943 sperm donors between January 2021 and March 2022. The result was positive for 140 donations, and the presence of SARS-CoV-2 could be studied in cryopreserved semen from 84 of these donors. This included 67 participants in whom the quality of fresh semen could be compared with the previous donation, the day of the PCR-positive nasopharyngeal sampling and the first subsequent donation. Semen donations were cryopreserved following total semen ($n = 26$) or ready-to-use ($n = 58$) protocols. The presence of SARS-CoV-2 in cryopreserved samples was determined by reverse transcription PCR. Semen quality (volume, concentration and progressive motility) was evaluated in accordance with World Health Organization 2010 recommendations.

Results: SARS-CoV-2 virus was not detected in any cryopreserved total semen or ready-to-use samples. No significant differences in semen volume, concentration or progressive motility were observed between the last previous donation, the day of the positive PCR nasopharyngeal sampling and the first subsequent donation.

Conclusions: The lack of detection of SARS-CoV-2 in semen samples from asymptomatic individuals infected with SARS-CoV-2 supports the safety of assisted human reproduction treatments using this type of sample.

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KEY WORDS

Asymptomatic donors
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Vaccination

INTRODUCTION

In March 2020 the *World Health Organization (WHO)* designated coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), as a pandemic disease. Multiple investigations are underway on its pathogenicity, prevention and treatment. There has also been growing interest in studying the impact of SARS-CoV-2 in the field of assisted human reproduction (*Cavalcante et al., 2020*).

The mechanism of cell infection is based on transmembrane protein serine-protease 2 (TMPRSS2) and the angiotensin-converting enzyme 2 (ACE2) receptor. The viral spike protein binds to the cellular ACE2 receptor to promote fusion of the viral and cell membranes. Importantly, testes have been found to contain different cell types expressing ACE2 and TMPRSS2, such as spermatogonia and Leydig and Sertoli cells. ACE2 expression has also been demonstrated in seminal vesicle glandular cells (*Hikmet et al., 2020; Wang and Xu, 2020*) and co-expression of ACE2 and TMPRSS2 occurs in the prostate (*Tur-Kaspa et al., 2021*).

Recommendations for SARS-CoV-2 screening in semen donors include triage and study of the presence of the virus in nasopharyngeal samples at different time points during the donation period (*Ata et al., 2022*). Numerous scientific societies have published guidelines on the management of semen in patients undergoing assisted reproductive techniques during the COVID-19 pandemic. Many of these societies recommended the cancellation of cycles in symptomatic patients and in those with the presence of SARS-CoV-2 in nasopharyngeal samples (*SEF-ASEBIR, 2022*).

These recommendations are aimed at symptomatic patients, whose ready identification has favoured their recruitment into clinical trials, leading to a bias towards symptomatic patients in studies on the presence of the virus in semen (*Wang et al., 2023*). Consequently, hardly any data are available on the presence of virus in semen or on the quality of semen in asymptomatic individuals with SARS-CoV-2 (Kteily et al., 2021), who are likely to be increasingly encountered in assisted reproduction

centres with the spread of less aggressive but more contagious variants. The study of semen quality in these patients is of particular interest, given reports of a decrease in semen quality in patients with febrile processes, typically observed in COVID-19 disease (*Abdelhamid et al., 2023*). With this background, the objective of the present study was to investigate the presence of SARS-CoV-2 in semen from infected asymptomatic individuals.

MATERIALS AND METHODS

Study design and ethics

A retrospective observational study was conducted between January 2021 and March 2022. All participating donors signed their informed consent to COVID-19 screening and to the utilization of their samples for research purposes. The study was approved by the regional ethics committee on 31 May 2022 and recorded in the minutes 5/22.

Study population

Donor semen samples ($n = 1943$) were obtained by the CEIFER Biobanco sperm bank from individuals aged 18–35 years accepted as donors under the sperm donation programme of the bank after meeting previously reported eligibility criteria (Molina et al., 2020). Sperm donors were selected in compliance with current Spanish legislation on assisted human reproduction (Law 14/2006, of 26 May 2006) and in accordance with the sperm bank criteria for minimum semen quality (sperm concentration $>50 \times 10^6$ spermatozoa/ml, progressive motility $>50\%$, sperm morphology [normal forms] $>4\%$, and semen volume >2 ml), which are much stricter than the reference limits set out by the WHO (WHO, 2010).

The COVID-19 screening strategy followed the recommendations of the Spanish Fertility Society (*SEF, 2020*), carrying out triage before each donation and performing PCR for SARS-CoV-2 in nasopharyngeal samples. The study included semen samples from asymptomatic donors with positive SARS-CoV-2 PCR results from nasopharyngeal swabs ($n = 140$).

Semen quality was analysed at three different time points: (i) the last donation before the day when the positive result was recorded for the nasopharyngeal PCR – designated as PCR(+) – with a mean interval between these dates of 22 days; (ii)

the day of the nasopharyngeal PCR(+); and (iii) the first semen donation after the PCR(+) day, with a mean interval between these dates of 57 days. Data were also collected on the COVID-19 vaccination status of semen donors (vaccinated/non-vaccinated) and their infective/non-infective status on the day of the nasopharyngeal PCR(+).

Nasopharyngeal PCR was performed in samples from 1943 donors, 140 of whom had a nasopharyngeal PCR(+) swab on the day of donation. Among these 140 donors, 33 were excluded for low semen quality according to the semen bank criteria and 23 due to the lack of a sample (semen straws had been discarded before analysis of the presence of virus). Out of the remaining 84 donors, 17 were excluded from the statistical analysis because they made no further donations, leaving a final total of samples from 67 donors (**FIGURE 1**).

Sampling processing

Donor semen samples were obtained by masturbation at the sperm bank after 3–5 days of sexual abstinence. Fresh semen quality was analysed at each donation after incubation at 37°C for 30 min to favour liquefaction of the sample. Information was collected on the ejaculate volume (ml), sperm concentration (millions/ml) and progressive motility (%), following WHO guidelines (WHO, 2010). The freezing protocol was initiated when the semen samples fulfilled the sperm bank's criteria for minimal semen quality, which are much stricter than the WHO criteria (*Molina et al., 2020*).

Semen samples were frozen at a controlled rate in liquid nitrogen freezers (Freeze Control Systems CryoLogic, Australia). Two freezing protocols were performed, one for total semen from fresh samples and the other for ready-to-use samples processed using density gradients. Under the total semen protocol, fresh semen samples ($n = 26$) were mixed in a 1:1 ratio with egg yolk cryopreservation medium (TEST-yolk, USA). In the ready-to-use protocol, semen samples ($n = 58$) underwent a semen preparation process using two density gradient tubes with 40% and 80% gradients (FertiPro, Belgium). After the density gradient process the recovery of motile sperm was analysed. Next, the spermatozoa were washed and resuspended in FertiCult Flushing culture medium (FertiPro, Belgium) and then mixed (3:1 ratio) with a commercial yolk-

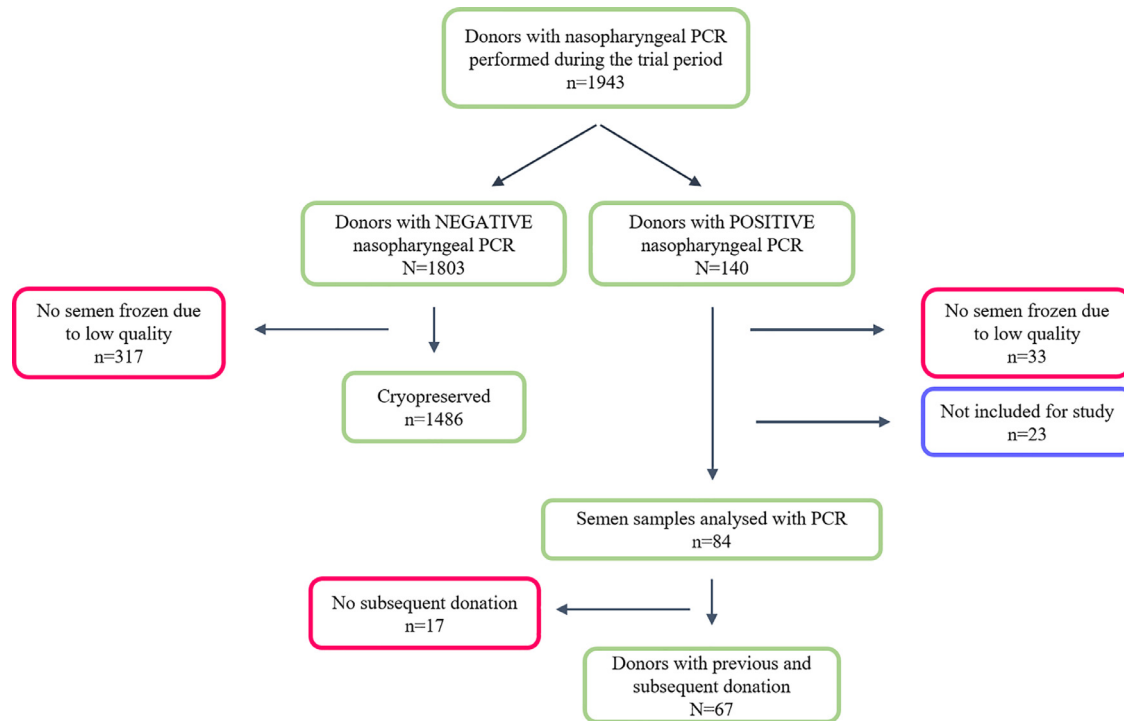


FIGURE 1 Flow chart depicting the selection of donors and donations in the study.

free cryoprotectant medium (SpermFreeze, FertiPro, Belgium).

Semen cryopreservation

The diluted samples with corresponding cryoprotectant were aliquoted in 0.5 ml biological safety straws of ionomer resin (Cryo Bio System, France). The freezing curve described by Mortimer ([Mortimer, 1994](#)) was used in both procedures, followed by direct immersion of the straws in liquid nitrogen. Before SARS-CoV-2 determination, semen straws were removed from the storage canister and incubated for 10 min at 37°C.

SARS-CoV-2 determination by nasopharyngeal swab

A nasopharyngeal swab was used to take a nasopharyngeal sample, following instructions from the qGenomics molecular biology laboratory (Barcelona, Spain). Swabs were sent for processing within 24 h of sampling. The criteria and procedures established by the US Centers for Disease Control and Prevention laboratories (CDC-006-00019, Revision 03) were followed for diagnosis by real-time reverse transcription quantitative PCR (RT-qPCR; COVID-19 RT-qPCR kit; qGenomics). This diagnostic procedure is based on the extraction of nucleic acids from the sample and simultaneous reverse transcription and PCR amplification using virus-specific fluorescent primers and

probes in real time. Specifically, this assay targets regions of the viral capsid genes (*N1* and *N2*) of SARS-CoV-2. Reverse transcription and amplification were performed in a single step (single-step RT-qPCR) using the TaqPath 1-Step RT-qPCR Master Mix reagent (Thermo Fisher Scientific, USA).

The study included ejaculates from donors with nasopharyngeal PCR(+) with a cycle threshold of less than 40 Ct from nasopharyngeal sampling, described as either infective (≤ 30 Ct) or non-infective (> 30 Ct) ([SEF, 2020](#)).

SARS-CoV-2 determination in semen

RT-PCR was used to detect SARS-CoV-2 in semen collected on the day of SARS-CoV-2-positive nasopharyngeal sampling, studying its presence in cryopreserved samples of total semen and ready-to-use samples from donors with an RT-PCR-positive nasopharyngeal swab (< 40 Ct).

RT-PCR was performed using the Cobas SARS-CoV-2 kit on the Cobas 6800 platform to detect the *E* and *ORF1ab* genes (Roche Diagnostics, Switzerland). Samples were previously diluted 1:10 with lysis reagent. A sarbecovirus-unrelated shielded RNA construct in the kit containing specific probe and primer sequence regions (non-infectious RNA in bacteriophage MS2) served as an internal

control. A microbial plasmid of non-infectious DNA containing SARS-CoV-2 sequences and another plasmid containing pan-sarbecovirus sequences were used as a positive control, and Tris buffer and poly (rA) RNA (synthetic) served as a negative control. The maximum number of cycles to consider a positive RT-PCR was 40.

Statistical methods

Qualitative variables were expressed as absolute and relative frequencies (%) and were compared using the chi-squared test, considering $P < 0.05$ as significant. The Agresti-Coull method ([Agresti and Coull, 1998](#)) was used to calculate the confidence interval (CI) of the proportions, truncating the lower confidence interval limit to zero.

Quantitative variables were expressed as means with standard deviations. The different semen quality parameters (volume, sperm concentration and percentage of progressively motile spermatozoa) were compared by one-factor multiple analysis of variance (SARS-CoV-2 infection) with repeated measures: pre-nasopharyngeal PCR(+), day of nasopharyngeal PCR(+) and post-nasopharyngeal PCR(+). This analysis was performed for the global study population, regardless of vaccination or infective status. Two-by-two comparisons were performed post hoc between the different measurement time points for each

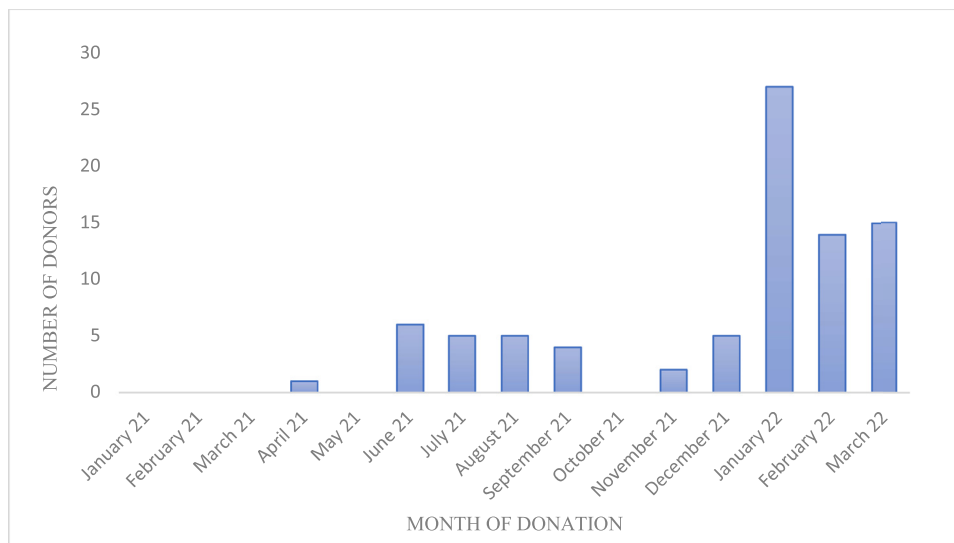


FIGURE 2 Number of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-positive cases detected in asymptomatic donors with a positive nasopharyngeal PCR result.

dependent variable analysed. The Bonferroni correction was calculated to establish the statistical significance.

To compare the motile sperm recovery for the three time points – pre-nasopharyngeal PCR(+), day of nasopharyngeal PCR(+) and post-nasopharyngeal PCR(+) – one-way analysis of variance was used.

Interactions between vaccination status and fresh semen quality at the different measurement time points were explored by conducting multiple analysis of variance with two factors: a between-subject factor with two levels (vaccinated/unvaccinated) and a within-subject factor (SARS-CoV-2 infection) with repeated measures and three levels – pre-nasopharyngeal PCR(+), day of nasopharyngeal PCR(+) and post-nasopharyngeal PCR(+). The effect of infective status on semen quality at the different time points was evaluated by performing a multiple analysis of variance with two factors, a between-subject factor with two levels (infective/non-infective) and a within-subject factor (SARS-CoV-2 infection) with repeated measures and three levels – pre-nasopharyngeal PCR(+), day of nasopharyngeal PCR(+) and post-nasopharyngeal PCR(+).

RESULTS

Nasopharyngeal PCR(+) donors

The number of donors with a positive nasopharyngeal swab for SARS-CoV-2 varied over the months under study

(**FIGURE 2**). The percentage of positive donors was significantly higher during the first quarter of 2022 (12.28%, 95% CI 9.6–15.6%) than in the other quarters studied (first quarter of 2021: 0%, 95% CI 0–1.4%, $P < 0.001$; second quarter of 2021: 1.39%, 95% CI 0.6–2.9%, $P < 0.001$; third quarter of 2021: 6.84%, 95% CI 4–11.2%, $P = 0.048$; and fourth quarter of 2021: 1.59%, 95% CI 0.7–3.3%, $P < 0.001$) (**FIGURE 3**).

Study of SARS-CoV-2 in semen using RT-PCR

SARS-CoV-2 virus was not detected in any of the semen samples collected on the day of the SARS-Cov2-positive nasopharyngeal result (**TABLE 1**).

Semen quality

The percentage of donations failing to meet the quality criteria for freezing did not differ significantly ($P = 0.096$) between donors with positive (33/140, 23.6%) or negative (317/1803, 17.6%) nasopharyngeal PCR results.

The one-factor multiple analysis of variance (SARS-CoV-2 infection) with repeated measures – pre-nasopharyngeal PCR(+), day of nasopharyngeal PCR(+) and post-nasopharyngeal PCR(+) – did not satisfy the sphericity assumption in relation to volume ($W = 0.281$; $P < 0.001$) or concentration ($W = 0.852$; $P < 0.05$). The multivariate Wilks' lambda was then calculated to evaluate the effect of SARS-CoV-2 infection on seminal parameters over time, yielding a non-significant result (Wilks' lambda = 0.916; $P = 0.363$). Hence,

the null hypothesis of equality of means was not rejected, and it was concluded that seminal parameters did not significantly differ between the three measurement time points.

Post-hoc two-by-two comparisons showed no statistically significant difference in semen volume, concentration or progressive motility between the different measurement time points (**TABLE 2**).

Among the 38 donors with motile sperm recovery available for the three time points, no significant difference in progressive motile sperm count was observed between the last donation before the day of nasopharyngeal PCR(+), the day of the nasopharyngeal PCR(+) and the first semen donation after the PCR(+) day ($P = 0.8607$; $58.07 \pm 23.77 \times 10^6$, $58.26 \pm 29.93 \times 10^6$ and $53.94 \pm 32.87 \times 10^6$ progressive sperm, respectively).

Analysis of fresh semen quality parameters in vaccinated and non-vaccinated donors on the day of nasopharyngeal PCR(+)

Among the 30 vaccinated donors, two doses of the Moderna vaccine had been received by 11 and three doses by 3, two doses of the Pfizer vaccine by 13, and a first dose of the Janssen vaccine and second of the Moderna vaccine by 1; no data were available on the date or type of vaccination for the remaining 2 donors. The mean time interval between first vaccination and positive nasopharyngeal PCR was 141 days.

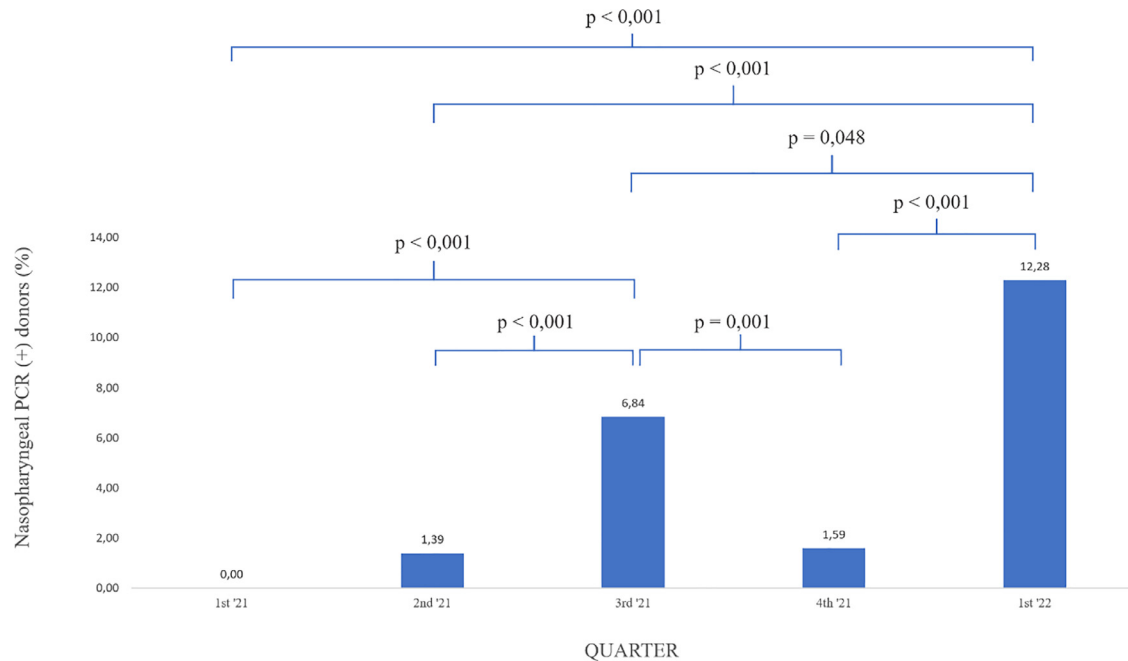


FIGURE 3 Donations from asymptomatic donors with a positive nasopharyngeal PCR test [PCR(+)] as a percentage of all donors tested.

TABLE 1 SARS-COV-2 IN CRYOPRESERVED DONOR SEMEN COLLECTED ON THE DAY OF THE NASOPHARYNGEAL PCR(+) RESULT

Donors	Total semen	Ready-to-use	Total
<i>n</i>	26	58	84
Semen PCR(+) (<i>n</i>)	0	0	0
Semen PCR(+) (%)	0	0	0
95% CI (%)	0–15.2	0–7.4	0–5.2

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Analysis of variance with two factors, a between-subject factor with two levels (vaccination status) and a within-subject factor (SARS-CoV-2 infection) with repeated measures and three levels (measurement time points), did not satisfy the sphericity assumption in relation to the volume ($W = 0.866$, $P < 0.05$) or concentration ($W = 0.816$; $P < 0.01$). Wilks' lambda was calculated and was not statistically significant for SARS-CoV-2 infection (Wilks' lambda = 0.966,

$P = 0.625$) or for the interaction of vaccine with SARS-CoV-2 infection (Wilks' lambda = 0.909, $P < 0.439$). It was concluded that the fresh semen quality parameters under study did not significantly differ between vaccinated ($n = 30$) and non-vaccinated ($n = 37$) donors at any time point.

TABLE 3 displays the semen parameter results on the day of donation – the day with nasopharyngeal PCR(+) – as a

function of the vaccination status of the donor.

Analysis of fresh semen quality parameters in infective and non-infective donors on the day of nasopharyngeal PCR(+)

In the multiple analysis of variance with two factors, the assumption of sphericity was not satisfied in relation to the volume ($W = 0.878$, $P < 0.05$) or concentration ($W = 0.800$, $P < 0.01$). Wilks' lambda was therefore calculated and was also not statistically significant for the SARS-CoV-2 infection factor (Wilks' lambda = 0.942, $P = 0.465$) or for the interaction of infective status with SARS-CoV-2 infection (Wilks' lambda = 0.900; $P < 0.345$). Hence, it was concluded that the fresh semen quality parameters did not significantly differ between infective (<30 Ct) donors ($n = 28$) and non-infective (>30 Ct) donors ($n = 39$) at any measurement time point.

TABLE 2 FRESH SEMEN QUALITY AT THE THREE MEASUREMENT TIME POINTS

Semen parameter	Last previous donation	Nasopharyngeal PCR(+) day	First subsequent donation
Volume (ml)	3.9 ± 1.5	3.8 ± 1.6	3.9 ± 1.7
Concentration (M/ml)	80.3 ± 33.16	84.6 ± 37.4	75.0 ± 33.1
Progressive motility (%)	47.2 ± 9.6	48.3 ± 12.2	46.6 ± 11.2

Data are reported for 67 donors with a semen sample available at each time point. Values are mean ± SD.

Results were not significant ($P = 0.363$).

TABLE 3 FRESH SEMEN QUALITY ON THE DAY OF THE NASOPHARYNGEAL PCR(+) RESULT ACCORDING TO SARS-COV-2 VACCINATION STATUS

Semen parameter	Vaccinated (n = 30)	Non-vaccinated (n = 37)	CI of difference in means (P-value)
Volume (ml)	3.9 ± 1.4	4.5 ± 3.70	−1.929 to 0.735 (P = 0.375)
Concentration (M/ml)	74.1 ± 27.4	82.98 ± 33.67	−22.791 to 5.176 (P = 0.214)
Progressive motility (%)	44.5 ± 9.2	48.32 ± 9.21	−7.891 to 0.371 (P = 0.74)

Data are reported as mean ± SD.

CI, confidence interval; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; M, Million.

TABLE 4 FRESH SEMEN QUALITY ON THE DAY OF THE NASOPHARYNGEAL PCR(+) RESULT ACCORDING TO DONOR INFECTIOUS STATUS

Semen parameter	Infective donors (n = 28)	Non-infective donors (n = 39)	CI of difference in means (P-value)
Volume (ml)	3.9 ± 1.5	3.94 ± 1.6	−0.686 to 0.772 (P = 0.981)
Concentration (M/ml)	80.3 ± 35.1	84.1 ± 38.6	−20.727 to 13.025 (P = 0.651)
Progressive motility (%)	48.5 ± 12.8	47.8 ± 10.9	−4.641 to 5.995 (P = 0.801)

Data are reported as mean ± SD.

CI, confidence interval; M, Million.

TABLE 4 shows the fresh semen quality results on the day of donation – day with nasopharyngeal PCR(+) – as a function of the infective status of the donor.

DISCUSSION

The results of this study confirm that SARS-CoV-2 virus is not present in the semen of asymptomatic semen donors infected with this virus, in agreement with previous findings in smaller patient samples (Kteily et al., 2021; Ma et al., 2020; Pavone et al., 2022; Song et al., 2020). These findings are reassuring for asymptomatic patients undergoing assisted reproductive techniques, given concerns that SARS-CoV-2 might cross the blood–testicular barrier and invade the male genital system due to the presence of ACE2 receptors in testicular cells.

Only two studies have reported the presence of SARS-CoV-2 in semen. In the first, Li and colleagues (Li et al., 2019) studied 38 individuals with the infection admitted to intensive care and detected virus in the semen of six patients (15.8%), who were in recovery or in the acute stage of the disease; however, the journal later issued a statement questioning the methodology after receiving multiple criticisms of its quality (Error in methods, 2020). In the other study, Purpura and co-workers (Purpura et al., 2022) described the presence of the virus in semen after

severe COVID-19 in one patient, who presented with severe oligozoospermia and generalized inflammation at 81 days after the disease. The patients in the above studies markedly differ from those in the present study and from the usual candidates for assisted reproduction during the COVID-19 pandemic, given that all individuals who tested positive would have been ruled out for donation and for assisted reproduction measures.

This evidence on the absence of virus in the semen of asymptomatic infected patients should impact on semen storage recommendations published during the pandemic. In this way, the biosafety risk level assigned by SEF-ASEBIR (2022) for the handling of samples from asymptomatic patients not tested for SARS-CoV-2 should be changed from high to low. The fact that the virus was not detected in total semen samples that had been directly frozen, without a washing or selection step, strongly suggests that it would not be detected in fresh semen either.

The significantly higher percentage of donors with nasopharyngeal PCR(+) during the first few months of 2022 coincided with an increase in the percentage of the general population infected with SARS-CoV-2 (<https://www.juntadeandalucia.es/institutodeestadisticaycartografia/salud/COVID19.html>).

The present findings on fresh semen quality are in agreement with some studies that found no alteration in patients with COVID-19, even with severe disease (Fraietta et al., 2021; Guo et al., 2020). However, others have described significantly lower sperm concentrations, total sperm count and total motility in infected patients than in healthy control participants, although the values were always within the normal ranges (Holtmann et al., 2020; Ruan et al., 2020). The meta-analysis of seven studies by Tiwari and colleagues (Tiwari et al., 2021) concluded that the semen quality was worse, with a lower number of spermatozoa per ejaculate, in patients who had recovered from COVID-19 than in those who had never been infected. Similar conclusions were drawn in the meta-analyses published by Sengupta and collaborators (Sengupta et al., 2021) and Wang and co-workers (Wang et al., 2023).

It might be considered that selection bias explains why this association was not observed in the present study of semen samples from donors with cryopreserved semen available for SARS-CoV-2 PCR testing, given that low-quality semen donations are discarded before freezing. However, this bias can be ruled out because no difference was found in the percentages of low-quality non-frozen donations between those donors who were nasopharyngeal PCR(+) or PCR(−). In addition, no effect on fresh sperm quality

was observed in any period studied, suggesting that the association between infection and semen quality described by other authors may correspond to an indirect effect related to the onset of symptoms rather than to the infectious process itself.

The present observation of no relationship between fresh semen quality and vaccination was also reported by other studies with a 1- to 2-month follow-up after vaccination (*Lifshitz et al., 2022; Olana et al., 2022; Safrai et al., 2022*). On the other hand, a recent study (*Gat et al., 2022*) described a transient decrease in semen quality at 3 months followed by a recovery at 6 months, indicating the need for studies with a longer post-vaccination follow-up.

Fresh semen quality was not significantly affected by the infective/non-infective status of the participants. This finding supports the proposition that previously reported decreases in semen quality might be more attributable to symptoms secondary to SARS-CoV-2 infection, the treatment received or sexual abstinence than to the presence of the virus itself (*Gacci et al., 2021*).

Participants in this study had very good fresh semen quality and were free of associated disease. Different results might, however, be obtained in other study populations. A further limitation is the short follow-up period, which does not allow subsequent involvement of the male genital tract to be ruled out (*Kayaaslan et al., 2020*). Longer follow-up studies of patients with COVID-19 are needed, as suggested by several authors (*Guo et al., 2020; Ma et al., 2020*).

In relation to detection of the virus in semen samples, the utilization of internal and positive controls makes a false-negative result highly unlikely, and other authors have validated similar procedures to detect SARS-CoV-2 in semen (*Chabrolles et al., 2022; Donders et al., 2022*). The analysis of frozen semen samples might be considered a potential limitation, although previous studies have demonstrated that other viruses can be accurately detected in frozen semen samples and that the detection of viral genetic material is not affected by the freezing of this type of sample (*Francis et al., 2022*). Finally, the time interval between donations was appreciably longer than usual due to the need to follow

recommendations by scientific societies and the national health system during the COVID-19 epidemic, including the triage of each donor at each donation.

In conclusion, the absence of SARS-CoV-2 in semen samples from asymptomatic individuals infected with SARS-CoV-2 supports their safe utilization in assisted human reproduction procedures. Any impact of SARS-CoV-2 infection on fresh semen quality may be related to associated symptoms or treatments of the disease or to sexual abstinence rather than to the SARS-CoV-2 infection itself.

DATA AVAILABILITY

Data will be made available on request.

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AUTHOR CONTRIBUTIONS

Conceptualization: G.A., M.M. and J.A.C.; Acquisition of data: M.M., N.B. and M.C.G.; data analysis: G.A., A.C., A.S., N.B., O.C. and J.A.C.; ethics: N.B. and J.A.C.; statistical analysis: G.A., N.B. and J.A.C.; original draft preparation: G.A., M.M., A.C., N.B. and O.C.; review and editing: G.A., M.M., A.C., M.C.G. and O.C. All authors have revised and agreed with the final version of the manuscript.

REFERENCES

- Abdelhamid, MHM, Fellah, AA, Elmarghani, A, Al Msellati, IA, 2023. An Assessment of Men Semen Alterations in SARS-CoV-2: Is Fever the Principal Concern? *Reprod. Sci.* 30, 72–80. <https://doi.org/10.1007/s43032-022-00889-z>.
- Agresti, A, Coull, B., 1998. Approximate is better than 'exact' for interval estimation of binomial proportions. *The American Statistician* 52, 119–126. <https://doi.org/10.2307/2685469>.
- Ata, B, Vermeulen, N, Mocanu, E, Gianaroli, L, Lundin, K, Rautakallio-Hokkanen, S, Tapanainen, JS, Veiga, A., 2022. SARS-CoV-2, fertility and assisted reproduction. *Hum. Reprod. Update.* 14, dmac037. <https://doi.org/10.1093/humupd/dmac037>.
- Cavalcante, MB, Sarno, M, da Silva, ACB, Barini, R., 2020. Letter: COVID-19 and human reproduction: hypothesis needs to be investigated. *Mol. Hum. Reprod.* 26, 549–550. <https://doi.org/10.1093/molehr/gaaa041>.
- Chabrolles, H, Pons-Rejraji, H, Chaput, L, Brebion, A, Fiot, M, Pereira, B, Brugnon, F, Henquell, C., 2022. Validation of a SARS-CoV-2 RT-PCR assay: a requirement to evaluate viral contamination in human semen. *Reprod. Biomed. Online.* 45, 1247–1254. <https://doi.org/10.1016/j.rbmo.2022.09.004>.
- Donders, G, Bosmans, E, Reumers, J, Donders, F, Jonckheere, J, Salembier, G, Stern, N, Jacquemyn, Y, Ombelet, W, Depuydt, C., 2022. Sperm quality and absence of SARS-CoV-2 RNA in semen after COVID-19 infection: a prospective, observational study and validation of the SpermCOVID test. *Fertil. Steril.* 117, 287–296. <https://doi.org/10.1016/j.fertnstert.2021.10.022>.
- Error in Methods, 2020. *JAMA. Netw. Open.* 3, e2010845. <https://doi.org/10.1001/jamanetworkopen.2020.10845>; Erratum for: *JAMA Netw Open.* 2020;13:e208292.
- Fraietta, R, de Carvalho, RC, Camillo, J, Groner, MF, Truzzi, JCCI, Petkov, CN, Barradas, V, Homsí, C, Bellei, W, Oehninger, S., 2021. SARS-CoV-2 is not found in human semen during mild COVID-19 acute stage. *Andrologia* 54, e14286. <https://doi.org/10.1111/and.14286>.
- Francis, J, Kärrberg, C, Hermansson, J, Lindh, M, Ganidou, S, Thurin-Kjellberg, A, Lundin, K, Akouri, R, Tsiartas, P., 2022. Seminal HPV detection: a pilot study comparing the preservation effectiveness and cost between a methanol-based solution and cryopreservation with liquid nitrogen. *Asian. J. Androl.* 24, 560–561. <https://doi.org/10.4103/aja202213>.
- Gacci, M, Coppi, M, Baldi, E, SebastianelliA, Zaccaro C, Morselli, S, Pecoraro, A, Manera, A, Nicoletti, R, Liaci, A, Bisegna, C, Gemma, L, Giancane, S, Pollini, S, Antonelli, A, Lagi, F, Marchiani, S, Dabizzi, S, Degl'Innocenti, S, Annunziato, F, Maggi, M, Vignozzi, L, Bartoloni, A, Rossolini, GM, Serni, S, 2021. Semen impairment and occurrence of SARS-CoV-2 virus in semen after recovery from COVID-19. *Hum. Reprod.* 36, 1520–1529. <https://doi.org/10.1093/humrep/deab026>.
- Gat, I, Kedem, A, Dviri, M, Umanski, A, Levi, M, Hourvitz, A, Baum, M., 2022. Covid-19 vaccination BNT162b2 temporarily impairs semen concentration and total motile count among semen donors. *Andrology.* <https://doi.org/10.1111/andr.13209>.
- Guo, L, Zhao, S, Li, W, Wang, Y, Li, L, Jiang, S, Ren, W, Yuan, Q, Zhang, F, Kong, F, Lei, J, Yuan, M., 2020. Absence of SARS-CoV-2 in

- semen of a COVID-19 patient cohort. *Andrology* 9, 42–47. <https://doi.org/10.1111/andr.12848>.
- Hikmet, F, Méar, L, Edvinsson, Å, Micke, P, Uhlén, M, Lindskog, C., 2020. The protein expression profile of ACE2 in human tissues. *Mol. Syst. Biol.* 16, e9610. <https://doi.org/10.15252/msb.20209610>.
- Holtmann, N, Edimiris, P, Andree, M, Doehmen, C, Baston-Buest, D, Adams, O, Kruessel, J, Biefeld, A, 2020. Assessment of SARS-CoV-2 in human semen—a cohort study. *Fertil. Steril.* 114, 233–238. <https://doi.org/10.1016/j.fertnstert.2020.05.028>.
- Kayaaslan, B, Korukluoglu, G, Hasanoglu, I, Kalem, AK, Eser, F, Akinci, E, Guner, R., 2020. Investigation of SARS-CoV-2 in Semen of Patients in the Acute Stage of COVID-19 Infection. *Urol. Int.* 104, 678–683. <https://doi.org/10.1159/000510531>.
- Li, D, Jin, M, Bao, P, Zhao, W, Zhang, S., 2019. Clinical Characteristics and Results of Semen Tests Among Men with Coronavirus Disease 2019. *JAMA Netw Open* 3, e208292. <https://doi.org/10.1001/jamanetworkopen.2020.8292>.
- Lifshitz, D, Haas, J, Lebovitz, O, Raviv, G, Orvieto, R, Aizer, A., 2022. Does mRNA SARS-CoV-2 vaccine detrimentally affect male fertility, as reflected by semen analysis? *Reprod Biomed Online* 44, 145–149. <https://doi.org/10.1016/j.rbmo.2021.09.021>.
- Ma, L, Xie, W, Li, D, Shi, L, Ye, G, Mao, Y, Xiong, Y, Sun, H, Zheng, F, Chen, Z, Qin, J, Lyu, J, Zhang, Y, Zhang, M., 2020. Evaluation of sex-related hormones and semen characteristics in reproductive-aged male COVID-19 patients. *J. Med. Virol.* 93, 456–462. <https://doi.org/10.1002/jmv.26259>.
- Molina, M, Yoldi, A, Navas, P, Gañán, M, Vaquero, Á, Del Pico, JL, Ramírez, JP, Castilla, JA., 2020. Carriers of cystic fibrosis among sperm donors: complete CFTR gene analysis versus CFTR genotyping. *Fertil. Steril.* 114, 524–534. <https://doi.org/10.1016/j.fertnstert.2020.04.016>.
- Mortimer, D., 1994. Laboratory standards in routine clinical andrology. *Reprod Med Review* 3, 97–111.
- Olana, S, Mazzilli, R, Salerno, G, Zamponi, V, Tarsitano, MG, Simmaco, M, Paoli, D, Faggiano, A., 2022. 4BNT162b2 mRNA COVID-19 vaccine and semen: What do we know? *Andrology*. <https://doi.org/10.1111/andr.13199>.
- Pavone, C, Giammanco, G, Cascino, A, Baiamonte, D, Pinelli, M, Cangelosi, E, Filizzolo, C, Sciortino, G, De Grazia, S, Bonura, F., 2022. Assessment of SARS-CoV-2 RNA shedding in semen of 36 males with symptomatic, asymptomatic, and convalescent infection during the first and second wave of COVID-19 pandemic in Italy. *Asian. J. Androl.* 24, 135. <https://doi.org/10.4103/aja2021103>.
- Purpura, LJ, Alulak, J, Chong, AM, Liu, L, Cantos, A, Shah, J, Medrano, N, Chang, JY, Tsujii, M, Mohri, H, Uhlemann, AC, Ho, D, Yin, MT., 2022. SARS-CoV-2 RNA Shedding in Semen and Oligozoospermia of Patient with Severe Coronavirus Disease 11 Weeks after Infection. *Emerging Infect. Dis.* 28, 196–200. <https://doi.org/10.3201/eid2801.211521>.
- Ruan, Y, Hu, B, Liu, Z, Liu, K, Jiang, H, Li, H, Li, R, Luan, Y, Liu, X, Yu, G, Xu, S, Yuan, X, Wang, S, Yang, W, Ye, Z, Liu, J, Wang, T., 2020. No detection of SARS-CoV-2 from urine, expressed prostatic secretions, and semen in 74 recovered COVID-19 male patients: A perspective and urogenital evaluation. *Andrology* 9, 99–106. <https://doi.org/10.1111/andr.12939>.
- Safrai, M, Herzberg, S, Imbar, T, Reubinoff, B, Dior, U, Ben-Meir, A., 2022. The BNT162b2 mRNA Covid-19 vaccine does not impair sperm parameters. *Reprod Biomed Online* 44, 685–688. <https://doi.org/10.1016/j.rbmo.2022.01.008>.
- SEF-ASEBIR, 2022. Sociedad Española de Fertilidad y Asociación para el Estudio de la Biología de la Reproducción. ASEBIR. Recomendaciones para la seguridad y reducción de riesgos ante la infección por coronavirus (SARS-CoV-2) en las clínicas de reproducción asistida. Versión 7. <https://www.sefertilidad.net/docs/noticias/recomendacionesSeguridad3.pdf>.
- Sengupta, P, Leisegang, K, Agarwal, A., 2021. The impact of COVID-19 on the male reproductive tract and fertility: A systematic review. *Arab. J. Urol.* 19, 423–436. <https://doi.org/10.1080/2090598X.2021.1955554>.
- SEF. Sociedad Española de Fertilidad, 2020. Casos especiales de reproducción asistida y SARS-CoV-2 Donantes de gametos. . <https://www.sefertilidad.net/docs/grupos/donacion/Cribado-COVID.pdf>.
- Song, C, Wang, Y, Li, W, Hu, B, Chen, G, Xia, P, Wang, W, Li, C, Diao, F, Hu, Z, Yang, X, Yao, B, Liu, Y., 2020. Absence of 2019 novel coronavirus in semen and testes of COVID-19 patients. *Biol. Reprod.* 103, 4–6. <https://doi.org/10.1093/biolre/iaaa050>.
- Tiwari, S, Kc, N, Thapa, S, Ghimire, A, Bujukchhe, S, Sah, GS, Isnuwardana, R., 2021. Semen parameters in men recovered from COVID-19: a systematic review and meta-analysis. *Middle. East. Fertil. Soc. J.* 26, 44. <https://doi.org/10.1186/s43043-021-00089-w>.
- Tur-Kaspa, I, Tur-Kaspa, T, Hildebrand, G, Cohen, D., 2021. COVID-19 may affect male fertility but is not sexually transmitted: a systematic review. *F. S. Rev.* 2, 140–149. <https://doi.org/10.1016/j.xfnr.2021.01.002>.
- Wang, Z, Xu, X., 2020. scRNA-seq Profiling of Human Testes Reveals the Presence of the ACE2 Receptor, A Target for SARS-CoV-2 Infection in Spermatogonia. *Leydig and Sertoli Cells.* 9, 920. <https://doi.org/10.3390/cells9040920>.
- Wang, S, Zhang, A, Pan, Y, Liu, L, Niu, S, Zhang, F, Liu, X., 2023 Apr. Association between COVID-19 and Male Fertility: Systematic Review and Meta-Analysis of Observational Studies. *World. J. Mens. Health.* 41, 311–329. <https://doi.org/10.5534/wjmh.220091>.
- WHO laboratory manual for the examination and processing of human semen. Fifth edition. World Health Organization. Available at: http://whqlibdoc.who.int/publications/2010/9789241547789_eng.pdf.

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