



Case Report

Persistent Male Genital Tract Inflammation and Semen Impairment: A Long-Term Effect of SARS-Cov-2 Infection

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Abstract

Background: Recent reports evidenced an impairment of semen parameters in men affected by coronavirus disease 2019 (COVID-19). In particular, our group previously reported that 1 over 4 COVID-19 healed men were found to be crypto- / azoo-spermic. Moreover, most patients had elevated IL-8 semen levels at sperm analysis. The aim of our study was to assess semen parameters and inflammation by evaluating a panel of sperm cytokine levels (IL-1, IL-4, IL-6, IL-8, IL-17, INF gamma, TNF-alpha) on average 1 month after the second SARS-CoV-2 negative nasopharyngeal swab and 3 months later. **Methods:** Ten men who showed normozoospermia (n=3), oligozoospermia (n=3) or crypto/azoospermia (n=4) 1 month after healing from COVID-19 in our previous study, were re-called and re-evaluated 3 months after the first semen analysis. Semen parameters were evaluated according to WHO manual and seminal plasma cytokine levels by an ELISA method. **Results:** At 3-months follow-up, 8 men showed an overall increase of semen parameters compared to levels assessed after 1 month. In particular, of

the 4 crypto-/azoo-spermic men 1 month after healing, 2 resulted oligozoospermic, 1 normozoospermic and only 1 remained azoospermic. Two of the 3 oligozoospermic men turned normozoospermic. Semen cytokine levels were remarkably high one month after healing and remained elevated after 3 months, with the exception of IL-6. **Conclusions:** This is the first longitudinal, prospective study comparing semen parameters and semen inflammatory markers one and three months after recovering from COVID-19. Our data indicate an overall tendency to an improvement of semen parameters although a genital tract inflammatory condition appears to persist at least 3 months after COVID-19 recovery. This condition could have an impact on male fertility requiring a careful follow up of these patients.

Keywords: Covid-19; SARS-Cov-2; Sperm; Semen, Inflammation, Fertility

Introduction

Male reproductive system is vulnerable to several viral infections, including those from ZIKA, Mumps, hepatitis B and C virus or human immunodeficiency virus, which exert different impacts on male fertility [1]. The main types of male gonadal cells (spermatogonia, Leydig and Sertoli cells) express Angiotensin-Converting Enzyme 2 (ACE2) receptor and transmembrane serine protease 2 (TMPRSS2) on their membrane [2]. Several reports suggest a role for ACE2 and TMPRSS2 as the cellular receptor for SARS-CoV-2, allowing the access of the virus to the male reproductive organs, with a consequent risk of testicular impairment in men with Covid-19 [3]. In a recent series of 34 Chinese men (median age: 37) recovering from COVID-19, SARS-CoV-2 was not detected in the semen 1 month after COVID-19 diagnosis [4]. However, subjects with a moderate infection from Covid-19 showed an impairment of sperm quality, even if SARS-CoV-2 RNA is not detected in semen [5]. Gonadal impairment could be due either to a direct effect of SARS-CoV-2 on testicular cells and/or to the high inflammatory response consequent to the disease [3]. In particular, the COVID-19 cytokine storm may produce a widespread tissue damage, resulting in multi-organ failure with the potential involvement of the reproductive system as well [3,6]. We previously demonstrated that at least one-fourth of men recently recovered from COVID-19, presented oligo/crypto/azoospermia and such condition was related to the severity of the illness [7]. In addition, most of these men, showed elevated semen levels of IL-8, a surrogate marker of male genital tract inflammation [7]. Alterations of semen quality in patients healed from COVID-19 have been demonstrated in other studies [8,9]. Even if some evidence indicates the loss of testicular architecture at post-mortem examination of COVID-19 severe cases, data regarding the long-term impact of COVID-19 on male reproductive function and semen quality after recovery are lacking [3,10]. Aim of the present study was the evaluation of semen parameters (Sperm Concentration, Total Spermatozoa Number, Progressive Motility) and semen inflammatory cytokine levels (IL-1, IL-4, IL-6, IL-8, IL-17, INF gamma, TNF-alpha), in 10 sexually active men one and 3 months after recovery from COVID-19.

Materials and Methods

Study Design

A prospective cohort study was designed following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines. Current study was approved by Ethics Committee Area Vasta Centro - based at AOU Careggi in June 2020, under the code 17104. Subsequently It was registered in clinicaltrials.gov with the identifier NCT04446169. It was designed and conducted according to the Declaration of Helsinki. All enrolled participants signed an informed consent. The study inclusion criteria are: male patients, between 18 and 65 years of age recovered from covid 19 and confirmed by molecular swab of sars-cov 2 infection. We re-contacted 10 subjects, crypto / azoospermic (n = 4), normozoospermic (n = 3) and oligozoospermic (n = 3) evaluated by spermiogram performed on average 1 months after recovery from COVID-19 according to our previous study [7] and offered them a reassessment of sperm parameters 3 months after the first one. Everyone agreed to participate in the study. Data recording, specimen collection and SARS-CoV-2 RNA detection. Clinical data were collected according to the previously reported scheme [7]. Clinical data included: patient demographics, comorbidities, medications, hospitalization time and features (including intensive care need), laboratory tests and treatments (including oxygen therapy) [11].

Semen Analysis

Semen was collected after a period of 2-7 days of abstinence. WHO guidelines [12] were strictly followed for semen analysis. Sperm concentration was determined using an improved Neubauer hemocytometer. The percentages of progressive, non-progressive and immotile spermatozoa were evaluated on 200 spermatozoa/sample by phase contrast microscopy. Sperm morphology was assessed after Diff-Quick staining on 200 spermatozoa at 1000x bright field microscopy. Semen leukocytes were quantified by counting the number of round cells/ml and evaluating the percentage of leukocytes after May-Grunwald staining of the sample.

Immunoplex Assay Semen Plasma

Frozen - thawed samples of seminal plasma were

centrifuged at 800 g for 10 minutes to remove particulates. Detection and quantification of cytokines was performed using the Milliplex® Map kit Human Cytokine/Chemokine/Growth Factor Panel A Magnetic Bead Panel (Merck KGaA, Darmstadt, Germany) following the manufacturer's protocols. The plate was analyzed with the Luminex 200 MAGPIX® and data were then analyzed using a 5-parameter logistic curve-fitting method for calculating analyte concentrations in samples. All samples were processed on the same day of collection or stored at -80°C until further analysis. Nucleic acids from samples were extracted with Microlab Nimbus IVD system (Seegene Inc, Seoul, South Korea) using the Starmag Universal Cartridge and amplified with the multiplex RT-PCR Allplex™ 2019-nCoV assay (Seegene Inc), targeting RdrP, E and N genes, according to Manufacturers' instructions.

Statistical Analysis

Continuous and categorical variables were reported as median (interquartile range) and number (percentage), accordingly. Patients were then divided according to their semen analysis results, thus normo-, oligo- and azoo-spermic. Statistical comparisons between groups were conducted with Mann-Whitney u-test and univariate ANOVA for continuous variables, and with χ^2 and Fisher's Exact test for categorical variables, as appropriate according to sample size. Intragroup comparison from baseline to follow-up were done with Wilcoxon test for continuous variables. Statistical significance was set with a p-value < 0.05. All statistical analyses were performed using IBM SPSS version 20.0 (SPSS Inc, Chicago, IL, USA).

Results

Patient characteristics and the received treatments during COVID-19 infection are reported in Table 1 and 2, respectively. Table 3 reports semen parameters and seminal plasma IL-8 levels 1 month and 3 months after recovery. Levels of the other evaluated cytokines, 1 and 3 months after recovery, are reported

in Table 4. In the latter table, semen levels of cytokines in healthy controls and in patients with chronic prostatitis according to a previous study where cytokine levels were measured in the same laboratory¹³ are shown for comparison. Eight out of the 10 patients showed an increase in sperm count (Figure 1, panels A-B and Table 3) after three months. Interestingly, 1 normozoospermic patient at the time of recovery (#2) tested oligozoospermic 3 months later. He is a young (32-years-old), healthy man, who was treated with remdesivir, hydroxychloroquine and heparin during hospitalization. One oligozoospermic patient (#6), who was treated with remdesivir and hydroxychloroquine during hospitalization, showed a small increase in sperm concentration (from 1.1 to 7.6 x10⁶/mL). Progressive motility (Figure 1, panel C) showed an improvement in 5 out of 10 patients, although only 4 patients (2 normozoospermic [#2, #3], 1 oligozoospermic [#4] and 1 crypto/azoospermic [#10]) reached values above the 5th percentile of WHO reference [12], at 3 months follow up. None of the enrolled patients required new medications or hospitalization during the follow-up period. IL-8 levels were above the cut-off value of 3.8 ng/ml [13] in all enrolled patients at the time of recovery (Table 3). Three months later, the levels of IL-8 were still high, with the exception of 1 patient (#1) (Figure 2). Overall, we demonstrate a halving of IL-8 levels between first (median: 95) and second (median: 9,) semen analysis, although more than 3 months after healing from COVID-19, IL-8 levels remained more than double of cut-off values (8) in the majority of patients. Semen cytokines were all elevated 1 month after recovery and were persistently high after 3 months, with the exception of IL-6 (Figure 3). Mean cytokines concentrations in post COVID-patients were all higher when compared with healthy population [13] both 1 month after recovery and after further 3 months (Table 4). Interestingly, the mean levels of INF γ and TNF α were significantly higher as compared to those reported in men with proven prostatitis¹³ (Table 4).

Figure 1

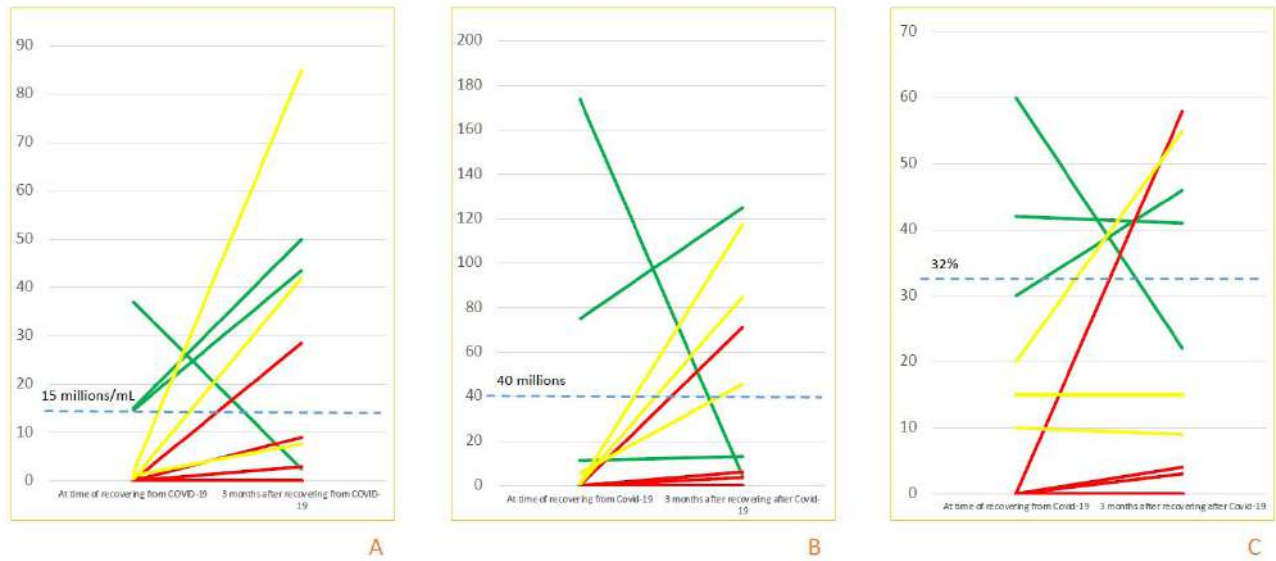


Figure 2

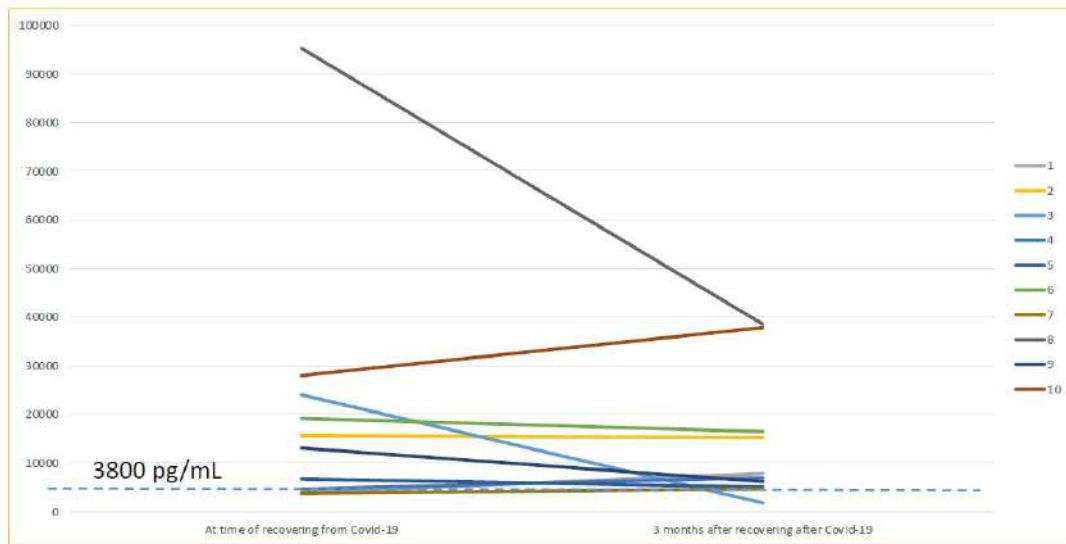


Figure 3

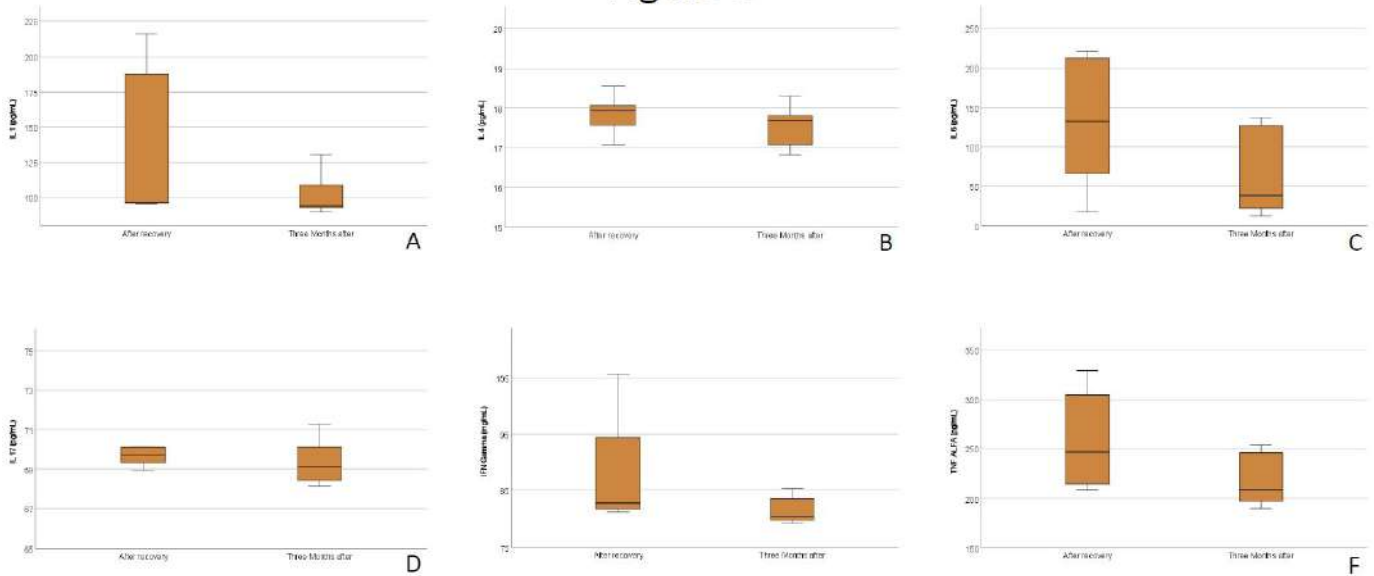


Figure 1: Semen changes from one month after COVID-19 recovering to after further three months after recovering, Dashed lines represent referral values, Green = normospermic patients, Yellow= oligospermic patients, Red = azospermic patients. 1 A: Sperm Concentration (Millions/mL), 1 B: Total Spermatozoa Number (Millions), 1 C: Progressive Motility (a+b) (%).

Figure 2: Interleukin 8 changes from one month after COVID-19 recovering to after further three months after recovering, Dashed line is the referral cut-off value for Interleukin 8 levels in semen, 3800 pg/mL.

Figure 3: Box plot comparison of Cytokines and Chemokines values “One Month After Recovery” and “After further three months”. Box represents interquartile range, while lines maximum and minimum values. All values are expressed as pg/mL. A: Interleukin 1β; B: Interleukin 4; C: Interleukin 6; D: Interleukin 17; E: Interferon γ; F: Tumor Necrosis Factor α.

	Normozoospermic			Oligozoospermic			Crypto-azoospermic			
	1	2	3	4	5	6	7	8	9	10
Age, years	35	32	47	44	60	58	62	64	49	40
BMI, Kg/m2	29,2	23,6	23,1	21,8	22,9	23,2	27,4	30,1	27,4	28,7
Smoke	No	No	No	No	No	No	No	No	Former	No
IPSS	6	6	2	5	9	2	10	12	6	3
IPSS QoL	2	1	2	0	2	0	1	3	2	0
IIEF-5	25	24	25	24	15	23	15	22	25	22

Paternity	0	0	1	0	2	2	1	1	2	1
BMI: Body Mass Index (calculated as weight in kilograms divided by height in meters squared); IQR: Interquartile Range. ^a International Prostatic Symptoms Score; ^b International Prostatic Symptoms Score Quality of Life; ^c International Index of Erectile Function Questionnaire.										

Table 1: Patients' Characteristics.

	Normozoospermic (n=3)	Oligozoospermic (n=3)	Crypto-azoospermic (n=4)
Non-Hospitalized (n, %)	1 (33.3)	0 (0)	1 (25.0)
Hospitalized (n, %)	2 (66.7)	3 (100)	1 (25.0)
Intensive Care (n, %)	0 (0)	0 (0)	2 (50.0)
Antibiotics ^a (n, %)	0 (0)	0 (0)	3 (75)
Antivirals (n, %)	2 (66.7)	3 (100)	3 (75)
Steroids (n, %)	0 (0)	0 (0)	1 (25)
Heparin (n, %)	2 (66.7)	2 (66.7)	3 (75)
Hydroxychloroquine (n, %)	3 (100)	3 (100)	3 (75)
Oxygen therapy ^b	2 (66.7)	1 (33.3)	1 (25)
Low Flow O2 (n, %)	1 (33.3)	2 (66.7)	1 (25)
Invasive Vent. (n, %)	0 (0)	0 (0)	2 (50)

Abbreviations: COVID-19: coronavirus disease 2019.

^aAntibiotics include amoxicillin and clavulanic acid, piperacillin and tazobactam, azythromycin.

^bLow Flow Oxygen Therapy includes nasal cannula, simple face mask and partial rebreather mask while High Flow Oxygen includes Trans Tracheal Catheters, Venturi Mask, Aerosol Mask, Tracheostomy collars, non-rebreathing mask with reservoir and one way valve and high humidity face tents.

Table 2: Patients' treatments during COVID-19 infection, according to semen analysis.

	Normozoo spermic (n=3)		Oligozoospermic (n=3)		Crypto- azoospermic (n=4)	
	1 month after recovering	3 Months later	1 month afterrecovering	3 Months later	1 month after recovering	3 Months later
Volume (ml) (median)	4.7	1.7	2.2	3	0.5	1.4
Number (millions) (median)	75	13.1	0.8	85	NA	6
Concentration (millions/ml) (median)	15	43.5	1	42	NA	6

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pH (median)	7.4	7.2	8	8	6.9	7
Prog. Motility (a+b) (%) (median)	42	41	15	15	NA	58
Morphology (NF) (%) (median)	4	5	4	5	NA	1
Leucocytes (103/ml) (median)	0.2	0	0	0	NA	0
IL-8 (ng/ml) (median)	15.7	7.9	6.7	7	20.6	22
Abbreviations: IQR: interquartile range. aTime needed to carry the samples to laboratory.						

Table 3: Patients' semen characteristics, according to time of analysis.

Cytokines (pg/ml) Median (Interquartile Range)	Healthy controls [13]	Covid Patients – One month after recovery (n=10)	CP IIIA13	Covid Patients – After further three months (n=10)
IL8	1984 (1164-2444)	14387 (4488-25113)	15240 (10630-19501)	7045 (4328-16160)
IL-1 β	17 (10-41)	96 (95-215)	61 (22-108)	94 (93-110)
IL-4	NA	18 (17-18)	NA	18 (17-18)
IL6	16 (10-26)	132 (43-221)	99 (31-130)	38 (20-132)
IL-17 α	NA	70 (69-70)	NA	69 (68-70)
INF γ	25 (9-55)	83 (82-104)	44 (17-63)	80 (80-84)
TNF α	33 (22-65)	246 (211-329)	58 (37-95)	209 (197-250)
13 Penna et al, Eur Urol, 2007 (https://doi.org/10.1016/j.eururo.2006.07.016) Legend: CP IIIA= Chronic Prostatitis patients stage IIIa				

Table 4: Semen Cytokines levels in Covid-19 recovered patients at recovery and three months after, together with healthy controls and Chronic Prostatitis patients stage IIIa.

Discussion

Our study indicates that alterations of semen parameters observed shortly after recovering from COVID-19 may be transient as most patients show an improvement of semen quality after three months although an inflammatory status of the male genital tract seems to persist. Several viruses, including HBV, HCV, HPV, HSV, Mumps, may cause an impairment of testicular function and can be detected in semen [14]. Some viruses such as MuV, HIV and SARS-CoV can affect testicular cells, resulting in severe orchitis, which can compromise male fertility [15]. During the very first weeks after recovery from Covid-19, the majority of men show high semen levels of IL-8, and at least one-fourth of these men, show alterations of semen parameters [7]. A recently published systematic review based on data collected from 70 studies confirms these data [16]. In particular, the Authors suggest that COVID-19 may cause an inflammatory condition of the testis which is correlated to the severity of the disease [16]. SARS-CoV-2 is very contagious and has already infected a higher proportion of young men during the second pandemic wave, as compared with the first one [17]. Since the highest expression of ACE2 in testicular cells occurs at about thirty years of age, the interaction between SARS-CoV-2 and ACE2 receptor and TMPRSS may cause severe damage to the testis in young males, rising concerns about male fertility [18]. Impaired gonadal function can be due to inflammation an autoimmune response [12] as well as high fever and medications during the course of the disease [19]. In particular, a significant increase of semen levels of inflammatory cytokines, such as IL-6 and TNF- α was observed in COVID-19 patients [1]. Furthermore, evidence suggests that both testicular function and semen quality may be reduced following severe COVID-19, due to several aspects of the disease including high fever and medications, indicating the need for andrological evaluation of recovered men [7,9]. Such a recommendation is also indicated in the official website of the government of Hubei Province which posted a bulletin inviting all men recovered from SARS-CoV-2 to undergo fertility checks, suggesting a big concern for a possible link between this novel disease and the male reproductive system [20].

In the present study, we report a longitudinal prospective case series of sexually active men based on two consecutive sperm analysis performed after healing from COVID-19 and 3 months later, after a complete new cycle of spermatogenesis. Overall, 3 out of 7 (43%) oligo-crypto-azoospermic patients at the time of recovery, showed an improvement of testicular function with an increase of sperm number, 3 months after healing, suggesting that the detrimental effects of COVID-19 could be temporary. However, in few cases, no improvement in sperm number has been achieved. Since semen quality of these men before COVID-19 was not available, whether or not persistence of crypto/azoospermia

is due to the illness cannot be ascertained. However, 6 out of 7 patients (86%) reported paternity before COVID-19 [7]. In most patients, an improvement of sperm progressive motility was observed, but values still remained below the 5th percentile of the WHO reference values in 6 of them. As previously reported, IL-8 is a cytokine associated with inflammation of prostate, seminal vesicles and epididymis [13]. Semen IL-8 concentrations 1 month after recovery from COVID-19 were related with severity of the illness, including the need of hospitalization, intensive care, oxygen therapy and invasive ventilation [7]. Interestingly, semen IL-8 levels remain high (more than double of the cut-off value of 3.8 ng/ml) in most of the enrolled patients also 3 months after COVID-19 healing, suggesting the persistence of an inflammatory condition in the male genital tract that may require further assessment. Similarly, semen levels of 7 cytokines which are part of the SARS-CoV-2 cytokine storm [21] resulted elevated both one and 3 months after COVID-19 recovery in all patients recruited in this study, with the exception of IL-6 which decreased significantly after 3 months. In particular, levels of some cytokines (IL-8, IL-1, IL-6, INF gamma and TNF-alpha), resulted higher respect to those found in patients affected by prostatitis (Table 4) [13], indicating that the inflammatory status of the male genital tract of patients recovering from COVID-19 may be similar or even more compromised. A limitation of our study regards the lack of semen parameters before COVID-19 diagnosis. Another limitation regards the small cohort of evaluated patients. However, our intent was to determine whether those patients that showed alterations of semen quality 1 month after healing from COVID-19 [7], could recover after 3 months, representing an entire spermatogenetic cycle. Our study has also strengths. To our knowledge, this is the first study comparing semen parameters in healed men from COVID-19 at 1 and 3 months after recovery, although in a small number of subjects. In addition, we evaluated a wide panel of seminal plasma cytokines involved in male genital tract inflammation. In conclusion, our study demonstrates that, despite an overall amelioration of semen quality, COVID-19 induced inflammation in the male reproductive tract may persist 3 months after recovery, with a possible detrimental effect on male fertility. Further studies are needed to understand the link between the cytokine storm during COVID-19 and the damage to reproductive system in order to minimize the potential sequelae on male fertility. Our study indicates that a careful follow up is needed for patients in reproductive age recovering from COVID-19. The dataset(s) supporting the conclusions of this article is(are) included within the article.

Declarations

Ethics approval and consent to participate: Current study was approved by our Institutional Review Board in June 2020, under the code 17104. Subsequently It was registered

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in clinicaltrials.gov with the identifier NCT04446169. It was designed and conducted according to the Declaration of Helsinki. All enrolled participants signed an informed consent.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests. The authors report no involvement in the research by the sponsor that could have influenced the outcome of this work.

Authors' contributions: Conception and Design: M. Gacci, S. Serni, E. Baldi, F. Annunziato, M. Maggi, L. Vignozzi; Acquisition of data: A. Manera, A. Pecoraro, R. Nicoletti, A. Liaci; Analysis and interpretation of data: A. Sebastianelli, M. Gacci, E. Baldi, S. Morselli; Drafting the manuscript: M. Gacci, A. Sebastianelli; Critical revision of the manuscript: M. Gacci, S. Serni, E. Baldi, F. Annunziato, M. Maggi, L. Vignozzi; Statistical analysis: S. Morselli, M. Gacci, E. Baldi, S. Pollini; Administrative, technical, or material support: A. Antonelli, S. Pollini, S. Marchiani, Rastrelli G.; Supervision: S. Serni, C. De Nunzio, A. Fanelli; Ethical approval: S. Morselli, C. Zaccaro. All authors have read and approved the manuscript

References

1. Li H, Xiao X, Zhang J, Zafar MI, Wu C, et al. (2020) Impaired spermatogenesis in COVID-19 patients. *EClinicalMedicine*. 28: 100604.
2. Patel DP, Guo J, Hotaling JM. (2020) The jury is still out: COVID-19 and male reproduction. *Fertil Steril*. 114: 257-258.
3. Dong M, Zhang J, Ma X, Tan J, Chen L, et al. (2020) ACE2, TMPRSS2 distribution and extrapulmonary organ injury in patients with COVID-19. *Biomed Pharmacother*. 131: 110678.
4. Pan F, Xiao X, Guo J, Song Y, Li H, et al. (2020) No evidence of severe acute respiratory syndrome-coronavirus 2 in semen of males recovering from coronavirus disease 2019. *Fertil Steril*. 113: 1135-1139.
5. Holtmann N, Edimiris P, Andree M, Doehmen C, Baston-Buest D, et al. (2020) Assessment of SARS-CoV-2 in human semen—a cohort study. *Fertil Steril*. 114: 233-238.
6. Ragab D, Salah Eldin H, Taeimah M, Khattab R, Salem R. (2020) The COVID-19 Cytokine Storm; What We Know So Far. *Front Immunol*. 11: 1446.
7. Gacci M, Coppi M, Baldi E, Sebastianelli A, Zaccaro C, et al. (2021) Semen impairment and occurrence of SARS-CoV-2 virus in semen after recovery from COVID-19. *Hum Reprod*. 1: deab026.
8. Holtmann N, Edimiris P, Andree M, Doehmen C, Baston-Buest D, et al. (2020) Assessment of SARS-CoV-2 in human semen—a cohort study. *Fertil Steril*. 114: 233-238.
9. Ruan Y, Hu B, Liu Z, Liu K, Jiang H, et al. (2021) 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.
10. Hallak J, Teixeira TA, Bernardes FS, Carneiro F, Duarte SAS, et al. (2020) SARS-CoV-2 and its relationship with the genitourinary tract: Implications for male reproductive health in the context of COVID-19 pandemic. *Andrology*. 9: 73-79.
11. Zaccaro C, Lotti F, Morselli S, Sebastianelli A, Frizza F, et al. (2020) SECRET® - SEXual Chronicle REcording Table: Validation and reliability. *Andrology*.
12. World Health Organization, (Eds.), 2010. WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th ed. Cambridge University Press, Cambridge, UK.
13. Penna G, Mondaini N, Amuchastegui S. (2007) Seminal plasma cytokines and chemokines in prostate inflammation: interleukin 8 as a predictive biomarker in chronic prostatitis/chronic pelvic pain syndrome and benign prostatic hyperplasia. *Eur Urol*. 51: 524-533.
14. Batiha O, Al-Deeb T, Al-Zoubi E, Alsharu E. (2020) Impact of COVID-19 and other viruses on reproductive health. *Andrologia*. 52: e13791.
15. Xu J, Qi L, Chi X, Yang J, Wei X, et al. (2006) Orchitis: a complication of severe acute respiratory syndrome (SARS). *Biol Reprod* 74: 410-416.
16. 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*.
17. Seligmann H, Iggui S, Rachdi M, Vuillerme N, Demongeot J. (2020) Inverted Covariate Effects for First versus Mutated Second Wave Covid-19: High Temperature Spread Biased for Young. *Biology (Basel)*. 9: 226.
18. Huang HH, Wang PH, Yang YP, Chou SJ, Chu PW, et al. (2020) A review of severe acute respiratory syndrome coronavirus 2 infection in the reproductive system. *J Chin Med Assoc*. 83:895-897.
19. Meng TT, Dong RJ, Li TG. (2021) Relationship between COVID-19 and the male reproductive system. *Eur Rev Med Pharmacol Sci*. 25: 1109-1113.
20. Gacci M, Coppi M, Baldi E, Sebastianelli A, Zaccaro C, et al. (2021) Reply: COVID-19: semen impairment may not be related to the virus. *Hum Reprod*. 1:deab083.
21. Degauque N, Haziot A, Brouard S, Mooney N. (2021) Endothelial cell, myeloid, and adaptive immune responses in SARS-CoV-2 infection. *FASEB J*. 35: e21577.