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ORIGINAL ARTICLE

Semen Analysis

Semen parameters in men recovered from COVID-19

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The novel coronavirus disease (COVID-19) pandemic is emerging as a global health threat and shows a higher risk for men than women. Thus far, the studies on andrological consequences of COVID-19 are limited. To ascertain the consequences of COVID-19 on sperm parameters after recovery, we recruited 41 reproductive-aged male patients who had recovered from COVID-19, and analyzed their semen parameters and serum sex hormones at a median time of 56 days after hospital discharge. For longitudinal analysis, a second sampling was obtained from 22 of the 41 patients after a median time interval of 29 days from first sampling. Compared with controls who had not suffered from COVID-19, the total sperm count, sperm concentration, and percentages of motile and progressively motile spermatozoa in the patients were significantly lower at first sampling, while sperm vitality and morphology were not affected. The total sperm count, sperm concentration, and number of motile spermatozoa per ejaculate were significantly increased and the percentage of morphologically abnormal sperm was reduced at the second sampling compared with those at first in the 22 patients examined. Though there were higher prolactin and lower progesterone levels in patients at first sampling than those in controls, no significant alterations were detected for any sex hormones examined over time following COVID-19 recovery in the 22 patients. Although it should be interpreted carefully, these findings indicate an adverse but potentially reversible consequence of COVID-19 on sperm quality.

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Keywords: COVID-19; semen; sex hormones; sperm count; sperm motility; spermatogenesis

INTRODUCTION

Since the initial outbreak in December 2019, the novel coronavirus disease (COVID-19) has been rapidly spread and developed into an unprecedented global health emergency that affects people in many aspects of everyday life. The World Health Organization (WHO) has announced that to date, over 143 million individuals worldwide have been confirmed with COVID-19. The coronavirus responsible for this epidemic,[1] termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by WHO, shares about 79% sequence identity with the human coronavirus SARS-CoV that caused the SARS pandemic in 2002.[2,3] Similar to SARS-CoV, SARS-CoV-2 can also utilize angiotensin-converting enzyme II (ACE2) as the receptor to enter host cells.[2] Analyses of human single-cell-RNA expression profiles revealed ACE2 expression in spermatogonia, Leydig and Sertoli cells,[4] but at a low level,[5] as well as in prostate epithelial cells.[6] Recently, several studies have reported that SARS-CoV-2 RNAs were not detected in semen samples from acute-infected, recovering or recovered patients.[5,7–10] In another study, SARS-CoV-2 RNAs were detected in semen samples of four patients who were at the acute phase and two patients who were recovering;[11] however, this finding was considered to result from contamination owing to methodological issues.[12,13] Noticeably, in one study, six of 34 patients had scrotal discomfort concerning viral orchitis during COVID-19 infection.[5] SARS-CoV-2 was detected by reverse transcription-polymerase chain reaction (RT-PCR) in the testes in only 1 of 12 patients who died of COVID-19 (for which the virus detected probably came from blood rather than testicular tissue) and in none by electron microscopy. Nonetheless, significant impairment in testicular histology was observed, including morphological changes in Sertoli cells, loss and sloughing into the lumen of tubular cells, reduction in the number of Leydig cells, and mild lymphocytic inflammation, suggesting testicular injuries during the disease course.[14] Hence, it appears that COVID-19 can adversely affect the male reproduction system. However, to date, the consequences of COVID-19 on sperm parameters remain barely investigated.

Here, we recruited 41 men of reproductive age who had recently recovered from COVID-19 and analyzed their semen and sex hormones to determine the consequences of COVID-19 on male reproduction.

PARTICIPANTS AND METHODS

Study participants

This study was approved by and conducted in accordance with the ethical committee of University of Science and Technology China (USTC), Hefei, China (approval number: 2020-XG(H)-022). We offered enrollment to all the COVID-19 male Chinese patients (18–45 years old) admitted to hospitals located in Anhui Province,

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China, from January to March 2020, and those who were willing to participate were all recruited in this study. A total of 41 men were recruited, among whom 22 were willing to have a second sampling approximately 1 month later. All these participants had been tested positive for SARS-CoV-2 viral RNAs in throat swabs or respiratory specimens by RT-PCR assays through a two-step confirmation strategy.[15] According to the New Coronavirus Pneumonia Prevention and Control Program published by the National Health Commission of China, the patients were classified into mild, moderate, severe, and critical subtypes on the basis of severity of the illness.[16] All the patients recruited for this study filled in a questionnaire regarding their marriage status, occupations, history of diseases and familial diseases, and any exposure to factors that are potentially harmful to the male reproductive system. The control group was composed of 50 Chinese men of matching ages, who had not suffered from COVID-19. Informed consent forms were obtained from all participants. All the participants underwent comprehensive clinical examination of the genitals and secondary sexual characteristics, all had normal secondary sexual characteristics and normal testicular sizes, and varicoceles were found in four patients and eight control men.

Semen analyses

Semen samples were obtained by masturbation. After the semen reached complete liquefaction at 37°C, semen parameters were assessed. Briefly, semen volumes were calculated from the sample weights according to the instructions of the WHO laboratory manual.[17] Sperm concentration and sperm motility were assessed by computer-assisted sperm analysis (CASA) under phase contrast microscopy (CX43, Olympus corporation, Tokyo, Japan) equipped with SAS-II system (Beijing Precise Instrument Co., Ltd., Beijing, China) at 10× magnification. The morphology of at least 200 spermatozoa was examined after Diff-Quick staining (Ankebio, Hefei, China) with a light microscope (UB100i, Aopuguangdian, Chongqing, China) at 100× magnification in accordance with the WHO laboratory manual.[16] Sperm vitality was assessed by eosin Y staining method in semen, following the WHO guidelines.[17]

Evaluation of sex hormone levels

Sera were obtained from participants' peripheral blood samples. The levels of estradiol, follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone, testosterone (T), prolactin, anti-Müllerian hormone (AMH) and inhibin B were assessed by chemiluminescent immunoassays, using commercial kits (Shenzhen Yahuilong Biotechnology, Shenzhen, China) measured by YHLO iFlash 3000-H immunoassay analyzer for inhibin B and using commercial kits (Beckman Coulter, Brea, CA, USA) measured by Beckman Coulter Unicell DXI 800 immunoassay analyzer for the other hormones.

Statistical analyses

Owing to the small numbers of participants, data are presented as median (interquartile range [IQR]). Data were compared between controls and men recovered from COVID-19 using the non-parametric Mann-Whitney-Wilcoxon test for semen characteristics, the Student's *t*-test for quantitative variables (ages, days after the appearance of symptoms, days after hospital discharge, and sex hormone levels), and the Chi-squared test or Fisher's exact test for categorical variables (percentages of patients with semen volume <1.5 ml, oligozoospermia, asthenozoospermia, and teratozoospermia). Data were compared between the first and second samplings using the Wilcoxon signed-rank sum test for semen characteristics and Student's *t*-test for sex hormone levels. All statistical tests were two-tailed and a $P < 0.05$ was considered statistically significant for all analyses.

RESULTS

Physical and clinical summaries of patients recovered from COVID-19

A total of 41 men with a median age of 26.0 (IQR: 22.0–34.0) years old who had been admitted to hospital with confirmed COVID-19 were enrolled in the study. The physical information and clinical characteristics related to COVID-19 for these patients are shown in **Supplementary Table 1** and **2**.

Among the 41 patients, 22 (53.7%) were unmarried and had no child, 18 (43.9%) were married and had at least one child, and one (2.4%) who had been married for six months had no child yet. Eight (19.5%) patients had light to moderate alcohol drinking and tobacco smoking habits. None of these patients reported having any exposure to toxic chemicals, radiations, or high temperature over the past 6 months, or having any genetic diseases in their families. Noticeably, the median body mass index (BMI) for these patients was 25.1 (IQR: 21.9–28.0) kg m^{-2}, above the upper limit of normal range (18.5–23.9 kg m^{-2}) for adult men according to the prevention and control guide for overweight and obesity in Chinese adult. All the patients were symptomatic, exhibiting at least one of the typical COVID-19 symptoms[18] during the SARS-CoV-2 infection period. One patient had blood in his urine and one felt burning around the eyes during infection. Twenty-nine patients were categorized as mild, ten as moderate and two as severe cases. They had been hospitalized for a median of 17.0 (IQR: 13.0–26.0) days and discharged after viral clearance. Of the 41 patients, one exhibited only very mild symptoms and did not take any medicine, six patients received corticosteroid therapy during hospitalization, seven patients received only conventional antiviral or anti-inflammatory medication, and the others took a variety of medicines, such as conventional antiviral or anti-inflammatory drugs, lopinavir-ritonavir, diamine glycyrrhizinate, bicyclol tablets, polyene phosphatidylcholine capsules, interferons, and traditional Chinese medicine.

All the patients were physically normal with respect to external genitalia, testicular volume and texture, and male secondary sex characteristics. No epididymal or testicular tenderness was found during physical examination of the genitourinary system. None of the patients reported having any scrotum-related symptoms since the appearance of COVID-19 symptoms. All the patients self-reported suffering from psychological stress, such as fear, tension, and anxiety, since appearance of COVID-19 symptoms.

Semen characteristics of patients recovered from COVID-19

Semen samples from the 41 recovered patients were obtained and assessed at a median time of 76.0 (IQR: 73.0–86.5) days after appearance of symptoms and 56.0 (IQR: 49.0–72.0) days after hospital discharge. The semen characteristics of each patient and control are shown in **Supplementary Table 3** and **4**.

Semen parameter values were compared between patients and controls who had not suffered from COVID-19 (**Table 1**). Of the 41 patients, 27 men (65.9%) showed an abnormal value for at least one semen parameter as judged from the reference defined by WHO,[17] in contrast to 40.0% (20 out of 50, data not shown) in controls ($P = 0.0248$; Chi-squared test). The median total sperm count per ejaculate and the median value of sperm concentration for the patients were significantly lower than those for the controls ($P = 0.0322$). Compared with controls, patients had significantly lower percentages of motile spermatozoa ($P = 0.0233$) and progressively motile spermatozoa ($P = 0.0280$) and a higher ratio of asthenozoospermia

Table 1: Semen characteristics and sex hormones in controls and patients recovered from coronavirus disease 2019

Parameter	*Reference values*	*Controls (n=50)*	*Patients (n=41)*	*P*
Age (year), median (IQR)	NA	26.5 (25.0–34.0)	26.0 (22.0–34.0)	0.8284
Days after the appearance of symptoms[a], median (IQR)	NA	NA	76.0 (73.0–86.5)	NA
Days after hospital discharge[a], median (IQR)	NA	NA	56.0 (49.0–72.0)	NA
Semen analyses[b]				
Sexual abstinence (day), median (IQR)	2–7	4.0 (3.0–4.8)	3.0 (3.0–5.0)	0.3410
Semen volume (ml), median (IQR)	≥1.5	3.0 (2.0–4.1)	3.0 (2.3–3.9)	0.9650
Men with semen volume <1.5 ml, % (*n*/total)	–	12.0 (6/50)	9.8 (4/41)	0.9970
Sperm concentration ($\times10^6$ ml^{-1}), median (IQR)	≥15.0	86.8 (56.1–130.6)	49.6 (32.1–95.3)	0.0115*
Total sperm count (millions per ejaculate), median (IQR)	≥39.0	226.8 (112.5–376.6)	148.9 (74.2–238.2)	0.0322*
Abnormal sperm morphology (%), median (IQR)	≤96.0	93.0 (92.0–95.0)	93.0 (91.0–96.0)	0.6944
Progressive sperm motility (%), median (IQR)	≥32.0	46.7 (36.1–55.8)	37.1 (25.4–49.1)	0.0233*
Sperm motility (%), median (IQR)	≥40.0	53.8 (42.5–64.9)	44.4 (30.5–56.1)	0.0280*
Sperm vitality (%), median (IQR)	≥58.0	81.0 (67.0–89.0)	81.0 (63.0–88.0)	0.9630
Men with oligozoospermia, % (*n*/total)	–	8.0 (4/50)	9.8 (4/41)	1.0000
Men with teratozoospermia, % (*n*/total)	–	14.0 (7/50)	24.4 (10/41)	0.3197
Men with asthenozoospermia, % (*n*/total)	–	22.0 (11/50)	43.9 (18/41)	0.0450*
Estradiol[c] (pg ml^{-1}), median (IQR)	20.0–55.0	27.0 (18.0–35.0)	31.0 (21.0–37.0)	0.1966
FSH (U l^{-1}), median (IQR)	1.5–12.4	4.6 (3.5–6.0)	4.6 (3.7–5.6)	0.9550
LH (U l^{-1}), median (IQR)	1.2–8.6	3.6 (2.6–4.6)	3.6 (3.0–4.8)	0.1903
Progesterone (μg dl^{-1}), median (IQR)	1.3–9.7	8.4 (4.1–10.8)	4.6 (2.0–7.0)	0.0050**
Testosterone (ng ml^{-1}), median (IQR)	1.8–7.9	3.5 (2.8–4.3)	3.6 (2.8–4.4)	0.9336
Prolactin (ng ml^{-1}), median (IQR)	2.6–18.1	11.0 (8.0–14.0)	14.5 (9.5–19.7)	0.0088**
AMH (ng ml^{-1}), median (IQR)	0.7–19.0	10.6 (7.7–13.4)	9.1 (5.8–13.8)	0.3606
Inhibin B (pg ml^{-1}), median (IQR)	87.4–299.9	116.0 (85.3–128.5)	103.0 (74.8–145.8)	0.5784

[a]When semen analyses were performed. [b]Reference values were suggested by WHO. [c]Reference values were suggested by clinical laboratory. According to WHO fifth edition, oligozoospermia, <15 $\times10^6$ sperm per ml or <39 million sperm per ejaculate; teratozoospermia, >96% of sperm with abnormal morphology; asthenozoospermia, <40% with sperm motility or <32% with progressive sperm motility.[17] NA: not applicable; FSH: follicle-stimulating hormone; LH: luteinizing hormone; AMH: anti-Müllerian hormone; IQR: interquartile range; WHO: World Health Organization; –: not applicable. *P<0.05; **P<0.01

(P = 0.0450). Among the 41 patients, 18 patients had percentages of progressively motile or motile spermatozoa below the WHO reference values, while in controls, 11 men had low percentages of progressively motile or motile spermatozoa (P = 0.0450; Chi-squared test). Semen volume, sperm vitality, percentage of abnormal sperm morphology, and percentage of men with teratozoospermia were all comparable between patients and controls with no significant between-group difference.

Sex hormones in patients recovered from COVID-19

Since sex-related steroids can also be used to evaluate the status of the male gonad, and in order to understand better the after-effects of SARS-COV-2 infection on male reproductive function, we next assessed sex hormone levels in the sera of these 41 patients (**Table 1** and **Supplementary Table 5**).

The levels of T, LH, FSH, and AMH were largely normal for all the patients. Statistically significantly higher prolactin (P = 0.0088) and lower progesterone levels (P = 0.0050) were observed in patients than those in controls. Though estradiol levels were below the reference range in nine patients, the median value in patients was not significantly different from that in controls. Inhibin B is predominantly secreted by Sertoli cells, correlates positively with sperm concentration, sperm count and testicular volume, and its level is used as a valuable index for spermatogenic function.[19-21] Inhibin B levels in 13 of the 38 patients examined fell below the reference range and the median value was 103.0 (IQR: 74.8–145.8) pg ml^{-1} in patients, in comparison with 116.0 (IQR: 85.3–128.5) pg ml^{-1} in controls, but the difference between patient and control groups did not reach statistical significance.

Comparison of semen characteristics and sex hormones at different time points after hospital discharge

A second sampling was conducted for 22 of the 41 patients at 84.0 (IQR: 74.0–89.0) days after hospital discharge and the median time between the first and second sampling was 29.0 (IQR: 28.0–32.8) days (**Supplementary Table 6**). The values of sperm parameters and sex hormones for these two samplings were compared (**Table 2**). There were significant increases in total sperm count (P = 0.0029), sperm concentration (P = 0.0066), and motile sperm count (P = 0.0391) at the second sampling, compared with those at the first sampling. The percentage of spermatozoa with abnormal sperm morphology was significantly reduced at the second sampling than that at the first sampling (P = 0.0333). No significant alterations in sex hormones were observed.

DISCUSSION

We have described the semen characteristics and sex hormones in a cohort of 41 patients after recovery from COVID-19 and for the first time, to our knowledge, to undertake a longitudinal assessment of spermatogenesis following recovery from COVID-19. Though several pieces of evidence have suggested that the testes could be a target organ of COVID-19,[2,4,5,11,14,22] so far there is only limited information available regarding the association between COVID-19 and spermatogenesis. Recently, Holtmann *et al.*[8] reported a negative influence of COVID-19 on sperm quality observed in four recovered patients in need of hospital care during COVID-19 course, but not in 18 recovered patients with mild symptoms when home care was possible, but the cohort size was small and the consequences of COVID-19 on spermatogenesis remain to be ascertained. Here, our findings present direct evidence

Table 2: Semen characteristics and sex hormones of the 22 patients at different time points after recovery from coronavirus disease 2019

Parameter	*Reference values*	*First sampling*	*Second sampling*	*P*
Days after the appearance of symptoms[a], median (IQR)	NA	75.0 (71.0–76.0)	106.0 (101.8–126.8)	<0.0001****
Days after hospital discharge[a], median (IQR)	NA	52.5 (46.8–56.0)	84.0 (74.0–89.0)	<0.0001****
Semen analyses[b]				
Sexual abstinence (day), median (IQR)	2–7	3.0 (2.3–4.0)	3.0 (3.0–5.0)	0.2813
Semen volume (ml), median (IQR)	≥1.5	3.0 (2.3–3.6)	3.3 (2.7–3.7)	0.0649
Sperm concentration ($\times10^6$ ml^{-1}), median (IQR)	≥15.0	39.2 (26.6–70.0)	59.0 (45.7–112.5)	0.0066**
Total sperm count (millions per ejaculate), median (IQR)	≥39.0	100.9 (57.0–207.2)	169.4 (146.7–332.6)	0.0029**
Abnormal sperm morphology (%), median (IQR)	≤96.0	94.5 (91.5–97.0)	92.5 (90.3–95.8)	0.0333*
Progressive sperm motility (%), median (IQR)	≥32.0	35.1 (20.4–42.0)	37.0 (29.4–50.1)	0.5661
Sperm motility (%), median (IQR)	≥40.0	39.8 (27.0–50.5)	42.2 (34.7–54.1)	0.6237
Progressively motile sperm count (millions per ejaculate), median (IQR)	NA	42.9 (8.5–87.5)	70.0 (41.6–147.5)	0.0587
Motile sperm count (millions per ejaculate), median (IQR)	NA	50.1 (11.3–111.8)	83.4 (49.1–165.1)	0.0391*
Sperm vitality (%), median (IQR)	≥58.0	81.0 (60.5–86.5)	81.5 (69.8–90.0)	0.5920
Estradiol[c] (pg ml^{-1}), median (IQR)	20.0–55.0	28.5 (15.0–38.8)	23.0 (17.3–40.8)	0.9178
FSH (U l^{-1}), median (IQR)	1.5–12.4	5.1 (3.9–6.0)	4.6 (3.4–5.8)	0.4546
LH (U l^{-1}), median (IQR)	1.2–8.6	3.6 (3.1–4.4)	3.8 (3.1–4.5)	0.8862
Progesterone (μg dl^{-1}), median (IQR)	1.3–9.7	5.7 (2.5–8.1)	4.9 (2.7–6.9)	0.7979
Testosterone (ng ml^{-1}), median (IQR)	1.8–7.9	3.5 (2.9–4.0)	3.15 (2.6–4.8)	0.6175
Prolactin (ng ml^{-1}), median (IQR)	2.6–18.1	16.1 (10.7–25.9)	15.8 (9.8–20.0)	0.5546
AMH (ng ml^{-1}), median (IQR)	0.7–19.0	9.6 (5.4–12.7)	9.9 (5.7–14.1)	0.7683
Inhibin B (pg ml^{-1}), median (IQR)	87.4–299.9	102.5 (74.0–126.5)	122.0 (77.3–141.0)	0.3191

[a]When semen analyses were performed. [b]Reference values were suggested by WHO. [c]Reference values were suggested by clinical laboratory. NA: not applicable; FSH: follicle-stimulating hormone; LH: luteinizing hormone; AMH: anti-Müllerian hormone; IQR: interquartile range; WHO: World Health Organization; *P<0.05; **P<0.01; ****P<0.0001

of temporary reductions in total sperm count, sperm concentration and numbers of motile and progressively motile sperm in patients who had recovered from COVID-19 after hospitalization, suggesting an adverse but potentially reversible consequence of COVID-19 on testicular function.

When compared with controls, the sperm concentration, motility, and progressive motility were all significantly reduced in the patients at the first sampling that was conducted at a median of 56 days after hospital discharge, while when comparing sperm characteristics over time following COVID-19 recovery, total sperm count, sperm concentration, and numbers of motile and progressively motile spermatozoa in the patients were all significantly increased at the second sampling that was conducted at a median of 84 days after hospital discharge than those at the first sampling for the 22 patients examined, suggesting a potential recovery of sperm numbers. There were also some increases in percentages of motile and progressively motile spermatozoa observed at the second sampling, but the increasements were not statistically significant, which suggest that a longer time may be needed for the recovery of the sperm motility or that these patients had pre-existing low sperm motility before COVID-19. Since the spermatogenic cycle in human is estimated as taking approximately 74 days,[23,24] these findings suggest that the after-effects of COVID-19 may last for one spermatogenic cycle.

Consistent with a preliminary study (preprint) on sex hormones in patients with SARS-CoV-2 infection,[22] higher prolactin levels were observed in our patients who had recovered from COVID-19, which could result from multiple physiological or pathological conditions, or medication.[25] On the other hand, the higher prolactin levels could be preexisting in these patients, as we did not observe any evidence of recovery in patients at the second sampling. In this previous study, the authors also found increased LH levels, along with decreased T/LH and FSH/LH ratios in patients with SARS-CoV-2 infection,[22] while in our study, all the patients have normal levels of LH, and the LH levels, and the ratios of T/LH and FSH/LH in the patients did not differ significantly from those in controls. These differences may be due to the timing of blood sampling - their samples were collected from patients with SARS-CoV-2 infection, while our samples were collected at least 36 days after hospital discharge; this, however, needs to be confirmed by studies on the same patients sampled at different time points after SARS-CoV-2 infection. We also noticed reduced inhibin B levels in patients at the first sampling compared with controls, and increased inhibin B levels at the second sampling than that at first sampling for the 22 patients examined, though the differences did not meet statistical significance. Inhibin B is predominantly produced by Sertoli cells, which support and nurse germ cells and are essential for spermatogenesis, its level positively correlates with Sertoli cell function and sperm number.[19,26] Hence, the low inhibin B levels at the first sampling may also support the observation of worse semen quality in our patients.

Though we found that the progesterone levels in the patients were lower than those in controls, we did not observe any significant change between the two samplings conducted for the 22 patients. Thus, we think that the finding of lower progesterone levels in patients than those in controls may result from variations in men (for example, compared with controls, our patients may have pre-existing lower progesterone levels) given our small cohort size, but other than a consequence of COVID-19.

In particular, in our opinion, the temporary sperm parameter alterations (at the first sampling) in the patients may not be solely attributable to a specific pathogenic mechanism of SARS-CoV-2. There are other plausible explanations of the COVID-19 after-effects on male reproduction. First, all our patients self-reported that they suffered from emotional and mental stresses since SARS-CoV-2 infection and a large retrospective cohort study also showed that COVID-19 was linked to higher rates of mental health problems,[27] while existing clinical evidence suggests a negative association between psychological stress and spermatogenesis.[28,29] Second, in our patients, 85% had fever and several reports have observed temporarily decreased semen

parameter values in patients who had experienced febrile illness.[30,31] In a recent study, sperm concentration, sperm number and motile sperm number in recovered patients with fever symptoms during COVID-19 course were lower than those without fever, but only 18 patients were included for this comparison.[8] Hence, the impact of fever needs to be ascertained by future studies on a larger scale. Third, though we did not observe any obvious correlation between the medications, therapies or the severity of the COVID-19 and the sperm quality in our patients, Holtmann *et al.*[8] reported that the negative influence of COVID-19 on sperm count and sperm motility was not detected in subjects recovered from mild symptoms when home care was possible, but was detected in patients in need of hospital care. Thus, the possibility that these medications or therapies may be harmful to testicular functions cannot be definitely ruled out.

However, our findings of the consequences of COVID-19 on male reproduction should be interpreted cautiously, as the lack of pre-COVID-19 sperm and hormone parameter values are a huge limitation and sperm concentration and sperm motility were determined mainly using CASA, which is not the standard procedure for semen analyses but an optional choice as suggested by the WHO.[17] The data were obtained from a small cohort size of 41 men who had recovered from COVID-19 for a median time of 56 days at the first sampling and controls lack a second sampling at a similarly spaced time point, while sperm parameters could be physiologically subject to intra-patient variability and fluctuations. Subsequent long-term follow-ups on more patients and elucidation of the underlying pathological mechanism and pathways would help a better understanding of the after-effects of COVID-19 on spermatogenesis and to find ways to diminish the consequences during coronavirus infections.

AUTHOR CONTRIBUTIONS

QHS and JPW conceived and designed the study. THG, MYS, SB, HM, YYW, BX, HC, XHJ, YWZ, XFX, and CJG recruited the participants, carried out examinations, and analyzed the data. JPW, XYZ, and SHL provided clinical information of the patients. HM, SB, and QHS wrote the manuscript. QHS and JPW edited the manuscript and provided comments and feedback. QHS supervised the study. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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Supplementary Table 1: Physical and clinical characteristics of the 41 patients

Patient ID	*Age (year)*[a]	*Height (cm)*	*Body weight (kg)*	*BMI (kg m^{-2})*	*Testis volume (left/ right, ml)*	*Occupations*	*Years of marriage*[a]	*Number of children*	*Comorbidity*	*Days before hospitalization after symptom appearance*	*Duration of hospitalization (day)*	*Subgroup of COVID-19*	*Treatment*		
													Corticosteroid therapy	Other medications	Remarks
1	24	176	75	24.2	16/16	Worker	0.5	0	No	3	19	Mild	Methylprednisolone	Antiviral and anti-inflammatory drugs	–
2	28	180	60	18.5	20/20	Dealer	3	1	No	NA	26	Moderate	No	Lopinavir-ritonavir, anti-inflammatory drugs, diammonium glycyrrhizinate, bicyclol, traditional Chinese medicine, and vitamins	–
3	26	174	65	21.5	15/15	Office staff	–	0	Varicocele	3	3	Mild	No	Antiviral, and anti-inflammatory drugs	Drinking and smoking
4	22	175	67	21.9	16/16	Student	–	0	Varicocele	5	34	Mild	No	Lopinavir-ritonavir, anti-inflammatory drugs, and diammonium glycyrrhizinate	–
5	22	169	90	31.5	16/16	Office staff	–	0	No	2	32	Moderate	No	Lopinavir-ritonavir, anti-inflammatory drugs, diammonium glycyrrhizinate, bicyclol, traditional Chinese medicine, and vitamins	–
6	22	170	60	20.8	15/15	Student	–	0	No	6	25	Mild	No	No	-
7	22	180	75	23.1	16/16	Student	–	0	No	0	19	Mild	No	Lopinavir-ritonavir, anti-inflammatory drugs, and lianhuaqingwen capsules	–
8	37	175	75	24.5	20/20	Office staff	8	3	No	7	16	Mild	No	Lopinavir drugs, ambroxol and interferons - ritonavir, anti-inflammatory	–
9	22	170	87	30.1	16/16	Student	–	0	No	2	27	Mild	No	Diammonium glycyrrhizinate, antiviral and anti-inflammatory drugs, polyene phosphatidylcholine capsules, and traditional Chinese medicine	–
10	29	178	105	33.1	20/20	Office staff	–	0	Chronic nephritis	1	16	Severe	Metacortandracin	Lopinavir-ritonavir, oxygen therapy, Jinshuibao capsule, polyene phosphatidylcholine capsules, and antihypertensive drugs	–
11	44	174	85	28.1	16/16	Courier	21	2	No	0	27	Mild	No	Antiviral drugs	Drinking occasionally
12	30	173	65	21.7	15/15	Office staff	2	1	No	5	5	Mild	No	Lopinavir-ritonavir and anti-inflammatory drugs	Smoking
13	21	182	115	34.7	16/16	Student	–	0	No	2	23	Moderate	Prednisone and Methylprednisolone	Lopinavir-ritonavir, oxygen therapy, antiviral and anti-inflammatory drugs, diammonium glycyrrhizinate, and probiotics	–

Contd...

Supplementary Table 1: Contd...

Patient ID	*Age (year)*[a]	*Height (cm)*	*Body weight (kg)*	*BMI (kg m^{-2})*	*Testis volume (left/ right, ml)*	*Occupations*	*Years of marriage*[a]	*Number of children*	*Comorbidity*	*Days before hospitalization after symptom appearance*	*Duration of hospitalization (day)*	*Subgroup of COVID-19*	*Treatment*		
14	23	173	75	25.1	20/20	Quality inspector	–	0	No	5	8	Mild	No	Lopinavir-ritonavir, antiviral and anti-inflammatory drugs	
15	24	175	65	21.2	25/25	Courier	–	0	No	3	13	Mild	No	Antiviral drugs	–
16	30	170	78	27.0	25/25	Dealer	5	1	No	2	15	Mild	No	Lopinavir-ritonavir	Smoking
17	38	169	80	28.0	15/15	Office staff	14	2	Asthma	6	42	Moderate	No	Lopinavir-ritonavir, interferons, antiviral and anti-inflammatory drugs, and probiotics	–
18	24	182	68	20.5	15/15	No	–	0	No	8	10	Mild	No	Antiviral drugs, interferons and Shufengjiedu capsules	–
19	23	176	84	27.1	20/20	Student	–	0	No	3	17	Mild	No	Antiviral and anti-inflammatory drugs, Asmeton, and traditional Chinese medicine	–
20	19	185	112	32.7	20/20	Student	–	0	No	0	27	Mild	No	Antiviral and anti-inflammatory drugs	–
21	37	181	77	23.5	20/20	Office staff	5.5	2	Varicocele	7	28	Moderate	No	Ambroxol and interferons	–
22	24	175	74	24.2	16/16	Freelance	–	0	No	1	27	Mild	Prednisone	Antiviral and anti-inflammatory drugs	–
23	23	175	100	32.7	16/16	Nurse	–	0	No	3	18	Mild	No	Antiviral and anti-inflammatory drugs	–
24	21	180	71	21.9	16/16	Student	–	0	No	2	10	Mild	No	Antiviral and anti-inflammatory drugs	–
25	21	170	60	20.8	16/16	Student	–	0	No	1	17	Mild	No	Antiviral drugs	–
26	21	178	65	20.5	16/16	Freelance	–	0	No	0	13	Mild	No	Interferons	–
27	43	168	80	28.3	16/16	Cook	15	2	No	7	12	Mild	No	Antiviral and anti-inflammatory drugs	–
28	31	182	90	27.2	20/20	Freelance	5	1	No	4	7	Mild	No	Lopinavir-ritonavir	Drinking occasionally
29	42	162	65	24.5	15/15	Office staff	14	2	No	0	21	Moderate	No	Antiviral and anti-inflammatory drugs, lopinavir-ritonavir, and traditional Chinese medicine	–
30	34	171	65	22.2	15/15	Freelance	10	2	No	8	13	Mild	Methylprednisolone	Lopinavir-ritonavir and levofloxacin	–
31	24	179	87	27.2	20/20	Office staff	–	0	No	3	14	Moderate	No	Lopinavir-ritonavir, anti-inflammatory drugs, diammonium glycyrrhizinate, Ambroxol, and traditional Chinese medicine	–
32	29	177	80	25.5	20/20	Freelance	–	0	Varicocele	1	24	Moderate	No	Lopinavir-ritonavir	–
33	20	174	52	17.2	NA	Student	–	0	No	1	15	Mild	No	Antiviral drugs, interferons and traditional Chinese medicine	Smoking

Contd...

Supplementary Table 1: Contd...

Patient ID	*Age (year)*[a]	*Height (cm)*	*Body weight (kg)*	*BMI (kg m^{-2})*	*Testis volume (left/ right, ml)*	*Occupations*	*Years of marriage*[a]	*Number of children*	*Comorbidity*	*Days before hospitalization after symptom appearance*	*Duration of hospitalization (day)*	*Subgroup of COVID-19*	*Treatment*		
34	44	180	80	24.7	20/20	Dealer	22	2	No	3	40	Mild	No	Antiviral and anti-inflammatory drugs, lopinavir-ritonavir, interferons and traditional Chinese medicine	Drinking and smoking
35	21	175	72	23.5	20/20	Office staff	–	0	No	NA	15	Mild	No	Antiviral drugs, interferons and traditional Chinese medicine	–
36	44	171	75	25.6	16/16	Farmer	22	2	Hepatitis B	NA	15	Moderate	No	Lopinavir-ritonavir and Lianhuaqingwen capsules	–
37	28	173	111	37.1	16/16	Manual workers	7	3	No	2	17	Mild	No	Lopinavir-ritonavir and Lianhuaqingwen capsules	–
38	35	170	82	28.3	16/16	Office staff	13	2	No	2	33	Mild	No	Lopinavir-ritonavir, anti-inflammatory drugs and traditional Chinese medicine	–
39	34	179	88	27.5	15/15	Worker	10	2	Renal cyst	8	19	Moderate	No	Lopinavir-ritonavir, interferons and traditional Chinese medicine	–
40	26	173	75	25.1	15/15	Dealer	5	1	No	1	13	Mild	No	Lopinavir-ritonavir, interferons, anti-inflammatory drugs and traditional Chinese medicine	Smoking
41	36	168	79	28.0	20/20	Office staff	6	1	Prostatitis	1	22	Severe	Methylprednisolone	Lopinavir-ritonavir, interferons and γ globin	

[a]When samples were collected. Dashes indicate that the patient is unmarried. BMI: body mass index; NA: not available; COVID-19: coronavirus disease 2019

Supplementary Table 2: Physical and clinical characteristics in patients recovered from coronavirus disease 2019

	Patients (n=41)
Age (years), median (IQR)	26.0 (22.0–34.0)
Men aged 18–30 (%)	65.9
Men aged 31–45 (%)	34.1
BMI (kg m^{-2}), median (IQR)	25.1 (21.9–28.0)
Men with BMI 18.5–23.9 (%)	36.6
Men with BMI 24–27.9 (%)	34.1
Men with BMI ≥28 (%)	29.3
COVID-19 subtypes (%)	
Mild	70.7
Moderate	24.4
Severe	4.9
Critical	0
Hospital days due to COVID-19, median (IQR)	17.0 (13.0–26.0)
Patients receiving corticosteroid therapy (%)	14.6
COVID-19 symptoms (%)	
Fever	85.0
Cough	56.1
Difficulty breathing	14.6
Sore throat	26.8
Muscle aches	24.4
Digestive issues	34.1
Abnormal chest CT	61.1

IQR: interquartile range; BMI: body mass index; CT: computed tomography; COVID-19: coronavirus disease 2019

Supplementary Table 3: Semen characteristics in patients recovered from coronavirus disease 2019

Patient ID	*Days after symptoms appearance*[a]	*Days after hospital discharge*[a]	*Semen volume (ml)*	*Sperm concentration (millions ml^{-1})*	*Total sperm count (millions per ejaculate)*	*Abnormal sperm morphology (%)*	*Progressive sperm motility (%)*	*Sperm motility (%)*	*Sperm vitality (%)*
1	74	52	3.5	95.2	333.4	91	56.1	64.5	75
2	NA	66	1.7	15.1	25.6	97	34.8	37.3	87
3	57	51	2.3	49.6	114.0	98	35.4	43.4	60
4	75	36	3.1	101.0	312.9	98	43.0	45.5	62
5	83	49	5.4	32.9	177.3	93	63.9	70.4	91
6	76	45	0.5	135.3	67.6	95	4.0	5.0	40
7	73	54	4.8	25.5	122.4	97	39.3	44.9	95
8	77	54	0.6	121.0	72.6	90	48.1	58.0	83
9	72	43	5.0	42.9	214.3	94	25.0	28.9	79
10	74	57	1.4	6.5	9.0	95	14.4	16.0	80
11	66	39	1.5	102.1	153.1	93	43.0	48.7	91
12	65	55	3.0	17.8	53.4	94	20.3	24.3	83
13	71	46	2.9	1.5	6.0	97	37.1	45.8	92
14	85	72	3.9	31.8	123.9	95	33.6	36.3	62
15	77	61	2.5	29.7	74.2	97	37.8	41.7	85
16	81	64	5.2	33.2	172.4	89	48.9	56.1	85
17	76	40	4.0	41.8	167.3	96	27.2	30.3	60
18	68	50	0.9	49.1	44.2	94	18.9	26.3	33
19	75	56	3.1	93.3	289.2	93	30.4	32.5	79
20	76	49	2.2	84.5	186.0	91	57.4	63.3	83
21	79	44	3.7	64.4	238.2	97	50.5	55.1	82
22	68	40	2.3	43.0	18.7	92	25.4	30.5	79
23	77	56	2.4	33.5	80.4	90	20.7	37.8	89
24	65	53	2.3	35.4	81.4	89	72.3	80.3	85
25	75	57	3.3	71.9	237.3	87	36.2	52.1	36
26	66	53	3.6	14.6	52.6	91	7.1	21.4	57
27	76	58	2.4	36.6	87.8	99	4.7	8.0	59
28	104	93	4.6	170.7	785.1	93	65.1	74.0	81
29	88	67	4.1	143.3	587.4	90	32.9	36.9	71
30	106	85	2.9	51.3	148.9	96	13.5	14.9	32
31	108	91	4.3	100.9	433.7	89	70.3	77.0	91
32	102	77	2.7	127.2	343.4	92	44.5	53.2	69
33	87	71	3.7	69.7	258.0	91	50.9	59.8	92
34	85	42	3.1	36.9	114.5	97	38.1	44.4	75
35	NA	80	2.7	75.7	204.5	92	49.1	55.2	96
36	NA	77	3.0	33.4	100.3	93	31.5	43.2	91
37	73	54	3.0	24.0	72.1	94	54.7	64.5	85
38	107	72	4.0	154.9	619.7	86	44.3	50.0	80
39	104	77	4.2	185.9	780.8	92	26.4	31.7	63
40	102	88	1.8	53.2	95.7	97	64.1	68.6	95
41	100	78	3.0	65.58	196.7	98	11.8	13.8	60

[a]When semen analyses were performed. All the semen samples were alkaline for pH and liquefied within 30 min after ejaculation. NA: not available

Supplementary Table 4: Semen characteristics in controls

Patient ID	*Age*[a]	*Height (cm)*	*Body weight (kg)*	*Varicocele*	*Semen volume (ml)*	*Sperm concentration (millions ml^{-1})*	*Total sperm count (millions per ejaculate)*	*Abnormal sperm morphology (%)*	*Progressive motility sperm (%)*	*Sperm motility (%)*	*Sperm vitality (%)*
1	18	178	80	No	3.8	38.2	145.2	97	9.5	10.8	21
2	25	180	76	Yes	5.5	14.0	77.1	93	44.7	49.8	60
3	26	169	71	No	1.0	56.8	56.8	92	68.2	75.0	93
4	25	175	85	No	2.7	187.7	506.8	93	69.1	81.2	90
5	26	180	120	No	4.0	225.7	902.6	91	62.9	73.4	91
6	26	170	77	No	2.9	103.3	299.5	94	62.0	72.8	88
7	25	185	105	No	3.3	110.0	363.0	89	62.7	70.0	85
8	27	171	66	No	1.3	214.8	279.3	97	33.2	39.2	56
9	27	173	84	No	0.7	156.2	109.3	93	48.6	59.3	79
10	25	178	80	No	5.5	68.7	377.7	95	38.7	45.5	82
11	22	175	60	No	1.4	95.3	133.4	91	43.4	48.2	ND
12	26	174	63	Yes	4.2	185.1	777.5	92	63.9	75.2	90
13	25	177	80	No	2.2	263.1	578.8	92.5	65.6	71.5	91
14	23	170	70	No	2.2	110.5	243.1	96	49.1	53.3	84
15	25	178	63	Yes	6.4	22.8	146.0	91	40.7	43.4	67
16	25	168	70	No	2.6	214.8	558.5	92	79.3	91.0	92
17	24	172	80	Yes	1.7	58.4	99.2	94	50.5	54.4	ND
18	33	175	66	No	3.0	75.7	227.0	93	37.0	46.9	79
19	34	179	74	No	4.3	129.9	558.4	94	49.5	58.9	77
20	34	170	69	No	5.5	67.8	373.0	96	33.7	37.1	57
21	29	170	70	No	2.1	89.6	188.1	92	75.2	82.7	95
22	34	171	80	No	1.7	114.5	194.7	90	44.7	57.5	89
23	38	172	61	No	4.4	130.9	576.0	93	51.3	58.2	ND
24	27	179	76	No	3.5	93.8	328.3	94	45.1	54.9	69
25	29	169	68	No	3.2	40.7	130.1	96	31.4	35.3	71
26	27	173	74	No	4	45.8	183.1	95	58.8	71.8	80
27	35	168	70	Yes	4.5	282.7	1272.2	94	58.8	71.0	84
28	36	170	76	No	1.5	55.9	83.8	97	30.7	39.3	58
29	47	175	95	No	2	61.0	122.0	92	35.8	43.4	59
30	28	175	70	No	4.7	14.3	67.4	92	49.5	53.2	ND
31	36	178	84	No	1.6	135.7	217.1	97	19.6	27.7	ND
32	36	173	72	No	2.6	182.9	475.6	95	52.4	66.4	83
33	40	177	77	No	3.3	4.2	14.0	98	42.9	49.0	ND
34	44	170	71	No	1.4	126.7	177.3	95	49.7	61.5	88
35	40	171	69	No	4.4	62.0	272.7	93	48.8	54.6	92
36	34	160	52	No	2.7	18.5	50.0	91	28.2	33.4	61
37	32	167	61	No	4.6	98.4	452.5	95	48.3	56.7	77
38	25	176	67	No	2.6	35.4	92.0	96	27.6	32.0	63
39	24	172	60	No	2	67.0	133.9	90	40.7	44.1	80
40	23	170	70	No	2.7	104.8	283.0	95	40.5	45.9	78
41	22	179	77	No	1.8	51.3	92.3	89	40.2	42.3	86
42	26	173	70	No	2.7	83.9	226.6	99.5	19.2	21.3	27
43	22	170	65	No	2.3	31.8	73.2	92.5	49.7	60.0	89
44	27	168	70	No	1.2	89.9	107.8	88	33.2	35.9	81
45	26	178	75	No	3.9	60.0	234.1	93	55.1	59.4	91
46	28	174	72	Yes	3.1	134.0	415.4	94	56.1	62.8	90
47	26	176	58	Yes	3.7	161.4	597.1	97	31.0	33.0	ND
48	26	173	57	No	4.1	83.9	344.0	96	11.7	12.9	ND
49	24	176	79	Yes	4	83.9	335.6	93	59.4	65.5	ND
50	38	170	70	No	6.6	3.7	24.6	92	38.9	44.4	63

[a]When semen analyses were performed. All the semen samples were alkaline for pH and liquefied within 30 min after ejaculation. ND: not detected

Supplementary Table 5: Levels of sex hormones in patients recovered from coronavirus disease 2019

Patient ID	*Hormone analysis*[a]							
	Estradiol (pg ml^{-1}) Ref: 20.0–55.0	*FSH (U l^{-1}) Ref: 1.5–12.4*	*LH (U l^{-1}) Ref: 1.2–8.6*	*Progesterone (μg dl^{-1}) Ref: 1.3–9.7*	*T (ng ml^{-1}) Ref: 1.8–7.9*	*Prolactin (ng ml^{-1}) Ref: 2.6–18.1*	*AMH (ng ml^{-1}) Ref: 0.7–19.0*	*Inhibin B (pg ml^{-1}) Ref: 87.4–299.9*
1	13.0	4.4	4.2	3.7	4.0	34.7	21.8	113.0
2	21.0	4.1	3.8	0.9	4.0	21.2	12.5	101.0
3	50.0	3.9	5.9	1.4	3.2	14.8	12.7	108.0
4	32	1.6	3.5	8.4	3.9	20.6	9.1	163.0
5	16	6.2	2.7	6.4	2.2	14.5	2.4	52.8
6	11	5.4	4.5	1.4	4.0	17.7	7.2	74.1
7	14	3.7	6.6	8.4	5.9	44.4	20.4	119.0
8	35	6.0	3.1	1.2	4.6	7.3	5.3	84.3
9	42	5.6	6.9	8.9	3.7	40.5	8.9	71.8
10	21	2.8	3.4	1.2	2.6	19.0	14.4	149.0
11	42	5.0	6.1	2.3	2.8	18.1	8.0	75.5
12	15	11.2	3.7	5.4	2.9	15.6	6.6	74.5
13	31	4.6	2.7	10.6	2.6	27.5	10.4	64.6
14	27	2.3	2.6	2.8	4.2	13.2	16.0	92.9
15	61	5.3	3.1	2.3	3.2	16.1	11.4	104.0
16	37	2.3	3.2	7.2	3.6	8.7	8.4	249.0
17	27	8.4	2.7	2.0	2.2	9.9	4.7	215.0
18	38	6.7	7.9	3.0	4.9	31.9	4.4	316.0
19	6	3.4	3.8	4.9	4.0	10.2	10.3	119.0
20	39	3.9	3.6	6.1	6.7	32.2	15.6	156.0
21	9	5.5	3.6	7.0	3.2	7.5	10.0	364.0
22	27	4.6	4.6	1.5	2.8	7.2	8.6	195.0
23	42	6.9	3.2	3.5	2.8	5.8	5.4	72.7
24	41	7.9	5.1	14.1	2.8	9.6	17.4	73.6
25	36	5.7	2.9	13.3	5.1	16.1	5.5	90.2
26	15	4.6	2.1	6.0	5.2	12.0	5.0	94.7
27	30	4.9	3.5	6.4	3.2	9.2	4.6	157.0
28	30	5.3	5.1	2.0	5.5	8.0	14.9	84.5
29	21	4.0	3.0	3.3	2.8	18.2	5.8	117.0
30	26	7.2	3.5	4.6	4.4	19.7	9.5	102.0
31	34	3.7	4.6	19.1	3.1	13.5	7.0	136.0
32	29	2.7	4.8	1.1	4.4	25.5	13.8	208.0
33	24	3.3	3.7	4.8	4.8	13.5	20.6	126.0
34	5	7.5	2.4	0.2	1.2	14.3	2.5	68.7
35	37	3.9	5.1	8.1	4.0	26.5	10.9	117.0
36	37	3.5	1.2	5.7	3.0	8.4	2.8	ND
37	37	3.1	2.6	5.2	2.2	9.5	13.9	ND
38	47	3.4	7.1	2.5	4.5	9.8	8.0	ND
39	36	5.1	5.6	9.0	3.8	18.3	14.1	94.2
40	33	6.3	3.2	1.0	2.7	7.6	11.9	69.5
41	31	5.3	3.4	2.2	2.6	7.9	5.8	66.5
Median (IQR)	31.0 (21.0–37.0)	4.6 (3.7–5.6)	3.6 (3.0–4.8)	4.6 (2.0–7.0)	3.6 (2.8–4.4)	14.5 (9.5–19.7)	9.1 (5.8–13.8)	103.0 (74.8–145.8)
Percentage (number) of men with abnormal values	22.0 (9/41)	0 (0/41)	0 (0/41)	24.4 (10/41)	2.4 (1/41)	34.1 (14/41)	4.8 (2/41)	39.5 (15/38)

[a]Reference values were suggested by clinical laboratory. FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone; AMH: anti-Müllerian hormone; ND: not determined; IQR: interquartile range

Supplementary Table 6: Semen characteristics in 22 patients recovered from coronavirus disease 2019 at second sampling

Patient ID	*Days after hospital discharge*[a]	*Days between first and second sampling*	*Semen volume (ml)*	*Sperm concentration (millions ml^{-1})*	*Total sperm count (millions per ejaculate)*	*Abnormal sperm morphology (%)*	*Progressively motile sperm (%)*	*Motile sperm (%)*	*Sperm vitality (%)*
1	84	32	3.5	125.2	438.3	90	68.5	77.1	90
2	109	43	1.9	117.8	223.8	91	37.8	41.9	79
3	125	74	2.0	71.5	143.0	96	54.2	60.3	79
4	67	31	3.4	93.8	318.6	99	37.5	42.4	75
5	73	24	3.3	42.7	157.8	96	35.8	40.7	90
6	70	25	0.6	50.3	30.2	94	2.7	3.5	9
7	84	30	5.5	65.1	357.8	96	51.3	58.5	96
9	73	30	6.2	54.4	337.2	90	46.5	51.9	66
10	90	33	3.3	48.7	160.7	92	51.6	54.8	93
12	85	30	3.6	45.3	163.0	89	20.5	25.5	49
13	69	23	3.0	14.1	42.2	92	41.7	48.2	88
15	130	69	2.7	60.9	164.5	93	33.1	36.9	91
17	67	27	3.7	42.7	157.8	96	35.8	40.7	67
18	77	27	1.3	96.8	125.9	93	24.5	32.8	79
19	84	28	3.2	44.6	139.4	93	28.5	34.0	84
20	77	28	1.6	180.4	288.6	89	32.7	41.0	96
21	106	62	3.7	47.0	173.9	96	27.2	31.3	68
23	84	28	3.0	57.1	171.4	92	36.4	47.0	94
24	81	28	4.4	136.3	599.8	90	32.1	40.0	85
25	124	67	2.8	128.1	358.6	92	69.1	75.1	87
26	81	28	4.9	13.9	34.2	88	13.9	17.9	75
27	86	28	3.8	145.6	553.3	98	42.7	49.5	60

[a]When the second sampling was conducted. All the semen samples were alkaline for pH and liquefied within 30 min after ejaculation