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ORIGINAL ARTICLE

Semen Analysis

Effects of the COVID-19 pandemic on semen quality in male partners of infertile couples: a hospital-based observational study

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The effects of the coronavirus disease 2019 (COVID-19) pandemic on male fertility have received considerable attention because human testes contain high levels of angiotensin-converting enzyme-2 receptors, through which severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can enter. Early studies showed decreases in semen quality during and after recovery from COVID-19. However, no semen quality studies have examined the effects of widespread subclinical and mild disease, as well as changes in lifestyle, psychosocial behavior, intake of dietary supplements, and stress. This cross-sectional study compared semen quality parameters in male partners of infertile couples between men who underwent semen analysis before the COVID-19 pandemic (prepandemic group) and men who underwent semen analysis during the pandemic period (pandemic group); the analysis sought to clarify the overall effects of the pandemic. No participants in the pandemic group had experienced clinically overt disease. Among the 239 participants, mean body weight ($P = 0.001$), mean body mass index ($P < 0.001$), median sperm concentration ($P = 0.014$), total sperm count ($P = 0.006$), and total percentages of motile ($P = 0.013$) and abnormal cells ($P < 0.001$) were significantly greater in the pandemic group ($n = 137$) than those in the prepandemic group ($n = 102$). Among abnormal cells, the percentages of cells with excess residual cytoplasm ($P < 0.001$), head defects ($P < 0.001$), and tail defects ($P = 0.015$) were significantly greater in the pandemic group than those in the prepandemic group. With the exception of morphology, the overall semenogram results were better in the pandemic group than those in the prepandemic group.

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INTRODUCTION

Since its initial outbreak as a cluster of pneumonia cases of unknown origin in December 2019, the coronavirus disease 2019 (COVID-19) has spread rapidly throughout the world to reach pandemic proportions. The causative pathogen was identified as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Thus far, more than 500 million confirmed cases of COVID-19 have been reported.[1] However, the overall burden of COVID-19 is estimated to be much higher than the numbers of diagnosed and reported cases because individuals with mild or subclinical disease do not seek medical care.[2] One study estimated that over 40% of people worldwide had been infected with SARS-CoV-2 at least once by mid-November 2021; one-third of these infected individuals were in southern Asia, including India.[3] This estimate was supported by the findings of the Fourth National Serosurvey conducted by the Indian Council of Medical Research (New Delhi, India), which revealed a seroprevalence of >75% in Bihar, the location of the present study.[4] This seroprevalence serves as an indicator of the extent of subclinical exposure to SARS-CoV-2.

COVID-19 mainly affects the respiratory system, but it can affect other systems (*e.g.*, heart, kidneys, liver, and brain).[5] Additionally, COVID-19 is associated with excessive cytokine production, generalized inflammation, endothelitis, and hypercoagulability.[6,7] SARS-CoV-2 enters host cells by binding to angiotensin-converting enzyme-2 receptors;[8] these receptors are abundant in many tissues, including the Leydig cells, Sertoli cells, and spermatogonia of adult human testes.[9–11] Thus, the human testes have been regarded as potential targets of SARS-CoV-2; such infections could have substantial effects on semen quality and fertility. Thus far, studies of semen quality in patients with COVID-19 have supported this hypothesis. These studies have shown overall decreases in semen quality, regardless of whether semen samples exhibited SARS-CoV-2.[12–15] However, the results of these studies have not clarified whether subclinical or mild exposure to SARS-CoV-2 affects semen quality parameters.

Furthermore, there have been reports of pandemic-related changes in lifestyle (*e.g.*, food habits, physical activity, sleep, and stress levels) that can affect body weight and body mass index (BMI); such changes have been greatest during lockdown periods.[16–18] There have also been reports of pandemic-related increases in the consumption of dietary supplements such as multivitamins, probiotics, and ayurvedic preparations.[16,18] Some studies showed increases in body weight and BMI,[19–21] whereas others showed increases in healthy eating habits (*e.g.*, consumption of fruits, vegetables, nutritional supplements, and immunity boosters such as ginger and garlic)[17,18,22–24] during the

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pandemic period compared with the prepandemic period. Modifiable lifestyle factors (*e.g.*, level of physical activity) and changes in dietary patterns have been reported to affect semen quality.[25–29] Similarly, psychosocial stress and grief can have negative effects on semen quality.[30,31] Previous studies have shown changes in sperm morphology during periods of stress[31–33] and improvements in semen quality parameters during the consumption of nutritional supplements.[29,34,35]

As noted above, the prevalence of subclinical and mild disease, along with pandemic-related increases in stress, anxiety, and disturbed sleep cycles, may have adverse effects on semen quality and fertility. Conversely, increases in healthy eating habits may have positive effects. In the present study, we compared the semen quality parameters of men who presented for semen analysis during the COVID-19 pandemic with those of men who presented for semen analysis before the pandemic.

PARTICIPANTS AND METHODS

This cross-sectional observational study included patients referred to the Andrology Laboratory of All India Institute of Medical Sciences (Patna, India) for semen quality assessment as part of the routine evaluation of infertile couples between January 2019 and February 2022. The patients were divided into prepandemic and pandemic groups according to whether semen analysis was conducted before or during the COVID-19 pandemic. This study is part of a larger study in which semen quality in the included patients is under investigation with respect to lifestyle, anthropometric, and demographic variations. The study was approved by the Institutional Ethics Committee of All India Institute of Medical Sciences (approval No. IEC2019372). During the initial clinical examination, patients provided written informed consent to participate in the study. In India, the first COVID-19 case was detected in January 2020; this is regarded as the beginning of the first wave.[36] However, the first COVID-19 case in Bihar, India, was reported in the last week of March 2020.[37] This wave was mild and the rate of infection with SARS-CoV-2 was low. The second wave began in March 2021; it was much more infectious and severe.[36] Considering these temporal characteristics, the prepandemic period was defined as January 2019 to February 2020, whereas the pandemic period was defined as March 2021 to February 2022. All patients who provided consent to participate and had no known causes of infertility were included in the study. Patients were excluded if they exhibited urological diseases or any other known causes of infertility, if they reported semen spillage out of the container, or if they had an abstinence period of <2 days or >7 days. Among 239 participants in the study, 102 were in the prepandemic group and 137 were in the pandemic group. Detailed sociodemographic histories and anthropometric measurements were recorded by trained healthcare staff. All patients who met the inclusion criteria were asked to return on a later date; they were also provided instructions regarding the appropriate abstinence period.

Semen analysis

All semen samples were obtained by masturbation in the laboratory facility; the samples were collected in sterile and nontoxic containers. Semen analysis was conducted manually, in accordance with World Health Organization recommendations.[38,39] The viscosities of collected samples were measured; samples were incubated at 37°C for a sufficient duration to achieve liquefaction. After liquefaction and within 1 h of collection, semen samples were analyzed to determine semen volume, pH, total sperm motility, sperm concentration, and sperm morphology.[39] Sperm concentrations were assessed after appropriate dilution using a modified Neubauer chamber (Rohem Instruments Private Limited, Nashik, India). Assessments of sperm motility were conducted by examining wet preparations at a total magnification of ×400 (ECLIPSE E200LED MV, Nikon Corporation, Tokyo, Japan). For morphological assessment, smears were fixed and stained; 200 spermatozoa were examined at a total magnification of ×1000. Spermatozoa were classified as normal or abnormal based on abnormalities in all parts of the spermatozoon including the head, midpiece, tail, and cytoplasmic residue. All semen samples were analyzed by two laboratory technicians using the same apparatus. Internal quality control was routinely performed to ensure that there were no significant differences between the results of the two technicians.

Statistical analyses

Data were analyzed using SPSS software version 22.0 (SPSS Inc., Chicago, IL, USA). First, the normality of the data was analyzed in each group. All normally distributed continuous parameters are presented as means and standard deviations; nonnormally distributed continuous parameters are presented as medians and interquartile ranges. All categorical variables are presented as numbers and percentages. Independent samples *t*-tests and the Mann–Whitney U test were used to analyze statistical significance. All statistical tests were two-tailed, and $P < 0.05$ was considered statistically significant.

RESULTS

Among 239 semen samples, 102 were in the prepandemic group and 137 were in the pandemic group. The age and anthropometric parameters of the two groups are presented in **Table 1**. There was no significant difference in mean age between the two groups ($P = 0.401$). The mean body weight ($P = 0.001$) and mean BMI ($P < 0.001$) were significantly greater in the pandemic group than those in the prepandemic group. The semen parameters of the prepandemic and pandemic groups are shown in **Table 2**. The median sperm concentration ($P = 0.014$), total sperm count ($P = 0.006$), percentage of total motility ($P = 0.013$), and percentage of abnormal cells ($P< 0.001$) were significantly greater in the pandemic group than those in the prepandemic group. The overall differences in the distributions of sperm concentration, total motility, and normal spermatozoa in the prepandemic and pandemic groups are shown in **Figure 1**. Among abnormal cells, the percentages of head defects ($P < 0.001$), tail defects ($P = 0.015$), and spermatozoa with excess residual cytoplasm ($P < 0.001$) were significantly greater in the pandemic group than those in the prepandemic group.

DISCUSSION

In this study, anthropometric and semen parameters in the male partners of infertile couples were compared between prepandemic and pandemic groups. Mean body weight, mean BMI, median

Table 1: Comparison of anthropometric parameters between coronavirus disease 2019 pandemic and prepandemic groups

Parameter	*Overall (n=239)*	*Prepandemic group (n=102)*	*Pandemic group (n=137)*	*P*
Age (year)	33.1 (6.7)	32.7 (6.1)	33.4 (7.0)	0.401
Weight (kg)	65.5 (11.3)	62.7 (10.5)	67.7 (11.5)	0.001
Height (cm)	166.0 (6.7)	165.8 (7.4)	166.2 (6.2)	0.674
BMI (kg m^{-2})	23.7 (3.4)	22.7 (3.2)	24.4 (3.4)	<0.001
Waist circumference (cm)	88.9 (10.5)	88.3 (10.0)	89.4 (10.9)	0.492
Hip circumference (cm)	94.1 (8.5)	93.3 (7.7)	94.7 (9.0)	0.269
Waist:hip ratio	0.95 (0.07)	0.95 (0.07)	0.95 (0.07)	0.859

Data are shown as mean (s.d.). *P* values were determined by independent samples *t*-tests. BMI: body mass index; s.d.: standard deviation

Table 2: Comparison of semen parameters between coronavirus disease 2019 pandemic and prepandemic groups

Parameter	*Overall (n=239)*	*Prepandemic group (n=102)*	*Pandemic group (n=137)*	*P*
Abstinence (day), mean (s.d.)	4.3 (1.5)	4.1 (1.4)	4.5 (1.5)	0.030
Semen volume (ml), median (IQR)	3.0 (2.0)	2.5 (1.0)	3 (2.0)	0.054[a]
Liquefaction time (min), median (IQR)	30.0 (15.0)	30.0 (30.0)	30.0 (18.0)	0.015[a]
Viscosity (cm), median (IQR)	2.0 (4.0)	2.0 (3.0)	2.0 (4.0)	0.722[a]
pH, median (IQR)	8.0 (0.0)	8.0 (1.0)	8.0 (0.0)	<0.001[a]
Sperm concentration (×10^6 cells per ml), median (IQR)	54.0 (75.2)	46.0 (60.9)	78.0 (75.9)	0.014[a]
Total sperm count (×10^6 cells), median (IQR)	139.5 (250.8)	112.9 (176.5)	184.4 (276.2)	0.006[a]
Total motility (%), median (IQR)	58.0 (24.0)	55.5 (30.0)	60.0 (21.0)	0.013[a]
Immotile sperm (%), mean (s.d.)	40.0 (18.8)	41.9 (21.0)	38.5 (17.0)	0.202
Normal cells (%), median (IQR)	10.0 (12.0)	12.5 (31.0)	8.0 (8.0)	<0.001[a]
Abnormal cells (%), median (IQR)	89.0 (13.0)	82.0 (39.0)	90.0 (9.0)	<0.001[a]
Head defect (%), median (IQR)	40.0 (16.0)	36.0 (18.0)	45.0 (17.0)	<0.001[a]
Midpiece defect (%), median (IQR)	16.0 (17.0)	19.0 (21.0)	15.0 (13.0)	0.131[a]
Tail defect (%), median (IQR)	17.0 (15.0)	15.0 (14.0)	19.0 (13.0)	0.015[a]
Residual cytoplasm (%), median (IQR)	4.0 (6.0)	2.0 (4.0)	5.0 (6.0)	<0.001[a]

P values were determined by independent samples *t*-tests or [a]the Mann–Whitney U test. s.d.: standard deviation; IQR: interquartile range

sperm concentration, total sperm count, percentage of total motility, percentage of cells with residual cytoplasm, and the percentages of head and tail defects were significantly greater in the pandemic group than those in the prepandemic group.

The effects of the COVID-19 pandemic on semen quality could be directly caused by SARS-CoV-2; they may also result from the indirect effects of widespread inflammation and associated oxidative stress, as well as changes in lifestyle, dietary patterns, and increased stress levels. The greater body weight and BMI in the pandemic group in our study are consistent with the findings in previous studies[19–21,40–43] that showed weight gain during self-quarantine and lockdown periods because of inadequate sleep, stress, anxiety, lack of dietary restraint, and inadequate physical activity.[20,21,40] In our study, although the mean body weight and mean BMI were greater in the pandemic group, the mean BMI was within the normal range indicating that the weight gain was not unhealthy; thus, improvements in health parameters could be expected.

Because SARS-CoV-2 enters host cells through angiotensin-converting enzyme-2 receptors, which are abundantly expressed on human testicular germ cells and somatic cells, it is expected to invade the testes, then exert both direct and indirect effects related to inflammation and increased levels of oxidative stress.[8–11,44] The results of studies concerning the presence of SARS-CoV-2 in semen samples have been inconsistent. Thus far, all semen samples containing SARS-CoV-2 have been collected from patients in the acute phase of severe COVID-19. It is unclear whether the virus invades the testes of each infected individual. A study comprising postmortem evaluations of the testes of patients with COVID-19 revealed a significant reduction in Leydig cell number and substantial damage to seminiferous tubules, but most of the samples did not contain SARS-CoV-2.[45] Another study showed a higher SARS-CoV-2 positivity rate in the semen of patients with acute COVID-19 than those in the semen of patients in remission.[12] Other studies have demonstrated the absence of SARS-CoV-2 RNA in semen samples after variable periods of recovery from COVID-19.[14,15]

In our study, the median sperm concentration, total sperm count, and total motility were greater in the pandemic group than those in the prepandemic group. Previous studies concerning specific semen quality parameters have yielded conflicting results. Some studies showed decreases in sperm count,[15,46–49] percentage of total motility,[48–50]

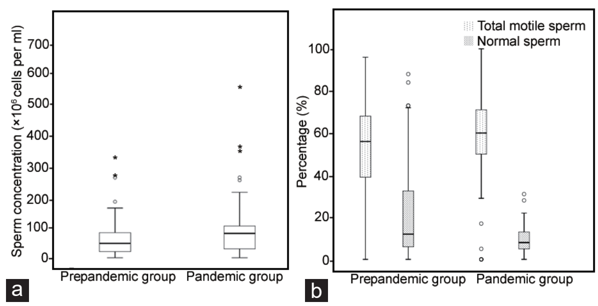

Figure 1: Box and whisker plots illustrating the distributions of different semen parameters in the prepandemic and pandemic groups. (**a**) Sperm concentration; (**b**) percentage of total motile sperm and percentage of normal sperm. The circles (o) indicate outlying values between 1.5 and 3 box lengths from the upper or lower edge of the box. The asterisks (*) represent the extreme values lying more than 3 box lengths from the upper or lower edge of the box.

and percentage of cells with normal morphology[46] in semen samples from patients who had recovered from COVID-19. Other studies have shown no changes in the percentages of total motility[46] or cells with normal morphology.[48] Additionally, Holtmann *et al.*[14] reported impaired semen parameters in individuals with moderate COVID-19 compared with healthy controls, but these impairments were not present in individuals with mild COVID-19. Similar findings were observed by Erbay *et al.*,[49] who reported worse semen quality in men with moderate COVID-19 compared with their samples prior to the onset of COVID-19. Furthermore, analyses of changes in semen parameters among serial semen samples showed improvements with increasing length of recovery from COVID-19.[15,48] These results suggest that the effect of COVID-19 on semen parameters is linked to disease severity; these changes appear to be reversible after recovery from COVID-19.

In our study, no participants in the pandemic group reported receiving a diagnosis of COVID-19. However, there was a high seroprevalence rate, according to the Fourth National Serosurvey.[4] Thus, we suspect that a large proportion of participants in the pandemic group encountered SARS-CoV-2 without developing clinically overt disease. In this context, the normal semen quality parameters in the pandemic group are consistent with findings in previous studies that suggested a severity-based effect on semen parameters. Thus, mild exposure to SARS-CoV-2 did not adversely affect semen quality parameters in the pandemic group in our study. However, we observed increases in sperm concentration, total sperm count, and total motility in the pandemic group, along with worse sperm morphology, compared with the prepandemic group; the underlying cause of these findings remains unclear.

Several studies concerning lifestyle and dietary habits during the COVID-19 pandemic have identified positive dietary changes, including increased consumption of home-cooked foods, fruits, and vegetables; the consumption of immunity boosters such as ginger, garlic and turmeric; the consumption of dietary supplements such as multivitamins, vitamins C and D, and zinc; and the use of ayurvedic preparations.[16,18,23,24] Vitamin C, vitamin D, and zinc have immune-modulating, anti-inflammatory, antioxidant, and antiviral properties.[51–54] Other studies have found improvements in semen quality after the use of nutritional supplements such as vitamin C, zinc, and multivitamins.[34,35] Improvements in semen parameters have also been observed in individuals with increased consumption of fruits and vegetables, as well as individuals who engage in regular exercises.[29]

Dawson *et al*.[55] reported a 140% increase in sperm count among infertile men after 1 week of supplementation with 1000 mg of vitamin C daily. In that study, all 30 infertile participants successfully impregnated their partners after 30 days of vitamin C, but no change was observed in the placebo group. An animal study by Akinyemi *et al*.[56] demonstrated significant improvements in systolic blood pressure, sperm motility, testosterone level, and antioxidant status in drug-induced hypertensive male rats after treatment with ginger and turmeric rhizomes. In another study, a prudent dietary pattern (*i.e.*, a pattern that involved high intakes of whole grains, legumes, vegetables, fish, and poultry) was associated with a higher testosterone level and a higher sperm concentration.[27] Considering the results of the above studies, we suspect that the significantly better semen quality that we observed in the pandemic group, compared with the prepandemic group, resulted from lifestyle changes that included changes in dietary habits and the widespread use of dietary and herbal supplements. However, controlled studies are needed to characterize the quantitative and qualitative effects of these foods and supplements on semen parameters.

We also observed worse sperm morphology in the pandemic group than that in the prepandemic group. Some previous studies have shown a decreased percentage of cells with normal morphology[46,49] among samples collected after SARS-CoV-2 infection compared with the samples collected before infection; another study has found no significant difference between the two groups.[48] Guo *et al*.[48] reported an increase in the percentage of cells with abnormal morphology in the second semen sample collected after recovery from COVID-19, compared with the first sample collected after recovery from COVID-19. In that study, the first sample was collected at a median of 56 days postrecovery, and the second sample was collected 29 days after the first sample. In contrast, in a similar study, Sarier *et al*.[57] observed no significant differences in semen parameters between prepandemic and pandemic groups. However, in that study, the mean age was significantly lower in the pandemic group than that in the prepandemic group, which is a potential confounding factor.

The COVID-19 pandemic also induced psychosocial stress related to a fear of disease, potential job loss, and the loss of loved ones. These increases in stress and grief during the pandemic period may also adversely affect sperm morphology. Fenster *et al*.[33] reported an increase in abnormal sperm morphology associated with work-related or personal stress; the association was stronger in individuals who had experienced the recent death of a close family member. They also observed decreases in linear velocity and the percentage of progressively motile spermatozoa in these individuals, compared with individuals who had not experienced a recent death in the family. Although they did not observe differences in sperm concentration or total motility between these groups, they observed an increase in the frequency of abnormal spermatozoa in men with any form of stress (work-related or personal). A similar work-related stress-induced increase in the number of cells with abnormal morphology was also reported by Auger *et al*.[31] Abu-Musa *et al*.[32] reported similar changes in sperm morphology because of stress related to the Lebanese civil war. In our study, the significant increase in the number of abnormal spermatozoa in the pandemic group may have resulted from increased levels of personal and work-related stress, or the experience of grief related to the loss of close family members. Additional studies are needed to test these hypotheses.

In conclusion, our analysis of samples from 239 patients suggests that, with the exception of sperm morphology, semen quality parameters were better in the pandemic group than those in the prepandemic group. The factors that contributed to these differences require further investigation.

AUTHOR CONTRIBUTIONS

TK conceived the study and drafted the manuscript. KJ participated in the collection of the data and helped draft the manuscript. MZ participated in the study design, as well as the collection, interpretation, and analysis of data. KN participated in the interpretation and analysis of the data, as well as critical revision of the manuscript for important intellectual content. YK participated in the collection of the data and helped draft the manuscript. KS participated in the collection, interpretation, and analysis of the data. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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