How Does COVID-19 affect male fertility?

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ABSTRACT

Background:

The SARS COVID-19 pandemic, caused by the zoonotic coronavirus, has posed significant threats to human health, with emerging concerns about its impact on male fertility. In response to this pressing issue, our study sought to investigate the effects of SARS COVID-19 and vaccination on male fertility parameters, particularly sperm DNA integrity, in individuals with normal male reproductive factors. We conducted a meticulous analysis of 320 semen samples obtained from 160 patients referred to the Fertility Clinic at the International Islamic Center for Population Studies and Research (IICPSR), Al-Azhar University. Our results unveiled distinctive patterns across four groups, shedding light on the intricate relationship between SARS COVID-19 and male reproductive health. Notably, individuals in the SARS COVID-19 group exhibited significant declines in sperm concentration, motility, and an increase in morphological abnormalities compared to controls. Furthermore, those who received vaccination displayed stability in certain parameters but showed heightened levels of abnormal forms and oxidative stress markers. These findings underscore the necessity for continued monitoring of male reproductive health, even in uninfected individuals, given the observed alterations in seminal parameters over time. Adhering to rigorous ethical standards and World Health Organization protocols, our comprehensive analysis strengthens the validity of our conclusions and emphasizes the importance of a nuanced understanding of SARS COVID-19's impact on reproductive health. **In conclusion**, our study contributes valuable insights to the ongoing discourse surrounding the broader repercussions of the pandemic on reproductive medicine and public health. As we navigate this global health crisis, our findings underscore the imperative for ongoing research and surveillance to guide healthcare practices and inform future interventions.

Key words: SARS COVID-19 pandemic, Oxidative stress markers, Fertility assessment, Semen quality, Vaccination and Male infertility

Background:

A zoonotic coronavirus illness called COVID-19 has become a pandemic, putting humans and the world economy in jeopardy [1]. Men are more prone to infections during this epidemic than women are, and they also have a greater fatality rate from COVID-19 coronavirus illness [2]. The etiological agent of COVID-19 is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is mostly transmitted by respiratory droplets, direct contact, and indirect contact. Angiotensin-converting enzyme 2 (ACE2) is utilized by both SARS-CoVs as their receptor, and genomic study reveals that SARS-CoV-2 and SARS-CoV are 79% genetically similar. Additionally, transmembrane protease, serine 2 (TMPRSS2) may facilitate viral entry mediated by ACE2 [3,4]. The SARS-CoV-2 receptor identification mechanism's structural underpinnings show that it has a stronger affinity for human ACE2, and its effects are more severe than those of other coronaviruses [3]. Despite serving as a "gate" for viruses to enter cells, ACE2 also protects against several pathophysiological functions [5]. The tissue distribution of ACE2 and the clinical signs of COVID-19 are highly correlated. In addition to lung tissue, ACE2 is expressed in the kidney, gut, testis, heart, and kidney, and it results in associated clinical symptoms [6]. A possible reason for the male-predominant infection and higher

mortality rates is that testosterone can enhance the expression of ACE2 and TMPRSS2, and in addition to the human immune response, lifestyle choices, and other factors also influence the progression and prognosis of COVID-19 [7]. There are a number of ideas on the functional change of several organs as a result of the SARS-CoV-2 epidemic. It is yet unknown how directly this virus affects a man's urogenital system. However, there are already several theories regarding the biological similarities between SARS-CoV and SARS-CoV2, particularly in andrology. In addition to SARS-CoV, SARSCoV-2 enters human cells via the 'Angiotensin Converting Enzyme-2' (ACE2). It was discovered that the testicles, namely the Leydig and Sertoli cells, contain ACE2, angiotensin, and its MAS receptors [8]. According to the first theory, the virus might enter the testis and change how they operate. The second theory is that the virus's attachment to the ACE2 receptor may result in an overproduction of ACE2 and trigger a normal inflammatory response. Leydig and Sertoli cell function may be hampered by inflammatory cells. Both possibilities should be examined and verified to potentially track fertility in COVID-19 patients [9]. Does COVID-19 or immunization have an impact on sperm DNA disintegration and male fertility in individuals with normal malefactor?

Patient and methods:

320 samples of semen were collected from patients who have been referred to the fertility clinic at the International Islamic Center for Population Studies and Research (IICPSR), Al-Azhar University, for use in this research. Patients in this trial, which had 160 patients, provided written permission before being split into 4 groups:

Group 1: A cohort of fertile men without a history of SARS COVID-19 infection or vaccination was included as a control group, comprising 40 cases.

Group 2: Another set of fertile men, totaling 40 cases, was considered, and this group had a history of contracting SARS COVID-19.

Group 3: A group consisting of 40 cases involved fertile men who received The Sinopharm COVID-19 vaccination against SARS COVID-19.

Group 4: Among the fertile men studied, there were 40 cases in which individuals had both received Sinopharm COVID-19 vaccination and contracted SARS COVID-19.

All patients subjected to Inclusion criteria: Age between 25-45 years old, male factor without high DNA fragmentation index (DFI), no history of chronic medical illness, and no previous testicular or scrotal operation.

Ethical approval: Approval for this study was granted by the Quality Education Assurance Unit of the Faculty of Medicine at Al-Azhar University in Egypt, under the Research Ethics Committee (REC) number 00000405. The approval process adhered to the ethical guidelines outlined in the 1964 Helsinki Declaration, along with subsequent equivalent ethical standards or amendments. Additionally, the study conformed to the ethical standards set forth by national and/or institutional research committees. To ensure participant understanding and compliance, all couples involved in the study completed informed consent forms.

I- The technical methodology employed for the semen analysis of male subjects adheres to the guidelines outlined by the World Health Organization (WHO) in 2010.

1-Sample collection: After 2 to 7 days of abstinence, semen samples are obtained through masturbation. The container was sterile, clean, and wide-mouthed to reduce collecting errors, and it needs to be from a batch that has been shown to be safe for spermatozoa. Within an hour after collection, the semen specimen should be kept at body temperature or at room temperature [10].

2-Physical and microscopic examination: as regards the appearance of the ejaculate, liquefaction, viscosity, volume, odor, and semen pH, concentration, motility, and abnormal forms.

II- Histological examination by Halo sperm G_2 stains to detect DNA fragmentation:

Principle of the method: The SCD test is the method's foundation. Intact, unfixed spermatozoa are submerged on a prepared slide in an inert agarose microgel. In those sperm cells with fractured DNA, a first acid therapy denatures the DNA. The lysing solution then eliminates most of the nuclear protein and, in the lack of significant DNA damage, creates nucleoids with significant haloes of spread DNA loops that emerge from a central core. However, the dispersion halo in the nucleoids from spermatozoa with fragmented DNA is either absent or hardly visible [11]. Sperm classification: Score at least 300 sperm in each sample in accordance with the standards:

Sperm with fragmented DNA:

1- Sperm with a narrow halo: the halo's width is equal to or less than 1/3 of the minor core diameter.
2- Halo-free sperm.
3- Degraded sperm are those that lack a halo and have an irregularly or faintly pigmented center.

Sperm without fragmented DNA:

1- Sperm with a large halo: the halo width equal to or greater than the minor core diameter.
2- Sperm with a medium-sized halo: this kind of sperm has a halo size that falls between big and extremely tiny.

III- Assessment of Reactive Oxygen Species (ROS) level by Oxisperm kit:

The principle guiding the technique: When natural antioxidant defenses fail to stop active ROS, damaging ROS, a notion linked to oxidative stress, is present. The interesting outcome is that various amounts of cellular damage are created. Potential targets of ROS include somatic and germ cell cells. An increase in oxidative stress directly affects male fertility when it affects germ line cells [12]. To figure out the sample: The spermatozoa concentrations are divided by 1000. The result is the volume that must be combined with a precise amount of RG (proportion 1:1; Semen –RG). The intensity levels have been pre-classified into four levels (L). L1 is low, L2 is low-medium, L3 is medium, and L4 is high. The sample's hue was contrasted with the newly designated color scheme.

Statistical analysis

Statistical analysis was done by SPSS v26 (IBM Inc., Chicago, IL, USA). Quantitative variables were presented as mean and standard deviation (SD) and compared between the two groups utilizing unpaired Student's t-test and compared between the four groups utilizing ANOVA (F) test with post hoc test (Tukey). A two tailed P value < 0.05 was considered statistically significant. [13].

Results:

I- The demographic data

Regarding the demographic data, the age, BMI, and pH were comparable between the studied groups. On the other hand, the volume was significantly higher in group 3 compared to group 2 and was significantly higher in group 4 compared to group 3. **(Table 1).**

Table 1: comparison between the demographic data & physical seminal characteristics between groups:

	Group 1	Group 2	Group 3	Group 4	P value	POST HOC
Age	33.74±1.4	33.0±3	33.08±3.02	34.0±3	0.173	P1=0.620 P2=0.94 P3=0.260
BMI	32.3±1.2	31.5±2.4	30.6±2.39	31.2±2.38	0.002	P1=0.311 P2=0.168 P3=0.559
pH	7.05±.63	7.14±.6	7.19±.61	7.23±.6	0.469	P1=0.867 P2=0.977 P3=.988
Volume	1.56±.18	1.6±.11	2.1±.12	2.55±.1	<0.001	P1=0.346 P2<.001* P3<.001*

Data are presented as mean ± SD. BMI: body mass index. * p value < 0.05 is statistically significant. P1: significance between control group and COVID-19 group, p2: significance between COVID-19 group and vaccinated group, p3: significance between the Vaccinated group and the COVID-19 vaccinated group.

II- Comparing the effect of Covid-19 and vaccination after three months in each group by t- test

In the control group, the seminal concentration was significantly lower after three months compared to baseline. **(Table 2)**. Regarding sperm motility, the total and progressive motility were significantly lower after three months compared to baseline (p= <0.001). **(Table 2)**. Regarding sperm morphology, there was no statistically significant difference between baseline and after three months regarding abnormal forms, head defects, midpiece defects, and tail defects. Moreover, there was insignificant difference in DNA index and ROS index between baseline and after three months.

Table 2: comparison between seminal parameters in group 1:

	Group 1		
	Baseline	3 months	P value
Count	26.37±.096	25.25± 0.82	<.001
Motility	52.5±1.45	51.32±1.63	<.001
Progressive	31.54±1.4	29.9 ±1.17	<.001
Abnormal forms	91.52±1.78	91.64 ±1.56	0.721
Head defects	45.14 ± 6.7	44.7±5.8	0.728
Midpiece defects	28.74 ± 4.8	28.7± 4.87	1
Tail defects	17.6 ± 3.1	17.8 ± 3.2	0.753
ROS	1.16 ± 0.11	1.18± 0.11	0.547
DNA%	19.6 ± 6.2	19.9±6.29	0.800

Data are presented as mean ± SD. * p value < 0.05 is statistically significant. ROS: reactive oxygen species.

In the Covid-19 group, the seminal concentration was significantly higher at the baseline (25.7±8.4 sperm/ml) compared to after three months (19.54±6.9 sperm/ml) (p<0.001). **(Table 3)**. Regarding sperm motility, the total motility was insignificantly different at baseline and after three months. On the other hand, the progressive motility was lower after three months (26.5±7.7) compared to baseline (30.7±8.5) (p= 0.011). Regarding sperm morphology, the abnormal forms, head defects, midpiece defects, and tail defects were significantly higher after three months compared to the baseline. Moreover, DNA index and ROS index increased significantly after three months compared to baseline (p<0.001, and 0.003, respectively). **(Table 3)**.

Table 3: Comparison between seminal parameters in group 2:

	Group 2		
	Baseline	3 months	P value
Count	25.7±8.4	19.54±6.9	<.001
Motility	51.5±12.5	49.9±12.3	0.523
Progressive	30.7±8.5	26.5±7.7	0.011
Abnormal Forms	92.9±.8	95.92±.804	<.001
Head defects	43.3±4.8	47.3±4.9	<.001
Midpiece defects	26.6±4.6	28.6±4.7	0.035
Tail defects	30.12±3.2	22.1±3.1	<.001
ROS	1.06±.11	1.66±.15	<.001
DNA	18.7±6.9	21.8±7.1	0.03

Data are presented as mean ± SD. * p value < 0.05 is statistically significant. ROS: reactive oxygen species.

In the Vaccinated group, the seminal concentration showed insignificant difference between baseline and after three months (p= 0.024). **(Table 4)**. Regarding sperm motility, there was no statistically significant difference in total motility and progressive motility between baseline and after three months. Regarding sperm morphology, the abnormal forms were significantly higher after three months compared to baseline (p<0.001). The head defects, and midpiece defects were comparable between baseline and after three months. On the other hand, tail defects were significantly lower after three months (23.2±3) compared to the baseline (27.22±2.9) (p<0.001). Moreover, the ROS index, and DNA index increased significantly after three months compared to baseline (p< 0.001).

Table 4: comparison between seminal parameters in group 3:

	Group 3		
	Baseline	**3 months**	**P value**
Count	47±15.8	43.9±15.7	0.33
Motility	50.7±13.3	50.4±13.7	0.912
Progressive	31.2±9.6	29.9±8.2	0.471
Abnormal forms	93.04±.78	94±.7	<.001
Head defects	44.3±4.9	45.8±4.7	0.126
Midpiece defects	28.56±4.7	29.96±5	0.160
Tail defects	27.22±2.9	23.2±3	<.001
ROS	1.76±.11	2.06±.11	<.001
DNA	20.46±6.2	24.9±6.8	0.001

Data are presented as mean ± SD. * p value < 0.05 is statistically significant. ROS: reactive oxygen species.

In the vaccinated, and contracted COVID-19 group, the seminal concentration was significantly higher at the baseline (24.56±4.7 sperm/ml) compared to after three months (15.86±4.3 sperm/ml) (p<0.001). **(Table 5).** Regarding sperm motility, the total and progressive motility were significantly lower after three months compared to the baseline (p<0.001). Regarding sperm morphology, the abnormal forms, and head defects were significantly higher after three months compared to the baseline (p<0.001). On the other hand, midpiece defects, and tail defects were significantly lower after three months compared to the baseline (p<0.001). Moreover, the ROS index, and DNA index increased significantly after three months compared to baseline (p< 0.001).

Table 5: comparison between seminal parameters in group 4:

	Group 4		
	Baseline	**3 months**	**P value**
Count	24.56±4.7	15.86±4.3	<.001
Motility	52.7±13.3	42.7±13.1	<.001
Progressive	31.2±9.6	26.2±9.5	0.011
Abnormal forms	94.04±.7	98±.78	<.001
Head defects	42.4±5	50.3±4.9	<.001
Midpiece defects	30.72±4.8	20.56±4.7	<.001
Tail defects	30.32±2.8	19.98±2.7	<.001
ROS	1.9±.11	2.1±.1	<.001
DNA	20.46±6.2	29.6±6.1	<.001

Data are presented as mean ± SD. * p value < 0.05 is statistically significant. ROS: reactive oxygen species.

III- Comparing the effect of Covid-19 and vaccination after three months in all group by One Way Anova followed by post-hoc

Regarding sperm concentration after three months, there was statistically significant difference between groups. Compared to the normal control group, the COVID-19 group showed lower sperm concentrations (p=0.009). Moreover, group 3 had significantly higher concentration compared to the group 2. On the other hand, group 4 had significantly lower concentration compared to group 3. Moreover, the sperm total motility was comparable between control, COVID-19, and vaccinated groups. However, the COVID-19 vaccinated group had significantly lower total motility compared to the vaccinated group (p= 0.05). After three months, the progressive motility was showed insignificant difference between the studied groups. **(Table 6- figure 1)**

Table 6: comparison between concentration and motility in the studied groups after three months:

	Group 1	Group 2	Group 3	Group 4	P value	Post hoc
Count	**25.25**±.82	19.54±6.9	**43.9**±15.7	15.86±4.3	**<.001***	**P1=.009* P2<.001* P3<.001***
Motility	**51.32**±1.63	49.9±12.3	**50.4**±13.7	42.7±13.1	**.001**	**P1=.917 P2=.996 P3=.005**
Progressive	**29.9**±1.17	26.5±7.7	**29.9**±8.2	26.2±9.5	**.011**	**P1=.106 P2=.107 P3=.067**

Data are presented as mean ± SD. * p value < 0.05 is statistically significant. P1: significance between control group and COVID-19 group, p2: significance between COVID-19 group and vaccinated group, p3: significance between the Vaccinated group and the COVID-19 vaccinated group.

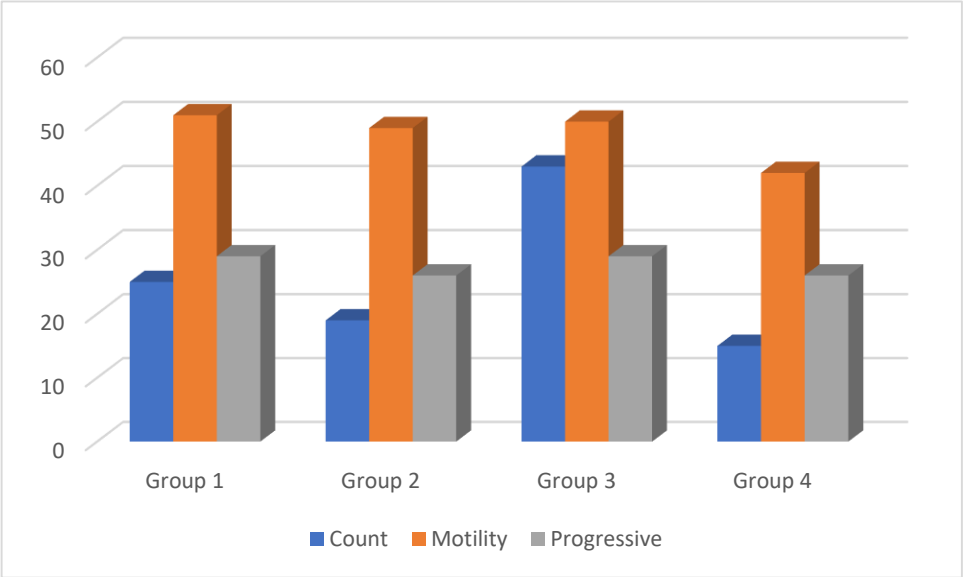

Figure (1): Comparison between sperm concentration and motility among the studied groups after three months

Regarding sperm morphology after three months, the COVID-19 group had significantly higher abnormal forms compared to the control group (p<0.001). additionally, the vaccinated group showed significantly lower abnormal forms compared to COVID-19 group. Moreover, the COVID-19 vaccinated group showed significantly higher abnormal forms compared to the vaccinated group (p< 0.001). Head defect, and midpiece defects showed no statistically significance difference between control, COVID-19, and vaccinated groups. However, group 4 showed significantly higher head defects and lower midpiece defects compared to group 3. **(Table 7- Figure 2)**. Tail defects were significantly higher in the COVID-19 group compared to the control group with no significant difference between COVID-19 and vaccinated groups. On the other hand, group 4 had significantly lower tail defects compared to the group3 (p<0.00). **(Table 7- Figure 2)**

Table 7: comparison between morphological parameters in the studied groups after three months:

	Group 1	Group 2	Group 3	Group 4	P value	Post hoc
Abnormal forms	**91.64**±1.56	95.92±.804	**94**±.7	98±.78	**<.001***	**P1<.001* P2<.001* P3<.001***
Head defects	**44.7**±5.8	47.3±4.9	**45.8**±4.7	50.3±4.9	**<.001***	**P1=.057 P2=.461 P3<.001***
Midpiece defects	**28.7**±4.87	28.6±4.7	**29.96**±5	20.56±4.7	**<.001***	**P1=.999 P2=.502 P3<.001***
Tail defects	**17.8**±3.2	22.1±3.1	**23.2**±3	19.98±2.7	**<.001***	**P1<.001* P2=.271 P3<.001***

Data are presented as mean ± SD. * p value < 0.05 is statistically significant. P1: significance between control group and COVID-19 group, p2: significance between COVID-19 group and vaccinated group, p3: significance between the Vaccinated group and the COVID-19 vaccinated group.

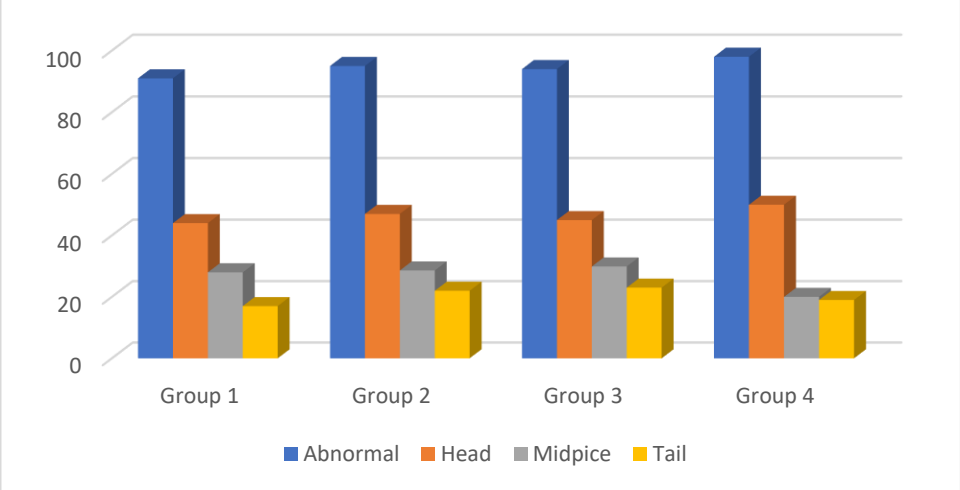

Figure (2): Comparison between sperm morphology among the studied groups after three months

IV- Result of human spermatozoa stained with Halo sperm G2 kit and oxisperm kit.

Regarding DNA fragmentation by with Halo sperm G2 kit, the sperms with big halo were observed in the control group and few sperms with small halo. In the COVID-19 group, sperms with medium-sized halo and sperms without halo and degraded were observed. In both vaccinated group and COVID-19 vaccinated group, sperm with big halo, sperm with medium-sized halo, and sperm with small halo were observe. The spermatozoa affected by oxidative stress was stained by oxisperm kit, the higher intensity of the blue color indicates the increased deposition of free radicals on the sperm surface. The control group showed null effect and the reaction increased in the COVID-19 group, vaccinated group and COVID-19 vaccinated group.

There was statistically significant difference between groups regarding ROS index ($p<0.001$). COVID-19 group had significantly higher ROS index compared to the control group ($p<0.001$). Vaccinated group had higher ROS index compared to COVID-19 group and the COVID-19 vaccinated group had higher ROS index compared to vaccinated group. **(Table 8- figure 3)**. Moreover, there was no statistically significant difference between the control, COVID-19, and vaccinated groups regarding DNA index. On the other hand, the COVID-19 vaccinated group had higher DNA index compared to the vaccinated group. **(Table 8- figure 3)**

Table 8: Comparison between DNA fragmentation and ROS in the studied groups after three months

	Group 1	Group 2	Group 3	Group 4	P value	POST HOC
ROS	**1.18**±.11	1.66±.15	**2.06**±.11	2.1±.1	**<.001***	**P1<.001* P2<.001* P3<.001***
DNA	**19.9**±6.29	21.8±7.1	**24.9**±6.8	29.6±6.1	**<.001***	**P1=.493 P2=.095 P3=.002***

Data are presented as mean ± SD. * p value < 0.05 is statistically significant. P1: significance between control group and COVID-19 group, p2: significance between COVID-19 group and vaccinated group, p3: significance between the Vaccinated group and the COVID-19 vaccinated group. ROS: reactive oxygen species

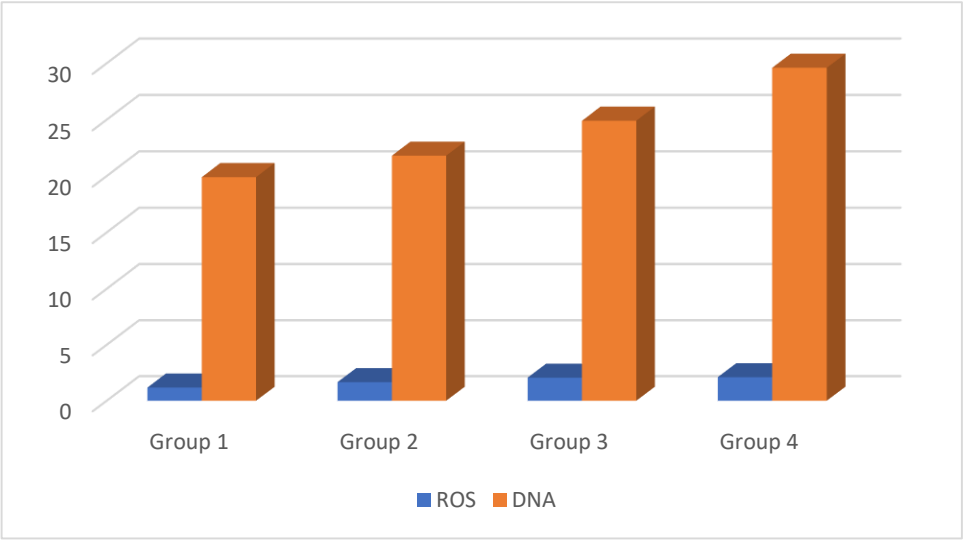

Figure (3): Comparison between DNA index and ROS index among the studied groups after three months

Discussion:

The findings of the current research were consistent with those of He *et al*., 2020, who showed that the expression of ACE2 in the testes and male genital tract indicated that the testis is also an organ sensitive to SARS-CoV-2 infection. He'd studied the impact of SARS-CoV-2 on male reproductive health. According to them, the SARS-CoV-2 that is targeting human sperm may react with these receptors, altering sperm motility and fertilization in a variety of ways and resulting in male infertility [14]. In agreement with our results study by Sharma, 2021 suggested that infection with SARS− COV-2 may result in possible reproductive problems [15], Additionally, research on the COVID-19 Pandemic and male fertility based on other CoVs revealed that testicular injury and eventual infertility may be caused by direct viral invasion or a secondary immune or inflammatory response, both of which may have a deleterious impact on adult fertility [16], Also, our results are in agreement with the results of Tur-Kaspa, Ilan, *et al*, 2021 **who** revealed that although COVID-19 is not a sexually transmitted disease (STD), it may have an impact on male fertility. Even though the ACE2 receptor is present in the reproductive organs, the absence of the TMPRSS2 modulatory protein, which is necessary for SARS-CoV-2 cell entry, in testicular cells, sperm, or oocytes refutes the idea that SARS-CoV-2 is transmitted through gametes. He suggests that the recovered COVID-19 patients, especially those who are infertile, should be assessed and monitored for their ovarian and testicular function [17], A study by Adel Abdel Moneim 2021, revealed that male reproductive health might be impacted in many ways by the current COVID-19 epidemic. The author implied that the impairment of testicular function may be brought on by the SARS-CoV-2 infection. Patients infected with SARS-CoV2 may have testicular impairment since the human testes' ACE2 gene is largely expressed in spermatogonia and Leydig and Sertoli cells. Additionally, inflammatory reactions, fever-related inflammation, and drugs used in extreme situations may all contribute to testicular injury [18], Research on COVID-19's impact on men's reproductive systems, as a result, they provide concrete proof that COVID-19 may cause orchitis via immunological or inflammatory responses, perhaps harm spermatogenesis, reduce sperm quality in individuals with mild infections, and further harm male fertility. Concerns about the long-term effects of pattern reproduction are raised by the fact that COVID-19 damages several organs, including the reproductive system [14], in keeping with our findings study by Maryam Hezavehei *et al*. 2021 reported that in testicular cells, the ACE2 protein functions as a receptor for SARS-CoV-2. Sertoli cells also exhibit significant levels of TMPRSS2 receptor expression. As a result, male gonads may be susceptible to SARS-CoV-2 [19]. There are still questions about the presence of viral mRNA in the reproductive tissue and follicular fluid of asymptomatic individuals using assisted reproductive technology (ART), despite several studies reporting the existence of SARS-CoV-2 mRNA in the reproductive system at or after acute COVID-19 symptomatic infections [20]. In an investigation of the SARS-CoV-2 virus in semen after recovery from COVID-19, our data indicated that the probability of SARSCoV-2 sexual transmission after recovery in stable partners appears to be negligible. However, caution should be used when handling the semen of healed COVID-19 patients during supported reproduction and cryopreservation. The severity of the condition was tightly correlated with oligo crypto-azoospermia and symptoms of inflammation in the male genital tract in 25% of individuals who recovered from COVID-19. Currently, in males evaluated more than a month after infection with COVID-19, sperm quality metrics were less prominent, and two months or more after infection, they were almost normal. Sperm quality attributes were most seriously affected when measured during the first month following infection with COVID-19 [21,22]. According to published evidence, people with COVID-19 have worse sperm quality and are more likely to develop orchitis. These patients also have lower sperm counts, slower sperm movement, and higher DNA fragmentation indices. SARS-CoV-2 can infect spermatogonia, spermatids, Leydig cells, and Sertoli cells, yet there is presently no proof that it may spread via semen. Additionally, supporting the findings of Xiaoping Li *et al.*, 2022, who revealed that by altering the semen characteristics and causing orchitis, SARS-CoV-2 infection may harm male

reproductive functions [23]. On the other hand, future studies suggest that should involve a long-term follow-up of COVID-19 patients and use sperm DFI as a potential indicator of male infertility in addition to other approaches like semen analysis and sex-related hormones in the examination of male infertility. [24].

Conclusion:

We concluded that the incidence of DNA index and ROS index were higher after 3 months compared to at the beginning of the study. So, COVID-19 affects male fertility negatively.

Abbreviation:

IICPSR: International Islamic Center for Population Studies and Research
COVID-19: Coronavirus disease-19
ACE2: Angiotensin-converting enzyme 2
TMPRSS2: transmembrane protease, serine 2
DFI: DNA fragmentation index
SCD: Sperm Chromatin Dispersion test
ROS: reactive oxygen species
STD: a sexually transmitted disease

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Declarations:

Ethical approval: The study was approved by the quality education assurance unit, faculty of medicine, Al-Azhar University, Egypt (**REC number: 00000405**). This was done following the ethical standards of the 1964 Helsinki Declaration and its later comparable ethical standards or amendments, as well as the ethical standards of the national and/or the institutional research committee. All couples filled out the informed consent forms for this study.
Consent for publication: Not applicable.

Competing interests: The authors declare that they have no competing interests.

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