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THE IMPACT OF ASYMPTOMATIC AND MILD COVID-19 ON SPERM CHARACTERISTICS

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Background: *Although the pandemic of the new coronavirus infection (COVID-19) is characterized by mostly mild and asymptomatic cases (more than 80%), it appears important to assess the potential risks for the male reproductive system, in particular, for the parameters of the sperm.* ***Aim:*** *To study the effect of a mild and asymptomatic course of the new coronavirus infection (COVID-19) on the male sperm's parameters.* ***Methods:*** *The study included 397 patients who applied for a spermogram. All the patients underwent a spermogram study according to the WHO protocol, with the additionally measured degree of sperm DNA fragmentation, acrosine activity, fructose level, zinc level, citric acid level, neutral-glucosidase activity. A test for sperm binding to hyaluronic acid (HBA test) was performed, and the serum antibodies (IgM and IgG) against SARS-CoV-2 were analyzed. The study was conducted twice with an interval of 3–5 months.* ***Results:*** *At the first examination, normozoospermia was found in 33.5% (n=133) of patients, pathospermia — in 66.5% (n=264). All the patients had no antibodies against SARS-CoV-2 (COVID-19). When the analysis was repeated after 3–5 months, antibodies (IgG) against SARS-CoV-2 were detected in 144 patients: in 14.6% (n=21) the coronavirus infection was mild, and in 85.4% (n=123) it was asymptomatic. Those patients who received some type of a treatment (andrologic, anticovid, and/or other) were excluded from the further study. The subsequent analysis of the data from the untreated patients (131 patients without antibodies against SARS-CoV-2 and 93 patients with antibodies (IgG) against SARS-CoV-2) was performed using the Student's paired test. For all the parameters of the standard spermogram according to the WHO protocol, the found changes after the mild and asymptomatic form of COVID-19 were not statistically significant. Also, the changes in the degree of fragmentation of sperm DNA, the enzymatic activity of acrosine, and other estimated parameters of the sperm (levels of fructose, citric acid, zinc, and the activity of neutral-glucosidase) were not statistically significant. The results of the test for the spermatozoa's interaction with hyaluronic acid (HBA test) worsened and had statistically significant differences after the coronavirus infection in both the mild form (p=0.006) and asymptomatic form (p=0.001). There were no statistically significant changes in the sperm parameters (including those from the HBA test) in the patients who had not had the new coronavirus infection.* ***Conclusions:*** *No statistically significant data were obtained on the effect of mild and asymptomatic forms of the course of the new coronavirus infection on the parameters of the standard spermogram, on the degree of fragmentation of spermatozoa's DNA and the enzymatic activity of acrosin. Mild and asymptomatic forms of the new coronavirus infection course can negatively affect the interaction of spermatozoa with hyaluronic acid (HBA test), that indicates the impairment of the spermatozoa binding to the zona pellucida. One should pay attention to the HBA test when examining the sperm of patients who have suffered the new coronavirus infection.*

Keywords: *semen quality analysis; fragmentation DNA; SPERM-hyaluronan binding assay; acrosin; COVID-19; antibodies specific to SARS-CoV-2.*

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BACKGROUND

The coronavirus disease 2019 (COVID-19)[1] pandemic has become a real challenge for the entire healthcare system in general and reproductive medicine in particular. During the pandemic, the Russian population significantly decline. The difficult demographic situation is exacerbated by a decrease in the number of children born in Russia during the pandemic compared with the same period before the pandemic.

Both in Russia and around the world, attempts are being made to assess the potential risks and consequences of the effect of COVID-19 on the male reproductive system, specifically on sperm parameters [1–3]. However, the effect on the male reproductive system is mainly investigated in patients with moderate and severe COVID-19 [4, 5]. Meanwhile, mild and asymptomatic forms of infection are the most widespread (>80%). These forms of COVID-19 occurred most often in men of reproductive age; therefore, mild and asymptomatic COVID-19 may require the most careful assessment of their possible effects on the morphological and functional characteristics of the spermatozoa and the biochemical parameters of sperm.

This study aimed to analyze the effect of mild and asymptomatic COVID-19 on the parameters of sperm.

METHODS

Study design

A multicenter observational cohort prospective study was conducted.

Eligibility criteria

The inclusion criteria were male sex, absence of antibodies (IgM and IgG) to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and no history of previous COVID-19 and vaccination against COVID-19.

The non-inclusion criteria were patients with a history of COVID-19 or those vaccinated against COVID-19 before the initial examination, and/or had serum antibodies to SARS-CoV-2, and presence of asymptomatic COVID-19 but without detectable antibody levels.

The exclusion criteria were andrological treatment between the initial and repeated examinations, moderate and severe COVID-19, treatment with drugs that can induce a negative effect on male fertility [4, 6], COVID-19, and/or vaccination.

Study conditions

The study was performed at the Center for Family Health and Reproduction, Alexandro-Mariinsky Regional Clinical Hospital, S.M. Kirov City Clinical Hospital No. 3, and laboratory "Diamed-express" of REPRODIAMED, Astrakhan.

Study duration

The study was conducted from May 2020 to February 2022, twice with an interval of 3–5 months.

Medical intervention

Patients who applied for a spermogram underwent a comprehensive semen analysis and determination of antibodies (IgM and IgG) to SARS-CoV-2 (COVID-19) in the blood serum.

In addition to performing a standard spermogram according to the protocol of the World Health Organization (WHO) [7], a comprehensive analysis of sperm was performed, including the mixed agglutination reaction (MAR) test to detect antisperm antibodies in the ejaculate immunoglobulins of isotypes G, A, and M, determination of zinc in sperm plasma, citric acid, fructose, activity of neutral α-glucosidase and acrosin, degree of DNA fragmentation of spermatozoa, and interaction of spermatozoa with hyaluronic acid.

To perform the MAR test, commercial kits SpermMar IgA and SpermMar IgG (FertiPro, Belgium) and ImmunoSpheres Anti-IgM (Bioscreen Inc., USA) were used. The study was conducted in accordance with WHO guidelines [7].

The level of zinc in the sperm plasma was determined by spectrophotometric method in accordance with the recommendations of the WHO guidelines [7]. We used a commercial kit Zinc Sp-DAC.Lq (DAC-SpectroMed s.r.l., Republic of Moldova) with 2-(5-nitro-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)-phenol (nitro-PAPS) as a chromogen. The optical density was determined at a wavelength of 570 nm.

The citric acid level in the sperm plasma was determined by the spectrophotometric method using the commercial Citric Acid Test kit (FertiPro, Belgium). The optical density was determined at a wavelength of 405 nm.

The spectrophotometric method was used to determine the level of fructose in the sperm plasma [7]. The commercial Fructose Test kit (FertiPro, Belgium) was also used. The optical density was determined at a wavelength of 492 nm.

The activity of neutral α-glucosidase was determined by the spectrophotometric method [8] with

[1] World Health Organization [Internet]. Coronavirus disease (COVID-19) weekly epidemiological update and weekly operational update. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports.

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journal of clinical practice 2022 Vol 13 №3

p-nitrophenyl-α-D-glucopyranoside as a substrate. The commercial EpiScreen Plus kit (FertiPro, Belgium) was used. The optical density was determined at a wavelength of 405 nm.

Chromatin dispersion was assessed to determine the degree of DNA fragmentation in the spermatozoa [8]. The commercial GoldCyto DNA kit (Guangzhou Jinsaite Trading, China) was used.

The hyaluron-binding assay (HBA) test for the binding of spermatozoa to hyaluronic acid was performed on slides with immobilized hyaluronic acid according to the standard procedure [9, 10] using the commercial HBA Assay kit (Biocoat Inc., USA).

Acrosin activity was determined by the standard spectrophotometric method with N-α-benzoyl-DL-arginine-p-nitroanilide as a substrate [11] using the commercial kit AcroScreen (Bioscreen Inc., USA). The optical density was determined at a wavelength of 405 nm.

Antibodies (IgM and IgG) to SARS-CoV-2 (COVID-19) in the blood serum were determined by enzyme-linked immunosorbent assay using commercial kits SARS-CoV-2-IgG-ELISA-BEST (Vector-Best, Russia) and SARS-CoV-2 IgM Screen (IMBIAN, Russia).

Ethical considerations

The study was performed in accordance with the ethical standards of the Declaration of Helsinki of the World Medical Association "Ethical Principles for Medical Research Involving Human Subjects" as amended in 2013. Informed consent was obtained from all the patients examined in the study and the use of anonymized health data for scientific purposes.

Statistical analysis

Student's *t*-test for related samples (paired Student's *t*-test) was used. Statistical analyses were performed using MedCalc Ver.19.7 (MedCalc Software Ltd., Belgium). A *p* value of <0.01 was taken as the threshold level of statistical significance.

RESULTS

Study participants

A total of 397 patients aged 18–55 (mean age, 32.86±6.16) years were examined. All these patients had no antibodies (IgM and IgG) to SARS-CoV-2 (COVID-19) in their blood serum. According to the results of the initial examination using WHO criteria, normozoospermia was detected in 33.5% (*n*=133) of the patients, and pathospermia was identified in 66.5% (*n*=264) [7]. Subsequent examination was performed with the same group of patients 3–5 months later.

Primary results

During the follow-up period, 36.27% of the patients (*n*=144, aged 19–55 years, mean age 33.19±6.02 years) had COVID-19 (IgG antibodies to SARS-CoV-2 were detected in the blood serum during the examination), including 81.25% (*n*=117, aged 19–55 years, mean age 33.11±5.83 years) who were asymptomatic, 15.28% (*n*=22, aged 23–47 years, mean age 32.50±5.68 years) who had mild COVID-19, and 3.47% (*n*=5, aged 30–55 years, mean age 38.00±10.15 years) had moderate or severe COVID-19 (including those requiring hospitalization). The remaining 63.73% of the patients (*n*=253, aged 18–55 years, mean age 32.68±6.32 years) did not have COVID-19 during the follow-up period (when examination, serum IgG and/or IgM antibodies to SARS-CoV-2 were not detected).

According to the eligibility criteria, 173 patients were excluded from further analysis, and their data were not taken into account further.

Of the remaining 224 patients who did not receive any treatment, 58.48% (group 1, *n*=131, aged 19–53 years, mean age 32.82±6.12 years) did not have a new COVID-19, 34.82% (group 2, *n*=78, aged 19–52 years, mean age 32.52±5.83 years) had asymptomatic COVID-19, and 6.70% (group 3, *n*=15, aged 25–39 years old, mean age 33.06±4.45 years) had mild COVID-19.

The data of the standard spermogram at the start of the examination were compared and analyzed pairwise using Student's *t*-test for related samples with a repeated spermogram after 3–5 months.

In group 1, the number of degrees of freedom (f) was 130 with 131 noted, and the critical value of Student's *t*-test (t_{cr}) was 1.980. The analysis showed that for all parameters (t_{not}) of the standard spermogram, the condition $t_{not}<t_{cr}$ was fulfilled; therefore, the changes in the signs were statistically not significant. Similarly, no statistically significant changes were registered for other assessed parameters (MAR test, levels of zinc, citric acid, and fructose, neutral α-glucosidase activity, degree of spermatozoa DNA fragmentation, interaction of spermatozoa with hyaluronic acid, and acrosin activity).

In group 2, with 78 cases, the number of degrees of freedom (f) was 77, and the critical value of Student's *t*-test (t_{cr}) was 1.992. The analysis showed that for all parameters (t_{not}) of the standard spermogram, the condition $t_{nab}<t_{cr}$ was fulfilled, i.e., changes in signs were not statistically significant. Similarly, no statistically significant changes were registered for the other evaluated parameters (MAR test, levels of zinc, citric acid, and fructose, neutral α-glucosidase activity, degree of spermatozoa DNA fragmentation, and

acrosin activity), except for the HBA test (assessment of the interaction of spermatozoa with hyaluronic acid), for which the paired Student's *t*-test (t_{not}) was 3.587, i.e., $t_{not}>t_{cr}$; therefore, changes in the trait were statistically significant (p=0.001).

In group 3, the number of degrees of freedom (f) was 14 with 15 noted, and the critical value of Student's *t*-test (t_{cr}) was 2.145. The analysis showed that for all parameters (t_{not}) of the standard spermogram, the condition $t_{not}<t_{cr}$ was fulfilled, i.e., changes in signs were not statistically significant. Similarly, no statistically significant changes were observed for the remaining parameters (MAR test, levels of zinc, citric acid, and fructose, neutral α-glucosidase activity, sperm DNA fragmentation, and acrosin activity), except for the HBA test, for which the paired Student's *t*-test (t_{not}) was 3.270, i.e., $t_{not}>t_{cr}$; therefore, changes in the trait were statistically significant (p=0.0016).

Further analysis showed that the results of the interaction of spermatozoa with hyaluronic acid within the normative values (HBA test ≥80%) and below the normative values (HBA test <80%) were comparable in all three groups at the initial examination (Table 1). Upon repeated examinations, the ratio of patients with HBA test results within the normative values and those below the normative values did not change in group 1 compared with groups 2 and 3 where this ratio changed toward an increase in the HBA test results below the normative value (Table 1).

DISCUSSION

The effect of COVID-19 on the male reproductive system is actively investigated [5, 12]. Preliminary studies have indicated the undoubted direct or indirect effects of COVID-19 on male reproductive health [4, 13]. In moderate and severe COVID-19, it is impossible to exclude the influence of factors such as hyperthermia, cytokine storm, systemic oxygenation decrease, and toxic effects of drugs [5, 6]. In this case, the study of mild and asymptomatic COVID-19 will help identify the possible direct effects of SARS-CoV-2 on spermatogenesis, morphofunctional state of the spermatozoa, and their biochemical environment.

Our data indicate that in the most common (asymptomatic and mild) forms of COVID-19, the parameters of a standard spermogram do not change significantly. No significant effect was found on the MAR test results, levels of citric acid, zinc, and fructose in the ejaculate, and activities of neutral α-glucosidase and acrosin. According to our data, COVID-19 had no significant effect on the degree of sperm DNA fragmentation. Sperm DNA damage, which was reported by other authors [4, 14] in moderate and severe COVID-19 cases, was probably associated with oxidative stress [15–17] and/or the toxic effects of drugs [6], but not with direct pathogenic influence.

Among the parameters evaluated, COVID-19 (mild and asymptomatic forms) in some cases significantly negatively affects only the interaction of spermatozoa with hyaluronic acid. Since other possible influence factors were excluded as much as possible, this can refer to the direct possibly specific effect of SARS-CoV-2, i.e., with COVID-19, there is a risk of disruption of the interaction of specific sperm receptors responsible for binding to hyaluronic acid, *zona pellucida*, and, accordingly, a negative effect on the fertilization process.

In our opinion, at least three possible causes may affect the results of the HBA test: (1) a decrease in the number of specific receptors present on the surface of spermatozoa, (2) a change in the spatial configuration of specific receptors, and (3) blocking specific receptors. The exact mechanism, which leads to the disruption of the interaction of spermatozoa with hyaluronic acid, remains unclear and requires further investigation.

Study limitations

The sample was formed mainly from men who applied to urologists-andrologists because of the absence

Table 1

Evaluation of the interaction of spermatozoa with hyaluronic acid (HBA test)

Groups	HBA test results				Change with respect to the initial examination	
	Initial examination		Repeated examinations			
	≥80%*	<80%	≥80%*	<80%	≥80%*	<80%
Group 1, *n*=131	93.13 (*n*=122)	6.87 (*n*=9)	93.13 (*n*=122)	6.87 (*n*=9)	0	0
Group 2, *n*=78	93.59 (*n*=73)	6.41 (*n*=5)	87.18 (*n*=68)	12.82 (*n*=10)	↓	↑
Group 3, *n*=15	93.33 (*n*=14)	6.67 (*n*=1)	80.0 (*n*=12)	20.0 (*n*=3)	↓	↑

Note: * Corresponds to the standard value.

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journal of clinical practice 2022 Vol 13 №3

of pregnancy in their partners despite 4–12 months of regular sexual activity without contraceptive use. In this study, <5% of the sample were in the process of preparing for a planned pregnancy. Thus, most of the patients included in the study may have had reproductive disorders, such as subfertility or infertility even before COVID-19.

CONCLUSIONS

Mild and asymptomatic COVID-19 do not affect the parameters of a standard spermogram, degree of sperm DNA fragmentation, MAR test parameters, levels of citric acid, zinc, and fructose in the ejaculate, and the activity of neutral α-glucosidase and acrosin. Moreover, mild and asymptomatic COVID-19 can lead to a disruption in the interaction of spermatozoa with hyaluronic acid. Patients who have had COVID-19 may be additionally recommended to undergo an HBA test when performing a semen analysis, especially if they are expected to participate in programs using assisted reproductive technologies, including in vitro fertilization and embryo transfer. Additional research on the molecular mechanisms of the influence of SARS-CoV-2 on the interaction of spermatozoa with hyaluronic acid is needed.

ADDITIONAL INFORMATION

Author contribution. *D.L. Lutsky* — research concept and design, statistical data analysis, manuscript writing; *R.M. Makhmudov* — research concept and design, statistical data analysis; *A.M. Lutskaya* — collection and processing of material, manuscript writing; *S.V. Vybornov, E.S. Kalashnikov, V.V. Lozovskii, V.Vl. Lozovskiy, L.M. Shishkina* — collection and processing of the date; *D.M. Nikulina, A.A. Nikolaev* — manuscript editing. The authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

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