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Male Reproductive Tract Involvement and Sperm Parameters in SARS-CoV-2 Patients: A Systematic Review and Meta-Analysis

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Purpose: There is a growing concern regarding the impact of SARS-CoV-2 infection on the male reproductive tract due to ACE2 receptor expression, however, its impact remains unclear. We performed this review to evaluate whether SARS-CoV-2 infection affects the male reproductive system.

Materials and Methods: We conducted a search in the Embase, Scopus, and MEDLINE databases, adhering to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guideline. Eligible studies comprised articles reporting viral RNA presence in semen, sperm parameters, and orchitis or orchiepididymitis occurrence in SARS-CoV-2 patients. Observational studies' quality was determined using the Newcastle–Ottawa Scale (NOS). Case reports were assessed using the Joanna Briggs Institute (JBI)'s checklist.

Results: A total of 32 relevant articles were included. Viral RNA was found in 7% of infected patients' semen (95% CI, -0.01 to 0.15) from 3 studies. There were also only 7% of patients with orchitis or orchiepididymitis clinical manifestations (95% CI, 0.05–0.10). The semen volume and concentration were 2.34 mL (95% CI, 1.87–2.81) and 51.73 million/mL (95% CI, 31.60–71.85). The progressive and total motility percentages were 36.11% (95% CI, 28.87–43.35) and 43.07% (95% CI, 28.57–57.57), respectively. The morphology was 6.03% (95% CI, -1.05 to 13.10). There is a difference in semen volume between moderate and severe infections (MD, 0.52; 95% CI, 0.27–0.76; $p < 0.0001$) and concentration between mild and moderate (MD, 18.74; 95% CI, 1.02–36.46; $p = 0.04$), mild and severe (MD, 43.50; 95% CI, 13.86–73.14; $p = 0.004$), as well as moderate and severe (MD, 22.25; 95% CI, 9.33–35.17; $p = 0.0007$).

Conclusions: SARS-CoV-2 infection may result in decreased sperm concentration in severe cases and the mechanism relates to potential reproductive tract inflammation. The absence of large viral RNA detection in the semen indicates a systemic effect, although this is largely unproven.

Keywords: COVID-19; Healthy lifestyle; Orchitis; SARS-CoV-2; Sperm

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INTRODUCTION

Since its emergence in December 2019, the coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has caused global morbidity and mortality at an unprecedented rate [1]. Currently, there has been a growing number of cases in many areas of the world since the second wave of the pandemic in India [2]. Many significant discoveries have been made regarding the etiology, epidemiology, diagnosis, and treatment strategies regarding COVID-19 [3-5]. Even though much is known regarding the morbidity and mortality of the clinical disease, less is still currently known about the pathobiology [6]. Studies suggest that the basic pathogenesis of the disease involves the role of the angiotensin-converting enzyme-2 (ACE2) receptor. The virus infects cells by binding its spike protein to ACE2 receptors on target cells [7]. It initiates with the proteases-mediated priming of viral spike proteins the receptor *via* the transmembrane protease, serine 2 (TMPRSS2) cleaving the ACE2 receptor, thus aiding the virus to enter the cell [8]. The disease commonly manifests as an acute respiratory illness; however, due to the presence of the receptor in other tissues, it can also affect the kidney, heart, liver, and testes [9]. The disease manifestations in several organs beyond the classical findings of the infection are increasingly being appreciated as important data to understand the disease further [10]. Many significant discoveries have been made regarding the potential manifestation of the virus in the reproductive system. Reports indicate that approximately 58% of SARS-CoV-2 infected patients are male, suggesting that the male sex is one of the risk factors for the disease [11]. During the 2003 SARS outbreak, studies had already proposed the possible link between the disease and inflammation of the male reproductive system as there is a high ACE2 expression level in the testes [12]. Several reports also suggested that the virus may be present in the seminal fluid, which could have reproductive implications [13]. The knowledge of other viruses, aside from the SARS-CoV-2 virus, present in the semen provides precedence for evaluating the semen and spermatozoa of infected patients. More than 25 viruses, generally considered to be non-sexually transmitted, have been discovered in the human semen [14]. Additionally, a few prior studies of other viruses reported evidence of increased sperm abnormalities and

inflammatory cells present in the semen, suggesting damage to the reproductive tract [15]. However, current information regarding SARS-CoV-2 infection on male reproductive tract involvement and sperm parameters are still limited. Based on the aforementioned theories and reports, we aimed to investigate the presence of SARS-CoV-2 in semen and its impact on sperm parameters, as well as the presence of male reproductive tract inflammation among infected patients. Previous narrative and systematic reviews pondering a similar question have been published; however, a meta-analysis of the topic hasn't been published as of the conduction of this review.

MATERIALS AND METHODS

1. Search strategy

We performed a systematic search in the Embase, Scopus, and MEDLINE databases, adhering to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guideline from late 2019 to the date of the search on November 2021 [16]. The guideline is recognized as a standard for reporting evidence in systematic reviews and meta-analyses.

The overall conduction and writing of this systematic review and meta-analysis were performed in accordance with the PRISMA guideline [17]. The language of the included studies was restricted to English only. Search strategies were generated based on these keywords: "SARS-CoV-2", "Severe acute respiratory syndrome coronavirus 2", "coronavirus", "COVID-19", "coronavirus disease 2019", "sperm", "sperm parameters", "sperm analysis", "semen analysis", "spermatogenesis", "testis", "testes", "testicle", "orchitis", "epididymitis", "orchiepididymitis", "fertility", and "male fertility". The references of obtained studies and previous reviews were also evaluated to identify eligible studies, ensuring that the most relevant studies were not missed. Three independent reviewers (YPK, FH, and ZAR) and two verifiers (EC and LH) were involved in the screening process. Any disagreements will be resolved with a discussion between the reviewers and justified by the verifiers.

2. Inclusion and exclusion criteria

Eligible studies comprised studies reporting male reproductive tract involvement and sperm parameters of SARS-CoV-2 positive patients diagnosed with a real-

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time reverse transcription-polymerase chain reaction (rRT-PCR) examination or other modalities which were considered sufficient at the time of the study's conduction. Studies fulfilling the following criteria were included: (1) observational studies including cohort, case-control, and cross-sectional studies, (2) case reports and case series, (3) evaluated SARS-CoV-2 patients of all ages, (4) reporting at least one of the following outcomes: reproductive tract inflammation (orchitis, epididymitis, or orchiepididymitis) incidence, viral RNA in seminal fluid, and sperm analysis. The following studies were excluded: (1) narrative or systematic reviews, (2) commentary articles, (3) posthumous studies, (4) basic science studies, and (5) animal studies. Case reports and case series were included in the qualitative analysis only. Observational studies, including retrospective and prospective studies, were included in both a qualitative and quantitative analysis, presented in pooled descriptive and analytical forest plots. Previous reviews were excluded from the analysis, however, a few notable ones were discussed in the discussion section.

3. References management

The obtained results were imported and merged into the Mendeley reference manager software (version 1.19.8, 2000; Mendeley Ltd., London, UK). Duplicates were automatically identified by the software and inspected manually by the reviewers before being removed.

4. Quality assessment

Case-control and cohort studies' quality was determined using the Newcastle–Ottawa Scale (NOS) [18]. Cross-sectional studies were evaluated using a modified version of NOS [19]. Case reports and case series were assessed using the Joanna Briggs Institute (JBI)'s checklist for case reports [20].

5. Data extraction

The extraction of data of eligible studies was performed by the three reviewers using a Microsoft Excel Spreadsheet (version 16.52, 2021; Microsoft Corporation, Redmond, WA, USA). Baseline characteristics data, including demographic data, sample characteristics, and study results, were extracted from the studies. Data used for the analysis included reproductive tract inflammation incidence, presence of viral RNA in semi-

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nal fluid, seminal volume, sperm concentration, sperm progressive motility, sperm total motility, and sperm morphology. Incidence and presence were analyzed as dichotomous data, whereas sperm parameters were analyzed as continuous data.

6. Qualitative and statistical analysis

A qualitative synthesis was performed on all included studies and described in the discussion section. Quantitative synthesis was performed both descriptively and analytically. Descriptive analyses were performed *via* pooled analyses of the outcomes. The occurrence of reproductive tract inflammation and viral RNA presence was pooled using a meta-analysis of proportions. The sperm analysis outcomes were displayed by pooling the average results from each study.

An analytical, statistical analysis was performed to determine the mean difference (MD) in sperm parameters based on the severity of the SARS-CoV-2 infection, classified into mild, moderate, and severe. The effects model for the analysis was performed based on the potential heterogeneity among the studies. Heterogeneity was determined using the I^2 statistic, which is the proportion of total variation observed between the trials attributable to differences between trials. A value of less than 25% was regarded as low heterogeneity, whereas an I^2 of more than 75% is considered high heterogeneity. A fixed-effects model was chosen for outcomes with homogeneity among the studies, whereas a random-effects model was chosen to account for potential heterogeneity among the studies. All analyses were reported with a 95% confidence interval (CI), and z-statistic was used to determine the effect size's significance. A p-value of less than 0.05 indicates statistical significance. Publication bias was controlled *via* visual assessment of funnel plot asymmetry and Egger's test [21]. Several results from the studies were obtained from raw data transformation in accordance with the Cochrane Handbook [16]. All pooled analyses were performed using the StataSE software (version 16.1, 2019; StataCorp LLC, College Station, TX, USA) and comparative analytical analyses were performed using the Review Manager (RevMan) software (version 5.4, 2020; The Cochrane Collaboration, London, UK).

7. Protocol registration

This systematic review and meta-analysis protocol

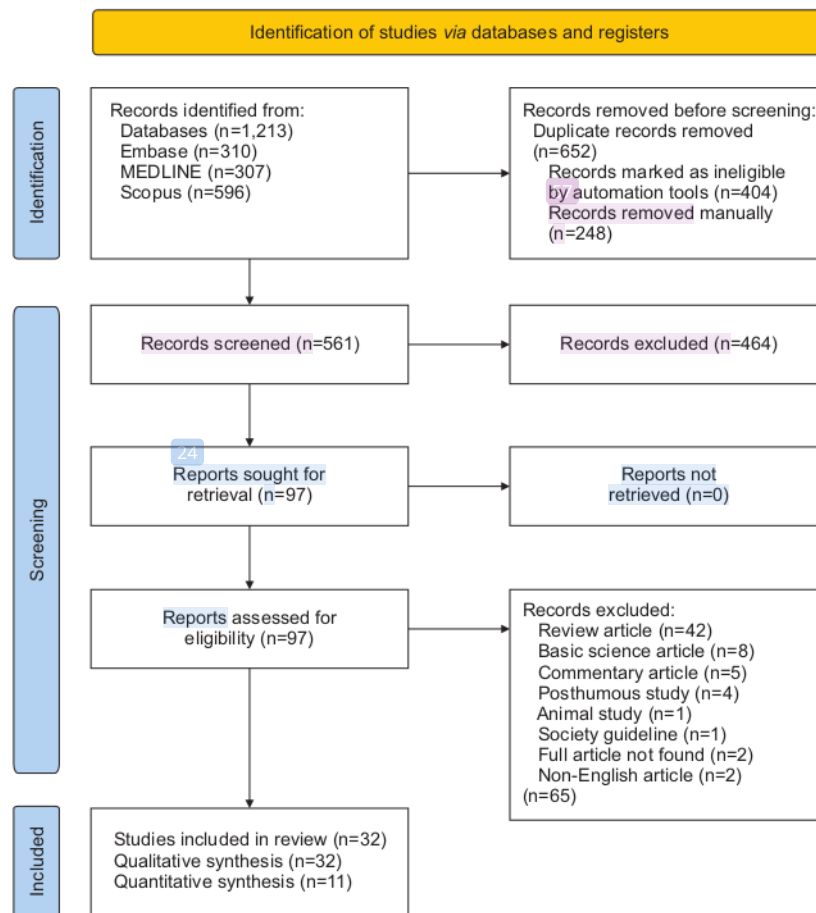


Fig. 1. Systematic search and screening based on the 2020 PRISMA Flowchart in the Embase, MEDLINE, and Scopus databases.

has been registered in The International Prospective Register of Systematic Reviews (PROSPERO) database (CRD42021266157).

RESULTS

1. Systematic search results

The initial search based on the keywords resulted in 310 articles from Embase, 307 articles from MEDLINE, and 596 articles from Scopus databases, yielding 1,213 studies as shown in Fig. 1. Before the screening, a total of 652 duplicate records were removed, 404 duplicates were removed using Mendeley, whereas 248 duplicates were excluded manually. Duplicates consisted of the same article extracted from different databases or similarly titled studies published in different sources, which are essentially the same articles. We performed a primary screening on 561 articles, in which 464 stud-

ies were removed based on the title and abstract. We retrieved the full paper of 97 studies to assess their eligibility. A total of 32 studies were considered eligible for qualitative synthesis. The reasons for the other studies' exclusion are listed in Fig. 1. A quantitative analysis was performed on 11 studies, whereas the data of the other studies were only extracted qualitatively due to insufficient quantitative data for analysis. The keywords used during the systematic search were based on "SARS-CoV-2", "COVID-19", "sperm", "sperm parameters", "semen analysis", "orchitis", "epididymitis", "orchiepididymitis" keywords and medical subject headings (MeSH) terms.

2. Studies' characteristics

The 32 included studies in this review are displayed in Table 1. Most studies consisted of cohort and cross-sectional studies. Several case reports were also in-

Table 1. Basic characteristics of the included studies

Study	Month, year	Country	Study design	Sample size
Bridwell et al [22]	April, 2021	USA	Case report	1
Erbay et al [39]	April, 2021	Turkey	Retrospective cohort	69
Gacci et al [46]	June, 2021	Italy	Prospective cross-sectional	43
Machado et al [23]	February, 2021	USA	Cohort cross-sectional	15
Li et al [28]	May, 2020	China	Cohort	38
Best et al [24]	September 2020	USA	Prospective cohort	40
Hajizadeh Maleki and Tartibian [47]	March, 2021	Germany	Prospective, longitudinal cohort	84
Burke et al [25]	March, 2021	USA	Cohort	18
Haydar et al [40]	July, 2021	Syria	Case report	1
Paoli et al [48]	April, 2021	Italy	Cross-sectional	4
Rawlings et al [26]	August, 2020	USA	Cohort	6
Li et al [29]	November, 2020	China	Case control and cross-sectional cohort	23
Holtmann et al [49]	August, 2020	Germany	Prospective cohort	18
Temiz et al [41]	November, 2020	Turkey	Cross-sectional	20
Guo et al [31]	June, 2020	China	Cohort	23
Ruan et al [32]	November, 2020	China	Cross-sectional	74
Pan et al [33]	June, 2020	China	Cross-sectional	34
Chen et al [34]	November, 2020	China	Retrospective cohort	142
Ma et al [35]	July 2020	China	Retrospective cohort	12
Ma et al [36]	March, 2020	China	Retrospective cohort	81
Gagliardi et al [50]	August, 2020	Italy	Case report	1
Kim et al [27]	March, 2020	USA	Case report	1
La Marca et al [51]	July, 2020	Italy	Case report	1
Alkhatatbeh et al [42]	July, 2020	Jordan	Retrospective cohort	253
Ediz et al [43]	October, 2020	Turkey	Prospective cohort	91
Kayaaslan et al [44]	August, 2020	Turkey	Prospective cohort	16
Pavone et al [52]	August, 2020	Italy	Prospective cohort	9
Song et al [37]	April, 2020	China	Prospective cohort	12
Quan et al [38]	March, 2020	China	Cohort cross-sectional	18
Zhang et al [30]	June, 2020	China	Cross-sectional	10
Özveri et al [45]	July, 2020	Turkey	Case report	1
Nicastri et al [53]	March, 2020	Italy	Case report	1

cluded. Six studies were conducted in the USA [22-27], 11 were conducted in China [28-38], seven were reported by Middle-Eastern countries [39-45], and eight were conducted in Western European countries [46-53]. All studies were published between March 2020 and July 2021 with a total of 1,160 subjects. The methods of specimen collection and processing adhering to a standard protocol have been described in each study.

3. Baseline characteristics

The baseline characteristics of sperm analysis and reproductive tract involvement are presented in Tables 2 and 3. The average age of the patient was 38.58±10.93

years old. Several studies and case reports reporting the incidence of orchitis and orchiepididymitis also included children patients. The Average days from diagnosis to specimen collection were 20.91±19.55 days. Kayaaslan et al [44] collected the semen of several patients as soon as the first day. Ruan et al [32] included patients whose specimen were collected 125 days after being diagnosed. To evaluate the long-term consequence of the infection after recovery, several studies purposely collected the specimen of patients after a negative rRT-PCR result. The average days from a negative rRT-PCR result to specimen collection were 59.81±40.39 days.

Table 2. Sperm analysis results of SARS-CoV-2 patients reported by the included studies

Study	Infection classification (n)	Age (y)	Time from confirmed infection to sample collection (d)	SARS-CoV-2 RNA in semen (n)	Time from last negative RT-PCR swab result to sperm analysis (d)	Sperm analysis				
						Semen volume (mL)	Sperm concentration (millions/mL)	Sperm progressive motility (%)	Sperm total motility (%)	Sperm morphology (%)
Erbay et al [39]	Mild (26)	30.04±4.8	-	-	119.42 (94-144)	3.08±0.8	28.62±12.4	20.92±9.1	33.41±12.3	-
	Moderate (43)	31.06±4.2	-	-	127.66 (96-190)	2.74±0.9	30.63±17.2	21.40±10.1	31.42±13.3	-
Gacciari et al [46]	Not hospitalized (12)	44 (33-49)	-	1 (43)	30 (23-39)	2.5 (1.5-3.5)	65.8 (23.8-71.0)	36.0 (26.0-58.0)	-	2 (2-5)
	Hospitalized without ICU (26)	52 (48-58)	-	-	37 (26-49)	2.0 (0.8-2.5)	17.8 (5.5-70.0)	25.0 (12.0-42.0)	-	3 (1-4)
	ICU (5)	59 (56-59)	-	-	24 (23-32)	1.5 (1-2)	0.0 (0.0-3.5)	27.0 (27.0-27.0)	-	0 (0-0)
Machado et al [23]	Mild, moderate	23.26 (19-43)	14	1 (15)	-	-	-	-	-	-
Li et al [28]	-	20s-50s	10.67±3.3	6 (38)	-	-	-	-	-	-
Best et al [24]	-	40 (IQR=24.75)	37	-	-	2.1 (IQR=1.23)	11.5 (IQR=26.8)	-	-	-
Hajizadeh Maleki and Tartibian [47]	Mild (1) Moderate (23) Severe (27) Critical (33)	34.7±6.3	13.2±4.9	0 (84)	-	-	-	-	-	-
Burke et al [25]	Asymptomatic (1) Mild (2) Moderate (15)	36.52±13.29	10.21 ±10.15	0 (19)	-	-	-	-	-	-
Paoli et al [48]	Mild (2) Recovered (2)	51.5±13.61	17.25± 25.30	0 (4)	-	-	-	-	-	-
Rawlings et al [26]	-	38	7-21	0 (6)	-	-	-	-	-	-
Li et al [29]	Mild (9) Moderate (14)	40.8±8.5	25.8	0 (23)	-	-	13.8 (5.2-36.8) 10.9 (3.0-39.1)	-	-	-
Holtmann et al [49]	Mild (14) Moderate (4)	42.7±10.4 40.8±8.7	43.5±6.2 47±5.3	0 (18)	34.9±11.7 25.5±8.3	2.5±1 1.4±0.7	95.9±50.5 16.2±22.4	46.1±21.1 20±0	57.5±24.1 42.5±24.7	- -
Temiz et al [41]	Before treatment (10) After treatment (10)	38.00±8.28	2-3	0 (20)	-	1.25±1.13	57.00±36.62	39.50±15.50	27.97±14.88	1.50±2.00
		37.00±8.69				1.60±1.63	45.10±36.90	33.00±14.75	23.54±18.53	1.00±1.00

Table 2. Continued

Study	Infection classification (n)	Age (y)	Time from confirmed infection to sample collection (d)	SARS-CoV-2 RNA in semen (n)	Time from last negative RT-PCR swab result to sperm analysis (d)	Sperm analysis				
						Semen volume (mL)	Sperm concentration (millions/mL)	Sperm progressive motility (%)	Sperm total motility (%)	Sperm morphology (%)
Guo et al [31]	Mild (18) Moderate (5) Severe (35)	23	32 (27.5-33)	0 (23)	-	2.3 (1.35-3.0)	95 (56-155.5)	50 (37.5-60)	65 (57.5-76)	16 (12-22)
Ruan et al [32]	Mild (11) Moderate (31) Severe (32)	30.5 (27-35.5)	3-125	0 (70)	80 (64-93)	3.01±1.22	66.41±31.82	43.64±12.37	48.89±13.72	10.52±6.69
Pan et al [33]	Mild and moderate	37 (31-49)	31 (29-36)	0 (34)	-	-	-	-	-	-
Ma et al [35]	Mild	39 (20-49)	78.5 (56-109)	-	-	3.58±1.45	65.5±42.38	37.29±19.83	41±20.91	5.23±1.29
Ma et al [36]	Mild (2) Moderate (70) Severe (7) Critical (2)	38 (34.5-42.5)	-	0 (81)	-	-	-	-	-	-
Kayaaslan et al [44]	Mild (11) Moderate (5)	33.5 (18-54)	1 (0-7)	0 (16)	-	-	-	-	-	-
Pavone et al [52]	Mild	41.11±10.57	6.67±2.75	0 (9)	-	-	-	-	-	-
Quan et al [38]	Mild	60 (20-60)	3-14	0 (18)	-	-	-	-	-	-
Zhang et al [30]	Mild	57 (29-76)	11 (8-17)	0 (10)	-	-	-	-	-	-
Song et al [37]	Asymptomatic (1) Mild (11)	22-38	-	0 (12)	-	-	-	-	-	-
Nicastri et al [53]	-	20s	-	0 (1)	-	-	-	-	-	-

IQU: intensive care unit, IQR: interquartile range, RT-PCR: reverse transcription-polymerase chain reaction, -: not available.

Table 3. Testicular involvement in SARS-CoV-2 patients based on clinical and imaging results

Study	Infection classification (n)	Age (y)	Testicular manifestations (n)		Time from confirmed COVID-19 infection to testicular involvement signs and symptoms (d)
			Clinical	Imaging (USG)	
Bridwell et al [22]	Mild	37	Bilateral scrotal discomfort, erythema without epididymal tenderness	Bilateral orchitis	15
Best et al [24]	-	40 (IQR=24.75)	Bilateral scrotal pain (1)	-	37 (IQR=23)
Haydar et al [40]	-	7	Left scrotal redness, increased warmth and swelling with testicular tenderness and pain	Left orchiepididymitis	-
Holtmann et al [49]	Mild (14)	42.7±10.4	None	-	43.5±6.2
	Moderate (4)	40.8±8.7	Testicular discomfort (1)	-	47±5.3
Pan et al [33]	Mild and moderate	37 (31-49)	Scrotal discomfort suggestive of orchitis (6/34)	-	31 (29-36)
Chen et al [34]	Nonsevere	58.3 (43.0-73.0)	Scrotal swelling or pain (3/142)	Orchitis (3/142), epididymitis (7/142), orchiepididymitis (5/142)	7-30
	Severe		Scrotal swelling or pain (10/142)	Orchitis (7/142), epididymitis (4/142), orchiepididymitis (10/142)	
Gagliardi et al [50]	-	14	Scrotal swelling and pain	Orchiepididymitis of the right testis	14
Kim et al [27]	-	42	Testicular pain	-	2
La Marca et al [51]	-	43	Testicular pain	Epididymitis	3.5
Alkhatatbeh et al [42]	Asymptomatic (53)	43 (1-78)		Orchitis signs or symptoms (0/253)	-
	Mild (152)				
	Severe (48)				
Ediz et al [43]	-	No testicular pain (37±28)		Testicular pain and swelling (10/91)	-
		Testicular pain (46±31.5)			
Özveri et al [45]		49	Left spermatic cord tenderness and tenderness	Spermatic cord inflammation	2

IQR: interquartile range, USG: ultrasonography, -: not available.

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Table 4. Assessment of both prospective and retrospective cohorts using the Newcastle–Ottawa Scale for cohort studies

Study	Selection				Comparability	Exposure			Total	Interpretation
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8		
Erbay et al [39]	*	*	*	*	**	*	*	*	9	High quality
Gacci et al [46]	*	*	*	*	**	*	*	*	9	High quality
Machado et al [23]	*	*	*	*	*	*	*	*	8	High quality
Li et al [28]	*	*	*	*	*	*	*	-	7	High quality
Best et al [24]	*	*	*	*	**	*	-	-	7	High quality
Burke et al [25]	*	*	*	*	**	*	*	*	9	High quality
Rawlings et al [26]	*	*	*	*	**	*	*	*	9	High quality
Li et al [29]	*	*	*	*	**	*	*	*	9	High quality
Holtmann et al [49]	*	*	*	*	**	*	*	*	9	High quality
Guo et al [31]	*	*	*	*	**	*	*	*	9	High quality
Chen et al [34]	*	*	*	*	**	*	*	*	9	High quality
Ma et al [35]	*	*	*	*	**	*	*	*	9	High quality
Ma et al [36]	*	*	*	*	*	*	*	*	8	High quality
Alkhatatbeh et al [42]	*	*	*	*	**	*	*	*	9	High quality
Ediz et al [43]	*	*	*	*	**	*	*	*	9	High quality
Kayaaslan et al [44]	*	-	*	*	**	*	*	*	8	High quality
Pavone et al [52]	*	-	*	*	*	*	*	*	7	High quality
Song et al [37]	*	-	*	*	*	*	*	*	7	High quality
Quan et al [38]	*	-	*	*	*	*	*	*	7	High quality

:- not available.

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Table 5. Assessment of cross-sectional using the modified Newcastle–Ottawa Scale for cross-sectional studies

Study	Selection				Comparability	Exposure		Total	Interpretation
	Q1	Q2	Q3	Q4	Q5	Q6	Q7		
Paoli et al [48]	*	*	*	**	**	**	*	10	Good quality
Temiz et al [41]	*	*	*	**	**	**	*	10	Good quality
Ruan et al [32]	*	*	*	**	**	**	*	10	Good quality
Pan et al [33]	*	*	-	*	-	*	*	7	Good quality
Zhang et al [30]	*	*	-	*	-	*	*	7	Good quality

:- not available.

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Table 6. Assessment of included case reports using the Joanna Briggs Institute critical appraisal for case reports

Study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Total	Interpretation
Bridwell et al [22]	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	7	Good quality
Haydar et al [40]	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	7	Good quality
Gagliardi et al [50]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Good quality
Kim et al [27]	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	7	Good quality
La Marca et al [51]	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	7	Good quality
Özveri et al [45]	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	7	Good quality
Nicastri et al [53]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Good quality

4. Studies' quality

The overall quality for both prospective and retrospective cohort studies is shown in Table 4. Table 5 showed the quality assessment of cross-sectional stud-

ies. The analysis of case reports using the JBI checklist is shown in Table 6. Overall, all studies and case reports showed a sufficient quality for a reliable interpretation.

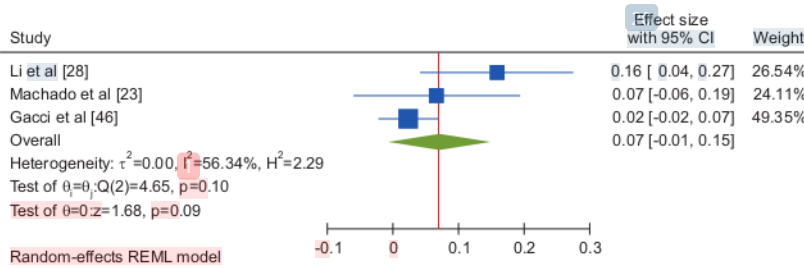
5. Semen and sperm parameters

Three studies reported positive viral RNA in the semen of six out of 38 patients, one out of 15 patients respectively, and one out of 43 patients indicating a 7% rate of positive findings (95% CI, -0.01 to 0.15) among the studies with positive findings as shown in Fig. 2. The other 17 studies, consisting of both observational studies and case reports, reported no viral RNA in the semen. The mean semen volume of the patients reported by eight studies in Fig. 3 was 2.34 mL (95% CI, 1.87–2.81), indicating normal values. The sperm con-

centration in Fig. 4, reported by eight studies, was 51.73 million/mL (95% CI, 31.60–71.85), which was within normal limits. The percentage of both the progressive and complete sperm motility in Fig. 5 were within normal limits, 36.11% (95% CI, 28.87–43.35) and 43.07% (95% CI, 28.57–57.57) respectively. The average sperm morphology percentage in Fig. 6 was 6.03% (95% CI, -1.05 to 13.10), which was within the normal range.

6. Infection severity and sperm parameters

Based on the comparison based on the infection



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Fig. 2. Pooled analysis result of viral RNA detection rate in the semen of SARS-CoV-2 patients.

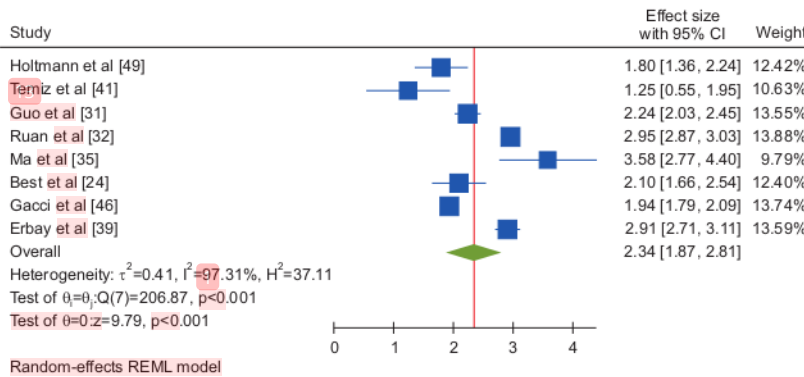


Fig. 3. Pooled analysis result of mean semen volume of SARS-CoV-2 patients.

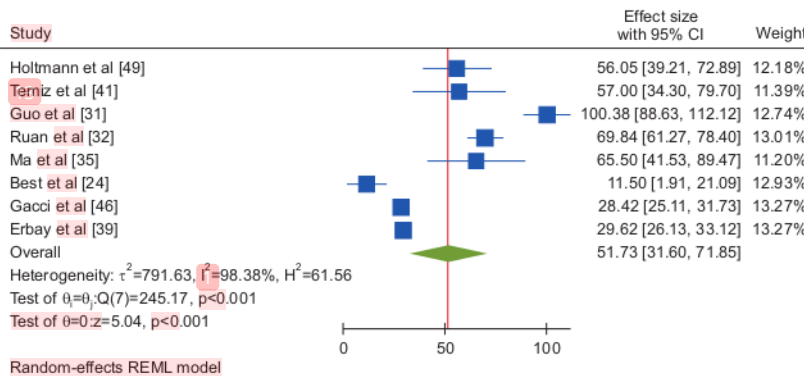


Fig. 4. Pooled analysis of mean sperm concentration of SARS-CoV-2 patients.

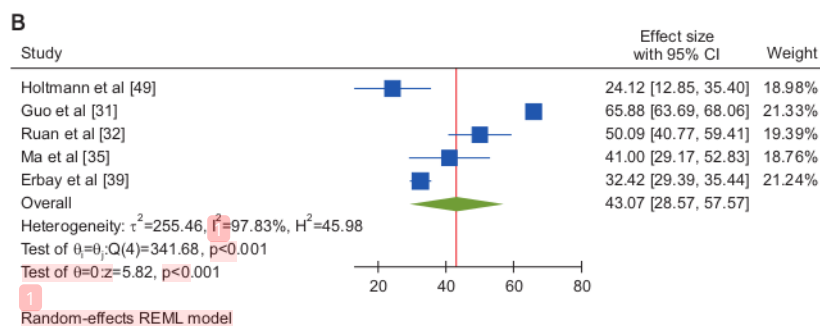
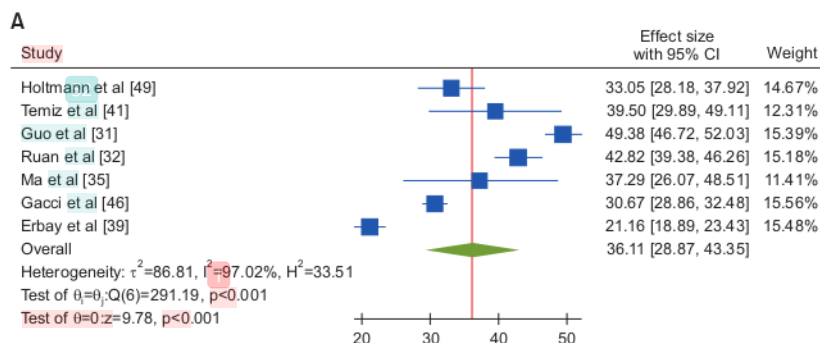


Fig. 5. Pooled analysis of (A) progressive and (B) complete sperm motility of SARS-CoV-2 patients.

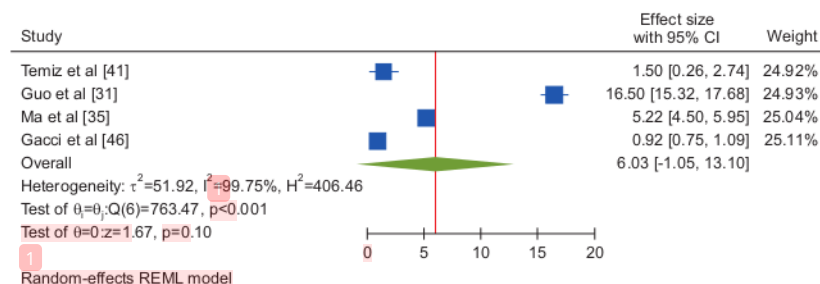


Fig. 6. Pooled analysis of the average sperm morphology percentage of SARS-CoV-2 patients.

severity reported by studies, there are five studies that classified the patients based on the severity. Fig. 7 showed that there is no difference in mean semen volume between mild and moderate ($p=0.47$) and mild and severe ($p=0.55$) infections. There is a significant difference, however, between the moderate and severe infections (MD, 0.52; 95% CI, 0.27–0.76; $p<0.0001$). There is a significant mean sperm concentration difference, shown in Fig. 8, in the comparison between mild and moderate (MD, 18.74; 95% CI, 1.02–36.46; $p=0.04$), mild and severe (MD, 43.50; 95% CI, 13.86–73.14; $p=0.004$), as well as moderate and severe (MD, 22.25; 95% CI, 9.33–35.17; $p=0.0007$). Fig. 9 showed the insignificant difference of progressive motility between mild and moder-

ate ($p=0.11$), mild and severe ($p=0.43$), and moderate and severe ($p=0.40$) infections. The mean total motility could only be evaluated between the mild and moderate infections due to insufficient data (Fig. 10). The comparison showed an insignificant difference ($p=0.48$). The impact of severity on sperm morphology could not be assessed quantitatively due to the lack of data from the included studies.

7. Reproductive tract involvement

There were only 7% of patients with orchitis or orchepididymitis clinical manifestations (95% CI, 0.05–0.10) from observational studies reporting positive findings in infected patients, shown in Fig. 11. However,

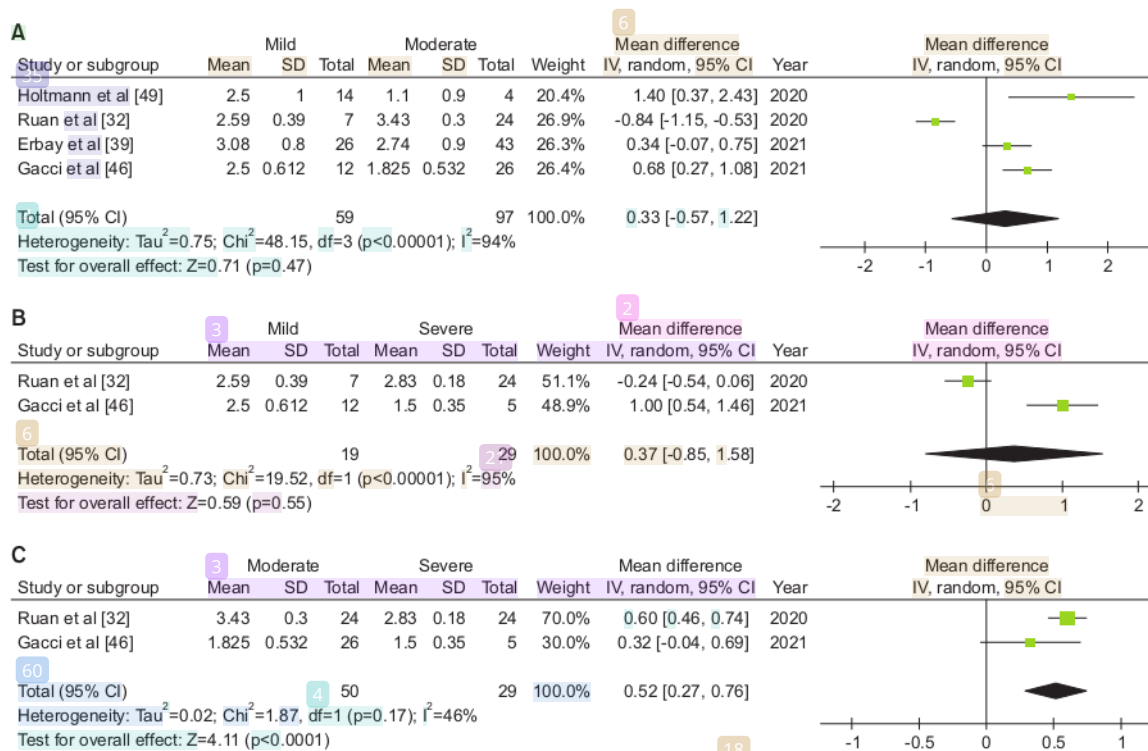


Fig. 7. Forest plot analysis of mean semen volume between (A) mild and moderate, (B) mild and severe, (C) moderate and severe infections of SARS-CoV-2 patients.

there are many case reports included in this study that reported positive clinical findings on infected patients. Only one study reported positive ultrasonography (USG) findings of orchitis among the patients. Other diagnoses made based on USG findings were reported by case reports

8. Publication bias

The funnel plot and Egger's test results for each outcome are displayed in the Supplement File. All analyses showed a low risk of publication bias of the included studies.

DISCUSSION

Even though several reviews discussing the potential involvement of male genitalia tract and affected sperm parameters in SARS-CoV-2 infected patients have been published, to the best of our knowledge, this is the first pooled and analytical meta-analysis on the matter. The findings of this review offered an additional interest-

ing insight regarding the theorized potential target of the virus in the reproductive tract including the testis and epididymis. The virus itself is a single-stranded RNA virus, named after the crown-like appearance of the glycoprotein spikes surrounding the envelope. Since its discovery in 1965, several strains of the virus have been identified and classified into 46 different species, affecting both animals and humans [54]. Several genera are able to affect humans and mammals by infecting the respiratory system [55]. Even though generally coronavirus causes mild flu-like symptoms in humans, certain types such as the SARS-CoV-1, Middle Eastern Respiratory Syndrome (MERS)-CoV, and the SARS-CoV-2 viruses gained massive attention due to the high rate of infections and mortality [56]. As it became apparent that the virus additionally affects other organs based on the presence of the ACE2 receptors, more studies began evaluating the clinical implications of this potential manifestation [57]. The ACE2 receptors are expressed in germ cells, Sertoli cells, and Leydig cells of the testis [8]. They are also highly expressed in

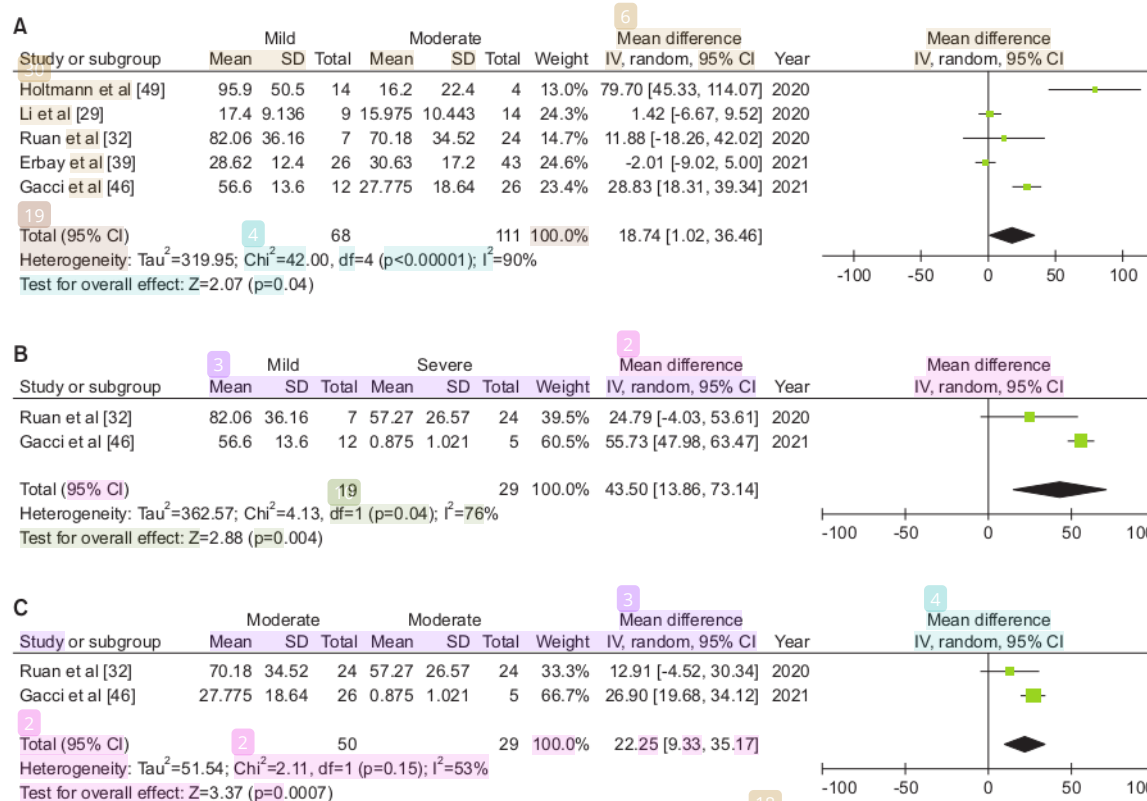


Fig. 8. Forest plot analysis of mean sperm concentration between (A) mild and moderate, (B) mild and severe, (C) moderate and severe infections of SARS-CoV-2 patients.

the epididymis [58]. In this review, we discovered three studies reporting positive viral RNA in the semen, indicating a 7% rate of positive findings (95% CI, 0.01 to 0.15) among the reporting studies. In addition to the small proportion, 17 other studies reported no occurrence of the virus in the semen of patients. The patients from each study varied greatly in terms of basic characteristics. The participants in the study Song et al [37] were given antiviral therapies, which could affect the results of the study. Administering antibiotics, corticosteroids, interferons, and immunoglobulins could also affect the results of a study [49]. Even with the low number of viral RNA present in the seminal fluid, the reports have shown that the virus can indeed be present in the semen, albeit rare. Li et al [28] reported six patients with positive results of virus in their semen, including four patients during the acute stage of infection and two recovering patients. Similar reports were reported by Machado et al [23], in which the virus

was present in one out of 15 patients' semen. Gacci et al [46] reported in their cohort study that the occurrence of the virus in semen was demonstrated in a very small percentage of sexually active samples of men who have recovered from the infection. Nevertheless, a minor risk could not be taken lightly regarding potential fertility issues occurring in an otherwise healthy couple [49]. Higher blood viral loads may be related to disease severity, increasing the change of the virus to reach body fluids including semen, either by alteration of the blood-testis barrier or by excretion to the prostatic fluid [59]. Therefore, the negative results among the samples might be attributed to the fact that most men in the reports were suffering from a mild infection. One autopsy study of an infected patient revealed normal testes [60]. Another post-mortem study evaluating the pathological changes and the viral presence of 12 deceased patients discovered a significant seminiferous tubular injury, decreased Leydig cells,

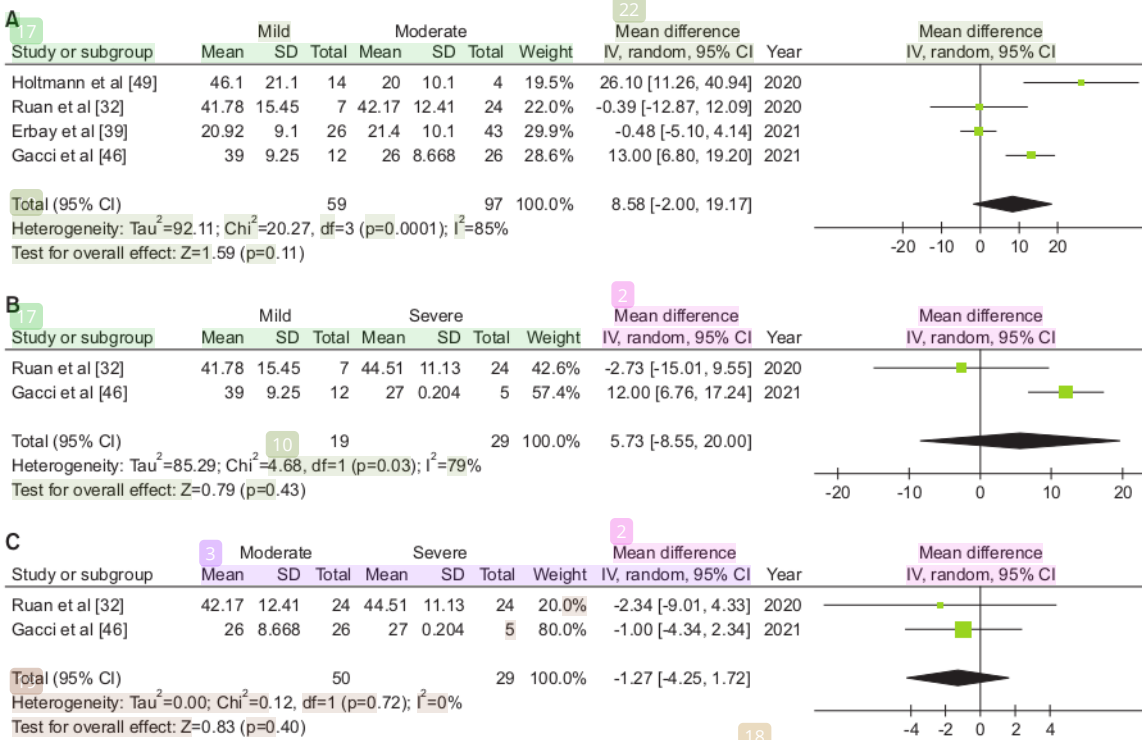


Fig. 9. Forest plot analysis of mean progressive motility between (A) mild and moderate, (B) mild and severe, (C) moderate and severe infections of SARS-CoV-2 patients.

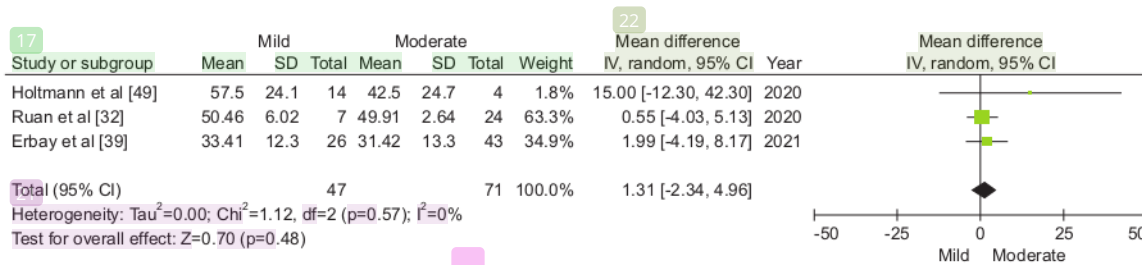


Fig. 10. Forest plot analysis of mean progressive motility between mild and moderate infections of SARS-CoV-2 patients.

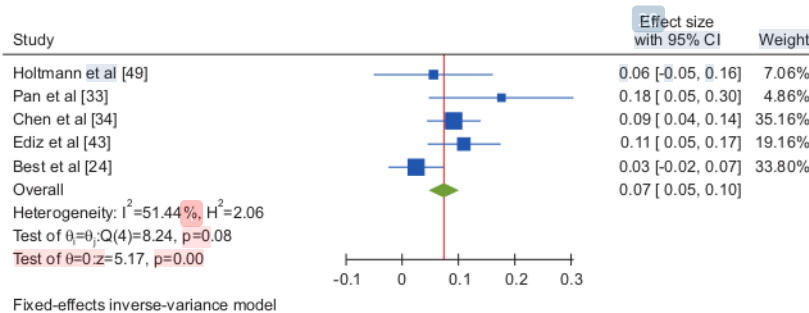


Fig. 11. Pooled analysis of clinical Orchitis or Orchiepididymitis percentage in SARS-CoV-2 patients.

and lymphocytic inflammation [61]. Despite the injuries, they did not find evidence of the virus in the testes in the majority of patients. It is possible that the virus operates through multiple mechanisms which may lead to male reproductive disruption. The low percentage of viral presence in the seminal fluid indicated that sexual transmission is unlikely. As of the conduction of this review, there have been no reported cases of SARS-CoV-2 transmission *via* human cells, tissues, or cellular and tissue-based products. A previous systematic review by Tur-Kaspa et al [62] claimed that COVID-19 is not a sexually-transmitted disease. The United States Food and Drug Administration (FDA) does not recommend screening blood donor candidates for SARS-CoV-2. In the acute phase of the disease, avoiding sexual intercourse for two to four weeks may be beneficial, however, the already-practiced universal precautions worldwide to prevent the transmission of the virus are likely already sufficient [62]. Based on these low results, several studies also discussed whether “low” is an acceptable risk for semen samples’ cryopreservation during the pandemic. As an RNA virus, SARS-CoV-2 is speculated to be able to remain viable if warmed after being cryopreserved. However, there hasn’t been a report of viral cross-contamination between cryopreserved clinical semen samples. Therefore, the risk of contamination is currently negligible [63]. The low presence of virus in the semen may be related to the low number of reproductive tract inflammation occurrence in this review, in which there are only 7% of patients with orchitis or orchiepididymitis clinical manifestations (95% CI, 0.05–0.10) from observational studies reporting positive findings in infected patients. However, there are many case reports included in this study that reported positive clinical findings on infected patients. The main concern of SARS-CoV-2-induced orchitis is its impact on male fertility due to fibrosis. Only one observational study by Chen et al [34] reported positive USG findings of orchitis among the patients. Some studies only reported clinical symptoms due to the limitations of using a doppler USG during the pandemic. However, several case reports included in this study provided evidence of orchitis and orchiepididymitis based on USG findings. It is crucial to distinguish between the receptors’ localization, localized inflammation, and virulence since many studies reported patients with negative viral presence in the semen but positive signs of inflammation. This phenome-

non was also noted in men with orchitis during the 2003 SARS epidemic [12]. Post-humous studies of deceased patients showed only testicular inflammation and reduced spermatogenesis. Biopsies of said patients showed lymphocytic inflammation, decreased spermatogenesis, and increased fibrosis with intact tubules, suggesting that the pathological findings are due to an inflammatory response and not directly of the virus [37,61]. However, Ma et al [64] reported two testis samples with positive RT-PCR results in a post-mortem study. Other theories suggest that the virus may attack the testicular tissue early on but is cleared from the testes later on during the course of the infection [33,61]. This is difficult to investigate, as the diagnosis and specimen collection duration varies between studies. Recent reports suggested that while the ACE2 receptor is present in reproductive organs, TMPRSS2 is not expressed in testicular cells or sperm. As TMPRSS2 is also necessary for viral entry, these findings suggested that the virus is unlikely to enter through an ACE2/TMPRSS2-mediated mechanism [7,65]. On the contrary, Wang and Xu [8] claimed that TMPRSS2 expression is concentrated in spermatogonia and spermatids. Its high expression suggested a high local infection in the human testes. However, due to concrete evidence reported by several studies, the direct mechanism of the virus attack on reproductive tissues requires further investigations. Nevertheless, the pooled sperm parameters of the studies are within normal limits based on the World Health Organization (WHO) reference values for human semen characteristics [66]. However, long-term clinical investigations in multiple large cohort studies are necessary to evaluate the impact of COVID-19 on spermatogenesis fully [67]. The blood-testis barrier, consisting of the Sertoli cells’ junctional complex, is vital for the regulation of nutrients, molecules, and immune compartments for seminiferous tubules. Damage to it can cause immune deficit and spermatogenesis problems, leading to infertility [68,69]. The seemingly normal results of the pooled analysis might be caused by the combined sperm parameters of asymptomatic and mild patients. Holtmann et al [49] noticed that no negative influence of SARS-CoV-2 infection on sperm parameters was noted in recovered subjects with mild symptoms in their study. However, patients with moderate infection had reduced sperm quality. Comparing the results between patients with different degrees of severity in this review showed

that patients with more severe infections had worse sperm analysis results, both in concentration between mild and moderate (MD, 18.74; 95% CI, 1.02–36.46; $p=0.04$), mild and severe (MD, 43.50; 95% CI, 13.86–73.14; $p=0.004$), as well as moderate and severe (MD, 22.25; 95% CI, 9.33–35.17; $p=0.0007$) infections and mean semen volume between moderate and severe infections (MD, 0.52; 95% CI, 0.27–0.76; $p<0.0001$). One of the included studies by Ruan et al [32] also compared the patients with an age-matched healthy control group. They discovered that the sperm parameters of the patients were slightly declined despite being within the normal reference limit of the WHO. The other included studies by Holtmann et al [49], Gacci et al [46], and Erbay et al [39] in the analysis of this review also noted the decrease of sperm quality with the increase of disease severity. Aside from the local damage caused by the virus, systemic damage is also theorized by several studies. One of which is elevated body temperature, known to have a deleterious effect on sperm count and motility. This might have caused the reduction in sperm count and motility from moderate and severe infections [70]. As previously mentioned, there are patients from several studies who received intense antiviral treatment and hydroxychloroquine. Some studies speculated a possible impact due to these drugs on sperm parameters. However, this is unlikely because the drugs were only given for a few days [49]. Additionally, there has yet to be evidence that said drugs could impact the male fertility [71,72]. Yang et al [61] reported histopathology results of deceased SARS-CoV-2 patients showing swelling Sertoli cells with vacuolation and cytoplasmic rarefaction, detachment from the tubular basement membranes, loss, and sloughing into the lumens of intratubular cells. Leydig cells reduction and inflammatory infiltrate in the interstitial were also noted. Since Sertoli cells are responsible for the homeostasis of seminiferous tubules and spermatogenesis and Leydig cells are involved in androgen production, the observed pathology may lead to seminiferous tubule damage and endocrine abnormality, which could affect spermatogenesis [73].

1. Strengths

This systematic review and meta-analysis evaluated the possibility of male reproductive tract involvement and decreased sperm quality in SARS-CoV-2 patients. To the best of our knowledge, this is the first meta-

analysis on this topic. Previous narrative and systematic reviews have discussed studies evaluating a similar issue, however, a quantitative synthesis has not been performed and reported. This review extends the discussion of previous reviews by adding a pooled result of all studies and an analytical comparison of sperm parameters based on disease severity. The strengths of the current review include the following: (1) evaluated a highly relevant and serious urological issue related to the COVID-19 pandemic, (2) included all observational studies and case reports with a higher quantity of studies compared to previous reviews, (3) conducted a quantitative synthesis in pooled results and comparative forest plots based on disease severity, and (4) utilized a broad and reproducible search strategy in major databases, examined by investigators with interpersonal discussions for disagreements.

2. Limitations

However, there are many limitations in the studies included in this review. Most of the studies are observational studies where the end-points are not designed to test if SARS-CoV-2 actually directly affects spermatogenesis. Testicular histological confirmation was absent from the studies despite the observed changes in sperm concentration. Histopathological examination of the testicular tissue was performed in post-mortem studies, which are not included in this review. Some studies performed a sperm analysis on recovered patients, while others performed a semen analysis on patients with active infection. Due to the limited reports, the pooled analyses combined these results. Different duration from diagnosis to specimen collection and the duration from negative RT-PCR swab results to specimen collection could also affect the accuracy of the findings. As the pandemic continues, some studies with different periods of publication might have different classifications for severity. One study classified asymptomatic patients as mild while another study separated asymptomatic patients from mild patients. Only a few studies report complete sperm analysis results based on disease severity to be compared quantitatively. More studies that directly compare sperm parameters between mild and moderate and comparing the parameters between infected and healthy patients should also be performed. General modifications of sperm parameters due to injury or inflammation may be seen only after three months of time; thus a repeated examina-

tion a few months after the first examination may be necessary to fully evaluate the long-term consequence of the infection on sperm parameters. Future reviews should also evaluate endocrine function damage due to the infection. A damaged endocrine function may be represented by significantly lower serum testosterone to luteinizing hormone ratios.

CONCLUSIONS

This systematic review and meta-analysis showed that SARS-CoV-2 may result in decreased sperm concentration in severe cases and the postulated mechanism relates to potential sperm reproductive tract inflammation in these patients. The absence of large viral RNA detection in the seminal fluid obtained in most patients indicates the likelihood of a systemic effect from the SARS-CoV-2 although this is largely unproven.

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Conflict of Interest

The authors have nothing to disclose.

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Author Contribution

Conceptualization: YPK, LH. Data curation: EC, LH. Formal analysis: YPK, FH, ZAR, LH. Funding acquisition: none. Investigation: YPK, FH, ZAR. Methodology: YPK, LH. Project administration: YPK, LH, EC. Resources: YPK, LH. Software: YPK, LH. Supervision: LH, EC. Validation: LH, EC. Visualization: YPK, LH, ZAR. Writing – original draft: YPK, LH. Writing – review & editing: YPK, LH, EC.

Supplementary Material

Supplementary material can be found via <https://doi.org/10.5534/wjmh.220019>.

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