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Investigating the mode of transmission of COVID-19 through genital secretions, semen, the birth canal, and lactation: A systematic review

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Abstract:

In a global pandemic, the coronavirus has brought new challenges to reproductive and sexual health. This systematic review has been conducted with the aim of investigating a) the transmission of coronavirus disease 2019 (COVID-19) through female-to-male sexual secretions and vice versa, b) vertical transmission to the fetus, c) transmission to the newborn through the birth canal, and d) transmission through breast milk. In this review study, to find related articles, databases were searched using English and Persian keywords from the beginning of 2019 to the end of 2023. Based on the entry and exit criteria and the qualitative evaluation of the studies based on the STROBE criterion, the final studies were summarized. According to the initial search, 5970 articles were reviewed. After removing duplicate articles and the inappropriateness of the title, the abstract of the article was not related to the objectives of the project, there was no relevant study, and retrospective studies were excluded. Finally, 120 articles were selected as final articles. The general results show that transmission of the virus through the vagina to the blood of the fetus causing septicemia, vertical transmission from the mother to the fetus, transmission through the water bag, genital secretions, and ejaculate fluid are considered rare and ruled out. Transmission through rectal secretions during anal or oral sex requires more extensive studies. It is not possible to transmit the virus through breast milk. The long-term reproductive impact of COVID-19 on the reproductive systems of women and men is unclear.

Keywords:

Birth canal, COVID-19, sexual health, sexual secretions, transmission, women

Introduction

The spread of the coronavirus disease 2019 (COVID-19) and deaths in more than 160 countries caused the declaration of an epidemic around the world.^[1-5] Restrictions on access to family planning services, reduction in sexual relationships in the form of group sex relationships, reduction in sexual relationships among transsexuals, and the educational and communication gap between healthcare services and mother and

child were the most important concerns during the COVID-19 era.^[6] In different methods of sexual intercourse, having anal, oral, or combined sexual intercourse can cause the transmission of the virus during marital intercourse.^[7-9] Rubin ES *et al.* 2020 shows that COVID-19 can be detected in the vulva,^[10] however, it confirms the evidence of vaginal transmission of the virus and the role of the sexual route in the transmission of the disease.^[11] The transmission of the virus in saliva and the rectum through oral and anal sex has also been reported during sexual intercourse.^[12] In connection

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with the transmission of the virus from mother to baby, Ashraf *et al.*^[13] (2020) raised the possibility of vertical transmission from mother to baby. Schwartz *et al.*^[14] (2020) confirmed the presence of the coronavirus in vaginal secretions. Then, contradictory studies were reported about transmission from vaginal secretions.^[15-17] Currently, the status of transmission of this virus through the reproductive system is still controversy regarding perinatal outcomes in infants, but vertical transmission of COVID-19 during vaginal delivery has been confirmed. Therefore, the possibility of contamination of the nasopharynx of the baby, possible infection of the baby, an increase in the rate of cesarean section, a lowering of the decision threshold for cesarean section,^[18,19] an increase in the maternal and fetal outcomes of cesarean section, prematurity of the baby, and the need for neonate intensive care (NICU) admission and significant radiographic signs of the baby's lungs can be expected.^[20] Increasing the financial burden of treatment, increasing the duration of hospitalization,^[3,19] and increasing perinatal complications (such as preeclampsia) are among the long-term consequences of COVID-19 transmission during pregnancy and childbirth.^[19] The increase in the number of screening requests for sexually transmitted diseases and the increase in the number of questions about sexual transmission related to COVID-19 are among the most important issues related to the presence of the virus in vaginal fluid.^[20-23] Considering the possibility of the spread of new strains of the virus in the future, the unpredictable pathogenic behavior of the virus, the performance of the health system in dealing with COVID-19, and the sixth position among the 15 determined and leveled ranks,^[24] the researchers decided to consider a complete compilation of reliable information in this field in the continuation of the systematic review process.

Materials and Methods

The aim of this study was to investigate a) the transmission of COVID-19 through female-to-male sexual secretions and vice versa, b) vertical transmission to the fetus, c) transmission to the newborn through the birth canal, and d) transmission through breast milk. This article was put on the agenda as a review and in a systematic way. A systematic search of previous research in a purposeful and focused manner on a specific topic that is mentioned above is performed. In this regard, the combination and evaluation of research are performed using specific and predetermined criteria, which ultimately lead to the presentation of results. The method of searching in this study is performed using keywords related to the subject in several specific and valid databases. In such studies, quality filters are also applied in the selection of previous research studies. Also, sampling strategies, type of the study, study period, and their limitations are examined.

The statistical population of this study includes published scientific articles that refer to the issue of transmission of COVID-19 through female-to-male sexual secretions and vice versa, vertical transmission to the fetus, transmission to the newborn through the birth canal, and transmission through breast milk. *Protocol:* This review study was conducted after approval by the research council and obtaining the ethical code approved by Islamic Azad University (ir.iau.ps.rec. 1400.199). This study was completed based on the protocols registered on the PROSPERO website with the code 251736, according to the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) format, and using the The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist. *Search strategy:* In this study, the following databases were used to find related studies in line with the goals of the project: Cochrane, IranMedx, Scientific Information Database (SID), PubMed, Google Scholar, Scopus, ScienceDirect, UpToDate, and Web of Science. Studies were reviewed from the beginning of the coronavirus epidemic 2019 until 2023 and based on the inclusion and exclusion criteria. The English keywords included sexual transmission, sexual, oral sex, rectal sex, Sexual Behavior, Sexual Partners, heterosexual, Sexual Abstinence, Sexual Health, Sexual Maturation, Vagina, Vaginal Smears, Vaginal Discharge Vaginal, normal vaginal delivery, covid-19, corona virus, sars-covid-2, cesarean section, infant, baby, fetus, newborns, placenta, fetomaternal, transmission, breastfeeding, neonate intensive care (NICU), which were investigated by combining AND and OR. *Selection of studies:* The selection of studies was put on the agenda based on inclusion and exclusion criteria and relevance. The studies in Persian and English and the format of a full article were finally reviewed. Also, the list of sources used in all published articles in this field was checked. *Study selection criteria* are as follows: 1. studies that are in English and Farsi and have a full article format; 2. studies that have been conducted on sexual contact (heterosexual, oral sex, and rectal sex) transmission through semen and vaginal secretions; 3. studies that mention transmission through oral and anal sex; and 4. articles in the form of case reports and original research studies that have been refereed. *Study exclusion criteria* are as follows: 1. studies that do not get the corresponding points according to the checklists related to the meta-analysis of descriptive studies; 2. studies that are related to the infection of people with multiple partners; 3. texts presented in the form of letters to the head secretary, short articles, and articles presented in congresses and conferences. *Excavating Information:* According to the initial search, 5970 articles were reviewed, and after removing duplicate articles, 1018 articles were reviewed. Among them, 603 articles were removed due to the inappropriateness of the title. Then, 415 articles were analyzed. Twenty articles were excluded because the abstract of the article was not

related to the objectives of the project, and 275 articles were excluded because they were not relevant. Finally, 120 articles were selected as final articles and entered the quality review stage [Figure A]. *Checking the quality of studies*: The information about the articles was prepared based on the checklist form and extracted and entered into Excel. To prevent bias, all the steps of extracting and checking the sources were performed by two authors independently of each other, and if the sources were rejected, the relevant reason was mentioned. In case of disagreement between two researchers, the review of the article was performed by a third person. In all stages of searching and using references, ethical principles were observed. Then, to obtain the data from the articles, data extraction worksheets were designed and adjusted in the form of eight indicators, and based on coding, each of the worksheets was completed by the researchers. The investigated indicators in each paper were article title, first author, type of the study, investigated variable,

year of publication, demographic information related to each title, quality of the study, and final conclusion of the study. The quality of the studies was evaluated according to the checklist of descriptive studies in STROBE epidemiology. The revised checklist consists of 11 items, and a maximum of one point is assigned for each methodological element. The total quality score is finally 22. Scores of 16–22 are high quality, 15–8 are average quality, and less than 8 means low quality studies.^[25] In this research, studies that had poor quality were deleted. After reviewing and comparing the worksheets specific to each of the extracted articles, the obtained data were compiled and the final description and analysis were performed based on the objectives of the project.

Result

The following practical findings were presented in a classified manner with separate thematic axes. The aim

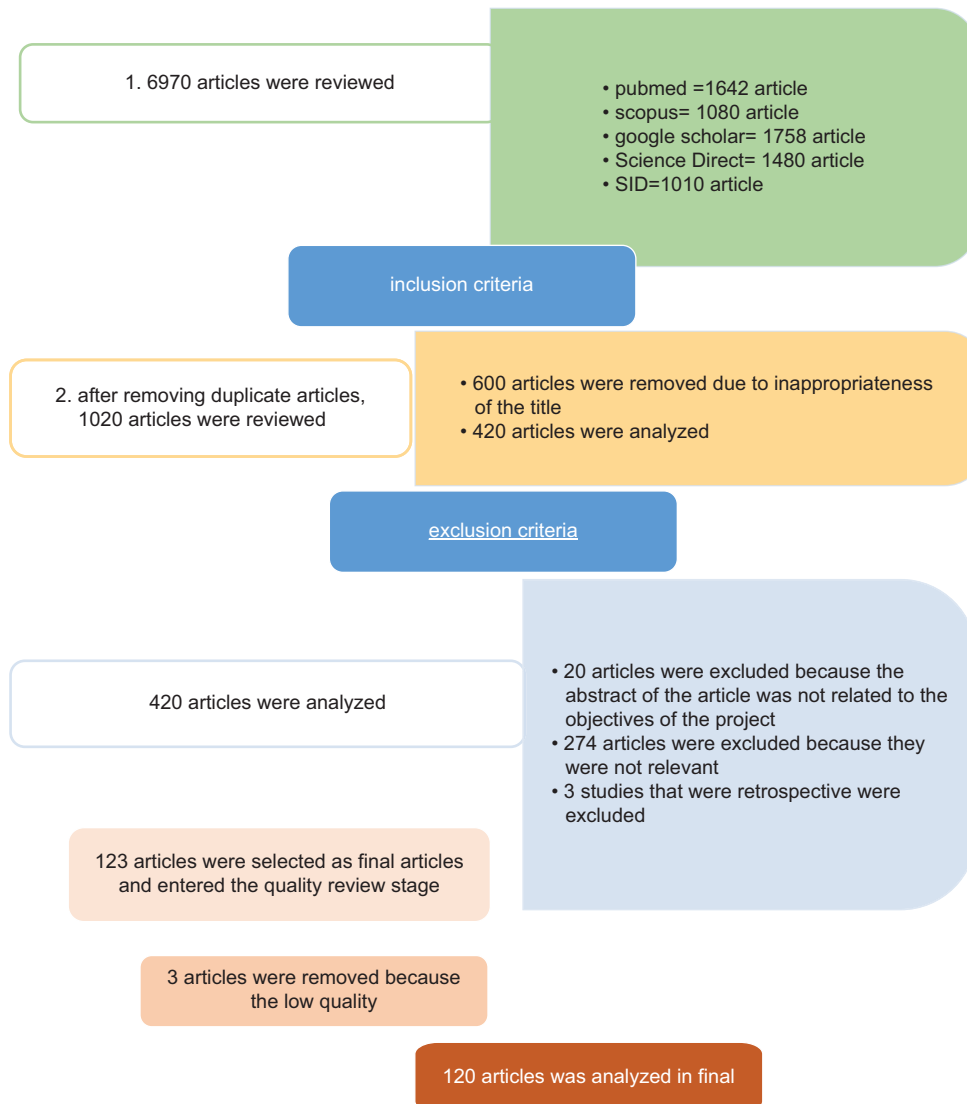


Figure A : Study algorithm

of this study was to investigate a) the transmission of COVID-19 through female-to-male sexual secretions and vice versa, b) vertical transmission to the fetus, c) transmission to the newborn through the birth canal, and d) transmission through breast milk.

Discussion

Later studies related to the possibility of vertical transmission from mother to fetus were reported, some in favor and some in opposition. The COVID-19 pandemic has affected all aspects of reproductive and sexual health. The current review was conducted with a systematic review of 120 articles. Various studies related to the transmission of the virus through genital secretions concluded that droplets, contact, aerosol, and fecal-oral transmission are the main ways of transmitting the infection of COVID-19, and this initial finding was confirmed in all the primary articles.^[135,136] In the future, due to the progress of the disease at a high speed and similar distribution in all parts of the world, the ability to detect COVID-19 in the lower genital tract of women will be noticed.^[10,15-17,31,34,137] According to Tables 1 and 2 In this part, the researcher categorized and emphasized a the transmission of COVID-19 through female-to-male sexual secretions and vice versa. Other studies were designed in response to the transmission of COVID-19, among which the study of Rubin (2020) reported a pregnant woman infected with the virus who had vulvovaginal lesions and raised the possibility of transmission through these lesions.^[10] In opposition to the above findings, other studies show that the SARS coronavirus 2 has been associated with oral and genital ulcers and describes a potential reactive dermatologic

manifestation of COVID-19, but the transmission of the virus through an ulcer is not mentioned.^[138,139] Later, sexual contact was proposed as a way to transmit Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and researchers sought to confirm or disconfirm the issue.^[75] Kotlyar (2021) showed in their research that the rectal swap test is positive even after the nasopharynx is negative. This possibility has been raised in other studies as well.^[140-143] In relation to the possibility of virus transmission through men’s semen, most of the studies showed the absence of virus transmission through ejaculate,^[43,44,47,49,51,54] but Li (2020) showed in their study that there were positive results of SARS-CoV-2 in the semen of six patients (15.8%).^[42] In this regard, Machado (2021) also found the virus in the semen of 6.66% of patients.^[53] Some studies point to the short-term and long-term effects of COVID-19 on the male reproductive system, such as the effect of COVID-19 on the amount of male sex hormone secretions that were discussed in the study by Ling Ma *et al.* (2021).^[46] Oligospermia, azoospermia, and sperm quality disorders have also been shown in Gacci *et al.* (2021).^[52] Groner *et al.*^[48] (2020) consider the effect of this virus on the reproductive system and reproductive health of men to be probable and suggest the need for more research. In favor of the result of this research, Nori and Salman (2023) reported that COVID-19 has a direct hematogenic spread to the testis and triggers the immunological response, which decreases sperm motility and counts^[144] and changes the hormonal level, and the formation of antisperm antibodies is reported.^[145,146] The other findings of this research are vaginal secretions that do not transmit the virus, but rectal secretions can be considered a route of transmission, which requires more

Table 1: Frequency distribution of articles according to the topic and objectives of the study

Row	Purpose	Abundance	Percentage
1	The relationship between the transmission of COVID-19 through vaginal secretions	23	19.16
2	Correlation between transmission of COVID-19 through ejaculate	19	15.83
3	The relationship between vertical transmission of COVID-19 around and around childbirth through the birth canal	57	47.5
4	Transmission of COVID-19 through genital secretions and umbilical cord blood	0	0/0
5	Transmission of COVID-19 through vaginal secretions to the baby's blood	1	0.83
6	Transmission of COVID-19 through breast milk	20	16.66
7	Total	120 articles	100/0

Type of study		
The reviewed articles have been published between 2019 and 2023		
10 prospective studies	5 observational studies	
7 retrospective studies	26 review studies	
3 systematic review studies and meta-analysis	9 experimental studies	
2 clinical trials	18 case report studies and 4 case series	
2 case-control studies	12 cross-sectional studies	
3 case reports and reviews	7 cohort studies	
1 narrative review	15 systematic review studies	

Quality of studies		
11 studies had excellent quality	109 studies had average quality	Studies that had poor quality are deleted

Table 2: Determining all of the types of transmission of COVID-19

Row	Author	Aim of research	Type of the study	Population	Sample size	Data collecting	Quality	Results	Type of transmission
1	Rubin et al., 2020 ^[10]	COVID-19 in vulvar lesions	Case report	Pennsylvania	1	PCR test	9	COVID-19 can be detected in vulva	Vaginal secretions and ejaculation (study from 1-42)
2	A. L. Tatu et al., 2020 ^[26]	Methods of sexual transmission	Observational	Romania	-	PCR test	11	Oral-anal contact may also play a role in transmission	"
3	Qiu, L et al., 2020 ^[27]	COVID-19 in vaginal fluid	Experimental	Beijing Tongji Zhongda Hospital	10	PCR test	14	SARS-CoV-2 was not found in vaginal fluid	"
4	Mohseni Amir Hossein et al., 2020 ^[28]	COVID-19 in body fluids	A review study	China	-	PRISMA	15	The vagina can be infected by HCoV-229E	"
5	P Cui et al., 2020 ^[29]	COVID-19 in the lower genital tract of women	Case series	Tongji and French	35	RT-PCR test	8	SARS-CoV-2 was not found in vaginal fluid	"
6	Mehmet Musa Aslan et al., 2020 ^[16]	COVID-19 in vaginal secretions	A cross-sectional study	Turkey	13	Nucleic acid tests on vaginal swabs	12	SARS-CoV-2 was not found in vaginal fluid	"
7	MF Ahmad et al., 2020 ^[30]	COVID-19 in vaginal secretions	Experimental	Kuala Lumpur	35	RT-PCR test	16	SARS-CoV-2 was not found in vaginal secretions	"
8	Angela Patri et al., 2020 ^[12]	COVID-19 in sexual contact	A review study	-	-	PRISMA	13	COVID-19 was found in saliva and can be transmitted through oral and anal sex	"
9	Anat Schwartz et al., 2020 ^[14]	COVID-19 in vaginal secretions	A prospective study	Israel	35	Vaginal RT-PCR swabs	9	The possibility of vaginal colonization should be considered at the time of delivery in patients with active SARS-CoV-2 infection	"
10	Wu, y et al., 2020 ^[17]	COVID-19 in vaginal secretions and breastfeeding	Cohort study	China	13	Stool samples, vaginal secretion samples, throat, and anal swabs	9	SARS-CoV-2 was not found in vaginal discharge samples	"
11	Lowe B and Bopp B, 2020 ^[31]	Vaginal delivery in a woman infected with coronavirus	Case report	Australian	1	Observation and PCR test	14	An uncomplicated vaginal delivery	"
12	Andrea Roberto Carosso et al., 2020 ^[32]	COVID-19 and vaginal delivery	Case report	Italy	35	Vaginal secretion samples, throat, and anal swabs	10	Potential transmission during vaginal delivery in a woman with positive rectal and fecal swabs for SARS-CoV-2 is underestimated	"
13	Aitken RJ, 2021 ^[25]	Sexual transmission of COVID-19	A review study	-	-	PRISMA	9	Risk of sexual transmission is currently unknown	"
14	Kavita Khoiwal et al., 2021 ^[33]	COVID-19 in vaginal secretions	A prospective study	India	15	RT-PCR	11	In the vaginal fluid of three (20%) participants, the virus of COVID-19 was detected	"
15	Hila Uslu Yuvasi et al., 2021 ^[34]	COVID-19 in vaginal secretions	A cross-sectional study	Sakarya	18	RT-PCR	13	SARS-CoV-2 was not found in vaginal fluid	"
16	Mehmet Musa Aslan et al., 2022 ^[16]	COVID-19 in the female genital system	A cross-sectional study	Turkey	13	Laboratory tests, imaging tests, and nucleic acid tests on vaginal swabs	15	SARS-CoV-2 was not found in lower genital tract samples	"

Contd...

Table 2: Contd...

Row	Author	Aim of research	Type of the study	Population	Sample size	Data collecting	Quality	Results	Type of transmission
17	Eiad Barber et al., 2021 ^[365]	COVID-19 in the female genital system	A prospective study	Israel	51	Vaginal swabs and RT-PCR	14	*SARS-CoV-2 was not found in the vaginal secretions	"
18	Mukta Agarwal et al., 2021 ^[366]	COVID-19 in vaginal secretions	A prospective observational study	India	35	History and clinical records along with laboratory findings of the patient was recorded	13.5	SARS-CoV-2 was not found in the cervical and vaginal secretions	"
19	Zoleikha Atarod et al., 2022 ^[367]	COVID-19 in the vagina and rectum	A cross-sectional study	Mazandaran, Iran	80	Vaginal PCR and rectal PCR	14	COVID-19 can infect the vagina and rectum of women	"
20	Deniz Erdem et al., 2023 ^[368]	COVID-19 in vaginal fluid	Prospective cross-sectional study	Ankara	30	RT-PCR in vaginal swab	12	SARS-CoV-2 was not detected in any vaginal samples	"
21	Pankush Gupta et al., 2023 ^[369]	COVID-19 in cervicovaginal secretions	A cross-sectional study	India	11	RT-PCR for SARS-CoV-2	15	SARS-CoV-2 does not shed in the cervicovaginal secretions of women with mild COVID-19 infection	"
22	Kavita Khoiwal et al., 2022 ^[40]	COVID-19 in the lower genital tract	A prospective study	India	61	RT-PCR in nasopharyngeal (NP) and oropharyngeal (OP) swabs	14.5	COVID-19 was found in the lower genital tract of women with active COVID-19	"
23	Pedro Ernesto Carvalho de Cillo, et al., 2023 ^[41]	COVID-19 in vaginal secretions	A cross-sectional study	Brazil	44	RT-PCR in vaginal swab	16	SARS-CoV-2 was not found in vaginal secretion	"
24	Diangeng Li et al., 2020 ^[42]	COVID-19 in semen	Cohort study	Shanghai Municipal Hospital	50	RT-PCR of nasal and pharyngeal swabs and semen sample for SARS-CoV-2 testing	13	Semen test results showed that six patients (15.8%) had positive results for SARS-CoV-2	"
25	Ci Song et al., 2020 ^[43]	COVID-19 in the semen and testicles of patients	Experimental	China	12	Semen PCR for SARS-CoV-2	13	SARS-CoV-2 was not found in the semen and testicles of patients	"
26	Feng Pan et al., 2020 ^[44]	COVID-19 in semen	Observational, cross-sectional study	China	34	RT-PCR of single-ejaculated semen samples	12	SARS-CoV-2 was not found in the semen of men who have recovered from coronavirus disease	"
27	Liqiang Guo et al., 2021 ^[45]	COVID-19 in semen	Experimental	China	23	Semen PCR for SARS-CoV-2	15	CoV-2 RNA was negative in semen samples	"
28	Ling Ma et al., 2021 ^[46]	COVID-19 in semen and examining sex hormones	Cohort	Wuhan	119	Semen PCR for SARS-CoV-2 and semen characteristic analysis	15	CoV-2 was not found in semen samples, but abnormal sex hormone secretion among patients with COVID-19 is seen	"

Contd...

Table 2: Contd...

Row	Author	Aim of research	Type of the study	Population	Sample size	Data collecting	Quality	Results	Type of transmission
29	Yajun Ruan et al., 2021 ^[47]	COVID-19 in semen	Clinical trial	Tongji	74	Semen samples were collected for SARS-CoV-2 RNA and semen quality and hormonal profiles were analyzed	9	SARS-CoV-2 was not found in the urogenital secretions	"
30	Matheus Ferreira Gröner et al., 2020 ^[48]	Effects of COVID-19 on the male reproductive system	A review study	-	-	PRISMA	8	The virus has already been found in the semen of infected patients, but its effects on male reproductive health have not yet been investigated	"
31	Nora Holtmann et al., 2020 ^[49]	COVID-19 in semen	Cohort study	America	34	Semen and a blood sample for SARS-CoV-2	13	SARS-CoV-2 was not found in semen	"
32	Donatella Paoli et al., 2021 ^[50]	COVID-19 in semen	A review study	-	-	PRISMA	9	This study shows a low risk of sperm infection. It may have important implications for sperm freezing	"
33	Kayaaslan B et al., 2020 ^[51]	COVID-19 in semen	Experimental	Ankara, Turkey	16	Routine laboratory and radiological tests, semen sample for SARS-CoV-2	13	SARS-CoV-2 was not found in all semen samples	"
34	M Gacci et al., 2021 ^[52]	Semen disorder in men with COVID-19	A prospective cross-sectional study	Italy	43	Biological fluid samples, namely saliva, pre-ejaculation urine, semen, and post-ejaculation urine, were tested for the SARS-CoV-2 genome	16	Azoospermia was significantly associated with the severity of COVID-19	"
35	Bruno Machado et al., 2021 ^[53]	COVID-19 in semen	Cross-sectional description	United States	15	Semen PCR for SARS-CoV-2	14	The SARS-CoV-2 viral RNA was present in 1 of 15 patients [6.66%]	"
36	Mustafa Zafer Temiz et al., 2020 ^[54]	COVID-19 in semen	Cross-sectional study	Turkey	30	Semen PCR for SARS-CoV-2	15	SARS-CoV-2 was not found in semen samples	"
37	Pankush Gupta et al., 2021 ^[55]	COVID-19 in semen	A cross-sectional study	India	37	RT-PCR and semen analysis	15	SARS-CoV-2 was not found in semen	"
38	Chuan Huang et al., 2021 ^[56]	COVID-19 in semen	Retrospective cohort study	China	100	Semen and a blood PCR for SARS-CoV-2	14.5	SARS-CoV-2 was not found in semen and blood samples	"
39	Pavone et al., 2022 ^[57]	COVID-19 in semen	Cross-sectional study	Italy	116	RT-PCR and nested PCR in semen for SARS-CoV-2	12	SARS-CoV-2 was not found in the semen	"
40	Lucie Delaroché et al., 2021 ^[58]	COVID-19 in semen	Cross-sectional study	France	342	SARS-CoV-2 PCR in semen and seminal plasma fractions	14	Only one presented positive SARS-CoV-2 PCR in semen and seminal plasma fractions, although the spermatozoa pellet was negative. Viral cultures were all negative	"

Contd...

Table 2: Contd...

Row	Author	Aim of research	Type of the study	Population	Sample size	Data collecting	Quality	Results	Type of transmission
41	Donders GG <i>et al.</i> , 2022 ^[69]	COVID-19 in semen	Prospective cohort study	Belgium	120	SARS-CoV-2 PCR in semen	16.5	Semen is not infectious with SARS-CoV-2 at 1 week or more after COVID-19 infection (mean, 53 days). However, couples with a desire for pregnancy should be warned that sperm quality after COVID-19 infection can be suboptimal	"
42	Zhongwei Huang <i>et al.</i> , 2022 ^[60]	COVID-19 in semen	Clinical trial study	Singapore	63	Male hormone profile level and expression of SARS-CoV-2 RNA, ACE2, and TMPRSS2 in human semen	15	SARS-CoV-2 was not found in the semen	"
43	Mohammad Ali Ashraf <i>et al.</i> , 2020 ^[13]	COVID-19 and Vertical transfer	Systematic review	-	-	PRISMA	15	Vertical transmission is possible	Vertical transmission through the birth canal (study from 43-99)
44	Lan Dong <i>et al.</i> , 2020 ^[61]	Vertical transfer	Case report	China	1	RT-PCR/nucleic acid/IgM and IgG/cytokine/RT-PCR testing of vaginal	10	There is a possibility of vertical transmission	"
45	Weiyong Liu <i>et al.</i> , 2020 ^[62]	Vertical transfer	Case series study	Wuhan, China	17	Testing procedure for SARS-CoV-2 infection using oropharyngeal swab, placenta tissue, vaginal mucus, and breast milk of mothers, and oropharyngeal swab, umbilical cord blood, and serum of newborns was conducted	13	There is no vertical transmission	"
46	Farida Elshateey <i>et al.</i> , 2020 ^[63]	Vertical transfer	A review study	-	-	PRISMA	13	There is no vertical transmission	"
47	Rozychi HJ and Kotechas 2020 ^[64]	Vertical transfer	A review study	USA	311	PRISMA	13	The probability of vertical transmission is unknown	"
48	Giulia Trippella <i>et al.</i> , 2020 ^[65]	Vertical transfer	A systematic review	-	-	PRISMA	15	Vertical transmission cannot be ruled out yet	"
49	Schwartz DA, 2020 ^[66]	Vertical transfer and pregnancy outcomes	A review study	China	-	PRISMA	12	There is no vertical transmission	"
50	E. Mullins <i>et al.</i> , 2020 ^[67]	Vertical transfer and pregnancy outcomes	A review study	-	-	PRISMA	13	There is no vertical transmission	"

Contd...

Table 2: Contd...

Row	Author	Aim of research	Type of the study	Population	Sample size	Data collecting	Quality	Results	Type of transmission
51	Andrea Carosso <i>et al.</i> , 2020 ^[68]	Vertical transfer	A review study	-	-	PRISMA	11	The risk of vertical transmission during vaginal delivery is currently unknown	"
52	Yan Chen <i>et al.</i> , 2020 ^[69]	Vertical transfer	Case series study	Wuhan, China	4	Clinical symptoms and abnormal radiologic or hematologic evidence	12	None of the babies tested positive	"
53	Na Li <i>et al.</i> , 2020 ^[70]	Vertical transfer and neonatal outcomes	Case-control	China	6	Control of WBCs, neutrophils, eosinophils, and CRP	16	Vertical transmission is not considered	"
54	Dongmei Cao <i>et al.</i> , 2020 ^[71]	Vertical transfer	Case report	Wuhan, China	1	Chest CT images and PCR for SARS-CoV-2	12	Vaginal delivery does not cause COVID-19 infection	"
55	Schwartz DA and Graham AL, 2020 ^[72]	Vertical transfer	A review study	-	-	PRISMA	12	There is no vertical transmission	"
56	Pablo Duran <i>et al.</i> , 2020 ^[73]	Vertical transfer	Systematic review	-	-	PRISMA	14	There is no vertical transmission	"
57	Gréssya Cavalcante de Melo and Araújo, 2020 ^[74]	Vertical transfer, low birth weight, and premature delivery	Systematic review and meta-analysis	-	-	PRISMA	13	Among 432 newborns, 10 cases with positive results for early SARS-CoV-2 were reported, and several reports indicated vertical transmission of SARS-CoV-2 to newborns with the occurrence of preterm delivery or birth weight of newborns	"
58	J. Juan <i>et al.</i> , 2020 ^[75]	Vertical transfer and neonatal-perinatal outcomes	Systematic review	-	-	PRISMA	12	There is no vertical transmission	"
59	Fadia Thamer Ahmed and Jabbar, 2020 ^[76]	Vertical transfer	Systematic review	-	-	PRISMA	12	There is no vertical transmission	"
60	Elicia Pettrosso <i>et al.</i> , 2020 ^[77]	Vertical transfer	A review study	-	-	PRISMA	12	Vertical transfer is possible	"
61	Feride Marim <i>et al.</i> , 2020 ^[78]	Vertical transfer and other perinatal complications	A review study	-	-	PRISMA	12	There is no clear evidence of vertical transmission of SARS-CoV-2 during childbirth	"
62	Petra Zimmermann <i>et al.</i> , 2020 ^[79]	Vertical transmission and perinatal and neonatal outcomes	A review study	-	-	PRISMA	11.5	Vertical transmission cannot be ruled out	"
63	Maria Claudia Alzamora, <i>et al.</i> , 2020 ^[80]	Vertical transfer	Case report	American	1	RT-PCR and immunoglobulin	14	The possibility of vertical transmission is seen	"

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Row	Author	Aim of research	Type of the study	Population	Sample size	Data collecting	Quality	Results	Type of transmission
64	Xiaolin Hu et al., 2020 ^[81]	Vertical transfer	Experimental	China	7	PCR test for SARS-CoV-2	11.5	Vertical transmission of SARS-CoV-2 infection from mothers with COVID-19 in the last days of pregnancy is possible	"
65	Sudip Sheth et al., 2020 ^[82]	Vertical transfer	A review study	-	-	PRISMA	14	There is minimal vertical transmission	"
66	Dani Dumitriu, et al., 2021 ^[83]	Vertical transfer	Retrospective cohort analysis	New York	101 neonates born and 100 mothers	Clinical evidence and PCR for SARS-CoV-2	15	There is no vertical transmission	"
67	Mohamed Joma et al., 2021 ^[84]	Vertical transfer	Systematic review	The population of England and the United States	-	PRISMA	14	There is a possibility of vertical transmission	"
68	Zheng He et al., 2021 ^[85]	Vertical transfer and infant kidney damage	Experimental	Wuhan, China	22	CT scan and SARS-CoV-2-IgM, IgG antibodies	13	Possibility of transmission of SARS-CoV-2 through the mother and the fetus	"
69	Roya Farhadi et al., 2021 ^[86]	Vertical transfer	Case report	Iran	1	RT-PCR for SARS-CoV-2	12	The newborn showed early infection with SARS-CoV-2	"
70	Rabia M. Palalioglu et al., 2021 ^[87]	Vertical transfer	Case report	Turkey	1	Samples from the placenta, amniotic fluid, cord blood, and postoperative breast milk	12	Vertical transfer is not possible	"
71	Hongwu Jing et al., 2021 ^[88]	Vertical transfer	Cohorts	United States	62	Samples included maternal blood, urine, amniotic fluid, cord blood, placenta, and fetal membrane tissue lysates	14	ENDOU may potentially play a role in the vertical transmission of SARS-CoV-2	"
72	K Kumari et al., 2021 ^[89]	Vertical transfer	An observational cross-sectional study	India	28 women, 29 neonates	RT-PCR test and neonate's nasopharyngeal swabs	12	There is no possibility of vertical transmission	"
73	K. Marie Douglass et al., 2021 ^[90]	Vertical transfer	Case series	California	2	RT-PCR swab IgG antibodies and CXR	10	Both infants had negative swabs, but one infant was positive for IgG antibodies to COVID-19	"
74	Maryam Rabiei et al., 2021 ^[91]	Vertical transfer	Case report	Iranian woman	1	Nasopharyngeal swab tests	10	The nasopharyngeal swab tests for COVID-19 were negative for the first and third babies, but positive for the second baby	"
75	Ibrahim A Abdelazim et al., 2021 ^[92]	Vertical transfer and premature birth	Case report	Kuwait	1	PCR swab	10	There is no reliable evidence of vertical transmission of COVID-19	"

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Row	Author	Aim of research	Type of the study	Population	Sample size	Data collecting	Quality	Results	Type of transmission
76	Paula Candice Alves De Assis Pereira et al., 2021 ^[93]	Vertical transfer	A review study			PRISMA	11	There is no evidence of vertical transmission of the coronavirus	"
77	Mehmet Yekta Oncel et al., 2021 ^[94]	Vertical transfer	Cohort	Turkey	120	RT-PCR test	15	4 infants of 120 infants (3.3%) had positive RT-PCR results	"
78	Gennady Sukhikh et al., 2021 ^[95]	Vertical transfer	Case report	Russia	1	Immunohistochemical analysis of placental tissue, umbilical cord blood, and baby blood	14	Demonstrate vertical transmission of SARS-CoV-2 from mother to fetus	"
79	Xin Lei Goh et al., 2021 ^[96]	Vertical transfer	Meta-analysis	-	-	PRISMA	11	There is a risk of vertical transmission of SARS-CoV-2	"
80	Bibechan Thapa et al., 2021 ^[97]	Vertical transfer	Case report and review	Nepal	1	PRISMA and RT-PCR for SARS-CoV-2	10	Vertical transmission is possible in COVID-19	"
81	J. J. Hijona Elosegui et al., 2021 ^[98]	Vertical transfer	Experimental	Spain	4	RT-PCR for SARS-CoV-2	14	SARS-CoV-2 cannot pass from an infected mother to the amniotic fluid	"
82	S. Paramanathan et al., 2021 ^[99]	Vertical transfer	Case report	Denmark	1	RT-PCR for SARS-CoV-2	12	No vertical transmission occurred	"
83	Lemi Belay Tolu et al., 2021 ^[100]	Vertical transfer	A review study	-	-	PRISMA	16	15 people (4.4%) were RT-PCR positive in terms of throat swab, five people (33.3%) had placenta, amniotic fluid, and umbilical cord blood samples at the same time, and only one amniotic fluid sample was positive for RT-PCR. Five infants had elevated IgG and IgM. Four infants had chest imaging suggestive of COVID-19 pneumonia	"
84	Carbayo-Jiménez et al., 2021 ^[101]	Vertical transfer	Case report	Denmark	1	Nasopharyngeal swab and the antibodies for SARS-CoV-2	12	This is a case of vertical transmission of SARS-CoV-2	"
85	Huijun Chen et al., 2021 ^[2]	Vertical transfer	Retrospective	China	9	Clinical records, laboratory results, and chest CT scans	12	There is no evidence of vertical transmission	"
86	Xiali Xiong et al., 2020 ^[102]	Vertical transfer	A review study	-	-	PRISMA	14	There is no evidence of vertical transmission	"
87	Shobo Sheikahmadi et al., 2021 ^[103]	Vertical transfer	Case series	Iran	8	t CT scan and CRP and PCR test	13	There is a possibility of vertical transmission	"
88	Al Asadi J and Mousa SM, 2021 ^[104]	Vertical transfer	A review study	-	-	PRISMA	14	There is a possibility of vertical transmission	"

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Row	Author	Aim of research	Type of the study	Population	Sample size	Data collecting	Quality	Results	Type of transmission
89	Ritu Sharma et al., 2021 ^[105]	Vertical transfer	Retrospective and prospective	India	44	Samples of cord blood, placental membrane, vaginal fluid, amniotic fluid, peritoneal fluid, and breast milk for COVID-19 reverse	12	There is a possibility of vertical transmission, but it is insignificant	"
90	Jianghui Cai et al., 2021 ^[106]	Vertical transfer	A review study	-	-	PRISMA	18	There is no sufficient evidence supporting that the cesarean section in preventing possible vertical transmission	"
91	Mendoza-Hernández M et al., 2021 ^[107]	Vertical transfer	Case report	Mexico	1	RT-PCR	10	Vertical transmission is very likely in this case	"
92	Mariane de Fátima Yukie Maeda et al., 2021 ^[108]	Vertical transfer	Cohort	Brazil	1044	Biological samples: cord blood, amniotic fluid, colostrum, and/or oropharyngeal swab of the neonate	15	There is a possibility of vertical transmission and it depends on a shorter interval between maternal symptoms and delivery is the influencing factor	"
93	Shahla Chaichian et al., 2021 ^[109]	Vertical transfer	Case series study	Iran	14	Computed tomography (HRCT) or RT-PCR tests	14	There is no evidence of vertical transmission	"
94	Selcan Sinaci et al., 2021 ^[110]	Vertical transfer	A prospective cross-sectional study	Turkey	63	RT-PCR tests and blood tests for antibodies	15	SARS-CoV-2 can be detectable in the placenta or vaginal secretions of pregnant women	"
95	Miriam Lopian et al., 2021 ^[111]	Vertical transfer	Cohort study	Israel	21	RT-PCR	16	There is no evidence of vertical transmission and increasing the risk of neonatal infection	"
96	Vandana Gupta et al., 2022 ^[112]	Vertical transfer	A hospital-based retrospective observational study	Ayodhya	37	RT-PCR and clinical signs	15.5	There is no evidence of vertical transmission, but SARS-CoV-2 infection during late pregnancy may have a maternal and neonatal impact	"
97	Deniz Akyıldız & Zühal Çamur, 2022 ^[113]	Vertical transfer	A case-control study	Turkey	202	Nasopharyngeal swab samples	15	There was no vertical contamination according to the nasopharyngeal swab samples of the newborns	"
98	Marzieh Zamaniyan et al., 2023 ^[114]	Vertical transfer	Case series study	Sari, Iran	7	PCR for amniotic fluid and neonatal throat samples	16	The prevalence of preterm labor, admission to the neonatal intensive care unit, and vertical transmission were significantly high in pregnant women with COVID-19 and positive PCR results for amniotic	"

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Row	Author	Aim of research	Type of the study	Population	Sample size	Data collecting	Quality	Results	Type of transmission
99	Neela Sunuwar <i>et al.</i> , 2022 ^[115]	Vertical transfer	Retrospective cross-sectional study	Nepal	104	RT-PCR and clinical signs	15	No significant differences were found in the outcomes of NVD and C/S	"
100	Petra Zimmermann <i>et al.</i> , 2020 ^[79]	Perinatal and neonatal consequences of COVID-19	A review study	Australia	-	PRISMA	12	Vertical transmission could not be excluded	Vaginal secretions to the baby's blood
101	Radu Marian Florea and Sultana, 2021 ^[116]	COVID-19 in breast milk	A review study	Romania	-	PRISMA	16	Vertical transmission through breast milk seems unlikely	Transmission through breast milk (study from 101 to 120)
102	Ceulemans M <i>et al.</i> , 2021 ^[117]	COVID-19 in breast milk and during pregnancy	A review study	Netherlands	-	PRISMA	14	There is currently no conclusive evidence of absence of intrauterine transmission of the virus; the virus has not been detected in breastmilk in most studies, although passage into breastmilk cannot be completely excluded	"
103	X. Gao <i>et al.</i> , 2020 ^[118]	COVID-19 in the milk of mothers with COVID-19	Observational	China	14	Clinical characteristics and serial specimens of the mother–infant	15	No neonatal infections were observed. No infants developed COVID-19 during breastfeeding	"
104	Elizabeth Centeno-Tablante, <i>et al.</i> , 2020 ^[119]	COVID-19 in breast milk and the breastfeeding process	Meta-analysis	New York	-	PRISMA	13	Currently, there is no evidence of SARS-CoV-2 transmission through breast milk	"
105	Marcelino Pérez-Bermejo <i>et al.</i> , 2021 ^[120]	COVID-19 and its relationship and impact on breast milk	A systematic review	Spain	-	PRISMA	12	Breastfeeding by mothers infected with SARS-CoV-2 is highly recommended for infants	"
106	Christine Bäuerl, <i>et al.</i> , 2022 ^[121]	COVID-19 in antibody in the milk of mothers with COVID-19	A retrospective longitudinal study	Spain	60	SARS-CoV-2- specific immunoglobulins in breast milk samples	13	The safety of breastfeeding highlights the relevance of transmission of virus-specific SARS-CoV-2 antibody	"
107	Ummu Aiman <i>et al.</i> , 2021 ^[122]	COVID-19 in breast milk	A review study	Indonesia	-	PRISMA	11	Vertical transmission through breast milk is unlikely	"
108	Emilia Vassilopoulou, <i>et al.</i> , 2021 ^[123]	Breastfeeding in the COVID-19 pandemic	A systematic review	Greece	--	PRISMA	12	Information is currently available on the transmissibility of SARS-CoV-2 during breastfeeding	"
109	Yvonne Peng Mei Ng, <i>et al.</i> , 2020 ^[124]	Breastfeeding during the COVID-19 pandemic	Review study	Singapore	-	PRISMA	14	The evidence and knowledge about COVID-19 and breastfeeding are still evolving	"

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Row	Author	Aim of research	Type of the study	Population	Sample size	Data collecting	Quality	Results	Type of transmission
110	X. Gao et al., 2020 ^[118]	Immunological benefits of breast milk in patients with COVID-19	Observational	China	14	Clinical characteristics and serial specimens of the mother–infant	15	Continuing breastfeeding with prudent precautions is encouraged	"
111	Jia Ming Low et al., 2022 ^[125]	COVID-19 antibodies in breast milk	A systematic review	Singapore	-	PRISMA	12	Human milk of lactating individuals after COVID-19 infection contains anti-SARS-CoV-2- specific IgG, IgM, and/or IgA, even after mild or asymptomatic infection	"
112	Hethyshi R, 2020 ^[126]	Breastfeeding in possible cases of COVID-19	Narrative review	India	-	PRISMA	10	Currently, there is no universal consensus on managing the issue of breastfeeding with rooming-in of the neonates	"
113	Augusto Pereira et al., 2020 ^[127]	Breastfeeding a baby by a mother infected with COVID-19	Case series	Spain	22	Observation and RT-PCR	15	Breastfeeding in newborns of mothers with COVID-19 is safe	"
114	Kimberly A. Lackey et al., 2020 ^[128]	COVID-19 in breast milk	A systematic review	America	-	PRISMA	12	Breastfeeding in newborns of mothers with COVID-19 is safe	"
115	Fuxing Lou, et al., 2021 ^[129]	Benefits and risks of breastfeeding in cases of transmission risk	Review study	China	-	PRISMA	11	Human breastfeeding should be encouraged	"
116	Weima Lubbe et al., 2020 ^[130]	COVID-19 and Breastfeeding	A review study	South Africa	-	PRISMA	13	Breastfeeding should be encouraged	"
117	Hillary L. Fry et al., 2020 ^[131]	COVID-19 in breast milk	Cross-sectional	Canada	335	Observation	14	There is no evidence of COVID-19 transmission through breastfeeding	"
118	Hayder M. Al-Kuraishy et al., 2021 ^[132]	COVID-19 in breast milk	A systematic review	Iraq	-	PRISMA	12	International organizations encourage breastfeeding	"
119	Bgee Kunjumon et al., 2021 ^[133]	COVID-19 in breast milk	Prospective cohort study	Brooklyn	19	Breast milk samples	15	Breast milk samples from 18 mothers tested negative for SARS-CoV-2, and one was positive for SARS-CoV-2 RNA	"
120	Gyanesh Mishra et al., 2022 ^[134]	COVID-19 in breast milk	A case series	Bhilai, Chhattisgarh	1	SARS-CoV-2 from nasal swab	16	*No detection of SARS-CoV-2 seen from the neonates From the nasopharyngeal swabs *No transmission of SARS-CoV-2 from breast milk to the neonates	"

RT-PCR: Reverse transcription polymerase chain reaction, HCoV-229E: Human coronavirus 229E, RNA: Ribonucleic acid, ACE2: Angiotensin-converting enzyme 2, TMPRSS2: Transmembrane serine protease 2, WBC: white blood cells, CRP: C-reactive protein, CT: Computed tomography, ENDOU: Endonuclease, poly(U) specific, CXR: Chest radiography, NVD: normal vaginal delivery, C/S: Caesarean section

extensive studies. Sexual contacts such as oral-anal contact play a role in the transmission of COVID-19. In favor of the above finding, even in Carosso A *et al.* (2020), the risk of virus transmission in feces during childbirth was mentioned.^[168] Some of this result of the search is in favor and some is in opposition to each of the research aims. In this part, the researcher categorized and emphasized b) vertical transmission to the fetus. This research finding showed in Fornari's^[147] study (2020) that some children born to mothers infected with COVID-19 at the end of pregnancy and near the time of delivery tested positive for COVID-19, but there was no transmission. This issue has been confirmed in the studies of Yuan J *et al.* (2021) and Najafi, T. F *et al.* (2021).^[148,149] Special attention is still being paid to the fact that women are not infected with COVID-19 at the time of trying to get pregnant and are not to be infected in the first trimester of pregnancy.^[150] This is opposed to De Luca's^[151] (2023) study that mentions the possibility of transmission of COVID-19 via pregnancy. Although Goulioumis A *et al.*^[152] (2022) have reported that the intrauterine infection remains controversial, anyway the expression of the ACE2 receptor on the placenta, the detection of IgM antibodies, and the COVID-19 genome in fetuses make the vertical transmission tenable. However, this article based on Hu X *et al.* (2020) showed in their findings that the vertical transmission of SARS-CoV-2 infection from mothers infected with COVID-19 in the last days of pregnancy is possible, but it is relatively low, and one baby of seven was infected (14.3%).^[81] The findings of Sukhikh G *et al.*^[95] (2021) showed that PCR and immunohistochemical analysis of placental tissue, umbilical cord blood, and child's blood jointly showed vertical transmission of SARS-CoV-2 from mother to fetus. In connection with the possibility of vertical transmission of COVID-19, the review of 27 articles shows the absence of vertical transmission of this virus through the birth canal and amniotic sac from mother to fetus and newborn.^{[2,62,63,66-71,73,75-78,83,84,87-89,92,93,98,99,102,106,10}

^{9]} In favor of the above finding, Kim YK and Kim EH *et al.*^[153] (2023) showed that vertical transmission in pregnancy (every trimester) is rare. This finding is consistent with that of Muhidin S *et al.*^[154] (2020), who reported that although the rate of cesarean delivery, preterm delivery, simultaneous multi-organ involvement in the pregnant mother, and the chance of hospitalization in the intensive care unit increased, no vertical transmission from mother to baby was reported. However, in this research, Zamaniyan M *et al.*^[114] (2023) showed that the high risk of maternal and neonatal outcomes of COVID-19 during pregnancy and the high probability of vertical transmission were seen. This report is consistent with the study by Melo GCd, and Araújo KCGMd^[74] (2020) that showed that of 432 newborns born to mothers infected with COVID-19,

10 cases had positive results for primary SARS-CoV-2. Also, some reports indicated the vertical transmission of SARS-CoV-2 to newborns. The other studies, such as Alzamora MC *et al.* (2020), reported a severe case of COVID-19 that was delivered by cesarean section, the baby was immediately separated, and the baby's PCR test was positive.^[80] Some of this result in this search are in favor and some are in opposition to each of the research aims. In this part, the researcher had categorized and emphasized c) transmission to the newborn through the birth canal. Zimmermann P and Curtis N^[79] (2020), by reviewing 11 articles and examining 333 babies, completely reject the possibility of virus transmission from vaginal secretions to the baby's blood causing septicemia. In vaginal secretions, COVID-19 has rarely been seen. In the study by Wu *et al.*^[17] in 2020, during further tests and investigations of mothers with COVID-19 and their babies, transmission to the baby through secretions of the birth canal was ruled out. In this regard, studies were conducted on the possible effect of elective cesarean delivery on reducing transmission through birth canal secretions and improving neonatal outcomes, but the elective cesarean section was not recommended in patients with COVID-19.^[106] In the same way as this research finding, Yanting Wu *et al.*^[155] (2020) suggested that vaginal delivery can be safe, and it does not seem that virus contamination happens through the vaginal secretion. Some of this result of the search is in favor and some is in opposition to each of the research aims. In this part, the researcher had categorized and emphasized d) transmission through breast milk.^[81,147] Transmission through breast milk was investigated, and no transmission was seen. It seems that the nursing mother infected with COVID-19 infects the baby through her respiratory droplets, rather than the virus being transmitted through breast milk.^[116,120-123,125-128] However, vaccination during the breastfeeding period cannot cause serious complications for the baby.^[156,157] However, inter-departmental transfer of pregnant mothers or transferring them to other related departments for continuing care is associated with an increase in the possibility of contact between pregnant mothers, nursing mothers, and babies with the virus. Therefore, it is recommended to consider this issue in midwifery care.^[154,158-162] The results of this study are in line with the results of Koç and Dilli^[163] (2022) who highly advise breastfeeding while using proper infection control methods. The most important limitation of the plan was not having sufficient information or studies in the field of all aspects of the transmission of COVID-19 due to its emerging nature. Another limitation of this study was not mentioning the detailed research information in the selected articles. Cohort studies in this field did not exist at the beginning of the researchers' work, and if they existed, they would have provided the possibility

of more powerful arguments. The information obtained from the articles is generally retrospective in nature, and the researchers hope that this research will pave the way for future research in the field of emerging diseases.

Conclusion

The results of this systematic review study, which was conducted with the aim of investigating the mode of transmission of COVID-19 through sexual secretions and the birth canal, showed that COVID-19 is not transmitted through vaginal secretions or ejaculate, but rectal secretions can be considered a route of transmission, which requires more extensive studies. Oral–anal sexual relations have played a vague role in the transmission of COVID-19 and have been reported in the form of uncertainty. Reproductive outcomes, including male sex hormone secretion disorders, oligospermia and azoospermia, and sperm quality, have been mentioned in studies. However, the viral attack on women’s reproductive system and other direct and indirect effects on men’s reproductive system in the long term are unclear and require a more comprehensive investigation. It is not possible to transfer COVID-19 from vaginal secretions to the baby’s blood and cause septicemia, vertical transmission, transmission through the birth canal and water sac, and transmission through the flow of breast milk, but there is a need for a more comprehensive investigation regarding the risk of transmission through contaminated feces to the baby during the delivery process. The most important limitations of the study were not having enough information or studies on all aspects of the transmission of COVID-19, not mentioning detailed research information in the selected articles, and lack of cohort studies on the aspects investigated by the researchers. According to the results of the research, it is necessary to put all-round investigations in the field of possible ways of disease transmission and public education about definite ways of transmission on the agenda of healthcare workers.

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Conflicts of interest

There are no conflicts of interest.

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