Mother-to-child transmission of SARS-CoV-2: review of reviews, systematic review and meta-analysis

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A thesis submitted to the University of Birmingham for the degree of M.Sc. in Metabolism and Systems research

Submitted: March 2022

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Abstract

Objective: To conduct a review of reviews on existing systematic reviews reporting on mother-to-child transmission (MTCT) of SARS-CoV-2 and to review the existing classification systems of MTCT. To assess the rates of SARS-CoV-2 neonatal positivity in babies born to SARS-CoV-2 infected mothers and the rate of vertical transmission if any and to determine the risk factors associated with offspring SARS-CoV-2 positivity.

Design: Review of reviews, Systematic review and meta-analysis

Results: From the 68 included systematic reviews, the review of reviews was able to show the lack of use of a classification system to ascertain the timing of SARS-CoV-2 transmission. Systematic reviews need to consider previously published research to avoid data duplication and minimize research waste. The World Health Organization (WHO) classification system provides robust and detailed information to classify babies born to mothers with SARS-CoV-2 infection. Of the included 472 studies, the overall rate of babies born to mothers with SARS-CoV-2 infection was 1.8%. Fourteen babies out of 592 had confirmed mother-to-child transmission, with seven *in utero,* two intrapartum and five in early postnatal period.

Conclusion: High-quality systematic reviews are needed in order to synthesis evidence allowing the determination of the true extent of vertical transmission of SARS-CoV-2. The WHO standardised definition and categorisation system allows the evaluation of timing and routes of possible transmission. The rates of SARS-CoV-2 positivity are low in babies born to SARS-CoV-

2 infected mothers. While there is evidence for vertical transmission, the occurrence is rare and the risk factors of neonatal positivity do not appear to be associated with breastfeeding, rooming-in or mode of delivery.

Acknowledgements

I would firstly like to offer my deepest appreciations to Professor Shakila Thangaratinam for allowing me to be part of such an incredible research project. Thank you for making me realise how exciting research can be.

I would also like to thank Dr John Allotey, JS, HL, OA and the rest of the PregCov team for their continued guidance and support.

Finally, I want to thank my parents, especially my mother for her words of encouragement and care.

Let's hope my next research venture is just as exciting as this one has been!

Publications associated with this Thesis

Ansari K, Kew T, Allotey J, Thangaratinam S. Mother-to-child transmission of severe acute respiratory syndrome coronavirus 2: review of classification systems and systematic reviews. Current Opinion in Obstetrics & Gynecology. 2021;33(5):391-399. doi:10.1097/GCO.0000000000000742

Allotey J, Stallings E, Bonet M, Yap M, Chatterjee S, Kew T, Debenham L, Clavé Llavall A, Dixit A, Zhou D, Balaji R, Ing Lee S, Oiu X, Yuan M, Coomar D, Sheikh J, Lawson H, **Ansari K**, van Wely M, van Leeuwen E, Kunst H, Khalil A, Tiberi S, Brizuela V, Broutet N, Kara E, Rahn Kim C, Thorson A, Escuriet R, Oladapo OT, Mofenson L, Zamora J, Thangaratinam S. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis *BMJ* 2020; 370 :m3320 doi:10.1136/bmj.m3320

Yap M, Debenham L, Kew T On behalf of PregCOV-19 Consortium, *et al.* Clinical manifestations, prevalence, risk factors, outcomes, transmission, diagnosis and treatment of COVID-19 in pregnancy and postpartum: a living systematic review protocol *BMJ Open* 2020;**10:**e041868. doi: 10.1136/bmjopen-2020-04186

Table of abbreviations

ACE 2	Angiotensin-converting Enzyme 2
ARDS	Acute Respiratory Distress Syndrome
CNKI	China National Knowledge Infrastructure
COVID-19	Coronavirus Disease 2019
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LSR	Living Systematic Review
MERS	Middle East Respiratory Syndrome
MTCT	Mother-To-Child Transmission
PICO	Population, Intervention, Comparison, Outcome
RCOG	Royal College of Obstetricians and Gynaecologists
RT-PCR	Real-time Reverse Transcription Polymerase Chain Reaction
SARS	Severe Acute respiratory syndrome
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus-2
TMPRSS2	Transmembrane Protease Serine 2
WHO	World Health Organization

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1. Background

1.1 SARS-CoV-2 and COVID-19

1.1.1 Global burden of SARS-CoV-2 in general population and pregnant women

Coronavirus disease 2019 (COVID-19) was first identified in Wuhan, China (1,2). Coronaviruses are large enveloped single-stranded RNA viruses and are a family of viruses that can cause less severe diseases such as the common cold (3), or more severe diseases like the Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS) (1,4). SARS-CoV-2 is the third coronavirus to cause severe disease; the first coronavirus was SARS, followed by MERS (5). An earlier outbreak of SARS occurred in 2003, where genomic sequencing shows that SARS and SARS-CoV-2 have a 70 to 85% genomic similarity (4,6).

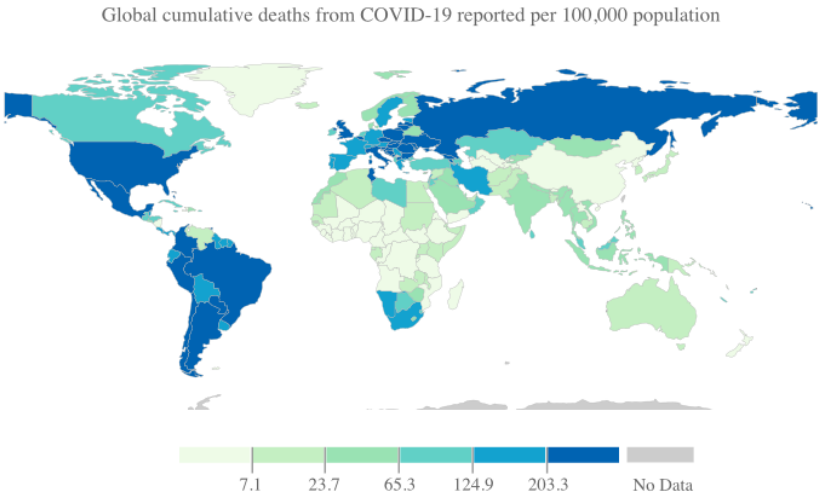

Figure 1: Image taken from https://covid.cdc.gov/covid-data-tracker/#global-counts-rates to depict the cumulative deaths worldwide caused by COVID-19.

SARS-CoV-2 first emerged in December 2019 and spread at an exponential rate globally (7). The COVID-19 outbreak was declared a global pandemic by the World Health Organization (WHO) on March 11th, 2020, (8). As of March 11th, 2022, the WHO COVID-19 dashboard reported 450,229,635 confirmed cases of COVID-19 and 6,019,085 deaths worldwide (9) **(Figure 1).**

Research on COVID-19 has shown that specific individuals are at higher risk of a more severe disease presentation than others (10). Pregnant women are in the high-risk group, due to the impact of COVID-19 on maternal and neonatal health, during or after pregnancy (11).

Thus far, numerous publications have reported on the adverse maternal and neonatal outcomes associated with COVID-19 during pregnancy (12). One such publication has reported 124 deaths in Brazil from February 2020 to June 2020 (13). While some of the research remains inconclusive, it is evident that there is a greater need for understanding the risks associated with COVID-19 in pregnancy to ensure effective clinical measures are actioned to better maternal and neonatal health.

1.1.2 Pathophysiology

COVID-19 in general population

The spread of COVID-19 globally has impacted morbidity and mortality worldwide. The clinical spectrum of COVID-19 ranges from mild, moderate to severe illness (14). Patients infected with COVID-19 can either be asymptomatic (display no symptoms but still carry the disease) or present symptomatically (14,15). The disease symptoms of COVID-19 in the general population are discussed further in section 1.1.3.

COVID-19 affects the upper and lower respiratory tract (16). The lungs are primarily affected as the virus accesses host cells through a receptor for the enzyme 'Angiotensin-Converting Enzyme' (ACE2) (**Figure 2**) (17). SARS-CoV and SARS-CoV-2 bind to ACE2 receptors and infect human cells (18). ACE2 receptors are located in abundance on the surface of type II alveolar cells of the lung (17). They are also expressed on the ciliated epithelium of the nasopharynx and upper respiratory tract (19) . The expression of ACE2 receptors is gradient in the respiratory tract, where expression is higher in the upper respiratory tract (19) .

The mode of action of the ACE2 receptors causes viral spike proteins to undergo proteolytic cleavage. This cleavage is catalysed by transmembrane protease serine 2 (TMPRSS2), which act as a hold membrane-anchored protein (20) The TMPRSS2 undergoes a conformational change in the spike protein, allowing membrane fusion of the host and virus. This, in turn, allows the virus

to release its RNA genome into the host cell to begin replication following a spike-mediated fusion process (15).

The risk of a more severe disease outcome of COVID-19 is increased in those with compromised immune systems, individuals with cardiovascular disease, the elderly population and pregnant women (21).

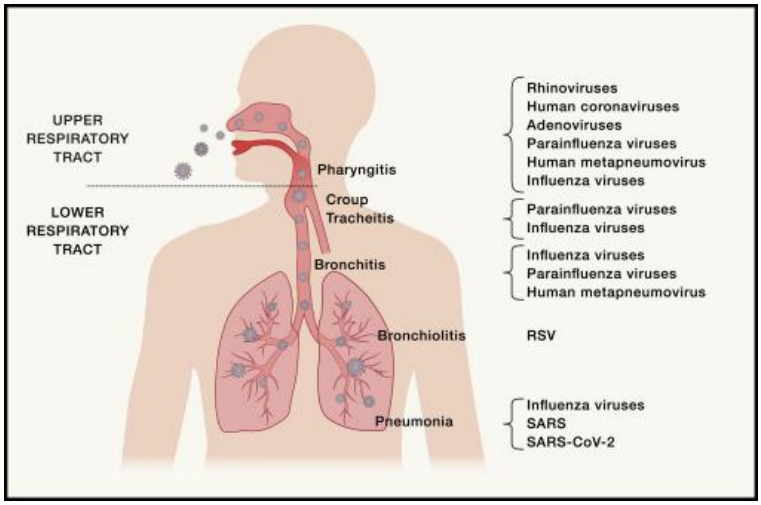

Figure 2: Image taken from https://www.sciencedirect.com/science/article/pii/S1074761320302120. An anatomical representation of the main organ target sites of COVID-19.

The most severe clinical characteristic of COVID-19 is the development of Acute Respiratory Distress Syndrome (ARDS) (22). ARDS is caused due to the release of excessive cytokines because of COVID-19 infection. Cytokines are small proteins necessary for cell signalling and play an essential role in self-defence against any infection, i.e., a cytokine storm (23). A cytokine storm is when the body suddenly releases an abundance of cytokines into the bloodstream at a quick rate (24), which tends to be due to a response to infection, disease or an autoimmune condition. As a

result, this causes a drastic increase in leukocyte recruitment to multiple body organs, specifically lung cells, leading to severe or life-threatening conditions or lead to ARDS (23,25). ARDS prevents sufficient oxygen from crossing the alveoli into the blood (26). As a result, patients affected by ARDS are put onto mechanical ventilators to increase oxygenation to the lungs (23).

COVID-19 and Pregnancy

COVID-19 can cause respiratory symptoms in all populations, such as cough, shortness of breath, and difficulty breathing (27,28). However, pregnant women infected with the virus have additional concerns about pregnancy and the health of their neonates. The main one is the risk of mother-to-child transmission (MTCT) of SARS-CoV-2 via the placenta and maternal body fluids during delivery and breastfeeding (29,30). The increased concerns are due to the occurrence of previous viruses causing congenital birth syndromes in neonates through mother-to-child transmission. Additionally concerns include ICU admission, maternal death, preterm birth and invasive ventilation.

Current studies provide inconclusive evidence of the exact extent of the virus's impact on pregnant women. However, some studies have shown an increased risk of more severe COVID-19 complications in pregnant women compared to non-pregnant women (11,29,31).

Pregnant women undergo various physiological changes to accommodate the growing foetus in the uterus (gravid uterus) (31). These physiological changes can impact almost every organ system, including; respiratory, immune and cardiovascular systems (31,32) **(Figure 3).**

The impact of modulations of the maternal immune system means it may affect the body's response to fighting infections, including viruses (31,33). Thus, it could make the mother more susceptible to catching the virus and have more severe complications (32,33). As well as immunological modulations, anatomical changes also occur in the respiratory system (31,32,34). Diaphragmatic splinting is the process of alterations to the chest shape and elevation of the diaphragm by the gravid foetus; this, in turn, causes changes in respiratory function (35,36). This reduction in chest volume results in a decreased functional residual capacity (31). The reduction of total lung capacity and the inability to clear secretions effectively can increase a pregnant woman's susceptibility to severe respiratory infections (31,35). Changes also occur in the cardiovascular system, where the cardiac output increases by 40% to 50% which can cause left ventricular hypertrophy in pregnancy (35).

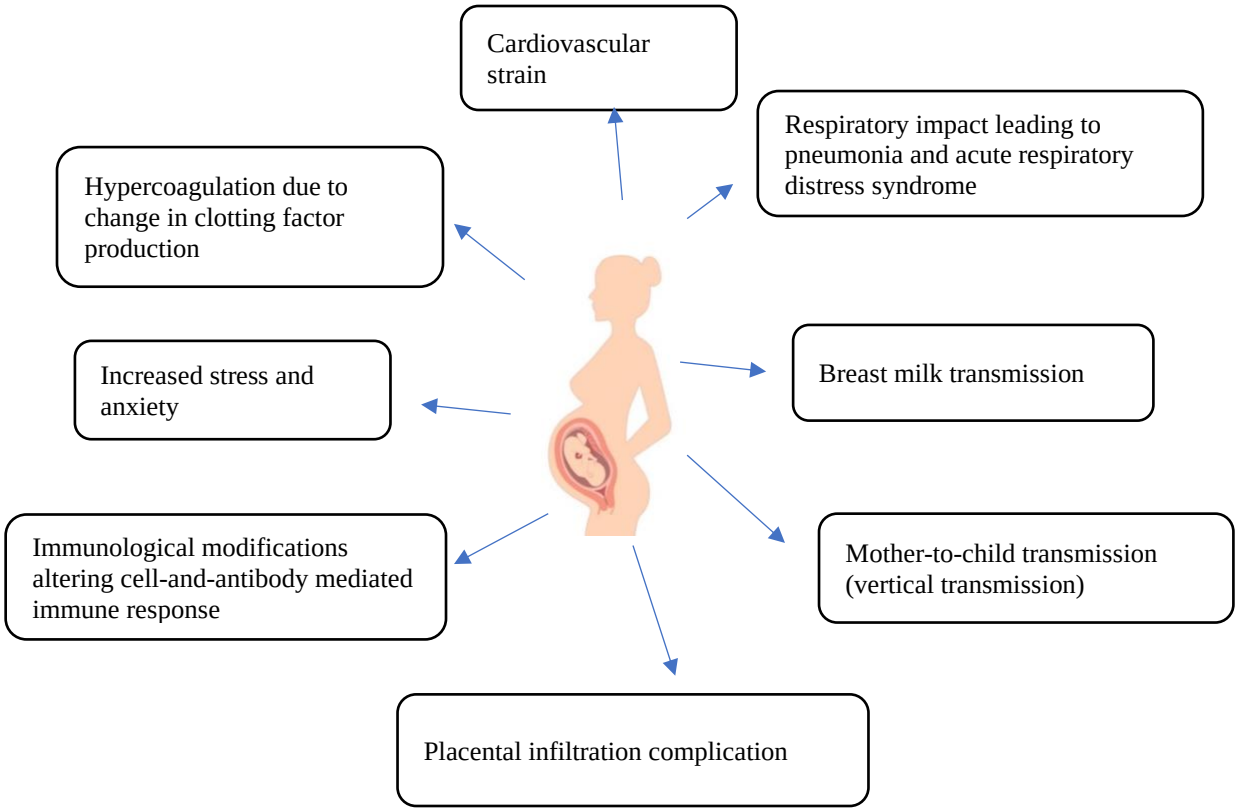

Figure 3: Image adapted from Wastnedge et al. (31) *. The range of physiological changes during pregnancy impacting Coronavirus disease.*

Mother-to-child transmission is facilitated by vertical transmission, which can occur at three-time points; *in utero*, intrapartum and postnatally (11,37). Existing literature on vertical transmission of SARS-CoV-2 and its implications on foetal development and neonatal outcomes is limited and remains to be discerned. In the past, there have been reports of transplacental, intrapartum and breast milk transmission for several infectious pathogens discussed in section 1.2.4.

Further details on MTCT are given in section 1.3.

1.1.3 Clinical presentation of SARS-CoV-2

The common symptoms of COVID-19 include fever (temperature above 38 degrees Celsius or higher), muscle pain, cough, headache, sore throat and a loss of taste (ageusia) or smell (anosmia) (38,39). In the more severe disease instances, due to overwhelming lung infections, the sign of difficulty in breathing arises due to pneumonia (40) (41). In many of the first reported cases of COVID-19, the symptoms described correlated with viral pneumonia (41).

The symptom presentation of COVID-19 can range from mild disease with coughs and cold to asymptomatic presentation with no symptoms, but patients are still carriers of the infection (42).

The disease presentation of SARS-CoV-2 seems multi-systemic; while research efforts are underway, the reason for this diversity in presentation remains unclear (43).

1.1.4 Transmission of SARS-CoV-2

General population

The dominant transmission mode of SARS-CoV-2 is a respiratory transmission. The respiratory tract of infected individuals releases aerosol droplets or large droplets on which virions suspend (44). Droplets are particles larger than 5 µm, and aerosol droplets are smaller than 5 µm; aerosol

droplets are able to persist in the air for prolonged periods (44–47). In areas with poor ventilation, these particles can be expelled in exhaled breath and transmitted to other individuals residing in close proximity (48). The understanding that respiratory transmission is the primary mode of transmission is supported by evidence showing that wearing masks and implementing a two-metre social distance, significantly reduced the risk of transmission (46,47).

SARS-CoV-2 has also been detected in blood, faeces, and urine, but whether the virus is active and able to infect at a significant rate needs to be researched further (49).

Details on transmission from mother to the foetus are given in section 1.3.

1.1.5 Tests to detect SARS-CoV-2

The current gold standard for SARS-CoV-2 testing is the method of Reverse Transcription Polymerase Chain Reaction (RT-PCR) (38). A Nucleic Acid Amplification Test (NAAT) is used in RT-PCR tests to amplify nucleic acids (genetic material) and detect the virus (50). These are high sensitivity and high specificity tests for diagnosing SARS-CoV-2 infection. The test detects one or more viral ribonucleic acid (RNA) genes and indicates whether the individual is currently infected or was recently infected (51).

Additionally, antigen tests are also used which are immunoassays that detect the presence of a specific viral antigen (52). The test also requires a nasopharyngeal swab specimen. The specificity is the same as NAATs but is less sensitive (50,52), however, results are provided within minutes and can aid as effective screening programs.

Antibody testing is a different diagnostic method of SARS-CoV-2, where serological assays are used to detect antibodies produced by the human body in response to infection with SARS-CoV-2 (50,51). According to the WHO brief on diagnostic testing for SARS-CoV-2, antibody testing should not be used as a standalone diagnostic method (53). Any interpretations should be made by an expert and should consider additional factors like timing of the disease, clinical morbidity, the epidemiology and prevalence, type of test used, validation method and the reliability of the results (53).

Testing during pregnancy for COVID-19 is given in section 1.3.

1.2 COVID-19 and pregnancy

Current studies have shown an increased risk of severe COVID-19 complications in pregnant women when compared to the general population (29). Therefore, clinicians and researchers need to determine whether there is a possibility of mother-to-child transmission with SARS-CoV-2 and, if so, recognise the extent and what protective measures need to be taken to protect both mother and child.

1.2.1 Presentation in pregnancy

The clinical presentation of COVID-19 in pregnancy differs slightly from the general non-pregnant population (54). The Royal College of Obstetricians and Gynaecologists have stated that two-thirds of pregnant women with COVID-19 have no symptoms at all, and those that do manifest symptoms have very mild, flu-like symptoms (11). A study by Allotey *et al.* (29) supports this, stating that pregnant and recently pregnant women were less likely to manifest symptoms such as fever, cough, myalgia or dyspnoea. However, pregnant women with COVID-19 are twice as likely to have early labour, thus exposing their new-born to prematurity (11,29). Reports have also summarised that affected pregnant women were at a higher risk for ICU admission or invasive ventilation (55).

Furthermore, pregnant women with pre-existing comorbidities are at an increased risk of developing severe COVID-19 symptoms, requiring hospitalisation. These risk factors include non-

white ethnicity, pre-existing comorbidities, pre-existing diabetes, high maternal age, high body mass index and chronic hypertension (29,55).

1.2.2 COVID-19 and clinical outcomes

The prevalence of SARS-CoV-2 in pregnancy has been studied by Allotey *et al.* (29) , where the study reported that 1 in 10 pregnant women and recently pregnant women attending or admitted to hospital for any reason test positive for COVID-19 (29). This study (29) supports that pregnant women are at an increased risk of severe COVID-19 complications than non-pregnant women with COVID-19. Also, pregnant women are no more likely to get COVID-19 than other healthy adults but are at an increased risk of becoming severely unwell if they contract COVID-19, leading to complications like preterm or stillbirths (56).

A protocol by Yap *et al.* (30) reported the outcomes of COVID-19 in pregnant women. The findings showed that 4% of pregnant women with COVID-19 were admitted to an intensive care unit, 3% required invasive ventilation and 0.2% required extracorporeal membrane oxygenation (ECMO) (30). The study compared these clinical outcomes in pregnant women to the non-pregnant population with COVID-19 and found a higher incidence of these outcomes in pregnant women with COVID-19 (30).

1.2.3 Vertical transmission

COVID-19 in pregnancy raises the concern of whether the mother can pass COVID-19 to her offspring during or after pregnancy. This process is called vertical transmission and is defined as transmitting an infectious pathogen from mother to child, *in utero*, intrapartum or postnatally (11,37,57).

The relevance of understanding if mother-to-child transmission is possible with SARS-CoV-2 is vital as previous viruses like ZIKA, CMV, and HSV have been shown to severely impact the foetus by causing congenital birth deformities like microcephaly (58–60).

While transmission of the pathogen from mother to child can be vertical, it is also possible for horizontal transmission to occur. Here, the infant could contract the virus through contact with infected caregivers, healthcare workers, or breastfeeding and rooming-in with suspected or positive COVID-19 mothers (61) **(Figure 4).**

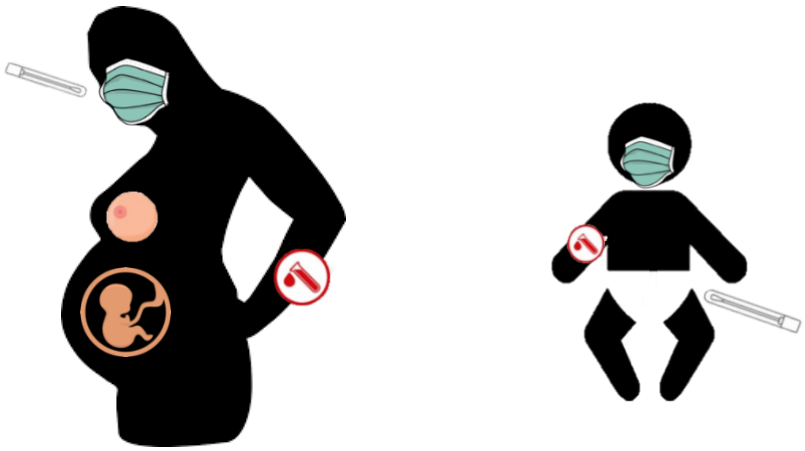

Figure 4: Vertical transmission of mother-to-child transmission – infection can be tested in both mother and child via blood samples and swabs of various maternal and neonatal serology. MTCT can occur in-utero, intrapartum or postnatally. Through swabbing neonatal samples like stool and nasopharyngeal samples, the presence of viral RNA could be determinant of MTCT of SARS-CoV-2.

1.2.4 Vertical transmission during pregnancy: other viruses

Vertical transmission of viruses has been seen in past decades with pathogens such as cytomegalovirus (CMV), herpes simplex virus (HSV), *Toxoplasma gondii*, Zika virus (ZIKV) (58,59,62,63). These viruses have been shown to cause congenital syndromes in neonates - the severity of which depends on the stage of pregnancy (58,62) **(figure 5).**

The placenta plays an active role in ensuring that infections from the mother do not get passed to the foetus (64). However, some viruses have been able to circumvent this placental barrier and cause congenital birth syndromes in newborns, e.g., CMV, MERS, ZIKV, all of which caused

congenital syndromes in the newborns (31,63). An extensive range of mechanisms could be facilitating vertical transmission of these pathogens; however, these remain ambiguous.

1.2.4.1 Middle East Respiratory Syndrome virus – MERS-CoV

The Middle Eastern Respiratory Syndrome virus, first isolated in 2012, is commonly found in the Arabian Peninsula (65). Common coexisting medical conditions include hypertension, diabetes and solid organ malignancy, and symptoms include cough and fever (65). Respiratory infectious diseases like MERS have shown an increased risk of adverse maternal obstetrical complications compared to non-pregnant women (5), i.e., preterm births, miscarriage and preeclampsia. This correlation is also seen within SARS-CoV-2 infected pregnant women (5,66).

1.2.4.2 Zika Virus

Zika virus, first isolated in 1947 in Uganda, is a mosquito-borne and sexually transmitted flavivirus closely related to Dengue virus, i.e., yellow fever (67). The symptoms of the Zika virus are mainly asymptomatic but can present as fever, rash, conjunctivitis, and joint pain (67). The ability of the virus to transmit from mother-to-child during pregnancy makes the Zika virus a cause for concern (68). The virus has been found in the amniotic fluid of mothers whose foetuses had cerebral abnormalities (69). The virus has also been found in brain tissues and placentas of neonates born with microcephaly (70). Zika virus during pregnancy, i.e., congenital Zika virus syndrome (CZS)

affects new-borns by causing microcephaly, intracranial calcifications, microphthalmia, and hearing loss (70).

1.2.4.3 Cytomegalovirus (CMV)

Cytomegalovirus is related to the viruses that cause chickenpox, herpes simplex and mononucleosis (71). *Cytomegalovirus* is a common virus that the body retains for life once infected. CMV rarely causes health problems in healthy individuals, allowing it to go unnoticed for a period of time (72). However, for pregnant women and those with a weakened immune system, it can be a cause for concern (60). In pregnant women, CMV can be passed to the neonate by spreading in breastmilk and blood (60). Before birth, new-borns infected with CMV are classified as congenital CMV, and those infected after birth are perinatal CMV (includes babies infected via breast milk) (73). Babies with congenital CMV can appear healthy at birth but can begin to develop signs of CMV in following months or years. These include hearing loss and developmental delay, seizures, low birth weight, microcephaly and pneumonia (74).

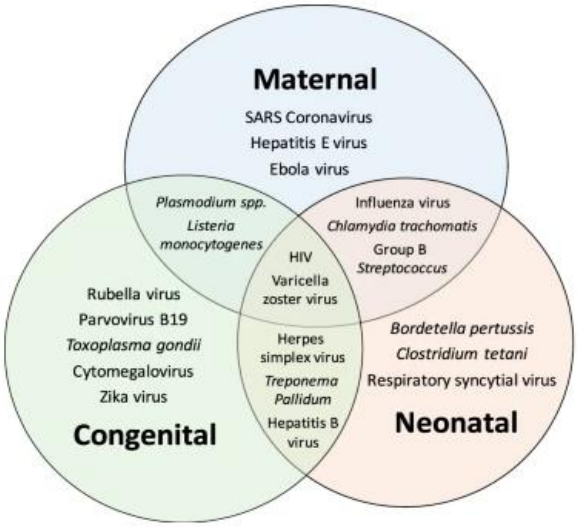

Figure 5: Image taken from Vermillion M and Klein S (75)*. The different modalities of transmission have been split into congenital, neonatal and maternal. The diagram illustrates examples of viruses that transmit during and prior to pregnancy.*

1.3 Mother-to-child transmission (MTCT) of SARS-CoV-2

In light of the risk of vertical transmission seen in other infectious pathogens (ZIKA, CMV, HSV), there are ongoing efforts to elucidate the possibility of SARS-CoV-2 MTCT. Many studies have reported on the possibility of SARS-CoV-2 MTCT, with results detecting viral RNA in the placenta, amniotic fluid, breastmilk and umbilical cord samples (76–81) **(table 1).** Whether the detection of viral RNA relates to active infection still remains to be determined.

Robust literature on SARS-CoV-2 MTCT, will allow healthcare professionals to take protective measures like repeat testing and using a classification system to determine timing of infection. In doing so, adverse neonatal and maternal outcomes can be prevented i.e., congenital birth deformities, preterm birth, stillborn and ARDS (70).

1.3.1 Mechanisms of mother-to-child transmission of SARS-CoV-2

The mechanisms for MTCT can either occur through vertical transmission or horizontal transmission (61). Horizontal transmission is the transmission of a pathogen from caregivers to the infant (82). This occurs in the postnatal period and can result from health check-ups on the infant or visitation from family members. Vertical transmission is the transmission of a viral pathogen from mother-to-child (37,45). This transmission occurs in three main modalities; *in utero*, intrapartum and postnatally (37) **(table 2).**

In utero transmission of SARS-CoV-2 occurs when the pathogen is present in the maternal blood and so is able to reach and breach the placental barrier, infecting the foetus (83). This mainly occurs through placental infection via the haematogenous route (63). SARS-CoV-2 viremia (virus present in the bloodstream) has been rarely reported and may be more common in infected individuals with a more severe disease presentation (84).

Maternal RT-PCR samples	Pharyngeal swab	Amniotic fluid	Placenta	Breast milk	Vaginal fluid
Neonatal RT-PCR samples	Cord blood PCR	Anal swab PCR	Neonatal stool sample	Blood test	Pharyngeal swab

Table 1: the maternal and neonatal samples that can be tested with RT-PCR are mentioned in this table. RT-PCR of the above samples can allow detection of SARS-CoV-2 viral RNA particles.

Intrapartum transmission requires foetal or infant exposure to the pathogen during labour and delivery. This is a result of ascending infection, where the foetus comes into contact with infected secretions during passage through the birth canal (83). The incidence of finding SARS-CoV-2 in vaginal fluids is rare but has not been ruled out (85). SARS-CoV-2 RNA particles have more frequently been found in the faeces of infected individuals (86). Faecal contamination of the vaginal canal and nearby area during labour and childbirth could lead to viral infection of the neonate immediately after birth, particularly during vaginal delivery as opposed to caesarean (87).

In the postnatal period, infants may be exposed to SARS-CoV-2 through various routes. These may include contact with infected mothers during infant skin-to-skin contact, rooming-in, breastfeeding

or via other infected caregivers/family members. As the routes of infection in the postnatal period are vast, identifying the direct source of postnatal infection, should it occur, is difficult to determine (29,30).

Postnatal transmission through breastfeeding requires infant exposure to the infectious pathogen in breast milk and infection of the infant through the oral/gastrointestinal route. However, the occurrence of detecting SARS-CoV-2 in RT-PCR samples of breast milk is infrequently reported, with no replication competent virus being detected (88,89).

Mode of transmission	Details
In utero	Through the mother's bloodstream via cord blood. Breaches in the placental barrier means infection can reach the foetus.
Intrapartum	Vaginal delivery means foetus is exposed to maternal vaginal fluid and stool. If these are infected, it could lead to infection in the neonate.
Postnatal	Breastfeeding, rooming-in, maternal respiratory droplets – viral RNA in breastmilk could infect neonate, as well as rooming-in as this increases exposure to maternal respiratory droplets.

Table 2: Summary of the various modes of transmission and their mechanism details.

1.3.2 Methods to ascertain MTCT

Various methods have been developed to ascertain the possibility of mother-to-child transmission of COVID-19. These diagnostic methods include using RT-PCR tests, testing serological samples and virological samples (**figure 4**).

Maternal diagnosis

In section 1.1.5, the various methods used to test for SARS-CoV-2 in the general population are mentioned above– these also apply for maternal diagnosis. In addition, an RT-PCR or antigen test can be used to identify maternal COVID-19 positivity (90).

Aside from this, maternal serology can also be tested for viral antigen. These include stool, blood, vaginal secretion and breastmilk samples. The presence of viral antigen in these samples can assist in determining the potential of vertical transmission to the infant.

Foetal and Neonatal diagnosis

Neonatal diagnosis of SARS-CoV-2

The tests mentioned in section 1.1.4 are applicable for neonatal SARS-CoV-2 diagnosis and the general population. However, some additional samples and mechanisms can assist in neonatal diagnosis. RT-PCR testing is the gold standard used in neonatal diagnosis via a nasopharyngeal or

an oropharyngeal swab. Additionally, the collection of neonatal samples to test for viral antigen presence is also used. These samples include anal and faecal samples, or blood PCR can also be used. Studies have reported the presence of the SARS-CoV-2 viral antigen in neonatal stool samples (91).

By collecting neonatal blood samples, the presence of immunoglobins like IgG and IgM can also detect for SARS-CoV-2 infection. IgG is a smaller antibody and can cross the placental barrier and hence the mother is able to give this antibody to her offspring passively. However, IgM is a larger pentameter structure, and so if there is IgM present in the neonatal blood samples, this means the neonate has made the antibody themselves in response to infection, potentially COVID-19. Therefore, the presence of these antibodies alone is not a determinate of neonatal SARS-CoV-2 infection and should always be combined with repeat confirmatory RT-PCR of a nasopharyngeal swab.

Foetal diagnosis of SARS-CoV-2

Invasive procedures pose a risk of vertical transmission; therefore, minimally invasive procedures, i.e., amniocentesis or fetoscopy, are recommended (92) Amniocentesis is when a hollow needle is inserted into the uterus to collect amniotic cells and these cells are then screened for foetal abnormalities (92). *Fetoscopy* is an endoscopic procedure that involves surgically accessing the foetus, the amniotic cavity, the foetal side of the placenta and umbilical cord (93). An incision is made in the abdomen through which the endoscope is inserted (93).

Through these procedures, it is possible to test for viral antigens and in doing so, early intervention is possible to introduce treatment options early.

Samples tested

All samples collected to test for viral antigen presence have to be sterile. A samples sterility is established by where it has been sampled, thus determining the likelihood of a true positive result. Non-sterile samples have a higher risk of contamination, making it difficult to determine whether the neonate was infected before birth or if the sample taken from the neonate is contaminated.

Sample sterility

Sterile samples include neonatal blood, lower respiratory tract samples and cerebrospinal fluid. These samples are a more vital determinant of viral detection than non-sterile samples, i.e., neonatal nasopharyngeal swab, saliva, and stool, as these can get contaminated (37).

Obtaining a positive SARS-CoV-2 RT-PCR result in infants can be either mean; active infection with replicating virus, or residual non-infectious viral gene fragments ('dead virus'), or the sample is contaminated (where the virus is present on the surface of the skin/mucus membrane but is not causing active infection in the infant) (94).

To avoid wrongly classifying neonates as positive or negative, a robust classification system to confirm the timing of transmission is needed to standardise and universalise definitions of the timing of vertical transmission and neonatal positivity.

Virological testing

Virological testing is based on the detecting the presence of viral nucleic acid, this includes viral RNA or viral DNA (95). This can be done by collecting neonatal samples such as placental, neonatal cord blood, and neonatal peripheral blood samples (37). These samples are sterile, so a positive RT-PCR of these samples is an accurate indication of infection. The World Health Organization provides a classification system that can be used to assess sample sterility (53). The reason for ensuring the samples is sterile, is to avoid the incidence of false-negative results. These can arise due to improper sample collection, handling, transport, as well as the stage of the disease (e.g., if the specimen is obtained when viral load is very low).

Serological testing

Serological testing is based on the demonstration of virus-specific IgM antibodies or a significant increase in the levels of specific IgG antibodies. Immunoassays are a common method used for serological assays (96). IgM antibodies are larger antibodies and hold a pentameter structure (90) and are the first type of antibodies to be made in response to an infection. IgG antibodies work by controlling infection of body tissue (90) through binding to various kinds of pathogens such as viruses, bacteria and fungi and are also associated with immune memory and producing long-term protection against the immune system (97).

IgM cannot cross the placental barrier due to its structure and so is not passively given to the infant by the mother's immune system (90,97). The presence of IgM antibodies in serological samples such as cord blood is therefore indicative of viral presence, thus suggests potential infection (98).

1.3.3 Classification of MTCT

MTCT can be classified into three main categories – *in utero*, intrapartum and postnatal transmission (37). As mentioned above, a robust classification system allows the determination of the exact timing of transmission.

Further details on the classification system have been reviewed in later sections.

1.3.4 Existing research on MTCT of COVID-19 in pregnant women

The existing literature on vertical transmission of SARS-CoV-2 is not definite. Some studies refute the possibility due to a lack of research, large sample size and no follow up studies. While other studies provide evidence to support the mechanism. Many studies also failed to incorporate an adequate classification system to ascertain the timing of infection.

1.3.4.1 In utero transmission

In utero transmission of SARS-CoV-2 occurs when the pathogen is present in the maternal bloodstream allowing it to reach and circumvent the placental barrier and infect the foetus (45,99). Transplacental transmission of SARS-CoV-2 is a possible route of infection and happens through placental infection via the haematogenous route (99). COVID-19 is also associated with hyper-

coagulopathy, which causes placental disruption, thus compromising the placental barrier and assisting viral passage to the foetus without actual placental infection (100).

The cell-membrane associated angiotensin-converting enzyme 2 (ACE2) receptor and Transmembrane Protease Serine 2 (TMPRSS2) are associated with SARS-CoV-2 cell entry. ACE2 and TMPRSS2 are expressed in placental maternal-foetal interface cells (101). Although co-expression may be limited, if viremia occurs, placental cell infection could arise and allow passage of the virus to the foetus (101). Additionally, placental disruption, possibly due to hyper-coagulopathy, could allow viral passage to the foetus without actual placental infection. ACE2 and TMPRSS2 can be found on the foetal lung, heart, and liver, suggesting foetal infection is possible should the virus reach the foetus (102,103).

The disadvantage with looking at viral load in samples is that, in situations where there are not high enough levels, this can go undetected, producing a false negative (104). Therefore, positivity and negativity should not be ruled out purely based on this method but confirmed through RT-PCR.

1.3.4.2 Intrapartum transmission

Intrapartum transmission relies on the presence of the pathogen in various maternal secretions. During labour and delivery, there is a high chance of the mothers' fluids coming into contact with the infant. SARS-CoV-2 have rarely been found in vaginal fluids but is more frequently found in infected individuals' faeces (105).

Faecal contamination of the vaginal canal and nearby environment during labour and childbirth could lead to viral infection in the neonate immediately after birth, particularly during vaginal delivery as opposed to caesarean section (106). Due to this contamination, it may make it difficult to differentiate infant viral infection from a viral infection acquired during passage through the birth canal and from an infection that occurred horizontally in the immediate postnatal period from caregivers and healthcare workers. While some studies (107) have stated that the mode of delivery does not associate with SARS-CoV-2 positivity in the neonate (108), the possibility of the neonate coming into contact with maternal secretions, like stool, blood, or vaginal secretions, is increased during vaginal delivery.

1.3.4.3 Postnatal transmission

The postnatal period allows the transmission to occur vertically and horizontally. Possible transmission routes include infection from positive mothers during maternal-infant-skin-to-skin contact and rooming-in the infant with positive mothers or breastfeeding. Horizontal transmission can be caused by caregivers/family members or the neonate's environment. As there are an array of possible transmission routes postnatally, it can make it difficult to determine the exact cause of neonatal positivity in this time period.

Postnatal transmission via breastfeeding requires the infectious pathogen to be present in the breast milk and causes infection to the infant via the oral/gastrointestinal route. However, the incidence of SARS-CoV-2 in breastmilk samples is low, and to date, there has been no competent replication virus detectable (88). Furthermore, SARS-CoV-2-specific immunoglobulin (IgG, IgM, and IgA) have been detected in breast milk, but whether SARS-CoV-2 antibodies in breast milk plays a protective role against infection in the infant is not known (88).

The WHO's brief (109) on breastfeeding summarises that breastmilk samples can test positive for SARS-CoV-2, but the neonate could still test negative. Detection of SARS-CoV-2 viral RNA in breastmilk is not the same as finding the viable and infective virus, *"Transmission of COVID-19 requires replicative and infectious virus to reach target sites in the infant by overcoming the and infant's defence systems"* (109). The brief states how the benefits of breastfeeding are crucial in the early stages of an infant's life. For this reason, with correct infection prevention methods, i.e.,

PPE, strict hygiene routine and face masks, breastfeeding can be continued and is not a cause for concern in terms of vertical transmission (109).

1.4 World Health Organization MTCT classification

The WHO classification is an international consensus classification that outlines definitions for determining infant SARS-CoV-2 vertical infection and the timing of such infection (37). The data was obtained from the WHO COVID-19 Living Evidence Synthesis (LENS) and a WHO expert consultation panel **(appendix 6).**

Transmission is categorised into four groups: *in utero* transmission in the case of live birth; *in utero* transmission in the case of foetal demise; intrapartum transmission; and early postnatal transmission (37). These categories are further delineated into mutually exclusive categories based on the likelihood of infection; confirmed, possible, unlikely or indeterminate (94). These are based on results of confirmatory investigations or absent testing procedures **(table 3).**

Sampling modalities are further distinguished by sterility. Sterile samples consist of neonatal blood, cerebrospinal fluid or lower respiratory secretions (37). While non-sterile samples include upper respiratory tract nasopharyngeal, oropharyngeal, or faecal swabs as these are more likely to present transient contamination (37).

In utero in the case of a live birth, stipulates the need for evidence of maternal infection anytime during pregnancy and *in utero* foetal SARS-CoV-2 exposure and SARS-CoV-2 persistence or immune response in the neonate.

In utero transmission in the case of foetal demise, requires evidence of maternal SARS-CoV-2 infection anytime during pregnancy and detection of SARS-CoV-2 in foetal tissue, amniotic fluid, or placental specimens.

Intrapartum SARS-CoV-2 transmission requires evidence of maternal SARS-CoV-2 infection near birth and a lack of *in utero* foetal SARS-CoV-2 exposure and SARS-CoV-2 intrapartum exposure with viral persistence of immune response in the infant.

Finally, the early postnatal transmission of SARS-CoV-2 covers neonatal age of greater than 48 hours to 28 days old. This category also requires evidence of maternal infection near the time of birth and evidence of a lack of *in utero* and intrapartum exposure and SARS-CoV-2 early postnatal exposure and viral persistence or immune response in the infant (37).

	Summary of WHO classification system categorizing the timing of MTCT.
Date of publication	07/02/2021
Categorisation of mother-to-child transmission	• *In utero* transmission in the case of a live birth • *In utero* transmission in the case of foetal demise • Intrapartum transmission • Early postnatal transmission (age > 48 hours – 28 days) The likelihood of infection is further classified into confirmed, possible, unlikely and indeterminate.
Definition of maternal SARS-CoV-2 infection	*In utero* transmission: mothers with confirmed COVID-19 infection anytime during pregnancy. Intrapartum and postnatal transmission: mothers with confirmed COVID-19 diagnosed near the time of delivery.
Samples for virologic or immunologic testing	Sterile samples • Neonatal lower respiratory tract samples • Neonatal blood • Foetal organs (e.g., lung, liver, brain) • Cerebrospinal fluid • Amniotic fluid (only if collected prior to membrane rupture or via amniocentesis) Non-sterile samples • Neonatal upper respiratory tract samples • Saliva, or stool • Placental tissue or surface swab • Foetal swab

Table 3: the WHO classification system on how to categorise timing of MTCT. The categorisation, definitions and examples of sterile & non-sterile samples.

1.5 Rationale of thesis

COVID-19 in pregnancy is of global concern due to the inconclusive evidence on how it affects both mother and child. The rate of neonatal positivity is important to discern to understand the true burden of SARS-CoV-2 in neonates. Furthermore, respiratory viruses like MERS have previously attributed to adverse neonatal and maternal outcomes. With these factors in mind, it is imperative to understand all these gaps in literature to provide clinicians with robust evidence-based medicine.

By using a robust classification system like the one generated by the WHO, studies will be able to classify MTCT in a universal classified approach. In addition, using a classification system that allows the categorisation of transmission into exact time points allows for even more accurate preventative measures. This means that if neonates are testing positive more during a particular period, e.g., *in utero*, appropriate preventative measures can be implemented.

Therefore, this thesis will collate evidence on MTCT of SARS-CoV-2 by reviewing existing systematic reviews on MTCT and the classification systems used to categorise and ascertain the timing of mother-to-child transmission. This thesis will also undertake a systematic review and meta-analysis to determine the rate of neonatal SARS-CoV-2 positivity and the rate of vertical transmission, if any. As well as this, any risk factors associated with increasing neonatal positivity will be determined.

1.5.1 Hypotheses

There is enough evidence surrounding previous respiratory viruses causing congenital birth deformities in neonates due to their ability to transmit vertically, i.e., MERS (5). For this reason, it is reasonable to hypothesise that SARS-CoV-2, being a respiratory virus, can also transmit from mother-to-child through vertical transmission, either *in utero*, intrapartum or postnatally and in doing so, can cause adverse neonatal and maternal outcomes. In addition to this, exposing the neonate to certain risk factors such as breastfeeding, mode of delivery, and rooming-in increases the rate of neonatal positivity.

1.6 Aims and Objectives of Thesis

1.6.1 Aim

I aim to map existing evidence on MTCT of SARS-CoV-2 and various classification systems. Additionally, I plan to assess the risk of SARS-CoV-2 MTCT by evaluating its impact on offspring positivity and risk factors associated with offspring positivity.

1.6.2 Objectives

1) To undertake a review of reviews on systematic reviews reporting on SARS-CoV-2 MTCT.
2) To review the various classification systems for reporting MTCT of SARS-CoV-2 and assess their quality.
3) Assess the rates of neonatal positivity and rate of vertical transmission, if any, for SARS-CoV-2
4) To determine risk factors that increase the rate of neonatal positivity i.e., breastfeeding, vaginal delivery and rooming-in.

1.6.3 Outline of Approach

The hypothesis presented in this thesis will be tested by conducting a systematic review and meta-analysis of data collected between 1 December 2019, and 3 August 2021, from the LSR undertaken by the PregCOV-19 consortium. Student X is actively involved in this and thus shares ownership of the dataset. Student X also independently undertook a review of reviews on systematic reviews reporting on MTCT of SARS-CoV-2 and reviewed MTCT classification systems. Student X is currently assisting in weekly searches on Stage 1 of screening.

2. Methodology

2.1 Pregnancy and COVID-19 Living Systematic Review Consortium

This thesis forms a part of a wider Pregnancy and COVID-19 (PregCOV-19) project, evaluating research questions relating to COVID-19 and pregnancy (110). The Pregnancy and COVID-19 Living Systematic Review (LSR) consortium (PregCOV-19) is based in the WHO Collaborating Centre for Global Women's Health, University of Birmingham (110) **(Appendix 4).** The PregCOV-19 LSR is a registered protocol on PROSPERO. The project aims to conduct multiple LSR's investigating pregnant and recently pregnant women with suspected or confirmed COVID-19. In addition, the LSR aims to synthesize evidence relating to MTCT of COVID-19, maternal and perinatal outcomes, prevalence, and risk factors. Novel findings have been published in the British Medical Journal (BMJ) (29,30), and due to the evolving nature of the LSR, continuous updates are posted on the website.

For my masters, I independently undertook a review of reviews on MTCT and a review of classification systems (94). As part of the wider group, I have contributed to both publications.

Due to the vast array of studies and time constraints of search results, this study was supported by fellow researchers as part of the Pregnancy and COVID-19 project. Through this collaboration, I have been involved in completing a living systematic review and meta-analysis, reported in

accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2009 guidelines.

2.1.1 PregCov-19 LSR process overview

The LSR functions through a set routine through a fortnightly cycle, where every cycle is termed a 'round'. The initial phase of the LSR takes the form of a traditional systematic review and includes a literature search, title and abstract screening, full-text screening, data extraction and data analysis. The rounds are repeated, allowing the combination of new evidence with previous evidence thus producing updated data. **Figure 6** depicts the stages of the PregCOV-19 LSR project.

The LSR further splits the team into Stage 1 and Stage 2 screening, where the former is involved in title and abstract screening and the latter on full-text review. Two independent reviewers undertake all screening stages **(figure 7).** This minimizes the risk of evidence selection bias as well as human error. The data analysis occurs every week, while the main analysis occurs every two to four months. Thus, results are published by the BMJ tri-annually. In the situation where significant evidence emerges, the analysis updates sooner.

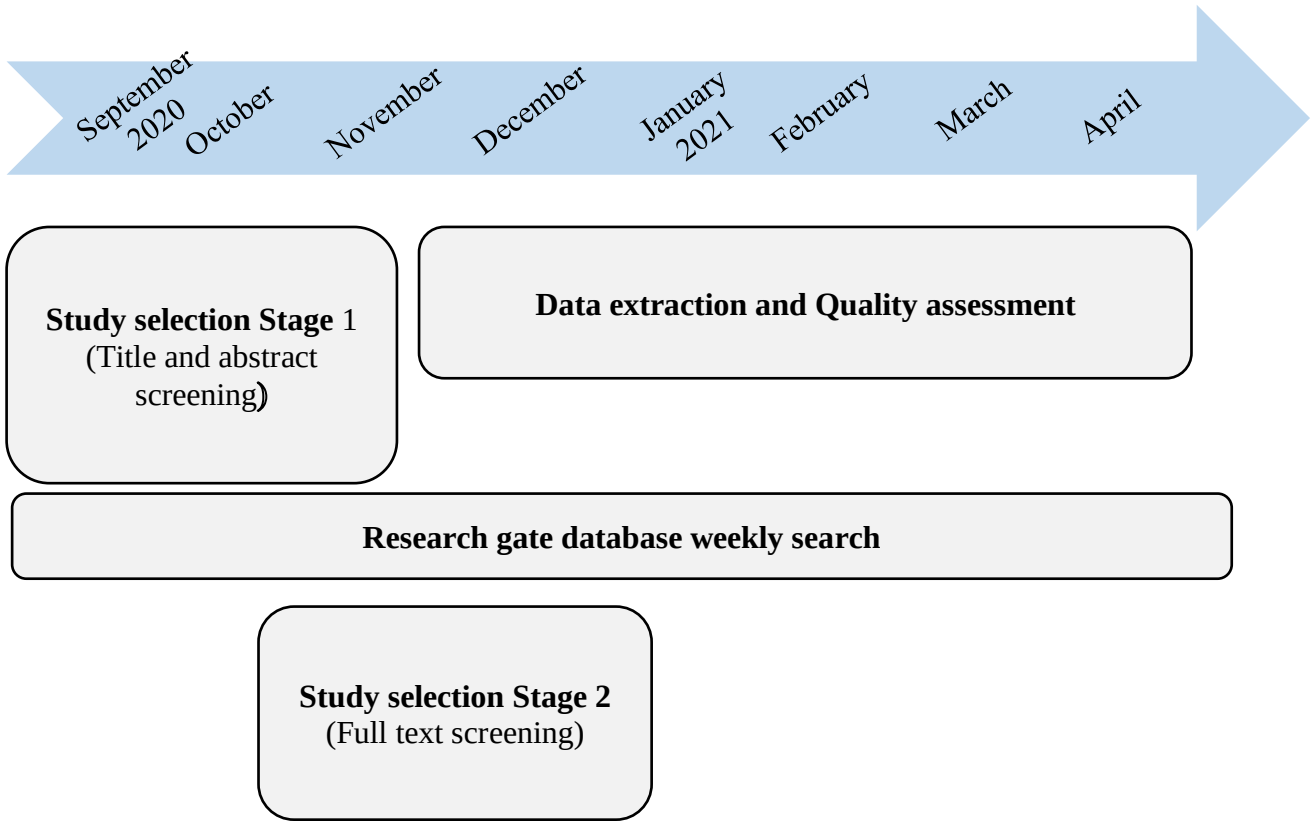

Figure 6: The flowchart summarises the involvement of Student X in the ongoing PregCOV-19 living systematic review research group.

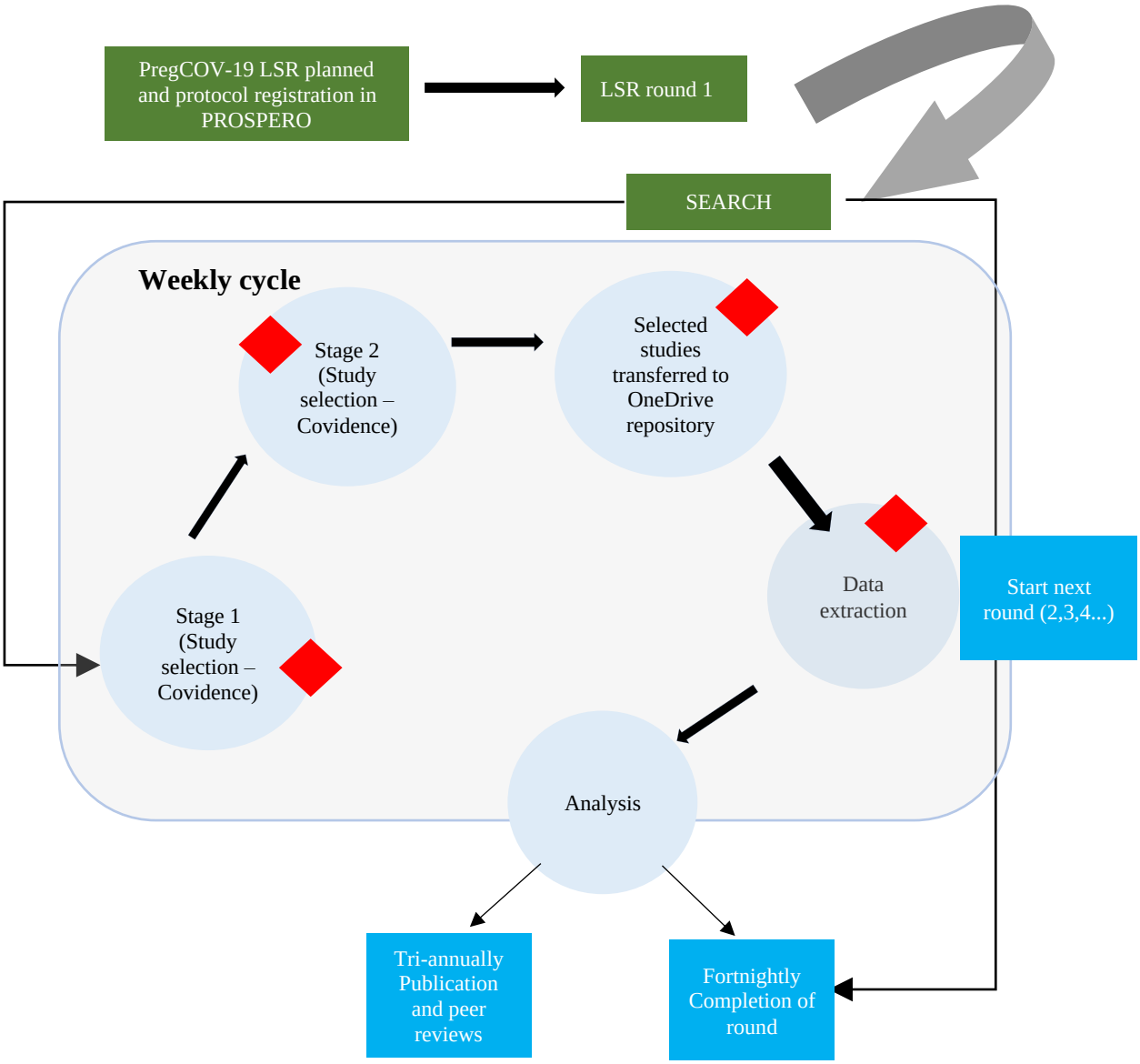

Figure 7: Depiction of the steps involved in the PregCOV-19 LSR also displaying author involvement with a red diamond.

2.1.2 Author involvement in PregCOV-19 LSR

Student X joined the PregCOV-19 LSR research group in late September 2020 to add to the student's master's degree. Student X's involvement in the project is highlighted in **Figures 6 and 7.**

- Worked on stage 1 title and abstract screening.
- Moved onto stage 2, trained, and led along with another team member
- Training on data extraction of mother-to-child transmission and then moved onto being one of the reviewers
- Worked on quality assessment of studies
- Weekly literature check of Research Gate
- Contacted corresponding authors of original studies for clarification of data for MTCT data extraction
- Published a review of reviews for the journal Current Opinions of Obstetrics and Gynaecology.

2.2 Review of reviews

A review of reviews on the classification systems for MTCT of SARS-CoV-2 and existing evidence on systematic reviews reporting on MTCT of SARS-CoV-2 was conducted. This is incorporated into the thesis to answer the research questions in section 2.5. A narrative descriptive approach was implemented as it was not possible to pool data.

2.2.1 Search Strategy

The data obtained for the review of reviews was begun by collating systematic reviews for SARS-CoV-2 and MTCT. Between December 2019 and March 2021, 68 systematic reviews were published on SARS-CoV-2 MTCT.

2.2.2 Databases searched

The following databases were systematically screened for relevant studies with no language restrictions; MEDLINE, The WHO database of publications on COVID-19, the Excerpta Medica database (EMBASE), Cochrane library databases, China National Knowledge Infrastructure (CNKI), PubMed, Norwegian Institute of Public Health, Wangfan and preprint databases (ArXiv, BiorXiv, medRxiv, search.bioPreprint), the Evidence for Policy and Practise Information and Co-ordinating Centre (EPPI-Centre) map of current evidence on COVID-19.

Grey Literature

John Hopkins centre for Humanitarian health, preprint servers, guidelines, specialized social media blogs; Professor Jim Thornton blog, ResearchGate – COVID-19 Research Community) and The Living Overview of the Evidence (L•OVE) (111).

2.2.3 Search Terms

Various search terms were used for each database as multiple databases were used. For example, PubMed search strategy used various keywords; ‘preg’, ‘pregnancy’, ‘COVID-19’, ‘coronavirus’, ‘placenta’, ‘transmission’, ‘vertical’, ‘maternal’, ‘foetal’, ‘neonatal’, ‘cord’, ‘amniotic’, ‘blood’, ‘hospitalisation’, ‘SARS-CoV-2’.

Search limits

There were no limits on language or date of publication. By doing so, no eligible studies could be missed from inclusion in the review.

2.2.4 Study Selection

Studies were selected on the basis of being a systematic review of SARS-CoV-2 MTCT.

2.2.5 Quality assessment

The AMSTAR-2 tool (A MeaSurement Tool to Assess Systematic Reviews) (112) was developed to produce robust evidence-based medicine.

The AMSTAR-2 tool is a critical appraisal tool to assess the methodological quality of systematic reviews. The tool is comprised of 16 questions, each was applied to the 68 systematic reviews. Results of the quality assessment were checked by another reviewer to avoid selection bias or human error. The purpose of using a critical appraisal tool is to allow reviewers to identify high-quality systematic reviews. Also, review authors can use this tool to self-assess their study and use the appraisal tool as a checklist.

I will use the AMSTAR tool for this thesis based on Shea *et al.* 's recommendation (113). While there are 16 questions, I will only hone in on seven critical domains. The reason for narrowing down to seven questions is because these have been identified as questions that can critically affect the validity of a review (113). These seven critical domains are AMSTAR questions 1,2,4,7,9,11 and 13 **(Table 4).** Unlike Shea *et al.* 's proposal (113) I have decided to add AMSTAR 1 as a critical domain. This is because it is essential to streamline research based on a question, that PICO, can aid in developing.

Current papers that have incorporated the AMSTAR-2 tool as a quality assessment tool and ranked each study with an overall score. For example, a 'yes' was scored a point of one, and a 'no' response

was scored a zero. However, for the purpose of this thesis, an overall score will not be given to studies as these tend to be poor predictors of results (114).

1. Did the research questions and inclusion criteria for the review include the components of PICO?	Yes/No/Cannot answer/Not applicable
2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	Yes/No/Cannot answer/Not applicable
3. Did the review authors explain their selection of the study designs for inclusion in the review?	Yes/No/Cannot answer/Not applicable
4. Did the review authors use a comprehensive literature search strategy?	Yes/No/Cannot answer/Not applicable
5. Did the review authors perform study selection in duplicate?	Yes/No/Cannot answer/Not applicable
6. Did the review authors perform data extraction in duplicate?	Yes/No/Cannot answer/Not applicable
7. Did the review authors provide a list of excluded studies and justify the exclusions?	Yes/No/Cannot answer/Not applicable
8. Did the review authors describe the included studies in adequate detail?	Yes/No/Cannot answer/Not applicable
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	Yes/No/Cannot answer/Not applicable
10. Did the review authors report on the sources of funding for the studies included in the review?	Yes/No/Cannot answer/Not applicable
11. If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?	Yes/No/Cannot answer/Not applicable
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	Yes/No/Cannot answer/Not applicable
13. Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?	Yes/No/Cannot answer/Not applicable
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	Yes/No/Cannot answer/Not applicable
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	Yes/No/Cannot answer/Not applicable
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Yes/No/Cannot answer/Not applicable

Table 4: AMSTAR-2 appraisal tool is comprised of 16 questions assessing the methodological quality of systematic reviews.

2.3 Systematic review and meta-analysis

This thesis also incorporates a systematic review and meta-analysis to determine the rates of offspring SARS-CoV-2 positivity and determine the risk factors for SARS-CoV-2 positivity in neonates by looking at comparative cohorts.

According to the Cochrane guidelines, systematic reviews are often used to address health decisions and produce high quality, accessible and up to date information (115). Evidence-based care (EBC) depends on the amalgamation of clinical expertise and research evidence (116) .

A systematic review allows a comprehensive, unbiased synthesis of the results of multiple studies (117). Based on a clearly formulated question, it identifies studies through search strategies and appraises study quality through quality assessment (115,117). The quality assessment and heterogeneity assessment allow transparent and high-quality evidence reporting (118). This, in turn, improves the level of evidence in the hierarchy of evidence **(figure 8)** (119). The strict methodological criteria and protocol registration minimises bias and provides robust data on epidemiological debates (115,117,118).

Systematic reviews can formulate the basis of evidence and guidelines for clinical practice. For this reason, including the study characteristics allows further interpretation of findings.

A meta-analysis encompasses the statistical processing of data by combining several statistical methods for the clear presentation of results, e.g., a forest plot (120). When a large body of research

exists, conducting a meta-analysis can aid in deriving conclusions by systematically assessing previous studies, using a quantitative, epidemiological study design (121). Hence, conducting a meta-analysis is useful when assessing the strength of evidence present on disease and treatment, making them good tools for evidence- based medicine (121,122). When a group of studies are heterogenous conducting a meta-analysis is not recommended. A meta-analysis should only be considered when the participants, intervention and outcomes of a group of studies are sufficiently homogenous to allow a meaningful summary (115). It enables the ability to answer questions that were not directly posed by individual studies and can settle conflicting claims (122).

A subgroup analysis can prove useful as a means in investigating heterogenous results. The participant data splits into subgroups to allow direct comparisons between them. Likewise, a sensitivity analysis can be used to take out certain low-quality studies to re-assess the heterogeneity (123) and thus see how conclusions may alter having high risk of bias studies excluded.

2.4 Living Systematic Review (LSR)

While systematic reviews are a prominent feature in producing evidence-based practise, the biggest challenge is keeping up with a high turnover rate of clinical trials. A study by Elbers *et al.* (124) looks at the low incidence of updates systematic reviews once published making the results out of date; this is where a living systematic review (LSR) can assist in. A LSR is almost a hybrid concept where it holds the same idea of the structure of a traditional systematic review, but also allows incorporation of emerging new evidence as and when it becomes available (115).

A LSR focuses on clinical questions where the preliminary research evidence is open to evolving. This makes a LSR the ideal methodology to study the COVID-19 pandemic. This is because immediate research and results are needed to make urgent clinical decisions, despite incomplete evidence. The COVID-19 pandemic is novel and rapidly evolving, with new evidence continuously emerging. A LSR can synthesise this vast number of evidence quickly to identify if any immediate changes to healthcare policies and management need to be incorporated.

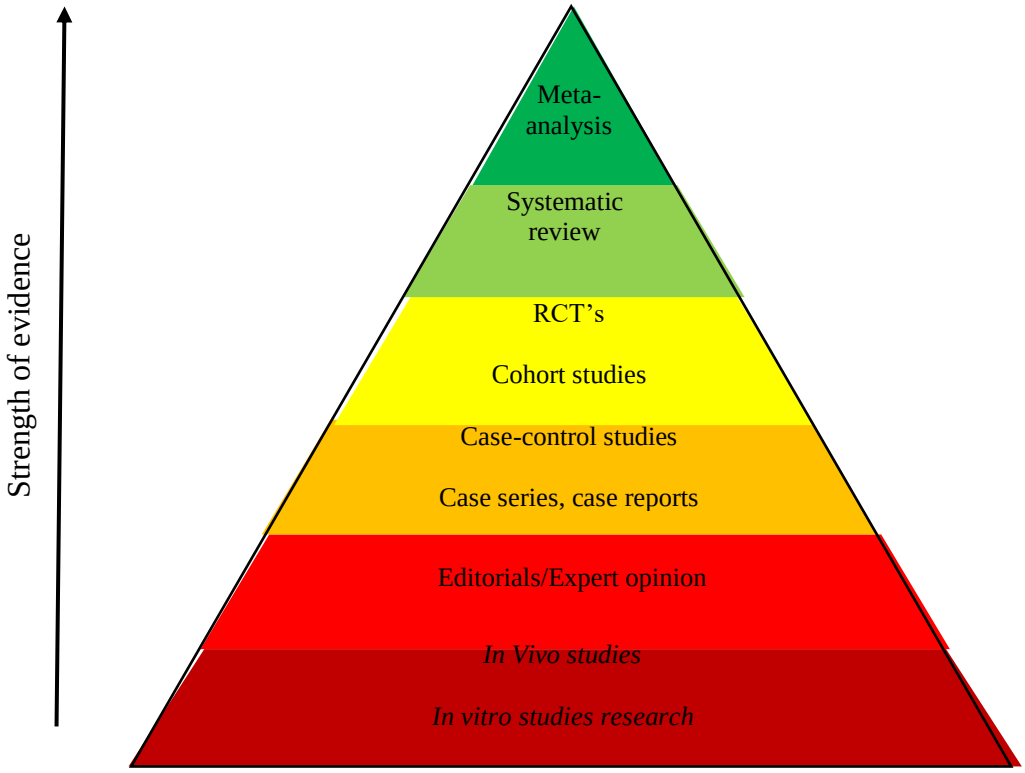

Figure 8: A hierarchical representation of the strength of evidence in different study designs. Taken and modified from Murad et al. (119)

2.5 Research questions of thesis

3. *What is the available evidence on SARS-CoV-2 MTCT?*
4. *What are the various classification systems for SARS-CoV-2 MTCT?*
5. *What are the rates of offspring SARS-CoV-2 positivity?*
6. *What are the risk factors for SARS-CoV-2 positivity in neonates?*

2.5.1 PICOS – Population, Intervention, Comparison and Outcomes

PICO 1 - What is the available evidence on SARS-CoV-2 MTCT?

Population	Pregnant and recently pregnant women with suspected or confirmed COVID-19 infection. *('Recently pregnant women' are postpartum women up to 42-day post-delivery).*
Intervention(s)/ exposure(s)	Exposure to SARS-CoV-2 *Exposure is either clinical or confirmed by laboratory testing*
Comparator(s)/ control	N/A
Outcome(s)	MTCT of SARS-CoV-2
Study design	Review of reviews

PICO 2 - What are the various classification systems for MTCT?

Population	Pregnant and recently pregnant women with suspected or confirmed COVID-19 infection. *('Recently pregnant women' are postpartum women up to 42-day post-delivery).*
Intervention(s)/ exposure(s)	Exposure to SARS-CoV-2 *Exposure is either clinical or confirmed by laboratory testing*
Comparator(s)/ control	N/A
Outcome(s)	Review the various classification systems for MTCT of SARS-CoV-2.
Study design	Review of reviews

PICO 3 - What are the rates of offspring SARS-CoV-2 positivity?

Population	Pregnant and recently pregnant women with suspected or confirmed COVID-19 infection. *('Recently pregnant women' are postpartum women up to 42-day post-delivery).*
Intervention(s)/ exposure(s)	Exposure to SARS-CoV-2 *Exposure is either clinical or confirmed by laboratory testing*
Comparator(s)/ control	Non-comparative analysis – no comparator group
Outcome(s)	Assess the rates of neonatal positivity and vertical transmission, if any, for SARS-CoV-2
Study design	Systematic review and meta-analysis of observational studies

PICO 4 – What are the risk factors for SARS-CoV-2 positivity in neonates?

Population	Pregnant and recently pregnant women with suspected or confirmed COVID-19 infection. *('Recently pregnant women' are postpartum women up to 42-day post-delivery).*
Intervention(s)/ exposure(s)	Exposure to SARS-CoV-2 *Exposure is either clinical or confirmed by laboratory testing*
Comparator(s)/ control	Neonates that have not been breastfed vs breastfed, rooming-in vs quarantined, vaginal delivery vs caesarean
Outcome(s)	Neonatal positivity
Study design	Systematic review and meta-analysis of comparative cohorts

2.6 Search Strategy

The literature search was conducted as per a cycle of a two-week period. The search was conducted systematically from December 1st, 2019, to August 3rd, 2021. Many sources were searched to maximise the findings of published literature and reduce selection bias. The search strategy was the same for all PICOS; however, study selection was limited for review reviews as this only included systematic reviews reporting on MTCT. However, the PICOS on the rates of offspring SARS-CoV-2 positivity and risk factors for SARS-CoV-2 positivity in neonates included cohort studies.

2.6.1 Databases searched

As mentioned above in the review of reviews section, the same method was used for this thesis section.

The following databases were systematically screened for relevant studies with no language restrictions; MEDLINE, The WHO database of publications on COVID-19, the Excerpta Medica database (EMBASE), Cochrane library databases, China National Knowledge Infrastructure (CNKI), PubMed, Norwegian Institute of Public Health, Wangfan and preprint databases (ArXiv, BiorXiv, medRxiv, search.bioPreprint), the Evidence for Policy and Practise Information and Co-ordinating Centre (EPPI-Centre) map of current evidence on COVID-19.

Grey Literature

In order to find additional literature that the main search may have overlooked, grey literature was also searched. John Hopkins centre for Humanitarian health (125) preprint databases (ArXiv, BioXiv, medRxiv and search.bioPreprint) guidelines, specialised social media blogs e.g., Professor Jim Thornton blog, these assisted in identification of primary scientific reports, online repositories of COVID-19 studies (Researchgate – COVID-19 Research Community (126)) and The Living Overview of the Evidence(L•OVE) (111). The L•OVE platform is a system that allows mapping and organisation of the evidence related to health making decisions.

Research Groups

Established groups like the WHO Maternal, New-born, Child and Adolescent Health (MNCAH) (127) COVID-19 research network, the International Network of Obstetric Survey Systems (INOSS) (128), the United States Centers for Disease Control and Prevention (CDC), and the European Centre for Disease Prevention and Control, were contacted on published and upcoming data.

2.6.2 Search Terms

The PregCOV-19 LSR research consortium developed and reviewed the search terms used. The search strategy used is outlined in the PROSPERO registration (PROSPERO CRD42020178076) and published protocol. Various search terms were used for each database as multiple databases were used. For example, PubMed search strategy used various keywords; 'preg', 'pregnancy', 'COVID-19', 'coronavirus', 'placenta', 'transmission', 'vertical', 'maternal', 'foetal', 'neonatal', 'cord', 'amniotic', 'blood', 'hospitalisation', 'SARS-CoV-2' **(appendix 5).**

Search limits

There were no limits on language or date of publication. By doing so, no eligible studies could be missed from inclusion in the review.

2.7 Study Selection

As mentioned in section 2.3.1, study selection compromises stage 1 and stage 2 (**Figures 5 and 6)**. Any discrepancies over inclusion or exclusion of studies were resolved with a third reviewer. The online platform 'Covidence' was utilised for study selection; Cochrane Collaboration recommends the website for efficient study screening, and access was provided through the WHO department of Reproductive Health and Research. Studies selected for PICSO's 1 and 2 were systematic reviews, and for PICO's 3 and 4, cohort studies were selected.

2.7.1 Title and Abstract Screening

As mentioned in section 2.7, title and abstract screening were completed on the Covidence platform. Two independent reviewers were involved in voting either 'yes', 'no', or 'unsure', on studies based on their titles and abstracts. If both reviewers voted 'yes', the paper automatically passed to stage 2 for the full-text review. If the paper was voted as 'unsure' or the reviewers had opposing opinions, the study passed to the conflicts folder, where the two reviewers discussed their reasoning and involved a third reviewer if necessary.

2.7.2 Full-Text Screening

The full-text screening process is a two-stage process. The first stage – part 1- involves accessing the full-text articles and subsequently uploading them to an online shared folder on Microsoft

OneDrive (accessible to all LSR team members). The University of Birmingham library service – findit@bham was used to retrieve full articles to upload. In the case where access was denied to articles, details on the paper were uploaded to a shared Google Document where a team member was responsible for sourcing the information. Two reviewers were tasked with assessing full-text articles, and any disagreements over the inclusion of studies were resolved with a third reviewer.

Part 2 of the process involved labelling the studies in the OneDrive folder. Studies were first named based on the format 'Surname Initials Year'. In the case where authors shared the same name, the nomenclature was 'Surname, Initials (1) Year'. The labels added were only to excluded studies, the number ranged from 1-7. Each number represents an exclusion code, as illustrated in **table 5** with an explanation of each code. This process allowed for studies to be grouped into included and excluded studies. The excluded studies were not eligible for data extraction and quality assessment.

2.7.3 Exclusion criteria

Studies that did not report on SARS-CoV-2 infection in pregnant or recently pregnant women were excluded. Cohort studies that reported on SARS-CoV-2 offspring status, and risk factors of breastfeeding, mode of delivery and rooming-in were included.

Studies that met all eligibility criteria against the PICOS table were included to extract data.

Code	Label	Explanation
1	Duplicate	This study has already been included in a previous round.
2	Animal study	The study population are not human.
3	Wrong design	The study is not a cohort
4	Wrong population	The study does not include pregnant or recently pregnant women with suspected or confirmed COVID-19 infection
5	Wrong exposure	The study population do not have COVID-19 exposure
6	Wrong outcome	The outcomes of the study are not COVID-19 related.

Table 5: Exclusion criteria for the LSR with exclusion codes and an explanation for the labels.

2.8 Study quality assessment and data extraction

2.8.1 Data extraction

Weekly data extraction was conducted using a pre-piloted form on a Microsoft Excel sheet **(Appendix 11 and 12).** Two independent reviewers conducted this stage, and any discrepancies were resolved through consensus or with a third reviewer. As two independent reviewers are handling the data, the risk of error through inputting data from different sources is minimised. It also allows checking for duplication by looking into the characteristics of the study, e.g., participants and hospital names.

The information extracted from the included studies on the spreadsheet includes author, study design, hospital name, the total number of women, the number confirmed by PCR, and serology or clinical/radiological method. Maternal samples were also recorded, such as amniotic fluid RT-PCR test, placenta, vaginal fluid, maternal stool and breast milk. Information on neonates was also obtained, including neonatal pharyngeal swab, neonatal stool RT-PCR, neonatal blood IgM/IgG test, number of foetuses in the study and how many babies were tested. A separate sheet was also used to record data on positive babies exclusively, i.e., mode of delivery, trimester of birth and timing of the nasopharyngeal swab **(Appendix 12).**

Data extraction for PICO's 1 and 2 was in the form of a narrative synthesis for reviews. Information on study design and period of literature search, maternal samples tested (breast milk, placenta,

amniotic fluid), neonatal samples tested (umbilical cord blood, urine/faecal swabs, peripheral blood, pharyngeal swab), number of SARS-CoV-2 positive mothers, number of neonates tested, the definition of neonatal positivity, number of positive neonates and any other additional information was collected **(table 8)**. There was no numerical data extracted for PICO's 1 and 2, and the data was descriptive. For PICO's 3 and 3, numerical data was obtained.

2.8.2 Quality Assessment

To assess the internal and external validity of non-comparative cohort studies, the validated tool developed by Hoy *et al.* was utilised (129). In doing so, the quality and risk of bias of studies were assessed **(appendix 7).** This tool incorporates ten questions, where four domains focus on the external validity (population, sampling frame, selection and non-response), and the remaining six domains focus on internal validity (data collection, case definition, measurement, differential verification, adequate follow-up and appropriate numerator and denominator). Every domain scores one point per question, giving score ranges of 0-3 as low risk, 4-6 as moderate, or 7-10 as high risk of bias (130).

Studies were considered low risk of bias if data were collected from clinical records or research case-report forms, if outcomes were clearly defined, SARS-CoV-2 infection was confirmed using laboratory-based tests, if the same mode of data collection was used in all participants, and if there was sufficient follow-up with appropriate numerator and denominator. Regarding the external validity, studies were considered a low risk of bias if they were representative of the national population for relevant variables, representative of the target population, undertook a consensus, and had more than 75% response rate in individuals with and without the outcome.

For the comparative cohort studies included, the methodological quality was assessed using the Newcastle Ottawa Scale (NOS) . NOS is based on a ‘star system’ and assesses the quality of non-randomised studies **(appendix 8)**. Two independent reviewers used a pre-piloted form created on

Microsoft Excel for quality assessment of included studies. The framework of how the Newcastle Ottawa Scale works is illustrated in **table 6**.

Selection	**Comparability**	**Outcome ascertainment**	**Risk of bias**
****	**	***	LOW
***/**	*	**	MEDIUM
*/-	-	*/-	HIGH

Table 6: The Newcastle Ottawa Scale (NOS) assessment framework for study quality assessment.

2.9 Data analysis

The rates of offspring SARS-CoV-2 positivity was summarised and identified by anti-SARS-CoV-2 IgM alone and/or RT-PCR as a proportion of all babies born to mothers with SARS-CoV-2 infection in cohort studies. The rates were calculated with 95% confidence intervals (CI) using DerSimonian and Laird's random-effects meta-analysis after transforming data using Freeman-Tukey double arcsine transformation. The assessment of heterogeneity was reported as I^2 estimates.

The sensitivity analysis was carried out for SARS-CoV-2 positivity rates in babies by restricting the analysis to low risk of bias studies, babies born to women diagnosed with SARS-CoV-2 infection antenatally and babies tested at less than 24 hours after birth. The rates of SARS-CoV-2 positivity was also evaluated by subgroups of studies involving mothers and babies from various World Bank Regions.

To summarize the association between maternal and perinatal characteristics and SARS-CoV-2 exposure status in babies, comparative dichotomous data were pooled as odds ratio (OR) and 95% CI by random-effects meta-analysis. Subgroup analysis was carried out for SARS-CoV-2 positivity in babies by risk factors.

Meta-analysis was carried out for SARS-CoV-2 positivity in babies and risk factors associated with SARS-CoV-2 positivity (postnatal factors). In situations where meta-analysis would not be an

appropriate tool, a narrative description approach was used to summarize the evidence, i.e., review of reviews on MTCT of SARS-CoV-2 and review of classification systems for MTCT.

Furthermore, all statistical analysis was done using Stata (version 16).

2.9.1 Heterogeneity

Heterogeneity refers to the variability in the data. This would be evidently found when collating various studies and analysing them via meta-analysis (121). Regarding statistical heterogeneity, this is variation found in intervention effects or results. Heterogeneity is measured using I^2, where I^2 statistic refers to the variation across studies due to heterogeneity as opposed to chance, thus refers to the inconsistency found in studies (131).

Heterogeneity in this study was assessed using the I^2 statistic and if the P value was of <0.05, this was considered statistically significant. According to the Cochrane handbook (121), an I^2 value of 0-40% was taken as an unimportant result, 30-60% represented a moderate level of heterogeneity, 50-90% represented substantial heterogeneity and 70-100% was considerable heterogeneity (121).

Tau^2 Is another heterogeneity measure that allows the between-study variance to be estimated based on the underlying distribution of true effect sizes. While in this study the I^2 value was used to determine heterogeneity, using Tau^2 is another useful tool (132).

3 Results

3.1 Review of reviews on MTCT of SARS-CoV-2

Through ongoing work on the PregCoV-19 research project, 68 systematic reviews on SARS-CoV-2 were identified, 66 studies were traditional systematic reviews, and two were living systematic reviews that evaluated the presence of SARS-CoV-2 specific antibodies in neonates born to SARS-CoV-2 infected mothers and the potential of this transmitting through breastmilk (88,133). The studies varied in the number of primary studies included in the review, ranging from 6-121 studies. The number of SARS-CoV-2 positive neonates ranged from 0-39 and was not always explained through the publication date of the review.

The reviews showed varied outcomes in reporting results despite some including the same primary studies. Also, most studies did not incorporate a classification system to confirm the persistence of the virus in the neonate with repeat testing i.e., collecting appropriate samples at appropriate times. Out of the 68 included studies, only one study (134) used a classification system to determine whether infant SARS-CoV-2 assay positivity was indicative of true infant infection.

The results and quality assessment of the 68 studies have been narratively reported. The study characteristics of the systematic reviews included in the review of reviews can be seen in Table 8. The variables looked at were the Author name and year of publication, the number of studies used as well as the study design incorporated i.e. case report, case series, cohort study, descriptive study

etc. The period of the literature search was also noted as well as the number of SARS-CoV-2 positive mothers.

The population characteristics comprise of the inclusion and exclusion criteria. All studies included were systematic reviews reporting on the transmission of COVID-19 from mother to child. The mothers in the studies had to be positive with COVID-19 during any period of pregnancy i.e. either before, during or after delivery. However, one study did not report how many mothers were positive but was still included as they reported on neonatal positivity. The mode of testing should be confirmed via RT-PCR or serology however where the mode of testing for SARS-CoV-2 positivity was not available, these studies were still included. The neonates in the studies had to have tested positive for SARS-CoV-2 also by the same methods as for maternal diagnosis. The studies also had to report on at least one mode of assessment of vertical transmission. This could either be via maternal samples like breast milk and amniotic fluid or neonatal samples i.e. pharyngeal swab or umbilical cord blood (**table 8**).

Of the 68 reviews, four did not report the period of literature search. The range of number of studies included was between six and 121. The study design used was specified in 58 studies, with ten either not specifying or not reporting. The range of positive SARS-CoV-2 mothers ranged between 16 to 27,237. The definition of neonatal positivity was done either through RT-PCR, serology, RT-PCR or serology, or was not reported. Thirteen studies did not report on the definition of neonatal positivity. Forty-five used RT-PCR alone, nine used RT-PCR or serology and one study used nasopharyngeal RT-PCR to define positivity. Fifty-two studies did not report on breast milk sample as an assessment of vertical transmission, 54 studies did not report on placenta samples and 53 did

not report on amniotic fluid samples. In regard to neonatal samples, 55 did not report on umbilical cord blood, 62 did not report on urine/faecal samples and 55 did not report on peripheral blood/other samples. Pharyngeal swabs were also used as a means to detect vertical transmission in neonates, of the 68 studies 38 studies did not report on this as a method to ascertain transmission.

3.1.1 AMSTAR of systematic reviews

The methodological quality of systematic reviews was assessed using the AMSTAR-2 critical appraisal tool, comprising of 16 questions applied to 68 systematic reviews **(appendix 9)** .

	YES	NO	PARTIAL YES	N/A	TOTAL	YES (%)	NO (%)	PARTIAL YES (%)	N/A (%)
1. Inclusion of PICO	64	4	0	0	68	94.1	5.9	0.0	0.0
2. Inclusion of protocol	9	44	15	0	68	13.2	64.7	22.1	0.0
3. Selection of study design for inclusion	1	67	0	0	68	1.5	98.5	0.0	0.0
4. Comprehensive literature search strategy	2	21	45	0	68	2.9	30.9	66.2	0.0
5. Study selection in duplicate	57	11	0	0	68	83.8	16.2	0.0	0.0
6. Data extraction in duplicate	58	10	0	0	68	85.3	14.7	0.0	0.0
7. List of excluded studies and justification	0	68	0	0	68	0.0	100.0	0.0	0.0
8. Detail provided of included studies	19	34	15	0	68	27.9	50.0	22.1	0.0
9. Satisfactory technique to assess RoB	10	42	16	0	68	14.7	61.8	23.5	0.0
10. Reporting on sources of funding for included studies	1	67	0	0	68	1.5	98.5	0.0	0.0
11. Methods for statistical combination of results (MA)	7	12	0	49	68	10.3	17.6	0.0	72.1
12. Assessment of RoB in individual studies (MA)	13	6	0	49	68	19.1	8.8	0.0	72.1
13. Discussion of RoB in individual studies	25	42	0	1	68	36.8	61.8	0.0	1.5
14. Explanation for heterogeneity	15	53	0	0	68	22.1	77.9	0.0	0.0
15. Investigation of publication bias (MA)	16	5	0	47	68	23.5	7.4	0.0	69.1
16. Reporting on sources of conflict of interest and funding for review	54	14	0	0	68	79.4	20.6	0.0	0.0

Table 7: AMSTAR-2 results of systematic reviews. Number of studies are displayed in grey. Percentage values are represented in the orange columns

The results obtained are illustrated in **table 7**. The grey columns show the actual number of systematic reviews in each category, while the orange columns show the percentage values of these results. The full extraction sheet for the study characteristics shows details for each individual study **(table 8).**

Critical domain one had 64/68 studies (94.1%) reporting 'yes' and 4/68 (5.9%) of studies scoring 'no'. For critical domain two, 9/68 studies (13.2%) reported 'Yes', 44/68 (64.7%) reported 'No', and 15/68 (22.1%) reported 'Partial yes'. For critical domain four, 2/68 (2.9%) reported 'Yes', 21/68 (30.9%) reported 'No', and 45/68 (66.2%) reported 'Partial yes'. For critical domain seven, 0/68 (0.0%) reported 'Yes', and 68/68 (100.0%) reported 'No'. For critical domain nine, 10/68 (14.7%) reported 'Yes', 42/68 (61.8%) reported 'No', and 16/68 (23.5%) reported 'Partial yes'. For critical domain 11, 7/68 (10.3%) reported 'Yes', 12/68 (17.6%) reported 'No' and 49/68 (72.1%) reported 'N/A'. Lastly, for critical domain 13, 25/68 (36.8%) reported 'Yes', 42/68 (61.8%) reported 'No' and 1/68 (1.5%) reported 'N/A' **(figure 9).**

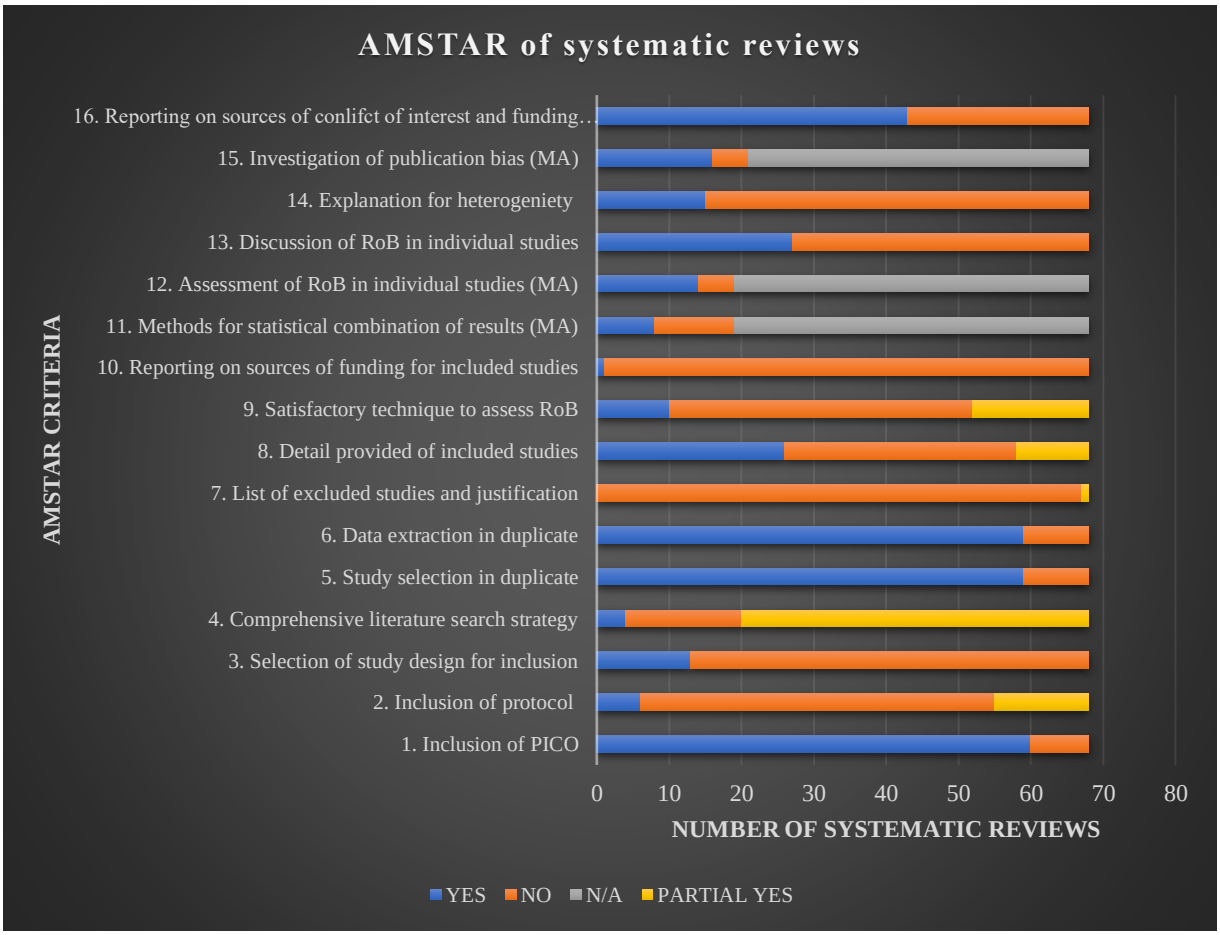

Figure 9: AMSTAR-2 quality assessment tool applied to all 68 systematic reviews. The question number is on the' y-axis' with a brief explanation. The' x-axis' is the number of systematic reviews. The key is blue for' yes' , orange for 'no' , yellow for 'partial yes' and grey for 'N/A'.

Study				Population			Assessment of vertical transmission							
							Maternal samples				Neonatal samples			
Author, Year	No. of studies	Study design	Period of literature search	No. of SARS-CoV-2 positive mothers	No. of neonates (no. tested)	Definition of neonatal positivity	No. of positive neonates	Breast milk	Placenta	Amniotic fluid	Other	Pharyngeal swab	Umbilical cord blood	Urine/faecal swabs	Peripheral blood/other
Christian et al, 2020	8	4CS/CR, 2RC, 2CS	Not reported	185	n/a (127)	RT-PCR	1	Not reported	Not reported	Not reported	Not reported	1/127	Not reported	Not reported	Not reported
Mirbeyk et al, 2020	37	5CS, 3CC, 17CR, 6CS, 3RC, 2CHS, 1CRS	Not reported	364	302 (219)	RT-PCR	11	Not reported	Not reported	Not reported	Not reported	11/219	0/219 cord blood	Not reported	Not reported
Shrestha et al, 2020	21	7CR, 8CS, 3CHS, 3CC	December 2019 - April 2020	230	229 (161)	RT-PCR	8/161	2/12	2/13	0/8	1/16 vaginal swab 1/9 stool	8/161	0/8 cord blood, 0/1 tissue	1/8 anal/stool swab	2/4 peripheral blood PCR 0/2 foetal tissue
Yee et al, 2020**	9	6 CHS, 3 CS	Not reported	93	103 (68)	RT-PCR	4	Not reported	Not reported	Not reported	Not reported	4/68	Not reported	Not reported	Not reported
Raschetti et al, 2020	74	37 CS, 34 CR, 2 RC, 1 CRS	Not reported	Not reported	176 (176)	RT-PCR or serology	176	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Yang N et al, 2020	6	5CR, 1CS	Inception - March 2020	16	16 (14)	RT-PCR	0	0/13	Not reported	Not reported	Not reported	0/14	Not reported	Not reported	Not reported
Sun et al, 2020**	17	19CR, 15 CRSDS, 6ACRS, 1CC, 1CHS	Inception - 11/03/2020	41	n/a (29)	RT-PCR	0	0/6	0/4	0/6	Not reported	0/29	0/7	0/1 faeces	0/1 peripheral blood PCR
Della Gatta An et al, 2020	6	5RC, 1RC w/ control group	14/03/2020 - 16/03/2020	51	46 (46)	RT-PCR	1	Not reported	Not reported	Not reported	Not reported	1/46	Not reported	Not reported	Not reported
Kasraeian et al, 2020**	9	Not specified	Inception - 18/03/2020	87	86 (n/a)	Not reported	0	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Alqahtani et al, 2020	9	9 CS	01/01/2020 - 20/03/2020	74	74 (64)	RT-PCR	1	Not reported	Not reported	Not reported	Not reported	1/64	Not reported	Not reported	Not reported
Trocado et al, 2020	8	1CHS, 2CR, 5CS	17/03/2020 - 20/03/2020	95	51 (48)	RT-PCR	1	Not reported	Not reported	Not reported	Not reported	1/48	Not reported	Not reported	Not reported
Smith et al, 2020	9	5 CR, 3CS, 1RS	01/11/2019 - 28/03/2020	92	60 (18)	RT-PCR	1	Not reported	Not reported	0/9	Not reported	1/18	0/9	Not reported	0/6 nasogastric content
Vigil-De Gracia et al, 2020	14	CR/CS	Inception – 30/03/2020	83	84 (n/a)	RT-PCR	4	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported

Chi H et al, 2020	14	Not specified	Inception - 31/03/2020	107	105 (91)	RT-PCR or serology	8 (5 via RT-PCR, 3 via serology)	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Direkvand-Moghadam et al, 2020	6	Not reported	1/12/2019 - 1/04/2020	50	41 (n/a)	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Lopes de Sousa et al, 2020	12	4CR, 5DS, 3ACRS	01/04/2020 - 02/04/2020	40	30 (29)	RT-PCR	1	Not reported	Not reported	Not reported	Not reported	1/29	Not reported	Not reported	Not reported
Hassanipour et al, 2020**	10	8CS, 2CC	Inception - 07/04/2020	135	n/a	Not specified	1	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Rodrigues et al, 2020	30	Not reported	Inception - 8/04/2020	212	185 (70)	RT-PCR or serology (IgG, IgM)	4 (via RT-PCR)	0/13	0/21	0/14	Not reported	4/70	0/13	Not reported	3/7 IgM 6/7 IgG
Trad et al, 2020	16	5CR, 5CS, 6CHS	25/03/2020 - 10/04/2020	155	118 (95)	RT-PCR	1	Not reported	Not reported	Not reported	Not reported	1/95	Not reported	Not reported	Not reported
Banaei et al, 2020**	16	6 RC, 1C, 5CS/CR	Inception - 10/04/2020	123	123 (n/a)	RT-PCR	5	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Yoon et al, 2020	28	16CS, 12CR	10/02/2020 - 13/04/2020	223	201 (167)	RT-PCR	4	Not reported	Not reported	Not reported	Not reported	4/167	Not reported	Not reported	Not reported
Ashraf et al, 2020	21	CS and CR, no CHS	Inception - 14/04/2020	90	92 (86)	RT-PCR	4	Not reported	Not reported	Not reported	Not reported	4/86	Not reported	Not reported	Not reported
Rodriguez-Blanco et al, 2020	20	12 CS, 7CR, 1CC	02/03/2020 - 14/04/2020	79	73 (n/a)	Not reported	Not reported	0/6	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Capobianco et al, 2020**	13	5CR, 2CS, 6OB	1/12/2019 - 15/04/2020	114	n/a	RT-PCR	4	Not reported	Not reported	Not reported	Not reported	4/4	Not reported	Not reported	Not reported
Giampiero et al, 2020**	13	5CR, 2CS, 6OB	1/12/2019 - 15/04/2020	114	108 (n/a)	Not reported	4	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Chamseddine et al, 2020	20	19CR, 1 study outside range	20/12/2019 - 18/04/2020	164	128 (44)	RT-PCR	3	Not reported	Not reported	Not reported	Not reported	3/44	Not reported	Not reported	Not reported
Chi J et al, 2020	20	15CR, 4RC, 1CC	Inception - 18/04/2020	230	156 (128)	RT-PCR	5	0/25	0/35 placental blood 0/9 placental tissues	0/32	0/13 vaginal secretion	5/128	Not reported	Not reported	Not reported
Trippella et al, 2020	37	18CS, 19CR 1CC,	01/12/2019 - 18/04/2020	275	248 (191)	RT-PCR	16	0/25	0/6	0/24	0/7 vaginal/cervical fluid	16/191	0/30	5/28 faecal/anal swabs	3/26 IgM/IgG
Elshafeey et al, 2020	33	16CR, 16CS	Inception - 19/04/2020	385	256 (n/a)	RT-PCR	4	0/26	0/12	0/23	Not reported	Not reported	0/30	Not reported	Not reported

Oltean et al, 2021	41	4RS, 4CS, 6CR, 2PS	Inception - 19/04/2020	315	332 (331)	RT-PCR	8	Not reported	Not reported	Not reported	Not reported	8/331	Not reported	Not reported	Not reported
Islam et al, 2020	47	9SR, 4SR+MA 9CS,	01/01/2020 - 20/04/2020	235	236 (n/a)	RT-PCR	0	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Juan et al, 2020	24	15CR	Inception - 20/04/2020	324	237 (155)	RT-PCR	3	0/22	1/4	1/32	0/6 vaginal mucus	3/155	0/34	0/19 faeces 0/19 urine	0/19 gastric juice
Yang Z et al, 2020	22	Not reported	Inception - 20/04/2020	83	83 (n/a)	RT-PCR or serology	9 (3 via RT-PCR, 6 via serology)	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Sepulveda-Martinez et al, 2020**	14	1CR, 13CS	Inception to 24/04/2020	292	223 (n/a)	NP RT-PCR	5	0/22	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Novoa et al, 2020**	37	4RS, 4CS, 6CR, 2PS	01/12/2020 - 27/04/2020	322	196 (138)	RT-PCR	4	0/17	0/15	0/25	1/16 vaginal swab 0/1 cervical secretion 2/3 sputum 0/2 faeces 0/1 serum 6/8 IgM 7/8 IgG	4/138	0/16	3/6 anal 0/2 faeces 0/1 urine	3/8 IgM 6/8 IgG 0/4 serum 0/1 gastric juice
Debrabandere et al, 2020	36	18CR, 1CC, 17CS	1/12/2019 - 29/04/2020	203	206 (n/a)	RT-PCR	8	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Huntley et al, 2020	13	13CR	Inception - 29/04/2020	538	310 (n/a)	RT-PCR	0	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Berbari et al, 2020**	24	8RC, 10CR, 4CS, 1 CC	01/12/2019 - 30/04/2020	136	94 (n/a)	RT-PCR	2	Not reported	0/94	Tested	Not reported	Not reported	Tested	Not reported	Not reported
Diriba et al, 2020**	39	14RC, 4CS, 14CR, 2CHS, 3CRS, 1PS, 1CC	1/01/2003 - 30/04/2020	1271	n/a	Not specified	0	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Thomas et al, 2020**	18	7CR, 7RS, 1PS, 2CS, 1CC	January - May 2020	157	160 (81)	RT-PCR	5	Not reported	Not reported	Not reported	Not reported	5/81	Not reported	Not reported	Not reported
Lakhkar et al, 2020	26	5CR, 20CS, 1LE 19CR, 15 CRSDS,	01/12/2019 - 1/05/2020	558	455 (452)	RT-PCR	13	0/9	0/3	Not reported	0/56 reproductive tissue	13/452	Not reported	Not reported	11/15 IgG 4/15 IgM
Sousa et al, 2020	42	6ACRS, 1CC, 1CHS	01/05/2020 - 02/05/2020	650	511 (n/a)	Not specified	8	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Gajbhiye et al, 2020	50	?	Inception - 03/05/2020	441	391 (313)	RT-PCR or serology	24 (17 via RT-PCR, 7 via serology)	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	4/8 IgM 7/8 IgM/IgG

Melo et al, 2020	38	4CC, 11CR, 10SR, 11CRS, 2CHS	Inception - 04/05/2020	520	n/a (432)	RT-PCR	16	Not reported	Not reported	Not reported	Not reported	16/432	Not reported	Not reported	Not reported
Di Toro et al, 2020**	23	17CS, 5CHS, 1CC	Inception - 08/05/2020	1100	n/a (444)	RT-PCR	19	Not reported	Not reported	Not reported	Not reported	19/444	Not reported	Not reported	1/5 IgM/IgG
Bwire et al, 2020	33	21CR, 10RC, 2PS	01/12/2019 - 18/05/2020	205	205 (n/a)	RT-PCR	13	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	10/11 IgM/IgG
Kumar et al, 2021**	46	34CS, 12CR	01/12/2019 - 20/05/2020	116	n/a	Not reported	n/a	10/88	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Walker et al, 2020	49	CR/CS	08/04/20 - May 2020	655	666 (n/a)	RT-PCR	28	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
González et al, 2020	42	26CS, 16CR	01/11/2019 - 21/05/2020	1098	875 (n/a)	RT-PCR	18	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Sampieri et al, 2020	17	Not reported	27/03/2020 - 21/05/2020	65	65 (n/a)	Not reported	8	2/32	6/34	1/38	Not reported	Not reported	Not reported	Not reported	Not reported
Pettirosso et al, 2020	60	Not reported	18/04/2020 - 23/05/2020	1287	717 (655)	RT-PCR	19	0/45	Not reported	Not reported	Not reported	19/655	Not reported	Not reported	Not reported
Kotlyar et al, 2020**	69	30CR, 39CHS/CS	Inception - 28/05/2020	1564	936 (936)	RT-PCR	27	Not reported	2/26	0/51	Not reported	27/936	1/28	0/17 urine 3/31 faecal/ rectal swab	3/81 IgM
Turan et al, 2020	63	28CS, 31CR, 4RS	26/03/2020 - 29/05/2020	637	479 (405)	RT-PCR	8	1/19	1/6	0/20	Not reported	8/405	0/29	Not reported	Not reported
Deniz et al, 2020	50	Not specified	1/02/2020 - 1/06/2020	714	606 (n/a)	RT-PCR or serology	20 (17 via RT-PCR, 3 via serology)	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Patnaik et al, 2020	8	8CS	Inception - June 2020	71	n/a	Not reported	1	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Khalil et al, 2020**	6	Not specified	06/04/2020 - 08/06/2020	2567	751 (n/a)	RT-PCR	19	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Thamir Ahmed et al, 2020	19	Research articles, LE, CR, CS	01/01/2020 - 20/06/2020	124	125 (n/a)	RT-PCR	10	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Najafi et al, 2020	20	6RC, 2PS, 1CHS, 9CR, 2CS	Inception - July 2020	145	145 (n/a)	RT-PCR or serology	9 (6 via RT-PCR, 3 via serology)	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Centeno-Tablante et al, 2020	37	28CR, 9CS	Inception - 07/07/2020	77	77 (n/a)	RT-PCR	8	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported

Dubey et al, 2020**	61	27CR, 34CS	Inception - 8/07/2020	790	548 (n/a)	Not specified	6	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Sharps et al, 2020	42	19CR, 8CS, 11 CHS	Inception - 23/07/2020	325	326 (n/a)	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Arroyo-Sánchez et al, 2021	30	10 LE, 8 NR, 12 SR	26/06/2020 - 30/07/2020	476	477 (n/a)	RT-PCR	9	Not reported	7/19	2/8	Not reported	Not reported	2/9	Not reported	Not reported
Papapanou et al, 2021	39	39 CHS	Inception - 10/09/2020	27,237	n/a	RT-PCR	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Peripheral blood
Caparros-Gonzalez et al, 2020	49	Not specified	25/08/2020 - 15/09/2020	329	331 (n/a)	RT-PCR	15	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Jafari et al, 2020**	121	RC, PS, CR, CS	Inception - October 2020	10,000	n/a	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Yuan et al, 2020	29	13CS, 16CR	01/01/2000-25/10/2020	564	555 (549)	RT-PCR or serology	18	Not reported	Not reported	Not reported	Not reported	18/549	Not reported	Not reported	Not reported
do Amaral et al, 2020	70	7CS, 21RC, 2OB, 9DS, 35CR, 2CHS, 1LE	1/12/2019 - 31/10/2020	1457	998 (n/a)	RT-PCR	39	Not reported	Not reported	Tested	Not reported	Not reported	Not reported	Not reported	Not reported
Naz et al, 2020	16	4RS, 4CS, 6CR, 2PS	March 2020-31/10/2020	498	471 (n/a)	RT-PCR or serology	23	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported

*Table 8: Study characteristics and data extracted on 68 systematic reviews for inclusion in review of reviews. (N/A: data not available, CS: case series, CR: case report, LE: letter to editor, NR: narrative review, SR: systematic review, CHS: cohort study, RC: retrospective cohort study, PC: prospective cohort study, C: case study, CC: case control, OB: observational study, CRS: cross-sectional study, DS: descriptive study, MA: meta-analysis, ACRS: analytical cross-sectional study. ** Denotes the review conducted a meta-analysis.*

3.2 Review of classification systems

The existing evidence surrounding MTCT of SARS-CoV-2 *in utero*, intrapartum or postnatally is sparse. The 68 systematic reviews differed in specificity and sensitivity. In these circumstances, the use of a classification system can provide a helpful structure to aid healthcare practitioners to evaluate appropriate infection control measures for pregnant patients **(table 9).**

Due to the differing recommendations on MTCT of SARS-CoV-2 in pregnancy, clinicians are unable to determine a common ground on which recommendations to follow. With the added complication that a pregnant woman can get infected with SARS-CoV-2 at any time during pregnancy. Infection in the early stages of pregnancy may present differently when compared to infection in the weeks before delivery (135).

3.2.1 Shah *et al.*

The Shah *et al.* (136) classification systems created five mutually exclusive categories that allow categorisation of maternal and infant infection to determine the likelihood of infection. These five categories are; (a) confirmed, (b) probable, (c) possible, (d) unlikely and (e) not infected.

Categories (a) and (e) are considered absolute, while category (c) implies suggestive evidence of infection but is incomplete. Category (d) means there is little support for diagnosis, but infection

is not entirely ruled out. The authors comment on how a case could be assigned a particular category but then move to a different category upon further available information.

Furthermore, the authors confirm that by defining these distinct categories, terminology of 'vertical' or 'horizontal' transmission can be avoided so the focus is solely on the exact pathological process rather than unknown directions of transmission.

The system to classify transmission is reported as a neonatal infection acquired intrapartum; neonatal infection acquired postpartum; congenital infection with intrauterine foetal demise; and congenital infection in the live-born neonate.

While the authors did not report how they decided these proposed definitions they did report that it was based on existing evidence of perinatal infection, and reported how the testing of serological samples, i.e., breast milk, maternal skin swab or rectal swabs, was not done due to the unclear nature of these swabs at the time of writing their paper.

3.2.2 **Blumberg *et al.***

The classification system by Blumberg *et al.* (137) is based on underlying assumptions that allow the proposal for definitions of vertical SARS-CoV-2 transmission.

The assumptions are that the incubation period is 1 to 14 days; intrauterine transplacental infection can occur via a haematogenous route or transmission by the neonate swallowing amniotic fluid. The authors also report that maternal viremia is unlikely during the incubation period (>48 hours before symptom onset), and the likelihood of positive SARS-CoV-2 infection through RT-PCR in blood samples is low in COVID-19 patients.

Intrapartum transmission of SARS-CoV-2 is possible due to exposure to maternal blood, faeces or vaginal secretions. An early postnatal infection could occur through respiratory routes or direct contact with the infected mother (infected breastmilk) or healthcare workers. Lastly, the authors also assume that SARS-CoV-2 may be transiently detected for up to 24 hours after birth due to superficial contamination or transient viremia.

Based on these assumptions, the authors categorise vertical SARS-CoV-2 transmission into three categories; intrauterine transmission, intrapartum or early postnatal transmission and superficial exposure to SARS-CoV-2 or transient viremia. The classification does not consider maternal or infant symptoms and does not include categorisation by the strength of the classification of the timing of infection.

Intrauterine transmission of SARS-CoV-2 during the peripartum period has likely occurred with both early exposure and persistence. The mother is positive for SARS-CoV-2 14 days before delivery and two days after delivery. By 'early exposure', the authors mean the virus is detected in either the amniotic fluid, umbilical cord blood, a neonatal respiratory tract swab or neonatal blood samples in the first 24 hours of life. Then, 'persistence' refers to a positive neonatal respiratory tract swab after 24 hours of postnatal life, or in the first seven days of postnatal life, the neonate has a positive SARS-CoV-2 IgM assay.

Intrapartum or early postnatal transmission were combined as the classification system assumes it would be difficult to distinguish between an intrapartum infection that has occurred via exposure to infected vaginal secretions, maternal blood or faeces during the passage of the neonate through the birth canal. As opposed to infection caused by the environment experienced soon after birth via the respiratory route of the neonate and direct contact with the mother/healthcare workers or potential transmission through breastmilk.

The intrapartum/early postnatal transmission category is defined as; when an infection has likely occurred due to a lack of *in utero* exposure, but there is evidence of intrapartum or early postnatal transmission. The author reports that at least one item of the following three categories needs to be filled: the mother or another person (healthcare worker/family) is positive for SARS-CoV-2 between 14 days prior to birth and two days after birth; a neonatal respiratory tract swab is negative in the first 24 hours of life, and persistence is defined as a neonatal respiratory tract swab being positive between 24 hours and two weeks of postnatal life, or the neonate is positive for SARS-CoV-2 IgM assay in the first 2-3 weeks of postnatal life.

Superficial exposure to SARS-CoV-2 or transient viremia is likely if the neonate is asymptomatic and satisfied one item of the following; the mother is positive for SARS-CoV-2 in the 14 days prior to birth and two days after birth; early exposure of the virus is detected in amniotic fluid, umbilical cord blood, neonatal blood samples or neonatal respiratory tract swab in the first 24 hours of life; and no evidence of *persistence* or immune response if the neonatal respiratory tract swab is negative between 24 and 48 hours of life or in the first 2 to 3 weeks the neonate tests negative for a SARS-CoV-2 IgM assay.

3.2.3 WHO classification system

The WHO (World Health Organization) classification system is an international consensus founded on evidence synthesis and a WHO expert consultation panel (37). The WHO COVID-19 LENS (Living Evidence Synthesis) group consolidated evidence on pregnancy and COVID-19 on potential mechanisms of vertical transmission of infectious pathogens, data related to interpreting positive SARS-CoV-2 virological and serological neonatal tests, the feasibility of vertical transmission of SARS-CoV-2, lessons from diagnosis of other congenital infections and the proposal of definitions to classify the timing of vertical transmission of SARS-CoV-2.

The WHO classification system begins by categorising the mechanisms of vertical transmission of infectious pathogens. These are split into *in utero* transmission, intrapartum transmission and postnatal transmission **(Appendix 6).**

As previously mentioned, *in utero* transmission is when pathogens are transmitted *in utero* through the haematogenous route. Intrapartum transmission occurs during labour and childbirth and would require the pathogen to be present in maternal blood, faeces and vaginal secretions during the birth process (37). Finally, postnatal transmission can occur when the infant is exposed to breast milk containing an infectious pathogen. It may also occur via an infected mother's respiratory droplets or other infectious, maternal secretions and can also occur through contact of the infant with other infected caregivers or family.

The WHO classification system categorises the timing of SARS-CoV-2 vertical transmission through a basis of three elements; tests to evaluate the likelihood of early *in utero* or intrapartum exposure; tests to evaluate later exposure/persistence of the virus (or virus-specific immune response) in the foetus/neonate; and maternal infection that has been documented using the WHO COVID-19 case definitions (at any point of pregnancy for *in utero* transmission and near the time of birth for intrapartum and early postnatal infection).

The timing of vertical transmission is further classified into mutually exclusive categories; confirmed, possible (evidence is suggestive but not confirmed for infection), unlikely (infection cannot be ruled out despite little supporting evidence), and indeterminate (tests used to define classification have not been performed).

The importance of harbouring a classification system in systematic reviews is so a universal consensus can be agreed on, where the timing and the method of assessing vertical transmission are compared against a tool used universally. This will allow clinicians to make more informed decisions about the actual disease burden and effective clinical measures that need to be taken.

	Shah *et al.* (113)	**Blumberg *et al.*** (114)	**WHO** (37)
Date of publication	11/04/2020	05/06/2020	07/02/2021
Categorisation of mother-to-child transmission	• Congenital (*in utero*) infection in intrauterine death/ stillbirth • Congenital (*in utero*) infection in live born • Neonatal infection acquired intrapartum • Neonatal infection acquired postpartum Likelihood of infection is further classified into confirmed, probable, possible, unlikely and not infected.	• Intrauterine (*in utero*) transmission • Intrapartum or early postnatal transmission • Superficial exposure to SARS-CoV-2 or transient viremia	• In utero transmission in the case of a live birth • In utero transmission in the case of foetal demise • Intrapartum transmission • Early postnatal transmission (age > 48 hours – 28 days) Likelihood of infection is further classified into confirmed, possible, unlikely and indeterminate.
Definition of maternal SARS-CoV-2 infection	Mothers with suspected or confirmed COVID-19 at the time of delivery.	Mothers with a positive SARS-CoV-2 test between 14 days before to 2 days after delivery.	In utero transmission: mothers with confirmed COVID-19 infection anytime during pregnancy. Intrapartum and postnatal transmission: mothers with confirmed COVID-19 diagnosed near the time of delivery.
Samples for virologic or immunologic testing	• Neonatal respiratory tract swab • Foetal tissue or surface swab • Placental tissue or surface swab: maternal or foetal side • Umbilical cord blood • Neonatal blood • Amniotic fluid • Skin/vaginal/ rectal swab	• Neonatal respiratory tract swab • Amniotic fluid • Umbilical cord blood • Neonatal blood	Sterile specimens • Neonatal lower respiratory tract samples • Cerebrospinal fluid • Neonatal blood • Amniotic fluid (only if collected prior to membrane rupture or via amniocentesis) • Foetal organs (e.g., lung, liver, brain) Non-sterile specimens • Neonatal upper respiratory tract samples • Placental tissue or surface swab • Foetal swab • Saliva, or stool

Table 9: A summary of the key features of the classification systems (Shah et al. , Blumberg et al. and WHO classification system) depicting the categorisation of MTCT in each, and the definition of maternal SARS-CoV-2 infection and the samples needed for virological and immunological testing.

3.3 The rate of SARS-CoV-2 positivity in exposed babies in cohort studies

The 569,232 identified articles consisted of 472 studies made up of 206 cohort studies, 266 case reports and series. Of the 206 cohort studies, 144 reported on offspring SARS-CoV-2 positivity status in 14,518 exposed babies. Across all studies included, 988 babies tested positive for SARS-CoV-2 (247 studies; 113 cohorts, 134 case series or reports). Regarding reports on offspring SARS-CoV-2 positivity, 6147 mother-baby dyads were found in 67 comparative cohorts **(figure 10).**

Characteristics of the included studies

Of the studies included, most were from Central Asia and Europe (145/472, 31%) and North America (87/472, 18%). East Asia and Pacific at 15% (73/472), the Middle East and North Africa at 13% (60/472); Latin America and the Caribbean at 11% (51/472), South Asia made up 51/472 studies at 11% and finally, Sub-Saharan Africa made up 1% of selected studies with 5/472 studies selected. The ascertainment of maternal infection was confirmed by RT-PCR in 99% (467/472) of studies. The most used test to ascertain offspring infection was RT-PCR in 97% of cohort studies (140/144); 10% (15/144) of cohort studies used either anti-SARS-CoV-2 IgM test alone or with RT-PCR.

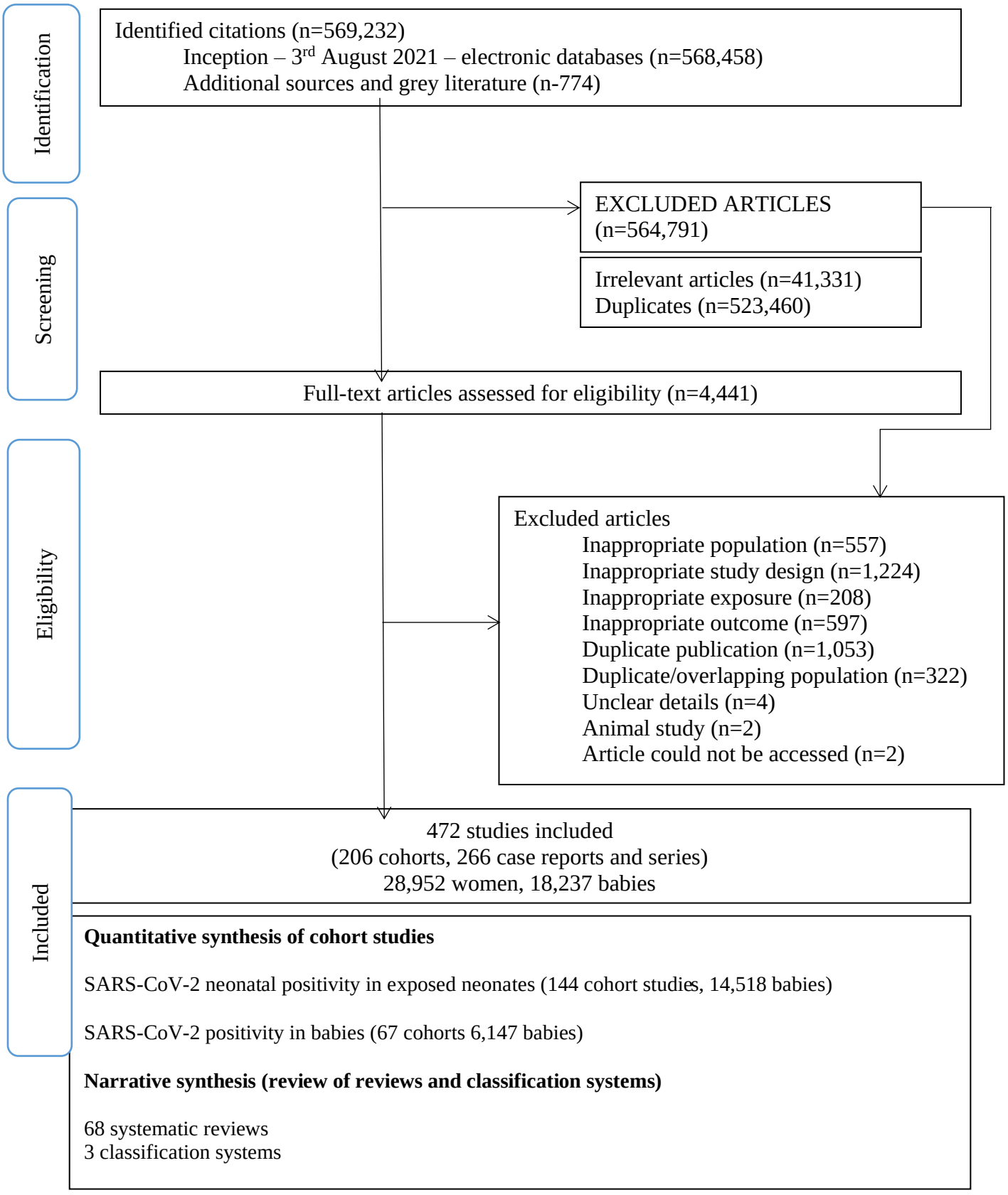

Figure 10: Study selection process of the LSR.

Quality of the included studies

Through using the Hoy *et al.* study quality assessment tool, the internal validity of non-comparative cohorts showed a low risk of bias for case definition in 62% (122/197) of the studies, for data collection in 98% (194/197) of the studies, for differential verification in 97% (192/197), for measurement in 100% (197/197), for adequate follow-up in 60% (119/197), and appropriate numerator and denominator in 92% (182/197).

The external validity of studies also had a low-risk bias for sampling in 24% (47/197) of the studies, representativeness in 9% (17/197), 98% for non-response (194/197) and 88% for selection (173/197).

To assess the overall risk of bias, the Newcastle Ottawa Scale was adopted to assess included comparative cohort studies. The overall risk was low in 99% of studies (66/67); 97% had a low risk of bias for study selection (65/67), 78% for outcome assessment (52/67) and 28% (19/67) for comparability of cohorts.

SARS-CoV-2 positivity in exposed babies in cohort studies

From 140 cohort studies, 14,271 babies were included, the overall SARS-CoV-2 positivity using RT-PCR was observed in 1.8% (95% CI 1.2-2.5%) of all babies born to mothers diagnosed with SARS-CoV-2 infection. In the case where studies used either RT-PCR or anti-SARS-CoV-2 IgM tests, 1.9% (95% CI 1.3-2.7%) tested positive (144 studies, 14,518 babies). Furthermore, of all

exposed babies who were tested (15 studies, 583 babies), anti-SARS-CoV-2-specific IgM antibodies were demonstrated in 2.6% (95% CI 0.52-5.55%). The SARS-CoV-2 RT-PCR positivity rate was 1.7% (95% CI 1.1-2.5%) in babies born to mothers infected with SARS-CoV-2 through sensitivity analysis limited to high-quality studies, which rendered similar to the main analysis. When limiting the analysis to just babies of mothers diagnosed with SARS-CoV-2 infection in the antenatal period, the positivity rate was 1.3% (95% CI 0.62-2.23%) **(figure 11).** The positivity rate was 0.9% (95% CI 0.15-2.12%) when limited to babies tested in the first 24 hours after birth. The rates of offspring SARS-CoV-2 RT-PCR positivity rates varied, through subgroup analysis, between regions, ranging from 0.1% (95% CI 0.00-0.34%) in studies from North America to 5.7% (95% CI 3.2%-8.7%) in studies from Latin America and the Caribbean.

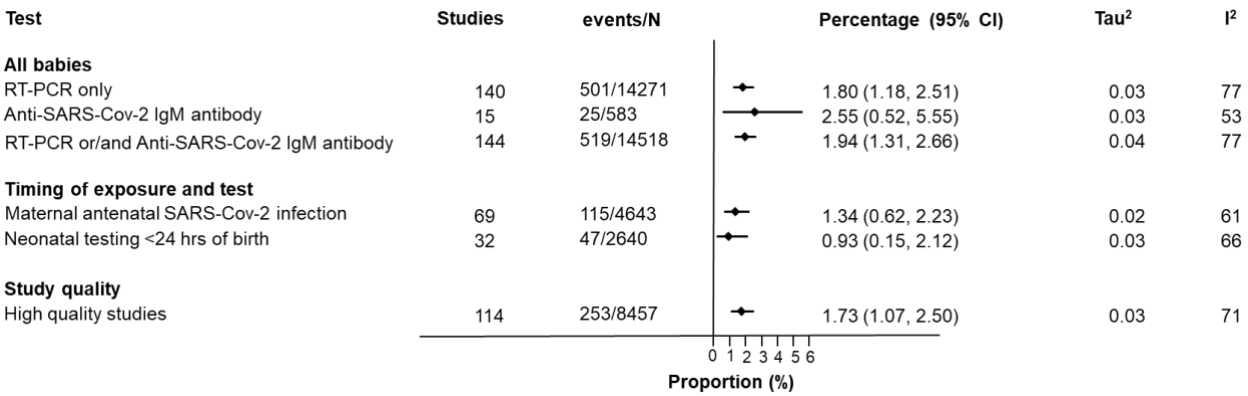

Figure 11: The rates of SARS-CoV-2 neonatal positivity born to mothers having active or recently diagnosed with SARS-CoV-2 infection, seeking hospital care for any reason.

3.4 What are the risk factors for SARS-CoV-2 positivity in neonates?

The risk factors hypothesised to be associated with SARS-CoV-2 neonatal positivity are vaginal delivery, breastfeeding and rooming-in **(table 10).**

A total of 49 studies were looked at for delivery mode, comprising of 4814 mother-baby dyads. The number of positive babies/total risk factor (vaginal birth) was 159/2429. While the number of positive babies/total no risk factors (caesarean) was 99/2385 (95% CI 0.97-1.95).

Not separated vs separation at birth included 11 studies comprising of 1617 mother-baby dyads. The number of positive babies/total risk factor (not separated) was 42/658. Meanwhile, the number of positive babies/total no risk factor (separated) was 48/959 (95% CI 0.47-3.98) **(figure 12).**

The breastfed vs not breastfed group included 13 studies comprising 1545 mother-baby dyads. The number of positive babies/total risk factors (breastfed) was 43/783, while the number of positive babies/total no risk factors (not breastfed) was 39/762 (CI 95% 0.34-1.62) **(figure 13).**

Risk factors	No. of studies	No. of mother-baby dyads	Positive babies/total risk factor	Positive babies/total no risk factors	OR (95% Confidence Interval)	I^2
Caesarean vs vaginal birth	49	4814	159/2429	99/2385	1.38 (0.97-1.95)	18%
Not separated vs separation at birth	11	1617	42/658	48/959	1.37 (0.47-3.98)	64%
Breastfed vs not breastfed	13	1545	43/783	39/762	0.74 (0.34-1.62)	29%

Table 10: The risk factors associated with SARS-CoV-2 neonatal positivity. The included risk factors are mode of delivery, separation at birth, and breastfed vs not breastfed.

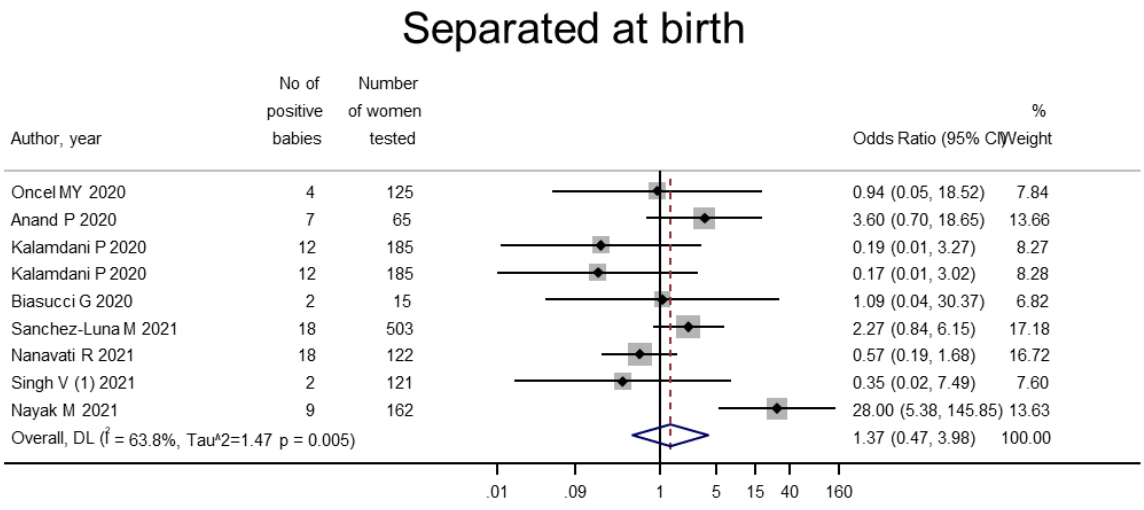

Figure 12: Forest plot of risk factor separation at birth. Overall significance is demonstrated by blue diamond.

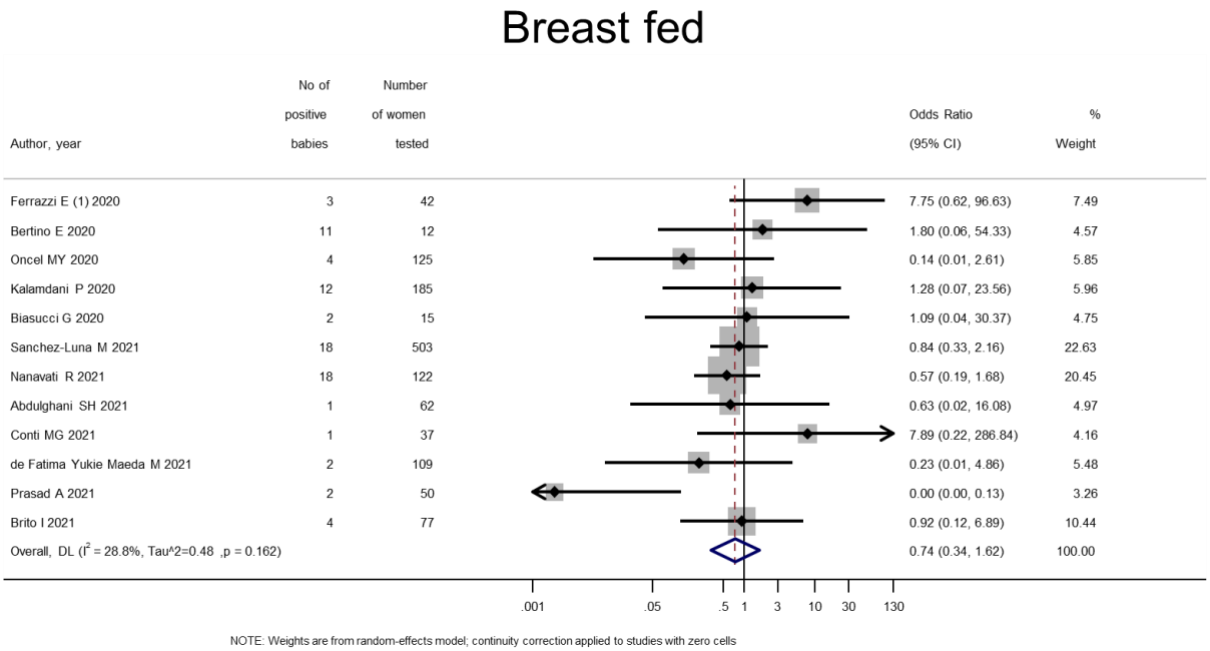

Figure 13: Forest plot of risk factor breastfeeding. Overall significance is demonstrated by blue diamond.

4 Discussion

4.1 Summary of results

A systematic review and meta-analysis were conducted to determine the rate of SARS-CoV-2 neonatal positivity and rate of vertical transmission if any and to establish if the risk factors of breastfeeding, rooming-in or vaginal delivery were contributing to offspring positivity. Furthermore, a review of reviews on existing systematic reviews on MTCT of SARS-CoV-2 was carried out to collate existing evidence. Additionally, reviews on classification systems on MTCT of SARS-CoV-2 were also narratively described.

The hypothesis of this thesis is partially accepted, as the systematic review and meta-analysis showed that the risk factors stated above had no association to increasing neonatal positivity rates. However, the hypothesis of ascertaining a rate, if any, for vertical transmission was proven and hence accepted. While the occurrence is rare, viral RNA particles have been detected in serological samples, confirming the incidence of vertical transmission. However, the presence of SARS-CoV-2 RNA in amniotic fluid, vaginal fluid, breastmilk, placenta, and its membrane does not necessarily correlate to active infection of the newborn.

Furthermore, the overall rate of SARS-CoV-2 positivity was estimated to be less than 2% in babies (tested using RT-PCR) of mothers seeking hospital care for any reason. The rate was even lower at 1% in babies with antenatal or intrapartum exposure to the virus.

Additionally, the evidence narratively synthesised from the 68 systematic reviews reporting on MTCT of SARS-CoV-2 showed that more high-quality systematic reviews are needed that incorporate larger cohort studies. Also, many failed to use an appropriate classification, such as the WHO classification system, therefore not allowing for a universal and standardised approach to understanding results. Many systematic reviews also failed to register their protocol with PROSPERO or database as such, resulting in data duplication and research waste.

4.1.1 Review of reviews

Existing evidence on MTCT of SARS-CoV-2 was searched for in systematic reviews. Between December 2019 to March 2021, 68 systematic reviews were published and are included in this thesis.

The main inclusion criteria for these papers were reporting on vertical transmission of SARS-CoV-2, which can be detected through viral SARS-CoV-2 RNA in serological samples. Data extraction of the 68 studies was divided into maternal (breast milk, placenta, amniotic fluid) and neonatal samples (pharyngeal swab, umbilical cord blood, urine/faecal swabs and peripheral blood/other) **(table 8).**

Sixteen studies tested breastmilk samples, of which four studies (138–141) reported eight positive neonates confirmed by pharyngeal swab out of 161 tested neonates. While there was viral RNA in maternal vaginal and stool swabs, the author attributed these to possible contamination, making it challenging to prove vertical transmission. Kumar *et al.* (139) reported 10/88 positive breastmilk samples. However, despite having more positive samples than Shrestha *et al.* , Kumar *et al.* (139) also reported that viral SARS-CoV-2 RNA presence in breastmilk does not equate to an active infection and encourages breastfeeding practise.

Lastly Sampieri *et al.* (140) reported 2/32 positive breastmilk samples, 6/34 positive placenta samples and 1/38 positive umbilical cord samples. However, the author reported zero positive

neonates and concluded the same as Shrestha *et al.* (138) and Kumar *et al.* , (139) that further, more extensive studies are needed to locate in situ RNA SARS-CoV-2 protein.

Various studies reported positive maternal placenta samples, as shown in **table 8.** Turan *et al.* (141) reported one positive placenta swab out of 16 samples but concluded that further evidence is needed to confirm vertical transmission. However, differing conclusions from Kotlyar *et al.* (57) report a pooled proportion of 3.2% for vertical transmission and positive placental sample. The study also reports positive umbilical cord, faecal/stool swab and peripheral blood swab testing positive for SARS-CoV-2 RNA. The study (57) concludes that vertical transmission of SARS-CoV-2 is possible and that a few cases show that it occurs more in the third trimester of pregnancy.

Arroyo-Sanchez *et al.* (142) and Kotlyar *et al.* (57) were the only studies to report positive umbilical cord samples. Arroyo-Sanchez *et al.* (142) reported 2/9 positive umbilical cord samples and confirmed that these samples coexisted with positivity in neonates. However, the authors (142) dispute that vertical transmission has not been conclusively demonstrated as there was much heterogeneity in the study's results. Like other authors (138–140) have agreed, more research is required incorporating homogenous studies.

Further authors confirmed neonatal positivity through RT-PCR testing or serology and did not test maternal or neonatal samples. Chi H *et al.* (143) reported 8/91 positive neonates and concluded that the evidence surrounding vertical transmission is scarce, but its risk should still be considered. In summary, the presence of SARS-CoV-2 RNA alone is not indicative of disease and could be attributed to other factors like contamination.

As systematic reviews are subject to a range of biases, a quality assessment tool that can be applied to differentiate between high and low-quality reviews is essential (115). The methodological quality of the systematic reviews was checked against the AMSTAR-2 tool as it covers a range of aspects quickly and comprehensively and provides user guidance (112).

The AMSTAR-2 tool is not purposed to generate an overall score but acts more like a checklist in identifying high-quality systematic reviews (113). Additionally, studies have shown that scoring papers might be counterproductive, so it has been recommended to avoid doing so (144)

As mentioned in the methodology section, in this thesis, the AMSTAR-2 questions were narrowed down to seven critical domains that critically impact the review's validity (113).

The first critical domain is the inclusion of PICO (population, intervention, comparison and outcome). This domain had the highest percentage score, with 64 systematic reviews scoring 'yes' to include PICO's. The PICO tool is used in producing evidence-based medicine to answer clinical or healthcare-related questions. It is essential in formulating a research question, and so it is crucial to implement in systematic reviews to streamline the process (115).

The subsequent critical domain checks if a protocol was registered before the commencement of the review process. The relevance of a protocol registration is to allow transparency in research, avoid duplication and reduce potential research bias (145). To score a 'partial yes', the review authors should have a written a protocol including the review question(s), search strategy,

inclusion/exclusion criteria, and risk of bias assessment. In order to score a 'yes', the protocol should be registered and specify a meta-analysis/synthesis plan and a plan for investigating causes of heterogeneity. Similarly, The Cochrane handbook also recommends that a protocol should include eligibility criteria, choice of comparators, and outcomes (115).

The 68 systematic reviews showed that only 13.2% scored 'yes', and 22.1% scored a 'partial yes'. This low rate is supported by Moher *et al.* 's study (146), where out of 300 systematic reviews, only 1/3 reported on the quality of included studies (146) . Many studies that did not report a protocol were 'non-Cochrane' reviews. The author concludes that many systematic reviews vary, and readers should not accept conclusions of this systematic review uncritically (146).

Next, domain four checks whether review authors used a comprehensive literature search strategy. To qualify for a 'partial yes', the review authors should have searched at least two databases (relevant to the research question), provided keyword and search strategy and justified publication restrictions (e.g., language). For 'yes', the authors should have searched reference lists/bibliographies of included studies, searched trials/study registries, consulted experts in the field and conducted the search within 24 months of completion of the review.

It is essential to conduct adequate literature searches in systematic reviews to ensure the study does not miss critical findings and reduces bias. Also, for authors to understand how previous literature has explored this topic, review authors can then tailor their research questions to fill research gaps by consolidating what is already known in the research domain (115) . The results showed that 2.9% of studies scored 'yes' and 30.9% scored 'no', and 66.2% scored 'partial yes'. The low

occurrence of studies scoring 'yes' may be attributed to the extensive list of criteria AMSTAR-2 had set out.

As 21/68 studies reported 'no', this could mean that bias in these studies is high and thus invalidate conclusions (115,147). However, contradicting evidence by Egger *et al.* (148) reports how conducting a comprehensive literature search is not the only mitigating factor in reducing study bias. The authors (148) found that comprehensive searches were still missing out studies and maybe introducing bias instead of preventing it. While Eggar *et al.* (148) argue that grey literature may be a waste of time and low quality, the Cochrane handbook counters this and recommends that grey literature sources should be extensively searched to reduce the risk of publication bias and allow as much evidence collection as possible (115).

The criteria for domain seven was based on whether review authors provided a list of excluded studies and justified these exclusions. Out of 68 studies, zero studies provided this information. Not accounting for these excluded studies increases the risk of not showing how these studies impacted the review, thus reducing transparency. However, while none of the 68 studies included this AMSTAR critical domain, there is the possibility that many studies had publishing guidelines of a word count or limits on how much content can be published. For this reason, it may be that the authors have decided to remove certain aspects of the review process. Again, this reiterates why the AMSTAR-2 tool should not be used as a scoring tool as it can be misleading (149,150).

AMSTAR critical domain nine queries the use of a satisfactory assessment for the risk of bias of individual studies included in the review. Forty-two studies reported no risk assessment of bias,

and ten reported ‘yes’. This domain coincides with domain 13 (did review authors account for the risk of bias in primary studies when interpreting/discussing the review?). Forty-three studies reported not discussing risk of bias, and only 25 studies did.

The relevance of this domain is that review authors should explicitly reference any potential risk of biases when interpreting and discussing results as systematic reviews use primary data from other primary sources. The bias assessment is only as good as the primary data's bias assessment. Hence, Cochrane and AMSTAR advise conducting a risk of bias on individual studies (150).

Lastly, critical domain 11 is only relevant for studies that conducted a meta-analysis and questions whether review authors used an appropriate method for statistical combination of results. The criteria for this domain are that for randomized control trials, it requires authors to use an appropriate weighted technique to combine study results, adjust for heterogeneity, and investigate its causes. For non-randomized studies of intervention, the authors have to justify the same as RCT, but also show statistically combined effect estimates from NRSI that were adjusted for confounding and also report separate summary estimates for RCT and NRSI separately if both are included in the review.

Of the 68 studies, 49 did not conduct a meta-analysis, and of the studies that did, seven scored a 'yes', and 12 scored a 'partial yes'. The importance of using appropriate meta-analytical methods is to allow reviews to quantify and synthesise outcomes and obtain data on statistical significance and relevance. If reviewers did conduct a meta-analysis, the inclusion of non-randomised control trials

would increase heterogeneity due to the complexity of the analysis. Only one study (151) explained a meta-analysis was not conducted due to the sample size and level of missing data.

4.1.2 Review of the classification system

The following subsection of this thesis reviewed existing classification systems on MTCT of SARS-CoV-2. Understanding the exact timing of vertical transmission can be difficult to ascertain, therefore classification systems have been developed to aid in this.

Classification systems and case definitions prove valuable tools for clinicians, researchers and epidemiologists. Furthermore, by utilising a universal approach, data quality and analysis are improved in accuracy and allow comparisons with a more expansive range of studies from different geographic locations and centres (152,153).

Classification systems allow for the categorisation of patients into distinct groups. In doing so, clinically similar patients can be provided with similar decisions on disease management (153). Likewise, in knowing the categorisation of MTCT SARS-CoV-2 transmission, the correct management and preventative measures can be adopted.

The possibility of mother-to-child transmission of SARS-CoV-2 has been highly debated due to the adverse neonatal and maternal outcomes (136); therefore, obstetricians, gynaecologists, paediatricians, and other healthcare providers need to understand the true extent of the transmission. The current evidence is sparse due to limitations in the sensitivity and specificity of diagnostic tests and classifying patients based on test results. Additionally, collecting differing samples and varying recommendations have made it difficult to determine which samples should be collected and how to distinguish between sterile and non-sterile samples.

Furthermore, the exact timing of vertical transmission is also vital, as a pregnant woman could be infected at any point during pregnancy. Some studies have found a link between the trimester of infection impacting maternal and neonatal outcomes (57,154). Therefore, the appropriate preventative measures can be taken by understanding if a particular time point during pregnancy renders a higher occurrence of vertical transmission.

The various diagnostic mechanisms surrounding the identification of virological RNA or through serological testing come with sensitivity and specificity issues (155). Therefore, to alleviate the ambiguity surrounding diagnostic techniques and interpretation of results, incorporating a classification system will allow the correct determination of appropriate infection control measures (136).

The three current classification systems used to categorise the timing of mother-to-child transmission of SARS-CoV-2 are The World Health Organization (WHO) (37) expert consensus classification system, the Blumberg *et al.* (137) and Shah *et al.* classifications (136). The classification systems work similarly; based on the timing and type of maternal and neonatal testing. However, they vary on the criteria they are set upon when characterising the timing of transmission. They all broadly categorise SARS-CoV-2 MTCT into three possible exclusive modalities of *in utero*, intrapartum and postnatal transmission.

Out of the three classification systems mentioned in this thesis, the WHO classification system developed by international consensus (37) combined the strength of classification of the timing of

transmission with the persistence of positivity and considered the sterility of the evaluated samples if preferred (94).

One of the main aspects of the 68 systematic reviews was that only one incorporated a classification system. Also, many of the studies determined neonatal positivity based on one sample. Through incorporating the WHO classification system to categorise the timing of infection, repeat testing at various time points is required. By combining the initial test with a repeat test, a more accurate determination of infection occurrence and timing can be established.

4.1.3 Offspring SARS-CoV-2 positivity

The overall rate of SARS-CoV-2 positivity using RT-PCR was observed in 1.8% of all babies born to SARS-CoV-2 diagnosed mothers. Therefore, it can be expected that the overall risk of SARS-CoV-2 transmission to exposed babies is very low. From the results of the subgroup analysis, it was also evident that the rate of offspring SARS-CoV-2 positivity varied between World Bank regions. The rate in studies from North America was at 0.1%, and 5.7% in studies from Latin America and the Caribbean. The variation in rates of SARS-CoV-2 across countries could be attributed to the different policies around maternal screening for COVID-19 (156). Supporting findings by Hashim *et al.* reports that universal testing of pregnant women with COVID-19 is higher in Western regions (157).

This thesis hypothesised that SARS-CoV-2 could transmit from mother-to-child through vertical transmission either *in utero*, intrapartum or postnatally, causing adverse neonatal and maternal outcomes. Based on the results of this thesis, the hypothesis is accepted, as there is evidence for vertical transmission of SARS-CoV-2. While the rates are low and the occurrence is rare, it is not impossible. Furthermore, the rate of neonatal positivity is estimated as 1.8% positivity in babies born to mothers diagnosed with SARS-CoV-2.

The findings of this thesis are in concordance with existing research on the rates of SARS-CoV-2 positivity. Fenizia *et al.* (158) found SARS-CoV-2 genome in the vaginal mucosa of pregnant women, term placentas, umbilical cord blood and milk specimens. This study incorporated the Shah *et al.* classification system to determine that *in utero* transmission is possible for SARS-CoV-

2 positive pregnant women, with a rate of 6%. Further studies (159) also report on vertical transmission rate. Sheth *et al.* (135) report a rate of 3% in a study of 326 SARS-CoV-2 positive mothers and 23 SARS-CoV-2 positive neonates. This study concluded that while vertical transmission was possible, the rate was low, which is coherent with the findings of this thesis.

Both Raschetti *et al.* (134) and Angelidou *et al.* (160), report on neonatal SARS-CoV-2 positivity rates. Raschetti *et al.* (134) found that 55% of infected neonates developed COVID-19 by looking at 176 meta-analyses on neonatal SARS-CoV-2 infections. Angelidou *et al.* (160) report a 2.2% rate of neonatal SARS-CoV-2 positivity. The authors (160) also conclude that the rate of SARS-CoV-2 neonatal positivity was enhanced by SARS-CoV-2 disease severity in the mother.

A further scoping review (161) studied 157 mothers and 160 neonates and reported a 6% positivity rate with five neonates testing positive for SARS-CoV-2. However, the author ensures to state that the positivity could be due to horizontal transmission rather than vertical transmission; an RT-PCR test was conducted at 16 hours after birth, and only one neonate, when re-tested, was positive but negative at birth.

While there are debates around the sensitivity and specificity of SARS-CoV-2 diagnostic tools, determining whether the infection has occurred *in utero*, intrapartum or postnatally is difficult; this hurdle can be solved by using a classification system such as the WHO classification for MTCT transmission (37). In doing so, researchers will obtain accurate timing and ascertainment of SARS-CoV-2 exposure increasing data accuracy.

However, it is essential to note that while biological samples may have SARS-CoV-2 RNA detected, the detection of the virus alone may not necessarily indicate infection of the baby.

The findings of this thesis are coherent with current literature in that the rate of neonatal SARS-CoV-2 positivity and vertical transmission remains relatively low. They are supported by studies (162,163) stating that evidence surrounding the vertical transmission of SARS-CoV-2 is limited and weak, therefore requiring further research efforts.

4.1.4 Risk factors associated with neonatal SARS-CoV-2 positivity

This thesis hypothesised that the risk factors associated with neonatal positivity include breastfeeding, vaginal delivery and rooming-in mother and child after birth. However, the findings of this thesis show that these factors did not impact neonatal positivity. Therefore, this hypothesis is rejected.

Vaginal delivery was hypothesised as a risk factor for increasing neonatal positivity due to the increased risk of vertical transmission. During vaginal delivery, the neonate comes into contact with maternal fluids from the vagina and rectum (164). Pathogens like Group B Streptococcus and Human Papilloma Virus (HPV) have been shown to infect neonates through this route (165). Therefore, if these maternal fluids are infected with SARS-CoV-2, it is safe to hypothesise that this could infect the neonate through entry via the oral or nasopharynx during vaginal delivery. Forty-nine studies reported on the mode of delivery, 159/2429 babies tested positive when delivered vaginally, and 99/2385 tested positive when delivered via caesarean section. The 95% confidence interval range of this was 0.97 to 1.95. As the range includes the number 1.0, it would cross over the line of no effect, so the results are not statistically significant.

Furthermore, separation of the infant from the mother after birth was hypothesised to reduce the rates of neonatal SARS-CoV-2 positivity. The reason is that if an infected mother is in close proximity with her newborn, infected aerosol droplets or contamination from touching surfaces may cause neonatal infection. However, from 11 included studies, 42/658 neonates tested positive when roomed-in with the mother, and 48/959 neonates tested positive when separated at birth.

However, the overall significance ranged from 0.47 to 3.98. As this again crosses over the line of no effect, the results are not statistically significant.

Breastfeeding infants was hypothesised as an additional risk as it allows potentially infected breastmilk to infect the infant. There have been reports on the presence of viral SARS-CoV-2 RNA particles in breastmilk samples (77,88,162,166), so it was hypothesised that breastfeeding is a risk factor contributing to neonatal positivity rates. The results for this risk factor included 13 studies, 43/783 neonates tested positive when breastfed, and 39/762 neonates tested positive when not breastfed. However, the overall significance of the results (**figure 12**) shows a confidence interval range of 0.34 to 1.62. As this crosses over the line of no effect (at number 1), the results are insignificant (**figure 12).**

A study by Walker *et al.* (163) incorporated 666 neonates. Of these, 28/666 (4%) tested positive in the postnatal period. The neonates born vaginally had a positivity rate of 8/292 (2.7%), whereas neonates born via caesarean route were positive at 20/374 (5.3%). The comparison between breastfed babies and non-breastfed babies (formula-fed babies) was 7/148 (4.7%) and 3/56 (5.3%), respectively. Finally, the positivity rates in babies separated from mothers were 6/46 (13%) and 4/107 (3.7%) in those that roomed in with mothers.

A study by Rottenstreich *et al.* (167) conducted a multicentre prospective analysis in which it was concluded that the rates of vaginal delivery were high and had favourable outcomes with no cases of neonatal COVID-19 positivity. Likewise, a study by Ferrazzi *et al.* (168) reported outcomes on 42 women eligible for the study. Vaginal delivery occurred in 60% of women, and it was

concluded that a low risk of intrapartum SARS-CoV-2 transmission was associated with vaginal delivery.

Raschetti *et al.* (134) report that breastfeeding and neonatal SARS-CoV-2 positivity rates are not related and that viral transmission through milk is rare. Therefore, supporting RCOG (11) and WHO (11,45) advice to continue breastfeeding even in COVID-19 positive mothers, but to wear protective masks adopt a strict hygiene routine (11). The findings are supported by a further study (89) where 110 samples of breastmilk were analysed by RT-PCR. SARS-CoV-2 viral RNA was found in seven samples and in 6/65 samples where the mother tested positive for SARS-CoV-2. However, infectious virus was not detected in any culture, and thus the study concludes that breastfeeding from women proven or suspected to have COVID-19 is not a hazard for their infants.

A systematic review by Centeno-Tablante *et al.* (88) reviewed 37 articles that reported breastmilk samples on 77 mothers who breastfed their children. Nineteen of the 77 children were confirmed COVID-19 positive, confirmed with RT-PCR. Furthermore, 9/68 breastmilk samples tested positive for SARS-CoV-2 RNA; of the six exposed infants, four tested positive, and two tested negative. The study concludes how currently there is no evidence of SARS-CoV-2 transmission through breastmilk. Studies including longer follow-up periods are needed to collect data on infant feeding practice to produce more accurate estimates on the viral presence of SARS-CoV-2 in breastmilk.

From results obtained in this thesis and results found in current literature, it is evident that the risk factors of breastfeeding, vaginal delivery and rooming-in are not associated with increasing SARS-

CoV-2 neonatal positivity; therefore, this hypothesis is rejected. The recommendation for pregnant women positive with SARS-CoV-2, is that COVID-19 infection in pregnancy should not influence the mode of delivery unless urgent respiratory intervention is needed for birth complications (164). Breastfeeding should be continued and encouraged, as should skin-to-skin contact with mother and child (11).

4.2 Strengths and limitations of thesis

Strengths

Several strengths are associated with adopting a systematic review and meta-analysis as a study design. Systematic reviews require various criteria, including a quality assessment tool to assess the validity of findings, a comprehensive literature search and a clear set of objects (115,120). These criteria ensure the risk of bias is reduced and thus increases the reliability of findings. In addition, meta-analyses allow for quantifying methods and more precise estimates of the effect or outcome (122,168).

Furthermore, the literature search was done as comprehensively as possible by including grey literature and contacting authors where possible for additional information, which therefore minimised the risk of missing out any relevant studies. Furthermore, an extensive de-duplication process was adopted during the review process to minimise the risk of using duplicated data in the review.

In the systematic review and meta-analysis, only cohort studies were included to estimate the rates of offspring positivity which minimised the risk of bias. Incorporating sensitivity and subgroup analysis in the study strengthened the conclusions and credibility of findings (169).

Each included study's methodological quality and risk of bias was assessed to provide a more robust platform to derive conclusions from and thus provide a strong evidence base to inform clinical guidelines.

Limitations

This thesis also possessed a few limitations. These are identified as limitations in heterogeneity which was a difficult aspect to avoid. The findings were limited by heterogeneity in populations, outcomes and tests.

Heterogeneity seen in populations referred to aspects where primary studies varied in their use of sampling frames to allow identification of women with COVID-19, comprised women with suspected and confirmed COVID-19, and primarily reported on pregnant women who required visits to hospital (including for childbirth) and thus affecting the replicability of the estimates.

Examples of heterogeneity in tests include factors like variation in timing and the type of tests used in mothers and babies to assess for SARS-CoV-2. Generally the timing of assessment of the clinical manifestations of disease was not available. The definitions of symptoms, tests and outcomes also introduced heterogeneity. There was also inconsistency surrounding the reporting of clinical outcomes of babies born to COVID-19 positive mothers. This increased the challenge of ascertaining whether the reported complications were in fact related to SARS-CoV-2 or were due to other factors like prematurity.

While examining the review of reviews, it was identified that the systematic reviews often pooled or meta-analysed case reports and case series along with cohort studies. This is known to contribute to biased estimates and results in an exaggeration of the actual burden of SARS-CoV-2 infection

in neonates, due to the selection bias in primary studies that include case reports or series (119). Not all studies used were of high quality, and where small studies were incorporated, introduced the issue of imprecise estimates.

Additionally, external factors like the COVID-19 pandemic impacting the day-to-day running of the healthcare system and the increased pressures and demands on the service meant service delivery and quality of care provided were often compromised (170,171). The lockdown also drastically changed the everyday routines of many individuals and impacted the rate of depression and anxiety in the general population including within pregnant women. There was also a decrease in physical activity, which relates to a reluctance towards attending hospital appointments thus increasing the risk of maternal and perinatal complications (172). Furthermore, many countries employed different healthcare systems with differences in resources – thus is a further factor that increased the level of heterogeneity.

(119)

4.3 Relevance for clinical practise and future research

This thesis estimated the rate of SARS-CoV-2 neonatal positivity as less than 2% in babies tested using RT-PCR and provided evidence for a low occurrence of vertical transmission.

The significance of these findings on future clinical practice should reinforce that while maternal or neonatal samples can test positive for viral SARS-CoV-2 RNA, this alone is not indicative of disease. These findings highlight the evident literature gap that more studies are needed to ascertain the true extent of vertical transmission. Also, the low rate of neonatal positivity should alleviate some of the concerns clinicians and healthcare workers have regarding the management of COVID-19 in pregnant patients.

To improve future diagnosis of COVID-19 in pregnancy, researchers should practise repeating confirmatory testing in positive foetuses/babies at various time points in relevant samples. i.e., vaginal fluid, placenta, cord blood, amniotic fluid, neonatal blood and respiratory and faecal samples. Collecting blood for SARS-CoV-2 IgM testing can also be obtained at multiple time points to allow confirmation of viral persistence/immune response starting at or near birth.

This thesis also demonstrated that breastfeeding, rooming-in and vaginal delivery are not significant enough risk factors to associate with increasing neonatal positivity. The impact of this on healthcare practise should be to encourage mother and baby skin-to-skin contact, and breastfeeding practice. While the mode of delivery should be based entirely on what is best for each patient on an individual basis.

Potential future research could include determining risk factors with enough significance, e.g., delayed cord clamping or trimester of pregnancy.

This thesis also portrayed the shared nature of not incorporating a classification system when determining the timing of MTCT. Improvements for future research should be to include a robust classification system to categorise patients. Implementing a universal classification system will allow improved comparisons of data sets on a larger scale. The WHO classification system is recommended as it provides the most detailed and comprehensive overview of accurately classifying babies born to mothers with/suspected SARS-CoV-2 infection.

Various systematic reviews also fail to incorporate a protocol and register studies. Future research should improve efforts to incorporate this to minimise research waste and data duplication. Researchers should also endeavour to report on individual study risk of bias allowing increased validity of results.

Future research areas in COVID-19 are endless due to the dynamic nature of the disease. Research efforts within vaccinations, prevalence and outcomes in different geographical areas and emerging variants of COVID-19 and their impact on MTCT are some areas requiring further research.

4.4 Conclusion

To conclude, the SARS-CoV-2 neonatal positivity and vertical transmission rate are low. The current data suggest that vertical transmission is possible due to viral RNA presence in biological samples; however, this alone does not confirm positivity in neonates. Clinicians and researchers should implement repeat testing with RT-PCR. Also, where possible, a classification system should be incorporated to categorise and ascertain the timing of neonatal infection.

Analysis of risk factors revealed that rooming-in, breastfeeding and vaginal delivery are not associated with increased neonatal SARS-CoV-2 positivity. Further investigation into risk factors is required in more extensive cohort studies with a follow-up period.

Next, the review showed that current systematic reviews on MTCT are of poor quality and variably report on the rates of SARS-CoV-2 positivity with considerable heterogeneity in definitions.

Lastly, the COVID-19 pandemic has exhibited a changing landscape in its disease presentation. With time, as the virus evolves, the impact of new variants or vaccines will be imperative to research to improve the management of pregnant women with confirmed COVID-19 and their neonates.

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6 Appendix

Appendix 1: Current Opinions in Obstetrics and Gynaecology publication

Mother-to-child transmission of severe acute respiratory syndrome coronavirus 2: review of classification systems and systematic reviews — University of Birmingham

Appendix 2: BMJ publication of the Living Systematic Review

Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis | The BMJ

Appendix 3: BMJ open publication of the Living systematic review protocol

Clinical manifestations, prevalence, risk factors, outcomes, transmission, diagnosis and treatment of COVID-19 in pregnancy and postpartum: a living systematic review protocol - PubMed (nih.gov)

Appendix 4: PregCOV-19 LSR website

https://www.birmingham.ac.uk/research/who-collaborating-centre/pregcov/index.aspx

Appendix 5: Details of search strategies used to include studies in the living systematic review

1. Cochrane Gynaecology and Fertility

Pubmed

1	Pregnancy/
2	pregnan*.tw.
3	neonatal.tw.

4	perinatal.tw.
5	mothers/.
6	mother.tw.
7	maternal.tw.
8	obstetric.tw.
9	infant, newborn/
10	infant.tw.
11	newborn.tw.
12	child*.tw.
13	or/1-12
14	COVID-19.tw.
15	COVID-2019.tw.
16	severe acute respiratory syndrome coronavirus 2.tw.
17	2019-nCoV.tw.
18	SARS-CoV-2.tw.
19	2019nCoV.tw
20	or/14-19
21	coronavirus.tw.
22	2019/12.pd
23	2020.pd.
24	or/22-23
25	21 and 24
26	or/20-25
27	13 and 24

Google Scholar and Google

Using the following text words (pregnancy OR neonatal OR perinatal OR maternal OR obstetric OR newborn) AND (COVID-19 or SARS-Cov-2)

2. EPPI Centre

The MEDLINE search strategy is the OVID Expert Search as developed by Wolters Kluwer and available at http://tools.ovid.com/coronavirus/

MEDLINE search strategy

1 exp Coronavirus/
2 exp Coronavirus Infections/
3 (coronavirus* or corona virus* or OC43 or NL63 or 229E or HKU1 or HCoV* or ncov* or covid* or sars-cov* or sarscov* or Sars-coronavirus* or Severe Acute Respiratory Syndrome Coronavirus*).mp.
4 (or/1-3) and ((20191* or 202*).dp. or 20190101:20301231.(ep).)
5 4 not (SARS or SARS-CoV or MERS or MERS-CoV or Middle East respiratory syndrome or camel* or dromedar* or equine or coronary or coronal or covidence* or covidien or influenza virus or HIV or bovine or calves or TGEV or feline or porcine or BCoV or PED or PEDV or PDCoV or FIPV or FCoV or SADS-CoV or canine or CCov or zoonotic or avian influenza or H1N1 or H5N1 or H5N6 or IBV or murine corona*).mp.
6 ((pneumonia or covid* or coronavirus* or corona virus* or ncov* or 2019-ncov or sars*).mp. or exp pneumonia/) and Wuhan.mp.
7 (2019-ncov or ncov19 or ncov-19 or 2019-novel CoV or sars-cov2 or sars-cov-2 or sarscov2 or sarscov-2 or Sars-coronavirus2 or Sars-coronavirus-2 or SARS-like coronavirus* or coronavirus-19 or covid19 or covid-19 or covid 2019 or ((novel or new or nouveau) adj2 (CoV on nCoV or covid or coronavirus* or corona virus or Pandemi*2)) or ((covid or covid19 or covid-19) and pandemic*2) or (coronavirus* and pneumonia)).mp.
8 COVID-19.rx,px,ox. or severe acute respiratory syndrome coronavirus 2.os.
9 ("32240632" or "32236488" or "32268021" or "32267941" or "32169616" or "32267649" or "32267499" or "32267344" or "32248853" or "32246156" or "32243118" or "32240583" or "32237674" or "32234725" or "32173381" or "32227595" or "32185863" or "32221979" or "32213260" or "32205350" or "32202721" or "32197097" or "32196032" or "32188729" or "32176889" or "32088947" or "32277065" or "32273472" or "32273444" or "32145185" or "31917786" or "32267384" or "32265186" or "32253187" or "32265567" or "32231286" or "32105468" or "32179788" or "32152361" or "32152148" or "32140676" or "32053580" or "32029604" or "32127714" or "32047315" or "32020111" or "32267950" or "32249952" or "32172715").ui.
10 or/6-9
11 5 or 10

The Embase search strategy as of 21st April 2020

1 exp Coronavirus Infections/
2 exp coronavirinae/

3 (coronavirus* or corona virus* or OC43 or NL63 or 229E or HKU1 or HCoV* or ncov* or covid* or sars-cov* or sarscov* or Sars-coronavirus* or Severe Acute Respiratory Syndrome Coronavirus*).mp.
4 or/1-3
5 4 not (SARS or SARS-CoV or MERS or MERS-CoV or Middle East respiratory syndrome or camel* or dromedar* or equine or coronary or coronal or covidence* or covidien or influenza virus or HIV or bovine or calves or TGEV or feline or porcine or BCoV or PED or PEDV or PDCoV or FIPV or FCoV or SADS-CoV or canine or CCov or zoonotic or avian influenza or H1N1 or H5N1 or H5N6 or IBV or murine corona*).mp.
6 ((pneumonia or covid* or coronavirus* or corona virus* or ncov* or 2019-ncov or sars*).mp. or exp pneumonia/) and Wuhan.mp.
7 (2019-ncov or ncov19 or ncov-19 or 2019-novel CoV or sars-cov2 or sars-cov-2 or sarscov2 or sarscov-2 or Sars-coronavirus2 or Sars-coronavirus-2 or SARS-like coronavirus* or coronavirus-19 or covid19 or covid-19 or covid 2019 or ((novel or new or nouveau) adj2 (CoV on nCoV or covid or coronavirus* or corona virus or Pandemi*2)) or ((covid or covid19 or covid-19) and pandemic*2) or (coronavirus* and pneumonia)).mp.
8 6 or 7
9 5 or 8

3. WHO COVID-19 database

The WHO COVID-19 database contained articles on the novel coronavirus from the following sources:

- Web of Science
- Oxford Academic Journals
- Pubmed NIH
- Ishiyaku
- J Stage
- Cinii articles
- Ichushi Web – JAMAS
- Science Direct
- Wiley Online Journals
- JAMA Network
- British Medical Journal
- Mary Ann Liebert
- New England Journal of Medicine
- Sage Publications
- Taylor and Francis Online

- Springer Link
- Biomed Central
- MDPI
- ASM
- PLOS
- The Lancet
- Cell Press
- Cell Press Search Interface
- EMBASE
- KoreaMed
- Global Index Medics
- MMWR
- Epidemiology and Health
- American Chemical Society
- Eurosurvellance
- Cambridge Press
- LWW
- Airiti
- JIMR
- Emerging Infectious Diseases
- Osong Public Health & Research Perspectives
- BASE Bielefeld
- LitCOVID

An additional step using the following search terms was added to the WHO search from 12th May 2020

tw:(newborn* OR mother* OR bab* OR wom* OR pregnan* OR postpart* OR neonat* OR foetus OR foetal OR newborn OR mother OR bab*)

Appendix 6: WHO categorisation of MTCT SARS-CoV-2 transmission in neonates.

	Type of sample and test			
Category	**Foetal tissue, RT-PCR, or ISH**	**Foetal tissue, IHC, microscopy or foetal swab RT-PCR**	**Amniotic fluid (sterile)**	**Placental tissue (RT-PCR, ISH, IHC or microscopy) or placental swab RT-PCR**
Confirmed	POS	POS	POS	POS
	POS	NEG or ND	POS	POS
	POS	POS	POS	NEG or ND
	POS	POS	NEG or ND	POS
	POS	POS	NEG or ND	NEG or ND
	POS	NEG or ND	POS	NEG or ND
	POS	NEG or ND	NEG or ND	POS
	POS	NEG or ND	NEG or ND	NEG or ND
Possible	ND	POS	POS	POS
	ND	NEG or ND	POS	POS
	ND	POS	NEG or ND	POS
	ND	POS	POS	NEG or ND
	ND	POS	NEG or ND	NEG or ND
	ND	NEG or ND	POS	NEG or ND
	ND	NEG or ND	NEG or ND	POS
	NEG	NEG or ND	POS	NEG or ND
Unlikely	NEG	POS	NEG or ND	POS
	NEG	POS	NEG or ND	NEG or ND

	NEG	NEG or ND	NEG or ND	POS

Appendix 6: CS: caesarean section; IHC: immunohistochemistry; ISH: in situ hybridization; ND = not done; RT-PCR: reverse transcription polymerase chain reaction; ROM: rupture of membranes; + = positive test (meets category if one or more tests positive); NEG = negative test (meets category if all tests that were performed are negative); [1] Foetal tissues from sterile site such as foetal organ (e.g. lung, live, brain), testing of multiple specimens recommended; [2] Amniotic fluid from sterile collection prior to rupture of membranes. Table taken and adapted from WHO/2019-nCoV/mother-to-child transmission/2021.1. Source: Definition and catagorization of the timing of Mother-to-child transmission of SARS-CoV-2. Scientific brief., COVID-19: Scientific briefs © World Health Organization 2021.

Appendix 7: **Quality assessment for risk of bias in non-comparative cohort studies using the tool by Hoy *et al.***

	External Validity				Internal validity						Summary
Study	Representativeness	Sampling frame	Selection	Non-response	Data collection	Case definition	Measurement	Differential verification	Adequate follow up	Appropriate numerator and denominator	Summary
2020 July Informe Epidemiológico Embarazadas y Puerperas sem28, 2020	LOW	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	LOW	LOW
Abdulghani SH 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Abedzadeh-Kalahroudi M 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Adhikari EH 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Agarwal N 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Ajith S 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	HIGH	MODERATE
Al-Matary A 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Alay I 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Aliaga CD (1) 2020	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Alnashry LM 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Anand P 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Angelidou A 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Antsaklis P 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Arakaki T 2021	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Argueta LB 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW

Arora D 2021	HIGH	LOW	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Artymuk N 2021	HIGH	HIGH	HIGH	LOW	LOW	HIGH	LOW	LOW	HIGH	LOW	MODERATE
Askary E 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Aslan MM 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Ayed A 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Barber E 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	LOW	MODERATE
Beharier O 2021	LOW	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Bender WR (1) 2020	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Berry M 2021	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Bertero L (1) 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Bertino E 2020	HIGH	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Biasucci G 2020	HIGH	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Bozkurt F 2021	HIGH	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	MODERATE
Brandt JS 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Briana DD 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Brito I 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Buhimschi CS 2020	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Cakirca TD 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	HIGH	MODERATE
Cardona-Perez JA 2021	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	HIGH	LOW
Chaichian S 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Charki S 2021	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Cheng B 2020	HIGH	HIGH	HIGH	LOW	LOW	LOW	LOW	HIGH	HIGH	LOW	MODERATE
Chowdhury L 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Clemente MJ 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	LOW	MODERATE
Cojocaru L 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Colson A 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Conti MG 2021	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW

Cosma S (2) 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Cribiu FM (1) 2021	HIGH	LOW	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	HIGH	LOW
Cubas JAC (1) 2020	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
de Fatima Yukie Maeda M 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
de Vasconcelos Gaspar A 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Dhuyvetter A 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Diaz-Corvillon P 2020	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Dingom MAN 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Donadieu D 2020	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	LOW	MODERATE
Doria M 2020	HIGH	LOW	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	LOW	LOW
Dumitriu D 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Egerup P 2020	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Elenga N 2021	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
ElHalik M 2020	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Facchetti F 2020	HIGH	HIGH	HIGH	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	MODERATE
Farghaly MAA 2020	HIGH	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Farhat AS 2020	HIGH	HIGH	HIGH	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	MODERATE
Fenizia C 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Ferrazzi E (1) 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Flannery DD (1) 2020	HIGH	LOW	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Flores-Pliego A 2021	HIGH	LOW	HIGH	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Gao J 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Garcia-Ruiz I 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Ghema K 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW

Grechukina O 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Griffin I 2020	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	LOW	MODERATE
Gulersen M (1) 2020	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Hadar E 2021	HIGH	HIGH	HIGH	HIGH	LOW	HIGH	LOW	LOW	HIGH	LOW	MODERATE
Halici-Ozturk F 2021	HIGH	LOW	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Hassan N 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Haye MT 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	HIGH	MODERATE
Hazari K 2020	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	HIGH	LOW
Hcini N 2020	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Hernandez OB 2020	LOW	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Hosseini MS 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Hu X (1) 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Huang W 2020	HIGH	HIGH	HIGH	HIGH	LOW	HIGH	LOW	LOW	HIGH	LOW	MODERATE
Huerta Saenz IH 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Jang WK 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Jani S 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Janssen O 2020	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Joseph NT (3) 2021	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Joshi SD 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Kalamdani P 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Kamali A 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Karasu D 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Kayem G 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Kest H 2020	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Khan S (1) 2020	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW

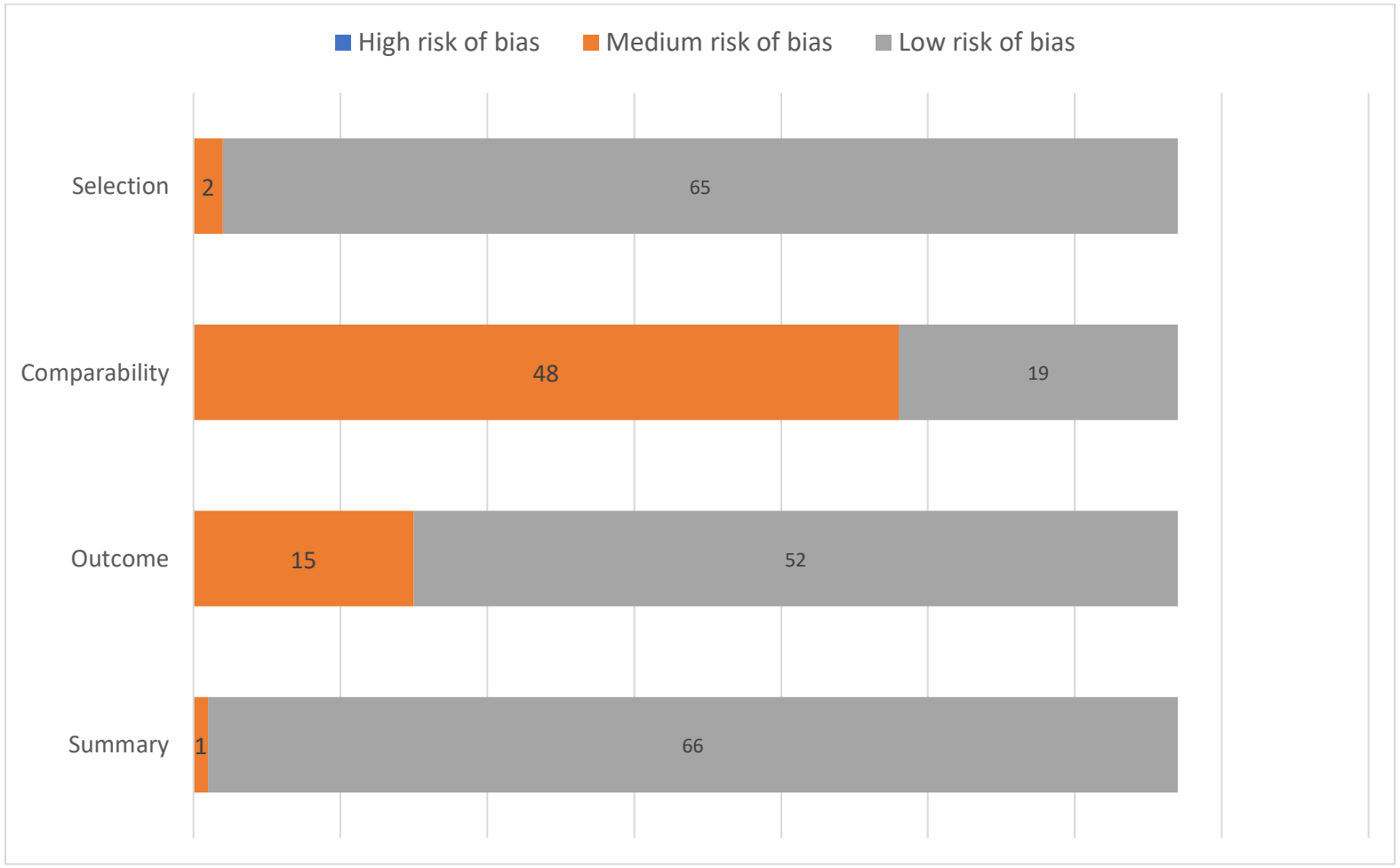
Khoury R 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Khushdil A 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Knight M 2020	LOW	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Korkmaz MF 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Kumari K 2021	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Lang LK 2020	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	LOW	MODERATE
Levitan D 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Liu P 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Lizama O 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Llorca J 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Lokken EM (1) 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	HIGH	MODERATE
Luo Q 2020	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Mahajan N (2) 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Malik S 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Malshe N 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Mand N 2021	LOW	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Maraschini A 2020	LOW	HIGH	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW	LOW
Marin Gabriel MA (2) 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Martinez-Perez O 2020	HIGH	HIGH	LOW	LOW	HIGH	LOW	LOW	HIGH	LOW	LOW	MODERATE
Masmejan S 2020	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Mattar CNZ 2020	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Mattern J 2020	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Mohaghegh Z 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Molina EO 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Moreira LMO 2021	HIGH	LOW	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW

Morhart P 2021	HIGH	HIGH	HIGH	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Morioka I 2021	LOW	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Mourad M (1) 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Murphy C 2020	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Nanavati R 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Nayak M 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Nethoss Juni 2020	LOW	HIGH	LOW	LOW	HIGH	HIGH	LOW	HIGH	HIGH	LOW	MODERATE
Ngalame AN 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Nizyaeva NV 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	LOW	MODERATE
Norman M 2021	LOW	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Ogamba I 2020	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Omrani AS 2020	LOW	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	LOW	LOW
Oncel MY 2020	LOW	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Oxana Z 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Ozsurmeli M 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Patanè L 2020	HIGH	LOW	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Patberg ET 2020	HIGH	LOW	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Pathak S 2020	HIGH	HIGH	HIGH	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	MODERATE
Pawar R 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Pecks U 2020	LOW	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	LOW	LOW
Penfield C 2020	HIGH	LOW	HIGH	LOW	LOW	HIGH	LOW	LOW	HIGH	LOW	MODERATE
Pineles BL 2020	HIGH	LOW	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Pissarra S 2020	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Poon L 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	HIGH	MODERATE
Prasad A 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Preßler J 2020	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	LOW	MODERATE
Puneet G 2021	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Qadri F 2020	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	LOW	MODERATE
Rathberger K 2021	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW

Rebutini PZ 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Remaeus K 2020	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Resta L 2021	HIGH	LOW	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Romagano MP 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	HIGH	MODERATE
Rosen H 2021	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Salvatore CM 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Santhosh J 2020	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	HIGH	MODERATE
Sastry SR 2020	HIGH	LOW	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	LOW	LOW
Sattari M 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Sayeed SK 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Schwartz DA (3) 2020	HIGH	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Sehra R 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Semeshkin AA 2020	HIGH	LOW	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Servei Catala 29/05	LOW	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	HIGH	MODERATE
Shah PT 2020	HIGH	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	MODERATE
Sharma N 2021	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Sharma R 2021	HIGH	LOW	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Shmakov R 2020	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Shook LL (1) 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Sibia P 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	HIGH	MODERATE
Sinaci S 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Singh V (1) 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	HIGH	MODERATE
Sola A 2020	LOW	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Solis-Garcia G 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Song D 2021	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Sri Sri G 2020	HIGH	LOW	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	LOW	LOW

Steffen HA 2021	HIGH	LOW	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Suyuthi FP 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Tadas MP 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Tallarek A 2021	HIGH	HIGH	HIGH	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Tang F 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Tasca C 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Teixeira MLB 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	HIGH	LOW	MODERATE
Thanigainathan S 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Vaezi M 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	LOW
Vera Loyola EM 2021	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Vizheh M 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	HIGH	HIGH	MODERATE
Vousden N 2020	LOW	HIGH	LOW	LOW	HIGH	LOW	LOW	LOW	HIGH	HIGH	MODERATE
Wiyati PS 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Wu H 2021	HIGH	HIGH	HIGH	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Wu YT 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Xu S (1) 2020	HIGH	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	HIGH	LOW	MODERATE
Yadav V 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Yaman A 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Yang H (1) 2020	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Yang H (2) 2020	HIGH	HIGH	HIGH	LOW	LOW	LOW	LOW	HIGH	HIGH	LOW	MODERATE
Yu X 2020	HIGH	HIGH	HIGH	LOW	LOW	HIGH?	LOW	LOW	LOW	LOW	MODERATE
Zaharie G 2020	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Zeng L 2020	HIGH	HIGH	HIGH	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	MODERATE
Zgutka K 2021	HIGH	HIGH	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW	LOW
Zhang P 2020	HIGH	LOW	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW
Zlochiver V 2021	HIGH	HIGH	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	LOW	LOW

Appendix 8: Quality assessment for risk of bias in comparative cohort studies using the Newcastle-Ottawa Scale



Appendix 9: AMSTAR of systematic reviews on MTCT included in review of review

Study	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Alqahtani et al, 2020	No	No	No	No	No	No	No	No	No	No	N/A	N/A	No	No	N/A	Yes
Arroyo-Sánchez et al, 2021	Yes	Yes	No	Partial yes	Yes	Yes	No	Yes	No	No	N/A	N/A	Yes	No	N/A	Yes
Ashraf et al, 2020	Yes	No	No	No	Yes	Yes	No	Partial yes	Partial yes	No	N/A	N/A	No	No	N/A	Yes
Banaei et al, 2020**	Yes	No	No	No	Yes	Yes	No	No	Partial yes	No	N/A	N/A	No	No	N/A	Yes
Berbari et al, 2020**	Yes	No	No	No	No	No	No	Partial yes	Yes	No	No	No	Yes	No	No	Yes
Bwire et al, 2020	Yes	Partial yes	No	No	Yes	Yes	No	No	No	No	N/A	N/A	No	No	N/A	Yes
Caparros-Gonzalez et al, 2020	Yes	Partial yes	No	Partial yes	Yes	Yes	No	Yes	No	No	N/A	N/A	No	No	N/A	Yes
Capobianco et al, 2020**	Yes	No	No	Partial yes	Yes	Yes	No	Partial yes	Partial yes	No	Yes	No	No	Yes	Yes	Yes
Centeno-Tablante et al, 2020	Yes	Partial yes	No	No	Yes	Yes	No	No	No	No	N/A	N/A	No	No	N/A	Yes
Chamseddine et al, 2020	Yes	No	No	No	No	No	No	No	No	No	N/A	N/A	No	No	N/A	No
Chi H et al, 2020	Yes	No	No	Partial yes	Yes	Yes	No	Partial yes	Partial yes	No	No	No	No	No	No	No
Chi J et al, 2020	Yes	No	No	No	Yes	Yes	No	No	No	No	N/A	N/A	No	No	N/A	Yes
Christian et al, 2020	Yes	No	No	No	Yes	Yes	No	Partial yes	No	No	N/A	N/A	No	No	N/A	Yes
Debrabandere et al, 2020	Yes	No	No	No	No	Yes	No	No	Partial yes	No	N/A	N/A	Yes	No	N/A	Yes
Della Gatta An et al, 2020	Yes	No	No	No	Yes	Yes	No	No	Partial yes	No	N/A	N/A	No	No	N/A	No
Deniz et al, 2020	Yes	No	No	No	No	No	No	Yes	No	No	N/A	N/A	No	No	N/A	No
Di Toro et al, 2020**	Yes	Partial yes	No	Partial yes	Yes	Yes	No	No	Yes	No	No	Yes	Yes	Yes	Yes	Yes
Direkvand-Moghadam et al, 2020	Yes	No	No	Partial yes	Yes	Yes	No	No	No	No	N/A	N/A	No	No	No	Yes
Diriba et al, 2020**	Yes	No	No	Partial yes	Yes	Yes	No	Partial yes	Yes	No	No	No	Yes	Yes	Yes	Yes
do Amaral et al, 2020	Yes	Partial yes	No	Partial yes	Yes	Yes	No	Yes	No	No	N/A	N/A	No	No	N/A	Yes
Dubey et al, 2020**	Yes	No	No	Partial yes	Yes	Yes	No	Yes	Partial yes	No	No	Yes	Yes	Yes	Yes	Yes
Elshafeey et al, 2020	Yes	No	No	Partial yes	Yes	Yes	No	No	No	No	N/A	N/A	No	No	N/A	Yes

Gajbhiye et al, 2020	Yes	No	No	Partial yes	Yes	Yes	No	No	No	No	N/A	N/A	No	No	N/A	Yes
Giampiero et al, 2020**	Yes	Partial yes	No	Partial yes	Yes	Yes	No	Partial yes	Partial yes	No	Yes	Yes	Yes	Yes	Yes	Yes
González et al, 2020	No	No	No	Partial yes	Yes	Yes	No	Partial yes	No	No	N/A	N/A	No	No	N/A	No
Hassanipour et al, 2020**	Yes	No	No	Partial yes	Yes	Yes	No	No	Partial yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Huntley et al, 2020	Yes	Yes	No	No	Yes	Yes	No	Yes	Yes	No	N/A	N/A	Yes	Yes	N/A	Yes
Islam et al, 2020	Yes	No	No	Partial yes	Yes	Yes	No	No	No	No	N/A	N/A	No	No	N/A	Yes
Jafari et al, 2020**	Yes	Partial yes	No	Partial yes	Yes	Yes	No	No	Yes	No	No	Yes	Yes	Yes	Yes	Yes
Juan et al, 2020	Yes	Partial yes	No	Partial yes	Yes	Yes	No	Partial yes	No	No	N/A	N/A	Yes	No	N/A	Yes
Kasraeian et al, 2020**	Yes	No	No	Partial yes	No	No	No	No	Partial yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Khalil et al, 2020**	Yes	Yes	No	Partial yes	Yes	Yes	No	Yes	No	No	No	Yes	Yes	Yes	Yes	Yes
Kotlyar et al, 2020**	Yes	Yes	No	Partial yes	Yes	Yes	No	Yes	Partial yes	No	No	Yes	Yes	No	No	Yes
Kumar et al, 2021**	Yes	Partial yes	Yes	Partial yes	Yes	Yes	No	Yes	Partial yes	No	Yes	No	No	Yes	Yes	No
Lakhkar et al, 2020	Yes	No	No	Partial yes	Yes	Yes	No	No	No	No	N/A	N/A	N/A	No	N/A	No
Lopes de Sousa et al, 2020	Yes	Yes	No	Partial yes	Yes	Yes	No	Yes	Partial yes	No	N/A	N/A	No	No	N/A	Yes
Melo et al, 2020	Yes	Partial yes	No	Partial yes	Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No
Mirbeyk et al, 2020	Yes	No	No	Partial yes	Yes	Yes	No	No	No	No	N/A	N/A	No	No	N/A	Yes
Najafi et al, 2020	Yes	Yes	No	Partial yes	Yes	Yes	No	Yes	No	Yes	N/A	N/A	No	No	N/A	Yes
Naz et al, 2020	Yes	Partial yes	No	No	Yes	Yes	No	No	No	No	N/A	N/A	No	No	N/A	No
Novoa et al, 2020**	Yes	Yes	No	Partial yes	No	No	No	No	Partial yes	No	No	Yes	Yes	Yes	Yes	Yes
Oltean et al, 2021	Yes	Partial yes	No	Partial yes	Yes	Yes	No	Yes	No	No	N/A	N/A	Yes	No	N/A	Yes
Papapanou et al, 2021	Yes	Partial yes	No	Partial yes	Yes	Yes	No	No	No	No	N/A	N/A	No	No	N/A	Yes
Patnaik et al, 2020	Yes	No	No	Partial yes	Yes	Yes	No	No	No	No	N/A	N/A	No	No	N/A	Yes
Pettirosso et al, 2020	No	No	No	No	Yes	Yes	No	Partial yes	No	No	N/A	N/A	Yes	No	N/A	No
Raschetti et al, 2020	Yes	Partial yes	No	Partial yes	Yes	Yes	No	No	No	No	No	No	Yes	No	No	No
Rodrigues et al, 2020	Yes	No	No	Partial yes	Yes	Yes	No	No	No	No	N/A	N/A	No	No	N/A	Yes
Rodriguez-Blanco et al, 2020	Yes	No	No	Partial yes	Yes	Yes	No	Partial yes	No	No	N/A	N/A	No	No	N/A	Yes
Sampieri et al, 2020	Yes	No	No	Partial yes	Yes	Yes	No	Partial yes	No	No	N/A	N/A	No	No	N/A	No

Sepulveda-Martinez et al, 2020**	Yes	No	No	Partial yes	No	No	No	No	Yes	No	No	Yes	No	No	Yes	Yes
Sharps et al, 2020	Yes	No	No	Partial yes	Yes	Yes	No	Yes	No	No	N/A	N/A	No	No	N/A	Yes
Shrestha et al, 2020	Yes	No	No	Partial yes	Yes	Yes	No	No	No	No	N/A	N/A	No	No	N/A	Yes
Smith et al, 2020	Yes	No	No	No	Yes	Yes	No	Partial yes	Partial yes	No	N/A	N/A	No	No	N/A	Yes
Sousa et al, 2020	Yes	No	No	Partial yes	Yes	Yes	No	Yes	No	No	N/A	N/A	No	No	N/A	Yes
Sun et al, 2020**	Yes	Yes	No	Yes	Yes	Yes	No	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Thamir Ahmed et al, 2020	Yes	No	No	No	No	No	No	Yes	No	No	N/A	N/A	No	No	N/A	No
Thomas et al, 2020**	Yes	No	No	No	No	No	No	No	Yes	No	N/A	N/A	Yes	No	N/A	Yes
Trad et al, 2020	Yes	No	No	Partial yes	Yes	Yes	No	Yes	No	No	N/A	N/A	No	No	N/A	Yes
Trippella et al, 2020	Yes	No	No	Partial yes	Yes	Yes	No	Yes	No	No	N/A	N/A	No	No	N/A	Yes
Trocado et al, 2020	Yes	Yes	No	No	Yes	Yes	No	Partial yes	No	No	N/A	N/A	Yes	No	N/A	Yes
Turan et al, 2020	Yes	No	No	No	Yes	Yes	No	No	No	No	N/A	N/A	Yes	No	N/A	Yes
Vigil-De Gracia et al, 2020	Yes	No	No	No	No	No	No	No	No	No	N/A	N/A	No	No	N/A	Yes
Walker et al, 2020	Yes	Partial yes	No	Partial yes	Yes	Yes	No	No	No	No	N/A	N/A	No	No	N/A	Yes
Yang N et al, 2020	Yes	No	No	Yes	Yes	Yes	No	No	Partial yes	No	N/A	N/A	Yes	No	Yes	Yes
Yang Z et al, 2020	No	No	No	Partial yes	Yes	Yes	No	No	No	No	N/A	N/A	Yes	No	N/A	No
Yee et al, 2020**	Yes	No	No	Partial yes	Yes	Yes	No	No	Yes	No	Yes	Yes	No	Yes	Yes	Yes
Yoon et al, 2020	Yes	No	No	Partial yes	Yes	Yes	No	Partial yes	No	No	N/A	N/A	No	No	N/A	Yes
Yuan et al, 2020	Yes	No	No	Partial yes	Yes	Yes	No	Yes	No	No	N/A	N/A	No	No	N/A	Yes

Appendix 10: Summary of answers for each AMSTAR domain and percentage of responses.

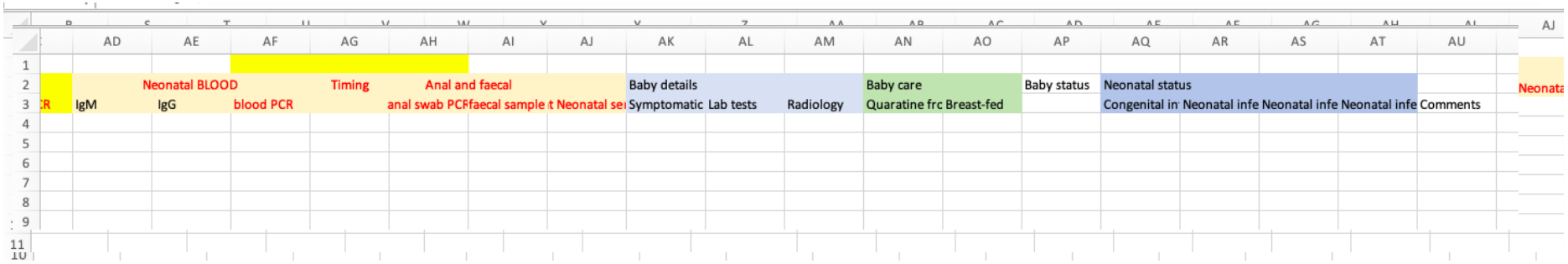
	YES	NO	PARTIAL YES	N/A	TOTAL	YES (%)	NO (%)	PARTIAL YES (%)	N/A (%)
1. Inclusion of PICO	64	4	0	0	68	94.1	5.9	0.0	0.0
2. Inclusion of protocol	**9**	**44**	**15**	**0**	**68**	**13.2**	**64.7**	**22.1**	**0.0**
3. Selection of study design for inclusion	1	67	0	0	68	1.5	98.5	0.0	0.0
4. Comprehensive literature search strategy	**2**	**21**	**45**	**0**	**68**	**2.9**	**30.9**	**66.2**	**0.0**
5. Study selection in duplicate	57	11	0	0	68	83.8	16.2	0.0	0.0
6. Data extraction in duplicate	58	10	0	0	68	85.3	14.7	0.0	0.0
7. List of excluded studies and justification	**0**	**68**	**0**	**0**	**68**	**0.0**	**100.0**	**0.0**	**0.0**
8. Detail provided of included studies	19	34	15	0	68	27.9	50.0	22.1	0.0
9. Satisfactory technique to assess RoB	**10**	**42**	**16**	**0**	**68**	**14.7**	**61.8**	**23.5**	**0.0**
10. Reporting on sources of funding for included studies	1	67	0	0	68	1.5	98.5	0.0	0.0
11. Methods for statistical combination of results (MA)	**7**	**12**	**0**	**49**	**68**	**10.3**	**17.6**	**0.0**	**72.1**
12. Assessment of RoB in individual studies (MA)	13	6	0	49	68	19.1	8.8	0.0	72.1
13. Discussion of RoB in individual studies	**25**	**42**	**0**	**1**	**68**	**36.8**	**61.8**	**0.0**	**1.5**
14. Explanation for heterogeneity	15	53	0	0	68	22.1	77.9	0.0	0.0
15. Investigation of publication bias (MA)	**16**	**5**	**0**	**47**	**68**	**23.5**	**7.4**	**0.0**	**69.1**
16. Reporting on sources of conflict of interest and funding for review	54	14	0	0	68	79.4	20.6	0.0	0.0

Appendix 11: Template of data extraction sheets for mother-to-child transmission

	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL	AM	AN
1	Ascertainment of MTCT transmission overall in the study														Positive signs of MTCT (amniotic fluid, breast milk, cord blood, neonatal blood, vaginal fluid, placenta)	FETUS/NEONATE				
2	No of women who had maternal stool RT-PCR test	No of women who had skin swab RT-PCR test	No of women who had breast milk RT-PCR	Other tests	No of babies had RT-PCR pharyngeal swab at birth	No of babies had RT-PCR pharyngeal swab at any time	No of babies who had neonatal stool RT-PCR test	No of babies had neonatal blood test (PCR)	No of babies had neonatal blood IgM test	No of babies had neonatal blood IgG test	No of babies who had Cord blood test (PCR)	No of babies who had cord blood IgM test	No of babies who had cord blood IgG test	Other test		No. of fetus/neonate in study	No. of babies tested	No. of fetus/neonate with suspected or confirmed COVID	Unlikely or not infected	Comment
3																				
4																				
5																				
6																				
7																				
8																				
9																				
10																				

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1											No. of women with confirmed COVID									
2	Round	First Author and initial, Yr	Study Design	Country	Hospital	Study title	Total no. of women	Mothers with confirmed diagnoses (RT-PCR)	Mothers with probable diagnoses (serology only)	Mothers with possible diagnoses (only clinical or radiological	1st tri	2nd tri	3rd tri	postnatal	CS	Vaginal delivery	No of women who had Amniotic fluid RT-PCR test	No of women who had Placenta (any side) RT-PCR test	No of women who had Placenta (baby side) RT-PCR test	No of women who had vaginal fluid RT-PCR test
3																				
4																				
5																				
6																				
7																				
8																				
9																				

Appendix 12: Template of data extraction sheet for MTCT positive babies



	AD	AE	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU
2		Neonatal BLOOD		Timing	Anal and faecal			Baby details			Baby care		Baby status	Neonatal status				
3	IgM	IgG	blood PCR		anal swab PCR	faecal sample t	Neonatal ser	Symptomatic	Lab tests	Radiology	Quaratine frc	Breast-fed		Congenital in	Neonatal infe	Neonatal infe	Neonatal infe	Comments

Appendix 13: Subgroup analysis of the different World Bank Region for the rates of SARS-CoV-2 positivity in babies by RT-PCR and RT-PCR or anti-SARS-CoV-2 IgM

World Bank Region	Studies	events/N	Proportion (95% CI)	Tau^2	I^2
North America					
RT-PCR only	32	19/2229	0.06 (0.00, 0.34)	0.0000	0
RT-PCR or/and IgM	32	25/2364	0.16 (0.00, 0.56)	0.0022	14
South Asia					
RT-PCR only	29	126/3365	2.87 (1.18, 5.12)	0.0696	89
RT-PCR or/and IgM	30	126/3378	2.75 (1.09, 4.95)	0.0690	88
Europe and Central Asia					
RT-PCR only	38	231/6079	1.50 (0.54, 2.76)	0.0234	75
RT-PCR or/and IgM	39	233/6101	1.59 (0.61, 2.86)	0.0232	75
Latin America and Caribbean					
RT-PCR only	14	83/1461	5.68 (3.21, 8.67)	0.0248	71
RT-PCR or/and IgM	15	83/1473	5.34 (2.97, 8.23)	0.0241	69
East Asia and Pacific					
RT-PCR only	12	22/502	3.15 (0.47, 7.31)	0.0500	65
RT-PCR or/and IgM	12	28/502	4.27 (0.78, 9.55)	0.0741	74
Middle east and North Africa					
RT-PCR only	15	20/635	2.16 (0.26, 5.19)	0.0372	58
RT-PCR or/and IgM	16	24/700	2.42 (0.47, 5.32)	0.0353	59
Overall					
RT-PCR only	140	501/14271	1.80 (1.18, 2.51)	0.0348	77
RT-PCR or/and IgM	144	519/14518	1.94 (1.31, 2.66)	0.0352	77

Heterogeneity between groups:
RT-PCR only (p=0.0000)
RT-pcr or/and IgM (p=0.0000)