A COMPARISON OF MATERNAL AND NEONATAL OUTCOMES OF PREGNANT WOMEN WITH AND WITHOUT EVIDENCE OF SARS-CoV-2 INFECTION IN THE WESTERN CAPE, SOUTH AFRICA

A mini-thesis in partial fulfilment of requirements for the MMed: Public Health Medicine, University of Cape Town

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DECLARATION

I, ……Vanessa Mudaly……, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Acknowledgements, format and contributions

I am extremely grateful to my supervisor Prof Emma Kalk for her patient mentorship and astute feedback throughout this journey, as well as her willingness to set time aside in her busy schedule to meet to discuss my progress regularly. Your expertise and encouragement have shaped my research and enriched my learning experience. I am also very thankful to Prof Mary-Ann Davies for her insightful guidance that helped me to see the wood for the trees so that I could compile a more focused analysis.

I am thankful for the encouragement I received from my colleagues at the Department of Health and Wellness, especially from my Director- Dr Hilary Goeiman**,** whose flexibility and understanding has helped me to balance work and academic commitments (at least some of the time).

I would like to thank my family for encouraging me to follow my passion and embark on this endeavour late in my career. To my very awesome children- thank you for being so patient and understanding while I spent hours upon hours on this document. Your endless love and encouragement have been the foundation of my academic journey, and I appreciated every single cup of tea that you made for me.

To my incredible friends, your unwavering belief in my abilities, encouragement and support were invaluable.

Format

This mini-dissertation is presented in the format of a publication-ready manuscript for publication in the South African Medical Journal (SAMJ).

Contributions

I acknowledge the Provincial Health Data Centre (PHDC) for providing the data according to my specifications, in particular Ms Nicole Chetty and Ms Florence Phelanyane for their kind assistance with clarifying data sources and definitions of variables.

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List of Terms and Abbreviations

ANC	Antenatal care
CDC	Centre for Disease Control and Prevention
CI	Confidence Interval
CFR	Case Fatality Rate
COVID-19/ C-19	Coronavirus disease 2019
DATCOV	Daily Hospital Surveillance system
HIV	Human Immunodeficiency Virus
ICU	Intensive Care Unit
NHLS	National Health Laboratory Service
NICD	National Institute for Communicable Diseases
OR	Odds ratio
aOR	Adjusted Odds ratio
PCR	Polymerase chain reaction
PHDC	Provincial Health Data Centre
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
WC	Western Cape

Abstract

Background: SARS-CoV-2 infection in pregnancy has been associated with poor pregnancy and neonatal outcomes. While SARS-CoV-2 infection itself could partly account for high maternal mortality rates observed in the Western Cape, indirect effects of the pandemic such as movement restrictions and health service pressure may also have played a role. There is limited data on the impact of having a SARS-CoV-2 diagnosis during pregnancy on maternal and neonatal outcomes in this setting.

Objectives: We compared the characteristics and outcomes of pregnant women with and without a SARS-CoV-2 diagnosis with pregnancy outcomes between 1 March 2020 and 31 January 2022 within the Western Cape public healthcare sector.

Methods: A retrospective cohort analysis was conducted using routine electronic data collated from public health sources. We compared demographic and clinical characteristics, pregnancy and maternal outcomes and neonatal outcomes by SARS-CoV-2 diagnosis status, gestational timing of diagnosis and by timing of pregnancy outcome during a COVID-19 wave to account for both direct and indirect effects of the pandemic. We used descriptive statistics, Chi-squared tests, Fisher Exact tests, and logistic regression models for analysis.

Results: We included 226,336 pregnancies with 193,195 linked live births. Prevalence of a maternal SARS-CoV-2 diagnosis was 2.5%. Increased odds of SARS-CoV-2 diagnosis or hospitalization were associated with age categories ≥25 years compared to 15-24 years, pre-existing and gestational hypertension compared to no hypertension, pre-existing and gestational diabetes compared to no diabetes, current and previous tuberculosis compared to no tuberculosis and HIV positive status compared to HIV negative or unknown status. These factors would likely be more common in admitted patients where testing coverage was higher. In analyses adjusted for these factors, the odds of maternal death were higher in women with a SARS-CoV-2 diagnosis (aOR=11.42; 95% CI 8.46-15.43) than those without. The odds of miscarriage were higher with an early diagnosis (<28 weeks gestation) (aOR=2.18; 95% CI 1.91-2.48) and the odds of stillbirth were higher with a late diagnosis (≥28 weeks gestation) (aOR=1.31; 95% CI 1.02-1.67) compared to no diagnosis. Increased odds of low birth weight and neonatal intensive care unit (ICU) admission were found among infants of women who had a late SARS-CoV-2 diagnosis (aOR=1.22; 95% CI 1.10-1.34 and aOR=2.56; 95% CI 2.18-3.01, respectively) compared to infants of women without a SARS-CoV-2 diagnosis.

Conclusion: This study found that that older age, diabetes, hypertension, current and previous tuberculosis and HIV were risk factors for a SARS-CoV-2 diagnosis or hospitalization in

pregnancy during the COVID-19 pandemic period in our setting. Adverse outcomes associated with a maternal SARS-CoV-2 diagnosis included miscarriage, stillbirth, maternal death, low birth weight and neonatal ICU admission. However, it was not possible to determine the extent to which these outcomes were associated with SARS-CoV-2 infection itself, as SARS-CoV-2 testing was likely much higher in women admitted during pregnancy, and these outcomes would be strongly associated with admission. Nonetheless, these findings coupled with demonstrated benefits of COVID-19 vaccination, highlight the need to prioritise both pregnant women and women of child-bearing age for vaccination and boosting in order to maintain protective benefits.

Word count 500

DISSERTATION:

PUBLICATION-READY MANUSCRIPT

Formatted for submission to the South African Medical Journal (SAMJ)

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Acknowledgements: We acknowledge the Provincial Health Data Centre (PHDC) for providing the data according to our specifications, and for clarifying data sources and definitions of variables.

Funding sources: None

Abstract

Background: SARS-CoV-2 infection in pregnancy has been associated with poor pregnancy and neonatal outcomes. While SARS-CoV-2 infection itself could partly account for high maternal mortality rates observed in the Western Cape, indirect effects of the pandemic may also have played a role. There is limited data on the impact of having a SARS-CoV-2 diagnosis during pregnancy in this setting.

Objectives: We compared the characteristics and outcomes of pregnant women with and without a SARS-CoV-2 diagnosis with pregnancy outcomes between 1 March 2020 and 31 January 2022 within the Western Cape public healthcare sector.

Methods: A retrospective cohort analysis was conducted using routine data collated from public health sources. We compared maternal characteristics, pregnancy and maternal outcomes and neonatal outcomes by SARS-CoV-2 diagnosis status, gestational timing of diagnosis and timing of pregnancy outcome during a COVID-19 wave to account for both direct and indirect effects of the pandemic. We used descriptive statistics, Chi-squared tests, Fisher Exact tests, and logistic regression models for analysis.

Results: We included 226,336 pregnancies with 193,195 linked live births. Prevalence of a maternal SARS-CoV-2 diagnosis was 2.5%. Increased odds of SARS-CoV-2 diagnosis or hospitalization were associated with age categories ≥25 years compared to 15-24 years and pre-existing and gestational hypertension, pre-existing and gestational diabetes, current and previous tuberculosis, and HIV compared to no corresponding comorbidity. These factors would likely be more common in admitted patients where testing coverage was higher. In adjusted analyses, the odds of maternal death were higher in women with a SARS-CoV-2 diagnosis (aOR=11.42; 95% CI 8.46-15.43) than those without. The odds of miscarriage and stillbirth were higher with an early and late diagnosis respectively (aOR=2.18; 95% CI 1.91-2.48 and aOR=1.31; 95% CI 1.02-1.67, respectively) compared to no diagnosis. The odds for low birth weight and neonatal intensive care unit (ICU) admission were higher among infants of women with a late SARS-CoV-2 diagnosis compared to those of women without a diagnosis.

Conclusion: This study found that older age, diabetes, hypertension, current and previous tuberculosis and HIV were risk factors for a SARS-CoV-2 diagnosis or hospitalization in pregnancy during the COVID-19 pandemic period in our setting. Adverse outcomes associated with a maternal SARS-CoV-2 diagnosis included miscarriage, stillbirth, maternal death, low birth weight and neonatal ICU admission. However, it was not possible to determine the extent to which these outcomes were associated with SARS-CoV-2 infection itself. Nonetheless, these findings highlight the need to prioritise both pregnant women and women of child-bearing age for COVID-19 vaccination.

Word count: 400

Background

The recent global outbreak of SARS-CoV-2 has resulted in over 770 million known cases and over 7 million deaths [1]. Early observational studies found that whilst pregnant women were more likely to be tested for SARS-CoV-2, they displayed similar severity of symptoms compared to non-pregnant women [2,3]. However, in later studies SARS-CoV-2 infection has been associated with poor maternal and neonatal outcomes including pre-eclampsia, pre-term labour, caesarean delivery, maternal death, low birth weight, and neonatal admission to intensive care units (ICU) [4].

In South Africa, four distinct waves of SARS-CoV-2 infection were experienced from March 2020 to January 2022, each driven by a different SARS-CoV-2 sub-lineage. The fourth wave, driven by the Omicron BA.1/2 sub-lineage, was associated with less severe disease than the first 3 waves driven be the ancestral, Beta and Delta sub-lineages respectively [5]. In the Western Cape, owing to resource constraints, access to SARS-CoV-2 testing was limited during the peaks of waves except for patients at high risk of severe disease or those who were hospitalized. Universal SARS-CoV-2 testing of this group was practiced in many facilities because admission of pregnant women to COVID-19 (C-19) wards allowed for cohorting and alignment with infection control policies. Therefore, SARS-CoV-2 testing was sometimes performed in asymptomatic pregnant women, especially if being admitted to hospital. Women undergoing routine antenatal care and uncomplicated delivery at primary health facilities were much less likely to be tested, even if mildly symptomatic.

According to data from a national C-19 hospital admissions surveillance system (DATCOV), among women of child-bearing age with a C-19 diagnosis, the cumulative incidence of admissions in pregnant women was nearly double that of non-pregnant women during the first year of the pandemic. The Western Cape had the highest incidence (1567.4 per 100,000) of C-19 admission in pregnant women compared to other provinces [6] This may have been due to provincial differences in testing practices and indications for hospitalization of infected pregnant women, as well as the ability to ascertain pregnancy in women with a SARS-2-diagnosis by inference at the Provincial Health Data Centre (PHDC) [7]. However, the provincial maternal mortality ratio per 100,000 live births also increased from 50.77 to 102.28 between 2019 to 2021 [8].

It is difficult to assess the impact of pregnancy on C-19 severity from these data as pregnant women are much more likely to be admitted to hospital than non-pregnant women and the higher observed mortality was likely directly due to SARS-CoV-2 disease, as well as reduced access to routine antenatal services due to lockdowns and health service pressure during the peaks of the waves, which may have impacted care for comorbidities and pregnancy-related complication [5, 9. 10]. Data on specific outcomes of pregnancies of women with a SARS-CoV-2 diagnosis are lacking in this setting.

Aim and Objectives

We aimed to compare the characteristics and outcomes of pregnant women accessing healthcare services within the public healthcare sector who had been diagnosed with SARS-CoV-2 infection between 1 March 2020 and 31 January 2022 and those of pregnant women in whom SARS-CoV-2 infection was not diagnosed over the same period in the Western Cape province. We compared demographic and clinical characteristics, and pregnancy and maternal outcomes, as well as neonatal outcomes of infants borne to these two groups of women.

Methods

Study design

This was a retrospective cohort analysis. The primary exposure was a diagnosis of SARS-CoV-2 during pregnancy, which was determined by evidence of a positive laboratory test result or a notification report from the National Institute of Communicable Diseases (NICD). To assess the combined effect of infection, health service pressure and reduced access, we also examined the exposure of having a pregnancy outcome during a C-19 wave (versus interwave period). A wave was defined by the NICD [11] as "the period from when COVID-19 weekly incidence is equal to or greater than 30 cases per 100 000 persons until the weekly incidence is equal or below 30 cases per 100 000 persons" in a geographic area. A list of exposure and outcome variables is shown in Table 1.

We included exposures (specific comorbidities) that were associated with an increased susceptibility to SARS-CoV-2 infection [12, 13] and consequent increased likelihood of C-19 testing as potential confounding variables. "Booking status" was used as a proxy for prior access to antenatal care: lack of antenatal care during pregnancy ("unbooked") has been associated with poor pregnancy outcomes [14] and may have also been associated with reduced access to SARS-CoV-2 testing. We inferred timing of the SARS-CoV-2 diagnosis (before/after 28 weeks gestation) using an estimated gestation start date. We hypothesized that women infected with SARS-CoV-2 in their third trimester of pregnancy would be more susceptible to COVID pneumonia, as well as complications associated with delivery. We also included maternal C-19 hospitalization status to account for variable SARS-CoV-2 testing and admission practices. Specific pregnancy, maternal and neonatal outcomes were evaluated.

Table 1: List of Exposure and Outcome Variables

Exposure Variables	
Maternal SARS-CoV-2 diagnosis	Evidence of laboratory test positivity or case received from National Institute of Communicable Diseases (NICD)
Timing of diagnosis	Early (<28 weeks gestation), late (≥ 28 weeks gestation).
Wave of Infection	Timing of pregnancy outcome by C-19 wave experienced in the Western Cape: Wave 1 = 03/05/20-16/08/20; Wave 2 = 08/11/20-07/02/21; Wave 3 = 23/05/21-19/09/21 Wave 4 = 28/11/21-30/01/22 and Inter-wave period (pregnancy outcome date not within a wave period).
Maternal age category	<15 years, 15-24 years, 25-34 years, ≥ 35 years
Comorbidities	Pre-existing and gestational hypertension, pre-existing and gestational diabetes, current and previous TB, HIV status*.
Prior access to antenatal care	Evidence of prior access to antenatal care during pregnancy*.
Outcome Variables	
Pregnancy outcomes	Live birth, Ectopic pregnancy, Elective termination of pregnancy (TOP), Miscarriage (pregnancy loss <28 weeks) and stillbirth (pregnancy loss ≥28 weeks).
Maternal outcomes	Maternal C-19 hospitalization (admission to a C-19 ward, or C-19 intensive care unit (ICU)), Maternal death (death during pregnancy and up to 42 days after pregnancy outcome).
Neonatal outcomes (<28 days after birth)	Preterm delivery (<37 weeks gestation), Low birth weight <2 500g and very low birth weight <1 500g, neonatal SARS-CoV-2 diagnosis, neonatal admission to ICU, neonatal death.

* We only included data with high confidence evidence to minimize the risk of misclassification bias.

Study Population and Data Sources

Data was obtained from the Provincial Health Data Centre (PHDC), which is a province-wide health information exchange that collates and links routine electronic data from multiple sources in the public health system including databases from hospitals, laboratories, pharmacies and primary health facilities. Linkage of an individual's clinical history is possible through a folder number which serves as a unique identifier [15]. This allows for the generation of large cohorts with longitudinal data and linkage of mother-infant dyads. Contact episodes with the health system at various points, related health conditions such as pregnancy, and outcomes of these encounters are inferred based on a weighting and scoring system of all the evidence collated.

For this study, the source population was all women accessing antenatal or obstetric health services at a health facility in the public sector in the Western Cape, referred to as the "maternity cascade". The study population was selected from this group as all women with high confidence evidence of inferred pregnancy [7] and evidence of a pregnancy outcome between 1 March 2020 to 31 January 2022. We included twin pregnancies but excluded other multiple pregnancies as they were more likely to be associated with complicated outcomes. The PHDC was able to link positive SARS-CoV-2 results collated from laboratories conducting Polymerase Chain Reaction (PCR) tests and facilities reporting rapid C-19 antigen test results to the individuals in the study population. Data relating to pre-existing and newly diagnosed comorbidities, namely diabetes, hypertension, HIV and tuberculosis were also included a priori as risk factors for severe C-19 disease.

Data analysis

Data were summarized using descriptive statistics. Statistical tests used for numerical variables were means and medians, with standard error and interquartile range (IQR), respectively, depending on the distribution. Statistical tests used for categorical variables were Chi-squared ($\chi 2$) test or Fisher Exact test (for data of 5 or less observations per group) with 95% confidence intervals (CIs). We generated multiple logistic regression models to assess associations between SARS-CoV-2 diagnosis and hospitalization with pregnancy and maternal outcomes during different C-19 waves with adverse outcomes of miscarriage, stillbirth, maternal death, preterm delivery, neonatal ICU admission and neonatal death. Variables were evaluated independently and then added or removed consecutively to build robust multivariable models incorporating timing of diagnosis and C-19 wave periods. Odds ratios (ORs) and adjusted odds ratios (aORs) with 95% CI's and p-values (<0.05) were calculated. Data were analysed using Stata version 13.

Ethical considerations

The PHDC operates under strict governance procedures and is fully adherent to South African legislation and National Department of Health regulations [14]. Datasets were de-identified, encrypted and password-protected. The study was approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (HREC Reference 113/2022) and the Provincial Health Research Committee (WC_202205_004). The requirement for informed consent was waived for this secondary analysis of de-identified data.

Results

A total of 230,140 pregnancies were identified between 1 March 2020 and 31 January 2022. Duplicate entries were removed. One set of quadruplets and 34 sets of triplets were also removed as they were deemed to represent pregnancy episodes with very high risk of adverse outcomes. Twin pregnancies were represented by two pregnancy episodes in the database,

corresponding to each infant. Only one episode was retained in the cohort used for analysis of pregnancy and maternal outcomes in order to avoid duplicating maternal data. Both episodes were included in the neonatal cohort if associated with a live birth. Of the 226,336 pregnancy episodes included in the final maternal cohort, 193,195 were linked to an outcome of a live birth and included in the cohort for the analysis of neonatal outcomes (Figure 1).

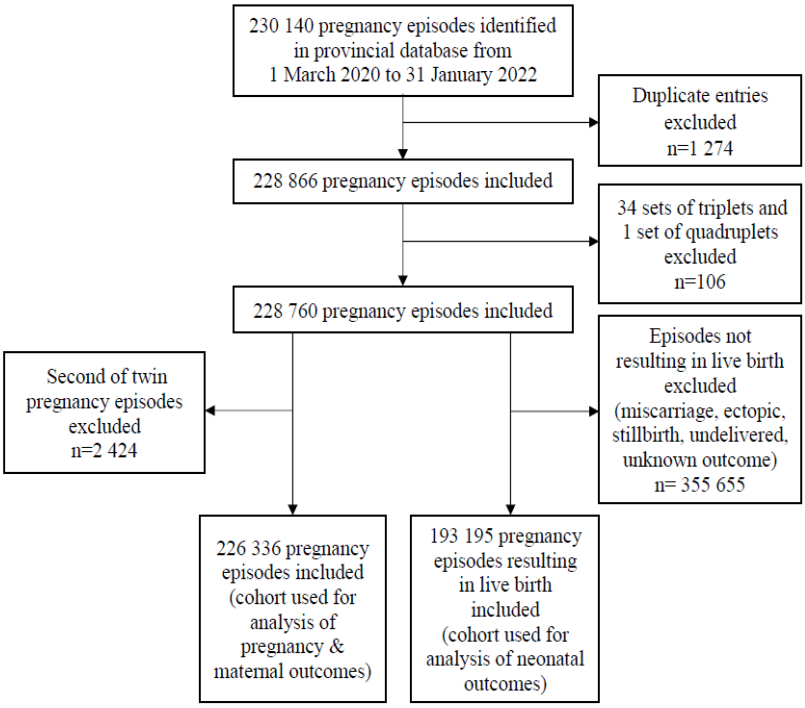

Figure 1 : Flowchart showing inclusion and exclusion of pregnancy episodes in the study

There was evidence of a SARS-CoV-2 diagnosis in 5,627 (2.5%) pregnant women. There were 2,818 hospital admissions in those with a SARS-CoV-2 diagnosis; 2,621 to a general C-19 ward and 197 to a C-19 ICU. A larger proportion of women with a late diagnosis were hospitalized compared to women with an early diagnosis (Figure 2). Since women may have been admitted to a C-19 ward for cohorting and infection control rather than due to C-19 disease itself, we were not able to differentiate between admissions for severity of SARS-CoV-2 disease and admissions for obstetric indications in the former group.

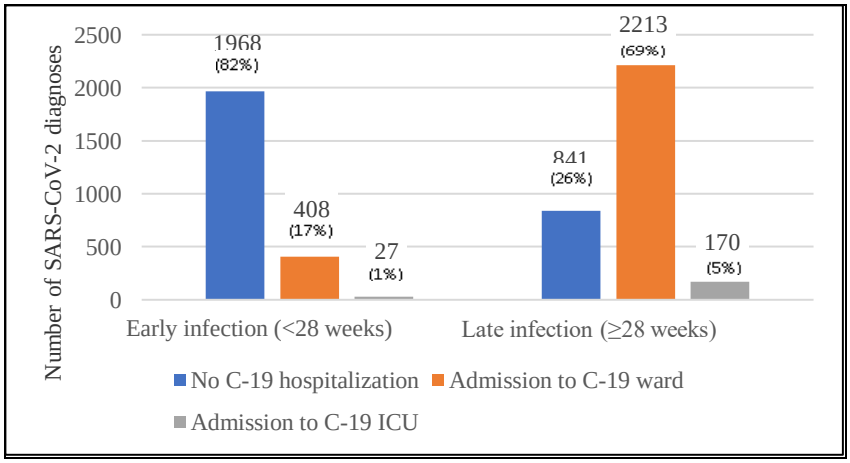

Figure 2: Relationship between gestational timing of SARS-CoV-2 diagnosis and COVID-19 hospitalization *(ICU: intensive care unit)*

The mean parity and gravidity were 1 and 2, respectively in women with and without a SARS-CoV-2 diagnosis. The median age was higher in women with compared to women without a diagnosis. The median age was higher in women with compared to women without a diagnosis (29 years (interquartile rage (IQR):24-34) vs 27 years (IQR:23-32)). Maternal characteristics and pregnancy outcomes are shown in Table 2. Out of the 232 deaths recorded, 60 (25.9%) were associated with a SARS-CoV-2 diagnosis. Of these, 27 deaths occurred in women who had been admitted to C-19 ICU.

Table 2: Comparison of Maternal Characteristics and Pregnancy Outcomes between pregnancies with and without a SARS-CoV-2 diagnosis

	Total pregnancies N= 226 336	Pregnancies with no SARS-CoV-2 diagnosis n= 220 709	Pregnancies with a SARS-CoV-2 diagnosis n=5 627	p-value
Maternal Age Category				
<15 years	646 (0.3%)	636 (0.3%)	10 (0.2%)	<0.01
15-24 years	75 598 (33.4%)	74 127 (33.6%)	1 471 (26.1%)	
25-34 years	111 518 (49.3%)	108 549 (49.2%)	2 969 (52.8%)	
≥35years	38 574 (17.0%)	37 397 (16.9%)	1 177 (20.9%)	
Hypertension				
None	216 613 (95.7%)	211 370 (95.8%)	5 243 (93.2%)	<0.01
Pre-existing	9 222 (4.1%)	8 857 (4.0%)	365 (6.5%)	
Gestational	501 (0.2%)	482 (0.2%)	19 (0.3%)	
Diabetes				
None	219 100 (96.8%)	213 822 (96.9%)	5 278 (93.8%)	<0.01
Pre-existing	3 438 (1.5%)	3 290 (1.5%)	148 (2.6)	
Gestational	3 798 (1.7%)	3 597 (1.6%)	201 (3.6)	
Current Tuberculosis				
None	225 053 (99.4%)	219 494 (99.5%)	5 559 (98.8%)	<0.01
Current	1 283 (0.6%)	1 215 (0.6%)	68 (1.2%)	
Previous Tuberculosis				
No	215 401 (95.2%)	210 106 (95.2%)	5 295 (94.1%)	<0.01
Yes	10 935 (4.8%)	10 603 (4.8%)	332 (5.9%)	
HIV status				
Negative/unknown	183 603 (81.1%)	179 061 (81.1%)	4 542 (80.7%)	0.436
Positive	42 733 (18.9%)	41 648 (18.9%)	1 085 (19.3%)	
Received prior antenatal care				
Yes	136 683 (60.4%)	132 728 (60.1%)	3 955 (70.3%)	<0.01
No	89 653 (39.6%)	87 981 (39.9%)	1 672 (29.7%)	
Pregnancy Outcomes				
Live birth	190 895 (84.4%)	185 909 (84.3%)	4 986 (88.6%)	<0.01
Miscarriage	13 213 (5.8%)	12 936 (5.9%)	277 (4.9%)	<0.01
Elective TOP	14 598 (6.5%)	14 422 (6.5%)	176 (3.1%)	<0.01
Ectopic pregnancy	3 961 (1.8%)	3882 (1.8%)	79 (1.4%)	0.044
Stillbirth	3 359 (1.5%)	3 259 (1.5%)	100 (1.8%)	0.067
Maternal Outcomes				
Maternal death	232 (0.1%)	172 (0.1%)	60 (1.1%)	<0.01

TOP: Termination of pregnancy

In multivariable analyses (Figures 3 and 4), increased odds of SARS-CoV-2 diagnoses was associated with age categories ≥25 years compared to 15-24 years, pre-existing hypertension compared to no hypertension, pre-existing and gestational diabetes compared to no diabetes. and current or previous tuberculosis compared to no tuberculosis. Except for age category and previous tuberculosis, these variables were also associated with comparatively higher odds of C-19-related hospitalization. The likelihood of being diagnosed with SARS-CoV-2 and having a related hospitalisation progressively increased with having a pregnancy outcome in later C-19 waves.

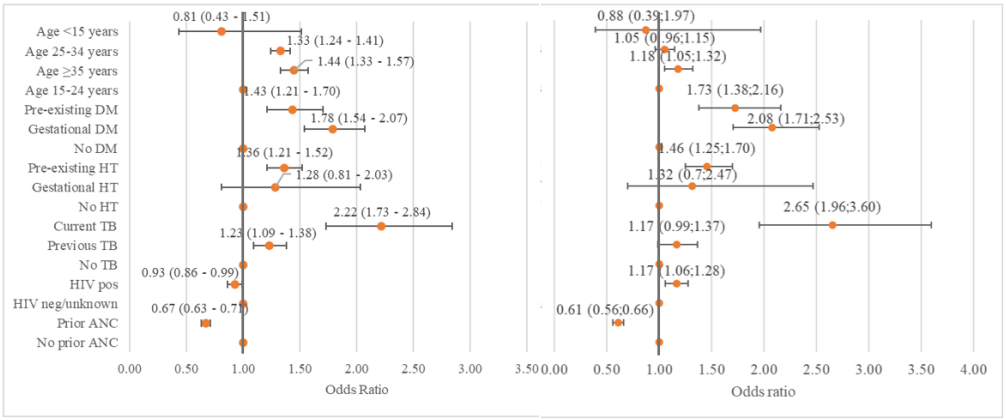

Figure 2: Forest plot comparing adjusted odds ratios of associations between maternal characteristics, SARS-CoV-2 diagnosis and COVID-19 hospitalization

(DM: diabetes mellitus; HT: hypertension; TB: tuberculosis; pos: positive; neg: negative; ANC: antenatal care)

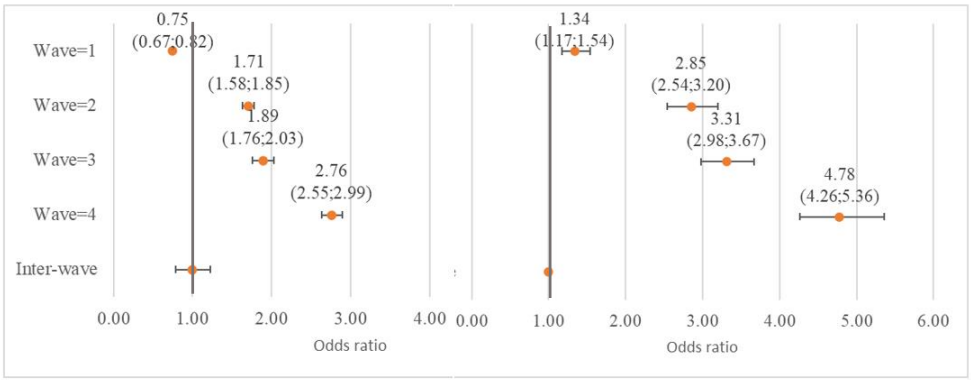

Figure 3: Forest plot comparing adjusted odds ratios of associations between COVID-19 waves, SARS-CoV-2 diagnosis and COVID-19 hospitalization

The odds of miscarriage were higher in women with an early SARS-CoV-2 diagnosis compared to those without a diagnosis (aOR=2.18 95% CI 1.91-2.48) (Supplementary table 1). An increased odds of stillbirth was also found in women with a late diagnosis compared to those without a diagnosis (aOR=1.31 95% CI 1.02-1.67) (Supplementary table 2). The odds for maternal death in women with a SARS-CoV-2 diagnosis was higher compared to those without a diagnosis (aOR=11.42 95% CI 8.46-15.43) (Supplementary table 3). A late diagnosis was more strongly associated with maternal death than an early diagnosis (aOR=4.53 95% CI 2.44-8.40 (early) versus aOR=17.16 95% CI 12.39-23.76 (late)) compared to no diagnosis (Supplementary table 4).

Among all women in the study regardless of C-19 diagnosis status, there were noteworthy associations with adverse pregnancy and maternal outcomes and having a pregnancy outcome in specific waves (Supplementary tables 1-3): the odds of miscarriage were higher in those with a pregnancy outcomes in the 3rd and 4th waves vs inter-wave periods (aOR=1.13 95% CI 1.08-1.19 and aOR=1.20 95% CI=1.13-1.28, respectively); the odds of stillbirth were higher in the 2nd wave (aOR=1.15 95% CI=1.03-1.27) and the odds of maternal death were higher in the first three waves (aOR=1.64 95% CI 1.13-2.37, aOR=2.22 95% CI 1.56-3.15 and aOR=1.68 95% CI 1.18-2.40 respectively) compared to inter-wave periods.

Neonatal Outcomes

There were 193,195 live born infants linked to pregnancies included in this study. The mothers of 5,074 (2.63%) infants had evidence of a SARS-CoV-2 diagnosis during pregnancy. Neonatal characteristics and outcomes are shown in Table 3. There was no significant difference between the proportion of preterm delivery in infants of mothers diagnosed with SARS-CoV-2 versus those who were not. However, there was a greater proportion of infants with low birth weight in the former group; 91.2% of whom had also had a preterm delivery. Of the 124 neonatal SARS-CoV-2 diagnoses recorded, 108 (87.1%) were in infants born during a wave period. The proportion of deaths in infants of mothers with and without a SARS-CoV-2 diagnosis was similar.

Table 3: Neonatal characteristics and outcomes

	Total live births N= 193 195	No maternal SARS-COV2 diagnosis n=188 121	Maternal SARS-COV2 diagnosis n=5 074	p-value
Neonatal Characteristics				
Male Gender	91 954 (49.2%)	89 520 (49.2%)	2 434 (49.9%)	0.317
Birth weight (g) median (IQR)	3 100 (2 720-3 440)	3 100 (2 720-3 440)	3 105 (2 680-3 480	0.298
Neonatal Outcomes				
Preterm delivery	31 476 (16.3%)	30 603 (16.3%)	873 (17.2%)	0.074
Low Birth Weight	27 900 (15.4%)	27 100 (15.3%)	800 (17.2%)	<0.01
Neonatal SARS-CoV-2 diagnosis	124 (0.1%)	104 (0.1%)	20 (0.4%)	<0.01
Neonatal ICU admission	3 745 (1.9%)	3 540 (1.9%)	205 (4.0%)	<0.01
Neonatal death	1 570 (0.8%)	1 522 (0.8%)	48 (1.00%)	0.255

IQR: interquartile range; ICU: intensive care unit

In the logistic regression analyses, no association between preterm delivery and maternal SARS-CoV-2 diagnosis was identified, however an increased odds of low birth weight was found in infants of mothers who had a late SARS-CoV-2 diagnosis (aOR=1.22 95% CI 1.10-1.34) compared to infants of mothers without a diagnosis (Supplementary table 5). A maternal SARS-CoV-2 diagnosis was also associated with increased odds of neonatal ICU admission compared to no maternal diagnosis (aOR-2.02 95% CI 1.75-2.34), with higher odds in infants whose mothers had a late diagnosis (aOR=2.56 95% CI 2.18-3.01) compared to infants of mothers with an early diagnosis (aOR=1.13 95% CI 0.83-1.53) (Supplementary table 6). A maternal diagnosis of SARS-COV-2 was not significantly associated with an increased odds of neonatal death compared to no diagnosis (aOR=1.18 95% CI 0.89-1.58), nor was there any association with a late diagnosis.

Discussion

In our study of >230,000 pregnancies during the C-19 pandemic period, maternal death was strongly associated with having a SARS-CoV-2 diagnosis (versus not) even after adjusting for other factors associated with diagnosis. It is difficult to assess the true effect of SARS-CoV-2 infection itself on maternal death due to admitted women being far more likely to be tested for C-19 and at greater risk of maternal death both from C-19 and due to other reasons. Nonetheless, the finding of increased maternal mortality in those with pregnancy outcomes during C-19 waves 1-3 which were driven by sub-lineages known to be associated with severe C-19 disease, suggests that C-19 infection itself likely played a role in increasing maternal mortality in this period.

We found that women ≥25 years old, with pre-existing diabetes or hypertension, gestational diabetes or current and previous tuberculosis were more likely to be diagnosed with SARS-CoV-2 during their pregnancies than younger women, those without comorbidities and those living with HIV. Furthermore, women ≥35 years old and those with the aforementioned risk factors were more likely to have a C-19-related hospitalisation. We also observed that women living with HIV were more likely to have a C-19-related hospitalisation compared to HIV negative women, corresponding with findings from other studies that HIV is associated with increased susceptibility to severe C-19 disease[12.16,17], which may be due to increased risk of non-communicable comorbid diseases at younger ages, viral non-suppression or persistent immune dysfunction even if virally suppressed, and previous or current tuberculosis.

While the risk factors that we identified for a SARS-CoV-2 diagnosis and related hospitalization were similar to those found in other studies, we could not account for the impact of variable clinical management policies applicable to pregnant women during the pandemic, which were likely to favour testing of women with high-risk pregnancies and those admitted with pregnancy-related complications or in labour. Our findings of progressively increased SARS-CoV-2 diagnosis and related hospitalization in those with a pregnancy outcome in later waves strongly suggests that testing of admitted women irrespective of C-19 symptoms became more widespread with each consecutive wave as a result of evolving mitigative strategies in the Western Cape. We were unable to distinguish between admissions to a C-19 ward for severe C-19 disease versus for cohorting and infection control.

Our findings corresponded with others showing that SARS-CoV-2 infection is associated with greater risk of maternal death [18,19,20]. Historically, increased maternal mortality rates have been recorded during many other viral epidemics [18]. Many factors contribute to susceptibility to critical SARS-CoV-2 infection with pneumonia, hypercoagulability and other complications [21,22]. Pregnancy itself is associated with changes in functioning of the immune system and altered hormone levels [23]. Pregnancy also induces physiological and anatomical changes which are more pronounced in the last trimester, reducing lung capacity and ability to clear secretions during infection. Women with SARS-CoV-2 infection in late pregnancy are more likely to be admitted to ICU to receive mechanical ventilation than non-pregnant women [24].

Data on associations between maternal SARS-CoV-2 infection and pregnancy loss are variable and may reflect challenges in ascertaining pregnancy loss especially for those that occur in early pregnancy and are not associated with a medical intervention. An early systematic review and metanalysis [25] found the prevalence of miscarriage to be 14.5% in women with SARS-CoV-2 infection, whereas a more recent systematic review [26] found a lower prevalence of 9.3%-10.8%, with a non-significant association between SARS-CoV-2 infection and

miscarriage. Many studies have reported increased rates of stillbirth during the pandemic [27, 28], while a systematic review and metanalysis [29] reported a two-fold increase in odds of stillbirth associated with SARS-CoV-2 infection. Our finding of a significant association between SARS-CoV-2 diagnosis and both miscarriage and stillbirth outcomes may be attributable to SARS-CoV-2 directly but must also be interpreted in the context of the SARS-CoV-2 testing bias, as women with miscarriage or stillbirth are much more likely to be admitted to hospital where testing coverage was higher. Nonetheless, our findings concur with those from two large studies demonstrating higher rates of miscarriage [30] and stillbirth [31] during peak periods of the COVID-19 pandemic which could be attributable to both direct effects of infection and service delivery factors such as reduced access to antenatal care.

Although increased rates of pregnancy loss may be due to severe maternal SARS-CoV-2 disease, many viral infections have been associated with placental infiltration which may play a role in pregnancy loss, [17, 23]. Placental tissue may be vulnerable because angiotensin-converting enzyme 2, which is highly expressed in maternal-fetal interface cells, is also the receptor for SARS-CoV-2. Significant findings from examination of placental tissue include fibrin deposition around placental villi and increase in features of maternal vascular malperfusion (MVM) which is a risk factor for pre-eclampsia [32]. SARS-CoV-2 infection has been associated with a significant increase in risk of preeclampsia [33] especially in the presence of maternal hypertension or diabetes.[30].

Data on adverse outcomes of infants born to SARS-CoV-2 infected women are limited and variable across different settings. Initially, no related adverse effects were identified [34]. More recently, a range of serious sequelae have been linked to infants of infected mothers, including sepsis, pneumothorax, neonatal respiratory distress (NRDS), asphyxia, preterm labour, premature rupture of membranes (PROMs) and intra-uterine growth retardation [21, 34,35]. In a systematic review, preterm delivery rates in SARS-CoV-2-infected women were reported as ranging from 14.3% to 63.8% [4]. Spontaneous preterm birth rates were lower than medically indicated rates (6.4% vs 18.4%).

A study from South Africa that included only hospitalised women with a SARS-CoV-2 diagnosis, found that 35% of deliveries were preterm deliveries, of which 63% were caesarean deliveries indicated for a maternal medical condition [24]. Comparing preterm rates between infected and uninfected women, one study of hospitalized pregnant women, in which universal SARS-CoV-2 screening with a SARS-CoV-2 PCR test was performed, found higher rates of induced labour (37.6% vs 32.5 %, p=<0.001) and caesarean section (27.7% vs 20.4%, p<0.001) in infected women [36]. Variability in reported adverse neonatal outcomes could be attributable to multiple contextual factors, including testing strategies, prevalent comorbidities and obstetric management practices.

While our finding of higher odds for low birth weight and neonatal ICU admission in infants of women with a SARS-CoV-2 diagnosis suggests that preterm delivery was a related adverse event, it was not possible to isolate the impact of SARS-CoV-2 infection on preterm delivery due to the strong testing biases among admitted women who would be much more at risk of having a preterm delivery. Evidence of maternal to infant transmission of SARS-CoV-2 both intrapartum and postpartum has emerged, however, transmission has been associated with severity of maternal disease and iatrogenic factors whereas vertical transmission is postulated to be uncommon [37]. We could not conclusively determine mode of transmission for any of the incidental neonatal diagnoses in our study, and therefore could not evaluate this phenomenon.

Limitations

There were three main limitations in this study. Firstly, there were several potential sources of residual confounding, including unmeasured clinical risk factors for SARS-CoV-2 morbidity and mortality (such as obesity, cardiovascular disease and renal disease); service delivery factors (including variable testing strategies and admission practices), socio-economic status and C-19 exposure (geographic location and timing of pregnancy outcomes in relation to the waves of C-19 infection, which all varied by virulence and pathogenicity [38]). A decrease in attendance of routine antenatal visits during the pandemic compared to before the pandemic as reported in other studies [39] may have impacted on pregnancy outcomes, adding to complexity in this analysis.

Another challenge was data quality and availability. Nearly 60% of all pregnancy episodes had an unspecified delivery method recorded, therefore this variable could not be included in our analyses. Due to use of routine health service data, there were missing and potentially incorrectly captured fields with possible misclassification bias [40]. Our analysis may also not be generalizable to all healthcare sectors, as data relating to pregnancies managed in the private healthcare sector were not included.

Thirdly, C-19 vaccination was introduced into the public sector in June 2021, however the impact of this could not be evaluated because data on vaccination status was not available. A systematic review and metanalysis comparing outcomes of vaccinated and unvaccinated pregnant women showed markedly reduced risks of SARS-CoV-2 infection, C-19 hospitalization during pregnancy and C-19 ICU admission as well as reductions in the rates of stillbirth, preterm delivery and neonatal ICU admission in vaccinated women [41].

Conclusion

We found that older age, diabetes, hypertension, current and previous tuberculosis, and HIV were risk factors for SARS-CoV-2 diagnoses or hospitalization in pregnancy during the C-19 pandemic in our setting. Several adverse outcomes were associated with a SARS-CoV-2 diagnosis including miscarriage, stillbirth, maternal death, low birth weight and neonatal ICU admission. However, it was not possible to determine the extent to which these outcomes were associated with SARS-CoV-2 infection itself, as SARS-CoV-2 testing was likely much higher in women admitted during pregnancy, and these outcomes would be strongly associated with admission. Nonetheless, these findings coupled with demonstrated benefits of C-19 vaccination, highlight the need to prioritise both pregnant women and women of child-bearing age for C-19 vaccination and boosting in order to maintain protective benefits.

References

1. WHO COVID-19 Dashboard https://data.who.int/dashboards/covid19/cases?n= c. Accessed 1 February 2024.
2. Pollán M, Pérez-Gómez B, Pastor-Barriuso R, et al. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. Lancet 2020;396(10250):535-544. https://doi.org/10.1016/S0140-6736(20)31483-5.
3. Molteni E, Astley CM, Ma W, Sudre CH, Magee LA, Murray B, et al. Symptoms and syndromes associated with SARS-CoV-2 infection and severity in pregnant women from two community cohorts. SCI REP-UK 2021;11(1):6928. https://doi.org/10.1038/s41598-021-86452-3.
4. Papapanou M, Papaioannou M, Petta A, et al. Maternal and Neonatal Characteristics and Outcomes of COVID-19 in Pregnancy: An Overview of Systematic Reviews. International journal of environmental research and public health 2021;18(2):596. https://doi.org/ 10.3390/ijerph18020596.
5. Davies MA, Morden E, Rousseau P, et al. Outcomes of laboratory-confirmed SARS-CoV-2 infection during resurgence driven by Omicron lineages BA.4 and BA.5 compared with previous waves in the Western Cape Province, South Africa. Int J Infect Dis. 2023 Feb;127:63-68. https//:doi.org/10.1016/j.ijid.2022.11.024.
6. National Institute for Communicable Diseases (NICD). Clinical characteristics, outcomes and epidemiology of pregnant women hospitalised with COVID-19 in South Africa. Volume 18, Supplementary issue 8. 5 March 2021. https://www.nicd.ac.za/wpcontent/ uploads/2021/03/ COVID-19-Special-Public-Health- Surveillance-Bulletin-March-2021-002.pdf.
7. Johnson LF, Mutemaringa T, Heekes A, Boulle A. Effect of HIV Infection and Antiretroviral Treatment on Pregnancy Rates in the Western Cape Province of South Africa. Supplementary materials. Journal of Infectious Diseases 2020;221(12):1953-1962. https://doi.org/10.1093/infdis/jiz362
8. Saving Mothers report: Executive Summary 2020-2022. National Department of Health, South Africa. 2023. https://www.health.gov.za/annual-reports/.
9. Jassat W, Mudara C, Ozougwu L, et al. DATCOV author group. Difference in mortality among individuals admitted to hospital with COVID-19 during the first and second waves in South Africa: a cohort study. Lancet Glob Health. 2021 Sep;9(9):e1216-e1225. https//:doi.org/10.1016/S2214-109X(21)00289-8.
10. Davies MA, Kassanjee R, Rousseau P, et al. Western Cape and South African National Departments of Health in collaboration with the National Institute for Communicable Diseases in South Africa Affiliations. Outcomes of laboratory-confirmed SARS-CoV-2 infection in the Omicron-driven fourth wave compared with previous waves in the Western Cape Province, South Africa. Trop Med Int Health. 2022 Jun;27(6):564-573. https//:doi.org/10.1111/tmi.13752.
11. Communicable Diseases Communiqué November 2021, Vol. 20 (11). https://www.nicd.ac.za/ wp-content/uploads/2021/11/Proposed-definition-of-COVID-19-wave-in-South-Africa.pdf.
12. Booth A, Reed AB, Ponzo S, et al. Population risk factors for severe disease and mortality in COVID-19: A global systematic review and meta-analysis. PloS one 2021;16(3):e0247461. https//:doi.org/10.1371/journal.pone.0247461.
13. Davies M, Vundle Z, Paleker M, et al. Risk Factors for Coronavirus Disease 2019 (COVID-19) Death in a Population Cohort Study from the Western Cape Province, South Africa. Clinical infectious diseases 2021;73(7). https//:doi.org/10.1093/cid/ciaa1198.

14. Hoque A, Buckus S, Hoque M & Van Hak G. Deciphering the Association of Antenatal Care Visits and Pregnancy Outcomes from South Africa. European Journal of Medical and Health Sciences 2022; 4 (3). https://doi.org/10.24018/ejmed.2022.4.3.1113.
15. Boulle A, Heekes A, Tiffin N, et al. Data centre profile: The provincial health data centre of the Western Cape province, South Africa. International journal of population data science; Int J Popul Data Sci 2019;4(2):1143. https://doi.org/10.23889/ijpds.v4i2.1143.
16. Parker A, Barit S, Belot Z, Beukes G, Botha A, Bradbury J, et al. Risk factors for COVID-19-related in-hospital mortality in a high HIV and tuberculosis prevalence setting in South Africa: a cohort study. The Lancet HIV 2021;8(9). https://doi.org/10.1016/S2352-3018(21)00151-X.
17. Pandit U, Shrestha N, Khanal G. Effects of COVID-19 Virus Infection on Pregnancy Outcomes: a Systematic Review and Meta-analysis. Journal of Nepal Health Research Council 2023;21(2):187-196. https://doi.org/10.33314/jnhrc.v21i02.4488.
18. Allotey J, Stallings E, Bonet M, et al. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis. BMJ (Online) 2020;370:m3320. https://doi.org/ 10.1136/bmj.m3320.
19. Chmielewska B, Barratt I, Townsend R, et al. Effects of the COVID-19 pandemic on maternal and perinatal outcomes: a systematic review and meta-analysis. The Lancet Global Health 2021;9(6):e759-e772.https://doi.org/10.1016/S2214-109X(21)00079-6.
20. Martinez-Portilla RJ, Sotiriadis A, Chatzakis C, et al. Pregnant women with SARS-CoV-2 infection are at higher risk of death and pneumonia: propensity score matched analysis of a nationwide prospective cohort (COV19Mx). Ultrasound in obstetrics & gynecology 2021;57(2):224-231. https://doi.org/ 10.1002/uog.23575.
21. Cruz Melguizo S, de la Cruz Conty M, Carmona Payán P, et al. Pregnancy Outcomes and SARS-CoV-2 Infection: The Spanish Obstetric Emergency Group Study. Viruses 2021;13(5):853. https://doi.org/ 10.3390/v13050853.
22. Martinez-Portilla RJ, Sotiriadis A, Chatzakis C, et al. Pregnant women with SARS-CoV-2 infection are at higher risk of death and pneumonia: propensity score matched analysis of a nationwide prospective cohort (COV19Mx). Ultrasound in obstetrics & gynecology 2021;57(2):224-231. https://doi.org/ 10.1002/uog.23575.
23. Wastnedge EAN, Reynolds RM, van Boeckel SR, et al. Pregnancy and COVID-19. Physiol Rev 2021;101(1):303-318. https://doi.org/ 10.1152/physrev.00024.2020.
24. Budhram S, Vannevel V, Botha T, et al. Maternal characteristics and pregnancy outcomes of hospitalized pregnant women with SARS-CoV-2 infection in South Africa: An International Network of Obstetric Survey Systems-based cohort study. Int J Gynecol Obstet. 2021;155:455–465. https://doi.org/0.1002/ijgo.13917.
25. Diriba K, Awulachew E, Getu E. The effect of coronavirus infection (SARS-CoV-2, MERS-CoV, and SARS-CoV) during pregnancy and the possibility of vertical maternal-fetal transmission: a systematic review and meta-analysis. Eur J Med Res 2020;25(1):39. https://doi.org /10.1186/s40001-020-00439-w.
26. van Baar JAC, Kostova EB, Allotey J, et al. COVID-19 in pregnant women: a systematic review and meta-analysis on the risk and prevalence of pregnancy loss. Hum Reprod Update 2023. https://doi.org/039 10.1093/humupd/dmad030.
27. Ashish KC, Gurung R, Kinney MV, et al. Effect of the COVID-19 pandemic response on intrapartum care, stillbirth, and neonatal mortality outcomes in Nepal: a prospective observational study. The Lancet Global Health 2020;8(10):e1273-e1281. https://doi.org/10.1016/S2214-109X(20)30345-4.
28. Khalil A, Kalafat E, Benlioglu C, et al. SARS-CoV-2 infection in pregnancy: A systematic review and meta-analysis of clinical features and pregnancy outcomes. EClinicalMedicine 2020;25:100446. https://doi.org/10.1016/j.eclinm.2020.100446.

29. Wei SQ, Bilodeau-Bertrand M, Liu S, Auger N. The impact of COVID-19 on pregnancy outcomes: a systematic review and meta-analysis. Can Med Assoc J 2021;193(16):E540-E548. https://doi.org/10.1503/cmaj.202604.
30. Gajbhiye RK, Tilve A, Kesarwani S, et al. Letters to the Editor: Increased rate of miscarriage during second wave of COVID-19 pandemic in India. Ultrasound Obstet Gynecol 2021; 58: 943–953. https://doi.org/10.1002/uog.24784.
31. Dandona R, Kumar GA,Akbar M, et al. Substantial increase in stillbirth rate during the COVID-19 pandemic: results from a population-based study in the Indian state of Bihar. BMJ Glob Health 2023;8:e013021. https://doi.org/ 10.1136/bmjgh-2023-013021.
32. Alberca RW, Pereira NZ, Oliveira LMDS, et al. Pregnancy, Viral Infection, and COVID-19. Frontiers in immunology 2020;11:1672. https://doi.org/ 10.3389/fimmu.2020.01672.
33. Vlachodimitropoulou Koumoutsea E, Vivanti AJ, Shehata N, et al. COVID-19 and acute coagulopathy in pregnancy. Journal of thrombosis and haemostasis 2020;18(7):1648-1652. https://doi.org/ 10.1111/jth.14856.
34. Vizheh M, Allahdadian M, Muhidin S, et al. Impact of COVID-19 Infection on Neonatal Birth Outcomes. Journal of tropical pediatrics (1980) 2021;67(5). https://doi.org/ 10.1093/tropej/fmab094.
35. Simbar M, Nazarpour S, Sheidaei A. Evaluation of pregnancy outcomes in mothers with COVID-19 infection: a systematic review and meta-analysis. Journal of obstetrics and gynaecology 2023;43(1):2162867. https://doi.org/ 10.1080/01443615.2022.2162867.
36. Martinez-Perez O, Prats Rodriguez P, Muner Hernandez M, et al. The association between SARS-CoV-2 infection and preterm delivery: a prospective study with a multivariable analysis. BMC pregnancy and childbirth 2021;21(1):273. https://doi.org/ 10.1186/s12884-021-03742-4.
37. Allotey J, Kew T, Fernández-García S, Gaetano-Gil A, Yap M, Sheikh J, et al. SARS-CoV-2 positivity in offspring and timing of mother-to-child transmission: living systematic review and meta-analysis. BMJ 2022;376. https://doi.org/ 10.1136/bmj-2021-067696.
38. Bradshaw D, Dorrington R, Laubscher R, Groenewald P, Moultrie T. COVID-19 and all-cause mortality in South Africa – the hidden deaths in the first four waves. S Afr J Sci. 2022;118(5/6). https://doi.org/ 10.17159/sajs.2022/13300.
39. Townsend R, Chmielewska B, Barratt I, et al. Global changes in maternity care provision during the COVID-19 pandemic: A systematic review and meta-analysis. 2021. https://doi.org/ 10.1016/j.eclinm.2021.100947.
40. Benchimol EI, Smeeth L, Guttmann A, et al. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) Statement: e1001885. PLoS medicine 2015;12(10). https://doi.org/ 10.1371/journal.pmed.1001885.
41. Rahmati M, Yon DK, Lee SW, et al. Effects of COVID-19 vaccination during pregnancy on SARS-CoV-2 infection and maternal and neonatal outcomes: A systematic review and meta-analysis. Rev Med Virol 2023;33(3):e2434-n/a. https://doi.org/ 10.1002/rmv.2434.

Word Count 3 694 (excluding figures, tables and references)

SUPPLEMENTARY TABLES

Supplementary Table 1: Association between Miscarriage, Maternal SARS-CoV-2 diagnosis and COVID-19 Wave									
	Univariable analysis			Multivariable analysis: Timing of SARS-CoV-2 diagnosis			Multivariable analysis: C-19 Wave		
	OR	95% CI	p-value	aOR	95% CI	p-value	aOR	95% CI	p-value
Maternal SARS-CoV-2 diagnosis									
No SARS-CoV-2 diagnosis	Ref	-	-	Ref	-	-	-	-	-
Early diagnosis	2.09	1.84 -2.37	<0.01	2.18	1.91-2.48	<0.01	-	-	-
COVID-19 Wave									
Inter-wave	Ref	-	-	-	-	-	Ref	-	-
Wave 1	0.80	0.75-0.84	<0.01	-	-	-	0.79	0.74-0.83	<0.01
Wave 2	0.94	0.89-0.99	0.042	-	-	-	0.98	0.92-1.04	0.459
Wave 3	1.14	1.08-1.19	<0.01	-	-	-	1.13	1.08-1.19	<0.01
Wave 4	1.23	1.16-1.31	<0.01	-	-	-	1.20	1.13-1.28	<0.01
Maternal Age									
15-24 years	Ref	-	-	Ref	-	-	Ref	-	-
<15 years	1.30	0.94-1.80	0.107	1.23	0.89-1.71	0.209	1.23	0.89-1.71	0.209
25-34 years	1.19	1.14-1.24	<0.01	1.26	1.20-1.31	<0.01	1.26	1.20-1.31	<0.01
≥35 years	1.78	1.70-2.87	<0.01	1.93	1.84-2.04	<0.01	1.93	1.84-2.04	<0.01
Hypertension									
No HT	Ref	-	-	Ref	-	-	Ref	-	-
Pre-existing HT	1.32	1.22-1.43	<0.01	1.38	1.27-1.50	<0.01	1.38	1.27-1.51	<0.01
Gestational HT	0.89	0.60-1.33	0.575	1.08	0.72-1.62	0.714	1.08	0.23-1.43	0.707
Diabetes									
No DM	Ref	-	-	Ref	-	-	Ref	-	-
Pre-existing DM	1.55	1.37-1.74	<0.01	1.77	1.56-2.01	<0.01	1.77	1.56-2.01	<0.01
Gestational DM	0.17	0.12-0.23	<0.01	0.31	0.23-0.43	<0.01	0.31	0.23-0.43	<0.01

Current Tuberculosis									
No	Ref	-	-	Ref	-	-	Ref	-	-
Yes	0.73	0.56-0.96	0.024	0.80	0.6 -1.05	0.113	0.80	0.6 -1.06	0.117
Previous Tuberculosis									
No	Ref	-	-	-	-	-	-	-	-
Yes	1.07	0.99-1.16	0.095	-	-	-	-	-	-
HIV status									
HIV neg/unknown	Ref	-	-	Ref	-	-	Ref	-	-
HIV pos	1.05	1.00-1.10	0.038	0.99	0.94-1.04	0.626	0.99	0.94-1.04	0.619
Antenatal care received during pregnancy									
Yes	Ref	-	-	Ref	-	-	Ref	-	-
No	5.01	4.81-5.21	<0.01	5.11	4.91-5.33	<0.01	5.14	4.94-5.36	<0.01

Supplementary Table 2: Associations between Stillbirth, Maternal SARS-CoV-2 Diagnosis and COVID-19 Wave*									
	Univariable			Multivariable: Timing of Maternal SARS-CoV-2 diagnosis			Multivariable: C-19 Wave		
	OR	95% CI	p-value	aOR	95% CI	p-value	aOR	95% CI	p-value
Maternal SARS-CoV-2 diagnosis									
No Maternal SARS-CoV-2 diagnosis	Ref	-	-	Ref	-	-	-	-	-
Early SARS-CoV-2 diagnosis	1.02	0.73- 1.43	0.913	1.01	0.71-1.41	0.978	-	-	-
Late SARS-CoV-2 diagnosis	1.31	1.02-1.68	0.032	1.31	1.02-1.67	0.034			
COVID -19 Wave									
Inter-wave period	Ref	-	-				Ref	-	-
Wave=1	1.05	0.95 -1.16	0.363				1.05	0.95-1.15	0.391
Wave=2	1.14	1.03-1.26	0.014				1.15	1.03-1.27	0.010
Wave=3	0.97	0.88-1.07	0.571				0.98	0.88-1.08	0.624
Wave=4	1.08	0.95-1.23	0.229				1.08	0.96-1.23	0.212
Maternal Age Category									
15-24 years	Ref	-	-	Ref	-	-	Ref	-	-
<15 years	1.07	0.57-2.01	0.825	1.10	0.59-2.06	0.765	1.10	0.58-2.07	0.762
25-34 years	0.99	0.92-1.07	0.840	0.93	0.86-1.00	0.060	0.93	0.86-1.00	0.059
≥35 years	1.17	1.06-1.29	<0.01	1.00	0.90-1.11	0.963	1.00	0.90-1.11	0.955
Hypertension									
No HT	Ref	-	-	Ref	-	-	Ref	-	-
Pre-existing HT	1.67	1.45-1.92	<0.01	1.55	1.34-1.80	0.000	1.56	1.35-1.80	<0.01
Gestational HT	3.73	2.51-5.55	<0.01	3.58	2.40-5.33	0.000	3.59	2.41-5.36	<0.01
Diabetes									
No DM	Ref	-	-	Ref	-	-	Ref	-	-
Pre-existing DM	2.33	1.92-2.83	<0.01	2.21	1.81-2.70	0.000	2.21	1.81-2.70	<0.01
Gestational DM	1.27	1.00-1.60	0.049	1.33	1.05-1.69	0.018	1.34	1.06-1.70	0.016
Current Tuberculosis									

No	Ref	-	-	Ref	-	-	Ref	-	-
Yes	1.68	1.18-2.39	<0.01	1.53	1.07-2.18	0.019	1.54	1.08-2.19	0.018
Previous Tuberculosis									
No	Ref	-	-	Ref	-	-	Ref	-	-
Yes	1.18	1.01-1.37	0.032	1.08	0.93-1.26	0.301	1.08	0.93-1.26	0.301
HIV Status									
HIV neg/unknown	Ref	-	-	Ref	-	-	Ref	-	-
HIV pos	1.36	1.26-1.48	<0.01	1.36	1.25-1.48	<0.01	1.36	1.25-1.48	<0.01
Antenatal care received during pregnancy									
Yes	Ref	-	-	Ref	-	-	Ref	-	-
No	1.27	1.19-1.36	<0.01	1.34	1.25-1.43	<0.01	1.34	1.25-1.43	<0.01

*Women not at risk of stillbirth (ie those who had experienced the outcome of miscarriage) in the group of women without a SARS-CoV-2 diagnosis were excluded from this analysis

Supplementary Table 3: Associations between Maternal Death, SARS-CoV-2 Diagnosis and COVID-19 Wave									
	Univariable			Multivariable: Maternal SARS-CoV-2 diagnosis			Multivariable: C-19 Wave		
	OR	95% CI	p-value	aOR	95% CI	p-value	aOR	95% CI	p-value
Maternal SARS-CoV-2 diagnosis									
No Maternal SARS-CoV-2 diagnosis	Ref	-	-	Ref	-	-	-	-	-
Maternal SARS-CoV-2 diagnosis	13.82	10.29-18.56	<0.01	11.42	8.46-15.43	<0.01	-	-	-
COVID-19 Wave									
Inter-wave period	Ref	-	-	-	-	-	Ref	-	-
Wave=1	1.61	1.12-2.34	0.011	-	-	-	1.64	1.13-2.37	0.009
Wave=2	2.24	1.58-3.19	<0.01	-	-	-	2.22	1.56-3.15	<0.01
Wave=3	1.63	1.15-2.33	0.007	-	-	-	1.68	1.18-2.40	0.004
Wave=4	0.61	0.31-1.22	0.161	-	-	-	0.62	0.31-1.23	0.172
Maternal Age Category									
15-24 years	Ref	-	-	Ref	-	-	Ref	-	-
<15 years	6.52	1.57-27.13	0.10	7.59	1.82-31.72	0.005	7.14	1.71-29.82	0.007
25-34 years	2.22	1.53-3.23	<0.01	1.80	1.23-2.63	0.002	1.90	1.30-2.77	0.001
≥35 years	4.14	2.79-6.16	<0.01	2.74	1.81-4.16	<0.01	2.94	1.94-4.45	<0.01
Hypertension									
No HT	Ref	-	-	Ref	-	-	Ref	-	-
Pre-existing HT	2.72	1.78-4.14	<0.01	1.70	1.09-2.66	0.019	1.82	1.17-2.83	0.008
Gestational HT	1.00	-	-	1.00	-	-	1.00	-	-
Diabetes									
No DM	Ref	-	-	Ref	-	-	Ref	-	-
Pre-existing DM	3.09	1.63-5.82	<0.01	1.99	1.03-3.85	0.039	2.01	1.04-3.88	0.038
Gestational DM	4.19	2.48-7.09	<0.01	2.85	1.66-4.90	<0.01	3.48	2.04-5.95	<0.01
Current Tuberculosis									
No	Ref	-	-	Ref	-	-	Ref	-	-
Yes	21.59	14.20-32.81	<0.01	15.71	10.03-24.62	<0.01	18.85	12.19-29.17	<0.01

Previous Tuberculosis									
No Yes	Ref 1.96	- 1.25-3.07	- <0.01	Ref 1.67	- 1.05-2.65	- 0.030	Ref 1.70	- 1.07-2.70	- 0.024
HIV Status									
HIV neg/unknown HIV pos	Ref 2.40	- 1.83-3.14	- <0.01	Ref 1.68	- 1.26-2.24	- <0.01	Ref 1.67	- 1.26-2.23	- <0.01
Prior Antenatal Care									
Yes No	Ref 0.77	- 0.59-1.01	- 0.063	Ref -	- -	- -	Ref -	- -	- -

Supplementary Table 4: Association between Maternal Death and Timing of Maternal SARS-CoV-2 diagnosis						
Maternal Death	Univariable			Multivariable		
	OR	95% CI	p-value	aOR	95% CI	p-value
Maternal SARS-CoV-2 diagnosis						
No Maternal SARS-CoV-2 diagnosis	Ref	-	-	Ref	-	-
Early SARS-CoV-2 diagnosis:	5.90	3.20-10.86	<0.01	4.53	2.44-8.40	<0.01
Late SARS-CoV-2 diagnosis	19.79	14.38-27.23	<0.01	17.16	12.39-23.76	<0.01
Maternal Age Category						
15-24 years	Ref	-	-	Ref	-	-
<15 years	6.52	1.57-27.13	0.10	7.85	1.88-32.84	0.005
25-34 years	2.22	1.53-3.23	<0.01	1.87	1.28-2.73	0.001
≥35 years	4.14	2.79-6.16	<0.01	2.86	1.88-4.33	<0.01
Hypertension						
No HT	Ref	-	-	Ref	-	-
Pre-existing HT	2.72	1.78-4.14	<0.01	1.72	1.10-2.68	0.017
Gestational HT	-	-	-	-	-	-
Diabetes						
No DM	Ref	-	-	Ref	-	-
Pre-existing DM	3.09	1.63-5.82	<0.01	2.03	1.05-3.92	0.035
Gestational DM	4.19	2.48-7.09	<0.01	2.76	1.61-4.74	<0.01
Current Tuberculosis						
No	Ref	-	-	Ref	-	-
Yes	21.59	14.20-32.81	<0.01	16.31	10.41-25.56	<0.01
Previous Tuberculosis						
No	Ref	-	-	Ref	-	-
Yes	1.96	1.25-3.07	<0.01	1.69	1.07-2.68	0.026
HIV Status						
HIV neg/unknown	Ref	-	-	Ref	-	-
HIV pos	2.40	1.83-3.14	<0.01	1.65	1.24-2.21	<0.01
Prior Antenatal Care						
Yes	Ref	-	-	Ref	-	-
No	0.77	0.59-1.01	0.063	-	-	-

Supplementary Table 5: Associations between Low Birth Weight and Maternal SARS-CoV-2 diagnosis						
	Univariable analysis			Multivariable analysis		
	OR	95% CI	p-value	aOR	95% CI	p-value
Maternal SARS-CoV-2 diagnosis						
No SARS-CoV-2 diagnosis	Ref	-	-	Ref	-	-
Early Maternal SARS-CoV-2 diagnosis	0.99	0.87-1.13	0.842	0.94	0.83-1.08	0.432
Late Maternal SARS-CoV-2 diagnosis	1.25	1.14-1.38	<0.01	1.22	1.10-1.34	<0.01
Maternal Age Category						
15-24 years	Ref	-	-	Ref	-	-
<15 years	0.84	0.63-1.16	0.228	0.85	0.64-1.13	0.274
25-34 years	0.97	0.94-0.99	0.025	0.92	0.89-0.95	<0.01
≥35 years	1.30	1.25-1.35	<0.01	1.15	1.11-1.20	<0.01
Maternal Hypertension						
No HT	Ref	-	-	Ref	-	-
Pre-existing HT	1.95	1.85-2.06	<0.01	1.86	1.76-1.97	<0.01
Gestational HT	3.13	2.55-3.84	<0.01	3.01	2.45-3.69	<0.01
Maternal Diabetes						
No DM	Ref	-	-	Ref	-	-
Pre-existing DM	1.36	1.23-1.50	<0.01	1.14	1.03-1.26	0.011
Gestational DM	1.17	1.07-1.27	<0.01	1.10	1.01-1.20	0.032
Maternal Current Tuberculosis						
No	Ref	-	-	Ref	-	-
Yes	2.31	2.02-2.64	<0.01	2.29	2.00-2.61	<0.01
Maternal Previous Tuberculosis						
No	Ref	-	-	Ref	-	-
Yes	1.69	1.60-1.78	<0.01	1.62	1.54-1.70	<0.01
Maternal HIV status						
HIV neg/unknown	Ref	-	-	Ref	-	-
HIV pos	1.23	1.19-1.26	<0.01	1.14	1.10-1.18	<0.01
Antenatal care received during pregnancy						
Yes	Ref	-	-	Ref	-	-
No	1.06	1.03-1.09	<0.01	1.10	1.07-1.13	<0.01

Supplementary Table 6: Associations between Neonatal ICU admission and Maternal SARS-CoV-2 Diagnosis									
	Univariable analysis			Multivariable analysis: Maternal SARS-CoV-2 diagnosis			Multivariable analysis: Timing of Maternal SARS-CoV-2 diagnosis		
	OR	95% CI	p-value	aOR	95% CI	p-value	aOR	95% CI	p-value
Maternal SARS-CoV-2 diagnosis									
No Maternal SARS-CoV-2 diagnosis	Ref	-	-	Ref	-	-	-	-	-
Maternal SARS-CoV-2 diagnosis	2.20	1.90 - 2.53	<0.01	2.02	1.75-2.34	<0.01	-	-	-
Early Maternal SARS-CoV-2 diagnosis	1.24	0.92-1.68	0.166	-	-	-	1.13	0.83-1.53	0.444
Late Maternal SARS-CoV-2 diagnosis	2.76	2.35-3.24	<0.01	-	-	-	2.56	2.18-3.01	<0.01
Maternal Age Category									
15-24 years	Ref	-	-	Ref	-	-	Ref	-	-
<15 years	0.93	0.46-1.87	0.838	0.95	0.47-1.92	0.890	0.96	0.47-1.93	0.897
25-34 years	1.03	0.96-1.11	0.433	0.93	0.86-0.99	0.046	0.93	0.86-1.00	0.057
≥35 years	1.39	1.26-1.52	<0.01	1.07	0.97-1.18	0.156	1.08	0.98-1.19	0.129
Maternal Hypertension									
No HT	Ref	-	-	Ref	-	-	Ref	-	-
Pre-existing HT	2.21	1.97-2.49	<0.01	1.72	1.51-1.95	<0.01	1.72	1.52-1.95	<0.01
Gestational HT	3.38	2.27-5.04	<0.01	2.68	1.79-4.01	<0.01	2.66	1.78-3.98	<0.01
Maternal Diabetes									
No DM	Ref	-	-	Ref	-	-	Ref	-	-
Pre-existing DM	3.64	3.11-4.26	<0.01	2.91	2.47-3.43	<0.01	2.92	2.48-3.44	<0.01
Gestational DM	3.05	2.63-3.53	<0.01	2.59	2.23-3.01	<0.01	2.59	2.23-3.01	<0.01
Maternal Current Tuberculosis									
No	Ref	-	-	-	-	-	-	-	-
Yes	1.18	0.80-1.75	0.401	-	-	-	-	-	-
Maternal Previous Tuberculosis									

No	Ref	-	-	-	-	-	-	-	-
Yes	1.01	0.87-1.17	0.908	-	-	-	-	-	-
Maternal HIV status									
HIV neg/unknown	Ref	-	-	Ref	Ref	-	Ref	-	-
HIV pos	1.14	1.05-1.24	<0.01	1.13	1.13	1.04-1.23	1.13	1.04-1.23	<0.01
Antenatal care received during pregnancy									
Yes	Ref	-	-	Ref	-	-	Ref	-	-
No	0.71	0.66-0.77	<0.01	0.80	0.74-0.86	<0.01	0.80	0.74-0.86	<0.01

APPENDICES

Appendix 1: Approval for Study by Human Research Ethics Committee, University of Cape Town (HREC-UCT)

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room 45 E-52-E-Floor- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-submissions@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

18 February 2022

HREC REF: 113/2022

Dr E Kalk
Centre for Infectious Disease Epidemiology & Research
Falmouth Building-FHS
Email: emma.kalk@uct.ac.za
Student: Vanessa.mudaly@westerncape.gov.za

Dear Dr Kalk

PROJECT TITLE: A COMPARISON OF MATERNAL & NEONATAL OUTCOMES OF PREGNANT WOMEN WITH & WITHOUT EVIDENCE OF SARS-COV-2 INFECTION IN THE WESTERN CAPE, SOUTH AFRICA-MMED CANDIDATE-DR VANESSA MUDALY

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, our letter dated 02 February 2022 provides guidance found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Approval is granted for one year until the 28 February 2023.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms

The HREC acknowledge that the student: Dr Vanessa Mudaly will also be involved in this study.

Please quote the HREC REF 113/2022 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Appendix 2: Renewal of Approval for Study by HREC-UCT (2023)

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

HUMAN RESEARCH ETHICS COMMITTEE
27 FEB 2023
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	28.02.2024
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	27/2/2023

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	24/02/2023		
HREC REF Number	113/2022	Current Ethics Approval was granted until	28/02/2023
Protocol title	A COMPARISON OF MATERNAL & NEONATAL OUTCOMES OF PREGNANT WOMEN WITH & WITHOUT EVIDENCE OF SARS-COV-2 INFECTION IN THE WESTERN CAPE, SOUTH AFRICA		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes	✓ ☐ No	
If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Dr Emma Kalk		
Department / Office Internal Mail Address	Centre for Infectious Disease Epidemiology and Research School of Public Health and Family Medicine		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Appendix 3: Renewal of Approval for Study by HREC-UCT (2024)

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	28.02.2025
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	3/4/2024

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

HUMAN RESEARCH ETHICS COMMITTEE
- 1 FEB 2024
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	31/01//2023		
HREC REF Number	113/2022	Current Ethics Approval was granted until	28/02/2024
Protocol title	A COMPARISON OF MATERNAL & NEONATAL OUTCOMES OF PREGNANT WOMEN WITH & WITHOUT EVIDENCE OF SARS-COV-2 INFECTION IN THE WESTERN CAPE, SOUTH AFRICA		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes	✓ ☐ No	
If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Dr Emma Kalk		
Department / Office Internal Mail Address	Centre for Infectious Disease Epidemiology and Research School of Public Health and Family Medicine		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Appendix 4: Study Approval from Provincial Health Research Committee (PHRC)

Western Cape Government
Health

STRATEGY & HEALTH SUPPORT
Health.Research@westerncape.gov.za
tel: +27 21 483 0866: fax: +27 21 483 6058
5th Floor, Norton Rose House,, 8 Riebeek Street, Cape Town, 8001
www.capegateway.gov.za)

REFERENCE: WC_202205_004
ENQUIRIES: Dr Sabela Petros

University of Cape Town
Anzio Road
Observatory
Cape Town
7925

For attention: Dr Emma Kalk

Re: A COMPARISON OF MATERNAL & NEONATAL OUTCOMES OF PREGNANT WOMEN WITH & WITHOUT EVIDENCE OF SARS-COV-2 INFECTION IN THE WESTERN CAPE, SOUTH AFRICA

Thank you for submitting your proposal to undertake the above-mentioned study. We are pleased to inform you that the department finds you study feasible and has provisionally granted you approval for your research. This is subject to the custodian of the datasets ability to process your request. Please note that you are not guaranteed any datasets.

Please contact the following people to assist you with any further enquiries in accessing or obtaining the requested datasets:

Epidemiology and Disease Surveillance EPI.SURV@westerncape.gov.za

Kindly ensure that the following are adhered to:

1. Researchers, in accessing provincial health facilities or datasets, are expressing consent to provide the department with an electronic copy of the final feedback (**Annexure 9**) within six months of completion of research. This can be submitted to the provincial Research Co-ordinator (Health.Research@westerncape.gov.za).
2. In the event where the research project goes beyond the *estimated completion* date which was submitted, researchers are expected to complete and submit a progress report (**Annexure 8**) to the provincial Research Co-ordinator (Health.Research@westerncape.gov.za).
3. The reference number above should be quoted in all future correspondence.

Yours sincerely

Signed by candidate

PROF. V ZWEIGENTHAL
DIRECTORATE: HEALTH INTELLIGENCE
DATE: 14 October 2022
CC

Appendix 5: Study Protocol

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Executive Summary

Background: The global outbreak of SARS-CoV-2 has resulted in over 245 million known cases and 5 million deaths. An emerging concern has been that COVID-19 is associated with adverse effects on pregnant women and their babies. SARS-CoV-2 infection has been found to have a range of presentations, varying from being asymptomatic to exhibiting multi-system symptoms ranging in severity from mild to severe. Certain demographic factors and comorbidities have been associated with the severity of symptoms and risk of mortality. Pregnant women appear to be more likely to be admitted to intensive care units (ICU) and receive mechanical ventilation than non-pregnant women. SARS-CoV-2 infection has been associated with poor maternal and neonatal outcomes including pre-eclampsia, pre-term labour, caesarean delivery, maternal death, low birth weight, and neonatal admission to ICU,

Gap in knowledge: Current literature based on studies in multiple settings indicates that pregnant women infected with SARS-CoV-2 are at greater risk of poor outcomes. Observational research has shown that these associations may be related to patient characteristics, socio-demographic factors and pregnancy itself. Data are lacking on maternal and neonatal outcomes of pregnant women infected with SARS-CoV-2 in South Africa, where there is a high prevalence of HIV infection and tuberculosis (current and past infection) in addition to obesity, hypertension and diabetes mellitus.

Research Aim: To compare the characteristics and outcomes of pregnant women accessing healthcare services within the public sector who have been diagnosed with SARS-CoV-2 infection between 1 March 2020 and 31 January 2022 and those of pregnant women in whom SARS-CoV-2 infection was not diagnosed over the same period in the Western Cape province.

Research Objectives: 1. To compare demographic and clinical characteristics of pregnant women at district level diagnosed with SARS-CoV-2 infection and those not diagnosed with SARS-CoV-2 infection over the specified time period. 2. To compare maternal and pregnancy outcomes and patient characteristics at district level (ICU admission-COVID-related, ICU admission-non-COVID-related, death, live birth, still birth, elective termination of pregnancy, miscarriage, premature delivery, delivery by caesarean section) between these two groups. 3. To compare neonatal outcomes of low birth weight, neonatal ICU admission, neonatal SARS-CoV-2 infection and neonatal mortality of babies between these two groups.

Study Design: This is a retrospective cohort analysis. The exposure is evidence of SARS-COV-2 infection during pregnancy, and the outcomes of interest are specific maternal (ICU admission-COVID-related, ICU admission-non-COVID-related, death, live birth, still birth, elective termination of pregnancy, miscarriage, premature delivery, delivery by caesarean

section) and neonatal outcomes (low birth weight, neonatal ICU admission, neonatal SARS-CoV-2 infection and neonatal mortality).

Data collection: The Provincial Health Data Centre (PHDC) collates and links routine data from multiple sources in the public health system including databases from hospitals (CLINICOM), laboratories (NHLS-TRACK), pharmacies (JAC) and primary health facilities (PHCIS, SINJANI). Linkage is possible through a folder number which serves as a unique identifier. Antenatal and obstetric patient episodes are collated in the "maternity cascade". SARS-CoV-2 testing and admissions are collated in the "Covid cascade".

Population: The source population is all women accessing obstetric health services at a health facility in the public sector in the Western Cape with evidence of pregnancy between 1 March 2020 to 31 January 2022. From the "maternity cascade", the study population will be those with a known pregnancy outcome in this time period.

Inclusion and exclusion criteria: All women in the maternity cascade with high confidence evidence of pregnancy during the selected time-period with evidence of a pregnancy outcome at a public health facility will be included. Women with low confidence evidence of pregnancy or who did not have evidence of a pregnancy outcome at a public health facility will be excluded.

Data analysis: Data will be summarized using descriptive statistics. Statistical tests for numerical variables: calculation of means and medians, with standard error and IQR depending on the distribution. Statistical tests for categorical variables: Chi- squared ($\chi 2$) test or Fisher Exact test with 95% CI. Data will be analysed using Stata software version 17. Logistic regression will be used to construct multivariate models of associations with outcomes and calculate odds ratios.

Ethical considerations: Anonymised data will be provided for this study by the PHDC therefore the patient consent process will be waived. The PHDC operates under strict governance procedures and is fully adherence to South African legislation and Provincial Department of Health regulations. Datasets are encrypted and password-protected to restrict access to information.

1. Background

The global outbreak of SARS-CoV-2 has resulted in just over 245 million known cases and 5 million deaths. Since the start of the outbreak in Wuhan, China in late 2019, substantial data across multiple settings has been collated and evaluated to determine the predictors and risk factors of mortality and morbidity. Significant risk factors that have been identified include older age and comorbidities such as diabetes mellitus, renal disease, cardiovascular disease, tuberculosis and immunosuppressive conditions [1]. An emerging concern has been that COVID-19 (C-19) is associated with adverse effects on pregnant women and their babies. In addition, emergency restrictions on movement that were instituted in countries to contain transmission of SARS-CoV-2 infection, were known to have impacted on access and availability of health services, including ante-natal and peri-natal services.

In South Africa, four distinct waves of infection were experienced from early March 2020 to January 2022. At the peaks of the first three waves, strict lockdown restrictions were implemented. In the Western Cape province, over half a million cases of SARS-CoV-2 have been recorded, with a mortality rate of 289 per 100,000 population (1 January 2022) [2]. This ranks among the top mortality rates globally [3]. A concerning increase in maternal mortality was also noted for the year 2020 compared to 2019. Preliminary data suggest an increase in the maternal mortality ratio from 56.8 (2019) to 96.9 (2020) deaths per 100 000 live births [4]. In multiple countries, SARS-CoV-2 has been associated with poor maternal and neonatal outcomes, however this phenomenon has not been previously explored in the context of the Western Cape province.

2. Literature Review

Search Strategy

A review of literature was done using the Primo, Google Scholar and Pubmed search engines using SARS-CoV-2 OR COVID-19 OR coronavirus AND risk factors OR mortality; SARS-CoV-2 OR COVID-19 OR coronavirus AND pregnancy OR pregnant OR maternal outcomes OR maternal mortality OR neonatal outcomes OR neonatal mortality. Large cohort studies and systematic reviews were preferentially selected over cases series and small cohort studies as these were considered more generalisable to multiple settings.

Associations between SARS-CoV-2 infection and pregnancy outcomes

Disease severity in pregnant women

SARS-CoV-2 infection has been found to have a range of presentations, varying from being asymptomatic to exhibiting multi-system symptoms ranging in severity from mild to moderate to severe. Certain demographic factors and comorbidities have been associated with the severity of symptoms and risk of mortality. These include age >60 years, male gender, obesity, cardiovascular disease, renal disease, lung disease, cancer and immunosuppressive disorders. The severity of specific symptoms such as fever, dyspnoea and myalgia have also been associated with a higher risk of mortality [1].

In a Spanish cohort of pregnant women, Villalain et al [5] found asymptomatic infection in 28% of women who tested IgG positive for SARS-CoV-2 during pregnancy. This concurs with the estimate of asymptomatic infection in the general population of one third [6]. In an observational study using data collected from a smartphone application interface used widely in UK, USA and Sweden and large web-based survey conducted in the USA, Molteni et al [7] found that whilst pregnant women were more likely to be tested for SARS-CoV-2, they displayed similar severity of symptoms compared to non-pregnant women. In a systematic review of case series reports from multiple countries collated up to April 2020, Elshafeey et al [8] concurred with this finding.

Maternal risk factors associated with hospitalization with SARS-COV-2 infection

In the United States of America (USA), as part of COVID-19 surveillance, the Centre for Disease Control and Prevention (CDC) collated reports on infections in women of child-bearing age between January and July 2020 [9]. Reports of chronic lung disease, diabetes mellitus, and cardiovascular disease were more frequent among pregnant women with SARS-CoV-2 infection than among nonpregnant women. 31.5% of pregnant women were reported to have been hospitalized compared with just 5.8% of nonpregnant women. Pregnant women were also more likely to be admitted to the intensive care unit (ICU) (adjusted risk ratio (aRR) = 1.5, 95% confidence interval [CI] = 1.2–1.8) and receive mechanical ventilation (aRR = 1.7, 95% CI = 1.2–2.4). However, reports of deaths were low (0.2%) in both groups.

In a cohort of 427 women presenting to 194 obstetric units in the United Kingdom (UK) between March-April 2020, Knight et al [10] found the estimated incidence of admission with confirmed SARS-CoV-2 infection in pregnancy was high at 4.9 per 1000 maternity cases. Most

women who were admitted presented with symptoms were in the third trimester (median 34 weeks). Identified risk factors for admission were overweight or obese (69%), age 35 or over (41%), and pre-existing comorbidities (34%). While 10% of women needed respiratory support, deaths were low (1%). In a large systematic review and metanalysis of observational data on pregnancy and SARS-CoV-2 infection admissions, Khalil et al [11] also identified high rates of obesity (38%) and comorbidities (32%). However, admission to ICU and maternal mortality was low.

Associations with maternal and neonatal outcomes

In a small case control study comparing outcomes of pregnant women diagnosed with COVID-19 pneumonia with pregnant women not diagnosed with pneumonia between January and February 2020 in a hospital in China, pneumonia was associated with higher rates of pre-term delivery and delivery by caesarean section [12]. No severe maternal or neonatal complications were found to be associated with SARS-CoV-2 pneumonia. Findings in a cohort study from 5 Nordic countries [13] were very similar, with no maternal or neonatal deaths being reported in hospitalised pregnant women with SARS-CoV-2, however preterm delivery (25%) and caesarean delivery (43.8%) were increased. This was a similar finding in the study conducted by Khalil et al [11].

In a larger French national retrospective cohort study including nearly 250 000 pregnancies between January to June 2020 [14], women with SARS-COV-2 infection compared to women without known SARS-COV-2 infection had a higher frequency of admission to ICU (5.9% versus 0.1%, $p < 0.001$), mortality (0.2% versus 0.005%, $p < 0.001$), preeclampsia/eclampsia (4.8% versus 2.2%, $p < 0.001$), gestational hypertension (2.3% versus 1.3%, $p < 0.03$), postpartum haemorrhage (10.0% versus 5.7%, $p < 0.001$), preterm birth at <37 weeks of gestation (16.7% versus 7.1%, $p < 0.001$), induced preterm birth (5.4% versus 1.4%, $p < 0.001$), spontaneous preterm birth (11.3% versus 5.7%, $p < 0.001$), foetal distress (33.0% versus 26.0%, $p < 0.001$), and Caesarean section (33.0% versus 20.2%, $p < 0.001$).

A systematic review and metanalysis was conducted later by Wei et al [15]. This included 42 studies and 438 548 pregnant women. Compared with no infection in pregnancy, SARS-CoV-2 infection in pregnancy was associated with preeclampsia (OR 1.33, 95% CI 1.03 to 1.73); preterm birth (OR 1.82, 95% CI 1.38 to 2.39); stillbirth (OR 2.11, 95% CI 1.14 to 3.90), ICU admission (OR 4.78, 95% CI 2.03 to 11.25), lower birth weight (grams; mean difference –

68.96, 95% CI –130.22 to –7.69) and neonatal ICU admission (OR 3.69, 95% CI 1.39 to 9.82). Compared with mild COVID-19, severe COVID-19 was strongly associated with preeclampsia (OR 4.16, 95% CI 1.55 to 11.15), preterm birth (OR 4.29, 95% CI 2.41 to 7.63), gestational diabetes (OR 1.99, 95% CI 1.09 to 3.64), ICU admission (OR 15.46, 95% CI 5.79 to 41.23; I2 = 0%), mechanical ventilation (OR 19.31, 95% CI 9.38 to 39.72; I2 = 0%), caesarean delivery (OR 2.58, 95% CI 1.64 to 4.06), low birth weight (OR 1.89, 95% CI 1.14 to 3.12) and NICU admission (OR 3.95, 95% CI 1.43 to 10.95).

Premature birth may be mainly iatrogenic in babies born to women admitted with SARS-CoV-2. In a multicentre prospective cohort study using multivariate logistic regression analysis [16], SARS-CoV-2 infection in admitted pregnant women increased the odds of preterm birth (aOR 2.12, 95% CI 1.32– 3.36, p = 0.002), premature rupture of membranes at term (aOR 1.70, 95% CI 1.11–2.57, p = 0.013) and neonatal intensive care unit admissions (aOR 4.62, 95% CI 2.43–8.94, p < 0.001). None of the neonates born to infected mothers tested positive for SARS-CoV-2 infection in this study. In other studies, rates of neonatal SARS-CoV-2 infection vary between about 5% (7) to 0.8% [17].

Specific Factors associated with poor maternal and neonatal outcomes

Physiological and anatomical changes during pregnancy

Pregnancy itself may be associated with increased vulnerability to infections, due to changes in functioning of the immune system and hormone levels [18]. This includes changes in CD4 T cell population proportions and reductions in natural killer cells and plasmacytoid dendritic cells, coupled with increases in progesterone levels. Progesterone may be beneficial for lung repair post-influenza infection, but it may also reduce antibody and CD8 T cell production. Pregnancy induces physiological and anatomical changes, thus chest shape is altered and the diaphragm is elevated. This reduces lung capacity and the ability to clear secretions in the event of infection.

Historically, increased risks of maternal mortality from pneumonia have been recorded during viral epidemics [19]. During the Spanish Flu (1918), 27% of infected pregnant women succumbed to infection, while in the H5N1 outbreak in Minnesota (1957), 50% of deaths in women of child-bearing age occurred in pregnant women. Influenza and varicella outbreaks are both associated with increased mortality due to pneumonia in pregnant women. In recent times, other viruses that have resulted in increases in maternal mortality and morbidity include

SARS-CoV-1 (2003), H1N1 (Swine Flu) (2009) and Middle East Respiratory Syndrome (MERS-CoV) (2012).

Hypercoagulability

Pregnancy is associated with hypercoagulability, due to increased thrombin production, intravascular inflammation and circulating coagulation factors. SARS-CoV-2 infection is associated with increased thromboembolic events, therefore it is possible that infection during pregnancy carries an augmented risk of thrombotic events. Koumoutsea at al [20] documented rapidly progressive coagulopathy during the third trimester in two pregnant women with SARS-CoV-2. Very elevated levels of D-dimers were recorded - a finding that has been associated with poor outcomes in hospitalised patients with SARS-CoV-2.

Increased risk of pre-eclampsia

An association between endothelial dysfunction due to SARS-CoV-2 and increased risk of pre-eclampsia has been postulated [18]. In acute respiratory distress syndrome (ARDS) associated with SARS-CoV-2, endothelial damage has been demonstrated. Several risk factors for endothelial dysfunction such as increasing age, obesity, diabetes mellitus, and cardiovascular disease, are also associated with SARS-CoV-2. Other shared factors in the pathogenesis of pre-eclampsia and SARS-CoV-2 infection such as dysfunction in the renal-angiotension system, the imbalance of angiogenic/antiangiogenic factors, the presence of ACE genetic polymorphisms, and the shared histopathological characteristics of pre-eclamptic and SARS-CoV-2 placentas, make the association more plausible.

Placental infiltration

Alarmingly, many viral infections have been associated with placental infiltration, such as Ebola, Lassa, cytomegalovirus, varicella and Zika [18, 19]. Placental tissue may be vulnerable to SARS-CoV-2 infiltration because angiotensin-converting enzyme 2 (ACE2) is the receptor for SARS-CoV-2 and has a key role in human infection and ongoing transmission. ACE2 is highly expressed in maternal-foetal interface cells which includes stromal cells and perivascular cells of decidua and cytotrophoblast as well as syncytiotrophoblast within the placenta [21]. There are also preliminary unverified reports state that embryos may harbour SARS-CoV-2 receptors ACE2 and other proteases. This makes disruption of placental function and vertical transmission possible, with subsequent miscarriage, stillbirth and neonatal complications likely.

Placentas and other foetal and maternal tissues from infected women have been examined in cases studies [19]. Significant findings include fibrin deposition around placental villi (resulting in villous infarction in one case) and increase in features of maternal vascular malperfusion (MVM). The latter is a risk factor for gestational hypertension and pre-eclampsia. Evidence of viral transmission has also emerged. Fenezia et al [22] identified SARs-CoV-2 genome in specimens of umbilical cord blood, placental tissue, maternal vaginal tissue and breastmilk. Valdespino-Vázquez et al [23] observed viral particles consistent with coronavirus by electron microscopy in post-mortem foetal lung tissue and the placental parenchyma.

Access to health services

Access to healthcare services was impacted in many settings during the pandemic. In a systematic review and metanalysis including observational studies or research letters reporting primary data on the changes in maternity service use by pregnant women from multiple countries, Townsend R et al [24] found that most of the 25 studies with data on ante-natal visits reported decreases in attendance of routine antenatal visits during the pandemic compared to before the pandemic. The reduction was more pronounced in lower income settings, where many women cited difficulties with travel and fear of contracting SARS-CoV-2 at clinics as reasons for poor clinic attendance. Overall, there was a reduction of almost 40% in attendance at routine ante-natal visits in this study.

Social and demographic factors

Data are emerging on the impact of ethnic and socio-economic disparities on SARS-CoV-2 transmission and outcomes. Pineles B et al [25] retrospectively reviewed a cohort of women delivering at a hospital in Texas. Hispanic patients and patients with public insurance were more likely to test positive for SARS-CoV-2 than non-Hispanics (10.6% vs 5.5%, aRR 1.73, 95% confidence interval [CI] 1.05–2.85), and patients with private insurance (9.5% vs 2.5 %, aRR 3.11, 95 % CI 1.12–8.64) respectively. However, most patients diagnosed with SARS-CoV-2 were asymptomatic and no differences was observed in outcomes. Similar findings were observed in a study in Georgia [26], where in addition to ethnicity and uninsured status, high neighbourhood density was associated with higher prevalence of SARS-CoV-2 seropositivity.

Current literature on outcomes of pregnant women with SARS-CoV-2 infection in South African settings

The National Institute of Communicable Disease (NICD) in South Africa used data obtained from a hospital surveillance system to report on the clinical characteristics, outcomes and epidemiology of pregnant women hospitalised with SARS-CoV-2 between 5 March 2020 and 28 February 2021 [27]. The cumulative incidence of SARS-CoV-2 admissions in pregnant women was significantly higher than in non-pregnant women of child-bearing age: 543.3 versus 287.5 per 100,000 population. The highest cumulative incidence of SARS-CoV-2 admissions in pregnant women was observed in the Western Cape province (1567.4 per 100,000) - this was nearly 3 times the national average. This may be attributable the introduction of routine SARS-CoV-2 PCR testing of obstetric admissions in 2020.

Of the hospitalizations, pregnant women were generally younger than non-pregnant women of childbearing age and had a lower prevalence of comorbid disease. Admitted pregnant women with SARS-CoV-2 were more likely to be Black African than other races. More admissions of infected pregnant women occurred in the private sector (55%) than in the public sector. HIV prevalence was slightly lower in pregnant women compared to non-pregnant women. Obesity, was more prevalent in the non-pregnant admissions. Admission to ICU and the need for ventilation was greater in the non-pregnant admissions. The in-hospital case-fatality ratio (CFR) for pregnant women was 3.5%, which was lower than the CFR of 10% for non-pregnant women of childbearing age [27].

In another study focussing on hospitalized pregnant women, Budhram et al [28] found a higher CFR in women admitted with SARS-CoV-2 versus other causes (6.5% versus 1.8%). Interestingly, rates of caesarean section and neonatal outcomes were similar between these two groups. The only comorbidity associated with admission with SARS-CoV-2 was TB, which is not surprising given that it is a highly prevalent disease in SA and contributed significantly to maternal mortality in the pre-COVID era. Notably, a cohort study based on SARS-CoV-2 infections in the general population in the Western Cape identified an association between SARS-CoV-2 mortality and HIV infection (adjusted hazard ratio [aHR], 2.14; 95% confidence interval [CI], 1.70–2.70), and with current and previous diagnoses of TB (aHR,2.70 [95% CI, 1.81–4.04] and 1.51 [95% CI, 1.18–1.93], respectively) [29].

3. Gap in knowledge and Justification for study

Current literature based on observational studies in multiple settings indicates that pregnant women infected with SARS-CoV-2 are at greater risk of poor outcomes. There are compelling explanations for these findings. Data are lacking on maternal and neonatal outcomes of pregnant women infected with SARS-CoV-2 in South Africa, where there is a high prevalence of HIV infection and tuberculosis (current and past infection) in addition to obesity, hypertension and diabetes mellitus. The purpose of this study is to describe and quantify at a local level the impact (direct and indirect) of the SARS-CoV-2 pandemic on maternal and neonatal outcomes of pregnant women. Local data will inform the quality of ante-natal and peri-natal services, and the vaccination strategy for pregnant women in the Western Cape and South Africa.

4. Aim, Hypotheses and Objectives

Aim: To compare the characteristics and outcomes of pregnant women accessing healthcare services within the public sector who have had confirmed SARS-CoV-2 infection between 1 March 2020 and 31 January 2022 and those of pregnant women in whom SARS-CoV-2 infection was not diagnosed over the same period in the Western Cape province.

Hypotheses

- The risk of severe SARS-COV-2 is associated with similar risk factors to non-pregnant women- these include age, comorbidities such as hypertension, diabetes, renal disease, cardiovascular disease and respiratory disease. In SA, TB and HIV may be additional risk factors.
- The risk of admission is higher in women with SARS-COV-2 in late pregnancy, where there is an increased risk of COVID pneumonia. This is associated with ICU admission with ventilation and iatrogenic preterm delivery and delivery by caesarean section.
- The risk of poor maternal and neonatal outcomes is higher in women with severe SARS-CoV-2 disease than in those with asymptomatic or mild disease.
- Socio-demographic factors that may be associated with poor maternal and neonatal outcomes include health services factors such as access to antenatal care, socio-economic disparities and increased risk of transmission in high-density setting. District of residence may therefore influence associations between SARS-CoV-2 infection and pregnancy outcomes.

Objectives

Objective 1: To compare demographic and clinical characteristics of pregnant women at district level diagnosed with SARS-CoV-2 infection and those not diagnosed with SARS-CoV-2 infection over the time period.

Objective 2: To compare maternal and pregnancy outcomes and associated characteristics at district level (ICU admission-COVID-related, ICU admission-non-COVID-related, death, live birth, still birth, elective termination of pregnancy, miscarriage, premature delivery, delivery by caesarean section) between pregnant women with diagnosed SARS-CoV-2 infection and those in whom SARS-CoV-2 infection was not diagnosed.

Objective 3: To compare neonatal outcomes of low birth weight, neonatal ICU admission, neonatal SARS-CoV-2 infection and neonatal mortality of babies born to pregnant women with diagnosed SARS-CoV-2 infection and those in whom SARS-CoV-2 infection was not diagnosed.

4. Study Methodology

4.1 Study design

This is a retrospective cohort analysis. The exposure is evidence of SARS-COV-2 infection during pregnancy, and the outcomes of interest are specific maternal (ICU admission-COVID-related, ICU admission-non-COVID-related, death, live birth, still birth, elective termination of pregnancy, miscarriage, premature delivery, delivery by caesarean section) and neonatal outcomes (low birth weight, neonatal ICU admission and neonatal mortality).

4.2 Population and sampling

The source population (fig 1)(30) is all women accessing antenatal or obstetric health services at a health facility in the public sector in the Western Cape with evidence of pregnancy between 1 March 2020 to 31 January 2022. The Provincial Health Data Centre (PHDC) collates and links routine data from multiple sources in the public health system including databases from hospitals (CLINICOM), laboratories (NHLS-TRACK), pharmacies (JAC) and primary health facilities (PHCIS, SINJANI). Linkage is possible through a folder number which serves as a unique identifier (31). Patients using antenatal and obstetric services are collated in a sub-group referred to as the maternity cascade, from which we will define the "database population"(fig 1). From these records, the study population will be selected as those with a known pregnancy outcome in the time period of interest.

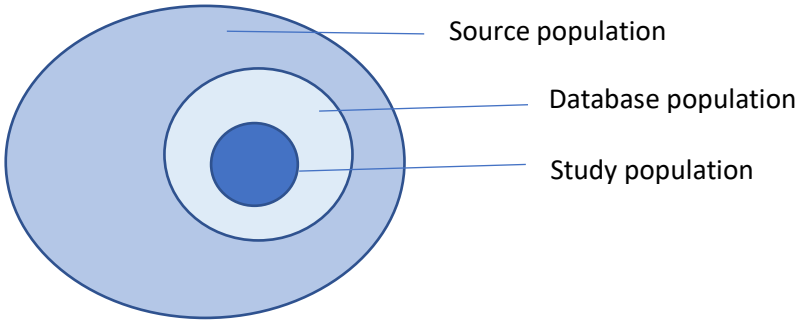


Figure 1: Population hierarchy in studies using routinely collected data sources
(Source: Benchimol et al The Reporting of studies conducted using observational routinely-collected health data (RECORD) statement [30])

The PHDC is able to provide information on comorbidities such as HIV, TB and COVID-19 by triangulation of data from the multiple sources available. Certainty levels are assigned for each health condition enumeration [31].

4.3 Inclusion and exclusion criteria

- **Inclusion criteria**

All women in the maternity cascade with high confidence evidence of pregnancy (according to PHDC criteria) during the selected time-period with evidence of a pregnancy outcome at a public health facility.

- **Exclusion criteria**

All women with low confidence evidence of pregnancy according to PHDC criteria.
All women with evidence of pregnancy who did not have evidence of a pregnancy outcome at a public health facility.

4.4 Measurements

4.4.1 Data Collection

The PHDC is able to collate and link data from multiple sources. These include:

- CLINICOM: collates data of patients admitted to hospitals. Includes ICD diagnostic codes, admission ward, comorbidities, procedures, outcomes including deaths
- NHLS-TRACK: collates results of patient haematology, biochemistry, virology and microbiology tests done at public health facilities

- JAC: collates data on medication issued to patients at public health facilities, both in-patient and out-patient
- PHCIS, SINJANI: collates data on patients attending primary health facilities. Includes appointment dates and reason for visit (e.g. visits to a midwife obstetric unit])
- ECCR reports: these are discharge summaries completed for patients being discharged from hospital
- SARS-CoV-2 PCR testing results from private pathology laboratories

4.4.2 Variables

Table 1: Variables for Patient Characteristics

Variable	Type of variable	Comments
Age	Continuous numerical	Calculate mean age
Age category cat 1:<15 years cat 2 15-24 years cat 3: 25-34 years cat 4 >35 years	Categorical	Women at the age extremes <15 years and >35 years are more likely to experience complicated pregnancies therefore represent a higher risk profile.
Antenatal booking status category cat 1: booked cat 2: unbooked/ no prior antenatal care	Categorical	Unbooked patients are more at risk of delivery complications and poor outcmes
District of antenatal booking category cat 1: City of Cape Town cat 2: Overberg cat 3: Central Karoo cat 4: Garden Route cat 5: Cape Winelands cat 6: West Coast	Categorical	May reflect differences in quality of antenatal services between districts.
Diabetes mellitus cat 1: no diabetes cat 2: pre-existing diabetes cat 3: new/ gestational diabetes	Categorical	Linkage will be to high certainty level episodes.
Hypertension (HT) cat 1: no HT cat 2: pre-existing HT cat 3: new/ gestational HT	Categorical	Linkage will be to high certainty level episodes.

Tuberculosis (TB) cat 1: no TB cat 2: current TB cat 3: previous TB	Categorical	Linkage will be to high certainty level episodes. WC has a high prevalence of TB, TB and previous TB are associated with poor maternal outcomes, may also increase risk of poor outcome with SAR-CoV-2.
HIV cat 1: HIV neg/unknown cat 2: HIV pos	Categorical	Linkage will be to high certainty level episodes. WC has a high prevalence of HIV, HIV is associated with poor maternal outcomes, may also increase risk of poor outcome with SAR-CoV-2.
SARS-CoV-2 diagnosis during pregnancy cat 1: negative result/ no diagnosis cat 2: positive result/ diagnosis	Categorical	SARS-CoV-2 diagnosis is evidence of a positive PCR or antigen test result, or clinical notes/ discharge summary indicating probable COVID.
Estimated gestational age at SARS-CoV-2 diagnosis (weeks)	Continuous numerical	Estimated gestational age is determined by triangulating data within the maternal cascade and estimating gestation start date.
Timing of SARS-CoV-2 diagnosis Cat 1: No infection Cat 2: Early (<28 weeks gestation) Cat 3: Late (≥28 weeks gestation)	Categorical	Early vs Late infection- may differ by disease severity and outcomes
Wave of COVID-19 Cat 1: Inter-wave Cat2: wave 1 Cat2: wave 2 Cat2: wave 3 Cat2: wave 4	Categorical	Wave 1 = 03/05/20-16/08/20; Wave 2 = 08/11/20-07/02/21; Wave 3 = 23/05/21-19/09/21 Wave 4 = 28/11/21-30/01/22
District of outcome cat 1: City of cape Town cat 2: Overberg cat 3: Central Karoo cat 4: Garden Route cat 5: Cape Winelands cat 6: West Coast	Categorical	May reflect differences in quality of services and access to SARS-CoV-2 testing between districts.

Table 2: Variables for Maternal Outcomes

Variable	Type of variable	Comments
Delivery outcome cat 1: live birth cat 2: stillbirth cat 3: TOP cat 4: miscarriage cat 5: maternal death	Categorical	
Mode of delivery cat 1: vaginal cat 2: caesarean sect	Categorical	
Maternal death cat1: no death cat 2: death	Categorical	Maternal death= death during pregnancy or within 42 days of outcome

Table 3: Variables for Neonatal Outcomes

Variable	Type of variable	Comments
Low Birth weight cat 1: Weight≥2 500g cat 2: Weight <2 500g	Categorical	
Preterm delivery cat 1: No preterm cat 2: Pre-term	Categorical	Gestational age is estimated by triangulating other data in the maternal cascade. Preterm =<37 weeks gestation
Neonatal ICU admission cat 1: not admitted ICU cat 2: nICU admission	Categorical	
Neonatal death cat 1: death cat 2: no death	Categorical	This is death that occurs within one week of delivery
Neonatal SARS-CoV-2 cat 1: l PCR test negative /no test done cat 2: PCR test positive	Categorical	

4.4.3 Validity and Reliability of measurements

- Data is collated from multiple operational sources and we cannot control for the quality of the data; therefore some information may be missing. This may result in misclassification bias. According to the RECORD statement[30] the risk of misclassification bias is amplified in studies using databases from large populations and affects validity of the study findings(ref). This will be mitigated by requesting inclusion of data with high levels of certainty.

- Some data in the "Maternity Cascade" (PHDC) are derived from multiple sources, increasing certainty in defining pregnancy.
- The outcome district is determined by the facility attended and not the physical address of the patient.
- Pregnancy outcome data from private sector facilities are not available to the PHDC therefore patients attending those facilities will be excluded.

4.4.4 Limitations of the Study

- Missing data (due to use of operational data) may be a source of misclassification bias. The advanced processes used by the PHDC to triangulate data using unique patient folder numbers will mitigate this risk.
- Exclusion of women with outcomes in private facilities may be a source of selection bias and results may not be generalizable beyond public health service-users.
- There may be various sources of confounding and bias in this setting: this includes socioeconomic status (SES) and increased risk of SARS-COV-2 infection associated with living in densely populated areas, varying degrees of herd immunity across districts, varying degrees of vaccination across insured and uninsured populations, higher risk of HIV and tuberculosis association with low SES (poorer outcomes in pregnancy and risk of severe SARS-COV-2 disease), limited access to health services and PCR/antigen testing and delays in health-seeking behaviour for various reasons leading to presentation with more advanced SARS-CoV-2 disease. Inclusion of district of booking and/or outcome will be included in the analysis.
- Patients with asymptomatic or mild infections may not have been tested leading to misclassification of infection status, however patients admitted for delivery were more likely to be tested since mandatory screening for admissions was introduced during 2020.

4.5 Data Management and Analysis

For objective 1, numerical and categorical data will be tabulated and compared between women with and without evidence of SARS-COV-2 infection during pregnancy (see dummy tables below).

Data will be summarized using descriptive statistics. Statistical tests for numerical variables: calculation of means and medians, with standard error and IQR, respectively depending on the

distribution of the data. Statistical tests for categorical variables: Chi- squared ($\chi 2$) test or Fisher Exact test with 95% CI.

Data will be analysed using Stata software version 17

Table 4: Dummy table for Objective 1

Variable	SARS-CoV-2 PCR or antigen test positive or clinical evidence of infection	SARS-CoV-2 PCR or antigen test negative or no evidence of infection	p-value
N=	n=	n=	
Mean Age (years)			
Age category 1-4			
Hypertension			
Gestational hypertension			
Pre-existing diabetes			
Gestational diabetes			
HIV status			
Current tuberculosis			
Previous tuberculosis			
Booked at ANC			
District of ANC booking cat 1-6			
District at outcome 1-6			

Table 5: Dummy table for Objective 2

Variable	SARS-CoV-2 PCR or antigen test positive or clinical evidence of infection	SARS-CoV-2 PCR or antigen test negative or no evidence of infection	95% CI and p-value
N=	n=	n=	
COVID-related ICU admission			
Non-COVID related ICU admission			
Live birth			
Stillbirth			
Miscarriage			
TOP			
Preterm birth <37 weeks			
Caesarean section			
Maternal death			

Table 6: Dummy table for Objective 3

Variables	SARS-CoV-2 PCR or antigen test positive or clinical evidence of infection	SARS-CoV-2 PCR or antigen test negative or no evidence of infection	95% CI and p-value
N=	n=	n=	
Mean birth weight (g)			
Low birth weight <2 500g			
Neonatal SARS-CoV-2 PCR pos			
Neonatal admission ICU			
Neonatal death			

For objectives 2 and 3, logistic regression will be used to calculate the crude and adjusted Odds Ratios with 95% CIs for each of the maternal and neonatal outcomes of interest. Models will be built to examine the association with each of the patient characteristics and a SARS-CoV-2 diagnosis to identify possible confounders. These will be considered for inclusion in multivariate models analysing the association between patient characteristics and each of the maternal and neonatal outcomes of interest. Analysis will also be stratified by wave and outcome district in order to account for indirect effects of the COVID-19 outbreak.

5. Ethical Considerations and Communication

5.1 Risks and Benefits of the Study

There are no direct risks to the study population as there will be no identification or contact with participants. The study will also not benefit the study population included directly. There is a risk that the study will uncover significant differences in outcomes between the different districts, and this may suggest variations in testing strategy as well as in the quality of service delivery. Results will be reported with sensitivity and in a non- accusatory manner. Any service delivery issues identified will be communicated to the relevant health services once the study is concluded. It is hoped that the findings will lead to improvements in delivery of antenatal services if indicated, and prioritisation of COVID-19 vaccination for pregnant and breast-feeding women in the future.

5.2 Informed consent process

Informed consent from patients is required for use of named data. However, patients do not explicitly consent to their data being included in the PHDC database [31]. Anonymised data will be provided for this study by the PHDC therefore we will apply for a waiver of individual informed consent. The rationale for this is as follows:

- The research is observational, part of standard of care, and involves no additional risk.
- Every effort will be made to protect the privacy and confidentiality of the women and newborns enrolled in the project as described below.
- The waiver does not adversely affect the rights and welfare of the women concerned, but rather, may plausibly improve the quality of care provided to pregnant women and newborns

- Obtaining retrospective individual consent from all women would not be possible given the size of the cohort
- This project represents a provincial and potentially national evaluation of health outcomes and services for a particularly vulnerable population of pregnant women and neonates. The findings of such surveillance would provide the provincial and national Department of Health with valuable information and insight that can be used to mitigate poor pregnancy outcomes.

5.3 Privacy and confidentiality

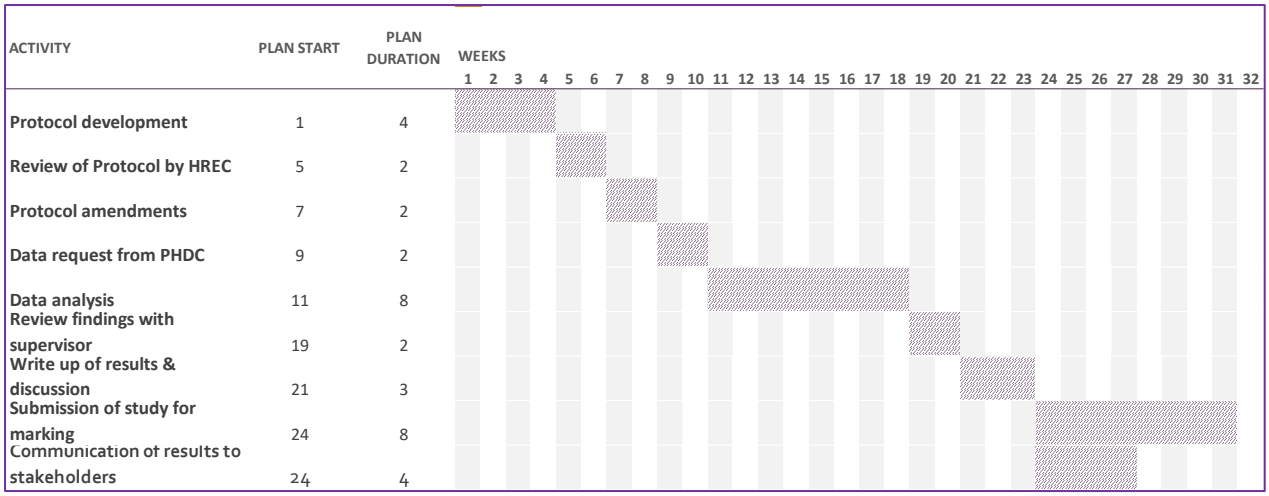
The PHDC operates under strict governance procedures and is fully adherence to South African legislation and Provincial Department of Health rules [31]. Datasets are encrypted and password-protected to limit access to information. All necessary data linkage will be conducted within the PHDC, falling within the ethical and legal assurances of the Western Cape Provincial Government Department of Health. No patient identifiers will be included in the datasets shared with the researchers and the risk of breach of confidentiality is low.

5.4 Communication with stakeholders

The results of the study will be communicated to the NDOH, WCDOH and the Maternal and Child Health (MCH) Primary-care Clinical Governance Committee (PCGC) in the Western Cape, which includes clinicians providing services at tertiary hospitals and primary health facilities.

6. Resources and Logistics

No additional resources are required. The protocol will be submitted to UCT Health Research Ethics Committee. Once approval is obtained, data will be requested from the PHDC. A lead-time of 2-4 weeks is envisaged. Once the data is received, analysis will be done. The analysis is expected to be completed within 2 months. The findings will be reviewed before the final write-up of results, discussion and recommendations. The mini-thesis will then be submitted for marking. The results of the study will be communicated to the relevant stakeholders.



Note: Deviations from protocol in final dissertation are as follow-

Data related to delivery methods was >60% incompletes therefore this variable (including caesarean section) was excluded. Inclusion of Outcome districts in the primary analysis was attempted, however results were subject to substantial information bias and the variable interactions with C-19 waves were difficult to interpret. Therefore, this variable was also excluded in the final analysis.

References

1. Booth A, Reed AB, Ponzo S, Yassaee A, Aral M, Plans D, et al. Population risk factors for severe disease and mortality in COVID-19: A global systematic review and meta-analysis. PLOS ONE 2021;16(3):e0247461.

2. Provincial Health Data Centre Western Cape. Daily COVID-19 Daily Summary Report. September 2022.

3. Coronavirus Resource Centre. Johns Hopkins University. https://coronavirus.jhu.edu/data/mortality . September 2022.

4. Western Cape Report on Maternal Mortality 2020- preliminary findings.

5. Villalaín C, Herraiz I, Luczkowiak J, Pérez-Rivilla A, Folgueira MD, Mejía I, et al. Seroprevalence analysis of SARS-CoV-2 in pregnant women along the first pandemic outbreak and perinatal outcome. PLOS ONE 2020;15(11):e0243029.

6. Pollán M, Pérez-Gómez B, Pastor-Barriuso R, Oteo J, Pérez-Olmeda M, Sanmartín J,L., et al. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. Lancet 2020;396(10250):535-544.

7. Molteni E, Astley CM, Ma W, Sudre CH, Magee LA, Murray B, et al. Symptoms and syndromes associated with SARS-CoV-2 infection and severity in pregnant women from two community cohorts. SCI REP-UK 2021;11(1):6928.

8. Elshafeey F, Magdi R, Hindi N, Elshebiny M, Farrag N, Mahdy S, et al. A systematic scoping review of COVID-19 during pregnancy and childbirth. Int J Gynecol Obstet 2020; 150: 47–52.

9. Ellington S, Strid P, Tong VT, Woodworth K, Galang RR, Zambrano LD, et al. Characteristics of women of reproductive age with laboratory-confirmed SARS-COV-2 infection by pregnancy status - United States, January 22-June 7, 2020. MMWR-MORBID MORTAL W 2020;69(25):769-775.

10. Knight M, Bunch K, Vousden N, Morris E, Simpson N, Gale C, et al. Characteristics and outcomes of pregnant women admitted to hospital with confirmed SARS-CoV-2 infection in UK: national population based cohort study. BMJ-BRIT MED J 2020;369:m2107.

11. Khalil A, Kalafat E, Benlioglu C, O'Brien P, Morris E, Draycott T, et al. SARS-CoV-2 infection in pregnancy: A systematic review and meta-analysis of clinical features and pregnancy outcomes. ECLINICALMEDICINE 2020;25:100446.

12. Li N, Han L, Peng M, Lv Y, Ouyang Y, Liu K, et al. Maternal and Neonatal Outcomes of Pregnant Women with Coronavirus Disease 2019 (COVID-19) Pneumonia: A Case-Control Study. Clin Infect Dis 2020;71(16):2035-2041.

13. Engjom H, Aabakke AJM, Klungsøyr K, Svanvik T, Äyräs O, Jonasdottir E, et al. COVID-19 in pregnancy—characteristics and outcomes of pregnant women admitted to hospital because of SARS-CoV-2 infection in the Nordic countries. ACTA OBSTET GYN SCAN 2021;100(9):1611-1619.

14. Epelboin S, Labrosse J, De Mouzon J, Fauque P, Gervoise-Boyer M, Levy R, et al. Obstetrical outcomes and maternal morbidities associated with COVID-19 in pregnant women in France: A national retrospective cohort study. PLoS medicine 2021;18(11):e1003857.

15. Wei SQ, Bilodeau-Bertrand M, Liu S, Auger N. The impact of COVID-19 on pregnancy outcomes: a systematic review and meta-analysis. Can Med Assoc J 2021;193(16):E540-E548.

16. Martinez-Perez O, Prats Rodriguez P, Muner Hernandez M, Encinas Pardilla MB, Perez Perez N, Vila Hernandez MR, et al. The association between SARS-CoV-2 infection and preterm delivery: a prospective study with a multivariable analysis. BMC PREGNANCY CHILDB 2021;21(1):273.

17. Vizheh M, Allahdadian M, Muhidin S, Valiani M, Bagheri K, Borandegi F, et al. Impact of COVID-19 Infection on Neonatal Birth Outcomes. J Trop Pediatr 2021;67(5).

18. Wastnedge EAN, Reynolds RM, van Boeckel S,R., Stock SJ, Denison FC, Maybin JA, et al. Pregnancy and COVID-19. Physiol Rev 2021;101(1):303-318.

19. Alberca RW, Pereira NZ, Oliveira, Luanda Mara Da Silva, Gozzi-Silva S, Sato MN. Pregnancy, Viral Infection, and COVID-19. FRONT IMMUNOL 2020;11:1672.

20. Koumoutsea EV, Vivanti AJ, Shehata N, Benachi A, Le Gouez A, Desconclois C, et al. COVID-19 and acute coagulopathy in pregnancy. J THROMB HAEMOST 2020;18(7):1648-1652

21. Bahadur G, Homburg R, Yoong W, Singh C, Bhat M, Kotabagi P, et al. Adverse outcomes in SAR-CoV-2 (COVID-19) and SARS virus related pregnancies with probable vertical transmission. JBRA assisted reproduction; JBRA Assist Reprod 2020;24(3):351-357.

22. Fenizia C, Biasin M, Cetin I, Vergani P, Mileto D, Spinillo A, et al. Analysis of SARS-CoV-2 vertical transmission during pregnancy. NAT COMMUN 2020;11(1):5128.

23. Valdespino-Vázquez M,Y., Helguera-Repetto C,A., León-Juárez M, Villavicencio-Carrisoza O, Flores-Pliego A, Moreno-Verduzco E,R., et al. Fetal and placental infection with SARS-CoV-2 in early pregnancy. J Med Virol 2021;93(7):4480-4487.

24. Townsend R, Chmielewska B, Barratt I, Kalafat E, van der Meulen J, Gurol-Urganci I, et al. Global changes in maternity care provision during the COVID-19 pandemic: A systematic review and meta-analysis. ECLINICALMEDICINE 2021;37:100947.

25. Pineles BL, Alamo IC, Farooq N, Green J, Blackwell SC, Sibai BM, et al. Racial-ethnic disparities and pregnancy outcomes in SARS-CoV-2 infection in a universally-tested cohort in Houston, Texas. Eur J Obstet Gynecol Reprod Biol 2020;254:329-330.

26. Joseph NT, Stanhope KK, Badell ML, Horton JP, Boulet SL, Jamieson DJ. Sociodemographic predictors of SARS-CoV-2 infection in obstetric patients, Georgia, USA. EMERG INFECT DIS 2020;26(11):2786-2789.

27. NICD. COVID-19 SPECIAL PUBLIC HEALTH SURVEILLANCE BULLETIN Vol 18, supplementary issue 8 https://www.nicd.ac.za/wp-content/uploads/2021/03/COVID-19-Special-Public-Health-Surveillance-Bulletin-5-March-2021.pdf

28. Budhram S, Vannevel V, Botha T, Chauke L, Bhoora S, Balie SM, et al. Maternal characteristics and pregnancy outcomes of hospitalized pregnant women with SARS-CoV-2 infection in South Africa: An International Network of Obstetric Survey Systems-based cohort study. Int J Gynecol Obstet. 2021;155:455–465.

29. Davies M, Vundle Z, Paleker M, Isaacs W, Allie T, Dane P, et al. Risk Factors for Coronavirus Disease 2019 (COVID-19) Death in a Population Cohort Study from the Western Cape Province, South Africa. Clinical infectious diseases 2021;73(7):e2005-e2015

30. Benchimol EI, Smeeth L, Guttmann A, Harron K, Hemkens LG, Moher D, et al. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) statement. 2016.

31. Boulle A, Heekes A, Tiffin N, Smith M, Mutemaringa T, Zinyakatira N, et al. Data centre profile: The provincial health data centre of the Western Cape province, South Africa. International journal of population data science; Int J Popul Data Sci 2019;4(2):1143.

Appendix 6: Guidelines for Submission to South African Medical Journal (SAMJ)

Authorship

Named authors must consent to publication. Authorship should be based on:
(i)substantial contribution to conceptualisation, design, analysis and interpretati on of data;
(ii) drafting or critical revision of important scientific content; or
(iii) approval of the version to be published. These conditions must all be met (uniform requirements for manuscripts submitted to biomedical journals; refer to www.icmje.org)

If authors' names are added or deleted after submission of an article, or the order of the names is changed, all authors must agree to this in writing.
Please note that co-authors will be requested to verify their contribution upon submission. Non-verification may lead to delays in the processing of submissions.
Author contributions should be listed/described in the manuscript.

Conflicts of interest

Conflicts of interest can derive from any kind of relationship or association that may influence authors' or reviewers' opinions about the subject matter of a paper. Theexistence of a conflict – whether actual, perceived or potential – does not preclude publication of an article. However, we aim to ensure that, in such cases, readers have all the information they need to enable them to make an informed assessment about a publication's message and conclusions. We require that both authors and reviewers declare all sources of support for their research, any personal or financial relationships (including honoraria, speaking fees, gifts received, etc) with relevant individuals or organisations connected to the topic of the paper, and any association with a product or subject that may constitute a real, perceived or potential conflict of interest. If you are unsure whether a specific relationship constitutes a conflict, please contact the editorial team for advice. If a conflict remains undisclosed and is later brought to the attention of the editorial team, it will be considered a serious issue prompting an investigation with the possibility of retraction.

Research ethics committee approval

Authors must provide evidence of Research Ethics Committee approval of the research where relevant. Ensure the correct, full ethics committee name and reference number is included in the manuscript.
If the study was carried out using data from provincial healthcare facilities, or required active data collection through facility visits or staff interviews, approval should be sought from the relevant provincial authorities. For South African authors, please refer to the guidelines for submission to the National Health ResearchDatabase. Research involving human subjects must be conducted according to the principles outlined in the Declaration of Helsinki. Please refer to the National Department of Health's guideline on Ethics in Health research: principles, processes and structures to ensure that the appropriate requirements for conducting research have been met, and that the HPCSA's General Ethical Guidelines for Health Researchers have been adhered to.

Copyright notice

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Ethnic/race classification

Use of racial or ethnicity classifications in research is fraught with problems. If you choose to use a research design that involves classification of participants based on race or ethnicity, or discuss issues with reference to such classifications, please ensure that you include a detailed rationale for doing so, ensure that the categories you describe are carefully defined, and that socioeconomic, cultural and lifestyle variables that may underlie perceived racial disparities are appropriately controlled for. Please also clearly specify whether race or ethnicity is classified as reported by the patient (self-identifying) or as perceived by the investigators. Please note that is not appropriate to use self-reported or investigator-assigned racial or ethnic categories for genetic studies.

General article format/layout

Accepted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction, which will delay publication.

General:

- Manuscripts must be written in UK English.
- The manuscript must be in Microsoft Word format. Text must be single-spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes).
- Please make your article concise, even if it is below the word limit.
- Qualifications, ***full*** affiliation (department, school/faculty, institution, city, country) and contact details of ALL authors must be provided in the manuscript and in the online submission process.

- Abbreviations should be spelt out when first used and thereafter used consistently, e.g. 'intravenous (IV)' or 'Department of Health (DoH)'.
- Include sections on Acknowledgements, Conflict of Interest, Author Contributions and Funding sources. If none is applicable, please state 'none'.
- Scientific measurements must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dL).
- Litres is denoted with an uppercase L e.g. 'mL' for millilitres).
- Units should be preceded by a space (except for % and ºC), e.g. '40 kg' and '20 cm' but '50%' and '19ºC'.
- Please be sure to insert proper symbols e.g. µ not u for micro, a not a for alpha, b not B for beta, etc.
- Numbers should be written as grouped per thousand-units, i.e. 4 000, 22 160.
- Quotes should be placed in single quotation marks: i.e. The respondent stated: '...'
- Round brackets (parentheses) should be used, as opposed to square brackets, which are reserved for denoting concentrations or insertions in direct quotes.
- If you wish material to be in a box, simply indicate this in the text. You may use the table format –this is the *only* exception. Please DO NOT use fill, format lines and so on.

Research Article

Guideline word limit: 4 000 words

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

Select figures and tables for your paper carefully and sparingly. Use only those figures that provided added value to the paper, over and above what is written in the text. Do not replicate data in tables and in text .

Structured abstract

- This should be 250-400 words, with the following recommended headings:

o **Background:** why the study is being done and how it relates to other published work.
o **Objectives:** what the study intends to find out

o **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.

o **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.

o **Conclusion:** must be supported by the data, include recommendations for further study/actions.

- Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors.
- Do not include any references in the abstracts.

Main article

All articles are to include the following main sections: Introduction/Background, Methods, Results, Discussion, Conclusions.

The following are additional heading or section options that may appear within these:

- Objectives (within Introduction/Background): a clear statement of the main aim of the study and the major hypothesis tested or research question posed
- Design (within Methods): including factors such as prospective, randomisation, blinding, placebo control, case control, crossover, criterion standards for diagnostic tests, etc.
- Setting (within Methods): level of care, e.g. primary, secondary, number of participating centres.
- Participants (instead of patients or subjects; within Methods): numbers entering and completing the study, sex, age and any other biological, behavioural, social or cultural factors (e.g. smoking status, socioeconomic group, educational attainment, co-existing disease indicators, etc)that may have an impact on the study results. Clearly define how participants were enrolled, and describe selection and exclusion criteria.
- Interventions (within Methods): what, how, when and for how long. Typically for randomised controlled trials, crossover trials, and before and after studies.
- Main outcome measures (within Methods): those as planned in the protocol, and those ultimately measured. Explain differences, if any.

Results

- Start with description of the population and sample. Include key characteristics of comparison groups.
- Main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm. Whenever possible, state absolute rather than relative risks.
- Do not replicate data in tables and in text.
- If presenting mean and standard deviations, specify this clearly. Our house style is to present this as follows:
- E.g.: The mean (SD) birth weight was 2 500 (1 210) g. Do not use the ± symbol for mean (SD).
- Leave interpretation to the Discussion section. The Results section should just report the findings as per the Methods section.

Discussion

Please ensure that the discussion is concise and follows this overall structure – sub-headings are not needed:

- Statement of principal findings
- Strengths and weaknesses of the study
- Contribution to the body of knowledge
- Strengths and weaknesses in relation to other studies
- The meaning of the study – e.g. what this study means to clinicians and policymakers
- Unanswered questions and recommendations for future research

Conclusions

This may be the only section readers look at, therefore write it carefully. Include primary conclusions and their implications, suggesting areas for further research if appropriate. Do not go beyond the data in the article.

Illustrations/photos/scans

If illustrations submitted have been published elsewhere, the author(s) should provide consent to republication obtained from the copyright holder.

Figures must be numbered in Arabic numerals and referred to in the text e.g.'(Fig. 1)'.

Each figure must have a caption/legend: Fig. 1. Description (any abbreviations in full).

All images must be of high enough resolution/quality for print.

All illustrations (graphs, diagrams, charts, etc.) must be in PDF or jpeg form.

Ensure all graph axes are labelled appropriately, with a heading/description and units (as necessary) indicated. Do not include decimal places if not necessary e.g. 0; 1.0; 2.0; 3.0; 4.0 etc.

Scans/photos showing a specific feature e.g.

Intermediate magnification micrograph of a low malignant potential (LMP) mucinous ovarian tumour. (HandE stain). –

include an arrow to show the tumour.

Each image must be attached individually as a 'supplementary file' upon submission (not solely embedded in the accompanying manuscript) and named Fig. 1, Fig. 2, etc.

Tables

- Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.
- Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author
- Embed/include each table in the manuscript Word file - do not provide separately as supplementary files.
- Number each table in Arabic numerals (Table 1, Table 2, etc.) and refer to consecutively in the text.
- Tables must be cell-based (i.e. not constructed with text boxes or tabs) and editable.

- Ensure each table has a concise title and column headings, and include units where necessary.
- Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ || then ** †† ‡‡ etc.

References

NB: *Only complete, correctly formatted reference lists in Vancouver style will be accepted. Reference lists must be generated manually and not with the use of reference manager software. Endnotes must* ***not*** *be used.*

- Authors must verify references from original sources.
- Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization,[2] and others.[3,4-6]
- All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).
- Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.
- Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.
- Volume and issue numbers should be given.
- First and last page, in full, should be given e.g.: 1215-1217 **not** 1215-17.
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