# Multiple Gestation as a Risk Factor for SARS-CoV-2-Associated Adverse Maternal Outcome: Data From the COVID-19 Related Obstetric and Neonatal Outcome Study (CRONOS)

## Mehrlingsschwangerschaft ist ein Risikofaktor für SARS-CoV-2-bedingtes ungünstiges mütterliches Outcome: Daten von der COVID-19 Related Obstetric and Neonatal Outcome Study (CRONOS)

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**Authors**

Marina Sourouni[1,2], Ariane Germeyer[1], Manuel Feißt[3], Alexandra Balzer[3], Helen Köster[4], Annemarie Minte[5], Dörthe Brüggmann[6], Celine Kohll[7], Kristin Reinhardt[8], Michaela Möginger[9], Anja Leonhardt[10], Constanze Banz-Jansen[11], Michael Bohlmann[12], Christiane Fröhlich[13], Clara Backes[14], Dietrich Hager[15], Lisa Kaup[16], Elsa Hollatz-Galuschki[17], Charlotte Engelbrecht[18], Filiz Markfeld-Erol[19], Carsten Hagenbeck[20], Hening Schäffler[21], Jennifer Winkler[22], Johannes Stubert[23], Katharina Rathberger[24], Laura Lüber[25], Linda Hertlein[26], Antonia Machill[27], Manuela Richter[28], Martin Berghäuser[29], Michael Weigel[30], Mirjam Morgen[31], Nora Horn[32], Peter Jakubowski[33], Bastian Riebe[34], Babett Ramsauer[35], Robert Sczesny[36], Ute Margaretha Schäfer-Graf[37], Susanne Schrey[38], Sven Kehl[39], Julia Lastinger[40], Sven Seeger[41], Olaf Parchmann[42], Antonella Iannaccone[43], Jens Rohne[44], Luise Gattung[45], Christine A. Morfeld[46], Michael Abou-Dakn[47], Markus Schmidt[48], Michaela Glöckner[49], Anja Jebens[50], Kathleen Marie Sondern[2], Ulrich Pecks[51], Ralf Schmitz[2], Mareike Möllers[2]

**Affiliations**

1  Gynäkologische Endokrinologie und Fertilitätsstörungen, University Hospital Heidelberg, Heidelberg, Germany
2  Gynecology and Obstetrics, University Hospital Münster, Münster, Germany
3  Universitätsklinikum Heidelberg, Institut für Medizinische Biometrie, Heidelberg, Germany
4  Gynecological practice, Berlin, Germany
5  Department of Obstetrics and Gynecology, Cristophorus Hospital, Coesfeld, Germany
6  Klinik für Frauenheilkunde und Geburtshilfe, Universitätsklinikum Frankfurt, Frankfurt am Main, Germany
7  Gynäkologie und Geburtshilfe, Klinikum rechts der Isar der Technischen Universität München, München, Germany
8  Geburtshilfe und Pränatalmedizin, Universitätsklinikum Halle, Halle, Germany
9  Klinik für Gynäkologie und Geburtshilfe, Universitätsklinikum Giessen und Marburg, Standort Marburg, Marburg, Germany
10  Klinik für Frauenheilkunde und Geburtshilfe, Klinikum Chemnitz gGmbH, Chemnitz, Germany
11  Gynäkologie und Geburtshilfe, Evangelisches Klinikum Bethel gGmbH, Bielefeld, Germany
12  Zentrum für Gynäkologie und Geburtshilfe, St. Elisabethen-Krankenhaus Lörrach gGmbH, Lörrach, Germany
13  Klinik für Gynäkologie und Geburtshilfe, Klinikum Rheine, Rheine, Germany
14  Klinik für Gynäkologie und Geburtshilfe, Schön Hospital Munich Harlaching, München, Germany
15  Klinik für Gynäkologie und Geburtshilfe, Thüringen-Kliniken Saalfeld-Rudolstadt, Thüringen, Germany
16  Dr. Geisenhofer Clinic for Gynecology and Obstetrics, München, Germany
17  Abteilung für Geburtshilfe und Pränatalmedizin, Klinik Hallerwiese, Nürnberg, Germany
18  Abteilung für Geburtshilfe, Charité Universitätsmedizin Berlin, Berlin, Germany
19  Frauenklinik, Universität Freiburg, Freiburg, Germany
20  Department of Gynecology and Obstetrics, University Hospital Düsseldorf, Heinrich Heine Universität Düsseldorf, Düsseldorf, Germany
21  Klinik für Gynäkologie und Geburtshilfe, Ulm University Hospital, Ulm, Germany
22  Klinik und Poliklinik für Gynäkologie und Geburtshilfe, University Hospital Carl Gustav Carus, Dresden, Germany
23  Dept. of Gynecology and Obstetrics, University of Rostock, Rostock, Germany
24  Klinik für Frauenheilkunde und Geburtshilfe, Hospital St. Hedwig of the Order of St. John, Regensburg, Germany
25  Klinik für Gynäkologie und Geburtshilfe, Oberschwaben Hospital Group, Ravensburg, Germany
26  Klinik und Poliklinik für Frauenheilkunde und Geburtshilfe, LMU, München, Germany
27  Department of Obstetrics and Gynecology, Vinzenz Pallotti Hospital, Bensberg, Germany

28 Neonatologie, Kinderkrankenhaus auf der Bult, Hannover, Germany

29 Klinik für Kinderheilkunde, Florence-Nightingale-Krankenhaus, Düsseldorf, Germany

30 Frauenklinik, Leopoldina-Krankenhaus der Stadt Schweinfurt GmbH, Schweinfurt, Germany

31 Klinik für Gynäkologie und Geburtshilfe, St. Vincenz-Krankenhaus, Datteln, Germany

32 Klinik für Gynäkologie und Geburtshilfe, Sana Klinikum Lichtenberg, Berlin, Germany

33 Department of Gynecology and Obstetrics, University of Tübingen, Tübingen, Germany

34 Klinik für Gynäkologie und Geburtshilfe, Klinikum Links der Weser gGmbH, Bremen, Germany

35 Klinik für Geburtsmedizin, Vivantes Klinikum Neukölln, Berlin, Germany

36 Klinik für Gynäkologie und Geburtshilfe, Marienhaus Hospital Saint Elisabeth Neuwied, Neuwied, Germany

37 Perinatalzentrum, St. Joseph Hospital Berlin, Berlin, Germany

38 Abteilung für Geburtsmedizin, Uniklinik Leipzig, Leipzig, Germany

39 Gynecology & Obstetrics, Universitätsklinikum Erlangen, Erlangen, Germany

40 Gynecology, Obstetrics and Gynecological Endocrinology, Kepler University Hospital, Linz, Austria

41 Klinik für Geburtshilfe, Krankenhaus St. Elisabeth und St. Barbara Halle, Halle, Germany

42 Klinik für Gynäkologie und Geburtshilfe, HELIOS Hospital Sangerhausen, Sangerhausen, Germany

43 Klinik für Gynäkologie und Geburtshilfe, University Hospital Essen, Essen, Germany

44 Klinik für Gynäkologie und Geburtshilfe, Maria Heimsuchung Caritas Klinik Pankow, Berlin, Germany

45 Klinik für Gynäkologie und Geburtshilfe, Bad Salzungen Hospital, Bad Salzungen, Germany

46 Frauenklinik, DIAKOVERE gGmbH, Hannover, Germany

47 Klinik für Gynäkologie, St. Joseph Krankenhaus Berlin-Tempelhof, Berlin, Germany

48 Gynecology & Obstetrics, Sana Kliniken Duisburg GmbH, Duisburg, Germany

49 Abteilung für Gynäkologie und Geburtshilfe, Hospital Memmingen, Memmingen, Germany

50 Abteilung für Gynäkologie und Geburtshilfe, Vivantes Klinikum im Friedrichshain, Berlin, Germany

51 Frauenklinik, UKSH, Kiel, Germany

## Correspondence

Dr. Marina Sourouni
Gynäkologische Endokrinologie und Fertilitätsstörungen
University Hospital Heidelberg
Im Neuenheimer Feld 440
69120 Heidelberg, Germany
marina.sourouni@med.uni-heidelberg.de
msourouni@gmail.com

Additional material is available at https://doi.org/10.1055/a-2196-6224.

## ABSTRACT

### Introduction

Studies have shown that pregnant women with COVID-19 have a higher risk of intensive care unit admission and invasive mechanical ventilation support than non-pregnant women. Pregnancy-associated physiological changes in respiratory function may contribute to the elevated risk. Alteration in lung volumes and capacities are attributed to the mechanical impediment caused by the growing fetus. Multiple pregnancies may therefore compromise functional lung capacity earlier than singleton pregnancies and contribute to severe respiratory symptoms of COVID-19.

### Materials and Methods

A total of 5514 women with a symptomatic SARS-CoV-2 infection during pregnancy registered in the COVID-19 Related Obstetric and Neonatal Outcome Study were included. The COVID-19-related adverse maternal outcomes were compared in 165 multiple versus 5349 singleton pregnancies. Combined adverse maternal outcome was defined as presence of COVID-19-related hospitalization and/or pneumonia and/or oxygen administration and/or transfer to ICU and/or death. Multivariate logistic regression was used to estimate the odds ratios and 95% confidence intervals were calculated.

### Results

The frequency of dyspnea, likelihood of developing dyspnea in a defined pregnancy week and duration of the symptomatic phase of the COVID-19 infection did not differ between the two groups. On average, COVID-19-related combined adverse outcome occurred earlier during pregnancy

in women expecting more than one child than in singleton pregnancies. The overall incidence of singular and combined COVID-19-associated adverse maternal outcomes was not significantly different between groups. However, regression analysis revealed that multiple gestation, preconceptional BMI > 30 kg/m$^2$ and gestational age correlated significantly with an increased risk of combined adverse maternal outcome. Conversely, maternal age and medically assisted reproduction were not significant risk factors for combined adverse maternal outcome.

### Conclusion

Our data show that multiple gestation alone is a risk factor for COVID-19-associated combined adverse maternal outcome. Moreover, severe courses of COVID-19 in women expecting more than one child are observed earlier in pregnancy than in singleton pregnancies.

### ZUSAMMENFASSUNG

#### Einleitung

Studien haben gezeigt, dass schwangere Frauen mit COVID-19 ein höheres Risiko für eine Aufnahme in die Intensivstation und invasive mechanische Beatmungsunterstützung haben, verglichen mit nicht schwangeren Frauen. Schwangerschaftsbedingte physiologische Änderungen der Atemfunktion tragen möglicherweise zu diesem höheren Risiko bei. Änderungen des Lungenvolumens und der Lungenkapazität werden der durch das fötale Wachstum verursachten mechanischen Behinderung zugeschrieben. Es ist daher vorstellbar, dass Mehrlingsschwangerschaften die funktionelle Lungenkapazität früher beeinträchtigen als Einlingsschwangerschaften und dass sie schwerwiegende Atemwegsbeschwerden bei COVID-19 verschärfen.

#### Material und Methoden

Insgesamt wurden 5514 Frauen mit symptomatischer SARS-CoV-2-Infektion während der Schwangerschaft, die in der COVID-19 Related Obstetric and Neonatal Outcome Study (CRONOS) registriert waren, in die Studie eingeschlossen. Es wurden die COVID-19-bedingten ungünstigen mütterlichen Outcomes bei 165 Mehrlings- und 5349 Einlingsschwangerschaften verglichen. Ein kombiniertes ungünstiges mütterliches Outcome wurde definiert als eine COVID-19-bedingte Krankenhausaufnahme und/oder eine Lungenentzündung und/oder eine Sauerstoffgabe und/oder eine Verlegung auf die Intensivstation und/oder Tod. Multivariate logistische Regression wurde zur Schätzung des Odds Ratios eingesetzt, und die 95%-Konfidenzintervalle wurden berechnet.

#### Ergebnisse

Es gab keine Unterschiede zwischen den 2 Gruppen hinsichtlich der Häufigkeit von Dyspnoe, der Wahrscheinlichkeit, Dyspnoe in einer bestimmten Schwangerschaftswoche zu entwickeln, oder der Dauer der symptomatischen Phase der COVID-19-Infektion. Im Schnitt trat ein COVID-19-bedingtes kombiniertes ungünstiges Outcome zu einem früheren Zeitpunkt der Schwangerschaft ein bei Frauen, die mehr als ein Kind erwarteten, verglichen mit Einlingsschwangerschaften. Insgesamt unterschied sich die Häufigkeit von einzelnen und kombinierten COVID-19-bedingten ungünstigen mütterlichen Outcomes nicht signifikant zwischen den Gruppen. Die Regressionsanalyse zeigte aber, dass Mehrlingsschwangerschaft, ein präkonzeptioneller BMI > 30 kg/m$^2$ und das Schwangerschaftsalter signifikant mit einem erhöhten Risiko für kombinierte ungünstige mütterliche Outcomes korrelierten. Dagegen waren mütterliches Alter und medizinisch unterstützte Fortpflanzung keine signifikanten Risikofaktoren für kombinierte ungünstige mütterliche Outcomes.

#### Schlussfolgerung

Unsere Daten zeigen, dass eine Mehrlingsschwangerschaft an sich bereits einen Risikofaktor für COVID-19-bedingte kombinierte ungünstige mütterliche Outcomes darstellt. Dazu kommt noch, dass ein schwerer Verlauf der COVID-19-Erkrankung früher in der Schwangerschaft auftritt bei Frauen, die mehr als ein Kind erwarten, als bei Einlingsschwangerschaften.

## Introduction

During the COVID-19 pandemic, concerns arose as to whether infections with SARS-CoV-2 adversely affect pregnancy outcomes. Indeed, observational cohort studies report that pregnant women infected with SARS-CoV-2 are more likely to be admitted to an intensive care unit (ICU) or need invasive ventilation than non-pregnant women [1]. To reduce risk of infection it has been suggested to wear face masks and to avoid crowds [2, 3, 4]. Data from the COVID-19 Related Obstetric and Neonatal Outcome Study (CRONOS) registry in Germany, which prospectively enrolled women with confirmed SARS-CoV-2 infection during their pregnancy, confirmed a high rate of severe COVID-19 cases requiring intensive care, mainly for respiratory support. The incidence correlated positively with increased gestational age at time of infection: amongst symptomatic women the risk of transfer to an ICU increased from < 0.3% in the first trimester to approximately 7% in the early third trimester, with the highest risk at about 30 weeks of gestation [5, 6].

Physiological respiratory system changes during pregnancy occur on every level and include

a) mucosal hyperemia and edema in nasopharynx, larynx, trachea and bronchi,
b) lower carbon dioxide response threshold of the respiratory center,
c) displacement of the diaphragm by the enlarged uterus and
d) expansion of the ribcage and increase of the subcostal angle, resulting in a decrease of functional residual capacity (FRC) by 20% to 30% [4].

The total lung capacity (TLC) is maintained to meet the increased demand for oxygen (by 20%) with an unchanged (or only slightly increased) respiratory rate, a higher tidal volume, and a steep increase in minute ventilation already in the first trimester [7]. Existing literature shows that mouth occlusion pressure increases progressively during pregnancy and falls significantly after delivery, indicating that effective respiratory impedance increases during pregnancy [8]. Staub et al. have shown that the symphysis-fundal height in twin pregnancies is substantially greater than in singleton pregnancies [9]. Therefore, multiple pregnancy could be a risk factor for early manifestation of adverse maternal outcome after SARS-CoV-2 infection in pregnancy.

In order to better counsel pregnant women in situations of increased risk for respiratory viral infection or COVID-19, we aimed to analyze COVID-19-specific outcome in women with multiple pregnancies. We hypothesized that women with multiple pregnancies are susceptible for severe COVID-19 earlier in gestation than singleton pregnancies, due to the earlier and more evident displacement of the diaphragm during pregnancy.

## Material and Methods

### Study design and setting

CRONOS is a multicentric, prospective, monitored observational study established by the German Society of Perinatal Medicine (DGPM) in April 2020 to rapidly provide data to counsel women with SARS-CoV-2 infection during pregnancy. The method is in detail described previously [10]. Information on the study is available at www.dgpm-online.org and the German Clinical Trials Register (DRKS00021208). Methodology and study results have been published recently [6, 11, 12, 13, 14, 15, 16, 17]. Approval of the Institutional Ethics Board was obtained for the study (University Hospital Schleswig-Holstein in Kiel, file number D 451/20) and for each study site separately. Women with confirmed SARS-CoV-2 infection any time during their pregnancy were eligible for inclusion in the CRONOS registry.

All German maternity hospitals were invited to participate in the CRONOS registry. The registry collected data between April 3rd, 2020 and February 10th, 2023 with 130 of 686 (18.9%) German obstetric hospitals and the Kepler University Hospital in Linz, Austria, actively participating. These maternity units attended approximately 250 000 deliveries per year, accounting for ⅓ of the births in Germany. CRONOS aimed at a complete recruitment of all eligible women.

### Data capture and study variables

A reporting form was developed for collecting data, initially using the cloud-based electronic data capture platform of the service provider castoredc.com (Amsterdam, Netherlands). The database was updated in December 2020 to include further data, e.g. vaccination status. Informed consent was either obtained in the antepartum period or waived in the postpartum period if COVID-19 had occurred in the current pregnancy. After the patients had given informed consent, information on the demographic characteristics, comorbidities, previous and current pregnancy characteristics, SARS-CoV-2-specific symptoms and treatments, pregnancy- and birth-specific events and neonatal outcomes were entered by each participating hospital in the data capture platform [6]. Primary endpoint of the study was the COVID-19-related combined adverse maternal outcome, which was defined as presence of COVID-19-related hospitalization and/or pneumonia and/or oxygen administration and/or transfer to ICU and/or death. The above-mentioned adverse outcomes were also assessed separately as secondary endpoints. Furthermore, prevalence of COVID-associated dyspnea among women with a symptomatic SARS-CoV-2 infection was assessed in the two groups, as a manifest symptom associated with respiratory tract infections.

### Statistical analysis

A multi-step analysis was performed in order to evaluate whether multiple pregnancy constituted a greater risk for COVID-19-related adverse maternal outcomes than singleton pregnancy. First, baseline data were analyzed to identify statistically significant differences in baseline characteristics between the two groups (▶ Table 1). Categorical variables were presented as absolute and relative frequencies, continuous baseline variables are shown as mean and standard deviation for each group. Statistical significance was tested for categorical baseline variables using the chi-square test, for continuous variables with Pearson's chi-squared test.

In a second step, it was evaluated whether COVID-19-associated symptoms and COVID-associated maternal outcome differed significantly between singleton and multiple pregnancies (▶ Table 2, Supplementary Table S2). Statistical significance for categorical variables was tested using Pearson's chi-squared test. In addition, P values, risk estimators (odds ratios [OR]) and corresponding 95% confidence intervals (95% CI) were calculated for the comparison of maternal outcomes between the two groups.

Thirdly, a multivariate regression analysis was conducted for COVID-19-associated combined adverse maternal outcome (▶ Table 3). The baseline variables which differed significantly between groups (maternal age, IVF, gestational age) or are known as potential risk factors for adverse outcome (BMI > 30 kg/m², maternal comorbidities, multiple gestation, smoking during pregnancy) were included into the model. SARS-CoV-2-vaccination status was not integrated in the logistic regression, due to great variability within the vaccinated population (vaccination before or during pregnancy, number and type of vaccinations, time since last vaccination). Virus variants were assumed in each case from date of infection and postal code, based on the given dominant virus variant according to the national institute for disease control, the Robert

▶**Table 1** Baseline and pregnancy characteristics of the study participants.

| Parameter | Multiple pregnancies (n = 165) | Singleton pregnancies (n = 5349) | P value |
|---|---|---|---|
| Maternal age (years) | 32 ± 4.6 | 31 ± 5.3 | 0.005[tt2] |
| Smoking (during pregnancy) | 5/161 (3%) | 209/5244 (4%) | 0.57[chi2] |
| BMI (kg/m$^2$) | 26 ± 5.3 (n = 155) | 26 ± 5.6 (n = 5014) | 0.05[tt2] |
| ART in this pregnancy | 47/143 (43%) | 174/4861 (4%) | |
| Maternal comorbidities (any) | 65 (39%) | 1919 (36%) | < 0.001[chi2] |
| ▪ Cardiovascular comorbidities | 6/163 (4%) | 218/5295 (4%) | 0.35[chi2] |
| ▪ Diabetes mellitus (preexisting) | 0/163 (0%) | 54/5295 (1%) | 0.78[chi2] |
| ▪ Pulmonary comorbidities | 5/163 (3%) | 181/5295 (3%) | 0.20[chi2] |
| Coagulation disorder or state after thrombosis | 1/163 (1%) | 126/5295 (2%) | 0.81[chi2] |
| Vaccinated against SARS-CoV-2 | 59/154 (38%) | 1550/5030 (31%) | 0.14[chi2] |
| Gestational age (week) at onset of COVID-19 symptoms | 25 ± 8.8 | 27 ± 9.7 | |
| ▪ 1$^{st}$ Trimester | 18 (11%) | 545 (10%) | 0.05[chi2] |
| ▪ 2$^{nd}$ Trimester | 77 (47%) | 1933 (36%) | 0.002[tt2] |
| ▪ 3$^{rd}$ Trimester | 70 (42%) | 2871 (54%) | 0.01[chi2] |

ART = assisted reproductive technology; BMI = body mass index
Data are shown as mean ± standard deviation or absolute/relative frequencies (percentage).
tt2 = Welch's two-sample t-test; chi2 = Pearson's chi-squared test

▶**Table 2** COVID-19-associated maternal outcome in singleton and multiple pregnancies.

| COVID-19-associated outcomes | Multiple pregnancies (n = 165) | Singleton pregnancies (n = 5349) | OR | 95% CI | P value |
|---|---|---|---|---|---|
| COVID-19-associated need for inpatient treatment | 31 (19%) | 748 (14%) | 1.42 | [0.96; 2.12] | 0.08[chi2] |
| Pneumonia | 8/161 (5%) | 260/5294 (5%) | 1.01 | [0.49; 2.08] | 0.97[chi2] |
| Oxygen supplementation | 8/164 (5%) | 328/5339 (6%) | 0.79 | [0.38; 1.60] | 0.51[chi2] |
| ICU admission | 8/164 (5%) | 195/5336 (4%) | 1.36 | [0.65; 2.79] | 0.41[chi2] |
| Mortality | 1/164 (1%) | 9/5332 (0%) | 3.62 | [0.46; 28.81] | 0.19[chi2] |
| Combined outcome* | 31 (19%) | 748 (14%) | 1.42 | [0.96; 2.12] | 0.08[chi2] |
| COVID-19-associated indication for caesarean section | 4/151 (3%) | 140/4733 (3%) | 0.80 | [0.33; 2.44] | 0.83[chi2] |

Data are shown as absolute/relative frequencies (percentage).
chi2 = Pearson's chi-squared test; ICU = intensive care unit
* The combined endpoint is composed of the following: COVID-19-associated need for inpatient treatment and/or pneumonia and/or oxygen supplementation and/or ICU admission and/or mortality.

Koch-Institute (RKI). However, this variable was also not integrated in the logistic regression due to partially small numbers within the subgroups (see **Supplementary Table S1**). Statistical analysis was performed using the R software version 4.2.2. All inferential statistics are intended to be exploratory (hypothesis generating), not confirmatory and are interpreted accordingly. Therefore, no power calculation was performed. The p values were considered to be statistically significant if p < 0.05.

▶**Table 3** Multivariate logistic regression analysis results for combined maternal outcome of women with COVID-19 during pregnancy.

| Combined Outcome | Level | OR | 95% CI | P value |
|---|---|---|---|---|
| Multiple gestation | present | 1.75 | [1.05; 2.80] | 0.02 |
| BMI > 30 (before pregnancy) | present | 1.52 | [1.23; 1.88] | < 0.001 |
| Gestational age in weeks | | 1.05 | [1.03; 1.06] | < 0.001 |
| Maternal age > 35 | present | 0.89 | [0.72; 1.09] | 0.26 |
| Maternal comorbidities | present | 0.98 | [0.81; 1.18] | 0.83 |
| Mode of conception | ART | 0.96 | [0.61; 1.46] | 0.86 |
| Smoking (during pregnancy) | present | 0.57 | [0.32; 0.94] | 0.04 |

ART = assisted reproductive technology; BMI = body mass index; CI = confidence interval; OR = odds ratio
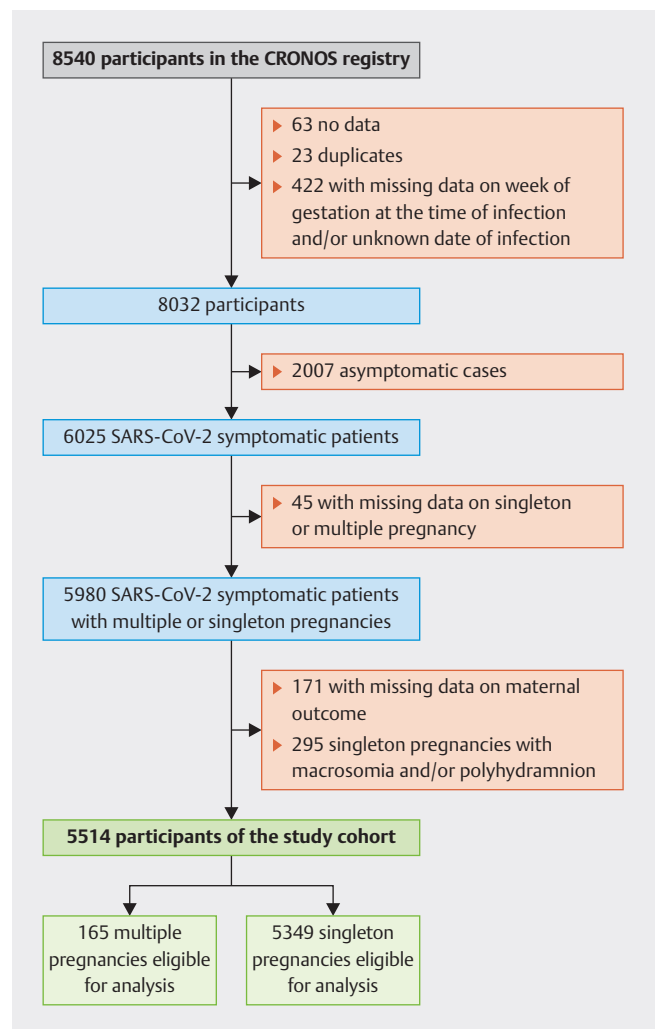
## Results

### Study population

By February 2023 in total 8540 women were registered. During the review of the registry and the plausibility check a total of 508 cases were excluded, consisting of 63 cases with no data, 23 confirmed duplicates, and 422 cases in which mandatory information was missing, either the week of gestation at the time of infection and/or the date of infection.

Of the remaining 8032 women with confirmed SARS-CoV-2 infection during pregnancy 6025 (75%) were symptomatic and 2007 (25%) asymptomatic. We excluded asymptomatic cases, cases in which data on singleton or multiple pregnancy was not provided (n = 45) and cases missing the date of COVID-related maternal outcome (n = 171). Singleton pregnancies with increased intra-abdominal pressure due to macrosomia (fetal weight greater than or equal to the 90 th percentile for gestational age) and/or polyhydramnios (n = 295) were also excluded (▶ **Fig. 1**).

The final study cohort consisted of 5514 patients with symptomatic SARS-CoV-2 infection, valid information on whether or not the patient was expecting multiple pregnancy, and on COVID-related maternal outcome. SARS-CoV-2 infection was confirmed via viral RNA detection with polymerase chain reaction testing in 4842 cases (88%), detection of maternal SARS-CoV-2 antibodies in 38 cases (1%) and antigen testing in 293 cases (5%). In 341 cases (6%) no information on the diagnostic test used to confirm SARS-CoV-2 infection was available.

The baseline maternal demographic and clinical characteristics of the final cohort of 5514 women with symptomatic SARS-CoV-2 infection during pregnancy, consisting of 165 women with multiple (162 twin and 3 triplet pregnancies) and 5349 with singleton pregnancies are presented in ▶ **Table 1**. Overall, women expecting more than one child were significantly older, achieved pregnancy more often after assisted reproductive technology (ART) and were infected earlier in pregnancy (p < 0.05). Around a quarter of women with a symptomatic COVID-19 infection developed dyspnea, the difference between multiple and singleton pregnancies was not significant (n = 38/157, 24% vs. n = 1248/5043, 25%, respectively, p = 0.877). The duration of the symptomatic phase, re-

**8540 participants in the CRONOS registry**

- ▶ 63 no data
- ▶ 23 duplicates
- ▶ 422 with missing data on week of gestation at the time of infection and/or unknown date of infection

**8032 participants**

- ▶ 2007 asymptomatic cases

**6025 SARS-CoV-2 symptomatic patients**

- ▶ 45 with missing data on singleton or multiple pregnancy

**5980 SARS-CoV-2 symptomatic patients with multiple or singleton pregnancies**

- ▶ 171 with missing data on maternal outcome
- ▶ 295 singleton pregnancies with macrosomia and/or polyhydramnion

**5514 participants of the study cohort**

**165 multiple pregnancies eligible for analysis**  **5349 singleton pregnancies eligible for analysis**

▶ **Fig. 1** Flow diagram describing patient inclusion.

ported by the patient, did not differ between the two groups (p = 0.594) and the vast majority (85% and 84%, respectively) had symptoms for less than 14 days (**Supplementary Table S2**).

## COVID-19-associated symptoms and adverse maternal outcomes in multiple vs. singleton pregnancies

The COVID-19-associated adverse maternal outcomes, including need for hospitalization, pneumonia, oxygen supplementation, transfer to intensive care unit and death, were not significantly different among the two groups (▶ **Table 3**). Also, the incidence of combined adverse maternal outcome was comparable between the two groups (n = 31, 19% vs. n = 748, 14%, p = 0.081, ▶ **Table 3**). Interestingly, although planned or emergency cesarean sections were more common in multiple pregnancies (70% vs. 34%, p < 0.001), the frequency of COVID-19 as indication for a cesarean section was identical, with 3% in either group (p = 0.825, OR 0.89 [0.33–2.44]).

A case was considered as symptomatic, if the patient reported experiencing one or more of the following symptoms in association to the COVID-19 infection: fever > 38 °C, cough, diarrhea, dyspnea, myalgia, fatigue, sore throat, general feeling of illness/chills, nasal respiratory obstruction, thoracic pain/chest pain, headache, dizziness or lightheadedness, altered sense of taste or smell, productive ejection/mucus, nausea or vomiting, or other (with the option to specify symptoms in free text). Patients were explicitly asked to report symptoms in temporal relation to their COVID-19 infection, in order to distinguish between dyspnea related to COVID-19 and breathing discomfort associated with pregnancy. The probability of COVID-19-related dyspnea or combined adverse maternal outcome among women with a symptomatic SARS-CoV-2 infection in a defined pregnancy week is depicted in ▶ **Fig. 2** and ▶ **Fig. 3**. Although there was no difference in the timing or frequency of dyspnea (mean gestational age 24.1 vs. 26.8 weeks, p = 0.12, ▶ **Fig. 2**), COVID-related combined adverse maternal outcome occurred significantly earlier in women with multiple pregnancies (mean gestational age 24.1 vs. 30.2 weeks, p = 0.001, ▶ **Fig. 3**).

Although the incidence of combined adverse maternal outcome was not significantly different in the two groups, multivariate logistic regression analysis showed that multiple gestation contributes significantly to the increased risk for combined adverse maternal outcome (OR 1.75; 95% CI 1.05–2.80; p = 0.02). Preconceptional BMI > 30 kg/m$^2$ (OR 1.52; 95% CI 1.23–1.88; p < 0.001) and gestational age (OR 1.05; 95% CI 1.03–1.06; p < 0.001) were also shown to be statistically significant predictors of combined adverse outcome. On the other hand, maternal comorbidities, maternal age > 35 years and ART were not significant risk factors (▶ **Table 3**). Multivariate logistic regression analysis was also conducted separately for the two groups, confirming preconceptional BMI > 30 kg/m$^2$ and higher gestational age as significant predictors for combined adverse maternal outcome in singleton pregnancies (**Supplementary Table S3**). However, none of these factors were shown to be significantly predictive in multiple pregnancies (**Supplementary Table S3**).

## Discussion

COVID-19-associated maternal outcomes (need for inpatient treatment, pneumonia, oxygen supplementation, ICU admission and maternal death), combined adverse maternal outcomes, prevalence of dyspnea and duration of the symptomatic phase of COVID-19 infection were descriptively comparable between multiple and singleton pregnancies. However, on average the combined adverse maternal outcome occurred earlier in multiple than in singleton pregnancies, possibly because pronounced uterine distension and elevation of the diaphragm result in superficial ventilation of the lungs. This may be observed especially at the end of the second trimester with increase of uterine volume and elevation of the uterine fundus beyond the level of the navel. Existing literature shows that respiratory impedance increases during pregnancy and that the symphysis-fundal height in twin pregnancies is substantially greater than in singleton pregnancies [8, 9]. Multivariate logistic regression revealed that multiple pregnancy, preconceptional BMI > 30 kg/m$^2$ and higher gestational age at time of infection are risk factors for combined adverse maternal COVID-19-specific outcome. Within the group of multiple pregnancies, no single variable was shown to be predictive.

Several cohort studies report an association between COVID-19 in pregnancy and substantially increased maternal and neonatal morbidity and mortality, compared to pregnant women without a diagnosis of COVID-19 [18]. Physiological pregnancy-associated changes, such as an altered immune response, an angiotensin-converting enzyme 2 (ACE2) receptor expression in the placenta and physiological changes in the respiratory and cardiovascular system during pregnancy are aspects that may potentially lead to a more severe course of SARS-CoV-2 infection in pregnant than in non-pregnant women [19]. However, insight into the maternal outcome of multiple pregnancies after COVID-19 is lacking. Changes in the respiratory system during pregnancy include the displacement of the diaphragm by the enlarged uterus. We hypothesized that alteration in lung volumes and capacities attributed to the greater mechanical impediment caused by the growth of multiple fetuses can lead to an earlier onset and/or to a worse maternal outcome after SARS-CoV-2 infection in pregnancy. Our findings support this hypothesis, as multiple gestation was proven to be a risk factor for combined adverse maternal outcome, and women expecting more than one child experienced COVID-19-related combined outcome on average 6 weeks earlier than singleton pregnancies. In this study, the probability of dyspnea and of combined adverse maternal outcome continuously increased during gestation, especially between the 20th and 36th week of pregnancy, following the fundal height with its maximum at around the 36th week of gestation. The sparse literature examining the differences in respiratory function in multiple and singleton pregnancies report that despite its higher physiological demands, multiple pregnancy does not appear to further compromise the respiratory system in healthy women [20, 21]. However, this may not be the case in pregnant women experiencing a symptomatic SARS-CoV-2 infection with clinical signs of lung involvement, such as dyspnea, pneumonia, admission to ICU, oxygen supplementation or death. In these cases, even slight differences in respiratory function may influence the outcome. In accordance
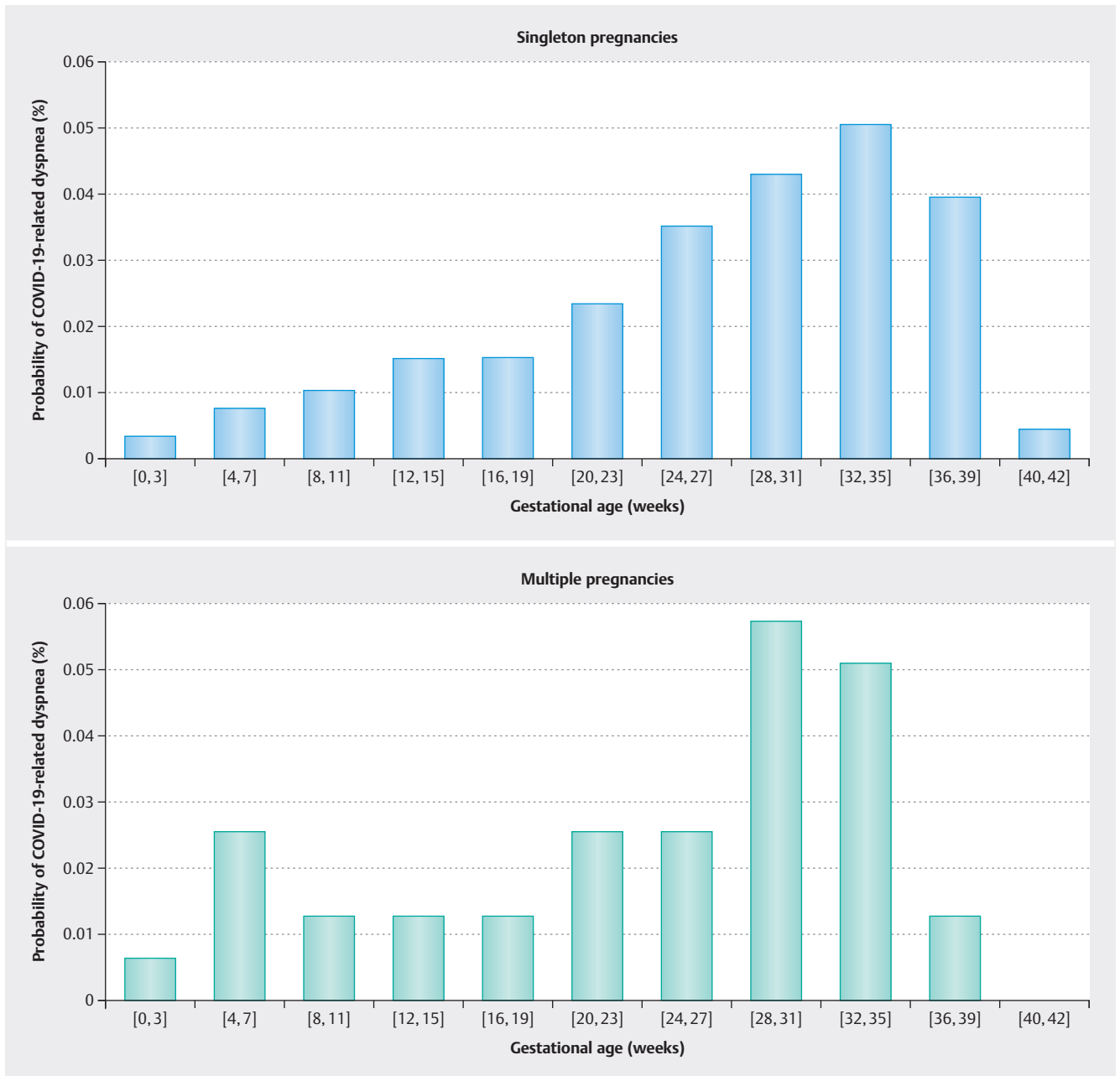
**Fig. 2** Probability of COVID-19-related dyspnea when infected with SARS-CoV-2 in a defined pregnancy week. There was no difference in the timing of occurrence of dyspnea between multiple and singleton pregnancies (Welch Two Sample t-test, mean gestational age singleton 26.8 vs. 24.1 multiple pregnancy, p value = 0.12).

with data already published, we confirmed that BMI > 30 kg/m² is a risk factor for COVID-19-related adverse maternal outcome in symptomatic women [16, 22] while ART is not [16]. Also, in conformance with existing literature, higher gestational age was a significant risk factor for adverse outcome [23]. Maternal age has previously been reported as a significant risk factor for worse maternal outcome in women infected with SARS-CoV-2 during pregnancy [16, 23]. This is in contrast to our observation, which is however based on a larger sample size (5514 vs. 1485 women). Women of reproductive age can generally be considered young

and minor age differences within the cohort may therefore not affect the outcome regarding COVID-19 [24]. Although several studies report an association of smoking or smoking history and COVID-19 [22, 25], in our study smoking during pregnancy was shown to be a protective factor. This paradox association can most likely be attributed to a selection bias, as women who smoke were more likely to receive obstetric care for associated complications, such as preterm delivery and fetal growth restriction. This bias led to a higher rate of smokers with mild symptoms who were identified by SARS-CoV-2 screening measures upon hospitalization for
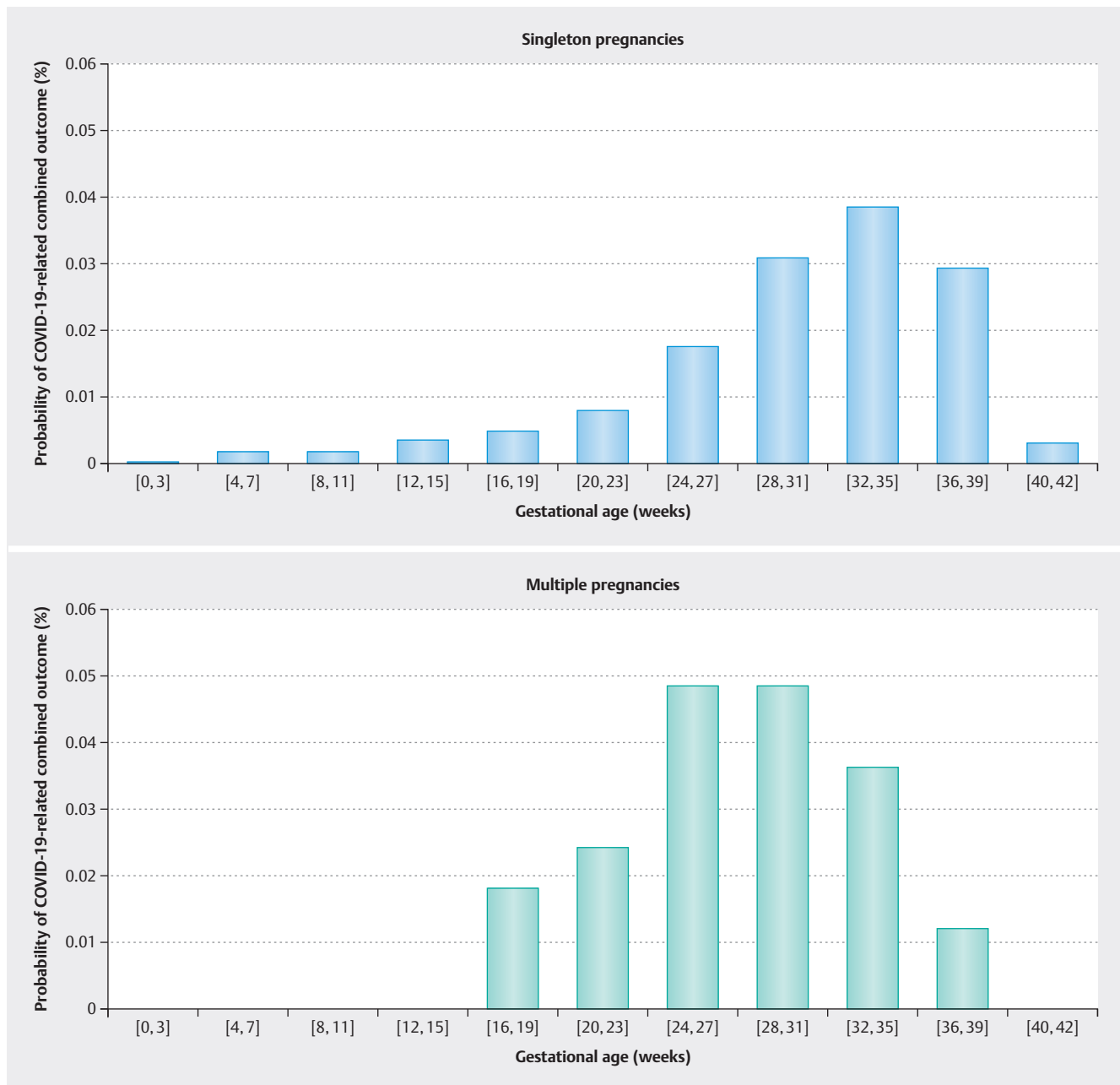
**▶ Fig. 3** Probability of COVID-19-related combined adverse maternal outcome when infected with SARS-CoV-2 in a defined pregnancy week. COVID-19 related combined outcome occurred on average significantly earlier during pregnancy in women expecting more than one child in comparison to singleton pregnancies (Welch Two Sample t-test, mean gestational age singleton 30.2 vs. 24.1 multiple pregnancy, p value = 0.001).

other reasons. In this context, we conclude that multiple gestation, gestational age and BMI > 30 kg/m² were the main predictors of combined adverse maternal outcome after SARS-CoV-2 infection in pregnancy.

Although planned or emergency cesarean sections were significantly more common in multiple pregnancies, the frequency of COVID-19 as indication for a cesarean section was identical between the two groups. COVID-19-associated reasons for a cesarean section included cases where delivery was deemed necessary for further maternal treatment due to severe COVID-19. These

were distinguished from cases categorized as obstetrically indicated termination of pregnancy or maternal request/no medical indication for pregnancy termination. However, it is possible that COVID-19 may have influenced obstetric complications leading to pregnancy termination (e.g. pathological cardiotocography, preterm contractions). Although this potential overlap is likely to affect both singleton and multiple pregnancies, the degree of uniformity across these groups remains undetermined.

The strengths of this work include high data quality as analyzed data were collected from a well-supervised prospective registry

study using a standardized electronic clinical report form. Hospitals participating in CRONOS cover approximately 33 % of all deliveries in Germany. Multiple pregnancies in our cohort were adequately represented (3.0 % of analyzed cases), as the proportion of multiple births in Germany and of multiple pregnancies after spontaneous conception (miscarriage not excluded) is 3.7 % and 4 % respectively [26]. Accordingly, the present data set is to be considered representative of the national collective.

As asymptomatic cases of SARS-CoV-2 infection are very likely documented due to clinical consultation for pregnancy-related complications, e.g. spontaneous preterm delivery, fetal growth restriction, or preeclampsia, they were considered as "incidental SARS-CoV-2 infection" and excluded from further analysis. However, women expecting more than one child visit the hospital more often and earlier during pregnancy due to higher risk of obstetric complications. This may have potentially led to earlier detection of mild symptomatic SARS-CoV-2 infection in multiple pregnancies than in singleton pregnancies. As this affects the denominator, the relative frequency of severe COVID-19-related outcomes may be underestimated in multiple pregnancies. Moreover, there were less cases attributed to the delta variant amongst unvaccinated women with multiple pregnancies compared to unvaccinated women with singleton pregnancies. As the delta variant is mainly responsible for severe courses of SARS-CoV-2 infection, this could theoretically mask multiple gestation as a risk factor for adverse maternal outcome. Despite this, multiple pregnancy was proven to be a risk factor in our cohort.

Our study has a variety of limitations, including the difference in cohort sizes. This factor makes it difficult to reliably show statistical correlations. Moreover, as immunity could only be assumed based on vaccination history and no antibody status was determined, the role of vaccination status was not thoroughly addressed in our work. Another conflicting point was the limited documentation of naturally acquired immunity due to previous infection in the registry. However, according to the national center of disease control RKI, the seroprevalence of SARS-CoV-2 antibodies at the time of national recommendation of vaccination in pregnancy (September 2021) was below 10 % [27]. Therefore, the effect on these results can be neglected. While the vaccination status was not included into our statistical models, it did not differ significantly between singletons and multiple pregnancies in our cohort (31% vs. 38%).

Virus variants were not separately addressed due to the relatively small sample size in the subgroups (**Supplementary Table S3**). Furthermore, while additional information on multiple pregnancy was requested, neonatal outcome data were available for only one of the newborns. Per definition, outcome data of the neonate with the worse outcome (for example neonate with lower birth weight, lower umbilical artery pH or neonatal death) was requested. Therefore, neonatal outcome cannot be meaningfully assessed and compared between multiple and singleton pregnancies in this study. Lastly, potential limitations associated with subjective reporting coming from patients or physicians, which cannot be objectively verified afterwards, pose a challenge in observational studies.

## Conclusion

In conclusion, our data shows that multiple gestation was associated with a substantially higher risk for combined adverse maternal outcome after onset of COVID-19, which was also observed earlier in pregnancy than in singleton pregnancies. This could result due to a more evident displacement of the diaphragm by the enlarged uterus in comparison to singleton pregnancies. These findings are of clinical importance during the ongoing pandemic, but also for similar respiratory viral infections. They can serve physicians when advising couples expecting more than one child or considering multiple embryo transfer after ART. Further research is needed to assess and quantify the respiratory stress in women with SARS-CoV-2 infection during multiple pregnancy and to evaluate whether a different therapeutic management than in singleton pregnancies is required, particularly if ventilatory support is needed.

## Supplementary Material

**Supplementary Table S1:** Frequency of different virus variants for unvaccinated and vaccinated women with singleton or multiple pregnancy.

**Supplementary Table S2:** Dyspnea due to COVID-19 infection and duration of symptomatic phase according to mother.

**Supplementary Table S3:** Multivariate logistic regression analysis results for combined maternal outcome in women with COVID-19 in multiple and singleton pregnancies.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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