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COVID-19 and first trimester spontaneous abortion: a case-control study of 225 pregnant patients

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AJOG at a Glance

A. **Why was the study conducted?** Limited research exists on the outcomes of pregnant women with COVID-19, mostly related to women in the second or the third trimester. Evidence about maternal and obstetric outcomes of women with SARS-COV-2 during the first trimester or about the risk of early pregnancy loss are lacking.

B. **What are the key findings?** We report the largest series of patients with COVID-19 in the first trimester of pregnancy to date. The study findings showed no significant difference in the cumulative incidence of COVID-19 in women who experienced spontaneous abortion (n=100) compared to those with ongoing pregnancies (n=125).

C. **What does this study add to what is already known?** These findings may reassure women residing in COVID-19 epidemic areas who are planning pregnancy and may provide obstetricians with a guide for preconceptional counseling.

ABSTRACT

Background

The disease caused by the "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) was named Coronavirus Disease 19 (COVID-19) and classified as a global public health emergency. The evidence related to the impact of COVID-19 on pregnancy are limited to the second and the third trimester of pregnancy, while data on the first trimester are scant. Many viral infections can be harmful to the fetus during the first trimester of pregnancy, and whether SARS-CoV-2 is one of them is still unknown.

Objective(s)

With this study we evaluated SARS-CoV-2 infection as a risk factor for early pregnancy loss in first trimester of pregnancy. Furthermore, COVID-19 course in the first trimester was assessed.

Study design

Between February 22 and May 21, 2020, we conducted a case-control study at S. Anna Hospital, Turin, among first trimester pregnant women, paired for last menstruation. The cumulative incidence of COVID-19 was compared between women with spontaneous abortion (case group, n=100) and those with ongoing pregnancy (control group, n=125). Current or past infection was determined by detection of SARS-CoV-2 from nasopharingeal swab and SARS-CoV-2 IgG/IgM antibodies in blood sample. Patient demographics, COVID-19-related symptoms, and the main risk factors for abortion were collected.

Results

Twenty-three of the 225 women (23/225, 10.2%) tested positive for COVID-19 infection. There was no difference in the cumulative incidence of COVID-19 between the cases (11/100, 11%) and the controls (12/125, 9.6%) (p=0.73). Logistic regression analysis confirmed that COVID-19 was not an independent predictor of early pregnancy loss (Odd Ratio 1.28, confidence interval 0.53-3.08). COVID-19 related symptoms in the first trimester were fever, anosmia, ageusia, cough, arthralgia and diarrhea; no pneumonia or Hospital admission due to COVID-19-related symptoms were recorded. No difference in the incidence of symptoms was noted between the two groups.

Conclusion(s)

SARS-CoV-2 infection during the first trimester of pregnancy does not appear to predispose to early pregnancy loss; its cumulative incidence did not differ between women with spontaneous abortion and women with ongoing pregnancy. COVID-19 appears to have a favorable maternal course at the beginning of pregnancy, consistent with what has been observed during the second and the third trimester.

Key Words

abortion; coronavirus; COVID-19; fetus; first trimester; miscarriage; pregnancy; pregnancy loss; preterm birth; SARS-CoV-2; seroprevalence; severe acute respiratory syndrome; vertical transmission

Introduction

The World Health Organization (WHO) named the new coronavirus (SARS-CoV-2) disease coronavirus disease-19 (COVID-19) and declared it a pandemic. Coronaviruses are enveloped, non-segmented positive-sense RNA usually responsible for mild illness such as the common cold in adults and children.[1] But in the last decade, coronaviruses have caused two important epidemics: the severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS). COVID-19 was first reported in Wuhan (China) in December 2019 followed by outbreaks across the world.[2] The first cases of COVID-19 in Italy were confirmed in January 2020, with a rapid rise in the number of cases in northern Italy starting in late February.

Despite the rapidly growing number of cases worldwide, data on COVID-19 during pregnancy remain limited, being derived mainly from small sample studies.[3–8] A systematic review of published reports on coronaviruses (COVID-19, SARS, MERS) reported higher rates of preterm birth, preeclampsia, cesarean section, and perinatal death.[9] The lack of data on spontaneous abortion due to COVID-19 during the first trimester precludes extrapolation of conclusive evidence for the effects of infection during early pregnancy. The paucity of reliable data has aroused concern in patients, while the disinformation reported by media may lead pregnant women to embrace dramatic choices such as voluntary abortion.[10]

The wide of clinical expression, the high rate of asymptomatic forms, the poor accuracy of nasopharyngeal swab testing and its limited availability have been the main barriers to gaining a real understanding of the prevalence of the infection and its impact on pregnancy. In this complex scenario, the development of serological tests for the detection of SARS-CoV-2 IgG and IgM could be useful to identify pregnant patients who were infected during early pregnancy. While the quantity and quality of data on test performance are still limited, the level of accuracy has been reportedly moderate/good, so that patients infected by SARS-CoV-2 can be traced.[11]

The aim of the present study was to evaluate the impact of COVID-19 on first trimester pregnancy loss by comparing the cumulative incidence of SARS-CoV-2 infection in a cohort of women who experienced early spontaneous abortion and that of women with ongoing pregnancy at 12 weeks of gestational age. Furthermore, COVID-19 course in the first trimester was evaluated.

Materials and methods

Women who had been referred to our Hospital for pregnancy loss care during the first 13 weeks of pregnancy, between February 22 and May 21, 2020 were contacted and enrolled (case group). All women who had access to our emergency room or to the pregnancy loss management service were contacted after being traced through our hospital's database. Women 12 weeks pregnant admitted to our Hospital for fetal nuchal translucency between April 16 and May 21, 2020 were the control group.

All pregnant women in Turin, Piedmont, are offered free of charge a comprehensive first trimester risk assessment, performed at gestational age 11-13 weeks as part of the public antenatal and obstetric health care service. The attendance rate is high. The risk assessment includes a double test (blood sample for pregnancy-associated plasma protein A [PAPP-A] and free beta human chorionic gonadotropin [β-hCG]) and an ultrasound nuchal translucency (NT) measurement (combined screening test) or NT measurement together with PAPP-A dosage and a further blood sample for α-fetoprotein, free estriol, and β-hCG at 15-18 weeks gestational age (integrated screening test).

The first reported case of COVID-19 infection in Piedmont was dated February 22, 2020. To exclude the possibility of COVID-19 seroconversion before pregnancy, only women with last menstruation before that date were considered

eligible for inclusion (Figure 1). This criterion allowed us to define seropositivity in the case group as a seroconversion that had occurred during pregnancy.

Blood tests were performed for the detection of IgG/IgM non neutralizing antibodies against SARS-CoV-2 and reverse transcriptase-polymerase chain reaction (RT-PCR) assays on nasopharingeal swabs. Patients testing positive at least one test were also tested for the determination of specific neutralizing antibodies. Blood samples were centrifuged at 3000 rpm for 5 min to separate serum and analyzed the same day of collection.

A rapid automated fluorescent lateral flow CE-approved immunoassay (AFIAS™ COVID-19, Boditech, Gang-won-do, Korea) was used for qualitative and semi-quantitative detection of IgG/IgM non neutralizing antibodies against the spike (S) and nucleocapsid (N) viral proteins; semi-quantitative results are expressed as the cut-off index (COI) in which a COI > 1.1 indicates a positive result. Chemiluminescence CE-approved immunoassay (CLIA) technology was used for the semi-quantitative determination of anti-S1 and anti-S2 specific IgG neutralizing antibodies to SARS-CoV-2 (Liaison® SARS-CoV-2 S1/S2 IgG, Diasorin, Saluggia, Italy): the antibody concentration is expressed as arbitrary units (AU/mL) and grades the results as positive when ≥ 15 AU/mL. Viral RNA extraction from the swab was performed on a MagNA Pure compact instrument (Roche, Mannheim, Germany) and analyzed using a RT-PCR assay (CFX-96, Bio-Rad, Milan, Italy) with the Liferiver Novel Coronavirus 2019-nCov real-time RT-PCR kit protocol, targeting genes N, E, and ORF1ab (Liferiver Bio-Tech, San Diego, CA, USA).

Sample size calculation was not possible because the expected prevalence of disease was unknown at the time of population enrollment and further recruitment beyond May 21 would have precluded the eligibility criterion for last menstruation.

Demographics, COVID-19-related symptoms, and data on exposure to possible risk factors for spontaneous abortion were collected by interview. The study was approved by the Institutional Review Board of the City of Health and Science of Turin (Reference number: 00171/2020). Written, informed consent was obtained from all participants. The results for quantitative variables are expressed as the mean ± standard deviation (SD) and qualitative categorical variables are expressed as frequency and percentages. Comparison of quantitative variables was performed using the t-test or Wilcoxon-Mann–Whitney test based on normal or not distribution, respectively. Qualitative variables were compared using the chi-square test or Fisher's exact test, as appropriate. When basic patient characteristics were present as confounding factors, regression analysis was performed to assess the relationship between COVID-19 infection and spontaneous abortion. Results are expressed as odds ratio (95% confidence interval [CI]). Statistical analyses were performed using SAS software ver. 9.4 for Windows (SAS Institute, Carey, NC, USA).

Results

A total of 225 women at first trimester of pregnancy, attending our Institute were included in the study. One hundred women in the case group and 125 women in the control group were enrolled. The patient attendance rate was 87% (100/115) and 88% (125/142), respectively. Table 1 reports the patients' characteristics at baseline; except for age, there were no statistically significant differences in demographics or risk factors for abortion between the two groups.

Twenty-three of the 225 women tested for anti-SARS-CoV-2 IgG and IgM antibodies were found to be seropositive or their nasopharyngeal swab tested positive for COVID-19, yielding an overall cumulative incidence of 10.2% in the first trimester. There was no significant difference in the cumulative incidence of COVID-19 between the case patients (11/100, 11%) and the controls (12/125, 9.6%) (p=0.73).

The age variable was entered into logistic regression analysis to evaluate COVID-19 infection in relation to confounders. There was no difference in the odd of being infected with SARS-CoV-2 between the two groups, indicating that COVID-19 infection was not an independent predictor of early pregnancy loss (1.282, CI 0.53-3.08).

Subgroup analysis of baseline characteristics of COVID-19-positive and -negative patients with early pregnancy loss (case group) showed no statistically significant differences in demographics or risk factors for spontaneous abortion between the two groups, except for body mass index (BMI) (26.4±5.2 vs 23.2±4.2; p=0.03).

In the case group, 5/11 (45.4%), 3/11 (27.2%), and 1/11 (9%) were positive for SARS-CoV-2 IgG, SARS-CoV-2 IgM, or both SARS-CoV-2 IgG and IgM, respectively; RT-PCR of the nasopharingeal swab resulted positive in 2/11 (18%) (Table 2). In the control group, 7/12 (58.3%), 3/12 (25%), and 2/12 (16.6%) were positive for SARS-CoV-2 IgG, SARS-CoV-2 IgM, or both SARS-CoV-2 IgG and IgM, respectively; RT-PCR of the nasopharingeal swab resulted positive in 5/12 (41.7%) (Table 3). No difference in positivity for IgG neutralizing antibodies was found between the case (6/11, 54.5%) and the control group (5/12, 41.7%) (p=0.53) (Table 1). There was no statistically significant difference between the two groups for average antibody titer, both non neutralizing (21.3 vs. 18.3 COI; p=0.42) and neutralizing antibodies (39.9 vs 46.9 AU/ml; p=0.69).

Twelve of the COVID-19 patients reported previous symptoms (12/23, 52.2%) including fever (7/12, 58.3%), anosmia and ageusia (5/12, 41.7%), cough (5/12, 41.7%), arthralgia (4/12, 33.3%), and diarrhea (1/12, 8.3%); no pneumonia or Hospital admission due to COVID-19-related symptoms was recorded. No difference in the incidence of symptoms was noted between the case (4/11, 36.4%) and the control group (8/12, 66.6%) (p=0.14).

Comment

Principal Findings

With this case-control study, we evaluated the impact of COVID-19 on early pregnancy loss in a cohort of pregnant women with SARS-CoV-2 infection confirmed by antibody testing or RT-PCR assay of nasopharyngeal swabs. The results show that the risk of first trimester spontaneous abortion is not impacted by SARS-CoV-2 infection, also after being adjusted for age. No severe cases or Hospital admission due to COVID-19-related symptoms was recorded, both in women who had ongoing pregnancies and in those with early pregnancy loss. To the best of our knowledge, this may be the largest cohort of Coronaviruses infection during early pregnancy published so far.

Results in context

Despite the large and rapidly growing number of cases worldwide, there is limited data on COVID-19 in pregnancy, mainly coming from case series and small sample studies, related to the second and third trimester of pregnancy. Concern is mounting about the impact of COVID-19 on pregnancy, possible vertical transmission,[12–15] and unfavorable obstetric outcomes in particular. Reproductive medicine societies advised delaying the start of assisted reproductive treatments[16] and guidelines on the prevention and control of COVID-19 among pregnant women have been issued.[17–19]

Currently, data on the impact of Coronaviruses on the first trimester of pregnancy are limited. Four of the seven patients who presented with SARS-CoV-1 infection during their first trimester had a spontaneous abortion, likely the result of the hypoxia caused by SARS-CoV-1-related acute respiratory distress.[20] Furthermore, one case of a woman with MERS during the first trimester has been reported. She was asymptomatic and went on to have a term delivery.[21] As for SARS-CoV-2, a single pregnancy loss during the second trimester of pregnancy in a woman with COVID-19 was probably related to placental infection.[22] Another study reported the first visualization by electron microscopy of the SARS-CoV-2 invading syncytiotrophoblasts in the placental villi.[23] This evidence could suggest a potential impact of SARS-CoV-2 on spontaneous abortion.

Clinical Implications

Our study findings may reduce concerns in patients during the first trimester of pregnancy. In the present cohort of women who experienced a spontaneous abortion during the first trimester the serological prevalence of antibodies was similar to that in the women with ongoing pregnancies. Furthermore, although viral infection at this stage could potentially affect embryogenesis and organ development, there is still no evidence for the intrauterine transmission of SARS-CoV-2. Our findings may reassure women who are planning a pregnancy in epidemic areas and may represent a guide for obstetricians during pre-conceptional counseling.

The course of COVID-19 varies widely: patients may remain asymptomatic or develop mild to severe symptoms leading to pneumonia, respiratory failure, and death.[24] In this cohort, however, few patients were symptomatic and not more numerous in the case group. Severe disease was never observed. The lower incidence of severe manifestations during the first trimester could be explained by the minimal alteration in respiratory dynamics during this phase of pregnancy. Despite these reassuring data, pregnancies in women with COVID-19 can still have an unfavorable obstetric outcome: inflammatory involvement of the placenta[25] can be associated with preterm delivery.[26] Obstetricians should discuss that although the first trimester seems not to expose the fetus to severe risks, pregnancy may still be complicated in the following weeks of gestation.

Research Implications

Serologic tests, in conjunction with SARS-CoV-2 RT-PCR assay, may offer a more feasible opportunity to identify both active and past infections and to evaluate the real spread of SARS-CoV-2, to the point that some governments have suggested their use in large-scale population tracking.[27] Determination of seroconversion in pregnant women could answer some concerns about unfavorable pregnancy outcomes, which are not otherwise resolvable. The non-negligible prevalence of infection in asymptomatic pregnant women reported in our cohort and elsewhere[8,28] makes universal screening of all pregnant patients appear desirable. Long-term follow-up of ongoing pregnancies will respond to other doubts about the impact of COVID-19 in pregnant patients.

Strengths and Limitations

One of the strengths of the present study is the enrollment of women with serologically confirmed COVID-19 by means of two different serological assays; the combined results of RT-PCR on nasopharyngeal swab samples is another major strength of the study. The high attendance rate to the study protocol limited confounding factors such as population selection bias. Antibodies to COVID-19 were detected in about one out of ten pregnant patients in the cohort; this finding should be carefully interpreted, however, as it cannot be generalized because derived from a single center located in a region with a high incidence of COVID-19.

While the number of COVID-19-positive patients in the case group is low, except for BMI, the group does not differ in baseline characteristics from the COVID-19-negative patients with early pregnancy loss. This suggests that the study conclusions may be extended to larger samples. Overweight among COVID-19 patients has been reported in other series of non-pregnant patients and is being increasingly described as an underappreciated risk factor for COVID-19.[29]A major limitation of the study is that we were unable to accurately backdate the time of infection in women with spontaneous abortion. In the absence of an IgG avidity test, we evaluated the time elapsed between the abortion and the blood test for antibody detection. The profile of antibodies against SARS-CoV-2 in this cohort was comparable with previous findings. Seroconversion of IgG or IgM within 20 days after symptom onset has recently been reported.[30] The median day of seroconversion for both IgG and IgM was 13 days with a synchronous or a discordant pattern. In light of this evidence, seroconversion during pregnancy could be excluded (or be controversial) only in one patient (no. 4, Figure 2) in the case group. The detection of IgM antibodies at 66 days after abortion does not preclude that

seroconversion might have occurred after the loss of pregnancy. In view of future research addressing the issue on the relationship between COVID-19 and spontaneous abortion, it will be difficult for researchers to precisely define the timing of infection and the effective seroconversion during pregnancy. Inclusion criteria, together with the beginning of the study at pandemic outbreak, allowed us to fairly overcome this issue.

Another limitation is that patients with very early pregnancy loss may not have been enrolled in our study because they did not require obstetric care, which is performed before the patient has her first obstetric visit. We believe, however, that there is no reason to think that within this small patient group the cumulative incidence of COVID-19 would have been so high as to question our results. It is difficult to hypothesize that "pre-clinical" abortions could be caused by SARS-CoV-2 in a stage when pregnancy loss is much more likely to occur due to chromosomal defects in the embryo rather than because of virus-induced detrimental effects at the maternal- fetal interface.[31–33] Recent evidence shows that SARS-COV-2 binds to angiotensin-converting enzyme (ACE) 2 receptors and the cellular transmembrane serine protease (TMPRSS) 2 to facilitate the fusion of viral and cellular target membranes. Because co-expression of ACE2 and TMPRSS2 at such early stage of pregnancy is negligible,[34] we believe our groups are reasonably representative and our analysis realistic.

Conclusions

In conclusion, our study provides reassuring findings for women who intend to become pregnant during the SARS-CoV-2 pandemic or who became infected during their first trimester of pregnancy. COVID-19 appears to have a favorable maternal course at the beginning of pregnancy, consistent with what has been observed during the third trimester when the clinical characteristics of COVID-19-positive pregnant women were similar to those found in women from the general population.[35] More importantly, no significant difference in the early pregnancy loss rate was observed.

Author contributions

Stefano Cosma and Andrea Carosso: Conceptualization, Data interpretation, Writing - Original Draft, Writing - Review & Editing. Jessica Cusato, Valeria Ghisetti, Antonio D'Avolio: Investigation, Resources. Claudia Filippini: Formal analysis. Fulvio Borella, Marco Carosso, Marialuisa Bovetti: Data curation. Giovanni Di Perri and Chiara Benedetto: Project administration, Supervision, Funding acquisition, Writing - Review & Editing. All authors reviewed and approved the final version of the manuscript.

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REFERENCES

1. Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. *Methods Mol Biol*. 2015;1282:1-23. doi:10.1007/978-1-4939-2438-7_1

2. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med*. 2020;382(8):727-733. doi:10.1056/NEJMoa2001017

3. Yan J, Guo J, Fan C, et al. Coronavirus disease 2019 in pregnant women: a report based on 116 cases. *Am J Obstet Gynecol*. Published online April 23, 2020. doi:10.1016/j.ajog.2020.04.014

4. Cao D, Yin H, Chen J, et al. Clinical analysis of ten pregnant women with COVID-19 in Wuhan, China: A retrospective study. *Int J Infect Dis*. 2020;95:294-300. doi:10.1016/j.ijid.2020.04.047

5. Ferrazzi E, Frigerio L, Savasi V, et al. Vaginal delivery in SARS-CoV-2-infected pregnant women in Northern Italy: a retrospective analysis. *BJOG*. Published online April 27, 2020. doi:10.1111/1471-0528.16278

6. Liao J, He X, Gong Q, Yang L, Zhou C, Li J. Analysis of vaginal delivery outcomes among pregnant women in Wuhan, China during the COVID-19 pandemic. *Int J Gynaecol Obstet*. Published online April 29, 2020. doi:10.1002/ijgo.13188

7. Breslin N, Baptiste C, Gyamfi-Bannerman C, et al. COVID-19 infection among asymptomatic and symptomatic pregnant women: Two weeks of confirmed presentations to an affiliated pair of New York City hospitals. *American Journal of Obstetrics & Gynecology MFM*. Published online April 9, 2020:100118. doi:10.1016/j.ajogmf.2020.100118

8. Sutton D, Fuchs K, D'Alton M, Goffman D. Universal Screening for SARS-CoV-2 in Women Admitted for Delivery. *New England Journal of Medicine*. 2020;0(0):null. doi:10.1056/NEJMc2009316

9. Di Mascio D, Khalil A, Saccone G, et al. Outcome of Coronavirus spectrum infections (SARS, MERS, COVID 1 -19) during pregnancy: a systematic review and meta-analysis. *American Journal of Obstetrics & Gynecology MFM*. Published online March 25, 2020:100107. doi:10.1016/j.ajogmf.2020.100107

10. Wu Y-T, Li C, Zhang C-J, Huang H-F. Is termination of early pregnancy indicated in women with COVID-19? *European Journal of Obstetrics and Gynecology and Reproductive Biology*. 2020;0(0). doi:10.1016/j.ejogrb.2020.05.037

11. Tré-Hardy M, Wilmet A, Beukinga I, Dogné J-M, Douxfils J, Blairon L. Validation of a chemiluminescent assay for specific SARS-CoV-2 antibody. *Clin Chem Lab Med*. Published online May 25, 2020. doi:10.1515/cclm-2020-0594

12. Alzamora MC, Paredes T, Caceres D, Webb CM, Valdez LM, La Rosa M. Severe COVID-19 during Pregnancy and Possible Vertical Transmission. *Am J Perinatol*. Published online April 18, 2020. doi:10.1055/s-0040-1710050

13. Carosso A, Cosma S, Borella F, et al. Pre-labor anorectal swab for SARS-CoV-2 in COVID-19 patients: is it time to think about it? *European Journal of Obstetrics & Gynecology and Reproductive Biology*. Published online April 14, 2020. doi:10.1016/j.ejogrb.2020.04.023

14. Dong L, Tian J, He S, et al. Possible Vertical Transmission of SARS-CoV-2 From an Infected Mother to Her Newborn. *JAMA*. Published online March 26, 2020. doi:10.1001/jama.2020.4621

15. Carosso A, Cosma S, Benedetto C. Vaginal delivery in COVID-19 pregnant women: anorectum as a potential alternative route of SARS-CoV-2 transmission. *Am J Obstet Gynecol*. Published online June 9, 2020. doi:10.1016/j.ajog.2020.06.012

16. Vaiarelli A, Bulletti C, Cimadomo D, et al. COVID-19 and ART: the view of the Italian Society of Fertility and Sterility and Reproductive Medicine. *Reprod Biomed Online*. Published online April 8, 2020. doi:10.1016/j.rbmo.2020.04.003

17. Poon LC, Yang H, Kapur A, et al. Global interim guidance on coronavirus disease 2019 (COVID-19) during pregnancy and puerperium from FIGO and allied partners: Information for healthcare professionals. *International Journal of Gynecology & Obstetrics*. 2020;149(3):273-286. doi:10.1002/ijgo.13156

18. Carosso A, Cosma S, Serafini P, Benedetto C, Mahmood T. How to reduce the potential risk of vertical transmission of SARS-CoV-2 during vaginal delivery? *European Journal of Obstetrics and Gynecology and Reproductive Biology*. 2020;0(0). doi:10.1016/j.ejogrb.2020.04.065

19. Rasmussen SA, Smulian JC, Lednicky JA, Wen TS, Jamieson DJ. Coronavirus Disease 2019 (COVID-19) and Pregnancy: What obstetricians need to know. *Am J Obstet Gynecol*. Published online February 24, 2020. doi:10.1016/j.ajog.2020.02.017

20. Wong SF, Chow KM, Leung TN, et al. Pregnancy and perinatal outcomes of women with severe acute respiratory syndrome. *Am J Obstet Gynecol*. 2004;191(1):292-297. doi:10.1016/j.ajog.2003.11.019

21. Alfaraj SH, Al-Tawfiq JA, Memish ZA. Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection during pregnancy: Report of two cases & review of the literature. *J Microbiol Immunol Infect*. 2019;52(3):501-503. doi:10.1016/j.jmii.2018.04.005

22. Baud D, Greub G, Favre G, et al. Second-Trimester Miscarriage in a Pregnant Woman With SARS-CoV-2 Infection. *JAMA*. Published online April 30, 2020. doi:10.1001/jama.2020.7233

23. Algarroba GN, Rekawek P, Vahanian SA, et al. Visualization of SARS-CoV-2 virus invading the human placenta using electron microscopy. *Am J Obstet Gynecol*. Published online May 13, 2020. doi:10.1016/j.ajog.2020.05.023

24. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020;395(10229):1054-1062. doi:10.1016/S0140-6736(20)30566-3

25. Shanes ED, Mithal LB, Otero S, Azad HA, Miller ES, Goldstein JA. Placental Pathology in COVID-19. *Am J Clin Pathol*. doi:10.1093/ajcp/aqaa089

26. Muhidin S, Behboodi Moghadam Z, Vizheh M. Analysis of Maternal Coronavirus Infections and Neonates Born to Mothers with 2019-nCoV; a Systematic Review. *Arch Acad Emerg Med*. 2020;8(1):e49.

27. Zullo F, Di Mascio D, Saccone G. COVID-19 Antibody Testing in Pregnancy. *Am J Obstet Gynecol MFM*. Published online May 18, 2020:100142. doi:10.1016/j.ajogmf.2020.100142

28. Vintzileos WS, Muscat J, Hoffmann E, et al. Screening all pregnant women admitted to labor and delivery for the virus responsible for coronavirus disease 2019. *American Journal of Obstetrics & Gynecology*. 2020;0(0). doi:10.1016/j.ajog.2020.04.024

29. Kass DA, Duggal P, Cingolani O. Obesity could shift severe COVID-19 disease to younger ages. *Lancet*. 2020;395(10236):1544-1545. doi:10.1016/S0140-6736(20)31024-2

30. Long Q-X, Liu B-Z, Deng H-J, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nature Medicine*. Published online April 29, 2020:1-4. doi:10.1038/s41591-020-0897-1

31. Massalska D, Zimowski JG, Bijok J, et al. First trimester pregnancy loss: Clinical implications of genetic testing. *J Obstet Gynaecol Res*. 2017;43(1):23-29. doi:10.1111/jog.13179

32. Choi TY, Lee HM, Park WK, Jeong SY, Moon HS. Spontaneous abortion and recurrent miscarriage: A comparison of cytogenetic diagnosis in 250 cases. *Obstet Gynecol Sci*. 2014;57(6):518-525. doi:10.5468/ogs.2014.57.6.518

33. Angiolucci M, Murru R, Melis G, Carcassi C, Mais V. Association between different morphological types and abnormal karyotypes in early pregnancy loss. *Ultrasound Obstet Gynecol*. 2011;37(2):219-225. doi:10.1002/uog.7681

34. Pique-Regi R, Romero R, Tarca AL, et al. Does the human placenta express the canonical cell entry mediators for SARS-CoV-2? *Elife*. 2020;9. doi:10.7554/eLife.58716

35. Yang Z, Wang M, Zhu Z, Liu Y. Coronavirus disease 2019 (COVID-19) and pregnancy: a systematic review. *J Matern Fetal Neonatal Med*. Published online April 30, 2020:1-4. doi:10.1080/14767058.2020.1759541

Table 1. Baseline characteristics, clinical findings, and COVID-19 cumulative incidence in case and control groups

Clinical findings		Case	Control	p-value
		N=100	N=125	
		No. (%) or mean (±SD)	No. (%) or mean (±SD)	
Age		35.5 (±4.7)	33.7 (±4.7)	0.001
BMI prior to pregnancy, Kg/m2		25.5 (±4.3)	22.6 (±4.1)	0.11
Pregnancy	0	51 (51)	77 (61.6)	0.34
	1	40 (40)	37 (29.6)	
	2	7 (7)	9 (7.2)	
	3	1 (1)	2 (1.6)	
	5	1 (1)	0 (0)	
Previous abortion	0	66 (66)	94 (75.2)	0.11
	1	27 (27)	21 (16.8)	
	2	6 (6)	7 (5.6)	
	3	0 (0)	3 (2.4)	
	6	1 (1)	0 (0)	
ART therapy		7(7)	12(9.6)	0.48
Smoking history		22 (22)	16 (12.8)	0.06
Thyroid disease		10 (10)	11 (8.8)	0.75
Autoimmune diseases		8 (8)	4 (3.2)	0.11
Thrombophilia		5 (5)	5 (4)	0.75
Uncontrolled DM		0	0	>0.99
Uterine abnormalities		8 (8)	9 (7.2)	0.82
COVID-19 disease		11 (11)	12 (9.6)	0.73

Ab, antibodies; ART, assisted reproductive technique; BMI: body mass index; DM, diabetes mellitus

Table 2. Antibody levels and SARS-Cov-2 detection in sera and nasopharyngeal swab samples from patients with abortion

Diagnostic Test	Positive result	Patient 1	2	3	4	5	6	7	8	9	10	11
Anti-NP IgM	COI>1.1	<1.1	2.11	<1.1	1.9	<1.1	<1.1	<1.1	<1.1	2.6	<1.1	2.9
Anti-NP IgG	COI>1.1	<1.1	18.9	<1.1	<1.1	19.4	<1.1	14.4	32.4	<1.1	21.7	<1.1
Anti-RBD IgG	≥15 AU/ml	<15	19.5	<15	<15	29.9	49.3	17.3	41	<15	82.9	<15
NS		pos	Neg	pos	neg	neg	neg	neg	neg	neg	neg	neg

NS, nasopharyngeal swab; NP, nucleoprotein; RBD, receptor-binding domain

Table 3. Antibody levels and SARS-CoV-2 detection in sera and nasopharyngeal swab samples from pregnant patients.

Diagnostic Test	Positive result	Patient 1	2	3	4	5	6	7	8	9	10	11	12
Anti-NP IgM	COI>1.1	<1.1	<1.1	<1.1	2.1	1.6	1.2	1.2	<1.1	1.2	<1.1	<1.1	<1.1
Anti-NP IgG	COI>1.1	19.3	19.3	15.6	<1.1	<1.1	21	<1.1	21.5	23.2	21.9	2.45	20.7
Anti-RBD IgG	≥15 AU/ml	<15	<15	<15	<15	<15	52.7	<15	21.1	103	30.5	<15	27.5
NS		neg	pos	neg	neg	neg	pos	neg	pos	neg	pos	neg	pos

NS, nasopharyngeal swab; NP, nucleoprotein; RBD, receptor-binding domain

Figure 1: Inclusion criteria and time of serological and molecular sampling in the case and the control group.

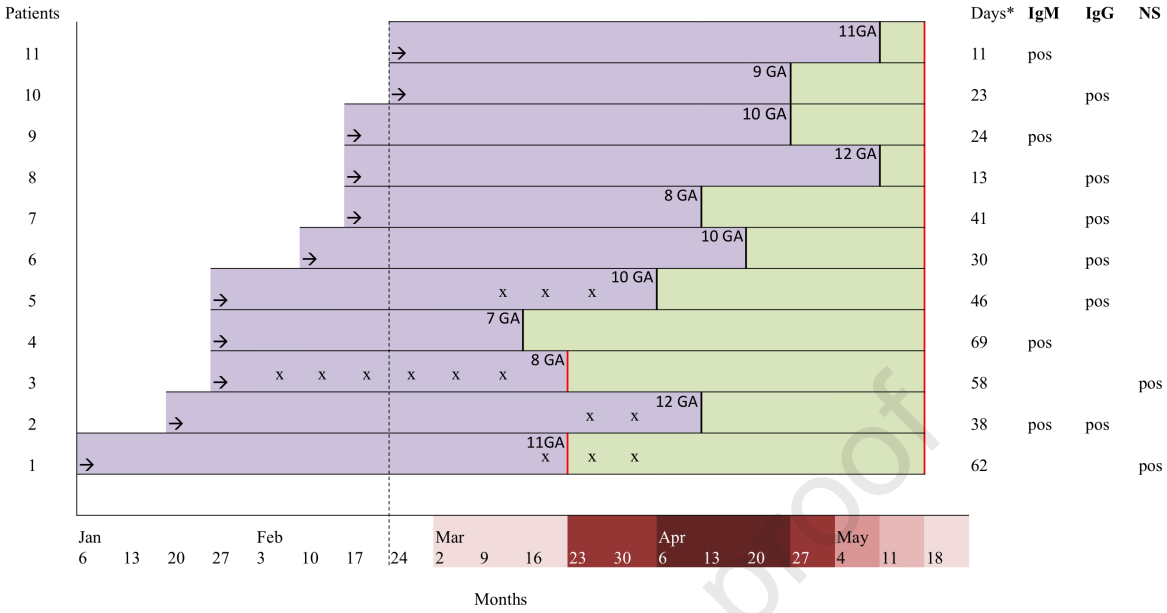
Blue line: time range for last menstruation inclusion; dotted red line: first reported case of COVID-19 in Piedmont, Italy; red line: time of sera and nasopharyngeal swab sample collection

COVID-19 outbreak cases in Piedmont Region: weekly case increase

Figure 2: Patients with first trimester pregnancy loss: time elapsed between spontaneous abortion care and diagnostic testing and seromolecular profiles.

Black arrow: last menstruation; black vertical line: hospital care for early pregnancy loss; dotted black line: first reported case of COVID-19 in Piedmont; NS: nasopharyngeal swab; rectangular green box: time elapsed between the spontaneous abortion and diagnostic testing; rectangular violet box: pregnancy; red line: serological and/or molecular sampling; x: reported COVID-19-related symptoms; * days elapsed between the spontaneous abortion and diagnostic testing

COVID-19 outbreak cases in Piedmont Region: weekly case increase



Days*	IgM	IgG	NS
11	pos		
23		pos	
24	pos		
13		pos	
41		pos	
30		pos	
46		pos	
69	pos		
58			pos
38	pos	pos	
62			pos