Cite as: Napieralska M, Modlinska A, Rybkiewicz T: Fatal outcomes of COVID-19 in postpartum patients and the clinical course of the early neonatal period: a case study. Pediatr Med Rodz 2026; 22 (1): 42–48

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Received: 10.10.2025
Accepted: 26.11.2025
Published: 16.04.2026

Fatal outcomes of COVID-19 in postpartum patients and the clinical course of the early neonatal period: a case study

Niekorzystne zejście zakażenia COVID-19 u pacjentek w połogu – obserwacja przebiegu wczesnego okresu noworodkowego ich dzieci (analiza przypadków)

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https://doi.org/10.15557/PiMR.2026.0006

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Abstract

In December 2019, a novel coronavirus was identified in Wuhan Province, China. This microorganism, known as SARS-CoV-2, is responsible for causing COVID-19, which can trigger a wide range of disorders in multiple organs and systems, and potentially lead to death. Pregnant women are among those particularly vulnerable to infection with this virus. Most studies published to date are based on case reports and data from hospital records, providing information on the health status of pregnant women infected with SARS-CoV-2 during the perinatal period. This paper presents four clinical cases of women infected with the virus who died during the postpartum period, and examines the health status of newborns born prematurely to these patients.

Keywords: premature birth, SARS-CoV-2 infection, perinatal health, perinatal mortality

Streszczenie

W grudniu 2019 roku w prowincji Wuhan w Chinach zidentyfikowano nową formę koronawirusa. Drobnoustrój ten, określony jako SARS-CoV-2, jest odpowiedzialny za wywołanie zakażenia COVID-19, które może wyzwalać szeroką gamę zaburzeń ze strony wielu narządów i układów, a także prowadzić do zgonu. Do grupy osób szczególnie narażonych na zakażenie tym wirusem należą kobiety ciężarne. Publikowane dotychczas prace opierają się na opisach przypadków i danych pochodzących z rejestrów szpitalnych, dostarczając informacji na temat stanu zdrowia kobiet ciężarnych zakażonych wirusem SARS-CoV-2 w okresie okołoporodowym. Celem niniejszej pracy jest omówienie czterech przypadków klinicznych kobiet zakażonych wirusem, które zmarły w okresie połogu, a także przedstawienie stanu zdrowia noworodków urodzonych przedwcześnie przez te pacjentki.

Słowa kluczowe: poród przedwczesny, zakażenie SARS-CoV-2, okołoporodowy stan zdrowia, śmiertelność okołoporodowa

INTRODUCTION

With the rising incidence of COVID-19 both nationally and worldwide, there has also been an increase in the number of infections in pregnant women, who are more likely to develop complications than non-pregnant women[1]. The mortality rate among pregnant women infected with SARS-CoV-2 is 0.8–1.3%, and according to some data, it may reach 2%[1,2]. Early diagnosis of infection may be difficult because many COVID-19 symptoms resemble natural physiological changes during pregnancy, particularly those affecting the circulatory and respiratory systems, especially when fatigue, tachycardia, and swelling of the lower limbs occur in the third trimester. The most common respiratory symptoms include shortness of breath (observed in up to 60–70% of pregnant women), cough, and tachypnoea. Tachypnoea, even with normal blood oxygen saturation (SpO_2 >95%), is an indicator of respiratory dysfunction in symptomatic SARS-CoV-2 infection[3].

Physiological changes in the immune system are necessary for the normal course of pregnancy. However, coronavirus infection strongly stimulates the immune system, which can lead to the development of generalised severe inflammatory reactions, reduced tolerance to hypoxia, placental ischaemia, and hypercoagulability. Laboratory tests show elevated inflammatory markers and increased transaminase levels. Symptoms may be more severe in obese, smoking, and diabetic individuals[1,2].

Significantly higher incidence and perinatal mortality rates are observed in newborns of mothers diagnosed with COVID-19 compared with those born to healthy mothers[4]. The vast majority of data on COVID-19 in newborns indicates that the most common form is mild respiratory failure. Cases of fever with sepsis, acute respiratory failure, lower Apgar scores at 5 minutes of life, and reduced birth weight have also been reported[4,5]. Between 31 August 2020 and 30 April 2021, 147 newborns were born at the Regional Specialist Hospital in Grudziądz to mothers with confirmed COVID-19. Only four (3%) newborns in this group tested positive for SARS-CoV-2 in PCR tests. Four women (2.7%) died during the postpartum period.

CASE DESCRIPTIONS

Case 1

A 29-year-old woman in her first pregnancy (G1) was admitted to the COVID-19 Infectious Diseases Ward (IDW) at 31 weeks' gestation due to cough and shortness of breath in the course of COVID-19. The pregnancy was complicated by pregnancy-induced hypertension, obesity, and increasing proteinuria. The pregnant woman required high-flow nasal oxygen (HFNO) therapy. Due to deteriorating clinical condition at 33 weeks' gestation, a decision was made to terminate the pregnancy by caesarean section. In the postoperative period, non-invasive respiratory support (continuous positive airway pressure, CPAP) was used. Additional tests revealed elevated levels of interleukin 6 (IL-6): 185 pg/mL (N <4.40) and D-dimers: 35.52 (N <0.5 mg/L FEU), and high C-reactive protein (CRP) levels: 45–110 mg/L. Chest computed tomography (CT) showed that the total area of consolidation area involved more than 90% of the volume of both lungs. Treatment included tocilizumab and blood pressure-lowering drugs. Despite the therapy, on the third day after the caesarean section, the patient developed cardiac arrest due to bradyarrhythmia. Clinical and echocardiographic features of pulmonary embolism were found. Cardiopulmonary resuscitation was unsuccessful.

A male newborn, delivered at 33 weeks' gestation and weighing 1,770 g, received an Apgar score of 7. The child required CPAP until the 8th day of life. Antibiotic therapy was initiated, and partial parenteral nutrition was started. A chest X-ray revealed patchy opacities in the lower right lung field adjacent to the heart. Swabs for SARS-CoV-2 were taken at 48-hour intervals; both results were negative. Due to the observed oxygen dependence, a repeat chest X-ray was performed, which showed no pathological changes. Systemic steroid therapy was initiated, enabling discontinuation of oxygen supplementation. During a cardiology consultation, an atrial septal defect and patent ductus arteriosus were diagnosed. Thermoregulation disorders were observed until the end of the first month of life. The child was fed formula milk and gained weight.

Case 2

A 29-year-old G1 patient at 34 weeks' gestation, was admitted to the IDW. The pregnant woman reported weakness, shortness of breath, and coughing for 7 days in the course of COVID-19. Due to her deteriorating health on the third day of hospitalisation, the pregnancy was terminated by caesarean section. CPAP support was used from the moment of anaesthesia for the procedure. After delivery, the patient was transferred to the Infectious Diseases Intensive Care Unit. Laboratory tests revealed elevated inflammatory markers: CRP increased from 52 mg/L to 409 mg/L, and D-dimers from 1.5 pg/mL to 35.6 pg/mL. From the third day after the caesarean section, the patient's condition continued to worsen. Serial chest X-rays showed expanding areas of interstitial thickening. A CT scan performed on the 26th day of hospitalisation revealed massive "ground-glass" opacities covering 100% of the lungs. On clinical examination, the patient's breathing was at the limit of capacity, and mechanical ventilation with high ventilator settings was initiated. Initially, CT angiography did not reveal any signs of pulmonary embolism, but in the following days, abnormalities appeared on echocardiography (ECHO). Fibrinolytic and renal replacement therapy, as well as steroid therapy, were introduced. On the 36th day of hospitalisation, the patient suffered cardiac arrest; cardiac function could not be restored, and she was pronounced dead.

 DOI: 10.15557/PiMR.2026.0006

A male newborn, delivered prematurely at 34 weeks' gestation, in good general condition, received 10 points on the Apgar scale. His birth weight was 2,450 g. From the first hours of life, increasing respiratory distress was observed. Non-invasive ventilation was initiated, and surfactant (Curosurf) was administered using the INSURE (intubation–surfactant–reintubation) method, resulting in improved respiratory efficiency. Empirical antibiotic therapy and parenteral nutrition were initiated. SARS-CoV-2 swabs were negative on two occasions. The newborn required respiratory support until day 6, followed by passive oxygen therapy in an incubator for an additional nine days. Thermoregulation disorders were observed until day 17 of life. Laboratory indicators of inflammation remained negative. Worsening anaemia required a supplementary transfusion on day 39 of life.

Case 3

A 33-year-old female G3 patient, at 34 4/7 weeks' gestation, was admitted due to increasing fever, cough, and moderate shortness of breath. Her medical history included pulmonary embolism in 2020, Graves' disease in the hyperthyroid phase, and obesity. Due to her deteriorating clinical condition, increased oxygen demand, and low saturation values despite HFNO, the pregnancy was terminated by caesarean section on the third day of hospitalisation. From the operating theatre, the patient was transferred to the intensive care unit, where she was placed on a ventilator. A chest CT scan revealed changes involving 75–90% of the lungs. In the following days, symptoms of respiratory failure increased, and bacterial pneumonia and multiple organ failure developed. Her neurological condition also deteriorated. Treatment included convalescent plasma, renal replacement therapy, and antibiotic therapy. Laboratory tests showed CRP levels of 90–620 mg/L and D-dimer levels of 0.5–1.7 pg/mL. From the fifth week of hospitalisation, due to severe cardiopulmonary failure, ECMO (extracorporeal membrane oxygenation) therapy was implemented after cardiac surgery consultation; however, the expected effect was not achieved. Cardiac arrest occurred in the form of asystole. Given the patient's critical condition, no resuscitation measures were performed.

A newborn delivered at 34 weeks' gestation, weighing 2,200 g, was in serious condition, with an Apgar score of 3. Physical examination revealed central cyanosis, decreased muscle tone, and tachycardia. Resuscitation measures were performed, resulting in improvement. The child required CPAP until day 2 of life. Antibiotic therapy was initiated, and parenteral nutrition was started. The newborn remained in complete isolation until two negative SARS-CoV-2 swab tests were obtained. After completing treatment, he was discharged home in good general condition.

Case 4

A 37-year-old female G4 patient at 30 weeks' gestation, was admitted to the ward due to weakness, cough, and shortness of breath in the course of SARS-CoV-2 infection. Antibiotic therapy and passive oxygen therapy were administered. On the second day of hospitalisation, due to foetal risk and increasing symptoms of infection, a decision was made to terminate the pregnancy by caesarean section. A sudden increase in blood pressure complicated the early postpartum period; improvement was achieved after the administration of antihypertensive drugs. On the second day after delivery, symptoms of respiratory failure appeared. The patient required respiratory support – initially assisted ventilation, followed by intubation and mechanical ventilation. A chest CT scan was performed, showing ground-glass opacity changes initially involving 75% of the lung parenchyma and, after another two weeks, 90% of the lung parenchyma. An increase in inflammatory markers (CRP values up to 592 mg/L) and in D-dimer levels (from 29.0 to 34.123 pg/mL) was observed. Mechanical ventilation was continued, and catecholamines, anticoagulant therapy, steroid therapy, and targeted antibiotic therapy were administered. Despite treatment, progressive multi-organ failure and systematic deterioration of ventilation parameters were observed. On the 33rd day of hospitalisation, cardiac arrest occurred due to asystole, and death was confirmed.

A premature baby was delivered at 30 weeks' gestation by caesarean section. The baby's condition was assessed as moderate, with an Apgar score of 7 and a birth weight of 1,360 g. He required respiratory support with NeoPuff. Then, due to increasing respiratory effort, he was intubated and placed on invasive mechanical ventilation. Surfactant (Curosurf) was administered. Two SARS-CoV-2 swabs were taken; both were negative. In the following weeks, the baby was treated in accordance with the standards of care for premature newborns. He was discharged home in the 9th calendar week of life.

DISCUSSION

Just as important as the impact of COVID-19 on the mother is its effect on the developing foetus. Due to the unknown nature of the coronavirus, pregnant patients often fear mother-to-child transmission, mutations and malformations, and even foetal death. According to current data, there appears to be no clear evidence of vertical transmission of SARS-CoV-2 to the foetus, although there are reports of a small percentage of infections in newborns[3,5,6]. Kotylar et al., in a systematic review of nearly 1,000 neonates born to mothers infected in the third trimester of pregnancy, demonstrated that vertical transmission of SARS-CoV-2 is possible. This analysis showed a 3.2% rate of positive PCR test results in neonates born to mothers with COVID-19, similar to studies conducted in China in 2020[5]. Numerous genetic mutations encoding SARS-CoV-2 proteins have led to the emergence of new viral strains with higher transmission rates, similar to congenital infections caused by other pathogens.

The four newborns described in this article, all delivered by caesarean section, were not infected with SARS-CoV-2.

DOI: 10.15557/PiMR.2026.0006

On the other hand, at the authors' hospital, among all children born to mothers with COVID-19, coronavirus genetic material was detected in swabs from four (3%) newborns, which is similar to data from Asian studies cited earlier.

In an American cohort study, swabs were taken from the newborns of 116 mothers infected with COVID-19; none tested positive on the first day of life. In a group of 82 newborns observed for 5–7 days (80% not separated from their mothers), repeat PCR testing for COVID-19 was negative in all newborns who remained with their mothers and were breastfed. This suggests that breastfeeding is safe and that vertical transmission between mother and child is limited[7].

Although the risk of vertical transmission of the virus during pregnancy can be considered low, infection of the child during and after delivery remains possible. Raschetti et al. analysed nearly 180 cases of SARS-CoV-2 infection in newborns, 55% of whom developed COVID-19. The most common symptoms were fever (44%), respiratory disorders (52%), gastrointestinal disorders (36%), and neurological symptoms (18%). In 64% of cases, abnormalities were found on lung imaging tests[8]. In a study by Bellos et al., fever was also the most common symptom in newborns (40%). In addition, shortness of breath (28%) and vomiting (24%) were observed. One in five SARS-CoV-2-infected newborns showed no symptoms of infection[9]. In a British study by Gale et al., 42% of infected newborns had a severe disease course. A quarter of these children were born prematurely, and in 26% of cases, they were children of mothers with known perinatal SARS-CoV-2 infection. Hospital-acquired infection was suspected in 12% of children[6]. The lack of separation between mother and newborn is considered a risk factor for late SARS-CoV-2 infection, while breastfeeding, as mentioned earlier, does not increase this risk[6].

The newborns described in this paper, born to mothers who died, were premature and presented symptoms typical of prematurity, including respiratory disorders requiring CPAP and oxygen dependence. In addition, thermoregulation disorders were observed. In these cases, a positive PCR test confirmed SARS-CoV-2 infection in the mothers, while the newborns tested negative twice.

Chest imaging plays an important role in assessing the clinical condition of patients diagnosed with COVID-19[4]. According to the American College of Radiology and the American College of Obstetricians and Gynecologists, chest X-rays in pregnant women do not pose a risk to the foetus. Radiological images of the lungs in pregnant patients with COVID-19 are similar to those observed in the general population (ground-glass opacities, multilobar infiltrates, interstitial thickening). In cases of clinical suspicion of pulmonary embolism, the preferred test is CT angiography of the lungs, which has a high sensitivity of up to 97%.

Similarly, in the women described in this study, enlarging areas of interstitial thickening and ground-glass opacities were observed, involving 75–100% of the lung surface.

Over 80% of pregnant women experience COVID-19 asymptomatically or mildly, while 3–16% develop severe disease. Other studies indicate that the proportion of asymptomatic cases among pregnant women ranges from 15 to 68%[2,5,9,10].

Lassi et al. included 31,000 women from 62 different studies in their meta-analysis. The most common symptoms were cough and fever (approximately 50% of pregnant women), as well as fatigue, malaise, loss of smell and taste, and shortness of breath (24%). Less frequently reported symptoms included muscle pain (21%), sore throat (18%) or dyspeptic symptoms (14%)[2].

The women described in this study had symptomatic SARS-CoV-2 infection. The most common symptoms were cough, shortness of breath (in all patients), and weakness. One of the pregnant women also reported fever.

The data obtained in this study were similar to the results of the analysis by Karimi et al., in which fever and cough were among the symptoms present in all fatal cases. Shortness of breath and muscle pain were also observed (50% of deaths). Sore throat and gastrointestinal symptoms were less common (8.3%)[1]. In most women with COVID-19 who died, the pregnancy was terminated by caesarean section (60%), and 17–24% of patients gave birth prematurely[1,2].

According to the available literature, SARS-CoV-2 infection is associated with a higher incidence of pre-eclampsia, eclampsia or HELLP syndrome, deep vein thrombosis and postpartum haemorrhage[4,6]. Twenty percent of women who died from COVID-19 had comorbidities[1]. In a study by Harel et al., nearly 2,500 women in their third trimester of pregnancy who gave birth in the second and third quarters of 2020 were evaluated. The most common findings were a higher incidence of gestational diabetes, heavier bleeding during delivery, and significantly reduced lymphocyte counts in laboratory tests[11]. In the meta-analysis cited by Lassi et al., comorbidities were absent in almost half of the women confirmed to be infected with SARS-CoV-2[2]. In another study, approximately 15% of women were asymptomatic[9].

In nearly 40% of pregnant women with initially moderate respiratory symptoms, the condition may progress to severe pneumonia[3]. In such cases, anticoagulants such as heparin and enoxaparin are used, as are antibiotics and antiviral drugs, including oseltamivir, ganciclovir, ribavirin, and interferon. In addition, immunotherapy and corticosteroid treatment are implemented[10,12].

A large meta-analysis by Lassi et al. described the management of complicated COVID-19 in pregnant women. Half of the women received anticoagulants (heparin, enoxaparin), as well as antibiotics, hydroxychloroquine, and antiviral drugs. A smaller proportion of patients were treated with immunotherapy and corticosteroids such as methylprednisolone, dexamethasone, and betamethasone. Approximately 20% of patients underwent oxygen therapy[2]. Pregnant women hospitalised in the department of the authors of this paper received similar treatment.

Pregnant patients may require respiratory support in the intensive care unit to maintain SpO_2 at 95% or higher,

 DOI: 10.15557/PiMR.2026.0006

relieve shortness of breath, and reduce respiratory rate. The higher the maternal partial pressure of oxygen in arterial blood (PaO_2), the better the oxygenation of the foetus. To reduce the risk of foetal hypoxaemia and acidosis, the World Health Organization recommends maintaining oxygen saturation in pregnant women between 92–95% and PaO_2 above 70 mm Hg[12]. According to various sources, mechanical ventilation is used in 1–8% of pregnant women infected with SARS-CoV-2[2,11]. The patients described in this paper received different forms of respiratory support for varying durations. Initially, passive oxygen therapy was used, followed by non-invasive and invasive ventilation, and ECMO in one case. None of the women regained full respiratory function. In a study by Harel et al., out of 172 pregnant women who tested positive for COVID-19, only one (0.6%) required mechanical ventilation, and none of the women in labour died[11]. In hospitalised pregnant women with confirmed COVID-19 and hypoxaemia below 94%, the use of remdesivir is permitted, but further clinical studies are needed to determine whether the drug can cross the placenta and what potential adverse effects it may have on the foetus[3]. In the studied population, 2.7% of 147 pregnant women infected with SARS-CoV-2 required mechanical ventilation.

Patients treated in intensive care units, including pregnant women, are at greater risk of death than other patients[8]. In addition, obesity and diabetes are risk factors for severe COVID-19 in all patient groups[3]. Pregnancy itself promotes thrombotic processes, which may further exacerbate the course of COVID-19 in pregnant women. According to available data, the increased risk of hypercoagulability occurs mainly in patients with critically severe disease[3,12]. In pregnant or postpartum women who are hospitalised due to a severe form of COVID-19, the use of low-molecular-weight or unfractionated heparin is recommended as prophylaxis for venous thromboembolism. Antithrombotic prophylaxis and treatment with heparin are indicated regardless of symptom severity[3]. Other treatments for COVID-19, such as antibodies and immunomodulatory drugs, may be considered in some patients with moderate COVID-19 to reduce the risk of progression to severe disease or death. Glucocorticosteroids have also been used in the treatment of severely ill patients requiring oxygen or respiratory support[3].

All women described in this paper received treatment in accordance with the recommendations cited in the literature, including dexamethasone, low-molecular-weight heparin, and monoclonal antibodies (tocilizumab), as well as antibiotic therapy modified based on bacteriological test results.

An important issue is the impact of SARS-CoV-2 infection on the duration of pregnancy. It is believed that any infection and fever may increase the likelihood of premature birth. However, data on this risk in pregnant women infected with SARS-CoV-2 vary widely. The rate of premature births is 12–17% in women infected with the coronavirus, and in those with severe COVID-19, it is up to twice as high. The risk of premature delivery increases several times in pregnant women with comorbidities such as hypertension, diabetes, and obesity[13]. In Israeli studies from the first months of the pandemic, the incidence of premature births did not differ significantly from that in the prepandemic period[7]. In contrast, American studies published in the second half of 2020, in which 3.7% of pregnant women tested positive, indicated that the course of pregnancy in these patients is associated with a higher risk of complications, including preterm birth[9]. Karimi et al. reported that 17% of women who died from COVID-19 gave birth prematurely[1].

In the cases presented, all newborns of mothers who died were born prematurely. In three cases, the gestational age was estimated at 33–34 weeks, and one newborn was born at 29 weeks, requiring invasive ventilation, surfactant administration, and transfer to a level III neonatal centre.

Karasek et al., like Villar et al., pointed out that the risk of delivery before 37 weeks' gestation was 40–60% higher among pregnant women with COVID-19[4,13]. Systematic reviews and population studies conducted among individuals hospitalised with COVID-19 in the early months of the pandemic did not find a clearly increased maternal mortality rate. In a 2020 analysis of the health status of 38 infected pregnant women and their newborns conducted by Schwartz et al., no maternal deaths related to SARS-CoV-2 infection were confirmed[14]. In later publications, the mortality rate among pregnant women infected with SARS-CoV-2 was 0.8–1.3% and even up to 2%[1,2]. The four obstetric deaths described in this paper account for 2.7% of all pregnant women giving birth at the authors' hospital.

Villar et al. noted that in pregnant women infected with coronavirus, compared with the general female population, there is an increase in the number of hospitalisations and admissions to the ICU requiring mechanical ventilation[4]. During the period covered by the cases described, no deaths were reported among women not infected with SARS-CoV-2, whereas among patients with COVID-19, six women developed severe infection, and four died.

Many obstetricians prefer to induce labour in infected patients for fear of unknown or unforeseen complications. These decisions are often based on caution rather than maternal or foetal indications for operative delivery. Each case should be considered individually after consultation with a multidisciplinary team. If the decision to perform a caesarean section is not indicated due to a direct threat to the life of the mother or child, the gestational age and the ability to rescue and monitor an immature newborn at a given centre should be prioritised.

No data support the induction of premature labour in women with few symptoms at a gestational age close to 34 0/7–36 6/7 weeks. Guidelines on the timing of delivery in women with COVID-19 have been published by the Society for Maternal-Fetal Medicine. A systematic review from the early stages of the pandemic reported that among 500 pregnant patients infected with COVID-19, caesarean section accounted

DOI: 10.15557/PiMR.2026.0006 PEDIATR MED RODZ Vol. 22 No. 1, p. 42–48

for 85% of deliveries[15]. Other cohort studies also indicate a higher-than-population incidence of caesarean sections in patients with COVID-19. A relationship was found between the severity of infection and a higher percentage of caesarean sections, which were performed in half of the patients with severe disease. A lower rate of operative deliveries was reported in asymptomatic and mild cases[11,15]. Israeli studies from 2020 did not show a significant increase in the number of caesarean deliveries in women with COVID-19. In a review by Karimi et al., the majority of women with infection who died had undergone caesarean section (58.3%)[1]. In the cases presented in this paper, a decision was made to terminate the pregnancy by caesarean section in all four women. The indication for operative delivery was the worsening course of COVID-19 in patients who were additionally burdened with obesity, hypertension, and gestational diabetes.

CONCLUSIONS

Determining the impact of SARS-CoV-2 infection on both the mother and the foetus requires further research and greater attention to prevention, diagnosis, and management by both gynaecologists and neonatologists. The specific physiological state of pregnancy and accompanying conditions mean that pregnant patients during the COVID-19 pandemic must be classified as a high-risk population. Diagnostic difficulties during the prenatal period are compounded by the fact that several symptoms are common to both pregnancy and COVID-19. The following symptoms should raise suspicion of infection: fever, shortness of breath, tachypnoea, cough, loss of smell and taste, fatigue, oedema, muscle aches and headaches. Properly performed swabs, isolation, use of personal protective equipment, close medical monitoring, early hospitalisation when indicated, and protection of pregnant women in the third trimester are measures that can effectively reduce the incidence of COVID-19.

Many pregnant women express concerns about the safety of vaccinations. However, extensive data indicate the effectiveness of immunity against various SARS-CoV-2 variants. There has been no reported increased risk of miscarriage, pre-eclampsia, or higher risk of death or hospitalisation of infants born after their mothers were vaccinated during pregnancy. Despite these recommendations, vaccination rates among pregnant women remain low in many countries, highlighting the importance of educating pregnant women and raising awareness of the need for vaccination. Successive waves of infection and the emergence of numerous SARS-CoV-2 mutations require continuous review of the literature and modification of treatment algorithms.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organisations which might negatively affect the content of this publication and/or claim authorship rights to this publication.

Author contribution

Original concept of study; collection, recording and/or compilation of data; analysis and interpretation of data: MN, TR. Writing of manuscript; critical review of manuscript; final approval of manuscript: AM, MN, TR.

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DOI: 10.15557/PiMR.2026.0006