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FROM TIMISOARA**

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# **DOCTORAL THESIS**

**PREVENTION STRATEGIES FOR PREGNANCY  
COMPLICATIONS ASSOCIATED WITH SARS-COV-2  
INFECTION**

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**Timisoara**

**2024**

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# **STUDY 1: THE INFLUENCE OF NUTRITIONAL SUPPLEMENTATION FOR IRON DEFICIENCY ANEMIA ON PREGNANCIES ASSOCIATED WITH SARS-COV-2 INFECTION.**

## **CONTEXT**

According to the World Health Organization, more than 2 billion people worldwide suffer from iron deficiency, and more than 38% of pregnant women worldwide suffer from anemia during pregnancy. Anemia affects only 20% of pregnancies in the United States due to intensive screening and empiric nutritional supplementation during pregnancy, but still, iron-deficiency anemia is the most frequent anemia of pregnancy and one of the most frequent complications during pregnancy in developed countries. Moreover, other studies researching anemia in pregnancy discovered that 42% of randomly chosen non-anemic first trimester women were iron deficient using conventional transferrin saturation and serum ferritin cut-off values; however, iron deficiency screening using serum ferritin testing is not regularly recommended in unselected pregnancies in the United States and the United Kingdom.

Iron deficiency is widespread, especially in women of childbearing age, and is mostly caused by menstrual blood loss and a lack of iron-rich foods consumed orally. This problem is exacerbated during pregnancy. Women need iron and folate throughout pregnancy to satisfy their own demands and those of the growing fetus. The issue is that if pregnant women become lacking in certain nutrients, they will be unable to provide them to the fetus in appropriate amounts. Iron deficiency anemia is defined by a low serum ferritin level, typically 15 µg/L. Additionally, a serum ferritin concentration of 30 µg/L suggests depleted iron reserves. Fetal growth requires around 800–850 mg of iron. Women who are already iron deficient and anemic throughout early pregnancy will exhaust residual iron reserves and become more anemic. In women who are not anemic but are iron deficient, continued depletion of iron reserves may result in anemia. Even women with adequate hemoglobin and iron reserves are at risk of developing an iron deficit later in pregnancy. Iron deficiency anemia is related to an increased risk of blood transfusion, preterm birth, cesarean delivery, and neonatal critical care unit hospitalization if present at delivery. Low folate intake before conception raises the chance of neural tube abnormalities in the infant. Inadequate iron and folate levels in women may result in anemia, which makes women weary, dizzy, and more susceptible to infections, such as the SARS-CoV-2, during the ongoing pandemic. As such, iron supplementation is suggested if this problem is detected during pregnancy or after delivery, since it is related to unfavorable maternal and newborn outcomes. Therefore, three possible ways to prevent and control the development of iron deficiency and iron deficiency anemia should be considered. These encompass dietary diversification, food fortification and individual oral iron supplementation, as the first line method. Considering the gastrointestinal upset caused by iron supplementation, this can be administered every other day with similar efficiency.

The pandemic caused by SARS-CoV-2 had a dramatic influence on healthcare systems, social institutions, and the global economy. The COVID-19 pandemic's detrimental impacts on maternal and perinatal health are not confined to the disease's direct morbidity and death. We expect that Romanian pregnant women who are left behind for prenatal monitoring and treatment as a result of the COVID-19 pandemic's limitations would have worse pregnancy outcomes, as it was recently demonstrated in a global analysis concluding that maternal and fetal outcomes have deteriorated as a result of the COVID-19 pandemic, with an increase in maternal deaths, stillbirths, and maternal depression. As growing concerns during the pandemic affect medical workers and mothers, we believe that anemia during pregnancy can be easily overlooked in these times, as much as it is still an understudied topic in correlation with COVID-19. Therefore, we aimed to identify potential unwanted outcomes of anemia during pregnancy that might be associated with maternal exposure to SARS-CoV-2 and determine the difference made by nutritional supplementation in these pregnancies.

## METHODS

This study was a retrospective population-based cohort analysis carried out between January 1, 2020, and January 1, 2022. It was conducted at the Department of Obstetrics and Gynecology, affiliated with Timis County Emergency Clinical Hospital, a tertiary hospital located in Timisoara, Romania. The research focused on pregnant women who met specific criteria, ensuring a comprehensive and relevant dataset for analysis. The criteria for inclusion in the study were as follows: (1) the provision of informed consent and agreement to participate in the study; (2) childbirth at the clinic, whether resulting in a live birth or stillbirth; (3) a documented history of pregnancy screening at the outpatient clinic, including at least one complete blood workup; and (4) undergoing testing for SARS-CoV-2 before or during hospitalization, following the standard RT-PCR protocol. To maintain the integrity of the study, 214 pregnant women who had follow-up histories at the outpatient clinic but chose to deliver in a private setting were excluded. Additionally, 109 pregnant women who delivered at the clinic during the study period but did not consent to participate in the research were also omitted. By the end of the data collection period, the study successfully included 351 pregnant women who tested negative for COVID-19 and 95 who tested positive for the virus, all meeting the specified inclusion criteria.

The study defined anemia in pregnancy according to international guidelines, using specific hemoglobin concentration thresholds. These thresholds were set at a hemoglobin concentration of 11.0 g/dL for the first trimester, 10.5 g/dL for the second and third trimesters, and 10.0 g/dL for the postpartum period. Additionally, serum ferritin levels were evaluated as an important indicator of the body's iron storage. The level of serum ferritin during early pregnancy is generally a reliable predictor of iron deficiency. For women, the reference range for serum ferritin is typically between 10 to 200 ng/mL. By employing these specific parameters, the study aimed to accurately identify and analyze cases of anemia among the pregnant women in the cohort.

Aside from hemoglobin and serum ferritin levels, the study also focused on a range of other variables. These included general maternal characteristics like age, gravidity (total number of pregnancies), and parity (number of births). Maternal pregnancy complications were also examined. In terms of neonatal outcomes, the study considered various factors such as neonatal characteristics, a full blood count for the newborn, and additional evaluations of direct and total bilirubin, birth weight, and neonatal complications. These complications included prematurity, neonatal death, congenital anomalies, and the need for red blood cell (RBC) transfusions. This comprehensive approach allowed for a detailed understanding of the maternal and neonatal health outcomes in relation to anemia and iron deficiency during pregnancy.

Upon diagnosing iron deficiency anemia and/or folate deficiency in the pregnant patients, appropriate supplementation was provided. All patients diagnosed with these conditions received a standard release iron supplement. The daily dosage of this supplement ranged between 30 mg and less than 60 mg of elemental iron. This dosage range was chosen to effectively address iron deficiency while minimizing potential side effects. Additionally, folic acid supplementation was administered in doses of 400 to 600 micrograms. These dosages align with the guidelines set by the American College of Obstetricians and Gynecologists, ensuring that the supplementation regimen was both effective and in accordance with established medical practices.

The study's approach to addressing iron deficiency anemia and folate deficiency in pregnant women demonstrates a commitment to following best medical practices and guidelines. By adhering to established international standards for diagnosing anemia and supplementing iron and folate deficiencies, the study aimed to provide valuable insights into the management of these conditions in pregnant women, particularly in the context of the COVID-19 pandemic. The comprehensive data collection, which included a wide range of

maternal and neonatal variables, allowed for an in-depth analysis of the effects of these nutritional deficiencies and their treatment on pregnancy outcomes. This research is not only relevant for understanding the impact of the COVID-19 pandemic on maternal health but also contributes to the broader field of obstetrics and gynecology by providing evidence-based insights into the management of anemia and folate deficiency in pregnancy.

## RESULTS

The general characteristics of the study participants revealed significant differences between groups stratified by COVID-19 status during pregnancy. Anemia was more prevalent in pregnant mothers with a history of SARS-CoV-2 infection during pregnancy (42.1% vs. 29.3%,  $p = 0.018$ ). Furthermore, their nutritional supplementation patterns differed significantly, with a higher proportion of pregnant mothers with COVID-19 self-medicating or being medically prescribed iron and folate daily supplements (81.1% vs. 70.7%,  $p = 0.009$ ). Notably, the greatest difference was observed in those taking both iron and folic acid supplements (52.6% vs. 33.9%). Newborns of mothers with anemia were also more likely to have anemia (31.6% vs. 21.7%,  $p = 0.043$ ). Birth weight and APGAR scores were statistically lower in newborns of mothers with COVID-19 ( $p = 0.027$  and  $p = 0.029$ , respectively).

Evaluating newborns' birth weight by COVID-19 status, the lowest median value was observed in newborns from mothers with COVID-19 who did not take iron or folate supplements (median birth weight = 2590 g), while the highest median birth weight was in newborns from mothers without COVID-19 who followed nutritional supplementation with iron and folate (median birth weight = 3340 g). Significant differences in birth weight were observed between groups stratified by nutritional supplementation ( $p = 0.022$ ).

The laboratory profile of pregnant women with anemia indicated that SARS-CoV-2 infection worsened anemia. Significant differences were observed between COVID-19 positive and negative patients, with lower levels of red blood cell count, hemoglobin, ferritin, sideremia, transferrin saturation, and reticulocyte count in COVID-19 positive pregnant women with anemia. Conversely, white blood cell count and haptoglobin levels were significantly elevated in the COVID-19 positive group.

Comparison by the status of iron deficiency anemia between mothers infected with SARS-CoV-2 during pregnancy revealed significant differences. Puerperal infections occurred at a significantly higher rate in mothers with anemia (52.5% vs. 27.3%,  $p = 0.015$ ), and emergency c-sections were more common in the anemia group (42.6% vs. 18.2%,  $p = 0.009$ ). Additionally, fetal growth showed a significant difference, with a higher percentage of newborns being small for gestational age in pregnancies with both anemia and SARS-CoV-2 infection (35.0% vs. 14.5%,  $p = 0.019$ ).

Lastly, correlation analysis revealed significant associations between maternal iron deficiency anemia and maternal and neonatal outcomes in both COVID-19 positive and negative groups. Notably, iron and folic acid supplementation had a strong negative association with iron deficiency in COVID-19 positive pregnancies ( $r = -0.646$ ,  $p = 0.005$ ). Anemia showed significant positive correlations with severe SARS-CoV-2 infection, puerperal infection, transfusion necessity, emergency c-section, and newborns being small for gestational age. Conversely, it was negatively correlated with birth weight and APGAR scores in COVID-19 positive mothers.

Figure 1 – Boxplot comparison of birth weight by nutritional supplementation in newborns from COVID-19 positive vs. COVID-19 negative mothers.

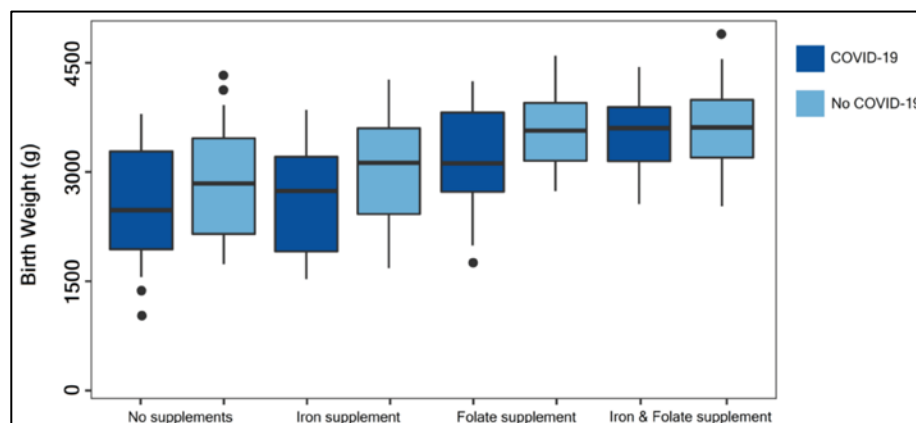
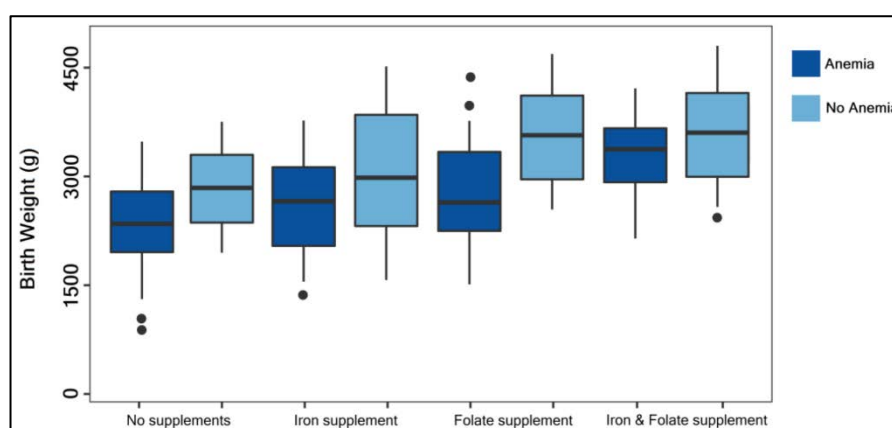


Figure 2 – Boxplot comparison of birth weight in newborns from COVID-19 mothers with and without anemia.



## CONCLUSIONS

This comprehensive study underscores the critical significance of meticulous management and nutritional supplementation in addressing iron deficiency anemia in pregnant women, particularly during the challenging backdrop of the COVID-19 pandemic. The findings conclusively demonstrate that the presence of SARS-CoV-2 infection in pregnant women exacerbates the already compromised maternal health associated with anemia. This synergistic effect significantly heightens the risks for adverse outcomes not only for the mother but also for the newborn. These results emphasize the imperative need for healthcare providers to adopt a multifaceted approach that includes rigorous screening for anemia, tailored nutritional supplementation, and vigilant monitoring, especially for pregnant women at risk for complications, including SARS-CoV-2 infection.

Furthermore, the study underscores the pivotal role of early intervention through iron and folic acid supplementation to mitigate the adverse impacts of anemia in pregnant women. The data reveal that proper supplementation can have a significant positive impact on birth weight and reduce the likelihood of anemia in newborns. This highlights the importance of proactive measures to ensure that pregnant women receive adequate nutritional support throughout their pregnancy journey.

# **STUDY 2: ASSESSING THE IMPACT OF COVID-19 VACCINATION ON PRETERM BIRTH: A SYSTEMATIC REVIEW WITH META-ANALYSIS.**

## **CONTEXT**

The SARS-CoV-2 virus, responsible for the COVID-19 pandemic, has significantly impacted public health worldwide, which extends to special population groups, such as pregnant women, where the interplay of viral infection and vaccination raises critical clinical questions. The physiological changes in pregnancy alter immune responses and may affect susceptibility to infections and their outcomes, making the study of COVID-19's impact in this demographic particularly vital.

Pregnant women with COVID-19 have been found to have an increased risk of severe illness and adverse pregnancy outcomes compared to their non-pregnant counterparts. These outcomes prominently include preterm birth, a significant concern given its implications for neonatal health. Studies have shown that COVID-19 infection during pregnancy increases the risk of preterm birth by up to 50% higher than the non-infected counterparts, necessitating a deeper understanding of its pathophysiology, implications, and risk factors.

Conversely, the role of COVID-19 vaccination during pregnancy has been a subject of extensive research. Studies have critically assessed the safety and efficacy of these vaccines in pregnant women. A significant finding from these studies is that COVID-19 vaccination during pregnancy does not increase the risk of adverse perinatal outcomes, rather it appears to provide protective effects against complications such as intensive care unit admissions and maternal SARS-CoV-2 infection. Thus, the potential of COVID-19 vaccination to reduce the risk of preterm birth is a critical area of focus. The interplay between maternal immunity, vaccine response, and neonatal outcomes underlines the need for targeted research in this domain.

The primary hypothesis of this study is that COVID-19 vaccination during pregnancy significantly reduces the risk of preterm birth. This review aims to analyze various vaccines' efficacy in different populations, providing a comprehensive overview of their impact on maternal and neonatal health. The objective is to inform clinical practice and public health policies, enhancing the care and safety of pregnant women during the ongoing pandemic.

## **METHODS**

In October 2023, a systematic review was conducted to evaluate the impact of COVID-19 vaccination on preterm birth, a crucial aspect of maternal and neonatal health. To achieve this, a comprehensive and systematic search was undertaken across several renowned electronic databases, including PubMed, Embase, and Scopus. The literature scope for this search was extensive, covering all relevant studies and publications up to October 2023. This extensive timeframe ensured the inclusion of the most recent and relevant data available on the subject, providing a robust basis for the review.

The review process was conducted adhering to the rigorous standards set by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. This adherence ensured that the review was structured, transparent, and methodologically sound. Additionally, the review was registered with the International Prospective Register of Systematic Reviews. Registering with PROSPERO provided an added layer of credibility and transparency to the review process, allowing for the systematic tracking of the review's progress and findings.

Overall, this systematic review represented a significant effort to collate and analyze the existing body of research on the crucial topic of COVID-19 vaccination during pregnancy and its impact on preterm birth. By employing a comprehensive and methodologically sound approach, the review aimed to provide valuable insights that could inform clinical practice and public health policies. The objective was to enhance the care and safety of pregnant women during the ongoing pandemic, addressing a critical gap in the understanding of the interplay between vaccination, maternal health, and neonatal outcomes.



The inclusion criteria for this review were carefully crafted and defined. First, the review focused on studies that explicitly investigated the impact of COVID-19 vaccination during pregnancy on the incidence of preterm birth. Secondly, it included research that examined pregnant populations vaccinated against COVID-19, with specific attention to outcomes related to preterm birth. Thirdly, the selected studies had to provide clear and comprehensive methodology regarding the assessment of vaccination status and timing during pregnancy. Lastly, the review sought research that offered explicit details on the criteria used to define and assess preterm birth in the context of COVID-19 vaccination. These criteria were essential to ensure that the studies included in the review were both relevant and of high methodological quality.

Conversely, the exclusion criteria for this review were equally stringent. Studies that did not focus on the impact of COVID-19 vaccination during pregnancy on preterm birth outcomes were excluded. This also included research that encompassed non-pregnant populations or failed to specifically analyze the link between COVID-19 vaccination and preterm birth. Additionally, studies that did not provide clear outcome measures related to preterm birth were omitted. Furthermore, the review excluded non-peer-reviewed articles, such as preprints, in-vitro studies, conference proceedings, general reviews, commentaries, and editorial letters. These criteria were pivotal in ensuring that the review was based on scientifically sound and peer-reviewed research.

For the purpose of this review, preterm birth was defined as any birth occurring before 37 completed weeks of gestation. This definition is in line with standard obstetric guidelines, which allowed for a consistent and clear understanding of the primary outcome measure across all the studies included in the review. This uniform definition was critical for the comparability and consistency of findings across the diverse range of studies analyzed.

COVID-19 vaccination, as considered in this review, encompassed any vaccination scheme from 1 to 3 doses of the Pfizer (BNT162b2), AstraZeneca (ChAdOx1-S/nCoV-19), Moderna (mRNA-1273), or Janssen (Ad26.COV2.S) vaccines. This comprehensive inclusion of various vaccines provided a broad perspective on the impact of different COVID-19 vaccines on preterm birth during pregnancy. Including multiple vaccine types in the review allowed for a more holistic understanding of the relationship between COVID-19 vaccination and preterm birth outcomes, considering the varied vaccination strategies implemented globally.

## **RESULTS**

The systematic review included six diverse studies originating from Israel, Romania, and the United States. Israel contributed three studies, published in 2021 and 2022, while the United States contributed two studies, both published in 2021 and 2022. Romania added one study published in 2022. These studies employed various study designs, with half using a prospective cohort design and the others adopting a retrospective cohort design. In terms of study quality, one study was rated as 'High', indicating a robust methodology, while the remaining studies were rated as 'Medium' or 'Low'.

The total number of patients across all studies was substantial, with the smallest cohort comprising 227 vaccinated and 608 unvaccinated patients, and the largest involving 24,288 patients. The age of the patients generally ranged around the early thirties. Various comorbidities were reported, including hypertensive disorders of pregnancy, gestational diabetes, and obesity, among others. The comparison groups typically consisted of unvaccinated individuals, with some studies categorizing patients based on their COVID-19 infection status or other characteristics, such as ethnicity, socioeconomic status, parity, and medical history.

The review highlighted a range of COVID-19 vaccines used across different studies, primarily BNT162b2, with variations in dosage and timing of vaccination. Citu et al. reported a significant increase in spike antibodies post-vaccination, with varying impacts on newborn features across studies, such as birth weight and APGAR scores. The majority of deliveries

occurred at or after 37 weeks' gestation, with low birth weight occurrences varying among studies.

Pregnancy complications varied among the studies, with preterm birth rates ranging from 0.6% to 6.1%. Neonatal outcomes, such as Small for Gestational Age (SGA) and NICU admissions, showed slight differences between vaccinated and unvaccinated groups. The risk of preterm birth, assessed through odds ratios, risk ratios, and hazard ratios, displayed varying values across studies, with some studies indicating no significant increase in risk following vaccination, while others reported differing risks depending on the trimester of vaccination. Additional risks assessed included adverse pregnancy outcomes, SGA, and specific neonatal complications.

A meta-analysis of the impact of COVID-19 vaccination on preterm birth risk revealed a pooled odds ratio of approximately 1.03, suggesting a marginal increase in the risk of preterm birth associated with vaccination. However, the confidence interval included the null value, indicating a lack of strong evidence for a significant effect. The analysis also showed little to no heterogeneity among the included studies, suggesting consistency in the study outcomes.

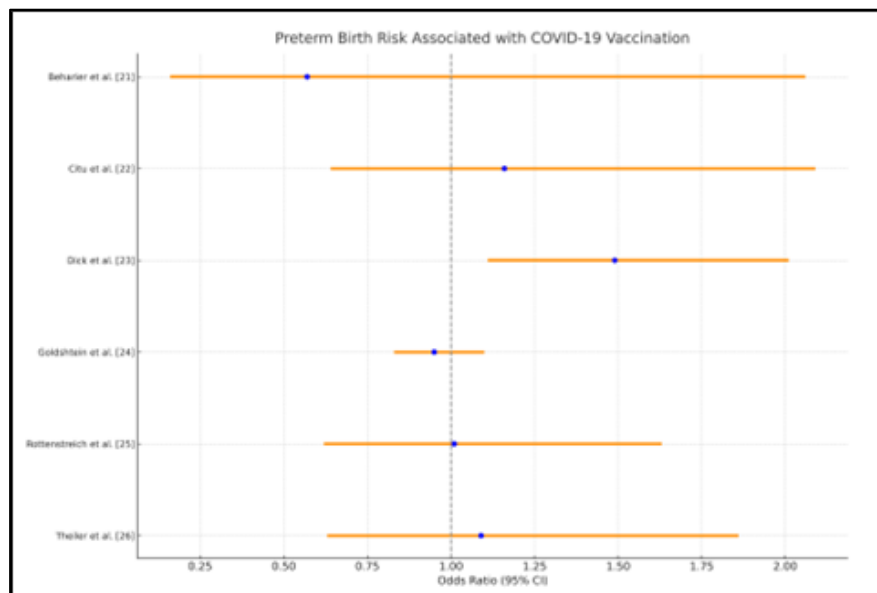


Figure 3 – Forest plot analysis for the risk of preterm birth after COVID-19 vaccination during pregnancy.

## CONCLUSIONS

The systematic review's key findings offer valuable reassurance regarding the safety of COVID-19 vaccination during pregnancy. The evidence suggests that the administration of COVID-19 vaccines to pregnant individuals does not significantly elevate the risk of preterm birth or lead to adverse pregnancy outcomes. Moreover, there is no substantial disparity in neonatal complications between vaccinated and unvaccinated groups, as indicated by comparable birth weights and APGAR scores. These findings collectively underscore the safety of COVID-19 vaccination for expectant mothers, alleviating concerns about potential harm to maternal or neonatal health.

The implications of these findings are significant for public health policies and healthcare providers. They provide a solid foundation upon which policymakers can continue to encourage COVID-19 vaccination among pregnant individuals, recognizing the potential benefits of maternal protection against COVID-19 without undue risks. Healthcare providers can confidently recommend vaccination to expectant mothers, emphasizing the safety of vaccination during pregnancy and its potential to protect both mother and baby.