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PHD THESIS

ASPECTS OF SARS-CoV-2 INFECTION AND COVID-19 VACCINATION DURING PREGNANCY - PERINATAL AND NEONATAL CONSIDERATIONS

A B S T R A C T

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Across the span of human history, contagious illnesses have consistently and significantly contributed to morbidity and death.

This doctoral thesis is anchored in a global context marked by the COVID-19 pandemic, an unprecedented health crisis that has profoundly affected all aspects of human life. As of February 7, 2024, more than 772 million confirmed cases of COVID-19 were reported to the World Health Organization (WHO), with 7 million deaths. Over 3.5 million cases and over 68 thousand deaths have been reported in Romania.

In this treacherous reality, every aspect of society, every field of science, every individual finds themselves in a relentless search for solutions to understand, manage and overcome this challenge with profound repercussions on global health.

It is within this complex and challenging landscape that the research in this thesis has addressed an area of great interest and importance: the interaction between SARS-CoV-2 infection and pregnancy, with a focus on the effects of anti-COVID vaccination at this crucial stage of a woman's life and fetal development.

The motivation behind the choice of this theme is twofold and is rooted in two distinct but closely interlinked directions. On the one hand, we are witnessing an acute need to shed light on the effects of SARS-CoV-2 infection on perinatal and neonatal health, in a context where scientific data are constantly evolving and where it is vital to establish appropriate medical protocols and interventions to protect the health of mothers and newborns. On the other hand, our interest is stimulated by a rapid increase in research in the field of COVID-19 vaccination in pregnancy, an area of investigation in which there are still many unknowns and few concrete data, and our results could contribute significantly to guiding medical practice and public health policy.

Therefore, this topic is in line with international and national concerns on maternal and neonatal health and is supported by a broad framework of scientific research and debate.

The main objectives of this doctoral research include:

- To investigate the effects of SARS-CoV-2 infection on pregnancy, fetus and newborn;
- To assess the impact of vaccination against SARS-CoV-2 during pregnancy on the mother and newborn;
- Analysis of perinatal and neonatal complications associated with SARS-CoV-2 infection compared to the vaccination and control groups.

This PhD thesis is divided into distinct parts, each dealing in depth with specific aspects of this vast topic:

The Special Part, the largest and most detailed section of the thesis, will be devoted to the presentation and analysis of four separate studies, each approaching the subject from a specific perspective and using varied methods to investigate various aspects of the interaction between SARS-CoV-2 infection and pregnancy, as well as the effects of anti-COVID vaccination in this vulnerable population.

To guide our investigation, we have defined three distinct stages, following the structure described by Charbonneau: (1) formulation of general hypotheses; (2) formulation of research hypotheses; and (3) formulation of statistical hypotheses.

The overarching hypothesis driving this research is that SARS-CoV-2 infection during pregnancy has a substantial impact on perinatal and neonatal outcomes. We hypothesize that infection can lead to negative consequences for both the mother and the newborn. Furthermore, we hypothesize that antenatal vaccination against SARS-CoV-2 could provide a protective effect against these adverse outcomes. The overall hypothesis encompasses the general notion that viral infection and vaccination status of pregnant individuals play a significant role in shaping perinatal and neonatal health.

First, we started from the fact that pregnant women who are vaccinated against SARS-CoV-2 have higher levels of antibodies against SARS-CoV-2 spike protein in serum and breast milk than unvaccinated pregnant women who contracted COVID-19.

Further research continued to determine the effects of SARS-CoV-2 infection on newborns. We therefore considered that newborns of mothers who were infected with SARS-CoV-2 during pregnancy have

different hematological and biochemical profiles than newborns born to unvaccinated mothers who contracted COVID-19 during pregnancy. Subsequently, brain, renal and cardiac changes were quantified in infants born to mothers with SARS-CoV-2 infection in pregnancy, based on the premise that there are major differences between infants born to mothers with proven infection during pregnancy compared to those born to mothers vaccinated in pregnancy against COVID-19, and compared to pregnancies without associated pathology. Last but not least, the study observed and described the second case in the literature of hydrops fetalis and intrauterine fetal death as a consequence of SARS-CoV-2 infection during pregnancy.

The first study from this thesis, validates the significance and advantages of vaccinating against SARS-CoV-2 while pregnant, resulting in the production and spread of anti-protein spike antibodies transferred to the fetus via the placenta and to the newborn through breastfeeding. A cohort study was carried out at Première Hospital (Regina Maria Health Network) in Timișoara, Romania, from May 2021 to February 2022. The study aimed to measure the levels of antibodies against the SARS-CoV-2 spike protein in the serum and breast milk of mothers and newborns who were fully vaccinated during pregnancy. The study included 91 pairs of mothers and newborns. The analysis included mothers who were fully vaccinated with the mRNA vaccine (BNT162b2 Pfizer/BioNTech) against COVID-19 during pregnancy. Significant correlations were found between maternal and neonatal antibodies ($r = 0.95$, $p < 0.001$) and between maternal serum and breast milk antibodies ($r = 0.82$, $p < 0.001$). This supports the transfer of antibodies from mother to child and suggests that mothers with a stronger immune response offer better protection to their infants. Our sample included 65

vaccinated mothers who did not contract COVID-19 and 26 vaccinated mothers who did. Mothers who experienced both infection and vaccination during pregnancy had a notably stronger immunological response compared to others, as indicated by higher levels of serum antibodies (mean difference = 9146.52; $t(89) = 9.92$, $p < 0.001$). The findings were consistent with the number of antibodies found in neonatal serum (mean difference = 8577.14, $t(89) = 8.95$, $p < 0.001$) and breast milk (mean difference = 83.91; $t(88) = 7.56$, $p < 0.001$). Maternal serum antibodies were significantly associated with the trimester of vaccination ($b = 971.91$, ß = 0.24, $t(60) = 1.95$, $p = 0.028$) in mothers who did not have COVID-19 during pregnancy. However, there was no significant association found between maternal age ($b = -160.25$, ß = -0.21, $t(60) = -1.62$, $p = 0.111$), number of births ($b = -50.42$, ß = -0.01, $t(60) = -0.07$, $p = 0.945$), or autoimmune thyroiditis ($b = -14.91$, ß = -0.00, $t(60) = -0.01$, $p = 0.989$). The level of antibodies in maternal serum rose by 971.91U/mL as the interval between vaccination and birth decreased. Pregnant women who had COVID-19 during pregnancy and received full immunization against SARS-CoV-2 transmitted higher levels of antibodies to their newborns compared to pregnant women who did not have the infection. Neonates born to vaccinated mothers who had COVID-19 had a mean neonatal antibody value of 11,414.90 U/mL, whereas neonates born to mothers who were only vaccinated had a mean value of 2837.76 U/mL. Antibody levels in breast milk of individuals without COVID-19 during pregnancy were influenced by the trimester of vaccination ($b = 13.60$, ß = 0.24, $t(58) = 1.95$, $p = 0.028$). Maternal age, gestational age, type and number of births did not affect the antibody titer in breast milk.

The second study aims to compare the hematological and biochemical test results of newborns born to mothers who were found to be COVID-19 positive during gestation with those born to unvaccinated mothers who did not acquire SARS-CoV-2 infection during pregnancy.

Thus, a retrospective cohort study was conducted on 367 babies born at Première Hospital (Regina Maria Health Network), Timișoara, Romania, over a 10-month period.

In terms of maternal laboratory investigations performed at the time of birth, the results did not show statistically significant variations between the two cohorts for most measurements, except for platelet counts. On the other hand, neonatal blood tests showed that leukocyte counts had a statistically significantly lower mean value in the group born to mothers without COVID-19 during pregnancy. Furthermore, the results indicate that there were no significant differences in leukocyte count ($p = 0.64$) and hemoglobin level ($p = 0.33$) between infants born to symptomatic and asymptomatic mothers. However, there was a significant difference in platelet count ($p = 0.04$), with neonates of symptomatic mothers having a higher median count of 307 (with an interquartile range of 73.75) compared with 278 (with an interquartile range of 51.50) in neonates of asymptomatic mothers. There were no significant differences in hematocrit level ($p = 0.35$) and erythrocyte count ($p = 0.18$) between the two groups. Therefore, the second study concludes that there were no significant differences between COVID-19 positive mothers and mothers without COVID-19 during pregnancy in terms of neonatal hematological and biochemical characteristics.

The third study had the main objective to compare both histopathological and immunohistochemical observations of placentas from mothers who tested positive for SARS-CoV-2 during pregnancy

with those without infection throughout gestation. We examined 44 placentas from patients who gave birth between November 2021 and August 2022, collected from mothers who were not vaccinated against SARS-CoV-2 infection before/during pregnancy. Macroscopic evaluation revealed distinct regions characterized by avascular villi (AV), a clear indication of compromised blood flow within these vital structures. In addition, thrombi were observed in both chorionic vessels and the umbilical cord, further affirming the presence of fetal vascular malperfusion. In the positive group, a remarkable finding was observed, detecting fetal thrombotic vasculopathy in a staggering 29% of cases, in stark contrast to placentas in the control group. The statistical significance of this discrepancy was confirmed with a p-value of 0.01. This compelling evidence strongly suggests that SARS-CoV-2 infection during pregnancy exerts a significant impact on coagulation disorders. Intervillous thrombosis, was seen in a significant proportion, accounting for approximately 21% of cases. This finding is particularly notable compared to the control group. However, the difference was not statistically significant ($p = 0.053$). Through our comprehensive study, we found an abundance of microscopic involvement that shows a strong correlation with visible placental appearances. Examining these microscopic details has provided us with invaluable information, allowing us to better understand the mechanisms underlying this vital organ. All placentas in the positive group showed strong positivity for SARS-CoV-2 in placental membranes, trophoblasts and macrophages of fetal villi. Placentas from the COVID-19 group showed a multitude of significant characteristics that demonstrated the impact of the virus on placental health. These features, such as placental infarction, pervillous fibrin deposition, intervillous fibrin, delayed placental maturation,

chorioangiosis, chorioamnionitis and the presence of meconium, provide crucial insight into the complexity of this condition. Among these characteristics, placental infarction was observed in 17% of cases in the COVID-19 positive group, compared to only 5% in the negative control group ($p = 0.4$). This notable difference highlights the potential influence of COVID-19 on placental vascular complications. Furthermore, perivillous fibrin deposition was found in approximately 29% of COVID-19 positive placentas.

The fourth study aims to identify newborns from pregnancies with SARS-CoV-2 infection and to investigate the extent of neonatal complications using cardiac, abdominal and brain ultrasound, hearing tests and indirect ophthalmoscopy. Our research compared the effects of SARS-CoV-2 infection during pregnancy with COVID-19 vaccination and normal pregnancies on the neonatal brain, heart and kidneys by ultrasonographic examinations during the first 4 days after birth, along with hearing screening and ophthalmological evaluation. A large number of newborns, namely 152 (91%), in the COVID-19 group had normal echocardiographic examinations compared to the vaccination group with 86 (95%) normal assessments and the control group with 190 (95%). Pathological findings were separated into two categories: patent foramen ovale (PFO) and congenital heart disease (CHD). For the persistent foramen ovale class, eight cases were found in the COVID-19 group (4.8%), three cases in the vaccination group (3.3%) and nine in the control group (4.5%). No association was obtained for this category ($p > 0.9$). Brain ultrasound detected several abnormalities, ranging from intraventricular hemorrhage (IVH) (grade 1, 2 and 3) to hypoxic ischemic encephalopathy (HIE) (grade 1, 2 and 3), choroid plexus cysts, severe ventriculomegaly and cerebral infarction. The most common abnormality

was grade 1 intraventricular hemorrhage and grade 1 hypoxic ischemic encephalopathy for all groups. Grade 1 intraventricular hemorrhage was the most common type of lesion, with 24 cases (14%) in the COVID-19 group, 12 cases in the control group (6%) and only 3 cases in the vaccine group (3.3%), which made a difference in the ultrasonographic changes of those born with SARS-CoV-2 infection during pregnancy ($p < 0.002$). Grade 2 and grade 3 IVH were not as common, with three findings among the COVID-19 group (1.8%) for grade 2 and one (0.6%) for grade 3 and almost none for the other groups.

In hypoxic-ischemic encephalopathy lesions, the differences were greater. We determined statistically significant data for patients with grade 1 HIE: 35 in the COVID-19 group (21%) versus 12 in the control group (6%) and 2 in the vaccine group (2.2%), resulting in a p-value of <0.001 and demonstrating how gestational infection can induce bleeding disorders.

Abdominal ultrasound noted a number of abnormalities such as unilateral and bilateral hydronephrosis, renal duplication, megaureter, pyeloureteral duplication, horseshoe kidney and septate gallbladder. The most common finding among patients in the COVID-19 group was unilateral grade 1 hydronephrosis, with 27 cases (16%), while the vaccine group had only three cases (3.3%) and the control group had 11 cases, or almost one third (5.5%), suggesting a possible correlation between SARS-CoV-2 infection during pregnancy and fetal and neonatal kidney ($p < 0.001$). For our study population, all patients were auditory tested using otoacoustic emission screening in conjunction with auditory brainstem response (EOAE-ABR). None of the COVID-19 group and the vaccinated group had a negative test result. The study found no ocular abnormalities in infants from mothers infected with

SARS-CoV-2 during pregnancy compared to the other groups evaluated, suggesting that infection during the gestation period has no effect on ocular abnormalities. The study concludes that the fetal heart did not appear to be adversely affected, although six cardiac malformations were found in the COVID-19 group, and no correlation was made compared to the vaccine and control groups. Grade 1 intraventricular hemorrhage and hypoxic ischemic encephalopathy were most common among infants from mothers with SARS-CoV-2 infection. The renal abnormality that was found to be most common in this group was unilateral grade 1 hydronephrosis. COVID-19 disease during gestation had no effect on hearing or visual function.

The thesis also presents a case of pregnancy complicated by hydrops fetalis that developed 7 weeks after recovery from SARS-CoV-2 infection, resulting in intrauterine fetal pathology in association with documented placental infection with SARS-CoV-2. To our knowledge, this is the second case reported in the literature of COVID-19 infection complicated by hydrops fetalis and intrauterine fetal death. More data on COVID 19-infected pregnant women and their fetuses are needed to create guidelines for clinical practice to prevent potentially negative outcomes and fetal complications. Until more definitive answers are available, increased surveillance of pregnant women and their fetuses is needed.

Finally, the future directions for the interaction of SARS-CoV-2 infection and pregnancy, with a focus on the effects of COVID vaccination at this critical stage of a woman's life and fetal development, remain highly promising and relevant in light of the ongoing COVID-19 pandemic and evolving scientific knowledge.

In this PhD thesis, personal contributions are set within a historical, scientific and global horizon that reflects the continuous evolution of human knowledge and medical practices in the context of the COVID-19 pandemic. From a scientific perspective, the contributions provide new data, analysis and insights into the interaction between SARS-CoV-2 infection, anti-COVID vaccination and pregnancy. Through the studies conducted in this thesis, important effects of infection on perinatal and neonatal health, as well as potential benefits of vaccination during pregnancy, have been highlighted.