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The Impact of Pro-Inflammatory Factors in Obstetrical Pathology

SUMMARY

Doctoral student:
CONSTANTIN CONDAC

PhD Supervisor:
Prof. Univ. dr. VICTORIA BIRLUTIU

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List of Abbreviations

ACE2 = Angiotensin-Converting Enzyme 2
AIP = Acute Inflammatory Pathology
DNA = Deoxyribonucleic Acid
ARN = Ribonucleic Acid
BPD = Bronchopulmonary Dysplasia
STD = Sexually Transmitted Diseases
CD44 = Cluster of Differentiation 44
CIP = Chronic Inflammatory Pathology
COVID-19 = coronavirus 2019
COX-2 = Cyclooxygenase-2
CVS = Chorionic Villi
E. Coli = *Escherichia coli*
FIGO = International Federation of Gynecology and Obstetrics
FVM = Fetal Vascular Malperfusion
GBS = Group B Streptococcus
H&E= Hematoxylin and Eosin
H_2O_2 = Hydrogen Peroxide
hCG = Human Chorionic Gonadotropin
IAI = Intra-amniotic Infection
IFITM1-3 = Interferon-Induced Transmembrane Protein 3
IHC = Immunohistochemistry
BMI = Body Mass Index
LBW = Low Birth Weight
M. hominis = *Mycoplasma hominis*
MERS = Middle East Respiratory Syndrome
MERS-CoV = Middle East Respiratory Syndrome Coronavirus
MVM = Maternal Vascular Malperfusion
NICU = Neonatal Intensive Care Unit
NP = Preterm Birth
OPN = Osteopontin
PCR = Polymerase Chain Reaction
PHH3 = Phosphohistone H3
PROM/PPROM = Premature Rupture of Membranes
PTB = Preterm Birth
RT-PCR = Reverse Transcription Polymerase Chain Reaction
SARS-CoV-2 = Severe Acute Respiratory Syndrome Coronavirus 2
SGA = Small for Gestational Age Fetuses
SNC = Central Nervous System
SYN = Multinucleated Syncytiotrophoblast
TMPRSS2 = Transmembrane Serine Protease 2
TNF = Tumor Necrosis Factor
U. urealyticum = *Ureaplasma urealyticum*
VDR = Vitamin D Receptor

Chapter 1

CURRENT STATE OF KNOWLEDGE

The leading cause of infant mortality in both developed and developing countries is preterm birth. Each year, approximately 15 million infants are born prematurely, and the ascending migration of bacteria from the vaginal tract into the uterine cavity is responsible for the majority of these cases. Even microorganisms associated with vaginal dysbiosis—such as *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Gardnerella vaginalis*—although characterized by low virulence, can trigger preterm birth by stimulating the production of proinflammatory cytokines. These inflammatory mediators promote premature rupture of the fetal membranes and induce uterine contractions, ultimately leading to preterm labor and delivery. **Premature rupture of membranes before 37 weeks of gestation occurs in approximately 30% of spontaneous preterm births.** The most common route of intrauterine infection involves the ascending migration of bacteria from the lower genital tract. This process most frequently occurs in the presence of ruptured membranes; however, it may also develop while the membranes remain intact. **The representative and most frequently encountered microbial flora in infections of the female genital tract includes** *Group B Streptococcus*, *Escherichia coli (E. coli)*, *Enterococcus faecalis*, *Enterobacter* spp., *Candida* spp., *Ureaplasma urealyticum (U. urealyticum)*, *Mycoplasma hominis (M. hominis)*, *Chlamydia trachomatis*, and bacterial vaginosis–associated species. These microorganisms are typically identified in vaginal environments characterized by a reduced or absent *Lactobacillus* population and, frequently, by the predominance of Gram-negative bacteria (Brown et al., 2019). **Intrauterine infection may be polymicrobial and, in most cases, results from a combination of aerobic and anaerobic organisms.** The pathogens most frequently isolated from the amniotic fluid of patients with chorioamnionitis are those commonly found in the vaginal flora, including *Gardnerella vaginalis*, *Ureaplasma urealyticum*, *Bacteroides bivius*, Group A, B, and D *Streptococcus*, *Peptococcus*, *Peptostreptococcus*, and *Escherichia coli*. Other routes of infection include hematogenous (blood-borne) or transplacental transmission, retrograde infection from the pelvic cavity, and transuterine infection secondary to medical procedures such as amniocentesis and chorionic villus sampling (CVS). However, all these pathways are considered relatively rare (Brown et al., 2019; Romero et al., 2007; Vogel et al., 2006).

The scientific literature emphasizes the pivotal role of the normal vaginal microflora and the detrimental impact of bacterial vaginosis during pregnancy. Studies have demonstrated that infections with *Candida* species and the presence of bacterial vaginosis during gestation significantly increase the risk of miscarriage and preterm birth. Furthermore, numerous reports have highlighted that vaginitis occurring during pregnancy may lead to serious complications such as spontaneous abortion, preterm delivery, premature rupture of membranes, chorioamnionitis, low birth weight, and postpartum endometritis (Brown et al., 2019; Peaceman et al., 2014).

Pregnancy is a physiological state characterized by multiple adaptive changes that enable mutual adjustment between the maternal organism and the developing fetus. These adaptations are primarily governed by hormonal fluctuations, which induce behavioral changes, physicochemical modifications of the mucosal surfaces, and structural and functional alterations of the genital tract.

All these factors lead to alterations in the structure and function of the microbiota, making it distinct from that of non-pregnant women. Moreover, evidence suggests the presence of microorganisms at the placental level, as well as indications that the composition of the maternal vaginal microbiota influences the establishment of the neonatal microbiome. A number of studies have investigated the association between vaginal microbiome composition and adverse pregnancy outcomes such as miscarriage and preterm birth. First-trimester miscarriage has been correlated with a reduction in *Lactobacillus* species and an increased variability in the composition of vaginal microorganisms.

The impact of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and the associated Coronavirus Disease 2019 (COVID-19) on pregnant women and newborns has become a subject of particular interest for obstetricians, pediatricians, and patients alike (Engjom et al., 2024). Coronaviruses are encapsulated, single-stranded RNA viruses. Most coronaviruses that commonly circulate in the human population cause mild upper respiratory tract infections, with severe disease occurring occasionally in immunocompromised individuals. Previous novel coronaviruses that have caused significant human infections include Severe Acute Respiratory Syndrome (SARS), caused by the SARS-CoV coronavirus, between 2002 and 2004, and Middle East Respiratory Syndrome (MERS), caused by the MERS-CoV

coronavirus, from 2012 to the present (Goyal et al., 2022). It is estimated that the SARS epidemic (which officially ended in 2023, although sporadic cases were reported until 2024) involved approximately 100 pregnant women worldwide. Several case series demonstrated that SARS infection during pregnancy was associated with severe maternal disease, an increased risk of maternal death, and spontaneous miscarriage.

The doctoral thesis entitled “The Impact of Proinflammatory Factors in Obstetrical Pathology” aimed to investigate the role of placental proinflammatory markers in pregnancy progression and the occurrence of obstetrical complications, in the context of SARS-CoV-2 infection and COVID-19 vaccination. The research sought to highlight how placental inflammation influences fetal development and maternal–fetal immunological adaptation.

The main objectives of the study were:

- To analyze the role and impact of proinflammatory factors on maternal health during pregnancy
- To investigate the expression patterns of placental cellular biomarkers in pregnancies affected by the COVID-19 pandemic
- To investigate the modulation of Vitamin D receptor (VDR) expression in pregnancy under COVID-19–related conditions
- To investigate the relationship between placental cellular biomarker expression and Vitamin D receptor (VDR) variation in pregnancies affected by the COVID-19 pandemic

Vaginal infections represent a common and frequent problem in medical practice and may involve various microorganisms, including both bacteria and fungi. The microorganisms most frequently associated with these infections include *Streptococcus*, *Escherichia coli (E. coli)*, *Enterococcus*, *Enterobacter*, *Gardnerella vaginalis*, *Ureaplasma*, and *Candida* species. Genital infections acquired during pregnancy may endanger both fetal development and maternal health, leading to complications such as chorioamnionitis, preterm birth, and premature rupture of membranes. These infections occur in the context of a compromised immunological state induced by pregnancy, as maternal immunity decreases in order to provide optimal conditions for fetal development (Brown et al., 2019; Dautt-Leyva et al., 2018; Gonçalves et al., 2022).

Placental pathology in the context of SARS-CoV-2 infection represents an active area of research, and our understanding of it continues to evolve. However, several findings have been reported in cases of placental pathology associated with SARS-CoV-2 infection (Prochaska et al., 2020). In some cases, placental examination has revealed features of maternal vascular malperfusion, characterized by abnormalities in the maternal blood vessels of the placenta. These may include alterations such as abnormal blood flow, thrombosis, and placental infarction. There have also been reported cases providing evidence of the presence of viral particles or SARS-CoV-2 genetic material within the placental tissue itself, suggesting the possibility of direct viral involvement (Prochaska et al., 2020). It is important to note that these findings are not universal and may vary from case to case. At present, it remains uncertain whether SARS-CoV-2 infection can be transmitted vertically. Case reports indicate the potential for both placental and neonatal infection, and associate maternal infection with specific placental morphological alterations.

Ongoing research and the development of early diagnostic strategies, along with the effective management of chorioamnionitis, are essential for reducing the risk of preterm birth and its associated complications. A multidisciplinary approach involving obstetricians, neonatologists, infectious disease specialists, and microbiologists is required to improve maternal and fetal outcomes in cases of chorioamnionitis and preterm delivery. Globally, the COVID-19 pandemic has had a profound impact on pregnant women and childbirth, leaving many aspects of this pathology still insufficiently understood. Even several years later, considerable effort is still required to fully elucidate its complex mechanisms and clinical implications

Significant information regarding both maternal and fetal health can be obtained through the histopathological examination of placental tissue. Viral infections during pregnancy have been associated with specific placental findings such as lymphoplasmacytic villitis with associated villous enlargement and intravillous hemosiderin deposition in cases of maternal *Cytomegalovirus* infection, as well as intervillositis observed in infections caused by *Zika* and *Dengue* viruses.

Preterm birth (PTB) represents one of the greatest challenges in modern obstetrics. The incidence of preterm birth remains relatively stable across various regions of the world, fluctuating between 5% and

12%, although in some countries it tends to increase - particularly in developing nations and regions of Latin America, where poverty negatively affects access to medical care. This situation is often associated with a higher prevalence of infections and the inappropriate use of antibiotics within this patient population (WHO, 2012). Preterm birth has been identified as the leading cause of infant mortality in both developed and developing countries. The microbial flora most frequently encountered in infections of the female genital tract includes *Group B Streptococcus*, *Escherichia coli (E. coli)*, *Enterococcus faecalis*, *Enterobacter* spp., *Candida* spp., *Ureaplasma urealyticum (U. urealyticum)*, *Mycoplasma hominis (M. hominis)*, *Chlamydia trachomatis*, and bacterial vaginosis–associated microorganisms. These pathogens are typically found in a vaginal environment characterized by a reduced or absent *Lactobacillus* population and by the predominance of Gram-negative bacteria. The scientific literature indicates that the prevalence of microorganisms in the female genital tract within a population varies according to several factors, including age, race, socioeconomic status, contraceptive use, menstruation, and pregnancy. The association between chorioamnionitis and preterm birth is well documented in the medical literature. Persistent inflammation and infection resulting from chorioamnionitis can lead to structural and functional alterations of the chorioamniotic membranes, including their thinning and weakening**.** Appropriate management of chorioamnionitis is essential for reducing maternal and fetal morbidity and mortality. Early diagnosis and prompt treatment of chorioamnionitis can help prevent severe complications, thereby contributing to a decreased risk of preterm birth and its associated adverse outcomes.

Chapter 2

IMMUNOHISTOCHEMICAL MARKERS

Osteopontin (OPN) is an extracellular glycoprotein with multiple roles in biological processes, including inflammation, extracellular matrix regulation, and immune cell interactions. This protein is expressed in various tissues and plays a key role in placental development and function. Placental OPN expression may be altered in cases of chorioamnionitis, characterized by inflammation of the fetal membranes and amniotic fluid. Previous studies have shown that OPN may be involved in placental inflammatory processes and play a role in the immune response to infection.

Phosphohistone H3 (PHH3) is a specific marker of mitosis, expressed during the late stages of mitosis and active cell division. By identifying PHH3-positive cells within the placental tissue, it is possible to assess mitotic activity and the level of cellular proliferation. PHH3 can therefore be used to determine the degree of placental development and maturation, as demonstrated in the study by Bermick et al. (2019).

The evaluation of placental PHH3 expression can provide valuable information regarding the mitotic activity of trophoblastic cells, which are responsible for the formation and remodeling of placental blood vessels. Elevated PHH3 levels may indicate abnormal cellular proliferation or deficient placental development (Hord et al., 2020).

The use of PHH3 as a tissue biomarker in patients with chorioamnionitis can offer additional insights into the degree of inflammation, cellular stress, and cellular activity within the affected placenta. However, it is important to note that the interpretation of results must be performed within the full clinical context, taking into account additional investigations and clinical signs associated with chorioamnionitis.

The Vitamin D Receptor (VDR) is involved in the regulation of immune function, and its placental expression may be altered in cases of chorioamnionitis. VDR is a nuclear receptor that binds to Vitamin D and acts as a transcription factor, regulating the expression of genes involved in various biological functions. Studies have shown that placental VDR expression can be modified in chorioamnionitis. Vitamin D and its receptor, VDR, are key components in the regulation of both innate and adaptive immunity, including the maintenance of placental epithelial barrier function and the modulation of inflammatory responses. In chorioamnionitis, an inflammatory condition, a decrease in VDR expression has been observed in the affected placenta (Cookson et al., 2018; Guevara et al., 2020; Knabl et al., 2017).

Reduced VDR expression may have negative consequences on immune function and inflammatory regulation at the placental level in cases of chorioamnionitis. For instance, decreased VDR levels may lead to excessive activation of the immune system and increased release of proinflammatory cytokines, which can exacerbate inflammation and impair maternal–fetal exchange within the placenta. It is important to emphasize that the interpretation of these findings should take into account other clinical aspects and complementary investigations related to vaginal infections and chorioamnionitis.

The role of Vitamin D in fetal brain development has been emphasized following the identification of Vitamin D receptors (VDR) in the fetal brain. Vitamin D has the ability to cross the blood–brain barrier by binding to VDR, thereby stimulating a wide range of neurobiological responses (Kesby et al., 2011).

CD44 is a cell adhesion molecule and a receptor for hyaluronan, an essential component of the extracellular matrix. CD44 plays a key role in inflammatory processes and tissue regeneration. This molecule is expressed in various tissues and is crucially involved in development, inflammation, and repair mechanisms.

The expression of CD44 in the placenta may be altered in cases of inflammation of the fetal membranes and amniotic fluid. CD44 is expressed in various placental cell types, including trophoblasts and stromal cells. This molecule fulfills multiple functions, including cell adhesion, migration, inflammation regulation, and interaction with the extracellular matrix. Moreover, CD44 may also participate in implantation, vascularization, and the development and proper functioning of the placenta (Marzioni et al., 2009). Placental CD44 expression may be increased in cases of chorioamnionitis, reflecting immune system activation and the interaction of inflammatory cells with the extracellular matrix. Alterations in CD44 expression may have significant consequences for placental function and may contribute to the severity of inflammation in chorioamnionitis.

COX-2 (Cyclooxygenase-2) is an enzyme involved in the production of prostaglandins, which are key mediators of inflammation and regulators of various physiological processes in the body. Prostaglandins synthesized by COX-2 can contribute to the amplification of inflammation and the induction of uterine contractions, which may influence pregnancy progression and lead to fetal and neonatal complications associated with infection-related preterm birth. In the placenta, COX-2 is expressed in various cell types and plays a crucial role in the development and normal functioning of this organ.

COX-2 may also influence placental barrier function and play a role in maternal–fetal interactions. On the other hand, chorioamnionitis can alter placental COX-2 expression and amplify inflammation within the maternal–fetal environment, leading to adverse pregnancy outcomes (Guzeloglu-Kayisli et al., 2015).

The immunohistochemical analysis of specific placental markers provides an in-depth understanding of the inflammatory and tissue adaptation mechanisms occurring during pregnancy. The markers CD44, osteopontin (OPN), cyclooxygenase-2 (COX-2), and the vitamin D receptor (VDR) represent key elements in the regulation of cellular interactions, trophoblastic differentiation processes, and local immune responses. CD44, a transmembrane glycoprotein involved in cell adhesion and trophoblast migration, plays an essential role in implantation and vascular remodeling. OPN, a multifunctional matrix protein, mediates cell–matrix interactions and contributes to the regulation of the pro- and anti-inflammatory balance. COX-2, an enzyme induced under inflammatory conditions, is involved in the synthesis of prostaglandins, which are essential for angiogenesis and the maintenance of placental homeostasis. VDR, the nuclear receptor of vitamin D, exerts a complex immunomodulatory effect by influencing gene expression involved in the inflammatory response and maternal–fetal immune tolerance. The evaluation of these biomarkers provides valuable insights into how inflammatory processes and the maternal immunological status can influence placental development and function under both physiological and pathological conditions.

Chapter 3

STUDY OF PLACENTAL EXPRESSION OF CELLULAR BIOMARKERS IN PREGNANT WOMEN IN THE CONTEXT OF THE COVID-19 PANDEMIC (NEGATIVE, VACCINATED, AND POSITIVE CASES)

To better understand the mechanism of mother-to-fetus transmission in previous emerging infections, anatomopathological examination of placentas has been employed, proving to be an extremely informative technique (Schwartz et al., 2021).

In all human tissues, macrophages mediate homeostasis, development, repair, and immune defense. During embryonic development, the first macrophages to appear are derived through a process known as primitive hematopoiesis and are referred to as Hofbauer cells. These cells primarily emerge in the stroma of the human placenta during the first trimester, approximately 18 days post-conception, and are similar to macrophages originating from the yolk sac.

The placenta represents a crucial barrier that protects the fetus from vertical infections. However, it is not yet fully elucidated whether Hofbauer cells possess the ability to detect and respond to microbial stimuli through Toll-like receptors, and to distinguish among different pathogen-associated molecular patterns (PAMPs) derived from various microorganisms, thereby demonstrating that these cells are not functionally

primitive. Although their function remains poorly characterized, Hofbauer cells secrete a range of factors—including osteopontin and matrix metalloproteinase-9—that influence angiogenesis and placental remodeling, potentially contributing to placental tissue repair (Thomas et al., 2021).

The study of CD44, OPN, and COX-2 expression in placental tissue may provide valuable insights into the effects of COVID-19 infection and vaccination during pregnancy. Each marker plays a distinct role in cellular processes and immune responses, underscoring their importance in understanding placental pathology under various conditions. The aim of this study was to evaluate the expression of CD44, OPN, and COX-2 in three groups of pregnant women—healthy and unvaccinated, COVID-19–positive and unvaccinated, and COVID-19–vaccinated but COVID-negative—in order to assess potential pathophysiological pathways underlying placental architectural alterations secondary to inflammatory responses, which may be influenced by viral infections or vaccine-induced immune modulation. Based on the understanding of the normal function of the previously discussed markers (CD44, OPN, and COX-2) during physiological pregnancy and the postpartum period, it becomes possible to anticipate and intervene in a timely manner when imbalances occur in these physiological processes. Consequently, major complications may be prevented, leading to a significant reduction in maternal and fetal morbidity and mortality.

This study is considered to be the first in Romania to investigate tissue-level biomarkers in pregnant patients, thereby representing a pioneering effort that may lay the foundation for future clinical guidelines aimed at preventing—and potentially enabling the early identification of—factors that could compromise the normal course of pregnancy.

The research conducted aimed to enhance current knowledge regarding the distinct role of each biomarker in promoting favorable cellular processes and immune responses, thereby emphasizing their importance in understanding placental pathology. Within this study, the involvement of the three biomarkers was analyzed in relation to specific obstetrical events in pregnant women, such as spontaneous abortion, premature rupture of membranes, preterm birth, and chorioamnionitis.

The study was conducted between January 2021 and January 2023. Tissue samples were collected from women who delivered at term, were over 18 years of age, capable of providing informed consent, and had no complications, associated diseases, or chronic treatments. The pregnant participants were divided into three groups: COVID-19–positive and unvaccinated women, COVID-19–negative and vaccinated women, and COVID-19–negative and unvaccinated women.

The COVID-19–positive and unvaccinated group included asymptomatic women in whom the disease was detected at any point during pregnancy, but at least 14 days prior to delivery. The diagnosis of SARS-CoV-2 infection was established using the polymerase chain reaction (PCR) test. The second group consisted of women vaccinated with the mRNA-based anti–SARS-CoV-2 vaccine (Pfizer), who had received either the first or second dose at least 14 days before delivery, had never tested positive for COVID-19 during the pandemic, and underwent repeated negative rapid COVID-19 testing every three weeks throughout pregnancy.

The control group included COVID-19–negative and unvaccinated women who delivered at term without complications or associated pathologies. All women in this third group had never tested positive for COVID-19 during the pandemic prior to inclusion in the study and had repeated negative rapid COVID-19 tests every three weeks throughout pregnancy.

Through immunohistochemistry, four tissue samples were collected from each placenta, one representative for each of the four quadrants. The sections were stained with hematoxylin and eosin (H&E) and examined anatomopathologically. IHC staining was performed on formalin-fixed, paraffin-embedded tissues using monoclonal antibodies against cluster of differentiation 44 (CD44), osteopontin (OPN), and cyclooxygenase-2 (COX-2).

All placental samples were examined to detect the presence of CD44, OPN, and COX-2. Cells showing brown or brownish-yellow nuclear staining within the epithelial and stromal compartments were considered positive for CD44, OPN, and COX-2, regardless of staining intensity or the number of positive cells. Pathological changes were observed upon examination of the H&E-stained sections. In COVID-19–positive and vaccinated patients, signs of inflammation such as villitis or intervillositis, increased fibrin deposition, and the possible presence of microthrombi were noted. These alterations indicated an immune response or vascular involvement associated with COVID-19 infection. In contrast, placentas from non-COVID and unvaccinated patients exhibited typical histological features, without specific inflammatory or vascular alterations. Interestingly, data analysis revealed a more intense CD44 expression on the surface of the placental connective stromal tissue in healthy, unvaccinated patients, whereas a subtle decrease in

CD44 expression was observed in the placental tissues of COVID-19–positive pregnant women. Comparatively, CD44 expression was lowest in the placental tissues of vaccinated patients with COVID-19. Furthermore, the analysis demonstrated an increased OPN expression in the placental tissues of COVID-19–vaccinated women, evident in both the epithelial and stromal compartments. COVID-19–negative and unvaccinated women showed a slight increase in OPN expression in placental tissues, whereas OPN expression in COVID-19–positive placentas was the lowest. Interestingly, both healthy and COVID-19–positive cases exhibited similar percentages of OPN within the stromal and epithelial compartments of the trophoblast, suggesting a relatively stable, non-inflammatory state. The most notable difference was observed in the vaccinated group, where the number of OPN-positive cases was twice that of the OPN-negative cases. In COVID-19–negative and unvaccinated placentas, increased anti-COX-2 expression was detected in trophoblasts, decidual cells, and infiltrating immune cells. Following COVID-19 infection, anti-COX-2 expression was modestly elevated compared to its low expression levels in vaccinated placentas, with no anti-COX-2–negative cases among healthy and vaccinated pregnant women. The results indicated that women in the vaccinated group were significantly more likely to exhibit positive OPN expression compared to those in the COVID-19–positive group, with no significant differences in OPN positivity between the control group and the COVID-19–negative group, and no significant impact of CD44 positivity on the likelihood of OPN being positive. The findings of this study reflected an alteration in placental structure and function as a result of modified expression of the studied biomarkers. High levels of CD44 and COX-2 expression were detected in normal, uninfected, and unvaccinated pregnancies, consistent with the physiological process of placentation, whereas a downregulation of these biomarkers was observed in women infected with COVID-19, with more subtle changes in vaccinated individuals. The OPN levels remained relatively similar, possibly due to COVID-19 infection occurring in the third trimester of pregnancy, when the placenta is already fully developed and no longer undergoes significant structural changes. However, in pregnant women who were vaccinated against COVID-19 during early pregnancy, subtle alterations in the proportion of OPN-positive cells were observed, with the number of positive cells being nearly twice that of the negative ones.

In conclusion, these findings highlight the need for closer obstetric monitoring due to the high risk of maternal and fetal complications associated with COVID-19 infection. Considering the results obtained, it appears that SARS-CoV-2 infection does not cause significant maternal or fetal alterations, except in cases complicated by COVID-19–related pathology, which may increase maternal and fetal morbidity and mortality.

Chapter 4

Interaction of Vitamin D Receptors in Pregnant Women in the Context of the COVID-19 Pandemic (Negative, Vaccinated, and Positive Cases)

By 2022, more than 197 million infections had been reported worldwide, with an estimated 4.2 million deaths since the beginning of the pandemic (Joshi et al., 2022). SARS-CoV-2 infection can have a detrimental impact on various organs and tissues, leading to a wide range of clinical manifestations (Bertero et al., 2021). Beyond respiratory symptoms, SARS-CoV-2 infection may impair gastrointestinal function, induce preeclampsia in pregnant women (characterized by lymphopenia, neutrophilia, and elevated LDH levels) (Valdespino-Vázquez et al., 2021), and adversely affect hepatic, renal, and cardiac function, as well as cause neurological, immune, and endocrine disturbances (El-Kassas et al., 2023; Tossetta et al., 2022. The virus gains entry into human cells through the angiotensin-converting enzyme 2 (ACE2), whose receptors are abundantly expressed on the placental syncytiotrophoblast, suggesting a potential link between infection and placental dysfunction (Di Girolamo et al., 2021; Garg et al., 2023). The impact of this pathology on pregnant women and infants is of particular interest to both healthcare professionals (obstetricians and pediatricians) and patients (Shanes et al., 2020). Anatomopathological analysis of placentas during previous epidemics has proven to be an extremely informative technique for understanding the mechanisms of disease transmission to the fetus (Schwartz et al., 2021). Although the pathophysiology is not yet fully elucidated, immature placentas may represent more vulnerable targets for virus-mediated injury compared to term, well-developed placentas, due to the severe systemic inflammatory response and the widespread presence of microthrombi on their surface (Patberg et al., 2021).

The average duration of SARS-CoV-2 infection is approximately 20 days, ranging from 5 to 60 days, making it possible for the virus to reach and affect the placenta before delivery. This implies that the virus may have been cleared and thus may no longer be detectable in a placental sample obtained at the time of birth (Debelenko et al., 2021). On the other hand, infection occurring in the late stages of gestation may also indicate the absence of evidence for vertical transplacental transmission of SARS-CoV-2, or, conversely, may reveal a significant impact on perinatal outcomes in newborns, both in mild and in more severe cases of infection (Giordano et al., 2021).

Fetal multi-organ infection and inflammation (including pulmonary and renal involvement) caused by SARS-CoV-2 during early pregnancy should alert clinicians to the need for thorough evaluation and monitoring of pregnant women regarding potential fetal consequences and adverse perinatal outcomes (Valdespino-Vázquez et al., 2021). Even with an apparently normal short-term outcome, long-term sequelae—such as neurodevelopmental consequences in newborns—cannot be excluded (Sarno et al., 2022).

Studies indicate that the rate of neonatal infection is higher when delivery occurs by cesarean section (5.3%) compared to vaginal birth (2.7%).

The histopathological analysis of placentas collected in each case included a comprehensive macroscopic examination, standard hematoxylin and eosin (H&E) staining, immunohistochemistry, fluorescence in situ hybridization (FISH), transmission electron microscopy, and detection of viral sequences using reverse transcriptase polymerase chain reaction (RT-PCR) (Di Girolamo et al., 2021).

The study included 70 healthy pregnant women, 65 COVID-19–positive women diagnosed during pregnancy (with COVID-19 confirmed at least 14 days prior to delivery), and 35 vaccinated pregnant women (who received the first or second dose of the Pfizer vaccine at least 14 days before delivery), with samples collected between January 2021 and January 2023. The inclusion criteria comprised healthy pregnant women, women with SARS-CoV-2 infection during pregnancy, and vaccinated pregnant women who had received the Pfizer vaccine. Samples were collected from healthy women who delivered at term without complications, associated diseases, or chronic treatments. Patients with malignant disorders, diabetes, depression, genetic syndromes, infectious or autoimmune diseases, smoking history, therapies affecting bone or mineral metabolism, or vitamin D supplementation within three months prior to pregnancy were excluded.

IHC staining was performed on formalin-fixed tissues that were subsequently embedded in paraffin. The anti–vitamin D receptor (VDR) antibody was diluted 1:3000 and incubated overnight at 4°C. Human jejunum tissue was used as a positive control for VDR to ensure the specificity and sensitivity of the staining, to validate the results, and to exclude false-positive or false-negative findings. For the negative control, tissue samples were treated in a manner that should not result in staining, ensuring that the tissue did not express the antigen of interest. A total of 170 placental samples were examined for the presence of VDR. Cells displaying brown or brownish-yellow nuclear staining within the epithelial and stromal compartments were considered VDR-positive, regardless of staining intensity or the number of positive cells.

Immunohistochemical analysis revealed differences in VDR expression among the groups. The group of COVID-19–positive pregnant women exhibited the lowest VDR expression, and a significant positive correlation (38.2%) was observed between gestational age at delivery and newborn birth weight.

Unvaccinated pregnant women had an average age approximately three years lower than that of vaccinated women (28 vs. 31 years). When analyzing the groups based on urban or rural residence, it was observed that in the COVID-19–positive group, 73% of the included cases were from urban areas, and nearly 69% in the vaccinated group, whereas in the healthy unvaccinated group, the distribution by residence was equal.

Placental histological lesions were classified according to the Amsterdam criteria (Giordano et al., 2021). Considerable effort and extensive research have been undertaken to elucidate the physiological and pathological mechanisms of COVID-19, with the aim of limiting the spread of infection and its consequences. Vitamin D plays multiple roles in the human body, including calcium homeostasis, bone formation, and the modulation of both the innate and adaptive immune systems (Cyprian et al., 2019).

The vitamin D receptor (VDR) is a nuclear receptor that binds to vitamin D and functions as a transcription factor, regulating the expression of genes involved in various biological processes (Knabl et al., 2017). Its effects on cytokine production and activity have been extensively investigated, demonstrating the regulation of suppressor T lymphocytes in complicated pregnancies, as well as the suppression of B lymphocyte proliferation and immunoglobulin production (Cyprian et al., 2019). Despite the widespread use of prenatal vitamins, vitamin D deficiency remains common among pregnant women (Cyprian et al., 2019).

Initially, vitamin D metabolism was believed to occur primarily in the kidneys; however, it has been demonstrated that other organs, such as the pituitary gland, hypothalamus, uterus, oviduct, ovaries, mammary glands, and placenta, are also involved. Vitamin D supports placental development and function by regulating calcium transport, which is essential for the maintenance of pregnancy and for preventing a maternal immune response against the embryo (Cyprian et al., 2019). Moreover, due to its expression in various cell types, including trophoblasts and immune cells, vitamin D plays a role in regulating inflammation and immunity within the maternal–fetal environment, being involved in the barrier function of the placental epithelium. In cases of chorioamnionitis, an inflammatory condition, a decreased VDR expression has been observed in the affected placenta (Cookson et al., 2018; Guevara et al., 2020; Knabl et al., 2017).

Decreased VDR expression may have negative consequences on immune function and inflammation at the placental level. For instance, reduced VDR levels can lead to excessive activation of the immune system and increased release of proinflammatory cytokines, as well as diminished resistance to infections within the maternal–fetal environment, which may exacerbate inflammation and impair maternal–fetal exchange at the placental interface.

Given the important protective effects of vitamin D in fetal development, screening for vitamin D levels during the preconception period and the first trimester of pregnancy should be recommended for women at increased risk of hypovitaminosis D—such as those with a high body mass index, dark skin, or autoimmune diseases—in order to initiate early and appropriate treatment and thus prevent adverse obstetric outcomes (Cyprian et al., 2019).

The research conducted within this doctoral thesis aimed to enhance current knowledge regarding the microorganisms involved in the development of various maternal and fetal pathologies, as well as to contribute to the development of diagnostic, treatment, and prevention protocols for such infections.

I consider this study to be the first in Romania to investigate placental biomarkers in pregnant patients, thus representing a pioneering effort that may lay the foundation for future prevention guidelines and, potentially, for the early identification of factors that contribute to the favorable progression of pregnancy. In this way, maternal–fetal complications arising from SARS-CoV-2 infection associated with pregnancy—which carries a high risk of maternal and fetal morbidity and mortality—may be reduced.

The doctoral study analyzed the involvement of COVID-19 in triggering adverse events among pregnant women, which may lead to spontaneous abortion, oligohydramnios, fetal growth restriction, premature detachment of a normally inserted placenta, postpartum hemorrhage, intrauterine fetal demise, low Apgar scores, congenital viral syndromes, fetal congenital malformations, maternal preeclampsia, preterm birth, and the need for delivery by cesarean section.

The study included 70 healthy pregnant women, 65 COVID-19–positive pregnant women (with the COVID-19 diagnosis established at least 14 days before delivery), and 35 vaccinated pregnant women (who had received the first or second dose of the Pfizer mRNA vaccine at least 14 days prior to delivery), with samples collected between January 2021 and January 2023.

For patients admitted with threatened preterm labor or miscarriage, spontaneous abortion in the second trimester, preterm labor, or premature rupture of membranes, sample collection is intended for PCR testing for COVID-19 and for determining serum vitamin D levels, in order to assess the involvement of these factors in preterm birth and potentially develop treatment protocols for such cases based on serum or amniotic fluid biomarker levels.

The age group distribution of the pregnant women included in the study showed that the groups positive for COVID-19 infection exhibited the lowest levels of vitamin D receptor (VDR) expression on the cell surface. Immunohistochemical analysis revealed differences in VDR expression among the groups. Thus, the group of COVID-19–positive pregnant women demonstrated the lowest VDR expression, and a significant positive correlation was observed between gestational age at delivery and neonatal birth weight.

The mean gestational age at delivery showed significant differences among the three groups: COVID-19–positive and COVID-19–vaccinated pregnant women had a lower gestational age at birth (an average of 38 weeks) compared with the healthy unvaccinated group, which had an average of 40 weeks. This statistically significant difference among the three groups is also evident when analyzing newborn birth weight, with infants from the healthy unvaccinated group having a higher average birth weight (approximately 3500 g) compared to those born to vaccinated or COVID-19–positive pregnant women (approximately 3200 g). However, when analyzing the immediate postnatal status of the newborns, as reflected by the Apgar score, no significant differences were found among the three groups.

Regarding the evaluation of serum infection and inflammation markers, no statistically significant differences were identified in C-reactive protein (CRP) levels among the pregnant participants. However,

pregnant women without prior infection or vaccination exhibited significantly higher leukocyte counts, with similar values observed among those who had experienced infection. Likewise, no statistically significant differences were found in hemoglobin or hematocrit values, either before or after delivery, among the three groups.

A significant difference was observed among the three analyzed groups, with a markedly higher number of VDR-negative cases in placental tissue samples from COVID-19–vaccinated women. The number of VDR-positive cases was higher in healthy unvaccinated pregnant women compared to both COVID-19–positive and vaccinated groups. The results of the present study indicated similar C-reactive protein (CRP) levels among the three studied groups and higher leukocyte counts in the COVID-19–negative and unvaccinated groups. The source of this inconsistency may be attributed to the limited number of excluded cases due to the strict inclusion and exclusion criteria, or possibly to regional particularities.

Compared to placentas from uninfected and unvaccinated pregnant women, no significant differences were observed in the macroscopic or microscopic placental appearance, including placental weight, abnormal umbilical cord insertion, or maternal and fetal vascular malperfusion. However, since most of the analyzed placentas were uninfected and the majority of newborns tested negative for coronavirus infection, the findings largely derived from the examination of healthy placentas from uninfected fetuses (Schwartz et al., 2021). The histopathological analysis of the placenta included gross examination, hematoxylin–eosin (H&E) staining, immunohistochemistry, and reverse transcriptase–polymerase chain reaction (RT-PCR).

Although we observed a lower birth weight in fetuses born to COVID-19–positive and COVID-19–vaccinated pregnant women compared to the control group, the difference was not statistically significant. However, we found increased expression of the vitamin D receptor (VDR) in placental tissue from healthy unvaccinated women compared with the other two study groups—the infected and the vaccinated. These two findings appear to be interconnected and may serve as a starting point for future management strategies of this condition in pregnant women.

Chapter 5

Placental Molecular Expression in Various Vaginal Infections with Pathogenic Potential

One of the most common pathologies encountered during pregnancy is vaginal infection, which plays a significant role in the occurrence of adverse maternal and fetal outcomes, increasing both neonatal and maternal morbidity and mortality (Daskalakis et al., 2023). Vaginal infections may lead to endometritis, preterm birth (PTB), premature rupture of membranes (PROM) or preterm premature rupture of membranes (PPROM), spontaneous abortion, intrauterine growth restriction (IUGR), low birth weight (LBW), fetal infection, and—in severe cases—fetal death (Daskalakis et al., 2023; Tita & Andrews, 2010; Williams et al., 2000).

Chorioamnionitis may develop before labor, during labor, or after delivery, and it can present as an acute, subacute, or chronic infection. This condition is associated with chronic lung disease in infants, retinopathy of prematurity, very low birth weight, and impaired brain development in preterm infants, as well as with the vertical transmission of the infectious process (Chansamouth et al., 2016; Dautt-Leyva et al., 2018).

A wide variety of microorganisms are involved in genital tract infections in women during pregnancy, including Group B Streptococcus (GBS), *Escherichia coli* (E. coli), *Neisseria gonorrhoeae*, *Enterococcus faecalis*, *Enterobacter* spp., *Trichomonas vaginalis*, *Candida* spp., and bacterial vaginosis (BV). Microorganisms responsible for pelvic inflammatory disease, such as *Ureaplasma urealyticum*, *Mycoplasma hominis*, and *Chlamydia trachomatis*, also contribute significantly (Daskalakis et al., 2023). In particular, *Mycoplasma* and *Ureaplasma* produce cytotoxic substances that amplify inflammation by inducing cyclooxygenase-2 (COX-2) synthesis and the subsequent formation of prostaglandins. Hormonal changes during pregnancy increase susceptibility to *Candida* spp., with vaginal colonization observed in approximately 40% of pregnant women. Although this generally leads to less severe complications compared to other infections, it may still result in late spontaneous abortion, preterm birth (PTB), and chorioamnionitis (Daskalakis et al., 2023). This research was conducted to identify the underlying causes of the inflammatory response by evaluating four closely related molecules with significant roles in

inflammation, as well as in placental structure and function: the Vitamin D Receptor (VDR), Cluster of Differentiation 44 (CD44), Osteopontin (OPN), and Cyclooxygenase-2 (COX-2).

This research aimed to investigate how different types of vaginal infections during pregnancy alter the expression of VDR, CD44, OPN, and COX-2, thereby contributing to structural and functional placental changes. By analyzing these markers, we hope to obtain a plausible explanation as to why some microorganisms exert a more significant negative impact on maternal and fetal outcomes than others.

Vaginal cultures (standard and special media cultures) were collected from patients prior to the initiation of antibiotic therapy, at the time of hospital admission. The samples were obtained without the use of antiseptics, and microbiological cultures were subsequently performed.

Four tissue samples from each placenta, one representative from each quadrant, were collected. Hematoxylin and eosin (H&E) staining and immunohistochemical (IHC) analysis were performed and examined histopathologically. The IHC staining was conducted on formalin-fixed, paraffin-embedded tissues using monoclonal antibodies against VDR, CD44, OPN, and COX-2.

This research aimed to investigate how different types of vaginal infections during pregnancy alter the expression of VDR, CD44, OPN, and COX-2, thereby contributing to structural and functional placental changes. Through the analysis of these markers, we aim to obtain a plausible explanation for why certain microorganisms exert a more pronounced negative impact on maternal and fetal outcomes than others.

The patients were grouped according to maternal age and gestational age at the time of admission. Accordingly, three experimental groups were established:

- Group 1 – 28–32 weeks of gestation
- Group 2 – 33–36 weeks of gestation
- Group 3 – more than 37 weeks of gestation

Vaginal cultures (standard and specialized media) were collected from patients prior to the initiation of antibiotic therapy, at the time of hospital admission. The samples were obtained without the use of antiseptics, and cultures were subsequently performed. Control group specimens were collected from women who delivered at term, without vaginal infections, complications, associated diseases, or chronic treatment. Women with obstetrical and/or other medical complications were excluded from the analysis. For the detection of *Ureaplasma* and *Mycoplasma*, bioMérieux kits were used, with cultures performed on selective media. For the detection of other bacteria involved in genital infections in pregnant women, selective and differential culture media were employed. For fungal detection, the ELITech CANDIFAST® kit was used. In addition to the vaginal cultures collected, blood samples were also taken to assess infection or chorioamnionitis (white blood cell count, C-reactive protein, and erythrocyte sedimentation rate). A total of four tissue samples were collected from each placenta, one representative from each quadrant. Hematoxylin and eosin (H&E) sections and immunohistochemical (IHC) analyses were performed and examined in the pathology laboratory. All placental samples were evaluated for the presence of VDR, CD44, OPN, and COX-2.

Patient sampling was performed as follows: seventy healthy term pregnant women were included in the control group, and seventy-eight third-trimester pregnant women with vaginal infection caused by a single pathogen identified through vaginal culture were included in the vaginal infection group. Statistical analysis revealed significant differences in gestational age at delivery. Pregnant women without vaginal infection delivered, on average, five weeks later than those in the vaginal infection group. Consequently, the mean birth weight between the two groups also differed by approximately 1,000 grams. As expected, the mean CRP value was also statistically significant, being eight times higher in pregnant women with vaginal infections.

The results revealed a wide range of pathogens involved in vaginal infections, with *E. coli* and Group B *Streptococcus* (GBS) occupying the first two positions, followed closely—at similar percentages—by *Candida* spp., *Enterococcus*, and *Ureaplasma urealyticum*, while the lowest incidence was recorded for vaginal infections caused by *Klebsiella*.

The analysis of the biomarkers assessed in this study revealed variability in their expression. Pregnant women with vaginal infections exhibited lower CD44 levels compared to controls, while OPN expression varied depending on the pathogen involved, showing a significantly higher expression in the vaginal infection group compared with the control group. COX-2 was expressed uniformly in almost all cases, whereas VDR was positive in three-quarters of all vaginal infection cases included in the study. Infections caused by *E. coli*, *Klebsiella*, Group B *Streptococcus* (GBS), *Enterococcus faecalis*, *Candida* spp., and *Ureaplasma urealyticum* can significantly alter the placental expression of VDR, CD44, OPN, and COX-2. The findings indicate that tissue expression of VDR was generally variable but often decreased,

while CD44 expression was elevated in most infections. OPN expression was largely absent, whereas COX-2 expression was increased in the majority of vaginal infection cases.

Our results suggest that pregnant women have a 1.67-fold increased risk of developing a vaginal infection if they are CD44-negative, a 1.102-fold increased risk if they are VDR-negative, and a 2.933-fold higher risk of having a vaginal infection if they are OPN-positive.

To date, many variables remain unknown regarding maternal and fetal outcomes in pregnant women with various vaginal infections. Oh et al. emphasized the limitations of studies in identifying infections through standard vaginal culture techniques in women with preterm birth and clinical chorioamnionitis (Witt et al., 2020).

The present study raises ongoing debates in the scientific literature regarding these aspects, aiming to provide a plausible histological explanation for the existing discrepancies. Given that the vaginal microbiome is highly diverse and influenced by numerous intrinsic and extrinsic factors, we chose to investigate key placental surface molecules to identify the actual placental impact of vaginal infection. The clinical manifestations of the women with vaginal infections included in the study varied greatly, ranging from signs and symptoms characteristic of chorioamnionitis to completely asymptomatic cases.

Consistent with the present observations, studies by Al-Adnani et al. and N.J. Sebire et al. reported septic infarcts, intervillous thrombi, and necrotizing villitis accompanied by perivillous inflammation in women with vaginal infections, in contrast to the findings observed in the control group during placental H&E examination (Al-Adnani & Sebire, 2007; Heerema-McKenney, 2018).

In accordance with findings from the scientific literature, our results indicate a lower gestational age and consequently a lower birth weight in women with PROM and PPROM who had positive vaginal cultures (Holliday et al., 2023; H. Li et al., 2022). The vaginal infection group also exhibited a slightly lower Apgar score compared to the control group. The minimal difference may be attributed to our inclusion criteria, as only pregnancies beyond 28 weeks of gestation with positive vaginal cultures and term pregnancies with negative vaginal cultures in the control group were selected. The vaginal infection group showed a higher leukocyte count compared to the control group. However, the difference was not statistically significant. A possible explanation could be that the women presented to the hospital within 12 hours after PROM/PPROM, at which time vaginal cultures and blood samples were collected and empirical antibiotic therapy was initiated. Nevertheless, the difference in CRP values between the two groups was statistically significant, with higher levels observed in the vaginal infection group.

The placenta is essential for fetal development and well-being. Despite extensive research on its numerous roles and functions, many aspects remain largely unknown (Cao et al., 2021). The four molecules studied—VDR, CD44, OPN, and COX-2—are involved in many placental processes (Baines et al., 2023; Cao et al., 2021; Choi et al., 2006; Gonzalez et al., 2007). Our results show that GBS and *Ureaplasma urealyticum* exert the most significant impact on the four markers analyzed, with elevated placental tissue levels of VDR, CD44, OPN, and COX-2. Moreover, *Enterococcus*, *E. coli*, and the absence of vaginal infection in the control group exhibited a nearly similar impact on the levels of the aforementioned markers, with OPN being the least affected. Vaginal infections caused by *Candida* resulted in minor alterations in the tissue expression of these markers, lower than those observed in the control group. The least significant impact was exerted by *Klebsiella* infection. Therefore, according to the observations reported in this study, the most aggressive microorganisms remain GBS and *Ureaplasma urealyticum*.

Tissue samples from pregnant women included in the study, both from the vaginal infection group and the control group, showed similarly strong COX-2 positivity. Thus, the expression of this molecule appears to be more closely related to inflammation than to infection. The elevated COX-2 levels observed in both groups may be explained by the fact that placental tissue for the control group was collected from term pregnancies near the onset of labor, when COX-2 expression and prostaglandin production are naturally increased to initiate delivery (Urrego et al., 2019).

Vitamin D, mediated through VDR, plays key roles in reproductive tissues, including the vagina. It regulates antimicrobial molecules that provide protection against bacterial infections and may also influence defensin production and neutrophil function (Anderson et al., 2020). Researchers have made significant observations regarding vitamin D supplementation, highlighting its beneficial effects, including a decreased frequency of vaginal *E. coli* infections. Furthermore, as suggested by the study of Cao et al. (2021), vitamin D downregulates COX-2 signaling and expression, thereby reducing inflammation (Cao et al., 2021). We identified elevated placental VDR levels in cases with vaginal infections caused by GBS, *Ureaplasma urealyticum*, and *Enterococcus*.

Tissue samples from the control group without vaginal infections showed VDR expression levels similar to those observed in women with vaginitis caused by *Candida* and *E. coli*. These results may be explained by the opportunistic pathogenicity of both *E. coli* and *Candida* (d'Enfert et al., 2021; O'Brien et al., 2019).

Analyzing OPN distribution in the samples, we detected similar levels of this inflammation- and immunity-modulating molecule in both the control group samples and those infected with Gram-negative enterobacteria. These observations are consistent with those of Salvi et al., who highlighted elevated OPN levels in Gram-positive infections that activate Toll-like receptor (TLR) 2, and lower levels in Gram-negative pathogens that trigger TLR 3 and 4 (Salvi et al., 2013).

All three markers, except COX-2, appear to correlate with the severity of vaginal infection and its causative pathogen in the last trimester of pregnancy. Vitamin D supplementation could be extremely beneficial in reducing host susceptibility to vaginal infections by lowering placental VDR expression. Moreover, if these findings are confirmed by larger population-based studies, it may be possible to develop local or systemic anti-CD44 and anti-OPN agents to help control the severity of the host immune response in chorioamnionitis.

The main limitations of this study lie in the relatively small number of cases included. Despite the similarity with findings reported in the literature, the results described and discussed above should be interpreted with caution until validated by larger studies. Another limitation arises from the inclusion of vaginitis cases caused by a single pathogen, whereas many vaginal infections are polymicrobial in nature, involving two or more pathogens. Additionally, the control group consisted exclusively of term pregnancies, which may limit the generalizability of the findings.

Variations were successfully detected in placental adhesion, angiogenesis, cell migration, differentiation, and the expression of immune-modulating molecules (VDR, CD44, OPN, and COX-2) across different vaginal infections. Although they exert significant effects during infection, gram-negative pathogens are able to evade the host immune system. This phenomenon was also evident in the present results, showing lower IHC positivity levels for the aforementioned molecules in vaginal infections caused by *E. coli*, *Enterobacter*, and *Klebsiella*. The pathogens showing the highest IHC positivity for VDR, CD44, OPN, and COX-2 were GBS and *Ureaplasma urealyticum*. In contrast, the most common vaginal infection during pregnancy — that caused by *Candida* spp. — was associated with low IHC expression of these tissue markers.

CONCLUSIONS

The expression of placental cellular biomarkers (Osteopontin, Phosphohistone 3, Vitamin D Receptor, CD44, and COX-2) is significantly influenced by SARS-CoV-2 infection status, with clear differences observed among negative, vaccinated, and positive pregnant women.

SARS-CoV-2 infection during pregnancy can lead to significant histopathological placental changes, including maternal and fetal vascular hypoperfusion, acute and chronic inflammation, intervillous thrombosis, and increased fibrin deposition. Placental Vitamin D Receptor (VDR) levels showed considerable variability depending on the immune status induced by COVID-19 infection or vaccination, supporting the hypothesis of a significant immunomodulatory role of this receptor during pregnancy.

The study demonstrated significant alterations in placental structure and function, reflected by modified expression of the CD44, COX-2, and OPN markers. An increased expression of CD44 and COX-2 was observed in the placentas of uninfected and unvaccinated pregnant women, indicating a normal physiological placentation process. In contrast, these expressions were significantly reduced in SARS-CoV-2–infected patients, suggesting a virus-induced inflammatory placental dysfunction. The vaccinated group showed intermediate values, which may indicate a partial protective effect of vaccination on the trophoblast.

The results revealed notable variations in the expression of VDR, CD44, OPN, and COX-2 in the placentas of women with vaginal infections during the third trimester of pregnancy. Gram-negative pathogens (E. coli, Enterobacter, Klebsiella) were associated with reduced levels of these markers, indicating an enhanced ability to evade maternal immunity. In contrast, Streptococcus agalactiae (GBS) and Ureaplasma urealyticum showed the highest levels of immunohistochemical expression.

The study provides a foundation for updating clinical protocols related to the monitoring of pregnant women in the context of viral and bacterial infections, highlighting the importance of early vaginal flora screening and immunomodulatory intervention in high-risk pregnancies. Moreover, the findings support the

hypothesis that disturbances of the vaginal microbiota and genital infections act as triggering factors for adverse obstetric events such as preterm birth, premature rupture of membranes, and chorioamnionitis.

The immunohistochemical analysis of the placenta in the context of the COVID-19 pandemic provides valuable insights into the inflammatory mechanisms involved in obstetric pathology, serving as an important tool for both diagnosis and prevention.

The results suggest that maternal immunization contributes not only to the prevention of severe viral infection but also to the maintenance of optimal placental function. Vaccination during pregnancy was associated with a placental histological profile more similar to that of uninfected patients, supporting a protective role of immunization in the context of SARS-CoV-2 infection..

Therefore, vaginal infections caused by the studied pathogens had a significant impact on maternal, fetal, and neonatal morbidity and mortality, ultimately affecting quality of life from both social and emotional perspectives.

REFERINȚE

Al-Adnani, M., & Sebire, N. J. (2007). The role of perinatal pathological examination in subclinical infection in obstetrics. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *21*(3), 505–521. https://doi.org/10.1016/j.bpobgyn.2007.02.001

Anderson, S. M., Thurman, A. R., Chandra, N., Jackson, S. S., Asin, S., Rollenhagen, C., Ghosh, M., Daniels, J., Vann, N. C., Clark, M. R., & Doncel, G. F. (2020). Vitamin D Status Impacts Genital Mucosal Immunity and Markers of HIV-1 Susceptibility in Women. *Nutrients*, *12*(10), 3176. https://doi.org/10.3390/nu12103176

Baines, K. J., Klausner, M. S., Patterson, V. S., & Renaud, S. J. (2023). Interleukin-15 deficient rats have reduced osteopontin at the maternal-fetal interface. *Frontiers in Cell and Developmental Biology*, *11*. https://doi.org/10.3389/fcell.2023.1079164

Bermick, J., Gallagher, K., denDekker, A., Kunkel, S., Lukacs, N., & Schaller, M. (2019). Chorioamnionitis exposure remodels the unique histone modification landscape of neonatal monocytes and alters the expression of immune pathway genes. *The FEBS Journal*, *286*(1), 82–109. https://doi.org/10.1111/febs.14728

Bertero, L., Borella, F., Botta, G., Carosso, A., Cosma, S., Bovetti, M., Carosso, M., Abbona, G., Collemi, G., Papotti, M., Cassoni, P., & Benedetto, C. (2021). Placenta histopathology in SARS-CoV-2 infection: analysis of a consecutive series and comparison with control cohorts. *Virchows Archiv*, *479*(4), 715–728. https://doi.org/10.1007/s00428-021-03097-3

Brown, R. G., Al-Memar, M., Marchesi, J. R., Lee, Y. S., Smith, A., Chan, D., Lewis, H., Kindinger, L., Terzidou, V., Bourne, T., Bennett, P. R., & MacIntyre, D. A. (2019). Establishment of vaginal microbiota composition in early pregnancy and its association with subsequent preterm prelabor rupture of the fetal membranes. *Translational Research*, *207*, 30–43. https://doi.org/10.1016/j.trsl.2018.12.005

Cao, Y., Jia, X., Huang, Y., Wang, J., Lu, C., Yuan, X., Xu, J., & Zhu, H. (2021). Vitamin D stimulates miR-26b-5p to inhibit placental COX-2 expression in preeclampsia. *Scientific Reports*, *11*(1), 11168. https://doi.org/10.1038/s41598-021-90605-9

Chansamouth, V., Thammasack, S., Phetsouvanh, R., Keoluangkot, V., Moore, C. E., Blacksell, S. D., Castonguay-Vanier, J., Dubot-Pérès, A., Tangkhabuanbutra, J., Tongyoo, N., Souphaphonh, P., Sengvilaipaseuth, O., Vongsouvath, M., Phommasone, K., Sengdethka, D., Seurbsanith, A., Craig, S. B., Hermann, L., Strobel, M., & Newton, P. N. (2016). The Aetiologies and Impact of Fever in Pregnant Inpatients in Vientiane, Laos. *PLOS Neglected Tropical Diseases*, *10*(4), e0004577. https://doi.org/10.1371/journal.pntd.0004577

Choi, C. H., Roh, C. R., Kim, T.-J., Choi, Y.-L., Lee, J.-W., Kim, B.-G., Lee, J.-H., & Bae, D.-S. (2006). Expression of CD44 adhesion molecules on human placentae. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *128*(1–2), 243–247. https://doi.org/10.1016/j.ejogrb.2006.01.017

Cookson, M., Ryan, S., Seedorf, G., Dodson, R., Abman, S., & Mandell, E. (2018). Antenatal Vitamin D Preserves Placental Vascular and Fetal Growth in Experimental Chorioamnionitis Due to Intra-amniotic Endotoxin Exposure. *American Journal of Perinatology*, *35*(13), 1260–1270. https://doi.org/10.1055/s-0038-1642033

Cyprian, F., Lefkou, E., Varoudi, K., & Girardi, G. (2019). Immunomodulatory Effects of Vitamin D in Pregnancy and Beyond. *Frontiers in Immunology*, *10*. https://doi.org/10.3389/fimmu.2019.02739

Daskalakis, G., Psarris, A., Koutras, A., Fasoulakis, Z., Prokopakis, I., Varthaliti, A., Karasmani, C., Ntounis, T., Domali, E., Theodora, M., Antsaklis, P., Pappa, K. I., & Papapanagiotou, A. (2023). Maternal Infection and Preterm Birth: From Molecular Basis to Clinical Implications. *Children*, *10*(5), 907. https://doi.org/10.3390/children10050907

Dautt-Leyva, J. G., Canizalez-Román, A., Acosta Alfaro, L. F., Gonzalez-Ibarra, F., & Murillo-Llanes, J. (2018). Maternal and perinatal complications in pregnant women with urinary tract infection caused by <scp> *Escherichia coli* </scp>. *Journal of Obstetrics and Gynaecology Research*, *44*(8), 1384–1390. https://doi.org/10.1111/jog.13687

Debelenko, L., Katsyv, I., Chong, A. M., Peruyero, L., Szabolcs, M., & Uhlemann, A.-C. (2021). Trophoblast damage with acute and chronic intervillositis: disruption of the placental barrier by severe acute respiratory syndrome coronavirus 2. *Human Pathology*, *109*, 69–79. https://doi.org/10.1016/j.humpath.2020.12.004

d'Enfert, C., Kaune, A.-K., Alaban, L.-R., Chakraborty, S., Cole, N., Delavy, M., Kosmala, D., Marsaux, B., Fróis-Martins, R., Morelli, M., Rosati, D., Valentine, M., Xie, Z., Emritloll, Y., Warn, P. A., Bequet, F., Bougnoux, M.-E., Bornes, S., Gresnigt, M. S., … Brown, A. J. P. (2021). The impact of the Fungus-Host-Microbiota interplay upon *Candida albicans* infections: current knowledge and new perspectives. *FEMS Microbiology Reviews*, *45*(3). https://doi.org/10.1093/femsre/fuaa060

Di Girolamo, R., Khalil, A., Alameddine, S., D'Angelo, E., Galliani, C., Matarrelli, B., Buca, D., Liberati, M., Rizzo, G., & D'Antonio, F. (2021). Placental histopathology after SARS-CoV-2 infection in pregnancy: a systematic review and meta-analysis. *American Journal of Obstetrics & Gynecology MFM*, *3*(6), 100468. https://doi.org/10.1016/j.ajogmf.2021.100468

El-Kassas, M., Alboraie, M., Elbadry, M., El Sheemy, R., Abdellah, M., Afify, S., Madkour, A., Zaghloul, M., Awad, A., Wifi, M.-N., Al Balakosy, A., & Eltabbakh, M. (2023). Non-pulmonary involvement in COVID-19: A systemic disease rather than a pure respiratory infection. *World Journal of Clinical Cases*, *11*(3), 493–505. https://doi.org/10.12998/wjcc.v11.i3.493

Garg, R., Agarwal, R., Yadav, D., Singh, S., Kumar, H., & Bhardwaj, R. (2023). Histopathological Changes in Placenta of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Cov-2) Infection and Maternal and Perinatal Outcome in COVID-19. *The Journal of Obstetrics and Gynecology of India*, *73*(1), 44–50. https://doi.org/10.1007/s13224-022-01666-3

Giordano, G., Petrolini, C., Corradini, E., Campanini, N., Esposito, S., & Perrone, S. (2021). COVID-19 in pregnancy: placental pathological patterns and effect on perinatal outcome in five cases. *Diagnostic Pathology*, *16*(1), 88. https://doi.org/10.1186/s13000-021-01148-6

Gonzalez, J. M., Xu, H., Ofori, E., & Elovitz, M. A. (2007). Toll-like receptors in the uterus, cervix, and placenta: is pregnancy an immunosuppressed state? *American Journal of Obstetrics and Gynecology*, *197*(3), 296.e1-296.e6. https://doi.org/10.1016/j.ajog.2007.06.021

Gonçalves, B. P., Procter, S. R., Paul, P., Chandna, J., Lewin, A., Seedat, F., Koukounari, A., Dangor, Z., Leahy, S., Santhanam, S., John, H. B., Bramugy, J., Bardají, A., Abubakar, A., Nasambu, C., Libster, R., Sánchez Yanotti, C., Horváth-Puhó, E., Sørensen, H. T., … Mahtab, S. (2022). Group B streptococcus infection during pregnancy and infancy: estimates of regional and global burden. *The Lancet Global Health*, *10*(6), e807–e819. https://doi.org/10.1016/S2214-109X(22)00093-6

Guevara, M. A., Lu, J., Moore, R. E., Chambers, S. A., Eastman, A. J., Francis, J. D., Noble, K. N., Doster, R. S., Osteen, K. G., Damo, S. M., Manning, S. D., Aronoff, D. M., Halasa, N. B., Townsend, S. D., & Gaddy, J. A. (2020). Vitamin D and Streptococci: The Interface of Nutrition, Host Immune Response, and Antimicrobial Activity in Response to Infection. *ACS Infectious Diseases*, *6*(12), 3131–3140. https://doi.org/10.1021/acsinfecdis.0c00666

Guzeloglu-Kayisli, O., Kayisli, U. A., Semerci, N., Basar, M., Buchwalder, L. F., Buhimschi, C. S., Buhimschi, I. A., Arcuri, F., Larsen, K., Huang, J. S., Schatz, F., & Lockwood, C. J. (2015). Mechanisms of chorioamnionitis-associated preterm birth: interleukin-1β inhibits progesterone receptor expression in decidual cells. *The Journal of Pathology*, *237*(4), 423–434. https://doi.org/10.1002/path.4589

Heerema-McKenney, A. (2018). Defense and infection of the human placenta. *APMIS*, *126*(7), 570–588. https://doi.org/10.1111/apm.12847

Holliday, M., Uddipto, K., Castillo, G., Vera, L. E., Quinlivan, J. A., & Mendz, G. L. (2023). Insights into the Genital Microbiota of Women Who Experienced Fetal Death in Utero. *Microorganisms*, *11*(8), 1877. https://doi.org/10.3390/microorganisms11081877

Hord, T. K., Aubone, A. M. P., Ali, A., Templeton, H. N., Evans, R., Bruemmer, J. E., Winger, Q. A., & Bouma, G. J. (2020). Placenta specific gene targeting to study histone lysine demethylase and androgen signaling in ruminant placenta. *Animal Reproduction*, *17*(3). https://doi.org/10.1590/1984-3143-ar2020-0069

Joshi, B., Chandi, A., Srinivasan, R., Saini, S. S., Prasad, G. R. V., Puri, G. D., Bhalla, A., Suri, V., & Bagga, R. (2022). The placental pathology in Coronavirus disease 2019 infected mothers and its impact on pregnancy outcome. *Placenta*, *127*, 1–7. https://doi.org/10.1016/j.placenta.2022.07.009

Knabl, J., Vattai, A., Ye, Y., Jueckstock, J., Hutter, S., Kainer, F., Mahner, S., & Jeschke, U. (2017). Role of Placental VDR Expression and Function in Common Late Pregnancy Disorders. *International Journal of Molecular Sciences*, *18*(11), 2340. https://doi.org/10.3390/ijms18112340

Kesby, J. P., Eyles, D. W., Burne, T. H. J., & McGrath, J. J. (2011). The effects of vitamin D on brain development and adult brain function. *Molecular and Cellular Endocrinology*, *347*(1–2), 121–127. https://doi.org/10.1016/j.mce.2011.05.014

Marzioni, D., Crescimanno, C., Zaccheo, D., Coppari, R., Underhill, C., & Castellucci, M. (2009). Hyaluronate and CD44 expression patterns in the human placenta throughout pregnancy. *European Journal of Histochemistry*, *45*(2), 131. https://doi.org/10.4081/1623

O'Brien, V. P., Gilbert, N. M., Lebratti, T., Agarwal, K., Foster, L., Shin, H., & Lewis, A. L. (2019). Low-dose inoculation of Escherichia coli achieves robust vaginal colonization and results in ascending infection accompanied by severe uterine inflammation in mice. *PLOS ONE*, *14*(7), e0219941. https://doi.org/10.1371/journal.pone.0219941

Patberg, E. T., Adams, T., Rekawek, P., Vahanian, S. A., Akerman, M., Hernandez, A., Rapkiewicz, A. V., Ragolia, L., Sicuranza, G., Chavez, M. R., Vintzileos, A. M., & Khullar, P. (2021). Coronavirus disease 2019 infection and placental histopathology in women delivering at term. *American Journal of Obstetrics and Gynecology*, *224*(4), 382.e1-382.e18. https://doi.org/10.1016/j.ajog.2020.10.020

Peaceman, A., Lai, Y., Rouse, D., Spong, C., Mercer, B., Varner, M., Thorp, J., Ramin, S., Malone, F., O'Sullivan, M., & Hankins, G. (2014). Length of Latency with Preterm Premature Rupture of Membranes before 32 Weeks' Gestation. *American Journal of Perinatology*, *32*(01), 057–062. https://doi.org/10.1055/s-0034-1373846

Prochaska, E., Jang, M., & Burd, I. (2020). COVID-19 in pregnancy: Placental and neonatal involvement. *American Journal of Reproductive Immunology*, *84*(5). https://doi.org/10.1111/aji.13306

Romero, R., Dey, S. K., & Fisher, S. J. (2014). Preterm labor: One syndrome, many causes. *Science*, *345*(6198), 760–765. https://doi.org/10.1126/science.1251816

Salvi, V., Scutera, S., Rossi, S., Zucca, M., Alessandria, M., Greco, D., Bosisio, D., Sozzani, S., & Musso, T. (2013). Dual regulation of osteopontin production by TLR stimulation in dendritic cells. *Journal of Leukocyte Biology*, *94*(1), 147–158. https://doi.org/10.1189/jlb.0412194

Sarno, L., Locci, M., Fulgione, C., Perillo, F., Dell'Isola, A., Mantelli, D., Sibillo, C., Saccone, G., Maruotti, G. M., Terracciano, D., Bifulco, G., Guida, M., & D'Armiento, M. (2022). Characteristics of Placental Histopathology in Women with Uncomplicated Pregnancies Affected by SARS-CoV-2 Infection at the Time of Delivery: A Single-Center Experience. *Biomedicines*, *10*(12), 3003. https://doi.org/10.3390/biomedicines10123003

Schwartz, D. A., Bugatti, M., Santoro, A., & Facchetti, F. (2021). Molecular Pathology Demonstration of SARS-CoV-2 in Cytotrophoblast from Placental Tissue with Chronic Histiocytic Intervillositis, Trophoblast Necrosis and COVID-19. *Journal of Developmental Biology*, *9*(3), 33. https://doi.org/10.3390/jdb9030033

Shanes, E. D., Mithal, L. B., Otero, S., Azad, H. A., Miller, E. S., & Goldstein, J. A. (2020). Placental Pathology in COVID-19. *American Journal of Clinical Pathology*, *154*(1), 23–32. https://doi.org/10.1093/ajcp/aqaa089

Tita, A. T. N., & Andrews, W. W. (2010). Diagnosis and Management of Clinical Chorioamnionitis. *Clinics in Perinatology*, *37*(2), 339–354. https://doi.org/10.1016/j.clp.2010.02.003

Tossetta, G., Fantone, S., delli Muti, N., Balercia, G., Ciavattini, A., Giannubilo, S. R., & Marzioni, D. (2022). Preeclampsia and severe acute respiratory syndrome coronavirus 2 infection: a systematic review. *Journal of Hypertension*, *40*(9), 1629–1638. https://doi.org/10.1097/HJH.0000000000003213

Thomas, J. R., Appios, A., Zhao, X., Dutkiewicz, R., Donde, M., Lee, C. Y. C., Naidu, P., Lee, C., Cerveira, J., Liu, B., Ginhoux, F., Burton, G., Hamilton, R. S., Moffett, A., Sharkey, A., & McGovern, N. (2021). Phenotypic and functional characterization of first-trimester human placental macrophages, Hofbauer cells. *Journal of Experimental Medicine*, *218*(1). https://doi.org/10.1084/jem.20200891

Urrego, D., Liwa, A. C., Cole, W. C., Wood, S. L., & Slater, D. M. (2019). Cyclooxygenase inhibitors for treating preterm labour: What is the molecular evidence? *Canadian Journal of Physiology and Pharmacology*, *97*(3), 222–231. https://doi.org/10.1139/cjpp-2018-0380

Valdespino-Vázquez, M. Y., Helguera-Repetto, C. A., León-Juárez, M., Villavicencio-Carrisoza, O., Flores-Pliego, A., Moreno-Verduzco, E. R., Díaz-Pérez, D. L., Villegas-Mota, I., Carrasco-Ramírez, E., López-Martínez, I. E., Giraldo-Gómez, D. M., Lira, R., Yocupicio-Monroy, M., Rodríguez-Bosch, M., Sevilla-Reyes, E. E., Cortés-Bonilla, M., Acevedo-Gallegos, S., Merchant-Larios, H., Cardona-Pérez, J. A., & Irles, C. (2021). Fetal and placental infection with SARS-CoV-2 in early pregnancy. *Journal of Medical Virology*, *93*(7), 4480–4487. https://doi.org/10.1002/jmv.26965

Vogel, I., Thorsen, P., Hogan, V. K., Schieve, L. A., Jacobsson, B., & Ferre, C. D. (2006). The joint effect of vaginal *Ureaplasma urealyticum* and bacterial vaginosis on adverse pregnancy outcomes. *Acta Obstetricia et Gynecologica Scandinavica*, *85*(7), 778–785. https://doi.org/10.1080/00016340500442423

Williams, M. C., O'Brien, W. F., Nelson, R. N., & Spellacy, W. N. (2000). Histologic chorioamnionitis is associated with fetal growth restriction in term and preterm infants. *American Journal of Obstetrics and Gynecology*, *183*(5), 1094–1099. https://doi.org/10.1067/mob.2000.108866