UNIVERSITÄTSKLINIKUM HAMBURG-EPPENDORF

Zentrum für Geburtshilfe, Kinder- und Jugendmedizin
Klinik für Geburtshilfe und Pränatalmedizin

Prof. Dr. med. Kurt Hecher

Maternal and placental galectin signature in normal and pathological pregnancies

Dissertation

zur Erlangung des Grades eines Doktors der Medizin
an der Medizinischen Fakultät der Universität Hamburg.

vorgelegt von:

Fangqi Zhao
aus Beijing China

Hamburg 2023

(wird von der Medizinischen Fakultät ausgefüllt)

Angenommen von der
Medizinischen Fakultät der Universität Hamburg am:
20.02.2024

Veröffentlicht mit Genehmigung der
Medizinischen Fakultät der Universität Hamburg.

Prüfungsausschuss, der/die Vorsitzende:
PD Dr. Kerstin Cornils

Prüfungsausschuss, zweite/r Gutachter/in:
Prof. Dr. Sandra Blois

Table of Contents

frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 05 July 2023
DOI 10.3389/fimmu.2023.1196395

OPEN ACCESS

EDITED BY
Ricardo Silvestre,
University of Minho, Portugal

REVIEWED BY
Patrícia Gonzalez-Dias,
University of Oxford, United Kingdom
Nora Heisterkamp,
Beckman Research Institute, United States

*CORRESPONDENCE
Sandra M. Blois
s.blois@uke.de

RECEIVED 29 March 2023
ACCEPTED 06 June 2023
PUBLISHED 05 July 2023

CITATION
Zhao F, Tallarek A-C, Wang Y, Xie Y, Diemert A, Lu-Culligan A, Vijayakumar P, Kittmann E, Urbschat C, Bayo J, Arck PC, Farhadian SF, Dveksler GS, Garcia MG and Blois SM (2023) A unique maternal and placental galectin signature upon SARS-CoV-2 infection suggests galectin-1 as a key alarmin at the maternal–fetal interface. *Front. Immunol.* 14:1196395. doi: 10.3389/fimmu.2023.1196395

A unique maternal and placental galectin signature upon SARS-CoV-2 infection suggests galectin-1 as a key alarmin at the maternal–fetal interface

Fangqi Zhao[1], Ann-Christin Tallarek[1], Yiru Wang[1], Yiran Xie[1], Anke Diemert[1], Alice Lu-Culligan[2], Pavithra Vijayakumar[3], Enrico Kittmann[1], Christopher Urbschat[1], Juan Bayo[4], Petra C. Arck[1], Shelli F. Farhadian[5], Gabriela S. Dveksler[6], Mariana G. Garcia[1] and Sandra M. Blois[1]*

[1]Department of Obstetrics and Fetal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [2]Department of Immunobiology, Yale School of Medicine, New Haven, CT, United States, [3]Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale School of Medicine, New Haven, CT, United States, [4]Gene Therapy Laboratory, Instituto de Investigaciones en Medicina Traslacional, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) - Universidad Austral, Buenos Aires, Argentina, [5]Section of Infectious Diseases, Department of Internal Medicine, Yale School of Medicine, Yale University, New Haven, CT, United States, [6]Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD, United States

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic imposed a risk of infection and disease in pregnant women and neonates. Successful pregnancy requires a fine-tuned regulation of the maternal immune system to accommodate the growing fetus and to protect the mother from infection. Galectins, a family of β-galactoside–binding proteins, modulate immune and inflammatory processes and have been recognized as critical factors in reproductive orchestration, including maternal immune adaptation in pregnancy. Pregnancy-specific glycoprotein 1 (PSG1) is a recently identified gal-1 ligand at the maternal–fetal interface, which may facilitate a successful pregnancy. Several studies suggest that galectins are involved in the immune response in SARS-CoV-2–infected patients. However, the galectins and PSG1 signature upon SARS-CoV-2 infection and vaccination during pregnancy remain unclear. In the present study, we examined the maternal circulating levels of galectins (gal-1, gal-3, gal-7, and gal-9) and PSG1 in pregnant women infected with SARS-CoV-2 before vaccination or uninfected women who were vaccinated against SARS-CoV-2 and correlated their expression with different pregnancy parameters. SARS-CoV-2 infection or vaccination during pregnancy provoked an increase in maternal gal-1 circulating levels. On the other hand, levels of PSG1 were only augmented upon SARS-CoV-2 infection. A healthy pregnancy is associated with a positive correlation between gal-1 concentrations and gal-3 or gal-9; however, no correlation was observed between these lectins during SARS-CoV-2 infection. Transcriptome analysis of the placenta showed that gal-1, gal-3, and several PSG and glycoenzymes responsible for the synthesis of gal-1-binding glycotopes (such as linkage-specific N-acetyl-glucosaminyltransferases (MGATs)) are upregulated in pregnant women infected with SARS-CoV-2. Collectively, our findings identify a

dynamically regulated "galectin-specific signature" that accompanies the SARS-CoV-2 infection and vaccination in pregnancy, and they highlight a potentially significant role for gal-1 as a key pregnancy protective alarmin during virus infection.

KEYWORDS

galectin-1, galectin-3, galectin-9, galectin-7, PSG1, SARS-CoV-2, COVID-19

1 Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection poses a particular risk to pregnant women and their neonates, in which pregnant women have higher rates of severe coronavirus disease 2019 (COVID-19) disease than non-pregnant adults (1). Pregnant women experience immunologic and physiologic changes, such as swelling of the respiratory tract and restricted lung expansion, that make them less tolerant to viral respiratory infections, especially in the last trimester (2, 3). Maternal SARS-CoV-2 infection affects pregnancy outcomes, with increased incidence rates of intensive care admission, invasive ventilation, maternal death, iatrogenic preterm birth, and stillbirth (4–12). Furthermore, comorbidities such as obesity, diabetes, heart disease, advanced maternal age, or a lack of vaccination increase the risk of severe COVID-19 symptoms (3, 11). A recent study revealed no association between the gestational age at infection and COVID-19 morbidity and mortality, suggesting that a previously reported increase in morbidity and mortality in the third trimester may be attributable to other gestational age–affected variables for which adjustment was made in this study (4, 13). Therefore, there is a continuing challenge for clinicians to manage the SARS-CoV-2 infection during pregnancy (1, 9). In this regard, SARS-CoV-2 vaccines have shown efficacy in preventing symptomatic maternal illness and are considered safe for both the mother and infant (8, 9, 14–16). SARS-CoV-2 antibodies have been documented in umbilical cord blood and breast milk after maternal vaccination, suggesting protection for the fetus (9, 14, 17–23). On the basis of the growing evidence that supports the safety and efficacy of COVID-19 vaccination in pregnancy (8, 9, 15–25), most countries recommend full COVID-19 vaccination, regardless of pregnancy trimester.

Galectins are a family of endogenous carbohydrate-binding proteins characterized by a unique sequence motif in their carbohydrate recognition domain (CRD), with the ability to bind β-galactosidase residues (6, 26–28). Galectins are classified into three major types: prototype, which contain one CRD and form homodimers (e.g., gal-1 and gal-7); chimera containing a C-terminal CRD and a proline- and glycine-rich N terminal tail that mediate their oligomerization (gal-3); and tandem repeats that have two different CRDs in tandem connected by a linker of up to 70 amino acids (e.g., gal-9). Galectins contribute to healthy gestation by modulating multiple immune and inflammatory processes (6). In normal pregnancy, circulating maternal gal-1 levels increase from the first trimester, peaking during the second trimester, and remain similar until term (28). Being less abundant, upregulation of gal-3, gal-7, and gal-9 levels in maternal circulation occurs mainly in the second trimester (26, 27). In the extracellular compartment, galectins bind to the glycans decorating glycoproteins. One identified gal-1 ligand at the maternal–fetal interface is pregnancy-specific glycoprotein 1 (PSG1); glycans in the N- and A2 domains of PSG1 mediate the interaction between these two molecules (29). PSGs belong to the carcinoembryonic antigen family within the immunoglobulin (Ig) superfamily and are secreted to the maternal circulation by trophoblast cells (30, 31). PSG1 is one of 10 PSGs and is considered one of the most abundant trophoblastic proteins in maternal circulation during the third trimester of pregnancy (31). PSG1 interacts with soluble and membrane-bound ligands and participates in processes required for successful pregnancy (29). Functional studies with recombinant PSG1 showed that this protein has pro-angiogenic activity; inhibits the interaction of fibrinogen with platelets; and regulates extravillous trophoblast adhesion, migration, and invasion (32–34). In addition, PSG1 activates the latent form of the anti-inflammatory cytokines Transforming growth factor β (TGF-β1) and (TGF-β2) by binding to the respective latent associated peptides contributing to the establishment of maternal immune tolerance (30, 35, 36). Dynamic changes in PSG1 maternal circulation levels during virus infection remain unexplored.

A growing body of clinical data suggests that the cytokine release syndrome is one of the main reasons for the high mortality observed in COVID-19 patients (37). Changes in the cytokine profile of pregnant women correlate with the clinical severity of patients with COVID-19 (10, 38). Recent studies revealed that circulating gal-1, gal-3, and gal-9 levels are increased in non-pregnant patients with COVID-19 (39–43). Levels of gal-3 and gal-9 were reported to be upregulated only in patients with severe disease (40, 41), raising the possibility that circulating gal-3 or gal-9 can be valuable biomarkers for severe pneumonia in patients with COVID-19 (44, 45). Interestingly, exogenous gal-9 administration during acute SARS-CoV-2 infection has been reported to increase the survival rate inducing a robust innate and adaptive immune response in mice (46). More recently, an inhaled gal-3 inhibitor has been tested as a potential therapy for COVID-19 pneumonitis (47). However, despite considerable progress in dissecting the functions of individual members of the

galectin family, there is no comprehensive study of the galectin signature in the maternal circulation and placenta following SARS-CoV-2 infection and vaccination during pregnancy.

Therefore, we aimed to determine the galectin fingerprint in the maternal and placental compartment during COVID-19 disease and SARS-CoV-2 vaccination in pregnancy. Our findings indicate that gal-1 is uniquely upregulated at the maternal circulation during SARS-CoV-2 infection and following vaccination. These results underscore the importance of gal-1 as a key pregnancy protective alarmin during SARS-CoV-2 infection. In addition, we observed that the concentration of PSG1, a gal-1 ligand, was increased following infection, providing the first indication of potential regulation of this trophoblast-derived protein in response to insults to maternal health.

2 Material and methods

2.1 Participants and data collection: human subjects for the study of SARS-CoV-2 infection and vaccination during pregnancy

For the current study, we used two pregnancy cohorts as follows.

2.1.1 PRINCE cohort

The PRINCE study is a longitudinal prospective cohort of pregnant women and their children located at the University Medical Center Hamburg-Eppendorf. This cohort aims to identify prenatal factors influencing maternal and children's future immune development and health. Women were included if they were at least 18 years old, were not expecting twins, and were between 12th and 14th ± 6 weeks of pregnancy with regular checking up by a specialized obstetrician. They were excluded if the pregnancy was conceived medically assisted, an autoimmune disorder was diagnosed, or fetal pathologies were observed. The PRINCE COVID cohort was established in March 2020 and recruited pregnant women infected with SARS-CoV-2 at any point during pregnancy. The third cohort, PRINCE VACCINE, included SARS-CoV-2–negative women vaccinated twice within an interval of 6 weeks with 30 µg of BNT162b2 messenger RNA COVID-19 vaccine during pregnancy. All study subjects signed informed consent forms, and the ethics committee of the Hamburg Chamber of Physicians (Ärztekammer Hamburg) approved the study protocol under the registration numbers PV3694 (PRINCE), PV 7312-4710 (PRINCE COVID), and 2021-10647-BO-ff (PRINCE VACCINE).

2.1.2 Yale IMPACT cohort

In the Yale study, women who were in labor at Yale New Haven Hospital from 27 March 2020 to 1 June 2020 and tested positive for SARS-CoV-2 by nasopharyngeal (NP) swab Quantitative reverse transcription polymerase chain reaction (RT-qPCR) were recruited to the Yale IMPACT study. These participants provided informed consent for research studies of donated placental tissue and blood. The placenta and blood of the control group were selected from SARS-CoV-2–uninfected women (as determined by negative RT-qPCR testing of NP swab) at Yale New Haven Hospital with matching maternal age, gestational age, and maternal comorbidities to the COVID-19 placental cases, recruited during the same months as the SARS-CoV-2–infected participants. They also provided informed consent to serve as uninfected controls for transcriptomic studies. The study was approved by the Yale Institutional Review Board (protocol #2000027690).

2.2 Determination of circulating galectins and PSG1 levels

For both PRINCE and Yale IMPACT studies, blood was collected in serum separator tubes, and serum was aliquoted and stored immediately at −80°C for further analysis. Human galectins and PSG1 levels were measured in the serum by enzyme-linked immunosorbent assay (ELISA) as previously described (26, 28). Briefly, Corning® 96-Well High-Binding (Fisher Scientific) was coated overnight with either polyclonal anti-human gal-1, gal-3, gal-7, gal-9, or anti-PSG1 antibodies (500-P210; PeproTech, AF 1154, 842118, AF 2045, and DY6799-05; R&D Systems, USA, respectively) and washed with washing buffer. Plates were blocked with 1%–2% Bovine serum albumin (BSA) in Phosphate-buffered saline (PBS). Individual wells were incubated with serial dilutions of galectins or PSG1 standards or serum samples for 1–2 h at room temperature (RT). Wells were washed and incubated with biotinylated polyclonal anti-human galectins or PSG1 antibodies (500-P210; PeproTech, AF 1154, 842118, AF 2045, and DY6799-05; R&D Systems, USA, respectively). Plates were washed three to six times and incubated with horseradish peroxidase–conjugated streptavidin (189733; Calbiochem, USA). After three to eight additional washes, a colorimetric reaction was developed with the 3,3,5,5′-tetramethyl benzidine. The reaction was stopped by adding one volume of 4 N H_2SO_4, and absorbance at 450 nm was recorded.

2.3 RNA sequencing data analysis

Bulk RNA sequencing analysis was performed on placental RNA sequencing data previously described (48). For the present study, FASTQ files were normalized with Kallisto v0.46.188 using the "-b 100 and -t 20" options to obtain transcript abundances in transcript per million (TPM). Expression data for our selected genes were downloaded, and differential expression analysis was performed using the Welch's t-test. Genes were considered differentially expressed if P-value < 0.05 (Supplementary Table 1). The fold change in gene expression was represented with Z-score after being calculated from TPM values and visualized as a heatmap.

2.4 Statistical analyses

GraphPad PRISM version 9 and R Statistical Software were utilized for statistical analysis. Correlations between the serum galectin levels and the clinical parameters were conducted using

Spearman's correlation analysis. Statistical parameters, including sample sizes and dispersion, are reported in the figures and their legends. Statistical difference between two groups was determined by an unpaired, two tailed *t*-test. One-way analysis of variance with Bonferroni or Dunn's multiple comparisons was used to compare different groups. If data did not meet test prerequisites, equivalent non-parametric tests were utilized. Data were considered to be statistically significant if $P \leq 0.05$.

3 Results

3.1 Clinical characteristics of patients in the PRINCE COVID cohort

To explore the impact of SARS-CoV-2 infection or vaccination in the maternal galectin profile, women from the PRINCE COVID cohort were grouped as follows: pregnant women who were tested positive for SARS-CoV-2 by NP swab qRT-PCR any time during pregnancy, matched control pregnant women tested negative for SARS-CoV-2 during the whole gestation, pregnant women who received two doses of vaccination, and matched control vaccinated non-pregnant women. Most of SARS-CoV-2 infections (75%, 15 of 20) were diagnosed during the second trimester of pregnancy, and all these patients had a negative viral test (NP swab qRT-PCR) at the time of delivery. Of the 30 patients, 24 (80%) received their first vaccination dose in the second trimester. Maternal and neonatal characteristics are summarized in Table 1. No severe COVID-19 disease (intensive care unit stay or administration of supplemental oxygen required) was observed in the PRINCE COVID cohort. All pregnancies included in the cohort resulted in live births, with no complications related to SARS-CoV-2 infection or vaccination. There were no significant differences among healthy pregnant women, pregnant women with COVID-19, and pregnant women who were vaccinated in terms of maternal age, body mass index (BMI), gestational age, mode of delivery, neonatal outcomes, or comorbidities.

3.2 Maternal circulating gal-1 levels increase with SARS-CoV-2 infection or vaccination in pregnant women

We first measured the circulating gal-1, gal-3, gal-7, and gal-9 levels of SARS-CoV-2–infected pregnant women, uninfected and unvaccinated healthy pregnant women, pregnant women who were vaccinated against SARS-CoV-2, and healthy vaccinated women who were not pregnant in the PRINCE cohort. Our results showed that circulating gal-1 levels were increased in pregnant women with antecedent SARS-CoV-2 infection (*P*-value = 0.0185) and those who were vaccinated against SARS-CoV-2 (*P*-value = 0.0054) compared with control healthy unvaccinated pregnant patients

TABLE 1 Clinical characteristics of the PRINCE cohorts.

	Pregnant healthy controls (n=20)	Pregnant COVID- 19 patients (n=20)	Pregnant vaccinated women (n=30)	Non-pregnant vaccinated controls (n=30)
Age: mean (range)	33.6 (27-42)	31.6 (22-40)	34.0 (26-39)	32.3 (22-42)
BMI: mean (SD)	22.1 (1.81)	22.0 (2.36)	26.6 (4.75)	24.1 (4.93)
Gestational week: median (range)	39 + 0 (37-41)	40 + 5 (38-41)	39 + 5 (38-42)	
Mode of delivery (% CS)	10.0%	20.0%	22%	
Sex of infant (% male)	60%	45.5%	50%	
Gravidity: median (range)	1 (1-2)	2 (1-6)	1 (1-3)	
Parity: median (range)	1 (0-2)	1 (0-4)	1 (0-3)	
Neonatal Apgar, 5 min:	10 (7-10)	10 (9-10)	10 (9-10)	
Infant body weight (g): mean (SD)	3513.5 (427.9)	3525.2 (478.2)	3396.5 (287.2)	
Comorbidities				
Hypertension	0	0	0	3.4%
Preeclampsia	0	5%	0	0
Diabetes	0	0	0	0
COVID-19 features				
COVID-19 symptoms at time of delivery (%)		75%		
severe COVID-19		0		

BMI, body mass index previously pregnancy in pregnant healthy controls and pregnant COVID-19 patients and at the time of the sample collection in pregnant vaccinated patients; CS, cesarean section.

(Figure 1A). We further observed that vaccinated women displayed higher levels of circulating gal-1 (*P*-value = 0.0313) if they were pregnant (Figure 1A), whereas they had similar levels of gal-3 in all the compared groups (Figure 1B). In addition, no differences were observed in the circulating levels of gal-7 and gal-9 between healthy pregnant women and the SARS-CoV-2 previously infected pregnant women (Figures 1C, D). Increased gal-9 levels in circulation were noticed in vaccinated pregnant women compared with the ones who were not vaccinated (*P*-value = 0.0243, Figure 1D). Furthermore, pregnant vaccinated women presented higher serum levels of gal-7 (*P*-value < 0.0001) and lower concentrations of gal-9 (*P*-value = 0.0015) compared with non-pregnant vaccinated group (Figures 1C, D).

3.3 Yale IMPACT cohort studies

We next sought to assess the galectins/PSG1 signature in acute SARS-CoV-2 infection during pregnancy. Table 2 describes the clinical features of the Yale IMPACT cohort, which were previously described (48). In addition, SARS-CoV-2–uninfected women (as determined by negative RT-qPCR testing of NP swab) were recruited as control. Among SARS-CoV-2–infected women, 45.5% (5 of 11) had symptomatic COVID-19, and two cases of severe COVID-19 disease required supplemental oxygen administration. All pregnancies resulted in live births, with a median Apgar score of 9 (range, 4–9). No significant differences existed between cases and matched controls for maternal age, gestational age, demographics, and neonatal outcomes. However, the groups differed in rates of gestational hypertension (45.5% in infected versus 0% in uninfected), preeclampsia (36.4% versus 0%), and diabetes (18.2% versus 0%).

In the Yale IMPACT cohort, we analyzed the circulating levels of gal-1, gal-3, and gal-9 in SARS-CoV-2–infected pregnant women and healthy pregnant controls. We found significantly higher levels of gal-1 in the serum of patients with COVID-19 compared with healthy pregnant controls (*P*-value = 0.0156, Figure 1E). However, no significant differences were observed in the circulating levels of gal-3 and gal-9 among both groups (Figures 1F, G).

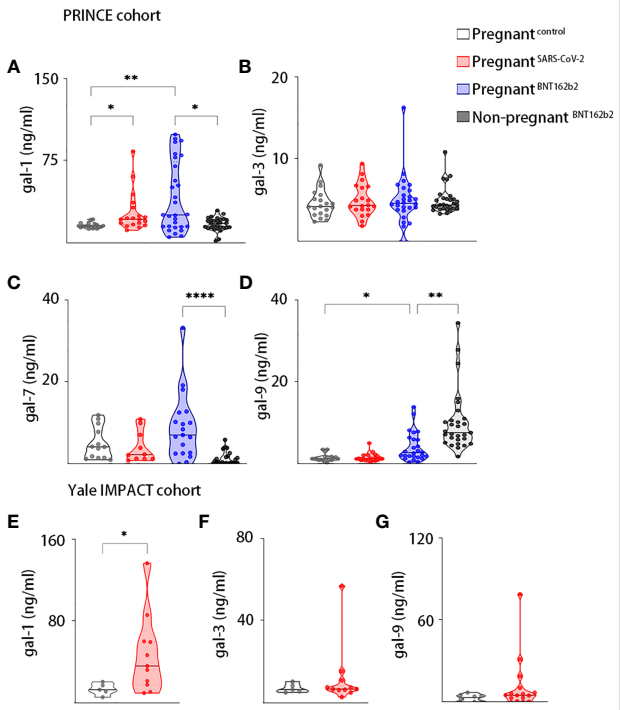


FIGURE 1
Galectins dynamics upon SARS-Cov-2 infection or vaccination during pregnancy. Maternal circulating levels of gal-1 **(A)**, gal-3 **(B)**, gal-7 **(C)**, and gal-9 **(D)** in the PRINCE cohort analyzed by ELISA in healthy pregnant women (pregnant^{control}, n = 20), pregnant women infected by SARS-CoV-2 (pregnant$^{SARS\text{-}CoV\text{-}2}$, n = 20), pregnant women vaccinated against SARS-CoV-2 (pregnant^{BNT162b2}, n = 30), and non-pregnant vaccinated women (non-pregnant^{BNT162b2}, n = 30). Circulating levels of gal-1 **(E)**, gal-3 **(F)**, and gal-9 **(G)** in the Yale IMPACT cohort analyzed by ELISA in healthy pregnant women (pregnant^{control}, n = 5) or pregnant women infected by SARS-CoV-2 (pregnant$^{SARS\text{-}CoV\text{-}2}$, n = 11). *$P$ < 0.05, **P < 0.01, and ****P < 0.0001 as analyzed by Kruskal–Wallis test or Welch's t-test. In all figures, circulating levels of galectins and PSG1 were determined in triplicate for each serum sample.

3.4 Correlation of maternal gal-1, gal-3, and gal-9 levels is altered following SARS-CoV-2 infection

We performed correlation analysis to explore the dynamics of galectin levels at the maternal circulation. During a healthy pregnancy, we found a significant positive correlation between gal-1 and gal-3 [*P*-value = 0.0331, Spearman correlation coefficient (ρ) = 0.478; Figure 2A] and between gal-1 and gal-9 (*P*-value = 0.0083, ρ = 0.571; Figure 2B). SARS-CoV-2 infection resulted in the loss of the observed correlation of gal-1 with both gal-3 and gal-9 (Figures 2A, B). On the other hand, vaccination only compromised the correlation between gal-1 and gal-9 (Figure 2B), whereas the correlation between gal-1 and gal-3 was maintained (*P*-value = 0.0433, ρ = 0.468; Figure 2A). No significant correlation was found between gal-1 and gal-7 in any of the analyzed groups (Figure 2C). Our results also demonstrated that gal-3 was positively associated with the levels of gal-9 in healthy pregnant women (*P*-value = 0.0103, ρ = 0.562; Figure 2D) and that this correlation was still present in the SARS-CoV-2–infected (*P*-value = 0.0010, ρ = 0.666; Figure 2D) and the SARS-CoV-2 vaccinated individuals (*P*-value = 0.0013, ρ = 0.704; Figure 2D).

Further correlation studies between galectins and clinical features in the PRINCE cohort were performed by analyzing general clinical parameters, including BMI, age, parity, gestational week of delivery, and the birth weight and height of the neonate. In addition, for each experimental group, we considered the time of infection (gestational week and trimester) and the gestational week in which women received the first and second doses of vaccination. Our results showed that the BMI of the patients was positively associated with gal-1 levels in maternal circulation ($P < 0.0001$, ρ = 0.439; Table 3) and gal-3 levels ($P = 0.0241$, ρ = 0.240; Table 3), and the age of the patients inversely correlated with gal-9 levels ($P = 0.0141$, ρ = −0.267; Table 3). In addition, when we analyzed the correlation between galectins and clinical parameters in the Yale

10.3389/fimmu.2023.1196395

TABLE 2 Clinical characteristics of the Yale IMPACT cohort.

	Pregnant healthy controls (n=5)	Pregnant COVID-19 patients (n=11)
Age: mean (range)	34.4 (30-42)	30.1 (20-40)
Gestational week: median (range)	39 + 2 (39-40)	39 + 5 (37-41)
Mode of delivery (% CS)	100%	45.5%
Sex of infant (% male)	60%	64%
Gravidity: median (range)	3 (1-4)	2 (1-4)
Parity: median (range)	1 (0-3)	1 (0-2)
Neonatal Apgar, 1 min: median (range)	9 (4-9)	9 (7-9)
Comorbidities		
Hypertension	0	45.5%
Preeclampsia	0	36.4%
Diabetes	0	18.2%
COVID-19 features		
COVID-19 symptoms at time of delivery (%)		45.5%
severe COVID-19		18.2%
SARS-CoV-2 PCR testing of NP swab CT value: median (range)		32.3 (17.1-32.5)

CS, cesarean section; CT, cycle threshold.

IMPACT cohort, our results showed that gal-1 was negatively associated with the parity of the pregnant women (P = 0.0029, ρ = −0.844; Table 4).

3.5 COVID-19 infection during pregnancy influences the PSG1 levels in maternal circulation

Because PSG1 is one of the most abundant trophoblastic proteins in maternal serum in the third trimester and binds gal-1 (29), we further examined the PSG1 levels in maternal circulation. Our results in the PRINCE cohort showed that pregnant women infected with SARS-CoV-2 displayed increased levels of PSG1 compared with non-infected pregnant women. A non-significant increase was observed in the vaccinated pregnant women (Figure 3A). However, we did not find any correlation between PSG1 and gal-1 (Figures 3C–E). The measurement of PSG1 levels in the Yale IMPACT cohort demonstrated similar circulating levels of PSG1 between control pregnant women and pregnant women with SARS-CoV-2 infection (Figure 3B).

3.6 Transcriptional placental changes of galectins, PSGs, and glycosylation pathways during maternal SARS-CoV-2 infection

To further explore the impact of SARS-CoV-2 infection on the placenta compartment, we analyzed RNA sequencing data (GSE171995) of placental villi from the Yale IMPACT cohort. The transcriptomic placental analysis of galectins and PSGs in pregnant women with SARS-CoV-2 infection and matched healthy controls indicated that, consistent with the serum protein measurements, the expression of gal-1 (*LGALS1*, P = 0.0077) and gal-3 genes (*LGALS3*, P = 0.0152) were increased in the placentas of pregnant women with SARS-CoV-2 infection compared with controls (Figure 4A). Moreover, several PSG genes were differentially expressed in the placentas: increased expression of *PSG1* (P = 0.0410), *PSG3* (P = 0.0456), *PSG5* (P = 0.0259), *PSG6* (P = 0.0482), *PSG8* (P = 0.0355), *PSG9* (P = 0.0209), and *PSG11* (P = 0.0462) were found in the SARS-CoV-2–infected individuals compared with controls (Figure 4A).

Extracellular biological functions of galectins rely on their capacity to bind specific glycans present in proteins on the cell membrane and in the extracellular matrix. Indeed, gal-1 can recognize N- or O-linked glycans containing multiple LacNAc units, which are generated by the enzymes N-acetylglucosaminyltransferases (MGAT genes) or β1,6-N-acetylglucosaminyltransferases (GCNT genes), respectively (Figures 4B–D) (6, 49, 50). Poly-LacNAc is also reported as a preferred ligand for gal-3 (51); meanwhile, gal-9 is considered to preferentially bind to internal LacNAc residues of a poly-LacNAc chain (52). We performed differential gene expression analysis to compare the transcriptomic profile of 84 glycoenzymes and generated a hierarchical clustering scheme. A total of 18 glycoenzymes were differentially expressed. Among them, eight are involved in the synthesis and modification of N-linked glycans (Figure 4C), five in the synthesis and processing of O-linked glycans (Figures 4D–E), and five in terminal extensions of sialylation and its degradation (Figures 4F, G). Overall our results showed upregulated expression of glycoenzymes in the placentas of SARS-CoV-2–infected

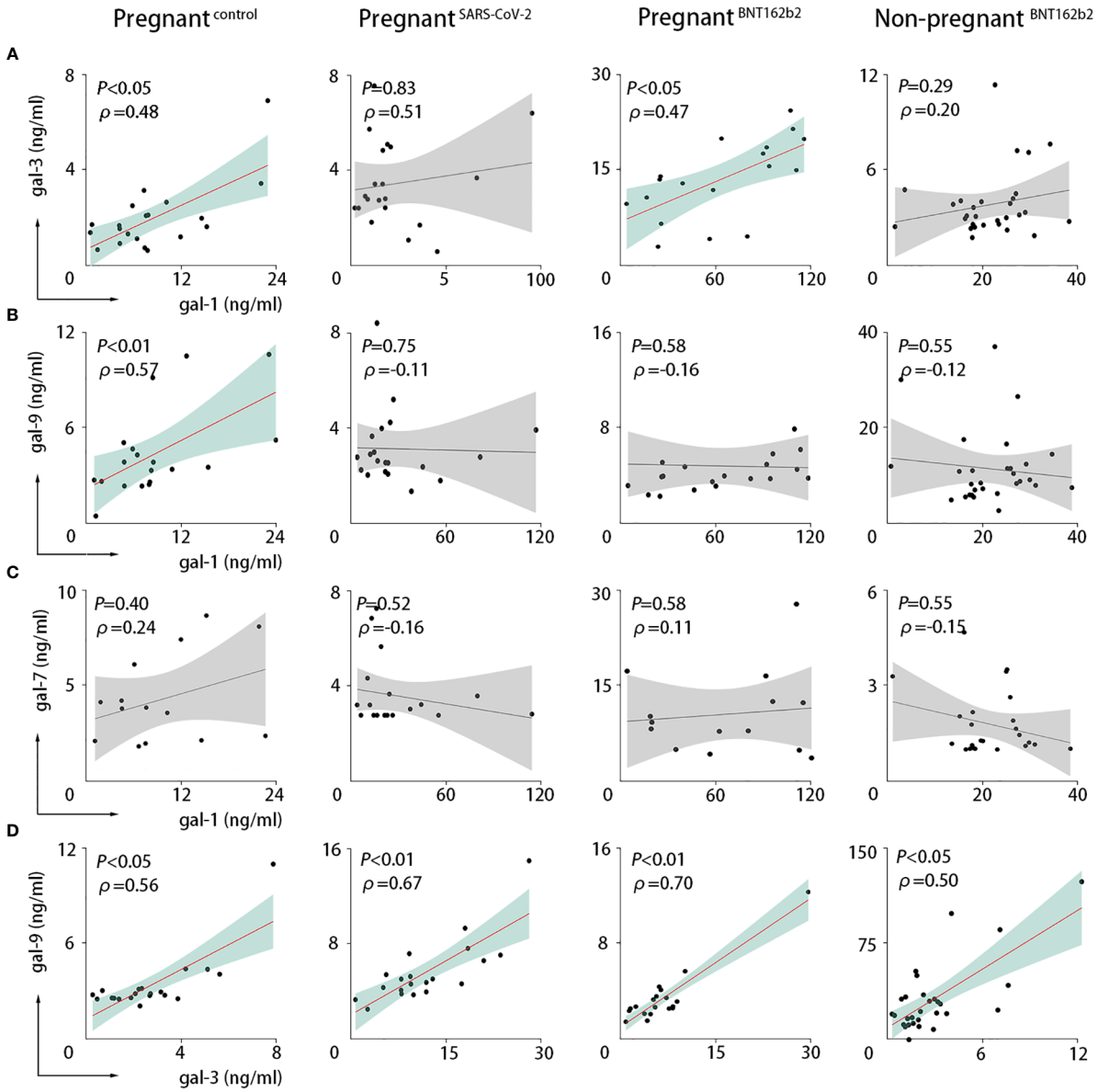


FIGURE 2
SARS-CoV-2 infection or vaccination altered the maternal circulating levels. Correlations in PRINCE cohort between serum values of gal-1 and gal-3 **(A)**, gal-1 and gal-9 **(B)**, gal-1 and gal-7 **(C)**, or gal-3 and gal-9 **(D)**. Pregnant^{control}, healthy pregnant women (n = 20); pregnant$^{SARS\text{-}CoV\text{-}2}$, pregnant women infected by SARS-CoV-2 (n = 20); pregnant^{BNT162b2}, pregnant women vaccinated against SARS-CoV-2 (n = 30); non-pregnant^{BNT162b2}, non-pregnant vaccinated women (n = 30). The Spearman correlation coefficient (ρ) is shown. $P < 0.05$ was considered statistically significant (green) and $P > 0.05$ not significant (gray) as analyzed with the Spearman statistical test.

individuals compared with healthy controls, which included the enzymes that take part in the initiation (*MAN1A2*, $P = 0.0011$), branched complex (*MGAT1*, $P = 0.0131$; *MGAT2*, $P = 0.0142$; *MGAT4B*, $P = 0.0260$; *MGAT5*, $P = 0.0143$; *MAN2B1*, $P = 0.0226$), and elongation (*B4GALT1*, $P = 0.0013$; *B4GALT3*, $P = 0.0004$) of N-linked glycans (Figure 4C). We also observed an upregulation of the expression of glycoenzymes that take part in the initiation (*GALNT1*, $P = 0.0015$; *GALNT2*, $P = 0.0172$; *GALNT7*, $P = 0.0023$) and core modification (*B4GALT5*, $P = 0.0302$; *C1GALTC1*, $P = 0.0171$) of O-linked glycans (Figure 4E) and enzymes linked to terminal extensions of sialylation and degradation including *ST3GAL1* ($P = 0.0034$), *MAN2B1* ($P = 0.0226$), *EDEM1* ($P = 0.0062$), *EDEM2* ($P = 0.0331$), and *HEXA* ($P = 0.0349$) (Figures 4F, G). These results suggest an enrichment of N-glycan– and O-glycan–associated enzymes compatible with the gal-1 binding in SARS-CoV-2–infected placentas.

4 Discussion

SARS-CoV-2 infection leads to a higher risk of severe disease in pregnant than in non-pregnant women. Considering that women of reproductive age make up more than 20% of the global population, studying the effects of SARS-CoV-2 infection and vaccination in this population is of great importance. We measured the concentration of

TABLE 3 PRINCE cohort correlations between galectins and clinical parameters.

	gal-1 (ng/ml)		gal-3 (ng/ml)		gal-7 (ng/ml)		gal-9 (ng/ml)	
	P	*ρ*	*P*	*ρ*	*P*	*ρ*	*P*	*ρ*
BMI	<0.001	0.439	0.024	0.240	0.599	0.068	0.600	-0.056
Age	0.203	-0.104	0.844	0.022	0.589	0.071	0.014	-0.267
Gestational week	0.753	-0.046	0.627	-0.072	0.058	-0.038	0.167	-0.200
Parity	0.123	-0.201	0.258	-0.150	0.356	0.154	0.817	0.030

BMI, body mass index.

several galectins and PSG1 in the circulation of pregnant women infected with SARS-CoV-2 or vaccinated against SARS-CoV-2 and also explored their placental expression (Figure 5). Our results demonstrated a dominant role of gal-1 within the galectin signature in pregnant women upon SARS-CoV-2 infection in both the PRINCE and Yale IMPACT cohorts. Our findings align with previous reports in non-pregnant individuals, indicating that SARS-CoV-2 infection increased gal-1 levels (41, 42). Moreover, one study showed a positive correlation between gal-1; levels of pro-inflammatory cytokines such as Interleukin-1 beta (IL-1β), IL-6, and IL-23; and COVID-19 severity, suggesting that gal-1 acts as an alarmin (42). In non-pregnant patients, gal-3 and gal-9 were found to be increased and correlated with COVID-19 severity (45, 53). However, we did not find increased levels of gal-3 or gal-9 in pregnant women with SARS-CoV-2 infection. This difference in results may be explained, at least, in part, by the fact that most of our cohort patients had mild or non-symptomatic COVID-19 disease. Furthermore, our results showed positive correlations between gal-1 and gal-3 or gal-9 levels only in healthy pregnancies. This positive correlation was expected because successful pregnancy is associated with a rise in maternal circulating levels of these three galectins from the first to the third trimester (28, 54, 55). The observation that only gal-1 was increased as a result of SARS-CoV-2 infection is likely responsible for the observed loss of correlation with the other two galectins in this group of patients.

The immune system plays a key role in mediating a successful pregnancy because a fine balance is necessary to promote maternal tolerance to the allogeneic fetus while protecting both the mother and the fetus from pathogens (56, 57). Several studies demonstrated that the maternal immune response tend to restrain inflammation through regulatory and anti-inflammatory mediators (58, 59), and galectins, especially gal-1, are involved in this process (6). Moreover, gal-1 has been described not only as a damage-associated molecular pattern molecule, amplifying the immune response, but also as a member of a diverse group of mediators collectively referred to as resolution-associated molecular pattern molecules with the capacity to resolve an acute inflammation process by counteracting the synthesis of pro-inflammatory cytokines (60, 61). The cytokine storm in the systemic circulation is responsible for the pathophysiology of SARS-CoV-2 infection and can lead to multi-organ damage (7, 62). Both systemic and inflammatory changes at the maternal–fetal interface were previously described during SARS-CoV-2 infection in pregnancy (48, 63). The analysis of bulk RNA sequencing of placental villi from the Yale IMPACT cohort demonstrated that COVID-19 cases presented increased expression of genes associated with an immune response, indicating a robust response at the maternal–fetal interface upon SARS-CoV-2 infection, even in the absence of localized placental infection (48). Another study evaluated the maternal systemic immune response, finding increased IL-8, IL-10, and IL-15 levels as well as a reduction in T-cell subsets, particularly of T helper cell 1 (Th1) and a subset of $CD8^+$ cells characterized by the production of IL-17 (Tc17)-like cells in pregnant women infected with SARS-CoV-2 (63). Gal-1 controls the fate of Th-1 and Th-17 cells through the glycan repertoire expressed by these cells that allow gal-1 binding (64). Therefore, the increase of gal-1 in maternal circulation during COVID-19 could be associated with the observed systemic decrease in Th-1 and Tc17-like cells. Gal-1 also induces apoptosis of activated $CD8^+$ T cells (65), which could explain the upregulation of gal-1 in SARS-CoV-2 infection. Thus, the increase of gal-1 observed in pregnant patients with COVID-19 could be related to its anti-inflammatory activity and its potential participation in the control of inflammation and tissue damage resulting from SARS-CoV-2 infection.

In our study, healthy pregnant women exposed to SARS-CoV-2 infection often exhibit simultaneously increased serum concentrations of gal-1 and PSG1. We have previously demonstrated that PSG1 binds to gal-1 and postulated that PSG1 protects gal-1 from inactivation by oxidation in the extracellular

TABLE 4 Yale cohort correlations between galectins and clinical parameters.

	gal-1 (ng/ml)		gal-3 (ng/ml)		gal-9 (ng/ml)	
	P	*ρ*	*P*	*ρ*	*P*	*ρ*
Age	0.136	-0.389	0.633	-0.129	0.279	-0.287
Gestational week	0.550	0.161	0.958	-0.015	0.715	-0.099
Parity	0.003	-0.844	0.749	-0.111	0.867	0.060

10.3389/fimmu.2023.1196395

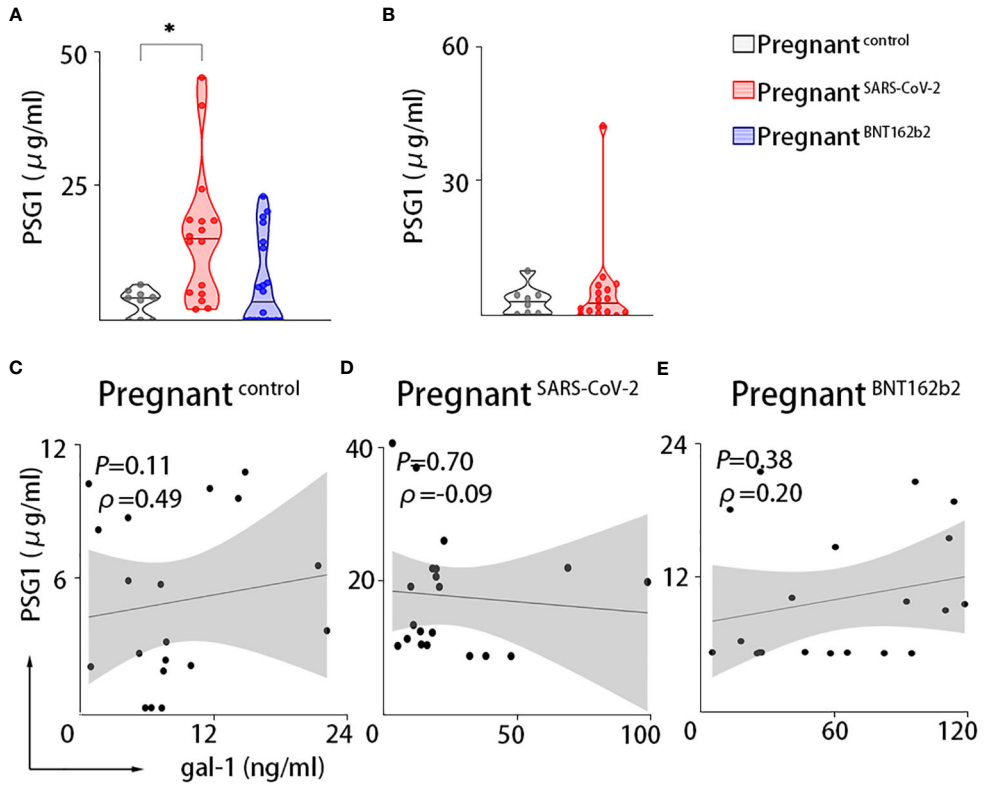


FIGURE 3
Maternal circulation of PSG1 is only upregulated upon SARS-CoV-2 infection during pregnancy. PSG1. **(A, B)** Maternal circulating levels of PSG1 in the PRINCE cohort **(A)** or the Yale IMPACT cohort **(B)**. **(C–E)** Correlations between serum values of gal-1 and PSG1 in the PRINCE cohort. Pregnant^{control}, healthy pregnant women; pregnant$^{SARS\text{-}CoV\text{-}2}$, pregnant women infected by SARS-CoV-2; pregnant^{BNT162b2}, pregnant women vaccinated against SARS-CoV-2. *$P < 0.05$ as analyzed by Kruskal–Wallis test or the Welch's t-test.

environment. Therefore, an increase in both PSG1 and gal-1 may extend the gal-1 activity once it is released from cells into the circulation (29). However, the correlation between these two proteins disappeared in infected pregnant women, likely due to the modest increase of PSG1 relative to gal-1. Unfortunately, we were unable to determine whether the concentration of other PSG family members, which can also interact with gal-1, is also increased in the circulation of SARS-CoV-2–infected pregnant women as suggested by the RNA sequencing data because specific validated ELISAs are only available to measure the concentration of PSG1. Future studies should be performed to determine the mechanism by which syncytiotrophoblast cells, the major cell type that secretes PSG1 into the maternal circulation, regulate PSG1 secretion following maternal SARS-CoV-2 infection. In contrast, PSG1 post-vaccination levels remain unchanged, suggesting that modulation of PSG1 secretion by the syncytiotrophoblast cells requires maternal SARS-CoV-2 infection. In the future, it would be interesting to determine whether an increase in PSG1 concentration is also observed following infection with other viruses that do not infect the PSG1-producing cells and whether this increase in PSG levels may contribute to the establishment of an anti-inflammatory environment.

The extracellular activity of gal-1 relies on its capacity to bind specific glycans. To further investigate the possible role of the gal-1 upregulation during the course of COVID-19, the expression of enzymes involved in relevant N- and O-glycosylation was analyzed in bulk placentas by RNA sequencing. Our results showed that genes involved in the initiation, hybrid complex formation, and elongation of N-glycans and in initiation and core modification of O-glycans were upregulated in patients with COVID-19. Gal-1 and gal-3 can bind β-1,6-GlcNAc–branched N-linked glycans on the cell surface or in secreted glycoproteins, and several enzymes are required for the biosynthesis of these glycotopes (51). Similar results were found for the enzymes involved in O-glycans synthesis, as initiation (GALNT1, GALNT2, and GALNT7) and core modification (C1GALT1C1 and B4GALT5) enzymes were also upregulated during SARS-CoV-2 infection. The addition of α2,6-linked sialic acids to the termini of glycans by ST6GAL1 inhibits the binding of gal-1 (6). However, our results demonstrated similar expression of ST6GAL1 in SARS-CoV-2–infected and control groups. More importantly, we observed an increase of ST3GAL1 expression signatures (responsible for the addition of α2,3-linked sialic acid, which is compatible which gal-1 high-affinity binding) within the placental compartment. These results suggest that both the expression of gal-1 and of the enzymes in the placenta involved in the N- and O-glycan modifications required for gal-1 to exert its function are increased following SARS-CoV-2 infection.

We also explored the galectin fingerprint of healthy pregnant women that were fully vaccinated. Several reports indicated that COVID-19 vaccines are safe for pregnant women and can effectively

10.3389/fimmu.2023.1196395

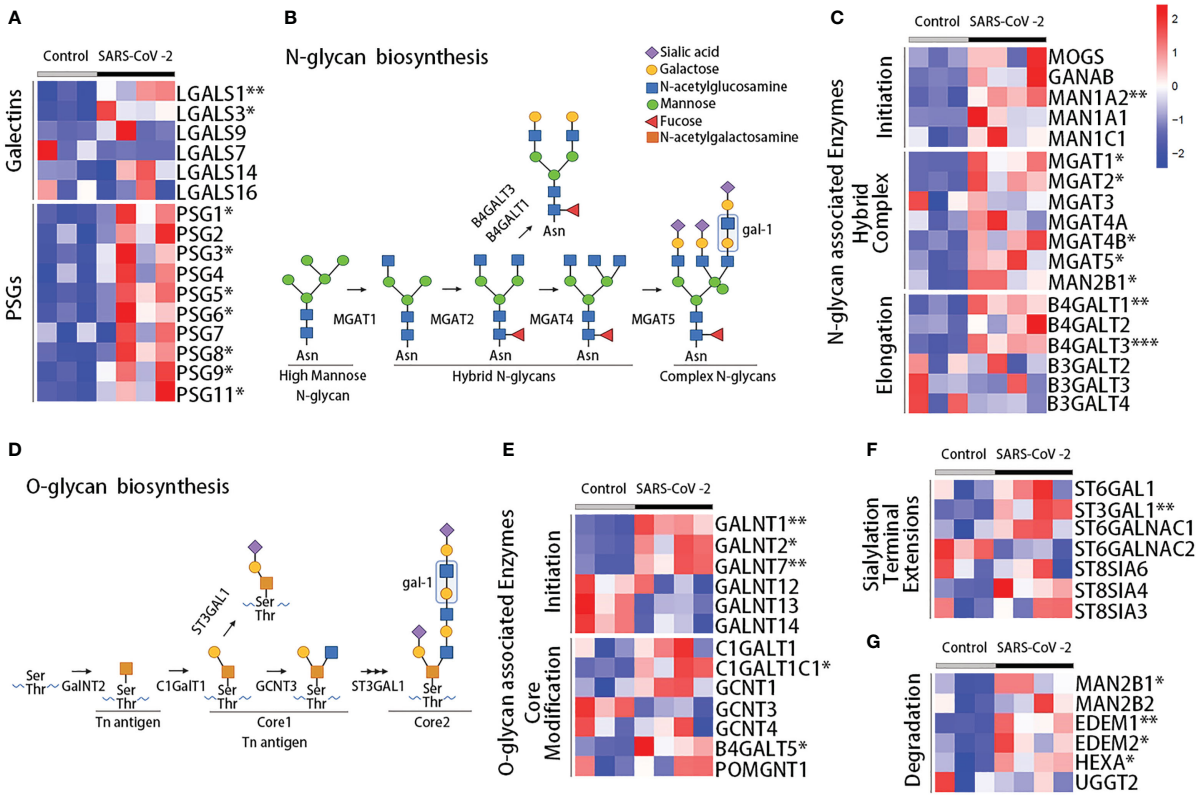


FIGURE 4
Transcriptome analysis of placenta during acute SARS-CoV-2 infection. **(A)** Heatmap showing the scaled expression of galectins and PSG genes from RNA sequencing data in the placenta of the Yale IMPACT cohort. Enzymes involved in the synthesis of N-glycans **(B)** and O-glycans **(D)**, showing the preferred ligand for gal-1. Heatmaps for the expression of the enzymes involved in the synthesis of N-glycans **(C)** and O-glycans **(E)**, involved in terminal extension **(F)** or glycan degradation **(G)**. Control, healthy pregnant women; SARS-CoV-2, pregnant women infected by SARS-CoV-2. *P < 0.05, **P < 0.01, and ***P < 0.001 as analyzed with the Welch's t-test.

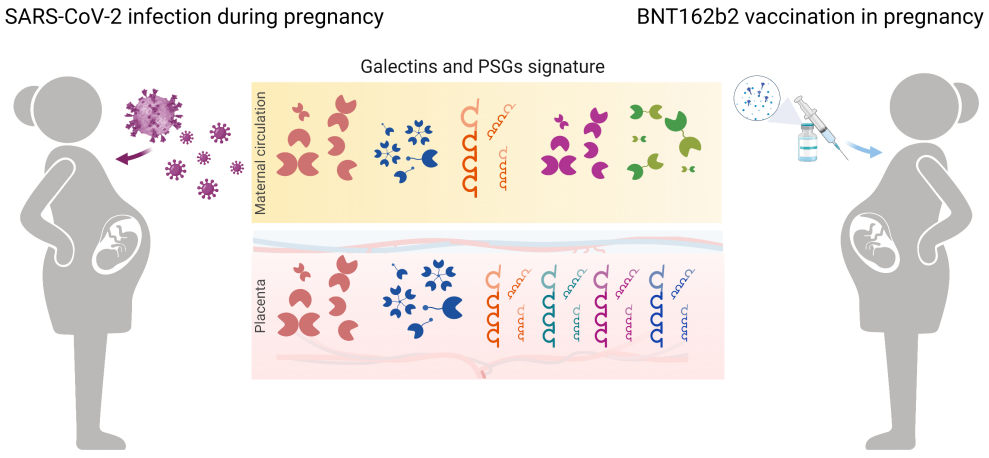


FIGURE 5
Schematic diagram highlighting galectin and PSGs fingerprints in pregnancy upon SARS-CoV-2 infection and in women subjected to vaccination. In maternal circulation, levels of gal-1 and PSG1 increased during COVID-19 disease in pregnancy. Vaccination with BNT162b2 caused an upregulation of gal-1 circulating levels mimicking the viral infection. During SARS-CoV-2 infection, placental transcription regulation of gal-1 and gal-3 dominates the galectin signature creating a protective microenvironment. In addition, upregulation of PSGs by the trophoblast cells contributes to immune modulation during COVID-19 disease.

protect them from getting severe COVID-19 and prevent maternal and fetal mortality (8, 66). Our results provide evidence that the status of the maternal immune system during vaccination modulates the galectin signature and could play a role for protection against COVID-19 disease. For instance, a simultaneous increase of gal-1 and gal-7 accompanied by a decrease of gal-9 in post- vaccination sera derived from pregnant individuals suggests a role of these lectins in the establishment of a protective response against the SARS-CoV-2 virus. Gal-1 is secreted by activated B cells, T cells, and macrophages, and activated lymphocytes secrete gal-7 (67, 68). We hypothesize that the increase of these two galectins in pregnant vaccinated patients could play a role in the effective immune system activation by the COVID-19 vaccine that results in the generation of neutralizing antibodies and virus-specific T-cell responses (69). Elevation of circulating levels of gal-9 in chronic and acute SARS-CoV-2 infection has been reported in non-pregnant individuals, and it was correlated with a severe disease outcome (43). On the basis of our findings, we hypothesized that a lack of increase in gal-9 levels during vaccination likely avoids a pro-inflammatory response during pregnancy vaccination that could result in detrimental effects at the maternal–fetal interface.

In conclusion, our study revealed a clear dominance of gal-1 within the maternal galectin fingerprint during SARS-CoV-2 infection and vaccination in pregnancy. One of the pregnancy-specific gal-1 ligands, PSG1, was also upregulated during the COVID-19 disease, suggesting a potential synergistic immunomodulatory role of these proteins at the maternal–fetal interface. Further work is needed to determine the functional relevance of the galectin signature in maternal circulation during the SARS-CoV-2 infection and vaccination in pregnancy. Specifically, it would be important to understand the molecular mechanism by which gal-1 may control the immune response to a virus during pregnancy.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Hamburg Chamber of Physicians (Ärztekammer Hamburg) approved the study protocol under the registration numbers PV3694 (PRINCE), PV 7312-4710 (PRINCE COVID) and 2021-10647-BO-ff (PRINCE VACCINE). The study was approved by the Yale Institutional Review Board (protocol #2000027690). The patients/participants provided their written informed consent to participate in this study.

Author contributions

SMB designed the study and secured grant funding. FZ, A-CT, YW, YX, AD, AL-C, PV, EK, CU, JB, PCA, SFF, GSD, and MGG performed experiments and/or analyzed data. The original draft of the manuscript was written by FZ, MGG, and SMB, and further writing, review, and editing were done by all authors. All authors contributed to the article and approved the submitted version.

Funding

This research was funded by the Deutsche Forschungsgemeinschaft (DFG) Heisenberg Program (BL1115/3-1, BL1115/7-1, and BL1115/11-1) to SMB; National Institute of Health (NIH) grants K23MH118999 and R01AI157488 to SFF; National Institute of Allergy and Infectious Diseases (NIAID)–NIH grant R21AI156058 to GSD. FZ, YX, and YW were supported by a China Scholarship Council (CSC) fellowship. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or the US Department of Defense. Figures 4B, D, 5 were created with BioRender.com.

Acknowledgments

We thank T. Andreas (Blois's Team), A. Wieczorek, N. Felber, G. Hansen, E. Ehrenburg (PRINCE Study Team), and the Yale IMPACT Team for their excellent technical assistance in generating this work.

Conflict of interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1196395/full#supplementary-material

References

1. Adam S, Pheiffer C, Dias S, Hlongwane T, Vannevel V, Soma-Pillay P, et al. Coronavirus and pregnancy: the challenges of the 21(st) century: a review. *Front Microbiol* (2022) 13:923546. doi: 10.3389/fmicb.2022.923546

2. Silasi M, Cardenas I, Kwon JY, Racicot K, Aldo P, Mor G. Viral infections during pregnancy. *Am J Reprod Immunol* (2015) 73(3):199–213. doi: 10.1111/aji.12355

3. Wastnedge EAN, Reynolds RM, van Boeckel SR, Stock SJ, Denison FC, Maybin JA, et al. Pregnancy and COVID-19. *Physiol Rev* (2021) 101(1):303–18. doi: 10.1152/physrev.00024.2020

4. Leung C, Simões ESAC, Oliveira EA. Are in-hospital COVID-19-related mortality and morbidity in pregnancy associated with gestational age? *Ultrasound Obstet Gynecol* (2022) 60(2):234–42. doi: 10.1002/uog.24931

5. Metz TD, Clifton RG, Hughes BL, Sandoval GJ, Grobman WA, Saade GR, et al. Association of SARS-CoV-2 infection with serious maternal morbidity and mortality from obstetric complications. *Jama* (2022) 327(8):748–59. doi: 10.1001/jama.2022.1190

6. Blois SM, Dveksler G, Vasta GR, Freitag N, Blanchard V, Barrientos G. Pregnancy galectinology: insights into a complex network of glycan binding proteins. *Front Immunol* (2019) 10:1166. doi: 10.3389/fimmu.2019.01166

7. Li N, Han L, Peng M, Lv Y, Ouyang Y, Liu K, et al. Maternal and neonatal outcomes of pregnant women with coronavirus disease 2019 (COVID-19) pneumonia: a case-control study. *Clin Infect Dis* (2020) 71(16):2035–41. doi: 10.1093/cid/ciaa352

8. Male V. SARS-CoV-2 infection and COVID-19 vaccination in pregnancy. *Nat Rev Immunol* (2022) 22(5):277–82. doi: 10.1038/s41577-022-00703-6

9. Marchand G, Patil AS, Masoud AT, Ware K, King A, Ruther S, et al. Systematic review and meta-analysis of COVID-19 maternal and neonatal clinical features and pregnancy outcomes up to June 3, 2021. *AJOG Global Rep* (2022) 2(1):100049. doi: 10.1016/j.xagr.2021.100049

10. Muthuka J, Kiptoo M, Oluoch K, Nzioki JM, Nyamai EM. Association of pregnancy with coronavirus cytokine storm: systematic review and meta-analysis. *JMIR Pediatr parenting.* (2022) 5(4):e31579. doi: 10.2196/31579

11. Nana M, Nelson-Piercy C. COVID-19 in pregnancy. *Clin Med (London England).* (2021) 21(5):e446–e50. doi: 10.7861/clinmed.2021-0503

12. Wainstock T, Yoles I, Sergienko R, Sheiner E. Prenatal maternal COVID-19 vaccination and pregnancy outcomes. *Vaccine* (2021) 39(41):6037–40. doi: 10.1016/j.vaccine.2021.09.012

13. Nana M, Hodson K, Lucas N, Camporota L, Knight M, Nelson-Piercy C. Diagnosis and management of covid-19 in pregnancy. *Bmj* (2022) 377:e069739. doi: 10.1136/bmj-2021-069739

14. Beharier O, Plitman Mayo R, Raz T, Nahum Sacks K, Schreiber L, Suissa-Cohen Y, et al. Efficient maternal to neonatal transfer of antibodies against SARS-CoV-2 and BNT162b2 mRNA COVID-19 vaccine. *J Clin Invest* (2021) 131(13):e150319. doi: 10.1172/JCI150319

15. Carbone L, Trinchillo MG, Di Girolamo R, Raffone A, Saccone G, Iorio GG, et al. COVID-19 vaccine and pregnancy outcomes: a systematic review and meta-analysis. *Int J Gynaecol Obstet* (2022) 159(3):651–61. doi: 10.1002/ijgo.14336

16. Rottenstreich A, Zarbiv G, Oiknine-Djian E, Vorontsov O, Zigron R, Kleinstern G, et al. The effect of gestational age at BNT162b2 mRNA vaccination on maternal and neonatal severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody levels. *Clin Infect Dis* (2022) 75(1):e603–e10. doi: 10.1093/cid/ciac135

17. Atyeo C, DeRiso EA, Davis C, Bordt EA, DeGuzman RM, Shook LL, et al. COVID-19 mRNA vaccines drive differential Fc-functional profiles in pregnant, lactating, and non-pregnant women. *Sci Transl Med* (2021) 617:eabi8631. doi: 10.1126/scitranslmed.abi8631

18. Ben-Mayor Bashi T, Amikam U, Ashwal E, Hershkovitz G, Attali E, Berkovitz-Shperling R, et al. The association of maternal SARS-CoV-2 vaccination-to-delivery interval and the levels of maternal and cord blood antibodies. *Int J Gynaecol Obstet* (2022) 156(3):436–43. doi: 10.1002/ijgo.14014

19. Bookstein Peretz S, Regev N, Novick L, Nachshol M, Goffer E, Ben-David A, et al. Short-term outcome of pregnant women vaccinated with BNT162b2 mRNA COVID-19 vaccine. *Ultrasound Obstet Gynecol* (2021) 58(3):450–6. doi: 10.1002/uog.23729

20. Citu IM, Citu C, Gorun F, Sas I, Tomescu L, Neamtu R, et al. Immunogenicity following administration of BNT162b2 and Ad26.COV2.S COVID-19 vaccines in the pregnant population during the third trimester. *Viruses* (2022) 14(2):307. doi: 10.3390/v14020307

21. Collier AY, McMahan K, Yu J, Tostanoski LH, Aguayo R, Ansel J, et al. Immunogenicity of COVID-19 mRNA vaccines in pregnant and lactating women. *Jama* (2021) 325(23):2370–80. doi: 10.1001/jama.2021.7563

22. Kugelman N, Nahshon C, Shaked-Mishan P, Cohen N, Sher ML, Gruber M, et al. Maternal and neonatal SARS-CoV-2 immunoglobulin G antibody levels at delivery after receipt of the BNT162b2 messenger RNA COVID-19 vaccine during the second trimester of pregnancy. *JAMA Pediat* (2022) 176(3):290–5. doi: 10.1001/jamapediatrics.2021.5683

23. Mithal LB, Otero S, Shanes ED, Goldstein JA, Miller ES. Cord blood antibodies following maternal coronavirus disease 2019 vaccination during pregnancy. *Am J Obstet Gynecol* (2021) 225(2):192–4. doi: 10.1016/j.ajog.2021.03.035

24. Nir O, Schwartz A, Toussia-Cohen S, Leibovitch L, Strauss T, Asraf K, et al. Maternal-neonatal transfer of SARS-CoV-2 immunoglobulin G antibodies among parturient women treated with BNT162b2 messenger RNA vaccine during pregnancy. *Am J Obstet Gynecol MFM* (2022) 4(1):100492. doi: 10.1016/j.ajogmf.2021.100492

25. Prabhu M, Murphy EA, Sukhu AC, Yee J, Singh S, Eng D, et al. Antibody response to coronavirus disease 2019 (COVID-19) messenger RNA vaccination in pregnant women and transplacental passage into cord blood. *Obstet Gynecol* (2021) 138 (2):278–80. doi: 10.1097/AOG.0000000000004438

26. Freitag N, Tirado-González I, Barrientos G, Cohen M, Daher S, Goldman-Wohl D, et al. The chimera-type galectin-3 is a positive modulator of trophoblast functions with dysregulated expression in gestational diabetes mellitus. *Am J Reprod Immunol* (2020) 84(6):e13311. doi: 10.1111/aji.13311

27. Li YH, Zhou WH, Tao Y, Wang SC, Jiang YL, Zhang D, et al. The galectin-9/Tim-3 pathway is involved in the regulation of NK cell function at the maternal-fetal interface in early pregnancy. *Cell Mol Immunol* (2016) 13(1):73–81. doi: 10.1038/cmi.2014.126

28. Tirado-Gonzalez I, Freitag N, Barrientos G, Shaikly V, Nagaeva O, Strand M, et al. Galectin-1 influences trophoblast immune evasion and emerges as a predictive factor for the outcome of pregnancy. *Mol Hum Reprod* (2013) 19(1):43–53. doi: 10.1093/molehr/gas043

29. Mendoza M, Lu D, Ballesteros A, Blois SM, Abernathy K, Feng C, et al. Glycan characterization of pregnancy-specific glycoprotein 1 and its identification as a novel galectin-1 ligand. *Glycobiology* (2020) 30(11):895–909. doi: 10.1093/glycob/cwaa034

30. Blois SM, Sulkowski G, Tirado-González I, Warren J, Freitag N, Klapp BF, et al. Pregnancy-specific glycoprotein 1 (PSG1) activates TGF-β and prevents dextran sodium sulfate (DSS)-induced colitis in mice. *Mucosal Immunol* (2014) 7(2):348–58. doi: 10.1038/mi.2013.53

31. Moore T, Williams JM, Becerra-Rodriguez MA, Dunne M, Kammerer R, Dveksler G. Pregnancy-specific glycoproteins: evolution, expression, functions and disease associations. *Reproduction* (2022) 163(2):R11–23. doi: 10.1530/REP-21-0390

32. Rattila S, Dunk CEE, Im M, Grichenko O, Zhou Y, Yanez-Mo M, et al. Interaction of pregnancy-specific glycoprotein 1 with integrin A5β1 is a modulator of extravillous trophoblast functions. *Cells* (2019) 8(11):1369. doi: 10.3390/cells8111369

33. Rattila S, Kleefeldt F, Ballesteros A, Beltrame JS, LR M, Ergün S, et al. Pro-angiogenic effects of pregnancy-specific glycoproteins in endothelial and extravillous trophoblast cells. *Reproduction* (2020) 160(5):737–50. doi: 10.1530/REP-20-0169

34. Shanley DK, Kiely PA, Golla K, Allen S, Martin K, O'Riordan RT, et al. Pregnancy-specific glycoproteins bind integrin αIIbβ3 and inhibit the platelet-fibrinogen interaction. *PloS One* (2013) 8(2):e57491. doi: 10.1371/journal.pone.0057491

35. Ballesteros A, Mentink-Kane MM, Warren J, Kaplan GG, Dveksler GS. Induction and activation of latent transforming growth factor-β1 are carried out by two distinct domains of pregnancy-specific glycoprotein 1 (PSG1). *J Biol Chem* (2015) 290(7):4422–31. doi: 10.1074/jbc.M114.597518

36. Kammerer R, Ballesteros A, Bonsor D, Warren J, Williams JM, Moore T, et al. Equine pregnancy-specific glycoprotein CEACAM49 secreted by endometrial cup cells activates TGFB. *Reproduction* (2020) 160(5):685–94. doi: 10.1530/REP-20-0277

37. Moore JB, June CH. Cytokine release syndrome in severe COVID-19. *Science* (2020) 368(6490):473–4. doi: 10.1126/science.abb8925

38. Rosen DB, Murphy EA, Gejman RS, Capili A, Friedlander RL, Rand S, et al. Cytokine response over the course of COVID-19 infection in pregnant women. *Cytokine* (2022) 154:155894. doi: 10.1016/j.cyto.2022.155894

39. De Biasi S, Meschiari M, Gibellini L, Bellinazzi C, Borella R, Fidanza L, et al. Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia. *Nat Commun* (2020) 11(1):3434. doi: 10.1038/s41467-020-17292-4

40. Patel H, Ashton NJ, Dobson RJB, Andersson LM, Yilmaz A, Blennow K, et al. Proteomic blood profiling in mild, severe and critical COVID-19 patients. *Sci Rep* (2021) 11(1):6357. doi: 10.1038/s41598-021-85877-0

41. Kazancioglu S, Yilmaz FM, Bastug A, Ozbay BO, Aydos O, Yücel Ç, et al. Assessment of galectin-1, galectin-3, and prostaglandin E2 levels in patients with COVID-19. *Japan J Infect Dis* (2021) 74(6):530–6. doi: 10.7883/yoken.JJID.2021.020

42. Markovic SS, Gajovic N, Jurisevic M, Jovanovic M, Jovicic BP, Arsenijevic N, et al. Galectin-1 as the new player in staging and prognosis of COVID-19. *Sci Rep* (2022) 12(1):1272. doi: 10.1038/s41598-021-04602-z

43. Bozorgmehr N, Mashhouri S, Rosero EP, Xu L, Shahbaz S, Sligl W, et al. Galectin-9, a player in cytokine release syndrome and a surrogate diagnostic biomarker in SARS-CoV-2 infection. *mBio* (2021) 12(3):e00384–21. doi: 10.1128/mBio.00384-21

44. Baykiz D, Emet S, Ayduk-Govdeli E, Kaytaz M, Yavuz ML, Karaca-Ozer P, et al. Galectin-3 as a novel biomarker for predicting clinical outcomes in hospitalized COVID-19 patients. *Clin Lab* (2022) 68(12). doi: 10.7754/Clin.Lab.2022.220134

45. Portacci A, Diaferia F, Santomasi C, Dragonieri S, Boniello E, Di Serio F, et al. Galectin-3 as prognostic biomarker in patients with COVID-19 acute respiratory failure. *Respir Med* (2021) 187:106556. doi: 10.1016/j.rmed.2021.106556

46. Yeung ST, Premeaux TA, Du L, Niki T, Pillai SK, Khanna KM, et al. Galectin-9 protects humanized-ACE2 immunocompetent mice from SARS-CoV-2 infection. *Front Immunol* (2022) 13:1011185. doi: 10.3389/fimmu.2022.1011185

47. Gaughan EE, Quinn TM, Mills A, Bruce AM, Antonelli J, MacKinnon AC, et al. An inhaled galectin-3 inhibitor in COVID-19 pneumonitis: a phase Ib/IIa randomized controlled clinical trial (DEFINE). *Am J Respir Crit Care Med* (2023) 207(2):138–49. doi: 10.1164/rccm.202203-0477OC

48. Lu-Culligan A, Chavan AR, Vijayakumar P, Irshaid L, Courchaine EM, Milano KM, et al. Maternal respiratory SARS-CoV-2 infection in pregnancy is associated with a robust inflammatory response at the maternal-fetal interface. *Med (New York NY).* (2021) 2(5):591–610.e10. doi: 10.1016/j.medj.2021.04.016

49. Grigorian A, Torossian S, Demetriou M. T-Cell growth, cell surface organization, and the galectin-glycoprotein lattice. *Immunol Rev* (2009) 230(1):232–46. doi: 10.1111/j.1600-065X.2009.00796.x

50. Kariya Y, Oyama M, Hashimoto Y, Gu J, Kariya Y. β4-Integrin/PI3K signaling promotes tumor progression through the galectin-3-N-Glycan complex. *Mol Cancer Res* (2018) 16(6):1024–34. doi: 10.1158/1541-7786.MCR-17-0365

51. Croci DO, Cerliani JP, Dalotto-Moreno T, Méndez-Huergo SP, Mascanfroni ID, Dergan-Dylon S, et al. Glycosylation-dependent lectin-receptor interactions preserve angiogenesis in anti-VEGF refractory tumors. *Cell* (2014) 156(4):744–58. doi: 10.1016/j.cell.2014.01.043

52. Giovannone N, Liang J, Antonopoulos A, Geddes Sweeney J, King SL, Pochebit SM, et al. Galectin-9 suppresses b cell receptor signaling and is regulated by I-branching of n-glycans. *Nat Commun* (2018) 9(1):3287. doi: 10.1038/s41467-018-05770-9

53. Gajovic N, Markovic SS, Jurisevic M, Jovanovic M, Arsenijevic N, Mijailovic Z, et al. Galectin-3 as an important prognostic marker for COVID-19 severity. *Sci Rep* (2023) 13(1):1460. doi: 10.1038/s41598-023-28797-5

54. Freitag N, Tirado-Gonzalez I, Barrientos G, Powell KL, Boehm-Sturm P, Koch SP, et al. Galectin-3 deficiency in pregnancy increases the risk of fetal growth restriction (FGR) via placental insufficiency. *Cell Death Dis* (2020) 11(7):560. doi: 10.1038/s41419-020-02791-5

55. Meggyes M, Miko E, Polgar B, Bogar B, Farkas B, Illes Z, et al. Peripheral blood TIM-3 positive NK and CD8+ T cells throughout pregnancy: TIM-3/galectin-9 interaction and its possible role during pregnancy. *PloS One* (2014) 9(3):e92371. doi: 10.1371/journal.pone.0092371

56. Abu-Raya B, Michalski C, Sadarangani M, Lavoie PM. Maternal immunological adaptation during normal pregnancy. *Front Immunol* (2020) 11:575197. doi: 10.3389/fimmu.2020.575197

57. Mor G, Aldo P, Alvero AB. The unique immunological and microbial aspects of pregnancy. *Nat Rev Immunol* (2017) 17(8):469–82. doi: 10.1038/nri.2017.64

58. Robertson SA, Care AS, Moldenhauer LM. Regulatory T cells in embryo implantation and the immune response to pregnancy. *J Clin Invest* (2018) 128 (10):4224–35. doi: 10.1172/JCI122182

59. Yockey LJ, Iwasaki A. Interferons and proinflammatory cytokines in pregnancy and fetal development. *Immunity* (2018) 49(3):397–412. doi: 10.1016/j.immuni.2018.07.017

60. Menkhorst E, Than NG, Jeschke U, Barrientos G, Szereday L, Dveksler G, et al. Medawar's PostEra: galectins emerged as key players during fetal-maternal glycoimmune adaptation. *Front Immunol* (2021) 12:784473. doi: 10.3389/fimmu.2021.784473

61. Sundblad V, Morosi LG, Geffner JR, Rabinovich GA. Galectin-1: a jack-of-All-Trades in the resolution of acute and chronic inflammation. *J Immunol* (2017) 199 (11):3721–30. doi: 10.4049/jimmunol.1701172

62. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. *Nat Rev Immunol* (2020) 20(6):363–74. doi: 10.1038/s41577-020-0311-8

63. Garcia-Flores V, Romero R, Xu Y, Theis KR, Arenas-Hernandez M, Miller D, et al. Maternal-fetal immune responses in pregnant women infected with SARS-CoV-2. *Nat Commun* (2022) 13(1):320. doi: 10.1038/s41467-021-27745-z

64. Toscano MA, Bianco GA, Ilarregui JM, Croci DO, Correale J, Hernandez JD, et al. Differential glycosylation of TH1, TH2 and TH-17 effector cells selectively regulates susceptibility to cell death. *Nat Immunol* (2007) 8(8):825–34. doi: 10.1038/ni1482

65. Kopcow HD, Rosetti F, Leung Y, Allan DS, Kutok JL, Strominger JL. T Cell apoptosis at the maternal-fetal interface in early human pregnancy, involvement of galectin-1. *Proc Natl Acad Sci USA* (2008) 105(47):18472–7. doi: 10.1073/pnas.0809233105

66. Magnus MC, Örtqvist AK, Dahlqwist E, Ljung R, Skår F, Oakley L, et al. Association of SARS-CoV-2 vaccination during pregnancy with pregnancy outcomes. *Jama* (2022) 327(15):1469–77. doi: 10.1001/jama.2022.3271

67. Luo Z, Ji Y, Tian D, Zhang Y, Chang S, Yang C, et al. Galectin-7 promotes proliferation and Th1/2 cells polarization toward Th1 in activated CD4+ T cells by inhibiting the TGFβ/Smad3 pathway. *Mol Immunol* (2018) 101:80–5. doi: 10.1016/j.molimm.2018.06.003

68. Sato S, St-Pierre C, Bhaumik P, Nieminen J. Galectins in innate immunity: dual functions of host soluble beta-galactoside-binding lectins as damage-associated molecular patterns (DAMPs) and as receptors for pathogen-associated molecular patterns (PAMPs). *Immunol Rev* (2009) 230(1):172–87. doi: 10.1111/j.1600-065X.2009.00790.x

69. Teijaro JR, Farber DL. COVID-19 vaccines: modes of immune activation and future challenges. *Nat Rev Immunol* (2021) 21(4):195–7. doi: 10.1038/s41577-021-00526-x

1 Publication

The paper is published in Frontiers in Immunology, section Immunological Tolerance and Regulation (doi: 10.3389/fimmu.2023.1196395).

2 Summary

2.1 Introduction

2.1.1 Galectins

Galectins (gal) are endogenous carbohydrate-binding proteins characterized by a unique sequence motif in their carbohydrate recognition domain (CRD), with the ability to bind soluble β-galactoside sugars. As of now, more than 13 family members have been identified in humans, displaying different intra- and extracellular localizations and biological functions [1-3].

2.1.1.1 Structures and functions of galectins

Galectins are classified based on their molecular structure as prototype, chimera or tandem-repeat type. The prototype contains one CRD per subunit and are biologically active as monomer [1, 4]. It comprises gal-1, gal-2, gal-5, gal-7, gal-10, gal-11, gal-13, gal-14 and gal-15 [5]. The chimera type, represented by gal-3, consists of a C-terminal CRD connected to a N-terminal and aggregates through its non-lectin domain as oligomer [1]. Finally, the tandem-repeat type contains two distinct CRDs connected by a short linker peptide in a single polypeptide. The gal-4, gal−6, gal−8, gal−9, and gal−12 are members in the tandem-repeat group [4, 6] (**Figure 1**).

Due to the absence of a classical secretory sequence, galectins are secreted and exported from cells by a non-classical secretion to the extracellular space where they bind to carbohydrate ligands on the cell surface or in the extracellular matrix [7, 8]. Following the synthesis in the cytoplasm, galectins can be translocated to the nucleus and participate in transcriptional regulation [1]. Galectins are presented in both intracellular and extracellular environments and exhibit a distinct combination of biological functions, including modulating cell growth, differentiation, and migration, as well as regulating cell adhesion, apoptosis, and immune responses [1-4, 6]. These functions either are regulated by the ability to engage in protein–protein interactions or depend on the interaction of their CRDs with N-acetyllactosamine (LacNAc) residues common in many cell-surface and extracellular matrix glycoproteins. Reduced levels and impaired functions of galectins are frequently associated with various pathologies [1, 4].

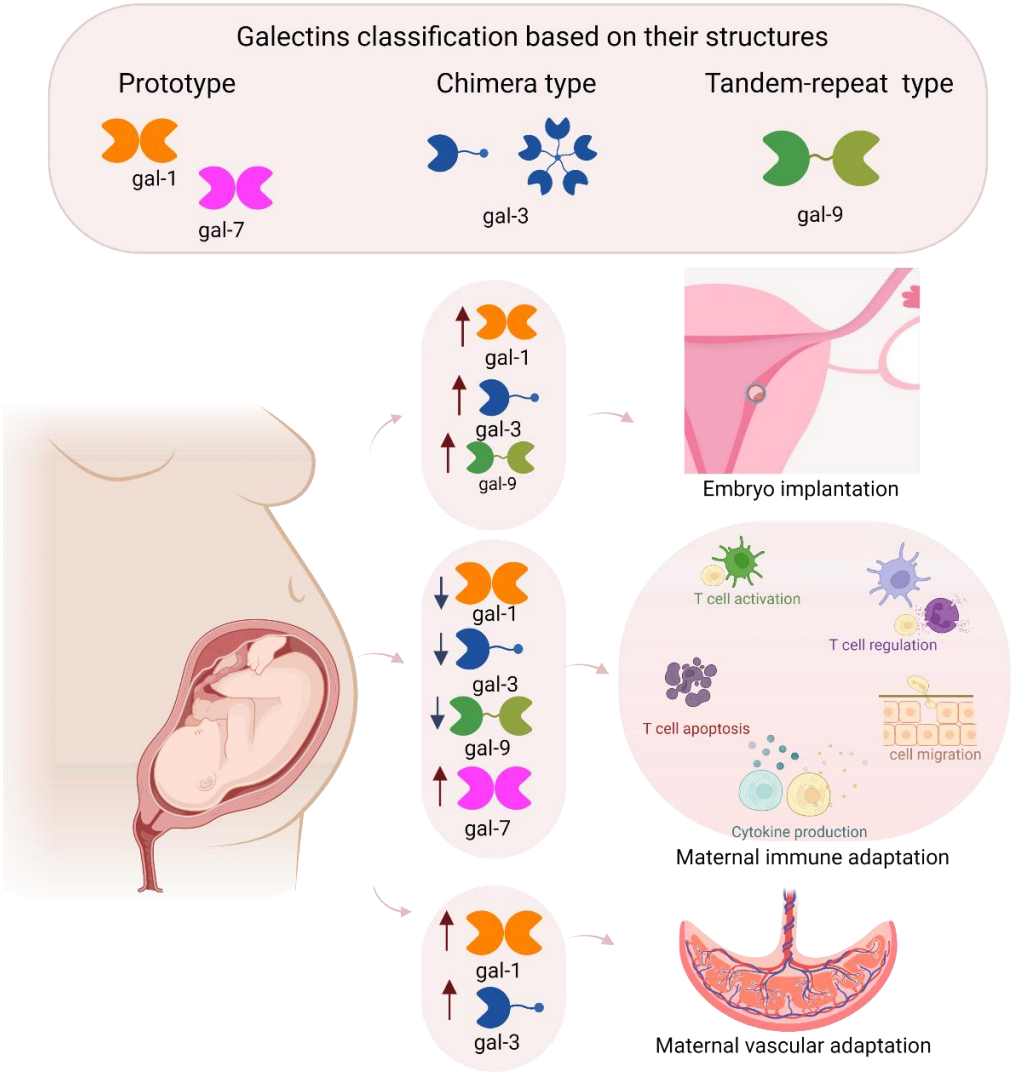


Figure 1: Galectins classification and their functions during pregnancy adaptation.

2.1.1.2 Galectins in pregnancy associated processes

Over the past decades, galectins have been recognized as contributing factors in the reproductive orchestration, which includes embryonic implantation, placental development, as well as maternal immune and vascular adaptation [1-3, 6, 9-12] (**Figure 1**). A brief description of the most abundant galectins at the maternal-fetal interface are summarized below:

Galectin-1

The expression of gal-1 in the endometrium undergoes changes during both the menstrual cycle and pregnancy. These changes are regulated by ovarian steroids, including estrogens and progesterone. Specifically, endometrial gal-1 rapidly increases during the late secretory phase endometrium and reaches even higher levels in the decidua as gestation progresses [5, 13]. Galectin-1 has been detected in human embryos as early as day 5 of development and may start

to influence the trophoblast differentiation prior to implantation [3]. During the window of embryo implantation, gal-1 influences uterine epithelial preparation for receptivity, as well as blastocyst activation. This is achieved through its binding to mucin-1 via the Thomsen-Friedenreich epitope, which is involved in the embryo-derived signals [14]. The maternal immune adaptation to pregnancy is a highly regulated process involving several galectins [1, 9, 12]. As an anti-inflammatory factor, gal-1 mediates immune cell activation and inflammation at the feto-maternal interface in order to sustain a local inflammatory balance that favors pregnancy maintenance [15, 16]. This delicate balance is crucial in safeguarding the developing fetus from harm while still allowing the mother to be protected against infections [17]. In particular, gal-1 induces the expression of tolerogenic dendritic cells and $CD4^+$ $CD25^+$ IL-10^+ regulatory T cells (Treg) in the decidua, shifting the T cell helper (Th)1/Th17 and Th2 balance into a Th2-dominant cytokine profile to sustain a successful pregnancy [11, 15, 16]. This was initially observed in a stress-challenged mouse model, which was accompanied by a significant reduction in the expression of gal-1 in the decidual tissue. Moreover, supplementing with recombinant gal-1 during early pregnancy prevents the rejection of the fetus by restoring the balance of Th1/Th2 cytokines in decidual mononuclear cells [15]. Furthermore, gal-1 blocks the stimulation of LPS on IL-6 production in human decidua cells *in vitro* and consequently regulates decidual immune cell populations to support a local anti-inflammatory microenvironment during pregnancy [18]. On the placenta niche, gal-1 modulates human leukocyte antigen G (HLA-G) expression on extravillous trophoblast cells, promoting maternal–fetal immune tolerance [3]. Moreover, cytotrophoblasts in human placenta also express gal-1, which has been suggested modulating maternal immune responses [5, 19]. Regarding maternal vascular adaptation, different steps of the angiogenic cascades and endothelial cell biology are influenced by gal-1 [15, 20-22]. For instance, gal-1 is involved in spiral arterial remodeling process by promoting endothelial cell activation, facilitating the transformation of spiral arteries into low-resistance vessels that can supply the growing placenta [21, 22]. This lectin (gal-1) also has proangiogenic functions, including the modulation of endothelial cell adhesion, migration, and proliferation when interacting with the neuropilin (NRP)-1/ vascular endothelial growth factor receptor (VEGFR)-2 signaling pathway. Such interaction is important in promoting angiogenesis during implantation, and placentation [15, 21, 23]. Our previous study shows that interfering with VEGFR2 signaling abrogates the protective effect of gal-1 in a model of reduced vascular expansion [21]. Thus, gal-1 plays a critical role during the angiogenesis processes in pregnancy and lack of gal-1 leads to complications with impaired angiogenesis such as preeclampsia (PE).

Galectin-3

During the peri-implantation period, the expression of gal-3 is increased, specifically in the endometrium. It can be detected in maternal decidual cells and all trophoblast lineages of the

placenta as the pregnancy progresses, indicating its significant role in the processes of implantation [24, 25]. Moreover, gal-3 is part of the trophoblast invasion machinery as well as a positive modulator of trophoblast cell functions involved in healthy placental development [25, 26]. The chimera type is considered as a pivotal modulator for innate and adaptive immune responses. It is involved in the activation and differentiation of diverse immune cells, including the induction of T cell apoptosis in the extracellular environment and inhibition of apoptosis when functioning intracellularly. Additionally, gal-3 modulates the activation and cytotoxicity of natural killer (NK) cells [27-30]. Moreover, gal-3 regulates inflammatory responses by influencing the production of pro-inflammatory cytokines, acting as a mediator in acute and chronic inflammatory processes [28]. Despite the function of gal-3 in the context of immune adaption during pregnancy is just emerging, gal-3 is proposed to be involved in inflammatory responses in infectious diseases during pregnancy [31]. For instance, increased gal-3 expression is reported in the amniotic epithelium in chorioamnionitis infection and in the preterm premature rupture of the membranes, indicating that gal-3 mediates the chorioamniotic inflammation [32, 33]. In addition, gal-3 can bind to VEGFR2 and enhance its phosphorylation, leading to the activation of downstream signaling cascades involved in angiogenesis [23, 34-37]. This interaction affects endothelial cell migration and capillary tube formation, which are important processes for placental development. The combined effect of exogenous gal-1 and gal-3 on angiogenesis-related events mediate VEGF activation by increasing the density of VEGFR1 and VEGFR2 on the cell surface, making them accessible to low levels of endogenous VEGF [38]. In mice, maternal gal-3 deficiency is associated with defective vascularization and impaired differentiation of trophoblast layers, resulting in placental insufficiency [39]. Pregnant women who experienced fetal growth restriction, a condition associated with placental insufficiency, exhibited lower circulating levels of gal-3. This decrease in gal-3 levels was observed starting from the first trimester and persisted throughout pregnancy, indicating the crucial involvement of gal-3 in placental development [39].

Galectin-7

During early pregnancy, gal-7 regulates the adhesion of the embryo to the endometrium [40], but is unlikely to be the key factor of blastocyst adhesion since it remains unchanged during the receptive phase of the menstrual cycle [41]. However, compared to normal healthy pregnancies, gal-7 is significantly higher at first trimester of gestation in women who miscarried. This abnormal increase in gal-7 alters endometrial function and allows embryos with lower quality to implant, and consequently leads to inappropriate blastocyst implantation and miscarriage [41]. Moreover, a recent study suggests that there is an increase in gal-7 expression in the endometrial epithelial cells of women who have a history of recurrent early pregnancy loss; the authors proposed that this is probably because gal-7 induces T cells towards a Th1 phenotype and alters the inflammatory environment in the endometrium [41, 42]. In addition, increased gal-7 is found

in chorionic villous samples and maternal serum during early stage of pregnancy from women who subsequently develop PE [35, 36]. Exogenous gal-7 alters renin-angiotensin-system (RAS), as well as triggers pro-inflammatory responses with elevated interleukin (IL)-1β and IL-6 in placenta biopsies of pregnant mice, which compromised the placental function and caused PE-like features [43]. Taken together, these studies suggest that the abnormal upregulation of gal-7 induces a pro-inflammatory state during early pregnancy. This consequently contributes to various complications related to impaired immune tolerance, including recurrent early pregnancy loss and PE.

Galectin-9

Endometrial gal-9 expression is detected mainly on epithelial cells and notably up-regulated in endometrium during the late-secretory phases and implantation, as well as in the decidua during early pregnancy, pointing out its potential role in embryo implantation [44]. Gal-9 is considered an immunomodulatory galectin [44-46]. The immune regulatory effects of gal-9 primarily rely on its interaction with the cell-surface receptor T-cell immunoglobulin and mucin domain 3 (Tim-3). Gal-9 acts as a negative regulator, restricting Th1 and Th17 immune responses and influencing the balance between Th1 and Th2 immune responses [46-49]. Moreover, Tim-3^{+} uterine NK (uNK) cells and the expression of gal-9 in placenta are reduced from mouse abortion-prone models [2]. Hence, the gal-9/Tim-3 pathway could have important roles in maternal immune adaptation. The dysregulation of gal-9 may be associated with an altered Th1/Th2 balance at the maternal-fetal interface and resulting in pathologic pregnancies such as spontaneous miscarriage or PE. Additionally, different gal-9 isoforms show inhibitory effects in angiogenesis *in vivo*, and the dominant isoform in endothelial cells gal-9 M exhibits a diphasic effect indicating its complex regulatory role in angiogenesis [50]. Moreover, the expression of six gal-9 splice variants have been reported in human decidua, among them, gal-9 D5 variant is considered to selectively suppresses interferon (IFN)-γ production by uNK cells, which could consequently influence the decidual arterial remodeling process [45].

2.1.1.3 Fluctuation of maternal circulating galectins during pregnancy

In normal pregnancy, serum gal-1 levels increase considerably during the first trimester, peaking in the second trimester and remain similar levels until the end of the third trimester [3]. This increase in peripheral gal-1 level is consistent with the period of placenta development during the first trimester, suggesting that trophoblast cells could be one of the sources of the circulating gal-1 [3, 15]. The increase of circulating level of gal-3 begins in the second trimester and continues in the third trimester [6, 31]. Serum gal-7 and gal-9 levels of pregnant women increase mildly compare to non-pregnant (Non-P) women in the second trimester [41, 51-53] (**Figure 2**).

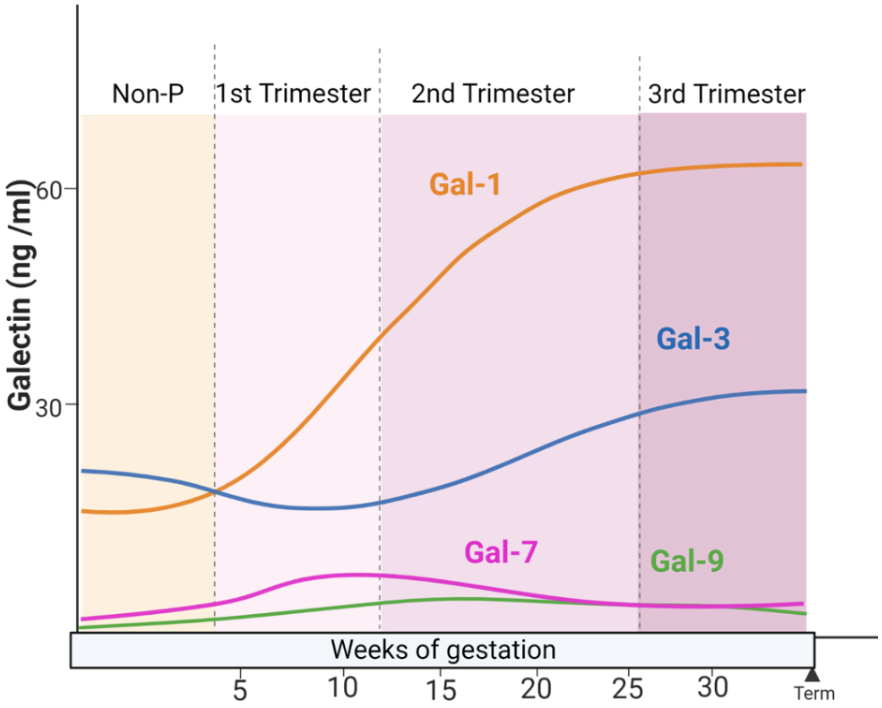


Figure 2: Fluctuation of maternal circulating gal-1, gal-3, gal-7, and gal-9 before and during the first, second and third trimester of healthy pregnancy. Non-P, Non-pregnant women.

2.1.1.4 Pregnancy specific glycoprotein 1

Native pregnancy-specific glycoprotein (PSG)-1 derived from healthy pregnant women has been recently identified as a novel gal-1 ligand, and the N and A2 domains of PSG1 are confirmed to interact with gal-1 [54] (**Figure 3**). PSGs belong to the carcinoembryonic antigen family within the immunoglobulin (Ig) superfamily [55]. They are generated by placental trophoblasts, mainly the syncytiotrophoblasts, and subsequently released into the maternal circulation. Notably, PSGs are the most abundant trophoblastic proteins in human maternal blood during pregnancy [54, 55].

PSG1 acts as a biological activator of transforming growth factor beta (TGF-β)2 through its interaction with latency-associated peptide, thereby promoting the generation of active TGF-β and facilitating its role in establishing maternal immune tolerance [56-58]. Additionally, PSG1 stimulates the secretion of VEGF-A, enhancing the endothelial tube formation and consequently participates in the regulation of maternal angiogenic adaption [59, 60]. Moreover, PSG1 inhibits the interaction between platelet and fibrinogen by binding to integrin αIIbβ3, which in turn antagonizes platelet formation and prevents thrombosis in the placenta [61].

Maternal serum concentration of PSG1 rapidly increases as pregnancy progresses, reaching the highest level at the end of the third trimester [54, 62, 63] (**Figure 3**). Low level of circulating PSGs concentrations in early pregnancy is related with pregnancy complications such as small-for-gestational-age fetuses, and spontaneous preterm delivery [64]. This suggests the high

steady state of PSG1 in maternal circulation could benefit both mother and fetus and ensure a successful pregnancy.

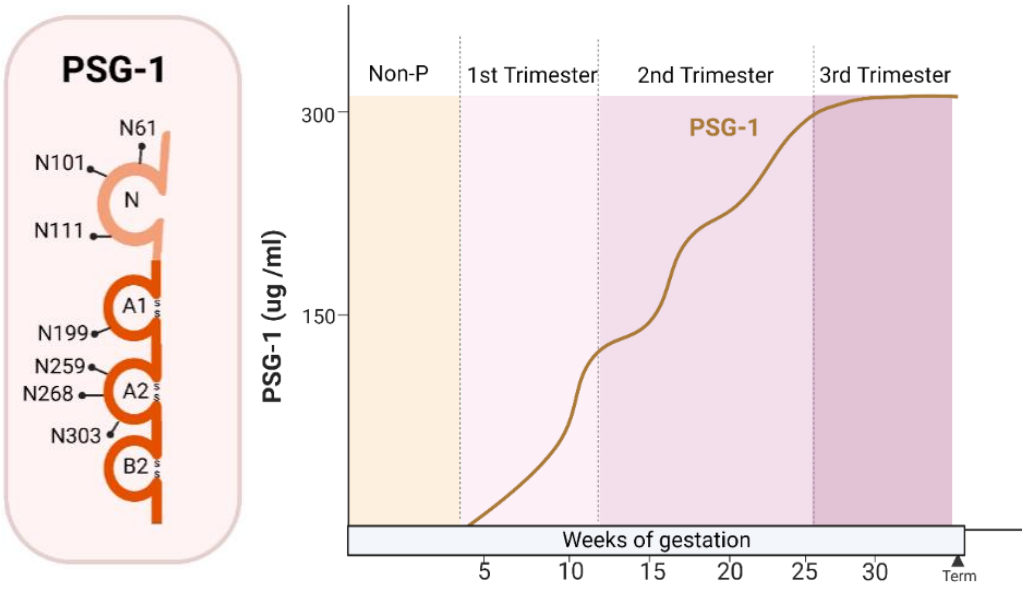


Figure 3: PSG1 structure and its fluctuation in maternal blood circulation during pregnancy. The domain organization of the human PSG1 contains one Ig variable-like domain (N-domain), three Ig constant-like domains (A1, A2 and B2) and a relatively hydrophilic tail. Several possible N-glycosylation sites are present in the PSG1 sequence, which are in the N- (Asn61, 104 and 111), A1- (Asn199) and A2- (Asn259, 268 and 303) domains [56, 62, 65].

2.1.2 Maternal adaptation to pregnancy

Maternal systems progressively adapt during pregnancy to accommodate the increasing demands of fetal growth and development [1, 4, 12]. It involves physiological and psychological changes, including the coordination of a series of simultaneous events occurring in the immune and cardiovascular system, as well as the awareness of the motherhood role in the patient's mind (**Figure 4**). These intricate elements collectively contribute to the overall success of the pregnancy.

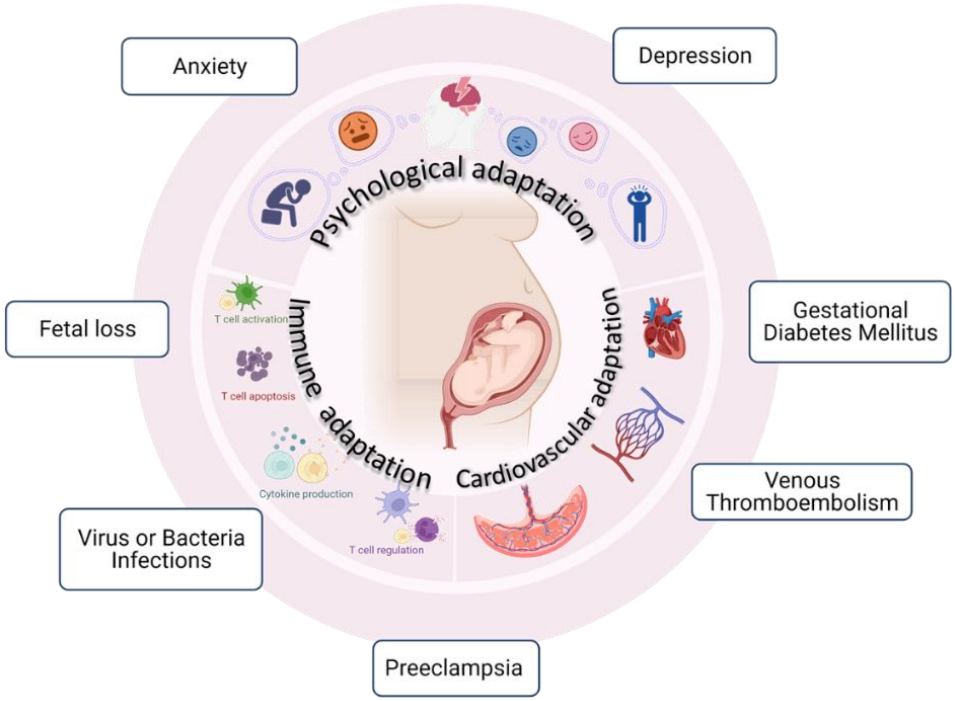


Figure 4: Maternal adaption during pregnancy and associated complications.

2.1.2.1 Immune system adaptation

During pregnancy, the maternal immune system undergoes complex adaptations to ensure the tolerance towards the semi-allogeneic fetus while still being able to effectively respond to foreign infectious agents and harmful substances. These adaptations aim to strike a balance between protecting the developing fetus and maintaining the ability to combat potential threats from the external environment such as infections [12]. Multiple factors are involved in the immunoregulatory processes at the feto-maternal interface to prevent the rejection of the allogeneic fetus and contribute to a successful pregnancy [1, 4]. Pregnancy-related changes in hormones, for example progesterone and estrogen, influence changes in immune cell function and cytokine production. For instance, both progesterone and estrogen promote the production of Th2 cytokine IL-6 and inhibit the production of the Th1 cytokine IL-2, shifting the Th1/Th2 balance towards an anti-inflammatory profile which is conducive for pregnancy [66].

The limited Th1 immune response facilitates trophoblasts invasion and promotes tissue remodeling and angiogenesis during the implantation period. Th1 secretes several cytokines including IL-2, which take part in the immune surveillance and hamper excessive trophoblast invasion [67, 68]. After the peri-implantation period, Th2 cells are activated at the maternal-fetal interface and infiltrate into the decidua basalis, promoting maternal-fetal tolerance by releasing Th2 cytokines such as IL-4 and IL-13 [69, 70]. In addition, uncontrolled Th1 immunity has been related to pregnancy losses and implantation failures [71], therefore the balance and mutual restrain of the T helpers are essential to a successful pregnancy.

As mentioned previously, galectins play a pivotal role in pregnancy establishment and maintenance due to their immunological effects and compromised galectins expressions are frequently observed in various pathologies associated with impaired immune responses [1, 4, 12, 72]. Gal-1 expression both in maternal circulation and at the feto-maternal interface is downregulated during pregnancy loss and is associated with compromised immune tolerance [15, 73]. Exogenous administration of gal-1 reduces fetal resorption rate in several mouse models of immunological abortions, probably by inducing tolerogenic dendritic cells and $CD4^+$ $CD25^+$ IL-10^+ Treg, or by restoring progesterone and progesterone-induced blocking factor serum levels [74, 75]. Regarding gal-3, patients with spontaneous abortion have decreased gal-3 expression in chorionic villi [31]. In gal-3 deficiency mice, increased inflammatory cytokine gene expression and aberrant NK cell infiltration are observed during pregnancy, resulting in fetal growth restriction [6]. In addition, elevated gal-7 levels are found at first trimester of gestation in women who subsequently experience early pregnancy loss [41]. In human patients, spontaneous abortion is associated with down-regulation of *LGALS*9 D5/10 splice variant [45]. Additionally, gal-1, gal-3, gal-7 and gal-9 are associated with the inadequate maternal immune adaptation in preeclampsia that will be discussed into details in the following section.

2.1.2.2 Cardiovascular system adaptation

Early in pregnancy, adaptions of the cardiovascular system are needed due to the increased metabolic demands of the mother and fetus. The cardiac output progressively increases from 6 weeks' gestation and reaches its peak values at 32 weeks and maintains until 37 weeks. The blood is redistributed to the uterus and placenta, kidneys, breasts, and skin, and 20% to 25% of the maternal cardiac output is distributed to the uteroplacental unit. The atrial and ventricular cardiac chambers enlarge with an enlarge over 30% of the atrial diameters while ventricular function is preserved. Maternal cardiac dysfunction is related to impaired uteroplacental flow and suboptimal fetal outcome [76].

Mean arterial blood pressure is directly proportional to cardiac output and systemic vascular resistance. The underlying mechanism of this decrease in systemic vascular resistance is likely multifactorial. Progesterone and nitric oxide likely exert some effects on relaxing vascular smooth muscle and contributing to changes in vascular resistance [76]. The blood pressure during pregnancy tends to be lower than nonpregnant value. Systemic and pulmonary vascular resistances decline and reach a lowest point at around 20 weeks' gestation followed by a gradual increase until term [76, 77].

Further adaptions in the maternal cardiovascular system may be observed in pregnancies with complications such as intrauterine growth restriction and preeclampsia due to the declined cardiac output and heart rate but increased vascular resistance of the mother [76-80].

2.1.2.3 Preeclampsia and pregnancy

The International Society for Studies in Gestational Hypertension define PE with systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, which should be measured at two separate occasions at least 4 to 6 hours apart. In addition, one of the conditions below must coexist: 1) Proteinuria (>300 mg/24h, or the ratio of proteinuria/creatininuria > 0.3 mg/mg, or the urine dipstick test ≥1+); 2) Insults of maternal organs (dysfunctions of kidney or liver, or neurological complications, or pain in the right hypochondrium, or hematological conditions); 3) Impairment of placenta and/or uterus [71]. PE imposes an increased -risk of cardiovascular and metabolic complications in mothers and children worldwide [81-83]. PE is usually classified depending on the gestational age as early-onset PE (<34weeks) and late-onset disease (>34weeks). Studies suggest that the late-onset PE is considered to occur as the consequence of impaired maternal vascular and immune adaptation to pregnancy, while the early-onset disease is mainly believed to associate with inadequate placentation [84-87]. However, this classification based solely on gestational age can hardly capture the full spectrum of preeclampsia subtypes and may overlook important differences in pathophysiology and maternal-fetal risks. A more recent classification based upon the placental transcriptomics identified PE into three types: canonical, maternal, and immunological. The canonical/placental type is characterized by impaired placental vascular function, including increased sFlt-1 expression and abnormal Doppler velocimetry, resulting in preterm birth and lower body weight of the neonate. Another type, maternal PE, is normally caused by chronic conditions of the mother with hardly any lesions in the maternal vasculature and mild influence on the fetus. Finally, the immunological type is associated with increased immune response and compromised maternal-fetal tolerance [88].

With regard to galectins in PE, serum gal-1 levels are reduced during early pregnancy in women who subsequently develop PE compared with women with an uneventful pregnancy [21, 73]. This is in line with our previous finding that gal-1 deficiency mice show compromised placentation, and consequently exhibits preeclampsia-like-features [21]. After diagnosis of late-onset PE, pregnant women show elevated gal-1 levels in maternal blood compare with healthy pregnant women [21, 73]. In contrast, gal-3 deficiency is associated only with impaired differentiation of trophoblast layers and placental insufficiency in mice [89]. Pregnant women with preeclampsia have higher gal-3 expression in placental tissues than the healthy ones, whereas whether altered maternal serum gal-3 levels in preeclampsia patients are controversial. In the study performed by Pankiewicz *et al.*, they exhibited higher gal-3 levels in patients with preeclampsia compared with healthy women. However, according to another research conducted in Bulgaria, there are not any noteworthy differences in gal-3 between the individuals with early-onset PE and those who had a normal pregnancy [89-91]. Women who have abnormally elevated gal-7 in serum or increased expression in chorionic villous samples during early pregnancy are more likely to subsequently develop early onset preeclampsia as the pregnancy progresses [43, 51]. Similarly, *in vivo* administration of exogenous gal-7 to pregnant

mice induces a pro-inflammatory state in the placenta and compromises the RAS homeostasis in maternal circulation, resulting in typical PE symptoms [43]. In addition, the expression and function of Tim-3/gal-9 was upregulated in decidual tissue in patients with preeclampsia [92].

2.1.2.4 Psychological adaptation

There is a rising attention for emotional adaptation during pregnancy and much of the existing literature focuses on risk factors for maternal mental health problems and stress during pregnancy [93, 94]. Even in a planned pregnancy, a woman may struggle to identify with the motherhood role or adapt to the physiological and psychological transformation [95]. Every woman undergoes and adapts to the pregnancy process uniquely. Pregnant women with previous mental health problems and social adversity are more likely to experience risks in their mental health and prenatal stress than women with positive psychological status during pregnancy [96]. Therefore, a healthy mental adaptation is a source of strength and resilience and provide promotive and protective benefits for maternal and infant outcomes. As a result, pregnancy is a sensitive period when a woman is vulnerable to the effects of adversity and sometimes correlates with increased vulnerability for the onset or relapse of a mental illness [93, 94, 97]. In pregnancy, maternal stress (i.e., perceived stress, depressive symptoms, racial discrimination, stressful life events, and pregnancy-specific anxiety) has been associated with preterm birth, low birth weight, risk of gestational hypertension, and adverse health and behavioral outcomes in offspring. Rise of physiological stress during pregnancy have been shown to increase the risk of maternal hypertension and diabetes, the susceptibility to infectious illnesses, as well as the emergence of depression and anxiety disorders [98-100]. Depression and anxiety are the most common psychiatric disorders during pregnancy [93, 101].

Several risk factors could influence the psychological adaptation to pregnancy and the motherhood role in pregnant women, including maternal characteristics like physical states and age, educational level, planned pregnancy, economic status and support from family, spouse, or government.

2.1.2.5 Antenatal anxiety and pregnancy

The exposure to maternal distresses, such as stress and anxiety during pregnancy has been associated with disturbing fetal glucocorticoid environment, altered physiological and neurocognitive development, and increased socio-emotional behavior problems in children [93, 94]. The links between maternal prenatal distress and child outcomes are part of the phenomenon known as fetal or prenatal programming because effects are often profound and long-lasting [101]. Moreover, there is a tendency to focus on physical health rather than mental health during pregnancy, and to misattribute emotional complaints to the physical and hormonal changes that occur during pregnancy [102]. Indeed, these women often present atypical symptoms of depression and unspecified somatic complaints such as fatigue, loss of energy, loss

of appetite, and sleep changes, rather than depressed mood. Therefore, it can be difficult to distinguish between physical pregnancy symptoms, which are common during pregnancy, and atypical somatic complaints, which may be related to depression or anxiety.

When the mother is exposed to stress, the hypothalamic–pituitary–adrenal (HPA) axis is activated, resulting in the release of multiple hormones, including cortisol. Cortisol is the end metabolite of the HPA axis and is essential in normal brain development [100].

Maternal cortisol levels increase during pregnancy and can be directly transported across the placenta to enter fetal circulation, accounting for approximately 40% of the variance in fetal cortisol concentrations [98]. However, due to pregnancy-related changes in the HPA axis, together with elevated cortisol level is associated with other factors, such as age, medical conditions, and different living habits, the association between stress and cortisol levels are not clear among pregnant women [93-95, 97, 101].

So far, there are lack of studies regarding the expression of galectins levels in maternal blood in human or mouse models related to anxiety [95]. Clearly, further studies are required to describe the expression of galectins in fetal-maternal interface and the potential role of galectins in maternal serum as a biomarker for pregnancy pathologies.

2.1.3 SARS-CoV-2 and pregnancy

In December 2019, a novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing the Coronavirus disease 2019 (COVID-19) pandemic, has an unprecedented effect on global health and affected the lives of millions of people around the world since its first identification in Wuhan, China [103, 104]. As of July 2023, a total of about 768 million cases along with 6.9 million deaths related to COVID-19 have been confirmed worldwide, which highlights the severity of SARS-CoV-2 infection [103-105]. The possible transmission route of this novel coronavirus is by contacting nasal and oral secretions of an infected individual, or by exposure to cough, sneeze, respiratory droplets of the infected patients [104-106]. The typical symptoms of a patient who has been infected with SARS-CoV-2 are fever, cough, myalgia, fatigue, normal or decreased leukocyte counts, and radiographic evidence of pneumonia. Up until now, despite the World Health Organization's predictions of an imminent end to the pandemic, COVID-19 continues to result in a significant number of intensive care unit cases and high mortality rates [103-108].

As with all viruses, mutations can occur leading to the emergence of new strains. To date, five of the new strains of the SARS-CoV-2 virus are of concern and have been termed as the Alpha, Beta, Gamma, Delta and Omicron variants. These variants have specific traits which may include increased transmissibility or more severe disease. The Delta variant seems to be associated with more severe disease [109, 110]. The Omicron variant, which was first reported in Botswana in November 2021, has been considered more infectious than the previous variant, with the ability to infect previously SARS-CoV-2-positive people. In Europe, even Omicron

variant causes milder symptoms than other previous variants, there are still considerable numbers of hospitalizations and mortality, especially in elderly people, pregnant women, and people with other comorbidities such as hypertension and obesity [111-113]. Owing to numerous mutations involving the spike protein, this variant has developed increased transmissibility compared with the Delta variant, rapidly becoming the predominant variant in many other countries.

2.1.3.1 COVID-19 infection and galectins

Recent studies revealed that circulating gal-1, gal-3 and gal-9 levels were increased in non-pregnant COVID-19 patients [114-118]. The increase of gal-1 may be because it has an anti-inflammatory activity to prevent and control the progression of inflammation and tissue destruction during SARS-CoV-2 infection, probably by preventing leukocyte invasion and migration [116, 117]. Moreover, gal-1 is considered as a disease prognostic marker for COVID-19 due to its positive correlation with clinical parameters of SARS-CoV-2 infection such as dry cough and chest radiographic findings [116, 117]. Levels of gal-3 and gal-9 were reported to be more pronouncedly increased in patients with severe disease compared to those ones with milder syndrome [115, 116], raising the possibility that circulating gal-3 or gal-9 can be valuable biomarkers for severe pneumonia in COVID-19 patients [119, 120]. Interestingly, exogenous gal-9 administration during acute SARS-CoV-2 infection has been shown to increase survival rate and induce a robust innate and adaptative immune response in mice [121]. More recently, an inhaled gal-3 inhibitor has been tested as a potential therapy for COVID-19 pneumonitis [122]. However, the galectin signature in SARS-CoV-2 infection and vaccination during pregnancy remain to be determined.

2.1.3.2 COVID-19 disease and pregnancy

Even though measuring the precise number of cases of SARS-CoV-2 infection in pregnant patients is difficult, approximately 20% of women are in the reproductive age and are pregnant at any given time. Consequently, we can deduce several millions of pregnant women were infected with SARS-CoV-2 during the past three years [123].

During pregnancy, especially in the last trimester, the upper respiratory tract of pregnant women is swollen, and their lung expansion are restricted [124]. In general, pregnant women experience immunologic and physiologic changes that make them less tolerant to viral respiratory infection, therefore pregnant women and their neonates are considered a higher risk group during and after pregnancy [125, 126].

The most common symptoms of COVID-19 disease in pregnant women are cough, fever, sore throat, dyspnea, myalgia, and loss of sense of taste. Studies showed that pregnant women have similar infection rate compared with the general population, and more than two-thirds of identified pregnant women have no symptoms [127]. After being infected, pregnant women are

more likely to develop cytokine storms in the pro-inflammatory stages, especially when they are infected during first trimester, which affected with respiratory changes may make them more susceptible to the effects of hypoxia [127]. There is growing evidence that pregnant women may be at increased risks of severe illnesses such as severe pneumonia, hospitalizations, admission to intensive care unit, invasive mechanical ventilation and mortality from SARS-CoV-2 infection compared with non-pregnant women, particularly in the second half of pregnancy [128]. In September 2020, a second wave of the pandemic appears to have more impact on pregnant women, including adverse maternal and neonatal outcomes, especially in unvaccinated pregnant women. The main reason could be that the variants in the second wave are considered more deadly than the ones from first wave (the Delta variant is associated with more severe diseases, and the Omicron variant is more contagious) [124].

Maternal SARS-CoV-2 infection affects pregnancy outcomes, with increased incidences of intensive care admission and invasive ventilation, iatrogenic preterm birth, stillbirth, severe disease, and maternal mortality [126, 129-134]. Pregnant infected women are potentially more vulnerable to cardiac complications such as arrhythmias, myocardial injury, thromboembolic complications, and development of preeclampsia . Women with comorbidities such as obesity, diabetes, heart disease, advanced maternal age, or those who are not vaccinated are considered at higher risk of severe COVID-19 [133, 135]. Even though the admission rate into hospitals is higher in the third trimester [136], a recent study pointed out that there is no association between the gestational age at infection and COVID-19 mortality and morbidity, suggesting that other gestational-age-related factors could be involved [129].

2.1.3.3 Pregnant women with vaccination against SARS-CoV-2

To date, the Pfizer-BioNTech and Moderna mRNA vaccines have the most published data of their use in pregnant women [123, 137]. The mRNA vaccines release mRNA which after being internalized by the cell, produces the SARS-CoV-2 spike protein and induces a response that provide immunity against the virus [137]. By contrast, in the DNA vaccines (i.e.: Oxford-AstraZeneca, and Johnson & Johnson-Janssen), a first copy of DNA into mRNA must be done into the cell. To date, both mRNA and DNA vaccines have been shown over 75% efficacy for preventing symptomatic illness in the non-pregnant people [123, 137].

SARS-CoV-2 antibodies have been documented in umbilical cord blood and breast milk after maternal full vaccination, suggesting protection to the fetus [126, 138-145]. Based on the growing evidence that supports the safety and efficacy of SARS-CoV-2 vaccination in pregnancy, most countries recommend full SARS-CoV-2 vaccination, including the boost dose for all pregnant populations regardless of pregnancy trimester [126, 131, 138, 140-149]. This is because the utmost goal of vaccination is to confer both the pregnant women and neonatal benefits by preventing adverse pregnancy outcomes related to severe maternal COVID-19 illness. In general, there are no known risks from receiving inactivated or recombinant vaccines

in pregnancy, or while breastfeeding. Therefore, there is no reason to suppose that the adverse effects from these SARS-CoV-2 vaccines should differ for pregnant women compared to non-pregnant women. Moreover, 98% of women admitted to hospitals and getting severe infection were unvaccinated [123].

Pregnant women are more likely to become seriously unwell when compared to non-pregnant women, and have a higher risk of their baby being born prematurely if they develop COVID-19 disease in their third trimester. Women should be offered both doses and a booster before giving birth if time allows, or before entering the third trimester, bearing in mind that it takes time for immunity to develop, and protection is higher after the second dose and a booster of the vaccine. A most recent study discovered a boosting effect of a third vaccine dose during pregnancy in women who were vaccinated in the first trimester [137]. Up until now, according to the US Vaccine Safety Datalink, up to 70% of adult pregnant women aged under 50 years old had been fully vaccinated with SARS-CoV-2 vaccines before or during pregnancy [123, 125, 137].

2.2 Material and methods

2.1 Materials

Table 1. Material

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Elisa Kits		
Gal-1 Human ELISA kit	PeproTech	Cat. # 500-P210
Gal-3 Human ELISA kit	R&D systems	Cat. # AF1154
Gal-7 Human ELISA kit	R&D systems	Cat. # 842118
Gal-9 Human ELISA kit	R&D systems	Cat. # AF2045
PSG-1 Human ELISA kit	R&D systems	Cat. # DY6799-05
Streptavidin-POD	Calbiochem	Cat. #189733
Software and Algorithms		
Prism Version 9.1.2	GraphPad Software,Inc.	https://www.graphpad.com/
Adobe Illustrator	Adobe	https://adobe.com/
R Statistical Software	Rstudio	https://www.r-project.org
BioRender	BioRender	https://app.biorender.com/
SkanIt RE 7.0	Thermofisher	https://www.thermofisher.com/de/de/home.html
Chemicals		
PBS Buffer	Thermofisher	Cat. # 10010031
Half area 96 microtiter plates	Corning	REF3690
Sodium chloride	Sigma-Aldrich	Cat. # S3014
Potassium Chloride	Sigma-Aldrich	Cat. # 60142
Disodium phosphate	Sigma-Aldrich	Cat. # S7907
Potassium dihydrogen phosphate	Sigma-Aldrich	Cat. # 529568

monobasic		
Tween 20	Sigma-Aldrich	Cat. # P7949
Bovine serum albumin	Sigma	Cat. # A2153-50G
3,3′,5,5′-Tetramethylbenzidin	Fluka	Cat. # 87748
Citric Acid	PanReac AppliChem	Cat. # 141018.1211
Ethanol	Roth	Cat. # 9056.3
Dimethyl sulfoxide	Sigma Aldrich	Cat. # D2650
Sulfuric acid	Thermofisher	Cat. # 10558620
Potassium hydroxide	Sigma Aldrich	Cat. # 221473

2.2 Methods

As described above in the publication (doi: 10.3389/fimmu.2023.1196395).

2.3 Results

2.3.1 Clinical characteristics of patients from the PRINCE cohorts

We first analyzed the pregnant women from different PRINCE cohorts in order to address our aim of determining the galectins signature in healthy and compromised pregnancies. We classified our patients into six groups as follows: patients with preeclampsia (PE, n=20), patients with higher blood cortisol (High cortisol, n=20), pregnant women who tested positive for SARS-CoV-2 by nasopharyngeal swab qRT-PCR during pregnancy (COVID, n=20), pregnant women who received two doses of vaccination (Vaccine, n=30), matching pregnant women with negative SARS-CoV-2 serology during the whole gestation, lower blood cortisol levels, and without preeclampsia (Pregnant control, n=20), and matching women who were not pregnant and received SARS-CoV-2 vaccinations (Non-pregnant vaccine control, n=30).

2.3.1.1 Clinical characteristics of patients in the PRINCE Cardio and High Cortisol cohorts

Fifteen (75%) of the patients in the PE group developed obstetric complications, 14 (70%) of them were hypertensive and three (15%) had vaginal bleeding during pregnancy. The pregnant women from the High cortisol group had a mean (± SD) circulating cortisol level of 19.17 ± 22.7pg/ml, which differed from healthy pregnant women, 1.96 ± 0.68pg/ml, (P value < 0.0001). There were no significant differences between PE group, High cortisol group, and Pregnant control group in terms of maternal age, gestational age, and neonatal outcomes. Table 2 described clinical maternal and neonatal characteristics of the patients from the PE and High cortisol groups.

Table 2. Clinical characteristics of women in the PE group and High cortisol group

	Pregnant PE patients (n=20)	Pregnant women with high blood cortisol (n=20)
Age: mean (range)	34.8 (25-46)	33.8 (26-39)
BMI: mean (SD)	26.5 (3.9)	23.2 (2.03)
Gestational week: median (range)	39 + 4 (37-41)	39 + 6 (38-42)
Infant body weight(g): mean (SD)	3439.1 (399.9)	3381.5 (379.1)
Sex of infant (% male)	60%	50%

* The BMI were before pregnancy in women with high blood cortisol, and at the time of the sample collection in PE patients.

2.3.1.2 Clinical features of patients in the PRINCE COVID and VACCINE cohort

As described above in the publication (doi: 10.3389/fimmu.2023.1196395).

2.3.2 Circulating gal-1 levels increasein pregnant women with PE

We initially measured the circulating gal-1, gal-3, gal-7, gal-9, and PSG1 levels of 20 pregnant women with PE, and compared them with matching healthy pregnant women. We found significantly higher levels of gal-1 in the serum of preeclampsia patients compared to healthy pregnant controls (**Figure 5A,** *P* value = 0.0216). However, minor differences were observed in the circulating levels of gal-3, gal-7, and gal-9 between analyzed groups (**Figure 5B-D**). To explore deeply about the dynamics of galectins, we then analyzed the correlation between endogenous lectins and clinical parameters. Concerning gal-1, we found it positively correlated gal-3 (**Figure 5E,** *P* value = 0.0331, Spearman correlation coefficient (ρ) = 0.478), and gal-9 (**Figure 5G,** *P* value = 0.0083, ρ = 0.571) in healthy pregnant women. However, only the correlation between gal-1 and gal-9 (**Figure 5K,** *P* value =0.0361, ρ = 0.472) remained in the preeclampsia patients. Moreover, we noticed that gal-3 strongly correlated with gal-9 in healthy pregnant women (**Figure 5H,** *P* value = 0.0103, ρ = 0.562), and the correlation persisted among patients with PE (**Figure 5L,** *P* value = 0.0010, ρ = 0.702). In addition, similar PSG1 levels were found in the maternal circulation in the PE patients and healthy pregnant women (**Figure 5M**), and there was no significant correlation between gal-1 and PSG1 in PE patients (**Figure 5N**). Furthermore, when we analyzed the correlation between galectins, PSG1, and general clinical parameters for all the women (the BMI, age, and delivery gestational week of the patients), no significant association were found as described in Table 3.

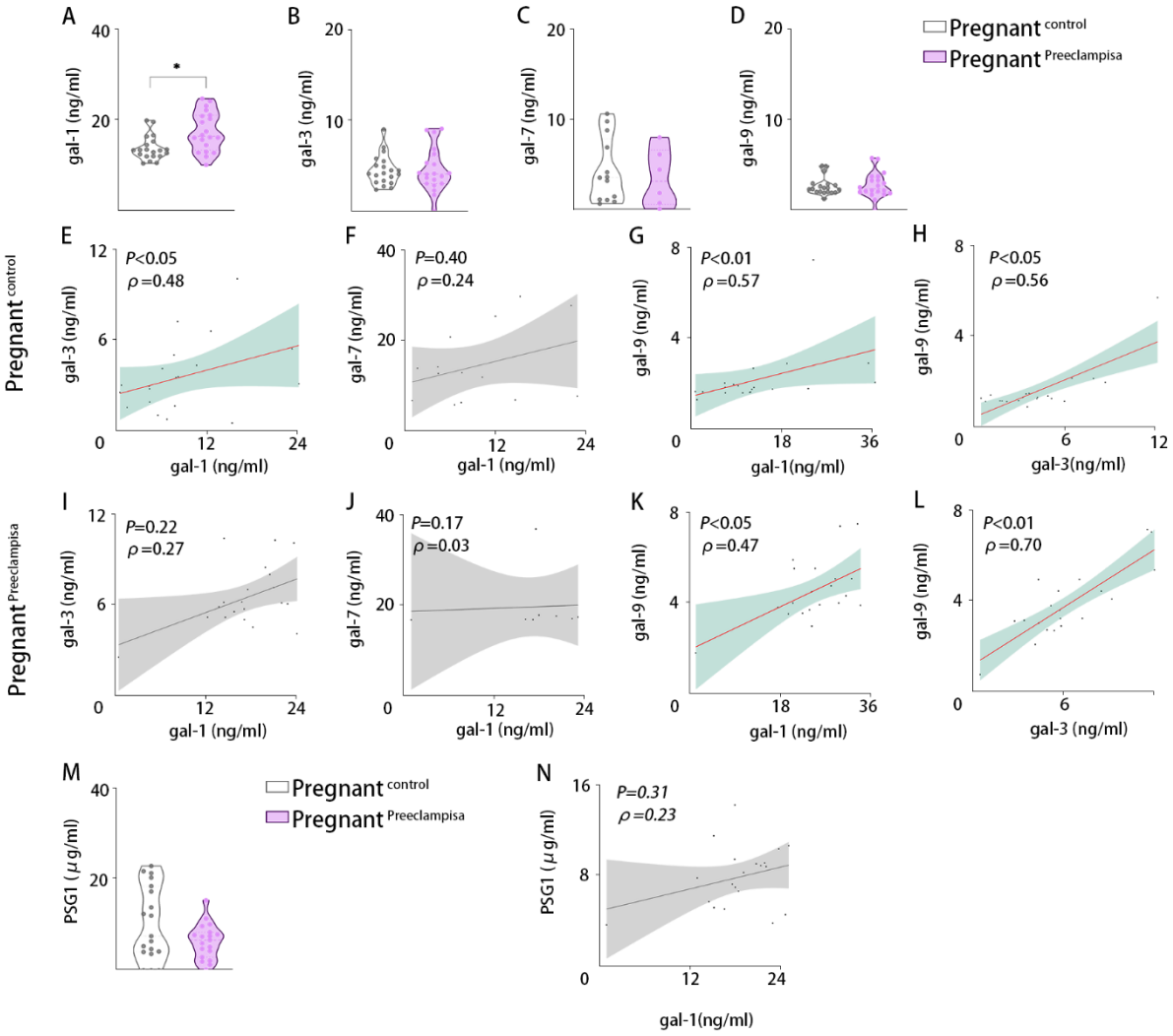


Figure 5. Serum values of gal-1 (A), gal-3 (B), gal-7 (C), gal-9 (D), and PSG1 (M) in PRINCE Cardio cohort were analyzed by ELISA in healthy pregnant women (pregnant ^{control}, n=20) or pregnant women with PE (pregnant ^{preeclampsia}, n=20). The statistical significance indicated by *P <0.05 as analyzed by adjusted t test. The correlations were examined between gal-1 and gal-3 (E), gal-1 and gal-7 (F), gal-1 and gal-9 (G), and gal-3 and gal-9 (H) in the maternal circulation of healthy pregnant women. The correlations between serum levels of gal-1 and gal-3 (I), gal-1 and gal-7 (J), gal-1 and gal-9 (K) or gal-3 and gal-9 (L), and gal-1 and PSG1 (N) were analyzed in pregnant women with PE. The Spearman correlation coefficient (ρ) was used, and statistical significance was denoted by $P < 0.05$ and $P < 0.01$ (green), while non-significant results ($P > 0.05$) were shown in grey as analyzed with the Spearman statistical test.

Table 3. Correlations between galectins and clinical characteristics of PRINCE Cardio cohort

	gal-1 (ng/ml)		gal-3 (ng/ml)		gal-7 (ng/ml)		gal-9 (ng/ml)		PSG1 (μg/ml)	
	P	***ρ***	***P***	***ρ***	***P***	***ρ***	***P***	***ρ***	***P***	***ρ***
BMI	0.72	-0.08	0.40	-0.19	0.22	-0.28	0.77	0.07	0.76	0.07
Age	0.50	0.16	0.64	0.11	0.52	0.15	0.28	0.28	0.10	0.39
GW	0.86	0.05	0.38	-0.21	0.48	-0.27	0.95	-0.01	0.74	0.01

*GW, Gestational week

2.3.3 Serum levels of galectins and PSG-1 remain unchanged in high cortisol patients

Subsequently, we investigated the levels of circulating galectins and PSG-1 in pregnant women exhibiting elevated cortisol levels. Our findings revealed similar levels of gal-1, gal-3, gal-7, gal-9, and PSG-1 in the maternal circulation of individuals with higher serum cortisol when compared to healthy pregnant controls (**Figure 6A-D, I**). Furthermore, pregnant women with higher cortisol levels showed no correlations between gal-1 and gal-3, as well as gal-1 and gal-9. Interestingly, gal-3 maintained a positive correlation with gal-9 (**Figure 6H,** *P* value = 0.0210, ρ = 0.51) in pregnant women with higher cortisol levels as the healthy control (**Figure 5H**). Additionally, serum cortisol levels exhibited a positive correlation with patients' BMI (**Figure 6K,** *P* value = 0.0287, ρ = 0.35). However, no further significant associations were observed in regards patients' serum cortisol levels, galectins, PSG-1 levels, and other clinical parameters as described in Table 4.

Table 4. Correlations between galectins and clinical features of PRINCE High Cortisol Cohorts

	gal-1 (ng/ml)		**gal-3 (ng/ml)**		**gal-7 (ng/ml)**		**gal-9 (ng/ml)**		**PSG1 (µg/ml)**	
	P	***ρ***	***P***	***ρ***	***P***	***ρ***	***P***	***ρ***	***P***	***ρ***
BMI	0.49	0.16	0.91	-0.03	0.70	0.09	0.11	0.37	0.93	0.02
Age	0.52	-0.15	0.10	0.11	0.62	0.15	0.29	0.48	0.65	0.11
Cortisol levels	0.94	0.02	0.53	-0.15	0.86	-0.04	0.46	-0.18	0.88	-0.04

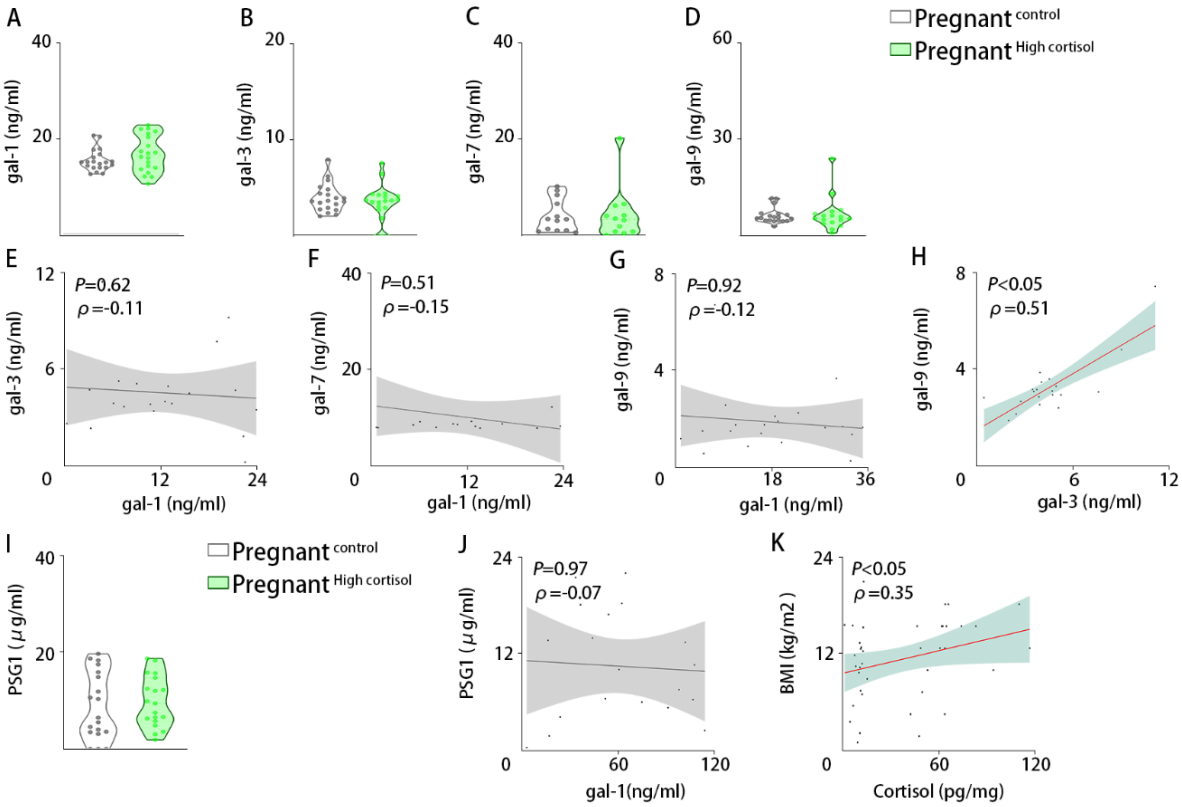


Figure 6. Circulating levels of gal-1 (A), gal-3 (B), gal-7 (C), gal-9 (D) and PSG1 (I) in PRINCE High Cortisol cohort analyzed by ELISA in healthy pregnant women (pregnant ^{control}, n=20), pregnant women with higher serum cortisol levels (pregnant $^{High\ cortisol}$, n=20). Correlations between serum levels of gal-1 and gal-3 (E), gal-1 and gal-7 (F), gal-1 and gal-9 (G), gal-3 and gal-9 (H), and gal-1 and PSG1 (J) were analyzed in pregnant women with higher serum cortisol levels. (K) Change in serum cortisol levels in relation to patients BMI in both groups. The Spearman correlation coefficient (ρ) is shown by $P < 0.05$ (green) and $P > 0.05$ (grey) as analyzed with the Spearman statistical test.

2.3.4 Circulating levels of galectins and PSG1 in patients with SARS-CoV-2 infection or vaccination

2.3.4.1 Higher gal-1 levels were found in patients with SARS-CoV-2 infection and vaccinated pregnant women

As described above in the publication (doi: 10.3389/fimmu.2023.1196395).

2.3.4.2 Positive correlations between gal-1/gal-3, and gal-1/gal-9 were compromised upon SARS-CoV-2 infection or vaccination

As described above in the publication (doi: 10.3389/fimmu.2023.1196395).

2.3.5 Galectins and PSG1 signature in the Yale IMPACT cohort

2.3.5.1 Clinical characteristics of patients in the Yale IMPACT cohort

As described above in the publication (doi: 10.3389/fimmu.2023.1196395).

2.3.5.2 Gal-1 increased in patients with SARS-CoV-2 infection in the Yale IMPACT cohort

As described above in the publication (doi: 10.3389/fimmu.2023.1196395).

2.3.6 Transcriptional changes of galectins, PSGs, and glycoenzymes during maternal acute SARS-CoV-2 infection

To further investigate the potential contribution of gal-1 for placental pathology during maternal SARS-CoV-2 infection, we next evaluated the transcriptional changes of galectins, PSGs, and glycoenzymes in placenta.

Women who tested positive for SARS-CoV-2 infection by qRT-PCR of a nasopharyngeal swab or saliva at the time of delivery or in the 1 month prior to delivery were prospectively enrolled and consented to donation of biospecimens in the Yale IMPACT cohort. Of the 11 total pregnant women with COVID-19 who delivered during the study period and we tested serum galectins and PSG-1 levels, 5 had placenta available for transcriptional profiling. We performed the analysis in gene expression level from acquired data of bulk RNA sequencing of placental villi to analyze the differences in placental gene expression between pregnant women with SARS-CoV-2 infection (n = 5) and uninfected pregnant women with matching maternal age, gestational age, maternal comorbidities, and mode of delivery (n = 3).

Subsequently, we used an unsupervised principal components analysis on our normalized database. This analysis involved plotting the samples on a two-dimensional scatterplot to generate a dissimilarity matrix using gene expression data from each group. The purpose was to assess the similarity between pairs of samples, with the distances on the plot reflecting the expression differences between the samples. As a result, we identified COVID2 sample as an outlier in multidimensional scaling (MDS) plot (**Figure 7**).

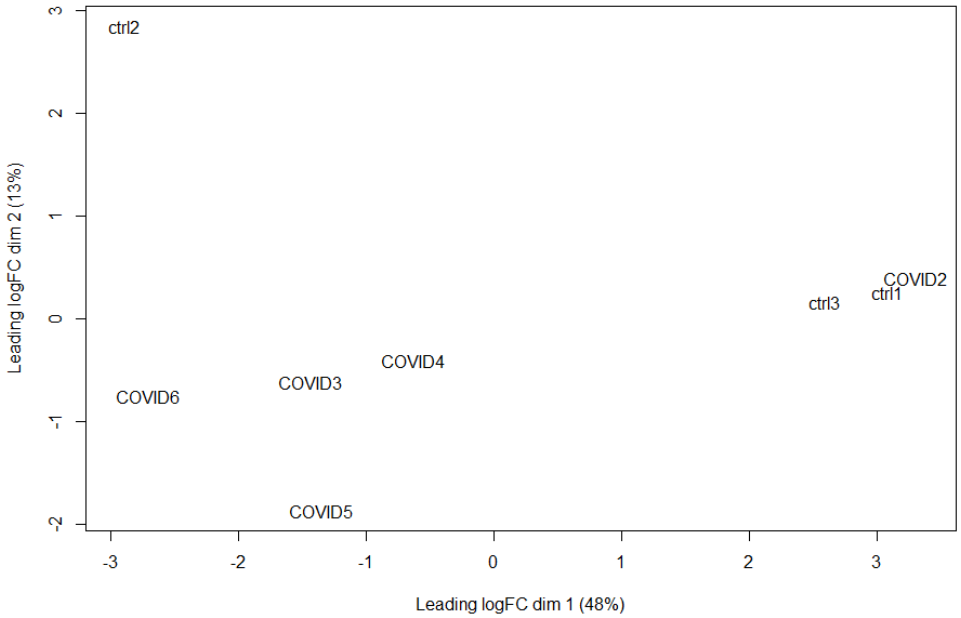


Figure 7. MDS plot showed the dissimilarity matrix of each group in the Yale IMPACT cohort.

2.3.6.1 Upregulation of Gal-1 and PSGs observed in the placenta during maternal SARS-CoV-2 infection.

As described above in the publication (doi: 10.3389/fimmu.2023.1196395).

2.3.6.2 Maternal acute SARS-CoV-2 infection in the placenta leads to transcriptional changes in glycoenzymes.

As described above in the publication (doi: 10.3389/fimmu.2023.1196395).

3.6.2.3 Cytokine profiles in the placenta showed differences during maternal SARS-CoV-2 infection.

We finally performed the transcriptomic profile for inflammatory cytokines in the placenta. While there were no significant altercations observed in the genes associated with Th1, Th2, and Th17 factors, which are known to be involved in immune response-related pathways, we observed a down-regulated tendency in these genes in COVID19 patients (**Figure 8A-C**). Moreover, the expression of genes related to inflammasome like *NFKB1* (*P* value = 0.0349) and *CASP3* (*P* value = 0.0006) were increased in patients with SARS-CoV-2 infection (**Figure 8D**).

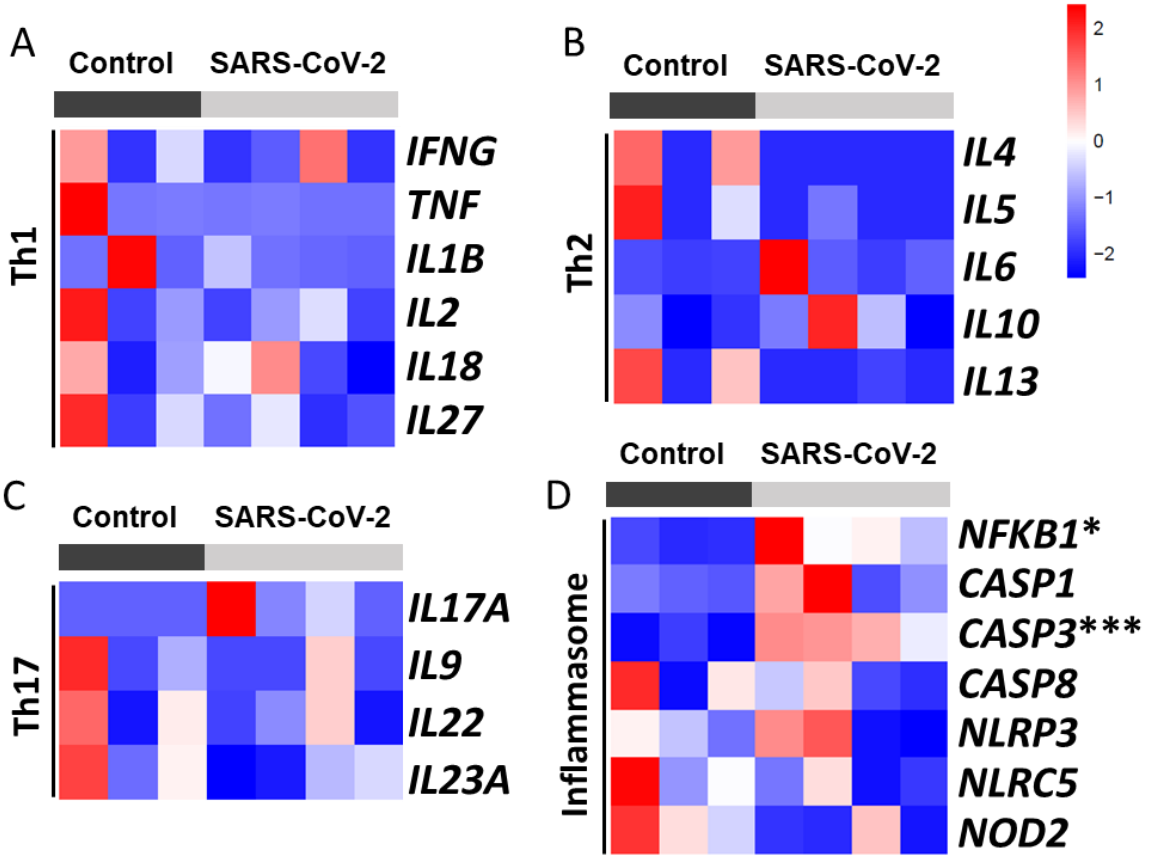


Figure 8. Heatmaps showing the genes involved in immune and inflammatory responses for Th1 (A), Th2 (B), Th17 (C), and inflammasome from RNA-seq data in the placenta of Yale IMPACT cohort. Control, healthy pregnant women, n=3; SARS-CoV-2, pregnant women infected by SARS-CoV-2, n=4. **P* <0.05, and ****P* <0.001 as analyzed by adjusted t test.

3.6.4 Correlations between galectins, PSGs, glycoenzymes, and cytokines during maternal COVID-19 infection

In addition, since the extracellular activity of galectins largely depend on their ability to bind specific glycans on cell surface proteins, we demonstrated the significant association between galectins, PSGs, glycoenzymes, and cytokines when considering them together in the COVID-19 patients. Specifically, our results suggested that *LGALS1* positively correlated with *LGALS3* (**Figure 9A**). Interestingly, *LGALS1* and *LGALS3* exhibited similar relation patterns. They both positively associated with LGALS8, various members in the PSG family (*PSG2*, *PSG3*, *PSG4*, *PSG5*, *PSG6*, *PSG8*, *PSG9*, and *PSG11*), and negatively correlated with *LGALS7* (**Figure 9A-C**). In addition, *LGALS1* and *LGALS3* were in positive relation to the genes that encode N-acetylglucosaminyl transferases (*MGAT1*, *MGAT2*, *MGAT4*), as well as some other enzymes that associated with N-linked glycans initiation (*MAN1A2*, *GANAB*) and elongation (*B4GALT3*, *B4GALT2*, *B4GALT1*). Similarly, *LGALS1* and *LGALS3* positively correlated with O-linked glycans associated enzymes, especially with the ones that modulate the initiation of O-linked

glycans (*GALNT1*, *GALNT2*) (**Figure 9A**). Interestingly, when we focus on the cytokines, we found that they negatively associated with *LGALS1* and *LGALS3*, while positively correlated with *LGALS7* (**Figure 9C**).

Shifting our attention to *PSG1*, we observed that in addition to its correlation with the PSG family, *PSG1* also displayed a positive correlation with genes encoding both N-linked and O-linked glycans. This includes *MGAT5*, which encodes an enzyme involved in generating beta1, 6GlcNAc-branched complex-type N-glycans and is known to bound to gal-1 and gal-3.

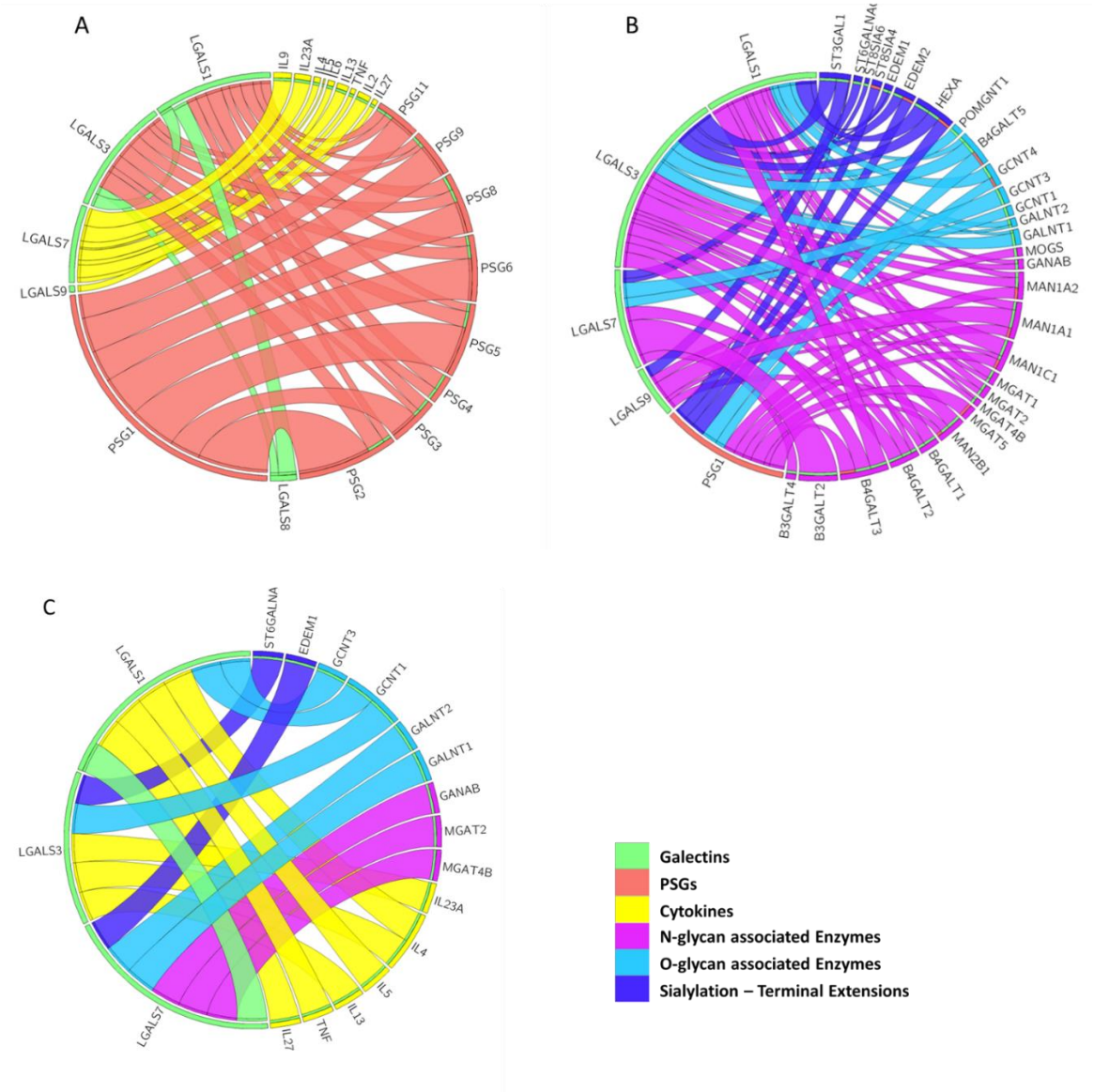


Figure 9. Chord diagrams were constructed to visually represent the associations between galectins, PSG1, cytokines, glycoenzymes, and PSGs based on the bulk RNA-seq data from the Yale IMPACT cohort. Plots (A) and (B) depicted the positive correlations among galectins, PSG1, cytokines, and glycoenzymes. Plot (C) illustrated the negative associations of galectins in relation to cytokines, and glycoenzymes. The width of the links representing the strength of the correlation coefficient (ρ). Only correlations with a significance level of $P < 0.05$ and $\rho > 0.5$, as determined by the Spearman statistical test, were included in the diagrams.

2.4 Discussion

Galectins modulate multiple biological processes and have been recognized as contributing factors in reproductive orchestration, including embryo implantation, immune tolerance, placental development, and maternal vascular adaptation. Pregnant women are at a higher risk of severe disease from SARS-CoV-2 infection compared to non-pregnant women. Understanding how SARS-CoV-2 infection and vaccination impact on pregnant women is of great importance. It allows healthcare professionals to manage their care effectively, develop appropriate preventive measures, and provide guidance on vaccination strategies. Preeclampsia is a significant cause of perinatal morbidity and mortality worldwide and is a leading factor in maternal deaths. Hence, it is important to identify biomarkers after the diagnosis of preeclampsia to identify women at higher risk and provide them a closer monitoring. Additionally, maternal distress during pregnancy has been largely linked to negative effects on fetal physiological and neurocognitive development, and increased maternal health issues, and there is a lack of studies investigating the levels of galectins in maternal blood in human or mouse models specifically related to maternal stress. To our best knowledge our study was the first one to decipher the galectin signature (gal-1, gal-3 gal-7, and gal-9) and PSG-1 in maternal circulation of pregnant women with complications during pregnancy (COVID-19 disease, stress, preeclampsia) and to further elucidate their impact on the disease, especially regarding on the maternal immune adaptation to pregnancy and the fetal-maternal interface.

2.4.1 Galectins and PSG-1 in patients with preeclampsia

The pathogenesis of late-onset type is considered to occur as consequence of incomplete spiral artery remodeling in the uterus, contributing to placental ischemia during placentation, and the release of antiangiogenic factors and inflammatory cytokines from the ischemic placenta into the maternal circulation, causing endothelial damages [150-152]. In the current study, our preeclampsia patients were all late-onset preeclampsia and the blood were collected after the disease onset. Our finding is consistent with our previous studies which showed circulating gal-1 levels were elevated in pregnant women that were diagnosed with late-onset preeclampsia compare to the healthy pregnant women [21, 73]. The overexpression of gal-1 after diagnosis in maternal circulation may serve as a compensatory mechanism aimed at mitigating the excessive systemic inflammatory response in affected patients. Interestingly, recent studies suggest that in early second trimester, low levels of gal-1 were detected in the patients who later develop PE [73]. The soluble fms-like tyrosine kinase (sFlt)-1 overexpression is considered a crucial cause of PE symptoms [153]. As a result, when sFlt-1 levels are elevated, the bioavailability of VEGF is down-regulated, leading to a decrease in VEGFR2 [154]. This disruption in the interaction could contribute to the pathogenesis of preeclampsia by interfering with the normal regulatory function of gal-1 in relation to the NRP-1/VEGFR2 pathway.

Another possibility is that the down-regulation of the anti-inflammatory mediator gal-1 may lead to an excessive maternal systemic inflammatory response and PE development [16].
Regarding gal-3, our result suggested similar levels between healthy women and PE patients. One of the reasons to explain the difference from the previous study is that in this study both early-onset and late-onset PE patients with an average delivery week of 35 weeks were recruited, while in our all late-onset PE patients with relatively more mild conditions and delivered after 37 weeks were included. Our finding that gal-3 and the immunomodulatory galectin gal-9 remained unchanged in late-onset PE patients. This suggests that gal-3 and gal-9 may not play a central role in modulating the excessive immune activation observed in late-onset PE patients. In a recent study, the serum level of gal-7 was found elevated during early pregnancy in patients who later develop PE [51], suggesting its predictive role for the onset of PE. This does not contradict our result showing similar levels of gal-7 after diagnosis of late-onset PE. However, it should be noted that a broader range of samples would be required in future studies. Collecting blood samples from patients at various time points throughout pregnancy would allow for a more comprehensive exploration of the dynamics of galectins and PSG1 in preeclampsia at different stages of gestation. The loss of correlation between gal-1 and gal-3 is likely because of the increase only observed in circulating gal-1 in the PE patients. An intriguing observation is the consistent strong correlation between the circulating levels of gal-3 and gal-9, both in patients with preeclampsia and in healthy pregnant women. This suggests that the functions of gal-3 and gal-9 in regulating maternal immune adaptations are closely interconnected, even after the occurrence of preeclampsia. Unchanged PSG-1 serum levels and the lack of correlation with gal-1 in the PE patients suggests a less sensitive PSG-1/gal-1 axis upon disease onset.

2.4.2 Galectins and PSG-1 in patients with high stress

Cortisol, known as the stress hormone, plays a crucial role in mediating psychological stress. Maternal stress during pregnancy has been suggested as a possible factor associated with various important outcomes related to pregnancy [155, 156]. Gal-1 and gal-3 involve in the pathology of neurobiology of depression and obsessive-compulsive-like disorder in mice model [151]. Based on our findings, there were no significant differences in circulating galectin levels and PSG-1 serum levels between women with higher cortisol levels and healthy pregnant women. It is important to note that cortisol levels can be influenced by various factors other than psychological stress, including age, nutrition, exercise, and medical conditions. As a result, it is reasonable that a direct link between galectins and cortisol levels was not observed in our study. Additional research should focus on elucidating the galectins dynamics in the stressed patients. This usually entails conducting psychological assessments on stressed individuals in accordance with the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) guidelines. Interestingly, our study pointed out cortisol levels of pregnant women are positively associated with BMI, which is in line with a recent study [152], suggesting that women with higher BMI

might experience higher perceived stress, and higher risk of obesity could lead to the increased risk of adverse pregnancy outcomes.

2.4.3 Galectins and PSG-1 in patients with COVID-19 infection or vaccination

As described above in the publication (doi: 10.3389/fimmu.2023.1196395).

2.4.4 Scientific significance and outlook

This MD Thesis presents the initial evidence demonstrating the functional role of galectins and PSG-1 in both maternal circulation and placental expression during pregnancy in individuals infected with SARS-CoV-2 and women who have been vaccinated against SARS-CoV-2. In our study, we present compelling evidence of significantly elevated levels of gal-1 and PSG-1 in pregnant women who have been exposed to SARS-CoV-2 infection within the PRINCE cohort. Furthermore, we observed a disruption in the positive correlations between gal-1 and gal-3, as well as gal-1 and gal-9, among individuals with SARS-CoV-2 infection, contributing to the overall increase in gal-1 levels. Remarkably, our findings demonstrate that fully vaccinated pregnant women exhibit even higher levels of gal-1 compared to both non-vaccinated pregnant women and non-pregnant vaccinated individuals, emphasizing the unique impact of vaccination on gal-1 levels. Additionally, vaccinated pregnant women also displayed elevated levels of gal-7 compared to vaccinated non-pregnant individuals. Notably, gal-9 levels increased in pregnant women after vaccination, while the highest levels were observed in non-pregnant vaccinated individuals. These elevated levels of galectins in vaccinated patients highlight their distinct functions in activating the immune system in response to the SARS-CoV-2 vaccine. Furthermore, we confirmed our findings in the validating Yale IMPACT cohort that gal-1 levels were increased in the pregnant women with SARS-CoV-2 infection. Next, our study pointed that gal-1, its ligand PSG-1, as well as several glycans which help gal-1 with its extracellular activity, took part in the placental immune responses of the patients with SARS-CoV-2 infection. In the context of changes at the maternal-fetal interface during SARS-CoV-2 infection, the immune responses are considered to protect the placenta and fetus from infection, preventing potential pathological changes with adverse consequences for developing fetuses [119, 152]. These observations enhance our knowledge of mediators between maternal immune response towards SARS-CoV-2 infection and uneventful pregnancy as well as the successful delivery. The provided data only covers pregnant women with mild COVID-19 disease, knowing that the gal-3 and gal-9 levels drastically amplified in non-pregnant patients with severe COVID-19 disease, and they both positively correlates with C-Reactive Protein (CRP), which is one of the markers for COVID-19 severity, it is important to explore the roles of galectins in the pregnant women with severe syndromes of SARS-CoV-2 infection. Additionally, we confirmed that gal-1 levels increase in maternal circulation after diagnosis with late-onset PE, while the expression of gal-3 remains similar, which are consistent with previous studies.

Future studies are needed to unravel the role of galectins and PSGs in viral pathogenesis, particularly in blocking viral replication and detrimental immune signaling pathways associated with SARS-CoV-2 and other similar viral infections. Further research efforts should also focus on determining the functional significance of these findings and investigating whether galectins play a role in mediating SARS-CoV-2 infection in pregnant women. Such studies will help in evaluating the potential use of galectins as targets for clinical trials and their application in developing effective interventions for pregnant women affected by SARS-CoV-2.

2.5 References

1. Blois, S.M., et al., *Pregnancy Galectinology: Insights Into a Complex Network of Glycan Binding Proteins.* Front Immunol, 2019. **10**: p. 1166.
2. Li, Y.H., et al., *The Galectin-9/Tim-3 pathway is involved in the regulation of NK cell function at the maternal-fetal interface in early pregnancy.* Cell Mol Immunol, 2016. **13**(1): p. 73-81.
3. Tirado-Gonzalez, I., et al., *Galectin-1 influences trophoblast immune evasion and emerges as a predictive factor for the outcome of pregnancy.* Mol Hum Reprod, 2013. **19**(1): p. 43-53.
4. Cooper, D.N., *Galectinomics: finding themes in complexity.* Biochim Biophys Acta, 2002. **1572**(2-3): p. 209-31.
5. Chen, M., et al., *Galectins: Important Regulators in Normal and Pathologic Pregnancies.* Int J Mol Sci, 2022. **23**(17).
6. Freitag, N., et al., *The chimera-type galectin-3 is a positive modulator of trophoblast functions with dysregulated expression in gestational diabetes mellitus.* Am J Reprod Immunol, 2020. **84**(6): p. e13311.
7. Seelenmeyer, C., et al., *Cell surface counter receptors are essential components of the unconventional export machinery of galectin-1.* J Cell Biol, 2005. **171**(2): p. 373-81.
8. Vasta, G.R., et al., *Functions of galectins as 'self/non-self'-recognition and effector factors.* Pathog Dis, 2017. **75**(5).
9. Blois, S.M. and G. Barrientos, *Galectin signature in normal pregnancy and preeclampsia.* J Reprod Immunol, 2014. **101-102**: p. 127-134.
10. Jovanović Krivokuća, M., et al., *Galectins in Early Pregnancy and Pregnancy-Associated Pathologies.* Int J Mol Sci, 2021. **23**(1).
11. Kopcow, H.D., et al., *T cell apoptosis at the maternal-fetal interface in early human pregnancy, involvement of galectin-1.* Proc Natl Acad Sci U S A, 2008. **105**(47): p. 18472-7.
12. Menkhorst, E., et al., *Medawar's PostEra: Galectins Emerged as Key Players During Fetal-Maternal Glycoimmune Adaptation.* Front Immunol, 2021. **12**: p. 784473.
13. Choe, Y.S., et al., *Expression of galectin-1 mRNA in the mouse uterus is under the*

control of ovarian steroids during blastocyst implantation. Mol Reprod Dev, 1997. **48**(2): p. 261-6.

14. Jeschke, U., et al., *The human endometrium expresses the glycoprotein mucin-1 and shows positive correlation for Thomsen-Friedenreich epitope expression and galectin-1 binding.* J Histochem Cytochem, 2009. **57**(9): p. 871-81.
15. Blois, S.M., et al., *A pivotal role for galectin-1 in fetomaternal tolerance.* Nat Med, 2007. **13**(12): p. 1450-7.
16. Garín, M.I., et al., *Galectin-1: a key effector of regulation mediated by CD4+CD25+ T cells.* Blood, 2007. **109**(5): p. 2058-65.
17. Blois, S.M., et al., *Depletion of CD8+ cells abolishes the pregnancy protective effect of progesterone substitution with dydrogesterone in mice by altering the Th1/Th2 cytokine profile.* J Immunol, 2004. **172**(10): p. 5893-9.
18. Gómez-Chávez, F., et al., *Galectin-1 reduced the effect of LPS on the IL-6 production in decidual cells by inhibiting LPS on the stimulation of IκBζ.* J Reprod Immunol, 2015. **112**: p. 46-52.
19. Dong, M., et al., *The effect of trophoblasts on T lymphocytes: possible regulatory effector molecules--a proteomic analysis.* Cell Physiol Biochem, 2008. **21**(5-6): p. 463-72.
20. Blois, S.M., et al., *Galectins in angiogenesis: consequences for gestation.* J Reprod Immunol, 2015. **108**: p. 33-41.
21. Freitag, N., et al., *Interfering with Gal-1-mediated angiogenesis contributes to the pathogenesis of preeclampsia.* Proc Natl Acad Sci U S A, 2013. **110**(28): p. 11451-6.
22. Than, N.G., et al., *Galectins: Double-edged Swords in the Cross-roads of Pregnancy Complications and Female Reproductive Tract Inflammation and Neoplasia.* J Pathol Transl Med, 2015. **49**(3): p. 181-208.
23. Douglas, N.C., et al., *Vascular endothelial growth factor receptor 2 (VEGFR-2) functions to promote uterine decidual angiogenesis during early pregnancy in the mouse.* Endocrinology, 2009. **150**(8): p. 3845-54.
24. Maquoi, E., et al., *Changes in the distribution pattern of galectin-1 and galectin-3 in human placenta correlates with the differentiation pathways of trophoblasts.* Placenta, 1997. **18**(5-6): p. 433-9.
25. von Wolff, M., et al., *Galectin fingerprinting in human endometrium and decidua during the menstrual cycle and in early gestation.* Mol Hum Reprod, 2005. **11**(3): p. 189-94.
26. Vićovac, L., M. Janković, and M. Cuperlović, *Galectin-1 and -3 in cells of the first trimester placental bed.* Hum Reprod, 1998. **13**(3): p. 730-5.
27. Moon, B.K., et al., *Galectin-3 protects human breast carcinoma cells against nitric oxide-induced apoptosis: implication of galectin-3 function during metastasis.* Am J Pathol, 2001. **159**(3): p. 1055-60.

28. Yu, F., et al., *Galectin-3 translocates to the perinuclear membranes and inhibits cytochrome c release from the mitochondria. A role for synexin in galectin-3 translocation.* J Biol Chem, 2002. **277**(18): p. 15819-27.
29. Karlsson, A., et al., *Galectin-3 functions as an opsonin and enhances the macrophage clearance of apoptotic neutrophils.* Glycobiology, 2009. **19**(1): p. 16-20.
30. Brittoli, A., et al., *"In vitro" studies on galectin-3 in human natural killer cells.* Immunol Lett, 2018. **194**: p. 4-12.
31. Farladansky-Gershnabel, S., et al., *Spontaneous Preterm Birth: Elevated Galectin-3 and Telomere Shortening May Reflect a Common Pathway of Enhanced Inflammation and Senescence.* Reprod Sci, 2022.
32. Kaya, B., et al., *Maternal serum galectin-1 and galectin-3 levels in pregnancies complicated with preterm prelabor rupture of membranes.* J Matern Fetal Neonatal Med, 2020. **33**(5): p. 861-868.
33. Stefanoska, I., et al., *Histological chorioamnionitis in preterm prelabor rupture of the membranes is associated with increased expression of galectin-3 by amniotic epithelium.* J Matern Fetal Neonatal Med, 2017. **30**(18): p. 2232-2236.
34. Markowska, A.I., F.T. Liu, and N. Panjwani, *Galectin-3 is an important mediator of VEGF- and bFGF-mediated angiogenic response.* J Exp Med, 2010. **207**(9): p. 1981-93.
35. Markowska, A.I., K.C. Jefferies, and N. Panjwani, *Galectin-3 protein modulates cell surface expression and activation of vascular endothelial growth factor receptor 2 in human endothelial cells.* J Biol Chem, 2011. **286**(34): p. 29913-21.
36. Machado, C.M., et al., *Galectin-3 disruption impaired tumoral angiogenesis by reducing VEGF secretion from TGFβ1-induced macrophages.* Cancer Med, 2014. **3**(2): p. 201-14.
37. Cano, I., et al., *Galectin-3 Enhances Vascular Endothelial Growth Factor-A Receptor 2 Activity in the Presence of Vascular Endothelial Growth Factor.* Front Cell Dev Biol, 2021. **9**: p. 734346.
38. D'Haene, N., et al., *VEGFR1 and VEGFR2 involvement in extracellular galectin-1- and galectin-3-induced angiogenesis.* PLoS One, 2013. **8**(6): p. e67029.
39. Freitag, N., et al., *Galectin-3 deficiency in pregnancy increases the risk of fetal growth restriction (FGR) via placental insufficiency.* Cell Death Dis, 2020. **11**(7): p. 560.
40. Unverdorben, L., et al., *Comparative analyses on expression of galectins1-4, 7-10 and 12 in first trimester placenta, decidua and isolated trophoblast cells in vitro.* Histol Histopathol, 2016. **31**(10): p. 1095-111.
41. Menkhorst, E.M., et al., *Galectin-7 acts as an adhesion molecule during implantation and increased expression is associated with miscarriage.* Placenta, 2014. **35**(3): p. 195-201.
42. Than, N.G., et al., *A primate subfamily of galectins expressed at the maternal-fetal*

interface that promote immune cell death. Proc Natl Acad Sci U S A, 2009. **106**(24): p. 9731-6.

43. Menkhorst, E., et al., *Galectin-7 Impairs Placentation and Causes Preeclampsia Features in Mice.* Hypertension, 2020. **76**(4): p. 1185-1194.
44. Popovici, R.M., et al., *Galectin-9: a new endometrial epithelial marker for the mid- and late-secretory and decidual phases in humans.* J Clin Endocrinol Metab, 2005. **90**(11): p. 6170-6.
45. Heusschen, R., et al., *Profiling Lgals9 splice variant expression at the fetal-maternal interface: implications in normal and pathological human pregnancy.* Biol Reprod, 2013. **88**(1): p. 22.
46. Seki, M., et al., *Galectin-9 suppresses the generation of Th17, promotes the induction of regulatory T cells, and regulates experimental autoimmune arthritis.* Clin Immunol, 2008. **127**(1): p. 78-88.
47. Li, F., et al., *Upregulation of Tim-3 expression at feto-maternal interface may explain embryo survival in the CBAxDBA/2 model of abortion.* Am J Reprod Immunol, 2018. **79**(1).
48. Zhu, C., et al., *The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity.* Nat Immunol, 2005. **6**(12): p. 1245-52.
49. Monney, L., et al., *Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease.* Nature, 2002. **415**(6871): p. 536-41.
50. Aanhane, E., et al., *Different angioregulatory activity of monovalent galectin-9 isoforms.* Angiogenesis, 2018. **21**(3): p. 545-555.
51. Menkhorst, E., et al., *Galectin-7 serum levels are altered prior to the onset of pre-eclampsia.* Placenta, 2014. **35**(4): p. 281-5.
52. Enninga, E.A.L., et al., *Immune checkpoint molecules soluble program death ligand 1 and galectin-9 are increased in pregnancy.* Am J Reprod Immunol, 2018. **79**(2).
53. Boroń, D.G., et al., *Galectin-1 and Galectin-9 Concentration in Maternal Serum: Implications in Pregnancies Complicated with Preterm Prelabor Rupture of Membranes.* J Clin Med, 2022. **11**(21).
54. Mendoza, M., et al., *Glycan characterization of pregnancy-specific glycoprotein 1 and its identification as a novel Galectin-1 ligand.* Glycobiology, 2020. **30**(11): p. 895-909.
55. Moore, T., et al., *Pregnancy-specific glycoproteins: evolution, expression, functions and disease associations.* Reproduction, 2022. **163**(2): p. R11-r23.
56. Ballesteros, A., et al., *Induction and activation of latent transforming growth factor-β1 are carried out by two distinct domains of pregnancy-specific glycoprotein 1 (PSG1).* J Biol Chem, 2015. **290**(7): p. 4422-31.
57. Blois, S.M., et al., *Pregnancy-specific glycoprotein 1 (PSG1) activates TGF-β and prevents dextran sodium sulfate (DSS)-induced colitis in mice.* Mucosal Immunol, 2014.

7(2): p. 348-58.
58. Kammerer, R., et al., *Equine pregnancy-specific glycoprotein CEACAM49 secreted by endometrial cup cells activates TGFB.* Reproduction, 2020. **160**(5): p. 685-694.
59. Ha, C.T., et al., *Human pregnancy specific beta-1-glycoprotein 1 (PSG1) has a potential role in placental vascular morphogenesis.* Biol Reprod, 2010. **83**(1): p. 27-35.
60. Lisboa, F.A., et al., *Pregnancy-specific glycoprotein 1 induces endothelial tubulogenesis through interaction with cell surface proteoglycans.* J Biol Chem, 2011. **286**(9): p. 7577-86.
61. Shanley, D.K., et al., *Pregnancy-specific glycoproteins bind integrin alphaIIbbeta3 and inhibit the platelet-fibrinogen interaction.* PLoS One, 2013. **8**(2): p. e57491.
62. Grudzinskas, J.G., et al., *Identification of high-risk pregnancy by the routine measurement of pregnancy-specific beta 1-glycoprotein.* Am J Obstet Gynecol, 1983. **147**(1): p. 10-2.
63. Moore, T. and G.S. Dveksler, *Pregnancy-specific glycoproteins: complex gene families regulating maternal-fetal interactions.* Int J Dev Biol, 2014. **58**(2-4): p. 273-80.
64. Pihl, K., et al., *First trimester maternal serum pregnancy-specific beta-1-glycoprotein (SP1) as a marker of adverse pregnancy outcome.* Prenat Diagn, 2009. **29**(13): p. 1256-61.
65. Towler, C.M., et al., *Plasma levels of pregnancy-specific beta1-glycoprotein in normal pregnancy.* Br J Obstet Gynaecol, 1976. **83**(10): p. 775-9.
66. AbdulHussain, G., et al., *Effects of Progesterone, Dydrogesterone and Estrogen on the Production of Th1/Th2/Th17 Cytokines by Lymphocytes from Women with Recurrent Spontaneous Miscarriage.* J Reprod Immunol, 2020. **140**: p. 103132.
67. Germain, S.J., et al., *Systemic inflammatory priming in normal pregnancy and preeclampsia: the role of circulating syncytiotrophoblast microparticles.* J Immunol, 2007. **178**(9): p. 5949-56.
68. Torchinsky, A., et al., *TNF-alpha protects embryos exposed to developmental toxicants.* Am J Reprod Immunol, 2003. **49**(3): p. 159-68.
69. Mitchell, R.E., et al., *IL-4 enhances IL-10 production in Th1 cells: implications for Th1 and Th2 regulation.* Sci Rep, 2017. **7**(1): p. 11315.
70. Piccinni, M.P., et al., *T helper cell mediated-tolerance towards fetal allograft in successful pregnancy.* Clin Mol Allergy, 2015. **13**(1): p. 9.
71. Kuroda, K., et al., *Increasing number of implantation failures and pregnancy losses associated with elevated Th1/Th2 cell ratio.* Am J Reprod Immunol, 2021. **86**(3): p. e13429.
72. Toscano, M.A., et al., *Differential glycosylation of TH1, TH2 and TH-17 effector cells selectively regulates susceptibility to cell death.* Nat Immunol, 2007. **8**(8): p. 825-34.
73. Hirashima, C., et al., *Galectin-1 as a novel risk factor for both gestational hypertension*

and preeclampsia, specifially its expression at a low level in the second trimester and a high level after onset. Hypertens Res, 2018. **41**(1): p. 45-52.

74. Hirota, Y., et al., *Galectin-1 markedly reduces the incidence of resorptions in mice missing immunophilin FKBP52.* Endocrinology, 2012. **153**(5): p. 2486-93.
75. Ramhorst, R.E., et al., *Galectin-1 confers immune privilege to human trophoblast: implications in recurrent fetal loss.* Glycobiology, 2012. **22**(10): p. 1374-86.
76. Kampman, M.A., et al., *Cardiac adaption during pregnancy in women with congenital heart disease and healthy women.* Heart, 2016. **102**(16): p. 1302-8.
77. Tan, E.K. and E.L. Tan, *Alterations in physiology and anatomy during pregnancy.* Best Pract Res Clin Obstet Gynaecol, 2013. **27**(6): p. 791-802.
78. Troiano, N.H., *Physiologic and Hemodynamic Changes During Pregnancy.* AACN Adv Crit Care, 2018. **29**(3): p. 273-283.
79. Christian, L.M., *Physiological reactivity to psychological stress in human pregnancy: current knowledge and future directions.* Prog Neurobiol, 2012. **99**(2): p. 106-16.
80. Pieper, P.G., et al., *Uteroplacental blood flow, cardiac function, and pregnancy outcome in women with congenital heart disease.* Circulation, 2013. **128**(23): p. 2478-87.
81. Paauw, N.D. and A.T. Lely, *Cardiovascular Sequels During and After Preeclampsia.* Adv Exp Med Biol, 2018. **1065**: p. 455-470.
82. Chandrasekaran, S. and R. Simon, *Hepatic Complications in Preeclampsia.* Clin Obstet Gynecol, 2020. **63**(1): p. 165-174.
83. Smith, M.A., *Preeclampsia.* Prim Care, 1993. **20**(3): p. 655-64.
84. El-Sayed, A.A.F., *Preeclampsia: A review of the pathogenesis and possible management strategies based on its pathophysiological derangements.* Taiwan J Obstet Gynecol, 2017. **56**(5): p. 593-598.
85. Tomimatsu, T., et al., *Preeclampsia: Maternal Systemic Vascular Disorder Caused by Generalized Endothelial Dysfunction Due to Placental Antiangiogenic Factors.* Int J Mol Sci, 2019. **20**(17).
86. Rana, S., et al., *Preeclampsia: Pathophysiology, Challenges, and Perspectives.* Circ Res, 2019. **124**(7): p. 1094-1112.
87. Roberts, J.M., et al., *Subtypes of Preeclampsia: Recognition and Determining Clinical Usefulness.* Hypertension, 2021. **77**(5): p. 1430-1441.
88. Than, N.G., et al., *Early pathways, biomarkers, and four distinct molecular subclasses of preeclampsia: The intersection of clinical, pathological, and high-dimensional biology studies.* Placenta, 2022. **125**: p. 10-19.
89. Pankiewicz, K., et al., *The association between serum galectin-3 level and its placental production in patients with preeclampsia.* J Physiol Pharmacol, 2020. **71**(6).
90. Kandel, M., et al., *Placental galectin-3 is reduced in early-onset preeclampsia.* Front Physiol, 2022. **13**: p. 1037597.

91. Nikolov, A., N. Popovski, and A. Blazhev, *Serum Galectin-3 Levels Are Unlikely to Be a Useful Predictive Marker for Early-onset Preeclampsia Development.* Prague Med Rep, 2020. **121**(3): p. 172-180.
92. Hao, H., et al., *Upregulation of the Tim-3/Gal-9 pathway and correlation with the development of preeclampsia.* Eur J Obstet Gynecol Reprod Biol, 2015. **194**: p. 85-91.
93. Bayrampour, H., et al., *Pregnancy-related anxiety: A concept analysis.* Int J Nurs Stud, 2016. **55**: p. 115-30.
94. Domínguez-Solís, E., M. Lima-Serrano, and J.S. Lima-Rodríguez, *Non-pharmacological interventions to reduce anxiety in pregnancy, labour and postpartum: A systematic review.* Midwifery, 2021. **102**: p. 103126.
95. Hadfield, K., et al., *Measurement of pregnancy-related anxiety worldwide: a systematic review.* BMC Pregnancy Childbirth, 2022. **22**(1): p. 331.
96. Barclay, M.E., et al., *Maternal Early Life Adversity and Infant Stress Regulation: Intergenerational Associations and Mediation by Maternal Prenatal Mental Health.* Res Child Adolesc Psychopathol, 2022.
97. García González, J., et al., *Effects of prenatal music stimulation on state/trait anxiety in full-term pregnancy and its influence on childbirth: a randomized controlled trial.* J Matern Fetal Neonatal Med, 2018. **31**(8): p. 1058-1065.
98. San Lazaro Campillo, I., et al., *Psychological and support interventions to reduce levels of stress, anxiety or depression on women's subsequent pregnancy with a history of miscarriage: an empty systematic review.* BMJ Open, 2017. **7**(9): p. e017802.
99. Santiváñez-Acosta, R., E.L.N. Tapia-López, and M. Santero, *Music Therapy in Pain and Anxiety Management during Labor: A Systematic Review and Meta-Analysis.* Medicina (Kaunas), 2020. **56**(10).
100. Smith, M.V., et al., *Perinatal depression and birth outcomes in a Healthy Start project.* Matern Child Health J, 2011. **15**(3): p. 401-9.
101. Grigoriadis, S., et al., *Maternal Anxiety During Pregnancy and the Association With Adverse Perinatal Outcomes: Systematic Review and Meta-Analysis.* J Clin Psychiatry, 2018. **79**(5).
102. Horvath, S. and C.A. Schreiber, *Unintended Pregnancy, Induced Abortion, and Mental Health.* Curr Psychiatry Rep, 2017. **19**(11): p. 77.
103. Davitt, E., et al., *COVID-19 disease and immune dysregulation.* Best Pract Res Clin Haematol, 2022. **35**(3): p. 101401.
104. Gralinski, L.E. and V.D. Menachery, *Return of the Coronavirus: 2019-nCoV.* Viruses, 2020. **12**(2).
105. Hussman, J.P., *Cellular and Molecular Pathways of COVID-19 and Potential Points of Therapeutic Intervention.* Front Pharmacol, 2020. **11**: p. 1169.
106. Li, H., Z. Liu, and J. Ge, *Scientific research progress of COVID-19/SARS-CoV-2 in the*

first five months. J Cell Mol Med, 2020. **24**(12): p. 6558-6570.
107. Bardají, A., et al., *The need for a global COVID-19 maternal immunisation research plan.* Lancet, 2021. **397**(10293): p. e17-e18.
108. Knight, M., et al., *Characteristics and outcomes of pregnant women admitted to hospital with confirmed SARS-CoV-2 infection in UK: national population based cohort study.* Bmj, 2020. **369**: p. m2107.
109. Kadiwar, S., et al., *Were pregnant women more affected by COVID-19 in the second wave of the pandemic?* Lancet, 2021. **397**(10284): p. 1539-1540.
110. Kalafat, E., et al., *COVID-19 booster doses in pregnancy and global vaccine equity.* Lancet, 2022. **399**(10328): p. 907-908.
111. Allotey, J., et al., *Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis.* Bmj, 2020. **370**: p. m3320.
112. Chen, H., et al., *Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records.* Lancet, 2020. **395**(10226): p. 809-815.
113. Piroth, L., et al., *Comparison of the characteristics, morbidity, and mortality of COVID-19 and seasonal influenza: a nationwide, population-based retrospective cohort study.* Lancet Respir Med, 2021. **9**(3): p. 251-259.
114. De Biasi, S., et al., *Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia.* Nat Commun, 2020. **11**(1): p. 3434.
115. Patel, H., et al., *Proteomic blood profiling in mild, severe and critical COVID-19 patients.* Sci Rep, 2021. **11**(1): p. 6357.
116. Kazancioglu, S., et al., *Assessment of Galectin-1, Galectin-3, and Prostaglandin E2 Levels in Patients with COVID-19.* Jpn J Infect Dis, 2021. **74**(6): p. 530-536.
117. Markovic, S.S., et al., *Galectin-1 as the new player in staging and prognosis of COVID-19.* Sci Rep, 2022. **12**(1): p. 1272.
118. Bozorgmehr, N., et al., *Galectin-9, a Player in Cytokine Release Syndrome and a Surrogate Diagnostic Biomarker in SARS-CoV-2 Infection.* mBio, 2021. **12**(3).
119. Baykiz, D., et al., *Galectin-3 as a Novel Biomarker for Predicting Clinical Outcomes in Hospitalized COVID-19 Patients.* Clin Lab, 2022. **68**(12).
120. Portacci, A., et al., *Galectin-3 as prognostic biomarker in patients with COVID-19 acute respiratory failure.* Respir Med, 2021. **187**: p. 106556.
121. Yeung, S.T., et al., *Galectin-9 protects humanized-ACE2 immunocompetent mice from SARS-CoV-2 infection.* Front Immunol, 2022. **13**: p. 1011185.
122. Gaughan, E.E., et al., *An Inhaled Galectin-3 Inhibitor in COVID-19 Pneumonitis: A Phase Ib/IIa Randomized Controlled Clinical Trial (DEFINE).* Am J Respir Crit Care

Med, 2023. **207**(2): p. 138-149.
123. Sadoff, J., et al., *Safety and Efficacy of Single-Dose Ad26.COV2.S Vaccine against Covid-19.* N Engl J Med, 2021. **384**(23): p. 2187-2201.
124. Abdelrahman, Z., M. Li, and X. Wang, *Comparative Review of SARS-CoV-2, SARS-CoV, MERS-CoV, and Influenza A Respiratory Viruses.* Front Immunol, 2020. **11**: p. 552909.
125. Birol Ilter, P., et al., *Clinical severity of SARS-CoV-2 infection among vaccinated and unvaccinated pregnancies during the Omicron wave.* Ultrasound Obstet Gynecol, 2022. **59**(4): p. 560-562.
126. Marchand, G., et al., *Systematic review and meta-analysis of COVID-19 maternal and neonatal clinical features and pregnancy outcomes up to June 3, 2021.* AJOG Glob Rep, 2022. **2**(1): p. 100049.
127. Novoa, R.H., et al., *Maternal clinical characteristics and perinatal outcomes among pregnant women with coronavirus disease 2019. A systematic review.* Travel Med Infect Dis, 2021. **39**: p. 101919.
128. Jafari, M., et al., *Clinical characteristics and outcomes of pregnant women with COVID-19 and comparison with control patients: A systematic review and meta-analysis.* Rev Med Virol, 2021. **31**(5): p. 1-16.
129. Leung, C., E.S.A.C. Simões, and E.A. Oliveira, *Are in-hospital COVID-19-related mortality and morbidity in pregnancy associated with gestational age?* Ultrasound Obstet Gynecol, 2022. **60**(2): p. 234-242.
130. Li, N., et al., *Maternal and Neonatal Outcomes of Pregnant Women With Coronavirus Disease 2019 (COVID-19) Pneumonia: A Case-Control Study.* Clin Infect Dis, 2020. **71**(16): p. 2035-2041.
131. Male, V., *SARS-CoV-2 infection and COVID-19 vaccination in pregnancy.* Nat Rev Immunol, 2022. **22**(5): p. 277-282.
132. Muthuka, J., et al., *Association of Pregnancy With Coronavirus Cytokine Storm: Systematic Review and Meta-analysis.* JMIR Pediatr Parent, 2022. **5**(4): p. e31579.
133. Nana, M. and C. Nelson-Piercy, *COVID-19 in pregnancy.* Clin Med (Lond), 2021. **21**(5): p. e446-e450.
134. Wainstock, T., et al., *Prenatal maternal COVID-19 vaccination and pregnancy outcomes.* Vaccine, 2021. **39**(41): p. 6037-6040.
135. Wastnedge, E.A.N., et al., *Pregnancy and COVID-19.* Physiol Rev, 2021. **101**(1): p. 303-318.
136. Nana, M., et al., *Diagnosis and management of covid-19 in pregnancy.* Bmj, 2022. **377**: p. e069739.
137. Baden, L.R., et al., *Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine.* N Engl J Med, 2021. **384**(5): p. 403-416.
138. Atyeo, C., et al., *COVID-19 mRNA vaccines drive differential Fc-functional profiles in*

pregnant, lactating, and non-pregnant women. bioRxiv, 2021.

139. Beharier, O., et al., *Efficient maternal to neonatal transfer of antibodies against SARS-CoV-2 and BNT162b2 mRNA COVID-19 vaccine.* J Clin Invest, 2021. **131**(19).
140. Ben-Mayor Bashi, T., et al., *The association of maternal SARS-CoV-2 vaccination-to-delivery interval and the levels of maternal and cord blood antibodies.* Int J Gynaecol Obstet, 2022. **156**(3): p. 436-443.
141. Bookstein Peretz, S., et al., *Short-term outcome of pregnant women vaccinated with BNT162b2 mRNA COVID-19 vaccine.* Ultrasound Obstet Gynecol, 2021. **58**(3): p. 450-456.
142. Citu, I.M., et al., *Immunogenicity Following Administration of BNT162b2 and Ad26.COV2.S COVID-19 Vaccines in the Pregnant Population during the Third Trimester.* Viruses, 2022. **14**(2).
143. Collier, A.Y., et al., *Immunogenicity of COVID-19 mRNA Vaccines in Pregnant and Lactating Women.* Jama, 2021. **325**(23): p. 2370-2380.
144. Kugelman, N., et al., *Maternal and Neonatal SARS-CoV-2 Immunoglobulin G Antibody Levels at Delivery After Receipt of the BNT162b2 Messenger RNA COVID-19 Vaccine During the Second Trimester of Pregnancy.* JAMA Pediatr, 2022. **176**(3): p. 290-295.
145. Mithal, L.B., et al., *Cord blood antibodies following maternal coronavirus disease 2019 vaccination during pregnancy.* Am J Obstet Gynecol, 2021. **225**(2): p. 192-194.
146. Carbone, L., et al., *COVID-19 vaccine and pregnancy outcomes: A systematic review and meta-analysis.* Int J Gynaecol Obstet, 2022. **159**(3): p. 651-661.
147. Nir, O., et al., *Maternal-neonatal transfer of SARS-CoV-2 immunoglobulin G antibodies among parturient women treated with BNT162b2 messenger RNA vaccine during pregnancy.* Am J Obstet Gynecol MFM, 2022. **4**(1): p. 100492.
148. Prabhu, M., et al., *Antibody Response to Coronavirus Disease 2019 (COVID-19) Messenger RNA Vaccination in Pregnant Women and Transplacental Passage Into Cord Blood.* Obstet Gynecol, 2021. **138**(2): p. 278-280.
149. Rottenstreich, A., et al., *The Effect of Gestational Age at BNT162b2 mRNA Vaccination on Maternal and Neonatal Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Antibody Levels.* Clin Infect Dis, 2022. **75**(1): p. e603-e610.
150. Bokslag, A., et al., *Preeclampsia; short and long-term consequences for mother and neonate.* Early Hum Dev, 2016. **102**: p. 47-50.
151. Braekke, K., et al., *Asymmetric dimethylarginine in the maternal and fetal circulation in preeclampsia.* Pediatr Res, 2009. **66**(4): p. 411-5.
152. Young, B.C., R.J. Levine, and S.A. Karumanchi, *Pathogenesis of preeclampsia.* Annu Rev Pathol, 2010. **5**: p. 173-92.
153. Maynard, S.E., et al., *Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia.* J

Clin Invest, 2003. **111**(5): p. 649-58.
154. Abel, T., et al., *Vascular Endothelial Growth Factor Receptor 2: Molecular Mechanism and Therapeutic Potential in Preeclampsia Comorbidity with Human Immunodeficiency Virus and Severe Acute Respiratory Syndrome Coronavirus 2 Infections.* Int J Mol Sci, 2022. **23**(22).
155. Galbally, M., et al., *The role of oxytocin in mother-infant relations: a systematic review of human studies.* Harv Rev Psychiatry, 2011. **19**(1): p. 1-14.
156. Seth, S., et al., *Maternal Prenatal Mental Health and Placental 11β-HSD2 Gene Expression: Initial Findings from the Mercy Pregnancy and Emotional Wellbeing Study.* Int J Mol Sci, 2015. **16**(11): p. 27482-96.

3 Zusammenfassung

Galektine modulieren zahlreiche biologische Prozesse und gelten als wichtige Faktoren bei der Regulierung der Reproduktion, einschließlich der Implantation des Embryos, der Immuntoleranz, der Plazentaentwicklung und der Angiogenese. Es wird angenommen, dass PSG-1, welches ein Ligand von gal-1 ist, an gal-1 bindet, um eine erfolgreiche Schwangerschaft zu ermöglichen. Ziel dieser Doktorarbeit war es daher, die Galektin-Signatur (gal-1, gal-3, gal-7 und gal-9) sowie PSG-1 im maternalen Blutkreislauf von Schwangeren mit Komplikationen während der Schwangerschaft (COVID-19-Krankheit, Stress, Präeklampsie) zu untersuchen und ihre Auswirkungen auf die Krankheit weiter zu ergründen. Hierbei sollten insbesondere die Anpassung des maternalen Immunsystems an die Schwangerschaft und die fötal-maternale Grenzfläche betrachtet werden. Unsere Studie zeigte erhöhte gal-1-Werte bei schwangeren Frauen, die mit dem SARS-CoV-2-Virus infiziert waren oder dagegen geimpft wurden. Die PSG-1-Werte waren nur bei schwangeren Frauen mit einer SARS-CoV-2-Infektion erhöht. Diese Daten stützen die Hypothese, dass gal-1 und PSG-1 im maternalen Blutkreislauf in Folge einer durch die SARS-CoV-2-Infektion ausgelösten Zytokinreaktion oder einer Immunreaktion auf die Impfung erhöht sind. Dies erfolgt vermutlich zur Modulation der Entzündungsreaktion gegen das Virus und zum Schutz der Schwangerschaft. Darüber hinaus ergab unsere Studie, dass die Korrelationen zwischen gal-1 und gal-3 sowie gal-1 und gal-9 bei Personen mit einer SARS-CoV-2-Infektion gestört waren, was zu einem Anstieg der gal-1-Spiegel geführt hat. Des Weiteren zeigten unsere Ergebnisse, dass vollständig geimpfte Frauen im Vergleich zu nicht geimpften Schwangeren und nicht schwangeren Geimpften höhere gal-1-Werte aufwiesen. Ebenso wiesen geimpfte Schwangere im Vergleich zu nicht geimpften Schwangeren höhere gal-7-Werte auf. Schwangere Frauen zeigten nach der Impfung zudem erhöhte Gal-9-Werte, wobei nicht schwangere geimpfte Patientinnen die höchsten Gal-9-Werte aufwiesen. Diese erhöhten Konzentrationen von Galektinen unterstreichen die unterschiedlichen Rollen, die diese bei der Aktivierung des Immunsystems infolge der Impfung gegen SARS-CoV-2 einnehmen. In unserer Studie wurde auch die Beteiligung von gal-1, seines Liganden PSG-1 und von mehreren Glykanen, die gal-1 bei seiner extrazellulären Aktivität unterstützen, an den plazentaren Immunreaktionen von Patienten mit einer SARS-CoV-2-Infektion identifiziert. Diese Erkenntnisse erweitern unser Wissen über die Mechanismen, die zwischen der maternalen Immunantwort auf eine SARS-CoV-2-Infektion und einer komplikationslosen Schwangerschaft stehen. Darüber hinaus konnte bestätigt werden, dass die Konzentration von gal-1 im maternalen Blutkreislauf nach der Diagnose einer spät auftretenden Präeklampsie ansteigt, wohingegen die Expression von gal-3 ähnlich bleibt, was mit den Erkenntnissen früherer Studien übereinstimmt.

Galectins modulate multiple biological processes and have been recognized as contributing factors in reproductive orchestration, including Embryo implantation, immune tolerance, placental development, and angiogenesis. PSG-1 which is a gal-1 ligand proteins is considered bind to gal-1 in order to facilitate a successful pregnancy. Thus, the aim of the MD thesis was to investigate the galectin signature (gal-1, gal-3 gal-7, and gal-9) and PSG-1 in maternal circulation of pregnant women with complications during pregnancy (COVID-19 disease, Stress, Preeclampsia) and to further elucidate their impact on the disease, especially regarding on the maternal immune adaptation to pregnancy and the fetal-maternal interface. Our study presented evidence of elevated gal-1 in pregnant women exposed to SARS-CoV-2 infection or those who were vaccinated against SARS-CoV-2. PSG-1 levels were increased only in pregnant women with SARS-CoV-2 infection. These data support the hypothesis that gal-1 and PSG-1 are increased in maternal circulation after the cytokine response caused by SARS-CoV-2 infection or after immune reaction of vaccination, probably to modulate the inflammatory response against this virus and to protect pregnancy. Moreover, our study revealed that the associations between gal-1 and gal-3, as well as gal-1 and gal-9, were disrupted in individuals with SARS-CoV-2 infection, contributing to the increase in gal-1 levels. Additionally, our findings showed that fully vaccinated women had higher gal-1 levels compared to both non-vaccinated pregnant women and non-pregnant vaccinated individuals. Similarly, vaccinated pregnant women exhibited higher gal-7 levels compared to vaccinated non-pregnant individuals. Furthermore, pregnant women displayed increased gal-9 levels after vaccination, whereas non-pregnant vaccinated patients had the highest gal-9 levels. These elevated levels of galectins highlight their distinct roles in activating the immune system in response to the SARS-CoV-2 vaccine. Our study also identified the involvement of gal-1, its ligand PSG-1, and several glycans that assist gal-1 in its extracellular activity in the placental immune responses of patients with SARS-CoV-2 infection. These observations enhance our knowledge of mediators between maternal immune response towards SARS-CoV-2 infection and uneventful pregnancy. Additionally, we confirmed that gal-1 levels increase in maternal circulation after diagnosis with late-onset PE, while the expression of gal-3 remains similar, which are consistent with previous studies.

4 List of abbreviations

B4GALT1	Beta-1,4-galactosyltransferase 1
B4GALT3	Beta-1,4-galactosyltransferase 3
BMI	Body mass index
C1GALTC1	Core 1 β1,3-galactosyltransferase specific chaperone 1
CRD	Carbohydrate recognition domain
COVID-19	Coronavirus disease 2019
EDEM1	ER degradation-enhancing alpha-mannosidase-like 1
ELISA	Enzymelinked-immunosorbent assay
Gal	Galectin
GALNT1	Polypeptide N-Acetylgalactosaminyltransferase 1
GALNT2	Polypeptide N-Acetylgalactosaminyltransferase 2
GALNT7	Polypeptide N-Acetylgalactosaminyltransferase 7
GANAB	Neutral alpha-glucosidase AB
GW	Gestational week
HEXA	Hexosaminidase A
HLA-G	Human leukocyte antigen G
HPA	Hypothalamic–Pituitary– Adrenal
HRP	Horseradish peroxidase
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
LacNAc	N-acetyllactosamine
MAN1A2	Mannosidase Alpha Class 1A Member 2
MAN2B1	Mannosidase Alpha Class 2B Member 1
MGAT1	Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase
MGAT2	Alpha-1,6-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase
MGAT4B	Alpha-1,3-Mannosyl-Glycoprotein 4-Beta-N-Acetylglucosaminyltransferase B
MGAT5	Alpha-1,6-Mannosylglycoprotein 6-Beta-N-Acetylglucosaminyltransferase
MOGS	Mannosyl-oligosaccharide glucosidase
MDS	Multidimensional scaling plots
NK	Natural killer
Non-P	Non-pregnant
NRP	Neuropilin
PE	Preeclampsia
PSG	Pregnancy specific glycoprotein
qRT-PCR	Real-time quantitative reverse transcription PCR
Treg	Regulatory T cells

RAS	Renin-angiotensin-system
RNA-seq	RNA sequencing
SARS-CoV-2	Severe acute respiratory syndrome coronavirus type 2
ST3GAL1	ST3 beta-galactoside alpha-2,3-sialyltransferase 1
ST8SIA	Alpha-N-acetylneuraminide alpha-2,8-sialyltransferase
Th	T cell helper
TIM-3	T cell immunoglobulin and mucin-domain containing-3
TMB	3,3,5,5′-tetramethyl benzidine
TGF-β	Transforming growth factor beta
uNK	Uterine natural killer cells
VEGF	Vascular endothelial growth factor

5 Author Contributions

SMB designed the study and secured grant funding. FZ, A-CT, YW, YX, AD, AL-C, PV, EK, CU, JB, PCA, SFF, GSD, and MGG performed experiments and/or analyzed data. The original draft of the manuscript was written by FZ, MGG, and SMB, and further writing, review, and editing were done by all authors. All authors contributed to the article and approved the submitted version.

6 Acknowledgment

I would like to take this opportunity to thank all the people who have contributed to the creation of this work.
First and foremost, I extend my heartfelt thanks to my dearest supervisor, Prof. Dr. Sandra Blois for her invaluable guidance and support throughout this research. Without her expertise and encouragement, this work would not have been possible.

I am also deeply grateful to Dr. Mariana Garcia, Enrico Kittmann, Yiran Xie, Yiru Wang, Charlotte Harms, and Thomas Andreas for their assistance in various aspects of this research. Their contributions have been essential to the success of this project. Through their commitment, motivation, and support, I have repeatedly faced new challenges over the last 2 years and have developed myself professionally and personally.

In addition, I would like to express my appreciation to Prof. Dr. Petra Arck and her team members for the cordial cooperation, their helpfulness and for the always open ear in professional and private matters.
I would also like to extend my gratitude to the Clinical Team of our department leaded by Prof. Anke Diemert and also participants who generously gave their time and shared their experiences, which were essential to this study's success.

Additionally, I am grateful to my friends and family for their unwavering support and encouragement throughout this journey. Their support has kept me motivated and focused.

Finally, I would like to express my sincere appreciation to my university Universität Hamburg, and Universitätsklinikum Hamburg-Eppendorf for providing me with the resources and opportunities that allowed me to undertake this research.

7 Curriculum vitae

PERSONAL INFORMATION

Family Name: Zhao

Given Name: Fangqi

Date of Birth: Sept. 29, 1994

Nationality: China

E-mail: f.zhao.ext@uke.de

EDUCATION

2017-2020 Capital Medical University (Master of Obstetrics and Gynecology)

2012-2017 Capital Medical University (Bachelor of Clinical Medicine)

CLINICAL EXPERIENCES

2017-2020 Clinical rotations, Anzhen Hospital, Beijing, China

QUALIFICATIONS

2020 Certificate of Resident Standardization Training of PR China

2018 Medical Practitioners Qualification of PR China

AWARDS and FUNDINGS

2021-2023 CSC Scholarship

2017-2020 Academic Scholarship of Capital Medical University

2015-2016 The Second-class Scholarship of Capital Medical University

2014-2015 The First-class Scholarship of Capital Medical University

2015 “Triple A Student” of Beijing & of Capital Medical University

PUBLICATIONS

1**.** Zhou X, Lu Y, **Zhao F**, Dong J, Ma W, Zhong S, Wang M, Wang B, Zhao Y, Shi Y, Ma Q, Lu T, Zhang J, Wang X, Wu Q. Deciphering the spatial-temporal transcriptional landscape of human hypothalamus development. Cell Stem Cell. 2022 Feb 3;29(2):328-343.e5.

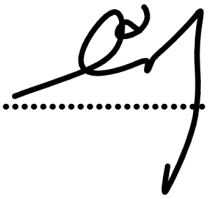
2.**Zhao Fangqi**, Shi Yingchao, Pan Na, Wu Qian, Wang Xiaoqun, Zhang Jun. The Spatial-Temporal Origin and Development of Human Cortical Interneuron. Chinese Journal of Cell Biology. 2018, 40(13): 2191–2197

3. An Y, Zhang J, Cheng X, Li B, Tian Y, Zhang X, **Zhao F**. miR-454 suppresses the proliferation and invasion of ovarian cancer by targeting E2F6. Cancer Cell Int. 2020 Jun 12;20:237.

4. **Fangqi Zhao**, Ann-Christin Tallarek, Yiru Wang, Yiran Xie, Anke Diemert, Alice Lu-Culligan, Pavithra Vijayakumar, Enrico Kittmann, Christopher Urbschat, Juan Bayo, Petra C. Arck, Shelli F Farhadian, Gabriela S. Dveksler, Mariana G. Garcia, Sandra M. Blois. (2023). A unique maternal and placental galectin signature upon SARS-CoV-2 infection suggests galectin-1 as a key alarmin at the maternal–fetal interface. Frontiers in Immunology. 14. 10.3389/fimmu.2023.1196395

8 Affidavit

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.
Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.
Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Unterschrift: .....  27.07.2023