IMMUNOLOGICAL CHALLENGES AROUND PREGNANCY COMPLICATIONS ASSOCIATED WITH FAILURES OF MATERNAL TOLERANCE TO THE FETUS

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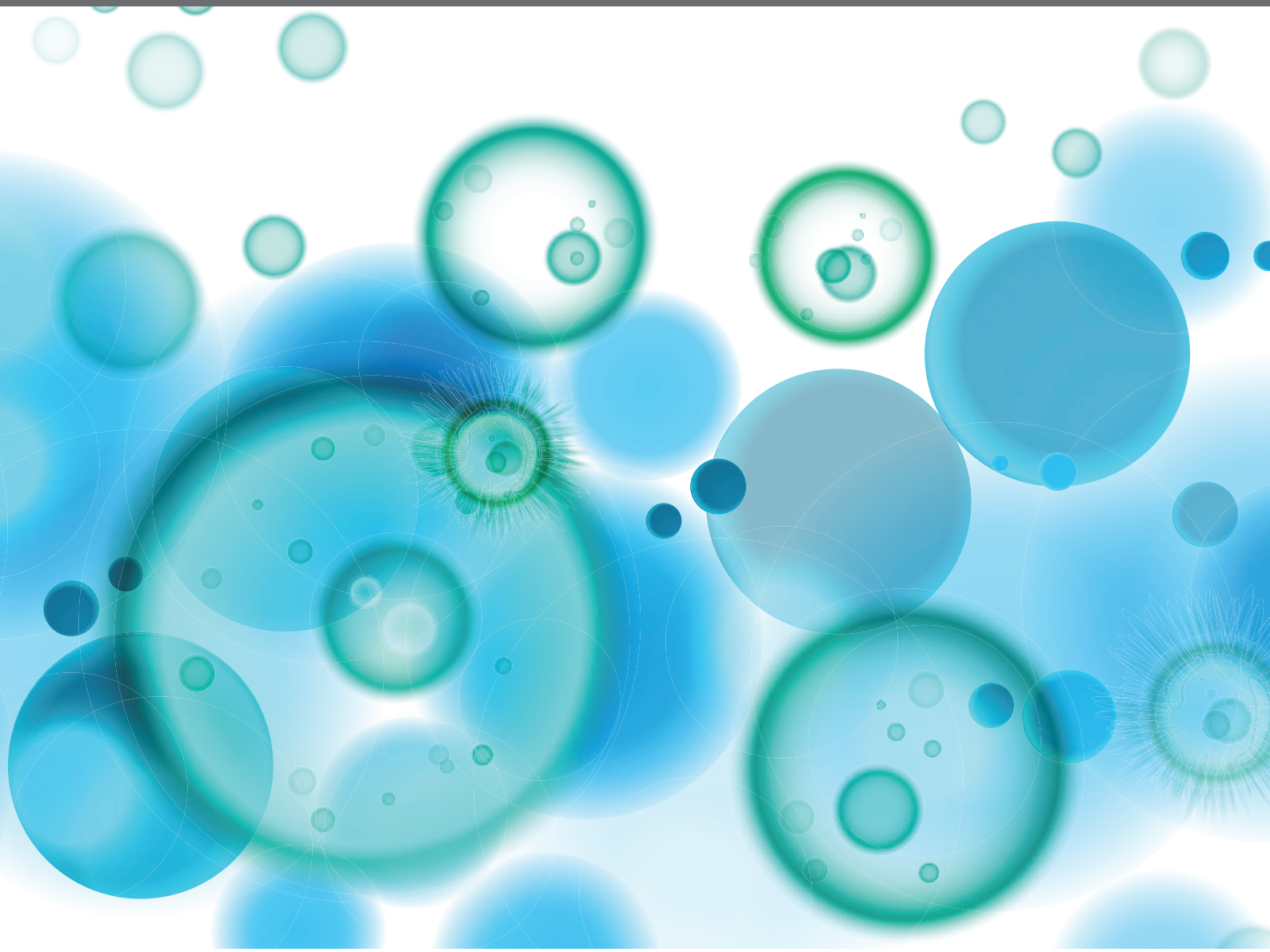

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IMMUNOLOGICAL CHALLENGES AROUND PREGNANCY COMPLICATIONS ASSOCIATED WITH FAILURES OF MATERNAL TOLERANCE TO THE FETUS

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Editorial: Immunological challenges around pregnancy complications associated with failures of maternal tolerance to the fetus

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Editorial on the Research Topic

Immunological challenges around pregnancy complications associated with failures of maternal tolerance to the fetus

Successful pregnancy provides an intriguing immunologic paradox. The fetus carries both paternal and maternal genes and therefore is 50% foreign to the mother, but is tolerized by the maternal immune system. Several immune regulatory mechanisms are at play at the maternal-fetal interface, which represents the location where fetal trophoblasts in the placenta come into contact with maternal immune cells. In early pregnancy, extravillous trophoblasts are relevant in plugging and remodeling maternal spiral arteries and thereby are relevant for the proper development of the placenta and the fetus. At the same time, fetal trophoblasts at the maternal-fetal interface need to be tolerized against immune attack by maternal T cells, myeloid cells (macrophages and dendritic cells), and decidual natural killer (NK) cells. One way of establishing this is the expression of HLA-G on the surface of trophoblasts and the secretion of soluble HLA-G. A second way is the generation and development of regulatory immune cells, including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs). These cell types mediate homeostasis by dampening effector immune cell mechanisms.

This Research Topic focuses on mechanisms that effectuate a tolerogenic microenvironment in healthy pregnancy and describes how these features may be disturbed in various types of pregnancy complications.

An experimental study by Dietz et al. uses a murine model lacking the Qa-2 antigen, a murine homologue-candidate for HLA-G, to show the effect of sHLA-G in reducing abortion rates through inducing Tregs and MDSCs. Both cell types are shown to suppress

the action of effector T cells. Interaction and cross-talk between the trophoblast and macrophage may lead to direct polarization of the latter, as reviewed by Ding et al.. Such effects mediating tolerogenicity can be driven either by direct cell-cell contact, excreted soluble factors or a combination of the two. Next to macrophages, dendritic cells make part of the myeloid cell lineage. These cells are the most efficient type of antigen-presenting cells, and as part of the decidual environment, they are thought to drive fetus-specific immunity by the maternal immune system. Zhang et al. show that the female steroid hormone progesterone has major effects on the characteristics of dendritic cells. Another regulatory mechanism at the maternal-fetal interface may be directed by surface molecules CD39 and CD73, which promote conversion of adenosine 5′-triphosphate (ATP) to adenosine and thereby change a pro-inflammatory to an anti-inflammatory environment. Zhu et al. demonstrate that trophoblasts express both molecules and also show that a high fraction of decidual NK cells are positive for CD39. Expression of both molecules was found to be decreased at the maternal-fetal interface in women with pregnancy loss, thereby emphasizing their importance in this disease.

The current Research Topic is further directed at pregnancy-related complications with possible involvement of the immune system, which are studied here. These include recurrent pregnancy loss (RPL), chronic histiocytic intervillositis (CHI), and preeclampsia.

As the fetal cells are tolerized by the maternal immune system in healthy pregnancy, immunological mechanisms and disturbances therein may underlie RPL. Women with RPL have had two or more spontaneous miscarriages. It affects approximately 3% of all fertile couples and in more than 50% of couples the underlying cause is not clear: a considerable part of such cases are suspected to have an immunologic nature. Li et al. outline how big data analysis of the maternal-fetal interface, as well as placenta and blood, from women with RPL may help elucidating the pathogenesis.

CHI is a poorly understood histopathological lesion of the placenta. CHI is significantly associated with miscarriage, fetal growth restriction, and intrauterine fetal death. In this disease, an intervillous infiltrate, which is of maternal origin, can be seen in every trimester throughout gestation. The interplay between maternal immune cells and fetal trophoblasts in CHI might point toward a breach in immune tolerance and is a topic for ongoing research. Cornish et al. present a detailed overview of prevalence, pathology, and clinical consequences of different gestational syndromes, including CHI, which are associated with adverse pregnancy outcomes. Brady et al. describe how application of immune modulatory medication in women with CHI may improve placental lesions and higher the chance of livebirth.

The third type of pregnancy-related complication discussed in this Research Topic is preeclampsia, which is a serious complication endangering the mother and the fetus. High blood pressure is an important indicator for diagnosing preeclampsia. Gan et al. found that systolic blood pressure of the mother at 12 weeks of gestation predicts the development of severe preeclampsia and of low birth weight of the baby. Green et al. present a systematic review concerning the role of Tregs in adverse pregnancy outcome. They show that decreased Treg numbers in the peripheral blood of pregnant women is significantly associated with the occurrence of pre-eclampsia. Acute atherosis is a lesion which can be present at the maternal spiral arteries, and which may be encountered in preeclampsia. Jacobsen et al. provide an extensive overview and discussion on acute atherosis, including its definition, underlying mechanism, and possible clinical consequences.

In summary, this Research Topic provides insights in the immunological challenges around pregnancy complications associated with failures of maternal tolerance to the fetus. The collection of articles highlights the function of specific immune cell populations and describes new mechanisms of pregnancy monitoring that can contribute to advanced basic research and clinical translation for various types of pregnancy complications.

Author contributions

ME, M-LH, DM-P, and UM composed the text, revised it, and approved the final draft.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Immunomodulatory Therapy Reduces the Severity of Placental Lesions in Chronic Histiocytic Intervillositis

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Chronic histiocytic intervillositis (CHI) is a rare, but highly recurrent inflammatory placental lesion wherein maternal macrophages infiltrate the intervillous space. Pregnancies with CHI are at high risk of fetal growth restriction, miscarriage or stillbirth. Presently, the diagnosis can only be made after histopathological examination of the placenta. Given its proposed immunological etiology, current treatments include aspirin, heparin, and immunomodulatory agents. However, the rationale for these medications is largely based upon small case series and reports as there is a lack of larger studies investigating treatment efficacy. Therefore, this study sought to determine whether inclusion of immunomodulatory medications was effective at reducing the severity of lesions and improving pregnancy outcomes in subsequent pregnancies. Thirty-three women with a history of CHI in at least one pregnancy (index case) were identified retrospectively through medical records. Twenty-eight participants presented with a first subsequent pregnancy and a further 11 with a second subsequent pregnancy at a specialist clinic for pregnancy after loss. Data on maternal demographics, medical history, medication, pregnancy outcome, and placental pathology was collected and compared between pregnancies. Twenty-seven (69%) subsequent pregnancies were treated with at least one or both of prednisolone and hydroxychloroquine. Inclusion of at least one immunomodulatory agent in treatment regimen resulted in an almost 25% increase in overall livebirth rate (61.5 vs. 86.2%). In women treated with immunomodulatory medication a greater proportion of placentas had reduced severity of lesions compared to those treated without (86.7 vs. 33.3%, respectively). A reduction in CHI severity was associated with a 62.3% improvement in livebirth rate compared to those where severity remained unchanged in relation to the index case. These data provide preliminary evidence that the use of immunomodulatory medication in the management of CHI improves histopathological lesions and the chance of livebirth in subsequent pregnancies. Due to CHI's rarity and ethical and feasibility issues,

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randomized controlled trials in affected women are challenging to conduct. As a result, collaboration between centers is required in future to increase study sample sizes and elucidate the mechanisms of hydroxychloroquine and prednisolone in reducing pathology.

Keywords: placental histopathology, prednisolone, hydroxychloroquine, stillbirth, treatment, miscarriage, outcomes

INTRODUCTION

Chronic histiocytic intervillositis (CHI), also known as chronic intervillositis or chronic intervillositis of unknown etiology (1), is a pregnancy disorder strongly associated with fetal growth restriction, miscarriage, stillbirth and neonatal death (2–5). Estimated to affect 6 in every 10,000 pregnancies over 12 weeks' gestation, CHI is characterized by maternal macrophage infiltration into the intervillous space of the placenta and has a 25–100% risk of recurrence in subsequent pregnancies (1, 3, 6, 7). Many cases also exhibit marked perivillous and/or intervillous fibrin deposition and trophoblast necrosis (8). Due to its asymptomatic nature and a lack of reliable associated biomarkers, currently a diagnosis of CHI can only be made following delivery by histopathological examination of the placenta. Management of CHI is further complicated by a lack of standardized treatment options proven to prevent recurrence.

Owing to the presence of maternal macrophages and the reported increased incidence in women with autoimmune disease, CHI has been hypothesized to be a disorder of failed maternal-fetal tolerance and excessive inflammation (9). On this basis and due to the presence of intervillous and perivillous fibrin, current treatments include thromboprophylactic agents such as aspirin and low-molecular-weight heparin (LMWH) as well as those aimed at suppressing inflammation e.g., corticosteroids and hydroxychloroquine (9). A systematic review of six observational studies conducted in 2010 found no significant improvement in pregnancy outcome with aspirin and LMWH alone. Though a growing number of case reports detail use of immunomodulatory agents such as prednisolone and hydroxychloroquine (9, 10), there remains a striking lack of larger studies supporting the efficacy of any treatment regime in reducing severity of CHI and improving pregnancy outcomes. Notably, a prior case series has highlighted that women with worse obstetric histories tend to be prescribed more therapeutic agents, despite their unproven efficacy (9). Due to the high rate of recurrence of CHI and severe consequences of the disorder, studies are urgently needed to determine effective therapies.

By retrospectively identifying women with a previous diagnosis of CHI, we aimed to investigate pregnancy outcomes and placental pathology in subsequent pregnancies referred to a specialist service following poor perinatal outcome, stillbirth or neonatal death. We hypothesized, due to the immunological nature of CHI, that treatment regimens where immunomodulatory agents were included would decrease the severity of the condition and consequently improve the chance of livebirth.

MATERIALS AND METHODS

Participant Recruitment and Data Collection

Women with a previous histopathological diagnosis of CHI between 2009 and 2021 were identified retrospectively from medical records at Manchester University NHS Foundation Trust, UK. The majority of these cases were identified after the death of a baby or late miscarriage when histopathological evaluation of the placenta is recommended practice. In other cases (following the birth of a live infant) the placenta is sent away for examination for a variety of clinical indications (e.g., FGR, fetal compromise at birth, placental abruption, and previous late pregnancy loss). CHI was diagnosed by a specialist perinatal pathologist in accordance with its initial description by Labarrere and Mullen as a placental lesion consisting of histiocytic (macrophage) infiltration into the intervillous space (8). Data on maternal demographics, medical history including results of tests for autoantibodies (lupus anticoagulant, anti-phospholipid, antinuclear, and anticardiolipin antibodies) and obstetric history were collected from the woman's case record. The first pregnancy diagnosed with CHI was classified as the "index" case, with data on any subsequent pregnancies recorded where applicable from retrospective medical records. Pregnancy outcomes consisted of liveborn and still living, liveborn at term and still living (>37 weeks' gestation), miscarriage (fetal death <24 weeks gestation, including spontaneous abortion), stillbirth (fetal death >24 weeks gestation), and termination of pregnancy for fetal anomaly (TOPFA). Cases of neonatal death (death of an infant within 28 days after birth) were also recorded. Fetal growth restriction (FGR) was defined as growth below the 3rd percentile, and small for gestational age (SGA) as between 3rd and 10th centile (11). Centiles were calculated for pregnancies >20 weeks' gestation using the GROW centile calculator, for cases where maternal demographics, pregnancy outcome and fetal sex were known (12).

The therapeutic agents used in our service evolved over time following publications from other researchers (3, 9, 10, 13) and following discussion with colleagues with expertise in lupus in pregnancy. Initially, pregnancies with CHI were managed using Aspirin and LMWH, but this evolved to a combination of Aspirin was given at a dose of 75–150 mg once a day, a prophylactic dose of LMWH (e.g., Tinzaparin 4,500iu) once a day, Hydroxychloroquine 200 mg twice a day and Prednisolone 20 mg once a day in the morning. Drug therapy was started from a viability scan at 6–7 weeks' gestation. Women underwent ultrasound assessment of uterine artery Doppler at 17 weeks' gestation, if this showed no abnormality then Prednisolone was

reduced by 5 mg per week. If there was evidence of uterine artery notching or raised pulsatility index Prednisolone was continued at 20 mg and then uterine artery Doppler's were reassessed after 2 weeks. If there was no improvement by 21 weeks' gestation the Prednisolone was reduced at this stage. Women underwent regular ultrasound assessment of fetal growth, amniotic fluid volume and umbilical artery Doppler after 23 week's gestation at a minimum frequency of 3-weekly intervals.

Where available, placental histopathology reports were analyzed for detail on histopathological features including the presence of villitis, increased fibrin deposition and recurrence and severity of CHI. Change in severity of CHI in subsequent pregnancies was in comparison to the severity of the index case of CHI. Focal CHI without accompanying fibrin deposition was classified as "mild," those with accompanying fibrin as "moderate," and diffuse, high-grade lesions classified as "severe."

Informed and written consent was obtained from all study participants and ethical approval granted by the NRES Committee North West—Greater Manchester West and NRES Committee London—City & East.

Statistical Analysis

Statistical analysis was conducted using GraphPad Prism Version 9.1.0. Chi-square and Kruskal-Wallis tests were performed for categorical and continuous variables, respectively. For statistical analysis, first and second subsequent pregnancies were combined, and pregnancy outcomes divided into liveborn and still living or liveborn at term and still living vs. adverse outcome (TOPFA, miscarriage, stillbirth and neonatal death). Where sample number precluded analysis by Chi-square, Fisher's Exact test was performed instead. Statistical analysis of treatment regimen was performed by dividing participants into those which received immunomodulatory therapy (one or both of prednisolone and hydroxychloroquine) and those without (one or both of aspirin and heparin, or untreated).

RESULTS

Participant Demographics

Thirty-three women with a history of at least one pregnancy affected by CHI between 2009 and 2021 were identified retrospectively from medical records. Twenty-eight women presented with a subsequent pregnancy and 11 of these with a further second subsequent pregnancy. Participant demographics, medical and obstetric history are shown in **Table 1**. Four Dichorionic-Diamniotic (DCDA) twin pregnancies were included in the study. Twenty-six women were White British (78.8%), with a smaller proportion of Asian ($n = 6$) and Black African women ($n = 1$). Four index CHI pregnancies (12.1%) were conceived using ART, compared to only one first subsequent pregnancy (3.6%) and no second subsequent pregnancies. Underlying autoimmune disease was present in four cases (12.1%), consisting of coeliac disease, hypothyroidism, autoimmune thrombocytopenia, and hypermobility syndrome, respectively. Seventeen women had been tested for antinuclear antibodies, three of which were positive (17.7%) and 25 and 22 women had testing for anti-cardiolipin antibodies and anti-phospholipid antibodies, respectively, none of which were positive. Of 26 women tested for lupus anticoagulant, one was positive (3.9%). Pre-existing hypertension was present in two women (6.1%).

TABLE 1 | Demographics and obstetric and medical history of women with a diagnosis of chronic histiocytic intervillositis (CHI).

	Index CHI	1st subsequent pregnancy	2nd subsequent pregnancy
***N* participants**	33	28	11
***N* fetuses**	34	30	12
Maternal age (years)	32 (19–38)	34 (22–41)	37 (26–40)
BMI	26 (20–47)	27 (20–47)	27 (20–40)
Ethnicity			
White British	26 (78.8%)		
Asian	6 (18.2%)		
Black African	1 (3.0%)		
Lifestyle			
Smoker	3 (9.4%)	1 (3.6%)	0
Unknown	1		
Alcohol consumption	0		
Unknown	1		
Obstetric history			
Previous livebirths	0 (0–5)		
Previous losses	0 (0–4)		
Primigravida	11 (33.3%)		
ART pregnancy	4 (12.1%)	1 (3.6%)	0
Twin pregnancy	1 (3.0%)	2 (7.1%)	1 (9.1%)
Medical history			
Autoimmune disease	4 (12.1%)		
Pre-existing hypertension	2 (6.1%)		
Anti-nuclear antibodies	3 (17.7%)		
Untested	16		
Lupus anticoagulant	1 (3.9%)		
Untested	7		

Index pregnancy was defined as a participant's first pregnancy diagnosed with CHI by placental histopathological examination following poor outcome. Subsequent pregnancies refer to those following diagnosis. Continuous variables are presented as median (range) and categorical variables N (percentage). ART, assisted reproductive technology; BMI, body mass index.

Pregnancy Outcomes

Outcomes of index pregnancies diagnosed with CHI and subsequent pregnancies are listed in **Table 2**. The proportion of infants born by Cesarean section increased from 27.3% (6/33) in index pregnancies to 59.1% (13/28) and 45.5% (5/11) in first and second subsequent pregnancies, respectively, though this was not statistically significant. Fetal sex did not differ significantly across pregnancies, and in two subsequent pregnancies fetal sex had not been determined due to loss early in gestation.

TABLE 2 | Pregnancy outcomes of index and subsequent pregnancies in women with a diagnosis of chronic histiocytic intervillositis (CHI).

	Index CHI	1st subsequent pregnancy	2nd subsequent pregnancy
***N* participants**	33	28	11
***N* fetuses**	34	30	12
Cesarean section (>24 weeks)	6 (27.3%)	13 (59.1%)	5 (45.5%)
Male fetus	13 (38.2%)	5 (53.6%)	7 (58.3%)
Unknown fetal sex	0	2	0
Pregnancy outcome			
Liveborn and still living	4 (11.8%)	21 (70.0%)	12 (100%)
Stillbirth	16 (47.1%)	1 (3.3%)	0
Miscarriage	5 (14.7%)	6 (20.0%)	0
TOPFA	5 (14.7%)	1 (3.3%)	0
Neonatal death	4 (11.8%)	1 (3.3%)	0
Gestation at delivery (weeks)	26 (17–41)	37 (12–39)	38 (35–39)
Birthweight centile	2.8 (0–68.4)	22.8 (0.4–99.9)	23.2 (3.8–89.6)
Complications			
FGR <3rd centile	13 (52.0%)	3 (13.6%)	0
SGA 3rd−10th centile	5 (20.0%)	2 (9.1%)	1 (9.1%)
Preterm <37 weeks (% of livebirths)	6 (75.0%)	5 (22.7%)	3 (25.0%)
Maternal comorbidities			
Gestational diabetes	1 (3.0%)	0	0

Index pregnancy was defined as a participant's first pregnancy diagnosed with CHI by placental histopathological examination following poor outcome. Subsequent pregnancies refer to those following diagnosis. Miscarriage was defined as fetal death <24 weeks gestation, and stillbirth as fetal death >24 weeks gestation. Neonatal death refers to the death of an infant within 28 days after birth. Continuous variables are presented as median (range) and categorical variables N (percentage). FGR, fetal growth restriction; SGA, small for gestational age; TOPFA, termination of pregnancy for fetal anomaly.

TABLE 3 | Histopathology of placentas from index and subsequent pregnancies in women with chronic histiocytic intervillositis (CHI).

	Index CHI	1st subsequent pregnancy	2nd subsequent pregnancy
N placentas	34	30	12
N placentas with pathology reports	34 (100%)	23 (76.7%)	8 (66.7%)
CHI	34 (100%)	11 (47.8%)	2 (25.0%)
Chronic villitis	5 (14.7%)	6 (26.1%)	2 (25.0%)
Increased fibrin deposition	18 (52.9%)	7 (30.4%)	2 (25.0%)
Small for gestational age	7 (20.6%)	4 (17.4%)	1 (12.5%)

Index pregnancy was defined as a participant's first pregnancy diagnosed with CHI by placental histopathological examination following poor outcome. Subsequent pregnancies refer to those following diagnosis. Variables are expressed as N (percentage).

The outcome of subsequent pregnancy/ies improved significantly, with 70% of first and 100% of second subsequent pregnancies resulting in a liveborn and still living infant, compared to 11.8% of index pregnancies ($p < 0.0001$). Gestation at delivery also increased significantly across pregnancies ($p = 0.002$), from a median of 26 weeks' gestation in index cases to 37 weeks' gestation in first subsequent ($p = 0.04$) and 38 weeks in second subsequent pregnancies ($p = 0.002$). Of infants who were liveborn, rates of neonatal death were significantly reduced from 50% (4/8) in index cases to 3.0% (1/33) of subsequent pregnancies ($p = 0.003$). TOPFA occurred in five index pregnancies (14.7%) and one first subsequent pregnancy (3.3%), after diagnosis of skeletal dysplasia ($n = 1$), severe FGR ($n = 3$), triploidy ($n = 1$) and neurological disorder ($n = 1$). In one case of stillbirth occurring in an index pregnancy, Trisomy 18 was diagnosed at post-mortem. For infants whose birthweight centiles could be calculated, rates of FGR <3rd centile decreased significantly between index and subsequent pregnancies from 52.0% (13/25) to 9.1% (3/33) ($p = 0.0007$). There were no significant differences in the incidence of SGA across pregnancies. Overall birthweight centiles increased significantly across pregnancies from a median of 2.8 in index cases to 22.8 and 23.2 in first and second subsequent pregnancies, respectively ($p < 0.0001$). In subsequent pregnancies, 23.5% of livebirths were preterm (8/34), significantly reduced from 75.0% (6/8) of index cases ($p = 0.01$). One participant was diagnosed with gestational diabetes in their index pregnancy (3.0%), and there were no cases of preeclampsia. Chorioamnionitis occurred in one index case (3.0%) alongside CHI, however since CHI recurred without infection in the participant's subsequent pregnancy this case was included in the study.

Placental Pathology

Findings of placental histopathology are shown in **Table 3**. By design, all index pregnancies had an accompanying placental pathology report. The number of cases with histopathological examination of the placenta decreased to 76.7% (23/30) and 66.7% (8/12) in first and second subsequent pregnancies as a result of placentas not having been sent for histopathological examination. The incidence of CHI significantly decreased from index pregnancies, with an overall recurrence rate of 41.9% (13/31) in subsequent pregnancies ($p < 0.0001$). There were no significant differences in the incidence of villitis, increased fibrin deposition and placentas classified as small for gestational age. Pregnancies with concurrent CHI and villitis in the placenta exhibited no differences in outcome compared to those with CHI alone.

The severity of CHI lesions in placentas from index and subsequent pregnancies as determined by histopathological examination is shown in **Figure 1**. Detail of CHI lesion severity was available for 28/34 (82.4%) and 30/31 (96.8%) placentas from index and subsequent pregnancies, respectively. Placentas from index cases exhibited mainly mild or moderate CHI [39.3% (11/28) and 39.3% (11/28)], followed by a smaller proportion

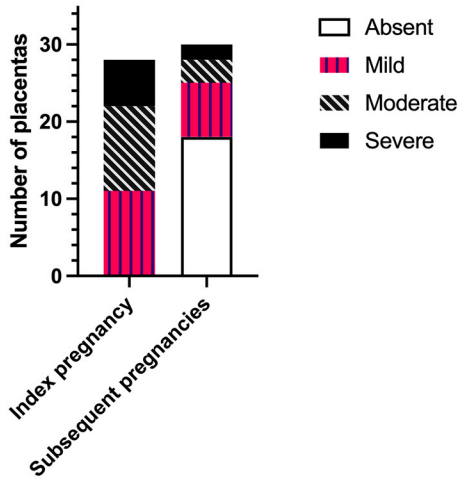

FIGURE 1 | Severity of chronic histiocytic intervillositis (CHI) lesions in placentas from index and subsequent pregnancies according to pathologist's report. Index CHI refers to a participant's first pregnancy diagnosed with CHI by placental histopathological examination following poor outcome. Subsequent pregnancies refer to those following diagnosis.

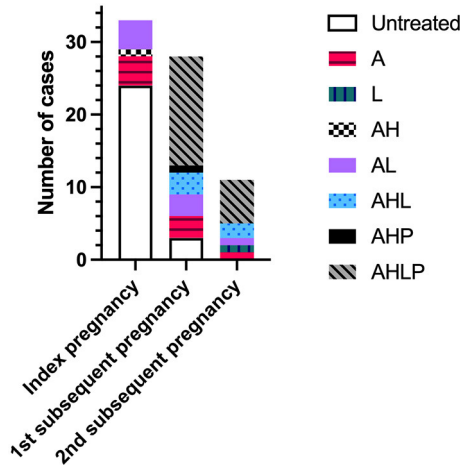

FIGURE 2 | Treatment regimen across index pregnancies with chronic histiocytic intervillositis (CHI) and first and second subsequent pregnancies. Index pregnancy was defined as a participant's first pregnancy diagnosed with CHI by placental histopathological examination following poor outcome. Subsequent pregnancies refer to those following diagnosis. A, aspirin; H, hydroxychloroquine; L, low-molecular-weight heparin; P, prednisolone.

of severe cases [21.4% (6/28). In comparison, CHI was absent in the majority of placentas from subsequent pregnancies (60% (18/30)], whilst 23.3% displayed mild lesions (7/30), 10.0% moderate (3/30), and 6.7% severe (2/30). Overall, severity of CHI was significantly reduced in subsequent pregnancies compared to index ($p < 0.0001$).

Treatment Effect

Treatment regimen across pregnancies is shown in **Figure 2**. The majority of index pregnancies had no medication (24/33, 72.7%), compared to 10.7% (3/28) of first subsequent pregnancies. No second subsequent pregnancies were untreated. In index pregnancies, the nine treated participants were on medication for pre-existing medical conditions or risk factors and took aspirin alone ($n = 4$), in combination with LMWH ($n = 4$), or hydroxychloroquine ($n = 1$). There was no significant difference in the livebirth rate of index pregnancies between women on medication and those without. In subsequent pregnancies, 69.2% (27/39) of all participants received immunomodulatory therapy with at least one or both of hydroxychloroquine or prednisolone, with the majority of participants 53.9% (21/39) receiving all four medications. All four women with a positive autoantibody screen were treated in their subsequent pregnancies ($n = 5$) with aspirin and LMWH ($n = 1$); aspirin, low-molecular-weight heparin and hydroxychloroquine ($n = 1$); aspirin, prednisolone, and hydroxychloroquine ($n = 1$) or a combination of all four medications ($n = 2$). These pregnancies all resulted in the birth of a live infant surviving past 28 days of life.

The overall rate of infants liveborn and still living (including infants born <37 weeks' gestation) was almost 25% higher in women receiving immunomodulatory treatment with one or both of hydroxychloroquine and prednisolone compared to those without [25/29 (86.2%) vs. 8/13 (61.5%)] (**Figure 3A**). However, this did not reach statistical significance due to the number of pregnancies included in the study ($p = 0.11$). Inclusion of immunomodulatory medication in treatment regimen also resulted in a 19% increase in the proportion of infants liveborn at term (>37 weeks) (**Figure 3B**) [65.5% (19/29) with immunomodulators vs. 46.2% (6/13) without], though again this was not statistically significant. There were no significant effects of immunomodulatory treatment on the incidence of FGR [8.33% (2/24) with vs. 12.5% (1/8) without], SGA [12.5% (3/24) with vs. 12.5% (1/8) without], or on overall birthweight centiles (median 20.9 with vs. 19.5 without).

Change in CHI severity following treatment regimen is shown in **Figure 4A**. 7/13 (53.8%) placentas from women treated without immunomodulatory medication were sent for histopathological examination, compared to 23/29 of those who were treated with it (82.8%). Change in severity of CHI in one subsequent pregnancy treated without immunomodulatory medication could not be determined due to a lack of detail on severity of the index case. The majority of remaining placentas from pregnancies treated without immunomodulatory medication exhibited no change in CHI severity compared to their index case [66.7% (4/6)]. In comparison, 86.7% (20/23) of cases treated with immunomodulators displayed a reduction in CHI severity or a lack of recurrence ($p = 0.02$). Decreased severity of CHI in subsequent pregnancies was associated with a 62.3% increase in livebirth rate compared to pregnancies where severity was unchanged [20/22 (90.9%) vs. 2/7 (28.6%), respectively] ($p = 0.003$; **Figure 4B**).

DISCUSSION

These data suggest that the use of one or both of prednisolone and hydroxychloroquine in the treatment of CHI resulted in a reduction of disease severity and a trend toward an increase in livebirth rate. Overall, a decrease in CHI severity was associated

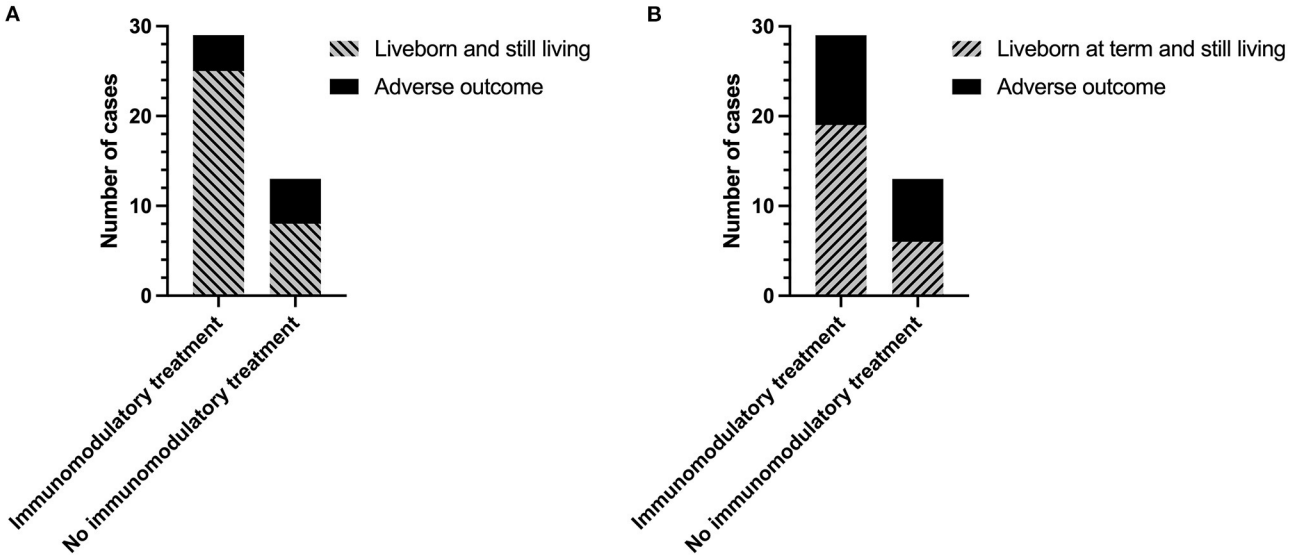

FIGURE 3 | Outcomes of subsequent pregnancies in women with a previous diagnosis of chronic histiocytic intervillositis (CHI) following treatment with or without immunomodulators. **(A)** Number of pregnancies resulting in a liveborn and still living infant (including those born <37 weeks' gestation) or adverse outcome (miscarriage, termination of pregnancy, stillbirth, or neonatal death). **(B)** Number of pregnancies resulting in a liveborn and still living infant at term (>37 weeks), or adverse outcome (miscarriage, termination of pregnancy, stillbirth, preterm birth, or neonatal death) Immunomodulatory treatment refers to a regimen including one or both of prednisolone and hydroxychloroquine in combination with either aspirin, heparin, or both. Pregnancies without immunomodulatory treatment were untreated or received aspirin or heparin or both.

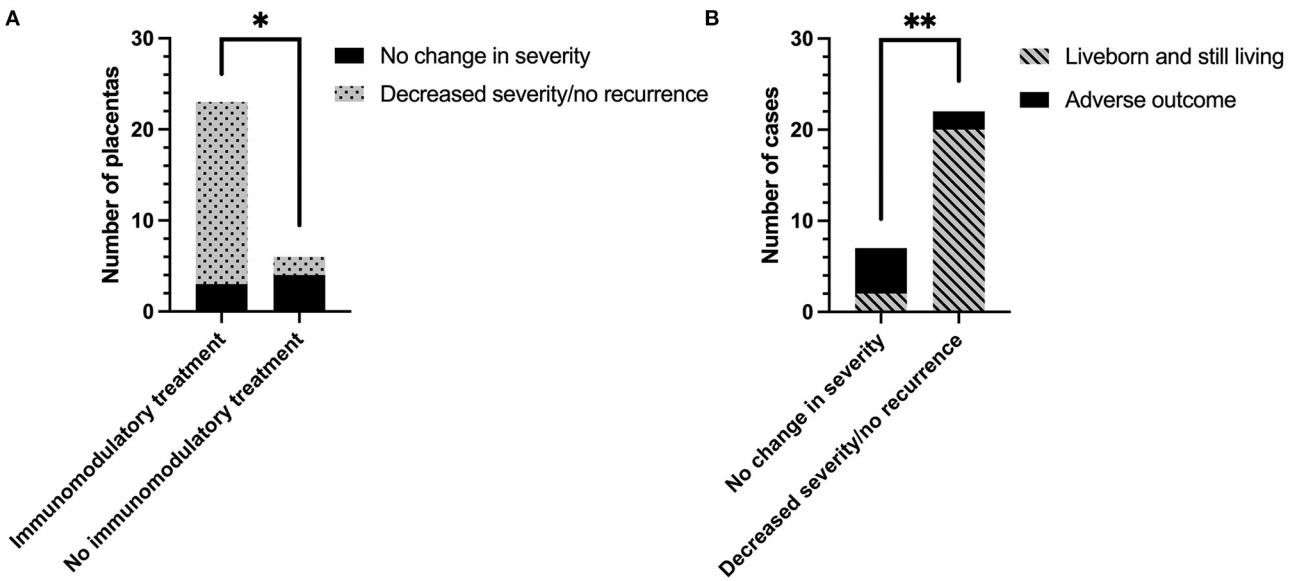

FIGURE 4 | The effect of immunomodulatory medication in subsequent pregnancies after a diagnosis of chronic histiocytic intervillositis (CHI). **(A)** Change in CHI lesion severity in pregnancies treated with or without immunomodulatory medication, compared to the participant's first placenta diagnosed with CHI from a previous pregnancy. Severity of CHI was determined by pathologist's report. Immunomodulatory treatment refers to a regimen including one or both of prednisolone and hydroxychloroquine in combination with either aspirin, heparin, or both. Pregnancies without immunomodulatory treatment were untreated or received aspirin or heparin or both. **(B)** Rate of infants liveborn and still living (past 28 days of life) in subsequent pregnancies related to change in CHI severity. *$p < 0.05$; **$p < 0.01$.

with a 62.3% reduction in pregnancy loss. These findings suggest that management of CHI in a specialist center for women pregnant after loss improves outcomes, with all second subsequent pregnancies resulting in the birth of a healthy infant.

Strengths and Limitations

Previously, treatment of CHI has been informed by case reports and small studies detailing successful pregnancy outcome following the use of prednisolone and other immunomodulatory agents (10), due to a lack of randomized controlled trials informing management. Given CHI's rarity and ethical and feasibility issues surrounding recruitment of women with such poor obstetric histories, it is unlikely that there will be a study where treatment is compared to placebo or a combination of agents known to be ineffective (e.g., aspirin and LMWH alone). As such, case series prove invaluable in guiding the care of women with a history of CHI. To date, this series is the largest to investigate the efficacy of immunomodulatory medication in the treatment of CHI and proposes a standardized protocol of medication to increase the chance of positive outcome in subsequent pregnancies.

Through analysis of medical records, we have been able to follow women through subsequent pregnancies following diagnosis of CHI and formulate a well-characterized cohort of a rare condition. However, we recognize that women who decided to become pregnant or became pregnant could differ from all women who have CHI. The demographic characteristics of our sample were similar to those from other case series (5, 14), suggesting that this potential selection bias had not significantly altered the nature of the sample.

In previous investigations, the rate of recurrence of CHI and accompanying outcome in treated pregnancies has been relatively well-documented, though there is a paucity of evidence regarding improvement in lesion severity. For the majority of pregnancies we included, detail of lesion severity was available in placental pathology reports, allowing any change to be related to an individual's index case. To our knowledge, this is the first time such an approach has been taken in a case series of treated pregnancies following CHI. Here, it was shown that although overall recurrence of CHI in participants treated with immunomodulatory treatment did not differ significantly compared to those treated without, the severity of lesions was greatly reduced. This indicates that inclusion of disease severity as an outcome in future studies on CHI may provide better insight into treatment effect rather than simple recurrence rate alone.

Due to the retrospective nature of this study, there were certain variables for which data was not available within medical records. For instance, many women whose index case of CHI occurred less recently had not received screening for all autoantibodies as this was a more recent practice. Additionally, the range of antibodies tested was limited, which may explain the low incidence of autoantibodies in this cohort in comparison to studies which looked more specifically at antibody status and have reported incidences between 29 and 58% (9). Since this suggestion of an autoimmune component to CHI, it has become standard practice within our center to offer women an antibody screen following referral. In future, this will allow better characterization of any possible role pre-existing antibodies may have within CHI and may inform immunosuppressive treatment if underlying conditions are present.

Several subsequent pregnancies included were without an accompanying placental histopathology report as a direct result of having not been sent for examination. In all these cases, all respective pregnancies had resulted in a livebirth. This is suggestive that there may have been a bias toward reporting pathology only in pregnancies resulting in poor outcome. In addition, pathologists were informed of patient's medical history which may have influenced diagnosis. In response to decreased rates of pathological examination in subsequent pregnancies, the importance of histopathological examination following a history of CHI is becoming increasingly recognized amongst midwives and clinicians within our center. Consequently, placental histopathology is now a routine requirement and is anticipated to improve sample size for prospective studies.

Clinical Context

Though the specific effects of prednisolone and hydroxychloroquine could not be individually determined here, our data is suggestive that inclusion of at least one of these medications in the treatment of CHI can significantly reduce the severity of macrophage infiltration into the intervillous space. Further to this, a reduction in CHI severity increased the likelihood of a live birth. This is in line with previous evidence that pregnancies where CHI is severe or diffuse in the placenta are more strongly associated with poor outcome (15). Pregnancy outcomes in CHI and severity following hydroxychloroquine use have not been well-documented previously, although a single study stated that four out of six pregnancies where it was included in treatment regimen resulted in a liveborn infant (9). Prednisolone has also in several case reports been associated with improved pregnancy outcome and reduced severity of both CHI and fibrin deposition compared to treatment with aspirin or heparin alone (2, 10, 16). Within our cohort, there was no significant difference in fibrin deposition between index and subsequent pregnancies, and the majority did not have an increase in fibrin noted in their pathology report. This is perhaps unsurprising as fibrin deposition has been classified previously as an accompanying feature which may or may not occur alongside CHI (1).

Whilst the exact cause of pathology in CHI is unknown, current evidence suggests that it is driven by excessive maternal inflammation. Inflammatory features characterized so far include the presence of partner-directed T lymphocytes and antibodies (17) and deposition of complement in the placenta (18). Our observation that administration of prednisolone and hydroxychloroquine aimed at suppressing the maternal immune response reduces CHI severity is therefore consistent with this hypothesis. The specific mechanism of hydroxychloroquine and prednisolone's anti-inflammatory effects in CHI are unknown, though in mouse and *in vitro* models of antiphospholipid syndrome, it has been suggested that hydroxychloroquine is able to reduce complement activation and antibody binding to the syncytiotrophoblast (19). In addition, hydroxychloroquine does not appear to have negative effects on placental explants and

increases release of anti-inflammatory cytokine interleukin-10 (19). As complement deposition and excessive inflammation is characteristic of CHI, further investigation into the mechanisms of immunomodulatory therapy in the condition is warranted.

Evidence of CHI as an alloimmune condition has also provided rationale for the use of other immunomodulatory and immunosuppressive routes of treatment, including intravenous immunoglobulin (IVIG) therapy, tumor necrosis factor (TNF) antagonists, and tacrolimus (20). In a case report by Abdulghani et al. (13), IVIG was used following the failure of prednisolone to produce successful outcome in a previous pregnancy, and resulted in two subsequent healthy pregnancies. Histopathological examination of both placentas revealed that CHI had not recurred in either case. Similarly, use of TNF antagonist adalimumab was reported in the pregnancy of a woman with recurrent intervillositis and has been proposed as a possible agent in the prevention of recurrent miscarriage (21). Though these case reports provide anecdotal evidence hinting at the benefits of these agents, there is still a lack of larger studies to justify their use and as such much controversy remains.

Despite the lack of comparative studies where the efficacy of immunomodulatory medication in treating CHI has been specifically investigated, a livebirth rate of 66.7% in 21 women receiving various combinations of aspirin, heparin, prednisolone and hydroxychloroquine has previously been reported by Mekinian et al. (9). Conversely, in the only systematic review of intervention in CHI to date, treatment was suggested to correlate with worse outcome, with a reported livebirth rate of 30.8% (3). Here, livebirth rate with inclusion of one or both of hydroxychloroquine and prednisolone was markedly higher than both studies at 86.2%. Importantly, the systematic review did not include any studies wherein hydroxychloroquine was administered, and treatment regimen was less consistent than that used within this case series, both of which may be factors in the differences observed. As in the aforementioned studies, differences in livebirth rates within this cohort were not proven to be significantly different between treatment groups, likely due to limited sample size considering that data did show a trend toward statistical significance. Given that the only systematic review is now a decade old, it is probable that an updated study of the literature is required to better characterize treatment combinations and their effect on pregnancy outcome.

In subsequent pregnancies, gestational age at delivery, fetal growth, and rates of preterm birth improved greatly, though these effects could not be significantly attributed to immunomodulatory treatment. It is possible that this is due to limited numbers, or an effect of treatment using LMWH and aspirin. Adverse outcomes may also have been reduced as a consequence of increased fetal monitoring following specialist care after previous pregnancy loss. As there were insufficient numbers of untreated women and all participants had attended the specialist clinic, it was not possible to distinguish whether these outcomes were a result of intervention or treatment. Increased fetal monitoring is unable to influence fetal growth, but delivery via Cesarean section showed a trend toward increasing across subsequent pregnancies despite lacking statistical significance. Other centers have reported low levels of spontaneous labor in cases of CHI and early delivery of a liveborn infant has been detailed in a case report following observations of fetal growth plateau (9, 10). Therefore, it is possible that electing for early delivery may have had a beneficial effect in select cases.

Recurrence rates of CHI in subsequent pregnancies vary widely between studies and have been reported between 25 and 100% (1, 3, 7). Within this cohort, recurrence of CHI was 41.9%, though the majority of pregnancies were treated which in 20 placentas reduced severity, some to the point where CHI was absent. In agreement with observations made by other groups, recurrence of CHI was not always associated with adverse outcome (9, 22). Bos et al. in their systematic review stated that incidence and recurrence of CHI may vary due to differing inclusion and exclusion criteria across studies (1). A controversial criterion noted was the exclusion of cases with chronic villitis or villitis of unknown etiology alongside CHI. As concurrent CHI and chronic villitis lesions have been reported in 25–47% of placentas (15, 23), those with combined lesions were not excluded here as they represented a similar and considerable proportion of cases. Of particular interest was the observation that the incidence of chronic villitis did not differ across pregnancies and therefore seemed unaffected by intervention within the participant group studied. In addition, though chronic villitis is itself associated with FGR (24), outcomes of pregnancies with concurrent CHI and chronic villitis were not significantly different, suggesting that chronic villitis in the presence of CHI may not have been a strong factor in adverse outcome.

CONCLUSION

The data from this retrospective study suggest that including at least one of hydroxychloroquine or prednisolone in treatment regimen of pregnancies following diagnosis of CHI is effective at reducing placental lesion severity. In turn, decreased severity of CHI improves the likelihood of livebirth in subsequent pregnancies. From this, we propose that a standardized treatment protocol including aspirin, heparin, prednisolone and hydroxychloroquine may prove beneficial in the management of CHI. Studies into the efficacy of both hydroxychloroquine and prednisolone as well as other immunomodulatory agents used within the condition is extremely limited, and feasibility of randomized controlled trials in affected women is low given their poor obstetric history. To overcome this challenge, collaboration between centers specializing in the management of CHI is required to increase sample sizes to allow sufficient evaluation of treatment regimen.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by NRES Committee North West—Greater Manchester West and NRES Committee London—City &

East. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CB, IC, and AH contributed toward the conception and design of the study. CB and CW organized the study database. CB, AH, and EC collected the data. CB performed the data analysis and wrote the manuscript. AH and IC assisted with data analysis and data presentation. GB, EC, CT, and AH assisted with manuscript proofreading and corrections. All authors contributed toward revision and approved the manuscript for submission.

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REFERENCES

1. Bos M, Nikkels PGJ, Cohen D, Schoones JW, Bloemenkamp KWM, Bruijn JA, et al. Towards standardized criteria for diagnosing chronic intervillositis of unknown etiology: a systematic review. *Placenta.* (2018) 61:80–8. doi: 10.1016/j.placenta.2017.11.012
2. Ozawa N, Yamaguchi K, Shibata M, Sugibayashi R, Yagi H, Sago H, et al. Chronic histiocytic intervillositis in three consecutive pregnancies in a single patient: differing clinical results and pathology according to treatment used. *J Obs Gynaecol Res.* (2017) 43:1504–8. doi: 10.1111/jog.13404
3. Contro E, deSouza R, Bhide A. Chronic intervillositis of the placenta: a systematic review. *Placenta.* (2010) 31:1106–10. doi: 10.1016/j.placenta.2010.10.005
4. Traeder J, Jonigk D, Feist H, Brocker V, Langer F, Kreipe H, et al. Pathological characteristics of a series of rare chronic histiocytic intervillositis of the placenta. *Placenta.* (2010) 31:1116–9. doi: 10.1016/j.placenta.2010.09.012
5. Marchaudon V, Devisme L, Petit S, Ansart-Franquet H, Vaast P, Subtil D. Chronic histiocytic intervillositis of unknown etiology: clinical features in a consecutive series of 69 cases. *Placenta.* (2011) 32:140–5. doi: 10.1016/j.placenta.2010.11.021
6. Boyd TK, Redline RW. Chronic histiocytic intervillositis: a placental lesion associated with recurrent reproductive loss. *Hum Pathol.* (2000) 31:1389–96. doi: 10.1016/S0046-8177(00)80009-X
7. Parant O, Capdet J, Kessler S, Aziza J, Berrebi A. Chronic intervillositis of unknown etiology (CIUE): relation between placental lesions and perinatal outcome. *Eur J Obs Gynecol Reprod Biol.* (2009) 143:9–13. doi: 10.1016/j.ejogrb.2008.06.012
8. Labarrere C, Mullen E. Fibrinoid and trophoblastic necrosis with massive chronic intervillositis: an extreme variant of villitis of unknown etiology. *Am J Reprod Immunol.* (1987) 15:85–91. doi: 10.1111/j.1600-0897.1987.tb00162.x
9. Revaux A, Mekinian A, Nicaise P, Bucourt M, Cornelis F, Lachassinne E, et al. Antiphospholipid syndrome and other autoimmune diseases associated with chronic intervillositis. *Arch Gynecol Obs.* (2015) 291:1229–36. doi: 10.1007/s00404-014-3536-6
10. Vardi L, Paterson H, Hung NA. Successful pregnancy following treatment of recurrent chronic histiocytic intervillositis. *BMJ Case Rep.* (2017) 2017:bcr2016217886. doi: 10.1136/bcr-2016-217886
11. Gordijn SJ, Beune IM, Thilaganathan B, Papageorghiou A, Baschat AA, Baker PN, et al. Consensus definition of fetal growth restriction: a Delphi procedure. *Ultrasound Obstet Gynecol.* (2016) 48:333–9. doi: 10.1002/uog.15884
12. Gardosi J, Williams A, Hugh O FA. *Customised Centile Calculator, GROW Version 2.1.6.1.* Gestation Network (2020). Available online at: www.gestation.net (accessed September 29, 2021).
13. Abdulghani S, Moretti F, Gruslin A, Grynspan D. Recurrent massive perivillous fibrin deposition and chronic intervillositis treated with heparin and intravenous immunoglobulin: a case report. *J Obstet Gynaecol Can.* (2017) 39:676–81. doi: 10.1016/j.jogc.2017.03.089
14. Mattuizzi A, Sauvestre F, André G, Poingt M, Camberlein C, Carles D, et al. Adverse perinatal outcomes of chronic intervillositis of unknown etiology: an observational retrospective study of 122 cases. *Sci Rep.* (2020) 10:12611. doi: 10.1038/s41598-020-69191-9
15. Nowak C, Joubert M, Jossic F, Masseau A, Hamidou M, Philippe HJ, et al. Perinatal prognosis of pregnancies complicated by placental chronic villitis or intervillositis of unknown etiology and combined lesions: about a series of 178 cases. *Placenta.* (2016) 44:104–8. doi: 10.1016/j.placenta.2016.04.017
16. Boog G, Le Vaillant C, Alnoukari F, Jossic F, Barrier J, Muller J-Y. [Combining corticosteroid and aspirin for the prevention of recurrent villitis or intervillositis of unknown etiology]. *J Gynecol Obstet Biol Reprod.* (2006) 35:396–404. doi: 10.1016/s0368-2315(06)76411-0
17. Reus AD, van Besouw NM, Molenaar NM, Steegers EA, Visser W, de Kuiper RP, et al. An immunological basis for chronic histiocytic intervillositis in recurrent fetal loss. *Am J Reprod Immunol.* (2013) 70:230–7. doi: 10.1111/aji.12125
18. Bendon RW, Coventry S, Thompson M, Rudzinski ER, Williams EM, Oron AP. Significance of C4d immunostaining in placental chronic intervillositis. *Pediatr Dev Pathol.* (2015) 18:362–8. doi: 10.2350/14-12-1582-OA.1
19. Scott RE, Greenwood SL, Hayes DJL, Baker BC, Jones RL, Heazell AEP. Effects of hydroxychloroquine on the human placenta—findings from *in vitro* experimental data and a systematic review. *Reprod Toxicol.* (2019) 87:50–9. doi: 10.1016/j.reprotox.2019.05.056
20. Williams DJ. Chronic histiocytic intervillositis and treatment to prevent recurrent pregnancy loss. In: *Royal College of Obstetricians & Gynaecologists' (RCOG) Virtual World Congress* (2021).
21. Mekinian A, Houfflin-Debarge V, Kolanska K, Cohen J, Abisror N, Bornes M, et al. Antagonists of TNFα for recurrent miscarriages: 2 Illustrative cases. *Eur J Obstet Gynecol Reprod Biol.* (2019) 236:263–4. doi: 10.1016/j.ejogrb.2019.02.036
22. Bos M, Harris-Mostert ETMS, van der Meeren LE, Baelde JJ, Williams DJ, Nikkels PGJ, et al. Clinical outcomes in chronic intervillositis of unknown etiology. *Placenta.* (2020) 91:19–23. doi: 10.1016/j.placenta.2020.01.001
23. Labarrere C, Althabe O. Chronic villitis of unknown aetiology in recurrent intrauterine fetal growth retardation. *Placenta.* (1987) 8:167–73. doi: 10.1016/0143-4004(87)90019-1
24. Derricott H, Jones RL, Heazell AEP. Investigating the association of villitis of unknown etiology with stillbirth and fetal growth restriction - a systematic review. *Placenta.* (2013) 34:856–62. doi: 10.1016/j.placenta.2013.07.003

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REVIEW
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Crosstalk Between Trophoblast and Macrophage at the Maternal-Fetal Interface: Current Status and Future Perspectives

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The immune tolerance microenvironment is crucial for the establishment and maintenance of pregnancy at the maternal-fetal interface. The maternal-fetal interface is a complex system containing various cells, including lymphocytes, decidual stromal cells, and trophoblasts. Macrophages are the second-largest leukocytes at the maternal-fetal interface, which has been demonstrated to play essential roles in remodeling spiral arteries, maintaining maternal-fetal immune tolerance, and regulating trophoblast's biological behaviors. Many researchers, including us, have conducted a series of studies on the crosstalk between macrophages and trophoblasts at the maternal-fetal interface: on the one hand, macrophages can affect the invasion and migration of trophoblasts; on the other hand, trophoblasts can regulate macrophage polarization and influence the state of the maternal-fetal immune microenvironment. In this review, we systemically introduce the functions of macrophages and trophoblasts and the cell-cell interaction between them for the establishment and maintenance of pregnancy. Advances in this area will further accelerate the basic research and clinical translation of reproductive medicine.

Keywords: maternal-fetal interface, trophoblast, macrophage, pregnancy, immune tolerance

INTRODUCTION

The establishment and maintenance of normal pregnancy is a complex process involving multiple cells and various molecules. Among them, the precise regulation at the maternal-fetal interface plays a pivotal role. The maternal-fetal interface comprises decidual immune cells, stromal cells, and trophoblasts, characterized by maintaining the defense against possible pathogens and immune tolerance to the allogeneic fetus (1). Increasing evidence demonstrates that interaction among these cellular components can influence the maternal-fetal interactive dialogue, thereby participating in

the regulation of the pregnancy program (1). Therefore, exploring the cell-cell crosstalk at the maternal-fetal interface will help us further understand pregnancy's physiological and pathological processes.

During early pregnancy, 30-40% of the decidual cells are leukocytes, including macrophages, natural killer (NK) cells, B cells, T cells, and dendritic cells (2). Macrophages, as antigen-presenting cells, comprise 20-30% of the leukocytes at the maternal-fetal interface (3), which participate in the complex regulation at the maternal-fetal interface by regulating the secrete cytokines, phagocytosis, and immune balance (4). Besides their effects on vascular remodeling, macrophages are actively associated with trophoblasts invasion and pregnancy maintenance (5). Macrophages could be divided into classically activated (M1) and alternatively activated (M2) subtypes based on their cytokine production and function (5). The polarization states of decidual macrophages undergo dynamic changes to changing microenvironment at different gestational ages, adjusting to the different stages of fetal development. It has been demonstrated that aberrantly activated macrophages at the maternal-fetal interface may be closely related to various pregnancy complications, including miscarriage, preeclampsia, preterm birth, fetal growth restriction, or demise (6–9). Accumulating evidence indicates that the polarization of macrophages is regulated by a variety of cytokines, chemokines, sex hormones, and cell-cell interactions at the maternal-fetal interface, among which trophoblasts exert pivotal roles (4, 10, 11). Trophoblasts are also important elements at the maternal-fetal interface, which invade the maternal myometrium and direct contact with maternal decidual stromal cells (12). Sufficient trophoblasts invasion could facilitate maternal spiral artery remodeling and placental blood flow, providing a favorable embryo implantation environment (12). Previous studies showed that the maternal microenvironment temporally and spatially controls trophoblast invasion at the maternal-fetal interface, including arteries, glands, decidual NK cells, macrophages and stromal cells (13). Trophoblasts can respond to various mediators secreted by polarized macrophages to regulate their biological behaviors (6, 14–16), while soluble cytokines produced by trophoblasts could induce macrophages polarization (17–19), involving in the regulation of normal pregnancy. Previously, our group conducted a series of studies on the crosstalk between macrophages and trophoblasts at the maternal-fetal interface: on the one hand, trophoblasts can regulate the polarization of the M2 macrophages by secreting IL-6 (20); on the other hand, M2 macrophages can promote the invasion and migration of trophoblasts by secreting G-CSF, thereby regulating the establishment and maintenance of normal pregnancy (21). In addition, macrophages could also secrete exosomes and deliver miRNAs to target and regulate the invasion and migration capabilities of trophoblasts, thereby participating in the occurrence of recurrent spontaneous abortion (RSA) (22). Although, the understanding of the interaction between trophoblasts and macrophages at the maternal-fetal interface is still insufficient and need to be further explored.

In the present review, we aimed to summarize the crosstalk between macrophages and trophoblasts at the maternal-fetal interface and the functions in normal pregnancy and pregnancy complications, providing the latest progress in the field and bringing more profound ideas to researches.

THE ROLE OF MACROPHAGES AT THE MATERNAL-INTERFACE

Generally, macrophages are classified as M1 and M2 phenotypes based on the different function and cytokine production, but in fact, there is a full spectrum of macrophage between M1 and M2 (2). Therefore, macrophages are highly plastic and heterogeneous, here we just focus on M1 and M2 as typical subtypes to explore their functions. M1 could be induced by toll-like receptor ligands, bacterial lipopolysaccharide (LPS) or other cytokines (including TNF-α, IFN-γ, and GM-CSF), which produces reactive oxygen species and high level of IL-1β, IL-6, IL-12 and IL-23 (3, 4). M2 can be polarized by cytokines like IL-4, IL-13, IL-10, immune complexes, LPS and M-CSF, producing high levels of TGF-β and IL-10. M2 plays important roles in clearing apoptotic cells, tissue repair and remodeling (4–6). M2 can be further divided into four subtypes, including M2a, M2b, M2c and M2d. M2a-Mφ could be induced by IL-4 and IL-13, which expresses high level of CD206, TGF-β, insulin-like growth factor (IGF) and IL-1 receptor (IL-R) (7). M2b-Mφ can be induced by immune complexes plus LPS or IL-1β, which expresses and secretes proinflammatory cytokine including TNF-α, IL-6 and IL-1β, and anti-inflammatory cytokine IL-10 (8). By contrast, M2c-Mφ is induced by IL-10 and releases high levels of IL-10 and TGF-β, exerting the function of phagocytosis of apoptotic cells (4). In addition, M2d-Mφ, also known as tumor-associated macrophages, can be induced by IL-6, or A2 adenosine receptor plus toll-like receptor ligands, mainly characterized by high expression level of TGF-β, IL-10 and vascular endothelial growth factor (VEGF), playing essential roles in cancer metastasis and angiogenesis (9).

In the context of reproduction, macrophages are present in the endometrium, decidua and placenta, which comprise 20-30% of the leukocytes. Affected by estrogen and progesterone, the number of macrophages fluctuates during the menstrual cycle (10, 11). During the embryo implantation window, macrophages exhibit M1 phenotype. As trophoblasts implant and invade the endometrium, macrophages transform into a mixed M1/M2 type, which lasts to the first trimester and the early stage of the second trimester (12). At the second trimester, macrophages are polarized to the M2 phenotype to prevent rejection of the fetus by the maternal system and maintain fetal growth until delivery (13). At the time of delivery, the macrophages appear to be M1 subtype (14). Although increasing evidence has demonstrated the roles and distribution of decidual macrophages (DMs), the classification and subtypes of DMs are still controversial. Based on the expression level of CD11c and CD209, DMs are divided into $CD11c^{low}$ and $CD11c^{high}$ subgroups (15), or $CD209^{-}$ and $CD209^{high}$ macrophages (16). Jiang et al. divided DMs into three

subtypes depending on the expression of CCR2 and CD11c, including $CCR2^{-}CD11c^{low}$, $CCR2^{-}CD11c^{high}$ and $CCR2^{+}CD11c^{high}$ (17). Evidence has demonstrated that M2-Mφ dominates at the maternal-fetal interface, maintaining the immune-suppressive environment toward the fetus (18).

Increasing studies demonstrate that macrophages play essential roles in the establishment and maintenance of normal pregnancy, including spiral artery remodel, apoptotic cell phagocytosis and trophoblasts functions (19–21). The polarization balance between M1-Mφ and M2-Mφ is important for various processes of normal pregnancy. Conversely, the dysregulated macrophages polarization is associated with a variety of pregnancy complications, including RSA, preeclampsia (PE), fetal growth restriction and preterm labor (14, 22–25). The increased number of M1-Mφ and the decreased of M2-Mφ at the maternal-interface is related with RSA (23) and PE (19), accompanied by a decrease in anti-inflammatory cytokines and an increase in pro-inflammatory cytokines. In addition, DMs differentiated into M1-Mφ is related to spontaneous preterm labor (14). Animal experiments demonstrate that induction of M1 macrophages polarization increases embryo resorption (26), while suppressing M1 macrophages polarization alleviates the opposite effect (27). Moreover, macrophages have high plasticity, and their polarization and function are affected by the surrounding environment (4). It has been reported that hormones, chemokines, cytokines, growth factors, and the crosstalk between macrophages and related cells are involved in the regulation (28). In addition, there is growing evidence confirming the microbiome colonization at the maternal-fetal interface (29, 30). Compounds originating from the microbiome might bind to aryl hydrocarbon receptor (AhR), which is widely expressed in adaptive and innate immune cells including macrophages (31). Indeed, AhR plays an important role in regulating macrophage responsiveness and the expression of AhR in macrophage is essential for homeostasis and inflammatory responses (31, 32).

THE ROLE OF TROPHOBLASTS AT THE MATERNAL-INTERFACE

In the human placenta, there are three trophoblast subpopulations: the cytotrophoblasts (CTBs), syncytiotrophoblasts (STBs) and extravillous cytotrophoblasts (EVTs) (33). After embryo implantation, the outermost layer of the blastocysts transforms into mononuclear CTBs. Proliferative CTBs make up the primary villi, then the primary villi further differentiates into secondary mesenchymal villi and mature tertiary villi. Cell fusion of villous CTBs generates STBs, which plays important roles in transport of oxygen and nutrients, production of pregnancy hormones and clearance of fetal waste products (34). STBs of the floating villi represent the transport units of the human placenta, while anchoring villi transforms into another trophoblast type, namely EVTs (35). EVTs are migratory and invasive trophoblasts, which can migrate into decidual lymphatics, veins and glands, participating in placental embedment and fetal development (36–38). Defects in spiral artery remodeling, and failure in the interactions of EVTs with uterine vessels might lead to pregnancy complications, as lower numbers of EVTs in lymphatic and venous vessels is found in RSA (37). Invasive EVTs remodel the maternal spiral arteries, ensuring an adequate maternal blood supply for normal fetal growth and development. The process reduces maternal blood pressure and flow velocity, meeting the increased uteroplacental perfusion required by the developing fetus (39). The arteries remain narrow at the openings, and blood enters the intervillous space at a higher velocity, which leads to damaged villi structure and impaired placental function (40). Trophoblast invasion is temporally and spatially controlled by the maternal uterus environment, including arteries, glands, decidual NK cells, macrophages and stromal cells. Insufficient EVTs invasion has been reported to be related with pregnancy complications including RSA, PE, stillbirth and fetal intrauterine growth restriction (41–44), while excessive invasion also might lead to pathological condition such as placenta accrete (45). In addition, trophoblasts serve as a member of innate immune system at the maternal-fetal interface, as trophoblasts could recognize and respond to viral and bacterial products (46), and trophoblasts might integrate microbial-derived signals *via* alternative pathways mediating the response to TLRs or epigenetic modifications (47, 48). Therefore, exploring the factors affecting trophoblasts functions is of great significance for further understanding of the normal pregnancy process and the pathogenesis of pregnancy complications.

CROSSTALK BETWEEN TROPHOBLASTS AND MACROPHAGES AT THE MATERNAL-FETAL INTERFACE

In this section, we summarized the interaction between trophoblasts and macrophages, and the role in the establishment and maintenance of normal pregnancy and pathological pregnancy (**Figure 1**).

The Effect of Macrophages on Trophoblasts Functions

Precise regulation of the proliferation, apoptosis, migration and invasion of trophoblasts are essential for the establishment and maintenance of pregnancy. In local microenvironment, abundant DMs are observed at the implantation site and the invasive front of EVTs (49). Thence, paracrine activity of macrophages exerts an important role in biological function of trophoblasts (15). Results from rhesus monkey indicated that the growth and differentiation of embryonic trophoblasts were regulated by macrophages (50). Here, we will elaborate on the effect of macrophages on proliferation, apoptosis, migration and invasion of trophoblasts in recent years.

Proliferation and Apoptosis

Trophoblast apoptosis may induce inflammatory events that might initiate trophoblasts dysfunction, leading to pregnancy

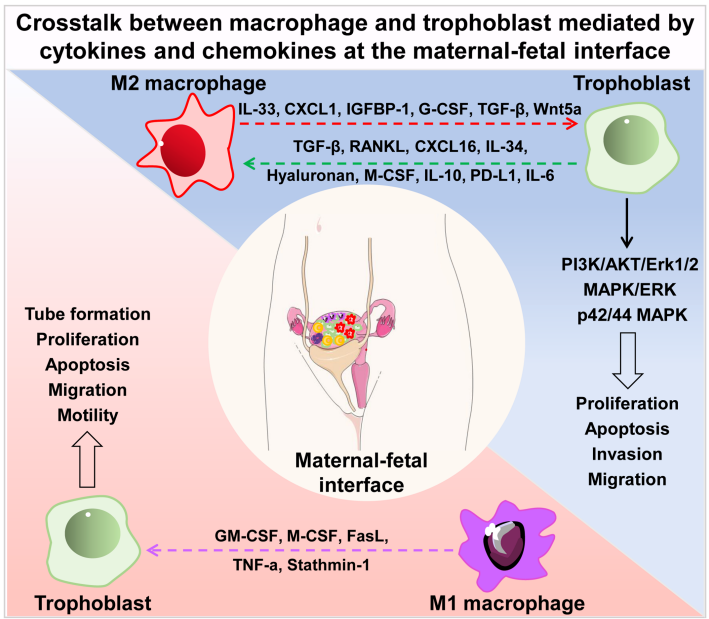

FIGURE 1 | Crosstalk between macrophage and trophoblast mediated by cytokines and chemokines at the maternal-fetal interface. Cytokines and chemokines secreted by M2 macrophages (IL-33, CXCL1, IGFBP1, G-CSF, TGF-β, Wnt5a) regulate the proliferation, apoptosis, invasion and migration of trophoblast *via* PI3K/AKT/Erk1/2, MAPK/ERK and p42/44 MAPK signal pathways. Trophoblast- derived secretory factors (TGF-β, RANKL, CXCL16, IL-34, hyaluronan, M-CSF, IL-10, PD-L1, IL-6) drive M2 macrophage polarization in turn. Soluble molecules secreted by M1 macrophages (GM-CSF, M-CSF, FasL, TNF-a, Stathmin-1) influence the tube formation, proliferation, apoptosis, migration and motility of trophoblast. IL, interleukin; CXCL, chemokine (C-X-C motif) ligand; IGFBP1, insulin-like growth factor-binding protein-1; G-CSF, granulocyte colony stimulating factor; TGF-β, transforming growth factor β; RANKL, receptor activator of NF-κB ligand; M-CSF, macrophage colony stimulating factor; PD-L1, programmed cell death 1 ligand 1; GM-CSF, granulocyte-macrophage colony stimulating factor; FasL, factor associated suicide ligand; TNF-α, tumor necrosis factor α.

complications (23, 51). Macrophages with different polarization statues have different effects on the motility and tube formation of trophoblasts: M1 macrophage exerted an inhibitory role, while M2 macrophages had the opposite effect (52). The Fas/FasL system is one of the major apoptotic pathways in tissues and cells. Results from our and others' group demonstrated that macrophages could induce trophoblasts apoptosis *via* FasL (23, 53). In addition, IL-33, as a member of the IL-1 family, has been demonstrated to promote the proliferation of cell column trophoblasts, villous cytotrophoblasts and primary trophoblasts, which was mediated by PI3K/AKT and MAPK/ERK signaling (54). Moreover, previous report also showed that macrophages could promote trophoblasts proliferation by secreting Wnt5a *via* p42/44 MAPK pathway (55). Meanwhile, decidual cells stimulated with pro-inflammatory cytokines including TNF-α and IL-1β significantly strengthened the promotion effect of macrophage-induced caspase-dependent trophoblasts apoptosis *via* GM-CSF and M-CSF (56).

Invasion and Migration

The regulatory effects of macrophages on the invasion and migration of trophoblasts are depend on their polarization states. Renaud et al. demonstrated that nonactivated macrophages had no effect on trophoblasts invasion, while macrophages activated by LPS inhibited the ability of trophoblasts to invade through extracellular matrix *in vitro* (57), and the effect could be reversed by IL-10 (58). In addition, M1 macrophage-derived TNF-a reduced the expression of stathmin-1 in trophoblasts, regulating the proliferation and invasion ability, *via* E-cadherin/β-catenin pathway (44). TGF-β is a soluble mediator produced by M2 macrophages, and our recent research shows that TGF-β secreted by M2 macrophage could induce trophoblast migration and invasion (59). Human leukocyte antigen G5 (HLA-G5) has been reported to play essential roles in immune tolerance during normal pregnancy. HLA-G5 is not expressed in the DMs, while secreted HLA-G5 could be detected in the exosomes of primary mononuclear cytotrophoblast cells and placental explants of first trimester (60). It has been confirmed that trophoblasts-derived HLA-G5 could difference monocytes into M2-Mφ, which in turn promotes the invasion ability of trophoblasts *via* secreting CXCL1 (61). Glycodelin-A is a glycoprotein abundantly present in the decidua, playing an important role in immune cell regulation at the maternal-fetal interface (62). Glycodelin-A could induce the differentiation of monocytes towards DMs-like phenotype by binding to Siglec-7, which promotes trophoblast invasion *via* the production of insulin-like growth factor-binding protein 1 (IGFBP-1) (63), a regulator of trophoblast functions and angiogenesis during pregnancy (64). Medroxyprogesterone is widely used for the

treatment of endometrium cancer, abnormal uterine bleeding and secondary amenorrhea (65). Recently, it has been proved that medroxyprogesterone could drive monocyte differentiation toward M2 macrophages *via* ERK phosphorylation, which promoted the invasion activity of trophoblasts and the decidualization of endometrial stromal cells (66). Granulocyte colony-stimulating factor (G-CSF) is secreted by multiple cells including macrophages (67, 68) and placental villous trophoblasts (69). Increasing evidence demonstrates the therapeutic effect of G-CSF in RSA, repeated implantation failure, luteinized unruptured follicle syndrome and women with thin endometrium (70–72). Previously, by using a co-cultured model, our study showed that trophoblasts-derived IL-6 promoted M2 macrophage polarization (73), which in turn induced the epithelial-mesenchymal transition of trophoblasts, thereby promoting migration and invasion though secreting G-CSF to activate PI3K/AKT/Erk1/2 signaling pathway (68).

The Effect of Trophoblasts on Macrophages Polarization

Macrophage polarization is a complex process and plastic to the environment surrounding or in contact with them, which is involved in multiple factors including cytokines, chemokines, growth factors, etc. Among which, cell-cell interaction exerts a vital role. DMs and EVTs are accumulated at the implantation site, and factors secreted by trophoblasts play important roles in regulating the differentiation and function of macrophages. However, the factors involved in this process has not been elucidated. In this part, we mainly focus on the effect of trophoblasts on the differentiation and function of macrophages.

Trophoblast debris have been reported to modulate the expression of cytokines in macrophages, upregulating the expression of programmed death-1 ligand 1 (PD-L1), indoleamine 2,3-dioxygenase (IDO), and anti-inflammatory cytokines including IL-1Ra, IL-6, and IL-10, while reducing the expression of costimulatory molecules (B7H3, CD40, CD80 and CD86), MHC-II molecules, IL-8 receptors, inter-cellular adhesion molecule 1 (ICAM-1), monocyte chemoattractant protein-1 (MCP-1), and pro-inflammatory cytokines including IL-8, IL-1β, and IL-12p70 (74). Aldo et al. reported that trophoblast-derived TGF-β could induce monocyte differentiation into $CD14^+/CD16^+$ macrophages, accompanied by high secretion levels of IL-1β, IL-10, and interferon-inducible protein-10 (IP-10), and increased phagocytosis capacity (75). By secreting IL-10 and M-CSF, trophoblasts could induce M2 macrophages polarization, sharing the phenotype of $CD163^+CD206^+CD209^+$ and producing high level of CCL18 and IL-10 (76). IL-34 is a second ligand for the M-CSF receptor, which is involved in the maintenance and development of other macrophage subsets, such as osteoclasts (77) and Langerhans cells (78). IL-34 is produced by both decidual stromal cells and trophoblasts, which could polarize monocytes into M2 macrophages with the similar cytokine secretion pattern with DMs (79). Recently, our group has demonstrated that trophoblasts-derived IL-6 could induce M2 macrophages polarization by activating STAT3 signal pathway, with high expression of CD206, CCL18, IL-10 and TGF-β (73). In addition, CXCL16 derived from trophoblasts differenced monocytes into M2 macrophages by binding to CXCR6, which exhibited decreased IL-15 production, facilitating the inactivation of NK cells and restricting the cytotoxicity of NK cells (80).

Moreover, it has been demonstrated that PD-1/PD-L1 pathway exerts an important role in regulation of immune cell homeostasis, especially macrophage polarization (26) and T cells activation (81). PD-L1 was reported to be expressed in the trophoblasts of normal placenta, and decreased expression of PD-L1 was confirmed in pregnancy complications such as RSA (26). PD-L1 expression/secretion of trophoblasts could be promoted by IFN-β, inducing PD-1/PD-L1-mediated M2 macrophage polarization (82). Similar to PD-L1, trophoblast-derived HLA-G5 could drive M2 macrophages polarization manifested by increased IDO 1 and IL-6 expression (61). In addition to cytokines and chemokines, the regulatory roles of other factors have been reported. Hyaluronan (HA) is an unbranched polymer and widely present in the extracellular matrix of mammalian tissues. Decreased expression of HA was detected in villi of miscarriage patients (83). Wang et al. demonstrated that HA derived from trophoblasts could induce M2 macrophages polarization by interacting with CD44 *via* PI3K/AKT-STAT3/STAT-6 pathway (84). It has been indicated that HA are divided into four subtypes depending on the molecular weight, high molecular weight HA, medium molecular weight HA, low molecular weight HA and HA oligomers (85). HA with different molecular weight might have different effects on macrophage polarization, low molecular weight HA drives M1 macrophage polarization featured with increased expression of CD80, iNOS2, TNF-α, NO and IL-12β, while high molecular weight HA induces M2 macrophage polarization charactered by increased expression of Arg1, MRC1, IL-10 and enhanced arginase activity (86, 87). As mentioned above, AhR is expressed in macrophages and plays an important role in regulating macrophage responsiveness (31, 32). Indeed, the expression of AhR in trophoblasts has been confirmed in 2018 (88). Therefore, AhR might play a role in the pregnancy program *via* regulating the crosstalk between trophoblasts and macrophages. What's more, lower expression of placental AhR was found in unexplained RSA women, and the activation of AhR might impair the proliferation and migration of trophoblasts (89).

Receptor activator of NF-κB ligand (RANKL) and its tumor necrosis factor (TNF)-family receptor RANK exert important roles in lymph node formation, bone remodeling, thymic microenvironment establishment, and mammary gland development during pregnancy. RANKL derived from trophoblasts could drive macrophage polarization to M2 subtype *via* activating AKT/STAT6-Jmjd3/IRF4 signaling pathway, and decreased RANKL in trophoblasts and RANK on DMs was observed in patients suffered from miscarriage (90). By using knockout mice, the authors further demonstrated that $RANKL^{-/-}$ mice displayed macrophage dysfunction and

increased fetal loss, while adoptive transfer of RANK$^+$ macrophages could relieve the fetal loss induced by macrophage depletion (90).

Altogether, results from the above studies confirm the role of trophoblasts on M2 macrophage polarization, which were based on trophoblasts from healthy pregnancy women or trophoblast cell lines. However, it is not known whether these roles were maintained in a pathological condition, as trophoblast dysfunction was related to pregnancy complications such as unexplained RSA. A previous study from an *ex vivo* model demonstrated that trophoblasts from unexplained RSA could promote an M2-like phenotype, indicating that trophoblasts from unexplained RSA had similar functional and proteomic properties with trophoblasts from healthy women (91). Cluster of differentiation 74 (CD74) was a high-affinity binding protein for the inflammatory cytokine macrophage migration inhibitory factor, which was released by trophoblasts to regulate monocyte activity (92). Przybyl et al. demonstrated that the adhesion of macrophages lacking CD74 to trophoblasts was decreased, exhibiting the pro-inflammatory phenotype when co-cultured with trophoblasts (24).

THE ROLE OF EXTRACELLULAR VESICLES IN CROSSTALK BETWEEN MACROPHAGES AND TROPHOBLASTS AT THE MATERNAL-FETAL INTERFACE

In addition to soluble factors, extracellular vesicles (EVs) are also demonstrated to be another important medium in communication network at the maternal-fetal interface in recent years (93). EVs, including microvesicles, exosomes and apoptotic bodies, are intricately involved in cell-cell communication, which are released by various types of cells, including macrophages and trophoblasts. EVs contain abundant molecules like lipids, proteins, DNA, mRNAs, long non-coding RNAs (LncRNA) and miRNAs, which are delivered to target cells (94). EVs could be detected in multiple biofluids including milk, urine, blood, saliva and amniotic fluid (95). EVs concentration in circulation is increased in pregnant women, with 5-folds higher than non-pregnant women (96), which could be detected in maternal plasma at 6 weeks of gestational age (97). Trophoblasts-released EVs are taken up by cells of maternal vascular and immune systems, regulating the maternal physiological system to adapt to the pregnancy process (93). Placental EVs has been proven to exert an important role in maintaining maternal-fetal immune tolerance *via* repression of modulatory signals in the maternal immune system and suppression of natural killer cells and T-lymphocyte activation (98). The release of EVs by EVTs has been confirmed as HLA-G$^+$ EVs were detected in the maternal circulation during pregnancy (99). In this section, we mainly summarized the latest progress about the role of EVs in crosstalk between macrophages and trophoblasts at the maternal-fetal interface (**Figure 2**).

In fact, little is known about EVs-mediated crosstalk between macrophages and trophoblasts up to now. Atay et al. firstly reported that trophoblast-derived exosomes could recruit and educate monocytes to produce G-CSF, granulocyte/monocyte colony-stimulating factor (GM-CSF), IL-1β, IL-6, TNF-α and Serpin-E1, in a cell-contact-independent manner, which were necessary for embryo implantation, stromal remodeling and angiogenesis (100). These authors further addressed the mechanism that IL-1β induction by exosome-associated fibronectin (101). EVs released by macrophages in turn modulate trophoblast's function. Holder et al. reported that macrophages-derived exosomes were internalized by human placenta in a time- and dose-dependent manner, *via* clathrin-dependent endocytosis, inducing the release of proinflammatory

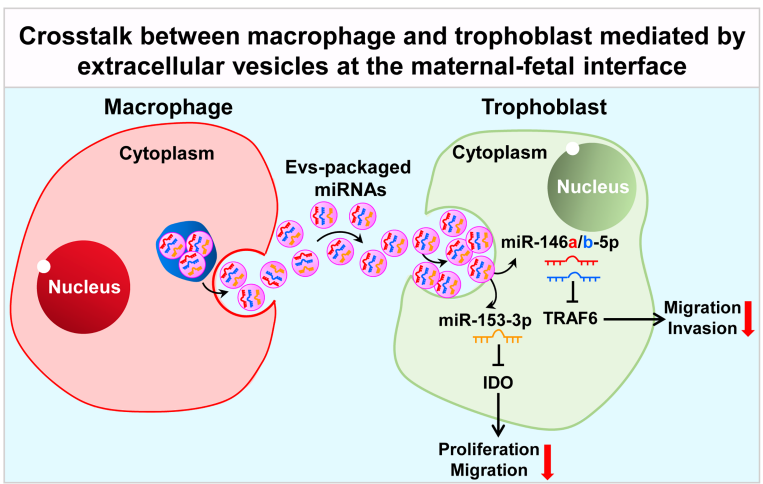

FIGURE 2 | Crosstalk between macrophage and trophoblast mediated by extracellular vesicles at the maternal-fetal interface. Macrophage-derived extracellular vesicles affect the expression of TRAF6 and IDO in trophoblast by delivering miR-146a-5p, miR-146b-5p and miR-153-3p, thereby affecting their invasion and migration capabilities, participating in the pathological process of RSA. TRAF6, TNF receptor associated factor 6; IDO, indoleamine 2, 3-dioxygenase.

cytokines, including IL-6, IL-8 and IL-10 (102). EVs exert biological functions mainly through the biologically molecules they conduct. For example, EVs released from DMs could deliver miR-153-3p to target IDO, inhibiting trophoblasts proliferation and migration *via* STAT3 pathway and contributing to occurrence of unexplained RSA (103). Recently, our group reported that EVs derived from M1-Mφ could deliver miR-146a-5p and miR-146b-5p to target TNF receptor associated factor 6 (TRAF6), suppressing trophoblast migration and invasion and contributing to the development of RSA (104). However, more evidence is needed to confirm the role of EVs in the establishment and maintenance of pregnancy.

CONCLUSIONS AND PERSPECTIVES

Taken together, current evidence suggests that trophoblasts and macrophages can establish extensive connections at the maternal-fetal interface and thus participate in regulating the physiological and pathological processes of pregnancy. Polarized macrophages can influence the biological behavior (including proliferation, apoptosis, invasion and migration) through the secretion of various cytokines and chemokines. Reciprocally, trophoblasts can also regulate the polarization state of macrophages through a variety of mechanisms, thus affecting the establishment and maintenance of immune tolerance microenvironment at the maternal-fetal interface.

In spite of this, the interaction between trophoblasts and macrophages still needs further study: 1) As above presented, the crosstalk reported for now between trophoblasts and macrophages are mainly mediated by cytokines and chemokines. EVs, as an emerging intercellular communication medium, have been gradually proven to exert role in mediating the mutual communication between cells. However, the role and potential mechanisms of EVs in the crosstalk between macrophages and trophoblasts at the maternal-fetal interface is rarely studied, which is an emerging field that needs to be explored urgently. 2) What' more, in addition to macrophages, there are also immune cells including T cells, NK cells and dendritic cells at the maternal-fetal interface, which also play important roles in regulating the biological behavior of trophoblasts. Then, whether other cells may also participate in the interaction between macrophages and trophoblasts, thereby establishing three-cell or multi-cell communication, and then participating in the establishment and maintenance of pregnancy, is also worth exploring in future research. 3) Additionally, as most of the current researches are based on cell lines, transforming the interaction of macrophages and trophoblasts into clinical research is also a direction worth studying.

AUTHOR CONTRIBUTIONS

CY and JY designed the review. JD, YZ, and XC drafted the manuscript and prepared the figures. LD helped to modify the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Xu L, Li Y, Sang Y, Li DJ, Du M. Crosstalk Between Trophoblasts and Decidual Immune Cells: The Cornerstone of Maternal-Fetal Immunotolerance. *Front Immunol* (2021) 12:642392. doi: 10.3389/fimmu.2021.642392
2. Mosser DM, Edwards JP. Exploring the Full Spectrum of Macrophage Activation. *Nat Rev Immunol* (2008) 8(12):958–69. doi: 10.1038/nri2448
3. Nathan CF. Mechanisms of Macrophage Antimicrobial Activity. *Trans R Soc Trop Med Hyg* (1983) 77(5):620–30. doi: 10.1016/0035-9203(83)90190-6
4. Shapouri-Moghaddam A, Mohammadian S, Vazini H, Taghadosi M, Esmaeili SA, Mardani F, et al. Macrophage Plasticity, Polarization, and Function in Health and Disease. *J Cell Physiol* (2018) 233(9):6425–40. doi: 10.1002/jcp.26429
5. Gordon S. Alternative Activation of Macrophages. *Nat Rev Immunol* (2003) 3(1):23–35. doi: 10.1038/nri978
6. Satoh T, Takeuchi O, Vandenbon A, Yasuda K, Tanaka Y, Kumagai Y, et al. The Jmjd3-Irf4 Axis Regulates M2 Macrophage Polarization and Host Responses Against Helminth Infection. *Nat Immunol* (2010) 11(10):936–44. doi: 10.1038/ni.1920
7. Wynn TA, Vannella KM. Macrophages in Tissue Repair, Regeneration, and Fibrosis. *Immunity* (2016) 44(3):450–62. doi: 10.1016/j.immuni.2016.02.015
8. Yue Y, Yang X, Feng K, Wang L, Hou J, Mei B, et al. M2b Macrophages Reduce Early Reperfusion Injury After Myocardial Ischemia in Mice: A Predominant Role of Inhibiting Apoptosis. *Via A20 Int J Cardiol* (2017) 245:228–35. doi: 10.1016/j.ijcard.2017.07.085
9. Atri C, Guerfali FZ, Laouini D. Role of Human Macrophage Polarization in Inflammation During Infectious Diseases. *Int J Mol Sci* (2018) 19(6):1801. doi: 10.3390/ijms19061801
10. Toniolo A, Fadini GP, Tedesco S, Cappellari R, Vegeto E, Maggi A, et al. Alternative Activation of Human Macrophages Is Rescued by Estrogen Treatment *In Vitro* and Impaired by Menopausal Status. *J Clin Endocrinol Metab* (2015) 100(1):E50–8. doi: 10.1210/jc.2014-2751
11. Tedesco S, Adorni MP, Ronda N, Cappellari R, Mioni R, Barbot M, et al. Activation Profiles of Monocyte-Macrophages and HDL Function in Healthy Women in Relation to Menstrual Cycle and in Polycystic Ovary Syndrome Patients. *Endocrine* (2019) 66(2):360–9. doi: 10.1007/s12020-019-01911-2
12. Das A, Sinha M, Datta S, Abas M, Chaffee S, Sen CK, et al. Monocyte and Macrophage Plasticity in Tissue Repair and Regeneration. *Am J Pathol* (2015) 185(10):2596–606. doi: 10.1016/j.ajpath.2015.06.001

13. Faas MM, de Vos P. Uterine NK Cells and Macrophages in Pregnancy. *Placenta* (2017) 56:44–52. doi: 10.1016/j.placenta.2017.03.001
14. Xu Y, Romero R, Miller D, Kadam L, Mial TN, Plazyo O, et al. An M1-Like Macrophage Polarization in Decidual Tissue During Spontaneous Preterm Labor That Is Attenuated by Rosiglitazone Treatment. *J Immunol* (2016) 196 (6):2476–91. doi: 10.4049/jimmunol.1502055
15. Houser BL, Tilburgs T, Hill J, Nicotra ML, Strominger JL. Two Unique Human Decidual Macrophage Populations. *J Immunol* (2011) 186(4):2633–42. doi: 10.4049/jimmunol.1003153
16. Svensson J, Jenmalm MC, Matussek A, Geffers R, Berg G, Ernerudh J. Macrophages at the Fetal-Maternal Interface Express Markers of Alternative Activation and Are Induced by M-CSF and IL-10. *J Immunol* (2011) 187 (7):3671–82. doi: 10.4049/jimmunol.1100130
17. Jiang X, Du MR, Li M, Wang H. Three Macrophage Subsets Are Identified in the Uterus During Early Human Pregnancy. *Cell Mol Immunol* (2018) 15 (12):1027–37. doi: 10.1038/s41423-018-0008-0
18. Jena MK, Nayak N, Chen K, Nayak NR. Role of Macrophages in Pregnancy and Related Complications. *Arch Immunol Ther Exp (Warsz)* (2019) 67 (5):295–309. doi: 10.1007/s00005-019-00552-7
19. Zhang Q, Shan F, Li Z, Gao J, Li Y, Shen L, et al. A Prospective Study on the Changes and Clinical Significance of Pre-Operative and Post-Operative Circulating Tumor Cells in Resectable Gastric Cancer. *J Transl Med* (2018) 16(1):171. doi: 10.1186/s12967-018-1544-1
20. Ning F, Liu H, Lash GE. The Role of Decidual Macrophages During Normal and Pathological Pregnancy. *Am J Reprod Immunol* (2016) 75(3):298–309. doi: 10.1111/aji.12477
21. Okabe Y, Medzhitov R. Tissue Biology Perspective on Macrophages. *Nat Immunol* (2016) 17(1):9–17. doi: 10.1038/ni.3320
22. Zhang YH, He M, Wang Y, Liao AH. Modulators of the Balance Between M1 and M2 Macrophages During Pregnancy. *Front Immunol* (2017) 8:120. doi: 10.3389/fimmu.2017.00120
23. Ding J, Yin T, Yan N, Cheng Y, Yang J. FasL on Decidual Macrophages Mediates Trophoblast Apoptosis: A Potential Cause of Recurrent Miscarriage. *Int J Mol Med* (2019) 43(6):2376–86. doi: 10.3892/ijmm.2019.4146
24. Przybyl L, Haase N, Golic M, Rugor J, Solano ME, Arck PC, et al. CD74-Downregulation of Placental Macrophage-Trophoblastic Interactions in Preeclampsia. *Circ Res* (2016) 119(1):55–68. doi: 10.1161/CIRCRESAHA.116.308304
25. Bezemer RE, Schoots MH, Timmer A, Scherjon SA, Erwich J, van Goor H, et al. Altered Levels of Decidual Immune Cell Subsets in Fetal Growth Restriction, Stillbirth, and Placental Pathology. *Front Immunol* (2020) 11:1898. doi: 10.3389/fimmu.2020.01898
26. Zhang Y, Ma L, Hu X, Ji J, Mor G, Liao A. The Role of the PD-1/PD-L1 Axis in Macrophage Differentiation and Function During Pregnancy. *Hum Reprod* (2019) 34(1):25–36. doi: 10.1093/humrep/dey347
27. Zhu X, Liu H, Zhang Z, Wei R, Zhou X, Wang Z, et al. MiR-103 Protects From Recurrent Spontaneous Abortion *via* Inhibiting STAT1 Mediated M1 Macrophage Polarization. *Int J Biol Sci* (2020) 16(12):2248–64. doi: 10.7150/ijbs.46144
28. Han X, Li W, Li P, Zheng Z, Lin B, Zhou B, et al. Stimulation of Alpha7 Nicotinic Acetylcholine Receptor by Nicotine Suppresses Decidual M1 Macrophage Polarization Against Inflammation in Lipopolysaccharide-Induced Preeclampsia-Like Mouse Model. *Front Immunol* (2021) 12:642071. doi: 10.3389/fimmu.2021.642071
29. Agostinis C, Mangogna A, Bossi F, Ricci G, Kishore U, Bulla R. Uterine Immunity and Microbiota: A Shifting Paradigm. *Front Immunol* (2019) 10:2387. doi: 10.3389/fimmu.2019.02387
30. Mor G, Kwon JY. Trophoblast-Microbiome Interaction: A New Paradigm on Immune Regulation. *Am J Obstet Gynecol* (2015) 213(4 Suppl):S131–7. doi: 10.1016/j.ajog.2015.06.039
31. Shinde R, McGaha TL. The Aryl Hydrocarbon Receptor: Connecting Immunity to the Microenvironment. *Trends Immunol* (2018) 39 (12):1005–20. doi: 10.1016/j.it.2018.10.010
32. Goudot C, Coillard A, Villani AC, Gueguen P, Cros A, Sarkizova S, et al. Aryl Hydrocarbon Receptor Controls Monocyte Differentiation Into Dendritic Cells *Versus* Macrophages. *Immunity* (2017) 47(3):582–96 e6. doi: 10.1016/j.immuni.2017.08.016
33. Maltepe E, Fisher SJ. Placenta: The Forgotten Organ. *Annu Rev Cell Dev Biol* (2015) 31:523–52. doi: 10.1146/annurev-cellbio-100814-125620
34. Aplin JD. Developmental Cell Biology of Human Villous Trophoblast: Current Research Problems. *Int J Dev Biol* (2010) 54(2-3):323–9. doi: 10.1387/ijdb.082759ja
35. James JL, Carter AM, Chamley LW. Human Placentation From Nidation to 5 Weeks of Gestation. Part I: What do We Know About Formative Placental Development Following Implantation? *Placenta* (2012) 33(5):327–34. doi: 10.1016/j.placenta.2012.01.020
36. Moser G, Weiss G, Sundl M, Gauster M, Siwetz M, Lang-Olip I, et al. Extravillous Trophoblasts Invade More Than Uterine Arteries: Evidence for the Invasion of Uterine Veins. *Histochem Cell Biol* (2017) 147(3):353–66. doi: 10.1007/s00418-016-1509-5
37. Windsperger K, Dekan S, Pils S, Golletz C, Kunihs V, Fiala C, et al. Extravillous Trophoblast Invasion of Venous as Well as Lymphatic Vessels Is Altered in Idiopathic, Recurrent, Spontaneous Abortions. *Hum Reprod* (2017) 32(6):1208–17. doi: 10.1093/humrep/dex058
38. He N, van Iperen L, de Jong D, Szuhai K, Helmerhorst FM, van der Westerlaken LA, et al. Human Extravillous Trophoblasts Penetrate Decidual Veins and Lymphatics Before Remodeling Spiral Arteries During Early Pregnancy. *PloS One* (2017) 12(1):e0169849. doi: 10.1371/journal.pone.0169849
39. Burton GJ, Woods AW, Jauniaux E, Kingdom JC. Rheological and Physiological Consequences of Conversion of the Maternal Spiral Arteries for Uteroplacental Blood Flow During Human Pregnancy. *Placenta* (2009) 30(6):473–82. doi: 10.1016/j.placenta.2009.02.009
40. Kingdom JC, Audette MC, Hobson SR, Windrim RC, Morgen E. A Placenta Clinic Approach to the Diagnosis and Management of Fetal Growth Restriction. *Am J Obstet Gynecol* (2018) 218(2S):S803–S17. doi: 10.1016/j.ajog.2017.11.575
41. DaSilva-Arnold S, James JL, Al-Khan A, Zamudio S, Illsley NP. Differentiation of First Trimester Cytotrophoblast to Extravillous Trophoblast Involves an Epithelial-Mesenchymal Transition. *Placenta* (2015) 36(12):1412–8. doi: 10.1016/j.placenta.2015.10.013
42. Ding J, Cheng Y, Zhang Y, Liao S, Yin T, Yang J. The miR-27a-3p/USP25 Axis Participates in the Pathogenesis of Recurrent Miscarriage by Inhibiting Trophoblast Migration and Invasion. *J Cell Physiol* (2019) 234(11):19951–63. doi: 10.1002/jcp.28593
43. Rugg-Gunn PJ. Epigenetic Features of the Mouse Trophoblast. *Reprod BioMed Online* (2012) 25(1):21–30. doi: 10.1016/j.rbmo.2012.01.012
44. Tian FJ, Qin CM, Li XC, Wu F, Liu XR, Xu WM, et al. Decreased Stathmin-1 Expression Inhibits Trophoblast Proliferation and Invasion and Is Associated With Recurrent Miscarriage. *Am J Pathol* (2015) 185(10):2709–21. doi: 10.1016/j.ajpath.2015.06.010
45. Jauniaux E, Collins S, Burton GJ. Placenta Accreta Spectrum: Pathophysiology and Evidence-Based Anatomy for Prenatal Ultrasound Imaging. *Am J Obstet Gynecol* (2018) 218(1):75–87. doi: 10.1016/j.ajog.2017.05.067
46. Abrahams VM, Visintin I, Aldo PB, Guller S, Romero R, Mor G. A Role for TLRs in the Regulation of Immune Cell Migration by First Trimester Trophoblast Cells. *J Immunol* (2005) 175(12):8096–104. doi: 10.4049/jimmunol.175.12.8096
47. Silasi M, Mor G. Decidual Stromal Cells as Regulators of T-Cell Access to the Maternal-Fetal Interface. *Am J Reprod Immunol* (2012) 68(4):279–81. doi: 10.1111/aji.12006
48. Alenghat T, Osborne LC, Saenz SA, Kobuley D, Ziegler CG, Mullican SE, et al. Histone Deacetylase 3 Coordinates Commensal-Bacteria-Dependent Intestinal Homeostasis. *Nature* (2013) 504(7478):153–7. doi: 10.1038/nature12687
49. Helige C, Ahammer H, Moser G, Hammer A, Dohr G, Huppertz B, et al. Distribution of Decidual Natural Killer Cells and Macrophages in the Neighbourhood of the Trophoblast Invasion Front: A Quantitative Evaluation. *Hum Reprod* (2014) 29(1):8–17. doi: 10.1093/humrep/det353
50. Rozner AE, Durning M, Kropp J, Wiepz GJ, Golos TG. Macrophages Modulate the Growth and Differentiation of Rhesus Monkey Embryonic Trophoblasts. *Am J Reprod Immunol* (2016) 76(5):364–75. doi: 10.1111/aji.12564
51. Teirila L, Heikkinen-Eloranta J, Kotimaa J, Meri S, Lokki AI. Regulation of the Complement System and Immunological Tolerance in Pregnancy. *Semin Immunol* (2019) 45:101337. doi: 10.1016/j.smim.2019.101337

52. Buckley RJ, Whitley GS, Dumitriu IE, Cartwright JE. Macrophage Polarisation Affects Their Regulation of Trophoblast Behaviour. *Placenta* (2016) 47:73–80. doi: 10.1016/j.placenta.2016.09.004
53. Guirelli PM, Angeloni MB, Barbosa BF, Gomes AO, Castro AS, Franco PS, et al. Trophoblast-Macrophage Crosstalk on Human Extravillous Under Toxoplasma Gondii Infection. *Placenta* (2015) 36(10):1106–14. doi: 10.1016/j.placenta.2015.08.009
54. Fock V, Mairhofer M, Otti GR, Hiden U, Spittler A, Zeisler H, et al. Macrophage-Derived IL-33 Is a Critical Factor for Placental Growth. *J Immunol* (2013) 191(7):3734–43. doi: 10.4049/jimmunol.1300490
55. Meinhardt G, Saleh L, Otti GR, Haider S, Velicky P, Fiala C, et al. Wingless Ligand 5a Is a Critical Regulator of Placental Growth and Survival. *Sci Rep* (2016) 6:28127. doi: 10.1038/srep28127
56. Wu ZM, Yang H, Li M, Yeh CC, Schatz F, Lockwood CJ, et al. Pro-Inflammatory Cytokine-Stimulated First Trimester Decidual Cells Enhance Macrophage-Induced Apoptosis of Extravillous Trophoblasts. *Placenta* (2012) 33(3):188–94. doi: 10.1016/j.placenta.2011.12.007
57. Renaud SJ, Postovit LM, Macdonald-Goodfellow SK, McDonald GT, Caldwell JD, Graham CH. Activated Macrophages Inhibit Human Cytotrophoblast Invasiveness. *Vitro Biol Reprod* (2005) 73(2):237–43. doi: 10.1095/biolreprod.104.038000
58. Renaud SJ, Macdonald-Goodfellow SK, Graham CH. Coordinated Regulation of Human Trophoblast Invasiveness by Macrophages and Interleukin 10. *Biol Reprod* (2007) 76(3):448–54. doi: 10.1095/biolreprod.106.055376
59. Wang J, Ding J, Zhang S, Chen X, Yan S, Zhang Y, et al. Decreased USP2a Expression Inhibits Trophoblast Invasion and Associates With Recurrent Miscarriage. *Front Immunol* (2021) 12:717370. doi: 10.3389/fimmu.2021.717370
60. Kshirsagar SK, Alam SM, Jasti S, Hodes H, Nauser T, Gilliam M, et al. Immunomodulatory Molecules Are Released From the First Trimester and Term Placenta *via* Exosomes. *Placenta* (2012) 33(12):982–90. doi: 10.1016/j.placenta.2012.10.005
61. Lee CL, Guo Y, So KH, Vijayan M, Guo Y, Wong VH, et al. Soluble Human Leukocyte Antigen G5 Polarizes Differentiation of Macrophages Toward a Decidual Macrophage-Like Phenotype. *Hum Reprod* (2015) 30(10):2263–74. doi: 10.1093/humrep/dev196
62. Lee CL, Lam KK, Vijayan M, Koistinen H, Seppala M, Ng EH, et al. The Pleiotropic Effect of Glycodelin-A in Early Pregnancy. *Am J Reprod Immunol* (2016) 75(3):290–7. doi: 10.1111/aji.12471
63. Vijayan M, Lee CL, Wong VHH, Wang X, Bai K, Wu J, et al. Decidual Glycodelin-A Polarizes Human Monocytes Into a Decidual Macrophage-Like Phenotype Through Siglec-7. *J Cell Sci* (2020) 133(14):jcs244440. doi: 10.1242/jcs.244400
64. Santos ED, Moindjie H, Serazin V, Arnould L, Rodriguez Y, Fathallah K, et al. Preimplantation Factor Modulates Trophoblastic Invasion Throughout the Decidualization of Human Endometrial Stromal Cells. *Reprod Biol Endocrinol* (2021) 19(1):96. doi: 10.1186/s12958-021-00774-5
65. Ayele H, Perner M, McKinnon LR, Birse K, Farr Zuend C, Burgener A. An Updated Review on the Effects of Depot Medroxyprogesterone Acetate on the Mucosal Biology of the Female Genital Tract. *Am J Reprod Immunol* (2021) 86(3):e13455. doi: 10.1111/aji.13455
66. Tsai YC, Tseng JT, Wang CY, Su MT, Huang JY, Kuo PL. Medroxyprogesterone Acetate Drives M2 Macrophage Differentiation Toward a Phenotype of Decidual Macrophage. *Mol Cell Endocrinol* (2017) 452:74–83. doi: 10.1016/j.mce.2017.05.015
67. Chang SF, Lin SS, Yang HC, Chou YY, Gao JI, Lu SC. LPS-Induced G-CSF Expression in Macrophages Is Mediated by ERK2, But Not Erk1. *PloS One* (2015) 10(6):e0129685. doi: 10.1371/journal.pone.0129685
68. Ding J, Yang C, Zhang Y, Wang J, Zhang S, Guo D, et al. M2 Macrophage-Derived G-CSF Promotes Trophoblasts EMT, Invasion and Migration *via* Activating PI3K/Akt/Erk1/2 Pathway to Mediate Normal Pregnancy. *J Cell Mol Med* (2021) 25(4):2136–47. doi: 10.1111/jcmm.16191
69. Scarpellini F, Klinger FG, Rossi G, Sbracia M. Immunohistochemical Study on the Expression of G-CSF, G-CSFR, VEGF, VEGFR-1, Foxp3 in First Trimester Trophoblast of Recurrent Pregnancy Loss in Pregnancies Treated With G-CSF and Controls. *Int J Mol Sci* (2019) 21(1):285. doi: 10.3390/ijms21010285
70. Eapen A, Joing M, Kwon P, Tong J, Maneta E, De Santo C, et al. Recombinant Human Granulocyte- Colony Stimulating Factor in Women With Unexplained Recurrent Pregnancy Losses: A Randomized Clinical Trial. *Hum Reprod* (2019) 34(3):424–32. doi: 10.1093/humrep/dey393
71. Wurfel W. Treatment With Granulocyte Colony-Stimulating Factor in Patients With Repetitive Implantation Failures and/or Recurrent Spontaneous Abortions. *J Reprod Immunol* (2015) 108:123–35. doi: 10.1016/j.jri.2015.01.010
72. Kamath MS, Kirubakaran R, Sunkara SK. Granulocyte-Colony Stimulating Factor Administration for Subfertile Women Undergoing Assisted Reproduction. *Cochrane Database Syst Rev* (2020) 1:CD013226. doi: 10.1002/14651858.CD013226.pub2
73. Ding J, Yang C, Cheng Y, Wang J, Zhang S, Yan S, et al. Trophoblast-Derived IL-6 Serves as an Important Factor for Normal Pregnancy by Activating Stat3-Mediated M2 Macrophages Polarization. *Int Immunopharmacol* (2021) 90:106788. doi: 10.1016/j.intimp.2020.106788
74. Abumaree MH, Chamley LW, Badri M, El-Muzaini MF. Trophoblast Debris Modulates the Expression of Immune Proteins in Macrophages: A Key to Maternal Tolerance of the Fetal Allograft? *J Reprod Immunol* (2012) 94(2):131–41. doi: 10.1016/j.jri.2012.03.488
75. Aldo PB, Racicot K, Craviero V, Guller S, Romero R, Mor G. Trophoblast Induces Monocyte Differentiation Into CD14+/CD16+ Macrophages. *Am J Reprod Immunol* (2014) 72(3):270–84. doi: 10.1111/aji.12288
76. Svensson-Arvelund J, Mehta RB, Lindau R, Mirrasekhian E, Rodriguez-Martinez H, Berg G, et al. The Human Fetal Placenta Promotes Tolerance Against the Semiallogeneic Fetus by Inducing Regulatory T Cells and Homeostatic M2 Macrophages. *J Immunol* (2015) 194(4):1534–44. doi: 10.4049/jimmunol.1401536
77. Chen Z, Buki K, Vaaraniemi J, Gu G, Vaananen HK. The Critical Role of IL-34 in Osteoclastogenesis. *PloS One* (2011) 6(4):e18689. doi: 10.1371/journal.pone.0018689
78. Wang Y, Szretter KJ, Vermi W, Gilfillan S, Rossini C, Cella M, et al. IL-34 Is a Tissue-Restricted Ligand of CSF1R Required for the Development of Langerhans Cells and Microglia. *Nat Immunol* (2012) 13(8):753–60. doi: 10.1038/ni.2360
79. Lindau R, Mehta RB, Lash GE, Papapavlou G, Boij R, Berg G, et al. Interleukin-34 Is Present at the Fetal-Maternal Interface and Induces Immunoregulatory Macrophages of a Decidual Phenotype *In Vitro*. *Hum Reprod* (2018) 33(4):588–99. doi: 10.1093/humrep/dey037
80. Wang XQ, Zhou WJ, Hou XX, Fu Q, Li DJ. Trophoblast-Derived CXCL16 Induces M2 Macrophage Polarization That in Turn Inactivates NK Cells at the Maternal-Fetal Interface. *Cell Mol Immunol* (2018) 15(12):1038–46. doi: 10.1038/s41423-018-0019-x
81. Salvany-Celades M, van der Zwan A, Benner M, Setrajcic-Dragos V, Bougleux Gomes HA, Iyer V, et al. Three Types of Functional Regulatory T Cells Control T Cell Responses at the Human Maternal-Fetal Interface. *Cell Rep* (2019) 27(9):2537–47 e5. doi: 10.1016/j.celrep.2019.04.109
82. Zhang YH, Aldo P, You Y, Ding J, Kaislasuo J, Petersen JF, et al. Trophoblast-Secreted Soluble-PD-L1 Modulates Macrophage Polarization and Function. *J Leukoc Biol* (2020) 108(3):983–98. doi: 10.1002/JLB.1A0420-012RR
83. Zhu R, Huang YH, Tao Y, Wang SC, Sun C, Piao HL, et al. Hyaluronan Up-Regulates Growth and Invasion of Trophoblasts in an Autocrine Manner *via* PI3K/AKT and MAPK/ERK1/2 Pathways in Early Human Pregnancy. *Placenta* (2013) 34(9):784–91. doi: 10.1016/j.placenta.2013.05.009
84. Wang S, Sun F, Han M, Liu Y, Zou Q, Wang F, et al. Trophoblast-Derived Hyaluronan Promotes the Regulatory Phenotype of Decidual Macrophages. *Reproduction* (2019) 157(2):189–98. doi: 10.1530/REP-18-0450
85. Tavianatou AG, Caon I, Franchi M, Piperigkou Z, Galesso D, Karamanos NK. Hyaluronan: Molecular Size-Dependent Signaling and Biological Functions in Inflammation and Cancer. *FEBS J* (2019) 286(15):2883–908. doi: 10.1111/febs.14777
86. Rayahin JE, Buhrman JS, Zhang Y, Koh TJ, Gemeinhart RA. High and Low Molecular Weight Hyaluronic Acid Differentially Influence Macrophage Activation. *ACS Biomater Sci Eng* (2015) 1(7):481–93. doi: 10.1021/acsbiomaterials.5b00181
87. Shi Q, Zhao L, Xu C, Zhang L, Zhao H. High Molecular Weight Hyaluronan Suppresses Macrophage M1 Polarization and Enhances IL-10 Production in

PM2.5-Induced Lung Inflammation. *Molecules* (2019) 24(9):1766. doi: 10.3390/molecules24091766
88. Wakx A, Nedder M, Tomkiewicz-Raulet C, Dalmasso J, Chissey A, Boland S, et al. Expression, Localization, and Activity of the Aryl Hydrocarbon Receptor in the Human Placenta. *Int J Mol Sci* (2018) 19(12):3762. doi: 10.3390/ijms19123762
89. Wu Y, Chen X, Chang X, Huang YJ, Bao S, He Q, et al. Potential Involvement of Placental AhR in Unexplained Recurrent Spontaneous Abortion. *Reprod Toxicol* (2016) 59:45–52. doi: 10.1016/j.reprotox.2015.11.005
90. Meng YH, Zhou WJ, Jin LP, Liu LB, Chang KK, Mei J, et al. RANKL-Mediated Harmonious Dialogue Between Fetus and Mother Guarantees Smooth Gestation by Inducing Decidual M2 Macrophage Polarization. *Cell Death Dis* (2017) 8(10):e3105. doi: 10.1038/cddis.2017.505
91. Bruno V, Lindau R, Jenmalm MC, Ticconi C, Piccione E, Pietropolli A, et al. First-Trimester Trophoblasts Obtained by Chorionic Villus Sampling Maintain Tolerogenic and Proteomic Features in Successful Pregnancies Despite a History of Unexplained Recurrent Pregnancy Loss. *Am J Reprod Immunol* (2020) 84(6):e13314. doi: 10.1111/aji.13314
92. Castro AS, Alves CM, Angeloni MB, Gomes AO, Barbosa BF, Franco PS, et al. Trophoblast Cells are Able to Regulate Monocyte Activity to Control Toxoplasma Gondii Infection. *Placenta* (2013) 34(3):240–7. doi: 10.1016/j.placenta.2012.12.006
93. Tannetta D, Masliukaite I, Vatish M, Redman C, Sargent I. Update of Syncytiotrophoblast Derived Extracellular Vesicles in Normal Pregnancy and Preeclampsia. *J Reprod Immunol* (2017) 119:98–106. doi: 10.1016/j.jri.2016.08.008
94. Statello L, Maugeri M, Garre E, Nawaz M, Wahlgren J, Papadimitriou A, et al. Identification of RNA-Binding Proteins in Exosomes Capable of Interacting With Different Types of RNA: RBP-Facilitated Transport of RNAs Into Exosomes. *PloS One* (2018) 13(4):e0195969. doi: 10.1371/journal.pone.0195969
95. Rezaie J, Rahbarghazi R, Pezeshki M, Mazhar M, Yekani F, Khaksar M, et al. Cardioprotective Role of Extracellular Vesicles: A Highlight on Exosome Beneficial Effects in Cardiovascular Diseases. *J Cell Physiol* (2019) 234 (12):21732–45. doi: 10.1002/jcp.28894
96. Salomon C, Torres MJ, Kobayashi M, Scholz-Romero K, Sobrevia L, Dobierzewska A, et al. A Gestational Profile of Placental Exosomes in Maternal Plasma and Their Effects on Endothelial Cell Migration. *PloS One* (2014) 9(6):e98667. doi: 10.1371/journal.pone.0098667
97. Sarker S, Scholz-Romero K, Perez A, Illanes SE, Mitchell MD, Rice GE, et al. Placenta-Derived Exosomes Continuously Increase in Maternal Circulation Over the First Trimester of Pregnancy. *J Transl Med* (2014) 12:204. doi: 10.1186/1479-5876-12-204
98. Mincheva-Nilsson L, Baranov V. Placenta-Derived Exosomes and Syncytiotrophoblast Microparticles and Their Role in Human Reproduction: Immune Modulation for Pregnancy Success. *Am J Reprod Immunol* (2014) 72(5):440–57. doi: 10.1111/aji.12311
99. Orozco AF, Jorgez CJ, Ramos-Perez WD, Popek EJ, Yu X, Kozinetz CA, et al. Placental Release of Distinct DNA-Associated Micro-Particles Into Maternal Circulation: Reflective of Gestation Time and Preeclampsia. *Placenta* (2009) 30(10):891–7. doi: 10.1016/j.placenta.2009.06.012
100. Atay S, Gercel-Taylor C, Suttles J, Mor G, Taylor DD. Trophoblast-Derived Exosomes Mediate Monocyte Recruitment and Differentiation. *Am J Reprod Immunol* (2011) 65(1):65–77. doi: 10.1111/j.1600-0897.2010.00880.x
101. Atay S, Gercel-Taylor C, Taylor DD. Human Trophoblast-Derived Exosomal Fibronectin Induces Pro-Inflammatory IL-1beta Production by Macrophages. *Am J Reprod Immunol* (2011) 66(4):259–69. doi: 10.1111/j.1600-0897.2011.00995.x
102. Holder B, Jones T, Sancho Shimizu V, Rice TF, Donaldson B, Bouqueau M, et al. Macrophage Exosomes Induce Placental Inflammatory Cytokines: A Novel Mode of Maternal-Placental Messaging. *Traffic* (2016) 17(2):168–78. doi: 10.1111/tra.12352
103. Ying X, Jin X, Zhu Y, Liang M, Chang X, Zheng L. Exosomes Released From Decidual Macrophages Deliver miR-153-3p, Which Inhibits Trophoblastic Biological Behavior in Unexplained Recurrent Spontaneous Abortion. *Int Immunopharmacol* (2020) 88:106981. doi: 10.1016/j.intimp.2020.106981
104. Ding J, Zhang Y, Cai X, Zhang Y, Yan S, Wang J, et al. Extracellular Vesicles Derived From M1 Macrophages Deliver miR-146a-5p and miR-146b-5p to Suppress Trophoblast Migration and Invasion by Targeting TRAF6 in Recurrent Spontaneous Abortion. *Theranostics* (2021) 11(12):5813–30. doi: 10.7150/thno.58731

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SYSTEMATIC REVIEW
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Regulatory T Cells in Pregnancy Adverse Outcomes: A Systematic Review and Meta-Analysis

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Background: Several studies report the role of Regulatory T-cells (Tregs) in the pathophysiology of pregnancy adverse outcomes.

Objective: The aim of this systematic review and meta-analysis was to determine whether there is an association between regulatory T cell levels and pregnancy adverse outcomes (PAOs), including pre-eclampsia and preterm birth (PTB).

Method: Literature searches were conducted in PubMed/MEDLINE, Embase, and Cochrane CENTRAL databases. Inclusion criteria were original articles (clinical trials, case-control studies and cohort studies) comparing Tregs, sampled from the decidua or maternal blood, in healthy pregnant women *versus* women with pre-eclampsia or PTB. The outcome was standardised mean difference (SMD) in Treg numbers. The tau-squared (Tau^2), inconsistency index (I^2), and chi-squared (χ^2) test quantified heterogeneity among different studies. Analyses were performed in RevMan software V.5.4.0 for Mac using a random-effects model with outcome data reported with 95% confidence intervals (CI). This study was prospectively registered with PROSPERO (CRD42020205469). PRISMA guidelines were followed.

Results: From 4,085 unique studies identified, 36 were included in qualitative synthesis, and 34 were included in quantitative synthesis (meta-analysis). In total, there were 1,783 participants in these studies: healthy controls=964, pre-eclampsia=759, PTB=60. Thirty-two studies compared Tregs in healthy pregnant women and women with pre-eclampsia, and 30 of these sampled Tregs from peripheral blood showing significantly higher Treg numbers in healthy pregnancies (SMD; 1.46; 95% CI, 1.03–1.88; I^2=92%). Four studies sampled Tregs from the maternal decidua showing higher Tregs in healthy pregnancies (SMD, 0.76; 95% CI, -0.13–1.65; I^2=84%). No difference was found in the number of Tregs between early *versus* late pre-eclampsia (SMD,-1.17; 95% CI, -2.79–0.44; I^2=94%).

For PTB, two studies compared Tregs sampled from the peripheral blood with a tendency for higher Tregs in healthy pregnancies but this did not reach significance (SMD, 2.18; 95% CI, -1.34–5.70; I^2=96%). Subcohort analysis using Treg analysis (flow cytometry *vs.* qPCR *vs.* immunofluorescence tissue staining) showed similar associations.

Conclusion: Lower Tregs in pregnancy, sampled from the maternal peripheral blood, are associated with pre-eclampsia. There is a need for further studies to confirm a relationship between low Tregs and PTB. As the precise mechanisms by which Tregs may mediate pre-eclampsia and PTB remain unclear, further fundamental research is necessary to elucidate the underlying processes and highlight the causative link.

Systematic Review Registration: PROSPERO, identifier CRD42020205469.

Keywords: regulatory T cells (Tregs), pregnancy, high blood pressure (hypertension), pre-eclampsia, pre-term birth (PTB), pregnancy adverse outcomes (PAO)

INTRODUCTION

Preterm Birth

Preterm birth (PTB) is defined by the World Health Organization (WHO) as birth prior to 37 weeks of gestation (1), further subdivided into extreme, very and moderate-to-late preterm occurring prior to 28 weeks, 28 to 32 weeks, and 32 to 37 weeks respectively. PTB has become the leading cause of perinatal morbidity and mortality in developed countries (2) and despite an overall decline in perinatal mortality, preterm infants face increased short-term morbidity and long-term neurodevelopmental, respiratory, and gastrointestinal complications (2).

The complex heterogeneity of PTB is due to its varying aetiology and pathogenesis, which result in idiopathic premature activation of the labour process, with or without pathological insults (2). Approximately 50% of PTBs are due to preterm labour (PTL), uterine contractions before 37 weeks' gestation that may or may not progress to delivery (i.e., PTB), with intact membranes or preterm premature rupture of membranes (PPROM, 25%) (3). Up to 40% of these cases are due to intrauterine infection. Other causes include inflammation, vascular disease, uterine overdistension, placental abruption or hormonal disruptions (4–6). 25% involve induced labours or caesarean deliveries as a result of maternal or fetal indications (7). Due to effects on placental blood supply and intrauterine growth, pre-eclampsia is a prime example of such an indication, accounting for up to 20% of PTBs (8).

Pre-eclampsia

Pre-eclampsia affects 3-5% of pregnancies, with incidence increasing due to a higher prevalence of risk factors including maternal obesity, older maternal age and diabetes mellitus (9). Pre-eclampsia is diagnosed in the presence of hypertension after 20 weeks' gestation accompanied by maternal acute kidney injury, liver dysfunction, neurological symptoms, haemolysis or thrombocytopenia, or fetal growth restriction (10). Further risk factors include first pregnancy, hypertensive disease in previous pregnancies and co-morbidities including autoimmune and renal disease. Proposed pathways of pre-eclampsia suggest immunological factors of genetic and environmental origin are involved in the pathogenesis (11).

Early and late-onset pre-eclampsia, defined as onset before 34 weeks of gestation and at or after 34 weeks respectively. These are important to differentiate as different pathogenic mechanisms and outcomes are implicated – whilst shallow trophoblast invasion is common in early onset type (12), exaggerated inflammatory responses may play a role in the development of late-onset disease (12). Furthermore, aspirin treatment prevents early onset pre-eclampsia but not late onset disease (13). Thus, it is important to compare Treg numbers between these subtypes as they may represent different disease entities (12).

Clinical diagnosis requires proteinuria, new-onset hypertension or signs of end-organ damage. The American College of Obstetricians and Gynaecologists (ACOG) (14) suggest diagnosis in the presence of proteinuria and new-onset hypertension, or new-onset hypertension with thrombocytopenia, renal insufficiency, impaired liver function or pulmonary oedema. Classification of severe disease involves severe hypertension (systolic BP ≥ 160mmHg, diastolic BP ≥ 110mmHg or both) or signs of end-organ damage.

Tregs in Pregnancy

Regulatory T-cells (Tregs) are a specialised subset of immunosuppressive cells defined by the expression of lineage-defining transcription factors FOXP3, CD25 and low or absent CD127 expression (15), subdivided into thymic/natural Tregs (nTregs) and peripherally induced Tregs (iTregs), which are thought to control autoimmune responses and mucosal immunity, respectively (16). Immunosuppressive function is primarily exerted by direct cell-cell interactions with the target cell, consumption of interleukin-2 (IL-2) and the release of anti-inflammatory molecules[14]. In addition to ensuring tolerance to self and non-inherited antigens (17), Tregs are essential in inducing transplantation tolerance (18–20).

During pregnancy, Tregs prevent rejection of the semi-allogeneic fetus by the maternal immune system (21, 22). Decidual Tregs, including nTregs and iTregs (23), create a tolerogenic microenvironment through the production of

soluble factors such as IL-10 (22). In healthy pregnancies, great diversity in Treg populations exists in both peripheral blood and at the maternal-fetal interface (22). High levels of $CD25^{hi}FOXP3^{+}$ Tregs are found in decidual tissues[21] and both FOXP3+ and FOXP3- Tregs are increased in the peripheral blood of pregnant women (22). The composition of fetal cells and maternal immune cells changes throughout gestation (22). T-cell frequencies increase during gestation, with local and systemic Treg expansion reaching its maximum in the 2^{nd} trimester (21). As labour progresses, the proportions of decidual Tregs once again decreases (24).

In PAO, maternal Tregs are altered (16). Treg maldistribution or functional impairment has been reported in implantation failure, miscarriage and pre-eclampsia (21, 22, 25, 26). In primary unexplained infertility, expression of FOXP3 mRNA is decreased in the uterine endometrium (27). In pre-eclampsia, Treg percentages are lower than in healthy pregnancies (28–30) and associated with spiral artery adaptation and defective maternal blood flow to the placenta (30). A reduction of decidual $CD4+CD25^{HI}$ FOXP3+ and HELIOS+ Tregs is observed in miscarriages (21–23, 31).

Impaired peripheral Treg signalling has also been found (22). Signalling pathways implicated in modulating T-cell function during pregnancy include the IL-2–dependent STAT5ab signalling pathways (32), the PD1-PDL1 pathway (23) and the TIM-3 pathway (24). Fetal Tregs are also implicated (33), however, this systematic review and meta-analysis focuses on maternal Tregs only.

Objective

Through this systematic review and meta-analysis, we aimed to determine whether there is an association between regulatory T cell levels and PAOs, including pre-eclampsia and preterm birth.

2 MATERIALS AND METHODS

Design

This study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (34). The review was prospectively registered with PROSPERO (CRD42020205469).

Outcomes

Primary outcome was standardised mean difference (SMD) in Treg numbers between healthy pregnant women and women with pre-eclampsia or PTB. The measures used to identify these differences include Tregs expressing CD4+/CD25+/CD127low or CD4+/CD125+/FOXP3+ sampled from the peripheral blood or maternal decidua.

Eligibility Criteria

Eligible for inclusion were original articles including clinical trials, case-control studies and cohort studies that examined the association between maternal Tregs in human pregnancy, sampled from the decidua or maternal blood, and the onset of pre-eclampsia and PTB. Studies were selected that compared these maternal co-morbidities with healthy age-matched pregnant individuals as control. No restrictions were made regarding population characteristics, such as age, ethnicity or setting. Studies examining fetal Tregs and studies that did not sample Tregs from the maternal blood or decidua were excluded. Duplicate studies were excluded from total counts.

Information Sources and Search Strategy

Three reviewers (SG, KSR and MP) searched PubMed/MEDLINE, Embase and Cochrane CENTRAL for eligible articles published between August 1st, 2010 and August 1st, 2020 using search terms specific for 'maternal' OR 'fetal' 'regulatory T-cells' AND 'pregnancy', 'pre-eclampsia', 'preterm birth', OR 'miscarriage' (**Appendix 1**). Results were restricted by article type (see **Appendix 1** for detailed search strategies), language (English), and species (Human).

Selection Process

For each article, title, abstract and full-text screening was performed independently by one of three reviewers (SG, KSR and MP). Screening results were reviewed by a senior author in the study (PS). Discrepancies were resolved through discussion in which a senior author was consulted (PS).

Data Collection Process and Data Items

For each article, data was extracted independently by one of four reviewers (SG, KSR, MP, ASVC) using a predefined data extraction form. Results were reviewed by a senior author in the study (PS). From each study, information was extracted regarding study design, location, population, participant demographics, baseline characteristics, details of intervention and control, interventions and attrition rate. Miscellaneous information, e.g. method of delivery, antenatal steroid use was also recorded.

Quality Assessment

A modified version (35) of the Newcastle–Ottawa Scale (NOS) (36) (**Supplementary Table 4**) was used to assess methodological quality of included studies. Studies were judged based on selection, comparability and outcome, with a maximum of 3, 2 and 2 stars, respectively, equating to a total score ranging from zero (worst) to 7 (best). ≥ 6 stars indicated high quality, 4–5 moderate quality and a high risk of bias and <4 indicated a very high risk of bias. Quality assessment was undertaken independently by two reviewers (KSR and MP), and inter-rater reliability was assessed.

Statistical Analysis

We estimated the SMD in Treg numbers, sampled from the decidua and peripheral blood, of healthy pregnant women *versus* pregnant women with pre-eclampsia or PTB along with 95% confidence intervals (CI) under a random-effects (RE) model using Review Manager Version 5.4 (V.5.4.0) for Mac.

We used the tau-squared (Tau^2), inconsistency index (I^2), and chi-squared (χ^2) test to quantify heterogeneity among different studies. Heterogeneity as defined by I^2 was considered to be minor if 0% to 40%, moderate if 30% to 60%, substantial if 50% to 90% and considerable if 75% to 100%. The percent heterogeneity was interpreted in the context of the magnitude of the effect size and the strength of evidence surrounding the heterogeneity (37). Potential publication bias was tested using the rank correlation test of funnel plot asymmetry [Begg's test (38) and Egger's test (39)].

RESULTS

Search Results

Figure 1 outlines the study selection process following PRISMA guidelines (40). The initial search identified 4,085 unique articles. 55 articles underwent full-text screening, with 36 studies included in qualitative synthesis and 34 in quantitative synthesis (meta-analysis). Treg populations between healthy pregnant women and pregnant women with pre-eclampsia was compared in 32 studies, 30 of which sampled Tregs from maternal peripheral blood (**Supplementray Table 1**). Four studies sample Tregs from maternal decidua (**Supplementary Table 2**), whilst an additional two studies compared Tregs, sampled from the peripheral blood, between healthy pregnant women and pregnant women who underwent PTL and PTB (**Supplementary Table 3**).

Characteristics of Studies Included in the Meta-Analysis (Quantitative Synthesis)

Supplementary Tables 1–3 feature the characteristics of included articles. Included studies were published between 2009 and 2019. Total sample sizes (including cases and controls) ranged from 20 to 108 pregnant subjects. Studies spanned 5 continents (Asia=19, Australia=1, Europe=9, North America=3 and South America=2), 12 countries (Australia=1, Bosnia and Herzegovina=1, Brazil=2, China=13, Czech Republic=1, Germany=1, Hungary=4, Iran=5, Japan=1, Mexico=1, Poland=2 and USA=2) and represented 1,783 participants (healthy controls=964, pre-eclampsia=759, PTB=60) of African American, Asian, Black, Caucasian, Hispanic, Latin and Persian ethnicity/race. The mean and median ages of women across studies ranged between 26.0 and 35.5 years. Treg analysis was typically performed in the second and third trimester of gestation. Most studies used flow cytometry (n=28) as the method of Treg analysis, while few reports using qPCR (n=5) and immunofluorescence tissue staining (n=1). Six studies considered and reported BMI measurements between cases and controls as an important confounder, and six studies considered and reported smoking status. All studies identified gestational age at the time of Treg analysis as a confounder, and 24 out of 34 studies (71%) considered gestational age at delivery. Birth weight was reported in 24 out of 34 studies (71%). A selection of different Treg markers were used in each study to identify Treg populations with $CD4^+$, $CD25^+$, $FOXP3^+$ as well as $CD4^+$, $CD25^+$, $CD127^{low}$ being the most common Treg marker combinations (**Supplementary Tables 1–3**). Gestational age at delivery is missing from 20 studies (**Supplementary Table 1**). Use of corticosteroids is not mentioned in 26 studies (**Supplementary Tables 1–3**).

Meta-Analysis Findings (Quantitative Synthesis)

Lower Number of Tregs in Peripheral Blood in the Peripheral Blood of Women Who Develop Pre-Eclampsia

30 studies were included in analysis exploring the association of pre-eclampsia and Treg populations in the peripheral blood. Twenty-six studies used flow cytometry to analyse Tregs populations (41–64) and four used qPCR (65–68). We analysed these two groups separately and combined them (**Figure 2**). In the qPCR group, the patients were matched for ethnicity and age group (Asian, <30 years old). The SMD of Treg numbers in the peripheral blood of healthy pregnant women compared to pregnant women with pre-eclampsia was 2.82 (95% CI, 0.81–4.83; I^2 = 96%; 4 studies), with healthy women reporting significantly higher Treg numbers in two studies and non-significant difference in two studies (**Figure 2.1.2**).

The overall SMD of Treg numbers in the peripheral blood of healthy pregnant women (using flow cytometry and qpcr) compared to pregnant women with pre-eclampsia was 1.46 (95% CI, 1.03–1.88; I^2 = 92%; 30 studies), with healthy women reporting significantly higher Treg numbers overall (**Figure 2**). We performed subgroup analysis based on their ethnic background and age. Testing for subgroup differences did not reveal any significant results (P=0.35, I^2 = 10.7%) (**Figure 2.1.2**, 2.1.3, 2.1.4, 2.1.5, 2.1.6). In addition changing the method of analysis from SMD to Mean Difference (MD) showed a MD of 2.49 (95% CI, 1.41-3.57; I^2 = 100%; 30 studies, data not shown). Testing for subgroup differences using MD did not show any significant results (P=0.14, I^2-41.9%, data not shown). In addition, we also performed subgroup analysis based on the year of publication. We divided the studies to the ones published before (n=10) and after 2015 (n=20). Testing for subgroups differences based on the year of publication did not show any significant results (P=0.66, I^2 = 0%, data not shown).

Tregs in the Decidua of Women With Pre-Eclampsia and Healthy Women

Four studies were included in the analysis to determine association of pre-eclampsia and Treg populations in the maternal decidua. Two (69, 70) used qPCR to analyse Treg populations, one (71) used immunofluorescence tissue staining and one used flow cytometry (43). We analysed these three subgroups separately and further analysis combined all four

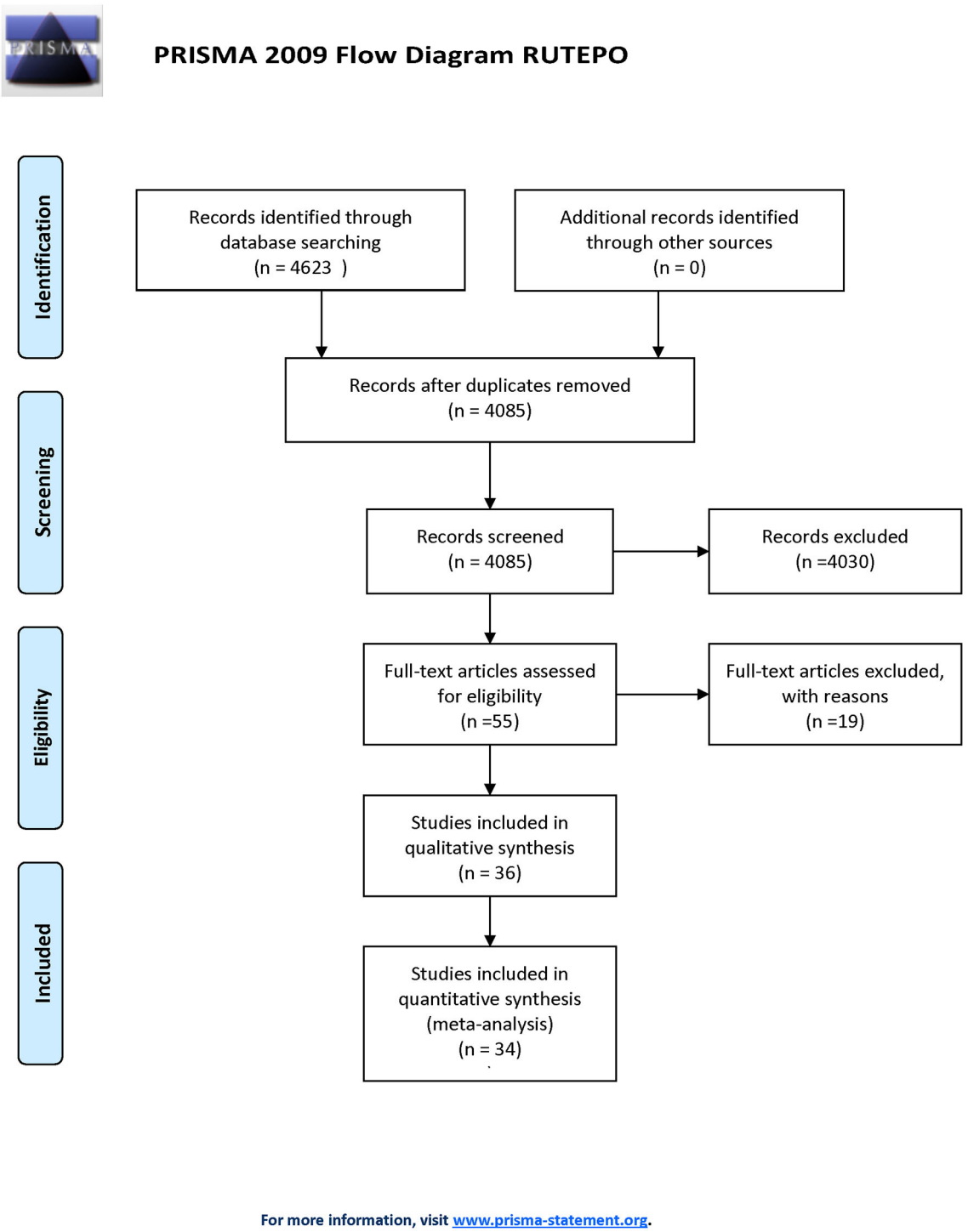

FIGURE 1 | PRISMA flowchart of study selection.

studies (**Figure 3**). For the qPCR group (69, 70), the SMD in Treg numbers in the decidua of healthy pregnant women compared to pregnant women with pre-eclampsia was 1.15 (95% CI, 0.61–1.68; $I^{2\ =}$ 0%; 2 studies), with healthy pregnant women reporting significantly higher Treg numbers in both studies (**Figure 3.1.1**). The immunofluorescence tissue staining study (71) also reported significantly higher Tregs in healthy pregnant women (SMD, 1.27; 95% CI, 0.52–2.02) (**Figure 3.1.2**). Flow cytometry (43)

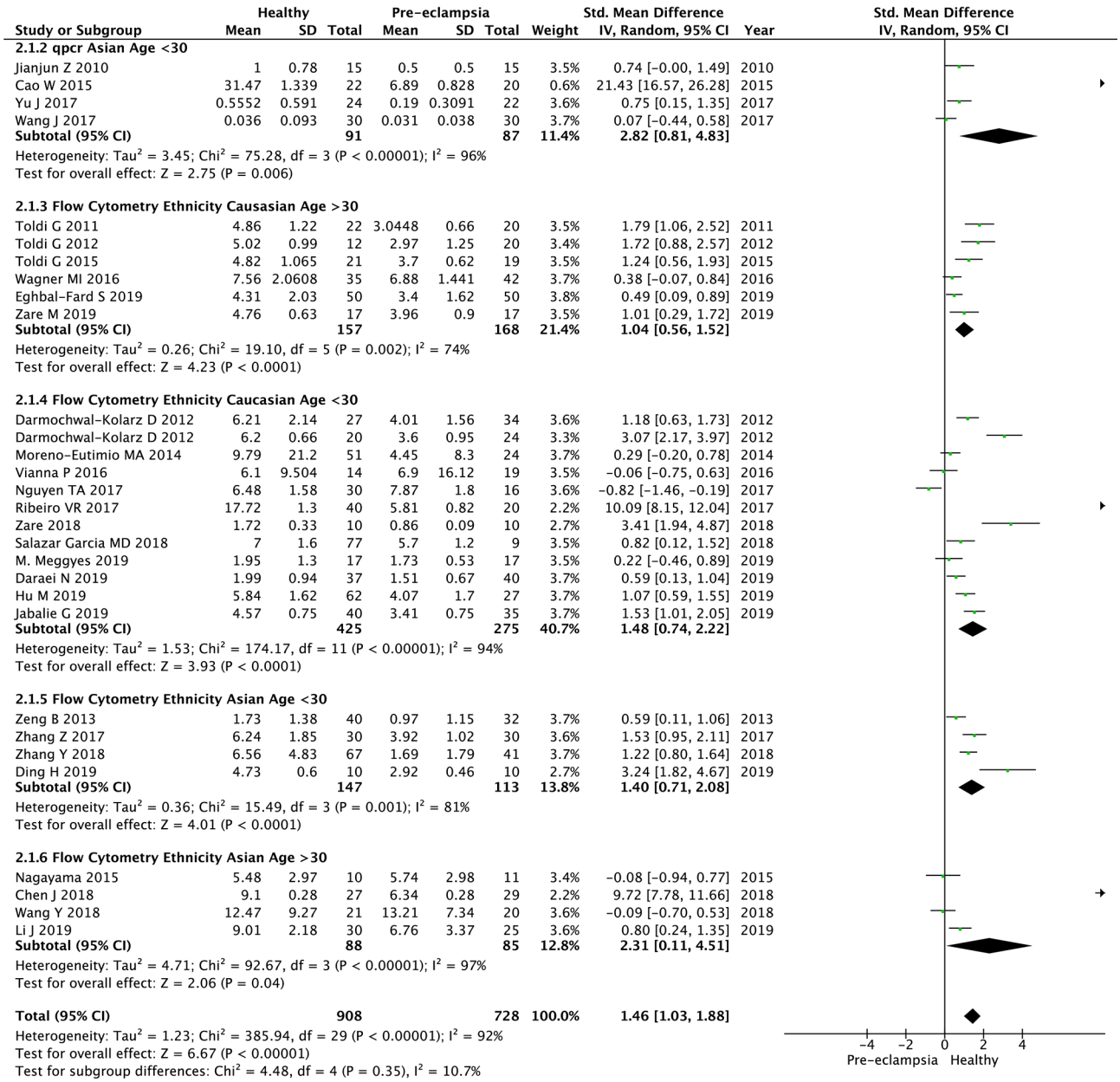

FIGURE 2 | Standardized mean difference of T regulatory cell numbers in the peripheral blood of healthy pregnant women and women with pre-eclampsia, in subgroups according to ethnicity, age and method of analysis. CI, confidence interval; SD, standard deviation; Std. Mean Difference, standardised mean difference; IV, inverse variance.

showed no significant difference in Treg numbers between healthy pregnancies and women with pre-eclampsia (SMD, -0.45; 95% CI, -1.06–0.16) (**Figure 3.1.3**). Analysing qPCR, immunofluorescence tissue staining and flow cytometry studies together, the SMD of Treg numbers in the decidua of healthy pregnant women compared to pregnant women with pre-eclampsia was 0.76 (95% CI, -0.13–1.65; I^2 = 84%; 4 studies), with healthy women reporting higher Treg numbers in 3 studies (65, 70, 71) and non-significant difference in 1 study (43). This result should be interpreted with caution as the 95% CI crosses the null value.

The Number of Tregs in Women With Early Pre-Eclampsia Are Similar to the Ones in Late Pre-Eclampsia

We identified studies, n=3 (44, 63, 68), which reported the number of Tregs separately in early *versus* late pre-eclampsia.

The SMD in Treg numbers in the peripheral blood of pregnant women with late pre-eclampsia compared to

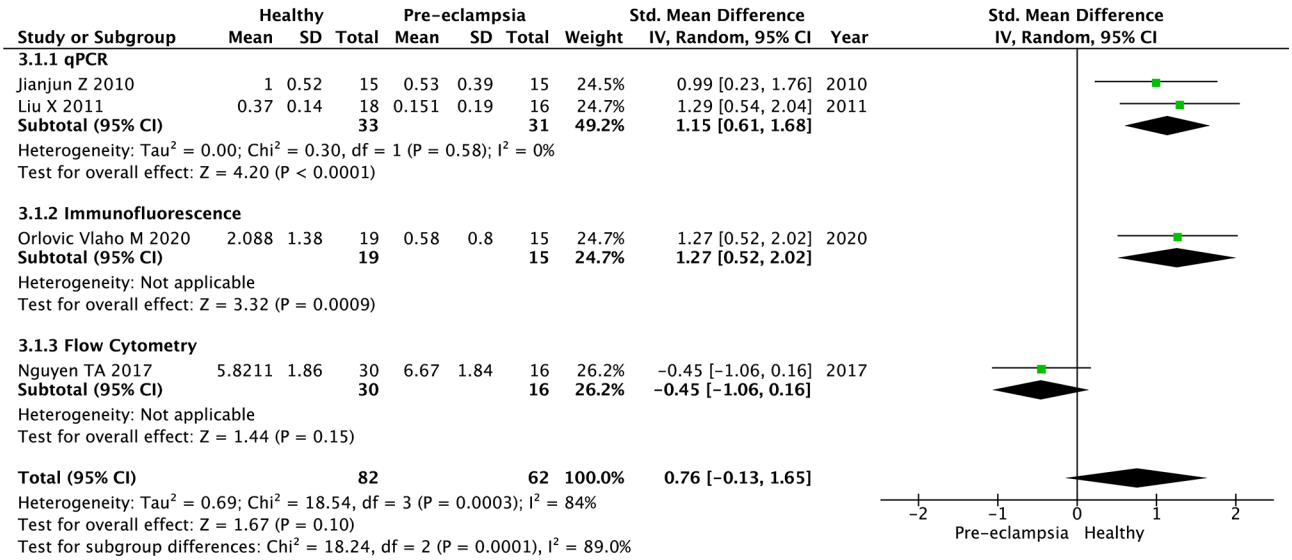

FIGURE 3 | Standardized mean difference of T regulatory cell numbers in the decidua of healthy pregnant women and women with pre-eclampsia. CI, confidence interval; SD, standard deviation; Std. Mean Difference, standardised mean difference; IV, inverse variance.

pregnant women with early pre-eclampsia was -1.17 [95% CI, -2.79–0.44; I^2 = 94%; 3 studies (44, 63, 68)], a non-significant difference with the 95% CI crossing the null value (**Figure 4**).

The Number of Tregs in Women Who Develop PTL and Had PTB Are Similar to Healthy Women

Only two studies (72, 73) reporting the association of PTB and Tregs in peripheral blood were included in the analysis. Both studies measured Tregs with flow cytometry. The SMD of Treg numbers in the peripheral blood of healthy pregnant women compared to pregnant women who underwent PTB was 2.18 (95% CI, -1.34–5.70; I^2 = 96%; 2 studies), with healthy pregnant women reporting higher Treg numbers overall in one study (72) and a non-significant difference in the second study (73) (**Figure 5**). However, the 95% CI crosses the null value.

Heterogeneity of the Studies

Heterogeneity was considerable in all meta-analyses, the 95% prediction intervals for individual studies crossing the null value (**Figures 2–5**). I^2 was 92% and 84% for the 30 and 4 respective studies investigating pre-eclampsia by sampling Tregs from maternal peripheral blood and decidua, respectively. Similarly, the I^2 was 96% for the two studies investigating PTB by sampling Tregs from maternal peripheral blood. Where I^2 could be estimated within subgroup analyses, age, ethnicity, year of publication and by the method of Treg analysis (flow cytometry *vs.* qPCR *vs.* immunofluorescence tissue staining), it remained considerable for all except the qPCR subgroup analysis of 2 studies (65, 70) investigating pre-eclampsia by sampling Tregs from the maternal decidua (I^2 = 0%) - 95% prediction intervals of neither of these studies crossed the null value (**Figure 3.1.1**). In the flow cytometry, ethnicity Caucasian, age >30 subgroup, of 6 studies (41, 52, 53, 56, 62, 63) the heterogeneity was relatively lower that the rest of the subgroups (I^2 = 74%) with only one study (63) crossing the null value. Heterogeneity should be considered as a confounder when interpreting the significance of results, particularly in relation to the analysis of pre-eclampsia studies sampling Tregs from the maternal decidua (**Figure 3**) and PTB studies sampling Tregs from the maternal peripheral blood (**Figure 5**) as the 95%

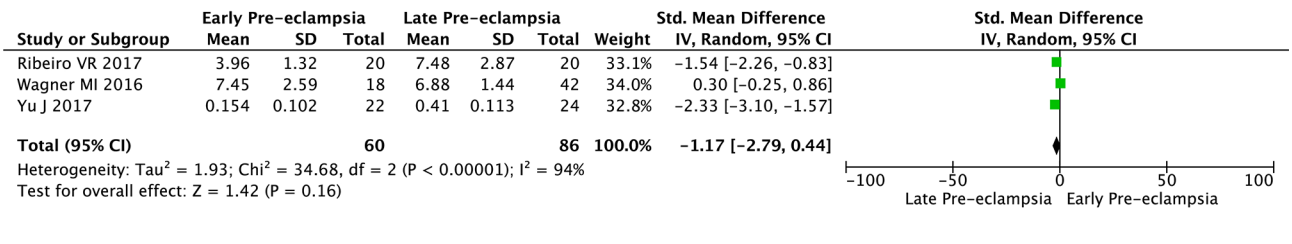

FIGURE 4 | Standardized mean difference of T regulatory cell numbers in the maternal blood of women with early and late pre-eclampsia. CI, confidence interval; SD, standard deviation; Std. Mean Difference, standardised mean difference; IV, inverse variance.

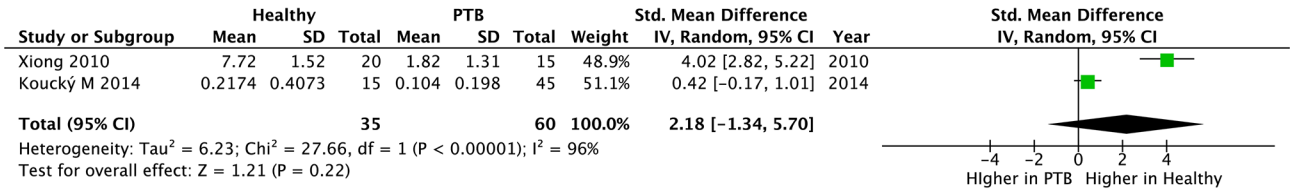

FIGURE 5 | Standardized mean difference of T regulatory cell numbers in the peripheral blood of healthy pregnant women and women who underwent preterm birth (PTB). PTB, preterm birth; CI, confidence interval; SD, standard deviation; Std. Mean Difference, standardised mean difference; IV, inverse variance.

CI crossed the null value in the end outcome of these analyses. This was not true for the analysis of pre-eclampsia studies sampling Tregs from the maternal peripheral blood (**Figure 2**).

Publication Bias

A funnel plot was used to graphically evaluate articles for publication bias. This was tested using the rank correlation test of funnel plot asymmetry [Begg's test (38) and Egger's test (39)]. SMD values were plotted against standard error (SE). Data from the 30 studies seemed to be roughly symmetrically distributed in an inverted funnel-shaped area (**Figure 6**).

Quality Assessment

The overall quality rating of the studies included in the meta-analysis was moderate, representing a high risk of bias. NOS scores ranged between 3–6 with a median score of 4 out of 7 (**Supplementray Table 5**). Quality ratings were impaired by poor population representativeness of the exposed cohort (due to hospital-based sampling), and inadequate follow up (<1 month after labour). Comparability was limited in several studies that did not adjust for all confounding variables (age, race, smoking and interpregnancy interval). The IRR was 100% regarding the assessment of 'selection' and 'outcomes' criteria; however, there were inter-rater discrepancies in the assessment of 'comparability' in 13 out of 36 studies (IRR=64%).

DISCUSSION

Findings

Our meta-analysis suggests that lower Treg cell numbers may be a potential independent risk factor for PAO, including pre-eclampsia and potentially PTL. Overall, healthy pregnant women have significantly higher Treg numbers than pregnant women with pre-eclampsia, evident by an SMD of 1.46 (95% CI, 1.03–1.88; I^2 = 92%; 30 studies) when sampling Tregs from the peripheral blood. In studies sampling Tregs from the maternal decidua, healthy pregnant women had higher, but non-

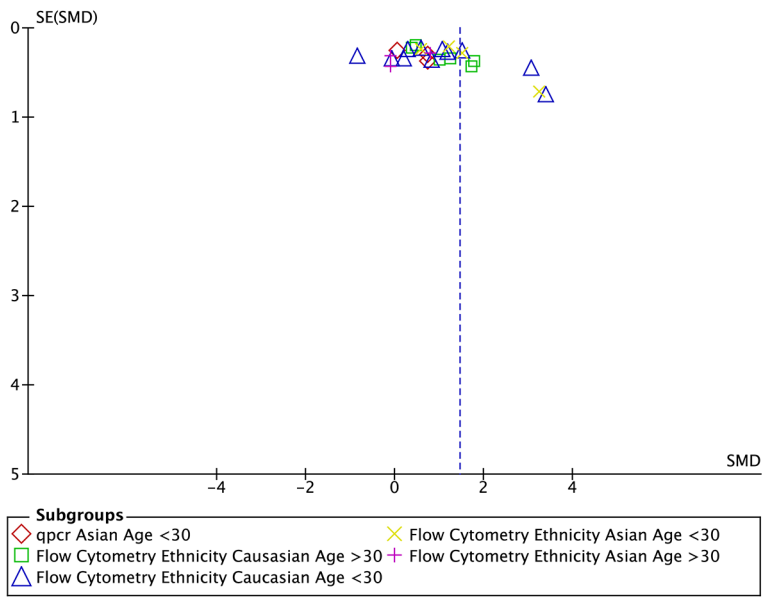

FIGURE 6 | Funnel plot for studies looking at the number of Tregs in the maternal blood included in the subgroup meta-analysis (n=30). SE, standard error; SMD, standardised mean difference.

significantly higher Treg numbers compared to women with pre-eclampsia, evident by an SMD of 0.76 (95% CI, -0.13–1.65; I^2 = 84%; 4 studies). This might be due to poor phenotyping of the decidual tissue. Healthy pregnant women also have a non-significant numerically higher Treg numbers compared to pregnant women who undergo PTL, with an SMD of 2.18 (95% CI, -1.34–5.70; I^2 = 96%; 2 studies) when sampling Tregs from the peripheral blood. Similar trends are observed in subcohort analysis when studies are grouped by the method of Treg analysis.

This is supported by previous research by Han et al (74) and Schober et al (75) who found an association between impaired Treg function and pre-eclampsia (74) and PTL (75). Han et al. (74), using high-dimensional mass cytometry immunoassay, suggests that specific aspects of peripheral immune system dynamics may be disrupted in preeclamptic pregnancies. Furthermore, Schober et al. (75), using flow cytometry, found that the suppressive activity of CD4+CD127low+/-CD25+-Treg cells was strongly diminished in PTL women and, to a lesser extent, in spontaneously term labouring women compared to term non-labouring women. This reduction in suppressive activity was due to Treg-cell deficiency but not due to CD4+-responder T (Tresp) cell resistance, with CD4+-T cells significantly reduced in term and preterm labouring women (75). There is a need for additional research, using tightly phenotyped PTL groups, before confirming any relationship and methods used by Han et al. and Schober et al. may be worthwhile.

Strengths

We identified and screened over 4,000 unique articles, the meta-analysis therefore including participants across five continents of different ethnicity, adding to the representability and generalizability of findings. We found similar results, in terms of Treg associations with healthy and adverse pregnancy outcomes, across studies that sampled Tregs from different sites (maternal decidua and maternal peripheral blood) as well as studies using other methods of Treg analysis (flow cytometry, qPCR and immunofluorescence tissue staining), increasing robustness of findings. Performance of subcohort analysis by age, ethnicity, year of publication, site of sampling and method of Treg analysis further supports the strength of association across different research conditions. Most studies attempted to adjust for confounding variables.

Limitations

Participant numbers were limited, especially for the PTB cohort (1739 pregnant women including 944 healthy controls, 735 pre-eclampsia, 60 PTB). Furthermore, each separate study had a small number of patients in each group. Indeed, only two studies investigating PTB were included in quantitative analysis; both sampling Tregs from maternal peripheral blood, none sampling decidua. In pre-eclampsia, only four studies tested Tregs from the maternal decidua, utilising three different methods of Treg analysis, with the flow cytometry study producing opposing findings to studies utilising qPCR or immunofluorescence tissue staining. Antenatal corticosteroids have been shown to alter T cell trafficking and cytokine production (76), yet use of corticosteroids prior to blood sampling was omitted in most studies (supplementary data). Additionally, whilst Treg cells are classified into naïve and effector Tregs, which express weak and powerful immunoregulation respectively, we did not discriminate between the two. Comparing Treg populations in early onset *versus* late onset pre-eclampsia is also important given the distinct underlying pathogenic processes (44, 63, 68, 77). In our cohort only 3 studies reported separately the Treg numbers in early *versus* late pre-eclampsia. In addition a subgroup analysis based on BMI would have been ideal since there is evidence the BMI alters the number of Tregs in obese individuals (78). Unfortunately only 7 studies reported BMI in their results, which was between 22-28 and no subgroup analysis could have been done (**Supplementary Tables 1–3**).

Methodological quality of studies was low to moderate. Nevertheless, all studies were included in the analysis regardless of quality assessment, none excluded based on high risk of bias. It may have been prudent to repeat the analysis, excluding studies with low NOS quality rating. Equally, meta-analyses could have been repeated after excluding studies with high heterogeneity (I^2) in which the 95% prediction intervals crossed the null value (Figs. 2-5). Overall, there was considerable heterogeneity across analyses. Indeed, this limited the significance of results in three of the meta-analyses, specifically for pre-eclampsia studies sampling Tregs from the maternal decidua and PTB studies sampling Tregs from the maternal peripheral blood as well as in the early *versus* late comparison (**Figure 4**). Assessing relative risk (RR) or odds ratio (OR) in addition to SMD may have demonstrated a stronger level of association to support outcomes.

Clinical Significance

Future research could investigate the potential of monitoring Treg numbers in peripheral blood of pregnant women as a possible biomarker to assess the risk of PAO, stratifying patients with high-risk pregnancies. Future preclinical and clinical models could investigate strategies to increase Treg numbers in pregnant women as a candidate therapeutic approach. Both of these suggestions, however, remain at a hypothesis stage and require further systematic evaluation.

CONCLUSION

This meta-analysis suggests an association between lower T-regulatory cell numbers and risk for pre-eclampsia and potentially for PTL (28, 29). Importantly, correlation does not imply causation and possibility of an underlying mechanism causing both low Treg numbers and pre-eclampsia and PTL must be considered. As the precise mechanisms by which Tregs may mediate pre-eclampsia and PTL remain unclear, further research is necessary to elucidate the underlying processes and highlight the causative link.

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.

AUTHOR CONTRIBUTIONS

PS, SG, MP, KR, and AS conceptualized the topic and structure of the systematic review. SG, MP, KR, and AS drafted and revised the manuscript. AE, NM, KD, CS, GL, RT, KN, and PS provided expert opinion, edited, and approved the final manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.737862/full#supplementary-material

REFERENCES

1. *Preterm Birth*. Available at: https://www.who.int/news-room/fact-sheets/detail/preterm-birth (Accessed April 26, 2021).
2. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and Causes of Preterm Birth. *Lancet* (2008) 371:75–84. doi: 10.1016/S0140-6736(08)60074-4
3. Moutquin J. Classification and Heterogeneity of Preterm Birth. *BJOG Int J Obstet Gynaecol* (2003) 110:30–3. doi: 10.1016/S1470-0328(03)00021-1
4. Agrawal V, Hirsch E. Intrauterine Infection and Preterm Labor. *Semin Fetal Neonatal Med* (2012) 17:12–9. doi: 10.1016/j.siny.2011.09.001
5. Lockwood CJ. The Diagnosis of Preterm Labor and the Prediction of Preterm Delivery. *Clin Obstet Gynecol* (1995) 38:675–87. doi: 10.1097/00003081-199538040-00002
6. Romero R, Espinoza J, Kusanovic J, Gotsch F, Hassan S, Erez O, et al. The Preterm Parturition Syndrome. *BJOG Int J Obstet Gynaecol* (2006) 113:17–42. doi: 10.1111/j.1471-0528.2006.01120.x
7. Sibai B, Dekker G, Kupferminc M. Pre-Eclampsia. *Lancet* (2005) 365:785–99. doi: 10.1016/S0140-6736(05)17987-2
8. Duley L. The Global Impact of Pre-Eclampsia and Eclampsia. *Semin Perinatol* (2009) 33:130–7. doi: 10.1053/j.semperi.2009.02.010
9. Lisonkova S, Joseph KS. Incidence of Preeclampsia: Risk Factors and Outcomes Associated With Early- *Versus* Late-Onset Disease. *Am J Obstet Gynecol* (2013) 209:544.e1–e12. doi: 10.1016/j.ajog.2013.08.019
10. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al. Hypertensive Disorders of Pregnancy. *Hypertension* (2018) 72:24–43. doi: 10.1161/HYPERTENSIONAHA.117.10803
11. Phipps E, Prasanna D, Brima W, Jim B. Preeclampsia: Updates in Pathogenesis, Definitions, and Guidelines. *Clin J Am Soc Nephrol* (2016) 11:1102–13. doi: 10.2215/CJN.12081115
12. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-Eclampsia. *Lancet* (2010) 376:631–44. doi: 10.1016/S0140-6736(10)60279-6
13. Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, de Paco Matallana C, et al. Aspirin *Versus* Placebo in Pregnancies at High Risk for Preterm Preeclampsia. *N Engl J Med* (2017) 377:613–22. doi: 10.1056/NEJMoa1704559
14. American College of Obstetricians and Gynecologists. Gestational Hypertension and Preeclampsia. *Obstet Gynecol* (2020) 135:1492–5. doi: 10.1097/AOG.0000000000003892
15. Rackaityte E, Halkias J. Mechanisms of Fetal T Cell Tolerance and Immune Regulation. *Front Immunol* (2020) 11:588. doi: 10.3389/fimmu.2020.00588
16. Miller D, Gershater M, Slutsky R, Romero R, Gomez-Lopez N. Maternal and Fetal T Cells in Term Pregnancy and Preterm Labor. *Cell Mol Immunol* (2020) 17:693–704. doi: 10.1038/s41423-020-0471-2
17. Burt TD. Fetal Regulatory T Cells and Peripheral Immune Tolerance *In Utero* : Implications for Development and Disease. *Am J Reprod Immunol* (2013) 69:346–58. doi: 10.1111/aji.12083
18. Velásquez-Lopera MM, Eaton VL, Lerret NM, Correa LA, Decresce RP, García LF, et al. Induction of Transplantation Tolerance by Allogeneic Donor-Derived CD4(+)CD25(+)Foxp3(+) Regulatory T Cells. *Transpl Immunol* (2008) 19:127–35. doi: 10.1016/j.trim.2008.02.003
19. van der Net JB, Bushell A, Wood KJ, Harden PN. Regulatory T Cells: First Steps of Clinical Application in Solid Organ Transplantation. *Transpl Int* (2016) 29:3–11. doi: 10.1111/tri.12608
20. Romano M, Tung SL, Smyth LA, Lombardi G. Treg Therapy in Transplantation: A General Overview. *Transpl Int* (2017) 30:745–53. doi: 10.1111/tri.12909
21. Tsuda S, Nakashima A, Shima T, Saito S. New Paradigm in the Role of Regulatory T Cells During Pregnancy. *Front Immunol* (2019) 10:573. doi: 10.3389/fimmu.2019.00573
22. Krop J, Heidt S, Claas FHJ, Eikmans M. Regulatory T Cells in Pregnancy: It Is Not All About Foxp3. *Front Immunol* (2020) 11:1182. doi: 10.3389/fimmu.2020.01182
23. Salvany-Celades M, van der Zwan A, Benner M, Setrajcic-Dragos V, Bougleux Gomes HA, Iyer V, et al. Three Types of Functional Regulatory T Cells Control T Cell Responses at the Human Maternal-Fetal Interface. *Cell Rep* (2019) 27:2537–2547.e5. doi: 10.1016/j.celrep.2019.04.109
24. Miller D, Gershater M, Slutsky R, Romero R, Gomez-Lopez N. Maternal and Fetal T Cells in Term Pregnancy and Preterm Labor. *Cell Mol Immunol* (2020) 17:693–704. doi: 10.1038/s41423-020-0471-2
25. Hsu P, Santner-Nanan B, Dahlstrom JE, Fadia M, Chandra A, Peek M, et al. Altered Decidual DC-SIGN+ Antigen-Presenting Cells and Impaired Regulatory T-Cell Induction in Preeclampsia. *Am J Pathol* (2012) 181:2149–60. doi: 10.1016/j.ajpath.2012.08.032
26. Santner-Nanan B, Peek MJ, Khanam R, Richarts L, Zhu E, Fazekas de St Groth B, et al. Systemic Increase in the Ratio Between Foxp3 + and IL-17-Producing CD4 + T Cells in Healthy Pregnancy But Not in Preeclampsia. *J Immunol* (2009) 183:7023–30. doi: 10.4049/jimmunol.0901154
27. Tsuda S, Zhang X, Hamana H, Shima T, Ushijima A, Tsuda K, et al. Clonally Expanded Decidual Effector Regulatory T Cells Increase in Late Gestation of Normal Pregnancy, But Not in Preeclampsia, in Humans. *Front Immunol* (2018) 9:1934. doi: 10.3389/fimmu.2018.01934
28. Sasaki Y, Darmochwal-Kolarz D, Suzuki D, Sakai M, Ito M, Shima T, et al. Proportion of Peripheral Blood and Decidual CD4+ CD25 Bright Regulatory T Cells in Pre-Eclampsia. *Clin Exp Immunol* (2007) 149:139–45. doi: 10.1111/j.1365-2249.2007.03397.x
29. Quinn KH, Parast MM. Decidual Regulatory T Cells in Placental Pathology and Pregnancy Complications. *Am J Reprod Immunol* (2013) 69:533–8. doi: 10.1111/aji.12077
30. Robertson SA, Green ES, Care AS, Moldenhauer LM, Prins JR, Louise Hull M, et al. Therapeutic Potential of Regulatory T Cells in Preeclampsia-Opportunities and Challenges. *Front Immunol* (2019) 10:478. doi: 10.3389/fimmu.2019.00478
31. Inada K, Shima T, Ito M, Ushijima A, Saito S. Helios-Positive Functional Regulatory T Cells Are Decreased in Decidua of Miscarriage Cases With Normal Fetal Chromosomal Content. *J Reprod Immunol* (2015) 107:10–9. doi: 10.1016/j.jri.2014.09.053

32. Aghaeepour N, Ganio EA, Mcilwain D, Tsai AS, Tingle M, Van Gassen S, et al. An Immune Clock of Human Pregnancy. *Sci Immunol* (2017) 2:eaan2946. doi: 10.1126/sciimmunol.aan2946
33. Rackaityte E, Halkias J. Mechanisms of Fetal T Cell Tolerance and Immune Regulation. *Front Immunol* (2020) 11:588. doi: 10.3389/fimmu.2020.00588
34. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 Statement: An Updated Guideline for Reporting Systematic Reviews. *BMJ* (2021) 372:n71. doi: 10.1136/bmj.n71
35. Kapadia MZ, Park CK, Beyene J, Giglia L, Maxwell C, McDonald SD. Weight Loss Instead of Weight Gain Within the Guidelines in Obese Women During Pregnancy: A Systematic Review and Meta-Analyses of Maternal and Infant Outcomes. *PloS One* (2015) 10:e0132650. doi: 10.1371/journal.pone.0132650
36. Luchini C, Stubbs B, Solmi M, Veronese N. Assessing the Quality of Studies in Meta-Analyses: Advantages and Limitations of the Newcastle Ottawa Scale. *World J Meta-Analysis* (2017) 5:80. doi: 10.13105/wjma.v5.i4.80
37. Ryan R. Planning the Analysis at Protocol Stage. *Cochrane Consum Commun Rev* (2013) 2016:2–9.
38. Begg CB, Mazumdar M. Operating Characteristics of a Rank Correlation Test for Publication Bias. *Biometrics* (1994) 50:1088–101. doi: 10.2307/2533446
39. Egger M, Davey Smith G, Schneider M, Minder C. Bias in Meta-Analysis Detected by a Simple, Graphical Test. *BMJ* (1997) 315:629–34. doi: 10.1136/bmj.315.7109.629
40. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 Statement. *Syst Rev* (2015) 4:1. doi: 10.1186/2046-4053-4-1
41. Toldi G, Rigó J, Stenczer B, Vásárhelyi B, Molvarec A. Increased Prevalence of IL-17-Producing Peripheral Blood Lymphocytes in Pre-Eclampsia. *Am J Reprod Immunol* (2011) 66:223–9. doi: 10.1111/j.1600-0897.2011.00987.x
42. Darmochwal-Kolarz D, Kludka-Sternik M, Tabarkiewicz J, Kolarz B, Rolinski J, Leszczynska-Gorzelak B, et al. The Predominance of Th17 Lymphocytes and Decreased Number and Function of Treg Cells in Preeclampsia. *J Reprod Immunol* (2012) 93:75–81. doi: 10.1016/j.jri.2012.01.006
43. Nguyen TA, Kahn DA, Loewendorf AI. Maternal—Fetal Rejection Reactions Are Unconstrained in Preeclamptic Women. *PloS One* (2017) 12:e0188250. doi: 10.1371/journal.pone.0188250
44. Ribeiro VR, Romao-Veiga M, Romagnoli GG, Matias ML, Nunes PR, Borges VTM, et al. Association Between Cytokine Profile and Transcription Factors Produced by T-Cell Subsets in Early- and Late-Onset Pre-Eclampsia. *Immunology* (2017) 152:163–73. doi: 10.1111/imm.12757
45. Zhang Z, Liu H, Shi Y, Xu N, Wang Y, Li A, et al. Increased Circulating Th22 Cells Correlated With Th17 Cells in Patients With Severe Preeclampsia. *Hypertens Pregnancy* (2017) 36:100–7. doi: 10.1080/10641955.2016.1239737
46. Salazar Garcia MD, Mobley Y, Henson J, Davies M, Skariah A, Dambaeva S, et al. Early Pregnancy Immune Biomarkers in Peripheral Blood may Predict Preeclampsia. *J Reprod Immunol* (2018) 125:25–31. doi: 10.1016/j.jri.2017.10.048
47. Chen J, Zhao L, Wang D, Xu Y, Gao H, Tan W, et al. Contribution of Regulatory T$\blacksquare$cells to Immune Tolerance and Association of microRNA−210 and Foxp3 in Preeclampsia. *Mol Med Rep* (2018) 19:1150–8. doi: 10.3892/mmr.2018.9733
48. Zare M, Doroudchi M, Gharesi-Fard B. Altered Frequencies of CD4+ CD25+ Foxp3+ and CD8+ CD25+ Foxp3+ Regulatory T Cells in Pre-Eclampsia. *Iran J Allergy Asthma Immunol* (2018) 17:540–7.
49. Wang Y, Liu Y, Shu C, Wan J, Shan Y, Zhi X, et al. Inhibition of Pregnancy-Associated Granulocytic Myeloid-Derived Suppressor Cell Expansion and Arginase-1 Production in Preeclampsia. *J Reprod Immunol* (2018) 127:48–54. doi: 10.1016/j.jri.2018.05.002
50. Daraei N, Ghafourian M, Ghadiri A, Amari A, Najafian M, Rokhafrooz S. Evaluation of Exhausted Regulatory T Cells in Preeclampsia. *Iran J Immunol* (2019) 16:163–9. doi: 10.22034/IJI.2019.80259
51. Ding H, Dai Y, Lei Y, Wang Z, Liu D, Li R, et al. Upregulation of CD81 in Trophoblasts Induces an Imbalance of Treg/Th17 Cells by Promoting IL-6 Expression in Preeclampsia. *Cell Mol Immunol* (2019) 16:302–12. doi: 10.1038/s41423-018-0186-9
52. Eghbal-Fard S, Yousefi M, Heydarlou H, Ahmadi M, Taghavi S, Movasaghpour A, et al. The Imbalance of Th17/Treg Axis Involved in the Pathogenesis of Preeclampsia. *J Cell Physiol* (2019) 234:5106–16. doi: 10.1002/jcp.27315
53. Toldi G, Saito S, Shima T, Halmos A, Veresh Z, Vásárhelyi B, et al. The Frequency of Peripheral Blood CD4+ CD25high FoxP3+ and CD4+ CD25- FoxP3+ Regulatory T Cells in Normal Pregnancy and Pre-Eclampsia. *Am J Reprod Immunol* (2012) 68:175–80. doi: 10.1111/j.1600-0897.2012.01145.x
54. Hu M, Eviston D, Hsu P, Mariño E, Chidgey A, Santner-Nanan B, et al. Decreased Maternal Serum Acetate and Impaired Fetal Thymic and Regulatory T Cell Development in Preeclampsia. *Nat Commun* (2019) 10:3031. doi: 10.1038/s41467-019-10703-1
55. Li J, Huang L, Wang S, Zhang Z. The Prevalence of Regulatory T and Dendritic Cells is Altered in Peripheral Blood of Women With Pre-Eclampsia. *Pregnancy Hypertens* (2019) 17:233–40. doi: 10.1016/j.preghy.2019.07.003
56. Zare M, Namavar Jahromi B, Gharesi-Fard B. Analysis of the Frequencies and Functions of CD4+CD25+CD127low/neg, CD4+HLA-G+, and CD8+HLA-G + Regulatory T Cells in Pre-Eclampsia. *J Reprod Immunol* (2019) 133:43–51. doi: 10.1016/j.jri.2019.06.002
57. Meggyes M, Miko E, Lajko A, Csiszar B, Sandor B, Matrai P, et al. Involvement of the PD-1/PD-L1 Co-Inhibitory Pathway in the Pathogenesis of the Inflammatory Stage of Early-Onset Preeclampsia. *Int J Mol Sci* (2019) 20:583. doi: 10.3390/ijms20030583
58. Darmochwal-Kolarz D, Saito S, Tabarkiewicz J, Kolarz B, Rolinski J, Leszczynska-Gorzelak B, et al. Apoptosis Signaling is Altered in CD4 +CD25 +FoxP3 + T Regulatory Lymphocytes in Pre-Eclampsi. *Int J Mol Sci* (2012) 13:6548–60. doi: 10.3390/ijms13066548
59. Zeng B, Kwak-Kim J, Liu Y, Liao A-H. Treg Cells Are Negatively Correlated With Increased Memory B Cells in Pre-Eclampsia While Maintaining Suppressive Function on Autologous B-Cell Proliferation. *Am J Reprod Immunol* (2013) 70:454–63. doi: 10.1111/aji.12154
60. Moreno-Eutimio MA, Tovar-Rodríguez JM, Vargas-Avila K, Nieto-Velázquez NG, Frías-De-León MG, Sierra-Martinez M, et al. Increased Serum Levels of Inflammatory Mediators and Low Frequency of Regulatory T Cells in the Peripheral Blood of Preeclamptic Mexican Women. *BioMed Res Int* (2014) 2014:1–8. doi: 10.1155/2014/413249
61. Nagayama S, Ohkuchi A, Shirasuna K, Takahashi K, Suzuki H, Hirashima C, et al. The Frequency of Peripheral Blood CD4 + FoxP3 + Regulatory T Cells in Women With Pre-Eclampsia and Those With High-Risk Factors for Pre-Eclampsia. *Hypertens Pregnancy* (2015) 34:443–55. doi: 10.3109/10641955.2015.1065884
62. Toldi G, Vásárhelyi ZE, Rigó J, Orbán C, Tamássy Z, Bajnok A, et al. Prevalence of Regulatory T-Cell Subtypes in Preeclampsia. *Am J Reprod Immunol* (2015) 74:110–5. doi: 10.1111/aji.12380
63. Wagner MI, Jöst M, Spratte J, Schaier M, Mahnke K, Meuer S, et al. Differentiation of ICOS + and ICOS – Recent Thymic Emigrant Regulatory T Cells (RTE T Regs) During Normal Pregnancy, Pre-Eclampsia and HELLP Syndrome. *Clin Exp Immunol* (2016) 183:129–42. doi: 10.1111/cei.12693
64. Vianna P, Mondadori AG, Bauer ME, Dornfeld D, Chies JAB. HLA-G and CD8+ Regulatory T Cells in the Inflammatory Environment of Pre-Eclampsia. *Reproduction* (2016) 152:741–51. doi: 10.1530/REP-15-0608
65. Jianjun Z, Yali H, Zhiqun W, Mingming Z, Xia Z. ORIGINAL ARTICLE: Imbalance of T-Cell Transcription Factors Contributes to the Th1 Type Immunity Predominant in Pre-Eclampsia. *Am J Reprod Immunol* (2009) 63:38–45. doi: 10.1111/j.1600-0897.2009.00763.x
66. Cao W, Wang X, Chen T, Zhu H, Xu W, Zhao S, et al. The Expression of Notch/Notch Ligand, IL-35, IL-17, and Th17/Treg in Preeclampsia. *Dis Markers* (2015) 2015:1–9. doi: 10.1155/2015/316182
67. Wang J, Wen ZQ, Cheng X-Y, Mei TY, Chen Z-F, Su L-X. siRNA-Mediated Knockdown of T-Bet and Rorγt Contributes to Decreased Inflammation in Pre-Eclampsia. *Mol Med Rep* (2017) 16:6368–75. doi: 10.3892/mmr.2017.7348
68. Yu J, Qian L, Wu F, Li M, Chen W, Wang H. Decreased Frequency of Peripheral Blood CD8 + CD25 + FoxP3 + Regulatory T Cells Correlates With IL-33 Levels in Pre-Eclampsia. *Hypertens Pregnancy* (2017) 36:217–25. doi: 10.1080/10641955.2017.1302470
69. Jianjun Z, Yali H, Zhiqun W, Mingming Z, Xia Z. ORIGINAL ARTICLE: Imbalance of T-Cell Transcription Factors Contributes to the Th1 Type Immunity Predominant in Pre-Eclampsia. *Am J Reprod Immunol* (2009) 63:38–45. doi: 10.1111/j.1600-0897.2009.00763.x
70. Liu X, Liu Y, Ding M, Wang X. Reduced Expression of Indoleamine 2,3-Dioxygenase Participates in Pathogenesis of Preeclampsia *via* Regulatory T Cells. *Mol Med Rep* (2011) 4:53–8. doi: 10.3892/mmr.2010.395
71. Orlovic Vlaho M, Tomic V, Vukojevic K, Vasilj A, Pejic R, Lesko J, et al. CD25 +FOXP3+ and CD4+CD25+ Cells Distribution in Decidual Departments of

Women With Severe and Mild Pre-Eclampsia: Comparison With Healthy Pregnancies. *Am J Reprod Immunol* (2020) 84:1–8. doi: 10.1111/aji.13281
72. Xiong H, Zhou C, Qi G. Proportional Changes of CD4+CD25+Foxp3+ Regulatory T Cells in Maternal Peripheral Blood During Pregnancy and Labor at Term and Preterm. *Clin Investig Med* (2010) 33:422. doi: 10.25011/cim.v33i6.14594
73. Koucký M, Malíčková K, Cindrová-Davies T, Germanová A, Pařízek A, Kalousová M, et al. Low Levels of Circulating T-Regulatory Lymphocytes and Short Cervical Length Are Associated With Preterm Labor. *J Reprod Immunol* (2014) 106:110–7. doi: 10.1016/j.jri.2014.04.001
74. Han X, Ghaemi MS, Ando K, Peterson LS, Ganio EA, Tsai AS, et al. Differential Dynamics of the Maternal Immune System in Healthy Pregnancy and Preeclampsia. *Front Immunol* (2019) 10:1305. doi: 10.3389/fimmu.2019.01305
75. Schober L, Radnai D, Schmitt E, Mahnke K, Sohn C, Steinborn A. Term and Preterm Labor: Decreased Suppressive Activity and Changes in Composition of the Regulatory T-Cell Pool. *Immunol Cell Biol* (2012) 90:935–44. doi: 10.1038/icb.2012.33
76. Whirledge S, Cidlowski JA. Glucocorticoids and Reproduction: Traffic Control on the Road to Reproduction. *Trends Endocrinol Metab* (2017) 28:399–415. doi: 10.1016/j.tem.2017.02.005
77. Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, de Paco Matallana C, et al. Aspirin *Versus* Placebo in Pregnancies at High Risk for Preterm Preeclampsia. *N Engl J Med* (2017) 377:613–22. doi: 10.1056/NEJMoa1704559
78. Wagner N-M, Brandhorst G, Czepluch F, Lankeit M, Eberle C, Herzberg S, et al. Circulating Regulatory T Cells Are Reduced in Obesity and may Identify Subjects at Increased Metabolic and Cardiovascular Risk. *Obesity* (2013) 21:461–8. doi: 10.1002/oby.20087

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APPENDIX 1 SEARCH TERMS

1. PubMed

Theme 1: Maternal T Cells in Pregnancy

- ((maternal) OR (mother)) AND ((regulatory T cells) OR (Tregs)) AND (pregnancy)

Theme 2: Fetal T Cells in Pregnancy

- ((Fetal) OR (Fetal)) AND ((Regulatory T Cells) OR (Tregs) OR (Regulatory T Lymphocyte)) AND (Pregnancy)

Theme 3: Adverse Outcomes

- ((Preeclampsia) OR (preterm birth) OR (miscarriage)) AND ((Regulatory T cells) OR (Tregs) OR (Regulatory T lymphocyte)) AND (pregnancy)

2. Embase

Theme 1: Maternal T Cells in Pregnancy

- ((maternal OR mother) AND 'regulatory t lymphocytes' OR 'regulatory t cells' OR 'tregs'/exp OR tregs) AND ('pregnancy'/exp OR pregnancy)

Theme 2: Fetal T Cells in Pregnancy

- ((((Fetal OR Fetal) AND 'Regulatory T Cells' OR Tregs OR Regulatory T Lymphocyte) AND Pregnancy))

Theme 3: Adverse Outcomes

- ((preeclampsia) OR 'preterm birth' OR 'preterm labor' AND 'tregs') AND ('pregnancy')

3. Cochrane

Theme 1: Maternal T Cells in Pregnancy

- (maternal):ti,ab,kw OR (mother):ti,ab,kw AND (Tregs):ti,ab,kw OR (T regulatory lymphocytes):ti,ab,kw OR (T regulatory cells):ti,ab,kw AND (pregnancy):ti,ab,kw

Theme 2: Fetal T Cells in Pregnancy

- (Fetal):ti,ab,kw OR (Fetal):ti,ab,kw AND (Tregs):ti,ab,kw OR (Regulatory T Cells):ti,ab,kw OR (Regulatory T Lymphocytes):ti,ab,kw AND (Pregnancy):ti,ab,kw

Theme 3: Adverse Outcomes

- (preeclampsia):ti,ab,kw OR (preterm birth):ti,ab,kw OR (miscarriage):ti,ab,kw AND (Tregs):ti,ab,kw OR (regulatory T lymphocyte):ti,ab,kw OR (regulatory T cells):ti,ab,kw AND (pregnancy):ti,ab,kw

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REVIEW
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Acute Atherosis Lesions at the Fetal-Maternal Border: Current Knowledge and Implications for Maternal Cardiovascular Health

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Decidua basalis, the endometrium of pregnancy, is an important interface between maternal and fetal tissues, made up of both maternal and fetal cells. Acute atherosis is a uteroplacental spiral artery lesion. These patchy arterial wall lesions containing foam cells are predominantly found in the decidua basalis, at the tips of the maternal arteries, where they feed into the placental intervillous space. Acute atherosis is prevalent in preeclampsia and other obstetric syndromes such as fetal growth restriction. Causal factors and effects of acute atherosis remain uncertain. This is in part because decidua basalis is challenging to sample systematically and in large amounts following delivery. We summarize our decidua basalis vacuum suction method, which facilitates tissue-based studies of acute atherosis. We also describe our evidence-based research definition of acute atherosis. Here, we comprehensively review the existing literature on acute atherosis, its underlying mechanisms and possible short- and long-term effects. We propose that multiple pathways leading to decidual vascular inflammation may promote acute atherosis formation, with or without poor spiral artery remodeling and/or preeclampsia. These include maternal alloreactivity, ischemia-reperfusion injury, preexisting systemic inflammation, and microbial infection. The concept of acute atherosis as an inflammatory lesion is not novel. The lesions themselves have an inflammatory phenotype and resemble other arterial lesions of more extensively studied etiology. We discuss findings of concurrently dysregulated proteins involved in immune regulation and cardiovascular function in women with acute atherosis. We also propose a novel hypothesis linking cellular fetal microchimerism, which is prevalent in women with preeclampsia, with acute atherosis in pregnancy and future cardiovascular and neurovascular disease. Finally, women with a history of preeclampsia have an increased risk of premature cardiovascular disease. We review whether presence of acute atherosis may identify women at especially high risk for premature cardiovascular disease.

Keywords: acute atherosis, inflammation, decidua basalis, preeclampsia, tolerance, cardiovascular disease, placenta, microchimerism

INTRODUCTION

Arterial lesions specific to the spiral arteries at the fetal-maternal border were first reported in 1945 (1). These lesions were later termed acute atherosis, described as lipid-laden foam cells within the intima, surrounded by fibrinoid necrosis and perivascular immune cell infiltrate (2, 3). Acute atherosis is associated with lower birthweight (4) and lower placental weight (5), and some studies show that acute atherosis may be correlated with an antiangiogenic profile (6, 7), all three of which are indicators of placental dysfunction. Moreover, the well documented high concomitance of acute atherosis and preeclampsia and other obstetric syndromes suggests shared underlying mechanisms (8–25).

Causal factors and effects of acute atherosis during pregnancy, as well as the long-term effects on maternal cardiovascular health, remain uncertain. There are several constraints on studying the acute atherosis lesions histologically. Systematic sampling of the decidua in large amounts following delivery is quite difficult, and a uniform, evidence-based research definition of acute atherosis lesions is historically lacking. As discussed below, both issues have been addressed by us (22, 26). However, even when these constraints are overcome, only a subset of the decidua can realistically be evaluated in any morphological tissue study, thus one can only with certainty determine the presence of acute atherosis, and never the definitive absence.

Acute Atherosis Sampling Methodology

The ideal method for studying the impact of decidual acute atherosis on placental function requires specimens of a challenging nature; there are today only some very rare hysterectomy specimens of severely preeclamptic women with the placenta still *in situ* (27). In one such published case report, acute atherosis of spiral arteries with severe narrowing of the vascular lumen were associated with substantial infarcted areas in the overlying parts of the placenta (28). Moreover, in this case the lesions could be traced as deeply as the inner myometrium, implying that the severity of the placental defects may be related to the depth of the lesions. It is also noteworthy in this study that remodeling of the placental bed spiral arteries, including the myometrial segments, was absolutely normal in a few invaded spiral arteries at the very center of the placental bed. This suggests that non-invaded, more laterally situated vessels run a higher risk of developing acute atherosis, again highlighting the need for uniform sampling of relevant tissues for the study of acute atherosis, preferably of the whole placental bed.

Several methods have been employed for sampling decidua basalis for research purposes. These include placental bed biopsies (29), biopsies from the basal plate of delivered placentas (30), and our method of vacuum suctioning the placental bed (31). Of these sampling methods, placental bed punch or knife biopsy is the most invasive, but has the advantage of providing myometrium, which is needed if the goal is to study spiral artery remodeling or other features of this tissue (32). Biopsies from delivered placentas is the least invasive sampling method, and will yield moderate samples of decidua basalis tissue. However, if the goal is to study decidua basalis alone, our vacuum suction technique is the superior method (32–34).

The vacuum suction technique is performed during caeserean section, after delivery of the placenta, by applying vacuum suction to the uterine wall. This method has the advantages of an unbiased sampling and a large tissue yield. It is also time efficient and without danger to maternal health (34). One drawback is that tissue orientation is lost due to suctioning. Still, the vacuum suction method provides tissue applicable for acute atherosis research. We showed that higher rates of acute atherosis detection was achieved using vacuum suction samples, as compared to routinely sampled basal surface placental tissue and fetal membrane roll biopsies from the same pregnancies (33). The rate of decidual acute atherosis is thus likely underestimated in most studies, and we recommend using the vacuum suction technique if the goal is to study the lesions independent of tissue orientation. Our large Oslo Pregnancy Biobank, consisting of decidual tissue collected during elective cesarean section, along with placental tissue biopsies, fetal (umbilical artery as well as umbilical vein) and maternal blood samples, amniotic fluid, and maternal muscle and fat tissue biopsies, has enabled multiple studies comparing the presence of decidual acute atherosis and dysregulated features of other anatomical compartments (20–26, 31, 33, 35–41).

Nonuniformity in Acute Atherosis Definitions

Historically, a uniform definition of acute atherosis has been lacking. This may have led to discrepancies in reported rates of acute atherosis across pregnancy groups. In addition, differences in patient populations studied as well as in tissue collection and evaluation methodology (e.g. antibody selection) may have contributed. Moreover, clear definitions of perivascular infiltrate (PVI) and fibrinoid necrosis have been lacking. We set out to address these issues by attempting to establish an evidence-based research definition of acute atherosis (22). After examining 278 decidua basalis samples, we observed that perivascular leukocyte infiltrates and increased fibrinoid did not always correlate with adjacent foam cell lesions. Instead, we concluded that these are features of the decidual pathology of preeclampsia, while $CD68^+$ foam cells are an essential aspect of acute atherosis (**Figure 1**). Thus, we proposed that acute atherosis should be diagnosed solely by the presence of foam cell lesions, defined as two or more intramural, adjacent, vacuolated $CD68^+$ cells. Nonetheless, throughout this review, we will include studies with other acute atherosis diagnosis criteria as well.

ACUTE ATHEROSIS ETIOLOGY

Hertig, who first described acute atherosis, proposed that vessel damage followed by lipophage infiltration is what initiates acute atherosis lesion development (1). Endothelial injury has long been suspected as integral to decidual lesion development by others as well (42). However, the lack of an association between acute

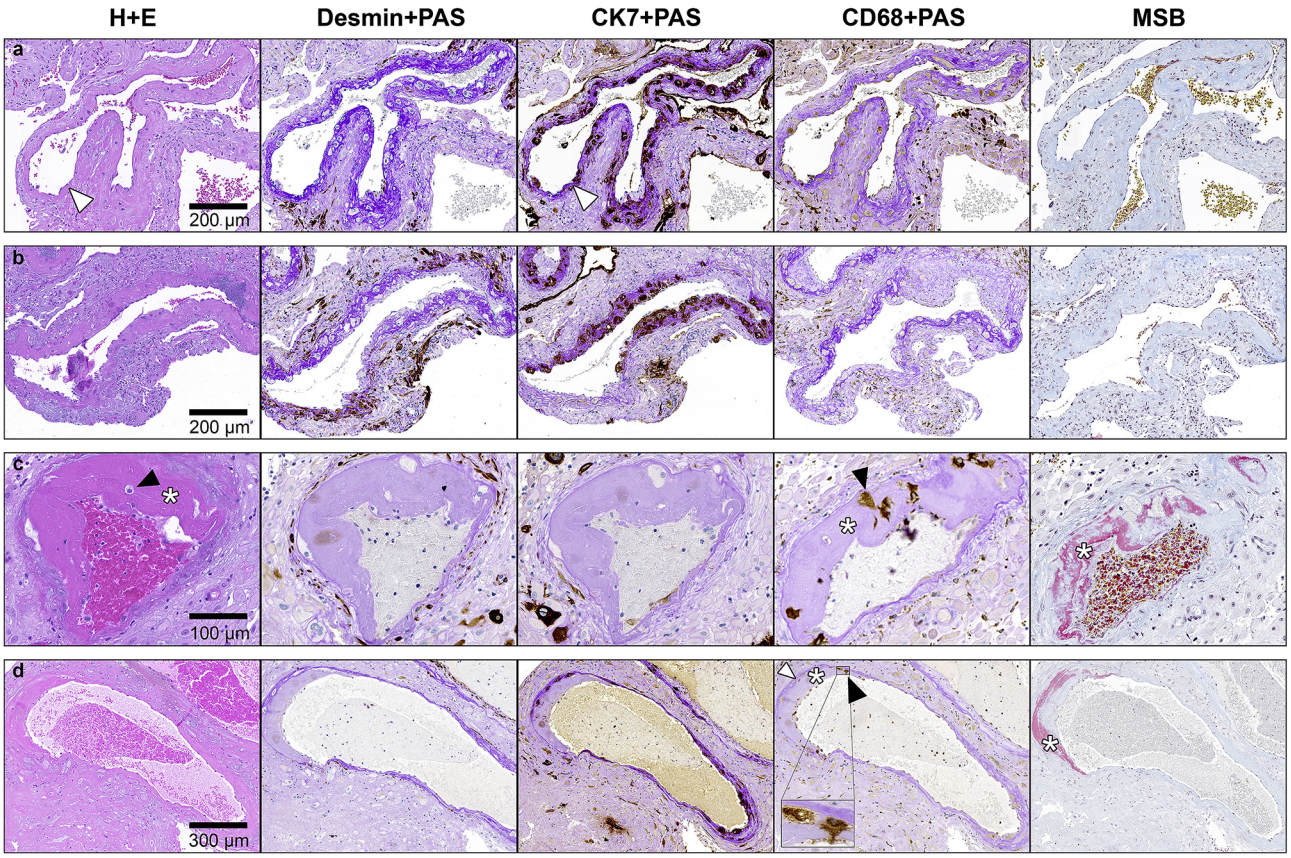

FIGURE 1 | Staining of serial FFPE sections of decidua basalis tissue to identify spiral arteries. Slides are stained with (from left to right) Hematoxylin + Eosin (H + E), desmin and Periodic acid–Schiff (PAS), cytokeratin 7 (CK7) and PAS, CD68 + PAS and Martius Scarlet Blue (MSB). Representative images of **(A)** spiral artery from a normotensive control with complete physiological transformation, characterized by presence of CK7-positive trophoblasts and intramural fibrinoid (bright purple upon PAS staining, white arrowhead) in the vessel wall, and complete absence of intramural smooth muscle cells (no desmin stain). **(B)** Spiral artery from a preeclampsia patient with partial physiological transformation characterized by intramural fibrinoid, trophoblasts and areas with traces of mural smooth muscle cells (desmin-positive). **(C)** Spiral artery with acute atherosis from same sample as in **(B)**, lacking bright purple fibrinoid and CK7-positive trophoblasts in the vessel wall. Traces of intramural smooth muscle cells (desmin positive) are seen. Fibrinoid necrosis is visible as a grey-pink material in the vessel wall (asterisk), which stains red upon MSB staining (asterisk). Erythrocytes in the lumen of the AA artery stains red-brown upon MSB staining. Intramural CD68-positive foam cells are present (black arrowhead). **(D)** Spiral artery from a preeclampsia patient with almost complete physiological transformation – evident by the lack of desmin-positive smooth muscle cells and the presence of CK7-positive trophoblasts – yet acute atherosis lesion is present (asterisk; fibrinoid necrosis, black arrowhead; foam cells, white arrowhead; purple physiological fibrinoid). Inset; higher power inset of foam cells. Reprinted from Fosheim et al. (35), with permission from the journal *Placenta*.

atherosis and the severity and duration of hypertension, or antihypertensive treatment, implies that hemodynamic forces alone are not adequate for lesion development (43). Similar to the heterogeneity of preeclampsia, acute atherosis is likely a multifactorial pathology with several pathways leading to an adverse uterovascular phenotype endpoint. We now have access to 75 years of research into the nature of the histological lesions known as acute atherosis, but have not finished elucidating the complexity of its etiological and mechanistic molecular constituents.

One such potential constituent, however, may be endothelin-1 (ET-1) (44). ET-1 is a highly potent vasoconstrictor (45). It is upregulated by mechanical stretch (46) and hypoxia (47), and plasma ET-1 is elevated in preeclampsia and gestational hypertension (48, 49). It exerts its effects by binding G-protein coupled receptors on vascular smooth muscle cells and endothelial cells (50), a byproduct of which may be intracellular lipid accumulation (51, 52). Thus, ET-1 may be a common trigger for lipid accumulation within endothelial cells in acute atherosis and in hepatocytes in the associated rare disease, acute fatty liver of pregnancy (44).

Another vasoconstrictor of interest is angiotensin II, which may play a role in the pathogenesis of atherosclerosis (53). We have postulated a role in acute atherosis for activating antibodies against the angiotensin II type 1 receptor ($AA\text{-}AT_1$), after demonstrating a clear association between $AA\text{-}AT_1$ and preeclampsia (54–56). Based on our cesarean delivery population, we were unable to demonstrate any association between $AA\text{-}AT_1$ and acute atherosis (21). However, angiotensin

II is known to work synergistically with ET-1 (57), and studying both of these vasoconstrictor systems simultaneously would shed more light on the possible involvement of G-protein cascades in acute atherosis development.

The regulator of G protein signaling 2 (RGS2) likely has implications for ET-1 and AA-AT_1 signaling (58). Interestingly, we have observed an association between acute atherosis and a genotype associated with lower RGS2 expression (38). If G-protein cascades indeed cause intracellular lipid accumulation, as proposed by Coffey (44), we would expect early acute atherosis lesions to contain lipid-laden endothelial or vascular muscle cells. Accordingly, arterial lesions containing vacuolated endothelial cells and myofibroblasts have been observed in first-trimester curetted endometrium samples from therapeutic and spontaneous abortions (59, 60). A higher incidence of such lesions was observed in primigravida as compared to multigravida (60). Primigravidity is associated with an increased risk of preeclampsia (36, 61), often considered due to excessive inflammation, although evidence is lacking (62). Whether these lesions are precursors to full-blown acute atherosis, and whether lipid accumulation within endothelial cells and myofibroblasts is indeed the insult that catalyzes intramural immune cell infiltration, remains to be investigated.

Acute atherosis shares morphological features with *early* atherosclerotic lesions, which is recognized as an inflammatory disease of the arterial walls (63). Both lesions present with increased numbers of intimal macrophages, lipid-laden foam cells, lipoprotein(a) throughout the vessel walls and extracellular droplets of lipid as well as similar expression of intracellular lipid-handling proteins (41, 64–67). Moreover, both acute atherosis and atherosclerosis are associated with preeclampsia and other states of systemic inflammation (68, 69).

However, there are several differences between acute atherosis and atherosclerosis. Firstly, vessel caliber differs enormously. Atherosclerosis is found in major arteries with a thick intimal layer, and the vessels are supplied with oxygen and nutrients from the vasa vasorum (70). Notably, the vasa vasorum may be instrumental as a source of lipids in the development of atherosclerosis (71–73). Spiral arteries are much smaller and do not have an external blood supply. Preeclampsia is associated with elevated lipid content in decidua basalis tissue, which may act as a source of lipid compounds for lesion development (26). Moreover, acute atherosis is not associated with *plasma* lipid contents, further indicating a local rather than a systemic excess of lipids (23). Secondly, research into the molecular composition of acute atherosis versus atherosclerosis reveals several dissimilarities. For instance, we have observed no LOX-1 positive endothelial cells or foam cells within the lesions of spiral arteries (41), while this lipid scavenger receptor is a key contributor to atherosclerotic development (74). Finally, endothelial activation is important in atherosclerosis (75), whereas evidence is conflicting with regards to endothelial status in acute atherosis. Although one study reported endothelial and interstitial extravillous trophoblast ICAM-1 expression in placentas with acute atherosis (76), we were unable to detect any ICAM-1 expression within the acute atherosis lesions (35). Moreover, the endothelial lining of the artery wall is often destroyed in acute atherosis and there is evidence of leakage of fibrin-like material from the circulation into the vessel walls (35, 42, 77). Accordingly, in a study of women with preeclampsia, we demonstrated elevated levels of thrombomodulin – a marker of endothelial dysfunction (78) or damage (79) – in those who had concomitant acute atherosis (39).

Acute atherosis is not found outside of the uterus (80). The lesions are focal and patchy, mainly localized downstream in the circulation, at the tips of the decidua basalis spiral arteries. The major remodeling problems occur upstream in the myometrium (67, 80). Yet, there is a link between acute atherosis formation and poor remodeling, as lesions are commonly found downstream of inadequately remodeled spiral arteries (77). The fully remodeled decidual segment of the spiral artery may be considered as being somewhat "naked" and is then likely more at risk for attacks both from the inside (by luminal, circulating factors) as well as from the outside (by components in the surrounding decidual tissue) in addition to being especially exposed to ischemia-reperfusion injury due to turbulent blood flow. Specifically, we postulate that these areas are especially exposed to local inflammatory signaling molecules. This may be compounded by their unique local environment close to the semi-allogenic fetal cells and the resulting inflammatory changes, potentially explaining why this uteroplacental location seems to be a prerequisite for the development of these particular atherosis lesions.

Inflammation does indeed appear to be clearly linked to acute atherosis development. Spiral arteries affected by acute atherosis contain huge deposits of IgM, as well as smaller amounts of IgG and IgA within the arterial wall. In addition to immunoglobulins, complement component 3 (C3) is often observed within acute atherosis lesions (81–86). In addition, early immunohistochemistry studies of the leukocyte infiltrate demonstrated T lymphocytes in acute atherosis (87). We later expanded on these findings, demonstrating increased concentrations of $CD3^+$, $CD8^+$ and $CD3^+CD8^-$ intramural T cells in the walls of spiral arteries with acute atherosis compared to arteries from samples without acute atherosis (37). Higher numbers of $CD3^+$ and $CD3+CD8^-$ T cells were also observed in the surrounding perivascular space. Furthermore, a study by Gill and colleagues conducting flow cytometry of basal plate samples demonstrated higher numbers of M1-macrophages in acute atherosis, displaying a pro-inflammatory phenotype (88). Fluorescence staining revealed M1-macrophage localization within vessel walls of spiral arteries affected by acute atherosis. Interestingly, a recent study from India showed that the incidence of acute atherosis – after exclusion of placental associated syndromes like fetal growth restriction, pregnancy hypertension and diabetes – was significantly higher in asymptomatic or mildly symptomatic SARS-CoV-2 positive pregnant women as compared to SARS-CoV-2 negative pregnant women (89). Finally, another support of acute atherosis representing an inflammatory lesion is its resemblance to systemic vasculitis, a general term applied to inflammation of vessel walls that progresses to fibrinoid necrosis (90, 91).

MANY ROADS TO DECIDUAL INFLAMMATION

We hypothesize that several mechanisms may trigger acute atherosis. Our hypothesis places inflammation at the center of lesion and as the final common pathway converged upon by these different triggers (69). We believe these mechanisms may act individually or in concert to produce acute atherosis, as illustrated in **Figure 2**.

Maternal Alloreactivity

Human pregnancy is a dynamic balancing act for the maternal immune system. The fetal allograft must peacefully coexist with the maternal immune and cardiovascular systems, whilst the

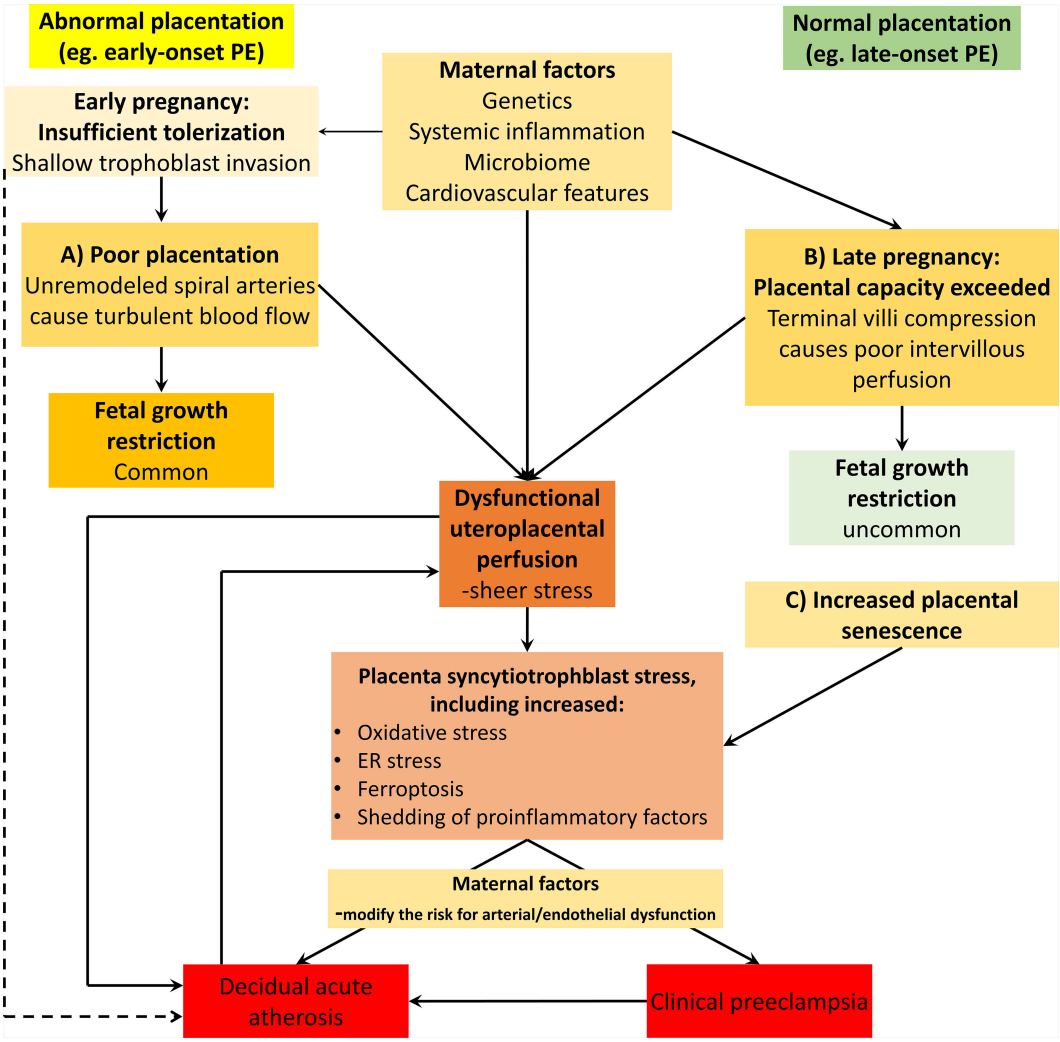

FIGURE 2 | Acute atherosis presence across the several forms of placental syndrome forms. Acute atherosis has been found in several forms of placental syndromes, many of these associated with spiral artery remodeling deficiencies, including early-onset preeclampsia (PE) (illustrated on the left side of the Figure). This figure also illustrates our concepts of how acute atherosis may be present also in late forms of preeclampsia (illustrated on the right side of the Figure), or indeed in any other forms of placental dysfunction without spiral artery remodeling defects. Preplacentation factors impacts the early and ensuing placentation processes, and includes both fetal-maternal tolerization processes as well as endometrial health (92). In this model, acute atherosis is seen as a consequence of any form of placental dysfunction and its underlying mechanisms (69). The triggers include both the uteroplacental malperfusion pathway secondary to spiral artery remodeling problems (as in early-onset preeclampsia) as well as the late-onset preeclampsia forms with other causes of placental malperfusion and syncytiotrophoblast stress (92, 93). Furthermore, this model also proposes that acute atherosis itself may represent a risk factor for placental dysfunction and preeclampsia. This is in line with acute atherosis developing very early in women with excessive vascular inflammation, such as in systemic lupus erythematosus, who also have a high risk for developing early-onset preeclampsia, severe fetal growth restriction and intrauterine death, all severe clinical aspects of placental dysfunction. The line from the tolerization box to acute atherosis illustrate how HLA-C/KIR interactions and alterations in level of immune-dampening molecules such as sHLA-G could contribute to lesion promotion at the maternal-fetal interface, as discussed in this review. The maternal factors promoting the clinical forms of preeclampsia (e.g. early- and late-onset) as well as atherosis development include cardiovascular, inflammatory and metabolic factors. Also, any form of clinical preeclampsia, with excessive circulation of proinflammatory factors and hypertension may add to the risk for atherosis development, although hypertension in itself is not a mandatory requirement.

mother and fetus simultaneously remain protected against microbial infections. Under any other circumstances, immune cells would quickly target genetically foreign tissue. However, a cascade of immune-modulating molecules acting throughout pregnancy enable the conceptus to evade rejection until parturition. The innate immune system is strengthened, while adaptive immunity is weakened (94). Serial blood samples collected at different time points during pregnancy have revealed precise timing of particular immunological changes (95). Aghaeepour and colleagues suggest deviations from this "immune clock of human pregnancy" could indicate pregnancy-related pathologies.

The fetus inherits half of its genetic material from the mother, and the other half from the father. Given the extremely high variability in major histocompatibility complex (MHC) genetics, it is unlikely that the paternally inherited fetal MHC alleles are identical to the maternally inherited fetal MHC alleles. Fetal trophoblasts circumvent this obstacle and avoid rejection by local maternal immune cells by not expressing most classical MHC class I or class II surface molecules (96). They do, however, express human leukocyte antigen (HLA) C and the non-classical MHC molecules HLA-G, HLA-E and HLA-F (96–98). HLA-C and HLA-G attenuate immune activation by binding to killer immunoglobulin like receptors (KIR), which are abundantly expressed on maternally derived immune cells (99, 100). KIR activation prevents cytotoxicity by alloreactive T-cells (101), and may induce apoptosis of activated T-cells and NK cells (102, 103). Invading extravillous trophoblasts rely on these KIR/HLA-interactions to avoid immune cell attacks. In fact, there is a positive correlation between the amount of surface HLA-G expression and the depth of trophoblast invasion into the decidua (104).

Several factors may influence these tolerogenic pathways. As HLA-C is the only polymorphic histocompatibility antigen expressed by fetal cells at the fetal-maternal interface, paternal HLA-C genotype is particularly important. In fact, HLA-C mismatched pregnancies are characterized by a higher percentage of activated maternal T-cells (105). Moreover, the combination of fetal HLA-C and maternal killer immunoglobulin like receptor (KIR) genotypes may greatly predispose pregnancies to preeclampsia (106). We have expanded on this finding by showing that the combination of fetal HLA-C2 with the maternal KIR-B haplotype was significantly associated with acute atherosis in preeclampsia (25).

Similarly, inadequate induction of tolerance by HLA-G is detrimental to pregnancy health (107). Membrane bound HLA-G expression is lower on trophoblasts from preeclamptic placentas (108, 109). Circulating soluble HLA-G (sHLA-G) is also lower in preeclampsia throughout all trimesters, compared to pregnancies that remain normotensive (110). We have shown that maternal sHLA-G inversely correlates with the level of placental dysfunction, the latter evaluated by maternal levels of the antiangiogenic factor sFlt-1, or by the sFlt-1/PlGF ratio (36), and that fetal polymorphisms in the 3'UTR region of HLA-G are associated with presence of acute atherosis in preeclampsia (24). Our hypothesis is that these polymorphisms lead to altered HLA-G expression in the decidua basalis, affecting local fetal-maternal immune tolerance and in turn promoting development of acute atherosis. Failure to establish fetal-maternal tolerance may also influence trophoblast invasion into the decidua. These extravillous trophoblasts are involved in the plugging and remodeling of uteroplacental spiral arteries in early pregnancy (80).

Ischemia-Reperfusion Injury

The first 10 weeks of pregnancy, the spiral arteries extending from the placenta to the endometrial surface of the decidua are effectively plugged, and the fetus exists in a state of physiological hypoxia (111, 112). At the end of the first trimester, the maternal vessels of the decidua open up and the placenta is submerged in maternal blood (111). This marks a dramatic shift in the fetoplacental exposure to the maternal cardiovascular and system.

Throughout pregnancy, uteroplacental blood flow increases, reaching up to 750 ml/minute at term, about 25% of maternal cardiac output (113). At the same time, the approximate 5-fold increase in diameter of the terminal coils of fully remodeled spiral arteries dramatically slows down the speed of the blood entering the intervillous space (111). Failure of proper spiral artery remodeling results in downstream placental malperfusion. The retention of smooth muscle within the arterial wall likely causes ischemia-reperfusion injury (114), and not impaired flow volume nor uteroplacental hypoperfusion. In addition, we argue that placental malperfusion may not be exclusively secondary to failure of spiral artery remodeling. Failed remodeling may be considered an "external" cause of placental malperfusion and is typically seen in early-onset preeclampsia. Late-onset preeclampsia affects a greater rate of women than the early-onset form, and spiral artery remodeling is rarely affected. In this setting, malperfusion may be caused by two "internal" pathways. In the one pathway, placental senescence causes a syncytiotrophoblast stress response as the pregnancy progresses towards term and thereafter. The other pathway occurs with particularly large placentas, such as in multiple gestations, in which compression leads to placental congestion and thereby malperfusion (93, 115). The ensuing disturbances in calcium-homeostasis may cause endoplasmic reticulum stress and initiate the unfolded protein response, ultimately leading to cell death (116, 117). Moreover, intracellular buildup of reactive oxygen species induces upregulation and secretion of the proinflammatory cytokines TNF and IL-1b (118, 119). ER stress and oxidative stress in placental tissues are both features of preeclampsia (31, 117, 120), and may also play a role in the strongly associated acute atherosis lesion development.

High pressure and turbulent blood flow may also damage the endothelial lining of the terminal coils of spiral arteries, as well as syncytiotrophoblasts coating placental villi. Analysis of hemodynamic forces on vascular endothelial cells has shown that disturbed blood flow and continuous low grade shear stress acting on the arterial wall may promote atherogenesis (121). This is in line with the increased incidence of atherosclerotic lesions at arterial branch points or sections with high curvature (122, 123). Endothelial cells possess surface molecules capable of detecting shear stress and inducing gene transcription through the Ras-

MAP kinase signaling pathway (124). Among several changes in gene expression is a transient upregulation of the monocyte chemotactic protein 1 (MCP-1) (125). Overexpression of MCP-1 attracts macrophages and may induce infiltration into vessel walls (126). Interestingly, endothelial MCP-1 is upregulated in preeclampsia (127, 128), possibly due to atherogenic blood flow, and could possibly be involved in $CD68^+$ cell recruitment to the sites of lesion development (129, 130).

Ferroptosis is a recently discovered mode of iron-dependent cell death (131, 132). Lipoxygenases and other enzymes may induce ferroptosis in a controlled manner (133). In addition, and more relevant to the topic at hand, ferroptosis may occur due to iron dysregulation and free-radical chain reactions, leading to hydroxyl and peroxyl radicals (133). Recently, ferroptosis has gained attention as a possible target against ischemia-reperfusion injury (134–136). Thus, ferroptosis may play a role in early-onset preeclampsia following incomplete spiral artery remodeling. Interestingly, huge amounts of iron have been observed in atherosclerotic lesions (137), and lipid peroxidation is known to play a significant role in atherogenesis (138). Moreover, animal experiments have shown alleviation of atherosclerosis through inhibition of ferroptosis (139). However, the role of iron-dependent cell death due to ischemia-reperfusion in acute atherosis development remains to be investigated.

Preexisting Systemic Inflammation

Systemic inflammation is associated with many chronic diseases, such as obesity, diabetes mellitus and cardiovascular disease, as reviewed in (140–142). Considering that even normal pregnancy is associated with elevated systemic inflammation (143), one would expect heightened baseline inflammation to be associated with higher rates of obstetric complications linked to the maternal immune system. This is indeed what is observed. Obesity, diabetes mellitus and high blood pressure are all substantial risk factors for miscarriage (144–146), preeclampsia (92, 147) and fetal neurodevelopmental disorders (148–150). In line with the effects of other chronic inflammatory conditions on pregnancy, pregnant women with autoimmune disease experience higher rates of hypertensive disorders of pregnancy, intrauterine growth restriction, preterm delivery and autism spectrum disorder in their offspring (151–154). Interestingly, autoimmune disorders are also commonly observed in association with acute atherosis (155, 156). In fact, lesions have been observed as early as the first trimester in women with systemic lupus erythematosus (157).

Microbial Infection

Many tissues first thought to be sterile have been shown to harbor dormant bacteria. This includes blood (158, 159), seminal fluid (160) and possibly the placenta (161) – although this latter claim is disputed (162). The source of these bacteria may be the gut (163), the oral cavity (164) or the urinary tract (165). The iron dysregulation and dormant microbes hypothesis proposes these bacteria may be resuscitated from dormancy by free iron and manifest a diverse range of chronic inflammatory diseases previously thought to not possess infectious properties such as preeclampsia and atherosclerotic disease (166). Viable bacteria release lipopolysaccharide (LPS) or lipoteichoic acid (LTA). This initiates a cascade of immune responses, including a dramatic upregulation of many circulating cytokines and other acute phase signaling molecules like serum amyloid A1 (SAA1) and C-reactive protein (167, 168). As extensively reviewed by Kell and Kenny, there are several lines of evidence pointing towards microbial contribution in the development of preeclampsia (169). There is high co-occurrence between bacterial infections and preeclampsia. Examples include *Chlamydia pneumoniae* (170, 171) and *Helicobacter pylori* (172). Several biomarkers associated with preeclampsia have also been linked to microbial infections, including sFlt-1/PlGF ratio (173, 174) and SAA1 (167). In support of the concept that preeclampsia development has a microbial component, is the virtual absence of preeclampsia in pregnancies with *Toxoplasma gondii* infection treated with anti-microbial medication (spiramycin) (175). Kell and others have also argued for the existence of a microbial component in atherosclerosis with quite compelling evidence (159, 166, 176). Patients with chronic bacterial infections are substantially more at risk for atherosclerosis (177, 178), and LPS is regularly used to generate animal models of the disease (179, 180). Moreover, atherosclerotic plaques contain bacterial DNA (181, 182) and elevated levels of iron (180), adding credence to the iron dysregulation and dormant microbes hypothesis. The similarities to atherosclerosis have led others to speculate that an infectious trigger underlies the development of acute atherosis as well (183, 184). However, this matter remains unsettled.

A NEW HYPOTHESIS LINKING CELLULAR FETAL MICROCHIMERISM WITH ACUTE ATHEROSIS IN PREGNANCY AND FUTURE CARDIOVASCULAR AND NEUROVASCULAR DISEASE

Cellular fetal microchimerism (cFMC) arises when cells of fetal origin are transferred to maternal blood and tissues during pregnancy (185). These cells are known to possess stem cell-like properties, capable of differentiating into endothelial cells, smooth muscle cells and even leukocytes (186, 187). During pregnancy, a lot of fetal material leaks into maternal circulation. While cell free fetal DNA and other debris is rapidly cleared following delivery (188) and likely completely absent from maternal systems postpartum, cFMC may persist for decades. In fact, fetal cells have been observed in maternal circulation up to 27 years postpartum, indicating that these cells may inhabit maternal systems throughout life (189). Restorative as well as detrimental effects have been attributed to cFMC, possibly tied to fetal-maternal histocompatibility (185). Of particular interest in the context of this review is the apparent detrimental effect of cFMC on autoimmunity (190, 191). The trigger has been proposed to be a maternal alloimmune response towards fetal cells expressing foreign HLA surface peptides (191). By far the majority of patients diagnosed with autoimmune disorders are women (192), which could partly be due to the acquisition of fetal cells during pregnancy.

In comparison to healthy pregnancies, circulating fetal microchimeric cells are more prevalent in pregnancies complicated by preeclampsia (193, 194) or severe fetal growth restriction combined with impaired placental perfusion (195). If this is due to increased cell transfer, reduced clearance or reduced migration from maternal blood into maternal tissues is unclear (185, 196). We hypothesize that when the placenta is dysfunctional, fetal cells leak more freely across into maternal blood and subsequently other tissues. These cells may then inhabit maternal vessels, in the presence or absence of endothelial damage, and induce a maternal anti-fetal immune response towards the vascular endothelium. This could explain the association between male cFMC and an increased cardiovascular mortality hazard ratio (197). However, this last observation was based on a total of only 5 cardiovascular deaths. A recent study on a much larger cohort found that male-origin microchimerism was associated with *reduced* risk of ischemic heart disease as well as no association between microchimerism and ischemic stroke (198).

Microchimerism is not unique to pregnancy. Low levels of donor cells may be acquired following solid organ transplantation (199). The presence of such microchimerism has been linked to graft acceptance (200) as well as graft rejection (201). As with autoimmune diseases and cFMC, the effect of donor microchimerism may depend on how well the host tolerates the graft. Interestingly, vascular lesions have been observed in arteries surrounding transplanted organs following kidney transplant rejection (202), after liver transplantation (203) and after heart transplantation (204). These lesions are characterized by large amounts of lipids, IgM and C3 (205), and thus bear striking resemblance to acute atherosis.

Our novel hypothesis states that placental dysfunction leads to augmented cFMC. If persistent in the circulation or alternatively engrafted in maternal endothelium, these semi-allogenic cells could cause further inflammation, particularly in vessel walls, and initiate development of inflammatory arterial lesions such as acute atherosis. As cFMC persist decades after pregnancy, there may be a role for these cells in the pathogenesis of chronic cardiovascular and neurovascular disease in the long term as well. We believe this hypothesis merits further testing in translational clinical studies.

CLINICAL TRANSLATION

During Pregnancy

Acute atherosis is associated with arterial thrombosis, placental infarction and perinatal death (22, 28, 30, 206, 207). This has led researchers to propose aspirin as a possible treatment for acute atherosis (208). Aspirin is an effective prophylactic treatment against thrombosis (209). Outside of pregnancy, aspirin is widely used for primary and secondary prevention of atherosclerotic CVD (210). Daily low-dose aspirin started during the first trimester has shown a substantially reduced risk of preeclampsia in women at high risk (211–213). Whether this effect is related to a reduced tendency of blood clot formation, and how this may relate to acute atherosis, remains to be investigated.

Acute atherosis lesions may also effectively reduce the diameter of uteroplacental spiral arteries, causing aberrant blood flow (69, 111). The evidence of this latter claim is, however, conflicting. Supporting this, an early study of only 6 cases showed a trend between acute atherosis and high uterine artery pulsatility index, which could reflect obstructed spiral artery blood flow (214). Furthermore, another research group showed that acute atherosis is associated with a higher incidence of placental lesions characteristic of maternal vascular hypoperfusion (215). Placental lesions are also associated with abnormal uterine velocimetry measurements among women with intrauterine fetal growth restriction (216). In contrast to these findings, one study found no association between uterine artery pulsatility index and acute atherosis (217). The reasons for these discrepant findings may be that the spiral arteries are not visualized directly by Doppler studies, but rather indirectly by studying the blood flow of the larger uterine arteries (214, 216–218). Uterine and spiral arteries differ much in structure, size and function. Uterine artery ultrasonography has historically been viewed as a reflection of spiral artery remodeling (219), but recent studies indicate that the radial arteries, as well as the maternal vascular system, may have a larger impact on uterine artery waveforms than the spiral arteries (218). Doppler ultrasonography is hence unlikely a reliable tool for diagnosing uteroplacental acute atherosis.

Postpartum: Targeting the Women at Highest Risk of Premature Cardiovascular Disease?

We have put forth the hypothesis that women with concomitant preeclampsia and acute atherosis are at especially high risk for developing atherosclerosis and premature cardiovascular disease (130). If pregnancy is a physiological stress test (220, 221), then preeclampsia is an unmasking of compromised maternal cardiovascular health. Time and time again preeclampsia has been linked to future maternal cardiovascular disease in one form or another (222–230). The severity of preeclampsia also correlates with ischemic heart disease incidence rate (231). Moreover, fetal manifestations of placental dysfunction, such as intrauterine growth restriction, further add to maternal risk of cardiovascular disease (232). Abnormal placentation also associates with infertility or subfertility, both of which are associated with poor long-term cardiovascular health (233, 234). As described above, acute atherosis may disturb placental perfusion (69, 111), and is associated with low birth weight (4) and low placental weight (5). Acute atherosis may thus play a role in producing and/or exacerbating maternal and fetal symptoms of placental dysfunction, associated with high cardiovascular disease risk.

There are striking similarities between acute atherosis and atherosclerosis, indicative of shared pathophysiology. We know that preeclampsia is associated with a substantial atherosclerotic load (235). Compared to normotensive women and women with gestational hypertension, women with preeclampsia have higher carotid intima-media thickness (CIMT) (236, 237) – increasingly used as a surrogate marker for preclinical atherosclerosis (238). In some studies, these differences remained evident up to 18 months postpartum (236, 237). However, other studies with longer follow-up did not corroborate these findings (239, 240).

Notably, one study unexpectedly reported *thinner* CIMT 7 Years postpartum in women with preeclampsia compared to controls (241). Instead, they found increased intima thickness as well as intima-media ratio in cases versus control. The authors suggest that these measures are preferable to the conventional CIMT for assessing cardiovascular disease risk in women with a history of preeclampsia. As lesions of the placental bed manifest during a shorter time span compared to atherosclerosis (possibly due to the proximity to the foreign fetus and the excessive inflammation of pregnancy), acute atherosis may possibly be used as an indicator of women with excessive atherosclerotic load (130).

Several studies have examined the link between decidual lesions and subsequent cardiovascular health. Two retrospective cohort studies by the same research group have revealed long-term cardiovascular consequences of decidual vasculopathy (242, 243). Decidual vasculopathy was in these studies defined by vascular fibrinoid necrosis and lipid-filled foam cells in the vascular wall, thus sharing some of the features traditionally used for acute atherosis (244). The first study examined cardiovascular parameters 2-74 months postpartum in women whose index pregnancies were complicated by preeclampsia. Women with concomitant decidual vasculopathy and preeclampsia had higher diastolic blood pressure, lower left ventricular stroke volume and higher total peripheral vascular resistance as compared to women with only preeclampsia (243). Decidual vasculopathy did, however, not correlate with circulating lipids or thrombophilia postpartum. The second study demonstrated a higher prevalence of chronic hypertension several years postpartum in women who had concomitant decidual vasculopathy and preeclampsia, compared to women who only had preeclampsia. These results remained significant after correcting for chronic hypertension before index pregnancy (242).

A small study comprising only 3 cases of acute atherosis showed higher levels of triglycerides and low-density lipoprotein in these women on the first day postpartum as compared to women without acute atherosis (245). Our group conducted a larger study where we also measured triglycerides and cholesterols (among other circulating biomarkers) the day of cesarean section. We observed no differences in circulating cardiovascular biomarkers between women with acute atherosis and women without. However, when restricting the analyses to women of advanced maternal age (age 36-44) we observed significantly elevated low-density lipoprotein and ApoB in women with acute atherosis (20). These studies highlight the potential use of acute atherosis in targeting women at particularly high risk of cardiovascular disease – a concept promoted by us and others previously (246, 247). Utilizing the more readily detected maternal vascular malperfusion lesions of the placenta has also been suggested (248), although some claim cardiovascular disease risk should be linked with atherosclerotic lesions of the uteroplacental artery instead of decidual basal artery or placental lesions (249).

FUTURE RESEARCH OPPORTUNITIES

As outlined above, there is substantial evidence backing up the concept of acute atherosis as a pregnancy-specific inflammatory arterial lesion. However, many uncertainties regarding acute atherosis remain. Firstly, the risk factors and triggers that initiate lesion development have not been fully elucidated, though there seems to be a consensus among researchers that endothelial damage is part of it. Damage could stem from ischemia-reperfusion injury, infections or excessive activation of G-protein cascades, to name a few. Further research into the molecular constituents of acute atherosis – in particular early lesion stages – could shed some light on this issue. Secondly, there are many candidates for drivers of inflammation and lesion development following endothelial insult. Further knowledge of which pathways play a substantial role could guide the development of prophylactic treatments of obstetric syndromes tightly associated with acute atherosis. This includes our suggestion of testing whether the use of anti-atherogenic statins during severe preeclampsia or fetal growth restriction, such as in women with systemic lupus, may ameliorate acute atherosis, improve uteroplacental perfusion and enhance pregnancy outcome (69). Thirdly, the long-lasting implications of pregnancy affected by acute atherosis on maternal health need further research. There is a clear lack of studies with hard endpoints to show if acute atherosis, as we have proposed, can be used to identify women at substantial risk for premature cardiovascular disease and death.

AUTHOR CONTRIBUTIONS

DPJ wrote the review. HEF, GMJ, IKF, KM, PA-K, RD, MS, and ACS revised the manuscript and gave expert scientific input on its content. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Hertig AT. Vascular Pathology in the Hypertensive Albuminuric Toxemias of Pregnancy. *Clinics* (1945) 4:602–14.
2. Zeek PM, Assali NS. Vascular Changes in the Decidua Associated With Eclamptogenic Toxemia of Pregnancy. *Am J Clin Pathol* (1950) 20 (12):1099–109. doi: 10.1093/ajcp/20.12.1099
3. Sexton LI, Hertig AT, Reid DE, Kellogg FS, Patterson WS. Premature Separation of the Normally Implanted Placenta; a Clinicopathological Study of 476 Cases. *Am J Obstet Gynecol* (1950) 59(1):13–24. doi: 10.1016/0002-9378(50)90335-8
4. Frusca T, Morassi L, Pecorelli S, Grigolato P, Gastaldi A. Histological Features of Uteroplacental Vessels in Normal and Hypertensive Patients in Relation to Birthweight. *Br J Obstet Gynaecol* (1989) 96(7):835–9. doi: 10.1111/j.1471-0528.1989.tb03324.x

5. Stark MW, Clark L, Craver RD. Histologic Differences in Placentas of Preeclamptic/Eclamptic Gestations by Birthweight, Placental Weight, and Time of Onset. *Pediatr Dev Pathol* (2014) 17(3):181–9. doi: 10.2350/13-09-1378-OA.1
6. Korzeniewski SJ, Romero R, Chaiworapongsa T, Chaemsaithong P, Kim CJ, Kim YM, et al. Maternal Plasma Angiogenic Index-1 (Placental Growth Factor/Soluble Vascular Endothelial Growth Factor Receptor-1) is a Biomarker for the Burden of Placental Lesions Consistent With Uteroplacental Underperfusion: A Longitudinal Case-Cohort Study. *Am J Obstet Gynecol* (2016) 214(5):629 e1– e17. doi: 10.1016/j.ajog.2015.11.015
7. Baltajian K, Hecht JL, Wenger JB, Salahuddin S, Verlohren S, Perschel FH, et al. Placental Lesions of Vascular Insufficiency are Associated With Anti-Angiogenic State in Women With Preeclampsia. *Hypertens Pregnancy* (2014) 33(4):427–39. doi: 10.3109/10641955.2014.926914
8. Ghidini A, Salafia CM, Pezzullo JC. Placental Vascular Lesions and Likelihood of Diagnosis of Preeclampsia. *Obstet Gynecol* (1997) 90(4 Pt 1):542–5. doi: 10.1016/S0029-7844(97)00360-8
9. Brosens I, Dixon HG, Robertson WB. Fetal Growth Retardation and the Arteries of the Placental Bed. *Br J Obstet Gynaecol* (1977) 84(9):656–63. doi: 10.1111/j.1471-0528.1977.tb12676.x
10. De Wolf F, Brosens I, Renaer M. Fetal Growth Retardation and the Maternal Arterial Supply of the Human Placenta in the Absence of Sustained Hypertension. *Br J Obstet Gynaecol* (1980) 87(8):678–85. doi: 10.1111/j.1471-0528.1980.tb04601.x
11. Althabe O, Labarrere C, Telenta M. Maternal Vascular Lesions in Placentae of Small-for-Gestational-Age Infants. *Placenta* (1985) 6(3):265–76. doi: 10.1016/S0143-4004(85)80056-4
12. McFadyen IR, Price AB, Geirsson RT. The Relation of Birthweight to Histological Appearances in Vessels of the Placental Bed. *Br J Obstet Gynaecol* (1986) 93(5):476–81. doi: 10.1111/j.1471-0528.1986.tb08657.x
13. Khong TY. Acute Atherosis in Pregnancies Complicated by Hypertension, Small-for-Gestational-Age Infants, and Diabetes Mellitus. *Arch Pathol Lab Med* (1991) 115(7):722–5.
14. Pijnenborg R, Anthony J, Davey DA, Rees A, Tiltman A, Vercruysse L, et al. Placental Bed Spiral Arteries in the Hypertensive Disorders of Pregnancy. *Br J Obstet Gynaecol* (1991) 98(7):648–55. doi: 10.1111/j.1471-0528.1991.tb13450.x
15. Meekins JW, Pijnenborg R, Hanssens M, McFadyen IR, van Asshe A. A Study of Placental Bed Spiral Arteries and Trophoblast Invasion in Normal and Severe Pre-Eclamptic Pregnancies. *Br J Obstet Gynaecol* (1994) 101 (8):669–74. doi: 10.1111/j.1471-0528.1994.tb13182.x
16. Jain K, Kavi V, Raghuveer CV, Sinha R. Placental Pathology in Pregnancy-Induced Hypertension (PIH) With or Without Intrauterine Growth Retardation. *Indian J Pathol Microbiol* (2007) 50(3):533–7.
17. Sousa FL, Sass N, Camano L, Stavale JN, Mesquita MR, Souza EV, et al. [Morphology of the Vascular Placental Bed in Chronic Arterial Hypertension]. *Rev Assoc Med Bras (1992)* (2008) 54(6):537–42. doi: 10.1590/S0104-42302008000600019
18. Milosevic-Stevanovic J, Krstic M, Radovic-Janosevic D, Stefanovic M, Antic V, Djordjevic I. Preeclampsia With and Without Intrauterine Growth Restriction-Two Pathogenetically Different Entities? *Hypertens Pregnancy* (2016) 35(4):573–82. doi: 10.1080/10641955.2016.1212872
19. Tateishi A, Ohira S, Yamamoto Y, Kanno H. Histopathological Findings of Pregnancy-Induced Hypertension: Histopathology of Early-Onset Type Reflects Two-Stage Disorder Theory. *Virchows Arch* (2018) 472(4):635–42. doi: 10.1007/s00428-018-2315-3
20. Moe K, Alnaes-Katjavivi P, Storvold GL, Sugulle M, Johnsen GM, Redman CWG, et al. Classical Cardiovascular Risk Markers in Pregnancy and Associations to Uteroplacental Acute Atherosis. *Hypertension* (2018) 72 (3):695–702. doi: 10.1161/HYPERTENSIONAHA.118.10964
21. Rieber-Mohn AB, Sugulle M, Wallukat G, Alnaes-Katjavivi P, Leite Storvold G, Bolstad N, et al. Auto-Antibodies Against the Angiotensin II Type I Receptor in Women With Uteroplacental Acute Atherosis and Preeclampsia at Delivery and Several Years Postpartum. *J Reprod Immunol* (2018) 128:23–9. doi: 10.1016/j.jri.2018.05.008
22. Alnaes-Katjavivi P, Lyall F, Roald B, Redman CW, Staff AC. Acute Atherosis in Vacuum Suction Biopsies of Decidua Basalis: An Evidence Based Research Definition. *Placenta* (2016) 37:26–33. doi: 10.1016/j.placenta.2015.10.020
23. Harsem NK, Roald B, Braekke K, Staff AC. Acute Atherosis in Decidual Tissue: Not Associated With Systemic Oxidative Stress in Preeclampsia. *Placenta* (2007) 28(8-9):958–64. doi: 10.1016/j.placenta.2006.11.005
24. Johnsen GM, Fjeldstad HES, Drabbels JJM, Haasnoot GW, Eikmans M, Storvold GL, et al. A Possible Role for HLA-G in Development of Uteroplacental Acute Atherosis in Preeclampsia. *J Reprod Immunol* (2021) 144:103284. doi: 10.1016/j.jri.2021.103284
25. Johnsen GM, Storvold GL, Drabbels JJM, Haasnoot GW, Eikmans M, Spruyt-Gerritse MJ, et al. The Combination of Maternal KIR-B and Fetal HLA-C2 is Associated With Decidua Basalis Acute Atherosis in Pregnancies With Preeclampsia. *J Reprod Immunol* (2018) 129:23–9. doi: 10.1016/j.jri.2018.07.005
26. Staff AC, Ranheim T, Khoury J, Henriksen T. Increased Contents of Phospholipids, Cholesterol, and Lipid Peroxides in Decidua Basalis in Women With Preeclampsia. *Am J Obstet Gynecol* (1999) 180(3 Pt 1):587–92. doi: 10.1016/S0002-9378(99)70259-0
27. Brosens I, Dixon HG. The Anatomy of the Maternal Side of the Placenta. *J Obstet Gynaecol Br Commonw* (1966) 73(3):357–63. doi: 10.1111/j.1471-0528.1966.tb05175.x
28. Brosens I, Renaer M. On the Pathogenesis of Placental Infarcts in Pre-Eclampsia. *J Obstet Gynaecol Br Commonw* (1972) 79(9):794–9. doi: 10.1111/j.1471-0528.1972.tb12922.x
29. Lyall F, Robson SC, Bulmer JN. Spiral Artery Remodeling and Trophoblast Invasion in Preeclampsia and Fetal Growth Restriction: Relationship to Clinical Outcome. *Hypertension* (2013) 62(6):1046–54. doi: 10.1161/HYPERTENSIONAHA.113.01892
30. Kim YM, Chaemsaithong P, Romero R, Shaman M, Kim CJ, Kim JS, et al. The Frequency of Acute Atherosis in Normal Pregnancy and Preterm Labor, Preeclampsia, Small-for-Gestational Age, Fetal Death and Midtrimester Spontaneous Abortion. *J Matern Fetal Neonatal Med* (2015) 28(17):2001–9. doi: 10.3109/14767058.2014.976198
31. Staff AC, Halvorsen B, Ranheim T, Henriksen T. Elevated Level of Free 8-Iso-Prostaglandin F2alpha in the Decidua Basalis of Women With Preeclampsia. *Am J Obstet Gynecol* (1999) 181(5 Pt 1):1211–5. doi: 10.1016/S0002-9378(99)70110-9
32. Burton GJ, Sebire NJ, Myatt L, Tannetta D, Wang YL, Sadovsky Y, et al. Optimising Sample Collection for Placental Research. *Placenta* (2014) 35 (1):9–22. doi: 10.1016/j.placenta.2013.11.005
33. Alnaes-Katjavivi P, Roald B, Staff AC. Uteroplacental Acute Atherosis in Preeclamptic Pregnancies: Rates and Clinical Outcomes Differ by Tissue Collection Methods. *Pregnancy Hypertens* (2020) 19:11–7. doi: 10.1016/j.preghy.2019.11.007
34. Harsem NK, Staff AC, He L, Roald B. The Decidual Suction Method: A New Way of Collecting Decidual Tissue for Functional and Morphological Studies. *Acta Obstet Gynecol Scand* (2004) 83(8):724–30. doi: 10.1111/j.0001-6349.2004.00395.x
35. Fosheim IK, Alnaes-Katjavivi P, Redman C, Roald B, Staff AC, Storvold GL. Acute Atherosis of Decidua Basalis; Characterization of Spiral Arteries, Endothelial Status and Activation. *Placenta* (2019) 82:10–6. doi: 10.1016/j.placenta.2019.04.006
36. Jacobsen DP, Lekva T, Moe K, Fjeldstad HES, Johnsen GM, Sugulle M, et al. Pregnancy and Postpartum Levels of Circulating Maternal sHLA-G in Preeclampsia. *J Reprod Immunol* (2021) 143:103249. doi: 10.1016/j.jri.2020.103249
37. Johnsen GM, Storvold GL, Alnaes-Katjavivi PH, Roald B, Golic M, Dechend R, et al. Lymphocyte Characterization of Decidua Basalis Spiral Arteries With Acute Atherosis in Preeclamptic and Normotensive Pregnancies. *J Reprod Immunol* (2019) 132:42–8. doi: 10.1016/j.jri.2019.03.003
38. Kvehaugen AS, Melien O, Holmen OL, Laivuori H, Oian P, Andersgaard AB, et al. Single Nucleotide Polymorphisms in G Protein Signaling Pathway Genes in Preeclampsia. *Hypertension* (2013) 61(3):655–61. doi: 10.1161/HYPERTENSIONAHA.111.00331
39. Lekva T, Sugulle M, Moe K, Redman C, Dechend R, Staff AC. Multiplex Analysis of Circulating Maternal Cardiovascular Biomarkers Comparing Preeclampsia Subtypes. *Hypertension* (2020) 75(6):1513–22. doi: 10.1161/HYPERTENSIONAHA.119.14580
40. Staff AC, Ranheim T, Halvorsen B. Augmented PLA2 Activity in Pre-Eclamptic Decidual Tissue–a Key Player in the Pathophysiology of 'Acute Atherosis' in Pre-Eclampsia? *Placenta* (2003) 24(10):965–73. doi: 10.1016/S0143-4004(03)00175-9

41. Fosheim IK, Johnsen GM, Alnaes-Katjavivi P, Turowski G, Sugulle M, Staff AC. Decidua Basalis and Acute Atherosis: Expression of Atherosclerotic Foam Cell Associated Proteins. *Placenta* (2021) 107:1–7. doi: 10.1016/j.placenta.2021.03.001
42. De Wolf F, Brosens I, Robertson WB. Ultrastructure of Uteroplacental Arteries. *Contrib Gynecol Obstet* (1982) 9:86–99. doi: 10.1159/000406847
43. Khong TY, Pearce JM, Robertson WB. Acute Atherosis in Preeclampsia: Maternal Determinants and Fetal Outcome in the Presence of the Lesion. *Am J Obstet Gynecol* (1987) 157(2):360–3. doi: 10.1016/S0002-9378(87)80172-2
44. Coffey CG. Cellular Bases for the Lipid-Related Aspects of Preeclampsia. *Med Hypotheses* (2003) 60(5):716–9. doi: 10.1016/S0306-9877(03)00044-6
45. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, et al. A Novel Potent Vasoconstrictor Peptide Produced by Vascular Endothelial Cells. *Nature* (1988) 332(6163):411–5. doi: 10.1038/332411a0
46. Macarthur H, Warner TD, Wood EG, Corder R, Vane JR. Endothelin-1 Release From Endothelial Cells in Culture is Elevated Both Acutely and Chronically by Short Periods of Mechanical Stretch. *Biochem Biophys Res Commun* (1994) 200(1):395–400. doi: 10.1006/bbrc.1994.1462
47. Rakugi H, Tabuchi Y, Nakamaru M, Nagano M, Higashimori K, Mikami H, et al. Evidence for Endothelin-1 Release From Resistance Vessels of Rats in Response to Hypoxia. *Biochem Biophys Res Commun* (1990) 169(3):973–7. doi: 10.1016/0006-291X(90)91989-6
48. Clark BA, Halvorson L, Sachs B, Epstein FH. Plasma Endothelin Levels in Preeclampsia: Elevation and Correlation With Uric Acid Levels and Renal Impairment. *Am J Obstet Gynecol* (1992) 166(3):962–8. doi: 10.1016/0002-9378(92)91372-H
49. Lu YP, Hasan AA, Zeng S, Hocher B. Plasma ET-1 Concentrations Are Elevated in Pregnant Women With Hypertension -Meta-Analysis of Clinical Studies. *Kidney Blood Press Res* (2017) 42(4):654–63. doi: 10.1159/000482004
50. Han SG, Ko S, Lee WK, Jung ST, Yu YG. Determination of the Endothelin-1 Recognition Sites of Endothelin Receptor Type A by the Directed-Degeneration Method. *Sci Rep* (2017) 7(1):7577. doi: 10.1038/s41598-017-08096-6
51. Lin CY, Lee TS, Chen CC, Chang CA, Lin YJ, Hsu YP, et al. Endothelin-1 Exacerbates Lipid Accumulation by Increasing the Protein Degradation of the ATP-Binding Cassette Transporter G1 in Macrophages. *J Cell Physiol* (2011) 226(8):2198–205. doi: 10.1002/jcp.22556
52. Stryer L, Bourne HR. G Proteins: A Family of Signal Transducers. *Annu Rev Cell Biol* (1986) 2:391–419. doi: 10.1146/annurev.cb.02.110186.002135
53. da Silva AR, Fraga-Silva RA, Stergiopulos N, Montecucco F, Mach F. Update on the Role of Angiotensin in the Pathophysiology of Coronary Atherothrombosis. *Eur J Clin Invest* (2015) 45(3):274–87. doi: 10.1111/eci.12401
54. Dechend R, Muller DN, Wallukat G, Homuth V, Krause M, Dudenhausen J, et al. Activating Auto-Antibodies Against the AT1 Receptor in Preeclampsia. *Autoimmun Rev* (2005) 4(1):61–5. doi: 10.1016/j.autrev.2004.07.002
55. Dechend R, Viedt C, Muller DN, Ugele B, Brandes RP, Wallukat G, et al. AT1 Receptor Agonistic Antibodies From Preeclamptic Patients Stimulate NADPH Oxidase. *Circulation* (2003) 107(12):1632–9. doi: 10.1161/01.CIR.0000058200.90059.B1
56. Herse F, Verlohren S, Wenzel K, Pape J, Muller DN, Modrow S, et al. Prevalence of Agonistic Autoantibodies Against the Angiotensin II Type 1 Receptor and Soluble Fms-Like Tyrosine Kinase 1 in a Gestational Age-Matched Case Study. *Hypertension* (2009) 53(2):393–8. doi: 10.1161/HYPERTENSIONAHA.108.124115
57. Lin YJ, Kwok CF, Juan CC, Hsu YP, Shih KC, Chen CC, et al. Angiotensin II Enhances Endothelin-1-Induced Vasoconstriction Through Upregulating Endothelin Type A Receptor. *Biochem Biophys Res Commun* (2014) 451(2):263–9. doi: 10.1016/j.bbrc.2014.07.119
58. Hao J, Michalek C, Zhang W, Zhu M, Xu X, Mende U. Regulation of Cardiomyocyte Signaling by RGS Proteins: Differential Selectivity Towards G Proteins and Susceptibility to Regulation. *J Mol Cell Cardiol* (2006) 41(1):51–61. doi: 10.1016/j.yjmcc.2006.04.003
59. Nadji P, Sommers SC. Lesions of Toxemia in First Trimester Pregnancies. *Am J Clin Pathol* (1973) 59(3):344–9. doi: 10.1093/ajcp/59.3.344
60. Lichtig C, Deutch M, Brandes J. Vascular Changes of Endometrium in Early Pregnancy. *Am J Clin Pathol* (1984) 81(6):702–7. doi: 10.1093/ajcp/81.6.702
61. Caritis S, Sibai B, Hauth J, Lindheimer M, VanDorsten P, Klebanoff M, et al. Predictors of Pre-Eclampsia in Women at High Risk. National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. *Am J Obstet Gynecol* (1998) 179(4):946–51. doi: 10.1016/S0002-9378(98)70194-2
62. Luo ZC, An N, Xu HR, Larante A, Audibert F, Fraser WD. The Effects and Mechanisms of Primiparity on the Risk of Pre-Eclampsia: A Systematic Review. *Paediatr Perinat Epidemiol* (2007) 21 Suppl 1:36–45. doi: 10.1111/j.1365-3016.2007.00836.x
63. Back M, Yurdagul AJr., Tabas I, Oorni K, Kovanen PT. Inflammation and its Resolution in Atherosclerosis: Mediators and Therapeutic Opportunities. *Nat Rev Cardiol* (2019) 16(7):389–406. doi: 10.1038/s41569-019-0169-2
64. Brosens I, Brosens JJ, Muter J, Benagiano G. Acute Atherosis and Diffuse Lipid Infiltration of the Placental Bed: A Review of Historical Lipid Studies. *Placenta* (2020) 97:36–41. doi: 10.1016/j.placenta.2020.06.012
65. Stary HC, Chandler AB, Glagov S, Guyton JR, Insull WJr., Rosenfeld ME, et al. A Definition of Initial, Fatty Streak, and Intermediate Lesions of Atherosclerosis. A Report From the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation* (1994) 89(5):2462–78. doi: 10.1161/01.cir.89.5.2462
66. Rath M, Niendorf A, Reblin T, Dietel M, Krebber HJ, Beisiegel U. Detection and Quantification of Lipoprotein(a) in the Arterial Wall of 107 Coronary Bypass Patients. *Arteriosclerosis* (1989) 9(5):579–92. doi: 10.1161/01.ATV.9.5.579
67. Meekins JW, Pijnenborg R, Hanssens M, van Assche A, McFadyen IR. Immunohistochemical Detection of Lipoprotein(a) in the Wall of Placental Bed Spiral Arteries in Normal and Severe Preeclamptic Pregnancies. *Placenta* (1994) 15(5):511–24. doi: 10.1016/S0143-4004(05)80420-5
68. Haukkamaa L, Moilanen L, Kattainen A, Luoto R, Kahonen M, Leinonen M, et al. Pre-Eclampsia is a Risk Factor of Carotid Artery Atherosclerosis. *Cerebrovasc Dis* (2009) 27(6):599–607. doi: 10.1159/000216834
69. Staff AC, Johnsen GM, Dechend R, Redman CWG. Preeclampsia and Uteroplacental Acute Atherosis: Immune and Inflammatory Factors. *J Reprod Immunol* (2014) 101-102:120–6. doi: 10.1016/j.jri.2013.09.001
70. Okuyama K, Yaginuma G, Takahashi T, Sasaki H, Mori S. The Development of Vasa Vasorum of the Human Aorta in Various Conditions. A Morphometric Study. *Arch Pathol Lab Med* (1988) 112(7):721–5.
71. Subbotin VM. Neovascularization of Coronary Tunica Intima (DIT) is the Cause of Coronary Atherosclerosis. Lipoproteins Invade Coronary Intima *via* Neovascularization From Adventitial Vasa Vasorum, But Not From the Arterial Lumen: A Hypothesis. *Theor Biol Med Model* (2012) 9:11. doi: 10.1186/1742-4682-9-11
72. Subbotin VM. Excessive Intimal Hyperplasia in Human Coronary Arteries Before Intimal Lipid Depositions is the Initiation of Coronary Atherosclerosis and Constitutes a Therapeutic Target. *Drug Discovery Today* (2016) 21(10):1578–95. doi: 10.1016/j.drudis.2016.05.017
73. Arcidiacono MV, Rubinat E, Borras M, Betriu A, Trujillano J, Vidal T, et al. Left Carotid Adventitial Vasa Vasorum Signal Correlates Directly With Age and With Left Carotid Intima-Media Thickness in Individuals Without Atheromatous Risk Factors. *Cardiovasc Ultrasound* (2015) 13:20. doi: 10.1186/s12947-015-0014-7
74. Pirillo A, Norata GD, Catapano AL. LOX-1, OxLDL, and Atherosclerosis. *Mediators Inflammation* (2013) 2013:152786. doi: 10.1155/2013/152786
75. Davies MJ, Gordon JL, Gearing AJ, Pigott R, Woolf N, Katz D, et al. The Expression of the Adhesion Molecules ICAM-1, VCAM-1, PECAM, and E-Selectin in Human Atherosclerosis. *J Pathol* (1993) 171(3):223–9. doi: 10.1002/path.1711710311
76. Labarrere CA, DiCarlo HL, Bammerlin E, Hardin JW, Kim YM, Chaemsaithong P, et al. Failure of Physiologic Transformation of Spiral Arteries, Endothelial and Trophoblast Cell Activation, and Acute Atherosis in the Basal Plate of the Placenta. *Am J Obstet Gynecol* (2017) 216(3):287 e1–e16. doi: 10.1016/j.ajog.2016.12.029
77. Robertson WB, Brosens I, Dixon G. Uteroplacental Vascular Pathology. *Eur J Obstet Gynecol Reprod Biol* (1975) 5(1-2):47–65. doi: 10.1016/0028-2243(75)90130-6
78. Drozdz D, Latka M, Drozdz T, Sztefko K, Kwinta P. Thrombomodulin as a New Marker of Endothelial Dysfunction in Chronic Kidney Disease in Children. *Oxid Med Cell Longev* (2018) 2018:1619293. doi: 10.1155/2018/1619293

79. Remkova A, Kovacova E, Prikazska M, Kratochvil'ova H. Thrombomodulin as a Marker of Endothelium Damage in Some Clinical Conditions. *Eur J Intern Med* (2000) 11(2):79–84. doi: 10.1016/S0953-6205(00)00066-2
80. Staff AC, Fjeldstad HE, Fosheim IK, Moe K, Turowski G, Johnsen GM, et al. Failure of Physiological Transformation and Spiral Artery Atherosis: Their Roles in Preeclampsia. *Am J Obstet Gynecol* (2020) 225:349. doi: 10.1016/j.ajog.2020.09.026
81. Hustin J, Foidart JM, Lambotte R. Maternal Vascular Lesions in Pre-Eclampsia and Intrauterine Growth Retardation: Light Microscopy and Immunofluorescence. *Placenta* (1983) 4 Spec No:489–98.
82. Labarrere C, Alonso J, Manni J, Domenichini E, Althabe O. Immunohistochemical Findings in Acute Atherosis Associated With Intrauterine Growth Retardation. *Am J Reprod Immunol Microbiol* (1985) 7(4):149–55. doi: 10.1111/j.1600-0897.1985.tb00344.x
83. Labarrere CA, Catoggio LJ, Mullen EG, Althabe OH. Placental Lesions in Maternal Autoimmune Diseases. *Am J Reprod Immunol Microbiol* (1986) 12 (3):78–86. doi: 10.1111/j.1600-0897.1986.tb00068.x
84. Yin L, Han J, Ma H. [Uterine and Placental Vascular Lesions in Pregnancy Induced Hypertension and its Relationship to Pregnancy Outcome]. *Zhonghua Fu Chan Ke Za Zhi* (1998) 33(8):459–61.
85. Hering L, Herse F, Verlohren S, Park JK, Wellner M, Qadri F, et al. Trophoblasts Reduce the Vascular Smooth Muscle Cell Proatherogenic Response. *Hypertension* (2008) 51(2):554–9. doi: 10.1161/HYPERTENSIONAHA.107.102905
86. Abramowsky CR, Vegas ME, Swinehart G, Gyves MT. Decidual Vasculopathy of the Placenta in Lupus Erythematosus. *N Engl J Med* (1980) 303(12):668–72. doi: 10.1056/NEJM198009183031204
87. Khong TY. Immunohistologic Study of the Leukocytic Infiltrate in Maternal Uterine Tissues in Normal and Preeclamptic Pregnancies at Term. *Am J Reprod Immunol Microbiol* (1987) 15(1):1–8. doi: 10.1111/j.1600-0897.1987.tb00141.x
88. Gill N, Leng Y, Romero R, Xu Y, Panaitescu B, Miller D, et al. The Immunophenotype of Decidual Macrophages in Acute Atherosis. *Am J Reprod Immunol* (2019) 81(4):e13098. doi: 10.1111/aji.13098
89. Jaiswal N, Puri M, Agarwal K, Singh S, Yadav R, Tiwary N, et al. COVID-19 as an Independent Risk Factor for Subclinical Placental Dysfunction. *Eur J Obstet Gynecol Reprod Biol* (2021) 259:7–11. doi: 10.1016/j.ejogrb.2021.01.049
90. Watts RA, Scott DG. Recent Developments in the Classification and Assessment of Vasculitis. *Best Pract Res Clin Rheumatol* (2009) 23(3):429–43. doi: 10.1016/j.berh.2008.12.004
91. Jennette JC, Falk RJ. Small-Vessel Vasculitis. *N Engl J Med* (1997) 337 (21):1512–23. doi: 10.1056/NEJM199711203372106
92. Staff AC. The Two-Stage Placental Model of Preeclampsia: An Update. *J Reprod Immunol* (2019) 134-135:1–10. doi: 10.1016/j.jri.2019.07.004
93. Redman CWG, Staff AC, Roberts JM. Syncytiotrophoblast Stress in Preeclampsia: The Convergence Point for Multiple Pathways. *Am J Obstet Gynecol* (2020). doi: 10.1016/j.ajog.2020.09.047
94. Kraus TA, Engel SM, Sperling RS, Kellerman L, Lo Y, Wallenstein S, et al. Characterizing the Pregnancy Immune Phenotype: Results of the Viral Immunity and Pregnancy (VIP) Study. *J Clin Immunol* (2012) 32(2):300–11. doi: 10.1007/s10875-011-9627-2
95. Aghaeepour N, Ganio EA, McIlwain D, Tsai AS, Tingle M, Van Gassen S, et al. An Immune Clock of Human Pregnancy. *Sci Immunol* (2017) 2(15). doi: 10.1126/sciimmunol.aan2946
96. Apps R, Murphy SP, Fernando R, Gardner L, Ahad T, Moffett A. Human Leucocyte Antigen (HLA) Expression of Primary Trophoblast Cells and Placental Cell Lines, Determined Using Single Antigen Beads to Characterize Allotype Specificities of Anti-HLA Antibodies. *Immunology* (2009) 127 (1):26–39. doi: 10.1111/j.1365-2567.2008.03019.x
97. Ellis SA, Sargent IL, Redman CW, McMichael AJ. Evidence for a Novel HLA Antigen Found on Human Extravillous Trophoblast and a Choriocarcinoma Cell Line. *Immunology* (1986) 59(4):595–601.
98. Shobu T, Sageshima N, Tokui H, Omura M, Saito K, Nagatsuka Y, et al. The Surface Expression of HLA-F on Decidual Trophoblasts Increases From Mid to Term Gestation. *J Reprod Immunol* (2006) 72(1-2):18–32. doi: 10.1016/j.jri.2006.02.001
99. Trowsdale J, Moffett A. NK Receptor Interactions With MHC Class I Molecules in Pregnancy. *Semin Immunol* (2008) 20(6):317–20. doi: 10.1016/j.smim.2008.06.002
100. King A, Hiby SE, Verma S, Burrows T, Gardner L, Loke YW. Uterine NK Cells and Trophoblast HLA Class I Molecules. *Am J Reprod Immunol* (1997) 37(6):459–62. doi: 10.1111/j.1600-0897.1997.tb00260.x
101. Dal Porto J, Johansen TE, Catipovic B, Parfiit DJ, Tuveson D, Gether U, et al. A Soluble Divalent Class I Major Histocompatibility Complex Molecule Inhibits Alloreactive T Cells at Nanomolar Concentrations. *Proc Natl Acad Sci U.S.A.* (1993) 90(14):6671–5. doi: 10.1073/pnas.90.14.6671
102. Contini P, Ghio M, Poggi A, Filaci G, Indiveri F, Ferrone S, et al. -B,-C and -G Molecules Induce Apoptosis in T and NK CD8+ Cells and Inhibit Cytotoxic T Cell Activity Through CD8 Ligation. *Eur J Immunol* (2003) 33(1):125–34. doi: 10.1002/immu.200390015
103. Spaggiari GM, Contini P, Carosio R, Arvigo M, Ghio M, Oddone D, et al. Soluble HLA Class I Molecules Induce Natural Killer Cell Apoptosis Through the Engagement of CD8: Evidence for a Negative Regulation Exerted by Members of the Inhibitory Receptor Superfamily. *Blood* (2002) 99(5):1706–14. doi: 10.1182/blood.V99.5.1706
104. Goldman-Wohl DS, Ariel I, Greenfield C, Hochner-Celnikier D, Cross J, Fisher S, et al. Lack of Human Leukocyte Antigen-G Expression in Extravillous Trophoblasts is Associated With Pre-Eclampsia. *Mol Hum Reprod* (2000) 6(1):88–95. doi: 10.1093/molehr/6.1.88
105. Tilburgs T, Scherjon SA, van der Mast BJ, Haasnoot GW, Versteeg VDV-MM, Roelen DL, et al. Fetal-Maternal HLA-C Mismatch is Associated With Decidual T Cell Activation and Induction of Functional T Regulatory Cells. *J Reprod Immunol* (2009) 82(2):148–57. doi: 10.1016/j.jri.2009.05.003
106. Hiby SE, Walker JJ, O'Shaughnessy KM, Redman CW, Carrington M, Trowsdale J, et al. Combinations of Maternal KIR and Fetal HLA-C Genes Influence the Risk of Preeclampsia and Reproductive Success. *J Exp Med* (2004) 200(8):957–65. doi: 10.1084/jem.20041214
107. Hviid TV. HLA-G in Human Reproduction: Aspects of Genetics, Function and Pregnancy Complications. *Hum Reprod Update* (2006) 12(3):209–32. doi: 10.1093/humupd/dmi048
108. Colbern GT, Chiang MH, Main EK. Expression of the Nonclassic Histocompatibility Antigen HLA-G by Preeclamptic Placenta. *Am J Obstet Gynecol* (1994) 170(5 Pt 1):1244–50. doi: 10.1016/S0002-9378(94)70134-2
109. Yie SM, Li LH, Li YM, Librach C. HLA-G Protein Concentrations in Maternal Serum and Placental Tissue are Decreased in Preeclampsia. *Am J Obstet Gynecol* (2004) 191(2):525–9. doi: 10.1016/j.ajog.2004.01.033
110. Beneventi F, Locatelli E, De Amici M, Martinetti M, Spinillo A. Soluble HLA-G Concentrations in Obese Women During Pregnancy and in Cord Blood. *J Reprod Immunol* (2017) 119:31–7. doi: 10.1016/j.jri.2016.11.005
111. Burton GJ, Woods AW, Jauniaux E, Kingdom JC. Rheological and Physiological Consequences of Conversion of the Maternal Spiral Arteries for Uteroplacental Blood Flow During Human Pregnancy. *Placenta* (2009) 30(6):473–82. doi: 10.1016/j.placenta.2009.02.009
112. Jauniaux E, Watson AL, Hempstock J, Bao YP, Skepper JN, Burton GJ. Onset of Maternal Arterial Blood Flow and Placental Oxidative Stress. A Possible Factor in Human Early Pregnancy Failure. *Am J Pathol* (2000) 157(6):2111–22. doi: 10.1016/S0002-9440(10)64849-3
113. Assali NS, Douglass RAJr., Baird WW, Nicholson DB, Suyemoto R. Measurement of Uterine Blood Flow and Uterine Metabolism. IV. Results in Normal Pregnancy. *Am J Obstet Gynecol* (1953) 66(2):248–53. doi: 10.1016/0002-9378(53)90560-2
114. Hung TH, Burton GJ. Hypoxia and Reoxygenation: A Possible Mechanism for Placental Oxidative Stress in Preeclampsia. *Taiwan J Obstet Gynecol* (2006) 45(3):189–200. doi: 10.1016/S1028-4559(09)60224-2
115. Redman CW, Sargent IL, Staff AC. IFPA Senior Award Lecture: Making Sense of Pre-Eclampsia - Two Placental Causes of Preeclampsia? *Placenta* (2014) 35 Suppl:S20–5. doi: 10.1016/j.placenta.2013.12.008
116. Xu C, Bailly-Maitre B, Reed JC. Endoplasmic Reticulum Stress: Cell Life and Death Decisions. *J Clin Invest* (2005) 115(10):2656–64. doi: 10.1172/JCI26373
117. Burton GJ, Yung HW, Cindrova-Davies T, Charnock-Jones DS. Placental Endoplasmic Reticulum Stress and Oxidative Stress in the Pathophysiology of Unexplained Intrauterine Growth Restriction and Early Onset Preeclampsia. *Placenta* (2009) 30 Suppl A:S43–8. doi: 10.1016/j.placenta.2008.11.003
118. Cindrova-Davies T, Spasic-Boskovic O, Jauniaux E, Charnock-Jones DS, Burton GJ. Nuclear Factor-Kappa B, P38, and Stress-Activated Protein Kinase Mitogen-Activated Protein Kinase Signaling Pathways Regulate

Proinflammatory Cytokines and Apoptosis in Human Placental Explants in Response to Oxidative Stress: Effects of Antioxidant Vitamins. *Am J Pathol* (2007) 170(5):1511–20. doi: 10.2353/ajpath.2007.061035
119. Hung TH, Charnock-Jones DS, Skepper JN, Burton GJ. Secretion of Tumor Necrosis Factor-Alpha From Human Placental Tissues Induced by Hypoxia-Reoxygenation Causes Endothelial Cell Activation *In Vitro*: A Potential Mediator of the Inflammatory Response in Preeclampsia. *Am J Pathol* (2004) 164(3):1049–61. doi: 10.1016/S0002-9440(10)63192-6
120. Hubel CA. Oxidative Stress in the Pathogenesis of Preeclampsia. *Proc Soc Exp Biol Med* (1999) 222(3):222–35. doi: 10.1046/j.1525-1373.1999.d01-139.x
121. Chiu JJ, Chien S. Effects of Disturbed Flow on Vascular Endothelium: Pathophysiological Basis and Clinical Perspectives. *Physiol Rev* (2011) 91 (1):327–87. doi: 10.1152/physrev.00047.2009
122. VanderLaan PA, Reardon CA, Getz GS. Site Specificity of Atherosclerosis: Site-Selective Responses to Atherosclerotic Modulators. *Arterioscler Thromb Vasc Biol* (2004) 24(1):12–22. doi: 10.1161/01.ATV.0000105054.43931.f0
123. Cornhill JF, Herderick EE, Stary HC. Topography of Human Aortic Sudanophilic Lesions. *Monogr Atheroscler* (1990) 15:13–9.
124. Chen KD, Li YS, Kim M, Li S, Yuan S, Chien S, et al. Mechanotransduction in Response to Shear Stress. Roles of Receptor Tyrosine Kinases, Integrins, and Shc. *J Biol Chem* (1999) 274(26):18393–400. doi: 10.1074/jbc.274.26.18393
125. Chien S. Molecular and Mechanical Bases of Focal Lipid Accumulation in Arterial Wall. *Prog Biophys Mol Biol* (2003) 83(2):131–51. doi: 10.1016/S0079-6107(03)00053-1
126. Namiki M, Kawashima S, Yamashita T, Ozaki M, Hirase T, Ishida T, et al. Local Overexpression of Monocyte Chemoattractant Protein-1 at Vessel Wall Induces Infiltration of Macrophages and Formation of Atherosclerotic Lesion: Synergism With Hypercholesterolemia. *Arterioscler Thromb Vasc Biol* (2002) 22(1):115–20. doi: 10.1161/hq0102.102278
127. Kauma S, Takacs P, Scordalakes C, Walsh S, Green K, Peng T. Increased Endothelial Monocyte Chemoattractant Protein-1 and Interleukin-8 in Preeclampsia. *Obstet Gynecol* (2002) 100(4):706–14. doi: 10.1016/s0029-7844(02)02169-5
128. Katabuchi H, Yih S, Ohba T, Matsui K, Takahashi K, Takeya M, et al. Characterization of Macrophages in the Decidual Atherotic Spiral Artery With Special Reference to the Cytology of Foam Cells. *Med Electron Microsc* (2003) 36(4):253–62. doi: 10.1007/s00795-003-0223-2
129. Lorentzen B, Henriksen T. Plasma Lipids and Vascular Dysfunction in Preeclampsia. *Semin Reprod Endocrinol* (1998) 16(1):33–9. doi: 10.1055/s-2007-1016250
130. Staff AC, Dechend R, Pijnenborg R. Learning From the Placenta: Acute Atherosis and Vascular Remodeling in Preeclampsia-Novel Aspects for Atherosclerosis and Future Cardiovascular Health. *Hypertension* (2010) 56 (6):1026–34. doi: 10.1161/HYPERTENSIONAHA.110.157743
131. Hirschhorn T, Stockwell BR. The Development of the Concept of Ferroptosis. *Free Radic Biol Med* (2019) 133:130–43. doi: 10.1016/j.freeradbiomed.2018.09.043
132. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, et al. Ferroptosis: An Iron-Dependent Form of Nonapoptotic Cell Death. *Cell* (2012) 149(5):1060–72. doi: 10.1016/j.cell.2012.03.042
133. Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, et al. Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. *Cell* (2017) 171(2):273–85. doi: 10.1016/j.cell.2017.09.021
134. Li Y, Feng D, Wang Z, Zhao Y, Sun R, Tian D, et al. Ischemia-Induced ACSL4 Activation Contributes to Ferroptosis-Mediated Tissue Injury in Intestinal Ischemia/Reperfusion. *Cell Death Differ* (2019) 26(11):2284–99. doi: 10.1038/s41418-019-0299-4
135. Yan HF, Tuo QZ, Yin QZ, Lei P. The Pathological Role of Ferroptosis in Ischemia/Reperfusion-Related Injury. *Zool Res* (2020) 41(3):220–30. doi: 10.24272/j.issn.2095-8137.2020.042
136. Lillo-Moya J, Rojas-Sole C, Munoz-Salamanca D, Panieri E, Saso L, Rodrigo R. Targeting Ferroptosis Against Ischemia/Reperfusion Cardiac Injury. *Antioxid (Basel)* (2021) 10(5):667. doi: 10.3390/antiox10050667
137. Altamura S, Muckenthaler MU. Iron Toxicity in Diseases of Aging: Alzheimer's Disease, Parkinson's Disease and Atherosclerosis. *J Alzheimers Dis* (2009) 16(4):879–95. doi: 10.3233/JAD-2009-1010
138. Chen X, Li X, Xu X, Li L, Liang N, Zhang L, et al. Ferroptosis and Cardiovascular Disease: Role of Free Radical-Induced Lipid Peroxidation. *Free Radic Res* (2021) 55:1–11. doi: 10.1080/10715762.2021.1876856
139. Bai T, Li M, Liu Y, Qiao Z, Wang Z. Inhibition of Ferroptosis Alleviates Atherosclerosis Through Attenuating Lipid Peroxidation and Endothelial Dysfunction in Mouse Aortic Endothelial Cell. *Free Radic Biol Med* (2020) 160:92–102. doi: 10.1016/j.freeradbiomed.2020.07.026
140. Ruparelia N, Chai JT, Fisher EA, Choudhury RP. Inflammatory Processes in Cardiovascular Disease: A Route to Targeted Therapies. *Nat Rev Cardiol* (2017) 14(5):314. doi: 10.1038/nrcardio.2016.185
141. Tsalamandris S, Antonopoulos AS, Oikonomou E, Papamikroulis GA, Vogiatzi G, Papaioannou S, et al. The Role of Inflammation in Diabetes: Current Concepts and Future Perspectives. *Eur Cardiol* (2019) 14(1):50–9. doi: 10.15420/ecr.2018.33.1
142. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and Inflammation: The Linking Mechanism and the Complications. *Arch Med Sci* (2017) 13(4):851–63. doi: 10.5114/aoms.2016.58928
143. Watts DH, Krohn MA, Wener MH, Eschenbach DA. C-Reactive Protein in Normal Pregnancy. *Obstet Gynecol* (1991) 77(2):176–80. doi: 10.1097/00006250-199102000-00002
144. Boots C, Stephenson MD. Does Obesity Increase the Risk of Miscarriage in Spontaneous Conception: A Systematic Review. *Semin Reprod Med* (2011) 29(6):507–13. doi: 10.1055/s-0031-1293204
145. Nobles CJ, Mendola P, Mumford SL, Naimi AI, Yeung EH, Kim K, et al. Preconception Blood Pressure Levels and Reproductive Outcomes in a Prospective Cohort of Women Attempting Pregnancy. *Hypertension* (2018) 71(5):904–10. doi: 10.1161/HYPERTENSIONAHA.117.10705
146. Wright AD, Nicholson HO, Pollock A, Taylor KG, Betts S. Spontaneous Abortion and Diabetes Mellitus. *Postgrad Med J* (1983) 59(691):295–8. doi: 10.1136/pgmj.59.691.295
147. Fox R, Kitt J, Leeson P, Aye CYL, Lewandowski AJ. Preeclampsia: Risk Factors, Diagnosis, Management, and the Cardiovascular Impact on the Offspring. *J Clin Med* (2019) 8(10):1625. doi: 10.3390/jcm8101625
148. Han VX, Patel S, Jones HF, Nielsen TC, Mohammad SS, Hofer MJ, et al. Maternal Acute and Chronic Inflammation in Pregnancy is Associated With Common Neurodevelopmental Disorders: A Systematic Review. *Transl Psychiatry* (2021) 11(1):71. doi: 10.1038/s41398-021-01198-w
149. Chen S, Zhao S, Dalman C, Karlsson H, Gardner R. Association of Maternal Diabetes With Neurodevelopmental Disorders: Autism Spectrum Disorders, Attention-Deficit/Hyperactivity Disorder and Intellectual Disability. *Int J Epidemiol* (2021) 50(2):459–74. doi: 10.1093/ije/dyaa212
150. Chen KR, Yu T, Kang L, Lien YJ, Kuo PL. Childhood Neurodevelopmental Disorders and Maternal Hypertensive Disorder of Pregnancy. *Dev Med Child Neurol* (2021) 63(9):1107–13. doi: 10.1111/dmcn.14893
151. Zhu Z, Tang S, Deng X, Wang Y. Maternal Systemic Lupus Erythematosus, Rheumatoid Arthritis, and Risk for Autism Spectrum Disorders in Offspring: A Meta-Analysis. *J Autism Dev Disord* (2020) 50(8):2852–9. doi: 10.1007/s10803-020-04400-y
152. Chen SW, Zhong XS, Jiang LN, Zheng XY, Xiong YQ, Ma SJ, et al. Maternal Autoimmune Diseases and the Risk of Autism Spectrum Disorders in Offspring: A Systematic Review and Meta-Analysis. *Behav Brain Res* (2016) 296:61–9. doi: 10.1016/j.bbr.2015.08.035
153. Chakravarty EF, Nelson L, Krishnan E. Obstetric Hospitalizations in the United States for Women With Systemic Lupus Erythematosus and Rheumatoid Arthritis. *Arthritis Rheum* (2006) 54(3):899–907. doi: 10.1002/art.21663
154. Kishore S, Mittal V, Majithia V. Obstetric Outcomes in Women With Rheumatoid Arthritis: Results From Nationwide Inpatient Sample Database 2003-2011(). *Semin Arthritis Rheum* (2019) 49(2):236–40. doi: 10.1016/j.semarthrit.2019.03.011
155. Labarrere C, Althabe O. Chronic Villitis of Unknown Etiology and Maternal Arterial Lesions in Preeclamptic Pregnancies. *Eur J Obstet Gynecol Reprod Biol* (1985) 20(1):1–11. doi: 10.1016/0028-2243(85)90077-2
156. Levy RA, Avvad E, Oliveira J, Porto LC. Placental Pathology in Antiphospholipid Syndrome. *Lupus* (1998) 7 Suppl 2:S81–5. doi: 10.1177/096120339800700218
157. Nayar R, Lage JM. Placental Changes in a First Trimester Missed Abortion in Maternal Systemic Lupus Erythematosus With Antiphospholipid Syndrome;

a Case Report and Review of the Literature. *Hum Pathol* (1996) 27(2):201–6. doi: 10.1016/S0046-8177(96)90377-9
158. Damgaard C, Magnussen K, Enevold C, Nilsson M, Tolker-Nielsen T, Holmstrup P, et al. Viable Bacteria Associated With Red Blood Cells and Plasma in Freshly Drawn Blood Donations. *PloS One* (2015) 10(3):e0120826. doi: 10.1371/journal.pone.0120826
159. Potgieter M, Bester J, Kell DB, Pretorius E. The Dormant Blood Microbiome in Chronic, Inflammatory Diseases. *FEMS Microbiol Rev* (2015) 39(4):567–91. doi: 10.1093/femsre/fuv013
160. Hou D, Zhou X, Zhong X, Settles ML, Herring J, Wang L, et al. Microbiota of the Seminal Fluid From Healthy and Infertile Men. *Fertil Steril* (2013) 100 (5):1261–9. doi: 10.1016/j.fertnstert.2013.07.1991
161. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The Placenta Harbors a Unique Microbiome. *Sci Transl Med* (2014) 6 (237):237ra65. doi: 10.1126/scitranslmed.3008599
162. de Goffau MC, Lager S, Sovio U, Gaccioli F, Cook E, Peacock SJ, et al. Human Placenta has No Microbiome But can Contain Potential Pathogens. *Nature* (2019) 572(7769):329–34. doi: 10.1038/s41586-019-1451-5
163. Swank GM, Deitch EA. Role of the Gut in Multiple Organ Failure: Bacterial Translocation and Permeability Changes. *World J Surg* (1996) 20(4):411–7. doi: 10.1007/s002689900065
164. Bhanji S, Williams B, Sheller B, Elwood T, Mancl L. Transient Bacteremia Induced by Toothbrushing a Comparison of the Sonicare Toothbrush With a Conventional Toothbrush. *Pediatr Dent* (2002) 24(4):295–9.
165. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary Tract Infections: Epidemiology, Mechanisms of Infection and Treatment Options. *Nat Rev Microbiol* (2015) 13(5):269–84. doi: 10.1038/nrmicro3432
166. Kell DB, Pretorius E. No Effects Without Causes: The Iron Dysregulation and Dormant Microbes Hypothesis for Chronic, Inflammatory Diseases. *Biol Rev Camb Philos Soc* (2018) 93(3):1518–57. doi: 10.1111/brv.12407
167. Lannergard A, Larsson A, Friman G, Ewald U. Human Serum Amyloid A (SAA) and High Sensitive C-Reactive Protein (hsCRP) in Preterm Newborn Infants With Nosocomial Infections. *Acta Paediatr* (2008) 97(8):1061–5. doi: 10.1111/j.1651-2227.2008.00814.x
168. Kell DB, Pretorius E. On the Translocation of Bacteria and Their Lipopolysaccharides Between Blood and Peripheral Locations in Chronic, Inflammatory Diseases: The Central Roles of LPS and LPS-Induced Cell Death. *Integr Biol (Camb)* (2015) 7(11):1339–77. doi: 10.1039/c5ib00158g
169. Kell DB, Kenny LC. A Dormant Microbial Component in the Development of Preeclampsia. *Front Med (Lausanne)* (2016) 3:60. doi: 10.3389/fmed.2016.00060
170. Xie F, Hu Y, Magee LA, Money DM, Patrick DM, Brunham RM, et al. Chlamydia Pneumoniae Infection in Preeclampsia. *Hypertens Pregnancy* (2010) 29(4):468–77. doi: 10.3109/10641950903242642
171. El-Shourbagy MA, El-Refaie TA, Sayed KK, Wahba KA, El-Din AS, Fathy MM. Impact of Seroconversion and Antichlamydial Treatment on the Rate of Pre-Eclampsia Among Egyptian Primigravidae. *Int J Gynaecol Obstet* (2011) 113(2):137–40. doi: 10.1016/j.ijgo.2010.11.014
172. Ponzetto A, Cardaropoli S, Piccoli E, Rolfo A, Gennero L, Kanduc D, et al. Pre-Eclampsia is Associated With Helicobacter Pylori Seropositivity in Italy. *J Hypertens* (2006) 24(12):2445–9. doi: 10.1097/HJH.0b013e3280109e8c
173. Shapiro NI, Yano K, Okada H, Fischer C, Howell M, Spokes KC, et al. A Prospective, Observational Study of Soluble FLT-1 and Vascular Endothelial Growth Factor in Sepsis. *Shock* (2008) 29(4):452–7. doi: 10.1097/SHK.0b013e31815072c1
174. Yano K, Liaw PC, Mullington JM, Shih SC, Okada H, Bodyak N, et al. Vascular Endothelial Growth Factor is an Important Determinant of Sepsis Morbidity and Mortality. *J Exp Med* (2006) 203(6):1447–58. doi: 10.1084/jem.20060375
175. Todros T, Verdiglione P, Ogge G, Paladini D, Vergani P, Cardaropoli S. Low Incidence of Hypertensive Disorders of Pregnancy in Women Treated With Spiramycin for Toxoplasma Infection. *Br J Clin Pharmacol* (2006) 61(3):336–40. doi: 10.1111/j.1365-2125.2005.02572.x
176. Sanchez-Rodriguez E, Egea-Zorrilla A, Plaza-Diaz J, Aragon-Vela J, Munoz-Quezada S, Tercedor-Sanchez L, et al. The Gut Microbiota and Its Implication in the Development of Atherosclerosis and Related Cardiovascular Diseases. *Nutrients* (2020) 12(3):605. doi: 10.3390/nu12030605
177. Kiechl S, Egger G, Mayr M, Wiedermann CJ, Bonora E, Oberhollenzer F, et al. Chronic Infections and the Risk of Carotid Atherosclerosis: Prospective Results From a Large Population Study. *Circulation* (2001) 103(8):1064–70. doi: 10.1161/01.CIR.103.8.1064
178. Serrano M, Moreno-Navarrete JM, Puig J, Moreno M, Guerra E, Ortega F, et al. Serum Lipopolysaccharide-Binding Protein as a Marker of Atherosclerosis. *Atherosclerosis* (2013) 230(2):223–7. doi: 10.1016/j.atherosclerosis.2013.07.004
179. Ostos MA, Recalde D, Zakin MM, Scott-Algara D. Implication of Natural Killer T Cells in Atherosclerosis Development During a LPS-Induced Chronic Inflammation. *FEBS Lett* (2002) 519(1-3):23–9. doi: 10.1016/S0014-5793(02)02692-3
180. Khedoe PP, Wong MC, Wagenaar GT, Plomp JJ, van Eck M, Havekes LM, et al. The Effect of PPE-Induced Emphysema and Chronic LPS-Induced Pulmonary Inflammation on Atherosclerosis Development in APOE*3-LEIDEN Mice. *PloS One* (2013) 8(11):e80196. doi: 10.1371/journal.pone.0080196
181. Koren O, Spor A, Felin J, Fak F, Stombaugh J, Tremaroli V, et al. Human Oral, Gut, and Plaque Microbiota in Patients With Atherosclerosis. *Proc Natl Acad Sci U.S.A.* (2011) 108 Suppl 1:4592–8. doi: 10.1073/pnas.1011383107
182. Ott SJ, El Mokhtari NE, Musfeldt M, Hellmig S, Freitag S, Rehman A, et al. Detection of Diverse Bacterial Signatures in Atherosclerotic Lesions of Patients With Coronary Heart Disease. *Circulation* (2006) 113(7):929–37. doi: 10.1161/CIRCULATIONAHA.105.579979
183. von Dadelszen P, Magee LA. Could an Infectious Trigger Explain the Differential Maternal Response to the Shared Placental Pathology of Preeclampsia and Normotensive Intrauterine Growth Restriction? *Acta Obstet Gynecol Scand* (2002) 81(7):642–8. doi: 10.1080/j.1600-0412.2002.810710.x
184. Barak S, Oettinger-Barak O, Machtei EE, Sprecher H, Ohel G. Evidence of Periopathogenic Microorganisms in Placentas of Women With Preeclampsia. *J Periodontol* (2007) 78(4):670–6. doi: 10.1902/jop.2007.060362
185. Fjeldstad HE, Johnsen GM, Staff AC. Fetal Microchimerism and Implications for Maternal Health. *Obstet Med* (2020) 13(3):112–9. doi: 10.1177/1753495X19884484
186. Schroder J, Tiilikainen A, de la Chapelle A. Fetal Leukocytes in the Maternal Circulation After Delivery. I. Cytological Aspects. *Transplantation* (1974) 17 (4):346–54.
187. Kara RJ, Bolli P, Karakikes I, Matsunaga I, Tripodi J, Tanweer O, et al. Fetal Cells Traffic to Injured Maternal Myocardium and Undergo Cardiac Differentiation. *Circ Res* (2012) 110(1):82–93. doi: 10.1161/CIRCRESAHA.111.249037
188. Lo YM, Zhang J, Leung TN, Lau TK, Chang AM, Hjelm NM. Rapid Clearance of Fetal DNA From Maternal Plasma. *Am J Hum Genet* (1999) 64(1):218–24. doi: 10.1086/302205
189. Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA. Male Fetal Progenitor Cells Persist in Maternal Blood for as Long as 27 Years Postpartum. *Proc Natl Acad Sci U.S.A.* (1996) 93(2):705–8. doi: 10.1073/pnas.93.2.705
190. Fugazzola L, Cirello V, Beck-Peccoz P. Microchimerism and Endocrine Disorders. *J Clin Endocrinol Metab* (2012) 97(5):1452–61. doi: 10.1210/jc.2011-3160
191. Adams Waldorf KM, Nelson JL. Autoimmune Disease During Pregnancy and the Microchimerism Legacy of Pregnancy. *Immunol Invest* (2008) 37 (5):631–44. doi: 10.1080/08820130802205886
192. Invernizzi P, Pasini S, Selmi C, Gershwin ME, Podda M. Female Predominance and X Chromosome Defects in Autoimmune Diseases. *J Autoimmun* (2009) 33(1):12–6. doi: 10.1016/j.jaut.2009.03.005
193. Gammill HS, Aydelotte TM, Guthrie KA, Nkwopara EC, Nelson JL. Cellular Fetal Microchimerism in Preeclampsia. *Hypertension* (2013) 62(6):1062–7. doi: 10.1161/HYPERTENSIONAHA.113.01486
194. Holzgreve W, Ghezzi F, Di Naro E, Ganshirt D, Maymon E, Hahn S. Disturbed Feto-Maternal Cell Traffic in Preeclampsia. *Obstet Gynecol* (1998) 91(5 Pt 1):669–72. doi: 10.1016/s0029-7844(98)00068-4
195. Al-Mufti R, Lees C, Albaiges G, Hambley H, Nicolaides KH. Fetal Cells in Maternal Blood of Pregnancies With Severe Fetal Growth Restriction. *Hum Reprod* (2000) 15(1):218–21. doi: 10.1093/humrep/15.1.218

196. Gammill HS, Nelson JL. Naturally Acquired Microchimerism. *Int J Dev Biol* (2010) 54(2-3):531–43. doi: 10.1387/ijdb.082767hg
197. Kamper-Jorgensen M, Hjalgrim H, Andersen AM, Gadi VK, Tjonneland A. Male Microchimerism and Survival Among Women. *Int J Epidemiol* (2014) 43(1):168–73. doi: 10.1093/ije/dyt230
198. Hallum S, Gerds TA, Sehested TSG, Jakobsen MA, Tjonneland A, Kamper-Jorgensen M. Impact of Male-Origin Microchimerism on Cardiovascular Disease in Women: A Prospective Cohort Study. *Am J Epidemiol* (2021) 190 (5):853–63. doi: 10.1093/aje/kwaa250
199. Eikmans M, van Halteren AG, van Besien K, van Rood JJ, Drabbels JJ, Claas FH. Naturally Acquired Microchimerism: Implications for Transplantation Outcome and Novel Methodologies for Detection. *Chimerism* (2014) 5 (2):24–39. doi: 10.4161/chim.28908
200. Starzl TE, Demetris AJ, Murase N, Ildstad S, Ricordi C, Trucco M. Cell Migration, Chimerism, and Graft Acceptance. *Lancet* (1992) 339 (8809):1579–82. doi: 10.1016/0140-6736(92)91840-5
201. Schlitt HJ, Hundrieser J, Ringe B, Pichlmayr R. Donor-Type Microchimerism Associated With Graft Rejection Eight Years After Liver Transplantation. *N Engl J Med* (1994) 330(9):646–7. doi: 10.1056/NEJM199403033300919
202. Dempster WJ, Harrison CV, Shackman R. Rejection Processes in Human Homotransplanted Kidneys. *Br Med J* (1964) 2(5415):969–76. doi: 10.1136/bmj.2.5415.969
203. Demetris AJ, Lasky S, Van Thiel DH, Starzl TE, Dekker A. Pathology of Hepatic Transplantation: A Review of 62 Adult Allograft Recipients Immunosuppressed With a Cyclosporine/Steroid Regimen. *Am J Pathol* (1985) 118(1):151–61.
204. Palmer DC, Tsai CC, Roodman ST, Codd JE, Miller LW, Sarafian JE, et al. Heart Graft Arteriosclerosis. An Ominous Finding on Endomyocardial Biopsy. *Transplantation* (1985) 39(4):385–8. doi: 10.1097/00007890-198504000-00009
205. Labarrere CA. Acute Atherosis. A Histopathological Hallmark of Immune Aggression? *Placenta* (1988) 9(1):95–108. doi: 10.1016/0143-4004(88)90076-8
206. De Wolf F, Carreras LO, Moerman P, Vermylen J, Van Assche A, Renaer M. Decidual Vasculopathy and Extensive Placental Infarction in a Patient With Repeated Thromboembolic Accidents, Recurrent Fetal Loss, and a Lupus Anticoagulant. *Am J Obstet Gynecol* (1982) 142(7):829–34. doi: 10.1016/S0002-9378(16)32527-3
207. Stevens DU, Al-Nasiry S, Bulten J, Spaanderman ME. Decidual Vasculopathy in Preeclampsia: Lesion Characteristics Relate to Disease Severity and Perinatal Outcome. *Placenta* (2013) 34(9):805–9. doi: 10.1016/j.placenta.2013.05.008
208. Khong TY, Mott C. Immunohistologic Demonstration of Endothelial Disruption in Acute Atherosis in Pre-Eclampsia. *Eur J Obstet Gynecol Reprod Biol* (1993) 51(3):193–7. doi: 10.1016/0028-2243(93)90034-A
209. Mistry DA, Chandratreya A, Lee PYF. A Systematic Review on the Use of Aspirin in the Prevention of Deep Vein Thrombosis in Major Elective Lower Limb Orthopedic Surgery: An Update From the Past 3 Years. *Surg J (N Y)* (2017) 3(4):e191–e6. doi: 10.1055/s-0037-1615817
210. Ittaman SV, VanWormer JJ, Rezkalla SH. The Role of Aspirin in the Prevention of Cardiovascular Disease. *Clin Med Res* (2014) 12(3-4):147–54. doi: 10.3121/cmr.2013.1197
211. Van Doorn R, Mukhtarova N, Flyke IP, Lasarev M, Kim K, Hennekens CH, et al. Dose of Aspirin to Prevent Preterm Preeclampsia in Women With Moderate or High-Risk Factors: A Systematic Review and Meta-Analysis. *PloS One* (2021) 16(3):e0247782. doi: 10.1371/journal.pone.0247782
212. Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, de Paco Matallana C, et al. Aspirin Versus Placebo in Pregnancies at High Risk for Preterm Preeclampsia. *N Engl J Med* (2017) 377(7):613–22. doi: 10.1056/NEJMoa1704559
213. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al. The Hypertensive Disorders of Pregnancy: ISSHP Classification, Diagnosis & Management Recommendations for International Practice. *Pregnancy Hypertens* (2018) 13:291–310. doi: 10.1016/j.preghy.2018.05.004
214. Olofsson P, Laurini RN, Marsal K. A High Uterine Artery Pulsatility Index Reflects a Defective Development of Placental Bed Spiral Arteries in Pregnancies Complicated by Hypertension and Fetal Growth Retardation. *Eur J Obstet Gynecol Reprod Biol* (1993) 49(3):161–8. doi: 10.1016/0028-2243(93)90265-E
215. Kim YM, Chaemsaithong P, Romero R, Shaman M, Kim CJ, Kim JS, et al. Placental Lesions Associated With Acute Atherosis. *J Matern Fetal Neonatal Med* (2015) 28(13):1554–62. doi: 10.3109/14767058.2014.960835
216. Ferrazzi E, Bulfamante G, Mezzopane R, Barbera A, Ghidini A, Pardi G. Uterine Doppler Velocimetry and Placental Hypoxic-Ischemic Lesion in Pregnancies With Fetal Intrauterine Growth Restriction. *Placenta* (1999) 20 (5-6):389–94. doi: 10.1053/plac.1999.0395
217. Aardema MW, Oosterhof H, Timmer A, van Rooy I, Aarnoudse JG. Uterine Artery Doppler Flow and Uteroplacental Vascular Pathology in Normal Pregnancies and Pregnancies Complicated by Pre-Eclampsia and Small for Gestational Age Fetuses. *Placenta* (2001) 22(5):405–11. doi: 10.1053/plac.2001.0676
218. Lloyd-Davies C, Collins SL, Burton GJ. Understanding the Uterine Artery Doppler Waveform and its Relationship to Spiral Artery Remodelling. *Placenta* (2021) 105:78–84. doi: 10.1016/j.placenta.2021.01.004
219. Pijnenborg R, Vercruysse L, Hanssens M. The Uterine Spiral Arteries in Human Pregnancy: Facts and Controversies. *Placenta* (2006) 27(9-10):939–58. doi: 10.1016/j.placenta.2005.12.006
220. Williams D. Pregnancy: A Stress Test for Life. *Curr Opin Obstet Gynecol* (2003) 15(6):465–71. doi: 10.1097/00001703-200312000-00002
221. Sattar N, Greer IA. Pregnancy Complications and Maternal Cardiovascular Risk: Opportunities for Intervention and Screening? *BMJ* (2002) 325 (7356):157–60. doi: 10.1136/bmj.325.7356.157
222. Staff AC, Redman CW, Williams D, Leeson P, Moe K, Thilaganathan B, et al. Pregnancy and Long-Term Maternal Cardiovascular Health: Progress Through Harmonization of Research Cohorts and Biobanks. *Hypertension* (2016) 67(2):251–60. doi: 10.1161/HYPERTENSIONAHA.115.06357
223. Bushnell C, McCullough LD, Awad IA, Chireau MV, Fedder WN, Furie KL, et al. Guidelines for the Prevention of Stroke in Women: A Statement for Healthcare Professionals From the American Heart Association/American Stroke Association. *Stroke* (2014) 45(5):1545–88. doi: 10.1161/01.str.0000442009.06663.48
224. Roberts JM, Gammill H. Pre-Eclampsia and Cardiovascular Disease in Later Life. *Lancet* (2005) 366(9490):961–2. doi: 10.1016/S0140-6736(05)67349-7
225. Cain MA, Salemi JL, Tanner JP, Kirby RS, Salihu HM, Louis JM. Pregnancy as a Window to Future Health: Maternal Placental Syndromes and Short-Term Cardiovascular Outcomes. *Am J Obstet Gynecol* (2016) 215(4):484 e1–e14. doi: 10.1016/j.ajog.2016.05.047
226. Irgens HU, Reisaeter L, Irgens LM, Lie RT. Long Term Mortality of Mothers and Fathers After Pre-Eclampsia: Population Based Cohort Study. *BMJ* (2001) 323(7323):1213–7. doi: 10.1136/bmj.323.7323.1213
227. Smith GC, Pell JP, Walsh D. Pregnancy Complications and Maternal Risk of Ischaemic Heart Disease: A Retrospective Cohort Study of 129,290 Births. *Lancet* (2001) 357(9273):2002–6. doi: 10.1016/S0140-6736(00)05112-6
228. Bellamy L, Casas JP, Hingorani AD, Williams DJ. Pre-Eclampsia and Risk of Cardiovascular Disease and Cancer in Later Life: Systematic Review and Meta-Analysis. *BMJ* (2007) 335(7627):974. doi: 10.1136/bmj.39335.385301.BE
229. Lykke JA, Langhoff-Roos J, Sibai BM, Funai EF, Triche EW, Paidas MJ. Hypertensive Pregnancy Disorders and Subsequent Cardiovascular Morbidity and Type 2 Diabetes Mellitus in the Mother. *Hypertension* (2009) 53(6):944–51. doi: 10.1161/HYPERTENSIONAHA.109.130765
230. Lazdam M, de la Horra A, Diesch J, Kenworthy Y, Davis E, Lewandowski AJ, et al. Unique Blood Pressure Characteristics in Mother and Offspring After Early Onset Preeclampsia. *Hypertension* (2012) 60(5):1338–45. doi: 10.1161/HYPERTENSIONAHA.112.198366
231. Wikstrom AK, Haglund B, Olovsson M, Lindeberg SN. The Risk of Maternal Ischaemic Heart Disease After Gestational Hypertensive Disease. *BJOG* (2005) 112(11):1486–91. doi: 10.1111/j.1471-0528.2005.00733.x
232. Newstead J, von Dadelszen P, Magee LA. Preeclampsia and Future Cardiovascular Risk. *Expert Rev Cardiovasc Ther* (2007) 5(2):283–94. doi: 10.1586/14779072.5.2.283
233. Sundheimer LW, Pisarska MD. Abnormal Placentation Associated With Infertility as a Marker of Overall Health. *Semin Reprod Med* (2017) 35 (3):205–16. doi: 10.1055/s-0037-1603570
234. Parikh NI, Cnattingius S, Mittleman MA, Ludvigsson JF, Ingelsson E. Subfertility and Risk of Later Life Maternal Cardiovascular Disease. *Hum Reprod* (2012) 27(2):568–75. doi: 10.1093/humrep/der400
235. Milic NM, Milin-Lazovic J, Weissgerber TL, Trajkovic G, White WM, Garovic VD. Preclinical Atherosclerosis at the Time of Pre-Eclamptic

Pregnancy and Up to 10 Years Postpartum: Systematic Review and Meta-Analysis. *Ultrasound Obstet Gynecol* (2017) 49(1):110–5. doi: 10.1002/uog.17367
236. Yuan LJ, Xue D, Duan YY, Cao TS, Yang HG, Zhou N. Carotid Arterial Intima-Media Thickness and Arterial Stiffness in Pre-Eclampsia: Analysis With a Radiofrequency Ultrasound Technique. *Ultrasound Obstet Gynecol* (2013) 42(6):644–52. doi: 10.1002/uog.12409
237. Brueckmann A, Seeliger C, Schlembach D, Schleussner E. PP048. Carotid Intima-Media-Thickness in the First Trimester as a Predictor of Preeclampsia. *Pregnancy Hypertens* (2013) 3(2):84. doi: 10.1016/j.preghy.2013.04.075
238. Willeit P, Tschiderer L, Allara E, Reuber K, Seekircher L, Gao L, et al. Carotid Intima-Media Thickness Progression as Surrogate Marker for Cardiovascular Risk: Meta-Analysis of 119 Clinical Trials Involving 100 667 Patients. *Circulation* (2020) 142(7):621–42. doi: 10.1161/CIRCULATIONAHA.120.046361
239. Blaauw J, Souwer ET, Coffeng SM, Smit AJ, van Doormaal JJ, Faas MM, et al. Follow Up of Intima-Media Thickness After Severe Early-Onset Preeclampsia. *Acta Obstet Gynecol Scand* (2014) 93(12):1309–16. doi: 10.1111/aogs.12499
240. Sandvik MK, Leirgul E, Nygard O, Ueland PM, Berg A, Svarstad E, et al. Preeclampsia in Healthy Women and Endothelial Dysfunction 10 Years Later. *Am J Obstet Gynecol* (2013) 209(6):569 e1– e10. doi: 10.1016/j.ajog.2013.07.024
241. Akhter T, Larsson M, Wikstrom AK, Naessen T. Thicknesses of Individual Layers of Artery Wall Indicate Increased Cardiovascular Risk in Severe Pre-Eclampsia. *Ultrasound Obstet Gynecol* (2014) 43(6):675–80. doi: 10.1002/uog.13289
242. Stevens DU, Smits MP, Bulten J, Spaanderman ME, van Vugt JM, Al-Nasiry S. Prevalence of Hypertensive Disorders in Women After Preeclamptic Pregnancy Associated With Decidual Vasculopathy. *Hypertens Pregnancy* (2015) 34(3):332–41. doi: 10.3109/10641955.2015.1034803
243. Stevens DU, Al-Nasiry S, Fajta MM, Bulten J, van Dijk AP, van der Vlugt MJ, et al. Cardiovascular and Thrombogenic Risk of Decidual Vasculopathy in Preeclampsia. *Am J Obstet Gynecol* (2014) 210(6):545 e1–6. doi: 10.1016/j.ajog.2013.12.029
244. Robertson WB, Brosens I, Dixon HG. The Pathological Response of the Vessels of the Placental Bed to Hypertensive Pregnancy. *J Pathol Bacteriol* (1967) 93(2):581–92. doi: 10.1002/path.1700930219
245. Veerbeek JH, Brouwers L, Koster MP, Koenen SV, van Vliet EO, Nikkels PG, et al. Spiral Artery Remodeling and Maternal Cardiovascular Risk: The Spiral Artery Remodeling (SPAR) Study. *J Hypertens* (2016) 34(8):1570–7. doi: 10.1097/HJH.0000000000000964
246. Kim JY, Kim YM. Acute Atherosis of the Uterine Spiral Arteries: Clinicopathologic Implications. *J Pathol Transl Med* (2015) 49(6):462–71. doi: 10.4132/jptm.2015.10.23
247. Staff AC, Dechend R, Redman CW. Review: Preeclampsia, Acute Atherosis of the Spiral Arteries and Future Cardiovascular Disease: Two New Hypotheses. *Placenta* (2013) 34 Suppl:S73–8. doi: 10.1016/j.placenta.2012.11.022
248. Parks WT, Catov JM. The Placenta as a Window to Maternal Vascular Health. *Obstet Gynecol Clin North Am* (2020) 47(1):17–28. doi: 10.1016/j.ogc.2019.10.001
249. Brosens I, Benagiano M, Puttemans P, D'Elios MM, Benagiano G. The Placental Bed Vascular Pathology Revisited: A Risk Indicator for Cardiovascular Disease. *J Matern Fetal Neonatal Med* (2019) 32(9):1556–64. doi: 10.1080/14767058.2017.1409718

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Identification of Profound Metabolic Alterations in Human Dendritic Cells by Progesterone Through Integrated Bioinformatics Analysis

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Maintaining the homeostasis of the decidual immune microenvironment at the maternal-fetal interface is essential for reproductive success. Dendritic cells (DCs) are the professional antigen-presenting cells and dominate this balance of immunogenicity and tolerance. Progesterone (P4) is highlighted as the "hormone of pregnancy" in most eutherian mammals because of its regulatory role in immune-endocrine behavior during pregnancy. Recent studies have shown that P4 is associated with the differentiation and function of DCs, however, the underlying mechanisms remain unidentified. In addition, while progress in the field of immunometabolism has highlighted the intimate connections between the metabolism phenotype and the immunogenic or tolerogenic fate of DCs, whether P4 can affect DCs metabolism and thus exert a functional manipulation has not yet been explored. In this study, we acquired human peripheral blood monocyte-derived DCs and conducted RNA sequencing (RNA-seq) on immature DCs (iDCs), P4-treated DCs (pDCs), and mature DCs (mDCs), aiming to comprehensively assess the effects of P4 on DCs. Our results showed pDCs performed a distinct differentially expressed genes (DEGs) profile compared with iDCs or mDCs. Further functional enrichment and weighted gene co-expression network (WGCNA) analysis found that these DEGs were related not only to the cellular components but also to the significant metabolic activities, including mitochondrial oxidative phosphorylation (OXPHOS) and fatty acid metabolism. In addition, these changes may be involved in the activation of various signaling pathways of PI3K/Akt/mTOR, AMPK/PGC1-α, and PPAR-γ. In summary, our work suggested that P4 induced profound metabolic alterations of mitochondrial OXPHOS and fatty acid metabolism in DCs. Our findings may provide new insights into the role of P4 in DCs.

Keywords: progesterone, dendritic cells, RNA-seq, OXPHOS, fatty acid metabolism

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INTRODUCTION

Pregnancy is a complex and highly coordinated event. Immunologically, the embryo is a semi-allograft that resides in an immune-competent mother, however, the distinct tolerable microenvironment at the maternal interface provides a guarantee for a successful pregnancy. The underlying mechanisms of maternal-fetal tolerance are multiple including the change of immune cells populations, induction of regulatory T cells, and the shift of Th1 pro-inflammatory to Th2 anti-inflammatory cytokine responses (1, 2). These alternations are sophisticatedly orchestrated under the immune-endocrine interactions. In particular, the female steroid hormone–progesterone (P4), is considered to occupy an important regulatory position in mediating immune response and maintaining pregnancy in humans (3).

P4 is mainly produced by the corpus luteum of the ovary in the menstrual cycle, and this function is subsequently taken over by the placenta after 8 weeks in pregnancy (4, 5). P4 is regarded as the "hormone of pregnancy" and its serum levels undergo a profound change ranging from 10^{-9} M to 10^{-6} M during pregnancy (6). P4 directly regulates gene transcription by binding specific receptors (7). Many studies have reported that P4 is widely involved in endometrial spiral artery remodeling, trophoblast cells adhesion, proliferation, and stromal cells decidualization (8, 9). In addition, studies have emphasized P4 as a communication bridge of endocrine-immune to mediate maternal tolerance to the fetus *via* acting on a series of decidual immune cells, including natural killer cells, T cells, and dendritic cells (DCs) (10–13). Remarkably, the effect of P4 on DCs has gained great attention because of its plastic talent in inducing antigen-specific immunity or tolerance at the maternal-fetal interface.

DCs are originally described as the most potent antigen-presenting cell, and play a central role in both innate and adaptive immunity, despite their small proportions. DCs are divided into two different developmental stages: immature DCs (iDCs) and mature DCs (mDCs) (14). iDCs are usually found to have a limited migratory and T cell priming capacity and serve as "surveillance police" to induce immune tolerance, whereas mDCs exhibit an exceptional ability for antigen presentation and T cell activation and therefore triggers a strong immune response (15). Moreover, iDCs can differentiate toward immunogenic mDCs when activated by various antigens *in vivo* or typical toll-like receptor (TLR) agonists such as lipopolysaccharide (LPS) *in vitro* (16). DCs are not only positively regulated for their maturation as described above, but also negatively responded to signals that prevent their activation, such as dexamethasone and vitamin D (17, 18). Recently, some studies reported that P4 can suppress the production of pro-inflammatory cytokines TNF-α and IL-1β, and reduce CD40 and CD80 expression in DCs (19–21). These studies emphasized that DCs were highly responsive to P4 and can be modified in terms of their activities and functions. However, the number of studies on this topic is still limited, and the underlying mechanism remains poorly understood.

Immunometabolism, an emerging field that explores how metabolism affects immune cell functions, has been well understood in the last decade (22, 23). Numerous studies have revealed that DCs performed preferred metabolism pathways in activated or resting status (24–26). Moreover, metabolism can also directly determine the immunogenicity or tolerability of DCs (27, 28). Interestingly, a recent study demonstrated that P4 can activate hypoxia-inducible factor 1α (HIF-1α) and c-Myc signal to maintain aerobic glycolysis and decidualization in decidualized cells (29). Therefore, it is reasonable to speculate that the metabolism phenotype of DCs may be regulated by P4. In this study, we aimed to determine the metabolic changes and key modules genes underlying human peripheral blood monocyte-derived DCs treated with P4 (pDCs) by RNA sequencing (RNA-seq) and weighted gene co-expression network analysis (WGCNA). Functional enrichment analysis was further applied to help in identifying the most important candidate genes involved in metabolism for quantitative real-time polymerase chain reaction (qRT-PCR) and immunoblot validation.

MATERIALS & METHODS

In Vitro Generation and Treatment of Human DCs

This study was approved by the Institutional Review Board of Reproductive Research Ethics Committees of Shenzhen Zhongshan Urology Hospital (Approval number: SZZSECHU-2020005). Informed consent was obtained following the Declaration of Helsinki. A protocol for the generation of human peripheral blood monocyte-derived DCs was based on previous research with some minor modifications (30). In brief, human monocytes were isolated from peripheral blood mononuclear cells (PBMCs) of healthy women volunteers by density centrifugation at 300 g for 10 minutes (Ficoll–Paque, GE Healthcare) and were purified by CD14 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany, purity >95%) according to the manufacturer's instructions. Then the cells were cultured in 24-well plates (Corning, China) with RPMI 1640 (Gibco, USA), supplemented with 10% FBS (Gibco, USA), 100 U/ml penicillin, and 100mg/ml streptomycin placed in an incubator under 5% CO_2 at 37°C. 10 ng/mL recombinant human IL-4 (R&D Systems) and 20 ng/mL recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) (R&D Systems) were also added to induce the differentiation of the cells. Refreshment of the culture medium and cytokines was performed on day 2. To obtain P4-induced DCs, cells were treated on day 4 with 10^{-8} M P4 (Sigma-Aldrich). Then cells were either treated with 1μg/mL LPS (Sigma-Aldrich) on day 5 or left untreated. On day 7, immature, mature, and P4-treated DCs were photographed with a High Content Analysis System (Operetta CLS, PerkinElmer) before being harvested for further experiments. The whole process was displayed in **Figure 1A**.

RNA Collection and Sequencing

Total RNA was isolated using RNA Purification Kit (Thermo Fisher) following the instructions of the manufacturer, and the purity and integrity were checked using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA) and RNA Nano 6000

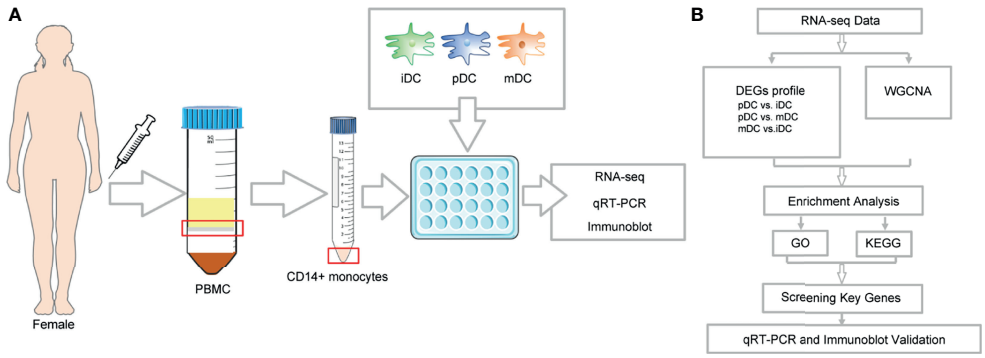

FIGURE 1 | Illustration of the study design framework and RNA-seq analysis flow.

Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA), respectively. Subsequently, a total amount of 3 μg RNA was used for the RNA sample preparations. First-strand and second-strand cDNA were synthesized according to the manufacturer of M-MuLV Reverse Transcriptase (RNase H^-), and DNA Polymerase I and RNase H. cDNA fragments of 250~300 bp in length were selected and purified with the AMPure XP system (Beckman Coulter, Beverly, MA, USA). Then 3 μl USER Enzyme (NEB, USA) was used with cDNA at 37°C for 15 min followed by 5 min at 95°C before PCR and products were purified with AMPure XP system. Library quality was assessed on the Agilent Bioanalyzer 2100 system. The clustering of the index-coded samples was performed on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumia) according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina Hiseq platform, and 125 bp/150 bp paired-end reads were generated. RNA-seq data have been deposited to NCBI's Sequence Read Archive with accession number PRJNA777391 (available at: https://www.ncbi.nlm.nih.gov/sra/). Finally, the overall workflow of data analysis was shown in **Figure 1B**.

Differentially Expressed Genes (DEGs) Analysis

The DESeq2 R package (1.16.1) was applied to perform DEGs analysis among iDCs, pDCs, and mDCs. The *P*-value was adjusted using Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted *P*-value of <0.05 were assigned as differentially expressed. The Venn diagram was drawn by using the online Venn diagrams tool (https://bioinfogp.cnb.csic.es/tools/venny/index.html) and volcano plots were represented with the R packages (1.16.1).

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichment Analysis

GO and KEGG enrichment analysis of DEGs were implemented by the cluster profile R package. The GO terms consist of the following three parts: biological process (BP), cell component (CC), and molecular function (MF). GO and KEGG terms with a corrected *P*-value of less than 0.05 were considered significantly enriched by DEGs.

WGCNA Analysis

WGCNA is a method for analyzing gene expression patterns of multiple samples, which can cluster genes with similar expression patterns and analyze the relationship between modules and specific traits or phenotypes. The genes with median absolute deviation (MAD) ≤ 0.01 were filtered, and the soft-power threshold of β = 13 was selected. WGCNA analysis was performed in R with the WGCNA package. The further GO and KEGG enrichment was performed using Metascape.

RNA Collection and qRT-PCR Analysis

Total RNA was acquired as above described. 500 ng total RNA was used to synthesize cDNA using the PrimeScript™ RT Reagent Kit with gDNA Eraser (Takara, Japan) with the Bio-Rad system. Synthesized cDNA was carried for qRT-PCR in a 10 μl reaction using Luna® Universal qPCR Master Mix (New England Biolabs) with primers specific to the genes. The reaction was performed in the QuantStudio 5 Real-Time PCR system (Applied Biosystems), following the manufacturer's instructions. The $2^{-\Delta\Delta Ct}$ method was determined to calculate and quantify the gene expression. All mRNA levels were normalized to β-actin. Primers were designed with computer assistance based on GeneBank, and the sequences were listed in **Table 1**.

Immunoblot Analysis

Cells were harvested and lysed with radioimmunoprecipitation (RIPA; Beyotime, Shanghai, China) lysis buffer, and the lysates were centrifuged at 4°C 12000g for 15 min to collect the supernatant. The BCA assay kit (Beyotime, Shanghai, China), was used to quantify protein concentrations. An automated Wes Capillary System (Protein Simple, San Jose, CA, 12-230 kDa kit cat. SM-W004) was used to detect the proteins levels of oxidative phosphorylation (OXPHOS) according to the manufacturer's protocol. Total protein loading was 0.75 μg/μl. Antibody signals were determined to be saturated at 1:50 for Total OXPHOS

TABLE 1 | Human forward and reverse primers for validation by qRT-PCR.

Primers	Forward primer (5′-3′)	Reverse primer (5′-3′)
MPC1	ATTTGCCTACAAGGTACAGCC	AGTCATCTCGTGTTTGATAAGCC
SDHC	TAGGTTCAAACCGTCCTCTGT	GAGAGACCCCTGCACTCAAAG
SDHB	TGAATAAGTGCGGACCTATGGTGTTG	GAGCCACAGATGCCTTCTCTACAAG
UQCRQ	CGCGAGTTTGGGAATCTGAC	TAGTGAAGACGTGCGGATAGG
ATP5A	ATGACGACTTATCCAAACAGGC	CGGGAGTGTAGGTAGAACACAT
COX II	CCATCCCTACGCATCCTTTAC	GTTTGCTCCACAGATTTCAGAG
NDUFB8	ACAGGAACCGTGTGGATACAT	CCCCACCCAGCACATGAAT
FASN	TCGTGGGCTACAGCATGGT	GCCCTCTGAAGTCGAAGAAGAA
ACACA	CATCAAATGCATCAGCAGAGACT	CTGCGTCATATGGATGATGGAAT
HADHA	AAATTGACAGCGTATGCCATGA	GCTTTCGCACTTTTTCTTCCACT
HADHB	CTGTCCAGACCAAAACGAAGAA	CGATGCAACAAACCCGTAAGC
CPT2	CTGGAGCCAGAAGTGTTCCAC	AGGCACAAAGCGTATGAGTCT
CPT1	TCCAGTTGGCTTATCGTGGTG	TCCAGAGTCCGATTGATTTTTGC
PI3K	AAGAAGCAAGCAGCTGAG	CTACAGAGCAGGCATAG
AKT	GCCTTTGCCGATCCGC	GCCGTAGCCGTTGTCG
mTOR	ATGACGAGACCCAGGCTAA	GCCAGTCCTCTACAATACGC
HIF-1α	GAAAGCGCAAGTCTTCAAAG	TGGGTAGGAGATGGAGATGC
AMPK	GGCACGCCATACCCTTGAT	TCTTCCTTCGTACACGCAAATAA
PGC1-α	TCTGAGTCTGTATGGAGTGACAT	CCAAGTCGTTCACATCTAGTTCA
PPAR-γ	CGGTGACTTATCCTGTGGTCC	CCGCAGATTCTACATTCGATGTT
β-actin	TCGTGCGTGACATTAAGGAG	GTCAGGCAGCTCGTAGCTCT

MPC1, mitochondrial pyruvate carrier 1; SDHB, succinate dehydrogenase complex iron sulfur subunit B; SDHC, succinate dehydrogenase subunit C; UQCRQ, ubiquinol-cytochrome c reductase complex III subunit VII; ATP5A, ATP synthase F1 subunit alpha; COX II, cytochrome c oxidase subunit II; NDUFB8, NADH ubiquinone oxidoreductase subunit B8; FASN, fatty acid synthase; ACACA, acetyl-CoA carboxylase-1; HADHA, hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha; HADHB, hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit beta; CPT2, carnitine palmitoyltransferase II; CPT1, carnitine palmitoyltransferase I; PI3K, phosphoinositide-3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; HIF-1α, hypoxia-inducible factor 1α; AMPK, Adenosine 5-monophosphate-activated protein kinase; PPAR-γ, peroxisome proliferator activated receptor gamma; PGC1-α, PPAR-γ coactivator 1 alpha; qRT-PCR, quantitative real-time polymerase chain reaction.

Human WB Antibody Cocktail (Abcam, ab110411) and 1:200 for vinculin antibody (Abcam, ab129002). Immunoblot analysis was executed using the Compass for Simple Western Program (Protein Simple).

Statistical Analysis

Continuous data with normal distribution were presented as the mean ± standard error of mean (SEM), and analyzed by analysis of variance (ANOVA) using SPSS 22.0 (IBM SPSS, USA). Continuous variables without normal distribution were presented as median and interquartile range and were analyzed by Kruskal-Wallis test, pairwise comparison. All *P*-values were two-tailed and significance was set as * *P*-value <0.05, ** *P*-value <0.01. All experiments were independently repeated at least three times.

RESULTS

Morphological Characterization of iDCs, pDCs, and mDCs

To investigate the alterations of human DCs exposed to P4, the morphological features of iDCs, pDCs, and mDCs were observed and photographed on day 7. Compared to the round-shaped iDCs, the mDCs showed more small dendrites, as previously reported (30). pDCs presented a completely different morphological feature from mDCs. These cells became larger with spindle-shaped dendrites (**Figure 2**). Moreover, all DCs appeared to be a typical characterization of the cell colony. The result showed that DCs developed a distinct morphological change after P4 treatment.

DEGs Identification of iDCs, pDCs, and mDCs

To investigate the effects of P4 on DCs, 5 samples in the iDCs, pDCs, and mDCs group were selected for gene sequencing, respectively. Genes with an adjusted *P*-value <0.05 were categorized as differentially expressed. The overall distribution of DEGs was shown in a Venn diagram (**Figure 3A**). After normalization, P4 induced 846 genes alterations, with 314 upregulated genes and 532 downregulated genes compared with iDCs (**Figure 3B**). Comparatively, pDCs showed a total of 7289 DEGs, which contain 3054 upregulated and 4235 downregulated genes versus mDCs (**Figure 3C**). We also detected 6978 differential genes between iDCs and mDCs, with 3740 upregulated and 3238 downregulated genes (**Figure 3D**). These results revealed that P4-treated DCs behaved a distinct gene expression profile, especially when compared with mDCs.

GO and KEGG Enrichment Analysis

To recognize the feature and function of these DEGs in detail, we conducted GO and KEGG enrichment analysis by the cluster Profiler R package, in which gene length bias was corrected. GO terms with a corrected *P*-value of less than 0.05 were considered to be significantly enriched. Based on this screening criterion, we identified the most significant GO annotation, which consists of biological process (BP), cell composition (CC), and molecular function (MF). Compared to iDCs, DEGs in pDCs were mainly enriched in the regulation of cellular components size,

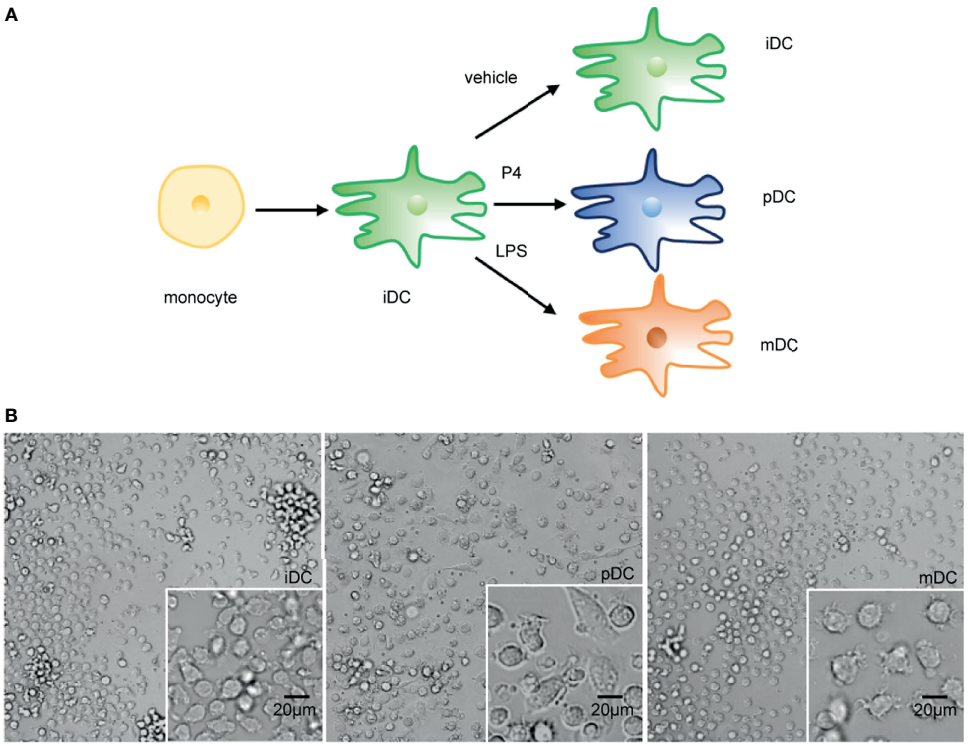

FIGURE 2 | Schematic diagram of DCs treatments and representative morphological pictures. **(A)** Schematic diagram of DCs treatments. **(B)** Representative monolayer cells pictures of iDC, pDC, and mDC on day 7. Magnification, x20; x40. Scale bar = 20 μm. P4, progesterone; LPS, lipopolysaccharide; iDC, immature DC; pDC, P4-treated DC; mDC, mature DC.

cytoskeleton organization, actin-based cell projection (**Figure 4A**). When compared with mDCs, pDCs showed a significant gene enrichment associated with metabolic behaviors, including OXPHOS, mitochondrial protein complex, mitochondrial respiratory chain, and NADH dehydrogenase activity (**Figure 4B**). These GO terms were also illustrated as dot plots, with the gene ratio denoted by size, and the significance denoted by color (**Figures 4C, D**). The detailed gene information of the top 10 GO annotations was also displayed in **Table 2**. To further investigate the potential pathway involved in these DEGs, we also selected the top 20 of KEGG enrichment results. We found that pDCs were mainly enriched in the regulation of actin cytoskeleton, phosphoinositide-3-kinase (PI3K)/protein kinase B (Akt) signaling, and glycerophospholipid metabolism compared with iDCs (**Figure 4E**). Interestingly, when compared with mDCs, pDCs presented significant enrichment involved in metabolic activities, including OXPHOS, citrate cycle, fatty acid elongation, and biosynthesis of unsaturated fatty acids (**Figure 4F**). We also listed the detailed gene information related to these metabolic pathways in **Table 3**. In addition, we observed several canonical metabolism-related signaling, including the peroxisome proliferator activated receptor (PPAR) signaling, mammalian target of rapamycin (mTOR) signaling, HIF-1α signaling, and adenosine 5-monophosphate-activated protein kinase (AMPK) signaling although the *P*-values were slightly above the 0.05 significance threshold (**Table S1**). Collectively, these data implied that compared with iDCs or mDCs, the DEGs of pDCs were associated with the cellular cytoskeleton and cellular metabolic events including OXPHOS and fatty acid metabolism.

WGCNA Analysis

To further explore the changes that DCs respond to P4, we also conducted a WGCNA analysis. A scale-free co-expression network was constructed with a soft threshold of 13 (**Figure 5A**). Genes in the same module had similar expression patterns in different samples, and modules were distinguished by different colors. A total of 26 modules were generated, of which the blue, red, and green-yellow modules showed the highest correlation and were selected for further analysis (**Figures 5B, C**). Our enrichment results showed that GO annotations in the red module were mainly related to fatty acid metabolism, lipid biosynthetic process, acetyl-CoA, and C-acyltransferase activity. The blue and green-yellow modules were enriched in mitochondrial biology, oxidoreductase activity, and the generation of cellular metabolites and energy (**Figure 5D**). Moreover, KEGG analysis of these three modules exhibited enrichment in metabolic activities, such as fatty acid elongation or oxidation (**Figure 5E**). These data suggested that P4 treatment induced the fatty acid metabolism and mitochondrial alterations of DCs.

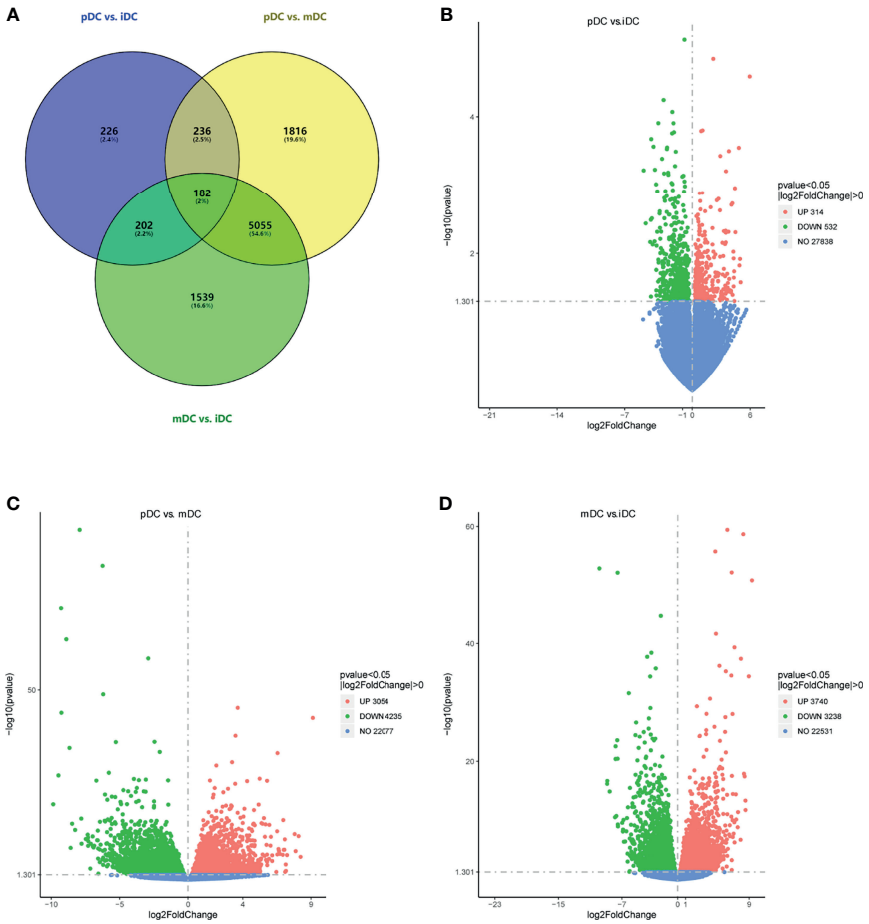

FIGURE 3 | DEGs analysis. **(A)** Venn diagram of DEGs (pDC vs. iDC, pDC vs. mDC, and mDC vs. iDC). **(B–D)** Volcano map of DEGs (pDC vs. iDC; pDC vs. mDC, and mDC vs. iDC, respectively). Red dots indicated upregulated DEGs, green dots indicated downregulated DEGs, and blue dots indicated genes that were not differentially expressed. DEGs, differentially expressed genes; iDC, immature DC; pDC, P4-treated DC; mDC, mature DC.

Validation of qRT-PCR and Immunoblot

To validate the RNA-seq data, we selected 20 genes related to the metabolism of all DEGs to perform qRT-PCR. Our results showed that the genes of mitochondrial complexes II-V (succinate dehydrogenase complex iron sulfur subunit B, SDHB; succinate dehydrogenase complex subunit C, SDHC; ubiquinol-cytochrome C reductase complex III subunit VII, UQCRQ; cytochrome c oxidase subunit II, COX II, and ATP synthase F1 subunit alpha, ATP5A) were highly expressed in pDCs compared with mDCs, though no significant differences were detected in complex I–NADH ubiquinone oxidoreductase subunit B8 (NDUFB8) (**Figure 6A**). These complexes are important components of the electron transport chain and the oxygen-coupled ATP synthesis in the mitochondrial OXPHOS process. Our data showed that the mRNA levels of mitochondrial pyruvate carrier 1 (MPC1) were upregulated in pDCs compared with mDCs, which is a key factor for mitochondrial pyruvate carrier and sequent OXPHOS (**Figure 6A**). We also demonstrated significantly increased protein levels of OXPHOS complexes in pDCs compared with mDCs (**Figures 6B, C**). Additionally, we found that hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit beta (HADHB), carnitine palmitoyltransferase I (CPT1), and II (CPT2), which are necessary for fatty acid oxidation (FAO), and acetyl-CoA carboxylase alpha (ACACA) and fatty acid synthase (FASN), which are involved in the fatty acid synthesis (FAS), were also increased in mRNA levels in pDCs compared with mDCs (**Figure 6A**). Moreover, we observed upregulated mRNA expression of AMPK, PPAR-γ coactivator 1 alpha (PGC1-α), PI3K, Akt, mTOR, and PPAR-γ in pDCs compared with mDCs, whereas the expression of HIF-1α had no change (**Figure 6A**). Taken together, these findings suggested DCs present a distinct phenotype following P4 treatment with general trends in association with enhanced OXPHOS and fatty metabolic activities.

DISCUSSION

P4 has long been considered as the master hormone of pregnancy. In recent years, P4 is put forward as a potential medium for regulating the maternal immune system *in vivo* and

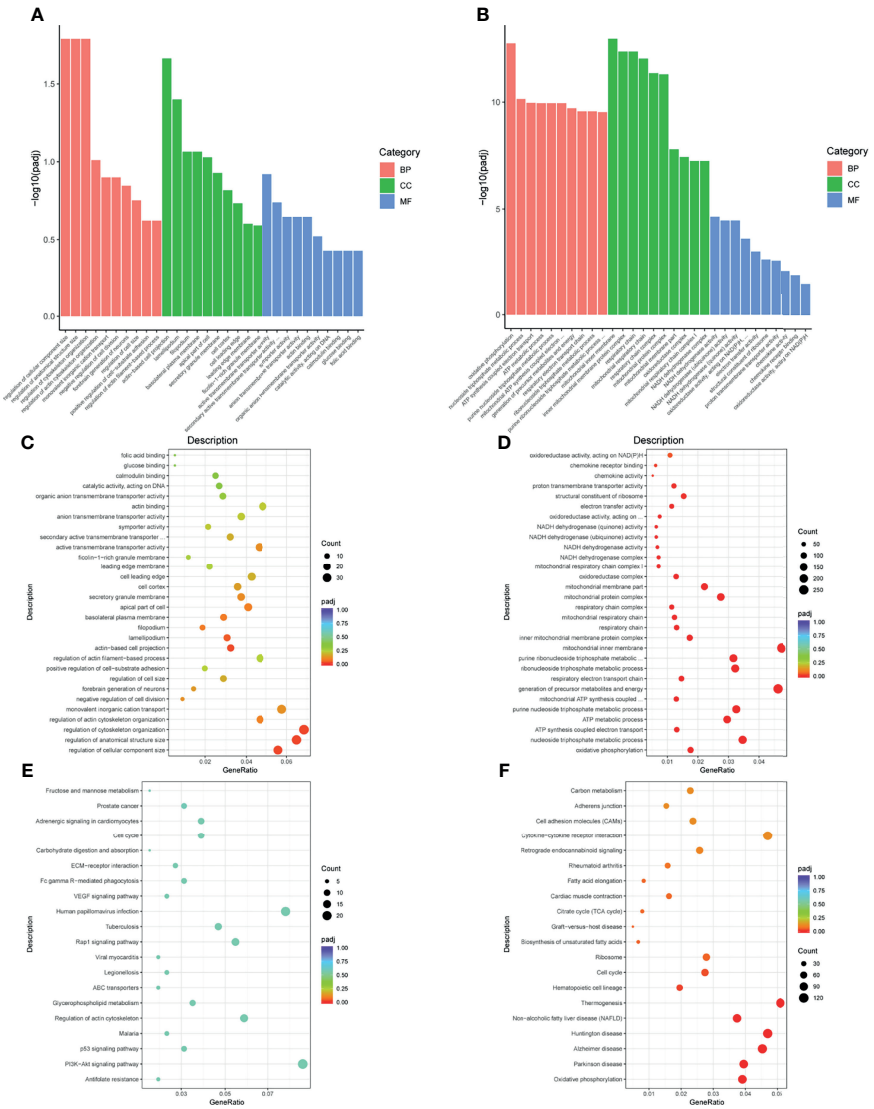

FIGURE 4 | GO and KEGG enrichment analysis. **(A, B)** Bars diagram of GO enrichment analysis (pDC vs. iDC and pDC vs. mDC, respectively). The X-axis denoted detailed annotation classes of GO ontologies by different colors and the Y-axis denoted the percentage of genes. **(C, D)** Dots diagram of GO enrichment (pDC vs. iDC and pDC vs. mDC, respectively). **(E, F)** Dots diagram of KEGG enrichment (pDC vs. iDC and pDC vs. mDC, respectively). The X-axis indicated the proportion of DEGs annotated to GO or KEGG terms to all GO or KEGG annotated DEGs and the Y-axis represented detailed classification of GO or KEGG. Size and color of dots represented the percentage of genes and significance level of adjusted *P*-value, respectively. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; CC, cellular component; MF, molecular function; DEGs, differentially expressed genes; iDC, immature DC; pDC, P4-treated DC; mDC, mature DC.

for maintaining maternal-fetal immune tolerance (13, 31, 32). These effects are achieved through interaction with a series of decidual immune cells, such as T cells, natural killer cells, macrophages, and DCs. Although some profound findings demonstrated the influence of P4 on lymphocytes, there are limited studies exploring the effects of P4 on DCs functions, and the mechanism underlying these effects (19, 33–35). DCs perform an extraordinary capacity to balance immunogenicity and tolerance for providing a protected environment during normal pregnancy. Therefore, it is interesting to explore the role of P4 on DCs. In the present study, we obtained an information-rich gene expression profile of human DCs treated with P4 by RNA-seq. Further analysis showed that P4 dramatically altered the expression of key genes and pathways involved in the metabolism of DCs, which may be a promising manipulator in modulating DCs tolerance. This current study provided the first evidence that P4 regulated the metabolism of DCs and helped to further improve our understanding of the effect of P4 on DCs.

In this study, we firstly analyzed the treatment and the corresponding gene data of DCs and found pDCs behaved a significant different DEGs profile from that of iDCs or mDCs. Compared to iDCs, the GO enrichment analysis in pDCs

TABLE 2 | Top 10 enriched GO terms.

Category	Description	*p*-value	Gene name
CC	Mitochondrial inner membrane	1.1E-13	ATP5MF/UQCR10/NDUFA2/COX5B/ATP5PB HADHB/UQCRC2/MPC1/UQCR11/HADHA COX18/SDHD/CPT2/MPC2/SDHB
BP	Oxidative phosphorylation	1.8E-13	NDUFB5/UQCR10/NDUFA2/COX5B/ATP5PB NDUFA1/UQCRC2/COX7C/NDUFB6/UQCRQ UQCRC1/COX6C/NDUFB9/COX5A/CYC1
CC	Inner mitochondrial membrane protein complex	4.3E-13	ATP5MF/UQCRFS1/NDUFA4/NDUFB2/UQCR10 NDUFA2/COX5B/ATP5PB/NDUFA1/UQCRC2 NDUFB6/UQCRQ/SDHC/ATP5F1B/ATP5MD
CC	Respiratory chain	4.3E-13	NDUFS4/SDHC/UQCR11/NDUFS5/NDUFS6 NDUFA3/NDUFB8/MTCO2/NDUFB10/NDUFV1 NDUFA10/NDUFS8/SDHAF4/COX7A1/SDHB
CC	Respiratory chain complex	4.4E-12	NDUFB5/UQCR10/COX7C/NDUFA8/COX8A NDUFA9/NDUFAB1/COX4I1/COX7B/NDUFS3 NDUFA5/SDHD/NDUFA12/SDHB/UQCRHL
CC	Mitochondrial protein complex	5E-12	UQCR10/NDUFA2/COX5B/ATP5PB/NDUFA1 TIMM13/UQCRC2/COXII/COX7C/NDUFS4 SDHC/ATP5F1B/ATP5MD/ATP5MPL/HADHA
BP	ATP metabolic process	1.1E-10	UQCRQ/NDUFB3/ATP5F1A/SLC25A33/UQCRH ENPP1/COX6B1/SDHC/COX4I1/LDHA ATP5PF/PKM/HK3/COX5A/HIF1A
BP	Mitochondrial ATP synthesis coupled electron transport	1.1E-10	COX4I1/COX7B/NDUFS3/NDUFA5/SDHD MT-ND4L/COX6C/SDHAF2/NDUFB8/MT-ND5 NDUFB7/CCNB1/CDK1/NDUFB1/NDUFC1
BP	Generation of precursor metabolites and energy	1.9E-10	COX8A/NDUFA9/ATP5ME/SDHD/DDIT4 ATP5F1C/MDH2/NUP35/COA6/HIF1A AOC2/DHA1/GPD1/PFKFB1/HK2
BP	Electron transport chain	4.7E-10	UQCRC2/COX7C/SDHD/AKR7A2/TSTA3 GPD2/NDUFB11/NDUFC2/TXNRD2/HSD17B6 COX7A2L/UGDH/COX7A1/HAAO/NDUFAF1

GO, Gene Ontology; BP, biological process; CC, cellular component; MF, molecular function.

TABLE 3 | Most significant KEGG metabolism pathways.

Terms	Description	*p*-value	Gene name
hsa00190	Oxidative phosphorylation	5.2E-38	ATP5MF/UQCRFS1/NDUFA4/NDUFB2/ATP5MC1/NDUFV3 NDUFB5/UQCR10/NDUFA2/COX5B/ATP5PB/NDUFA1 UQCRC2/COX7C/NDUFA8/ATP5MC3/COX8A/NDUFA9 ATP5ME/UQCRB/ATP5F1D/NDUFB6/UQCRQ/NDUFB3 ATP5F1A/UQCRC1/PPA2/COX7A2/UQCRH/COX6B1 NDUFS7/NDUFS4/SDHC/ATP5F1B/ATP6V1F/UQCR11 NDUFS5/NDUFAB1/ATP6V1D/COX4I1/COX7B/ATP5MG MTATP8/NDUFS3/ATP5MC2/NDUFA5/ATP6V0E1/SDHD ATP5F1C/ATP5F1E/MTND4L/COX6C/NDUFB9/COX17 ATP5PF/NDUFB4/COX5A/CYC1/ATP6V0D1/NDUFS6 NDUFA3/NDUFB8/MTND5/NDUFB7/NDUFB1/NDUFC1 MTCO2/NDUFB10/NDUFV1/ATP6V1G1/NDUFA10 NDUFS8/MTCO1/NDUFS1/ATP6V0A1/NDUFB11 NDUFC2/ATP6V0B/ATP6V1B2/COX7A2L/COX7A1 ATP5PD/ATP6V1E1/ATP6V1A/NDUFA12/SDHB UQCRHL/NDUFA6/ATP5PO/COX6A1
hsa00020	Citrate cycle	2.82E-06	IDH1/PC/MDH1/PDHB/SDHC/IDH3G/SUCLG1 SDHD/MDH2/FH/ACO2/PDHA1/IDH2/SUCLG2 SUCLA2/SDHB/IDH3B
hsa01040	Biosynthesis of unsaturated fatty acids	6.81E-05	SCD/FADS2/ACOT7/FADS1/HADHA/TECR ELOVL5/ACAA1/ACOT1/ACOT4/ACOX1 HSD17B12/ACOT2/HACD1
hsa00062	Fatty acid elongation	0.00472	ECHS1/ACAA2/HADHB/ACOT7/PPT1/HADHA ABHD17A/TECR/ELOVL5/ACOT1/ACOT4/HSD17B12 ACOT2/HACD1

KEGG, Kyoto Encyclopedia of Genes and Genomes.

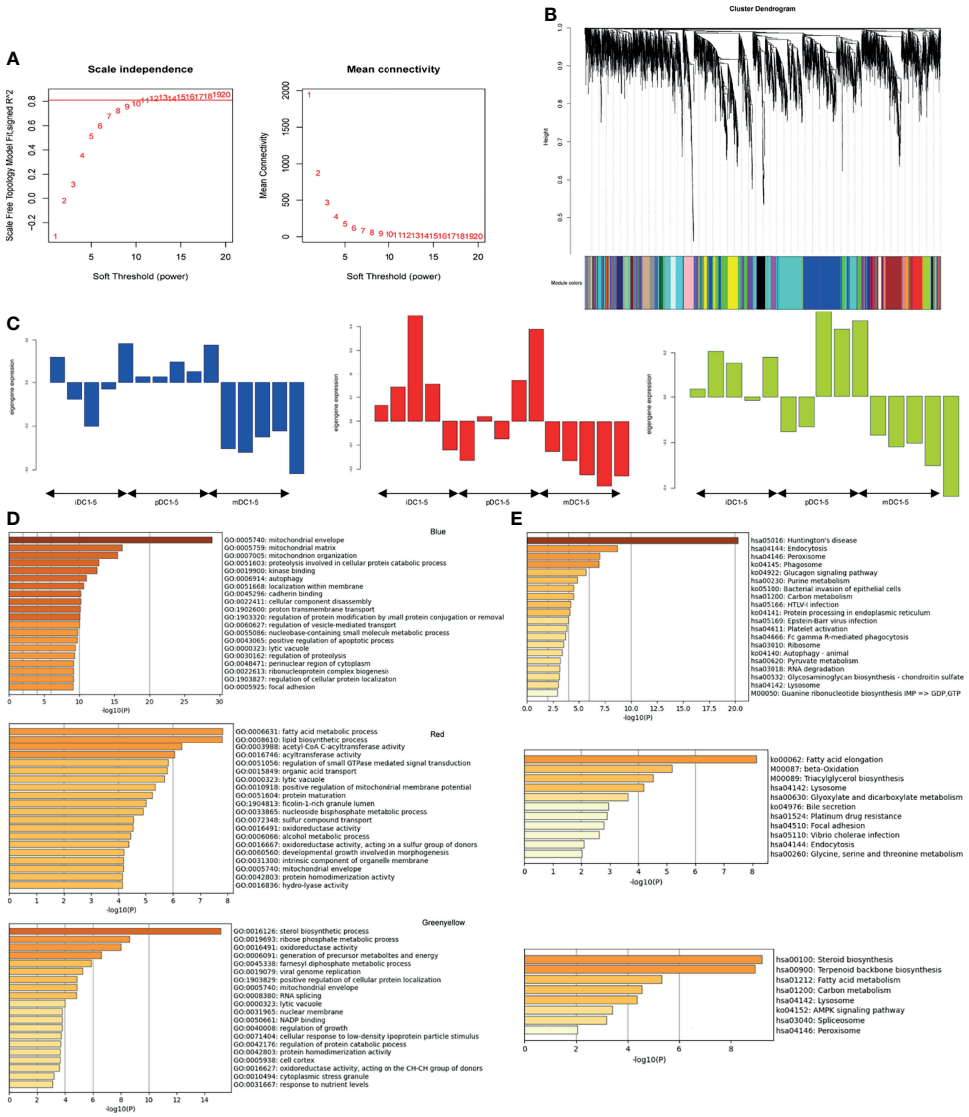

FIGURE 5 | WGCNA analysis. **(A)** Determination of soft-threshold power. **(B)** Cluster dendrogram of genes. **(C)** Expression pattern of the genes and eigengenes in the blue, red, and green-yellow modules. **(D)** GO enrichment results for genes in blue, red, and green-yellow modules. **(E)** KEGG enrichment results for genes in blue, red, and green-yellow modules. GO, Gene Ontology; KEGG, Kyoto encyclopedia of genes and genomes; iDC, immature DC; pDC, P4-treated DC; mDC, mature DC.

revealed enriched terms related to cellular components and cytoskeleton. Interestingly, the mitochondria were identified as the core of GO cellular component terms in pDCs, such as mitochondrial protein complex, mitochondrial inner membrane, mitochondrial respiratory chain, and electron transfer activity compared with mDCs. The mitochondrial respiratory chain, also known as the mitochondrial electron transport chain, consists of five enzyme complexes (complexes I-V) situated in the inner mitochondrial membrane that couple oxidation to phosphorylation, and provide ATP. KEGG analysis also showed that DEGs in pDCs were primarily concentrated in the OXPHOS process and fatty acid metabolism. These data suggested that P4 induced obvious changes in gene transcription levels that point to mitochondrial metabolism of DCs. Our qRT-PCR validation demonstrated that P4 upregulated the mRNA levels of mitochondrial complexes II-V (SDHB, SDHC, UQCRQ, ATP5A, COX II) in DCs. We also further confirmed increased protein levels of OXPHOS complexes, including SDHB, COX II, and ATP5A. In addition, we found upregulated mRNA levels of MPC1, which is essential for mitochondrial pyruvate transport and subsequent OXPHOS process. These findings together indicated that human DCs initiated early OXPHOS metabolic pathways at the mRNA and protein levels after exposure to P4. According to WGCNA results and literature research, we also selected genes related to FAO (CPT1, CPT2, HADHB) and FAS (FASN, ACACA) for

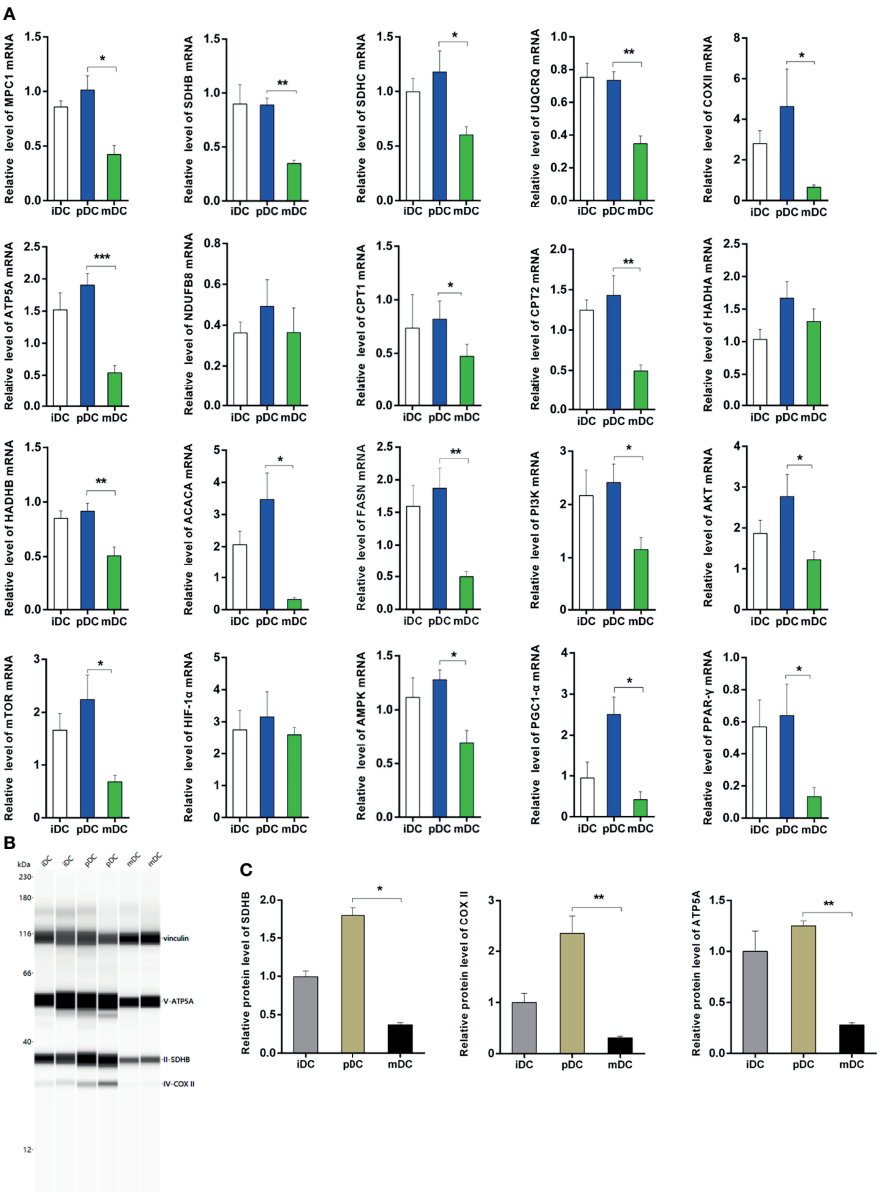

FIGURE 6 | Validation of qRT-PCR and immunoblot. **(A)** Expression of genes associated with OXPHOS, FAO, and FAS using qRT-PCR. All values were normalized against the mRNA expression level of β-actin. **(B, C)** Protein levels and quantification of OXPHOS. qRT-PCR, quantitative real-time polymerase chain reaction; OXPHOS, oxidative phosphorylation; FAO, fatty acid oxidation; FAS, fatty acid synthesis; iDC, immature DC; pDC, P4-treated DC; mDC, mature DC. *P < 0.05, **P < 0.01, ***P < 0.001.

validation, and the results showed that these genes were significantly upregulated in pDCs compared with mDCs. In detail, FAO involves two key steps of fatty acid transfer and β-oxidation. Both CPT1 and CPT2 are crucial to transfer activated long-chain fatty acids into mitochondria while HADHB acts in the subsequent β-oxidation process as one of the mitochondrial thiolates. In summary, our study identified profound mitochondrial and fatty metabolic alterations in human DCs regulated by P4.

It has long been acknowledged that the activation and function of immune cells correlate with, and are supported by alterations in their metabolic pathways (22, 23). Phenomena accompanying immunological responses involve changes in gene expression that are thermodynamically demanding, requiring fast metabolic adaptations, which is known as immunometabolism. Accumulating evidence has indicated that the metabolic programming of immune cells is an important determinant of their function (36, 37). DCs were reported to undergo metabolic

reprogramming in response to multiple environmental factors. To date, the metabolic features of immunogenic DCs have become more well characterized. Krawczyk et al. demonstrated that TLR agonists stimulated a profound metabolic transition to aerobic glycolysis in mouse DCs. This metabolic switch depended on the PI3K/Akt pathway, was antagonized by the AMPK, and was important for DCs maturation and function (38). Induction of DCs glycolysis was also essential for the anabolic demands that underpinned the production of membranes needed for expansion of the endoplasmic reticulum and Golgi to accommodate the stimulation *via* TLR agonists (39). Several studies have reported metabolic characteristics of tolerogenic DCs despite the limitations of the available research. Proteomic analysis of human DCs treated with vitamin D and dexamethasone, two well-known immunosuppressive agents that induced tolerogenic DCs, revealed increased expression of genes associated with mitochondrial metabolism and OXPHOS (17, 30). Malinarich et al. confirmed similar results that human tolerogenic DCs displayed a markedly augmented catabolic pathway, related to high oxidative phosphorylation and fatty acid metabolism levels (40). Another recent study showed that tolerogenic effects of vitamin D on DCs also involved FAS (18). Thus, a current generally accepted opinion shows that increased OXPHOS and FAO are tightly associated with the tolerogenic function of DCs (25, 28, 41, 42). The above findings emphasize that the activation and function of DCs are dictated by the type of metabolism these cells commit to. In our current study, P4-treated DCs performed high OXPHOS and fatty acid metabolism capacities. This may provide promising information that P4 may be another potential tolerogenic medium that coordinates the metabolism and immune tolerogenic function in DCs during pregnancy. These observations also point to a mechanism for rapid genome-wide reprogramming by modulation of underlying cellular metabolism during DC differentiation. Our findings may contribute to the identification of P4 as new metabolic targets for manipulating DC function. This knowledge may be used in the rational design of strategies to improve the immunogenicity or tolerogenic of DCs in clinically relevant settings.

Additionally, we also tentatively demonstrated that P4 upregulated mRNA levels of PI3K, Akt, mTOR, AMPK, PGC1-α, and PPAR-γ in DCs. The mTOR and its upstream PI3K/Akt have been well known as metabolism-related regulation signaling pathways in DCs (24). AMPK is a central regulator of catabolic metabolism and has been shown to activate the PGC-1α, which promotes mitochondrial biogenesis to increase mitochondrial OXPHOS (43). PPAR-γ, a sensor for fatty acids, was recently confirmed to be involved in the functional regulation of human DCs (27). Taken together, these data suggested that the above metabolic effects in pDCs may be mediated by these signaling pathways, however, further studies are needed to clarify the mechanism.

In summary, our current study revealed a comprehensive RNA-seq analysis and identified some critical molecules and pathways related to metabolism between P4 and DCs. Our results provided the first evidence that P4-treated DCs had a defined metabolic phenotype characterized by a prominent mitochondrial OXPHOS and high fatty acid metabolic phenotype. These data may provide a novel insight into the role of P4 in DCs.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, accession ID: PRJNA777391.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Institutional Review Board of Reproductive Research Ethics Committees of Shenzhen Zhongshan Urology Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YZ, TY, and SL supported the research. SZ and SL conceived the original idea and the structure of the manuscript. SZ performed the experiments and drafted the first version of the manuscript. LH assisted in the experiments and manuscript. XW collected the clinical samples. LD and SC provided critical feedback and helped revise the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. La Rocca C, Carbone F, Longobardi S, Matarese G. The Immunology of Pregnancy: Regulatory T Cells Control Maternal Immune Tolerance Toward the Fetus. *Immunol Lett* (2014) 162(1 Pt A):41–8. doi: 10.1016/j.imlet.2014.06.013
2. PrabhuDas M, Bonney E, Caron K, Dey S, Erlebacher A, Fazleabas A, et al. Immune Mechanisms at the Maternal-Fetal Interface: Perspectives and Challenges. *Nat Immunol* (2015) 16(4):328–34. doi: 10.1038/ni.3131
3. Piccinni MP, Raghupathy R, Saito S, Szekeres-Bartho J. Cytokines, Hormones and Cellular Regulatory Mechanisms Favoring Successful Reproduction. *Front Immunol* (2021) 12:717808. doi: 10.3389/fimmu.2021.717808
4. Stocco C, Telleria C, Gibori G. The Molecular Control of Corpus Luteum Formation, Function, and Regression. *Endocr Rev* (2007) 28(1):117–49. doi: 10.1210/er.2006-0022
5. Arck P, Hansen PJ, Mulac Jericevic B, Piccinni MP, Szekeres-Bartho J. Progesterone During Pregnancy: Endocrine-Immune Cross Talk in Mammalian Species and the Role of Stress. *Am J Reprod Immunol* (2007) 58(3):268–79. doi: 10.1111/j.1600-0897.2007.00512.x
6. Lydon JP, DeMayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery CAJr., et al. Mice Lacking Progesterone Receptor Exhibit Pleiotropic Reproductive Abnormalities. *Genes Dev* (1995) 9(18):2266–78. doi: 10.1101/gad.9.18.2266
7. Szekeres-Bartho J, Halasz M, Palkovics T. Progesterone in Pregnancy; Receptor-Ligand Interaction and Signaling Pathways. *J Reprod Immunol* (2009) 83(1-2):60–4. doi: 10.1016/j.jri.2009.06.262
8. Kim M, Park HJ, Seol JW, Jang JY, Cho YS, Kim KR, et al. VEGF-A Regulated by Progesterone Governs Uterine Angiogenesis and Vascular Remodelling During Pregnancy. *EMBO Mol Med* (2013) 5(9):1415–30. doi: 10.1002/emmm.201302618
9. Wang J, Liu S, Qin HM, Zhao Y, Wang XQ, Yan Q. Pregnancy-Associated Plasma Protein A Up-Regulated by Progesterone Promotes Adhesion and Proliferation of Trophoblastic Cells. *Int J Clin Exp Pathol* (2014) 7(4):1427–37.
10. Stites DP, Siiteri PK. Steroids as Immunosuppressants in Pregnancy. *Immunol Rev* (1983) 75:117–38. doi: 10.1111/j.1600-065x.1983.tb01093.x
11. Miyaura H, Iwata M. Direct and Indirect Inhibition of Th1 Development by Progesterone and Glucocorticoids. *J Immunol* (2002) 168(3):1087–94. doi: 10.4049/jimmunol.168.3.1087
12. Siiteri PK, Stites DP. Immunologic and Endocrine Interrelationships in Pregnancy. *Biol Reprod* (1982) 26(1):1–14. doi: 10.1095/biolreprod26.1.1
13. Shah NM, Imami N, Johnson MR. Progesterone Modulation of Pregnancy-Related Immune Responses. *Front Immunol* (2018) 9:1293. doi: 10.3389/fimmu.2018.01293
14. O'Doherty U, Peng M, Gezelter S, Swiggard WJ, Betjes M, Bhardwaj N, et al. Human Blood Contains Two Subsets of Dendritic Cells, One Immunologically Mature and the Other Immature. *Immunology* (1994) 82(3):487–93.
15. Hopkins RA, Connolly JE. The Specialized Roles of Immature and Mature Dendritic Cells in Antigen Cross-Presentation. *Immunol Res* (2012) 53(1–3):91–107. doi: 10.1007/s12026-012-8300-z
16. Reis e Sousa C. Dendritic Cells in a Mature Age. *Nat Rev Immunol* (2006) 6(6):476–83. doi: 10.1038/nri1845
17. Ferreira GB, Kleijwegt FS, Waelkens E, Lage K, Nikolic T, Hansen DA, et al. Differential Protein Pathways in 1,25-Dihydroxyvitamin D(3) and Dexamethasone Modulated Tolerogenic Human Dendritic Cells. *J Proteome Res* (2012) 11(2):941–71. doi: 10.1021/pr200724e
18. Garcia AM, Bishop EL, Li D, Jeffery LE, Garten A, Thakker A, et al. Tolerogenic Effects of 1,25-Dihydroxyvitamin D on Dendritic Cells Involve Induction of Fatty Acid Synthesis. *J Steroid Biochem Mol Biol* (2021) 211:105891. doi: 10.1016/j.jsbmb.2021.105891
19. Quispe Calla NE, Ghonime MG, Cherpes TL, Vicetti Miguel RD. Medroxyprogesterone Acetate Impairs Human Dendritic Cell Activation and Function. *Hum Reprod* (2015) 30(5):1169–77. doi: 10.1093/humrep/dev035
20. Mackern-Oberti JP, Jara EL, Riedel CA, Kalergis AM. Hormonal Modulation of Dendritic Cells Differentiation, Maturation and Function: Implications for the Initiation and Progress of Systemic Autoimmunity. *Arch Immunol Ther Exp (Warsz)* (2017) 65(2):123–36. doi: 10.1007/s00005-016-0418-6
21. Butts CL, Shukair SA, Duncan KM, Bowers E, Horn C, Belyavskaya E, et al. Progesterone Inhibits Mature Rat Dendritic Cells in a Receptor-Mediated Fashion. *Int Immunol* (2007) 19(3):287–96. doi: 10.1093/intimm/dxl145
22. O'Neill LA, Kishton RJ, Rathmell J. A Guide to Immunometabolism for Immunologists. *Nat Rev Immunol* (2016) 16(9):553–65. doi: 10.1038/nri.2016.70
23. Ganeshan K, Chawla A. Metabolic Regulation of Immune Responses. *Annu Rev Immunol* (2014) 32:609–34. doi: 10.1146/annurev-immunol-032713-120236
24. Pearce EJ, Everts B. Dendritic Cell Metabolism. *Nat Rev Immunol* (2015) 15(1):18–29. doi: 10.1038/nri3771
25. O'Neill LA, Pearce EJ. Immunometabolism Governs Dendritic Cell and Macrophage Function. *J Exp Med* (2016) 213(1):15–23. doi: 10.1084/jem.20151570
26. He Z, Zhu X, Shi Z, Wu T, Wu L. Metabolic Regulation of Dendritic Cell Differentiation. *Front Immunol* (2019) 10:410. doi: 10.3389/fimmu.2019.00410
27. Szatmari I, Töröcsik D, Agostini M, Nagy T, Gurnell M, Barta E, et al. PPARgamma Regulates the Function of Human Dendritic Cells Primarily by Altering Lipid Metabolism. *Blood* (2007) 110(9):3271–80. doi: 10.1182/blood-2007-06-096222
28. Sim WJ, Ahl PJ, Connolly JE. Metabolism Is Central to Tolerogenic Dendritic Cell Function. *Mediators Inflamm* (2016) 2016:2636701. doi: 10.1155/2016/2636701
29. Zuo RJ, Gu XW, Qi QR, Wang TS, Zhao XY, Liu JL, et al. Warburg-Like Glycolysis and Lactate Shuttle in Mouse Decidua During Early Pregnancy. *J Biol Chem* (2015) 290(35):21280–91. doi: 10.1074/jbc.M115.656629
30. Ferreira GB, van Etten E, Lage K, Hansen DA, Moreau Y, Workman CT, et al. Proteome Analysis Demonstrates Profound Alterations in Human Dendritic Cell Nature by TX527, an Analogue of Vitamin D. *Proteomics* (2009) 9(14):3752–64. doi: 10.1002/pmic.200800848
31. Szekeres-Bartho J. The Role of Progesterone in Feto-Maternal Immunological Cross Talk. *Med Princ Pract* (2018) 27(4):301–7. doi: 10.1159/000491576
32. Lissauer D, Eldershaw SA, Inman CF, Coomarasamy A, Moss PA, Kilby MD. Progesterone Promotes Maternal-Fetal Tolerance by Reducing Human Maternal T-Cell Polyfunctionality and Inducing a Specific Cytokine Profile. *Eur J Immunol* (2015) 45(10):2858–72. doi: 10.1002/eji.201445404
33. Xiu F, Anipindi VC, Nguyen PV, Boudreau J, Liang H, Wan Y, et al. High Physiological Concentrations of Progesterone Reverse Estradiol-Mediated Changes in Differentiation and Functions of Bone Marrow Derived Dendritic Cells. *PloS One* (2016) 11(4):e0153304. doi: 10.1371/journal.pone.0153304
34. Jones LA, Kreem S, Shweash M, Paul A, Alexander J, Roberts CW. Differential Modulation of TLR3- and TLR4-Mediated Dendritic Cell Maturation and Function by Progesterone. *J Immunol* (2010) 185(8):4525–34. doi: 10.4049/jimmunol.0901155
35. Butts CL, Candando KM, Warfel J, Belyavskaya E, D'Agnillo F, Sternberg EM. Progesterone Regulation of Uterine Dendritic Cell Function in Rodents Is Dependent on the Stage of Estrous Cycle. *Mucosal Immunol* (2010) 3(5):496–505. doi: 10.1038/mi.2010.28
36. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. The Biology of Cancer: Metabolic Reprogramming Fuels Cell Growth and Proliferation. *Cell Metab* (2008) 7(1):11–20. doi: 10.1016/j.cmet.2007.10.002
37. Donnelly RP, Loftus RM, Keating SE, Liou KT, Biron CA, Gardiner CM, et al. Mtorc1-Dependent Metabolic Reprogramming Is a Prerequisite for NK Cell Effector Function. *J Immunol* (2014) 193(9):4477–84. doi: 10.4049/jimmunol.1401558
38. Krawczyk CM, Holowka T, Sun J, Blagih J, Amiel E, DeBerardinis RJ, et al. Toll-Like Receptor-Induced Changes in Glycolytic Metabolism Regulate Dendritic Cell Activation. *Blood* (2010) 115(23):4742–9. doi: 10.1182/blood-2009-10-249540
39. Everts B, Amiel E, Huang SC, Smith AM, Chang CH, Lam WY, et al. TLR-Driven Early Glycolytic Reprogramming via the Kinases TBK1-IKKε Supports the Anabolic Demands of Dendritic Cell Activation. *Nat Immunol* (2014) 15(4):323–32. doi: 10.1038/ni.2833
40. Malinarich F, Duan K, Hamid RA, Bijin A, Lin WX, Poidinger M, et al. High Mitochondrial Respiration and Glycolytic Capacity Represent a Metabolic

Phenotype of Human Tolerogenic Dendritic Cells. *J Immunol* (2015) 194 (11):5174–86. doi: 10.4049/jimmunol.1303316
41. Everts B, Pearce EJ. Metabolic Control of Dendritic Cell Activation and Function: Recent Advances and Clinical Implications. *Front Immunol* (2014) 5:203. doi: 10.3389/fimmu.2014.00203
42. Zhuang Q, Cai H, Cao Q, Li Z, Liu S, Ming Y. Tolerogenic Dendritic Cells: The Pearl of Immunotherapy in Organ Transplantation. *Front Immunol* (2020) 11:552988. doi: 10.3389/fimmu.2020.552988
43. Hardie DG. AMP-Activated/SNF1 Protein Kinases: Conserved Guardians of Cellular Energy. *Nat Rev Mol Cell Biol* (2007) 8(10):774–85. doi: 10.1038/nrm2249

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Human Leucocyte Antigen G and Murine Qa-2 Are Critical for Myeloid Derived Suppressor Cell Expansion and Activation and for Successful Pregnancy Outcome

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During pregnancy, maternal immune system has to balance tightly between protection against pathogens and tolerance towards a semi-allogeneic organism. Dysfunction of this immune adaptation can lead to severe complications such as pregnancy loss, preeclampsia or fetal growth restriction. In the present study we analyzed the impact of the murine MHC class Ib molecule Qa-2 on pregnancy outcome *in vivo*. We demonstrate that lack of Qa-2 led to intrauterine growth restriction and increased abortion rates especially in late pregnancy accompanied by a disturbed trophoblast invasion and altered spiral artery remodeling as well as protein aggregation in trophoblast cells indicating a preeclampsia-like phenotype. Furthermore, lack of Qa-2 caused imbalanced immunological adaptation to pregnancy with altered immune cell and especially T-cell homeostasis, reduced T_{reg} numbers and decreased accumulation and functional activation of myeloid-derived suppressor cells. Lastly, we show that application of sHLA-G reduced abortion rates in Qa-2 deficient mice by inducing MDSC. Our results highlight the importance of an interaction between HLA-G and MDSC for pregnancy success and the therapeutic potential of HLA-G for treatment of immunological pregnancy complications.

Keywords: HLA-G, Qa-2, pregnancy, myeloid-derived suppressor cells, abortion, preeclampsia

INTRODUCTION

Premature termination of pregnancy either by abortion or by preterm delivery is the most important pregnancy complication. About 15% of clinically recognized pregnancies miscarry, however total reproductive losses are closer to 50% (1). Preterm birth, defined as birth before 37 weeks of gestation, affects 5% to 18% of successful pregnancies (2). Besides chromosomal or anatomic anomalies and

endocrinological disorders, immunological factors play an important role in pathogenesis of abortions and preterm delivery (2). During pregnancy, there is a close contact between maternal immune cells and fetal cells. Thus, the maternal immune system has to balance tightly between protection against pathogens and tolerance towards the semi-allogeneic fetus. Dysfunction of the immune adaptation to pregnancy can lead to severe complications such as pregnancy loss, preeclampsia, preterm birth or fetal growth restriction. The mechanisms facilitating maternal-fetal tolerance are only incompletely understood and therapeutic options are limited.

The major histocompatibility class Ib (MHC Ib) molecule human leucocyte antigen G (HLA-G) is a non-classical MHC I molecule with low allelic variation and a restricted peptide repertoire which was first described by Geraghty et al. in 1987 (3, 4). Under physiological conditions, HLA-G is mainly expressed by trophoblast cells at the maternal-fetal interface (5) and can be secreted in a soluble form (soluble HLA-G, sHLAG) to the circulation (4). *In vitro* studies show that HLA-G shapes the maternal immune system towards tolerance by modulating function of antigen-presenting cells (APCs) (6) and inhibition of T-cell activity (7) and natural killer cell (NK-cell) cytotoxicity (8). During an uncomplicated pregnancy, levels of sHLA-G first increase and then decrease until the third trimester. Undetectable sHLA-G levels or variation in the course of sHLA-G levels seem to be related with gestational complications such as pregnancy loss and preeclampsia (9, 10). However, so far there have been no *in-vivo* studies that clearly demonstrate a beneficial role of HLA-G for pregnancy success.

Qa-2 is a murine non-classical MHC-Ib molecule which controls the rate of preimplantation embryonic cleavage division and subsequent embryo survival. It is the product of the preimplantation embryo development (Ped) gene (11) and encoded by four genes, Q6, Q7, Q8 and Q9, of which only Q7 and Q9 are transcribed in mouse embryos (12, 13). Embryos expressing Q7 or Q9 or both show the *Ped fast* phenotype with high embryo cleavage rates, while mouse strains that have a deletion of both genes do not express Qa-2 protein and show the *Ped slow* phenotype with slowed preimplantation embryo development and survival (12, 14). Due to various similarities between human HLA-G and murine Qa-2, such as their occurrence as membrane-bound and soluble forms and profound immunoregulatory properties of both molecules, Qa-2 has been considered a possible candidate for a murine HLA-G correlate (11, 15–17). Functional characteristics of HLA-G and Qa-2 are very similar during pregnancy; both HLA-G and Qa-2 are expressed by preimplantation embryos and seem to play an important role for early embryo survival and fetal growth (18–21). However interestingly, also women with deletion of HLA-G and mice completely lacking Qa-2 can give birth to healthy offspring, suggesting that HLA-G and Qa-2 are not necessary for reproductive success but reduce it when absent (13).

The pioneer work by Carol Warner and colleagues originally characterized the Ped gene and its product Qa-2 more than 30 years ago (22, 23) and already showed that mice not expressing the Qa-2 antigen (Qa-2$^-$) have smaller litters, lighter fetuses and a shorter duration of gestation than Qa-2 expressing animals (20, 24). Furthermore, they showed that Qa-2$^-$ animals develop increased systolic blood pressure when they get older (25). However, until now, mechanisms mediating the protective role of Qa-2 during reproduction and especially the impact of Qa-2 on immune regulation during pregnancy are marginally understood.

Myeloid derived suppressor cells (MDSC) are myeloid cells with various immune suppressive properties. They mainly consist of two subtypes named granulocytic MDSC (GR-MDSC) with phenotypic characteristics of neutrophils and monocytic MDSC (MO-MDSC) with phenotypic similarities to monocytes. In mice, GR-MDSC are defined as CD11b$^+$/Ly6G$^+$/Ly6Clo and MO-MDSC as CD11b$^+$/Ly6G-/Ly6Chi cells (26). Primarily, MDSC accumulation has been described under tumor conditions where they suppress immune responses against tumor cells, thereby leading to disease progression (27). Later, MDSC have been shown to accumulate under various other pathologies like infection, trauma, autoimmune disease, obesity and transplantation (28). In recent years, however, there is increasing evidence that MDSC also play a physiological role during pregnancy by modulating maternal immune responses and protecting the fetus from rejection (29–33). Accumulation and activation of MDSC are driven by various factors (26). Recently, we demonstrated that HLA-G induced and activated GR-MDSC *in vitro* (34). Furthermore, it could be shown by us and others that the transcription factor hypoxia-inducible factor 1α (HIF-1α) plays a role for MDSC function during pregnancy and cancer (31, 35).

In the present study, we investigated the *in-vivo* role of HLA-G and Qa-2 for pregnancy success. Most inbred strains of mice, such as C57BL/6, carry all four genes encoding for Qa-2 (Q6/Q7/Q8/Q9) on each allelic chromosome, making a total of eight Qa-2 encoding genes and thus express the Qa-2 antigen widely in adult and embryonic tissues (12, 36). But there are also a few strains that are missing all eight genes, defining a null allele leading to lack of Qa-2 (13). We used such a murine model lacking the Qa-2 antigen (Qa-2 negative, Qa2$^-$, B6.K1) (37) and could show that Qa-2 deficiency led to intrauterine growth restriction and increased abortion rates with profound changes in uterine spiral artery remodeling and trophoblast invasion as well as trophoblast morphology, indicating a preeclampsia-like phenotype. Immunological adaptation to pregnancy was imbalanced with altered immune cell and especially T-cell homeostasis and reduced T_{reg} numbers in Qa-2$^-$ mice. Most impressively, there was a lack of MDSC accumulation and reduced functional activation of MDSC. We further showed that expression of Qa-2 on MDSC was regulated by the pregnancy hormone estrogen *via* the transcription factor HIF-1α. By application of sHLA-G to Qa-2$^-$ mice we could reduce their increased abortion rate, an effect that was abrogated by simultaneous MDSC-depletion. Conversely, adoptive transfer of *in vitro* generated MDSC also resulted in a reduced abortion rate in Qa-2$^-$ animals. Our results highlight the therapeutic potential of HLA-G for treatment of immunological pregnancy complications.

METHODS

Study Approval

All experiments were approved by the ethics committee of Tuebingen University (682/2016BO1) for human studies or the

regional council Tuebingen (K05/19M, K09/19M, K09/18G, K02/19G, K05/20G and K08/20G) for animal studies.

Mice

B6.K1 (B6.Cg-H2^{b3}/FlaCmwJ, Qa-2^{-}), HIF-1α^{flox} (B6.129-Hif1a^{tm3Rsjo}/J) mice and LysMcre (B6.129P2-Lyz2$^{tm1(cre)Ifo}$/J) mice were obtained from The Jackson Laboratory (Bar Harbour, Maine, USA). C57BL/6J (WT) mice, CBA/J and DBA/2J mice were obtained from Charles River (Sulzfeld, Germany). HIF-1α^{flox} mice and LysMcre mice were crossed to get animals with deletion of HIF-1α in myeloid cells (HIF-1α^{flox}/LysMcre, HIF-KO). The B6.K1 or Qa-2- mouse strain was developed in the 1976 by Dr. Lorraine Flaherty. It is originated spontaneously through intercross and backcross of the congenic strains B6.A-H2-T18^{a} (B6-Tla^{a}) and B6.AK-H2^{k} (37). All animals were maintained under pathogen-free conditions in the research animal facility of Tuebingen University, Tuebingen, Germany. All experimental animal procedures were conducted according to German federal and state regulations.

Syngeneic matings of Qa-2^{-}, HIF-KO and WT mice and allogeneic matings of CBA/J females with DBA/2J males were set up at 8-12 weeks of age. Male and female mice were mated 1:2 in the afternoon and plug control was carried out the next morning. Gestational ages were determined by visualizing of the presence of a vaginal plug (E0.5 = embryonic day).

Lack of Qa-2 expression in Qa-2^{-} mice was verified by flow cytometric analysis of blood leucocytes (**Supplementary Figure 1A**).

Abortion rates were determined by visual inspection of fetal-placental units and defined as ratio of resorbing units to the total number of implantation sites. Resorbing units were either dark, small and necrotic or pale, small and without visible fetus inside the amniotic cavity.

Fetal weight was determined by weighing of E18.5 fetuses immediately after removal from the amniotic cavity.

sHLA-G Application, MDSC-Depletion and Adoptive MDSC Transfer

Biotinylated HLA-G1 monomers bound to the peptide KGPPAALTL were kindly provided by Jianhong Cao, Fred Hutchinson Cancer Research Center, Seattle, USA. HLA-G1 tetramers were produced by binding to streptavidin (Biolegend, San Diego, USA).

To test the effect of sHLA-G on pregnancy outcome in Qa2^{-} mice, pregnant mice were injected intravenously at E10.5 and E14.5 with 1 µg/g body weight HLA-G1 tetramers in 100 µl PBS or with 100 µl PBS alone (control).

For depletion of MDSC, pregnant Qa-2^{-} mice were injected intravenously at E10.5 and E14.5 with 250µg/mouse Ly6G-antibody (BioXCell, Lebanon, USA) in 100µl dilution buffer (BioXCell) simultaneously with the HLA-G application.

For adoptive MDSC transfer, MDSC were *in vitro* generated from bone marrow cells. Bone marrow cells were collected from femora, tibiae and humeri of WT mice by rinsing with PBS. BM-cells were than cultivated for four days with 100ng/ml G-CSF and 2,5ng/ml GM-CSF (PeproTech, Hamburg, Germany). After four days MDSC were enriched by removing non-adherent cells and detaching adherent cells containing MDSC by Trypsin/EDTA. By this method a purity of >92% can be achieved. Pregnant Qa-2^{-} mice were injected intravenously at E10.5 and E14.5 with 4x10^{6} *in vitro* generated MDSC in 100µl PBS or with PBS alone (control).

To test the effect of sHLA-G on pregnancy outcome in abortion-prone DBA/2J-mated CBA/J mice, plug-positive mice were injected intravenously with 1 µg/g body weight sHLA-G tetramers in 100 µl PBS or with PBS alone (control) at E0.5, E3.5, E6.5 and E9.5.

Determination of Mouse Estrous Cycle

For identification of the mouse estrous cycle stage, female C57BL/6J mice were anesthetized with 1.5% isoflurane (CP-Pharma, Burgdorf, Germany). Blood was obtained by puncture of the retroorbital vein plexus or the tail vein and vaginal swabs were collected according to an established protocol (38). A cotton tipped swab (Applimed, Châtel-Saint-Denis, Switzerland) wetted with room-temperature physiological saline was inserted vaginally, gently turned, and then removed. The procedure was repeated twice for four consecutive days with 28 days in between.

The vaginal cells were transferred to a glass slide by rolling the swab over the slide. The slide was air dried, stained with hematoxylin-eosin (HE, Merck GmbH, Darmstadt, Germany) and viewed at 10x magnification under bright field illumination. The cycle stage was determined based on the presence or absence of leucocytes, cornified epithelial cells and nucleated epithelial cells according to (38).

Patients

The local ethics committee approved this study (682/2016BO1) and all women gave written informed consent. From August to October 2019 peripheral blood from pregnant women (aged 18-43 years, gestational age 10 – 36 weeks of gestation) was collected during routine blood sampling. Patients suffering from severe pregnancy complications (severe infection, preterm rupture of membranes, preterm labour, preeclampsia/eclampsia), chronic diseases (autoimmune diseases, malignancies, chronic infections) or receiving immune-suppressive therapy were excluded.

Mouse Tissue Collection and Single Cell Preparations

Non-pregnant and pregnant mice at gestational age E10.5 or E18.5 were euthanized by CO2 inhalation. Blood (0.5-1 ml) was collected immediately after death by intracardial puncture and placed into EDTA-tubes. Blood plasma was collected after centrifugation of whole blood at 400 rpm. Red blood cells were removed from whole blood by ammonium chloride lysis. Spleens were removed and tissue was pushed through a 100 µm filter (Greiner bio-one, Frickenhausen, Germany) using a syringe plunger. Red blood cells of the spleen were also removed by ammonium chloride lysis and the resulting cell suspension was again passed through a 40 µm filter (Greiner bio-one, Frickenhausen, Germany). Uterine horns were removed in toto. Fetuses and the fetal part of the placenta were dissected from uteri; blood vessels were removed. Uteri were placed into PBS, cut into 1 mm pieces and pushed through a 40 µm filter. Placentas were pushed through a 100 µm filter using a syringe plunger. Red blood cells were removed by ammonium chloride lysis

and the resulting cell suspension was then passed again through a 40 µm filter. All cell suspensions were then adjusted to 1-4x10^6 cells/ml in PBS or medium.

Cell Isolation and Culture

Human peripheral blood mononuclear cells (PBMC) were prepared from EDTA blood samples by Ficoll density gradient centrifugation (lymphocyte separation medium, Biochrom, Berlin, Germany).

To isolate GR-MDSC from murine splenocytes, cells were labeled with Gr-1 Biotin-Antibody and isolated over Streptavidin microbeads followed by a second isolation step using Ly6G Biotin-Antibody and Anti-Biotin microbeads (modified protocol of MDSC Isolation Kit mouse, Miltenyi, Bergisch-Gladbach, Germany). Purity of GR-MDSC after separation was >90%, as determined by flow cytometry.

For isolation of $CD4^+$ T-cells from murine splenocytes, cells were labeled with T cell Biotin-Antibody Cocktail followed by two consecutive Anti-Biotin magnetic bead separation steps (Miltenyi) according to the manufacturer´s instructions. Purity of $CD4^+$ T-cells after separation was >90%, as determined by flow cytometry.

In vitro generation of murine MDSC was performed according to previously established protocols (31, 39). For *in vitro* generation of MDSC, non-pregnant WT and Qa-2^- mice were euthanized and femora and tibia removed. Bone marrow was collected by flushing the bones with PBS using a syringe and a 25G needle. Bone marrow cells were then washed twice, adjusted to 5x10^5 cells/ml and cultured for 72 h at 37°C in Dulbecco´s modified eagle medium (DMEM, Thermo Fisher Scientific, Waltham, USA), supplemented with 10% fetal calf serum (FCS, Biochrom, Berlin, Germany) and 1% penicillin/streptomycin (P/S, Biochrom, Berlin, Germany) supplemented with 100 ng/ml recombinant murine granulocyte colony-stimulating factor (G-CSF, Peprotech, Hamburg, Germany) and 2,5 ng/ml recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF, Peprotech, Hamburg, Germany). After 72 h of culture, non-adherent cells were removed and adherent MDSC were detached using 0.5% trypsin/EDTA (Biochrom, Berlin, Germany) and a cell scraper. Purity of $Gr1^+/CD11b^+$ MDSC was >90%, as determined by flow cytometry.

For induction of T_{regs} by MDSC, *in vitro* generated MDSC from WT and Qa-2^- mice were co-cultured with freshly isolated murine $CD4^+$ T-cells at a ratio of 2:1 (500 000 T-cells and 250 000 MDSC) in RPMI 1640 with 10% FCS and 1% P/S in 24-well plates at 37°C and 5% CO2. After 3 days of culture, cells were harvested and intracellular Foxp3 staining was performed. $CD4^+$ T-cells cultured without MDSC served as control.

For analysis of the effect of anoxia, bacterial stimulation or stimulation with estrogen on Qa-2 expression on MDSC, splenocytes from pregnant WT animals or pregnant HIF-KO animals at E10.5 were isolated and cultured overnight in DMEM with 10% FCS and 1% P/S in 24-well plates at 37°C and 5% CO2. The next day, cells were stimulated for four hours either with anoxia by placing them into a hermetically sealed chamber with anaerobic gas generating sachets (Anaerogen 2.5l, Thermo Fisher Scientific, Waltham, USA), with E. coli at a MOI of 1:50, or with estrogen at concentrations of 1 nM, 10 nM and 100 nM (Merck GmbH, Darmstadt, Germany). After four hours, cells were harvested and extracellular staining was performed.

Bacterial Culture

E. coli DH5α, an encapsulated K12 laboratory strain was grown in Lennox-Lysogeny-Broth (LB)-medium (Invitrogen, Carlsbad, USA) until early logarithmic growth, resuspended in phosphate buffered saline (PBS, Biochrom, Berlin, Germany) and used immediately.

T-Cell Suppression Assay

Freshly isolated $CD4^+$ splenocytes were stained with carboxyfluorescein-succinimidyl ester (CFSE, Invitrogen, Carlsbad, USA) according to the manufacturer´s instructions. Cells were suspended in RPMI 1640 media containing 1% P/S and 10% FCS. CFSE-labelled $CD4^+$ T-cells (2x10^5) suspended in 100µl media were stimulated with 2x10^5 mouse T-Activator CD3/CD28 Dynabeads (Thermo Fisher Scientific, Waltham, USA) and 50 ng recombinant murine Interleukin-2 (rmIL-2, R&D Systems, Minneapolis, USA) under addition of ß Mercaptoethanol (Merck, Darmstadt, Germany) at a concentration of 50mM. MDSC isolated from spleens of pregnant WT and Qa-2^- animals at E10.5 also suspended in RPMI 1640 containing 1% P/S and 10% FCS were added in different ratios (1:2, 1:4 and 1:8). After 3 days of culture, $CD4^+$ T-cell proliferation was determined by flow cytometry using the CFSE dye dilution. Proliferation index, defined as the ratio of $CD4^+$ T-cell proliferation after addition of MDSC and $CD4^+$ T-cell proliferation without MDSC, was determined. $CD4^+$ T-cell proliferation without MDSC was set to a fixed value of 1.

Flow Cytometry

Human GR-MDSC were characterized as $CD66b^+/CD14^-/HLA\text{-}DR^{low/-}$ cells, according to previously established protocols for characterization of human MDSC (17). Antibodies used for extracellular staining of human cells were anti-CD66b-FITC (clone G10F, concentration 1 µl/1x10^5 cells), anti-HLA-G-PE (clone MEM-G/9, concentration 3µl/1x10^5 cells), anti-HLA-DR-PerCP-Cy5.5 (clone REA805, concentration 0.1 µl/1x10^5 cells) and CD14-APC (clone MφP9, concentration 1 µl/1x10^5 cells) [purchased from BD biosciences, Heidelberg, Germany (CD66b and CD14), Miltenyi (HLA-DR) and Exbio, Vestec, Czech Republic (HLA-G)].

For extracellular staining of mouse cells, freshly isolated cells were washed in FACS buffer and fluorescent-conjugated extracellular antibodies were added. Antibodies were purchased from BD Biosciences [CD3 FITC (145-2C11), CD3 PE (17A2), CD4 APC (RM4-5), CD8a APC-H7 (53-6.7), CD8 PE (53-6.7), CD11b Alexa (M1/70), CD19 PE (1D3), CD25 BB515 (PC61), CD44 BB700 (IM7), CD45 BV510 (30-F11), CD45 PerCp (30-F11), CD62L BV421 (MEL-14), CD183 BB700 (CXCR3-173), CD196 BV421 (CCR6), FSV700 Alexa Fluor700, NK1.1 APC (PK136), Gr-1 FITC (RB6-8C5), Gr-1 PerCp (RB6-8C5), Ly-6C FITC (AL-21), Ly-6G PE (1A8)] and Miltenyi [Qa2 PE (REA523)].

For immune cell quantification, cells were pre-gated to CD45. Among $CD45^+$ cells, cell types were identified as follows: T-cells $CD3^+$, T-Helper cells $CD3^+/CD4^+$, cytotoxic T-cells $CD3^+/CD8^+$,

B-cells CD3-/CD19$^+$, NK-cells CD3-/NK1.1$^+$, MDSC CD11b$^+$/Gr-1$^+$, MO-MDSC CD11b$^+$/Ly6C$^+$/Ly6G$^-$, GR MDSC CD11b$^+$/Ly6Clow/Ly6G$^+$ and monocytes CD11b$^+$/Gr-1$^-$.

For quantification of T-cell subsets cells were pre-gated to CD45, CD3 and CD4 or CD8. Among CD45$^+$/CD3$^+$/CD4$^+$ cells, cell types were identified as follows: naïve T-helper cells CD44$^-$/CD62L$^+$, effector memory T-helper cells CD44$^+$/CD62L$^-$, central memory T-helper cells CD44$^+$/CD62L$^+$, T-helper 1 cells CXCR3$^+$/CCR6$^-$, T-helper 2 cells CXCR3$^-$/CCR6$^-$, T-helper 17 cells CXCR3$^-$/CCR6$^+$, T_{reg} CD25$^+$. Among CD45$^+$/CD3$^+$/CD8$^+$ cells, cell types were identified as follows: naïve cytotoxic T cells CD44$^-$/CD62L$^+$, effector memory cytotoxic T cells CD44$^+$/CD62L$^-$, central memory cytotoxic T cells CD44$^+$/CD62L$^+$.

For intracellular staining of Foxp3 cells were extracellular stained with CD4 and CD25 for 30 minutes at 4°C and then incubated in Foxp3 Fixation/Permeabilization working solution (Thermo Fisher Scientific, Waltham, USA) for 60 minutes at room temperature and protected from light. Cells were washed in 1x Permeabilization buffer (Thermo Fisher Scientific) and stained with Foxp3 antibody in 1x permeabilization buffer for 30 minutes at room temperature.

Data acquisition was performed with a FACScalibur or LSR II flow cytometer (BD Bioscience) and analyzed *via* FlowJo V10 (FlowJo, LLC, Ashland, Oregon, USA).

RNA Isolation and Transcriptome Analyses

For transcriptome analyses of whole uterine lysates, uteri of pregnant WT and Qa 2$^-$ mice at E18.5 were collected, snap-frozen in liquid nitrogen and stored at -80°C until RNA isolation. For RNA isolation, frozen tissue (20-30 mg) was shredded using micro pestles (Sigma Aldrich, St.Louis, USA) and liquid nitrogen to obtain powder. RLT buffer was added and the solution was centrifuged at 8000 rcf. RNA isolation was then performed using the RNeasy Mini Kit (Qiagen, Hilden, Germany).

RNA quality was determined by measuring 260/280 and 230/260 absorbance ratio on a spectrophotometer (Nanodrop ND-1000; Peqlab, Erlangen, Germany), RNA concentration was determined using the Qubit Fluorometric Quantitation and RNA Broad-Range Assay (Thermo Fisher Scientific) and RNA Integrity Number RIN using the Fragment Analyzer 5300 and the Fragment Analyzer RNA kit (Agilent Technologies, Santa Clara, USA). For library preparation, mRNA fraction was enriched using polyA capture from 200 ng of total RNA using the NEB Next Poly(A) mRNA Magnetic Isolation Module (New England Biolabs, Frankfurt, Germany). Next, mRNA libraries were prepared using the NEB Next Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs) according to the manufacturer's instructions. Library molarity was determined by measuring the library size (approximately 400 bp) using the Fragment Analyzer 5300 and the Fragment Analyzer DNA HS NGS fragment kit (Agilent Technologies, Santa Clara, USA) and the library concentration (>0.5 ng/µl) using Qubit Fluorometric Quantitation and dsDNA High sensitivity assay (Thermo Fisher Scientific). In the first experiment, libraries were denaturated according to the manufacturer's instructions, diluted to 270 pM and sequenced as paired-end 100 bp reads on an Illumina NovaSeq 6000 (Illumina) with a sequencing depth >25 million clusters per sample.

Read quality of RNA-seq data in fastq files was assessed using ngs-bits (v.2020_06) to identify sequencing cycles with low average quality, adaptor contamination, or repetitive sequences from PCR amplification. Raw expression values were available for 55.421 genes in 4 samples. Raw gene expression was filtered by demanding a minimum expression value of 1 cpm (counts per million) in at least 2 samples. Filtered data contained expression values for 17.210 genes. Data analysis was performed using the STRING database (40).

Protein Isolation and Proteome Analyses

For proteome analyses, single cell suspensions were prepared from placentas of pregnant WT and Qa2$^-$ animals at E18.5. Cells were lysed by adding lysis buffer [5% 1M Tris/HCl pH 7,4, 2% 5M NaCl, 1% Triton X 100, 1% PMSF, 4% protease inhibitor cocktail (Sigma-Aldrich, St. Louis, USA)] on ice followed by snap-freezing in liquid nitrogen. Ten micrograms of each sample were digested in solution with trypsin as described in (41). After desalting using C18 stage tips, extracted peptides were separated on an Easy-nLC 1200 system coupled to a Q Exactive HFX mass spectrometer (Thermo Fisher Scientific) as described in (42) with slight modifications: The peptide mixtures were separated using a 90 minutes segmented gradient from to 10-33-50-90% of HPLC solvent B (80% acetonitrile in 0.1% formic acid) in HPLC solvent A (0.1% formic acid) at a flow rate of 200 nl/min. The 12 most intense precursor ions were sequentially fragmented in each scan cycle using higher energy collisional dissociation (HCD) fragmentation. Acquired MS spectra were processed with MaxQuant software package version 1.6.7.0 with integrated Andromeda search engine. Database search was performed against a target-decoy Mus musculus database obtained from Uniprot, containing 63.686 protein entries and 286 commonly observed contaminants. Peptide, protein and modification site identifications were reported at a false discovery rate (FDR) of 0.01, estimated by the target/decoy approach. The LFQ (Label-Free Quantification) algorithm was enabled, as well as match between runs and LFQ protein intensities were used for relative protein quantification. Data analysis was performed using the STRING v11 database (40).

Immunohistochemistry

The mouse placentas at E18.5 were fixed in 4.5% formaldehyde (Sigma Aldrich) and paraffin embedded (max. 48 hours). The samples were infiltrated with paraffin wax in a tissue processor (Leica, Wetzlar, Germany). 3-5 µm thick sections were cut with a sledge microtome (Leica, Wetzlar, Germany) and stained with hematoxylin-eosin (H&E), Periodic acid-Schiff (PAS) and PAS diastase (Merck GmbH, Darmstadt, Germany). Slides were analyzed using an Axioskop 2 plus Zeiss microscope (Zeiss, Oberkochen, Germany) equipped with a Jenoptik ProgRes C10 (Laser Optik System, Jena, Germany) plus camera and software. To quantify the placental phenotype the slides were further evaluated using a histo score with 0 (no aggregates), 1 (intermediate phenotype) and 2 (prominent aggregates) (**Supplementary Figure 5**).

For analysis of spiral artery remodeling at E10.5, uterine arteries were ligated by dental floss and the uterus was removed, placed on a polystyrene piece, fixed in 4.5% formaldehyde (Sigma Aldrich) and paraffin embedded as described in (43). 3-5 µm thick sections were

stained with H&E and the slides were scanned with the Ventana DP200 (Roche, Basel, Switzerland). Placentas from all animals were analyzed at the midsagittal point, given by the presence of the chorioallantoic attachment. The total vessel and luminal areas of the spiral arteries were measured in the central 2/4 of the decidua basalis (43). The 5 spiral arteries with the largest and roundest lumen in three consecutive sections (50um between sections) were used for analysis and the mean was calculated.

Statistical Analysis

Statistical analysis was done using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA). Data were analyzed for Gaussian distribution using D`Agostino and Pearson omnibus normality test. Unpaired and normally distributed data were analyzed using the unpaired t-test, unpaired and not normally distributed data were evaluated using the Mann-Whitney test. Paired and normally distributed data were analyzed using the paired t-test and paired and not normally distributed data were analyzed using the Wilcoxon matched pairs signed rank test. A p-value <0.05 was considered as statistically significant.

RESULTS

Qa-2 Deficiency in Mice Leads to Adverse Pregnancy Outcome

To evaluate the impact of Qa-2 on pregnancy outcome, we analyzed mice lacking the Qa-2 antigen (Qa-2$^-$, B6.K1). Compared to WT mice, we found significantly smaller litter sizes in Qa-2- animals (**Figure 1A**). At mid-pregnancy (E10.5), Qa-2$^-$ mice had similar numbers of intact fetuses compared to WT mice (**Supplementary Figure 1B**) and slightly increased abortion rates (**Supplementary Figures 1C, D**). At E18.5, lack of Qa-2 led to an abortion rate of 17% in comparison to 5% in WT animals (**Figures 1B, C**) and to an intrauterine growth restriction (IUGR) in surviving fetuses (**Figures 1D, E**).

Since IUGR and late abortions are often a sign of a disturbed blood supply to the fetus, we next analyzed uteri of Qa-2$^-$ and WT fetuses histologically. To assess spiral artery remodeling, which is a crucial step in hemodynamic adaptation to pregnancy taking place between E8.5 and E12.5 (43), we analyzed uteri at E10.5. Here, we found profound changes between Qa2$^-$ and WT animals; in Qa-2$^-$ animals we found large areas within the decidua with unorganized trophoblast distribution, while in WT animals, trophoblasts proper organized around the vessels, pointing to an abnormal trophoblast-migration in Qa-2$^-$ mice (**Figure 1F**). In addition, spiral arteries of Qa-2$^-$ animals had thicker vessel walls than that of WT mice, while luminal areas did not differ (**Figures 1G–I**). Corresponding to that, we found upregulation of genes encoding for proteins involved in "circulatory system development" in WT uteri in comparison to Qa-2$^-$ uteri in transcriptome analyses of whole uterine lysates (**Supplementary Figure 2**).

We further analyzed placenta histology of E18.5 old WT and Qa-2$^-$ fetuses; placentas from both genotypes showed similar cross-section areas (**Figure 1J**); however, while placentas from WT animals showed long and thin villi with proper morphology, placentas from Qa-2$^-$ animals showed irregular and short villi and abnormal vacuolization of the trophoblast and numerous eosinophilic aggregates. On a scale of 0 (no aggregates) to 2 (prominent aggregates) (**Supplementary Figure 3**), the phenotype of WT placentas was 0.2 ± 0.4 while that of Qa-2$^-$ placentas was 1.4 ± 1.0 (**Figures 1K, L**). These aggregates were still present in PAS-diastase staining, indicating that they were not glycogen (**Figure 1M**). Proteome analyses of placenta lysates from WT and Qa-2$^-$ animals showed strong enrichment in proteins involved in protein metabolism processes (GO:0019538), especially in translation (GO:0006412), proteolysis (GO:0006508), phosphorylation (GO:0016310) and dephosphorylation (GO:0006470) (**Supplementary Figure 4**) in Qa-2$^-$ placentas (184 of 655 proteins only detected in Qa-2$^-$), suggesting dysfunctional protein storage in trophoblasts of these animals. On the whole, Qa-2 deficiency led to increased abortions rates in late pregnancy and to disturbed placental vascularization.

Qa-2 Deficiency Leads to an Altered Immune Cell Composition During Pregnancy

Since it is known that HLA-G plays an important role in immune regulation during pregnancy (44), we next analyzed immune cell populations in spleens and uteri of Qa-2$^-$ and WT mice. **Figure 2A** shows gating strategy for immune cell populations. Myeloid cells increased and B-cells decreased in WT spleens and uteri at E18.5 compared to non-pregnant controls while in Qa-2$^-$ animals myeloid cells remained unchanged and splenic B-cells even increased. Splenic and uterine T-cell numbers did not change in WT mice during pregnancy but decreased in spleens and increased in uteri of pregnant Qa-2$^-$ mice compared to non-pregnant controls. NK-cells decreased in uteri of both WT and Qa-2$^-$ animals during pregnancy (**Figure 2B** and **Supplementary Figure 5**). No differences were observed in placental immune cell composition between Qa-2$^-$ and WT placentas (**Supplementary Figures 6A–E**).

We next investigated whether there were any differences in T-cell subpopulations between pregnant WT and Qa-2$^-$ animals at E18.5. Gating strategy for T-cell subpopulations is depicted in **Figure 2A**, and phenotyping strategy is depicted in **Supplementary Figure 7**. No differences were found in percentages of T-helper cells and cytotoxic T-cells between pregnant WT and Qa-2$^-$ animals (**Figures 2C, G**). Qa-2$^-$ animals had higher numbers of effector memory CD4$^+$ and CD8$^+$ T-cells and lower numbers of central memory CD4$^+$ and naïve CD8$^+$ T-cells (**Figures 2D–F, H–J**). Furthermore, Qa-2$^-$ animals had significantly less T_{reg}- and more Th17-cells, while there were no differences in numbers of Th1- and Th2-cells (**Figures 2K–P**). Decreased numbers of T_{regs} in Qa-2$^-$ animals were confirmed by intracellular staining of FoxP3 (**Figures 2Q, R**).

Qa-2 Deficiency Leads to Impaired Accumulation and Function of Myeloid Derived Suppressor Cells During Pregnancy

Since MDSC are critical for maintaining immune tolerance during pregnancy, we investigated whether there were differences in MDSC

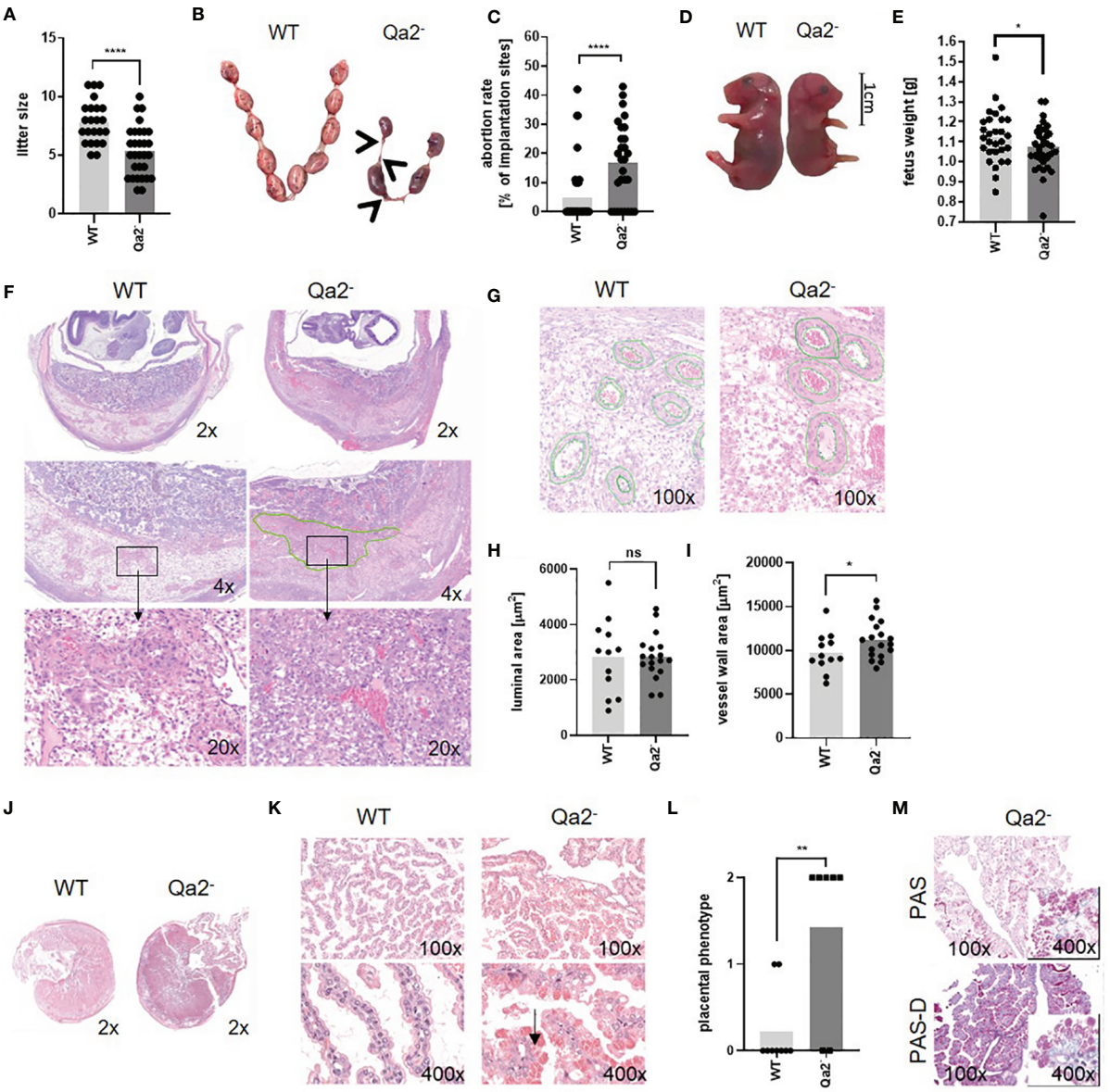

FIGURE 1 | Phenotype of Qa-2$^-$ mice during pregnancy. Wildtype (WT) and Qa-2 deficient mice (Qa2$^-$) were term-bred and the day when a vaginal plug was detected was defined as day E0.5. Mice delivered spontaneously and litter size was determined **(A)** or mice were euthanized at E18.5 and uteri containing feto-placental units were removed and inspected **(B–E)**. Total implantation sides and resorbing units were counted and fetuses were weighed. **(A)** Litter size of spontaneous delivered WT (n=23) and Qa2$^-$ mice (n=30). **(B)** Representative uteri containing feto placental units from WT and Qa-2$^-$ mice at gestational day E18.5. Arrows show resorbing units. **(C)** Abortion rate (percentage of resorbed fetuses per litter) of WT (n=39) and Qa-2$^-$ mice (n=28) at E18.5. **(D)** Representative WT and Qa-2$^-$ fetuses at E18.5. **(E)** Weight of WT (n=29) and Qa-2$^-$ fetuses (n=35) at E18.5. **(F–I)** Mice were euthanized at E10.5, uterine arteries were ligated and spiral arteries were analyzed. **(F)** Representative images of H&E stained WT and Qa-2$^-$ uteri showing cross-section areas of placenta and decidua in different magnifications. The green line shows an area with unorganized trophoblast distribution within the Qa-2$^-$ decidua. **(G)** Representative images of H&E stained WT and Qa-2$^-$ uteri showing quantification of spiral artery wall and luminal area. **(H)** Luminal area of spiral arteries from WT (n=5) and Qa-2$^-$ animals (n=5). **(I)** Vessel wall area of spiral arteries from WT and Qa-2$^-$ animals. **(J–M)** Mice were euthanized at E18.5 and placentas were analyzed. **(J)** Representative images of H&E stained cross-section areas of WT and Qa-2$^-$placentas. **(K)** Representative images of H&E stained chorionic villi from WT and Qa-2$^-$ placentas showing abnormal vacuoles with eosinophilic aggregates in trophoblasts of Qa-2$^-$ animals (arrow). **(L)** Grade of eosinophilic aggregation in trophoblasts of WT and Qa-2$^-$ animals. **(M)** Representative images of PAS and PAS-diastase stained chorionic villi from a Qa-2$^-$ placenta showing aggregates still present in PAS-diastase staining. Each symbol represents an individual mother **(A, C, L)**/fetus **(E)**/measurement (H+I) and the mean is indicated. ****p < 0.0001; **p < 0.01; *p < 0.05; ns, not significant. Mann-Whitney test **(A, C, L)** or unpaired t-test **(E, H, I)**.

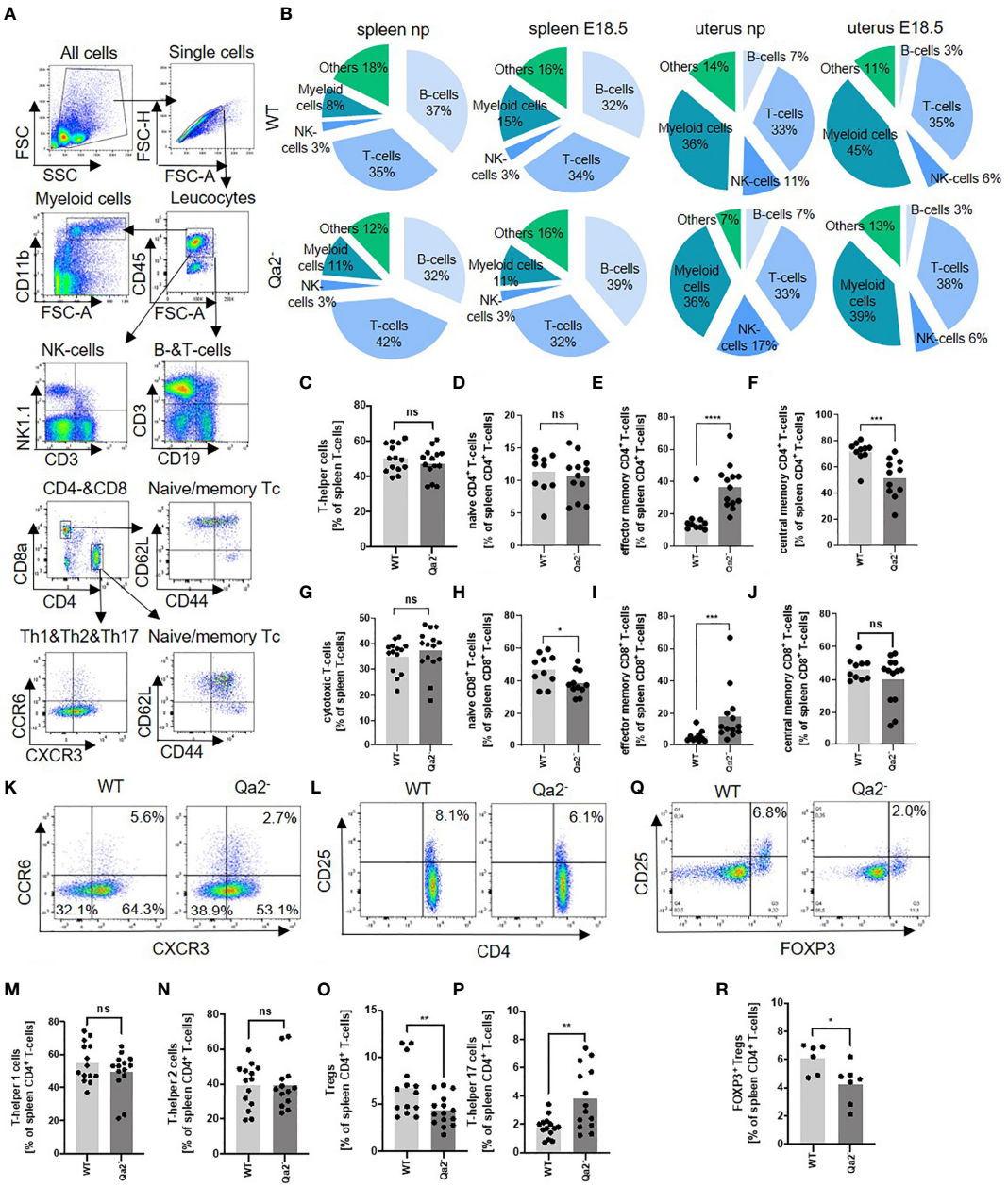

FIGURE 2 | Immune cell composition in WT and Qa-2 deficient animals. Wildtype (WT) and Qa-2 deficient mice (Qa2$^-$) were term-bred and the day when a vaginal plug was detected was defined as day E0.5. Spleen cells were analyzed by flow cytometry. **(A)** Gating strategy for gating immune cell subpopulations. **(B)** Proportions of the different cell types in spleens and uteri of non-pregnant (np) and E18.5 pregnant (E18.5) WT (upper diagrams, n=14-17 for np and n=16 for E18.5) and Qa-2$^-$ animals (lower diagrams, n=16-21 for npand n=12-13 for E18.5). **(C–J)** Percentages of all T-helper cells **(C)**, naïve T-helper cells **(D)**, effector memory T-helper cells **(E)**, central memory T-helper cells **(F)**, all cytotoxic T-cells **(G)**, naïve cytotoxic T-cells **(H)**, effector memory cytotoxic T-cells **(I)** and central memory cytotoxic T-cells **(J)** from all spleen leucocytes in WT (n=10-14) and Qa-2$^-$ animals (n=11-15). **(K)** Representative pseudocolor plots for CXCR3 versus CCR6 showing the populations of T-helper 1 cells (lower right quadrant), T-helper 2 cells (lower left quadrant) and T-helper 17 cells (upper left quadrant) in spleen leucocytes. Cells were pre-gated on CD45, CD3 and CD4. **(L)** Representative pseudocolor plots for CD4 versus CD25 showing the population of T_{reg} cells in spleen leucocytes in the upper right quadrant. Cells were pre-gated on CD45 and CD3. (M-P) Percentages of all T-helper 1 cells **(M)**, T-helper 2 cells **(N)**, T_{reg} cells **(O)** and T-helper 17 cells **(P)** from all spleen leucocytes in WT (n=10-14) and Qa-2$^-$ animals (n=11-15). **(Q)** Representative pseudocolor plots for Foxp3 versus CD25 showing the population of Foxp3+ T_{reg} cells in spleen leucocytes in the upper right quadrant. Cells were pre-gated on CD45, CD3 and CD4. **(R)** Percentages of Foxp3 T_{reg} cells from all spleen leucocytes in WT (n=6) and Qa-2$^-$ animals (n=7). Each symbol represents an individual animal and the mean is indicated. Light grey bars represent WT animals and dark grey bars represent Qa-2$^-$ animals. ****p < 0.0001; ***p < 0.001; **p < 0.01; *p < 0.05; ns, not significant. Mann-Whitney test.

accumulation and function between WT and Qa-2$^-$ animals. We observed a strong increase in total splenic MDSC, as well as in splenic GR-MDSC and MO-MDSC between non-pregnant WT animals and WT animals at E18.5. In Qa-2$^-$ animals however, there was only a marginal increase in total splenic MDSC at E18.5, while neither GR-MDSC nor MO-MDSC numbers increased (**Figures 3A–D**). Correspondingly, we found strongly increased numbers of uterine MDSC in WT animals at E18.5 in comparison to non-pregnant controls, but not in Qa-2$^-$ animals (**Figures 3E, F**). Interestingly, transcriptome analyses of whole uterine lysates also showed upregulation of genes encoding for proteins involved in "immune system processes" and among that especially in cytokine/chemokine signaling, myeloid cell differentiation, apoptosis regulation, leucocyte migration and lymphocyte activation in WT uteri in comparison to Qa-2$^-$ uteri (**Supplementary Figure 2**).

We further analyzed the functional capacity of MDSC from WT and Qa-2$^-$ animals and found a decreased capacity to induce T_{regs} *in vitro* (**Figures 3G, H**) and a slightly but not significantly reduced capacity of Qa-2$^-$ MDSC to inhibit T-cell proliferation in comparison to WT MDSC (**Figures 3I, J**).

Expression of Qa-2 on MDSC Is Regulated by Estrogen *via* HIF-1α

As Qa-2$^-$ MDSC had reduced functional capacity, we next asked how Qa-2$^-$ expression may be regulated. Flow cytometric analyses of Qa-2 expression on MDSC and T-cells revealed that in non-pregnant WT mice between 10% and 60% of MDSC and all T-cells expressed Qa-2, however Qa-2 expression on MDSC (MFI mean 33.8 ± 25.8) was much lower than on T-cells (MFI 241.7 ± 137.4); pregnancy increased the expression of Qa-2 on both MDSC and T-cells (**Figures 4A–C**). The same effect could be observed for HLA-G-expression on human MDSC (**Figures 4D, E**). To get hints on a potential hormonal regulation of Qa-2 expression on immune cells, we analyzed blood of female mice during the menstrual cycle and found increased Qa-2 expression on MDSC during proestrus and estrus, the phases with higher estrogen levels (23), than during metestrus and diestrus (**Figures 4F, G**). To further evaluate the effect of estrogen on Qa-2 expression on MDSC, we next stimulated spleen cells of WT mice with increasing concentrations of estrogen and showed that Qa-2 expression on MDSC increased upon estrogen stimulation in a concentration dependent manner, while Qa-2 expression on T-cells did not change (**Figures 4H–J**). Recent data showed that expression of HLA-G on tumor cells can be regulated by the transcription factor hypoxia-inducible factor 1α (HIF-1α) (45, 46) and that HIF-1α regulates MDSC function during murine pregnancy (31). We thus assumed that expression of Qa-2 on MDSC may be regulated by HIF-1α and stimulated spleen cells of WT mice with classic (anoxia) and alternative (Escherichia coli, E. coli) stimuli of HIF-1α. We found that both anoxia and E. coli stimulation led to an increased expression of Qa-2 on MDSC (**Figures 4K–M**), but not on T-cells (**Figures 4N, O**). Correspondingly, MDSC isolated from pregnant mice with targeted deletion of HIF-1α in myeloid cells (HIF-KO) expressed lower levels of Qa-2 than MDSC isolated from WT mice (**Figures 4P, Q**). Stimulation of myeloid HIF-KO MDSC with estrogen did not result in an upregulation of Qa-2 expression (**Figure 4R**). Taken together, our results show that expression of Qa-2 on MDSC is regulated by estrogen *via* HIF-1α.

Application of sHLA-G Improves Pregnancy Outcome via MDSC

Lastly, we asked whether we could restore pregnancy success in Qa-2$^-$ animals by application of sHLA-G. Pregnant Qa-2$^-$ mice received either 1μg/g bodyweight sHLA-G or PBS at E10.5 and E14.5. Application of sHLA-G led to a pronounced reduction in the abortion rate of Qa-2$^-$ animals (**Figure 5A**), accompanied by a partial restoration of normal trophoblast morphology (**Figure 5B**). Furthermore, it marginally increased splenic MDSC, but strongly increased uterine MDSC (**Figures 5C–E**). Simultaneous depletion of MDSC with sHLA-G application reversed the pregnancy-protective effect of sHLA-G (**Figure 5F**). Conversely, adoptive transfer of *in vitro* generated MDSC also resulted in a reduced abortion rate in Qa-2$^-$ animals (**Figure 5G**). To confirm the beneficial effect of sHLA-G on pregnancy outcome in another model, we treated abortion-prone DBA/2J-mated CBA/J mice (suffering from early abortions) with sHLA-G or PBS. Correspondingly, in this model, application of sHLA-G significantly reduced abortion rates (**Figures 5H, I**).

DISCUSSION

Our data show that the murine MHC-Ib molecule Qa-2 is relevant for pregnancy success and protects from late pregnancy loss by regulating immune adaptation to pregnancy in terms of modulating T-cell homeostasis and promoting MDSC accumulation. We further show that expression of Qa-2 on MDSC is relevant for their functionality and regulated by estrogen *via* HIF-1α. Lastly, we show that application of sHLA-G to abortion-prone Qa-2$^-$ mice decreases abortion rates *via* induction of MDSC. Adoptive transfer of *in vitro* generated MDSC had the same effect as HLA-G application.

It has been shown for a long time that HLA-G is highly expressed during pregnancy especially by trophoblast cells (3) mediating various immune-modulatory effects *in vitro* (4–6). Furthermore, alterations of HLA-G expression during pregnancy are associated with adverse pregnancy outcome such as preeclampsia (47–50). Until now, however, little *in vivo* evidence for a pregnancy-preserving role of HLA-G exists. Since Qa-2 is the most similar murine MHC-Ib molecule to human HLA-G described so far, we used Qa-2 deficient mice to evaluate the *in vivo* role of Qa-2 and HLA-G for pregnancy outcome.

Our first result of smaller litter sizes and growth restriction in surviving fetuses in Qa-2$^-$ animals in comparison to WT animals confirms previous studies also describing smaller litters and smaller offspring in Qa-2$^-$ animals (20, 25) and studies in humans showing an association of HLA-G gene polymorphisms and birth weight (21). In murine studies,

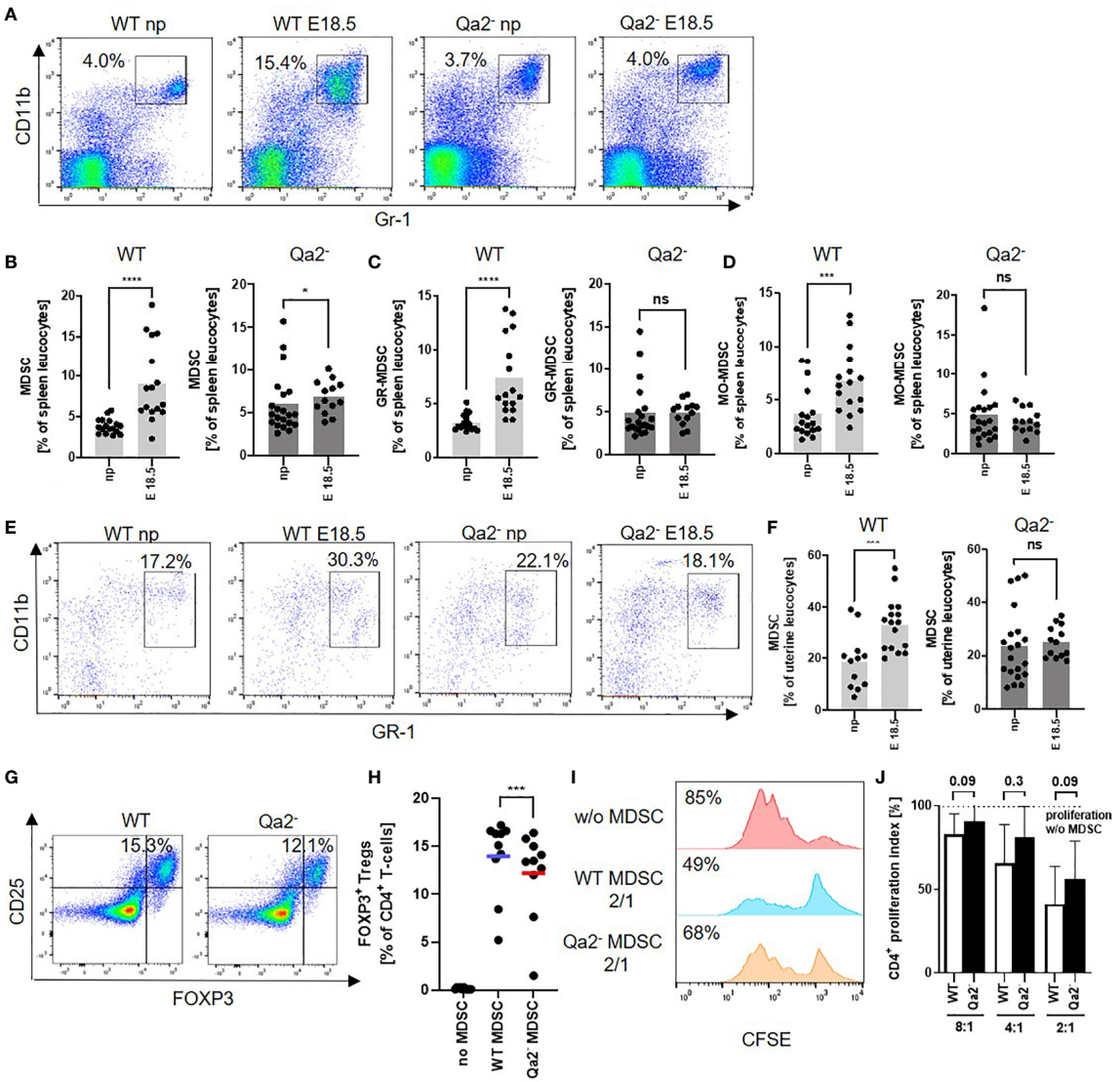

FIGURE 3 | Decreased accumulation and function of MDSC in Qa-2 deficient mice. Wildtype (WT) and Qa-2 deficient mice (Qa2$^-$) were term-bred and the day when a vaginal plug was detected was defined as day E0.5. Mice were euthanized at E18.5 and spleens and uteri were collected. Non-pregnant animals served as controls. Tissues were homogenized and filtered to obtain single cell suspensions and cells were analyzed by flow cytometry. **(A)** Representative pseudocolor plots for Gr-1 versus CD11b showing the population of MDSC in spleen leucocytes in the upper right quadrant. Cells were pre-gated on CD45. **(B–D)** Percentages of all MDSC **(B)**, GR-MDSC **(C)** and MO-MDSC **(D)** from all spleen leucocytes in non-pregnant animals (np, n=17 for WT and n=21 for Qa-2$^-$) and pregnant animals at E18.5 (n=16 for WT and n=13 for Qa-2$^-$). Light grey graphs represent WT animals and dark grey graphs represent Qa-2$^-$ animals. **(E)** Representative pseudocolor plots for Gr-1 versus CD11b showing the population of MDSC in uterus leucocytes in the upper right quadrant. Cells were pre-gated on CD45. **(F)** Percentages of MDSC from all uterus leucocytes in non-pregnant animals (np, n=12 for WT and n=20 for Qa-2-) and pregnant animals at E18.5 (n=16 for WT and n=13 for Qa-2$^-$). Light grey graph represents WT animals and dark grey graph represents Qa-2$^-$ animals. Each symbol represents an individual animal and the mean is indicated. ****p < 0.0001; ***p < 0.001; *p < 0.05; ns, not significant. Mann-Whitney test. MDSC were *in vitro* generated from bone marrow cells (G+H) or isolated by magnetic activated cell sorting from E18.5 pregnant WT and Qa-2$^-$ mice (I+J) and added to MACS isolated splenic CD4+ T-cells from WT mice. **(G)** Representative pseudocolor plots for Foxp3 versus CD25 showing the population of Foxp3+ T_{reg} cells after four days of co-culture with MDSC generated from WT and Qa-2$^-$ mice in a 2:1 (T-cells: MDSC) ratio in the upper right quadrant. Cells were pre-gated on CD45, CD3 and CD4. **(H)** Percentages of Foxp3$^+$ T_{reg} cells of all CD4$^+$ T-cells without addition of MDSC, with addition of MDSC generated from WT mice and with addition of MDSC generated from Qa-2$^-$ mice (n=10). Each symbol represents an individual animal and the mean is indicated. Bars represent pooled data from 6-7 independent experiments. **(I)** Representative histogram plots showing proliferation of CFSE-stained and anti-CD3/CD28 stimulated T-cells without addition of MDSC and with addition of MDSC generated from WT and Qa-2$^-$ mice in a 2:1 (T-cells: MDSC) ratio. **(J)** Inhibitory effect of MDSC from WT mice (white bars) and Qa-2$^-$ mice (black bars) on proliferation of CD4$^+$ T-cells in different ratios (T-cells:MDSC) (n=6-7). Dashed line shows proliferation of target CD4+ T-cells without addition of MDSC. Proliferation index was determined as ratio of T-cell proliferation with and without addition of MDSC. **p < 0.001; ns, not significant. Wilcoxon matched pairs signed rank test **(H)** and Mann-Whitney test **(J)**.

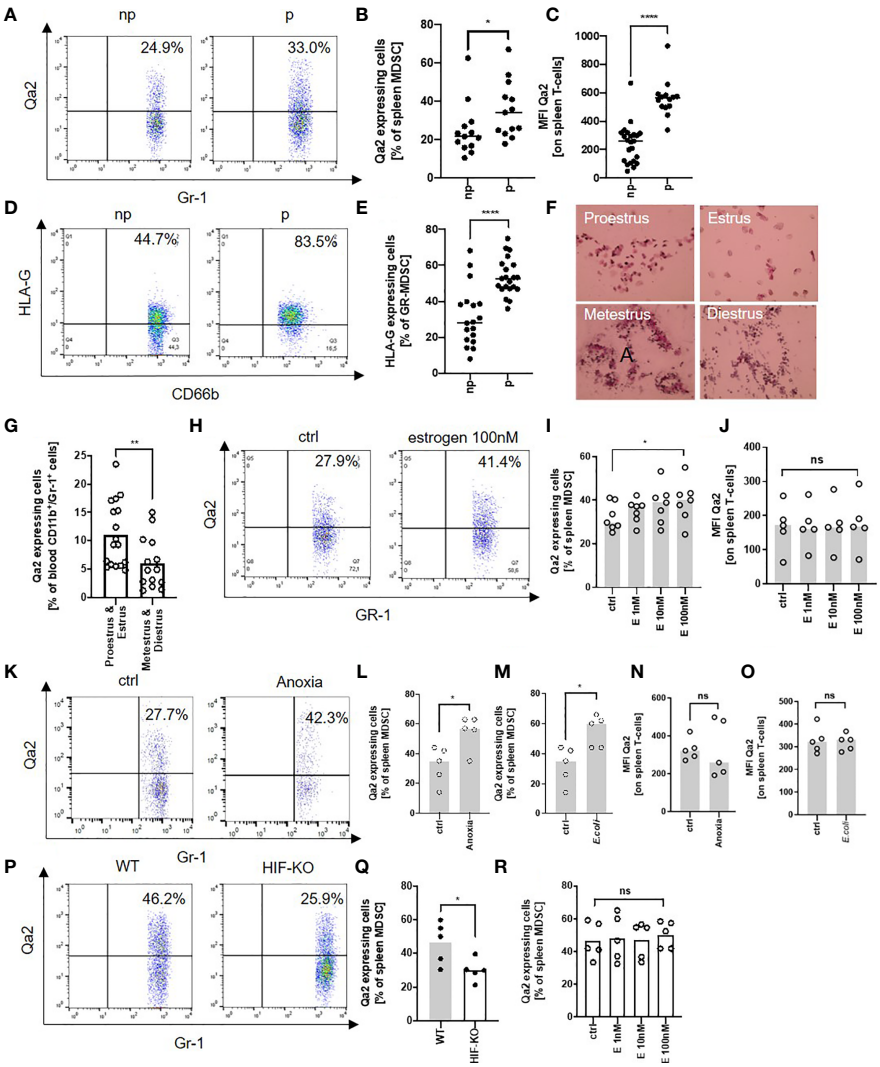

FIGURE 4 | Expression of Qa-2 on MDSC is regulated by estrogen *via* HIF-1α. **(A)** Representative pseudocolor plots for Gr-1 versus Qa-2 from splenocytes from non-pregnant (np) and pregnant (p) wildtype (WT) animals showing the percentage of Qa-2 expressing MDSC in the upper right quadrant. Cells were pre-gated on CD45 and CD11b. **(B)** Percentages of Qa-2 expressing MDSC from all spleen MDSC from non-pregnant (n=13) and pregnant WT mice (n=13). **(C)** MFI for Qa-2 on spleen T-cells from non-pregnant (n=23) and pregnant WT mice (n=14). **(D)** Representative pseudocolor plots for CD66b versus HLA-G from PBMC from non-pregnant (np) and pregnant (p) women showing the percentage of HLA-G expressing MDSC in the upper right quadrant. **(E)** Percentages of HLA-G expressing MDSC from all MDSC in the peripheral blood of non-pregnant (n=18) and pregnant women (n=21). **(F)** Representative images of H&E stained vaginal swabs from the four different phases of the mouse estrus cycle. **(G)** Percentages of Qa-2 expressing cells from all blood $CD11b^+/Gr\text{-}1^+$ cells in proestrus & estrus (n=14) and metestrus & diestrus (n=17). **(H)** Representative pseudocolor plots for Gr-1 versus Qa-2 from splenocytes from WT mice without stimulation (ctrl) and with stimulation with 100nM estrogen showing the percentage of Qa-2 expressing MDSC in the upper right quadrant. Cells were pre-gated on CD45 and CD11b. **(I)** Percentages of Qa-2 expressing MDSC from all spleen MDSC from WT mice without stimulation and with stimulation with estrogen in rising concentrations (n=7). **(J)** MFI for Qa-2 on spleen T-cells from wildtype mice without stimulation and with stimulation with estrogen in rising concentrations (n=5). **(K)** Representative pseudocolor plots for Gr-1 versus Qa-2 from splenocytes from wildtype (WT) mice cultured under normoxia (ctrl) or anoxia for 4 h showing the percentage of Qa-2 expressing MDSC in the upper right quadrant. Cells were pre-gated on CD45 and CD11b. **(L)** Percentages of Qa-2 expressing MDSC from all spleen MDSC from WT mice cultured under normoxia or anoxia (n=5). **(M)** Percentages of Qa-2 expressing MDSC from all spleen MDSC from WT mice without stimulation or with stimulation with **(E)** coli (n=5). **(N)** MFI of Qa-2 on spleen T-cells from WT mice cultured under normoxia or anoxia (n=5). **(O)** MFI of Qa-2 on spleen T-cells from wildtype mice without stimulation or with stimulation with **(E)** coli (n=5). **(P)** Representative pseudocolor plots for Gr-1 versus Qa-2 from splenocytes from pregnant WT animals and pregnant animals with targeted deletion of HIF-1α in myeloid cells (HIF-KO) showing the percentage of Qa-2 expressing MDSC in the upper right quadrant. Cells were pre-gated on CD45 and CD11b. **(Q)** Percentages of Qa-2 expressing MDSC from all spleen MDSC from WT mice (n=5) and from HIF-KO mice (n=5). **(R)** Percentages of Qa-2 expressing MDSC from all spleen MDSC from HIF-KO mice without stimulation and with stimulation with estrogen in rising concentrations (n=5). Each symbol represents an individual animal/women and the mean is indicated. ****$p < 0.0001$; **$p < 0.01$; *$p < 0.05$; ns, not significant. Mann-Whitney test **(B, C, E, G, Q)** and Wilcoxon matched-pairs signed rank test **(I, J, L–O, R)**.

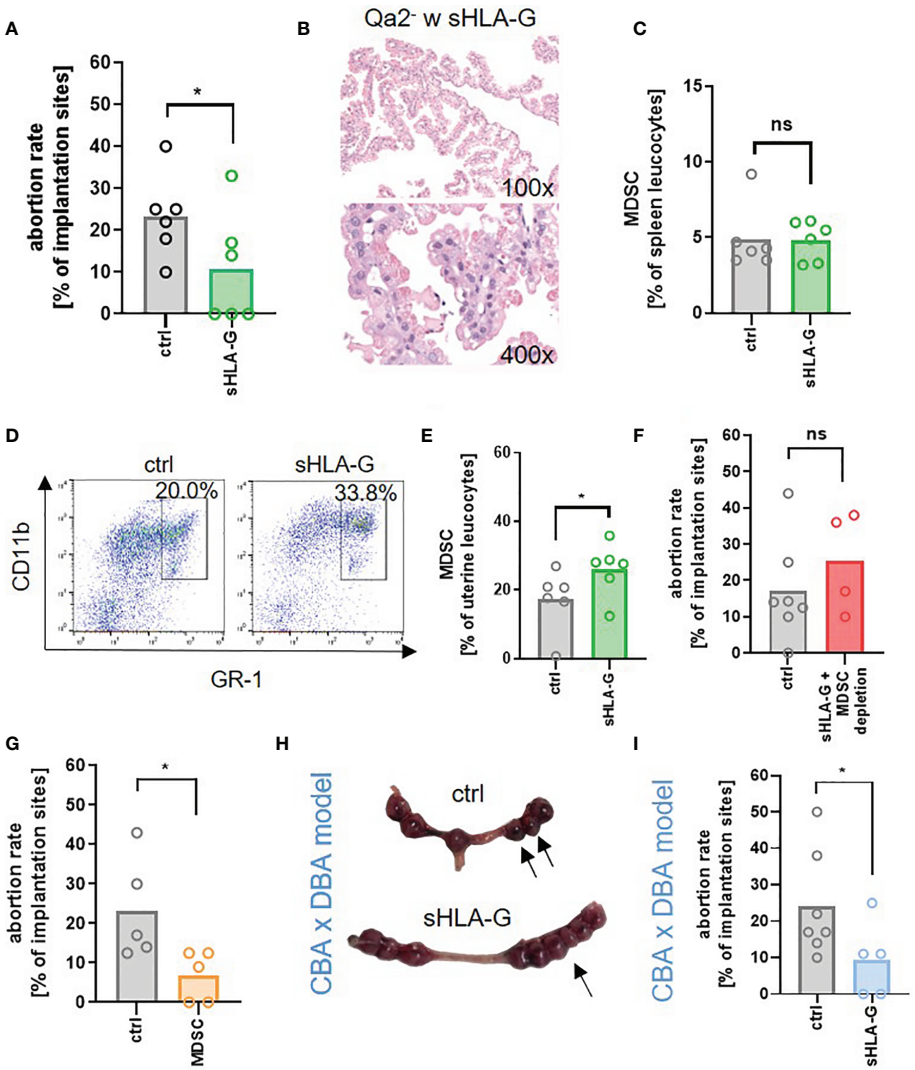

FIGURE 5 | sHLA-G improves pregnancy outcome. **(A–F)** Qa-2 deficient mice (Qa2$^-$) were term-bred and the day when a vaginal plug was detected was defined as day E0.5. At E10.5 and E14.5 mice received either PBS, 1µg/g bodyweight sHLA-G or 1µg/g bodyweight sHLA-G and anti-Ly6G-antibody. **(A)** Abortion rate (percentage of resorbed fetuses per litter) of Qa-2$^-$ mice after application of PBS (ctrl, n=6) or sHLA-G (n=6) at E18.5. **(B)** Representative images of H&E stained chorionic villi Qa-2$^-$ animals that received sHLA-G. **(C)** Percentages of MDSC in spleens of pregnant Qa-2- animals after application of PBS (ctrl, n=6) or sHLA-G (n=6) at E18.5. **(D)** Representative pseudocolor plots for Gr-1 versus CD11b showing the population of MDSC in uterus leucocytes of Qa-2$^-$ mice after application of PBS or sHLA-G in the upper right quadrant. Cells were pre-gated on CD45. **(E)** Percentages of MDSC in uteri of pregnant Qa-2$^-$ animals after application of PBS (ctrl, n=6) or sHLA-G (n=6) at E18.5. **(F)** Abortion rate of Qa-2 deficient mice after application of PBS (ctrl, n=7) or sHLA-G and anti-Ly6G-antibody (n=4) at E18.5. (G+H) Female CBA/J mice were term-bred with male DBA/2J mice and the day when a vaginal plug was detected was defined as day E0.5. At E0.5, E3.5, E6.5 and E9.5 mice received either PBS or 1µg/g bodyweight sHLA-G. **(G)** Abortion rate of Qa-2 deficient mice after application of PBS (ctrl, n=5) or MDSC (n=5) at E18.5. (H+I) Female CBA/J mice were term-bred with male DBA/2J mice and the day when a vaginal plug was detected was defined as day E0.5. At E0.5, E3.5, E6.5 and E9.5 mice received either PBS or 1ug/g bodyweight sHLA-G. **(H)** Representative uteri containing fetal-placental units from PBS (ctrl, n=7) and sHLA-G treated mice (n=5) at gestational day E10.5. Arrows show resorbed fetuses. **(I)** Abortion rate of CBA/J mice after application of PBS (ctrl, n=7) or sHLA-G (n=5) at E10.5. Each symbol represents an individual animal and the mean is indicated. *p < 0.05; ns, not significant. Mann-Whitney test.

expression of Qa-2 on the fetal side was found to be advantageous for survival, leading to a higher embryonic cleavage rate (20, 51, 52). Correspondingly, HLA-G expression in early embryos seems to be important for obtainment of pregnancy (18, 19) and maternal expression and haplotype of HLA-G may predict success of *in vitro* fertilization (53). Additionally, we now show increased rates of pregnancy loss in Qa-2$^-$ animals especially during late pregnancy accompanied by profound changes in maternal adaptation to pregnancy in comparison to WT mice, demonstrating that Qa-2 not only plays a local role in fetal tissue but is also systemically needed in the maternal organism to facilitate a successful pregnancy.

In pregnant Qa-2$^-$ animals we found an unorganized trophoblast invasion and altered spiral artery morphology with thicker vessel in comparison to WT animals. Remodeling of uterine spiral arteries driven by invasion of trophoblast cells to

the maternal decidua is one of the critical steps in maternal adaptation to pregnancy as it permits normal placental perfusion and fetal growth and development (54). Inadequate trophoblast invasion and spiral artery remodeling results in placental hypoxia and may lead to development of preeclampsia and fetal growth restriction (55). It should be mentioned at this point that the structure of human and murine placentas and thus also the localisation of HLA-G and Qa2 differ. HLA-G is expressed by extravillous trophoblast cells, which migrate into the maternal decidua, whereas Qa-2, which is expressed by trophoblast giant cells, is found more locally at the boundary layer between decidua and labyrinth (56, 57).

In Qa-2$^-$ placentas, we observed profound changes in trophoblast morphology in comparison to WT placentas with cytoplasmic storage of eosinophilic aggregates and enrichment of proteins involved in protein metabolism. Interestingly, it has been shown that during preeclampsia, misfolded proteins accumulate in urine, serum and placenta similar to the protein accumulation observed in neurodegenerative disorders like Alzheimer´s disease (58–60). Our findings of an altered trophoblast invasion and spiral artery remodeling, pathological protein storage in placenta, fetal growth restriction and late abortions may suggest the development of a preeclampsia-like phenotype in Qa-2$^-$ mice. This assumption is supported by recent data from others showing that injection of an anti-Qa-2 antibody led to preeclampsia symptoms in mice that could be abrogated by simultaneous injection of recombinant VEGF (61). It also corresponds to human data showing that an altered pattern of HLA-G expression on trophoblast cells is associated with preeclampsia (47, 50).

We further found significant differences in immunological adaptation to pregnancy between WT and Qa-2$^-$ animals. In pregnant Qa-2$^-$ mice but not in pregnant WT mice we found an increase in splenic T-cells in comparison to non-pregnant controls – an effect that may be associated with pregnancy loss (62). Analysis of T-cell subpopulations showed differences in numbers of effector memory T-cells, T_{regs} and Th17 cells between pregnant WT and Qa-2$^-$ animals. The CD44$^+$/CD62L-effector memory CD4$^+$ and CD8$^+$ T-cell subsets were increased in pregnant Qa-2$^-$ mice, while naïve CD8$^+$ and central memory CD4$^+$ T-cells were decreased. This points towards a higher activation status of T-cells in Qa-2$^-$ mice. A recent study showed that effector/activated T-cells led to adverse pregnancy outcome, i.e. preterm birth (63). Furthermore, patients with preeclampsia downregulate CD62L on T-cells (64).

Balance between Th1 and Th2 cells did not differ between WT and Qa-2$^-$ animals, while T_{regs} decreased and Th17 cells increased in pregnant Qa-2$^-$ in comparison to pregnant WT mice. This effect corresponds to the T-helper cell changes described in patients with preeclampsia (65). Studies in mice showed that expansion of T_{regs} was relevant for healthy pregnancy and that adoptive transfer of T_{regs} protected from abortions (66, 67).

Both, systemically and locally in the uterus, we observed a strong increase in MDSC in WT but not in Qa-2$^-$ mice. The accumulation of MDSC and its relevance for successful pregnancy has been described previously in different mouse models [reviewed in (68)]. However, all these studies focused on early- to mid-gestation (31–33). Our present results now illustrate that MDSC accumulation also seems to be relevant for pregnancy success in later stages of pregnancy. Interestingly, Ostrand-Rosenberg et al. showed that a single depletion of MDSC at E8.5 did not affect pregnancy outcome (32). However, since antibody-mediated MDSC depletion lasts only three days (69), one injection at E8.5 may be insufficient to examine the role of MDSC during late pregnancy. In correspondence to the increased accumulation of MDSC in uteri of WT mice we observed an upregulation of genes involved in myeloid differentiation, leucocyte migration and chemotaxis in transcriptome analyses of WT whole uterine lysates in comparison to Qa-2$^-$ animals. Corresponding to that, we previously showed that sHLA-G *in vitro* led to a quantitative and functional induction of MDSC (34). We thus assume that a combination of direct and indirect effects of Qa-2 attract MDSC to the pregnant uterus *in vivo*.

The exact mechanism by which Qa-2 interacts with MDSC during pregnancy remains unclear. There are two candidates for Qa-2 receptors on immune cells, the paired immunoglobulin-like inhibitory receptor (PIR-B) on myeloid cells and B-cells, which have been shown to interact with MHC class I (70) molecules and HLA-G (71, 72) and Ly49C on NK-cells (73). However, for both, clear evidence for a functional interaction with Qa-2 is lacking. MDSC from pregnant WT animals expressed PIR-B (data not shown) and therefore may be able to bind Qa-2/HLA-G. However, we did not go further into detail of this interaction for example by simultaneous blocking PIR-B and application of HLA-G.

Since the main effector function of MDSC is an inhibition of T-cell activation, one could hypothesize that increased activation of T-cells in Qa-2$^-$ animals results from a decreased MDSC influence. However, contrary to our results, MDSC have been shown to downregulate CD62L on T-cells (32, 74). As Qa-2 is also highly expressed on T-cells, the lack of Qa-2 itself may lead to differences in T-cell activation between WT and Qa-2$^-$ mice overlapping the effect of MDSC.

A crosstalk between T_{regs} and MDSC has been described extensively under tumor conditions (reviewed in (75)). Kang et al. described an induction of T_{regs} by MDSC *via* production of TGF-β *in vivo* during pregnancy (76), while we showed *in vitro* that fetal human MDSC as well as exosomes released by MDSC from pregnant women were able to induce T_{regs} (77, 78). We now demonstrate that induction of T_{regs} by MDSC was reduced in absence of Qa-2 on MDSC. This is in line with two studies showing an induction of T_{regs} by mesenchymal stem cells (MSCs) *via* HLA-G (79). T_{reg} induction by MDSC may explain the decreased numbers of T_{regs} in pregnant Qa-2- mice. Although the differences we measured in our functional assay are not huge, together with the lower Treg levels in pregnant Qa-2$^-$ mice, we consider this effect to be biologically relevant.

Due to the observed functional differences between WT and Qa-2$^-$ MDSC, we asked whether Qa-2 contributes to the functional activation of MDSC during pregnancy. Although

the main source of Qa-2 during pregnancy is embryonic tissue, it is known that Qa-2 is also expressed on immune cells (36). We found an obvious, albeit low, expression of Qa-2/HLA-G also on MDSC. Furthermore, we found that MDSC isolated from pregnant individuals (mice and women) expressed higher levels of Qa-2, respectively HLA-G, than MDSC from non-pregnant individuals and that Qa-2 expression on MDSC, but not on T-cells, could be stimulated by the pregnancy hormone estrogen. Immunomodulatory effects of estrogens have been repeatedly described, e.g. an expansion of T_{regs} and a modulation of Th-cell cytokine expression (80, 81). Furthermore, it could be shown that estrogen mediates expansion and functional activation of MDSC (82, 83). However, upregulation of Qa-2 expression on MDSC by estrogen is a yet unknown mechanism.

We further showed that the effect of estrogen on Qa-2 expression on MDSC during pregnancy was mediated through HIF-1α. This is in line with results from other groups showing that estrogen can activate HIF-1α (84) and that activation of HIF-1α stimulated HLA-G-expression in cancer cells (45, 46). We recently showed that expression of HIF-1α was relevant for MDSC accumulation and function during pregnancy and that targeted deletion of HIF-1α in myeloid cells (myeloid HIF-KO) led to pregnancy failure in terms of abortions (31). Our new results suggest that an impaired expression of Qa-2 on MDSC may at least partially be responsible for the adverse pregnancy outcome in myeloid HIF-KO mice.

Lastly, we aimed to investigate the therapeutic effect of sHLA-G on pregnancy outcome. We showed that application of sHLA-G reduced the abortion rate in Qa-2$^-$ animals, restored placental morphology and induced uterine MDSC accumulation. Simultaneous antibody-mediated depletion of MDSC nullified the protective effect of sHLA-G while adoptive transfer of *in vitro* generated MDSC protected from abortions as well as HLA-G application. Interestingly, the protective effect of sHLA-G on abortion rate was also observed in a mouse model with early abortions (CBA/J x DBA/2J mating model) pointing towards positive effects not only during late but also during early gestation. The fact that CBA/J mice also lack Qa-2 expression may explain their good response to HLA-G. Previous studies in mice showed protective effects of HLA-G on transplant rejection (71, 85) and collagen-induced arthritis (86); furthermore, sHLA-G was shown to allow tumor evasion from immunosurveillance (72, 87), with some of these effects being mediated by an expansion of MDSC (72, 87, 88). These results together with those reported here suggest that a mutual support of HLA-G and MDSC helps to protect allografts from immune rejection and that this interaction is helpful whenever tolerance is needed to survive (pregnancy, organ transplantation), but detrimental in case of tumor growth. Further studies are needed to work out the exact mechanisms behind this effect at different stages of pregnancy. As we aimed to demonstrate an *in vivo* protective effect of HLA-G on murine pregnancy outcome that could be transferred to human pregnancy, we did not include the application of recombinant Qa-2 in our experimental settings.

One limitation of our study is that we used a syngeneic mating model. Since allo antigens play an important role for immunological pregnancy complications and especially for preeclampsia it may be worth to investigate allogeneic pregnancy. However, in our case, allogeneic mating would have led to expression of Qa-2 by the fetuses making it impossible to investigate the effect of a total lack of Qa-2. Another limitation is that HLA-G is not endogenously expressed in mice. Thus, we used mice lacking Qa-2, the only homologue-candidate for HLA-G yet known, for analyzing its impact on pregnancy outcome. However, although HLA-G is a human MHC I molecule, it binds to the murine paired immunoglobulin-like inhibitory receptor (PIR-B) and mediates tolerogenic effects in mice making it possible to analyze its effects *in-vivo* (71, 72). As we observed relevant pregnancy losses in our model in the second half of pregnancy, we started HLA-G application only on day E10.5. Earlier HLA-G administration could have an additional positive effect, as it could possibly already influence the spiral artery remodeling in the uterus at earlier timepoints. Another point worth mentioning is that, in contrast to previous studies with Qa-2 mice (B6.K1) (24, 25), we did not use B6.K2 as a control strain, but normal C57BL6/J mice. To our best knowledge and the knowledge of the supplier, the only difference between B6.K2 and C57BL6/J is that B6.K1 and B6.K2 models were developed in parallel and B6.K2 mice might differ from C57BL6/J mice in the substrain. However this does not influence the haplotype, which is $H2^{b4}$ for both B6.K2 and C57BL6/J (carrying the allels K^b, D^b, Qa $2^{a(hi)}$ and Tla^b) and thus should not influence pregnancy outcome.

In conclusion, we here describe the impact of Qa-2 on maternal phenotype and immune adaptation during pregnancy, providing evidence that Qa-2 may prevent the development of preeclampsia and abortions by promoting MDSC accumulation and functional activation. We further show for the first time *in-vivo* that application of sHLA-G improves pregnancy outcome *via* MDSC induction. These results give reason to hope that synthetic sHLA-G may find a place in the prevention of immunological pregnancy complications like abortions and preeclampsia.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/geo/, accession ID: GSE186053.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethikkommission an der Medizinischen Fakultät der Eberhard-Karls-Universität Tübingen und am Universitätsklinikum Tübingen. The patients/participants provided their written

informed consent to participate in this study. The animal study was reviewed and approved by Regierungspräsidium Tübingen.

AUTHOR CONTRIBUTIONS

SD, JS, AV, IG-M, LQ-M, NC, AM, CG, and NK-G contributed to the acquisition of data, analysis, and interpretation of data. CP provided critical feedback on intellectual content. NK-G and CG conceived the study and wrote the paper. NK-G and CG contributed equally. All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.787468/full#supplementary-material

REFERENCES

1. Rai R, Regan L. Recurrent Miscarriage. *Lancet* (2006) 368(9535):601–11. doi: 10.1016/S0140-6736(06)69204-0
2. Romero R, Dey SK, Fisher SJ. Preterm Labor: One Syndrome, Many Causes. *Science* (2014) 345(6198):760–5. doi: 10.1126/science.1251816
3. Geraghty DE, Koller BH, Orr HT. A Human Major Histocompatibility Complex Class I Gene That Encodes a Protein With a Shortened Cytoplasmic Segment. *Proc Natl Acad Sci USA* (1987) 84(24):9145–9. doi: 10.1073/pnas.84.24.9145
4. Rebmann V, da Silva Nardi F, Wagner B, Horn PA. HLA-G as a Tolerogenic Molecule in Transplantation and Pregnancy. *J Immunol Res* (2014) 2014:297073. doi: 10.1155/2014/297073
5. Kovats S, Main EK, Librach C, Stubblebine M, Fisher SJ, DeMars R. A Class I Antigen, HLA-G, Expressed in Human Trophoblasts. *Science* (1990) 248 (4952):220–3. doi: 10.1126/science.2326636
6. LeMaoult J, Krawice-Radanne I, Dausset J, Carosella ED. HLA-G1-Expressing Antigen-Presenting Cells Induce Immunosuppressive CD4+ T Cells. *Proc Natl Acad Sci USA* (2004) 101(18):7064–9. doi: 10.1073/pnas.0401922101
7. Contini P, Ghio M, Poggi A, Filaci G, Indiveri F, Ferrone S, et al. -B,-C and -G Molecules Induce Apoptosis in T and NK CD8+ Cells and Inhibit Cytotoxic T Cell Activity Through CD8 Ligation. *Eur J Immunol* (2003) 33(1):125–34. doi: 10.1002/immu.200390015
8. Riteau B, Rouas-Freiss N, Menier C, Paul P, Dausset J, Carosella ED. HLA-G2, -G3, and -G4 Isoforms Expressed as Nonmature Cell Surface Glycoproteins Inhibit NK and Antigen-Specific CTL Cytolysis. *J Immunol* (2001) 166(8):5018–26. doi: 10.4049/jimmunol.166.8.5018
9. Rizzo R, Andersen AS, Lassen MR, Sorensen HC, Bergholt T, Larsen MH, et al. Soluble Human Leukocyte Antigen-G Isoforms in Maternal Plasma in Early and Late Pregnancy. *Am J Reprod Immunol* (2009) 62(5):320–38. doi: 10.1111/j.1600-0897.2009.00742.x
10. Gonzalez A, Rebmann V, LeMaoult J, Horn PA, Carosella ED, Alegre E. The Immunosuppressive Molecule HLA-G and Its Clinical Implications. *Crit Rev Clin Lab Sci* (2012) 49(3):63–84. doi: 10.3109/10408363.2012.677947
11. Comiskey M, Goldstein CY, De Fazio SR, Mammolenti M, Newmark JA, Warner CM. Evidence That HLA-G Is the Functional Homolog of Mouse Qa-2, the Ped Gene Product. *Hum Immunol* (2003) 64(11):999–1004. doi: 10.1016/j.humimm.2003.08.352
12. Cai W, Cao W, Wu L, Exley GE, Waneck GL, Karger BL, et al. Sequence and Transcription of Qa-2-Encoding Genes in Mouse Lymphocytes and Blastocysts. *Immunogenetics* (1996) 45(2):97–107. doi: 10.1007/s002510050177
13. Byrne MJ, Jones GS, Warner CM. Preimplantation Embryo Development (Ped) Gene Copy Number Varies From 0 to 85 in a Population of Wild Mice Identified as Mus Musculus Domesticus. *Mamm Genome* (2007) 18(11):767–78. doi: 10.1007/s00335-007-9067-8
14. Newmark JA, Sacher F, Jones GS, Warner CM. Ped Gene Deletion Polymorphism Frequency in Wild Mice. *J Exp Zool* (2002) 293(2):179–85. doi: 10.1002/jez.10117
15. da Silva IL, Montero-Montero L, Ferreira E, Quintanilla M. New Insights Into the Role of Qa-2 and HLA-G Non-Classical MHC-I Complexes in Malignancy. *Front Immunol* (2018) 9:2894. doi: 10.3389/fimmu.2018.02894
16. Warner CM, Brenner CA. Genetic Regulation of Preimplantation Embryo Survival. *Curr Top Dev Biol* (2001) 52:151–92. doi: 10.1016/S0070-2153(01)52011-6
17. Gomes AQ, Correia DV, Silva-Santos B. Non-Classical Major Histocompatibility Complex Proteins as Determinants of Tumour Immunosurveillance. *EMBO Rep* (2007) 8(11):1024–30. doi: 10.1038/sj.embor.7401090
18. Fuzzi B, Rizzo R, Criscuoli L, Noci I, Melchiorri L, Scarselli B, et al. HLA-G Expression in Early Embryos Is a Fundamental Prerequisite for the Obtainment of Pregnancy. *Eur J Immunol* (2002) 32(2):311–5. doi: 10.1002/1521-4141(200202)32:2<311::AID-IMMU311>3.0.CO;2-8
19. Hviid TV, Hylenius S, Lindhard A, Christiansen OB. Association Between Human Leukocyte Antigen-G Genotype and Success of *In Vitro* Fertilization and Pregnancy Outcome. *Tissue Antigens* (2004) 64(1):66–9. doi: 10.1111/j.1399-0039.2004.00239.x
20. Warner CM, Panda P, Almquist CD, Xu Y. Preferential Survival of Mice Expressing the Qa-2 Antigen. *J Reprod Fertil* (1993) 99(1):145–7. doi: 10.1530/jrf.0.0990145
21. Emmery J, Christiansen OB, Nilsson LL, Dahl M, Skovbo P, Moller AM, et al. Associations Between Fetal HLA-G Genotype and Birth Weight and Placental Weight in a Large Cohort of Pregnant Women - Possible Implications for HLA Diversity. *J Reprod Immunol* (2017) 120:8–14. doi: 10.1016/j.jri.2017.02.002
22. Warner CM. Genetic Manipulation of the Major Histocompatibility Complex. *J Anim Sci* (1986) 63(1):279–87. doi: 10.2527/jas1986.631279x
23. McElhinny AS, Kadow N, Warner CM. The Expression Pattern of the Qa-2 Antigen in Mouse Preimplantation Embryos and Its Correlation With the Ped Gene Phenotype. *Mol Hum Reprod* (1998) 4(10):966–71. doi: 10.1093/molehr/4.10.966
24. Warner CM, Brownell MS, Rothschild MF. Analysis of Litter Size and Weight in Mice Differing in Ped Gene Phenotype and the Q Region of the H-2 Complex. *J Reprod Immunol* (1991) 19(3):303–13. doi: 10.1016/0165-0378(91)90042-O
25. Watkins A, Wilkins A, Osmond C, Warner CM, Comiskey M, Hanson M, et al. The Influence of Mouse Ped Gene Expression on Postnatal

Development. *J Physiol* (2006) 571(Pt 1):211–20. doi: 10.1113/jphysiol.2005.099192
26. Gabrilovich DI. Myeloid-Derived Suppressor Cells. *Cancer Immunol Res* (2017) 5(1):3–8. doi: 10.1158/2326-6066.CIR-16-0297
27. Youn JI, Nagaraj S, Collazo M, Gabrilovich DI. Subsets of Myeloid-Derived Suppressor Cells in Tumor-Bearing Mice. *J Immunol* (2008) 181(8):5791–802. doi: 10.4049/jimmunol.181.8.5791
28. Pawelec G, Verschoor CP, Ostrand-Rosenberg S. Myeloid-Derived Suppressor Cells: Not Only in Tumor Immunity. *Front Immunol* (2019) 10:1099. doi: 10.3389/fimmu.2019.01099
29. Kostlin N, Kugel H, Spring B, Leiber A, Marme A, Henes M, et al. Granulocytic Myeloid Derived Suppressor Cells Expand in Human Pregnancy and Modulate T-Cell Responses. *Eur J Immunol* (2014) 44 (9):2582–91. doi: 10.1002/eji.201344200
30. Kostlin N, Hofstadter K, Ostermeir AL, Spring B, Leiber A, Haen S, et al. Granulocytic Myeloid-Derived Suppressor Cells Accumulate in Human Placenta and Polarize Toward a Th2 Phenotype. *J Immunol* (2016) 196 (3):1132–45. doi: 10.4049/jimmunol.1500340
31. Kostlin-Gille N, Dietz S, Schwarz J, Spring B, Pauluschke-Frohlich J, Poets CF, et al. HIF-1alpha-Deficiency in Myeloid Cells Leads to a Disturbed Accumulation of Myeloid Derived Suppressor Cells (MDSC) During Pregnancy and to an Increased Abortion Rate in Mice. *Front Immunol* (2019) 10:161. doi: 10.3389/fimmu.2019.00161
32. Ostrand-Rosenberg S, Sinha P, Figley C, Long R, Park D, Carter D, et al. Frontline Science: Myeloid-Derived Suppressor Cells (MDSCs) Facilitate Maternal-Fetal Tolerance in Mice. *J Leukoc Biol* (2017) 101(5):1091–101. doi: 10.1189/jlb.1HI1016-306RR
33. Pan T, Liu Y, Zhong LM, Shi MH, Duan XB, Wu K, et al. Myeloid-Derived Suppressor Cells Are Essential for Maintaining Feto-Maternal Immunotolerance *via* STAT3 Signaling in Mice. *J Leukoc Biol* (2016) 100 (3):499–511. doi: 10.1189/jlb.1A1015-481RR
34. Kostlin N, Ostermeir AL, Spring B, Schwarz J, Marme A, Walter CB, et al. HLA-G Promotes Myeloid-Derived Suppressor Cell (MDSC) Accumulation and Suppressive Activity During Human Pregnancy Through Engagement of the Receptor ILT4. *Eur J Immunol* (2017) 47(2):374–84. doi: 10.1002/eji.201646564
35. Corzo CA, Condamine T, Lu L, Cotter MJ, Youn JI, Cheng P, et al. HIF-1alpha Regulates Function and Differentiation of Myeloid-Derived Suppressor Cells in the Tumor Microenvironment. *J Exp Med* (2010) 207(11):2439–53. doi: 10.1084/jem.20100587
36. Ungchusri T, Chiang EY, Brown G, Chen M, Tabaczewski P, Timares L, et al. Widespread Expression of the Nonclassical Class I Qa-2 Antigens in Hemopoietic and Nonhemopoietic Cells. *Immunogenetics* (2001) 53(6):455–67. doi: 10.1007/s002510100347
37. Flaherty L. The Tla-Region of the Mouse: Identification of a New Serologically Defined Locus, Qa-2. *Immunogenetics* (1976) 3:533–9. doi: 10.1007/BF01576983
38. Byers SL, Wiles MV, Dunn SL, Taft RA. Mouse Estrous Cycle Identification Tool and Images. *PloS One* (2012) 7(4):e35538. doi: 10.1371/journal.pone.0035538
39. Koehn BH, Apostolova P, Haverkamp JM, Miller JS, McCullar V, Tolar J, et al. GVHD-Associated, Inflammasome-Mediated Loss of Function in Adoptively Transferred Myeloid-Derived Suppressor Cells. *Blood* (2015) 126(13):1621–8. doi: 10.1182/blood-2015-03-634691
40. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING V11: Protein-Protein Association Networks With Increased Coverage, Supporting Functional Discovery in Genome-Wide Experimental Datasets. *Nucleic Acids Res* (2019) 47(D1):D607–13. doi: 10.1093/nar/gky1131
41. Borchert N, Dieterich C, Krug K, Schutz W, Jung S, Nordheim A, et al. Proteogenomics of Pristionchus Pacificus Reveals Distinct Proteome Structure of Nematode Models. *Genome Res* (2010) 20(6):837–46. doi: 10.1101/gr.103119.109
42. Kliza K, Taumer C, Pinzuti I, Franz-Wachtel M, Kunzelmann S, Stieglitz B, et al. Internally Tagged Ubiquitin: A Tool to Identify Linear Polyubiquitin-Modified Proteins by Mass Spectrometry. *Nat Methods* (2017) 14(5):504–12. doi: 10.1038/nmeth.4228
43. Kieckbusch J, Gaynor LM, Colucci F. Assessment of Maternal Vascular Remodeling During Pregnancy in the Mouse Uterus. *J Vis Exp* (2015) 106: e53534. doi: 10.3791/53534
44. Lynge Nilsson L, Djurisic S, Hviid TV. Controlling the Immunological Crosstalk During Conception and Pregnancy: HLA-G in Reproduction. *Front Immunol* (2014) 5:198. doi: 10.3389/fimmu.2014.00198
45. Garziera M, Scarabel L, Toffoli G. Hypoxic Modulation of HLA-G Expression Through the Metabolic Sensor HIF-1 in Human Cancer Cells. *J Immunol Res* (2017) 2017:4587520. doi: 10.1155/2017/4587520
46. Yaghi L, Poras I, Simoes RT, Donadi EA, Tost J, Daunay A, et al. Hypoxia Inducible Factor-1 Mediates the Expression of the Immune Checkpoint HLA-G in Glioma Cells Through Hypoxia Response Element Located in Exon 2. *Oncotarget* (2016) 7(39):63690–707. doi: 10.18632/oncotarget.11628
47. Goldman-Wohl DS, Ariel I, Greenfield C, Hochner-Celnikier D, Cross J, Fisher S, et al. Lack of Human Leukocyte Antigen-G Expression in Extravillous Trophoblasts Is Associated With Pre-Eclampsia. *Mol Hum Reprod* (2000) 6(1):88–95. doi: 10.1093/molehr/6.1.88
48. Steinborn A, Varkonyi T, Scharf A, Bahlmann F, Klee A, Sohn C. Early Detection of Decreased Soluble HLA-G Levels in the Maternal Circulation Predicts the Occurrence of Preeclampsia and Intrauterine Growth Retardation During Further Course of Pregnancy. *Am J Reprod Immunol* (2007) 57 (4):277–86. doi: 10.1111/j.1600-0897.2007.00475.x
49. Yie SM, Li LH, Li YM, Librach C. HLA-G Protein Concentrations in Maternal Serum and Placental Tissue Are Decreased in Preeclampsia. *Am J Obstet Gynecol* (2004) 191(2):525–9. doi: 10.1016/j.ajog.2004.01.033
50. Hara N, Fujii T, Yamashita T, Kozuma S, Okai T, Taketani Y. Altered Expression of Human Leukocyte Antigen G (HLA-G) on Extravillous Trophoblasts in Preeclampsia: Immunohistological Demonstration With Anti-HLA-G Specific Antibody "87G" and Anti-Cytokeratin Antibody "CAM5.2". *Am J Reprod Immunol* (1996) 36(6):349–58. doi: 10.1111/j.1600-0897.1996.tb00185.x
51. McElhinny AS, Exley GE, Warner CM. Painting Qa-2 Onto Ped Slow Preimplantation Embryos Increases the Rate of Cleavage. *Am J Reprod Immunol* (2000) 44(1):52–8. doi: 10.1111/j.8755-8920.2000.440108.x
52. Exley GE, Warner CM. Selection in Favor of the Ped Fast Haplotype Occurs Between Mid-Gestation and Birth. *Immunogenetics* (1999) 49(7-8):653–9. doi: 10.1007/s002510050661
53. Kofod L, Lindhard A, Bzorek M, Eriksen JO, Larsen LG, Hviid TVF. Endometrial Immune Markers Are Potential Predictors of Normal Fertility and Pregnancy After In Vitro Fertilization. *Am J Reprod Immunol* (2017) 78 (3). doi: 10.1111/aji.12684
54. Ridder A, Giorgione V, Khalil A, Thilaganathan B. Preeclampsia: The Relationship Between Uterine Artery Blood Flow and Trophoblast Function. *Int J Mol Sci* (2019) 20(13):3263. doi: 10.3390/ijms20133263
55. Cui Y, Wang W, Dong N, Lou J, Srinivasan DK, Cheng W, et al. Role of Corin in Trophoblast Invasion and Uterine Spiral Artery Remodelling in Pregnancy. *Nature* (2012) 484(7393):246–50. doi: 10.1038/nature10897
56. Guleria I, Sayegh MH. Maternal Acceptance of the Fetus: True Human Tolerance. *J Immunol* (2007) 178(6):3345–51. doi: 10.4049/jimmunol.178.6.3345
57. Ander SE, Diamond MS, Coyne CB. Immune Responses at the Maternal-Fetal Interface. *Sci Immunol* (2019) 4(31):eaat6114. doi: 10.1126/sciimmunol.aat6114
58. Buhimschi IA, Nayeri UA, Zhao G, Shook LL, Pensalfini A, Funai EF, et al. Protein Misfolding, Congophilia, Oligomerization, and Defective Amyloid Processing in Preeclampsia. *Sci Trans Med* (2014) 6(245):245ra92. doi: 10.1126/scitranslmed.3008808
59. Cater JH, Kumita JR, Zeineddine Abdallah R, Zhao G, Bernardo-Gancedo A, Henry A, et al. Human Pregnancy Zone Protein Stabilizes Misfolded Proteins Including Preeclampsia- and Alzheimer's-Associated Amyloid Beta Peptide. *Proc Natl Acad Sci USA* (2019) 116(13):6101–10. doi: 10.1073/pnas.1817298116
60. Tong M, Cheng SB, Chen Q, DeSousa J, Stone PR, James JL, et al. Aggregated Transthyretin Is Specifically Packaged Into Placental Nano-Vesicles in Preeclampsia. *Sci Rep* (2017) 7(1):6694. doi: 10.1038/s41598-017-07017-x
61. Sulistyowati S, Bachnas MA, Anggraini ND, Yuliantara EE, Prabowo W, Anggraini NW, et al. Recombinant Vascular Endothelial Growth Factor 121 Injection for the Prevention of Fetal Growth Restriction in a Preeclampsia Mouse Model. *J Perinat Med* (2017) 45(2):245–51. doi: 10.1515/jpm-2016-0149
62. Du M, Yu N, Ding Q, Chen X, Frempong ST, Cai X, et al. Elevated Percentage of CD3(+)T Cells and Pregnancy Outcome in Women With Recurrent

Pregnancy Loss. *Clin Chim Acta* (2018) 486:341–6. doi: 10.1016/j.cca.2018.08.024
63. Arenas-Hernandez M, Romero R, Xu Y, Panaitescu B, Garcia-Flores V, Miller D, et al. Effector and Activated T Cells Induce Preterm Labor and Birth That Is Prevented by Treatment With Progesterone. *J Immunol* (2019) 202(9):2585–608. doi: 10.4049/jimmunol.1801350
64. Luppi P, Tse H, Lain KY, Markovic N, Piganelli JD, DeLoia JA. Preeclampsia Activates Circulating Immune Cells With Engagement of the NF-kappaB Pathway. *Am J Reprod Immunol* (2006) 56(2):135–44. doi: 10.1111/j.1600-0897.2006.00386.x
65. Hosseini A, Dolati S, Hashemi V, Abdollahpour-Alitappeh M, Yousefi M. Regulatory T and T Helper 17 Cells: Their Roles in Preeclampsia. *J Cell Physiol* (2018) 233(9):6561–73. doi: 10.1002/jcp.26604
66. Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T Cells Mediate Maternal Tolerance to the Fetus. *Nat Immunol* (2004) 5(3):266–71. doi: 10.1038/ni1037
67. Zenclussen AC, Gerlof K, Zenclussen ML, Sollwedel A, Bertoja AZ, Ritter T, et al. Abnormal T-Cell Reactivity Against Paternal Antigens in Spontaneous Abortion: Adoptive Transfer of Pregnancy-Induced CD4+CD25+ T Regulatory Cells Prevents Fetal Rejection in a Murine Abortion Model. *Am J Pathol* (2005) 166(3):811–22. doi: 10.1016/S0002-9440(10)62302-4
68. Kostlin-Gille N, Gille C. Myeloid-Derived Suppressor Cells in Pregnancy and the Neonatal Period. *Front Immunol* (2020) 11:584712. doi: 10.3389/fimmu.2020.584712
69. Ribechini E, Leenen PJ, Lutz MB. Gr-1 Antibody Induces STAT Signaling, Macrophage Marker Expression and Abrogation of Myeloid-Derived Suppressor Cell Activity in BM Cells. *Eur J Immunol* (2009) 39(12):3538–51. doi: 10.1002/eji.200939530
70. Takai T, Nakamura A, Endo S. Role of PIR-B in Autoimmune Glomerulonephritis. *J BioMed Biotechnol* (2011) 2011:275302. doi: 10.1155/2011/275302
71. Liang S, Baibakov B, Horuzsko A. HLA-G Inhibits the Functions of Murine Dendritic Cells *via* the PIR-B Immune Inhibitory Receptor. *Eur J Immunol* (2002) 32(9):2418–26. doi: 10.1002/1521-4141(200209)32:9<2418::AID-IMMU2418>3.0.CO;2-L
72. Loumagne L, Baudhuin J, Favier B, Montespan F, Carosella ED, Rouas-Freiss N. *In Vivo* Evidence That Secretion of HLA-G by Immunogenic Tumor Cells Allows Their Evasion From Immunosurveillance. *Int J Cancer* (2014) 135(9):2107–17. doi: 10.1002/ijc.28845
73. Goodall KJ, Nguyen A, Sullivan LC, Andrews DM. The Expanding Role of Murine Class Ib MHC in the Development and Activation of Natural Killer Cells. *Mol Immunol* (2019) 115:31–8. doi: 10.1016/j.molimm.2018.05.001
74. Hanson EM, Clements VK, Sinha P, Ilkovitch D, Ostrand-Rosenberg S. Myeloid-Derived Suppressor Cells Down-Regulate L-Selectin Expression on CD4+ and CD8+ T Cells. *J Immunol* (2009) 183(2):937–44. doi: 10.4049/jimmunol.0804253
75. Haist M, Stege H, Grabbe S, Bros M. The Functional Crosstalk Between Myeloid-Derived Suppressor Cells and Regulatory T Cells Within the Immunosuppressive Tumor Microenvironment. *Cancers (Basel)* (2021) 13(2):210. doi: 10.3390/cancers13020210
76. Kang X, Zhang X, Liu Z, Xu H, Wang T, He L, et al. Granulocytic Myeloid-Derived Suppressor Cells Maintain Feto-Maternal Tolerance by Inducing Foxp3 Expression in CD4+CD25-T Cells by Activation of the TGF-Beta/Beta-Catenin Pathway. *Mol Hum Reprod* (2016) 22(7):499–511. doi: 10.1093/molehr/gaw026
77. Kostlin N, Vogelmann M, Spring B, Schwarz J, Feucht J, Hartel C, et al. Granulocytic Myeloid Derived Suppressor Cells From Human Cord Blood Modulate T-Helper-Cell Response Towards an Anti-Inflammatory Phenotype. *Immunol* (2017) 152(1):89–101. doi: 10.1111/imm.12751
78. Dietz S, Schwarz J, Vogelmann M, Spring B, Molnar K, Orlikowsky TW, et al. Cord Blood Granulocytic Myeloid-Derived Suppressor Cells Impair Monocyte T Cell Stimulatory Capacity and Response to Bacterial Stimulation. *Pediatr Res* (2019) 86(5):608–15. doi: 10.1038/s41390-019-0504-7
79. Selmani Z, Naji A, Zidi I, Favier B, Gaiffe E, Obert L, et al. Human Leukocyte Antigen-G5 Secretion by Human Mesenchymal Stem Cells Is Required to Suppress T Lymphocyte and Natural Killer Function and to Induce CD4+CD25highFOXP3+ Regulatory T Cells. *Stem Cells* (2008) 26(1):212–22. doi: 10.1634/stemcells.2007-0554
80. Polanczyk MJ, Carson BD, Subramanian S, Afentoulis M, Vandenbark AA, Ziegler SF, et al. Cutting Edge: Estrogen Drives Expansion of the CD4+CD25+ Regulatory T Cell Compartment. *J Immunol* (2004) 173(4):2227–30. doi: 10.4049/jimmunol.173.4.2227
81. Wang C, Dehghani B, Li Y, Kaler LJ, Vandenbark AA, Offner H. Oestrogen Modulates Experimental Autoimmune Encephalomyelitis and Interleukin-17 Production *via* Programmed Death 1. *Immunology* (2009) 126(3):329–35. doi: 10.1111/j.1365-2567.2008.03051.x
82. Kozasa K, Mabuchi S, Matsumoto Y, Kuroda H, Yokoi E, Komura N, et al. Estrogen Stimulates Female Cancer Progression by Inducing Myeloid-Derived Suppressive Cells: Investigations on Pregnant and Non-Pregnant Experimental Models. *Oncotarget* (2019) 10(20):1887–902. doi: 10.18632/oncotarget.26711
83. Ozerova M, Nefedova Y. Estrogen Promotes Multiple Myeloma Through Enhancing the Immunosuppressive Activity of MDSC. *Leukemia lymphoma* (2019) 60(6):1557–62. doi: 10.1080/10428194.2018.1538511
84. Yang J, AlTahan A, Jones DT, Buffa FM, Bridges E, Interiano RB, et al. Estrogen Receptor-Alpha Directly Regulates the Hypoxia-Inducible Factor 1 Pathway Associated With Antiestrogen Response in Breast Cancer. *Proc Natl Acad Sci USA* (2015) 112(49):15172–7. doi: 10.1073/pnas.1422015112
85. Favier B, HoWangYin KY, Wu J, Caumartin J, Daouya M, Horuzsko A, et al. Tolerogenic Function of Dimeric Forms of HLA-G Recombinant Proteins: A Comparative Study In Vivo. *PloS One* (2011) 6(7):e21011. doi: 10.1371/journal.pone.0021011
86. Kuroki K, Hirose K, Okabe Y, Fukunaga Y, Takahashi A, Shiroishi M, et al. The Long-Term Immunosuppressive Effects of Disulfide-Linked HLA-G Dimer in Mice With Collagen-Induced Arthritis. *Hum Immunol* (2013) 74(4):433–8. doi: 10.1016/j.humimm.2012.11.060
87. Agaugue S, Carosella ED, Rouas-Freiss N. Role of HLA-G in Tumor Escape Through Expansion of Myeloid-Derived Suppressor Cells and Cytokinic Balance in Favor of Th2 Versus Th1/Th17. *Blood* (2011) 117(26):7021–31. doi: 10.1182/blood-2010-07-294389
88. Zhang W, Liang S, Wu J, Horuzsko A. Human Inhibitory Receptor Immunoglobulin-Like Transcript 2 Amplifies CD11b+Gr1+ Myeloid-Derived Suppressor Cells That Promote Long-Term Survival of Allografts. *Transplantation* (2008) 86(8):1125–34. doi: 10.1097/TP.0b013e318186fccd

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The Value of Prenatal First Systolic Blood Pressure Can Predict Severe Preeclampsia and Birth Weight in Patients With Preeclampsia

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Background: Preeclampsia is a serious complication of pregnancy that threatens the safety of the fetus and mother. We assessed the relationship between systolic blood pressure (SBP) in the early pregnancy stage (12 weeks) in patients with preeclampsia and the development of severe eclampsia and birth weight.

Methods: Patients were categorized based on the quartiles of the prenatal first SBP level. Logistic regression analysis was performed to assess whether prenatal first SBP was a risk factor for low birth weight and severe preeclampsia. The area under the receiver-operating characteristic curve (AUC) of sensitivity and specificity were used to predict the risk of low birth weight and severe preeclampsia.

Results: A total of 333 patients with preeclampsia were enrolled. There were 162 (48.6%) patients with severe preeclampsia and 270 (81.08%) cesareans. Group I patients with a prenatal first SBP $\leq$ 119 mmHg prenatal had a higher birth weight. Multiple logistic regression analysis showed that serum creatinine ($p = 0.025$), prenatal first SBP ($p = 0.029$), S-preeclampsia ($p = 0.003$), gestational age ($p < 0.001$), total cholesterol (TC) ($p < 0.001$), and low-density lipoprotein (LDL) ($p < 0.001$) were independent risk factors for low birth weight. Multiple logistic regression analysis showed that prenatal first SBP ($p = 0.003$), TC ($p = 0.002$), and B-type natriuretic peptide (BNP) ($p < 0.001$) were independent risk factors for severe preeclampsia. Compared with Group I (SBP $\leq$ 119 mmHg), the incidence of low birth weight for patients in groups III (131 $\leq$ SBP $\leq$ 138 mmHg) and IV (SBP $\geq$ 139 mmHg) was significantly higher. Even after correcting for age, gestational age, and biochemical indices, the difference remained statistically significant. The risk of diagnosed severe preeclampsia for patients in Groups IV (SBP $\geq$ 139 mmHg), III (131 $\leq$ SBP $\leq$ 138 mmHg), and II (120 $\leq$ SBP $\leq$ 130 mmHg) was significantly higher than that in Group I (SBP $\leq$ 119 mmHg). The AUC of the prenatal first SBP for predicting low birth weight and severe preeclampsia was 0.676 (95% *CI* 0.618–0.733, $p < 0.001$) and 0.727 (95% *CI* 0.673–0.781, $p < 0.001$), respectively, in patients with preeclampsia.

Conclusions: Prenatal first SBP was associated with birth weight and severe preeclampsia. Higher prenatal first SBP in patients with preeclampsia can predict low birth weight and severe preeclampsia.

Keywords: preeclampsia, severe preeclampsia, birth weight, systolic blood pressure, prenatal

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INTRODUCTION

Preeclampsia is a serious complication of pregnancy that threatens the safety of the fetus and mother. Preeclampsia and eclampsia, which vary greatly from region to region, cause approximately 50,000 maternal deaths worldwide every year (1). Although the application of magnesium sulfate has reduced the incidence rate of eclampsia (2), it remains an important reason for the threat of maternal life safety. Preeclampsia is mainly characterized by new hypertension and proteinuria and can develop into multiple organ damage, such as visceral, renal, and cerebrovascular damage. Its risk factors mainly include whether the mother has complications, such as chronic kidney disease, hypertension, obesity, familial preeclampsia, previous eclampsia, or fetal intrauterine growth restriction.

Blood pressure is an important indicator for the diagnosis of preeclampsia. Blood pressure higher than 160/110 mmHg is characteristic of the diagnosis of severe preeclampsia (3). Currently, the mechanism of preeclampsia is unclear. Placental ischemia may be one of the important mechanisms leading to preeclampsia, which may occur in the early stages of pregnancy. Hypoxia and energy demands are unbalanced during early pregnancy (4). Studies have reported that a decrease in vasodilation function, circulating nitrous oxide (NO), and an increased cholesterol level are important evidence that endothelial cell injury may precede preeclampsia (5). Currently, there is no relevant report on the relationship between changes in blood pressure in the early stage of pregnancy and the prognosis of patients with preeclampsia. Therefore, this study observed the systolic blood pressure (SBP) at early pregnancy (12 weeks) in patients with preeclampsia and defined the relationship between the SBP level and the development of severe eclampsia and birth weight.

METHODS

Patients

This study protocol was approved by the ethics committee of the First Affiliated Hospital of Fujian Medical University. All patients provided written informed consent to participate in this study. Patient inclusion criteria included providing written informed consent, aged >18 years, and diagnosed with preeclampsia between June 2015 and July 2021. Patients with infection, trauma, mental illness, hypertension, connective tissue disease, kidney disease, nervous system diseases, multiple pregnancies, congenital anomalies, preterm premature rupture of membrane, diabetes, and those who needed antibiotics, corticosteroids, or immunosuppressive agents within a month were excluded. Of the 362 patients, six were excluded due to connective tissue disease and use of corticosteroids or immunosuppressive agents, eight due to infection or antibiotic use within a month, nine due to chronic kidney disease, and six due to hypertension. Finally, a total of 333 individuals were included.

Clinical data were recorded, including the history of the disease, concomitant disease (kidney disease and hypertension), medications, prenatal first blood pressure, prenatal blood pressure, laboratory data, and delivery model. The primary endpoints were birth weight and severe preeclampsia. Severe preeclampsia was defined by any of the findings of severe features based on the American College of Obstetricians and Gynecologists (ACOG) 2013 criteria: blood pressure $\geq$ 160/110 mmHg (on two occasions at least 4 h apart), thrombocytopenia (platelet count <100,000/μl), progressive renal insufficiency (serum creatinine $\geq$ 1.1 mg/dl or doubling serum creatinine in the absence of other renal diseases), new-onset cerebral or visual disturbances, and pulmonary edema (3). Prenatal first blood pressure will be tested at 12 weeks of gestational age. Birth weight < 2,500 g was classified as low birth weight and $\geq$ 2500 g as normal.

Laboratory Tests

Blood samples were collected before the delivery. Blood biochemical indices, including average values of calcium (Ca^{2+}), phosphate(P^{3+}), total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, albumin (Alb), hemoglobin (Hb), B-type natriuretic peptide (BNP), fibrinogen, uric acid (UA), and lactate dehydrogenase (LDH) were measured in all patients.

Statistical Analysis

The Kolmogorov–Smirnov test was used to estimate the Gaussian distribution of the data, and $p > 0.05$ indicated a normal distribution. Normally distributed measurement data are represented as mean ± SD, and the Bonferroni test was used for pairwise comparisons among groups. Measurement data that were not normally distributed are represented as median and quartile, and the Mann–Whitney *U*-test was used for comparisons between groups. We categorized patients based on the quartiles of the prenatal first SBP levels within our study population. Comparisons between groups for quantitative data were conducted using the chi-square test or Fisher's exact test. Categorical variables are expressed as numbers (or percentages). A logistic regression analysis was performed to assess whether prenatal first SBP was a risk factor for low birth weight and severe preeclampsia. The area under the receiver-operating characteristic (ROC) curve (AUC) of sensitivity and specificity was used to predict the risk of low birth weight and severe preeclampsia. Statistical significance was set at $p < 0.05$. SPSS 15.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses and figures.

RESULTS

Clinical Manifestations and Biochemical Tests

Among the 333 patients with preeclampsia, the mean age was 32.00 ± 5.03 years, and the median birth weight was 2,800.00 (2,100.00, 3,500.00) g. There were 162 (48.6%) cases of severe preeclampsia and 270 (81.08%) cesarean sections. Based on the quartiles of prenatal first SBP, the patients were divided into four groups: group I had a prenatal first SBP $\leq$ 119 mmHg renatal f; group II had a prenatal first SBP between

120–130 mmHg, group III had a prenatal first SBP between 131 and 138 mmHg, and group IV had a prenatal first SBP ≥ 139 mmHg prenatal. **Table 1** shows that the birth weight in groups III and IV was significantly lower than that in group I ($p < 0.001$). The values of Hb, Scr, ACR, and 24 h protein urine were higher in Groups III and IV than that in Group I. Intravenous antihypertensive drugs were used more frequently in groups III and IV. There were no significant differences in LDL, HDL, fibrinogen, and BNP levels between the groups.

Analysis of Risk Factors for Low Birth Weight and Severe Preeclampsia

Univariate analysis showed that prenatal first SBP was correlated with low birth weight. Multiple logistic regression analysis showed that serum creatinine (hazard ratio [*HR*] = 0.380, 95% *CI* 0.163–0.884, $p = 0.025$), prenatal first SBP (*HR* = 2.652, 95% *CI* 1.107–6.357, $p = 0.029$), S-preeclampsia (*HR* = 4.123, 95% *CI* 1.620–10.493, $p = 0.003$), gestational age (*HR* = 0.043, 95% *CI* 0.017–0.110, $p < 0.001$), TC (*HR* = 0.093, 95% *CI* 0.026–0.329, $p < 0.001$), and LDL (*HR* = 22.816, 95% *CI* 6.208–88.859, $p < 0.001$) were independent risk factors for low birth weight (**Table 2**).

Univariate analysis showed that the first prenatal SBP was correlated with severe preeclampsia. Multinomial logistic regression analysis showed that prenatal first SBP (*HR* = 3.490, 95% *CI* 1.539–7.915, $p = 0.003$), TC (*HR* = 3.582, 95% *CI* 1.599–8.020, $p = 0.002$), BNP (*HR* = 9.167, 95% *CI* 3.737–22.486, $p < 0.001$) were independent risk factors for severe preeclampsia (**Table 3**).

TABLE 1 | Basic patient demographic characteristics and laboratory data by quartiles of prenatal first systolic blood pressure (SBP) in patients with preeclampsia.

	All	I ≤ 119 mmHg	II 120–130 mmHg	III 131–138 mmHg	IV ≥ 139 mmHg
General condition					
Number (*n*,%)	333 (100)	84 (25.23)	87 (26.11)	84 (25.23)	78 (23.43)
Age (years, x ± s)	32.00 ± 5.03	30.14 ± 4.27	30.76 ± 4.56	32.96 ± 4.56**	34.35 ± 5.64**
Gestational age (w)	37.57 (34.29, 39.14)	38.57 (38.14, 39.68)	38.29 (36.14, 39.71)	36.36 (30.46, 38.82)**	34.71 (30.14, 37.57)**
Cesarean (*n*, %)	270 (81.08)	57 (67.9)	75 (86.21)**	66 (78.57)	72 (92.3)**
Birth weight [g,M(1/4,3/4)]	2800.00 (2100.00, 3500.00)	3175.00 (2755.00, 3760.00)	3000.00 (2400.00, 3600.00)	2500.00 (1290.00, 3175.00)**	2050.00 (1120.00, 2850.00)**
S-preeclampsia (*n*,%)	162 (48.6)	15 (17.9)	45 (51.7)**	51 (60.7)**	51 (65.4)**
Lab tests					
Hb (g/l, x ± s)	122.58 ± 15.27	118.92 ± 13.89	119.62 ± 17.24	128.21 ± 10.95**	123.77 ± 16.57*
Scr (mmol/l, x ± s)	54.19 ± 14.67	49.85 ± 11.54	53.32 ± 15.22	55.38 ± 12.89*	58.10 ± 17.42**
UA (mmol/l, x ± s)	317.40 ± 82.22	374.38 ± 82.81	421.83 ± 74.48**	422.33 ± 85.78**	377.40 ± 84.23
ACR [g/mg,M(1/4,3/4)]	1240.76 (153, 334.14)	453.00 (67.87, 2733.13)	1133.85** (334.47, 1900.10)	1315.36** (101.67–3776.35)	2649.73** (309.96, 4322.81)
24 h protein urine [g/d,M(1/4,3/4)]	1.33 (0.47, 4.03)	0.68 (0.41, 1.82)	1.34** (0.42, 1.83)	1.52** (0.53, 2.84)	4.24** (0.58, 6.50)
Alb (g/l, x ± s)	30.45 ± 4.20	31.70 ± 2.95	31.44 ± 3.43	29.73 ± 5.55**	28.87 ± 3.71**
TG (mmol/l, x ± s)	3.25 ± 1.32	3.83 ± 1.98	2.76 ± 1.08**	3.15 ± 0.95*	3.51 ± 1.28
TC (mmol/l, x ± s)	5.94 ± 1.5	6.06 ± 1.76	5.37 ± 1.07*	6.06 ± 1.49	6.26 ± 1.60
LDL (mmol/l, x ± s)	3.33 ± 1.22	3.5 ± 1.44	2.99 ± 0.92	3.45 ± 1.21	3.39 ± 1.31
HDL (mmol/l, x ± s)	1.86 ± 0.43	1.75 ± 0.34	1.88 ± 0.48	1.84 ± 0.49	1.93 ± 0.35
Ca (mmol/l, x ± s)	2.03 ± 0.24	2.09 ± 0.25	2.01 ± 0.23*	2.03 ± 0.24	1.98 ± 0.21**
Fib (g/l, x ± s)	4.65 ± 0.83	4.51 ± 0.77	4.31 ± 0.85	4.29 ± 1.12	4.65 ± 0.83
BNP [pg/ml,M(1/4,3/4)]	54.15 (22.43, 92.19)	56.00 (21.00, 80.00)	64.43 (44.99, 129.93)	32.65 (19.33, 98.01)	48.03 (19.00, 74.78)
LDH (U/L, x ± s)	311.64 ± 156.38	256.46 ± 102.19	327.21 ± 151.16**	318.46 ± 169.26**	346.35 ± 181.42**
GPT (U/L, M1/4,3/4)	17.00 (12.00, 28.00)	16.00 (15.00, 30.00)	16.00 (11.00, 30.00)	21.00 (11.00, 27.25)	15.00 (12.00, 20.00)
Antihypertensive					
β-blocker (*n*, %)	201 (60.4)	21 (25)	48 (55.2)**	60 (71.4)**	72 (92.3)**
Intravenous (*n*, %)	42 (12.6)	0 (0)	3 (3.4)	21 (25)**	18 (23.1)**
CCB (*n*, %)	144 (43.2)	21 (25)	33 (37.8)	42 (50)**	48 (61.5)**

*Compared with Group I $p < 0.05$; **compared with group I $p < 0.001$; Hb, hemoglobin; Alb, serum albumin; Scr, serum creatinine; UA, uric acid; GPT, glutamic pyruvic transaminase; TG, triglyceride; TC, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; Ca, calcium; BNP, B-type natriuretic peptide; S-preeclampsia, severe preeclampsia; ACR, albumin creatinine ratio; Fib, fibrinogen; LDH, lactate dehydrogenase; Intravenous, intravenous drug application; CCB, calcium blocker.*

TABLE 2 | Logistic regression analysis risk factors for low birth weight in patients with preeclampsia.

	Univariate analysis		Multi variate analysis	
	HR	***P***	**HR**	***P***
Age (32 years-old)	3.048 (0.538–17.271)	0.208		
Hb (123 g/l)	2.693 (0.504–14.395)	0.247		
Scr (54.88 umol/l)	0.198 (0.033–0.970)	0.002	0.380 (0.163–0.884)	0.025
UA (391 umol/l)	0.154 (0.017–1.423)	0.099		
Alb (30.2 g/l)	0.029 (0.002–0.382)	0.007		
TG (3.41 mmol/l)	0.046 (0.006–0.383)	0.004		
TC (6.07 mmol/l)	0.037 (0.004–0.329)	0.003	0.093 (0.026–0.329)	<0.001
LDL (3.35 mmol/l)	37.766 (4.890–291.664)	<0.001	22.816 (6.208–83.859)	<0.001
HDL (1.89 mmol/l)	6.803 (0.961–48.177)	0.055		
Ca (1.92 mmol/l)	5.442 (0.939–31.518)	0.059		
Fibrinogen (4.4 g/l)	0.161 (0.025–1.023)	0.530		
BNP (54.3 pg/ml)	1.690 (0.365–7.823)	0.502		
24 h protein urine (1.66 g/d)	0.972 (0.298–3.167)	0.962		
Prenatal first SBP (130 mmHg)	5.793 (1.556–21.562)	0.009	2.652 (1.107, 6.357)	0.029
S–preeclampsia	2.400 (1.570–12.608)	0.005	4.123 (1.620–10.493)	0.003
Gestational age (35.7 weeks)	0.038 (0.028–0.243)	<0.001	0.043 (0.017–0.110)	<0.001

HR, hazard ratio; Hb, hemoglobin; Alb, serum albumin; Scr, serum creatinine; UA, uric acid; TG, triglyceride; TC, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; Ca, calcium; BNP, B-type natriuretic peptide; and S-preeclampsia, severe preeclampsia.

TABLE 3 | Logistic regression analysis risk factors for severe preeclampsia in patients with preeclampsia.

	Univariate analysis		Multi variate analysis	
	HR	***P***	**HR**	***P***
Age (32 years-old)	8.273 (2.204–31.057)	0.002		
Hb (123 g/l)	0.159 (0.042–0.598)	0.007		
Scr (54.88 umol/l)	0.11.932 (0.555–6.722)	1.932		
UA (391 umol/l)	0.173 (0.047–0.642)	0.009		
Alb (30.2 g/l)	2.487 (0.645–9.585)	0.186		
TG (3.41 mmol/l)	1.084 (0.328–3.580)	0.895		
TC (6.07 mmol/l)	5.674 (1.849–17.407)	0.002	3.582 (1.599–8.020)	0.002
LDL (3.35 mmol/l)	0.698 (0.128–3.809)	0.678		
HDL (1.89 mmol/l)	0.381 (0.078–1.874)	0.381		
Ca (1.92 mmol/l)	1.944 (0.601–6.287)	0.267		
Fibrinogen (4.4 g/l)	1.221 (0.342–5.223)	0.329		
BNP (54.3 pg/ml)	53.297 (8.836–321.485)	<0.001	9.167 (3.737–22.486)	<0.001
24 h protein urine (1.66 g/d)	4.097 (1.700–9.876)	0.002		
Prenatal first SBP (130 mmHg)	10.542 (2.705–41.090)	0.001	3.490 (1.539–7.915)	0.003
Birth weight	1.148 (0.304–4.337)	0.839		
Gestational age (35.7 weeks)	3.715 (0.835–16.520)	0.085		

HR, hazard ratio; Hb, hemoglobin; Alb, serum albumin; Scr, serum creatinine; UA, uric acid; TG, triglyceride; TC, cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; Ca, calcium; and BNP, B–type natriuretic peptide.

Relationship Between Prenatal First SBP and Low Fetal Weight, Severe Preeclampsia

Compared with patients in group I (≤ 119 mmHg), the risk of low birth weight for patients in group III (120–130 mmHg) and IV (≥ 139 mmHg) were significantly higher. After correcting for age, gestational age, and biochemical indices, the difference remained statistically significant. For patients in group IV (≥ 139 mmHg statistic), the risk of low birth weight significantly increased by 271.8% compared with that in Group I (≤ 119 mmHg hat in Gr) ($p < 0.001$) (**Table 4**).

Among the patients who were diagnosed with severe preeclampsia, the risk of severe preeclampsia diagnosed in Group IV (≥ 139 mmHg), III (120–130 mmHg), and II

TABLE 4 | Association of prenatal first SBP and low birth weight.

	I ≤ 119 mmHg	II 120–130 mmHg	III 131–138 mmHg	IV ≥ 139 mmHg
n with low birth weight/total	18/84	39/87	45/84	48/78
Unadjusted	1	1.970 (1.058–3.669) $P = 0.033$	2.436 (1.301–4.561) $P = 0.005$	5.730 (2.908–11.291) $P < 0.001$
Fully adjusted[a]	1	2.604 (1.160, 2.805) $P = 0.107$	2.534 (1.309–4.905) $P = 0.006$	3.718 (1.779–7.771) $P < 0.001$

[a]*Adjusted for age, Hb, Scr, Alb, TC, LDL, 24 h protein urine, and gestational age.*

TABLE 5 | Association of prenatal first SBP and severe preeclampsia.

	I ≤ 119 mmHg	II 120–130 mmHg	III 131–138 mmHg	IV ≥ 139 mmHg
n with s–preeclampsia /total	15/84	45/87	51/84	51/78
Unadjusted	1	4.929 (2.45, 9.915) $P < 0.001$	7.109 (3.497–14.454) $P < 0.001$	8.689 (4.197, 17.987) $P < 0.001$
Fully adjusted[a]	1	5.978 (2.133, 16.765) $P = 0.001$	6.125 (2.514, 14.919) $P < 0.001$	5.109 (1.971, 13.503) $P < 0.001$

[a]*Adjusted for age, Hb, Scr, Alb, TC, LDL, 24 h protein urine, and gestational age.*

(120–130 mmHg) was significantly higher than that in Group I (≤ 119 mmHg); this difference remained statistically significant even after correcting for all potential confounding factors (**Table 5**).

ROC Analysis Prenatal First SBP Predicting Low Birth Weight and Severe Preeclampsia

The AUC for the first prenatal SBP to predict low birth weight and severe preeclampsia was 0.676 (95% *CI* 0.618–0.733, $p < 0.001$) and 0.727 (95% *CI* 0.673–0.781, $p < 0.001$), respectively, in patients with preeclampsia, which showed that prenatal first SBP had a high accuracy for predicting low birth weight and severe preeclampsia. A cut-off value of 121.5 and 123 mmHg yielded good sensitivity and specificity for predicting low birth weight (88 and 60.7%, respectively) and severe preeclampsia (88.9 and 52.6%, respectively) (**Figures 1**, **2**).

DISCUSSION

Preeclampsia and eclampsia seriously threaten the safety of pregnant mothers and fetuses. The incidence rate of hypertensive disorders in pregnancy, such as preeclampsia and eclampsia, pregnancy with hypertension, and chronic hypertension, is 5.5% (6). Patients with preeclampsia or eclampsia have a 3–25-fold increased risk of serious complications during pregnancy, such as pulmonary edema, disseminated intravascular coagulation, placental abruption, preterm birth, and aspiration pneumonia. Preeclampsia risk factors include family history, genetic susceptibility, maternal smoking, pregnancy, maternal age, *in vitro* fertilization, and maternal physical conditions, such as hypertension, diabetes, chronic kidney disease, and obesity.

The ACOG divides gestational hypertension into four categories: preeclampsia and eclampsia, chronic hypertension, chronic hypertension combined with preeclampsia, and gestational hypertension. In this study, patients with preeclampsia had no history of chronic hypertension before pregnancy, and their blood pressure was monitored and recorded at the first follow-up of prenatal examination at 12 weeks. At 12 weeks, even if the blood pressure was within the normal range, the proportion of patients with severe preeclampsia and low birth weight was significantly higher in the SBP ≥ 120 mmHg group than in the SBP ≤ 119 mmHg group. The risk of low birth weight was 2.604–3.718 times and severe preeclampsia was 5.109–6.125 times. These abnormalities in women with preeclampsia suggest that the mechanisms that contribute to placental ischemia are set into motion very early in pregnancy. Thus, the concept of defective placentation and failure to transform uterine spiral arteries has emerged to be central to the pathogenesis of preeclampsia (7, 8).

Endothelial dysfunction may be an important cause of high blood pressure. Studies have shown that the imbalance of circulating angiogenic factors plays an important role in the etiology of maternal syndrome (9–11). High levels of anti-angiogenic factor soluble fms-like tyrosine kinase1 (sFLT1) produced in the placenta and released into the maternal circulation led to the dysfunction of maternal endothelial cells, leading to preeclampsia (12–16). sFLT1 is a soluble splice variant of the membrane-bound receptor vascular endothelial growth factor receptor 1 (VEGFR1) that binds to the proangiogenic

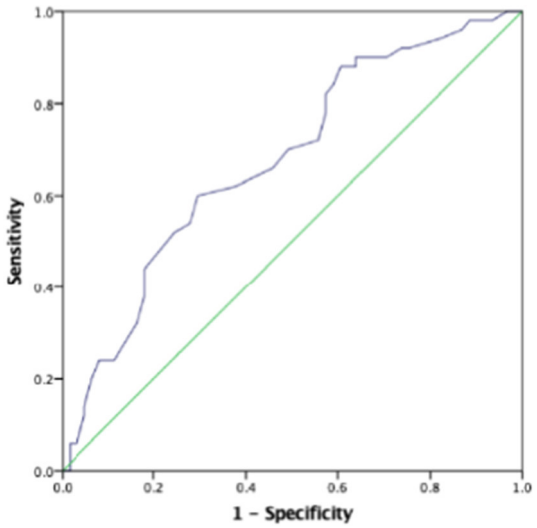

FIGURE 1 | The role of prenatal first systolic blood pressure (SBP) in predicting low birth weight. The receiver operating characteristic (ROC) curve illustrates prenatal first SBP. Areas under the curves (AUCs) are 0.676 (95% *CI* 0.618–0.733, $p < 0.001$) for the prenatal first SBP. A cut-off valuable of 121.5 mmHg yielded good sensitivity and specificity. The sensitivity and specificity are 88 and 60.7%, respectively.

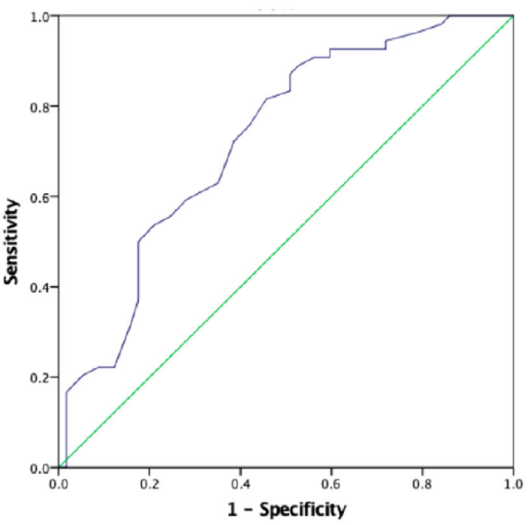

FIGURE 2 | The role of prenatal first SBP in predicting severe preeclampsia. The ROC curve illustrates prenatal first SBP. AUCs are 0.727 (95% *CI* 0.673–0.781, $p < 0.001$) for the prenatal first SBP. A cut-off valuable of 123.0 mmHg yielded good sensitivity and specificity. The sensitivity and specificity are 88.9 and 52.6%, respectively.

proteins VEGF and placental growth factor (PIGF) and mediates angiogenic signaling *via* cell surface receptors (10, 17). Patients with higher levels of sFLT1 may have more serious diseases and worse clinical prognoses (15, 18–20). NO is an effective vasodilator that mediates the effects of PIGF and VEGF *in vitro* (21–23). The circulating NO level of women with preeclampsia decreases may be relatively insufficient in the early stages of pregnancy (24–26). Hypertension in patients with preeclampsia is not mediated by the renin-angiotensin-aldosterone system. However, it may be mediated by antiangiogenic factors and agonistic autoantibodies that bind to the angiotensin II type 1 receptor (AT1-AAs) (21, 27). This antibody may exist in women with early preeclampsia (28), leading to an increased risk of cardiovascular events in preeclampsia, thus progressing to severe preeclampsia. In animal experiments, AT1-AAs in pregnant mice can upregulate sFLT1 and induce fetal growth restriction (29). In this study, among the patients with preeclampsia in the early stage of pregnancy, the birth weight in the group with an SBP $\geq$ 131 mmHg was significantly lower than that in the group with an SBP $\leq$ 119 mmHg, and AT1-AAs may appear in the early stage of pregnancy and lead to fetal growth retardation. Therefore, in the early stage of pregnancy in patients with preeclampsia, even if the blood pressure of the patient is within the normal range, patients with lower SBP have a higher birth weight, longer gestational weeks, and lower probability of severe preeclampsia.

Gestational age is closely related to birth weight. A study shows that patients with preeclampsia can predict birth weight and heavily influence perinatal survival. With the advancing gestational age, the different impacts on birth weight between patients with preeclampsia and healthy pregnant women become small (30). In this study, gestational age in patients with preeclampsia was close to the birth age. Logistic regression analysis showed that gestational age was an independent risk factor for low birth weight. In addition, we found that SBP in the early stages of pregnancy has a relationship with gestational age. The lower the systolic blood pressure in the early-stage pregnancy, the longer the gestational age they have. Therefore, monitoring the blood pressure in the early stages of pregnancy may predict the gestational age and low birth weight in patients with preeclampsia.

This study has some limitations. First, because AT1-AAs can upregulate the sFLT1 gene and induce maternal endothelial cell damage and fetal growth retardation, the levels of AT1-AAs and sFLT1 were not detected in the early stage of pregnancy to further prove the relationship between SBP and preeclampsia. Second, the population included in this study was patients with preeclampsia, and there were no healthy mothers as controls. It is not clear whether SBP in the early stages of pregnancy can predict adverse outcomes, such as preeclampsia. Finally, this was a retrospective observational study. The corrected confounding factors were limited to the current recognized and measured data. Therefore, we need to follow up with these patients to observe whether SBP in early pregnancy can predict maternal prognosis and complications.

In conclusion, high SBP in the early stages of pregnancy in patients with preeclampsia is closely related to the occurrence of severe preeclampsia and low birth weight. Even after adjusting for risk factors, SBP in the early stage of pregnancy is an independent risk factor for predicting the occurrence of severe preeclampsia and low birth weight. Closely monitoring pregnant women with high SBP in early pregnancy may reduce the incidence of severe preeclampsia, reduce the incidence of

newborns with low birth weight, and improve the survival rate of newborns.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee on human of the First Affiliated Hospital of Fujian Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BG wrote the manuscript, conceived the study, and participated in its design. XW made the statistics and enrolled the patients. JL and LL planed and supervised the study. XW and XL collected and enrolled patients. All authors read and approved the final manuscript.

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REFERENCES

1. Ghulmiyyah L, Sibai B. Maternal mortality from preeclampsia/eclampsia. *Semin Perinatol.* (2012) 36:56–9. doi: 10.1053/j.semperi.2011.09.011
2. Lo JO, Mission JF, Caughey AB. Hypertensive disease of pregnancy and maternal mortality. *Curr Opin Obstet Gynecol.* (2013) 25:124–32. doi: 10.1097/GCO.0b013e32835e0ef5
3. Hypertension in Pregnancy. Report on the American College of Obstetricians and Gynecologists' task force on hypertension in pregnancy. *Obstet Gynecol.* (2013) 122:1122–31. doi: 10.1097/01.AOG.0000437382.03963.88
4. Cindrova-Davies Tissot van patot M, Gardner L, Jauniaux E, Burton GJ, Charnock-jones DS. Energy status and HIF signalling in chorionic villi show no evidence of hypoxic stress during human early placental development. *Mol Hum Reprod.* (2015) 21:296–308. doi: 10.1093/molehr/gau105
5. Germain AM, Romanik M C, Guerra I, Solari S, Reyes MS, Johnson RJ, et al. Endothelial dysfunction: a link among preeclampsia, recurrent pregnancy loss, and future cardiovascular events? *Hypertension.* (2007) 49:90–5. doi: 10.1161/01.HYP.0000251522.18094.d4
6. Zhang J, Meikle S, Trumble A. Severe maternal morbidity associated with hypertensive disorders in pregnancy in the United States. *Hypertens Pregnancy.* (2003) 22:203–12. doi: 10.1081/PRG-120021066
7. Zhou Y, Fisher SJ, Janatpour M, Genbacev O, Dejana E, Wheelock M, et al. Human cyotrophoblasts adopt a vascular phenotype as they differentiate. a strategy for successful endovascular invasion? *J Clin Invest.* (1997) 99:2139–51. doi: 10.1172/JCI119387
8. Zhou Y, Damsky CH, Fisher SJ. Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome?*J Clin Invest.* (1997) 99:2152–64. doi: 10.1172/JCI119388
9. Karumanchi SA. Angiogenic factors in preeclampsia: from diagnosis to therapy. *Hypertension.* (2016) 67:1072–9. doi: 10.1161/HYPERTENSIONAHA.116.06421
10. Powe CE, Levine RJ, Karumanchi SA. Preeclampsia, a disease of the maternal endothelium: the role of antiangiogenic factors and implications for later cardiovascular disease. *Circulation.* (2011) 123:2856–69. doi: 10.1161/CIRCULATIONAHA.109.853127
11. Ahmad S, Ahmed A. Elevated placental soluble vascular endothelial growth factor receptor-1 inhibits angiogenesis in preeclampsia. *Circ Res.* (2004) 95:884–91. doi: 10.1161/01.RES.0000147365.86159.f5
12. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, et al. Excess placental soluble fms-tyrosine kinase 1(sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest.* (2003) 111:649–58. doi: 10.1172/JCI17189
13. Venkatesha S, Toporsian M, Lam C, Hanai JI, Mammoto T, Kim YM, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med.* (2006) 12:642–9. doi: 10.1038/nm1429
14. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med.* (2004) 350:672–83. doi: 10.1056/NEJMoa031884
15. Levint RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med.* (2006) 355:992–1005. doi: 10.1056/NEJMoa055352
16. Noori M, Donald AE, Angelakopoulou A, Hingorani AD, Williams DJ. Prospective study of placental angiogenic factors and maternal vascular function before and after preeclampsia and gestational hypertension. *Circulation.* (2010) 122:478–87. doi: 10.1161/CIRCULATIONAHA.109.895458
17. Sharon M, Franklin HE,S Ananth K. Preeclampsia and angiogenic imbalance. *Annu Rev Med.* (2008) 59:61–78. doi: 10.1146/annurev.med.59.110106.214058
18. Fangxian L, Monica L, Esther T, William M, Ayman AH, Garland DA, et al. The effect of over-expression of sFlt-1 on blood pressure and the occurrence of other manifestations of preeclampsia in unrestrained conscious pregnant mice. *Am J Obstet Gynecol.* (2007) 196:396e1–7. doi: 10.1016/j.ajog.2006.12.024
19. Zhihe L, Ying Z, Jing YM, Ann MK, Qiming S, Irene K, et al. Recombinant vascular endothelial growth factor 121 attenuates hypertension and improves kidney damage in a rat model of preeclampsia. *Hypertension.* (2007) 50:686–92. doi: 10.1161/HYPERTENSIONAHA.107.092098
20. Gabor S, Roberto R, Tinnakorn C, Yi X, Bing W, Hyunyoung A, et al. Full-length human placental sFlt-1-e15a isform induces distince maternal phenotypes of preeclampsia in mice. *PLoS One.* (2015) 10:e0119547. doi: 10.1371/journal.pone.0119547
21. Burke SD, Zsengeller ZK, Khankin EV, Lo AS, Rajakumar A, Dupont JJ, et al. Soluble fms-like tyrosine 1 promotes angiotensin II sensitivity in preeclampsia. *J Clin Invest.* (2016) 126:2561–74. doi: 10.1172/JCI83918
22. Osol G, Celia G, Gokina N, Barron C, Chien E, Mandala M, et al. Placental growth factor is a potent vasodilator of rat and human resistance arteries. *Am J Physiol Heart Circ Physiol.* (2008) 294:H1381–7. doi: 10.1152/ajpheart.00922.2007
23. Zhang HH, Chen JC, Sheibani,L, Lechuga TJ, Chen DB. Pregnancy augments VEGF-stimulated *in vitro* angiogenesis and vasodilator (NO and H2S)

production in human uterine artery endothelial cells. *J Clin Endocrinol Metab.* (2017) 102:2382–93. doi: 10.1210/jc.2017-00437
24. Pimentel AM, Pereira NR, Costa CA, Mann GE, Cordeiro VSC, Moura RS, et al. L-Arginine-nitric oxide pathway and oxidative stress in plasma and platelets of patients with pre-eclampsia. *Hypertens Res.* (2013) 36:783–8. doi: 10.1038/hr.2013.34
25. Sandrim VC, Palei ACT, Metzger IF, Gomes VA, Cavalli RC, Tanus-Santos-JE. Nitric oxide formation is inversely related to serum levels of antiangiogenic factors soluble fms-like tyrosin kinase-1 and soluble endogline in preeclampsia. *Hypertension.* (2008) 52:402–7. doi: 10.1161/HYPERTENSIONAHA.108.115006
26. Zeng Y, Li M, Chen Y, Wang S. Homocysteine, endothelin-1 and niteic oxide in patients with hypertensive disorders complicating pregnancy. *Int Clin Exp Pathol.* (2015) 8:15275–9.
27. Verdonk K, Vissern W, Van Den Meiracker AH, Danser AH. The renin-angiotensin-aldosterone system in pre-eclampsia: the delicate balance between good and bad. *Clin Sci.* (2014) 126:537–44. doi: 10.1042/CS20130455
28. Wallukat G, Homuth V, Fisher T, Lindschau C, Horstkamp B, Jupner A, et al. Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor. *J Clin Invest.* (1999) 103:945–52. doi: 10.1172/JCI4106
29. Xia Y, Kellems RE. Angiotensin receptor agonistic autoantibodies and hypertension: preeclampsia and beyond. *Circ Res.* (2013) 113:78–87. doi: 10.1161/CIRCRESAHA.113.300752
30. Annettee N, Jennifer ES, Sarah N, Imelda N, Musa S, Eve N, et al. Relative impact of pre-eclampsia on birth weight in a low resource setting: a prospective cohort study. *Pregnancy Hypertesion.* (2020) 21:1–6. doi: 10.1016/j.preghy.2020.04.002

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CD39/CD73 Dysregulation of Adenosine Metabolism Increases Decidual Natural Killer Cell Cytotoxicity: Implications in Unexplained Recurrent Spontaneous Abortion

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Unexplained recurrent spontaneous abortion (URSA) is believed to be associated with impaired immunosuppression at the maternal-fetal interface, but the detailed molecular mechanism remains unclear. The ATP-adenosine metabolic pathway regulated by CD39/CD73 has recently been recognized to be important in immunosuppression. This study aimed to investigate the regulation of decidual natural killer (dNK) cells and fetal extravillous trophoblast (EVT) cells by CD39 and CD73 in URSA, as well as the possible regulatory mechanism of CD39/CD73 *via* the TGF-β-mTOR-HIF-1α pathway using clinical samples and cell models. Fewer $CD39^+$ and $CD73^+$ cells were found in the URSA decidual and villous tissue, respectively. Inhibition of CD39 on dNK cells transformed the cells to an activated state with increased toxicity and decreased apoptosis, and changed their cytokine secretion, leading to impaired invasion and proliferation of the co-cultured HTR8/SVneo cells. Similarly, inhibition of CD73 on HTR8/SVneo cells decreased the adenosine concentration in the cell culture media, increased the proportion of $CD107a^+$ dNK cells, and decreased the invasion and proliferation capabilities of the HTR8/SVneo cells. In addition, transforming growth factor-β (TGF-β) triggered phosphorylation of mammalian

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target of rapamycin (mTOR) and Smad2/Smad3, which subsequently activated hypoxia-inducible factor-1α (HIF-1α) to induce the CD73 expression on the HTR8/SVneo cells. In summary, reduced numbers of $CD39^+$ and $CD73^+$ cells at the maternal-fetal interface, which may be due to downregulated TGF-β-mTOR-HIF-1α pathway, results in reduced ATP-adenosine metabolism and increased dNK cytotoxicity, and potentially contributes to URSA occurrences.

Keywords: URSA, dNK, CD39, CD73, adenosine, TGF-β

INTRODUCTION

Unexplained recurrent spontaneous abortion (URSA) is defined as two or more consecutive spontaneous abortions (1, 2). The mechanism remains unclear, but is thought to be associated with immune intolerance. During early pregnancy in humans, the fetal extravillous trophoblast (EVT) cells invade into decidua and remodel the uterine spiral arteries (3). Decidua consists of epithelial cells and immune cells. Successful pregnancy requires a unique immune tolerant environment where delicate and complex crosstalk between the fetal-derived EVT cells and the maternal-derived decidual cells takes place (4).

Natural killer (NK) cells, a population of innate lymphoid cells, can lyse cancer cells and virus-infected cells. They play an important role in controlling the adaptive immune response by producing pro-inflammatory and anti-inflammatory cytokines (5, 6). Decidual natural killer (dNK) cells are a special type of NK cells, which constitutes 70% of immune cells in the decidua (7). The other 30% include macrophages, dendritic cells, and T cells (8). dNK cells are different from peripheral blood NK (pNK) cells, in that they are identified as $CD56^{bright}CD16^-$, while the pNK cells are mainly (95%) $CD56^{dim}CD16^+$ (7). The origin of dNK cells remains uncertain, but it is possible that they are from a group of minor agranular $CD56^{bright}CD16^-$ NK cells in the blood, which migrate into the uterus and transform (8). The role of dNK cells in the adaptive immune response, particularly the production of proinflammatory and anti-inflammatory cytokines, has been characterized (5, 6). It has been reported that dNK cells exhibit lower cytotoxicity, but higher secretion potential, than pNK cells in physiological settings (9–11). The dNK cells express a variety of surface receptors, such as NKp44 (CD336), NKp30 (CD337) and NKG2D (CD314) (1, 2). When these receptors are activated, an array of cytokines and growth factors that regulate the immune tolerance and angiogenesis at the maternal-fetal interface are produced, including GM-CSF, TNF-α, IFN-γ, IL-10, IL-8, IL-2, interferon-inducible protein-10 (IP-10), hepatocyte growth factor (HGF), PLGF, CCL-3, CCL-4 and several vascular endothelial growth factor (VEGF) family members (2, 9).

Abbreviations: URSA, unexplained recurrent spontaneous abortion; dNK, decidual natural killer; pNK, peripheral blood natural killer; EVT, extravillous trophoblast; TGF-β, transforming growth factor-β; mTOR, mammalian target of rapamycin; HIF-1α, hypoxia-inducible factor-1α.

Adenosine and its phosphates ADP and ATP mediate several functions such as inflammation *via* binding to purine receptors on cell surfaces (12). Extracellular ATP and ADP concentrations are regulated by CD39 (NTPDase1) and CD73. CD39 is expressed on the extracellular surface of endothelial cells, particularly in human vascular and placental trophoblastic tissues (13). It is also expressed on the surface of certain immune cells such as neutrophils, monocytes, natural killer cells, and some subsets of T and B lymphocytes, where it inactivates nucleotides (12). CD39 hydrolyses ATP and ADP to produce AMP, and the membrane-bound ecto-5'-nucleotidase CD73 further hydrolyses AMP to produce adenosine (14, 15). Extracellular adenosine regulates the immune function of T lymphocytes (16–18), B lymphocytes (19–21) and NK cells (21–24) *via* binding to four different G protein–coupled purinergic receptors A1, A2A, A2B and A3. Thus, CD39 and CD73 can change pro-inflammatory immune cells driven by ATP to anti-inflammatory ones induced by adenosine (25). It has been demonstrated that CD39 and CD73 can mediate the growth and metastasis of tumor cells (24, 26–31). The adenosine effect mediated by CD39 and CD73 is considered one of the most important immunosuppressive regulatory pathways in the tumor microenvironment. Upregulated CD39/CD73 has been reported in a large number of solid cancer studies, and displays correlation with poor prognosis (32–35). Studies have shown that tumor cells and Treg cells co-express CD73 and CD39 and produce extracellular adenosine (36, 37). However, the effects of CD39 and CD73 on dNK and EVT cells, which are the main immune cells at the maternal-fetal interface maintaining immune tolerance, and the key cells for the remodeling of spiral arteries, respectively, have been seldom studied.

TGF-β is abundantly expressed in the endometrium and promotes its decidualization (38). It regulates the immunosuppression and immunoactivation balance *via* acting on type I (TbRI) and type II (TbRII) receptors that phosphorylate the downstream signal transducers Smad2 and Smad3 (39, 40). It also regulates the homeostasis of NK cells and inhibits their cytokine production and cytolytic activity (41, 42). TGF-β facilitates the transition of $CD16^+$ pNK cells to $CD16^-$ cells and inhibits the development and differentiation of human NK cells (43). It also transforms pNK cells into noncytotoxic and proangiogenic NK cells, a cell type similar to dNK cells, with the presence of hypoxia and a demethylating agent (34). In tumors, TGF-β signaling has been found to induce the generation of CD39/CD73 myeloid cells (44). However, dNK cells exist in a different microenvironment

from pNK cells and exhibit a different phenotype, and it remains unknown whether TGF-β regulates CD39 and CD73 expression on dNK and EVT cells, respectively, at the maternal-fetal interface.

This study aimed to investigate the role of CD39/CD73 in the crosstalk between dNK and EVT cells at the maternal-fetal interface, and its relationship with URSA. We first measured CD39 and CD73 levels at the maternal-fetal interface of URSA and normal tissues, and then investigated the impact of CD39 and CD73 on the adenosine production and functions of dNK and EVT (or HTR8/SVneo) cells. Furthermore, the impact of TGF-β on the CD73 expression in HTR8/SVneo cells was explored to find a possible cause for the CD39/CD73 imbalance.

MATERIALS AND METHODS

Clinical Sample Collection

Fresh decidual and villous samples were obtained at the First Affiliated Hospital of Chongqing Medical University from women with voluntary terminations of pregnancy (n = 30) or unexplained recurrent spontaneous abortions (URSA, n = 12). Abortions with genetic abnormalities detected by chorionic villus sampling or anatomical examination were excluded. Before the operation, informed consent from each participant was obtained. The study conformed to the Ethical Review Methods for Biomedical Research involving Humans adopted by the National Health Commission of the People's Republic of China. All the samples (**Table S1**) were put in ice-cold phosphate buffer saline (PBS) in sterile containers after collection, and immediately transferred to the laboratory.

Cell Lines

The human trophoblast HTR-8/SVneo cell line was purchased from the American Type Culture Collection (ATCC, USA), and cultured in Gibco™ RPMI 1640 medium (Thermo Fisher Scientific, USA) with L-glutamine, 10% fetal bovine serum (FBS, PAN-Biotech, Germany) and 1% penicillin-streptomycin. The K562 cells were purchased from the National Infrastructure of Cell Line Resource of China and cultured in the same medium. All the cells were grown in standard culture conditions (37°C and 5% CO_2 in humidified air).

Cell Preparation and Purification

The decidual tissue was first washed thoroughly with cold PBS, cut into pieces of about 1–3 mm, and digested by gentle shaking with 0.1% collagenase type IV (Catalog#: C5138, Millipore Sigma, USA) and 0.01% DNase I (Catalog#: 10104159001, Millipore Sigma, USA) for 1 h at 37°C. Afterwards, the mixture was sequentially filtered through nylon meshes of decreasing pore sizes (100, 200 and 400 mesh). The decidual mononuclear cells in the final filtrate were then concentrated by density gradient centrifugation (1000g, 20 min) with ficoll (Catalog#: 17144002, GE Healthcare, Sweden). After centrifugation, the mononuclear cells were collected, washed, and counted. The dNK cells from the decidual mononuclear cells were enriched using the human $CD56^+CD16^-$ NK Cell Isolation Kit (Catalog#: 130-092-661, Miltenyi Biotec, Germany) according to the manufacturer's instructions. The isolated dNK cells were cultured in RPMI 1640 medium containing recombinant human IL-15 (10 ng/mL, Catalog#:I8648, Sigma), and then immediately underwent various analyses such as flow cytometry.

Flow Cytometry Assays

The dNK cells were cultured in 6-well plates (5×10^5 cells/well) for 24 hours. The mononuclear cells were resuspended in staining buffer, and then immediately stained with a range of monoclonal antibodies, namely anti-CD56 TULY56 FITC (Catalog#: 11-0566-42, eBioscience, USA), anti-CD16 PE (Catalog#: 12-0168-42, eBioscience, USA), anti-CD39 APC (Catalog#: 17-0399-41, eBioscience, USA), anti-CD107 APC H4A3 (Catalog#: 560664, BD Pharmingen, USA), anti-CD314 PE (Catalog#: 557940, BD Pharmingen, USA), anti-CD336 PE (Catalog#: 558563, BD Pharmingen, USA), anti-CD337 PE (Catalog#: 558407, BD Pharmingen, USA), anti-CD73 PE (Catalog#: 550257, BD Pharmingen, USA), anti-Annexin V FITC (Catalog#: 556419, BD Pharmingen, USA), anti-DAPI (Catalog#: 564907, BD Pharmingen, USA) and anti-CD45 APCCY7 (Catalog#: 348805, BD Pharmingen, USA). After incubation at room temperature for 30 min, the cells were then washed and resuspended in PBS for flow cytometry analysis (CytoFLEX, eBioscience, USA). The strategy for multidimensional flow cytometry analysis is shown in the **Supplementary Material, Figure S1**.

ELISA

The concentrations of ATP (Catalog#: 14432H1, MEIMIAN) and adenosine (Catalog#: 1913H1, MEIMIAN) in the culture medium were determined with enzyme-linked immunosorbent assay (ELISA) Kits. The dNK cells were cultured in 24-well plates (2×10^5 cells/well) for 24 hours. The culture medium was collected and centrifuged, and the supernatant was stored at –80°C until assayed according to the manufacturer's protocols.

Luminex Assay

The isolated dNK cells were cultured in 24-well plates (2×10^5 cells/well) for 24 hours, and the medium was collected. A Luminex X200 System (Luminex, USA) was used to measure the cytokine levels in the medium. The cytokines TNF-α, IL-10, IL-4, IL-6, IL-8, IP-10, IL-5, IL-13, IFN-γ, IL-17, IL-1, IL-27, HGF, GM-CSF and Galectin-1were determined using human luminex discovery assays (LXSAHM-15). The concentrations were calculated based on the mean fluorescent intensity (MFI). Standard curves were generated for reference cytokines and used to calculate cytokine concentrations in the medium.

Western Blot

The proteins in the villous and decidual tissues as well as the cultured cells were extracted using the RIPA lysis buffer (Catalog#: P0013B, Beyotime Biotechnology, China) with PMSF (Catalog#: ST506, 1mM, Beyotime Biotechnology, China). After separation with 10% SDS-PAGE, the proteins were transferred to a PVDF membrane. The membranes were

blocked with TBST (TBS+Tween) containing 5% skimmed milk powder for 1 hour, and then incubated with the corresponding primary rabbit polyclonal antibodies in Primary Antibody Dilution Buffer (Catalog#: P0256, Beyotime Biotechnology, China) at 4°C overnight. The antibodies were CD39 (1:1000, Catalog#: ab223842, Abcam, UK), CD73 (1:1000, Catalog#: ab175396, Abcam, UK), TGF-β (1:2000, Catalog#: ab27969, Abcam, UK), Phospho-mTOR (1:1000, Catalog#: 5536, Cell Signaling Technology, USA), Phospho-Smad2 (1:1000, Catalog#: 3108, Cell Signaling Technology, USA), Phospho-Smad3 (1:1000, Catalog#: 9520, Cell Signaling Technology, USA), HIF-1α (1:1000, Catalog#: 36169, Cell Signaling Technology, USA) and β-actin (1:5000, Catalog#: GB11001, Servicebio, China). Then, the membranes were washed and incubated with HRP-conjugated secondary rabbit antibodies (1:10,000; Proteintech) at room temperature for 2 hours. Band signals were visualized and analyzed with enhanced chemiluminescent reagent (Millipore Sigma) and a Vilber Fusion image system (Fusion FX5 Spectra, France).

RT-PCR

Total RNA was extracted from HTR8/SVneo cells using Invitrogen™ TRIzol reagent (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. cDNA was synthesized using Roche Reverse Transcription Kit (Catalog#: 07912455001, Roche), and qPCR was performed using the SYBR Premix Ex Taq (TaKaRa Biomedical Technology, China) with a LightCycler™ 96 instrument (Roche, Switzerland). GAPDH was used as the internal gene control. Its primer pair was: forward: 5′CAGGAGGCATTGCTGATGAT3′, reverse: 5′ GAAGGCTGGGGCTCATTTT3′. The primer pairs of the tested genes were as follows: CD73 forward: 5′ACCAGCGAGGACTC CAGCAAG3′, reverse: 5′AGCAGCAGCACGTTGGGTTC3′, HIF-1α forward: 5′ATCAGACACCTAGTCCTTCCGATGG3′, reverse: 5′GTGGTAGTGGTGGCATTAGCAGTAG3′.

Immunohistochemistry

The villous and decidual tissues were first cut into 4 μm-thick slices. Immunohistochemical staining was then performed according to standard procedures (45). Primary antibodies for TGF-β (1:200, Catalog#: ab27969, Abcam), cytokeratin 7 (1:200, Catalog#: ET1609-62, HUABIO), CD39(1:200, Catalog#: ab223842, Abcam), CD73 (1:200, Catalog#: ab175396, Abcam), or NCAM (1:200, Catalog#: ET1702-43, HUABIO) was added and incubated overnight. The antibodies were quantified with 3,3'-diaminobenzidine (DAB) staining (ZSGB Biotech, China). ImageJ software was used to measure the average optical density (AOD) of positive signal in each view field.

Cell Invasion Assay

Transwell™ inserts (8 μm) containing polycarbonate membranes (Catalog#: 3428, Corning Incorporated, USA) were coated with an 8× dilution of Matrigel (Catalog#: 356234, Corning Incorporated, USA). HTR8/SVneo cells (5×10^4) were seeded into the upper chamber with serum-free culture medium and the isolated dNK cells (1×10^5) were placed in the lower chamber with culture medium supplemented with 10% FBS, and incubated for 24 hours. The cells were stained with crystal violet and observed with light microscopy (EVOS FL Auto Imaging System, Thermo Fisher Scientific, USA). The invasion rate was measured with the ImageJ software.

Cell Proliferation Assay and Measurement of Mitochondrial Membrane Potential

Approximately 5×10^3 HTR8/SVneo cells and 1×10^4 isolated dNK cells were co-cultured in the central part of each well in a 96-well plate for 24 hours. Then, the suspended dNK cells were discarded and fresh culture medium was added. After 24-hour incubation, 10 μL of CCK-8 solution was added into each well, and the plates were incubated for another 2 hours. A microplate reader (Thermo Fisher Scientific, USA) was used to measure the absorbance at 450 nm of each well.

Mitochondrial membrane potential (ΔΨm) is an important parameter of mitochondrial function (46, 47). Loss of ΔΨm is a sign of early apoptosis, and hence was also measured in dNK cells using the cationic probe JC-1 (Catalog#: C2006, Beyotime Biotechnology, China) according to the manufacturer's instructions. In short, the isolated dNK cells were cultured in 12-well plates (2×10^5 cells/well) for 12 hours. Then the cells were collected and incubated with JC-1 staining solution (5 μg/ml) at 37°C for 20 min. The cells were then washed twice with JC-1 staining buffer, and the fluorescence intensities of the mitochondrial JC-1 monomers (λex=514 nm, λem=529 nm) and aggregates (λex=585 nm, λem=590 nm) were measured with a fluorescence microscope (EVOS FL Auto Imaging System, Thermo Fisher Scientific, USA) and a flow cytometer (CytoFLEX, eBioscience, USA), respectively. The ΔΨm were calculated as the fluorescence ratio of red (i.e., aggregate) to green (i.e., monomer) signals.

Statistical Analysis

Statistical analysis was carried out with GraphPad Prism (version 8, GraphPad Software, USA). Comparisons between two groups were performed using unpaired two-tailed t tests, and those among three or more groups were using one-way analysis of variance (ANOVA). All data are presented as mean ± SEM; $P < 0.05$ was considered statistically significant.

RESULTS

Lower Levels of CD39 and CD73 Were Found in URSA Patients

To assess the expression of CD39 and CD73 in normal pregnancy and URSA patients, we performed immunohistochemical staining analysis. It was found that CD39 was present in the dNK cells that also express CD56, and CD73 was present in trophoblast cells that also express CK7 (**Figures 1A** and **S2**). The intensity values of CD39 in the decidua and those of CD73 in the villi from the URSA patients were both significantly lower than those from normal pregnancies (**Figure 1A**). Since there are multiple types of immune cells in the decidual tissue expressing CD39, we further used multicolor flow cytometry to measure the proportions of $CD39^+$ dNK cells in the

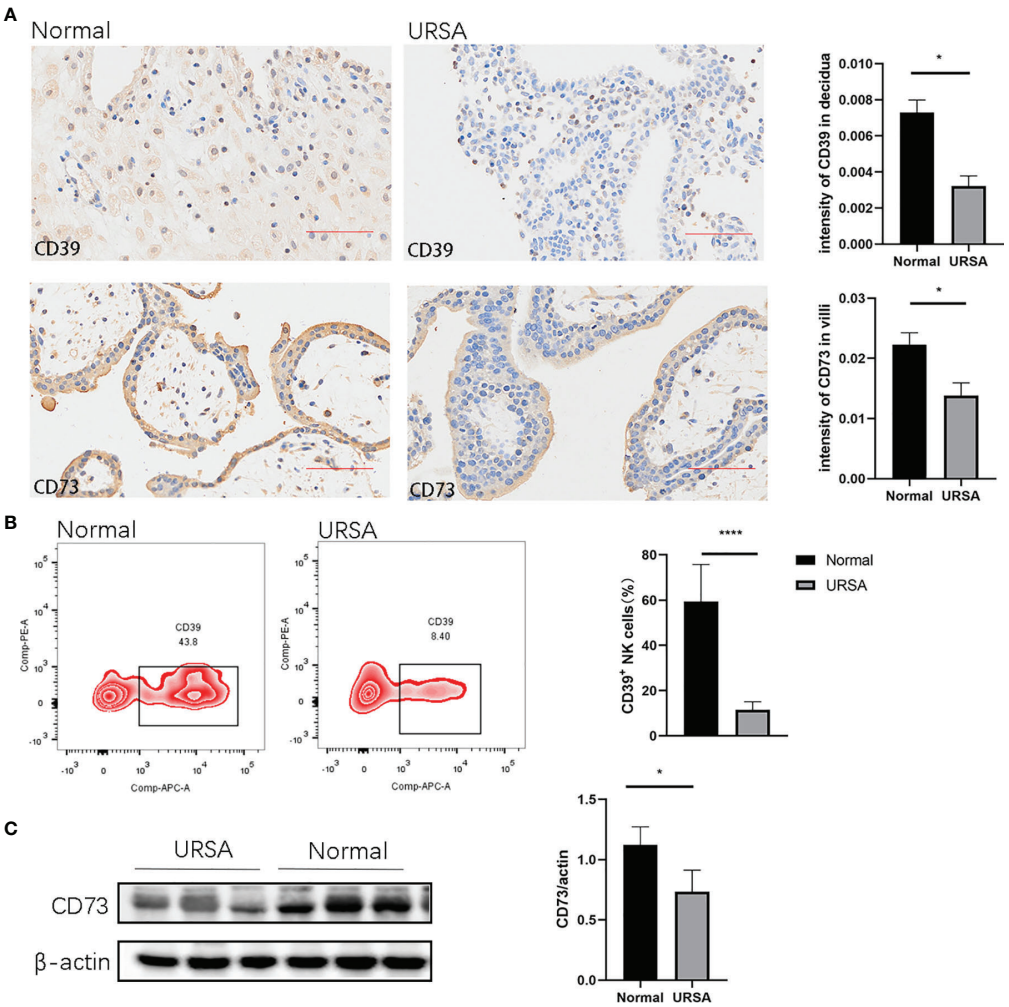

FIGURE 1 | Expression of CD39 and CD73 in normal pregnancy and URSA patients. **(A)** Immunohistochemical staining of CD39 in decidual tissues and CD73 in villous tissues in normal pregnancies (n=3) and URSA patients (n=3). **(B)** Percentages of $CD39^+$ dNK cells in the decidual tissues of normal pregnant women (n=6) and URSA patients (n=6). **(C)** Western blot analyses of CD73 in the villous tissue of normal pregnant women (n=3) and URSA patients (n=3). Results are shown as mean ± SEM, *P < 0.05, ****P < 0.0001 by two-tailed t tests. AOD, average optical density.

decidua of normal pregnancies and URSA patients. The proportions of $CD39^+$ dNK cells in URSA were significantly lower than those of normal specimens (**Figure 1B**). Western blot analysis also showed that the levels of CD73 in villous tissues of URSA patients were significantly lower than those of normal pregnancies (**Figure 1C**).

Impacts of CD39/CD73 on the Extracellular Levels of ATP and Adenosine

We first isolated dNK cells from the decidual tissue of normal pregnant women and URSA patients, and cultured them *in vitro* for 24 hours. The concentrations of ATP and adenosine in the media were determined using ELISA. The concentration of ATP was found to be significantly higher, while that of adenosine was significantly lower in the URSA medium, indicating a hampered metabolism from ATP to adenosine, which was catalyzed by CD39 on dNK cells from the URSA patients (**Figure 2A**).

We then further analyzed whether the abovementioned effects were related to CD39 of the dNK cells by adding the CD39 inhibitor ARL67156 into the media of dNK cells from normal pregnancies. Isolated dNK cells were cultured in the absence or presence of the CD39 inhibitor for 24 hours. Results showed the CD39 inhibitor increased the concentration of ATP and decreased that of adenosine (**Figure 2B**).

Additionally, we investigated the CD39/CD73 regulation on ATP and adenosine production at the maternal-fetal interface by co-culturing dNK and HTR8/SVneo cells, with or without ARL67156 and the CD73 inhibitor APCP. In this co-culture system, both individual and combinational addition of ARL67156 and APCP significantly attenuated adenosine production and increased ATP concentration (**Figure 2C**).

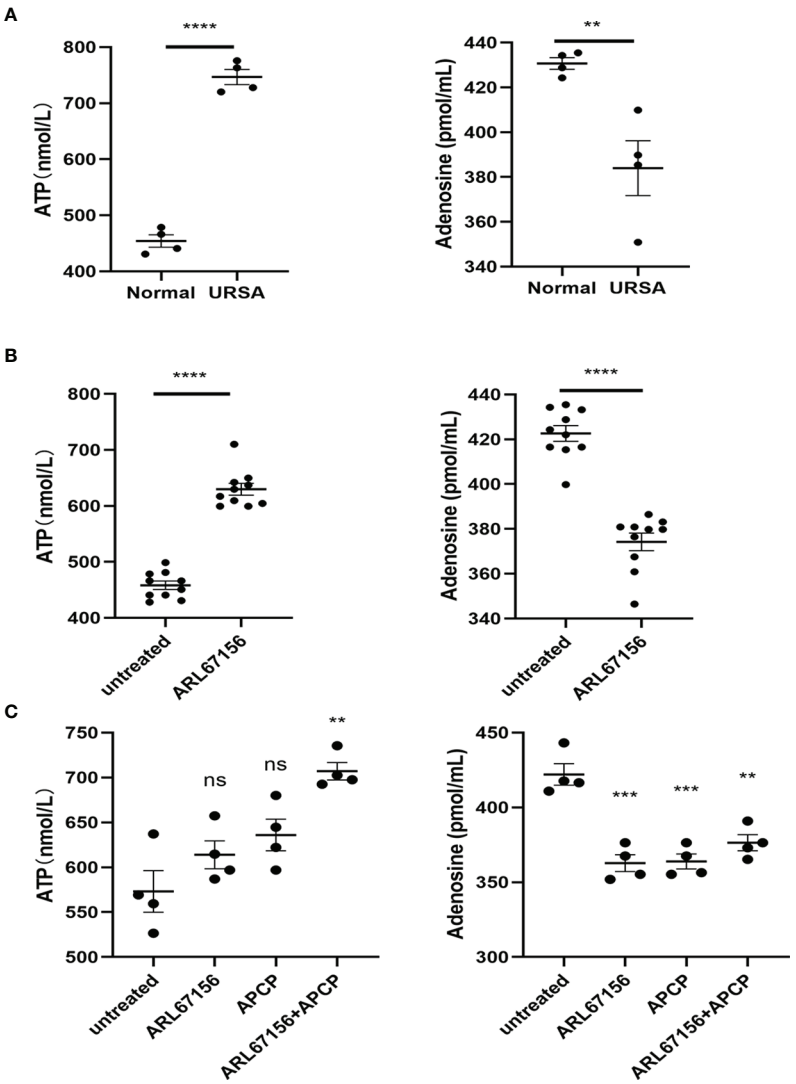

FIGURE 2 | Production of ATP and adenosine in dNK cells. **(A)** dNK cells were isolated from decidual tissues of normal pregnant women (n=4) and URSA patients (n=4), and then cultured in a 24-well plate (2×10^5 cells/well) for 24 hours. The concentrations of ATP and adenosine in the media were measured using ELISA (mean ± SEM, ****P < 0.0001, two-tailed t tests). **(B)** Freshly prepared dNK cells (n=10) were isolated from healthy decidua and incubated in the absence or presence of CD39 inhibitor (ARL67156) in a 24-well plate (2×10^5 cells/well) for 24 hours. The concentrations of ATP and adenosine in the media were measured using ELISA (mean ± SEM, **P < 0.01, ****P < 0.0001, two-tailed t tests). **(C)** dNK cells isolated from healthy decidua (n=4) were co-cultured with HTR-8/SVneo cells (dNK cells:HTR-8/SVneo cells = 2:1) and incubated in the absence or presence of the CD39 inhibitor ARL67156 and/or the CD73 inhibitor APCP in a 24-well plate (2×10^5 cells/well) for 24h. The concentrations of ATP and adenosine in the media were measured using ELISA (mean ± SEM, ns, statistically not significant, **P < 0.01, ***P < 0.001 compared with the untreated group, one-way ANOVA and *post hoc* tests).

Impacts of CD39/CD73 on the Apoptosis and Mitochondrial Function of Activated dNK Cells

We investigated the effect of CD39 on dNK cell apoptosis by culturing the activated dNK cells with or without the CD39 inhibitor ARL67156 for 12 or 18 hours, followed by staining with Annexin V and DAPI. The percentages of Annexin V^+ $DAPI^+$ dNK cells cultured with CD39 inhibitors were found to be lower than those untreated (**Figure 3**). This indicates that CD39 promotes apoptosis of dNK cells. We further investigated the effect of CD39 on $\Delta\Psi m$ of the dNK cells, the loss of which is an indicator of dNK apoptosis. It was observed that the JC-1 red/green ratio was higher in dNK cells treated with CD39 inhibitors, suggesting reduced $\Delta\Psi m$ caused by CD39.

Impacts of CD39/CD73 on Cytokine Secretion by dNK Cells

We first evaluated whether the cytokine secretions of dNK cells from normal pregnant women and those from URSA patients were different. The dNK cells isolated from the decidual tissues

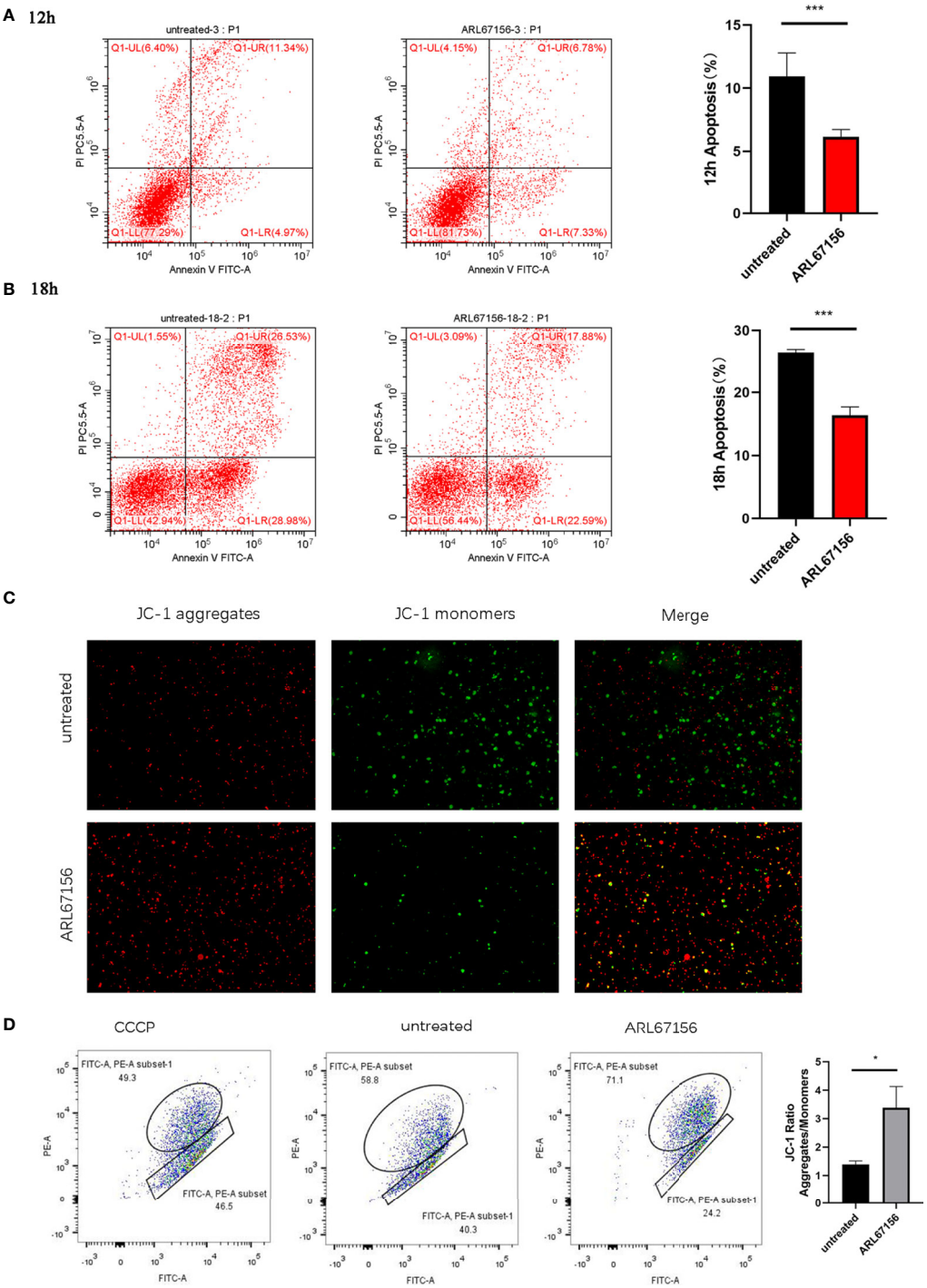

FIGURE 3 | The effect of CD39/CD73 inhibiting on the apoptosis and mitochondrial membrane potential ($\Delta\Psi$m) of dNK cells. dNK cells were isolated from healthy decidua of first-trimester pregnancies (n = 3), incubated in the absence or presence of the CD39 inhibitor ARL67156 for 12 hours **(A)** or 18 hours **(B)**, and then double stained with Annexin V and DAPI (mean ± SEM, ***P < 0.001, two-tailed t tests). **(C)** Microscope images taken from the JC-1 monomer fluorescence channel (green) and aggregate fluorescence channel (red) of dNK cells (n=5) with and without incubation with ARL67156 for 12 h. The monomeric JC-1 form was excited using a 525 nm laser, observed at an emission wavelengths of 514~529 nm, and is shown in green. The aggregate form was excited using a 566 nm laser, observed at 585~590 nm, and is shown in red. **(D)** Flow cytometry-based JC-1 assay as a measure of changes in mitochondrial membrane potential in dNK cells (n=5) induced by the CD39 inhibitor. The upper left quadrant indicates the cells with more JC-1 as aggregates with red fluorescence (i.e., normal $\Delta\Psi$m), the lower right quadrant indicates the cells with more JC-1 as monomers with green fluorescence (i.e. low $\Delta\Psi$m). (mean ± SEM, *P < 0.05 compared with the untreated group).

of both donor groups were cultured for 24 hours, and then the cytokine concentrations in the culture media were determined using a multiplex cytokine assay. The dNK cells from URSA patients secreted significantly higher amounts of GM-CSF, IL-1, IL-5 and IL-10, with a trend of more IL-13, but lower levels of IL-8 and IP-10, and a trend of less HGF, IFN-γ, IL-6, IL-17 and IL-27 than dNK cells from the normal group. There was no difference in the secretion of IL-4 and TNF-α between the two groups (**Figure 4A**).

Next, we isolated dNK cells from normal pregnancies, and cultured them in the absence or presence of the CD39 inhibitors ARL67156. The cytokines in the culture media were then analyzed. The results showed that dNK cells cultured with the CD39 inhibitor secreted significantly more IL-10, IL-4, GM-CSF, galectin-1 and IL-13, with a trend of more IL-5 than the untreated group. The secretion of TNF-α, HGF and IL-1, however, was suppressed in cells treated with the CD39 inhibitor, and IL-6, IL-8, IL-17 and IL-27 displayed a trend of decreased secretion. Little or no effect on the secretion of IP-10, IL-8 and IFN-γ was observed (**Figure 4B**).

Furthermore, we explored the influences of CD39 and CD73 on cytokine secretion in the co-culture system. Interestingly, co-cultivation of dNK and HTR-8/SVneo cells in the presence of only the CD39 inhibitor or both the CD39 and CD73 inhibitors decreased the secretion of IL-6, IL-8 and IL-4, but the CD73 inhibitor alone did not significantly affect the secretion of these cytokines. The secretion of GM-CSF and galectin-1 was significantly reduced only in the presence of both the CD39 and CD73 inhibitors. There was no significant difference in the secretion of TNF-α, IL-10, IP-10, HGF, IL-5, IL-13, IL-17, IFN-γ, IL-1 or IL-27 when the co-culture system was treated with either the CD39 or CD73 inhibitor (**Figure 4C**).

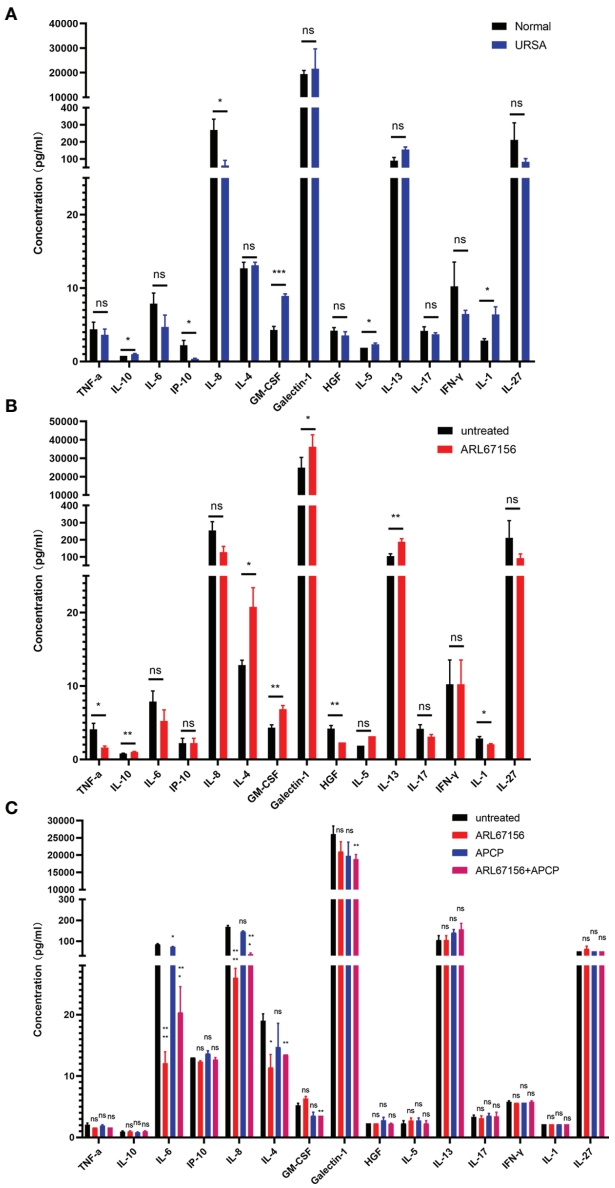

FIGURE 4 | The effect of CD39/CD73 inhibiting on the cytokine secretion of dNK cells. **(A)** dNK cells were isolated from decidual tissues of normal pregnant women (n=4) and URSA patients (n=4), and cultured in a 24-well plate (2×10^5 cells/well) for 24 hours. The cytokine concentrations in the media were determined using Luminex assay (mean ± SEM, ns, statistically not significant, *P < 0.05, ***P < 0.001, two-tailed t tests). **(B)** Cytokine concentrations in the media of dNK cells (n=5) cultured in the absence or presence of the CD39 inhibitor ARL67156 in 24-well plates for 24 hours (mean ± SEM, ns, statistically not significant, *P < 0.05, **P < 0.01, two-tailed t tests). **(C)** Cytokine concentrations in the media at 24 hours of co-culture of dNK and HTR-8/SVneo cells (n=3) (mean ± SEM, ns, statistically not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 compared with the untreated group, one-way ANOVA and *post hoc* tests).

Impacts of CD39/CD73 on the Cytotoxicity of Activated dNK Cells

We then investigated the effect of CD39 on the expression of dNK cell receptors. The expression of NKG2D, NKp30 and NKp44 on dNK cells isolated from normal pregnancies in the presence or absence of CD39 inhibitor for 24 hours was measured with flow cytometry. Increased expression of NKG2D, NKp30 and NKp44 in the dNK cells treated with the CD39 inhibitor was observed (**Figures 5A–C**).

Furthermore, flow cytometry was used to evaluate the CD107a (lysosomal associated membrane protein-1, LAMP-1) expression that reflects the cytotoxic degranulation ability of the dNK cells. The percentage of the activated dNK cells expressing CD107a was as low as 6.60% in the absence of other cells. However, after adding the NK-sensitive K562 cells, this percentage increased significantly to 15.1%. The percentage of $CD107a^+$ dNK cells further increased to 28.7% when the CD39 inhibitor was added (**Figures 5D, E**), which indicated that CD39 reduces degranulation of the dNK cells.

Additionally, the expressions of CD107a on dNK cells co-cultured with the HTR-8/SVneo cells, in the presence or absence of the CD39 and/or CD73 inhibitor was investigated. Interestingly, the percentage of $CD107a^+$ dNK cells decreased significantly to 2.83% when co-cultured with the HTR-8/SVneo cells, a result opposite to co-cultivation with the K562 cells. However, the percentage of dNK cells expressing CD107a in the co-culture system increased to approximately 5% in the presence

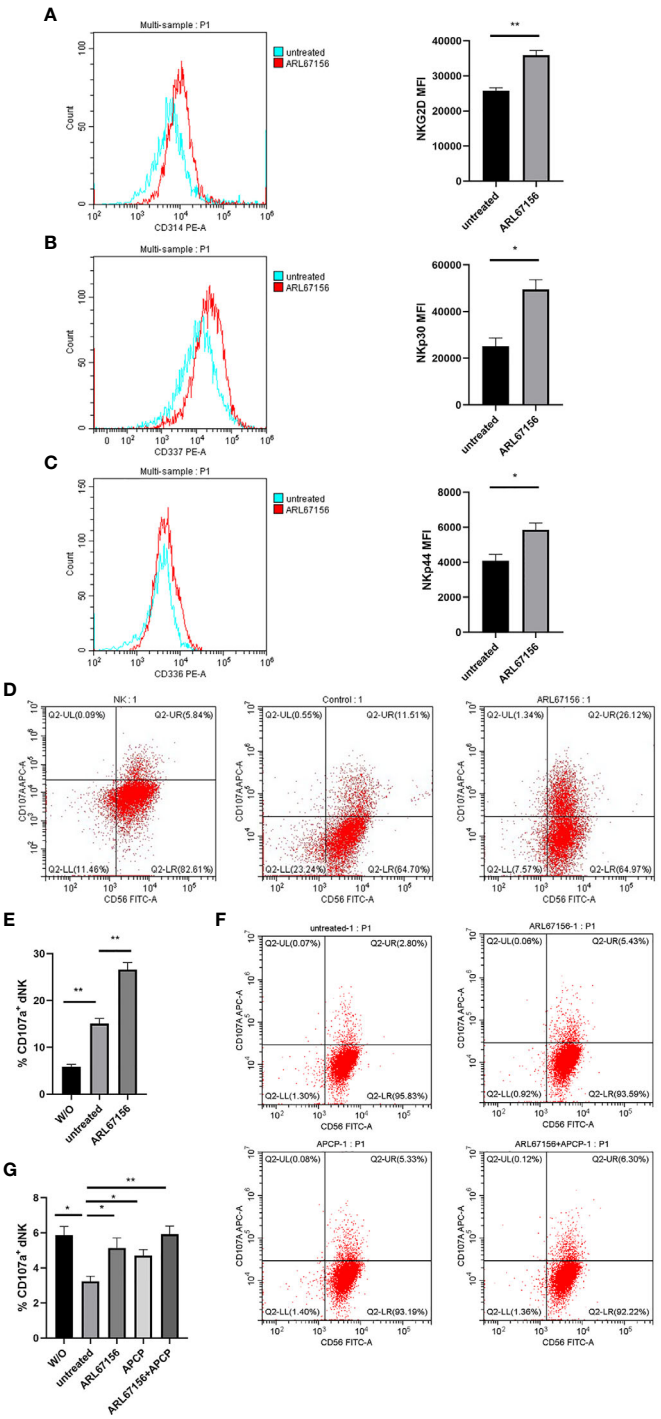

FIGURE 5 | The cytotoxicity of dNK cells was regulated by CD39/CD73 enzyme activity. **(A–C)** Freshly isolated dNK cells from healthy donors were incubated in the absence or presence of the CD39 inhibitor ARL67156 for 24 hours, and then stained for the surface expression of the cytotoxicity markers NKG2D **(A)**, NKp30 **(B)** and NKp44 **(C)**. All experiments were performed in triplicate (mean ± SEM, *P < 0.05, **P < 0.01 by two-tailed t tests). **(D, E)** Effects of CD39 on the degranulation of activated dNK cells from healthy donors. To quantify degranulation, the surface expression of CD107a was measured after activation of isolated dNK cells were incubated with or without (W/O) the NK-susceptible target cell line (K562 cells) in the absence or presence of the CD39 inhibitor ARL67156 for 24 hours (mean ± SEM, **P < 0.01 compared with the untreated group, one-way ANOVA and *post hoc* tests). **(F, G)** dNK cells isolated from healthy donors co-cultured with HTR-8/SVneo cells (dNK : HTR-8/SVneo cells = 2:1) in the absence or presence of the CD39 inhibitor ARL67156 and/or CD73 inhibitor APCP for 24 hours. The percentage of dNK cells expressing CD107a was used as the indicator of degranulation (mean ± SEM, *P < 0.05, **P < 0.01, one-way ANOVA and *post hoc* tests).

of the CD39 or CD73 inhibitor, and returned to the original value of about 6% when both CD39 and CD73 inhibitors were added (**Figures 5F, G**). These results indicated that HTR-8/SVneo cells suppress the degranulation of dNK cells, and this suppression is associated with CD39 and CD73.

Impacts of CD39/CD73 on the Invasion and Proliferation of HTR8/SVneo Cells

To evaluate changes in the invasion and proliferation abilities of the HTR8/SVneo cells, isolated dNK cells from normal pregnancies were co-cultured with HTR8/SVneo cells in the absence or presence of the CD39 inhibitor for 24 hours, and the matrigel transwell assay and CCK-8 assay were performed, respectively. It was found that the invasion of the HTR8/SVneo cells in the groups with CD39 or/and CD73 inhibitor was significantly less than that of the untreated group (**Figures 6A, B**). Compared with the untreated group, cell proliferation rates also fell in the CD39 or CD73 inhibitor groups, and the decrease was even more in the group exposed to both inhibitors (**Figure 6C**).

TGF-β Induces the Expression of CD73 on HTR-8/SVneo Cells *via* the mTOR-HIF-1α Pathway

We then explored the potential mechanism for the CD73 downregulation in URSA. Compared with the samples from normal pregnancies, the level of TGF-β in the decidual and villous tissues was significantly lower in the URSA pregnancies, as revealed by immunohistochemical staining (**Figure 7A**). Western blots showed the same result - the level of TGF-β in the villous tissue of URSA patients was significantly lower than that in tissues from normal pregnancies (**Figure 7B**).

To study whether and how TGF-β induces the expression of CD73 on the HTR-8/SVneo cells, we measured the levels of pmTOR (**Figures 7C, D**), the rapid phosphorylation products of downstream mTOR effectors pSmad2 and pSmad3 (**Figures 7E, F**), as well as CD73 (**Figures 7K–N**) in the HTR-8/SVneo cells after rhTGF-β treatment, and found increased expression of all the receptors and effectors. Moreover, these increases could be diminished by the addition of Smad2/3 inhibitor S525334, suggesting a TGF-β-mTOR signaling pathway in the HTR-8/SVneo cells.

We further analyzed the activation of HIF-1α, since it has been shown that CD73 is a direct target of HIF-1α (48). The hypoxia mimetic $CoCl_2$ was used to increase the level of HIF-1α in the HTR-8/SVneo cells (**Figure 7G**). TGF-β enhanced HIF-1α expression in the HTR-8/SVneo cells in the presence of $CoCl_2$ (**Figures 7H, I**). However, this TGF-β-induced HIF-1α expression was reduced by the addition of rapamycin. In addition, $CoCl_2$ treatment enhanced the CD73 expression, which was abrogated by the HIF-1α inhibitor MeoE2

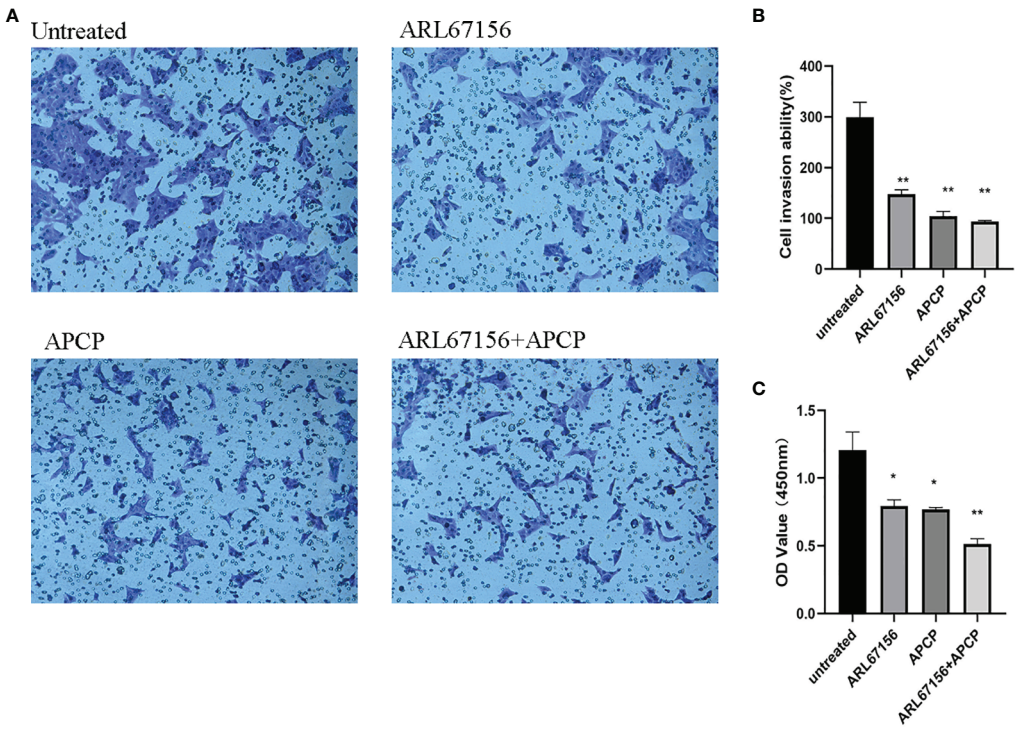

FIGURE 6 | The change of invasion and proliferation of HTR8/SVneo cells after inhibiting CD39 and CD73. Representative photos **(A)** and data **(B)** for the matrigel transwell assay of normal dNK cells from healthy donors co-cultured with the HTR-8/SVneo cells (dNK: HTR-8/SVneo cells = 2:1) in the absence or presence of the CD39 inhibitor ARL67156 and/or the CD73 inhibitor APCP. n = 3 for each group (mean ± SEM, **P < 0.01 compared with the untreated group, one-way ANOVA and *post hoc* tests). **(C)** The OD values of the HTR-8/SVneo cells in the CCK-8 assay, showing their proliferation capacities (mean ± SEM, *P < 0.05, **P < 0.01 compared with the untreated group, one-way ANOVA and *post hoc* tests).

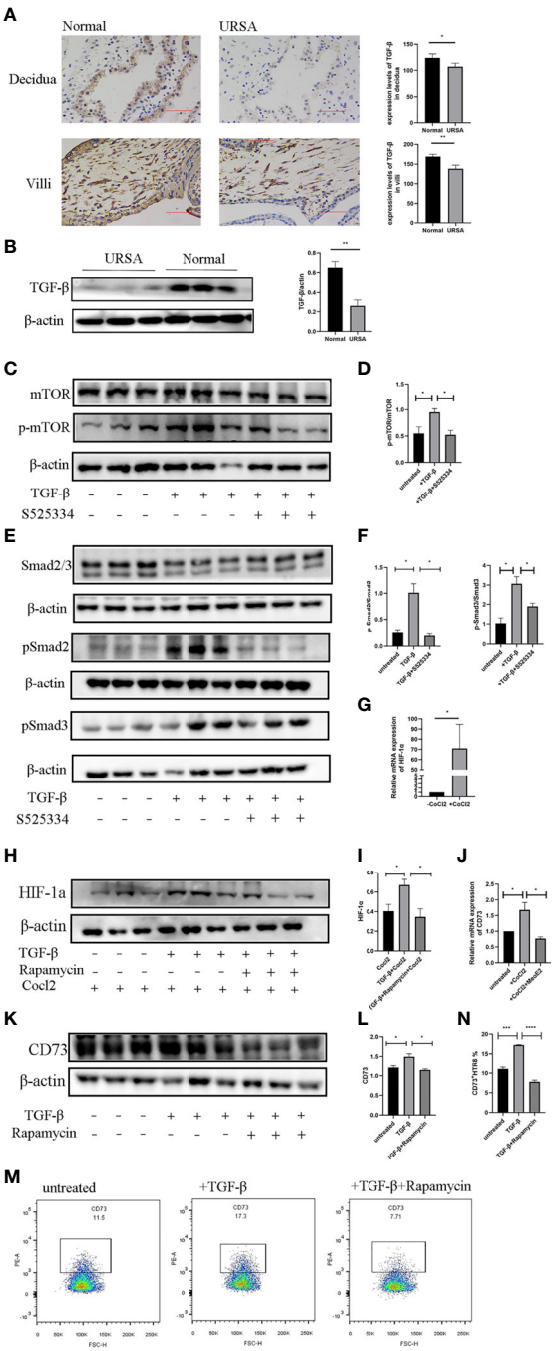

FIGURE 7 | TGF-b induces the expression of CD73 on HTR-8/SVneo cells *via* mTOR-HIF-1a. **(A)** Immunohistochemical staining and villous tissues from normal and URSA pregnancies. Results are shown as mean ± SEM, n = 3 (*P < 0.05, **P < 0.01, two-tailed t tests). **(B)** Western blot analysis of TGF-β in the villous tissues from normal and URSA pregnancies. Results are shown as mean ± SEM, n = 3 (**P < 0.01, two-tailed t tests). **(C, D)** Western blot analysis of mTOR and pmTOR in HTR-8/SVneo cells cultured with or without rhTGF-β (10 ng/mL) and the Smad2/3 inhibitor S525334. **(E, F)** Western blot analysis of Smad2/3 and pSmad2 and pSmad3 in HTR-8/SVneo cells cultured with or without rhTGF-β (10 ng/mL) and the Smad2/3 inhibitor S525334. **(G)** Relative mRNA levels of HIF-1α in the HTR-8/SVneo cells cultured with or without $CoCl_2$ (100 mM). **(H, I)** HTR-8/SVneo cells were treated with or without rapamycin (10 nM) for 1 hour, and then stimulated with rhTGF-β (10 ng/mL) (or vehicle) and $CoCl_2$ (100 mM) for 12 hours. The whole cell lysate was analyzed for HIF-1α by Western blot. **(J)** HTR-8/SVneo cells were cultured with or without $CoCl_2$ (100 mM) and the HIF-1α inhibitor MeoE2 (10 mM) for 24 hours. Relative mRNA levels of CD73 were measured by RT-PCR. **(K, L)** HTR-8/SVneo cells were treated with the mTOR inhibitor rapamycin (10 nM) for 1 hour and then with rhTGF-β (10 ng/mL) or vehicle for 24 hours. The whole cell lysate was analyzed for CD73 by Western blot. **(M, N)** The percentages of $CD73^+$ HTR-8/SVneo cells from the abovementioned experiment were further analyzed with flow cytometry. Means of three different experiments ± SEM are shown (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, two-tailed t tests or one-way ANOVA and *post hoc* tests).

(**Figure 7J**). When the mTOR pathway in the HTR-8/SVneo cells was suppressed by rapamycin, the TGF-β-mediated expression of CD73 also decreased (**Figures 7K, L**). This observation was confirmed with flow cytometry analyses (**Figures 7M, N**). Thus, our data indicated that TGF-β may induce CD73 expression in the HTR-8/SVneo cells *via* the mTOR-HIF-1α pathway.

DISCUSSION

In this study, we found marked decrease levels of CD39 and CD73 in the tissue from URSA patients compared with that from normal pregnancies. The decreases in CD39 and CD73 resulted in increased toxicity, decreased apoptosis, altered cytokine secretion of the dNK cells, as well as impaired invasion and proliferation of the co-cultured HTR8/SVneo cells, and may be caused by downregulated TGF-β-mTOR-HIF-1α pathway. To the best of our knowledge, this is the first study on CD39/CD73 at the maternal-fetal interface in URSA (**Figure 8**).

Three subgroups of dNK cells have been described, namely dNK1, dNK2 and dNK3 cells. All the three subgroups express CD49 and CD9. However, dNK1 cells also express CD39, CYP26A1 and B4GALNT1, dNK2 cells express ANXA1 and ITGB2, and dNK3 cells express CD160, KLRB1 and CD103, respectively. Studies have found that dNK1 cells contain more cytoplasmic granules than dNK2 and dNK3 cells. Also, dNK1 cells are more active in glycolytic metabolism and KIR gene expression, and produce higher levels of LILRB1 and cytoplasmic granule protein. This suggests a more critical crosstalk between the dNK1 and EVT cells (8). It could be speculated that CD39 on the surface of dNK1 may play a key role in communicating with EVT.

Both extracellular ATP and adenosine can induce apoptosis. ATP possibly induces apoptosis *via* activation of P2X7R (49, 50), while adenosine triggers apoptosis *via* A2A receptors. The potency of ATP and adenosine in induction of apoptosis may be different for different cells, depending on the receptors on the cell surfaces (51). For example, Wang, et al. found extracellular adenosine induces apoptosis of HGC-27 cells more strongly than ATP (52). In our study, A2A is present on dNK cells and hence the apoptosis may be triggered by adenosine rather than ATP. Extracellular cAMP may also be hydrolyzed to adenosine (53). However, no significant difference between cAMP concentration was observed between the groups (**Figure S3**), and hence the cAMP-adenosine pathway may not be involved in URSA. Therefore, it is likely that CD39 increases the level of intracellular adenosine and acts on A2A receptors, resulting in the increase of mitochondrial membrane potential and apoptosis of dNK cells.

The cytokine analyses showed that the dNK cells from URSA patients, which had lower levels of CD39, secreted higher levels of IL-10 than dNK cells from normal pregnancies. Samudra et al. have also shown that the production of IL-10 leads to miscarriage, which may be inhibited by CD39 (54). When CD39 was inhibited, dNK cells increased the production of IL-10 *in vitro*, suggesting a phenotypic shift to activated dNK cells (55). IL-10 is known as an anti-inflammatory cytokine and, in the majority of studies, CD39 is positively associated with IL-10 excretion (i.e. CD39 blockage reduces IL-10 levels). However, our study and the study by Samudra et al. (54) showed the opposite phenomenon, namely a negative association between CD39 and IL-10. This may be because that both studies investigated miscarriage associated with the maternal-fetal interface. Considering that NK cell-derived IL-10 is critical in

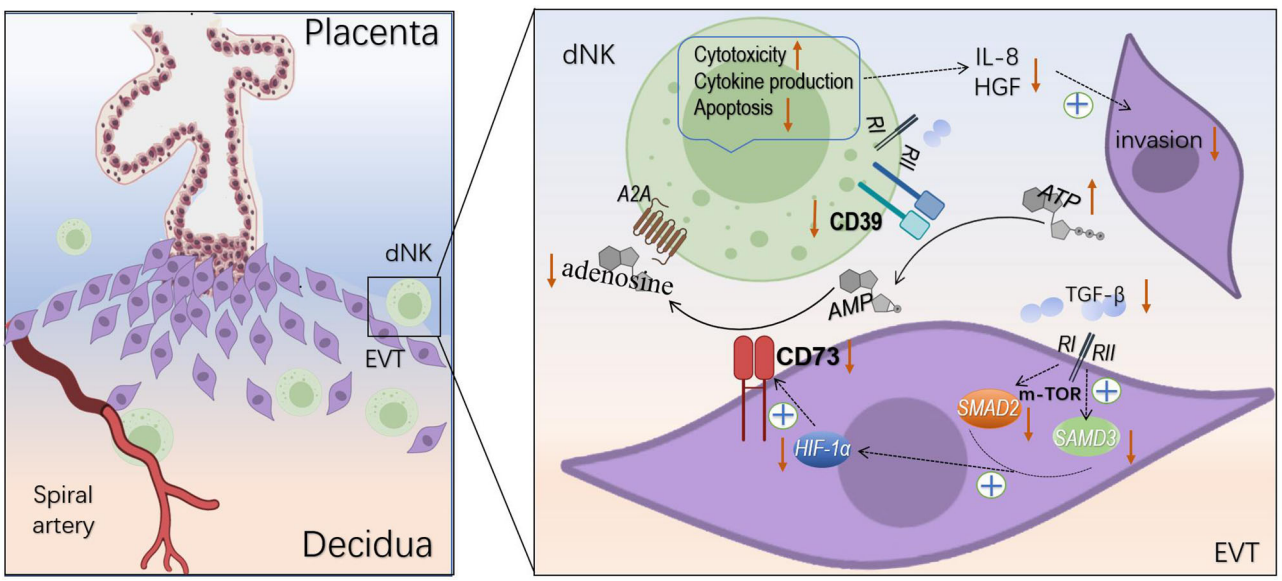

FIGURE 8 | Regulation of dNK cells and EVT cells by CD39 and CD73. CD39 and CD73 hydrolyze ATP to adenosine changed dNK cells cytotoxicity, apoptosis, cytokine production, and impaired invasion of EVT cells. TGF-β induces the expression of CD73 on the EVT cells *via* mTOR-HIF-1α pathway.

the DC-NK cell crosstalk (56), and that dNK cells exhibit lower cytotoxicity but higher secretion potential, it is possible that the regulatory mechanism for IL-10 secretion in dNK cells at the maternal-fetal interface is different from other cells, although this hypothesis requires further investigation. *In vitro* and *in vivo* studies have both shown that IL-8 and IP-10 regulate trophoblast invasion (57). We found the secretion of IL-8 was significantly reduced after inhibiting CD39 in the co-culture system, but the secretion of IP-10 was not affected by either CD39 or CD73 inhibition, suggesting the regulation of IP-10 may not rely on CD39 or CD73. The secretion of IFN-γ by dNK cells transforms spiral arteries from arterioles that are constricted, muscular, and vasoactive to vein-like structures with dilated thin-walls (58), but our data showed that CD39 has no regulatory effect on IFN-γ secretion by dNK cells. HGF promotes the trophoblast cells incorporation into the walls of endothelial tubes, indicating a role in the differentiation of trophoblast intravascular and the remodeling of vascular during pregnancy. HGF derived from dNK cells is likely to act as a paracrine factor of the decidua, guiding the differentiation of trophoblast cells along the invasion pathway (59). As expected, inhibition of CD39 reduced HGF secretion. In addition, our data showed that CD39 inhibitors reduced the invasion and proliferation of the HTR-8/SVneo cells (**Figure 6**). Taken together, the cytokine data suggest that $CD39^+$ dNK may promote the production of IL-8 and HGF to support the continuous invasion of EVT and the transformation of uterine spiral arteries.

Generally, dNK cells display lower cytotoxicity and higher cytokine secretion than pNK cells, which prevents immune attack of the fetal cells (7). Previous studies have shown that NKP30, NKP44 and NKG2D can increase the cytotoxicity of circulating NK cells *via* binding to related ligands (60, 61). Our results showed reduced expression of NKP30, NKP44 and NKG2D in $CD39^+$ dNK cells. It was likely that the loss of CD39 may transform dNK cells into a highly toxic state by promoting the expression of cytotoxicity receptors. In the co-cultivation experiments, the combined effects of CD39 and CD73 inhibited the cytotoxicity of dNK cells. Interestingly, HTR8/SVneo cells also showed the ability to suppress dNK cytotoxicity *in vitro*. Studies have shown that exogenous TGF-β can stimulate the expression of CD39 and CD73 in T cells and dendritic cells (62, 63). In this study, we found the villous tissues of URSA patients displayed lower level of TGF-β compared with the normal pregnancies. In the subsequent *in vitro* experiments, we found that TGF-β stimulated the CD73 expression in the HTR-8/SVneo cells. After adding exogenous TGF-β into the culture system, pSmad2, pSmad3 and pmTOR signals increased simultaneously in the HTR-8/SVneo cells. This observation indicated a link between Smad signaling and mTOR activation in the HTR-8/SVneo cells. We also found that the Smad2/3 inhibitor SB525334 inhibited TGF-β-induced phosphorylation of mTOR, which suggested that this phosphorylation was Smad2/3-dependent. In addition, we proved that the activation of HIF-1α induced by exogenous TGF-β is mTOR-dependent, and is essential for the induction of CD73 under normoxic conditions. On the contrary, mTOR activity is not required in hypoxia-induced HIF-1α activation. These results indicate that the TGF-β-mTOR-HIF-1α pathway has important significance in regulating immune tolerance *via* CD73. In addition, we found that the decidual tissue of URSA patients showed lower level of TGF-β, so it was possible that TGF-β may regulate the expression of CD39 in dNK cells in the same way.

Some limitations exist in this study. Firstly, although we found extracellular adenosine activated the dNK cells, which receptor the adenosine activated was uncertain. Among the four types of purinergic receptors (A1, A2A, A2B and A3), A2A is the one highly expressed on lymphocytes, and the immune regulation of NK cells by adenosine *via* the purinergic receptor A2A has been extensively reported (24, 64–66). In addition, activation of A2B receptors requires a high concentration of adenosine, which is not common in physiological conditions (67). The cAMP/protein kinase A (PKA)/cAMP response element binding protein (CREB) signaling pathway is positively regulated by adenosine acting on A2A receptor (53, 68–70). We believe the effects of adenosine on the dNK cells were also A2A-dependent. It was possible that CD39 increases the level of intracellular cAMP by increasing the level of extracellular adenosine and acting on A2A receptor, resulting in the subsequent effects of dNK cells. Secondly, although we observed reduced level of TGF- β in URSA, neither the source or the cause was revealed. The decidual tissue is rich in TGF-β (71), which may be excreted by many cell types including decidual stromal cells (72), NK cells (41, 73), and trophoblasts (74). This study focused on the regulatory pathway from TGF-β to adenosine, and hence the upstream and downstream mechanism may be investigated in future studies. Thirdly, only limited quantities of decidual tissue was obtained due to practical reasons, hence limiting the numbers of replicates for certain analyses. Fourthly, although HTR8/SVneo cells are often used as substitutes for EVT, they cannot fully represent EVTs under physiological conditions. Lastly, animal studies were not conducted in this study due to lack of an ideal model, although the clinical samples and the isolated dNK cells co-cultured with HTR8/SVneo cells provided sufficient evidence to support the major findings of this study.

The pathway found in this study may be an important immunoregulatory mechanism that causes URSA, and provides a potential new therapeutic target for the prevention of URSA. However, the cause for the downregulated TGF-β in URSA and the detailed signaling pathways within dNK cells in response to extracellular adenosine require future exploration.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Chongqing Medical University.

The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JZ wrote the original draft preparation. JZ, GS and XZ designed the experiment and method. BN, PB, T-LH and TM curated the data. JZ, HC and XY prepared the figures and collected the samples. CC, RC, RS, and HZ reviewed the data and the manuscript. All authors reviewed and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.813218/full#supplementary-material

REFERENCES

1. Male V, Sharkey A, Masters L, Kennedy PR, Farrell LE, Moffett A. The Effect of Pregnancy on the Uterine NK Cell KIR Repertoire. *Eur J Immunol* (2011) 41(10):3017–27. doi: 10.1002/eji.201141445
2. Marlin R, Duriez M, Berkane N, de Truchis C, Madec Y, Rey-Cuille MA, et al. Dynamic Shift From CD85j/ILT-2 to NKG2D NK Receptor Expression Pattern on Human Decidual NK During the First Trimester of Pregnancy. *PLoS One* (2012) 7(1):e30017. doi: 10.1371/journal.pone.0030017
3. Moffett A, Loke C. Immunology of Placentation in Eutherian Mammals. *Nat Rev Immunol* (2006) 6(8):584–94. doi: 10.1038/nri1897
4. Munoz-Suano A, Hamilton AB, Betz AG. Gimme Shelter: The Immune System During Pregnancy. *Immunol Rev* (2011) 241(1):20–38. doi: 10.1111/j.1600-065X.2011.01002.x
5. Moretta A, Marcenaro E, Sivori S, Della Chiesa M, Vitale M, Moretta L. Early Liaisons Between Cells of the Innate Immune System in Inflamed Peripheral Tissues. *Trends Immunol* (2005) 26(12):668–75. doi: 10.1016/j.it.2005.09.008
6. Martin-Fontecha A, Thomsen LL, Brett S, Gerard C, Lipp M, Lanzavecchia A, et al. Induced Recruitment of NK Cells to Lymph Nodes Provides IFN-Gamma for T(H)1 Priming. *Nat Immunol* (2004) 5(12):1260–5. doi: 10.1038/ni1138
7. Trinchieri G, Santoli D. Anti-Viral Activity Induced by Culturing Lymphocytes With Tumor-Derived or Virus-Transformed Cells. Enhancement of Human Natural Killer Cell Activity by Interferon and Antagonistic Inhibition of Susceptibility of Target Cells to Lysis. *J Exp Med* (1978) 147(5):1314–33. doi: 10.1084/jem.147.5.1314
8. Vento-Tormo R, Efremova M, Botting RA, Turco MY, Vento-Tormo M, Meyer KB, et al. Single-Cell Reconstruction of the Early Maternal-Fetal Interface in Humans. *Nature* (2018) 563(7731):347–53. doi: 10.1038/s41586-018-0698-6
9. Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, et al. Decidual NK Cells Regulate Key Developmental Processes at the Human Fetal-Maternal Interface. *Nat Med* (2006) 12(9):1065–74. doi: 10.1038/nm1452
10. Crespo AC, Mulik S, Dotiwala F, Ansara JA, Sen Santara S, Ingersoll K, et al. Decidual NK Cells Transfer Granulysin to Selectively Kill Bacteria in Trophoblasts. *Cell* (2020) 182(5):1125–39.e18. doi: 10.1016/j.cell.2020.07.019
11. Tilburgs T, Crespo AC, van der Zwan A, Rybalov B, Raj T, Stranger B, et al. Human HLA-G+ Extravillous Trophoblasts: Immune-Activating Cells That Interact With Decidual Leukocytes. *Proc Natl Acad Sci USA* (2015) 112 (23):7219–24. doi: 10.1073/pnas.1507977112
12. Yegutkin GG. Nucleotide- and Nucleoside-Converting Ectoenzymes: Important Modulators of Purinergic Signalling Cascade. *Biochim Biophys Acta* (2008) 1783(5):673–94. doi: 10.1016/j.bbamcr.2008.01.024
13. Kittel A, Csapo ZS, Csizmadia E, Jackson SW, Robson SC. Co-Localization of P2Y1 Receptor and NTPDase1/CD39 Within Caveolae in Human Placenta. *Eur J Histochem* (2004) 48(3):253–9. doi: 10.4081/894
14. Kaczmarek E, Koziak K, Sevigny J, Siegel JB, Anrather J, Beaudoin AR, et al. Identification and Characterization of CD39/vascular ATP Diphosphohydrolase. *J Biol Chem* (1996) 271(51):33116–22. doi: 10.1074/jbc.271.51.33116
15. Zimmermann H. 5'-Nucleotidase: Molecular Structure and Functional Aspects. *Biochem J* (1992) 285(Pt 2):345–65. doi: 10.1042/bj2850345
16. Huang S, Apasov S, Koshiba M, Sitkovsky M. Role of A2a Extracellular Adenosine Receptor-Mediated Signaling in Adenosine-Mediated Inhibition of T-Cell Activation and Expansion. *Blood* (1997) 90(4):1600–10. doi: 10.1182/blood.V90.4.1600
17. Schiedel AC, Lacher SK, Linnemann C, Knolle PA, Muller CE. Antiproliferative Effects of Selective Adenosine Receptor Agonists and Antagonists on Human Lymphocytes: Evidence for Receptor-Independent Mechanisms. *Purinergic Signal* (2013) 9(3):351–65. doi: 10.1007/s11302-013-9354-7
18. Takahashi HK, Iwagaki H, Hamano R, Kanke T, Liu K, Sadamori H, et al. Effect of Adenosine Receptor Subtypes Stimulation on Mixed Lymphocyte Reaction. *Eur J Pharmacol* (2007) 564(1-3):204–10. doi: 10.1016/j.ejphar.2007.02.005
19. Sakata N, Kawasome H, Terada N, Johnson GL, Gelfand EW. CD40 and Adenosine A2 Receptor Agonist-Cyclic Adenosine Monophosphate Rescue B-Cell Antigen Receptor-Induced Apoptosis Through Independent Pathways and Converge to Prevent Caspase Activation. *J Allergy Clin Immunol* (2000) 105(3):522–31. doi: 10.1067/mai.2000.104251
20. Napieralski R, Kempkes B, Gutensohn W. Evidence for Coordinated Induction and Repression of Ecto-5'-Nucleotidase (CD73) and the A2a Adenosine Receptor in a Human B Cell Line. *Biol Chem* (2003) 384 (3):483–7. doi: 10.1515/BC.2003.054
21. Rickles RJ, Pierce LT, Giordano TP3rd, Tam WF, McMillin DW, Delmore J, et al. Adenosine A2A Receptor Agonists and PDE Inhibitors: A Synergistic Multitarget Mechanism Discovered Through Systematic Combination

Screening in B-Cell Malignancies. *Blood* (2010) 116(4):593–602. doi: 10.1182/blood-2009-11-252668
22. Jeffe F, Stegmann KA, Broelsch F, Manns MP, Cornberg M, Wedemeyer H. Adenosine and IFN-{Alpha} Synergistically Increase IFN-Gamma Production of Human NK Cells. *J Leukoc Biol* (2009) 85(3):452–61. doi: 10.1189/jlb.0108046
23. Wallace KL, Linden J. Adenosine A2A Receptors Induced on iNKT and NK Cells Reduce Pulmonary Inflammation and Injury in Mice With Sickle Cell Disease. *Blood* (2010) 116(23):5010–20. doi: 10.1182/blood-2010-06-290643
24. Hausler SF, Montalban del Barrio I, Strohschein J, Chandran PA, Engel JB, Honig A, et al. Ectonucleotidases CD39 and CD73 on OvCA Cells Are Potent Adenosine-Generating Enzymes Responsible for Adenosine Receptor 2A-Dependent Suppression of T Cell Function and NK Cell Cytotoxicity. *Cancer Immunol Immunother* (2011) 60(10):1405–18. doi: 10.1007/s00262-011-1040-4
25. Eltzschig HK, Sitkovsky MV, Robson SC. Purinergic Signaling During Inflammation. *N Engl J Med* (2013) 368(13):1260. doi: 10.1056/NEJMc1300259
26. Bastid J, Regairaz A, Bonnefoy N, Dejou C, Giustiniani J, Laheurte C, et al. Inhibition of CD39 Enzymatic Function at the Surface of Tumor Cells Alleviates Their Immunosuppressive Activity. *Cancer Immunol Res* (2015) 3 (3):254–65. doi: 10.1158/2326-6066.CIR-14-0018
27. Yegutkin GG, Marttila-Ichihara F, Karikoski M, Niemela J, Laurila JP, Elima K, et al. Altered Purinergic Signaling in CD73-Deficient Mice Inhibits Tumor Progression. *Eur J Immunol* (2011) 41(5):1231–41. doi: 10.1002/eji.201041292
28. Wang L, Fan J, Thompson LF, Zhang Y, Shin T, Curiel TJ, et al. CD73 has Distinct Roles in Nonhematopoietic and Hematopoietic Cells to Promote Tumor Growth in Mice. *J Clin Invest* (2011) 121(6):2371–82. doi: 10.1172/JCI45559
29. Stagg J, Divisekera U, Duret H, Sparwasser T, Teng MW, Darcy PK, et al. CD73-Deficient Mice Have Increased Antitumor Immunity and Are Resistant to Experimental Metastasis. *Cancer Res* (2011) 71(8):2892–900. doi: 10.1158/0008-5472.CAN-10-4246
30. Stagg J, Divisekera U, McLaughlin N, Sharkey J, Pommey S, Denoyer D, et al. Anti-CD73 Antibody Therapy Inhibits Breast Tumor Growth and Metastasis. *Proc Natl Acad Sci USA* (2010) 107(4):1547–52. doi: 10.1073/pnas.0908801107
31. Jin D, Fan J, Wang L, Thompson LF, Liu A, Daniel BJ, et al. CD73 on Tumor Cells Impairs Antitumor T-Cell Responses: A Novel Mechanism of Tumor-Induced Immune Suppression. *Cancer Res* (2010) 70(6):2245–55. doi: 10.1158/0008-5472.CAN-09-3109
32. Perry C, Hazan-Halevy I, Kay S, Cipok M, Grisaru D, Deutsch V, et al. Increased CD39 Expression on CD4(+) T Lymphocytes has Clinical and Prognostic Significance in Chronic Lymphocytic Leukemia. *Ann Hematol* (2012) 91(8):1271–9. doi: 10.1007/s00277-012-1425-2
33. Wu XR, He XS, Chen YF, Yuan RX, Zeng Y, Lian L, et al. High Expression of CD73 as a Poor Prognostic Biomarker in Human Colorectal Cancer. *J Surg Oncol* (2012) 106(2):130–7. doi: 10.1002/jso.23056
34. Lu XX, Chen YT, Feng B, Mao XB, Yu B, Chu XY. Expression and Clinical Significance of CD73 and Hypoxia-Inducible Factor-1alpha in Gastric Carcinoma. *World J Gastroenterol* (2013) 19(12):1912–8. doi: 10.3748/wjg.v19.i12.1912
35. Loi S, Pommey S, Haibe-Kains B, Beavis PA, Darcy PK, Smyth MJ, et al. CD73 Promotes Anthracycline Resistance and Poor Prognosis in Triple Negative Breast Cancer. *Proc Natl Acad Sci USA* (2013) 110(27):11091–6. doi: 10.1073/pnas.1222251110
36. Kuang DM, Zhao Q, Peng C, Xu J, Zhang JP, Wu C, et al. Activated Monocytes in Peritumoral Stroma of Hepatocellular Carcinoma Foster Immune Privilege and Disease Progression Through PD-L1. *J Exp Med* (2009) 206(6):1327–37. doi: 10.1084/jem.20082173
37. Li JY, Duan XF, Wang LP, Xu YJ, Huang L, Zhang TF, et al. Selective Depletion of Regulatory T Cell Subsets by Docetaxel Treatment in Patients With Nonsmall Cell Lung Cancer. *J Immunol Res* (2014) 2014:286170. doi: 10.1155/2014/286170
38. Jones RL, Stoikos C, Findlay JK, Salamonsen LA. TGF-Beta Superfamily Expression and Actions in the Endometrium and Placenta. *Reproduction* (2006) 132(2):217–32. doi: 10.1530/rep.1.01076
39. Derynck R, Zhang YE. Smad-Dependent and Smad-Independent Pathways in TGF-Beta Family Signalling. *Nature* (2003) 425(6958):577–84. doi: 10.1038/nature02006
40. Liu X, Sun Y, Constantinescu SN, Karam E, Weinberg RA, Lodish HF. Transforming Growth Factor Beta-Induced Phosphorylation of Smad3 Is Required for Growth Inhibition and Transcriptional Induction in Epithelial Cells. *Proc Natl Acad Sci USA* (1997) 94(20):10669–74. doi: 10.1073/pnas.94.20.10669
41. Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. Transforming Growth Factor-Beta Regulation of Immune Responses. *Annu Rev Immunol* (2006) 24:99–146. doi: 10.1146/annurev.immunol.24.021605.090737
42. Yang L, Pang Y, Moses HL. TGF-Beta and Immune Cells: An Important Regulatory Axis in the Tumor Microenvironment and Progression. *Trends Immunol* (2010) 31(6):220–7. doi: 10.1016/j.it.2010.04.002
43. Allan DS, Rybalov B, Awong G, Zuniga-Pflucker JC, Kopcow HD, Carlyle JR, et al. TGF-Beta Affects Development and Differentiation of Human Natural Killer Cell Subsets. *Eur J Immunol* (2010) 40(8):2289–95. doi: 10.1002/eji.200939910
44. Ryzhov SV, Pickup MW, Chytil A, Gorska AE, Zhang Q, Owens P, et al. Role of TGF-Beta Signaling in Generation of CD39+CD73+ Myeloid Cells in Tumors. *J Immunol* (2014) 193(6):3155–64. doi: 10.4049/jimmunol.1400578
45. Liu K, Shen C, Chen X. Expression of Androgen Receptor in Coronary Artery in the Cases of Sudden Coronary Death. *Int J Clin Exp Pathol* (2015) 8 (4):3742–7.
46. Chou SHY, Lan J, Esposito E, Ning MM, Balaj L, Ji XM, et al. Extracellular Mitochondria in Cerebrospinal Fluid and Neurological Recovery After Subarachnoid Hemorrhage. *Stroke* (2017) 48(8):2231. doi: 10.1161/STROKEAHA.117.017758
47. Liu F, Lu JF, Manaenko A, Tang JJ, Hu Q. Mitochondria in Ischemic Stroke: New Insight and Implications. *Aging Dis* (2018) 9(5):924–37. doi: 10.14336/AD.2017.1126
48. Rozen-Zvi B, Hayashida T, Hubchak SC, Hanna C, Platanias LC, Schnaper HW. TGF-Beta/Smad3 Activates Mammalian Target of Rapamycin Complex-1 to Promote Collagen Production by Increasing HIF-1alpha Expression. *Am J Physiol Renal Physiol* (2013) 305(4):F485–94. doi: 10.1152/ajprenal.00215.2013
49. Salvestrini V, Orecchioni S, Talarico G, Reggiani F, Mazzetti C, Bertolini F, et al. Extracellular ATP Induces Apoptosis Through P2X7R Activation in Acute Myeloid Leukemia Cells But Not in Normal Hematopoietic Stem Cells. *Oncotarget* (2017) 8(4):5895–908. doi: 10.18632/oncotarget.13927
50. Costa-Junior HM, Mendes AN, Davis GH, da Cruz CM, Ventura AL, Serezani CH, et al. ATP-Induced Apoptosis Involves a Ca2+-Independent Phospholipase A2 and 5-Lipoxygenase in Macrophages. *Prostaglandins Other Lipid Mediat* (2009) 88(1-2):51–61. doi: 10.1016/j.prostaglandins.2008.09.004
51. Peyot ML, Gadeau AP, Dandre F, Belloc I, Dupuch F, Desgranges C. Extracellular Adenosine Induces Apoptosis of Human Arterial Smooth Muscle Cells *via* A(2b)-Purinoceptor. *Circ Res* (2000) 86(1):76–85. doi: 10.1161/01.RES.86.1.76
52. Wang MX, Ren LM. Growth Inhibitory Effect and Apoptosis Induced by Extracellular ATP and Adenosine on Human Gastric Carcinoma Cells: Involvement of Intracellular Uptake of Adenosine. *Acta Pharmacol Sin* (2006) 27(8):1085–92. doi: 10.1111/j.1745-7254.2006.00342.x
53. Su W, Chen X, Zhu W, Yu J, Li W, Li Y, et al. The cAMP-Adenosine Feedback Loop Maintains the Suppressive Function of Regulatory T Cells. *J Immunol* (2019) 203(6):1436–46. doi: 10.4049/jimmunol.1801306
54. Samudra AN, Dwyer KM, Selan C, Freddi S, Murray-Segal L, Nikpour M, et al. CD39 and CD73 Activity Are Protective in a Mouse Model of Antiphospholipid Antibody-Induced Miscarriages. *J Autoimmun* (2018) 88:131–8. doi: 10.1016/j.jaut.2017.10.009
55. Hausler SF, Del Barrio IM, Diessner J, Stein RG, Strohschein J, Honig A, et al. Anti-CD39 and Anti-CD73 Antibodies A1 and 7G2 Improve Targeted Therapy in Ovarian Cancer by Blocking Adenosine-Dependent Immune Evasion. *Am J Transl Res* (2014) 6(2):129–39.
56. Blois SM, Freitag N, Tirado-Gonzalez I, Cheng SB, Heimesaat MM, Bereswill S, et al. NK Cell-Derived IL-10 Is Critical for DC-NK Cell Dialogue at the Maternal-Fetal Interface. *Sci Rep* (2017) 7(1):2189. doi: 10.1038/s41598-017-02333-8
57. Ma L, Li G, Cao G, Zhu Y, Du MR, Zhao Y, et al. dNK Cells Facilitate the Interaction Between Trophoblastic and Endothelial Cells *via* VEGF-C and HGF. *Immunol Cell Biol* (2017) 95(8):695–704. doi: 10.1038/icb.2017.45
58. Burke SD, Barrette VF, Gravel J, Carter AL, Hatta K, Zhang J, et al. Uterine NK Cells, Spiral Artery Modification and the Regulation of Blood Pressure

During Mouse Pregnancy. *Am J Reprod Immunol* (2010) 63(6):472–81. doi: 10.1111/j.1600-0897.2010.00818.x
59. Yang Y, Wang Y, Zeng X, Ma XJ, Zhao Y, Qiao J, et al. Self-Control of HGF Regulation on Human Trophoblast Cell Invasion *via* Enhancing C-Met Receptor Shedding by ADAM10 and ADAM17. *J Clin Endocrinol Metab* (2012) 97(8):E1390–401. doi: 10.1210/jc.2012-1150
60. Dennehy KM, Klimosch SN, Steinle A. Cutting Edge: NKp80 Uses an Atypical Hemi-ITAM to Trigger NK Cytotoxicity. *J Immunol* (2011) 186(2):657–61. doi: 10.4049/jimmunol.0904117
61. Deguine J, Breart B, Lemaitre F, Bousso P. Cutting Edge: Tumor-Targeting Antibodies Enhance NKG2D-Mediated NK Cell Cytotoxicity by Stabilizing NK Cell-Tumor Cell Interactions. *J Immunol* (2012) 189(12):5493–7. doi: 10.4049/jimmunol.1202065
62. Chalmin F, Mignot G, Bruchard M, Chevriaux A, Vegran F, Hichami A, et al. Stat3 and Gfi-1 Transcription Factors Control Th17 Cell Immunosuppressive Activity *via* the Regulation of Ectonucleotidase Expression. *Immunity* (2012) 36(3):362–73. doi: 10.1016/j.immuni.2011.12.019
63. Schuler PJ, Schilling B, Harasymczuk M, Hoffmann TK, Johnson J, Lang S, et al. Phenotypic and Functional Characteristics of CD4+ CD39+ FOXP3+ and CD4+ CD39+ FOXP3neg T-Cell Subsets in Cancer Patients. *Eur J Immunol* (2012) 42(7):1876–85. doi: 10.1002/eji.201142347
64. Young A, Ngiow SF, Gao Y, Patch AM, Barkauskas DS, Messaoudene M, et al. A2AR Adenosine Signaling Suppresses Natural Killer Cell Maturation in the Tumor Microenvironment. *Cancer Res* (2018) 78(4):1003–16. doi: 10.1158/0008-5472.CAN-17-2826
65. Cekic C, Day YJ, Sag D, Linden J. Myeloid Expression of Adenosine A2A Receptor Suppresses T and NK Cell Responses in the Solid Tumor Microenvironment. *Cancer Res* (2014) 74(24):7250–9. doi: 10.1158/0008-5472.CAN-13-3583
66. Ohta A, Gorelik E, Prasad SJ, Ronchese F, Lukashev D, Wong MK, et al. A2A Adenosine Receptor Protects Tumors From Antitumor T Cells. *Proc Natl Acad Sci USA* (2006) 103(35):13132–7. doi: 10.1073/pnas.0605251103
67. Hasko G, Linden J, Cronstein B, Pacher P. Adenosine Receptors: Therapeutic Aspects for Inflammatory and Immune Diseases. *Nat Rev Drug Discov* (2008) 7(9):759–70. doi: 10.1038/nrd2638
68. Shao M, Zheng C, Ma X, Lyu F. Ecto-5'-Nucleotidase (CD73) Inhibits Dorsal Root Ganglion Neuronal Apoptosis by Promoting the Ado/cAMP/PKA/CREB Pathway. *Exp Ther Med* (2021) 22(6):1374. doi: 10.3892/etm.2021.10809
69. Waidmann O, Pleli T, Dvorak K, Baehr C, Mondorf U, Plotz G, et al. Inhibition of the Equilibrative Nucleoside Transporter 1 and Activation of A2A Adenosine Receptors by 8-(4-Chlorophenylthio)-Modified cAMP Analogs and Their Hydrolytic Products. *J Biol Chem* (2009) 284(47):32256–63. doi: 10.1074/jbc.M109.056622
70. Campo GM, Avenoso A, D'Ascola A, Prestipino V, Scuruchi M, Nastasi G, et al. Protein Kinase a Mediated Anti-Inflammatory Effects Exerted by Adenosine Treatment in Mouse Chondrocytes Stimulated With IL-1beta. *Biofactors* (2012) 38(6):429–39. doi: 10.1002/biof.1040
71. Radomska-Leśniewska DM, Białoszewska A, Kamiński P. Angiogenic Properties of NK Cells in Cancer and Other Angiogenesis-Dependent Diseases. *Cells* (2021) 10(7):1621. doi: 10.3390/cells10071621
72. Bruno A, Ferlazzo G, Albini A, Noonan DM. A Think Tank of TINK/TANKs: Tumor-Infiltrating/Tumor-Associated Natural Killer Cells in Tumor Progression and Angiogenesis. *J Natl Cancer Institute* (2014) 106(8):dju200. doi: 10.1093/jnci/dju200
73. Lash GE, Robson SC, Bulmer JN. Review: Functional Role of Uterine Natural Killer (uNK) Cells in Human Early Pregnancy Decidua. *Placenta* (2010) 31 Suppl:S87–92. doi: 10.1016/j.placenta.2009.12.022
74. Graham CH, Lysiak JJ, McCrae KR, Lala PK. Localization of Transforming Growth Factor-Beta at the Human Fetal-Maternal Interface: Role in Trophoblast Growth and Differentiation. *Biol Reprod* (1992) 46(4):561–72. doi: 10.1095/biolreprod46.4.561

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REVIEW
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Chronic Inflammatory Placental Disorders Associated With Recurrent Adverse Pregnancy Outcome

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Chronic inflammatory placental disorders are a group of rare but devastating gestational syndromes associated with adverse pregnancy outcome. This review focuses on three related conditions: villitis of unknown etiology (VUE), chronic histiocytic intervillositis (CHI) and massive perivillous fibrin deposition (MPFD). The hallmark of these disorders is infiltration of the placental architecture by maternal immune cells and disruption of the intervillous space, where gas exchange between the mother and fetus occurs. Currently, they can only be detected through histopathological examination of the placenta after a pregnancy has ended. All three are associated with a significant risk of recurrence in subsequent pregnancies. Villitis of unknown etiology is characterised by a destructive infiltrate of maternal CD8+ T lymphocytes invading into the chorionic villi, combined with activation of fetal villous macrophages. The diagnosis can only be made when an infectious aetiology has been excluded. VUE becomes more common as pregnancy progresses and is frequently seen with normal pregnancy outcome. However, severe early-onset villitis is usually associated with fetal growth restriction and recurrent pregnancy loss. Chronic histiocytic intervillositis is characterised by excessive accumulation of maternal CD68+ histiocytes in the intervillous space. It is associated with a wide spectrum of adverse pregnancy outcomes including high rates of first-trimester miscarriage, severe fetal growth restriction and late intrauterine fetal death. Intervillous histiocytes can also accumulate due to infection, including SARS-CoV-2, although this infection-induced intervillositis does not appear to recur. As with VUE, the diagnosis of CHI requires exclusion of an infectious cause. Women with recurrent CHI and their families are predisposed to autoimmune diseases, suggesting CHI may have an alloimmune pathology. This observation has driven attempts to prevent CHI with a wide range of maternal immunosuppression. Massive perivillous fibrin deposition is diagnosed when >25% of the intervillous space is occupied by fibrin, and is associated with fetal

growth restriction and late intrauterine fetal death. Although not an inflammatory disorder per se, MPFD is frequently seen in association with both VUE and CHI. This review summarises current understanding of the prevalence, diagnostic features, clinical consequences, immune pathology and potential prophylaxis against recurrence in these three chronic inflammatory placental syndromes.

Keywords: chronic placental inflammation, villitis of unknown etiology, chronic histiocytic intervillositis, massive perivillous fibrin deposition, CD8+ T lymphocytes, allograft rejection, fetal growth restriction, stillbirth

INTRODUCTION

Miscarriage, preterm birth, fetal growth restriction and stillbirth are complex multifactorial disorders that carry immense medical, psychological and economic impact (1–3). There are national and global imperatives to reduce their frequency (4, 5). Although almost half of intrauterine fetal deaths remain unexplained (6), a significant proportion (approximately one-third) are attributed to placental disorders detectable at routine histopathological examination (7). Within this category are the three conditions that form the subject of this review: villitis of unknown etiology (VUE), chronic histiocytic intervillositis (CHI) and massive perivillous fibrin deposition (MPFD). They are distinguished from other chronic inflammatory lesions of the placenta, such as chronic deciduitis and chronic chorioamnionitis, by their striking associations with adverse obstetric outcomes and recurrent pregnancy loss (8). It is their recurrent nature that makes them particularly important for patients and clinicians, despite their relative rarity. VUE affects 5-15% of placentas (9), CHI 0.2% (10), and MPFD 0.5% (11). The underlying disease mechanisms are poorly understood and there are no biomarkers or treatment protocols that can reliably predict or prevent recurrence in a subsequent pregnancy (12, 13).

Table 1 summarises the definitions, diagnostic criteria, grading systems and pregnancy outcomes for each of these disorders. It should be emphasised that these are not universally applied; some studies discussed in this review use different or unspecified definitions, and there is no universal consensus on diagnostic criteria.

Figure 1 shows the characteristic histological appearances of VUE, CHI and MPFD.

PLACENTAL INFLAMMATION IN HEALTHY AND PATHOLOGICAL PREGNANCY

Maternal Tolerance of the Semi-Allogeneic Fetus

Healthy term pregnancy requires maternal immunological tolerance of the semi-allogeneic fetus. This state is established rapidly in the decidua, where maternal innate immune cells [including macrophages, dendritic cells, natural killer cells and T cells (40–42)] are in direct contact with fetal extravillous trophoblast (EVT). The invading EVT infiltrates through the decidua into the myometrium, driving maternal recognition of

TABLE 1 | Definitions, diagnostic criteria, severity grading and key obstetric outcomes for three chronic inflammatory placental disorders associated with recurrence risk: villitis of unknown etiology (VUE), chronic histiocytic intervillositis (CHI) and massive perivillous fibrin deposition (MPFD).

	Villitis of unknown etiology (VUE)	Chronic histiocytic intervillositis (CHI)	Massive perivillous fibrin deposition (MPFD)
Definition	Infiltration of maternal CD8+ T lymphocytes into the chorionic villi, with proliferation of activated fetal macrophages (Hofbauer cells) (9, 14, 15)	Presence of a CD68+ maternal histiocytic infiltrate in the intervillous space, accompanied by varying degrees of fibrin deposition (16–18)	>25% of the intervillous space occupied by fibrin, leading to villous engulfment, atrophy and sclerosis (12, 19–21)
Diagnostic criteria	1. Villous infiltrate as above; 2. Absence of an infectious cause (9)	1. 80% of intervillous immune cells are CD68+ histiocytes; 2. ≥5% of the intervillous space occupied by the infiltrate; 3. Absence of clinical or histopathological signs of infection (22)	As above
Grading of lesion extent/ severity	Low-grade: involvement of <10 villi per focus High-grade: involvement of ≥10 villi per focus (9)	Low-grade: 5-50% of the intervillous space occupied by histiocytes and/or histiocyte-associated fibrin High-grade: ≥50% of the intervillous space occupied by histiocytes and/or histiocyte-associated fibrin (10, 23, 24)	Classic (severe): ≥3mm fibrin encasing the basal villi of the entire maternal surface Transmural (moderate): extending from the maternal surface towards the fetal surface and surrounding ≥50% of villi on ≥1 slide Borderline (mild): as with transmural, but only 25-50% villi affected (19)
Pregnancy outcome	Live birth in >90%; has also been associated with recurrent pregnancy loss in small series (25–27) Fetal growth restriction in 23-66% (25, 28–30)	Live birth: 55% Stillbirth: 38% Fetal growth restriction: 72% (22)	Live birth: 83-85% Stillbirth: 15-17% (11) Fetal growth restriction: wide range reported; precise risk uncertain (11, 31, 32)
Recurrence	15-55% (27, 33, 34)	25-100% (22, 35–37)	10-80% (11, 19, 21, 31, 38, 39)

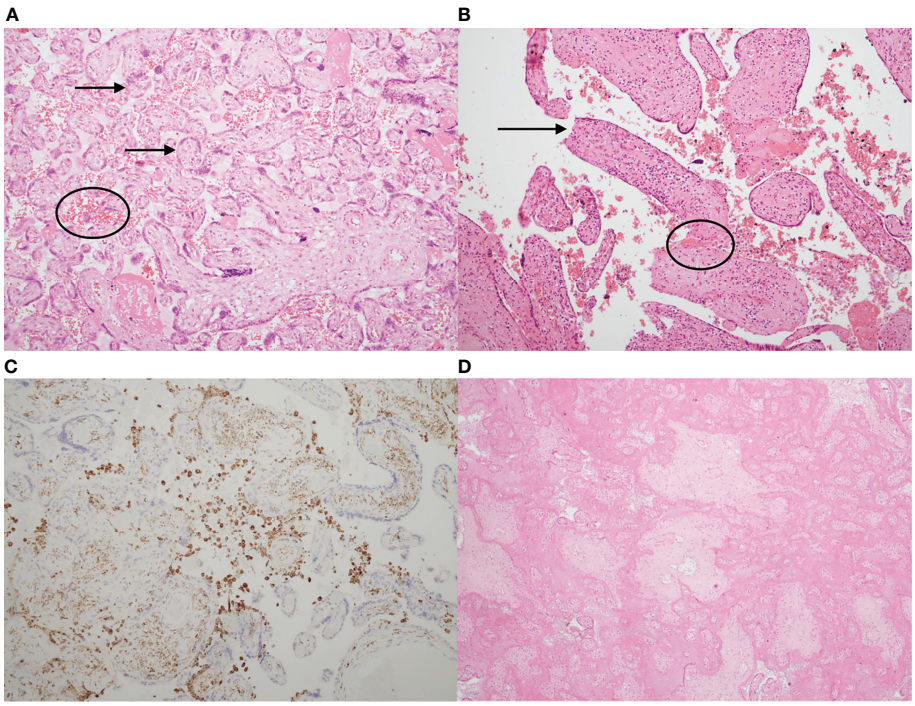

FIGURE 1 | Typical histological appearances of villitis of unknown etiology (VUE), chronic histiocytic intervillositis (CHI) and massive perivillous fibrin deposition (MPFD). **(A)** Haematoxylin & eosin stain of a slide showing normal placenta at 35 weeks' gestation, with normal chorionic villi (arrows) and maternal erythrocytes in the intervillous space (circled). **(B)** Haematoxylin & eosin stain demonstrating VUE. There is an excess of stromal T lymphocytes, villous agglutination (circled) and loss of trophoblast integrity (arrow). **(C)** Chronic histiocytic intervillositis. Immunohistochemistry demonstrates presence of CD68+ maternal histiocytes (brown) in the intervillous space. **(D)** Massive perivillous fibrin deposition (MPFD), with >95% of the intervillous space obliterated by fibrin. There is also villous agglutination and loss of nuclear integrity. **(A,B)**, x10. **(C)**, x2. **(D)**, x4.

fetal antigens. Successful implantation depends on an inflammatory milieu, which is established through production of pro-inflammatory cytokines by the decidual immune system (43, 44). Once implantation has occurred, the balance shifts. Immune crosstalk mediated by human leukocyte antigen (HLA)-G, which is expressed on the EVT (45), induces expansion of Foxp3+ regulatory T cells (T-regs) and tolerogenic macrophage and dendritic cell subsets. Anti-inflammatory cytokines such as IL-10 predominate and cytotoxic effector T cells are suppressed (46). This tolerogenic environment is maintained throughout the second and third trimesters under the influence of hormones, cytokines and soluble mediators (44).

At term gestation, an abrupt return to the pro-inflammatory state triggers the onset of labour. Circulating maternal neutrophils and macrophages are recruited to the decidua through chemotaxis and infiltrate the chorio-amniotic membranes, stimulating myometrial contraction and membrane rupture (47–49). Aberrant activation of this pathway in early gestation can lead to spontaneous preterm labour (49).

Placental Histopathology in Normal Pregnancy

The defining histological features of VUE, CHI and MPFD can all be found, to varying extents, in healthy pregnancy with normal fetal growth. Chronic villitis has been reported in up to 33% of term placentas, yet the vast majority have a normal clinical outcome (25, 50). Its prevalence increases with advancing gestational age. This suggests that as tolerance declines near term, there may be a loss of the regulatory mechanisms that protect the villi from infiltration by maternal T-effector cells (9). Similarly, the presence of maternal CD68+ histiocytes occupying up to 5% of the intervillous space can be associated with normal outcome (22, 51). Foci of fibrin deposition are found in all placentas, increasing with advancing gestational age, and perivillous fibrin deposition is a physiological response to trophoblast injury (19, 20, 52). Whether VUE, CHI and MPFD represent exaggerated versions of physiological responses, or distinct disease entities, remains unclear.

Determining the true prevalence of these features in normal pregnancies is difficult. It requires a prospective approach, access to specialist perinatal pathology services, and precise, objective case definitions. Retrospective studies of placentas sent to histopathology for clinical indications are influenced by selection bias and will invariably overestimate their frequency (53).

Two studies have attempted to overcome this challenge. In a prospective, unselected analysis of 1,153 women delivering at 34-43 weeks' gestation, VUE, CHI and MPFD were detected in 35/935 (3.7%), 2/935 (0.2%) and 2/935 (0.2%), respectively, of

pregnancies with normal obstetric and perinatal outcomes (53). The strengths of this study include its prospective design and the fact that the reporting pathologists were completely blinded to clinical outcomes. However, the small numbers of cases of chronic inflammatory conditions detected, the lack of any histological grading and the inclusion of preterm deliveries limit its generalisability (53).

In another study of 946 placentas from term deliveries of non-growth-restricted infants (birthweight 10-90th centile), 283/946 (30%) had a chronic inflammatory lesion and 176/946 (19%) had VUE. However, the majority of these were mild, whereas only 11/927 (1.2%) had high-grade VUE (54). The disparities in rates of VUE between the two studies probably reflect (1): The fact that the second study, which reports significantly higher prevalence, includes only term deliveries; and (2) Inconsistent definition and severity classification of VUE – the first study does not state its case definition for VUE. It is possible that only high-grade lesions were reported, which would make its prevalence (35/935, 3.7%) comparable to that reported in the second study (11/927, 1.2%) (53, 54).

These studies prove that chronic placental inflammation can occur in healthy pregnancy. They also highlight the uncertainties generated by lack of universal diagnostic criteria and severity grading systems. The key question is how to define the transition from physiology to pathology, and the mechanism by which this is provoked.

Chronic Placental Inflammation and Autoimmune Disease

VUE, CHI and MPFD have been reported in conjunction with several immune-mediated diseases, including systemic lupus erythematosus (SLE), Sjögren's syndrome and autoimmune thyroid disease (12, 35, 55, 56). In particular, they are associated with 2 other disorders with significant implications for pregnancy outcome: obstetric antiphospholipid syndrome (APS) and fetal-neonatal alloimmune thrombocytopenia (F-NAIT). Salafia et al. studied a cohort of 13 women with recurrent pregnancy loss due to APS and found VUE in 4/13 (31%) and MPFD in 6/13 (46%) of their placentas (57). In another retrospective series of 38 CHI pregnancies in 12 women, 4/12 (33%) affected women had APS (58). The over-representation of VUE and CHI in F-NAIT, a rare disorder affecting 1 in 1,000 live births (59), is especially pronounced. In one multi-centre case-control study, VUE and CHI were detected in 10/27 (37%) and 11/27 (41%) of F-NAIT placentas, respectively (60).

Antiphospholipid syndrome, in which maternal autoantibodies are associated with thrombosis and recurrent pregnancy loss, accounts for up to 25% of recurrent miscarriage (61). It is also strongly associated with fetal growth restriction (FGR), pre-eclampsia and stillbirth (62). APS is diagnosed according to international consensus criteria, summarised in **Table 2** (63).

Despite the clear association between APS and adverse pregnancy outcome, these criteria encompass a wide range of clinical phenotypes and the mechanism by which pregnancy loss occurs remains controversial. Early and late pregnancy loss have different aetiologies, reflecting the major adaptations in decidual immunity and utero-placental circulation that occur as pregnancy progresses. Two key mechanisms identified in APS are:

1. Autoantibody-driven thrombosis of the utero-placental circulation: levels of annexin-V, a potent anticoagulant, are significantly reduced in placental villous tissue from women with APS. Exposure of cultured trophoblast and endothelial cells to antiphospholipid antibodies leads to decreased annexin-V expression and accelerated thrombosis (64).
2. Complement-mediated pregnancy loss: in mice injected with human antiphospholipid antibodies, administration of a C3 convertase inhibitor (which blocks classical complement activation) protects offspring from FGR and intrauterine death (65). In women with antiphospholipid antibodies, complement activation in the first trimester is predictive of adverse pregnancy outcome (including FGR and stillbirth) (66). Deposition of the complement degradation product C4d is a hallmark of antibody-mediated rejection in solid organ transplants (67–69). It is generated through activation of the classical complement pathway in response to specific antibody-antigen binding. C4d deposition is significantly more common in placentas from women with antiphospholipid antibodies than those without (70).

TABLE 2 | Updated international consensus classification criteria for the diagnosis of antiphospholipid syndrome (APS). APS is diagnosed when at least 1 clinical criterion and at least 1 laboratory criterion are met (63).

Clinical criteria	Vascular thrombosis	One or more episode(s) of arterial, venous or small vessel thrombosis
	Pregnancy morbidity	a) One or more unexplained death(s) of a morphologically normal fetus at ≥10 weeks' gestation; or b) One or more preterm birth(s) of a morphologically normal baby <34 weeks' gestation due to severe pre-eclampsia, eclampsia or placental insufficiency; or c) Three or more consecutive spontaneous miscarriages before 10 weeks' gestation (in the absence of an anatomical, hormonal or chromosomal cause)
Laboratory criteria	Any of the following present on two or more occasions, 12 weeks apart: 1. Lupus anticoagulant 2. Anti-cardiolipin antibody 3. Anti-beta-2-glycoprotein-1 antibody	

APS provides an attractive paradigm for investigating an immunological aetiology in VUE, CHI and MPFD, given that all four disorders cause recurrent pregnancy loss. C4d deposition is also seen in VUE (71), CHI (72) and MPFD (73). However, there are several caveats to this comparison. Firstly, the pregnancy losses associated with VUE, CHI and MPFD usually occur in the second and third trimester, whereas APS primarily causes early first-trimester miscarriage (74). Secondly, although they overlap, the majority of women with VUE, CHI and MPFD do not have APS (56). Thirdly, C4d deposition is not specific to these conditions and has also been identified in unexplained recurrent miscarriage (75). Finally, there are clearly additional mechanistic factors involved given that neither anti-thrombotic therapy (low-molecular weight heparin) (76) nor non-specific immune suppression (hydroxychloroquine) can completely alleviate the risk of obstetric complications in APS (77).

Discordance of VUE, CHI and MPFD has been reported in dizygotic twin pregnancies (78–80). This suggests that fetal genetics contribute to the pathogenesis, with a specific feto-placental antigen stimulating maternal immune "rejection" of the placenta, rather than a general failure of tolerance at the decidua. However, other evidence implies that the primary problem is maternal, particularly in the case of CHI. Firstly, CHI carries a recurrence rate of up to 100% and worsens in successive pregnancies (36, 81–83). Secondly, when women with recurrent pregnancy loss due to CHI undergo IVF with their own oocyte and their partner's sperm, followed by implantation into a surrogate, CHI does not recur (84). The true underlying mechanism may therefore incorporate a double hit of both fetal immunogenetics and inappropriate maternal immune reactivity: perhaps a feto-placental antigen expressed in the first pregnancy "primes" a mother with a pre-existing propensity towards autoimmune disease to reject all subsequent pregnancies.

Chronic Placental Inflammation and Infection

Placental villitis is evident in response to various infections (85). In the absence of proven infection, VUE should be considered (86). While unidentified infections could be responsible for what we currently term VUE, this seems unlikely given that infectious villitis and VUE differ in both histological and clinical parameters. **Table 3** summarises the key differences between infectious villitis and VUE.

Similarly, the term "CHI" is used interchangeably with "chronic intervillositis of unknown etiology" (CIUE), defined by Bos et al. as an infiltrate of CD68+ mononuclear cells occupying ≥5% of the intervillous space "in the absence of clinical or histopathological signs of infection" (22). This is what distinguishes CHI from the many infectious causes of intervillositis – for example, Plasmodium falciparum (87), cytomegalovirus (88), dengue virus (89), Zika virus (90), parvovirus B19 (16), SARS-CoV-2 (91), Chlamydia psittaci (92) and Listeria monocytogenes (12, 55, 93, 94).

The association of all three conditions with SARS-CoV-2 has provided the opportunity to study rates of VUE, CHI and MPFD in thousands of infected placentas. This is in stark contrast to the handful of case reports describing their occurrence in certain infections prior to the COVID-19 pandemic. A systematic review of placental pathology in 1,008 pregnant women with SARS-CoV-2 infection identified chronic inflammatory pathology (including chronic villitis) in 26% and increased perivillous fibrin in 33% (95). Pregnancy outcomes were not reported in this study, meaning that the clinical consequences of this chronic inflammation are unclear (95). However, large cohort studies and meta-analyses have shown that SARS-CoV-2 infection in pregnancy is associated with significantly higher rates of preterm birth, stillbirth, perinatal and neonatal morbidity and mortality (96–99).

More specifically, a combination of CD68+ histiocytic intervillositis with chronic villitis, perivillous fibrin deposition and trophoblast necrosis has been described in case reports and small series of placental SARS-CoV-2 (91, 100–102). One group studied 39 placentas from SARS-CoV-2-positive mothers and found that all four that stained positive for the virus also had CHI, MPFD, chronic villitis and an unusual CD20+ B cell component in the intervillous infiltrate. This pattern was seen irrespective of severity of maternal symptoms and correlated with fetal distress (103). **Figure 2** demonstrates coexistent CHI and SARS-CoV-2 infection.

High SARS-CoV-2 viral loads in the placenta are unsurprising given that it enters host cells using the angiotensin-converting enzyme 2 (ACE-2), which is highly expressed in the decidua and trophoblast (104). But why would SARS-CoV-2 trigger intervillositis? Infection with other single-stranded RNA viruses such as dengue virus and SARS-CoV-1 is also associated with chronic intervillositis, but the mechanism by which this occurs is unknown (89, 105). It is uncertain whether

TABLE 3 | Comparison of clinical and histological features in infectious villitis and villitis of unknown etiology (VUE) (9).

	Infectious villitis	VUE
Prevalence	0.1-0.4%	5-15%
Histological features	Diffuse distribution	Focal or patchy distribution
	Histiocytic villitis	Lymphohistiocytic villitis
	Presence of villous plasma cells	Absence of plasma cells
	Haemosiderin deposition	Absence of haemosiderin
	Viral inclusion bodies	Absence of viral inclusion bodies
Symptomatic maternal infection during pregnancy	Common	Rare
Association with maternal autoimmune disease	Rare	Common
Recurrence	Rare	Common

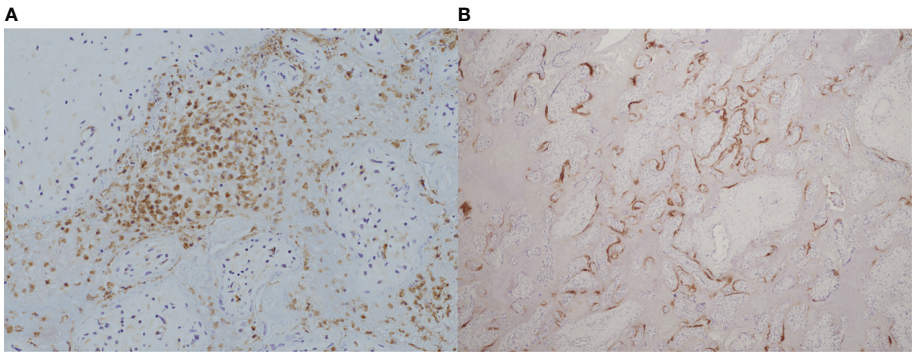

FIGURE 2 | Coexistence of chronic histiocytic intervillositis (CHI) with SARS-CoV-2 infection in a placenta from a late miscarriage at 20 weeks' gestation. **(A)** Immunohistochemical staining demonstrates CD68+ histiocytes (brown) in the intervillous space. **(B)** Positive expression of SARS-CoV-2 nucleocapsid protein (brown) by immunohistochemistry within the syncytiotrophoblast membrane of the same placenta. **(A)**, x20. **(B)**, x4.

the accumulation of maternal histiocytes in the intervillous space, adjacent to the SARS-CoV-2 nucleocapsid-positive syncytiotrophoblast, represents a specific response to that particular virus or a non-specific component of the maternal immune response to intra-placental infection.

The detection of this new viral aetiology for villitis and intervillositis highlights the critical importance of accurately excluding an infectious cause when making the diagnosis of VUE or CHI. This remains a conflicted area. One study showed no evidence of increased receptor specificity for common viral epitopes in T cells isolated from VUE placentas, concluding that VUE is more likely to represent a primary allograft rejection response (106). However, another group used viral nucleic acid enrichment combined with metagenomic sequencing to investigate occult viral infection in 20 placentas diagnosed with VUE, and detected at least 1 virus in 65% of them, compared to 35% of control placentas without chronic inflammatory lesions (107). Whatever the pathophysiology of infection-associated villitis or intervillositis, thorough placental examination and/or maternal serology should be used to rule out infection, as the infection-associated cases do not appear to recur.

In the absence of an infectious cause, attention should focus on an immune-mediated pathophysiology. Parallels with transplant immunology have led some groups to describe VUE, CHI and MPFD as analogous to maternal "rejection" of the fetal semi-allograft (13, 72, 83, 108, 109). However, unlike solid organ allografts, serial sampling of the placenta during pregnancy is impossible given the associated risks to both mother and fetus. The diagnosis of VUE, CHI or MPFD therefore rests entirely on placental histology from a single, postnatal timepoint. This makes it particularly difficult to elucidate the sequence of events that culminates in villitis, histiocytic infiltration of the intervillous space or deposition of perivillous fibrin.

Insights From Animal Studies

The inherent challenges of studying the human placenta in "real time" have prompted the use of alternative strategies, such as primary trophoblast cultures and commercially available cell lines (110–112). However, these have their own limitations: primary cytotrophoblast cells typically fail to replicate long-term in culture and cell line expression profiles diverge significantly from the trophoblast tissues they are intended to mimic (110, 112, 113).

As a result, animal studies have proved essential in building our understanding of human placental function and disease. There is enormous inter-species structural diversity in the placenta. Rodent, rabbit, primate and human placentas are classified as haemochorial, in which maternal blood is in direct contact with fetal-origin trophoblast (114). Of this category, mice have obvious practical advantages for laboratory work (they are small, with short gestation periods and large litter sizes) and have become the most widely used. Murine models of alloimmune disorders have provided valuable insights into pathogenesis and treatment, demonstrating that the neonatal Fc receptor (FcRn) is necessary for transport of maternal IgG into the fetal compartment in F-NAIT (115) and that activation of the classical complement pathway correlates with fetal death in APS (116).

Although they share haemochorial placentation, other critical discrepancies between murine and human placental development and immunology compromise the translational value of mouse models (117). The most important difference is in their capacity for trophoblast invasion. Successful human placentation depends on deep infiltration of the outer myometrium by migrating extravillous trophoblasts, which remodel maternal spiral arteries into dilated, low-pressure vessels to maximise placental blood flow. Inadequate invasion and remodelling leads to fetal growth restriction, pre-eclampsia and fetal death (118). Decidual natural killer (dNK) cells, which are the most abundant maternal leukocyte population at the time of implantation, play an essential role in orchestrating this invasion, through expression of killer cell immunoglobulin-like receptors (KIR) that interact directly with non-classical HLA on invading EVT (119–121). However, murine NK cells do not express KIR and trophoblast invasion is far more superficial, limited to the decidua basalis (117).

In female mice, pre-conception exposure to paternal antigen in seminal fluid stimulates a specific effector CD8+ T-cell

response (122). However, this response is transient: if the mouse subsequently becomes pregnant with an embryo that expresses the same antigen, these specific T-cell populations undergo clonal deletion to maintain tolerance to the developing fetus (123). By contrast, in humans, fetal-specific T cells are detectable several years after delivery (83, 124, 125). This persistence of anti-fetal T cells could account for the recurrence and worsening clinical severity observed in CHI and VUE, but murine models could not support investigation of this hypothesis.

Non-human primates share greater parallels with humans in terms of the structure and immunology of the maternal-fetal interface (126). The rhesus macaque has been particularly well studied (127–129). Although they do not exhibit trophoblast invasion to the same degree as humans (126), rhesus macaques express non-classical HLA-G homologs (130), possess a large population of dNK cells (131) and can develop pre-eclampsia (132, 133). Their clinical and histological response to certain viral infections in pregnancy is closely correlated with that seen in humans (134). Rhesus macaques infected with Zika virus during pregnancy show high rates of fetal loss with histiocytic intervillositis and massive perivillous fibrin deposition on placental histology (90, 135–137). Although idiopathic chronic placental inflammation has never been documented in primates, studying infection-associated CHI/MPFD in these models could reveal new insights into the underlying mechanisms involved.

Despite their potential, the ethical, logistic and financial restrictions on availability and maintenance of female rhesus macaques have limited translation of these shared features into reliable models of human placental disease (114).

The recent development of epithelial trophoblast organoids, which recapitulate the 3-dimensional villous architecture, HLA expression profiles and endocrine functions of the human placenta, could transform the study of placental development and disease (138). As with the other models described, the current organoids have intrinsic limitations in terms of their translatability to *in vivo* placental function in general and to chronic inflammatory placental conditions in particular. They are derived from first-trimester placental tissue [whereas the majority of VUE- and CHI-related pregnancy loss occurs in the third trimester (9, 22)], lack vascularisation, and develop without the influence of the decidual and fetal immune systems (139). Development of third-trimester placental organoids has so far proved impossible (140), but would represent a vital step towards the elusive *in vitro* model of the term human placenta in health and disease.

The following sections will summarise evidence for an immunological aetiology in VUE, CHI and MPFD and highlight current challenges in the efforts to understand how these conditions cause recurrent adverse pregnancy outcome.

VILLITIS OF UNKNOWN ETIOLOGY (VUE)

VUE: Definition, Prevalence, and Diagnosis

As mentioned above, VUE is evident in 5-15% of third-trimester placentas, with increasing frequency towards term (9, 141, 142). A maternal lymphohistiocytic infiltrate consisting primarily of CD8+ cytotoxic T cells invades the villous stroma, causing destructive inflammation that can lead to villous sclerosis and necrosis. In severe cases the villous inflammation can progress to "obliterative fetal vasculopathy", in which complete luminal occlusion of fetal stem villi leads to avascular villi and fetal hypoxia (143). There is also expansion of activated fetal macrophages (Hofbauer cells) within the villi (9, 12, 14, 15). VUE is classified according to the spatial pattern of villous involvement: distal, proximal or basal villi. It can be high-grade (>10 contiguous villi affected in more than one section) or low-grade (≤10 villi affected in any single focus) (9, 144). Key factors that distinguish it from infectious villitis include its non-uniform patchy distribution within the placental parenchyma and the absence of neutrophils and viral inclusions (9, 86, 145, 146).

VUE: Clinical Implications and Recurrence Risk

The majority of pregnancies affected by VUE end in live birth: 73/78 (94%) from a study in 2016, in which the average gestational age at birth was 36.7 weeks (25). However, it has been associated with recurrent pregnancy loss in small series and clinical severity appears to increase with recurrence (26, 27, 33). When VUE is associated with adverse outcome, the principal feature is fetal growth restriction, which occurs in up to two-thirds of cases (25, 28–30). VUE has also been associated with perinatal mortality, necrotising enterocolitis and abnormal neurodevelopmental outcome (143, 147, 148). The risk of recurrent VUE ranges from 17-54% (27, 33, 34) in individual studies but is generally quoted as 10-15% in expert review articles (9, 12).

Estimating the recurrence rate of VUE is challenging. The primary issue is that its incidence is very difficult to precisely determine: general (as opposed to specialist perinatal) pathologists commonly under-diagnose VUE (149) and its focal distribution means that insufficiently thorough placental sampling will lead to missed cases (12). Altemani et al. demonstrated that six blocks are required to detect 85-95% of cases of VUE, while using four blocks could lead to over 30% of cases being missed (150). Even if adequate sampling is performed, there is significant inter-observer variability even among experienced pathologists (151). These diagnostic difficulties are compounded by the small numbers and retrospective design in the majority of available studies. Attempts to determine recurrence rate can only be truly accurate if every subsequent pregnancy of each affected woman is studied, which is logistically unfeasible.

Two small cohorts have reported recurrence rates of 10/59 (17%) (33) and 7/19 (37%) (27). However, a much larger study designed specifically to investigate recurrence risk in VUE found a rate of 102/190 (54%) among 883 women who had had two placentas submitted for histological analysis during the study period (34). The significantly higher rate identified in this study likely reflects selection bias: only singleton live births in which there was a clinical indication for placental histology (e.g. FGR,

severe pre-eclampsia, placental abruption) were included. As the authors point out, low-grade VUE that occurs with normal pregnancy outcome may be less likely to recur; and these placentas would not have been included in the cohort (34).

The extent to which VUE causes FGR is, like its recurrence risk, difficult to ascertain. The high prevalence of VUE in term placentas with normal pregnancy outcome (54) suggests it could represent a physiological process that develops as tolerance wanes. However, VUE is consistently over-represented in cohorts of growth-restricted infants, implying a potential contribution to pathology (28, 152–154). Becroft et al. detected VUE in 88/509 (17%) placentas from infants with birthweight <10th centile vs. 62/529 (12%) placentas from appropriately-grown infants (145). This raises an important question: how could this common histological observation cause adverse outcome in a small minority, and account for high proportions of FGR? Some of this effect can be attributed to the grade of VUE: studies using methodology that only detects severe VUE are likely to report higher incidences of adverse outcomes. This is consistent with evidence that higher-grade VUE is more strongly associated with FGR and stillbirth than low-grade VUE (27).

Severe CHI and MPFD can lead to complete obliteration of the intervillous space through accumulation of CD68+ histiocytes and/or fibrin, respectively. It is easy to perceive how this leads to fetal hypoxia, growth restriction and demise (20, 55). However, the same cannot be said for VUE. Even in the most severe high-grade lesions, it is very rare for more than 10% of the chorionic villi to be affected (9). It seems surprising that involvement of such a small fraction of the placental parenchyma could compromise fetal growth (145). It may therefore be the fetal inflammatory response, rather than the percentage of the placental parenchyma infiltrated by maternal immune cells, that determines clinical outcome.

This hypothesis is supported by the discovery of elevated CXCL10 levels in fetal plasma in 22 pregnancies affected by VUE compared to 22 normal term pregnancies (108). Increased CXCL10 is a hallmark of rejection in renal and cardiac allografts (155–157). These findings reinforce the theory that VUE represents maternal rejection of the placenta, but larger studies are required.

Although more common at term, VUE has also been detected in preterm placentas (27, 29, 34). This could represent either accelerated decline of maternal tolerance, or a specific "rejection" event, but has not been specifically studied. Detailed comparisons of term and preterm VUE using advanced techniques such as placental transcriptomics may help to overcome the gaps in our knowledge.

Once we can distinguish physiological VUE at term from pathological preterm VUE with fetal growth restriction, we should be able to determine risk of recurrence more accurately. Until then, the broad estimates quoted above will remain frustrating for clinicians and patients alike. They highlight how little we know about VUE and the ways in which this common lesion can occasionally account for severe obstetric complications.

VUE: Evidence for an Immune Aetiology

Current hypotheses around the pathogenesis of chronic inflammation in VUE are divided into 2 categories: firstly, a response to an as yet unidentified infection; and secondly, a primary immunological mechanism analogous to allograft rejection (158). The latter theory will be the focus of this section. Evidence is summarised in **Figure 3**.

Linear C4d deposition has been detected on the syncytiotrophoblast of VUE placentas, but was absent in healthy placentas and those infected with cytomegalovirus (71, 161). The fact that VUE is significantly more common in donor-oocyte pregnancies (where the fetus is completely allogeneic) compared with own-oocyte IVF pregnancies lends support to the humoral rejection hypothesis (162). However, the placental alloantigen to which the mother raises an antibody, triggering complement activation, is unknown.

The HLA system on the semi-allogeneic placenta is a possible target, just as circulating anti-HLA antibodies are a major risk factor for graft rejection in transplant recipients (163). Villous trophoblast typically lacks HLA class II expression, but VUE placentas demonstrate abnormal upregulation of HLA class II molecules (158, 164). A group in Michigan found a significantly higher frequency of maternal anti-fetal-HLA seropositivity in pregnancies affected by VUE compared to those without: anti-HLA class I antibodies were present in 39/53 (74%) vs. 74/227 (33%), and anti-HLA class II antibodies were present in 23/53 (43%) vs. 29/227 (13%). The same findings were observed for chronic chorioamnionitis, which, like VUE, involves maternal cellular infiltration of fetal tissues – in this case, the chorio-amniotic membranes, rather than the placental parenchyma (165).

As well as humoral mechanisms, the possibility of cellular-mediated rejection has been explored. Kim et al. demonstrated significant upregulation of chemokines and chemokine receptors in 20 VUE placentas, compared to controls (108). The transcriptomic signature they identified is similar to that found in rejected allografts (166–168). These findings were corroborated in a larger study by the same group (169) and also in a report by Enninga et al., who found that 38% of the differentially expressed genes in VUE placentas as compared to normal gestational-age-matched control placentas mapped to allograft rejection pathways and that CXCL10 was the most strongly upregulated gene (158). The observation of increased T-regs in VUE infiltrates vs. normal placentas could indicate a compensatory attempt to suppress inflammation and restore the tolerogenic state (170).

Inappropriate T cell trafficking is another potential contributing mechanism in the pathogenesis of VUE. In healthy pregnancy, physiological suppression of intercellular adhesion molecule-1 (ICAM-1) expression helps to maintain tolerance towards the fetus (171). In VUE, however, ICAM-1 is upregulated on the syncytiotrophoblast, promoting transfer of maternal lymphocytes into the villi (159).

Defects in tolerogenic immune checkpoint signalling pathways may also have a role. Programmed death ligand-1 (PD-L1), a major suppressor of cytotoxic T cells that is pivotal in tumour immune evasion (172) and may be a key player in fetal tolerance (173), is expressed on the trophoblast in normal pregnancy (174) and circulates in maternal blood (175). Shahi et al. found that PD-L1 expression was significantly reduced in

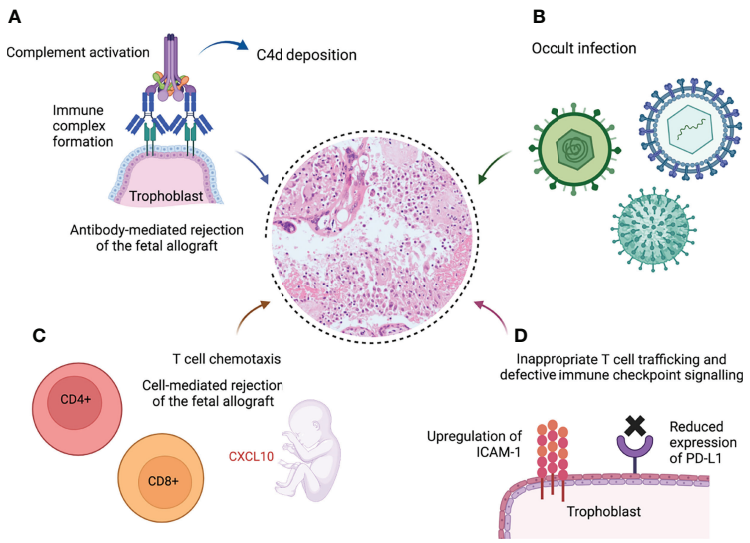

FIGURE 3 | Schematic demonstrating potential pathological pathways leading to villitis of unknown etiology (VUE) (9, 86, 107, 108, 159, 160). **(A)** Formation of immune complexes at the syncytiotrophoblast leads to complement activation and C4d deposition. **(B)** Undiagnosed infection is responsible for the chronic inflammation. **(C)** The fetal response to maternal infiltration of the villi includes elevated plasma levels of the T cell chemokine CXCL10, a hallmark of solid organ allograft rejection (8). **(D)** Excessive intercellular adhesion molecule-1 (ICAM-1) expression on the syncytiotrophoblast in VUE promotes infiltration of maternal T cells (159); programmed death ligand-1 (PD-L1), which suppresses cytotoxic T cell activity, is downregulated in VUE (160). Created with BioRender.com.

VUE placentas compared to both normal and CMV-infected placentas, suggesting a loss of tolerance unique to non-infectious villitis that may play a critical role in the disease process of VUE (160).

CHRONIC HISTIOCYTIC INTERVILLOSITIS (CHI)

CHI: Definition, Prevalence, and Diagnosis

CHI is defined as a CD68+ maternal histiocytic infiltrate occupying ≥5% of the intervillous space (22). This is accompanied by a small population of CD4+ and CD8+ T cells, varying degrees of perivillous fibrin deposition, villous agglutination and trophoblast necrosis (94, 176–178).

CHI is much less common than VUE. A large Canadian series analysed almost 30,000 placentas and reported a prevalence of 0.17% (10). However, CHI and VUE frequently coexist (179): in the first account of CHI published by Labarrere & Mullen in 1987, it was described as an "extreme variant" of VUE (17). Nowak et al., who reported outcomes from 76 cases of combined CHI-VUE, suggested that CHI might represent either a precursor lesion or a more aggressive variant of VUE (25). Although Labarrere later described phenotypic similarities in the cellular infiltrates (180), current consensus is that they represent two distinct syndromes, with the chronic inflammation of CHI confined to the intervillous space rather than infiltrating into the villi (13).

In 2018, Bos et al. proposed diagnostic criteria for CHI as follows: ≥5% of the intervillous space occupied by a mononuclear cell infiltrate, of which ≥80% are CD68+, in the absence of clinical or histopathological signs of infection (22). These have been widely adopted since and recently adapted to produce a grading system for lesion severity that correlated with overall perinatal survival in a cohort of 122 cases (181). Concurrent MPFD has been omitted from these criteria as grading of MPFD intensity is highly subjective and evidence regarding its correlation with clinical outcomes is conflicting (23, 55, 181, 182). Its reported incidence alongside CHI varies widely, from 16% (55) to 100% (176). In a recent large series, 51/111 (46%) of CHI cases were associated with MPFD (56).

CHI: Clinical Implications and Recurrence Risk

Unlike VUE, CHI is consistently associated with all forms of adverse pregnancy outcome (22, 25, 56). In a systematic review of 350 CHI pregnancies in 291 women, the live birth rate was just 135/246 (55%) overall. Fetal growth restriction affected 63/88 (72%), first-trimester miscarriage 62/256 (24%) and stillbirth 59/155 (38%) (22). Another series of 122 cases reported that 18/122 (15%) ended in medical termination due to severe FGR. In this cohort, 38/70 (54%) of live births were delivered preterm (from 24 to 36 + 6 weeks' gestation) (35).

The risk of recurrent CHI is high. However, reports of the exact recurrence rate vary from 25-100% in large, well-phenotyped cohorts (10, 22, 35–37, 94, 181, 183, 184). These disparities result from multiple factors (1): the rarity of the lesion and its variable appearance in different trimesters (2); the fact that some studies have excluded first-trimester miscarriages (3); under-diagnosis of CHI due to lack of familiarity even among specialist perinatal pathologists (4); the lack (until recently) of

consensus diagnostic criteria (5); the influence of selection bias in small case series of adverse outcomes; and (6) the recent observation that CHI secondary to infection, in particular SARS-CoV-2, does not seem to recur (12, 22, 56, 82, 185).

CHI: Evidence for an Immune Aetiology

Investigations into a potential immune aetiology for CHI largely mirror those conducted in VUE. C4d deposition on the syncytiotrophoblast is emerging as a consistent feature of CHI. The percentage of villi that stain positive for C4d is significantly higher in CHI placentas than in normal controls (182) and in VUE (72). The authors hypothesise that complement fixation localised to the microvillous border could represent a maternal humoral response to a paternally inherited placental alloantigen. This could induce expression of pro-inflammatory cytokines that recruit maternal macrophages into the intervillous space (72). However, placental C4d deposition is also evident in other autoimmune conditions such as SLE and APS, which can coexist with CHI (116). While the presence of C4d reinforces the theory that CHI results from humoral immune rejection, it does not of course identify the causative placental antigen. Benachi et al. confirmed diffuse villous staining for C4d in recurrent CHI and also noted focal deposition of the C5b-9 membrane attack complex, suggesting localised complement activation (83). **Figure 4** shows C4d deposition in CHI.

In parallel with VUE, one study showed increased frequency of partner-specific anti-HLA antibodies in women with CHI (3/4, 75%) compared to controls (0/7) (84). However, the numbers studied were very small (only 4 affected women). Moreover, formation of anti-partner-HLA antibodies is also seen in normal pregnancy and is a physiological maternal response to peri-conceptional exposure to paternal antigens (13, 186) and term birth (187).

An important and detailed immunophenotypic analysis of two women who had healthy full-term first pregnancies each followed by two subsequent pregnancies with histologically and clinically worsening CHI supports the theory that CHI is driven by an alloimmune mechanism. In both cases, anti-HLA antibodies that showed specific reactivity for epitopes harboured by all 3 fetuses were isolated. The authors concluded that the first pregnancy constitutes a sensitising event that establishes broad humoral reactivity against HLA-eplet-mismatched siblings (83). Although small, this study provides evidence of alloimmune sensitisation in CHI. A larger-scale study would be required to validate these findings.

Freitag et al. used placental transcriptomics to analyse inflammation- and angiogenesis-associated gene expression in 5 CHI placentas, 4 VUE placentas and 7 normal control placentas. Compared to controls, the CHI group showed significantly higher levels of transforming growth factor beta receptor 1 (TGFBR1) and matrix metallopeptidase-9 (MMP-9) (178). Although there is evidence that macrophage trafficking depends on MMP-9 (188), it is impossible to conclude from such a small cohort that MMP-9 overexpression drives histiocyte accumulation in CHI.

ICAM-1 expression is markedly higher in CHI than both VUE and control placentas (189). However, ICAM-1 promotes adhesion and transmigration of maternal cells into fetal tissues – for its expression to be higher in CHI, where maternal cells remain confined to the intervillous space, than in VUE, where maternal cells infiltrate and destroy the villi, therefore seems counter-intuitive. It is likely that increased ICAM-1 expression is sufficient to arrest maternal cells in the intervillous space, but whether or not they then invade across the syncytiotrophoblast barrier into fetal tissues depends on the cellular composition and properties of the infiltrate itself.

Differential expression of two other cell surface proteins in CHI has recently been reported. Firstly, there was a significant reduction in the percentage of villi staining positive for CD39 in 22 CHI placentas compared to 20 healthy controls (45% vs. 95%) (182). CD39 is a key contributor to T-reg-mediated tolerance and is expressed at lower levels in a wide range of autoimmune diseases (190). In this study, the extent to which CD39 was downregulated correlated directly with clinical outcome, with greater reductions in the subgroup of those with CHI who had FGR or intrauterine death. However, the usual caveat of poor quantification of protein expression when ascertained through immunostaining alone should be applied here (182).

Secondly, Clark et al. showed a significant reduction in CD200 expression intensity on villous trophoblast from a single CHI placenta compared to a healthy term control (191). CD200 is an anti-inflammatory immune checkpoint molecule that inhibits macrophage proliferation and promotes T-reg polarisation through interaction with its receptor, CD200R. CD200-/- knockout mice develop expansion of activated macrophage populations and increased susceptibility to autoimmunity (192, 193). However, there are a number of caveats to the study demonstrating reduced CD200 in CHI: firstly, its size (n=1); secondly, CD200R expression was also reduced; and thirdly, these reductions were only seen in villous trophoblast but not in the intervillous infiltrate (191). Although a mechanism of CD200 depletion appears consistent with the pathophysiology of CHI, this result should therefore be interpreted with caution.

The aggressive clinical phenotype of CHI is at odds with the characteristics of the histiocytic infiltrate. The maternal CD68+ macrophages that occupy the intervillous space overexpress CD163, indicating polarisation towards the anti-inflammatory M2 phenotype associated with matrix remodelling and tissue repair (194). This is consistent with the lack of fetal tissue infiltration, but inconsistent with the severity of the adverse obstetric outcomes in CHI. It is possible that the intervillous infiltrate is a consequence of an as yet undiscovered immune pathology, rather than the primary driver of disease.

MASSIVE PERIVILLOUS FIBRIN DEPOSITION (MPFD)

MPFD: Definition, Prevalence, and Diagnosis

The term MPFD describes a pathological process in which >25% of the intervillous space is occupied by fibrin. The fibrin forms a

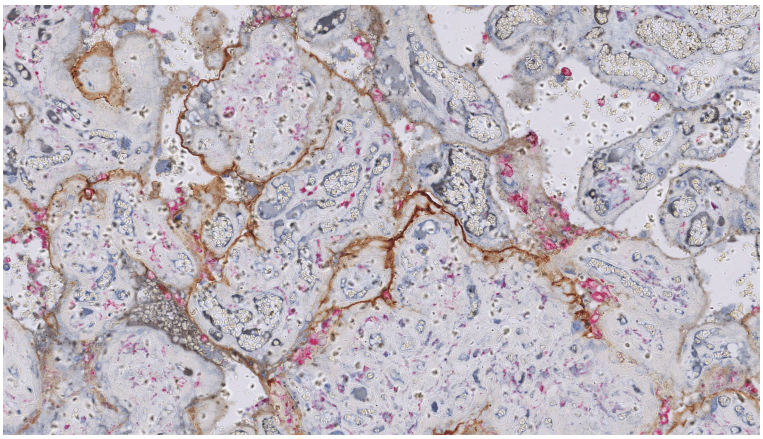

FIGURE 4 | Chronic histiocytic intervillositis (CHI). Dual staining demonstrates aggregates of CD68+ histiocytes in the intervillous space (pink) and linear deposition of C4d along the microvillous border (brown) in a case requiring delivery by emergency Caesarean section for severe fetal growth restriction with abnormal umbilical artery Dopplers at 24 weeks' gestation. Histology also showed extensive perivillous fibrin deposition.

lattice that suffocates the chorionic villi and impairs feto-maternal gas exchange. This is accompanied by foci of degenerative trophoblastic injury (20, 21). It can be categorised as classic, transmural or borderline according to the site and extent of deposition (see **Table 1**) (19).

In isolation, MPFD is uncommon. The largest published cohort found MPFD in 190/39,215 (0.48%) of placentas sent for histopathology (11) and the incidence is approximately 0.28 cases per 1,000 live births (21). As with VUE and CHI, estimations of prevalence are fraught with inaccuracy because of selection bias: histopathological examination of the placenta is not routine and is only conducted when adverse perinatal outcomes occur. Despite this uncertainty, it is clear that the frequency of MPFD varies with gestational age. Recent data demonstrate higher rates in first-trimester miscarriages: 2.7% (15/562) (73).

MPFD often overlaps with VUE/CHI rather than occurring as an isolated lesion, and how this influences clinical outcomes is difficult to ascertain. One study examined 318 first-trimester miscarriage specimens with normal karyotype and identified isolated CHI in 14/318 (4.4%), isolated MPFD in 17/318 (5.3%) and a combination of chronic inflammation with MPFD in 60/318 (19%) (195).

MPFD: Clinical Implications and Recurrence Risk

The reported risk of fetal death in MPFD ranges from 15-80% (11, 21, 31, 32, 109, 196). In general, studies quoting higher rates are smaller: 24/60 (40%) and 4/10 (80%) (31, 109). Studies analysing >50 cases quote lower rates that are likely to be more reliable: 32/190 (17%) (11) and 11/71 (15%) (32). This broad range is unhelpful for clinicians and makes it difficult to counsel patients appropriately. As with VUE and CHI, several factors contribute to this challenging ambiguity: small studies, subjectivity of reporting, a lack of consensus on diagnostic criteria and terminology, and failure to distinguish infectious vs. idiopathic cases.

MPFD is also associated with high rates of prematurity (26-58%) (11, 21, 31, 32) and fetal growth restriction (53-88%) (31, 32, 38, 197). It is independently associated with overall severe adverse neonatal outcome (198) and, in preterm infants, significantly higher rates of neurodevelopmental impairment at 24 months than in gestational age-matched cases without MPFD (197). Pregnancy outcomes appear to be worse with higher degrees of fibrin deposition. Intrauterine death, preterm birth and birthweight <3rd centile for gestational age were all significantly more common in a study comparing 39 cases of severe transmural MPFD to 32 cases of borderline MPFD and 142 controls (32).

The reported risk of recurrence in MPFD is highly variable and ranges from 12-78% (11, 19, 21, 31, 38, 39). As with VUE and CHI these statistics are of uncertain accuracy given the subjectivity of the diagnosis, the failure to consistently send placentas from subsequent pregnancies for histology and the exclusion of miscarriages from some studies (19, 21, 32).

MPFD: Aetiology

Theories about the aetiology of MPFD extend more broadly than those for VUE and CHI. As stated above, small amounts of fibrin deposition are a physiological finding in term placentas and reflect activation of the maternal coagulation cascade in response to turbulent intervillous blood flow (20, 199). However, the shift from physiological to pathological fibrin deposition that defines MPFD can arise in response to a large number of different insults. These include infection, maternal autoantibodies that provoke local complement activation, maternal thrombophilia, or direct cytotoxicity (which arises from excessive proliferation of extravillous trophoblast, leading to overproduction of major basic protein) (20, 200). All these sources of trophoblast injury could lead to localised inflammation, stasis within the

intervillous space, excessive activation of the coagulation pathway, and development of MPFD either in isolation or accompanied by VUE and/or CHI (80, 199, 201).

Accumulation of damaged and degenerating trophoblast exposes the basement membrane to the intervillous blood. This activates the coagulation system and leads to synthesis of fibrinoid extracellular matrix, which occludes the intervillous space (20, 21). As well as a specific response to trophoblast injury, MPFD could also represent the terminal phase of a secondary response to progressive trophoblast damage resulting from severe destructive villitis or intervillositis, since they often occur together (195). These theories are appealing given the frequent coexistence of the 3 disorders in question. However, the "snapshot" nature of placental pathology makes the temporal relationship between the lesions difficult to establish.

Determining the source of trophoblast injury is fundamental to the understanding of MPFD. In maternal infection, haematogenous spread brings pathogens into the intervillous space where they can cause direct trophoblast damage. MPFD has been reported in conjunction with enteroviruses, cytomegalovirus, SARS-CoV-2 and syphilis (100, 202–205). In one of these reports, a patient who had an intrauterine death at 36 weeks' gestation with MPFD (>80% of the intervillous space occupied) and Coxsackievirus A16 infection of the placenta went on to have an uneventful pregnancy 15 months later, though the placenta was not sent for histology (205). Perhaps unsurprisingly, infection-associated MPFD does not appear to recur (202, 205). It is the cause and management of recurrent cases in the absence of infection that pose the greatest challenge for clinicians.

Gogia et al. found a high prevalence of maternal thrombophilia in cases of MPFD, particularly protein S deficiency, which was present in a total of 9/26 (35%) of women tested 3 months after the pregnancy had ended. They speculated that this might promote dysfunctional activation of the coagulation system in the intervillous space (206). However, this was not replicated in a larger study in which none of the 71 women affected by MPFD had a thrombophilia (though the type being tested for was not specified) (32).

In parallel with their work on VUE, the Michigan group have demonstrated significantly increased rates of C4d deposition, maternal anti-fetal-HLA class I seropositivity and upregulation of the T cell chemokine CXCL10 in maternal plasma in women affected by MPFD, albeit in small numbers (73, 109, 207). In their 2013 study, they compared levels of plasma CXCL10 in serial antenatal samples taken every 2-4 weeks from women subsequently found to have MPFD (n=10) and controls who delivered a healthy term infant with normal placental histology (n=175). They demonstrated a longitudinal rise in plasma CXCL10 with advancing gestational age in women with MPFD. In contrast, CXCL10 levels declined steadily from 16 weeks' gestation in controls and were significantly lower than in women with MPFD by 20 weeks' gestation. This led the authors to speculate that CXCL10 could constitute an antenatal predictor for the development of MPFD (109). Although this finding holds some potential given the current lack of a reliable antenatal biomarker for MPFD, systemic elevation of a T cell chemokine cannot explain the fact that the pathology is limited to the intervillous compartment.

The prevalence of autoimmune disease in MPFD has not been well quantified. MPFD has been reported in conjunction with SLE and APS (32, 73, 199). This is consistent with an antiphospholipid antibody-induced decrease in trophoblast expression of the anticoagulant annexin-V, promoting a hyper-coagulable state within the placenta that could lead to excessive fibrin deposition (64). However, the most consistent association of MPFD is with polymyositis (201, 208–210). Autoantibodies, particularly anti-Jo-1, are detectable in the majority of cases of polymyositis and it is associated with adverse pregnancy outcome (201, 209). Skeletal muscle and placental syncytiotrophoblast both possess the unusual ability to form syncytia. It is possible that a shared antigen triggers T cell-mediated attack on both tissues simultaneously, accounting for the apparent association between polymyositis and MPFD (210).

IMMUNOSUPPRESSION TO PREVENT RECURRENT ADVERSE PREGNANCY OUTCOME

There is very little evidence available around optimal treatment approaches for prevention of recurrent adverse pregnancy outcomes due to chronic inflammatory placental disorders. The suspected alloimmune contribution to the pathogenesis of CHI and VUE has prompted case reports and small series describing variable clinical benefit in response to prednisolone, hydroxychloroquine, tacrolimus, intravenous immunoglobulin and adalimumab (10, 37, 73, 82, 184, 211–214). However, drug combinations and dosing regimens are highly variable and results are conflicting.

Early small studies of heterogeneous combinations of thromboprophylaxis and immunosuppression showed no reduction in adverse pregnancy outcomes (37). However, more organised and rational immunosuppressive regimens have shown promise.

A prospective study in 2015 reported on 24 patients with previous CHI who underwent subsequent pregnancies treated with various combinations of aspirin, LMWH, prednisolone and hydroxychloroquine. The live birth rate for the treated pregnancies was 16/24 (67%) overall, a significant rise from 24/76 (32%) in their previous pregnancies. The authors drew attention to the fact that 4/6 women receiving hydroxychloroquine had a live birth (184). However, larger and more recent studies are less confident in endorsing treatment: a French cohort of 122 cases found no evidence for benefit from aspirin (n=18), LMWH (n=7) or prednisolone (n=6) in comparison to the 90 untreated cases – but again, doses were not specified (56). Despite the lack of conclusive evidence, some authors now advocate routine consideration of immunosuppression (10) while others propose enhanced obstetric surveillance alone (56). These small-scale contradictory

reports have created considerable dilemmas for both patients and providers.

The largest cohort of women with previous CHI treated in 1-2 subsequent pregnancies is 39, with 27/39 (69%) receiving prednisolone and/or hydroxychloroquine. This combination significantly reduced histological severity of CHI: in the index pregnancy, 11/28 (39%) cases were mild, 11/28 (39%) were moderate and 6/28 (21%) were severe; whereas in subsequent pregnancies treated with prednisolone and/or hydroxychloroquine, CHI was absent in 18/30 (60%) and only severe in 2/30 (7%). It also translated into a non-significant trend towards higher rates of live birth in the prednisolone and/or hydroxychloroquine group, compared with those who received non-immunomodulatory treatment: 25/29 (86%) vs. 8/13 (62%), p=0.11 (179).

CHALLENGES AND LIMITATIONS

Clinical studies that have examined the treatment of chronic inflammatory placental disorders have obvious limitations. They use small numbers, lack randomisation and include heterogeneous patient groups with poorly defined histopathology. This reflects the lack of research into the conditions and the tendency to rely on low-quality studies. Additional caveats in the primary literature about VUE, CHI and MPFD include:

1) Proven significant inter-observer variability in the diagnosis of the three placental disorders in question (12, 23, 151, 181, 215);
2) Discrepancies in the definitions used for adverse clinical outcomes: there is no consensus in the data on what constitutes "early" or "late" miscarriage, whether FGR is defined as estimated fetal weight below the 3rd or 10th percentile for gestational age, and the method used to calculate these centiles;
3) The absence of clear, validated consensus criteria for stratification of lesion severity and the strength of its correlation with clinical outcome (10, 22, 23, 181);
4) The confounding effect of aspirin and low-molecular weight heparin, which are frequently recommended in women with recurrent adverse pregnancy outcome irrespective of the cause, in determining the role of maternal immunosuppression (5, 216);
5) The extent to which general improvements in obstetric care have influenced clinical outcomes: the UK stillbirth rate has fallen from 5.5 per 1,000 total births in 1985, when many of the early series describing VUE/CHI/MPFD were published, to 3.8 per 1,000 total births in 2019 (217). The global stillbirth rate is estimated to have fallen by 35% since 2000 (218). Alongside this, increased obstetric surveillance and various forms of treatment for women with VUE, CHI and MPFD have become the norm in several countries and it is difficult to ascertain which of these, if any, lead to improved outcome (179).

The availability and utilisation of perinatal pathology is another major challenge. VUE, CHI and MPFD are difficult to diagnose. Despite some limited evidence for an association with antenatal biochemical [raised serum alkaline phosphatase (35, 56), low PAPP-A (181)] and ultrasonographic [abnormal umbilical artery Doppler, oligohydramnios (56, 185)] biomarkers, these are non-specific and placental histopathology remains the only reliable method for diagnosis. But even in women with recurrent disease, specimens are still not reliably sent for histopathology. In the cohort of 56 CHI cases described by Simula et al. in 2020, there were 36 subsequent pregnancies but less than half (14/36) had placental analysis to assess for recurrent CHI (10). Availability of specialist perinatal pathology services is subject to financial constraints and regional disparities, exacerbated by the recent COVID-19 pandemic. Many obstetric units use their own local eligibility criteria, with variable adherence (179, 219, 220). This makes accurate quantification of recurrence risk and patient counselling extremely challenging.

Affected patients are well-informed, highly motivated, increasingly seek immunosuppressive therapy and are unlikely to find the prospect of an untreated pregnancy acceptable. Ensuring appropriate referral for histopathological analysis of the placenta is therefore paramount if we are to narrow the spectrum of reported recurrence rates and understand treatment efficacy in these conditions. We consider placental pathology essential for all women with a history of CHI, VUE, MPFD and/or SARS-CoV-2 infection in pregnancy, as it is for all cases of fetal growth restriction or demise.

CURRENT RESEARCH GAPS

Several unanswered questions remain in our knowledge of these conditions:

1. The histological features of VUE, CHI and MPFD can all be seen in normal pregnancy. Do the three conditions simply represent exaggerated forms of normal responses to advancing gestational age or to placental injury? Where along the continuum do we designate pathology, and what triggers the transition from physiology to disease?
2. Are there different clinical phenotypes of VUE/CHI/MPFD in infection-associated compared with immune-associated cases?
3. Do VUE, CHI and MPFD represent manifestations of maternal "rejection" of the feto-placental unit? If yes, are they antibody- or T-cell-mediated, what determines which disorder develops, and how does the fetal inflammatory response contribute to disease?
4. Exclusion of an infectious aetiology is essential to determine whether or not VUE, CHI and MPFD are likely to recur. How reliable is our screening for placental infection? How might new techniques help to exclude occult infection?
5. What is the mechanism underlying recurrent disease? Do women become sensitised against a specific placental antigen in a first pregnancy, or does recurrence arise due to non-specific cross-reactivity with antigens encountered during infection or exposure to a chromosomally abnormal pregnancy?

6. Is massive perivillous fibrin deposition a "final common pathway" in a non-specific response to trophoblast damage that obscures the initial pathology as it accumulates?
7. What is the role of maternal immunosuppression in preventing recurrence of VUE, CHI and MPFD? Which are the most effective drug regimens, and how should these be integrated with obstetric surveillance in a subsequent pregnancy?

CONCLUSION

The three placental disorders discussed in this article are strongly associated with recurrent adverse perinatal outcomes but remain incompletely understood and difficult to treat. Accumulating evidence in favour of an alloimmune "fetal rejection" aetiology and an increasing recognition of their clinical implications are likely to prompt wider experimentation with immunosuppressive therapy, but caution is essential until the pathogenesis of these syndromes is firmly established. Physiological gestational immune modulation already predisposes pregnant women to disproportionately severe clinical consequences of infection with SARS-CoV-2, among others (96). Systemic maternal immunosuppression in pregnancy should therefore only be initiated after careful consideration.

Given their rarity and severity, there is a clear imperative for international collaboration and application of novel scientific techniques in the investigation of exactly how CHI, VUE and MPFD cause adverse pregnancy outcome and how this might be prevented. A prospective registry of women diagnosed with these conditions would provide a highly valuable resource to determine whether and how their subsequent pregnancies are affected. We are in the process of establishing a multicentre network of clinicians and scientists interested in chronic inflammatory placental disorders in order to achieve this goal.

The recent discovery of their association with SARS-CoV-2 has given these neglected disorders new prominence in the obstetric community and in the medical literature. There is an opportunity to capitalise on this momentum, address the gaps in our knowledge and collaborate on larger, prospective studies that will elucidate the underlying mechanisms of disease.

AUTHOR CONTRIBUTIONS

EC and DW conceived the article. EC wrote the first draft of the manuscript. TM and DW provided critical revisions and edited the text. All authors contributed to manuscript revision, read, and approved the submitted version.

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REFERENCES

1. Heazell AE, Siassakos D, Blencowe H, Burden C, Bhutta ZA, Cacciatore J, et al. Stillbirths: Economic and Psychosocial Consequences. *Lancet* (2016) 387 (10018):604–16. doi: 10.1016/S0140-6736(15)00836-3
2. Farren J, Mitchell-Jones N, Verbakel JY, Timmerman D, Jalmbrant M, Bourne T. The Psychological Impact of Early Pregnancy Loss. *Hum Reprod Update* (2018) 24(6):731–49. doi: 10.1093/humupd/dmy025
3. Petrou S, Yiu HH, Kwon J. Economic Consequences of Preterm Birth: A Systematic Review of the Recent Literature (2009-2017). *Arch Dis Child* (2019) 104(5):456–65. doi: 10.1136/archdischild-2018-315778
4. Flenady V, Wojcieszek AM, Middleton P, Ellwood D, Erwich JJ, Coory M, et al. Stillbirths: Recall to Action in High-Income Countries. *Lancet* (2016) 387 (10019):691–702. doi: 10.1016/S0140-6736(15)01020-X
5. NHS England. *Saving Babies' Lives Version Two: A Care Bundle for Reducing Perinatal Mortality* (2019). Available at: https://www.england.nhs.uk/wp-content/uploads/2019/03/Saving-Babies-Lives-Care-Bundle-Version-Two-Updated-Final-Version.pdf.
6. Man J, Hutchinson JC, Heazell AE, Ashworth M, Levine S, Sebire NJ. Stillbirth and Intrauterine Fetal Death: Factors Affecting Determination of Cause of Death at Autopsy. *Ultrasound Obstet Gynecol* (2016) 48(5):566–73. doi: 10.1002/uog.16016
7. Man J, Hutchinson JC, Heazell AE, Ashworth M, Jeffrey I, Sebire NJ. Stillbirth and Intrauterine Fetal Death: Role of Routine Histopathological Placental Findings to Determine Cause of Death. *Ultrasound Obstet Gynecol* (2016) 48 (5):579–84. doi: 10.1002/uog.16019
8. Kim CJ, Romero R, Chaemsaithong P, Kim JS. Chronic Inflammation of the Placenta: Definition, Classification, Pathogenesis, and Clinical Significance. *Am J Obstet Gynecol* (2015) 213(4 Suppl):S53–69. doi: 10.1016/j.ajog.2015.08.041
9. Redline RW. Villitis of Unknown Etiology: Noninfectious Chronic Villitis in the Placenta. *Hum Pathol* (2007) 38(10):1439–46. doi: 10.1016/j.humpath.2007.05.025
10. Simula NK, Terry J, Kent NE, Robertson J, Purkiss S, Bloomenthal D, et al. Chronic Intervillositis of Unknown Etiology (CIUE): Prevalence, Patterns and Reproductive Outcomes at a Tertiary Referral Institution. *Placenta* (2020) 100:60–5. doi: 10.1016/j.placenta.2020.07.032
11. Naeye RL. Maternal Floor Infarction. *Hum Pathol* (1985) 16(8):823–8. doi: 10.1016/S0046-8177(85)80254-9
12. Chen A, Roberts DJ. Placental Pathologic Lesions With a Significant Recurrence Risk - What Not to Miss! *APMIS* (2018) 126(7):589–601. doi: 10.1111/apm.12796
13. Brady CA, Williams C, Sharps MC, Shelleh A, Batra G, Heazell AEP, et al. Chronic Histiocytic Intervillositis: A Breakdown in Immune Tolerance

Comparable to Allograft Rejection? *Am J Reprod Immunol* (2020) 85(3): e13373. doi: 10.1111/aji.13373
14. Redline RW, Patterson P. Villitis of Unknown Etiology Is Associated With Major Infiltration of Fetal Tissue by Maternal Inflammatory Cells. *Am J Pathol* (1993) 143(2):473–9.
15. Kim JS, Romero R, Kim MR, Kim YM, Friel L, Espinoza J, et al. Involvement of Hofbauer Cells and Maternal T Cells in Villitis of Unknown Aetiology. *Histopathol* (2008) 52(4):457–64. doi: 10.1111/j.1365-2559.2008.02964.x
16. Bos M, Harris-Mostert E, van der Meeren LE, Baelde JJ, Williams DJ, Nikkels PGJ, et al. Clinical Outcomes in Chronic Intervillositis of Unknown Etiology. *Placenta* (2020) 91:19–23. doi: 10.1016/j.placenta.2020.01.001
17. Labarrere C, Mullen E. Fibrinoid and Trophoblastic Necrosis With Massive Chronic Intervillositis: An Extreme Variant of Villitis of Unknown Etiology. *Am J Reprod Immunol Microbiol* (1987) 15(3):85–91. doi: 10.1111/j.1600-0897.1987.tb00162.x
18. Heller DS. CD68 Immunostaining in the Evaluation of Chronic Histiocytic Intervillositis. *Arch Pathol Lab Med* (2012) 136(6):657–9. doi: 10.5858/arpa.2011-0328-OA
19. Katzman PJ, Genest DR. Maternal Floor Infarction and Massive Perivillous Fibrin Deposition: Histological Definitions, Association With Intrauterine Fetal Growth Restriction, and Risk of Recurrence. *Pediatr Dev Pathol* (2002) 5 (2):159–64. doi: 10.1007/s10024-001-0195-y
20. Redline RW. Extending the Spectrum of Massive Perivillous Fibrin Deposition (Maternal Floor Infarction). *Pediatr Dev Pathol* (2021) 24(1):10–1. doi: 10.1177/1093526620964353
21. Bane AL, Gillan JE. Massive Perivillous Fibrinoid Causing Recurrent Placental Failure. *BJOG* (2003) 110(3):292–5. doi: 10.1046/j.1471-0528.2003.00119.x
22. Bos M, Nikkels PGJ, Cohen D, Schoones JW, Bloemenkamp KWM, Bruijn JA, et al. Towards Standardized Criteria for Diagnosing Chronic Intervillositis of Unknown Etiology: A Systematic Review. *Placenta* (2018) 61:80–8. doi: 10.1016/j.placenta.2017.11.012
23. Ongaro D, Terry J. Reproducibility of Grading in Chronic Intervillositis of Unknown Etiology. *Pediatr Dev Pathol* (2020) 23(3):210–4. doi: 10.1177/1093526619882522
24. Rota C, Carles D, Schaeffer V, Guyon F, Saura R, Horovitz J. Perinatal Prognosis of Pregnancies Complicated by Placental Chronic Intervillitis. *J Gynecol Obstet Biol Reprod (Paris)* (2006) 35(7):711–9. doi: 10.1016/s0368-2315(06)76468-7
25. Nowak C, Joubert M, Jossic F, Masseau A, Hamidou M, Philippe HJ, et al. Perinatal Prognosis of Pregnancies Complicated by Placental Chronic Villitis or Intervillositis of Unknown Etiology and Combined Lesions: About a Series of 178 Cases. *Placenta* (2016) 44:104–8. doi: 10.1016/j.placenta.2016.04.017
26. Russell P, Atkinson K, Krishnan L. Recurrent Reproductive Failure Due to Severe Placental Villitis of Unknown Etiology. *J Reprod Med* (1980) 24(2):93–8.
27. Feeley L, Mooney EE. Villitis of Unknown Aetiology: Correlation of Recurrence With Clinical Outcome. *J Obstet Gynaecol* (2010) 30(5):476–9. doi: 10.3109/01443611003802339
28. Salafia CM, Vintzileos AM, Silberman L, Bantham KF, Vogel CA. Placental Pathology of Idiopathic Intrauterine Growth Retardation at Term. *Am J Perinatol* (1992) 9(3):179–84. doi: 10.1055/s-2007-999316
29. Iskender C, Zergeroglu S, Kaymak O, Celen S, Danisman N. Villitis of Unknown Aetiology: Clinical Implications in Preterm Population. *J Obstet Gynaecol* (2016) 36(2):192–5. doi: 10.3109/01443615.2015.1036410
30. Kovo M, Ganer Herman H, Gold E, Bar J, Schreiber L. Villitis of Unknown Etiology - Prevalence and Clinical Associations. *J Matern Fetal Neonatal Med* (2016) 29(19):3110–4. doi: 10.3109/14767058.2015.1114090
31. Andres RL, Kuyper W, Resnik R, Piacquadio KM, Benirschke K. The Association of Maternal Floor Infarction of the Placenta With Adverse Perinatal Outcome. *Am J Obstet Gynecol* (1990) 163(3):935–8. doi: 10.1016/0002-9378(90)91100-Q
32. Devisme L, Chauviere C, Franquet-Ansart H, Chudzinski A, Stichelbout M, Houfflin-Debarge V, et al. Perinatal Outcome of Placental Massive Perivillous Fibrin Deposition: A Case-Control Study. *Prenat Diagn* (2017) 37(4):323–8. doi: 10.1002/pd.5013
33. Redline RW, Abramowsky CR. Clinical and Pathologic Aspects of Recurrent Placental Villitis. *Hum Pathol* (1985) 16(7):727–31. doi: 10.1016/s0046-8177(85)80159-3
34. Freedman AA, Miller GE, Ernst LM. Chronic Villitis: Refining the Risk Ratio of Recurrence Using a Large Placental Pathology Sample. *Placenta* (2021) 112:135–40. doi: 10.1016/j.placenta.2021.07.298
35. Mattuizzi A, Sauvestre F, Andre G, Poingt M, Camberlein C, Carles D, et al. Adverse Perinatal Outcomes of Chronic Intervillositis of Unknown Etiology: An Observational Retrospective Study of 122 Cases. *Sci Rep* (2020) 10 (1):12611. doi: 10.1038/s41598-020-69191-9
36. Parant O, Capdet J, Kessler S, Aziza J, Berrebi A. Chronic Intervillositis of Unknown Etiology (CIUE): Relation Between Placental Lesions and Perinatal Outcome. *Eur J Obstet Gynecol Reprod Biol* (2009) 143(1):9–13. doi: 10.1016/j.ejogrb.2008.06.012
37. Contro E, deSouza R, Bhide A. Chronic Intervillositis of the Placenta: A Systematic Review. *Placenta* (2010) 31(12):1106–10. doi: 10.1016/j.placenta.2010.10.005
38. Fuke Y, Aono T, Imai S, Suehara N, Fujita T, Nakayama M. Clinical Significance and Treatment of Massive Intervillous Fibrin Deposition Associated With Recurrent Fetal Growth Retardation. *Gynecol Obstet Invest* (1994) 38(1):5–9. doi: 10.1159/000292434
39. Redline RW. Maternal Floor Infarction and Massive Perivillous Fibrin Deposition: Clinicopathologic Entities in Flux. *Adv Anatomic Pathol* (2002) 9(6):372–3. doi: 10.1097/00125480-200211000-00010
40. Starkey PM, Sargent IL, Redman CW. Cell Populations in Human Early Pregnancy Decidua: Characterization and Isolation of Large Granular Lymphocytes by Flow Cytometry. *Immunol* (1988) 65(1):129–34.
41. Vince GS, Starkey PM, Jackson MC, Sargent IL, Redman CW. Flow Cytometric Characterisation of Cell Populations in Human Pregnancy Decidua and Isolation of Decidual Macrophages. *J Immunol Methods* (1990) 132(2):181–9. doi: 10.1016/0022-1759(90)90028-t
42. Ferry BL, Starkey PM, Sargent IL, Watt GM, Jackson M, Redman CW. Cell Populations in the Human Early Pregnancy Decidua: Natural Killer Activity and Response to Interleukin-2 of CD56-Positive Large Granular Lymphocytes. *Immunol* (1990) 70(4):446–52.
43. Forger F, Villiger PM. Immunological Adaptations in Pregnancy That Modulate Rheumatoid Arthritis Disease Activity. *Nat Rev Rheumatol* (2020) 16(2):113–22. doi: 10.1038/s41584-019-0351-2
44. Aghaeepour N, Ganio EA, McIlwain D, Tsai AS, Tingle M, Van Gassen S, et al. An Immune Clock of Human Pregnancy. *Sci Immunol* (2017) 2(15):1–11. doi: 10.1126/sciimmunol.aan2946
45. Apps R, Gardner L, Sharkey AM, Holmes N, Moffett A. A Homodimeric Complex of HLA-G on Normal Trophoblast Cells Modulates Antigen-Presenting Cells *via* LILRB1. *Eur J Immunol* (2007) 37(7):1924–37. doi: 10.1002/eji.200737089
46. Gregori S, Amodio G, Quattrone F, Panina-Bordignon P. HLA-G Orchestrates the Early Interaction of Human Trophoblasts With the Maternal Niche. *Front Immunol* (2015) 6:128. doi: 10.3389/fimmu.2015.00128
47. Yuan M, Jordan F, McInnes IB, Harnett MM, Norman JE. Leukocytes are Primed in Peripheral Blood for Activation During Term and Preterm Labour. *Mol Hum Reprod* (2009) 15(11):713–24. doi: 10.1093/molehr/gap054
48. Gomez-Lopez N, Estrada-Gutierrez G, Jimenez-Zamudio L, Vega-Sanchez R, Vadillo-Ortega F. Fetal Membranes Exhibit Selective Leukocyte Chemotaxic Activity During Human Labor. *J Reprod Immunol* (2009) 80(1-2):122–31. doi: 10.1016/j.jri.2009.01.002
49. Gomez-Lopez N, StLouis D, Lehr MA, Sanchez-Rodriguez EN, Arenas-Hernandez M. Immune Cells in Term and Preterm Labor. *Cell Mol Immunol* (2014) 11(6):571–81. doi: 10.1038/cmi.2014.46
50. Labarrere CA, McIntyre JA, Faulk WP. Immunohistologic Evidence That Villitis in Human Normal Term Placentas is an Immunologic Lesion. *Am J Obstet Gynecol* (1990) 162(2):515–22. doi: 10.1016/0002-9378(90)90421-3
51. Traeder J, Jonigk D, Feist H, Brocker V, Langer F, Kreipe H, et al. Pathological Characteristics of a Series of Rare Chronic Histiocytic Intervillositis of the Placenta. *Placenta* (2010) 31(12):1116–9. doi: 10.1016/j.placenta.2010.09.012
52. Weber MA, Nikkels PG, Hamoen K, Duvekot JJ, de Krijger RR. Co-Occurrence of Massive Perivillous Fibrin Deposition and Chronic Intervillositis: Case Report. *Pediatr Dev Pathol* (2006) 9(3):234–8. doi: 10.2350/06-01-0019.1
53. Pathak S, Lees CC, Hackett G, Jessop F, Sebire NJ. Frequency and Clinical Significance of Placental Histological Lesions in an Unselected Population at

or Near Term. *Virchows Arch* (2011) 459(6):565–72. doi: 10.1007/s00428-011-1157-z
54. Romero R, Kim YM, Pacora P, Kim CJ, Benshalom-Tirosh N, Jaiman S, et al. The Frequency and Type of Placental Histologic Lesions in Term Pregnancies With Normal Outcome. *J Perinat Med* (2018) 46(6):613–30. doi: 10.1515/jpm-2018-0055
55. Marchaudon V, Devisme L, Petit S, Ansart-Franquet H, Vaast P, Subtil D. Chronic Histiocytic Intervillositis of Unknown Etiology: Clinical Features in a Consecutive Series of 69 Cases. *Placenta* (2011) 32(2):140–5. doi: 10.1016/j.placenta.2010.11.021
56. Homatter C, Stichelbout M, Devisme L, Chudzinski A, Debarge V, Garabedian C, et al. Is Chronic Histiocytic Intervillositis a Severe Placental Disease? *A case-control study Placenta* (2020) 91:31–6. doi: 10.1016/j.placenta.2019.12.020
57. Salafia CM, Cowchock FS. Placental Pathology and Antiphospholipid Antibodies: A Descriptive Study. *Am J Perinatol* (1997) 14(8):435–41. doi: 10.1055/s-2007-994176
58. Revaux A, Mekinian A, Nicaise P, Bucourt M, Cornelis F, Lachassinne E, et al. Antiphospholipid Syndrome and Other Autoimmune Diseases Associated With Chronic Intervillositis. *Arch Gynecol Obstet* (2015) 291(6):1229–36. doi: 10.1007/s00404-014-3536-6
59. Kamphuis MM, Paridaans NP, Porcelijn L, Lopriore E, Oepkes D. Incidence and Consequences of Neonatal Alloimmune Thrombocytopenia: A Systematic Review. *Pediatrics* (2014) 133(4):715–21. doi: 10.1542/peds.2013-3320
60. Nedberg NH, Turowski G, Guz K, Przytula E, Uhrynowska M, Roald B, et al. Platelet Alloimmunization is Associated With Low Grade Chronic Histiocytic Intervillositis - A New Link to a Rare Placental Lesion? *Placenta* (2021) 112:89–96. doi: 10.1016/j.placenta.2021.07.291
61. Vinatier D, Dufour P, Cosson M, Houpeau JL. Antiphospholipid Syndrome and Recurrent Miscarriages. *Eur J Obstet Gynecol Reprod Biol* (2001) 96(1):37–50. doi: 10.1016/s0301-2115(00)00404-8
62. Saccone G, Berghella V, Maruotti GM, Ghi T, Rizzo G, Simonazzi G, et al. Antiphospholipid Antibody Profile Based Obstetric Outcomes of Primary Antiphospholipid Syndrome: The PREGNANTS Study. *Am J Obstet Gynecol* (2017) 216(5):525.e1–525.e12. doi: 10.1016/j.ajog.2017.01.026
63. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International Consensus Statement on an Update of the Classification Criteria for Definite Antiphospholipid Syndrome (APS). *J Thromb Haemost* (2006) 4(2):295–306. doi: 10.1111/j.1538-7836.2006.01753.x
64. Rand JH, Wu XX, Andree HA, Lockwood CJ, Guller S, Scher J, et al. Pregnancy Loss in the Antiphospholipid-Antibody Syndrome–a Possible Thrombogenic Mechanism. *N Engl J Med* (1997) 337(3):154–60. doi: 10.1056/NEJM199707173370303
65. Holers VM, Girardi G, Mo L, Guthridge JM, Molina H, Pierangeli SS, et al. Complement C3 Activation Is Required for Antiphospholipid Antibody-Induced Fetal Loss. *J Exp Med* (2002) 195(2):211–20. doi: 10.1084/jem.200116116
66. Kim MY, Guerra MM, Kaplowitz E, Laskin CA, Petri M, Branch DW, et al. Complement Activation Predicts Adverse Pregnancy Outcome in Patients With Systemic Lupus Erythematosus and/or Antiphospholipid Antibodies. *Ann Rheum Dis* (2018) 77(4):549–55. doi: 10.1136/annrheumdis-2017-212224
67. Murata K, Baldwin WM3rd. Mechanisms of Complement Activation, C4d Deposition, and Their Contribution to the Pathogenesis of Antibody-Mediated Rejection. *Transplant Rev (Orlando)* (2009) 23(3):139–50. doi: 10.1016/j.trre.2009.02.005
68. Djamali A, Kaufman DB, Ellis TM, Zhong W, Matas A, Samaniego M. Diagnosis and Management of Antibody-Mediated Rejection: Current Status and Novel Approaches. *Am J Transplant* (2014) 14(2):255–71. doi: 10.1111/ajt.12589
69. Loupy A, Haas M, Roufosse C, Naesens M, Adam B, Afrouzian M, et al. The Banff 2019 Kidney Meeting Report (I): Updates on and Clarification of Criteria for T Cell- and Antibody-Mediated Rejection. *Am J Transplant* (2020) 20(9):2318–31. doi: 10.1111/ajt.15898
70. Viall CA, Chamley LW. Histopathology in the Placentae of Women With Antiphospholipid Antibodies: A Systematic Review of the Literature. *Autoimmun Rev* (2015) 14(5):446–71. doi: 10.1016/j.autrev.2015.01.008
71. Rudzinski E, Gilroy M, Newbill C, Morgan T. Positive C4d Immunostaining of Placental Villous Syncytiotrophoblasts Supports Host-Versus-Graft Rejection in Villitis of Unknown Etiology. *Pediatr Dev Pathol* (2013) 16(1):7–13. doi: 10.2350/12-05-1195-OA.1
72. Bendon RW, Coventry S, Thompson M, Rudzinski ER, Williams EM, Oron AP. Significance of C4d Immunostaining in Placental Chronic Intervillositis. *Pediatr Dev Pathol* (2015) 18(5):362–8. doi: 10.2350/14-12-1582-OA.1
73. Kim EN, Lee JY, Shim JY, Hwang D, Kim KC, Kim SR, et al. Clinicopathological Characteristics of Miscarriages Featuring Placental Massive Perivillous Fibrin Deposition. *Placenta* (2019) 86:45–51. doi: 10.1016/j.placenta.2019.07.006
74. Cervera R, Piette JC, Font J, Khamashta MA, Shoenfeld Y, Camps MT, et al. Antiphospholipid Syndrome: Clinical and Immunologic Manifestations and Patterns of Disease Expression in a Cohort of 1,000 Patients. *Arthritis Rheumatol* (2002) 46(4):1019–27. doi: 10.1002/art.10187
75. Meuleman T, Cohen D, Swings GM, Veraar K, Claas FH, Bloemenkamp KW. Increased Complement C4d Deposition at the Maternal-Fetal Interface in Unexplained Recurrent Miscarriage. *J Reprod Immunol* (2016) 113:54–60. doi: 10.1016/j.jri.2015.12.001
76. Hamulyak EN, Scheres LJ, Marijnen MC, Goddijn M, Middeldorp S. Aspirin or Heparin or Both for Improving Pregnancy Outcomes in Women With Persistent Antiphospholipid Antibodies and Recurrent Pregnancy Loss. *Cochrane Database Syst Rev* (2020) 5:CD012852. doi: 10.1002/14651858.CD012852.pub2
77. Frishman M, Radin M, Schreiber K. Hydroxychloroquine and Antiphospholipid Antibody-Related Pregnancy Morbidity: A Systematic Review and Meta-Analysis. *Curr Opin Obstet Gynecol* (2020) 32(5):351–8. doi: 10.1097/GCO.0000000000000649
78. Jacques SM, Qureshi F. Chronic Villitis of Unknown Etiology in Twin Gestations. *Pediatr Pathol* (1994) 14(4):575–84. doi: 10.3109/15513819409023332
79. van der Meeren LE, Krop J, Dijkstra KL, Bloemenkamp KWM, Cornish EF, Nikkels PGJ, et al. One-Sided Chronic Intervillositis of Unknown Etiology in Dizygotic Twins: A Description of 3 Cases. *Int J Mol Sci* (2021) 22(9):4786. doi: 10.3390/ijms22094786
80. Faye-Petersen O, Sauder A, Estrella Y, Heller DS. Dichorionic Twins Discordant for Massive Perivillous Fibrinoid Deposition: Report of a Case and Review of the Literature. *Int J Surg Pathol* (2018) 26(1):41–6. doi: 10.1177/1066896917720029
81. Crawford A, Moore L, Bennett G, Savarirayan R, Manton N, Khong Y, et al. Recurrent Chronic Histiocytic Intervillositis With Intrauterine Growth Restriction, Osteopenia, and Fractures. *Am J Med Genet A* (2016) 170(11):2960–4. doi: 10.1002/ajmg.a.37856
82. Vardi L, Paterson H, Hung NA. Successful Pregnancy Following Treatment of Recurrent Chronic Histiocytic Intervillositis. *BMJ Case Rep* (2017) 2017: bcr2016217886. doi: 10.1136/bcr-2016-217886
83. Benachi A, Rabant M, Martinovic J, Bouchghoul H, Vivanti AJ, Leon J, et al. Chronic Histiocytic Intervillositis: Manifestation of Placental Alloantibody-Mediated Rejection. *Am J Obstet Gynecol* (2021) 225(6):662.e1–662.e116. doi: 10.1016/j.ajog.2021.06.051
84. Reus AD, van Besouw NM, Molenaar NM, Steegers EA, Visser W, de Kuiper RP, et al. An Immunological Basis for Chronic Histiocytic Intervillositis in Recurrent Fetal Loss. *Am J Reprod Immunol* (2013) 70(3):230–7. doi: 10.1111/aji.12125
85. Khong TY, Mooney EE, Nikkels PGJ, Morgan TK, Gordijn SJSpringerLink. *Pathology of the Placenta: A Practical Guide*. Cham: Springer International Publishing: Imprint: Springer (2019). XVI, 395 222 illus., 212 illus. in color. online resource. p. English.
86. Tamblyn JA, Lissauer DM, Powell R, Cox P, Kilby MD. The Immunological Basis of Villitis of Unknown Etiology - Review. *Placenta* (2013) 34(10):846–55. doi: 10.1016/j.placenta.2013.07.002
87. Ordi J, Ismail MR, Ventura PJ, Kahigwa E, Hirt R, Cardesa A, et al. Massive Chronic Intervillositis of the Placenta Associated With Malaria Infection. *Am J Surg Pathol* (1998) 22(8):1006–11. doi: 10.1097/00000478-199808000-00011
88. Taweevisit M, Sukpan K, Siriaunkgul S, Thorner PS. Chronic Histiocytic Intervillositis With Cytomegalovirus Placentitis in a Case of Hydrops Fetalis. *Fetal Pediatr Pathol* (2012) 31(6):394–400. doi: 10.3109/15513815.2012.659405

89. Ribeiro CF, Lopes VGS, Brasil P, Pires ARC, Rohloff R, Nogueira RMR. Dengue Infection in Pregnancy and its Impact on the Placenta. *Int J Infect Dis* (2017) 55:109–12. doi: 10.1016/j.ijid.2017.01.002
90. Martines RB, Bhatnagar J, de Oliveira Ramos AM, Davi HP, Iglezias SD, Kanamura CT, et al. Pathology of Congenital Zika Syndrome in Brazil: A Case Series. *Lancet* (2016) 388(10047):898–904. doi: 10.1016/S0140-6736(16)30883-2
91. Debelenko L, Katsyv I, Chong AM, Peruyero L, Szabolcs M, Uhlemann AC. Trophoblast Damage With Acute and Chronic Intervillositis: Disruption of the Placental Barrier by Severe Acute Respiratory Syndrome Coronavirus 2. *Hum Pathol* (2021) 109:69–79. doi: 10.1016/j.humpath.2020.12.004
92. Hyde SR, Benirschke K. Gestational Psittacosis: Case Report and Literature Review. *Mod Pathol* (1997) 10(6):602–7. doi: 10.1097/00019048-199706080-00005
93. Vawter GF. Perinatal Listeriosis. *Perspect Pediatr Pathol* (1981) 6:153–66.
94. Boyd TK, Redline RW. Chronic Histiocytic Intervillositis: A Placental Lesion Associated With Recurrent Reproductive Loss. *Hum Pathol* (2000) 31(11):1389–96. doi: 10.1053/hupa.2000.19454
95. Girolamo RD, Khalil A, Alameddine S, D'Angelo E, Galliani C, Matarrelli B, et al. Placental Histopathology After SARS-CoV-2 Infection in Pregnancy: A Systematic Review and Meta-Analysis. *Am J Obstet Gynecol MFM* (2021) 3 (6):100468. doi: 10.1016/j.ajogmf.2021.100468
96. Allotey J, Stallings E, Bonet M, Yap M, Chatterjee S, Kew T, 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:m3320. doi: 10.1136/bmj.m3320
97. Villar J, Ariff S, Gunier RB, Thiruvengadam R, Rauch S, Kholin A, et al. Maternal and Neonatal Morbidity and Mortality Among Pregnant Women With and Without COVID-19 Infection: The INTERCOVID Multinational Cohort Study. *JAMA Pediatr* (2021) 175(8):817–26. doi: 10.1001/jamapediatrics.2021.1050
98. Wei SQ, Bilodeau-Bertrand M, Liu S, Auger N. The Impact of COVID-19 on Pregnancy Outcomes: A Systematic Review and Meta-Analysis. *CMAJ* (2021) 193(16):E540–8. doi: 10.1503/cmaj.202604
99. Glele LSA, Simon E, Bouit C, Serrand M, Filipuzzi L, Astruc K, et al. Association Between SARS-Cov-2 Infection During Pregnancy and Adverse Pregnancy Outcomes: A Re-Analysis of the Data Reported by Wei Et al. (2021). *Infect Dis Now* (2022) 52:00042-2. doi: 10.1016/j.idnow.2022.02.009
100. Vivanti AJ, Vauloup-Fellous C, Prevot S, Zupan V, Suffee C, Do Cao J, et al. Transplacental Transmission of SARS-CoV-2 Infection. *Nat Commun* (2020) 11(1):3572. doi: 10.1038/s41467-020-17436-6
101. Hosier H, Farhadian SF, Morotti RA, Deshmukh U, Lu-Culligan A, Campbell KH, et al. SARS-CoV-2 Infection of the Placenta. *J Clin Invest* (2020) 130(9):4947–53. doi: 10.1172/JCI139569
102. Schwartz DA, Baldewijns M, Benachi A, Bugatti M, Collins RRJ, De Luca D, et al. Chronic Histiocytic Intervillositis With Trophoblast Necrosis are Risk Factors Associated With Placental Infection From Coronavirus Disease 19 (COVID-19) and Intrauterine Maternal-Fetal Systemic Acute Respiratory Coronavirus 2 (SARSCoV-2) Transmission in Liveborn and Stillborn Infants. *Arch Pathol Lab Med* (2020) 145(5):517–28. doi: 10.5858/arpa.2020-0771-SA
103. Husen MF, van der Meeren LE, Verdijk RM, Fraaij PLA, van der Eijk AA, Koopmans MPG, et al. Unique Severe COVID-19 Placental Signature Independent of Severity of Clinical Maternal Symptoms. *Viruses* (2021) 13 (8):1670. doi: 10.3390/v13081670
104. Li M, Chen L, Zhang J, Xiong C, Li X. The SARS-CoV-2 Receptor ACE2 Expression of Maternal-Fetal Interface and Fetal Organs by Single-Cell Transcriptome Study. *PloS One* (2020) 15(4):e0230295. doi: 10.1371/journal.pone.0230295
105. Ng WF, Wong SF, Lam A, Mak YF, Yao H, Lee KC, et al. The Placentas of Patients With Severe Acute Respiratory Syndrome: A Pathophysiological Evaluation. *Pathology* (2006) 38(3):210–8. doi: 10.1080/00313020600696280
106. Enninga EAL, Raber P, Quinton RA, Ruano R, Ikumi N, Gray CM, et al. Maternal T Cells in the Human Placental Villi Support an Allograft Response During Noninfectious Villitis. *J Immunol* (2020) 204(11):2931–9. doi: 10.4049/jimmunol.1901297
107. Ernst LM, Bockoven C, Freedman A, Wang V, Pellerite M, Wylie TN, et al. Chronic Villitis of Unknown Etiology: Investigations Into Viral Pathogenesis. *Placenta* (2021) 107:24–30. doi: 10.1016/j.placenta.2021.02.020
108. Kim MJ, Romero R, Kim CJ, Tarca AL, Chhauy S, LaJeunesse C, et al. Villitis of Unknown Etiology is Associated With a Distinct Pattern of Chemokine Up-Regulation in the Feto-Maternal and Placental Compartments: Implications for Conjoint Maternal Allograft Rejection and Maternal Anti-Fetal Graft-Versus-Host Disease. *J Immunol* (2009) 182(6):3919–27. doi: 10.4049/jimmunol.0803834
109. Romero R, Whitten A, Korzeniewski SJ, Than NG, Chaemsaithong P, Miranda J, et al. Maternal Floor Infarction/Massive Perivillous Fibrin Deposition: A Manifestation of Maternal Antifetal Rejection? *Am J Reprod Immunol* (2013) 70(4):285–98. doi: 10.1111/aji.12143
110. Apps R, Sharkey A, Gardner L, Male V, Trotter M, Miller N, et al. Genome-Wide Expression Profile of First Trimester Villous and Extravillous Human Trophoblast Cells. *Placenta* (2011) 32(1):33–43. doi: 10.1016/j.placenta.2010.10.010
111. Rothbauer M, Patel N, Gondola H, Siwetz M, Huppertz B, Ertl P. A Comparative Study of Five Physiological Key Parameters Between Four Different Human Trophoblast-Derived Cell Lines. *Sci Rep* (2017) 7(1):5892. doi: 10.1038/s41598-017-06364-z
112. Haider S, Meinhardt G, Saleh L, Kunihs V, Gamperl M, Kaindl U, et al. Self-Renewing Trophoblast Organoids Recapitulate the Developmental Program of the Early Human Placenta. *Stem Cell Rep* (2018) 11(2):537–51. doi: 10.1016/j.stemcr.2018.07.004
113. Nursalim YNS, Blenkiron C, Groom KM, Chamley LW. Growing Human Trophoblasts *In Vitro*: A Review of the Media Commonly Used in Trophoblast Cell Culture. *Reproduction* (2020) 160(6):R119–28. doi: 10.1530/REP-19-0605
114. Grigsby PL. Animal Models to Study Placental Development and Function Throughout Normal and Dysfunctional Human Pregnancy. *Semin Reprod Med* (2016) 34(1):11–6. doi: 10.1055/s-0035-1570031
115. Chen P, Li C, Lang S, Zhu G, Reheman A, Spring CM, et al. Animal Model of Fetal and Neonatal Immune Thrombocytopenia: Role of Neonatal Fc Receptor in the Pathogenesis and Therapy. *Blood* (2010) 116(18):3660–8. doi: 10.1182/blood-2010-05-284919
116. Cohen D, Buurma A, Goemaere NN, Girardi G, le Cessie S, Scherjon S, et al. Classical Complement Activation as a Footprint for Murine and Human Antiphospholipid Antibody-Induced Fetal Loss. *J Pathol* (2011) 225(4):502–11. doi: 10.1002/path.2893
117. Schmidt A, Morales-Prieto DM, Pastuschek J, Frohlich K, Markert UR. Only Humans Have Human Placentas: Molecular Differences Between Mice and Humans. *J Reprod Immunol* (2015) 108:65–71. doi: 10.1016/j.jri.2015.03.001
118. Brosens I, Puttemans P, Benagiano G. Placental Bed Research: I. The Placental Bed: From Spiral Arteries Remodeling to the Great Obstetrical Syndromes. *Am J Obstet Gynecol* (2019) 221(5):437–56. doi: 10.1016/j.ajog.2019.05.044
119. Hiby SE, Apps R, Chazara O, Farrell LE, Magnus P, Trogstad L, et al. Maternal KIR in Combination With Paternal HLA-C2 Regulate Human Birth Weight. *J Immunol* (2014) 192(11):5069–73. doi: 10.4049/jimmunol.1400577
120. Moffett A, Chazara O, Colucci F. Maternal Allo-Recognition of the Fetus. *Fertil Steril* (2017) 107(6):1269–72. doi: 10.1016/j.fertnstert.2017.05.001
121. Jabrane-Ferrat N. Features of Human Decidual NK Cells in Healthy Pregnancy and During Viral Infection. *Front Immunol* (2019) 10:1397. doi: 10.3389/fimmu.2019.01397
122. Robertson SA, Prins JR, Sharkey DJ, Moldenhauer LM. Seminal Fluid and the Generation of Regulatory T Cells for Embryo Implantation. *Am J Reprod Immunol* (2013) 69(4):315–30. doi: 10.1111/aji.12107
123. Moldenhauer LM, Hayball JD, Robertson SA. Utilising T Cell Receptor Transgenic Mice to Define Mechanisms of Maternal T Cell Tolerance in Pregnancy. *J Reprod Immunol* (2010) 87(1-2):1–13. doi: 10.1016/j.jri.2010.05.007
124. Verdijk RM, Kloosterman A, Pool J, van de Keur M, Naipal AM, van Halteren AG, et al. Pregnancy Induces Minor Histocompatibility Antigen-Specific Cytotoxic T Cells: Implications for Stem Cell Transplantation and Immunotherapy. *Blood* (2004) 103(5):1961–4. doi: 10.1182/blood-2003-05-1625

125. Piper KP, McLarnon A, Arrazi J, Horlock C, Ainsworth J, Kilby MD, et al. Functional HY-Specific CD8+ T Cells are Found in a High Proportion of Women Following Pregnancy With a Male Fetus. *Biol Reprod* (2007) 76 (1):96–101. doi: 10.1095/biolreprod.106.055426
126. Carter AM, Enders AC, Jones CJ, Mess A, Pfarrer C, Pijnenborg R, et al. Comparative Placentation and Animal Models: Patterns of Trophoblast Invasion – a Workshop Report. *Placenta* (2006) 27(Suppl A):S30–3. doi: 10.1016/j.placenta.2006.01.008
127. Martin CBJr., Ramsey EM, Donner MW. The Fetal Placental Circulation in Rhesus Monkeys Demonstrated by Radioangiography. *Am J Obstet Gynecol* (1966) 95(7):943–7. doi: 10.1016/0002-9378(66)90542-4
128. Myers RE. The Pathology of the Rhesus Monkey Placenta. *Acta Endocrinol Suppl (Copenh)* (1972) 166:221–57. doi: 10.1530/acta.0.071s221
129. Ragavendra N, Tarantal AF. Intervillous Blood Flow in the Third Trimester Gravid Rhesus Monkey (Macaca Mulatta): Use of Sonographic Contrast Agent and Harmonic Imaging. *Placenta* (2001) 22(2-3):200–5. doi: 10.1053/plac.2000.0605
130. Boyson JE, Iwanaga KK, Golos TG, Watkins DI. Identification of a Novel MHC Class I Gene, Mamu-AG, Expressed in the Placenta of a Primate With an Inactivated G Locus. *J Immunol* (1997) 159(7):3311–21.
131. Slukvin II, Watkins DI, Golos TG. Phenotypic and Functional Characterization of Rhesus Monkey Decidual Lymphocytes: Rhesus Decidual Large Granular Lymphocytes Express CD56 and Have Cytolytic Activity. *J Reprod Immunol* (2001) 50(1):57–79. doi: 10.1016/s0165-0378(00)00090-5
132. Combs CA, Katz MA, Kitzmiller JL, Brescia RJ. Experimental Preeclampsia Produced by Chronic Constriction of the Lower Aorta: Validation With Longitudinal Blood Pressure Measurements in Conscious Rhesus Monkeys. *Am J Obstet Gynecol* (1993) 169(1):215–23. doi: 10.1016/0002-9378(93)90171-e
133. Krugner-Higby L, Luck M, Hartley D, Crispen HM, Lubach GR, Coe CL. High-Risk Pregnancy in Rhesus Monkeys (Macaca Mulatta): A Case of Ectopic, Abdominal Pregnancy With Birth of a Live, Term Infant, and a Case of Gestational Diabetes Complicated by Pre-Eclampsia. *J Med Primatol* (2009) 38(4):252–6. doi: 10.1111/j.1600-0684.2009.00349.x
134. Boyle A, Preslar JP, Hogue CJ, Silver RM, Reddy UM, Goldenberg RL, et al. Route of Delivery in Women With Stillbirth: Results From the Stillbirth Collaborative Research Network. *Obstet Gynecol* (2017) 129(4):693–8. doi: 10.1097/AOG.0000000000001935
135. Dudley DM, Van Rompay KK, Coffey LL, Ardeshir A, Keesler RI, Bliss-Moreau E, et al. Miscarriage and Stillbirth Following Maternal Zika Virus Infection in Nonhuman Primates. *Nat Med* (2018) 24(8):1104–7. doi: 10.1038/s41591-018-0088-5
136. Hirsch AJ, Roberts VHJ, Grigsby PL, Haese N, Schabel MC, Wang X, et al. Zika Virus Infection in Pregnant Rhesus Macaques Causes Placental Dysfunction and Immunopathology. *Nat Commun* (2018) 9(1):263. doi: 10.1038/s41467-017-02499-9
137. Mohr EL, Block LN, Newman CM, Stewart LM, Koenig M, Semler M, et al. Ocular and Uteroplacental Pathology in a Macaque Pregnancy With Congenital Zika Virus Infection. *PloS One* (2018) 13(1):e0190617. doi: 10.1371/journal.pone.0190617
138. Turco MY, Gardner L, Kay RG, Hamilton RS, Prater M, Hollinshead MS, et al. Trophoblast Organoids as a Model for Maternal-Fetal Interactions During Human Placentation. *Nature* (2018) 564(7735):263–7. doi: 10.1038/s41586-018-0753-3
139. Sheridan MA, Fernando RC, Gardner L, Hollinshead MS, Burton GJ, Moffett A, et al. Establishment and Differentiation of Long-Term Trophoblast Organoid Cultures From the Human Placenta. *Nat Protoc* (2020) 15 (10):3441–63. doi: 10.1038/s41596-020-0381-x
140. Okae H, Toh H, Sato T, Hiura H, Takahashi S, Shirane K, et al. Derivation of Human Trophoblast Stem Cells. *Cell Stem Cell* (2018) 22(1):50–63.e6. doi: 10.1016/j.stem.2017.11.004
141. Knox WF, Fox H. Villitis of Unknown Aetiology: Its Incidence and Significance in Placentae From a British Population. *Placenta* (1984) 5 (5):395–402. doi: 10.1016/s0143-4004(84)80019-3
142. Russell P. Inflammatory Lesions of the Human Placenta. III. The Histopathology of Villitis of Unknown Aetiology. *Placenta* (1980) 1 (3):227–44. doi: 10.1016/s0143-4004(80)80005-1
143. Redline RW. Severe Fetal Placental Vascular Lesions in Term Infants With Neurologic Impairment. *Am J Obstet Gynecol* (2005) 192(2):452–7. doi: 10.1016/j.ajog.2004.07.030
144. Khong TY, Mooney EE, Ariel I, Balmus NC, Boyd TK, Brundler MA, et al. Sampling and Definitions of Placental Lesions: Amsterdam Placental Workshop Group Consensus Statement. *Arch Pathol Lab Med* (2016) 140 (7):698–713. doi: 10.5858/arpa.2015-0225-CC
145. Becroft DM, Thompson JM, Mitchell EA. Placental Villitis of Unknown Origin: Epidemiologic Associations. *Am J Obstet Gynecol* (2005) 192(1):264–71. doi: 10.1016/j.ajog.2004.06.062
146. Stanek J. Placental Infectious Villitis Versus Villitis of Unknown Etiology. *Pol J Pathol* (2017) 68(1):55–65. doi: 10.5114/pjp.2017.67616
147. Redline RW, O'Riordan MA. Placental Lesions Associated With Cerebral Palsy and Neurologic Impairment Following Term Birth. *Arch Pathol Lab Med* (2000) 124(12):1785–91. doi: 10.1043/0003-9985(2000)124<1785:PLAWCP>2.0.CO;2
148. Torrance HL, Bloemen MC, Mulder EJ, Nikkels PG, Derks JB, de Vries LS, et al. Predictors of Outcome at 2 Years of Age After Early Intrauterine Growth Restriction. *Ultrasound Obstet Gynecol* (2010) 36(2):171–7. doi: 10.1002/uog.7627
149. Sun CC, Revell VO, Belli AJ, Viscardi RM. Discrepancy in Pathologic Diagnosis of Placental Lesions. *Arch Pathol Lab Med* (2002) 126(6):706–9. doi: 10.5858/2002-126-0706-DIPDOP
150. Altemani A, Gonzatti A, Metze K. How Many Paraffin Blocks are Necessary to Detect Villitis? *Placenta* (2003) 24(1):116–7. doi: 10.1053/plac.2002.0875
151. Khong TY, Staples A, Moore L, Byard RW. Observer Reliability in Assessing Villitis of Unknown Aetiology. *J Clin Pathol* (1993) 46(3):208–10. doi: 10.1136/jcp.46.3.208
152. Bjoro KJr., Myhre E. The Role of Chronic Non-Specific Inflammatory Lesions of the Placenta in Intra-Uterine Growth Retardation. *Acta Pathol Microbiol Immunol Scand A* (1984) 92(2):133–7. doi: 10.1111/j.1699-0463.1984.tb04387.x
153. Nordenvall M, Sandstedt B. Placental Villitis and Intrauterine Growth Retardation in a Swedish Population. *APMIS* (1990) 98(1):19–24. doi: 10.1111/j.1699-0463.1990.tb00996.x
154. Orefice R, Kent A, Sethna F, Dahlstrom JE. Of Pregnancies Complicated by Small for Gestational Age Babies at Term, What Proportions Have Placental Findings With Implications for Future Pregnancies or Neonatal Outcomes? *J Matern Fetal Neonatal Med* (2019) 33(17):2990–5. doi: 10.1080/14767058.2019.1566899
155. Fahmy NM, Yamani MH, Starling RC, Ratliff NB, Young JB, McCarthy PM, et al. Chemokine and Chemokine Receptor Gene Expression Indicates Acute Rejection of Human Cardiac Transplants. *Transplantation* (2003) 75(1):72–8. doi: 10.1097/00007890-200301150-00013
156. Lazzeri E, Rotondi M, Mazzinghi B, Lasagni L, Buonamano A, Rosati A, et al. High CXCL10 Expression in Rejected Kidneys and Predictive Role of Pretransplant Serum CXCL10 for Acute Rejection and Chronic Allograft Nephropathy. *Transplantation* (2005) 79(9):1215–20. doi: 10.1097/01.tp.0000160759.85080.2e
157. Lo DJ, Weaver TA, Kleiner DE, Mannon RB, Jacobson LM, Becker BN, et al. Chemokines and Their Receptors in Human Renal Allotransplantation. *Transplantation* (2011) 91(1):70–7. doi: 10.1097/TP.0b013e3181fe12fc
158. Enninga EAL, Leontovich AA, Fedyshyn B, Wakefield L, Gandhi M, Markovic SN, et al. Upregulation of HLA-Class I and II in Placentas Diagnosed With Villitis of Unknown Etiology. *Reprod Sci* (2020) 27 (5):1129–38. doi: 10.1007/s43032-019-00101-9
159. Labarrere CA, Ortiz MA, Sosa MJ, Campana GL, Wernicke M, Baldridge LA, et al. Syncytiotrophoblast Intercellular Adhesion Molecule-1 Expression in Placental Villitis of Unknown Cause. *Am J Obstet Gynecol* (2005) 193 (2):483–8. doi: 10.1016/j.ajog.2004.12.090
160. Shahi M, Mamber Czeresnia R, Cheek EH, Quinton RA, Chakraborty R, Enninga EAL. Expression of Immune Checkpoint Receptors in Placentae With Infectious and Non-Infectious Chronic Villitis. *Front Immunol* (2021) 12:705219. doi: 10.3389/fimmu.2021.705219
161. Lee AK, Kim YW, Shim JY, Won HS, Lee PR, Kim A, et al. Distinct Patterns of C4d Immunoreactivity in Placentas With Villitis of Unknown Etiology, Cytomegaloviral Placentitis, and Infarct. *Placenta* (2013) 34(5):432–5. doi: 10.1016/j.placenta.2013.02.003

162. Rudenko EE, Kogan EA, Demura TA, Zharkov NV, Trifonova NS, Zhukova EV, et al. Immunomorphological Features of the Placenta in Allogeneic Pregnancy as the Background for the Development of Obstetric Complications. *Pathobiology* (2020) 87(4):232–43. doi: 10.1159/000506776
163. Zeevi A, Girnita A, Duquesnoy R. HLA Antibody Analysis: Sensitivity, Specificity, and Clinical Significance in Solid Organ Transplantation. *Immunol Res* (2006) 36(1-3):255–64. doi: 10.1385/IR:36:1:255
164. Labarrere CA, Faulk WP. MHC Class II Reactivity of Human Villous Trophoblast in Chronic Inflammation of Unestablished Etiology. *Transplantation* (1990) 50(5):812–6. doi: 10.1097/00007890-199011000-00014
165. Lee J, Romero R, Xu Y, Kim JS, Topping V, Yoo W, et al. A Signature of Maternal Anti-Fetal Rejection in Spontaneous Preterm Birth: Chronic Chorioamnionitis, Anti-Human Leukocyte Antigen Antibodies, and C4d. *PloS One* (2011) 6(2):e16806. doi: 10.1371/journal.pone.0016806
166. Panzer U, Reinking RR, Steinmetz OM, Zahner G, Sudbeck U, Fehr S, et al. CXCR3 and CCR5 Positive T-Cell Recruitment in Acute Human Renal Allograft Rejection. *Transplantation* (2004) 78(9):1341–50. doi: 10.1097/01.tp.0000140483.59664.64
167. Piper KP, Horlock C, Curnow SJ, Arrazi J, Nicholls S, Mahendra P, et al. CXCL10-CXCR3 Interactions Play an Important Role in the Pathogenesis of Acute Graft-Versus-Host Disease in the Skin Following Allogeneic Stem-Cell Transplantation. *Blood* (2007) 110(12):3827–32. doi: 10.1182/blood-2006-12-061408
168. Ahmed SS, Wang XN, Norden J, Pearce K, El-Gezawy E, Atarod S, et al. Identification and Validation of Biomarkers Associated With Acute and Chronic Graft Versus Host Disease. *Bone Marrow Transplant* (2015) 50(12):1563–71. doi: 10.1038/bmt.2015.191
169. Lee J, Romero R, Chaiworapongsa T, Dong Z, Tarca AL, Xu Y, et al. Characterization of the Fetal Blood Transcriptome and Proteome in Maternal Anti-Fetal Rejection: Evidence of a Distinct and Novel Type of Human Fetal Systemic Inflammatory Response. *Am J Reprod Immunol* (2013) 70(4):265–84. doi: 10.1111/aji.12142
170. Katzman PJ, Murphy SP, Oble DA. Immunohistochemical Analysis Reveals an Influx of Regulatory T Cells and Focal Trophoblastic STAT-1 Phosphorylation in Chronic Villitis of Unknown Etiology. *Pediatr Dev Pathol* (2011) 14(4):284–93. doi: 10.2350/10-09-0910-OA.1
171. Gaffuri B, Vigano P, Nozza A, Gornati G, Di Blasio AM, Vignali M. Expression of Intercellular Adhesion Molecule-1 Messenger Ribonucleic Acid and Protein in Human Term Placental Cells and its Modulation by Pro-Inflammatory Cytokines (Interleukin-1beta and Tumor Necrosis Factor Alpha). *Biol Reprod* (1998) 58(4):1003–8. doi: 10.1095/biolreprod58.4.1003
172. Juneja VR, McGuire KA, Manguso RT, LaFleur MW, Collins N, Haining WN, et al. PD-L1 on Tumor Cells is Sufficient for Immune Evasion in Immunogenic Tumors and Inhibits CD8 T Cell Cytotoxicity. *J Exp Med* (2017) 214(4):895–904. doi: 10.1084/jem.20160801
173. Guleria I, Khosroshahi A, Ansari MJ, Habicht A, Azuma M, Yagita H, et al. A Critical Role for the Programmed Death Ligand 1 in Fetomaternal Tolerance. *J Exp Med* (2005) 202(2):231–7. doi: 10.1084/jem.20050019
174. Petroff MG, Chen L, Phillips TA, Azzola D, Sedlmayr P, Hunt JS. B7 Family Molecules are Favorably Positioned at the Human Maternal-Fetal Interface. *Biol Reprod* (2003) 68(5):1496–504. doi: 10.1095/biolreprod.102.010058
175. Enninga EAL, Harrington SM, Creedon DJ, Ruano R, Markovic SN, Dong H, et al. Immune Checkpoint Molecules Soluble Program Death Ligand 1 and Galectin-9 are Increased in Pregnancy. *Am J Reprod Immunol* (2018) 79(2):e12795. doi: 10.1111/aji.12795
176. Labarrere C, Althabe O. Chronic Villitis of Unknown Aetiology in Recurrent Intrauterine Fetal Growth Retardation. *Placenta* (1987) 8(2):167–73. doi: 10.1016/0143-4004(87)90019-1
177. Jacques SM, Qureshi F. Chronic Intervillositis of the Placenta. *Arch Pathol Lab Med* (1993) 117(10):1032–5.
178. Freitag L, von Kaisenberg C, Kreipe H, Hussein K. Expression Analysis of Leukocytes Attracting Cytokines in Chronic Histiocytic Intervillositis of the Placenta. *Int J Clin Exp Pathol* (2013) 6(6):1103–11.
179. Brady CA, Williams C, Batra G, Church E, Tower CL, Crocker IP, et al. Immunomodulatory Therapy Reduces the Severity of Placental Lesions in Chronic Histiocytic Intervillositis. *Front Med (Lausanne)* (2021) 8:753220. doi: 10.3389/fmed.2021.753220
180. Labarrere CA, Hardin JW, Haas DM, Kassab GS. Chronic Villitis of Unknown Etiology and Massive Chronic Intervillositis Have Similar Immune Cell Composition. *Placenta* (2015) 36(6):681–6. doi: 10.1016/j.placenta.2015.03.008
181. Sauvestre F, Mattuizzi A, Sentilhes L, Poingt M, Blanco P, Houssin C, et al. Chronic Intervillositis of Unknown Etiology: Development of a Grading and Scoring System That Is Strongly Associated With Poor Perinatal Outcomes. *Am J Surg Pathol* (2020) 44(10):1367–73. doi: 10.1097/PAS.0000000000001549
182. Sato Y, Maekawa K, Aman M, Yamashita A, Kodama Y, Maki Y, et al. CD39 Downregulation in Chronic Intervillositis of Unknown Etiology. *Virchows Arch* (2019) 475(3):357–64. doi: 10.1007/s00428-019-02598-6
183. Capuani C, Meggetto F, Duga I, Danjoux M, March M, Parant O, et al. Specific Infiltration Pattern of FOXP3+ Regulatory T Cells in Chronic Histiocytic Intervillositis of Unknown Etiology. *Placenta* (2013) 34(2):149–54. doi: 10.1016/j.placenta.2012.12.004
184. Mekinian A, Costedoat-Chalumeau N, Masseau A, Botta A, Chudzinski A, Theulin A, et al. Chronic Histiocytic Intervillositis: Outcome, Associated Diseases and Treatment in a Multicenter Prospective Study. *Autoimmunity* (2015) 48(1):40–5. doi: 10.3109/08916934.2014.939267
185. Koby L, Keating S, Malinowski AK, D'Souza R. Chronic Histiocytic Intervillositis - Clinical, Biochemical and Radiological Findings: An Observational Study. *Placenta* (2018) 64:1–6. doi: 10.1016/j.placenta.2018.02.002
186. Regan L, Braude PR, Hill DP. A Prospective Study of the Incidence, Time of Appearance and Significance of Anti-Paternal Lymphocytotoxic Antibodies in Human Pregnancy. *Hum Reprod* (1991) 6(2):294–8. doi: 10.1093/oxfordjournals.humrep.a137325
187. Honger G, Fornaro I, Granado C, Tiercy JM, Hosli I, Schaub S. Frequency and Determinants of Pregnancy-Induced Child-Specific Sensitization. *Am J Transplant* (2013) 13(3):746–53. doi: 10.1111/ajt.12048
188. Hanania R, Sun HS, Xu K, Pustylnik S, Jeganathan S, Harrison RE. Classically Activated Macrophages Use Stable Microtubules for Matrix Metalloproteinase-9 (MMP-9) Secretion. *J Biol Chem* (2012) 287(11):8468–83. doi: 10.1074/jbc.M111.290676
189. Labarrere CA, Bammerlin E, Hardin JW, Dicarlo HL. Intercellular Adhesion Molecule-1 Expression in Massive Chronic Intervillositis: Implications for the Invasion of Maternal Cells Into Fetal Tissues. *Placenta* (2014) 35(5):311–7. doi: 10.1016/j.placenta.2014.02.006
190. Antonioli L, Pacher P, Vizi ES, Hasko G. CD39 and CD73 in Immunity and Inflammation. *Trends Mol Med* (2013) 19(6):355–67. doi: 10.1016/j.molmed.2013.03.005
191. Clark DA, Dmetrichuk JM, McCready E, Dhesy-Thind S, Arredondo JL. Changes in Expression of the CD200 Tolerance-Signaling Molecule and its Receptor (CD200R) by Villus Trophoblasts During First Trimester Missed Abortion and in Chronic Histiocytic Intervillositis. *Am J Reprod Immunol* (2017) 78(1):e12665. doi: 10.1111/aji.12665
192. Hoek RM, Ruuls SR, Murphy CA, Wright GJ, Goddard R, Zurawski SM, et al. Down-Regulation of the Macrophage Lineage Through Interaction With OX2 (Cd200). *Sci* (2000) 290(5497):1768–71. doi: 10.1126/science.290.5497.1768
193. Nathan C, Muller WA. Putting the Brakes on Innate Immunity: A Regulatory Role for CD200? *Nat Immunol* (2001) 2(1):17–9. doi: 10.1038/83124
194. Hussein K, Stucki-Koch A, Muller AM, Arnold R, Kreipe H, Feist H. Complement Receptor-Associated CD163(+)/CD18(+)/CD11c(+)/CD206(-)/CD209(-) Expression Profile in Chronic Histiocytic Intervillositis of the Placenta. *Placenta* (2019) 78:23–8. doi: 10.1016/j.placenta.2019.02.007
195. Redline RW, Zaragoza M, Hassold T. Prevalence of Developmental and Inflammatory Lesions in Nonmolar First-Trimester Spontaneous Abortions. *Hum Pathol* (1999) 30(1):93–100. doi: 10.1016/s0046-8177(99)90307-6
196. Mandsager NT, Bendon R, Mostello D, Rosenn B, Miodovnik M, Siddiqi TA. Maternal Floor Infarction of the Placenta: Prenatal Diagnosis and Clinical Significance. *Obstet Gynecol* (1994) 83(5 Pt 1):750–4.
197. Adams-Chapman I, Vaucher YE, Bejar RF, Benirschke K, Baergen RN, Moore TR. Maternal Floor Infarction of the Placenta: Association With

Central Nervous System Injury and Adverse Neurodevelopmental Outcome. *J Perinatol* (2002) 22(3):236–41. doi: 10.1038/sj.jp.7210685
198. Spinillo A, Gardella B, Muscettola G, Cesari S, Fiandrino G, Tzialla C. The Impact of Placental Massive Perivillous Fibrin Deposition on Neonatal Outcome in Pregnancies Complicated by Fetal Growth Restriction. *Placenta* (2019) 87:46–52. doi: 10.1016/j.placenta.2019.09.007
199. Sebire NJ, Backos M, Goldin RD, Regan L. Placental Massive Perivillous Fibrin Deposition Associated With Antiphospholipid Antibody Syndrome. *BJOG* (2002) 109(5):570–3. doi: 10.1111/j.1471-0528.2002.00077.x
200. Vernof KK, Benirschke K, Kephart GM, Wasmoen TL, Gleich GJ. Maternal Floor Infarction: Relationship to X Cells, Major Basic Protein, and Adverse Perinatal Outcome. *Am J Obstet Gynecol* (1992) 167(5):1355–63. doi: 10.1016/s0002-9378(11)91716-5
201. Al-Adnani M, Kiho L, Scheimberg I. Recurrent Placental Massive Perivillous Fibrin Deposition Associated With Polymyositis: A Case Report and Review of the Literature. *Pediatr Dev Pathol* (2008) 11(3):226–9. doi: 10.2350/07-06-0306.1
202. Feist H, Turowski G, Hussein K, Blocker T, Heim A. Massive Perivillous Fibrin Deposition of an Enterovirus A-Infected Placenta Associated With Stillbirth: A Case Report. *Pediatr Dev Pathol* (2019) 22(2):142–5. doi: 10.1177/1093526618798772
203. Taweevisit M, Tansatit M, Sutthiruangwong P, Siranart N, Thorner PS. Combined Placental Maternal Floor Infarction and Cytomegalovirus Placentitis: A Case Report. *Fetal Pediatr Pathol* (2020) 39:1–6. doi: 10.1080/15513815.2020.1857487
204. Taweevisit M, Thawornwong N, Thorner PS. Massive Perivillous Fibrin Deposition Associated With Placental Syphilis: A Case Report. *Pediatr Dev Pathol* (2021) 24(1):43–6. doi: 10.1177/1093526620957523
205. Yu W, Tellier R, Wright JRJr. Coxsackie Virus A16 Infection of Placenta With Massive Perivillous Fibrin Deposition Leading to Intrauterine Fetal Demise at 36 Weeks Gestation. *Pediatr Dev Pathol* (2015) 18(4):331–4. doi: 10.2350/15-01-1603-CR.1
206. Gogia N, Machin GA. Maternal Thrombophilias are Associated With Specific Placental Lesions. *Pediatr Dev Pathol* (2008) 11(6):424–9. doi: 10.2350/07-09-0345.1
207. Chaiworapongsa T, Romero R, Korzeniewski SJ, Chaemsaithong P, Hernandez-Andrade E, Segars JH, et al. Pravastatin to Prevent Recurrent Fetal Death in Massive Perivillous Fibrin Deposition of the Placenta (MPFD). *J Matern Fetal Neonatal Med* (2016) 29(6):855–62. doi: 10.3109/14767058.2015.1022864
208. Satoh M, Ajmani AK, Hirakata M, Suwa A, Winfield JB, Reeves WH. Onset of Polymyositis With Autoantibodies to threonyl-tRNA Synthetase During Pregnancy. *J Rheumatol* (1994) 21(8):1564–6.
209. Silva CA, Sultan SM, Isenberg DA. Pregnancy Outcome in Adult-Onset Idiopathic Inflammatory Myopathy. *Rheumatol (Oxf)* (2003) 42(10):1168–72. doi: 10.1093/rheumatology/keg318
210. Hung NA, Jackson C, Nicholson M, Highton J. Pregnancy-Related Polymyositis and Massive Perivillous Fibrin Deposition in the Placenta: Are They Pathogenetically Related? *Arthritis Rheumatol* (2006) 55(1):154–6. doi: 10.1002/art.21710
211. Boog G, Le Vaillant C, Alnoukari F, Jossic F, Barrier J, Muller JY. Combining Corticosteroid and Aspirin for the Prevention of Recurrent Villitis or Intervillositis of Unknown Etiology. *J Gynecol Obstet Biol Reprod (Paris)* (2006) 35(4):396–404. doi: 10.1016/s0368-2315(06)76411-0
212. Boog G. Chronic Villitis of Unknown Etiology. *Eur J Obstet Gynecol Reprod Biol* (2008) 136(1):9–15. doi: 10.1016/j.ejogrb.2007.06.018
213. Ozawa N, Yamaguchi K, Shibata M, Sugibayashi R, Yagi H, Sago H, et al. Chronic Histiocytic Intervillositis in Three Consecutive Pregnancies in a Single Patient: Differing Clinical Results and Pathology According to Treatment Used. *J Obstet Gynaecol Res* (2017) 43(9):1504–8. doi: 10.1111/jog.13404
214. Mekinian A, Houfflin-Debarge V, Kolanska K, Cohen J, Abisror N, Bornes M, et al. Antagonists of TNFalpha for Recurrent Miscarriages: 2 Illustrative Cases. *Eur J Obstet Gynecol Reprod Biol* (2019) 236:263–4. doi: 10.1016/j.ejogrb.2019.02.036
215. Redline RW, Vik T, Heerema-McKenney A, Jamtoy AH, Ravishankar S, Ton Nu TN, et al. Interobserver Reliability for Identifying Specific Patterns of Placental Injury as Defined by the Amsterdam Classification. *Arch Pathol Lab Med* (2021) 146(3):372–8. doi: 10.5858/arpa.2020-0753-OA
216. Kupferminc M, Rimon E, Many A, Maslovitz S, Lessing JB, Gamzu R. Low Molecular Weight Heparin Versus No Treatment in Women With Previous Severe Pregnancy Complications and Placental Findings Without Thrombophilia. *Blood Coagul Fibrinolysis* (2011) 22(2):123–6. doi: 10.1097/MBC.0b013e328343315c
217. Office of National Statistics. *Births in England and Wales: 2020* (2021). Available at: https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/livebirths/bulletins/birthsummarytablesenglandandwales/2020#stillbirths.
218. Hug L, You D, Blencowe H, Mishra A, Wang Z, Fix MJ, et al. Global, Regional, and National Estimates and Trends in Stillbirths From 2000 to 2019: A Systematic Assessment. *Lancet* (2021) 398(10302):772–85. doi: 10.1016/S0140-6736(21)01112-0
219. Turowski G, Tony Parks W, Arbuckle S, Jacobsen AF, Heazell A. The Structure and Utility of the Placental Pathology Report. *APMIS* (2018) 126(7):638–46. doi: 10.1111/apm.12842
220. Evans CC, Cox P. *The Royal College of Pathologists: Tissue Pathway for Histopathological Examination of the Placenta* (2019). Available at: https://www.rcpath.org/uploads/assets/ec614dfa-007c-4a93-8173cb202a071a72/G108-Tissue-pathway-for-histopathological-examination-of-the-placenta.pdf.

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REVIEW
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Multiomics Studies Investigating Recurrent Pregnancy Loss: An Effective Tool for Mechanism Exploration

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Patients with recurrent pregnancy loss (RPL) account for approximately 1%-5% of women aiming to achieve childbirth. Although studies have shown that RPL is associated with failure of endometrial decidualization, placental dysfunction, and immune microenvironment disorder at the maternal-fetal interface, the exact pathogenesis remains unknown. With the development of high-throughput technology, more studies have focused on the genomics, transcriptomics, proteomics and metabolomics of RPL, and new gene mutations and new biomarkers of RPL have been discovered, providing an opportunity to explore the pathogenesis of RPL from different biological processes. Bioinformatics analyses of these differentially expressed genes, proteins and metabolites also reflect the biological pathways involved in RPL, laying a foundation for further research. In this review, we summarize the findings of omics studies investigating decidual tissue, villous tissue and blood from patients with RPL and identify some possible limitations of current studies.

Keywords: omics, villus, decidua, blood, recurrent pregnancy loss

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INTRODUCTION

Recurrent pregnancy loss (RPL), defined as two or more consecutive clinically recognized spontaneous pregnancy losses before 20 weeks of gestation and includes embryonic or fetal loss, is a frequently occurring human infertility-related disease that affects 1-5% of parturients (1). Various factors have been proven to cause the occurrence and development of RPL, including chromosomal abnormalities, genital tract anomalies, immunological diseases, endocrine diseases, antiphospholipid syndrome, thrombophilic disorders and pathogen infections (2). Approximately 40-50% of cases remain unexplained, and the molecular mechanisms have not been fully explored. These cases are defined as unexplained recurrent pregnancy loss (URPL) (3). Although the diagnosis of RPL is relatively clear, the lack of standardized definitions, the uncertainties of its pathogenesis and the variable clinical manifestations still hamper progress in the treatment and prevention of RPL (4).

Omics studies generate a large amount of information concerning the biomarkers, molecular mechanisms and biological pathways involved in complex diseases (5). More recently, due to advances in the development and optimization of high-throughput techniques, numerous studies have applied omics approaches to the study of RPL (6–8). In this review, we summarize the results of omics-based studies conducted using human samples (decidua, villus, and blood) to explain the pathophysiological processes of RPL. The keywords in the included references were omics, omics technology, genomics, epigenomics, transcriptomics, proteomics, metabolomics, recurrent miscarriage, recurrent pregnancy loss, trophoblast, villi, decidua, and blood. We also attempted to perform an integrative analysis of omics data to obtain a global depiction of the complex relationships within and between different biological layers in RPL.

OVERVIEW OF OMICS

Omics technologies, including genomics, epigenomics, transcriptomics, proteomics (9, 10) and metabolomics (11), provide a holistic and integrative approach toward the study of biological systems (12). Through genomics technology, we can collectively characterize and quantitatively analyze all genes of an organism and then study the structure, function, location, and editing of the genome and their impact on the organism. In genomics, genotype arrays and next-generation sequencing are mostly used to obtain copy number variations, single nucleotide variations and small insertions or deletions (13, 14). Epigenomics refers to the study of all epigenetic modifications at the genome level, and epigenetic modifications are heritable. The most pivotal epigenetic modifications are DNA methylation, histone modifications and nucleosome remodeling. The current epigenetic studies investigating RPL mainly focus on DNA methylation (15). The main methods of DNA methylation analyses are bisulfite sequencing, array or bead hybridization, pyrosequencing, methylation-specific polymerase chain reaction (PCR), high -performance liquid chromatography-ultraviolet, and mass spectrometry (MS)-based approaches (16). The transcriptomics approach, including microarrays, bulk RNA sequencing (RNA-seq) and single-cell RNA-seq (scRNA-seq), is often used to study gene expression and regulation. Qualitative and quantitative information concerning mRNA and noncoding RNA is available due to these approaches (17). Proteomics is the systematic and holistic study of the types, structures and functions of proteins expressed in cells or tissues. Proteomics methods mainly include protein microarray, gel-based approaches [two-dimensional polyacrylamide gel electrophoresis (2-DE)], MS-based approaches (18), X-ray crystallography, nuclear magnetic resonance spectroscopy, SOMAmer-based technology, and quantitative techniques, including isotope-coded affinity tag labeling, stable isotope labeling with amino acids in cell culture and isobaric tags for relative and absolute quantitation (iTRAQ) (19, 20). However, most proteomics studies investigating RPL were performed using 2D-DIGE or quantitative techniques, such as iTRAQ combined with MS-based approaches. Metabolomics usually identifies and quantifies metabolites, such as amino acids, lipids, sugars and hormones, *via* nuclear magnetic resonance, gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry. By exploring the relative relationship between metabolites and physiopathological changes, it is possible to find biomarkers for the diagnosis of disease. By studying and integrating data obtained using different omics approaches, knowledge of the underlying molecular interactions and associated longitudinal effects may be discovered and understood more deeply (21). In some studies investigating RPL, omics technologies were used in combination with bioinformatics analyses, and key genes and molecular pathways affecting RPL were identified. Upregulated or downregulated genes have been found to participate in a variety of biological processes important for embryonic/fetal development and fertility (22–24).

ESTABLISHMENT AND MAINTENANCE OF EARLY PREGNANCY

A normal pregnancy starts with the successful implantation of a promising embryo into the receptive endometrium (25). In preparation for implantation, the endometrium undergoes remodeling, which is regulated by estrogen and progesterone. During this timepoint, the endometrial epithelium becomes permissive to the adhesion of embryonic trophectoderm cells (the outer cells of the blastocyst), and then, the embryo implants into the uterus (26). After embryo implantation, the endometrium is gradually decidualized under the stimulation of ovarian hormones and other inducing factors. Decidualization is the differentiation of endometrial stromal cells into secretory decidual stromal cells. This process involves balancing pro- and anti- inflammatory cytokines (27, 28), angiogenesis, and uterine spiral artery remodeling (29). Meanwhile, the aggregation of immune cells (including uterine natural killer cells, macrophages, T cells, and dendritic cells) in the endometrium is involved in regulating the microenvironment that sustains pregnancy (30, 31). Placenta formation is the key process responsible for maintaining the growth and development of the embryo. During this process, the trophectoderm cells of the blastocyst differentiate into the extravillous trophoblast and the villous trophoblast (comprising the cytotrophoblast and syncytiotrophoblast), which form the major cell lineages of the placenta. Cytotrophoblasts can further differentiate into invasive extravillous trophoblasts (32). The proliferation and invasion of extravillous trophoblast cells into the decidua and the uterine myometrium are necessary for uterine spiral artery remodeling and establishing maternal-fetal circulation (33). The villous trophoblast contacts the maternal blood directly, which affects the material exchange among the mother, placenta and fetus (34). Extravillous trophoblast invasion and placental development are also controlled by decidualization. The establishment and maintenance of early pregnancy are represented in **Figure 1**.

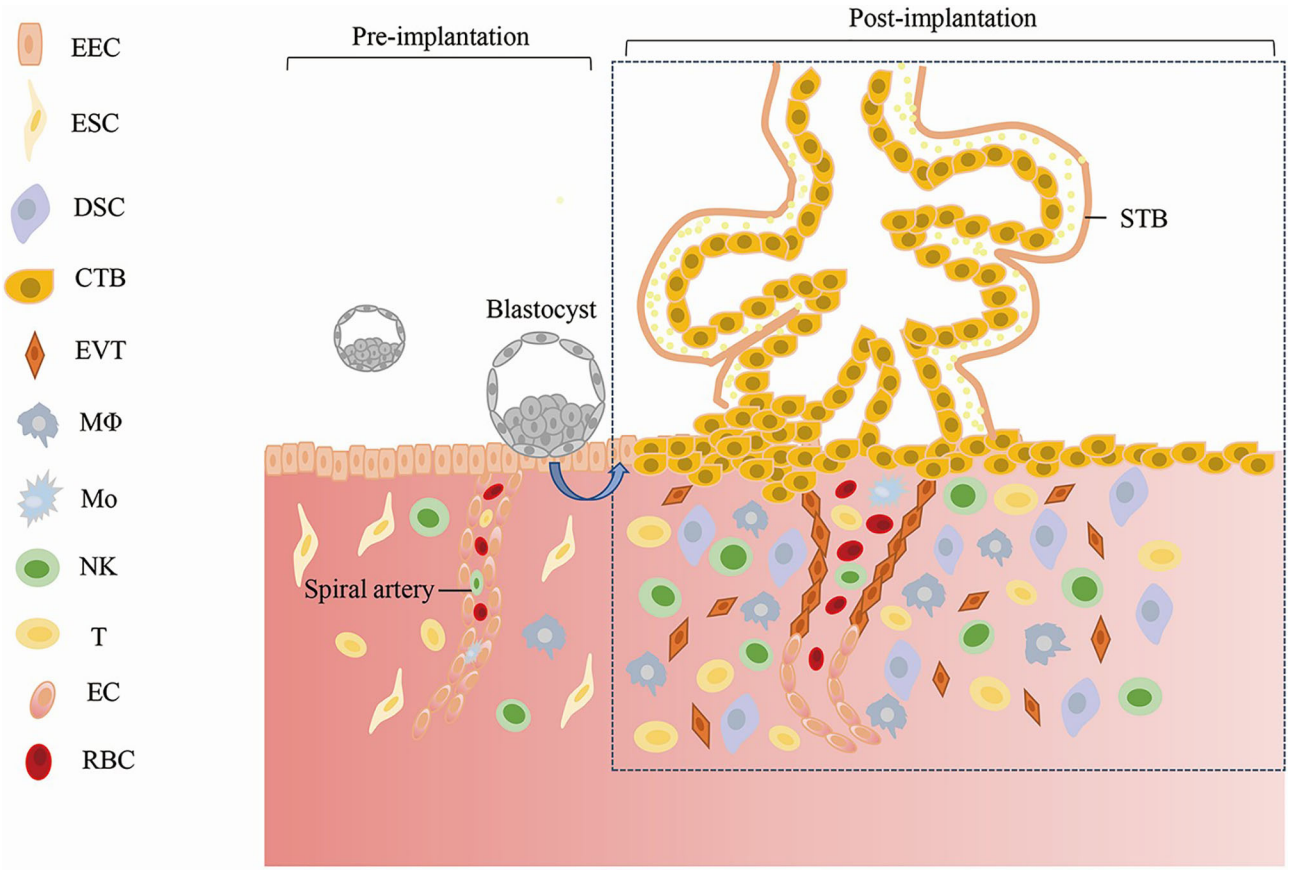

FIGURE 1 | Establishment and Maintenance of Early Pregnancy. EEC, Endometrial Epithelial; Cell ESC, Endometrial Stromal Cell; DSC, Decidual Stromal Cell; CTB, Cytotrophoblast; STB, Syncytiotrophoblast; EVT, Extravillous Trophoblast; MΦ, Macrophages; Mo, Monocyte; NK, Natural Killer Cell; EC, Endothelial Cell; RBC, Red Blood Cell.

OMICS STUDIES INVESTIGATING THE ESTABLISHMENT AND MAINTENANCE OF EARLY PREGNANCY

During early pregnancy, extravillous trophoblasts move upstream along the arterial wall and migrate to, invade, and replace vascular smooth muscle cells and endothelial cells, thereby remodeling the uterine spiral arteries (35). Several cytokines produced by the placenta, such as vascular endothelial growth factor-A (*VEGF-A*), insulin-like growth factor, Krüppel-like factor 17, atrial natriuretic peptide may play important roles in the formation of the maternal-fetal vasculature (35, 36). Amin et al. found that the heterozygous genotype GA was significantly associated with the overexpression and underexpression of *VEGF* mRNA, while the homozygous variant genotype AA only decreased the *VEGF* mRNA levels in RPL patients by genotyping and quantitative real-time PCR (qRT–PCR-PCR) (37). Another similar study indicated that the 3'-untranslated region of *VEGF* resulted in susceptibility to RPL in Korean women (38). A recent study applying RNA sequencing of spontaneously hypertensive stroke-prone rats (SHRSP) found that the gene expression pattern of pregnant SHRSP uterine arteries was dominated by increased reactive oxygen species and downstream effectors of the renin-angiotensin-aldosterone system. The disrupted pathway involved may contribute to adverse vascular remodeling and the resultant placental ischemia and systemic vascular dysfunction (39). Uterine spiral artery remodeling is essential for promoting blood flow to the placenta and fetal development, and omics could help us better understand this process.

Successful decidualization is necessary for a normal pregnancy. By using DNA microarray and cytological verification, Lucas et al. demonstrated that in RPL, the loss of an epigenetic signature was related to the reduced expression of endometrial *HMGB2* [associated with replicative senescence of human fibroblasts (40)], and then perturbed decidualization (41). Recently, scRNA-seq data of highly proliferative mesenchymal cells (hPMCs) in the midluteal human endometrium indicated that hPMC depletion was relevant to RPL. Vascular transmigration- and decidualization -related genes, including interleukin 1 receptor like 1 (42) and prolactin, were highly expressed in hPMC (43). hPMCs play an integral role in decidualization in pregnancy (44). A proteomic analysis also confirmed that decidualization in RPL patients differed from that in normal pregnant women. Dhaenens et al. identified 1416

differentially expressed proteins (DEPs), revealing the higher expression of serotransferrin in RPL samples compared with those in normal fertile samples (45). Another study by Harden et al. showed significant differences in endometrial metabolic profiles between decidualized and nondecidualized endometrium, which may be essential for successful embryo implantation (46).

The imbalance of immune tolerance at the maternal-fetal interface is an important factor for the occurrence of RPL (47). The immunologic events occurring at the maternal-fetal interface in early pregnancy are extremely complex and involve numerous immune cells and molecules with immunoregulatory properties (47). A genome-wide transcriptome profiling of splenic B cells in pregnant and nonpregnant mice found revealed 625 upregulated and 511 downregulated transcripts in B cells from pregnant mice compared with nonpregnant mice, suggesting that B cells acquire a state of hypo-responsiveness during gestation (48). Researchers performing single-cell transcriptomic profiling of decidual tissue revealed a dramatic difference in immune cell subsets and molecular properties in RPL cases (49). In RPL patients, a decidual NK (dNK) subset that supports embryonic growth was diminished in proportion, while the ratio of another dNK subset with cytotoxic and immune-active signatures (such as pro-inflammatory $CD56^{+}CD16^{+}$ dNK subset) was significantly increased (50). Chen et al. found that a subpopulation of $CSF1^{+}CD59^{+}$ KIR-expressing dNK cells was decreased in URPL decidua (51). Treg cells constitute another important immune cell type at the maternal-fetal interface, and the transcriptional and protein expression profiles of endometrial Tregs in RPL differ from those in normal pregnant women (52).

During early pregnancy, the changes in different genes, RNAs, proteins and metabolites associated with vascular remodeling, the abnormal expression of decidualized genes and phenotypic changes in various uterine immune cells all increase the possibility of the occurrence of RPL by affecting embryo implantation and blastocyst development (**Figure 2**). Although the pathogenesis of RPL is known to some extent, specific diagnostic biomarkers and candidate regulatory targets of RPL have not yet been identified. Thus, researchers have conducted various omics studies using decidual tissue, villi tissue, and blood from patients with RPL.

OMICS STUDIES OF DECIDUA IN RECURRENT PREGNANCY LOSS

It is of great significance to study the growth, degradation and functional regulation of maternal decidual tissue to clarify the mechanism of embryo implantation and fertility regulation.

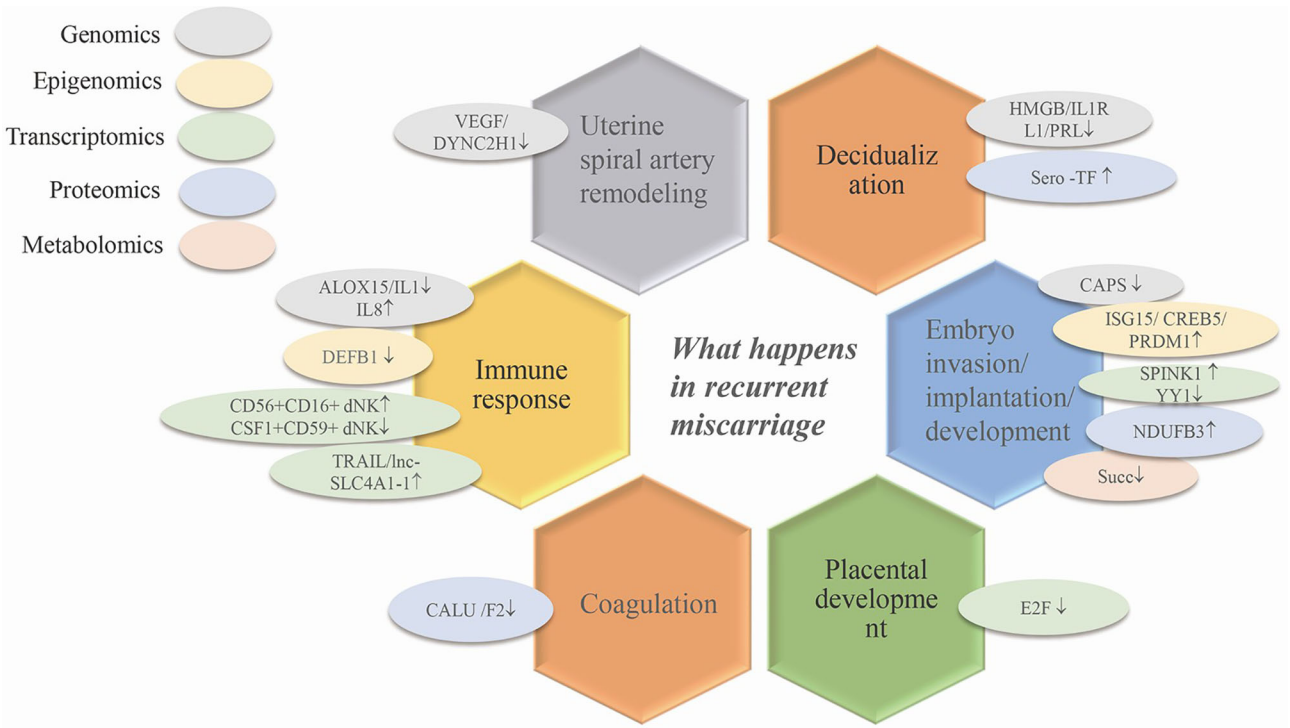

FIGURE 2 | The application of omics techniques in recurrent miscarriage. Ovals of different colors represent different omics studies. VEGF, vascular endothelial growth factor; HMGB, high mobility group box; IL1RL1, interleukin 1 receptor-like 1; PRL, prolactin; ISG15, interferon stimulated gene 15; SPINK1, serine peptidase inhibitor Kazal-type 1; CREB5, cAMP responsive element binding protein5; CAPS, calcyphsine; PRDM1, positive regulatory domain 1; YY1, yin and yang 1 transcription factor; NDUFB3, NADH dehydrogenase (ubiquinone) 1 beta subcomplex 3; E2F, one transcription factor family; IL1, interleukin 1; IL8, IL1, interleukin 1; CD56+CD16+dNK/CSF1+CD59+dNK, transcriptomics *via* single cell sequencing; TRAIL, TNF-related apoptosis-inducing ligand; lnc-SLC4A1-1, solute carrier family 4, anion exchanger, member 1; DEFB1, defensin beta 1; Succ, succinate; CALU, calumenin; F2, coagulation factor II.

Epigenomics has verified that RPL is associated with abnormal DNA modification in decidual cells. In an animal model, the abnormal methylation of the decidua has been demonstrated to be associated with pregnancy failure (53). Li et al. conducted a genome-wide screening of DNA methylation in decidual samples from women with RPL (54). The findings showed that the differentially methylated genes (*PRDM16*, *HLA-E*, *HLA-G*, and *ISG15*) were closely related to embryonic development (55–57). QRT-PCR verification showed that the mRNA expression levels of *ISG15*, *HLA-E*, and *HLA-G* were increased in RPL. Li et al. also verified the upregulated expression of *ISG15* in RPL by utilizing RNA-seq technology, and these authors found that *ISG15* was involved in the type I interferon signaling pathway by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses (54). *CREB5* regulates cell growth, proliferation, differentiation and the cell cycle and belongs to the cAMP response element (*CRE*)-binding protein family (58). The upregulation of *CREB5* has been shown to promote the invasion of tumor cells (59). Yu et al. conducted an analysis of DNA methylation and gene expression, and found that hypomethylation in the *GREB5* promoter regions upregulated the mRNA and protein expression levels of *CREB5* in RPL (60). These authors further proved that *CREB5* increased migration and apoptosis in the HTR8-S/Vneo (human chorionic trophoblast cell line *in vitro*) and JEG-3 (human choriocarcinoma cell) cell lines and prolonged the cell cycle (61). Some studies have shown that *CREB5* expression and methylation are related to the plasma interleukin-6 levels. Moreover, reduced *CREB5* expression in monocytes could cause immunosuppression by increasing the tumor necrosis factor alpha (TNF-α) levels and decreasing the interleukin-10 levels in plasma (62, 63). However, the immune regulation of *CREB5* at the maternal-fetal interface and its contribution to RPL require further verification.

The upregulation of matrix metallopeptidase 26 and the serine peptidase inhibitor Kazal-type 1 (64) are crucial for trophoblastic invasion by regulating the degradation of the extracellular matrix (65). Furthermore, Krieg et al. found that all genes in the interleukin-1 pathway (downregulated) and interleukin-8 pathway (upregulated) were differentially expressed (64), suggesting that the dysregulation of genes related to immunity might contribute to RPL. Transcriptomic data also suggest that the alteration of noncoding RNA (ncRNA) expression profiles in the decidua is related to RPL. NcRNAs refer to RNAs that do not have the potential to encode proteins, including microRNAs (miRNAs), long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs). NcRNAs have complex types and functions that regulate cell activity by controlling gene expression, transcription, and translation processes (66). In the decidua of RPL patients, GO and KEGG pathway analyses suggest that differentially expressed miRNAs are involved in the ErbB signaling pathway and p53 signaling pathway. LncRNAs are involved in the peroxisome proliferator-activated receptor pathway. These signaling pathways participate in the progression of RPL (67, 68). However, the function of ncRNAs in decidual cells has not been further verified. Transcriptomics data have shown the abnormal expression of genes related to embryo invasion, implantation, and development, and immune responses of decidual cells. RPL may be the result of the coregulation of these differentially expressed genes (DEGs). Unfortunately, the regulatory mechanisms of most genes or regulatory factors have not been further explored. In addition, the function of some DEGs in the decidua was further verified in HTR8-S/Vneo and JEG-3 cell lines rather than decidual cells, which is less rigorous.

Proteomics studies investigating decidua also revealed numerous DEPs and pathways involved in the pathogenesis of RPL. The complex I subunit NDUFB3 was involved in mitochondrial respiratory chain function (69), which was significantly increased in the decidua of RPL. The overexpression of NDUFB3 induced oxidative stress and apoptosis in decidual stromal cells by participating in the process of oxidative phosphorylation and affecting mitochondrial function (70). Xiong et al. suggested that 50 DEPs in the decidua were important for embryonic development and revealed that angiotensinogen (AGT) was the most important upstream regulator (71). A proteomic analysis suggested changes in protein levels related to decidual cell growth and embryonic development in decidual tissue of RPL patients, identified more biomarkers of RPL, and helped us better understand the pathogenesis of RPL.

Metabolomics provides an opportunity to study the metabolic state of tissues. Metabolic abnormalities in the decidua could result in immune dysfunction and nutritional disorders, leading to pregnancy complications, including RPL. A recent study found differentially expressed metabolites, including lipids, amino acids and organic acids, in the decidua of RPL (72), indicatingabnormalities in glucose, lipid and amino acid metabolism in decidual cells. Metabolomics studies of RPL decidua are currently limited. However, differentially expressed metabolites in patients with RPL and subsequent functional analyses could be helpful for identifying biomarkers of RPL. Omics studies investigating decidual tissue in RPL are shown in **Table 1**.

OMICS STUDIES OF VILLUS IN RECURRENT PREGNANCY LOSS

Placental trophoblast cells can promote embryo implantation, uterine spiral artery remodeling and placentation *via* their proliferation, invasion, and migration. Furthermore, these cells secrete numerous active substances to regulate maternal-fetal interactions to ensure the normal growth and development of the embryo and fetus (17). It is important to understand the changes in trophoblast cell function in RPL to explain its pathogenesis. Whole-exome sequencing of villi indicate that different pathogenic genes played a vital role in RPL. For example, one patient was found to have compound heterozygous mutations in dynein cytoplasmic 2 heavy chain 1, while another patient exhibited compound heterozygous variants in arachidonate 15-lipoxygenase (74). Mutations in these genes may be individually

TABLE 1 | Omics studies on decidual tissue of recurrent pregnancy loss patients and controls.

Reference(s)	Cell model	Omics strategy	Gestational age	Samples size	Main findings
Li et al., 2020 (54)	Decidua-	Epigenomics (DNA methylation chip)	6 to 12 weeks	RM vs NP 15 vs 15	↑ *ISG15*, *ABR*, *HLA-E*, *HLA-G*
Yu et al., 2018 (60)	Decidua	Epigenomics(DNA methylation chip)	7.715 ± 0.572 weeks	RPL vs NP 20 vs 20	↑ *CREB5*, *RBM24*, *IRF4*, *DPYSL4*
Wang et al., 2016 (67)	Decidua and villus	Transcriptomics (Small RNA deep-sequencing)	8.33 ± 1.80 weeks	RM vs NP 18 vs 15	↑ In decidua: *hsa-mir-516a-5p*, *-517a-3p*, *-519a-3p* and *-519d* ↑ In villus: *hsa-mir-100* and *-146a-5p* ↓ In villus: *hsa-mir-1* and *-372*
Li et al., 2021 (73)	Decidua	Transcriptomics (RNA sequencing)	28 to 82 days	RPL vs NP 15 vs 12	↑ *IFI27*, *ISG15*, *MX1*, *TNFRSF21*
Krieg et al., 2012 (64)	Decidua	Transcriptomics (RNA sequencing)	7 to 11 weeks	RPL vs NP 10 vs 6	↑ *MMP-26*, *SPINK1*, *IL8*, *IL17*, *SCGB2A1*, *HLA-DRB5* ↓ *ZNF*
Huang et al., 2021 (68)	Decidua	Transcriptomics (RNA sequencing)	First trimester	URPL vs NP 50 vs 50	↑ *Lnc-CES1-1*
Dhaenens et al., 2019 (45)	Decidua	Proteomics (LC-HDMS)	6 to 12 weeks	RPL vs NP 3 vs 4	↓ TF
Yin et al., 2021 (70)	Decidua	Proteomics (iTRAQ technology, LC-MS/MS)	57.25 ± 9.16 days	RPL vs NP 6 vs 6	↑ COX-2, NDUFB3
Wang et al., 2021 (72)	Decidua	Metabolomics (LC-ESI-MS/MS system)	63.83 ± 7.09 days	RPL vs NP 23 vs 30	↑ l-citrulline, SM ↓ NAAGA, CAR, PC, PE, PS, PG, LPC, LPE

RM, recurrent miscarriage; RPL, recurrent pregnancy loss; URPL, unexplained recurrent pregnancy loss; TA, elective terminations; NP, normal pregnancy; PI, primary infertility; ABR, activator of RhoGEF and GTPase; HLA-E, major histocompatibility complex, class I, E; HLA-G, major histocompatibility complex, class I, G; RBM24, RNA binding motif protein 24; IRF4, interferon regulatory factor 4; DPYSL4, dihydropyrimidinase like 4; IFI27, interferon alpha inducible protein 27; MX1, myxovirus resistance 1; TNFRSF21, tumor necrosis factor receptor superfamily, member 21; MMP-26, matrix metallopeptidase 26; SCGB2A1, secretoglobin family 2A member 1; HLA-DRB5, major histocompatibility complex, class II, DR beta 5; RPS25, 40S ribosomal protein S25; ACADVL, very long-chain specific acyl-CoA dehydrogenase; TF, serotransferrin; COX2, cytochrome c oxidase subunit 1; SM, sphingomyelin; NAAGA, N-acetylaspartylglutamic acid; CAR, carnitine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PG, phosphatidylglycerol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine.

↑, upregulation; ↓, downregulation.

or jointly involved in the occurrence of RPL by affecting inflammation, the oxidative stress response and angiogenesis (75, 76) at the maternal-fetal interface.,

Changes in DNA methylation affect the levels of gene expression. Positive regulatory domain 1 (*PRDM1*) is a transcription inhibitor that plays a role in embryonic development (95). In RPL, hypomethylation near the transcription start site of *PRDM1* can upregulate the expression of *PRDM1*, leading to increased apoptosis and migration of trophoblast cells (78). *PRDM1* hypomethylation plays a regulatory role by recruiting transcription factors, such as Forkhead boxA1 and GATA binding protein 2, and then regulating the differentiation and development of trophoblast cells (96). Another study found that in placental villus tissue of RPL patients, the β-defensinb1 gene involved in the innate immune response had decreased methylation at the promoter region (77, 97).

Transcriptomics is widely used in the study of villi tissue of RPL. RNA sequencing results showed that Yin Yang 1 (*YY1*) mRNA expression was reduced in the trophoblasts of RPL (83). *YY1* is a transcription factor involved in embryogenesis. *YY1* can enhance the invasion and proliferation of trophoblasts by directly binding the promoter region of the matrix metalloproteinase 2 gene, which is involved in extracellular matrix remodeling during trophoblast invasion (83, 98, 99). The mRNA expression levels of placental TNF-related apoptosis-inducing ligand and S100 calcium binding protein A8 were confirmed to be elevated in RPL (84). Both genes are associated with cell apoptosis and the immune response (100, 101), and their increased expression levels indicate fetal rejection and abnormalities in trophoblasts. Another study found that most of DEGs in trophoblastic cells of RPL could bind the transcription Factor E2F (82). E2F plays an important role in maintaining trophoblastic cell function and placental development, and the absence of E2F causes disruption in the placental transcriptional network and leads to fetal death (102). Other studies have focused on the role of ncRNAs in RPL, and the differentially expressed ncRNAs participate in biological pathways, including immunity, apoptosis and hormonal regulation (79, 80). Huang et al. identified increased *lnc-SLC4A1-1* expression in URPL patients, which activated interleukin-8 (IL-8), and then exacerbated the inflammatory response of trophoblastic cells by enhancing the release of TNF-α and IL-1β (81).Transcriptome studies have revealed that the altered expression of genes or regulatory factors was associated with trophoblast proliferation, invasion, migration, and apoptosis, resulting in placental dysfunction and embryo failure to survive. In addition, the function of trophoblasts during pregnancy is affected by the immune response at the maternal-fetal interface. DEGs involved in the balance between pro- and anti-inflammatory responses also contribute to the development of RPL.

Recently, researchers have attempted to use proteomics technology to screen proteins associated with RPL in villous tissue. Compared with the normal condition, numerous DEPs in RPL, including AGT, mitogen-activated protein kinase14

(MAPK14) and Prothrombin (F2), were associated with early embryonic development (85). Among these proteins, AGT belongs to the renin-angiotensin system, MAPK14 participates in MAPK pathways, and defects in F2 lead to susceptibility to thrombosis. All these biological pathways play a role in RPL (103–105). Proteins related to regulating the function of endothelial cells and coagulation, including calumenin (CALU) and enolase 1 (ENO1), were also differentially expressed in villi (86). Defects in CALU promote coagulation and thrombosis, and a lack of ENO1 renders the placenta intolerant to hypoxia, eventually resulting in RPL (106, 107).

Research concerning the metabolomics of villous tissue in RPL is limited. A recent study showed that low succinate accumulation in villi participated in the occurrence of RPL by reducing the invasion and migration of trophoblastic cells (87). Omics of the villi of RPL patients indicated new biomarkers for the diagnosis and treatment of RPL; however, further research is needed to reveal how these differential molecules work and how they are regulated. Omics studies investigating villous tissue in RPL are shown in **Table 2**.

OMICS STUDIES OF BLOOD IN RECURRENT PREGNANCY LOSS

To explore the mechanism of RPL, researchers have conducted omics studies using maternal blood from RPL patients to find disease-causing genes and biomarkers of RPL. Genomics data show that a homozygous frameshift mutation in calcyphosine (*CAPS*) might be the potential pathogenesis of RPL (90). *CAPS* encodes a Ca^{2+}-binding protein and is involved in the crosstalk between Ca^{2+} signaling and cAMP-protein kinase A pathways, which is crucial for embryo implantation and pregnancy maintenance (108, 109). Another molecule associated with embryo adhesion, trophinin, has also been found to be mutated in RPL. Functional defects in trophinin led to failed implantation and eventually evolved into RPL (110, 111). Genomics is a good way to identify potential targets or biomarkers for the diagnosis and treatment of RPL. These mutated genes are involved in various pathways, including cell adhesion between trophoblasts and the endometrium, embryo implantation, angiogenesis, and extracellular matrix remodeling.

TABLE 2 | Omics studies on villous tissue of recurrent pregnancy loss patients and controls.

Reference(s)	Cell model	Omics strategy	Gestational age	Samples size	Main findings
Qiao et al., 2016 (74)	Villus	Genomics (Whole exome sequencing)	First trimester	RPL vs NP 7 vs 2	Compound heterozygous mutations in *DYNC2H1* and *ALOX15*
Hanna et al., 2013 (77)	Villus	Epigenomics(DNA methylation chip)	9.5 ± 2.4 weeks	RM vs TA 33 vs 16	↑ *CYP1A2, AXL, H19/IGF2*, *ICR1* ↓*DEFB1* (marginally)
Du et al., 2019 (78)	Villus	Epigenomics, Transcriptomics (DNA methylation chip, RNA sequencing)	7.826 ± 0.630 weeks	RPL vs NP 27 vs 25	↑ *PRDM1*
Wang et al., 2016 (79)	Villus	Transcriptomics (lncRNA array)	3 to 6 weeks	RPL vs NP 5 vs 5	↑ *SCARNA9*, *DIO3OS*, *H2AZ2-DT*, *RP11-379F4.4* ↓ *PRINS*, *BMP1*, *TCL6*, *CTA833B7.2*, *RPINS*
Tang et al., 2015 (80)	Villus	Transcriptomics (miRNA microarray)	3 to 6 weeks	RPL vs NP 15 vs 15	↑ *MiR-149-3p*, *miR-4417, miR-4497* and *miR-3651* ↓ *MiR-181d*, *miR-29b-1-5p*, *miR-24-1-5p*
Huang et al., 2018 (81)	Villus	Transcriptomics (RNA sequencing)	9.83 ± 1.25 weeks	URPL vs NP 50 vs 50	↑ *Lnc-ERGIC1-4*, *lnc-MRPS30-5*, *lnc-RCAN1-1*, *lnc-SLC4A1-1*, *lnc-TMEM135-8*, *lnc-CES1-1* ↓ *Lnc-FGGY-4*, *lncPBK-2*, *lnc-SOX4-1*, *lncAC106873.4.1-8*
Sõber et al., 2016 (82)	Villus	Transcriptomics (RNA sequencing)	44 to 67 days	RPL vs NP 2 vs 8	↑ *ATF4*, *C3*, *PHLDA2*, *GPX4*, *ICAM1*, *SLC16A2* ↓ *HIST1H1B*, *HIST1H4A*
Tian et al., 2016 (83)	Villus	Transcriptomics (mRNA microarray)	6 to 12 weeks	RPL vs NP 31 vs 36	↑ *CDC20*, *CTSF*, *CCR7*, *NUF2* ↓ *IGFBP1*, *YY1*, *FGF7*, *CCNA2*
Rull et al., 2012 (84)	Villus	Transcriptomics (RNA microarray)	67.7 ± 6.6 days	RPL vs NP 13 vs 23	↑ *S100A8*, *TRAIL*
Pan et al., 2017 (85)	Villus	Proteomics (iTRAQ labeling, LC-ESI-MS/MS)	6 to 10 weeks	RPL vs NP 4 vs 4	↑ AGT, APOC1, SLC1A3, GOLT1B, PRELP ↓ REEP6, DNTTIP2, NOLC1, SEC11C, SRSF3
Gharesi et al., 2014 (86)	Villus	Proteomics (2D-PAGE, MALDI TOF/TOF technique)	14.8 ± 2.6 weeks	RPL vs NP 5 vs 5	↓ CALU andENO1 ↑CTSD, TUBB, TUBA1, GST, PHB, ACTB
Wang et al., 2021 (87)	Decidua and villus	Metabolomics (Nuclear magnetic resonance)	First trimester	RPL vs NP 30 vs 30	↓ Succinate ↑ SDHB

DYNC2H1, dynein cytoplasmic 2 heavy chain 1; ALOX15, arachidonate 15-lipoxygenase; CYP1A2, cytochrome P450 family 1 subfamily A member 2; AXL, receptor tyrosine kinase, H19, imprinted maternally expressed transcript; IGF2, insulin like growth factor 2; DEFB1, defensin beta 1; SCARNA9, small Cajal body-specific RNA 9; DIO3OS, opposite strand upstream RNA; PRINS, psoriasis associated non-protein coding RNA induced by stress; BMP1, bone morphogenetic protein 1; TCL6, T cell leukemia/lymphoma 6; PRINS, psoriasis associated non-protein coding RNA induced by stress; ATF4, activating transcription factor 4; C3, component 3, PHLDA2, pleckstrin homology like domain family A member 2; GPX4, glutathione peroxidase 4; ICAM1, intercellular adhesion molecule 1; SLC16A2, solute carrier family 16 member 2; HIST1H, H1.5 linker histone, cluster member; HIST1H4A, H4 clustered histone 1; CDC20, cell division cycle 20; CTSF, cathepsin F; CCR7, C-C motif chemokine receptor 7; NUF2, NUF2 component of NDC80 kinetochore complex; IGFBP1, insulin like growth factor binding protein 1; FGF7, fibroblast growth factor 7; CCNA2, cyclin A2; S100A8, S100 calcium binding protein A8; APOC1, apolipoprotein C1; SLC1A3, solute carrier family 1 member 3; GOLT1B,golgi transport 1B; PRELP, proline and arginine rich end leucine rich repeat protein; REEP6, receptor accessory protein 6; DNTTIP2, deoxynucleotidyltransferase terminal interacting protein 2; NOLC1, nucleolar and coiled-body phosphoprotein 1; SEC11C, homolog C, signal peptidase complex subunit; SRSF3, serine and arginine rich splicing factor 3; CTSD, cathepsin D; TUBB, tubulin beta; TUBA1, tubulin alpha 1; GST, glutathione S-transferas; PHB, prohibitin; ACTB, actin beta; SDHB, succinate dehydrogenase B.
↑, upregulation; ↓, downregulation.

However, the extent to which these genes play a role in RPL has not been further verified.

The study of epigenomics in the maternal blood of RPL females is limited. Only one study showed that the methylenetetrahydrofolate reductase (*MTHFR*) gene was specifically hypermethylated in RPL (91). *MTHFR* is a thrombophilic marker involved in global DNA methylation. The methylation of the *MTHFR* gene alters the transmethylation cycle and leads to other gene methylation abnormalities, ultimately contributing to the development of RPL (112).

Using a proteomics approach, researchers have found that DEPs, including CD45, pregnancy-specific glycoprotein 1, and peroxiredoxin-2, act as predictive and diagnostic biomarkers of RPL (92). These proteins affect the invasiveness of trophoblastic cells through the Fc gamma R-mediated phagocytosis pathway, and the regulation of reactive species oxygen. Furthermore, proteins, such as insulin-like growth factor-binding protein-related protein 1, dickkopf-related protein 3 and angiopoietin-2, are significantly decreased in RPL, but the mechanism by which these proteins contribute to RPL is unclear (93).

The study of metabolomics in patients with RPL is limited. The only data available show that the levels of metabolites related to the tricarboxylic acid cycle and phenylalanine metabolism in RPL females significantly differ from those in normal controls. Lactic acid was increased, while 5-methoxytryptamine was lower in RPL patients (94). Testing metabolomics in blood provides new clues for the understanding of disease. The dysfunction of glucose, lipid and amino acid metabolism in RPL can be revealed by comparing the metabolic profiles of blood in RPL with those in normal pregnant women. This disorder might cause dysfunction of the uterus and placenta and further inhibit the growth and development of the embryo. Additionally, analyzing biomarkers in blood could to some extent reflect the condition of the decidua and villi of females and provide a new perspective for explaining the abnormal function of trophoblasts and decidual cells in RPL patients. Omics studies investigating blood in RPL are shown in **Table 3**.

In this review, we attempted to show the common genes, proteins and metabolites by integrating and analyzing data from references (**Table 4**). However, due to the limited research available and the incomplete data provided in some references, the results we obtained are not optimistic. The commonly altered genes and proteins shown in **Table 4** were derived from the results of two or more studies.

ADVANTAGES AND LIMITATIONS OF OMICS STUDIES OF RECURRENT PREGNANCY LOSS

With the development of high-throughput technology, different omics technologies have been continuously improved, providing a new perspective for the mechanistic study of RPL. The genomics technique accurately identifies the disease-causing genes of RPL and discovers the types of defective genes. Epigenomics studies do not change the DNA sequence but allow us to understand the mechanism of RPL from the perspective of gene modification. Transcriptomics suggests the regulatory mechanism of RPL-related RNA. Proteomics and metabolomics provide new biomarkers and analyze the biological pathways, in which these molecules participate. By combining these omics findings, we infer that most differentially expressed molecules associated with RPL are involved in the processes of decidualization, embryo implantation, trophoblast cell differentiation, invasion and apoptosis, placental development, fetal development, the immune response, and coagulation. Omics research investigating RPL is an emerging field, and there are still some shortcomings in the related

TABLE 3 | Omics studies on blood of recurrent pregnancy loss patients and controls.

Reference(s)	Cell model	Omics strategy	Gestational age	Samples size	Results
Quintero et al., 2017 (88)	Blood leucocytes	Genomics (Whole exome sequencing)	Before 20 weeks	RPL: 49	Mutations in *MMP10* and *FGA*
Maddirevula et al., 2020 (89)	Blood	Genomics (Whole exome sequencing)	First trimester	RPL vs PI 61 vs 14	Homozygous variant in *CCDC68*, *CBX3*, *CENPH*, *PABPC1L*, *PIF1*, *PLK1*, and *REXO4*
Pan et al., 2018 (90)	Blood	Genomics (Whole exome sequencing)	First trimester	RPL vs NP 5 vs 5	The homozygous mutation in *CAPS*
Mishra et al., 2019 (91)	Blood	Epigenomics (DNA methylation chip)	Before 24 weeks	RPL vs NP 28 vs 39	↑ *MTHFR*
Cui et al., 2019 (92)	Blood	Proteomics (iTRAQ technology, LC-MS/MS)	55.80 ± 5.85 days	RPL vs NP 30 vs 30	↓ B4DTF1, PSBG-1 ↑ B4DF70
Wu et al., 2017 (93)	Blood	Proteomics (Antibody array assay)	First trimester	RPL vs NP 60 vs 20	↓ IGFBP-rp1/IGFBP-7, Dkk3, ANGPT2
Li et al., 2018 (94)	Blood	Metabolomics (GC-MS, LC-MS)	Less than 10 weeks	RPL vs NP 50 vs 51	↑ Lactic acid ↓ 5-methoxytryptamine

MMP-10, matrix metallopeptidase 10; FGA, fibrinogen alpha chain; CCDC68, coiled-coil domain containing 68; CBX3, chromobox 3; CENPH, centromere protein H; PABPC1L, poly(A) binding protein cytoplasmic 1 like; PIF1, 5′-to-3′ DNA helicase; PLK1, polo like kinase 1; REXO4, REX4 homolog, 3′-5′ exonuclease; B4DTF1, highly similar to Pregnancy-specific beta-1-glycoprotein 9; PSBG-1, pregnancy-specific beta-1-glycoprotein 1; B4DF70, highly similar to peroxiredoxin-2; IGFBP 7, insulin-like growth factor-binding protein 7; Dkk3, dickkopf-related protein 3; ANGPT2, angiopoietin-2.

↑, upregulation; ↓, downregulation.

TABLE 4 | The Common Genes, Proteins, Metabolites Changed in recurrent pregnancy loss patients compared with normal pregnancy females.

	Decidua	Villus	Blood
The Common Genes with Differentially Methylated Regions	*C2ORF54, TMEM161B, TBX3, SKI, TAL1, GPR137B, ITGA4* (54, 60)	*CYP1A2, DEFB1, APC, AXL, CDKN1C, MEG3, PLAGL1* (77, 78)	Only One Reference (91)
The Common mRNAs	*IFI44L, IFIT1, TNFRSF21, MMP26* (64, 73)	No Common mRNAs (82–84)	No Reference
The Common Proteins	ALB, NACA4P, KRT2, FARSA, CAT, TUBA8 (45, 70)	No Common Proteins (85, 86)	ITIH4, IGFBP4 (92, 93)
The Common metabolites	Only One Reference (72)	Only One Reference (87)	Only One Reference (94)

*Genomics data do not intersect and are therefore not represented in the **Table 4**. There are many types of non-coding RNAs while few related transcriptomic studies, the sequencing results cannot be intersected. C2ORF54, chromosome 2 open reading frame 54; TMEM161B, transmembrane protein 161B; TBX3, T-box transcription factor 3; SKI, v-ski avian sarcoma viral oncogene homolog; TAL1, T-cell acute lymphocytic leukemia 1; GPR137B, G protein-coupled receptor 137B; ITGA4, integrin subunit alpha 4; APC, adenomatosis polyposis coli; CDKN1C, cyclin dependent kinase inhibitor 1C; MEG3, maternally expressed 3; PLAGL1, pleiomorphic adenoma gene-like 1; IFI44L, interferon induced protein 44 like; IFIT1, interferon induced protein with tetratricopeptide repeats 1; TNFRSF21, TNF receptor superfamily member 21; MMP26, matrix metallopeptidase 26; ALB, albumin; NACA4P, putative nascent polypeptide-associated complex subunit alpha-like protein; KRT2, type II cytoskeletal 2 epidermal; FARSA, phenylalanine–tRNA ligase alpha subunit; CAT, catalase; TUBA8, tubulin alpha-8 chain; ITIH4, inter-alpha-inhibitor heavy chain 4; IGFBP4, insulin-like growth factor-binding protein 4.*

research. First, the sample sizes in most studies are small, the reproducibility of the data is poor, and it cannot be ruled out that these experimental results are caused by individual differences. Therefore, further studies with a larger sample size are needed to support the current results. Second, most studies were only performed with omics combined with a bioinformatics analysis. The results only show the potential pathways or regulation modules in which these molecules participate. To illustrate the specific mechanisms of these molecules in RPL, more basic research is needed. Third, most current studies were conducted at the single-omics level, and few studies integrated multiomics to verify the pathogenesis of RPL. Subsequent studies should perform multiple omics tests in patients with RPL, integrate information from different omics, and delve deeper into the pathogenesis of RPL. Finally, no studies performed proteomics in RPL to determine the posttranslational modifications of proteins, such as phosphorylation, ubiquitination, acetylation, and glycosylation, which can verify the biological processes more precisely. In addition, isotope labeling in differential metabolites can be used in a metabolic flux analysis to better understand the dynamics of metabolic systems in RPL. In summary, there is still a long way to go for research investigating the etiology, treatment and prevention of RPL based on high-throughput omics.

CONCLUSION

The pathogenesis of RPL is complex, and related to hormonal, environmental, and genetic factors. Furthermore, the causes of approximately 50% of RPL cases are unknown, and its incidence rate is increasing. Although assisted reproductive technology can be used to promote the pregnancy rate, the success rate is not very satisfactory. RPL patients are often under tremendous physical and psychological pressure. Therefore, in future studies, it is indispensable to comprehensively analyze the pathogenesis of RPL from different biochemical and biophysical processes. As omics investigates pathomechanisms from multiple dimensions, such as DNA, RNA, proteins, and metabolites, we hope that multiomics could provide more clues regarding the diagnosis and treatment of PRL.

AUTHOR CONTRIBUTIONS

JL, LW and JD contributed equally to the preparation and the writing of the manuscript. YC and LD critically provided critical feedback and helped shape the manuscript. LL, YZ and TY supervised and revised the manuscript. All authors approved the final version of the manuscript for publication.

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REFERENCES

1. Rai R, Regan L. Recurrent Miscarriage. *Lancet* (2006) 368(9535):601–11. doi: 10.1016/S0140-6736(06)69204-0
2. Li TC, Makris M, Tomsu M, Tuckerman E, Laird S. Recurrent Miscarriage: Aetiology, Management and Prognosis. *Hum Reprod Update* (2002) 8 (5):463–81. doi: 10.1093/humupd/8.5.463
3. Allison JL, Schust DJ. Recurrent First Trimester Pregnancy Loss: Revised Definitions and Novel Causes. *Curr Opin Endocrinol Diabetes Obes* (2009) 16(6):446–50. doi: 10.1097/MED.0b013e3283327fc5
4. Dimitriadis E, Menkhorst E, Saito S, Kutteh WH, Brosens JJ. Recurrent Pregnancy Loss. *Nat Rev Dis Primers* (2020) 6(1):98. doi: 10.1038/s41572-020-00228-z
5. Karahalil B. Overview of Systems Biology and Omics Technologies. *Curr Med Chem* (2016) 23(37):4221–30. doi: 10.2174/0929867323666160926150617

6. Rull K, Nagirnaja L, Laan M. Genetics of Recurrent Miscarriage: Challenges, Current Knowledge, Future Directions. *Front Genet* (2012) 3:34. doi: 10.3389/fgene.2012.00034
7. Hong L, Zhu YC, Liu S, Wu T, Li Y, Ye L, et al. Multi-Omics Reveals a Relationship Between Endometrial Amino Acid Metabolism and Autophagy in Women With Recurrent Miscarriagedagger. *Biol Reprod* (2021) 105 (2):393–402. doi: 10.1093/biolre/ioab101
8. Du L, Deng W, Zeng S, Xu P, Huang L, Liang Y, et al. Single-Cell Transcriptome Analysis Reveals Defective Decidua Stromal Niche Attributes to Recurrent Spontaneous Abortion. *Cell Prolif* (2021) 54(11): e13125. doi: 10.1111/cpr.13125
9. Hasin Y, Seldin M, Lusis A. Multi-Omics Approaches to Disease. *Genome Biol* (2017) 18(1):83. doi: 10.1186/s13059-017-1215-1
10. Manzoni C, Kia DA, Vandrovcova J, Hardy J, Wood NW, Lewis PA, et al. Genome, Transcriptome and Proteome: The Rise of Omics Data and Their Integration in Biomedical Sciences. *Brief Bioinform* (2018) 19(2):286–302. doi: 10.1093/bib/bbw114
11. Worheide MA, Krumsiek J, Kastenmuller G, Arnold M. Multi-Omics Integration in Biomedical Research - A Metabolomics-Centric Review. *Anal Chim Acta* (2021) 1141:144–62. doi: 10.1016/j.aca.2020.10.038
12. Conesa A, Beck S. Making Multi-Omics Data Accessible to Researchers. *Sci Data* (2019) 6(1):251. doi: 10.1038/s41597-019-0258-4
13. Boycott KM, Vanstone MR, Bulman DE, MacKenzie AE. Rare-Disease Genetics in the Era of Next-Generation Sequencing: Discovery to Translation. *Nat Rev Genet* (2013) 14(10):681–91. doi: 10.1038/nrg3555
14. Stamou MI, Cox KH, Crowley WFJr. Discovering Genes Essential to the Hypothalamic Regulation of Human Reproduction Using a Human Disease Model: Adjusting to Life in the "-Omics" Era. *Endocr Rev* (2015) 36(6):603–21. doi: 10.1210/er.2015-1045
15. Zhou Q, Xiong Y, Qu B, Bao A, Zhang Y. DNA Methylation and Recurrent Pregnancy Loss: A Mysterious Compass? *Front Immunol* (2021) 12:738962. doi: 10.3389/fimmu.2021.738962
16. Kurdyukov S, Bullock M. DNA Methylation Analysis: Choosing the Right Method. *Biol (Basel)* (2016) 5(1):3. doi: 10.3390/biology5010003
17. Jaremek A, Jeyarajah MJ, Jaju Bhattad G, Renaud SJ. Omics Approaches to Study Formation and Function of Human Placental Syncytiotrophoblast. *Front Cell Dev Biol* (2021) 9:674162. doi: 10.3389/fcell.2021.674162
18. Aslam B, Basit M, Nisar MA, Khurshid M, Rasool MH. Proteomics: Technologies and Their Applications. *J Chromatogr Sci* (2017) 55(2):182–96. doi: 10.1093/chromsci/bmw167
19. Shiio Y, Aebersold R. Quantitative Proteome Analysis Using Isotope-Coded Affinity Tags and Mass Spectrometry. *Nat Protoc* (2006) 1(1):139–45. doi: 10.1038/nprot.2006.22
20. Kroksveen AC, Jaffe JD, Aasebo E, Barsnes H, Bjorlykke Y, Franciotta D, et al. Quantitative Proteomics Suggests Decrease in the Secretogranin-1 Cerebrospinal Fluid Levels During the Disease Course of Multiple Sclerosis. *Proteomics* (2015) 15(19):3361–9. doi: 10.1002/pmic.201400142
21. Sun YV, Hu YJ. Integrative Analysis of Multi-Omics Data for Discovery and Functional Studies of Complex Human Diseases. *Adv Genet* (2016) 93:147–90. doi: 10.1016/bs.adgen.2015.11.004
22. Filges I, Nosova E, Bruder E, Tercanli S, Townsend K, Gibson WT, et al. Exome Sequencing Identifies Mutations in KIF14 as a Novel Cause of an Autosomal Recessive Lethal Fetal Ciliopathy Phenotype. *Clin Genet* (2014) 86(3):220–8. doi: 10.1111/cge.12301
23. Tsurusaki Y, Yonezawa R, Furuya M, Nishimura G, Pooh RK, Nakashima M, et al. Whole Exome Sequencing Revealed Biallelic IFT122 Mutations in a Family With CED1 and Recurrent Pregnancy Loss. *Clin Genet* (2014) 85 (6):592–4. doi: 10.1111/cge.12215
24. Ellard S, Kivuva E, Turnpenny P, Stals K, Johnson M, Xie W, et al. An Exome Sequencing Strategy to Diagnose Lethal Autosomal Recessive Disorders. *Eur J Hum Genet* (2015) 23(3):401–4. doi: 10.1038/ejhg.2014.120
25. Cha J, Sun X, Dey SK. Mechanisms of Implantation: Strategies for Successful Pregnancy. *Nat Med* (2012) 18(12):1754–67. doi: 10.1038/nm.3012
26. Evans J, Salamonsen LA, Winship A, Menkhorst E, Nie G, Gargett CE, et al. Fertile Ground: Human Endometrial Programming and Lessons in Health and Disease. *Nat Rev Endocrinol* (2016) 12(11):654–67. doi: 10.1038/nrendo.2016.116
27. Rytkonen KT, Erkenbrack EM, Poutanen M, Elo LL, Pavlicev M, Wagner GP. Decidualization of Human Endometrial Stromal Fibroblasts Is a Multiphasic Process Involving Distinct Transcriptional Programs. *Reprod Sci* (2019) 26(3):323–36. doi: 10.1177/1933719118802056
28. Erkenbrack EM, Maziarz JD, Griffith OW, Liang C, Chavan AR, Nnamani MC, et al. The Mammalian Decidual Cell Evolved From a Cellular Stress Response. *PloS Biol* (2018) 16(8):e2005594. doi: 10.1371/journal.pbio.2005594
29. Sato Y. Endovascular Trophoblast and Spiral Artery Remodeling. *Mol Cell Endocrinol* (2020) 503:110699. doi: 10.1016/j.mce.2019.110699
30. Wang F, Qualls AE, Marques-Fernandez L, Colucci F. Biology and Pathology of the Uterine Microenvironment and its Natural Killer Cells. *Cell Mol Immunol* (2021) 18(9):2101–13. doi: 10.1038/s41423-021-00739-z
31. Murata H, Tanaka S, Okada H. Immune Tolerance of the Human Decidua. *J Clin Med* (2021) 10(2):351. doi: 10.3390/jcm10020351
32. Lee BK, Kim J. Integrating High-Throughput Approaches and *In Vitro* Human Trophoblast Models to Decipher Mechanisms Underlying Early Human Placenta Development. *Front Cell Dev Biol* (2021) 9:673065. doi: 10.3389/fcell.2021.673065
33. Huppertz B. Traditional and New Routes of Trophoblast Invasion and Their Implications for Pregnancy Diseases. *Int J Mol Sci* (2019) 21(1). doi: 10.3390/ijms21010289
34. Lunghi L, Ferretti ME, Medici S, Biondi C, Vesce F. Control of Human Trophoblast Function. *Reprod Biol Endocrinol* (2007) 5:6. doi: 10.1186/1477-7827-5-6
35. Albrecht ED, Pepe GJ. Regulation of Uterine Spiral Artery Remodeling: A Review. *Reprod Sci* (2020) 27(10):1932–42. doi: 10.1007/s43032-020-00212-8
36. Wang C, Wang Z, He M, Zhou T, Niu Y, Sun S, et al. Kruppel-Like Factor 17 Upregulates Uterine Corin Expression and Promotes Spiral Artery Remodeling in Pregnancy. *Proc Natl Acad Sci USA* (2020) 117(32):19425–34. doi: 10.1073/pnas.2003913117
37. Amin I, Pandith AA, Manzoor U, Mir SH, Afroze D, Koul AM, et al. Implications of VEGF Gene Sequence Variations and its Expression in Recurrent Pregnancy Loss. *Reprod BioMed Online* (2021) 43(6):1035–44. doi: 10.1016/j.rbmo.2021.08.009
38. An HJ, Kim JH, Ahn EH, Kim YR, Kim JO, Park HS, et al. 3'-UTR Polymorphisms in the Vascular Endothelial Growth Factor Gene (VEGF) Contribute to Susceptibility to Recurrent Pregnancy Loss (RPL). *Int J Mol Sci* (2019) 20(13):3319. doi: 10.3390/ijms20133319
39. Scott K, Morgan HL, Delles C, Fisher S, Graham D, McBride MW. Distinct Uterine Artery Gene Expression Profiles During Early Gestation in the Stroke-Prone Spontaneously Hypertensive Rat. *Physiol Genomics* (2021) 53 (4):160–71. doi: 10.1152/physiolgenomics.00159.2020
40. Taniguchi N, Carames B, Ronfani L, Ulmer U, Komiya S, Bianchi ME, et al. Aging-Related Loss of the Chromatin Protein HMGB2 in Articular Cartilage is Linked to Reduced Cellularity and Osteoarthritis. *Proc Natl Acad Sci USA* (2009) 106(4):1181–6. doi: 10.1073/pnas.0806062106
41. Lucas ES, Dyer NP, Murakami K, Lee YH, Chan YW, Grimaldi G, et al. Loss of Endometrial Plasticity in Recurrent Pregnancy Loss. *Stem Cells* (2016) 34 (2):346–56. doi: 10.1002/stem.2222
42. Salker MS, Nautiyal J, Steel JH, Webster Z, Sucurovic S, Nicou M, et al. Disordered IL-33/ST2 Activation in Decidualizing Stromal Cells Prolongs Uterine Receptivity in Women With Recurrent Pregnancy Loss. *PloS One* (2012) 7(12):e52252. doi: 10.1371/journal.pone.0052252
43. Gellersen B, Brosens JJ. Cyclic Decidualization of the Human Endometrium in Reproductive Health and Failure. *Endocr Rev* (2014) 35(6):851–905. doi: 10.1210/er.2014-1045
44. Diniz-da-Costa M, Kong CS, Fishwick KJ, Rawlings T, Brighton PJ, Hawkes A, et al. Characterization of Highly Proliferative Decidual Precursor Cells During the Window of Implantation in Human Endometrium. *Stem Cells* (2021) 39(8):1067–80. doi: 10.1002/stem.3367
45. Dhaenens L, Lierman S, De Clerck L, Govaert E, Deforce D, Tilleman K, et al. Endometrial Stromal Cell Proteome Mapping in Repeated Implantation Failure and Recurrent Pregnancy Loss Cases and Fertile Women. *Reprod BioMed Online* (2019) 38(3):442–54. doi: 10.1016/j.rbmo.2018.11.022
46. Harden SL, Zhou J, Gharanei S, Diniz-da-Costa M, Lucas ES, Cui L, et al. Exometabolomic Analysis of Decidualizing Human Endometrial Stromal

and Perivascular Cells. *Front Cell Dev Biol* (2021) 9:626619. doi: 10.3389/fcell.2021.626619
47. Ticconi C, Pietropolli A, Di Simone N, Piccione E, Fazleabas A. Endometrial Immune Dysfunction in Recurrent Pregnancy Loss. *Int J Mol Sci* (2019) 20 (21):5332. doi: 10.3390/ijms20215332.
48. Valeff N, Muzzio DO, Matzner F, Dibo M, Golchert J, Homuth G, et al. B Cells Acquire a Unique and Differential Transcriptomic Profile During Pregnancy. *Genomics* (2021) 113(4):2614–22. doi: 10.1016/j.ygeno.2021.06.016
49. Guo C, Cai P, Jin L, Sha Q, Yu Q, Zhang W, et al. Single-Cell Profiling of the Human Decidual Immune Microenvironment in Patients With Recurrent Pregnancy Loss. *Cell Discov* (2021) 7(1):1. doi: 10.1038/s41421-020-00236-z
50. Wang F, Jia W, Fan M, Shao X, Li Z, Liu Y, et al. Single-Cell Immune Landscape of Human Recurrent Miscarriage. *Genomics Proteomics Bioinf* (2021) 19(2):208–22. doi: 10.1016/j.gpb.2020.11.002
51. Chen P, Zhou L, Chen J, Lu Y, Cao C, Lv S, et al. The Immune Atlas of Human Deciduas With Unexplained Recurrent Pregnancy Loss. *Front Immunol* (2021) 12:689019. doi: 10.3389/fimmu.2021.689019
52. Granne I, Shen M, Rodriguez-Caro H, Chadha G, O'Donnell E, Brosens JJ, et al. Characterisation of Peri-Implantation Endometrial Treg and Identification of an Altered Phenotype in Recurrent Pregnancy Loss. *Mucosal Immunol* (2021) 15(1):120–9. doi: 10.1038/s41385-021-00451-1.
53. Brown LY, Bonney EA, Raj RS, Nielsen B, Brown S. Generalized Disturbance of DNA Methylation in the Uterine Decidua in the CBA/J X DBA/2 Mouse Model of Pregnancy Failure. *Biol Reprod* (2013) 89(5):120. doi: 10.1095/biolreprod.113.113142
54. Pi L, Zhang Z, Gu Y, Wang X, Wang J, Xu J, et al. DNA Methylation Profiling in Recurrent Miscarriage. *PeerJ* (2020) 8:e8196. doi: 10.7717/peerj.8196
55. Gelmini GF, Costa CH, Nardi FD, Wowk PF, Mattar SB, Schuffner A, et al. Is HLA-E a Possible Genetic Marker Relevant for Natural Conception? *Am J Reprod Immunol* (2016) 76(6):439–42. doi: 10.1111/aji.12587.
56. Verloes A, Spits C, Vercammen M, Geens M, LeMaoult J, Sermon K, et al. The Role of Methylation, DNA Polymorphisms and microRNAs on HLA-G Expression in Human Embryonic Stem Cells. *Stem Cell Res* (2017) 19:118–27. doi: 10.1016/j.scr.2017.01.005
57. Horn KH, Warner DR, Pisano M, Greene RM. PRDM16 Expression in the Developing Mouse Embryo. *Acta Histochem* (2011) 113(2):150–5. doi: 10.1016/j.acthis.2009.09.006
58. He S, Deng Y, Liao Y, Li X, Liu J, Yao S. CREB5 Promotes Tumor Cell Invasion and Correlates With Poor Prognosis in Epithelial Ovarian Cancer. *Oncol Lett* (2017) 14(6):8156–61. doi: 10.3892/ol.2017.7234
59. Qi L, Ding Y. Involvement of the CREB5 Regulatory Network in Colorectal Cancer Metastasis. *Yi Chuan* (2014) 36(7):679–84. doi: 10.3724/SP.J.1005.2014.0679
60. Yu M, Du G, Xu Q, Huang Z, Huang X, Qin Y, et al. Integrated Analysis of DNA Methylome and Transcriptome Identified CREB5 as a Novel Risk Gene Contributing to Recurrent Pregnancy Loss. *EBioMedicine* (2018) 35:334–44. doi: 10.1016/j.ebiom.2018.07.042
61. Nevalainen T, Kananen L, Marttila S, Jylha M, Hervonen A, Hurme M, et al. Transcriptomic and Epigenetic Analyses Reveal a Gender Difference in Aging-Associated Inflammation: The Vitality 90+ Study. *Age (Dordr)* (2015) 37(4):9814. doi: 10.1007/s11357-015-9814-9
62. Long X, Li Y, Qiu S, Liu J, He L, Peng Y. MiR-582-5p/miR-590-5p Targeted CREB1/CREB5-NF-kappaB Signaling and Caused Opioid-Induced Immunosuppression in Human Monocytes. *Transl Psychiatry* (2016) 6: e757. doi: 10.1038/tp.2016.4
63. Lv XF, Sun LL, Cui CL, Han JS. NAc Shell Arc/Arg3.1 Protein Mediates Reconsolidation of Morphine CPP by Increased GluR1 Cell Surface Expression: Activation of ERK-Coupled CREB is Required. *Int J Neuropsychopharmacol* (2015) 18(9):pyv030. doi: 10.1093/ijnp/pyv030.
64. Krieg SA, Fan X, Hong Y, Sang QX, Giaccia A, Westphal LM, et al. Global Alteration in Gene Expression Profiles of Deciduas From Women With Idiopathic Recurrent Pregnancy Loss. *Mol Hum Reprod* (2012) 18(9):442–50. doi: 10.1093/molehr/gas017
65. Li Q, Wang H, Zhao Y, Lin H, Sang QA, Zhu C. Identification and Specific Expression of Matrix Metalloproteinase-26 in Rhesus Monkey Endometrium During Early Pregnancy. *Mol Hum Reprod* (2002) 8 (10):934–40. doi: 10.1093/molehr/8.10.934
66. Beermann J, Piccoli MT, Viereck J, Thum T. Non-Coding RNAs in Development and Disease: Background, Mechanisms, and Therapeutic Approaches. *Physiol Rev* (2016) 96(4):1297–325. doi: 10.1152/physrev.00041.2015
67. Wang JM, Gu Y, Zhang Y, Yang Q, Zhang X, Yin L, et al. Deep-Sequencing Identification of Differentially Expressed miRNAs in Decidua and Villus of Recurrent Miscarriage Patients. *Arch Gynecol Obstet* (2016) 293(5):1125–35. doi: 10.1007/s00404-016-4038-5
68. Huang Z, Yu H, Du G, Han L, Huang X, Wu D, et al. Enhancer RNA lnc-CES1-1 Inhibits Decidual Cell Migration by Interacting With RNA-Binding Protein FUS and Activating PPARgamma in URPL. *Mol Ther Nucleic Acids* (2021) 24:104–12. doi: 10.1016/j.omtn.2021.02.018
69. Calvo SE, Compton AG, Hershman SG, Lim SC, Lieber DS, Tucker EJ, et al. Molecular Diagnosis of Infantile Mitochondrial Disease With Targeted Next-Generation Sequencing. *Sci Transl Med* (2012) 4(118):118ra10. doi: 10.1126/scitranslmed.3003310.
70. Yin XJ, Hong W, Tian FJ, Li XC. Proteomic Analysis of Decidua in Patients With Recurrent Pregnancy Loss (RPL) Reveals Mitochondrial Oxidative Stress Dysfunction. *Clin Proteomics* (2021) 18(1):9. doi: 10.1186/s12014-021-09312-2
71. Xiong YM, Pan HT, Ding HG, He Y, Zhang J, Zhang F, et al. Proteomic and Functional Analysis of Proteins Related to Embryonic Development of Decidua in Patients With Recurrent Pregnancy Lossdagger. *Biol Reprod* (2021) 105(5):1246–56. doi: 10.1093/biolre/ioab140
72. Wang LL, Liu H, Zhao SJ, Shen L, Xie T, Luo J, et al. The Metabolic Landscape of Decidua in Recurrent Pregnancy Loss Using a Global Metabolomics Approach. *Placenta* (2021) 112: 45–53. doi: 10.1016/j.placenta.2021.07.001
73. Li Y, Wang R, Wang M, Huang W, Liu C, Fang Z, et al. RNA Sequencing of Decidua Reveals Differentially Expressed Genes in Recurrent Pregnancy Loss. *Reprod Sci* (2021) 28(8):2261–9. doi: 10.1007/s43032-021-00482-w
74. Qiao Y, Wen J, Tang F, Martell S, Shomer N, Leung PC, et al. Whole Exome Sequencing in Recurrent Early Pregnancy Loss. *Mol Hum Reprod* (2016) 22 (5):364–72. doi: 10.1093/molehr/gaw008
75. Goetz SC, Anderson KV. The Primary Cilium: A Signalling Centre During Vertebrate Development. *Nat Rev Genet* (2010) 11(5):331–44. doi: 10.1038/nrg2774
76. Ivanov I, Kuhn H, Heydeck D. Structural and Functional Biology of Arachidonic Acid 15-Lipoxygenase-1 (ALOX15). *Gene* (2015) 573(1):1–32. doi: 10.1016/j.gene.2015.07.073
77. Hanna CW, McFadden DE, Robinson WP. DNA Methylation Profiling of Placental Villi From Karyotypically Normal Miscarriage and Recurrent Miscarriage. *Am J Pathol* (2013) 182(6):2276–84. doi: 10.1016/j.ajpath.2013.02.021
78. Du G, Yu M, Xu Q, Huang Z, Huang X, Han L, et al. Hypomethylation of PRDM1 is Associated With Recurrent Pregnancy Loss. *J Cell Mol Med* (2020) 24(12):7072–7. doi: 10.1111/jcmm.15335
79. Wang L, Tang H, Xiong Y, Tang L. Differential Expression Profile of Long Noncoding RNAs in Human Chorionic Villi of Early Recurrent Miscarriage. *Clin Chim Acta* (2017) 464:17–23. doi: 10.1016/j.cca.2016.11.001
80. Tang L, Gao C, Gao L, Cui Y, Liu J. Expression Profile of Micro-RNAs and Functional Annotation Analysis of Their Targets in Human Chorionic Villi From Early Recurrent Miscarriage. *Gene* (2016) 576(1 Pt 2):366–71. doi: 10.1016/j.gene.2015.10.047.
81. Huang Z, Du G, Huang X, Han L, Han X, Xu B, et al. The Enhancer RNA lnc-SLC4A1-1 Epigenetically Regulates Unexplained Recurrent Pregnancy Loss (URPL) by Activating CXCL8 and NF-kB Pathway. *EBioMedicine* (2018) 38:162–70. doi: 10.1016/j.ebiom.2018.11.015
82. Sober S, Rull K, Reiman M, Ilisson P, Mattila P, Laan M. RNA Sequencing of Chorionic Villi From Recurrent Pregnancy Loss Patients Reveals Impaired Function of Basic Nuclear and Cellular Machinery. *Sci Rep* (2016) 6:38439. doi: 10.1038/srep38439
83. Tian FJ, Cheng YX, Li XC, Wang F, Qin CM, Ma XL, et al. The YY1/MMP2 Axis Promotes Trophoblast Invasion at the Maternal-Fetal Interface. *J Pathol* (2016) 239(1):36–47. doi: 10.1002/path.4694

84. Rull K, Tomberg K, Koks S, Mannik J, Mols M, Sirotkina M, et al. Increased Placental Expression and Maternal Serum Levels of Apoptosis-Inducing TRAIL in Recurrent Miscarriage. *Placenta* (2013) 34(2):141–8. doi: 10.1016/j.placenta.2012.11.032
85. Pan HT, Ding HG, Fang M, Yu B, Cheng Y, Tan YJ, et al. Proteomics and Bioinformatics Analysis of Altered Protein Expression in the Placental Villous Tissue From Early Recurrent Miscarriage Patients. *Placenta* (2018) 61:1–10. doi: 10.1016/j.placenta.2017.11.001
86. Gharesi-Fard B, Zolghadri J, Kamali-Sarvestani E. Alteration in the Expression of Proteins in Unexplained Recurrent Pregnancy Loss Compared With in the Normal Placenta. *J Reprod Dev* (2014) 60(4):261–7. doi: 10.1262/jrd.2013-096
87. Wang XH, Xu S, Zhou XY, Zhao R, Lin Y, Cao J, et al. Low Chorionic Villous Succinate Accumulation Associates With Recurrent Spontaneous Abortion Risk. *Nat Commun* (2021) 12(1):3428. doi: 10.1038/s41467-021-23827-0
88. Quintero-Ronderos P, Mercier E, Fukuda M, Gonzalez R, Suarez CF, Patarroyo MA, et al. Novel Genes and Mutations in Patients Affected by Recurrent Pregnancy Loss. *PloS One* (2017) 12(10):e0186149. doi: 10.1371/journal.pone.0186149
89. Maddirevula S, Awartani K, Coskun S, AlNaim LF, Ibrahim N, Abdulwahab F, et al. A Genomics Approach to Females With Infertility and Recurrent Pregnancy Loss. *Hum Genet* (2020) 139(5):605–13. doi: 10.1007/s00439-020-02143-5
90. Pan H, Xiang H, Wang J, Wei Z, Zhou Y, Liu B, et al. CAPS Mutations Are Potentially Associated With Unexplained Recurrent Pregnancy Loss. *Am J Pathol* (2019) 189(1):124–31. doi: 10.1016/j.ajpath.2018.09.010
91. Mishra J, Talwar S, Kaur L, Chandiok K, Yadav S, Puri M, et al. Differential Global and MTHFR Gene Specific Methylation Patterns in Preeclampsia and Recurrent Miscarriages: A Case-Control Study From North India. *Gene* (2019) 704:68–73. doi: 10.1016/j.gene.2019.04.036
92. Cui Y, He L, Yang CY, Ye Q. iTRAQ and PRM-Based Quantitative Proteomics in Early Recurrent Spontaneous Abortion: Biomarkers Discovery. *Clin Proteomics* (2019) 16:36. doi: 10.1186/s12014-019-9256-y
93. Wu Y, He J, Guo C, Zhang Y, Yang W, Xin M, et al. Serum Biomarker Analysis in Patients With Recurrent Spontaneous Abortion. *Mol Med Rep* (2017) 16(3):2367–78. doi: 10.3892/mmr.2017.6890
94. Li X, Yin M, Gu J, Hou Y, Tian F, Sun F. Metabolomic Profiling of Plasma Samples From Women With Recurrent Spontaneous Abortion. *Med Sci Monit* (2018) 24:4038–45. doi: 10.12659/MSM.907653
95. Ohinata Y, Payer B, O'Carroll D, Ancelin K, Ono Y, Sano M, et al. Blimp1 is a Critical Determinant of the Germ Cell Lineage in Mice. *Nature* (2005) 436 (7048):207–13. doi: 10.1038/nature03813
96. Paul S, Home P, Bhattacharya B, Ray S. GATA Factors: Master Regulators of Gene Expression in Trophoblast Progenitors. *Placenta* (2017) 60(Suppl 1): S61–6. doi: 10.1016/j.placenta.2017.05.005
97. King AE, Kelly RW, Sallenave JM, Bocking AD, Challis JR. Innate Immune Defences in the Human Uterus During Pregnancy. *Placenta* (2007) 28(11-12):1099–106. doi: 10.1016/j.placenta.2007.06.002
98. Calame K, Atchison M. YY1 Helps to Bring Loose Ends Together. *Genes Dev* (2007) 21(10):1145–52. doi: 10.1101/gad.1559007
99. Ferretti C, Bruni L, Dangles-Marie V, Pecking AP, Bellet D. Molecular Circuits Shared by Placental and Cancer Cells, and Their Implications in the Proliferative, Invasive and Migratory Capacities of Trophoblasts. *Hum Reprod Update* (2007) 13(2):121–41. doi: 10.1093/humupd/dml048
100. Bai X, Williams JL, Greenwood SL, Baker PN, Aplin JD, Crocker IP. A Placental Protective Role for Trophoblast-Derived TNF-Related Apoptosis-Inducing Ligand (TRAIL). *Placenta* (2009) 30(10):855–60. doi: 10.1016/j.placenta.2009.07.006
101. Kostakis ID, Cholidou KG, Kallianidis K, Perrea D, Antsaklis A. The Role of Calprotectin in Obstetrics and Gynecology. *Eur J Obstet Gynecol Reprod Biol* (2010) 151(1):3–9. doi: 10.1016/j.ejogrb.2010.03.006
102. Ouseph MM, Li J, Chen HZ, Pecot T, Wenzel P, Thompson JC, et al. Atypical E2F Repressors and Activators Coordinate Placental Development. *Dev Cell* (2012) 22(4):849–62. doi: 10.1016/j.devcel.2012.01.013
103. Tyers M, Mann M. From Genomics to Proteomics. *Nature* (2003) 422 (6928):193–7. doi: 10.1038/nature01510
104. Jeon YJ, Kim JH, Lee BE, Rah H, Shin JE, Kang H, et al. Association Between Polymorphisms in the Renin-Angiotensin System Genes and Prevalence of Spontaneously Aborted Fetuses. *Am J Reprod Immunol* (2013) 70(3):238–45. doi: 10.1111/aji.12110
105. Bell CE, Lariviere NM, Watson PH, Watson AJ. Mitogen-Activated Protein Kinase (MAPK) Pathways Mediate Embryonic Responses to Culture Medium Osmolarity by Regulating Aquaporin 3 and 9 Expression and Localization, as Well as Embryonic Apoptosis. *Hum Reprod* (2009) 24 (6):1373–86. doi: 10.1093/humrep/dep010
106. Coppinger JA, Cagney G, Toomey S, Kislinger T, Belton O, McRedmond JP, et al. Characterization of the Proteins Released From Activated Platelets Leads to Localization of Novel Platelet Proteins in Human Atherosclerotic Lesions. *Blood* (2004) 103(6):2096–104. doi: 10.1182/blood-2003-08-2804
107. Pringle KG, Kind KL, Sferruzzi-Perri AN, Thompson JG, Roberts CT. Beyond Oxygen: Complex Regulation and Activity of Hypoxia Inducible Factors in Pregnancy. *Hum Reprod Update* (2010) 16(4):415–31. doi: 10.1093/humupd/dmp046
108. Kusama K, Yoshie M, Tamura K, Imakawa K, Isaka K, Tachikawa E. Regulatory Action of Calcium Ion on Cyclic AMP-Enhanced Expression of Implantation-Related Factors in Human Endometrial Cells. *PloS One* (2015) 10(7):e0132017. doi: 10.1371/journal.pone.0132017
109. Salker MS, Singh Y, Durairaj RRP, Yan J, Alauddin M, Zeng N, et al. LEFTY2 Inhibits Endometrial Receptivity by Downregulating Orai1 Expression and Store-Operated Ca(2+) Entry. *J Mol Med (Berl)* (2018) 96(2):173–82. doi: 10.1007/s00109-017-1610-9
110. Lessey BA. Adhesion Molecules and Implantation. *J Reprod Immunol* (2002) 55(1-2):101–12. doi: 10.1016/s0165-0378(01)00139-5
111. Quintero-Ronderos P, Mercier E, Fukuda M, González R, Suárez CF, Patarroyo MA, et al. Novel Genes and Mutations in Patients Affected by Recurrent Pregnancy Loss. *PLoS One* (2017) 12(10):e0186149. doi: 10.1371/journal.pone.0186149
112. Dos Santos Nunes MK, Silva AS, de Queiroga Evangelista IW, Filho JM, Gomes C, do Nascimento RAF, et al. Hypermethylation in the Promoter of the MTHFR Gene is Associated With Diabetic Complications and Biochemical Indicators. *Diabetol Metab Syndr* (2017) 9:84. doi: 10.1186/s13098-017-0284-3

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