Maternal and Fetal CD4+CD25+CD^{127}\text{low/-} \text{Regulatory T Cells in Pregnancies with Gestational Diabetes, Preeclampsia, and Premature Rupture of Membranes}

Yuan-yuan Zhao
Qingdao University Medical College  https://orcid.org/0000-0001-5711-2394

Xiaolu Zhang
Qingdao University Medical College Affiliated Yantai Yuhuangding Hospital

Hong Sun
Qingdao University Medical College Affiliated Yantai Yuhuangding Hospital

Lei Chen
Qingdao University Medical College Affiliated Yantai Yuhuangding Hospital

Ding Ma
Qingdao University Medical College Affiliated Yantai Yuhuangding Hospital

Joanne Kwak-Kim
Rosalind Franklin University of Medicine and Science

Wen-juan Wang (✉️ 18678119343@163.com)
Qingdao University Medical College Affiliated Yantai Yuhuangding Hospital  https://orcid.org/0000-0003-2892-1330

Research

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Abstract

Background Regulatory T cells (Tregs) play a crucial role in maternal-fetal tolerance, but little is known about the characteristic of Tregs in peripheral blood (PB), maternal-fetal interface and cord blood (CB) in normal and complicated pregnancies with preeclampsia (PE), gestational diabetes mellitus (GDM) and premature rupture of membranes (PROM).

Methods PB, retro-placental blood (RPB), and CB were collected immediately after delivery in women with normal full-term pregnancy (NP), PE, GDM, and PROM. The proportion of CD4+ CD25+ CD127 low/- T cells (Tregs) and the expression of PD-1, GITR, HLA-G and CTLA-4 on T cell subsets were investigated by flow cytometric analysis. The data were analyzed based on sample origins (PB, RPB, and CB) and the obstetrical study groups (NP, PE, GDM, and PROM).

Results The proportions of Tregs in PB, RPB, and CB from NP were significantly higher than the other obstetrical groups (P < 0.01, respectively). However no significant differences were present among the PE, GDM and PROM groups (P = NS). The proportion of PD-1 + and GITR + Tregs in RPB and PB as well as PD-1 + Tregs in CB from NP were significantly higher than those of the same origin in each obstetrical study group (P < 0.01, respectively). There were no differences in HLA-G + and CTLA-4 + Tregs between different origins in each obstetrical study group. In NP, the proportion of PD-1 + Tregs was significantly decreased in CB as compared to those of PB and RPB (P < 0.05, respectively). Among the four groups, the proportion of GITR + Tregs were significantly higher in PB as compared to those of CB and RPB (P < 0.01, respectively). The proportion of HLA-G + Tregs in PB was significantly lower than that of CB and RPB (P < 0.01, respectively). The proportion of CTLA-4 + Tregs had no significant differences (P = NS).

Conclusions In conclusion, the proportion and characteristics of Tregs vary in the maternal, fetal and maternal-fetal junction at the time of delivery. The different feature and density of Tregs in maternal and fetal tissue may suggest the multiple and complicated roles of Tregs during pregnancy.

Background

The maternal immune system has various mechanisms to tolerate semi-allogenic pregnancy. Tregs have been reported to play an essential role in the maintenance of pregnancy. Tregs, identified by the surface markers CD4+, CD25+, and CD127dim/- [1], are increased in early pregnancy, peaked in mid-pregnancy and decreased in late pregnancy [2]. There are four main processes by which Tregs suppress immune responses: (1) modulation of dendritic cell (DC) function or maturation, (2) release of inhibitory cytokines, (3) cytolysis, and (4) metabolic disruption [3]. Accumulating evidence indicated that elevated levels of Tregs are detected in normal pregnancy [4], whereas deficiencies in their quantity and/or function have been demonstrated in obstetrical complications, such as recurrent pregnancy loss (RPL), preeclampsia (PE), gestational diabetes mellitus (GDM) and premature rupture of membrane (PROM) [5-8].

PE, which complicates approximately 10% of pregnancies, is a leading cause of maternal-fetal morbidity and mortality worldwide. There is strong evidence that disturbance in the immune response during early
pregnancy appears to be the central cause of later placental pathology and secondary systemic reaction, such as hypertensive disorder [9-12]. There is substantial evidence that many pregnant women with PE have fewer and less functionally competent Tregs [13, 14]. Therefore, it is conceivable that defective Tregs may be causatively involved in the development of PE. GDM is defined as carbohydrate intolerance with onset or first recognition during pregnancy. Recent evidence indicates that GDM is characterized not only by increased insulin resistance and glucose intolerance but by a state of low-grade systemic inflammation and dysregulation of the immune system, which induces an imbalance of Tregs [15, 16]. Like PE and GDM, the etiology of PROM is multifactorial. However, many studies have focused on the detection of evidence of inflammation during pregnancy [17]. It is generally believed that preterm PROM is causally linked to intra-amniotic inflammation and intra-amniotic infection [18]. Studies have shown that women with intra-amniotic infection had a higher number of total T and CD4+ T cells in amniotic fluid [19]. There are phenotypic changes in granulocytes and Tregs, which are consistent with the presence of intravascular inflammation in preterm PROM and preterm labor patients [20, 21]. The inflammatory response is crucial to the onset of labor [22]. Hence, abnormal expression of Tregs could explain the activation of maternal inflammatory cascade, leading to PROM and labor. Since immunosuppressive Tregs were shown to control excessive inflammation, and immoderate immune responses are known to be of vital importance for the successful course of pregnancy, it could be possible that functional deficiencies of such cells are involved in the pathogenesis of PROM.

Diverse expression of surface markers on Tregs may reflect the changes in their function and capacity to expand during pregnancy. There are several critical surface markers on Tregs, such as programmed cell death receptor-1 (PD-1/CD279), glucocorticoid-inducible TNF receptor family-related protein (GITR/CD357), human leucocyte antigen-G (HLA-G) and cytotoxic T lymphocyte antigen 4 (CTLA-4/CD152). PD-1 is a cell surface receptor belonging to the CD28 family. PD-1 cross-linking with PD-L1 results in the promotion of Tregs development and function [23]. The proportion of PD-1+ T lymphocytes were elevated in a normal healthy pregnancy, while a deficiency of PD-1 expression might cause the overreaction of T cells, which occurred in abnormal pregnancies [24-26]. It is reasonable to assume that PD-1 may play critical roles in regulating the immune response during pregnancy.

GITR, which plays inhibitory roles, is constitutively expressed on the cell surface of Tregs. The role of GITR triggering in the maturation/expansion of Tregs has been suggested by previous studies in mice and humans [27, 28]. It is also reported that blockade of GITR ligand reduces both Tregs numbers and immune suppressor phenotype [29-32], which demonstrate the crucial role of GITR in Tregs maturation and expansion. HLA-G, expressed on invasive trophoblast cells, is supposed to confer to maternal-fetal tolerance. Interaction of extra-villous trophoblasts (EVT) with CD4+ T cells resulted in increased numbers of CD4+CD25HiFoxp3+CD45RA+ resting Tregs and increased the expression level of the Treg specific transcription factor Foxp3 in these cells [33, 34]. It has been reported that HLA-G+/CD4+ T cells were significantly higher in PB from pregnant women [35], while the insufficient expression of HLA-G is correlated with PE and PRL [36, 37]. CTLA-4, which competes with the stimulatory molecule CD28 for CD80 and CD86 binding on antigen-presenting cells, serves an immune-regulatory function on T cell
activation. CTLA-4 is constitutively expressed on Tregs, and its ligation positively reinforces the immunosuppressive functions of Tregs.

Tregs from different origins may have unique phenotypes and various expressions of surface markers. The purpose of this study was to investigate the proportion of Tregs and their phenotypic characteristics in PB, RPB, and CB of normal pregnant women and women with PE, GDM, and PROM.

**Methods**

**Study population**

The study was approved by the ethics committee of Yuhuangding hospital, and all study subjects signed informed consent and permission form prior to entering the study. PB was drawn from 94 pregnant women, including 15 PE, 28 GDM, 23 PROM, and 28 NP. 15 CB and RPB were taken from each group separately. Immediately after the cord was clamped, CB and RPB were collected into EDTA-containing tubes for Tregs evaluation. All women with NP delivered vaginally without any complication. None had multiple gestations, pregnancies with chromosomal or fetal abnormalities, diabetes, pregnancy-related hypertension, autoimmune or chronic disease. PE was defined as the occurrence of hypertension; systolic blood pressure of $\geq 140$ mm Hg or as the diastolic blood pressure of $\geq 90$ mm Hg, plus albuminuria; presence of $\geq 300$ mg of protein in 24h urine sample after 20 weeks of gestation \[11\]. The diagnosis of GDM was made between 24 and 28 weeks of gestation by a positive 2-h 75 g oral glucose tolerance test (OGTT) with the following criteria: a fasting plasma glucose $\geq 5.1$ mmol/l (92 mg/dl), or a 1-h plasma glucose level of $\geq 10.0$ mmol/l (180 mg/dl), or a 2-h plasma glucose of $\geq 8.5$ mmol/l (153 mg/dl) \[38\]. The diagnosis of PROM was confirmed by the clinical findings of a posterior vaginal pool and the vaginal PH or ferning test. The clinical characteristics of women with normal and complicated pregnancies are presented in Table 1.

**Cells preparation and flow cytometry analysis**

The following mAbs were used: peridinin chlorophyll protein (PerCP)/Cy5.5-conjugated anti-CD3, fluorescein isothiocyanate FITC-anti-CD4, PE-conjugated anti-CD25, brilliant violet 510 anti-CD127, APC/Fire750 anti-CD279 (PD-1), and PE/Cy7 anti-CD357 (GITR), allophycocyanin APC anti-HLA-G, and brilliant violet421 anti-CD152 (CTLA-4). For surface staining, cells were incubated with the respective mAbs for 15 min at room temperature in the dark according to the manufacturer's instructions (eBioscience, San Diego, C.A, USA). After that, cells were washed twice and suspended in PBS before analysis.

Cells were analyzed on a FACS Canto II flow cytometer (BD Biosciences, USA); 200,000 events were recorded. The gating strategy for the detection of cells was as follows: The population of Tregs was characterized by the gating of CD3\(^+\), CD4\(^+\), CD25\(^+\), and CD127\(^{low/-}\) subsets. Then, within these subsets,
PD-1, GITR, HLA-G, or CTLA-4 cells were gated, respectively. The collected data were exported to FlowJo software for analysis (Tree Star, Ashland, OR).

Statistical analysis

Statistical analysis were performed using statistical software GraphPad Prism, version 5 (GraphPad, San Diego, CA, USA). Data were described as mean ± standard error (SEM). Normality tests were used first to determine if all the data sets are in normal distribution. Student t-test was applied to test the differences between the two groups. ANOVA was used to test the differences between multiple groups. The correlations of the proportion of Tregs with various surface markers between different groups were analyzed by linear correlation analysis. Statistical significance was set at $P < 0.05$.

Results

Clinical characteristics of the study population

The clinical characteristics of the obstetrical study populations, including age, obstetrical history, and outcome of the index pregnancies are listed in Table 1.

The proportions of CD3$^+$, CD4$^+$, and CD8$^+$ T cells in PB, RPB, and CB from NP, PE, GDM, and PROM

The proportion of CD3$^+$ T cells in PB was higher compared to RPB and CB in each obstetrical group ($P < 0.01$, respectively). CD3$^+$ T cells in each sample origin (PB, RPB, and CB) were the same among obstetrical study groups ($P = NS$, respectively).

The proportion of CD4$^+$ T cells in RPB was lower compared to those of PB and CB in each obstetrical group ($P < 0.01$, respectively). The proportion of CD4$^+$ T cells in each sample origin (PB, RPB, and CB) was the same among obstetrical study groups ($P = NS$, respectively).

The proportions of CD8$^+$ T cells in PB, RPB, and CB have no differences in each obstetrical group ($P = NS$, respectively). The proportions of CD8$^+$ T cells in each sample origin (PB, RPB, and CB) were the same among obstetrical study groups ($P = NS$, respectively) (Table 2).

The proportions of Tregs in PB, RPB, and CB from NP, PE, GDM, and PROM

The proportions of Tregs in PB, RPB, and CB from NP were significantly higher than those of other obstetrical study groups ($P < 0.01$, respectively). In NP, the proportion of Tregs in RPB (5.25% ± 0.33%) was significantly lower than those from CB (6.85% ± 0.35%) and PB (7.85% ± 0.53%) ($P < 0.01$, respectively).

There were no differences in Tregs proportions of each sample origin among PE, GMD, and PROM groups ($P = NS$, respectively) (Fig. 1A).
The Tregs reduction rates [(PB-RPB)/PB)] showed no differences among obstetrical study groups (P = NS,respectively) (Fig. 1B).

**Expression of PD-1, GITR, HLA-G, and CTLA-4 on CD4 T cells in PB, RPB and CB from NP, PE, GDM and PROM**

The proportions of PD-1+/CD4+ T cells in RPB and PB of NP were significantly higher than those of other obstetrical study groups (P < 0.01, respectively). In all obstetrical study groups, the proportions of PD-1+/CD4+ T cells from RPB were higher than those of CB (P < 0.01, respectively) while the proportions of PD-1+/CD4+ T cells from PB were higher than those of CB (P < 0.01, respectively).

In NP, the proportion of GITR+/CD4+ T cells from PB (3.01% ± 0.58%) was higher compared to CB (0.68% ± 0.26%) and RPB (0.91% ± 0.28%) (P < 0.01, respectively). The proportion of GITR+/CD4+ T cells in PB of NP was significantly higher than those of other obstetrical study groups (P < 0.01, respectively).

In all obstetrical study groups, the proportions of HLA-G+/CD4+ T cells in PB were significantly lower than those of CB (P < 0.05, respectively) and RPB (P < 0.01, respectively) while the proportions of HLA-G+/CD4+ T cells in RPB were significantly lower than those of CB (P < 0.05, respectively).

The proportions of CTLA-4+/CD4+ T cells had no statistical differences among the sample origin or obstetrical study groups (P = NS, respectively) (Table 3).

Similar results were found in CD3+ and CD8+ T cells (supplemental table 1, 2).

**Expression of PD-1, GITR, HLA-G, and CTLA-4 on Tregs in PB, RPB and CB from NP, PE, GDM and PROM**

The proportions of PD-1+ Tregs in PB, RPB, and CB of NP were significantly higher than those of other obstetrical study groups (P < 0.01, respectively). In NP, the proportion of PD-1+ Tregs in CB (6.16% ± 0.43%) was significantly lower than those of RPB (8.58% ± 0.79%) and PB (8.09% ± 0.81%) (P < 0.05, respectively).

GITR+ Tregs in PB and RPB of NP were significantly higher than those of other obstetrical study groups (P < 0.01, respectively). In NP, the proportion of GITR+ Tregs from PB (7.71% ± 0.87%) was significantly higher than those of CB (0.91% ± 0.18%) and RPB (2.51% ± 0.63%) (P < 0.01, respectively). The same findings were present in all obstetrical study groups.

The proportion of HLA-G+ Tregs in PB (2.94% ± 0.63%) of NP was significantly lower than those of CB (17.51% ± 3.61%) and RPB (25.19% ± 4.94%) (P < 0.01, respectively). The same findings were present in the other obstetrical study groups.

The proportions of CTLA-4+ Tregs had no statistical differences among the sample origin or obstetrical study groups (P = NS, respectively) (Table 4)
Correlations between Tregs subsets in PB, RPB, and CB from NP

There were significant positive correlations between the proportions of PD-1\(^+\) Tregs (\(r = 0.42, P < 0.05\)), GITR\(^+\) Tregs (\(r = 0.67, P < 0.05\)) and HLA-G\(^+\) Tregs (\(r = 0.43, P < 0.05\)) in CB and RPB. However, there were no significant correlations between the proportions of PD-1\(^+\) Tregs, GITR\(^+\) Tregs and HLA-G\(^+\) Tregs in PB and CB as well as PB and RPB (\(P = \text{NS}\), respectively). No significant correlations were present in proportions of CTLA-4\(^+\) Tregs among the sample origins (\(P = \text{NS}\), respectively).

Discussion

Our experimental results showed that the proportions of CD3\(^+\) T cells from NP, PE, GDM and PROM were significantly higher in PB than in RPB or CB. The proportions of CD4\(^+\) T cells in RPB from NP, GDM, PE, and PROM were significantly lower than those in PB or CB. Interestingly, there were no differences in CD8\(^+\) T cells among the sample origins or the obstetrical study groups. The RPB taken immediately after delivery may reflect maternal-fetal interface blood with the potential mixture of maternal blood [39]. We named it as "retro placental blood" in this study. During pregnancy, maternal placental circulation allows bidirectional passage of soluble antigens and nucleated blood cells through the maternal-fetal interface [40]. At the maternal-fetal junction, the fetal blood cells are proximity to the maternal blood, however the mixture of fetal and maternal blood cells does not occur because of the uteroplacental interface [41-43]. In this study, we report that CB retains a lower proportion of CD3\(^+\) T cells but the same proportion of CD4\(^+\) T cells with maternal PB. This finding was inconsistent with previous report which demonstrated that CD3\(^+\) and CD4\(^+\) T cells were increased in CB compared to PB [44]. More interestingly, our findings showed that CD3\(^+\) and CD4\(^+\) T cells in RPB were significantly lower than those in PB, which suggested that T cell-related immunity was suppressed at the maternal-fetal junction at the time of parturition.

CD3\(^+\) T cells account for 10% of maternal immune cells in the first-trimester human decidua, and about 30-45% of CD3\(^+\) T cells are CD4\(^+\) T cells. In contrast, CD3\(^+\) T cells represent about 80% of lymphocytes in peripheral blood, and approximately 65% of these CD3\(^+\) T cells are CD4\(^+\) T cells [45, 46]. These data indicate that CD3\(^+\) and CD4\(^+\) T cells are rare in the decidua during early pregnancy, possibly due to a reduced capacity for T cell accumulation [47]. Our findings showed that these cells were also decreased at the maternal-fetal junction during late pregnancy compared to those in PB regardless of obstetrical study groups.

Tregs play a role in modifying the maternal immune response to the fetoplacental ‘allograft’ within the uterus [41, 43, 48, 49]. Previously, we reported that the proportions of Tregs were decreased in PB and decidua of women with unexplained RPL when compared to normal early pregnant women [5, 38]. Contrarily, the temporary elevation of Tregs on the day of embryo transfer was associated with the higher embryo implantation rate [50]. The adoptive transfer of Tregs rescued pregnancy in abortion-prone mice model [51]. Few studies have investigated Tregs at the maternal-fetal junction and CB at the time of delivery [12, 52, 53]. In this study, we found that the proportions of Tregs were significantly decreased in
women with obstetrical complications as compared to NP regardless of sample origins. In addition, the proportions of Tregs in RPB of each obstetrical study group were lower than those in PB in all obstetrical study groups, including NP, and the reduction rates of Tregs were the same among the obstetrical study groups. Hence, the proportion of Tregs in RPB is determined by the proportion of PB Tregs, and the reduction rate of Tregs in RPB was not affected by the presence of obstetrical complications.

Data suggested that fetus-specific Tregs are specifically recruited from PB to the maternal-fetal interface [54]. Recruitment failure of CD4+ CD25bright Tregs to the maternal-fetal interface may play a role in the development of obstetrical complications. Both maternal factors and fetal antigenicity were reported to play a role in determining maternal Treg accumulation at the uteroplacental interface [55]. The postpartum decline in Tregs was consistent with the withdrawal of the immunological stimulus of the allograft [2]. In this study, we found that proportions of Tregs in RPB, and CB from PE, GDM and PROM were lower compared to NP, which was consistent with the previous study [56]. It may suggest that immune suppression in the maternal side, as well as the maternal-fetal interface, was less effective in obstetrical complications than those of normal pregnancies.

The characteristics of Tregs, determined by their surface expression of various markers, were different between PB, RPB, and CB in this study. Previous studies showed that PD-1 provided multiple and possibly redundant mechanisms to enhance the suppressive function and stability of Tregs and promote Tregs differentiation [57-59]. While the engagement of GITR on Tregs inhibited their suppressive function and in vivo GITR stimulation increased the numbers of Tregs [60-62]. Our experimental results showed that the proportion of PD-1+ and GITR+ Tregs were higher in PB and RPB than in CB from NP. Placental or trophoblast-derived soluble factors, including M-CSF, IL-10, TGF-β, and TRAIL may expand and activate Tregs in maternal side but not in fetal side [63]. Maternal PB and RPB contain possibly induced Tregs resulting from an encounter with foreign antigens, while Tregs in CB are functionally mature immune-regulatory population with naive phenotype [64]. Considering the naive phenotype and incomplete post thymic expansion of CB Tregs, the regulatory mechanisms engaged in the augmentation (PD-1 and GITR) of their suppressive activity might be acquired during the postnatal maturation process [65-67]. In this study, we found that the proportion of PD-1+/CD4+ and GITR+/CD4+ T cells as well as PD-1+ and GITR+ Tregs were significantly lower in CB compared to PB and RPB. Besides, the high proportion of PD-1+ and GITR+ Tregs from PB and RPB suggest that it may create a tolerogenic immune environment that allows the development of allogeneic placental and fetal tissues.

Studies have shown that PD-1+ T cells seem to be increased in healthy pregnant women compared to non-pregnant women [68], while decreased PD-1+ T cells were observed in PE and GDM[69-71]. In our studies, we found that PD-1+ Tregs in CB, PD-1+ Tregs and GITR+ Tregs in PB from PE, GDM, and PROM were lower compared to those of NP. These findings underscored the critical roles of PD-1 and GITR in regulating Treg function and maintaining immune suppression during pregnancy, whereas insufficient PD-1 and GITR expression might cause unsustainability in maternal-fetal immune tolerance and consequently, lead to pregnancy complications.
The higher proportions of HLA-G^+ Tregs from RPB and CB in NP, PE, GDM, and PROM are other significant findings of the present study. The expression of HLA-G is confined to immune-privileged sites, although the highest expression is shown in the placenta, where its primary role involves the protection of the fetus from maternal immunity, thus critically contributing to maternal-fetal tolerance [72]. Previous studies demonstrated that the HLA-G^+/CD4^+ T cells were expanded in the decidua compared to the periphery [35, 73, 74], which was in line with our results. In this study, we found a much higher proportion of HLA-G^+ Tregs at the maternal-fetal interface compared to PB. The high density of Tregs might be necessary for fetal acceptance at the site of the highest allogeneic stimulation inevitably presented by the fetus. However, it remains to be clarified whether the HLA-G^+ Tregs are recruited from the periphery or be induced at the maternal-fetal interface to promote tolerance. Considering the functions of HLA-G in pregnancy-associated immune tolerance, the increased expression of HLA-G on Tregs from RPB and CB may, at least in part, contribute to the restraint of undesirable maternal alloreactivity. HLA-G also stimulates the production of angiogenic factors and cytokines that favor embryo implantation, placental vascularization, and maternal-fetal tolerance [75]. Low blood concentrations of soluble HLA-G (sHLA-G) have been associated with increased risks of PE, GDM, and intrauterine growth restriction [76-78]. In this study, the proportion of HLA-G^+ Tregs in all sample origins of NP tends to be higher than those of PE, GDM and PROM, however the differences did not reach a statistically significant level. It may be due to small sample size, and further study is needed.

CTLA-4 is accepted as a crucial negative regulator of T-cell responses. Upon the stimulation of the TCR, CTLA-4 becomes stabilized on the surface of T cells, thus competing with CD28 for B7 binding and impairs the activation of T cells. It is reported that the expression of CTLA-4 on the cell surface is a key for Tregs to exert suppression by a critical mechanism [79-82]. In animal studies, treatment with CTLA-4-blocking antibody caused greater susceptibility to fetal loss with altered cytokine profiles by decidual CD4^+ T (dCD4^+ T) cells [83]. It is also reported that percentages of CTLA-4^+ cells were significantly increased in the endometrium of women with recurrent implantation failure (RIF) and RPL than those in controls [84]. This study showed that there was no difference in the proportions of CTLA-4^+ Tregs between the obstetrical study groups, which may indicate the decreased contribution of CTLA-4 in Tregs suppression during pregnancy.

In conclusion, Tregs possess different immunologic features in terms of phenotype and function in PB, RPB, and CB. Deregulated PD-1 and GITR expressions on Tregs may be associated with obstetrical complications, such as PE, GDM and PROM. HLA-G^+ Tregs in RPB may serve for the maternal-fetal tolerance during pregnancy. Further studies are needed to investigate the maternal factors and microenvironment, which affect the development and function of Tregs during pregnancy.

**Abbreviations**

Tregs: Regulatory T cells; PB: Peripheral blood; RPB: Retro-placental blood; CB: Cord blood; NP: Normal full-term pregnant; PE: Preeclampsia; GDM: Gestational diabetes mellitus; PROM: Premature rupture of...
Declarations

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Authors’ contributions

Z.Y.Y. was responsible for the laboratory operation, data analysis and manuscript drafting. Z.X.L. and S.H. was responsible for the data acquisition and analysis. C.L. and M.D. were responsible for the specimen collection, data interpretation and critical discussion. J.K.K and W.W.J. was responsible for the study design, data analysis and manuscript writing. All authors read and approved the final manuscript.

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Availability of data and materials

The primary data for this study is available from the authors on direct request.

Ethics approval and consent to participate

The study was approved by the Research Ethics Committee of Yantai Yuhuangding Hospital.

Consent for publication

Not applicable
Competing interests

The authors declare that they have no competing interests.

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Author details

1 Medical College of Qingdao University, Qingdao, 266071, P. R. China. 2 Reproduction Medical Center, the affiliated Yantai Yuhuangding Hospital of Qingdao University, 20 Yuhuangding East Road, Yantai, 264000, P. R. China. 3 Department of Clinical Laboratory, the Affiliated Yantai Yuhuangding Hospital of Qingdao University, 20 Yuhuangding East Road, Yantai, 264000, P. R. China. 4 Department of Obstetrics and Gynecology, the Affiliated Yantai Yuhuangding Hospital of Qingdao University, 20 Yuhuangding East Road, Yantai, 264000, P. R. China. 5 Reproductive Medicine and Immunology, Obstetrics and Gynecology, Clinical Sciences, Chicago Medical School, Rosalind Franklin University of Medicine and Science, Vernon Hills, IL, 60061, USA. 6 Microbiology and Immunology, Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, IL, 60064, USA.

References

1 Rodriguez-Perea AL, Arcia ED, Rueda CM, Velilla PA. Phenotypical characterization of regulatory T cells in humans and rodents. 2016; 185:281-91.

2 Somerset DA, Zheng Y, Kilby MD, Sansom DM, Drayson MT. Normal human pregnancy is associated with an elevation in the immune suppressive CD25+ CD4+ regulatory T-cell subset. Immunology. 2004; 112:38-43.

3 Vignali DA, Collison LW, Workman CJ. How regulatory T cells work. Nat Rev Immunol. 2008; 8:523-32.

4 Tsuda S, Nakashima A, Shima T, Saito S. New Paradigm in the Role of Regulatory T Cells During Pregnancy. Front Immunol. 2019; 10:573.

5 Wang WJ, Hao CF, Qu QL, Wang X, Qiu LH, Lin QD. The deregulation of regulatory T cells on interleukin-17-producing T helper cells in patients with unexplained early recurrent miscarriage. Hum Reprod. 2010; 25:2591-6.
6 Seol HJ, Oh MJ, Lim JE, Jung NH, Yoon SY, Kim HJ. The role of CD4+CD25bright regulatory T cells in the maintenance of pregnancy, premature rupture of membranes, and labor. Yonsei Med J. 2008; 49:366-71.

7 Daraei N, Ghafourian M, Ghadiri A, Amari A, Najafian M, Rokhafrooz S. Evaluation of Exhausted Regulatory T Cells in Preeclampsia. Iran J Immunol. 2019; 16:163-169.

8 Yang Y, Liu L, Liu B, Li Q, Wang Z, Fan S, Wang H, Wang L. Functional Defects of Regulatory T Cell Through Interleukin 10 Mediated Mechanism in the Induction of Gestational Diabetes Mellitus. DNA Cell Biol. 2018; 37:278-285.

9 Harmon AC, Cornelius DC, Amaral LM, Faulkner JL, Cunningham MW, Jr., Wallace K, LaMarca B. The role of inflammation in the pathology of preeclampsia. Clin Sci (Lond). 2016; 130:409-19.

10 Redman CW, Sargent IL. Immunology of pre-eclampsia. Am J Reprod Immunol. 2010; 63:534-43.

11 Ahn H, Park J, Gilman-Sachs A, Kwak-Kim J. Immunologic characteristics of preeclampsia, a comprehensive review. Am J Reprod Immunol. 2011; 65:377-94.

12 Queiroz AA, Franca EL, Hara CCP, Honorio MS, Fagundes DLG, Calderon IMP, Honorio-Franca AC. Phenotypic characterization of regulatory T cells populations in maternal blood, cord blood and placenta from diabetic mothers. J Matern Fetal Neonatal Med. 2019; 32:1098-1104.

13 Chen J, Zhao L, Wang D, Xu Y, Gao H, Tan W, Wang C. Contribution of regulatory T cells to immune tolerance and association of microRNA210 and Foxp3 in preeclampsia. Mol Med Rep. 2019; 19:1150-1158.

14 Prins JR, Boelens HM, Heimweg J, Van der Heide S, Dubois AE, Van Oosterhout AJ, Erwich JJ. Preeclampsia is associated with lower percentages of regulatory T cells in maternal blood. Hypertens Pregnancy. 2009; 28:300-11.

15 Lobo TF, Borges CM, Mattar R, Gomes CP, de Angelo AGS, Pendeloski KPT, Daher S. Impaired Treg and NK cells profile in overweight women with gestational diabetes mellitus. 2018; 79.

16 Sifnaios E, Mastorakos G, Psarra K, Panagopoulos ND, Panoulis K, Vitoratos N, Rizos D, Creatsas G. Gestational Diabetes and T-cell (Th1/Th2/Th17/Treg) Immune Profile. In Vivo. 2019; 33:31-40.

17 Tchirikov M, Schlabritz-Loutsevitch N, Maher J, Buchmann J, Naberezhnev Y, Winarno AS, Seliger G. Mid-trimester preterm premature rupture of membranes (PPROM): etiology, diagnosis, classification, international recommendations of treatment options and outcome. J Perinat Med. 2018; 46:465-488.

18 Salazar Garcia MD, Sung N, Mullenix TM, Dambaeva S, Beaman K, Gilman-Sachs A, Kwak-Kim J. Plasminogen Activator Inhibitor-1 4G/5G Polymorphism is Associated with Reproductive Failure: Metabolic, Hormonal, and Immune Profiles. Am J Reprod Immunol. 2016; 76:70-81.
Gomez-Lopez N, Romero R. Cellular immune responses in amniotic fluid of women with preterm labor and intra-amniotic infection or intra-amniotic inflammation. 2019: e13171.

Gervasi MT, Chaiworapongs T, Naccasha N, Pacora P, Berman S, Maymon E, Kim JC, Kim YM, Yoshimatsu J, Espinoza J, Romero R. Maternal intravascular inflammation in preterm premature rupture of membranes. J Matern Fetal Neonatal Med. 2002; 11:171-5.

Areia AL, Rodrigues P, Alarcao A, Ladeirinha A, Moura P, Carvalho L. Is Preterm Labor Influenced by the Maternal-Fetal Interface? Fetal Pediatr Pathol. 2017; 36:89-105.

Gilman-Sachs A, Dambaeva S, Salazar Garcia MD, Hussein Y, Kwak-Kim J, Beaman K. Inflammation induced preterm labor and birth. J Reprod Immunol. 2018; 129:53-58.

Zhang B, Chikuma S, Hori S, Fagarasan S, Honjo T. Nonoverlapping roles of PD-1 and FoxP3 in maintaining immune tolerance in a novel autoimmune pancreatitis mouse model. Proc Natl Acad Sci U S A. 2016; 113:8490-5.

Li G, Lu C, Gao J, Wang X, Wu H, Lee C, Xing B, Zhang Q. Association between PD-1/PD-L1 and T regulate cells in early recurrent miscarriage. Int J Clin Exp Pathol. 2015; 8:6512-8.

Wang S, Zhu X, Xu Y, Zhang D, Li Y, Tao Y, Piao H, Li D, Du M. Programmed cell death-1 (PD-1) and T-cell immunoglobulin mucin-3 (Tim-3) regulate CD4+ T cells to induce Type 2 helper T cell (Th2) bias at the maternal-fetal interface. Hum Reprod. 2016; 31:700-11.

Zhang Y, Liu Z, Tian M, Hu X, Wang L, Ji J, Liao A. The altered PD-1/PD-L1 pathway delivers the 'one-two punch' effects to promote the Treg/Th17 imbalance in pre-eclampsia. Cell Mol Immunol. 2018; 15:710-723.

Petrillo MG, Ronchetti S, Ricci E, Alunno A, Gerli R, Nocentini G, Riccardi C. GITR+ regulatory T cells in the treatment of autoimmune diseases. Autoimmun Rev. 2015; 14:117-26.

Scirka B, Szurek E, Pietrzak M, Rempala G, Kisielow P, Ignatowicz L, Miazek A. Anti-GITR Antibody Treatment Increases TCR Repertoire Diversity of Regulatory but not Effector T Cells Engaged in the Immune Response Against B16 Melanoma. Arch Immunol Ther Exp (Warsz). 2017; 65:553-564.

Pedroza-Gonzalez A, Zhou G, Singh SP, Boor PP, Pan Q, Grunhagen D, de Jonge J, Tran TK, Verhoef C, JN IJ, Janssen H, Biermann K, Kwekkeboom J, Sprengers D. GITR engagement in combination with CTLA-4 blockade completely abrogates immunosuppression mediated by human liver tumor-derived regulatory T cells ex vivo. Oncoimmunology. 2015; 4:e1051297.

Kim YH, Shin SM, Choi BK, Oh HS, Kim CH, Lee SJ, Kim KH, Lee DG, Park SH, Kwon BS. Authentic GITR Signaling Fails To Induce Tumor Regression unless Foxp3+ Regulatory T Cells Are Depleted. J Immunol. 2015; 195:4721-9.
31 Mahne AE, Mauze S, Joyce-Shaikh B, Xia J, Bowman EP, Beebe AM, Cua DJ, Jain R. Dual Roles for Regulatory T-cell Depletion and Costimulatory Signaling in Agonistic GITR Targeting for Tumor Immunotherapy. Cancer Res. 2017; 77:1108-1118.

32 Nowakowska DJ, Kissler S. Ptpn22 Modifies Regulatory T Cell Homeostasis via GITR Upregulation. J Immunol. 2016; 196:2145-52.

33 Tilburgs T, Crespo AC. Human HLA-G+ extravillous trophoblasts: Immune-activating cells that interact with decidual leukocytes. 2015; 112:7219-24.

34 Brown R, Kabani K, Favaloro J, Yang S, Ho PJ, Gibson J, Fromm P, Suen H, Woodland N, Nassif N, Hart D, Joshua D. CD86+ or HLA-G+ can be transferred via trogocytosis from myeloma cells to T cells and are associated with poor prognosis. Blood. 2012; 120:2055-63.

35 Hsu P, Santner-Nanan B, Joung S, Peek MJ, Nanan R. Expansion of CD4(+) HLA-G(+) T Cell in human pregnancy is impaired in pre-eclampsia. Am J Reprod Immunol. 2014; 71:217-28.

36 Prins JR, van der Hoorn MLP, Keijser R, Ris-Stalpers C, van Beelen E, Afink GB, Claas FHJ, van der Post JAM, Scherjon SA. Higher decidual EBI3 and HLA-G mRNA expression in preeclampsia: Cause or consequence of preeclampsia. Hum Immunol. 2016; 77:68-70.

37 Mosaferi E, Alizadeh Gharamaleki N. The Study of HLA-G Gene and Protein Expression in Patients with Recurrent Miscarriage. 2019; 9:70-75.

38 Lee SK, Kim JY, Hur SE, Kim CJ, Na BJ, Lee M, Gilman-Sachs A, Kwak-Kim J. An imbalance in interleukin-17-producing T and Foxp3(+) regulatory T cells in women with idiopathic recurrent pregnancy loss. Hum Reprod. 2011; 26:2964-71.

39 Djurisic S, Skibsted L, Hviid TV. A Phenotypic Analysis of Regulatory T Cells and Uterine NK Cells from First Trimester Pregnancies and Associations with HLA-G. Am J Reprod Immunol. 2015; 74:427-44.

40 Mor G, Aldo PA, Alvero AB. The unique immunological and microbial aspects of pregnancy. Nat Rev Immunol. 2017; 17:469-482.

41 Alijotas-Reig J, Llurba E, Gris JM. Potentiating maternal immune tolerance in pregnancy: a new challenging role for regulatory T cells. Placenta. 2014; 35:241-8.

42 Sharma S. Natural killer cells and regulatory T cells in early pregnancy loss. Int J Dev Biol. 2014; 58:219-29.

43 Ruocco MG, Chaouat G, Florez L, Bensussan A, Klatzmann D. Regulatory T-cells in pregnancy: historical perspective, state of the art, and burning questions. Front Immunol. 2014; 5:389.
44 Gomes Fagundes DL, Franca EL, da Silva Fernandes RT, Hara Cde C, Morceli G, Honorio-Franca AC, Calderon Ide M. Changes in T-cell phenotype and cytokines profile in maternal blood, cord blood and colostrum of diabetic mothers. J Matern Fetal Neonatal Med. 2016; 29:998-1004.

45 Mjosberg J, Berg G, Jenmalm MC, Ernerudh J. FOXP3+ regulatory T cells and T helper 1, T helper 2, and T helper 17 cells in human early pregnancy decidua. Biol Reprod. 2010; 82:698-705.

46 Tilburgs T, Claas FH, Scherjon SA. Elsevier Trophoblast Research Award Lecture: Unique properties of decidual T cells and their role in immune regulation during human pregnancy. Placenta. 2010; 31 Suppl:S82-6.

47 Nancy P, Tagliani E, Tay CS, Asp P, Levy DE, Erlebacher A. Chemokine gene silencing in decidual stromal cells limits T cell access to the maternal-fetal interface. Science. 2012; 336:1317-21.

48 Saito S, Shiozaki A, Sasaki Y, Nakashima A, Shima T, Ito M. Regulatory T cells and regulatory natural killer (NK) cells play important roles in feto-maternal tolerance. Semin Immunopathol. 2007; 29:115-22.

49 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; 162:41-8.

50 Wang WJ, Liu FJ, Zhang X, Liu XM, Qu QL, Li FH, Zhuang LL, Li XX, Hao CF. Periodic elevation of regulatory T cells on the day of embryo transfer is associated with better in vitro fertilization outcome. J Reprod Immunol. 2017; 119:49-53.

51 Wang WJ, Liu FJ, Xin L, Hao CF, Bao HC, Qu QL, Liu XM. Adoptive transfer of pregnancy-induced CD4+CD25+ regulatory T cells reverses the increase in abortion rate caused by interleukin 17 in the CBA/JxBALB/c mouse model. Hum Reprod. 2014; 29:946-52.

52 Fujimaki W, Takahashi N, Ohnuma K, Nagatsu M, Kurosawa H, Yoshida S, Dang NH, Uchiyama T, Morimoto C. Comparative study of regulatory T cell function of human CD25CD4 T cells from thymocytes, cord blood, and adult peripheral blood. Clin Dev Immunol. 2008; 2008:305859.

53 Salvany-Celades M, van der Zwan A, Benner M, Setrajcic-Dragos V, Bougleux Gomes HA, Iyer V, Norwitz ER, Strominger JL, Tilburgs T. Three Types of Functional Regulatory T Cells Control T Cell Responses at the Human Maternal-Fetal Interface. Cell Rep. 2019; 27:2537-2547.e5.

54 Tilburgs T, Roelen DL, van der Mast BJ, de Groot-Swings GM, Kleijburg C, Scherjon SA, Claas FH. Evidence for a selective migration of fetus-specific CD4+CD25bright regulatory T cells from the peripheral blood to the decidua in human pregnancy. J Immunol. 2008; 180:5737-45.

55 Wambach CM, Patel SN, Kahn DA. Maternal and fetal factors that contribute to the localization of T regulatory cells during pregnancy. Am J Reprod Immunol. 2014; 71:391-400.
56  Hsu P, Santner-Nanan B, Dahlstrom JE, Fadia M, Chandra A, Peek M, Nanan R. Altered decidual DC-SIGN+ antigen-presenting cells and impaired regulatory T-cell induction in preeclampsia. Am J Pathol. 2012; 181:2149-60.

57  Wang SC, Li YH, Piao HL, Hong XW, Zhang D, Xu YY, Tao Y, Wang Y, Yuan MM, Li DJ, Du MR. PD-1 and Tim-3 pathways are associated with regulatory CD8+ T-cell function in decidua and maintenance of normal pregnancy. Cell Death Dis. 2015; 6:e1738.

58  Chen X, Fosco D, Kline DE, Meng L, Nishi S, Savage PA, Kline J. PD-1 regulates extrathymic regulatory T-cell differentiation. Eur J Immunol. 2014; 44:2603-16.

59  Leung CS, Yang KY, Li X, Chan VW, Ku M, Waldmann H, Hori S, Tsang JCH, Lo YMD, Lui KO. Single-cell transcriptomics reveal that PD-1 mediates immune tolerance by regulating proliferation of regulatory T cells. 2018; 10:71.

60  Meiler S, Smeets E, Winkels H, Shami A, Pascutti MF, Nolte MA, Beckers L, Weber C, Gerdes N, Lutgens E. Constitutive GITR Activation Reduces Atherosclerosis by Promoting Regulatory CD4+ T-Cell Responses-Brief Report. Arterioscler Thromb Vasc Biol. 2016; 36:1748-52.

61  Stephens GL, McHugh RS, Whitters MJ, Young DA, Luxenberg D, Carreno BM, Collins M, Shevach EM. Engagement of glucocorticoid-induced TNFR family-related receptor on effector T cells by its ligand mediates resistance to suppression by CD4+CD25+ T cells. J Immunol. 2004; 173:5008-20.

62  Shimizu J, Yamazaki S, Takahashi T, Ishida Y, Sakaguchi S. Stimulation of CD25(+)CD4(+) regulatory T cells through GITR breaks immunological self-tolerance. Nat Immunol. 2002; 3:135-42.

63  Svensson-Arvelund J, Mehta RB, Lindau R, Mirrasekhian E, Rodriguez-Martinez H, Berg G, Lash GE, Jenmalm MC, Ernerudh J. The human fetal placenta promotes tolerance against the semiallogeneic fetus by inducing regulatory T cells and homeostatic M2 macrophages. J Immunol. 2015; 194:1534-44.

64  Takahata Y, Nomura A, Takada H, Ohga S, Furuno K, Hikino S, Nakayama H, Sakaguchi S, Hara T. CD25+CD4+ T cells in human cord blood: an immunoregulatory subset with naive phenotype and specific expression of forkhead box p3 (Foxp3) gene. Exp Hematol. 2004; 32:622-9.

65  Samstein RM, Josefowicz SZ, Arvey A, Treuting PM, Rudensky AY. Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. Cell. 2012; 150:29-38.

66  Mayer E, Bannert C, Gruber S, Klunker S, Spittler A, Akdis CA, Szepfalusi Z, Eiwegger T. Cord blood derived CD4+ CD25(high) T cells become functional regulatory T cells upon antigen encounter. PLoS One. 2012; 7:e29355.

67  Wing K, Lindgren S, Kollberg G, Lundgren A, Harris RA, Rudin A, Lundin S, Suri-Payer E. CD4 T cell activation by myelin oligodendrocyte glycoprotein is suppressed by adult but not cord blood CD25+ T cells. Eur J Immunol. 2003; 33:579-87.
68 Grozdics E, Berta L, Bajnok A, Veres G, Ilisz I, Klivenyi P, Rigo J, Jr., Vecsei L, Tulassay T, Toldi G. B7 costimulation and intracellular indoleamine-2,3-dioxygenase (IDO) expression in peripheral blood of healthy pregnant and non-pregnant women. BMC Pregnancy Childbirth. 2014; 14:306.

69 Tian M, Zhang Y, Liu Z, Sun G, Mor G, Liao A. The PD-1/PD-L1 inhibitory pathway is altered in pre-eclampsia and regulates T cell responses in pre-eclamptic rats. Sci Rep. 2016; 6:27683.

70 Meggyes M, Lajko A, Palkovics T, Totsimon A, Illes Z, Szereday L, Miko E. Feto-maternal immune regulation by TIM-3/galectin-9 pathway and PD-1 molecule in mice at day 14.5 of pregnancy. Placenta. 2015; 36:1153-60.

71 Ye X, Ju S, Duan H, Yao Y, Wu J, Zhong S, Chen L, Cao S, Xu Y, Zheng X, Wang H, Ge Y, Ju S. Immune checkpoint molecule PD-1 acts as a novel biomarker for the pathological process of gestational diabetes mellitus. Biomark Med. 2017; 11:741-749.

72 Carosella ED, Rouas-Freiss N, Tronik-Le Roux D, Moreau P, LeMaoult J. HLA-G: An Immune Checkpoint Molecule. Adv Immunol. 2015; 127:33-144.

73 Lynge Nilsson L, Djurisic S, Hviid TV. Controlling the Immunological Crosstalk during Conception and Pregnancy: HLA-G in Reproduction. Front Immunol. 2014; 5:198.

74 Pankratz S, Ruck T, Meuth SG, Wiendl H. CD4(+)HLA-G(+) regulatory T cells: Molecular signature and pathophysiological relevance. Hum Immunol. 2016; 77:727-33.

75 Rajagopalan S. HLA-G-mediated NK cell senescence promotes vascular remodeling: implications for reproduction. Cell Mol Immunol. 2014; 11:460-6.

76 Martinetti M, Beneventi F, Capittini C. The Immunosignature of Mother/Fetus Couples in Gestational Diabetes Mellitus: Role of HLA-G 14 bp ins/del and PAPP-A A/C Polymorphisms in the Uterine Inflammatory Milieu. 2017; 2017:4254750.

77 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; 191:525-9.

78 Smith GC, Stenhouse EJ, Crossley JA, Aitken DA, Cameron AD, Connor JM. Early pregnancy levels of pregnancy-associated plasma protein a and the risk of intrauterine growth restriction, premature birth, preeclampsia, and stillbirth. J Clin Endocrinol Metab. 2002; 87:1762-7.

79 Khalife E, Khodadadi A, Talaeizadeh A, Rahimian L, Nemati M, Jafarzadeh A. Overexpression of Regulatory T Cell-Related Markers (FOXP3, CTLA-4 and GITR) by Peripheral Blood Mononuclear Cells from Patients with Breast Cancer. Asian Pac J Cancer Prev. 2018; 19:3019-3025.

80 Yamaguchi T, Kishi A, Osaki M, Morikawa H, Prieto-Martin P, Wing K, Saito T, Sakaguchi S. Construction of self-recognizing regulatory T cells from conventional T cells by controlling CTLA-4 and IL
2 expression. Proc Natl Acad Sci U S A. 2013; 110:E2116-25.

81 Wing JB, Ise W, Kurosaki T, Sakaguchi S. Regulatory T cells control antigen-specific expansion of Tfh cell number and humoral immune responses via the coreceptor CTLA-4. Immunity. 2014; 41:1013-25.

82 Arce Vargas F, Furness AJS, Litchfield K, Joshi K, Rosenthal R, Ghorani E, Solomon I, Lesko MH, Ruef N, Roddie C, Henry JY, Spain L, Ben Aissa A, Georgiou A, Wong YNS, Smith M, Strauss D, Hayes A, Nicol D, O’Brien T, Martensson L, Ljungars A, Teige I, Frendeus B, Pule M, Marafioti T, Gore M, Larkin J, Turajlic S, Swanton C, Peggs KS, Quezada SA. Fc Effector Function Contributes to the Activity of Human Anti-CTLA-4 Antibodies. Cancer Cell. 2018; 33:649-663.e4.

83 Wang S, Chen C, Li M, Qian J, Sun F, Li Y, Yu M, Wang M, Zang X, Zhu R, Li D, Du M. Blockade of CTLA-4 and Tim-3 pathways induces fetal loss with altered cytokine profiles by decidual CD4(+)T cells. Cell Death Dis. 2019; 10:15.

84 Ding JL, Diao LH, Yin TL, Huang CY, Yin B, Chen C, Zhang Y, Li J, Cheng YX, Zeng Y. Aberrant expressions of endometrial Id3 and CTLA-4 are associated with unexplained repeated implantation failure and recurrent miscarriage. 2017; 78.

Tables

Table 1 Age, obstetrical history and outcome of the index pregnancies from NP women, women with PE, GDM or PROM
| Items                        | NP (n = 28) | PE (n = 15) | GDM (n = 28) | PROM (n = 23) | P   |
|-----------------------------|-------------|-------------|--------------|---------------|-----|
| Age (years)                 | 26.74 ± 1.18| 28.33 ± 1.87| 27.45 ± 1.25| 27.88 ± 1.76  | NS  |
| Gestation weeks (Wks)       | 39.58 ± 0.32| 39.75 ± 0.24| 39.59 ± 0.18| 39.45 ± 0.37  | NS  |
| Birth weight (g)            | 3329.34 ± 35.62| 2837.57 ± 32.68| 3634.66 ± 37.54| 3415.56 ± 36.59| <   |
|                            |             |             |              |               | 0.01|
| Primiparity                 |             |             |              |               |     |
| Yes                         | 16 (57%)    | 7 (46%)     | 15 (53%)     | 11 (47%)      | NS  |
| No                          | 12 (43%)    | 8 (54%)     | 13 (47%)     | 12 (53%)      |     |
| Mode of Delivery            |             |             |              |               |     |
| Vaginal delivery            | 28 (100%)   | 0           | 10 (35%)     | 12 (52%)      | <   |
|                            |             |             |              |               | 0.01|
| Cesarean section            | 0           | 15 (100%)   | 18 (65%)     | 11 (48%)      |     |
| Blood pressure (mmHg)       | ≤ 120/80    | ≥ 140/90    | ≤ 120/80     | ≤ 120/80      | <   |
|                            |             |             |              |               | 0.01|
| Proteinuria (mg/dL/day)     | ≤ 150       | ≥ 300       | ≤ 150        | ≤ 150         | <   |
|                            |             |             |              |               | 0.01|
| Fasting glucose (mg/dl)     | 85.76 ± 1.39| 86.37 ± 0.87| 89.48 ± 0.76| 86.23 ± 1.26  | <   |
|                            |             |             |              |               | 0.01|
| 1-hr plasma glucose after OGTT (mg/dl) | 100.67 ± 0.73| 99.25 ± 1.65| 169.99 ± 1.07| 101.53 ± 1.46| <   |
|                            |             |             |              |               | 0.01|

OGTT: oral glucose tolerance test, NS: no significance

**Table 2** Proportion of CD3⁺, CD4⁺, and CD8⁺ T cells in PB, RPB and CB from NP women, women with PE, GDM or PROM
| Items (%) | NP (n = 28) | PE (n = 15) | GDM (n = 28) | PROM (n = 23) | $P^a$ |
|-----------|-------------|-------------|--------------|---------------|------|
| **CD3**+  |             |             |              |                |      |
| T cells   |             |             |              |                |      |
| PB        | 76.77 ± 1.62 | 75.37 ± 1.69 | 75.46 ± 1.54 | 76.29 ± 1.78 | NS  |
| RPB       | 63.75 ± 1.01 | 63.73 ± 1.34 | 63.36 ± 1.42 | 64.27 ± 1.27 | NS  |
| CB        | 63.13 ± 1.87 | 62.78 ± 1.24 | 62.25 ± 1.34 | 63.33 ± 1.35 | NS  |
| $P^d$     | < 0.01      | < 0.01      | < 0.01       | < 0.01        |      |
| **CD4**+  |             |             |              |                |      |
| T cells   |             |             |              |                |      |
| PB        | 46.62 ± 1.17 | 46.25 ± 1.42 | 48.48 ± 1.33 | 47.55 ± 1.39  | NS  |
| RPB       | 38.45 ± 1.33 | 37.79 ± 1.54 | 39.28 ± 1.57 | 36.36 ± 1.11  | NS  |
| CB        | 50.34 ± 1.67 | 51.55 ± 1.58 | 51.66 ± 1.17 | 52.27 ± 1.54  | NS  |
| $P^d$     | < 0.01      | < 0.01      | < 0.01       | < 0.01        |      |
| **CD8**+  |             |             |              |                |      |
| T cells   |             |             |              |                |      |
| PB        | 25.44 ± 1.28 | 25.73 ± 1.43 | 26.4 ± 1.32  | 27.32 ± 1.41  | NS  |
| RPB       | 26.38 ± 1.50 | 26.34 ± 1.45 | 25.30 ± 1.83 | 26.43 ± 1.58  | NS  |
| CB        | 24.56 ± 1.40 | 24.25 ± 1.47 | 24.29 ± 1.33 | 25.55 ± 1.41  | NS  |
| $P^d$     | NS          | NS          | NS           | NS            |      |

*a* Comparison with NP, PE, GDM and PROM by ANOVA.

*b* indicates $P < 0.01$ when compared to PB and CB in the same obstetrical group.

*c* indicates $P < 0.01$ when compared to PB and RPB in the same obstetrical group.

*d* Comparison with PB, RPB and CB in the same obstetrical group by ANOVA.

*e* indicates $P < 0.01$ when compared to RPB and CB in the same obstetrical group.

Values are mean ± SEM. NS: no significance

### Table 3: Expression of PD-1, GITR, HLA-G and CTLA-4 on CD4+ T cells in PB, RPB and CB from from NP, PE, GDM or PROM

| Items (%) | NP (n = 28) | PE (n = 15) | GDM (n = 28) | PROM (n = 23) | $P^a$ |
|-----------|-------------|-------------|--------------|---------------|------|
| **PD-1**  |             |             |              |                |      |
| PB        | 5.85 ± 1.08 | 2.69 ± 0.15 | 2.54 ± 0.71  | 2.74 ± 0.12   | < 0.01 |
| RPB       | 7.29 ± 1.63 | 4.47 ± 0.42 | 4.29 ± 0.53  | 4.52 ± 0.33   | < 0.01 |
| CB        | 0.43 ± 0.07 | 0.15 ± 0.37 | 0.37 ± 0.46  | 0.23 ± 0.69   | NS   |
| $P^e$     | < 0.01      | < 0.01      | < 0.01       | < 0.01        |      |
| **GITR**  |             |             |              |                |      |
| PB        | 3.01 ± 0.58 | 0.65 ± 0.09 | 0.9 ± 0.26   | 0.55 ± 0.10   | < 0.01 |
| RPB       | 0.91 ± 0.28 | 0.86 ± 0.39 | 0.83 ± 0.58  | 0.73 ± 0.85   | NS   |
| CB        | 0.68 ± 0.26 | 0.77 ± 0.25 | 0.76 ± 0.33  | 0.64 ± 0.36   | NS   |
| $P^e$     | < 0.01      | < 0.01      | < 0.01       | < 0.01        |      |
| **HLA-G** |             |             |              |                |      |
| PB        | 0.55 ± 0.09 | 0.21 ± 0.04 | 0.21 ± 0.14  | 0.31 ± 0.05   | NS   |
| RPB       | 16.88 ± 3.18| 14.28 ± 3.54| 15.24 ± 4.72 | 14.27 ± 4.24  | NS   |
| CB        | 7.05 ± 2.42 | 5.62 ± 1.79 | 6.35 ± 2.79  | 5.73 ± 1.28   | NS   |
| $P^e$     | < 0.01      | < 0.01      | < 0.01       | < 0.01        |      |
| **CTLA-4**|             |             |              |                |      |
| PB        | 0.07 ± 0.04 | 0.06 ± 0.05 | 0.07 ± 0.02  | 0.07 ± 0.03   | NS   |
| RPB       | 0.08 ± 0.04 | 0.07 ± 0.11 | 0.05 ± 0.03  | 0.05 ± 0.08   | NS   |
| CB        | 0.07 ± 0.02 | 0.05 ± 0.03 | 0.06 ± 0.04  | 0.07 ± 0.02   | NS   |
| $P^e$     | NS          | NS          | NS           | NS            |      |
Comparison with NP, PE, GDM and PROM by ANOVA.

\( b^* \) indicates \( P < 0.05 \), \( b^{**} \) indicates \( P < 0.01 \) when compared to PB and CB in the same obstetrical group.

\( c \) indicates \( P < 0.01 \) when compared to NP.

\( d^* \) indicates \( P < 0.05 \), \( d^{**} \) indicates \( P < 0.01 \) when compared to RPB and CB.

Comparison with PB, RPB and CB by ANOVA.

\( f \) indicates \( P < 0.01 \) when compared to RPB and CB in the same obstetrical group.

Values are mean ± SEM. NS: no significance

Table 4: Expression of PD-1, GITR, HLA-G and CTLA-4 on Tregs in PB, RPB and CB from NP women, women with PE, GDM or PROM

| Tregs(%) | NP (n = 28) | PE (n = 15) | GDM (n = 28) | PROM (n = 23) | \( P^a \) |
|----------|-------------|-------------|-------------|--------------|---------|
| PD-1     |             |             |             |              |         |
| PB       | 8.09 ± 0.81 | 1.86 ± 0.26 | 1.42 ± 1.13 | 1.49 ± 0.28  | < 0.01  |
| RPB      | 8.58 ± 0.79 | 1.82 ± 0.38 | 1.33 ± 0.27 | 1.33 ± 0.27  | < 0.01  |
| CB       | 6.16 ± 0.43 | 1.79 ± 0.01 | 1.45 ± 0.25 | 1.38 ± 0.48  | < 0.01  |
| \( P^e \) | < 0.05      | < 0.05      | NS          | NS           |         |
| GITR     |             |             |             |              |         |
| PB       | 7.71 ± 0.87 | 3.07 ± 0.41 | 2.94 ± 1.3  | 2.84 ± 0.33  | < 0.01  |
| RPB      | 2.51 ± 0.63 | 0.28 ± 0.27 | 0.26 ± 0.27 | 0.27 ± 0.28  | < 0.01  |
| CB       | 0.91 ± 0.18 | 0.47 ± 0.25 | 0.36 ± 0.25 | 0.62 ± 1.15  | NS      |
| \( P^e \) | < 0.01      | < 0.01      | < 0.01      | < 0.01       |         |
| HLA-G    |             |             |             |              |         |
| PB       | 2.94 ± 0.63 | 1.36 ± 0.25 | 3.31 ± 0.90 | 1.47 ± 0.23  | NS      |
| RPB      | 25.19 ± 4.94| 27.37 ± 5.47| 26.46 ± 4.73| 26.46 ± 5.11 | NS      |
| CB       | 17.51 ± 3.61| 15.46 ± 4.67| 16.14 ± 4.67| 16.46 ± 4.16 | NS      |
| \( P^e \) | < 0.01      | < 0.01      | < 0.01      | < 0.01       |         |
| CTLA-4   |             |             |             |              |         |
| PB       | 0.12 ± 0.03 | 0.11 ± 0.01 | 0.13 ± 0.04 | 0.12 ± 0.05  | NS      |
| RPB      | 0.12 ± 0.11 | 0.11 ± 0.25 | 0.13 ± 0.25 | 0.12 ± 0.27  | NS      |
| CB       | 0.11 ± 0.02 | 0.14 ± 0.33 | 0.12 ± 0.24 | 0.12 ± 0.14  | NS      |
| \( P^e \) | NS          | NS          | NS          | NS          |         |

Comparison with NP, PE, GDM and PROM by ANOVA.

\( b^* \) indicates \( P < 0.01 \) when compared to NP.

\( c \) indicates \( P < 0.05 \) when compared to RPB and CB.

\( d^* \) indicates \( P < 0.05 \), \( d^{**} \) indicates \( P < 0.01 \) when compared to PB and CB.

Comparison with PB, RPB and CB by ANOVA.

\( f \) indicates \( P < 0.01 \) when compared to PB and RPB in the same obstetrical group.

Values are mean ± SEM. NS: no significance
Figures

Figure 2

Proportion of Tregs in PB, RPB and CB(A) and the reduction rate of Tregs(B) from NP women or women with GDM, PE and PROM. Values are mean ± SEM. *indicates significant differences from NP by Student’s T-test, P < 0.01. # indicates significant differences by one-way ANOVA followed by Tukey’s post-hoc test indicates when compared to RPB P < 0.05. ## indicates significant differences by one-way ANOVA followed by Tukey’s post-hoc test indicates when compared to RPB P < 0.01

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalTable.docx
- SupplementalTable.docx