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Inflammatory placental lesions are specifically observed in healthy oocyte donation pregnancies with extreme fetal-maternal incompatibility

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ABSTRACT

Introduction: Oocyte donation (OD) pregnancy is a risk factor for pre-eclampsia (PE). Due to a higher extent of fetal-maternal human leukocyte antigens (HLA) mismatching in OD pregnancies compared to naturally conceived (NC) and in vitro fertilization (IVF) pregnancies, the immune response in OD placentas is probably divergent and affects clinical outcomes. We hypothesized that placental pathology varies among diverse pregnancy conditions and is related to fetal-maternal HLA incompatibility.

Methods: Placental lesions were scored in four patient groups: OD-PE (n = 16), OD-healthy (n = 37), NC-PE (n = 45), and IVF-healthy (n = 17). All combinations were genotyped for HLA-A, -B, -C, -DR, and -DQ to calculate fetal-maternal HLA mismatches. Placentas showing chronic deciduitis with plasma cells were immunofluorescently stained with CD138 and the anti-inflammatory cytokine interleukin-10 (IL-10).

Results: The distribution and severity of placental lesions varied among groups. The OD-healthy group had the highest inflammation score and greatest extent of chronic deciduitis with plasma cells ($p < 0.05$). However, the majority of $CD138^+$ plasma cells (90%) in OD-healthy group expressed IL-10, in contrast to the OD-PE group (58%). The OD-healthy group was separated into semi-allogeneic (≤5 HLA mismatches) and fully allogeneic (>5 mismatches) subgroups. The elevated inflammatory pathology score and chronic deciduitis with plasma cells were found more often in the HLA-class-I fully allogeneic OD-healthy group than the IVF-healthy group ($p < 0.05$).

Discussion: Placental inflammatory lesions are most often present in uncomplicated OD pregnancies. Immune cells that infiltrate these lesions might play an immunosuppressive role to protect OD pregnancies from complications when facing a higher extent of fetal-maternal HLA mismatching.

1. Introduction

Oocyte donation (OD) is an indispensable part of modern assisted reproductive technology, and it has been proven to be an effective technique in achieving pregnancy for women with no functioning ovaries. However, OD pregnancy is a risk factor for pregnancy complications including pre-eclampsia [1–3], even after correction for maternal age and multiplets.

Pre-eclampsia is generally diagnosed as the new onset of hypertension and/or proteinuria at 20 weeks onwards of gestation, and it may affect multiple organs [4]. Abnormal placentation and maternal endothelial dysfunction are thought to be the two stages of the pathogenesis of pre-eclampsia [5]. The central characteristic of abnormal placentation is placenta ischemia, which can result from various factors,

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including poor spiral artery remodeling and imbalanced placental oxidative stress [6]. During placentation these procedures are influenced by decidual immune cells, as they accumulate around the degenerating vessels and also affect fetal extravillous trophoblasts (EVT) invading the basal plate [7,8].

Fetal and maternal cells come in contact at several interfaces after implantation [9]. Syncytiotrophoblast is directly exposed to maternal blood and invasive cytotrophoblast cells are attached to decidual tissue [10]. In OD pregnancies, the fetus can be fully allogeneic to the mother, with the consequence that decidual maternal cells are facing highly HLA-mismatched fetal cells. It is hypothesized that decidual immune cells play an important role in OD pregnancies to deal with the higher fetal-maternal gene dissimilarity and maintain healthy pregnancies [11]. It has been shown that in uncomplicated OD pregnancies, a higher level of fetal-maternal HLA matching is present than expected by chance [12]. Meanwhile, in uncomplicated OD pregnancies with a large extent of maternal-fetal HLA mismatching, a higher incidence of $FOXP3^+$ decidual Tregs was found [13].

Histologic assessment of the placenta may provide a clue to the pathophysiologic mechanisms and help explain adverse pregnancy outcomes. Lesions associated with maternal vascular malperfusion, such as decidual arteriopathy, are frequently found in the placentas from pre-eclampsia pregnancies and are related to the severity of symptoms [14,15]. Basal chronic villitis of unknown etiology (VUE), chronic membranitis, and chronic deciduitis are more likely present in the placentas of OD pregnancies than in those of non-donor in vitro fertilization (IVF) pregnancies [16,17]. VUE is characterized by infiltration of maternal T cells and activation of fetal Hofbauer cells in the villi, and it can be related to fetal growth restriction, pre-eclampsia, and preterm birth [18,19]. The presence of neutrophils within the chorion or amnion is defined as acute chorioamnionitis, whereas the infiltration of maternal T cells into the fetal membranes is indicated as chronic membranitis. Maternal lymphocytes and plasma cells are present in the placenta basal plate when diagnosed with chronic deciduitis [20]. Plasma cells are mainly known for antibody production and cell cytotoxicity. The infiltration of plasma cells in acute renal allograft rejection is usually associated with poor allograft survival [21], but in recent years immunosuppressive plasma cells expressing interleukin-10 (IL-10) have been identified [22,23]. The presence of plasma cells in deciduitis emphasizes its chronic nature and may result from fetal-maternal interactions [20].

In this study, we hypothesized that the frequency of pathology lesions is different among pregnancy conditions, including OD and pre-eclampsia, and that these differences relate to fetal-maternal HLA dissimilarity. We compared individual placental pathology lesions within pregnancy categories, namely OD, naturally conceived (NC) and IVF pregnancies, either healthy or complicated by pre-eclampsia. By analyzing the association between histology and fetal-maternal HLA mismatches we aimed to identify unique features that may reveal underlying pathophysiology.

2. Materials and methods

2.1. Patient selection

A total of 115 women who delivered in the Leiden University Medical Center (LUMC) were enrolled in this retrospective case-control study. Women were selected from a biobank of prospectively collected materials from every oocyte donation pregnancy delivered in our hospital without any selection or preference. Medical records of all patients were reviewed and clinical data were collected. Samples fell into four groups: OD pregnancies with pre-eclampsia (OD-PE, n = 16), uncomplicated OD pregnancies (OD-healthy, n = 37), NC pregnancies with pre-eclampsia (NC-PE, n = 45), and uncomplicated non-OD IVF pregnancies (IVF-healthy, n = 17). Pre-eclampsia was defined as de novo hypertension (systolic blood pressure of ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg) and proteinuria (≥300 mg in 24 h) presenting after 20 weeks of gestational age, or without proteinuria but with significant organ dysfunction [4]. Exclusion criteria were: maternal autoimmune disease, the presence of chromosomal abnormalities, the use of immunosuppressive medication, and gestational complications other than pre-eclampsia (maternal diabetes or gestational diabetes, stillbirth). Informed consent was obtained and the study was approved by the LUMC Ethics Committee (P16.048, P08.229/228, P10.009).

2.2. Histology examination

Placentas were collected after delivery. In both parenchyma and membrane a minimum of three locations (central, middle, and lateral) were selected. After fixation in 4% formaldehyde, tissue samples were embedded in paraffin. Serial sections (4-μm thick) were dried overnight at 37 °C. After staining with hematoxylin and eosin (H&E), slides were microscopically examined by a perinatal pathologist (N.G.), who was blinded for the group information and clinical data. Histopathology lesions were scored for their presence and severity. After reviewing by a second perinatal pathologist (L.M.), 21 lesions that were considered to be potentially associated with OD and/or pre-eclampsia were enrolled in this study (Supplementary Table 1). Based on Amsterdam Placental Workshop Group Consensus Statement [24], all lesions were divided into three categories: inflammatory, Maternal Vascular Malperfusion (MVM), and Fetal Vascular Malperfusion (FVM). Although intervillous thrombus and perivillous fibrin are not inflammatory lesions themselves, they are frequently seen with intervillositis and villitis, and evidence supports their relation to fetal-maternal interactions [25,26]. Therefore, they were categorized as inflammatory. Twelve lesions represented binary data as they were either present or absent. The other 9 lesions represented non-binary data for they also contained information of the severity.

2.3. Immunofluorescence

To investigate characteristics of plasma cells in chronic deciduitis, 10 placentas in the OD-healthy group and 3 placentas in the OD-PE group which were diagnosed with chronic deciduitis with plasma cells were studied for immunofluorescence. Additional placentas with chronic deciduitis with plasma cells were added. In total, 25 OD-healthy and 5 OD-PE placenta samples with chronic deciduitis with plasma cells were immunofluorescently stained. As plasma cells are the culprit in antibody-mediated transplant damage, as additional control, six kidney biopsy samples with acute rejection and/or chronic transplant dysfunction were enrolled for the study of plasma cells.

Paraffin-embedded placenta and kidney biopsy sections (4 μm) were deparaffinized in three consecutive xylol baths, then rehydrated in 100%, 70%, 50% ethanol, and two demi water baths, respectively.Antigen retrieval was performed via heat treatment in preheated 1x citrate buffer for 10 min, followed by a 5-min PBS washing. Then the sections were incubated with primary antibodies (CD138, mouse anti-human IgG1, 1:400, BioLegend, San Diego, CA; IL-10, rabbit anti-human IgG, 1:20, Hycult Biotech, Uden, the Netherlands) overnight in a dark humidified chamber, washed three times in PBS, and incubated with secondary antibodies (Alexa Fluor 488, goat-anti-mouse IgG1, 1:200, Life Technologies; Alexa Fluor 546, goat-anti-rabbit IgG, 1:200, Life Technologies, the Netherlands) for 30 min in the dark chamber. Sections were washed thrice in PBS before being mounted with Prolong Gold Antifade Mountant with DAPI (Invitrogen, Carlsbad, CA). A Zeiss LSM 700 confocal microscope was used for the imaging. The total number of $CD138^+$ cells and $CD138^+IL\text{-}10^+$ cells of one placenta/kidney section for each patient were manually counted at 200X magnification.

2.4. HLA typing

HLA typing was performed by the HLA typing laboratory of the LUMC. Maternal blood and fetal umbilical cord blood were used for

HLA-A, -B, -C, -DRB1, and -DQB1 2-digit typing at split level, using sequence specific polymerase chain reaction (PCR) priming. HLA class I mismatches were defined as the total of HLA-A, -B, -C mismatches; HLA class II mismatches were defined as the total of HLA-DRB1 and -DQB1 mismatches; total HLA mismatches were defined as the summary for all these five loci.

To determine the association between fetal-maternal HLA mismatches and pathology lesions, the OD-healthy group was divided into two subgroups according to the number of HLA mismatches. In the semi-allogeneic group, the number of mismatches was not higher than half of the antigens per HLA locus; in the fully allogeneic group, the number of mismatches was higher than half of the antigens per HLA locus, which is a situation unique for OD pregnancies.

2.5. Statistical analysis

Statistical analyses were performed using SPSS statistics 25 (IBM SPSS Software, Chicago USA), GraphPad Prism version 8 for Windows (GraphPad Software, San Diego California USA), and R version 4.0.3.

Singleton pregnancies (OD-healthy, n = 25; OD-PE, n = 3; IVF-healthy, n = 17; NC-PE, n = 45) were selected to perform analyses on. To enhance the statistical power for the OD-PE group, additional analysis was also performed on the whole cohort, including singleton and multiplets.

Agglomerative hierarchical clustering analysis was performed to visualize the distribution of pathology lesions among the groups. To present every detail of individual lesion/sample, non-binary data were used if lesions contained severity information. Since pathology lesions represented categorical data with limited categories, absolute values were used to build the matrix. Unsupervised clustering was performed on rows (samples) and columns (lesions) of the matrix, using Ward's hierarchical clustering method with Euclidean distance. A heatmap was made by "heatmap.2" from the package "gplots" in R. To visualize features clearly, several lesions were excluded from cluster analysis as they were observed in less than 10% of samples (chronic membranitis; intervillositis; chorionic plate vasculitis; microscopic parenchyma infarct; villous stromal karyorrhexis).

Scores of categories were summarized from the scores of lesions contained in each category. To equal each lesion, non-binary data were changed into binary data (different severities were all considered as "Yes"). The frequencies of individual inflammatory lesions were also analyzed to reveal remarkable lesions.

All data were non-normally distributed. Therefore, Mann Whitney U tests and Spearman's correlation were used to analyze continuous data and Fisher's exact test for nominal data. Logistic regression was used to evaluate whether inflammatory pathology score can influence the pregnant outcome independently, besides maternal age and gestational age. A value of $p < 0.05$ was considered to represent statistical significance.

3. Results

3.1. Patient characteristics

The results of the baseline characteristics of singleton pregnancies are summarized in Table 1. Two pre-eclampsia groups showed significantly higher diastole ($p < 0.01$), proteinuria ($p < 0.05$) and lower gestational age, birth weight, and vaginal delivery rate compared to two healthy groups ($p < 0.05$). The NC-PE group had the lowest maternal age among all the groups ($p < 0.05$) and demonstrated a significantly lower gestational age and fetal birth weight than the OD-PE group ($p < 0.01$). Patient characteristics of all samples are shown in Supplementary Table 2.

3.2. Cluster analysis

As shown in the heatmap (Fig. 1), unsupervised clustering separated all lesions (columns) mainly into two clusters: one included the MVM lesions and the other consisted mostly of inflammatory lesions. Two patient groups (Patient cluster I and Patient cluster II) demonstrated relatively different appearances in lesion categories. Patient cluster I contained samples that showed high intensity of placental inflammatory lesions, but lower intensity in MVM lesions. Patient cluster II had the opposite characteristics. The samples in these two clusters also exhibit different group aggregation. Nineteen out of 30 samples in Patient cluster I were from the OD-healthy group, while 36 out of 42 samples in Patient cluster II were from the NC-PE group. Vice versa, the majority of samples of the OD-healthy group fell into Patient Cluster I (19 out of 25) and the majority of samples of the NC-PE group fell into Patient Cluster II (36 out of 45).

3.3. Category analysis

Pathology categories analysis showed similar patterns as the

Table 1
Patient characteristics.

Clinical parameters		OD-healthy (n = 25)	OD-PE (n = 3)	IVF-healthy (n = 17)	NC-PE (n = 45)
Mother	Maternal age (year)	37 (27–49)	37 (36–44) #	36 (29–41) ##	30 (20–43) **
	Gravidity	2 (1–7)	1 (1–3)	2 (1–5)	1 (1–9)
	Parity	0 (0–2)	0 (0–1)	0 (0–2)	0 (0–5)
	Spontaneous miscarriage	0 (0–3)	0 (0–1)	0 (0–4)	0 (0–3)
	Gestational age (weeks)	40.1 (38.6–42.3)	36.3 (33.0–38.4) **/##	40.0 (36.7–42.0) ##/•	31.0 (27.1–38.7) **
	Highest diastole (mmHg)	80 (60–90)	105 (90–105) **	80 (70–95) ##/••	105 (75–160) **
	Proteinuria (dipstick grading from negative to ++++)	No (No - ±)	++ (+- +++) *	No (No - No) ##/••	++ (No - +++) **
	Vaginal delivery rate (number (percentage))	12 (48.0%)	1 (33.3%)	10 (58.8%) ##	3 (6.7%) **
Child	Gender (male) (number (percentage))	12 (48.0%)	1 (33.3%)	7 (41.2%)	19 (42.2%)
	Birth weight (gram)	3738 (3080–4260)	2620 (1600–3235) **/##	3450 (2440–4080) */##/•	1188 (657–1770) **
Donor	Related donor (number (percentage))	3 (12%)	0 (0%)		

Categorical variables (Spontaneous delivery rate, Gender and Related donor) are described using numbers and percentages. Fisher's exact tests were performed to determine p-value. Other variables are all non-normally distributed numerical variables, which are described by median with the minimum and maximum. Mann Whitney U tests were performed to determine p-value. Data are marked with *, #, or • for having significant differences between certain groups. Other data show no significant difference between groups.

OD: oocyte donation; PE: pre-eclampsia; IVF: in vitro fertilization; NC: naturally conceived.

*$p < 0.05$ vs. OD/healthy group.

**$p < 0.01$ vs. OD/healthy group.

#$p < 0.05$ vs. NC/PE group.

##$p < 0.01$ vs. NC/PE group.

••$p < 0.01$ vs. OD/PE group.

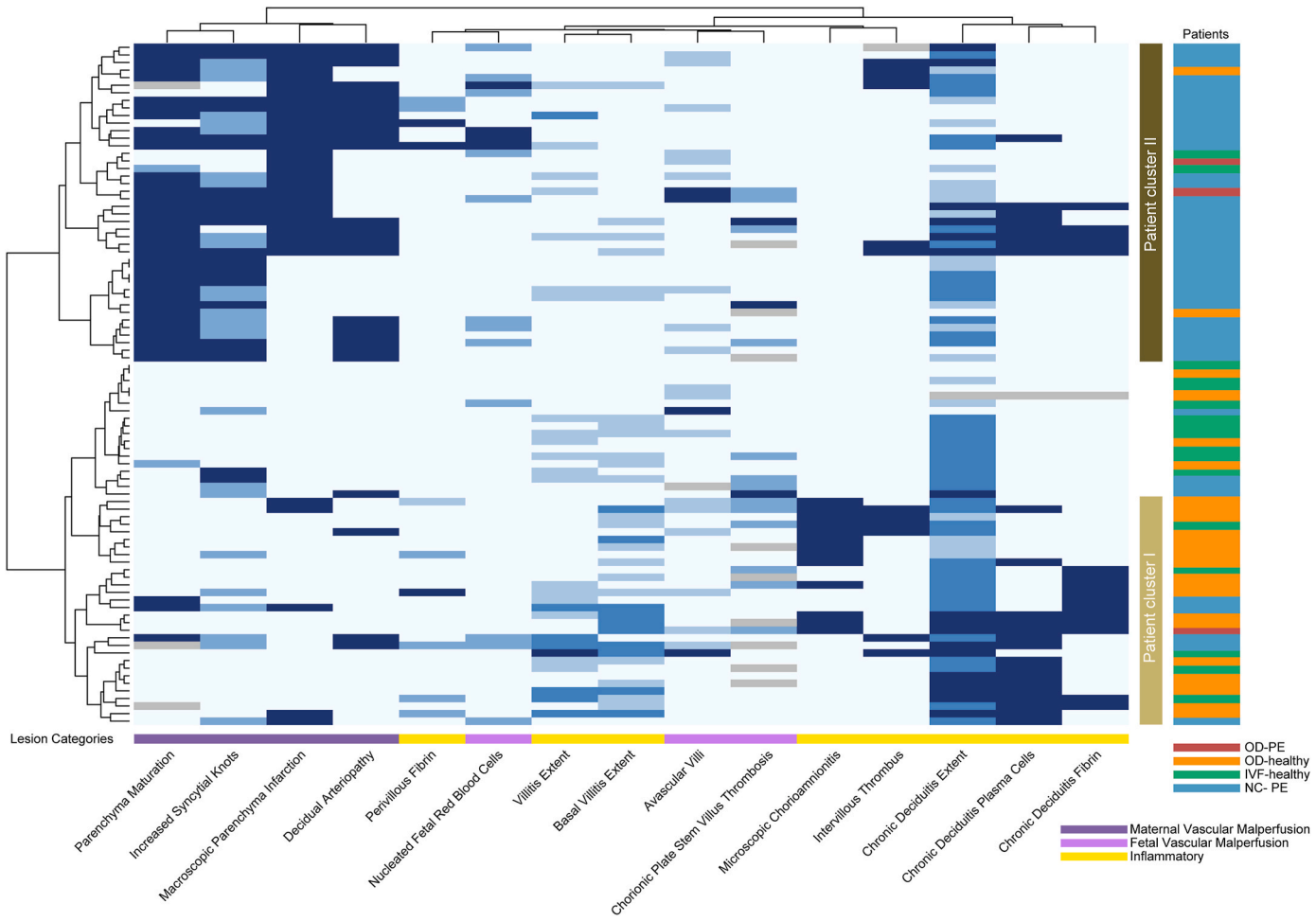


Fig. 1. Two-way hierarchical cluster analysis with Euclidean distance and Ward's aggregation method. Columns represent placenta lesions and rows represent patient samples. The intensity of lesions is indicated using a color scale, ranging from light blue (no lesion) to dark blue (severe lesion). Missing values are marked in grey. The groups of samples are shown on the right (red: OD-PE group, orange: OD-healthy group, green: IVF-healthy group, blue: NC-PE group). The categories of lesions are shown on the bottom (dark purple: Maternal Vascular Malperfusion, light purple: Fetal Vascular Malperfusion, yellow: Inflammatory). The dendrograms depicted at the top and on the left of the heatmap demonstrate the two dimensions of unsupervised clustering.

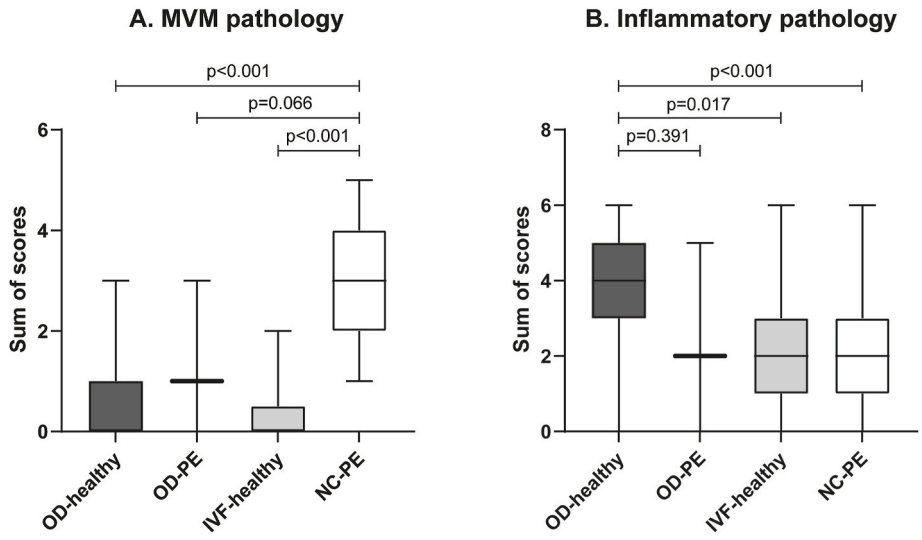


Fig. 2. Pathology lesion categories in the different pregnancy groups. OD-healthy, n = 25; OD-PE, n = 3; IVF-healthy, n = 17; NC-PE, n = 45. The middle horizontal line within the box indicates the median, the ends of the box correspond to the upper and lower quartiles of the data, and the whiskers indicate the minimum and maximum values. Mann-Whitney U tests were performed to identify differences between two groups. Results for statistical analyses results between groups are shown with p value.

clustering analysis of individual lesions. For the MVM pathology category, the NC-PE group showed significantly higher score than OD-healthy and IVF-healthy groups ($p < 0.001$; Fig. 2A). For the inflammatory pathology category, the OD-healthy group showed significantly higher score than IVF-healthy and NC-PE groups ($p < 0.05$; Fig. 2B). This higher inflammatory pathology score independently predicted pregnancy outcome and did not show significant correlation with maternal age or gestational age (data not shown). After including multiplets to expand the sample size, the OD-PE group showed significantly lower MVM score than the NC-PE group ($p < 0.001$) and lower inflammatory score than the OD-healthy group ($p = 0.046$) (see Supplementary Fig. 1A and B). The FVM pathology lesions score did not show significant difference between groups (data not shown).

3.4. Individual lesions analysis

The presence of microscopic chorioamnionitis was significantly higher in the OD-healthy and OD-PE group compared to the NC-PE group ($p = 0.013$ and $p < 0.001$; Fig. 3A,D). Basal chronic villitis showed significantly higher frequency in the OD-healthy group compared to the NC-PE group ($p = 0.002$; Fig. 3B, E). After including multiplets, the OD-healthy group showed significantly higher frequency of basal chronic villitis than the OD-PE group ($p = 0.035$, Supplementary Figure 1C). Although chronic villitis had significant correlation with basal chronic villitis (Spearman's correlation $r^2 = 0.16$, $p < 0.0001$), the extent of chronic villitis did not show significant difference between any groups. When compared with the IVF-healthy group, the OD-healthy group showed significantly higher percentage of chronic deciduitis with plasma cells ($p = 0.046$; Fig. 3C, F). Additionally, samples with basal chronic villitis had a significant higher chance to have chronic deciduitis with plasma cells on their placentas ($p < 0.001$) than those without basal chronic villitis.

In the OD-healthy group, basal chronic villitis had significantly negative correlation with patient gestational age (Spearman's correlation $r^2 = 0.40$, $p = 0.0010$). No significant correlation was found between the other two lesions and clinical parameters (data not shown). No clinical or histological signs of infection were present in patients with one of these three pathology lesions.

3.5. IL-10 producing plasma cells in chronic deciduitis

As plasma cells have been primarily regarded as having an adverse effect in the context of humoral immunity, it is remarkable that their presence is higher in healthy pregnancies after OD. We therefore further quantified the frequencies of these cells by immunofluorescence and determined their phenotype by investigating the expression of the immune regulatory cytokine IL-10. $CD138^{+}IL\text{-}10^{+}$ and $CD138^{+}IL\text{-}10^{-}$ plasma cells were both observed (Fig. 4A and B). Firstly, the number of $CD138^{+}$ plasma cells varied between samples and between groups. Seven samples in the OD-healthy group contained insufficient (<5) numbers of $CD138^{+}$ plasma cells (28%) but none in the OD-PE group (0%) (Fig. 4C). More importantly, after excluding samples with insufficient plasma cell numbers, the proportion of IL-10 producing plasma cells was significantly higher in the OD-healthy group (90%) than that in the OD-PE group (58%; $p = 0.037$; Fig. 4D). As additional and external control, we investigated kidney transplants with rejection and these also showed a significantly lower proportion of IL-10 producing plasma cells (Fig. 4D).

3.6. Association between pathology lesions and fetal-maternal HLA mismatches

We focused on the OD-healthy group since the inflammatory lesions were most pronounced in this group. For HLA class I mismatches, a significantly higher inflammatory pathology score was found in the fully allogeneic OD-healthy group than in the IVF-healthy group ($p < 0.05$;

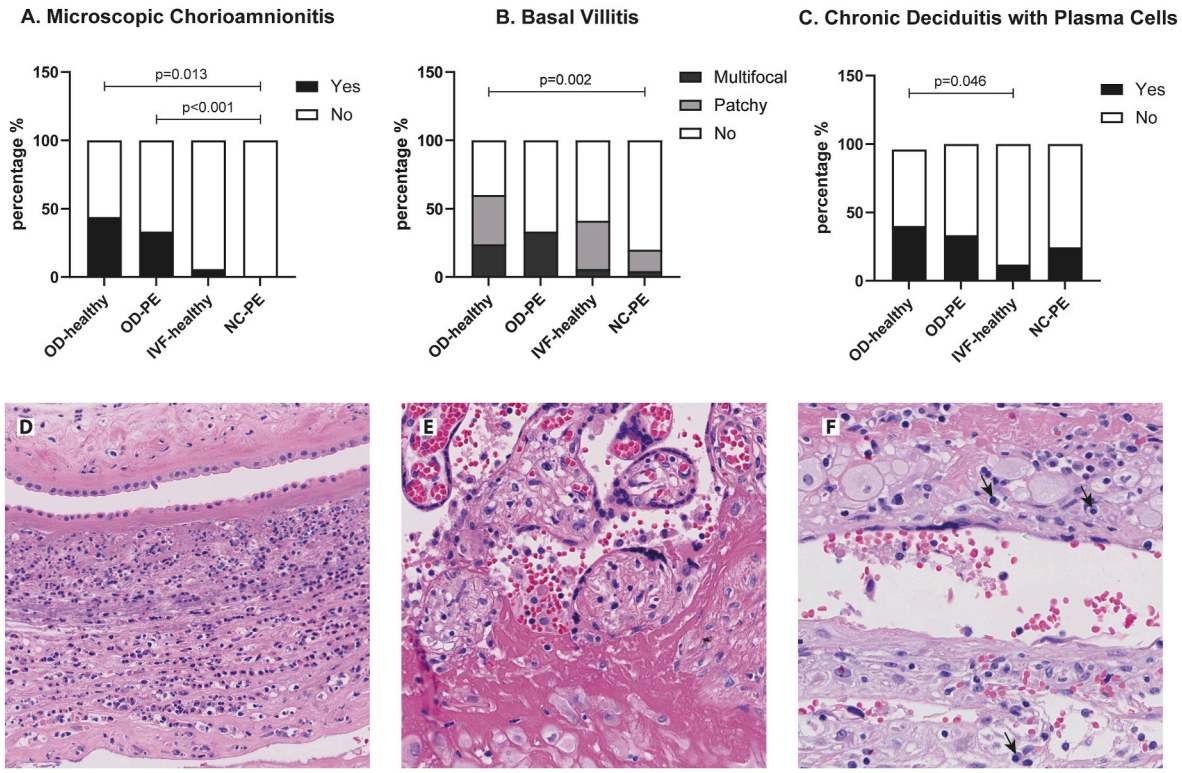


Fig. 3. A-C: Percentages of lesions are shown in histograms. OD-healthy, n = 25; OD-PE, n = 3; IVF-healthy, n = 17; NC-PE, n = 45. Fisher's exact tests were performed to identify differences in the proportion of lesions between groups. Significant differences are shown with p value on the top of the histogram; **D-F:** HE staining of microscopic chorioamnionitis (D), basal villitis (E), chronic deciduitis with plasma cells, arrows point to plasma cells (F). Magnification 400X.

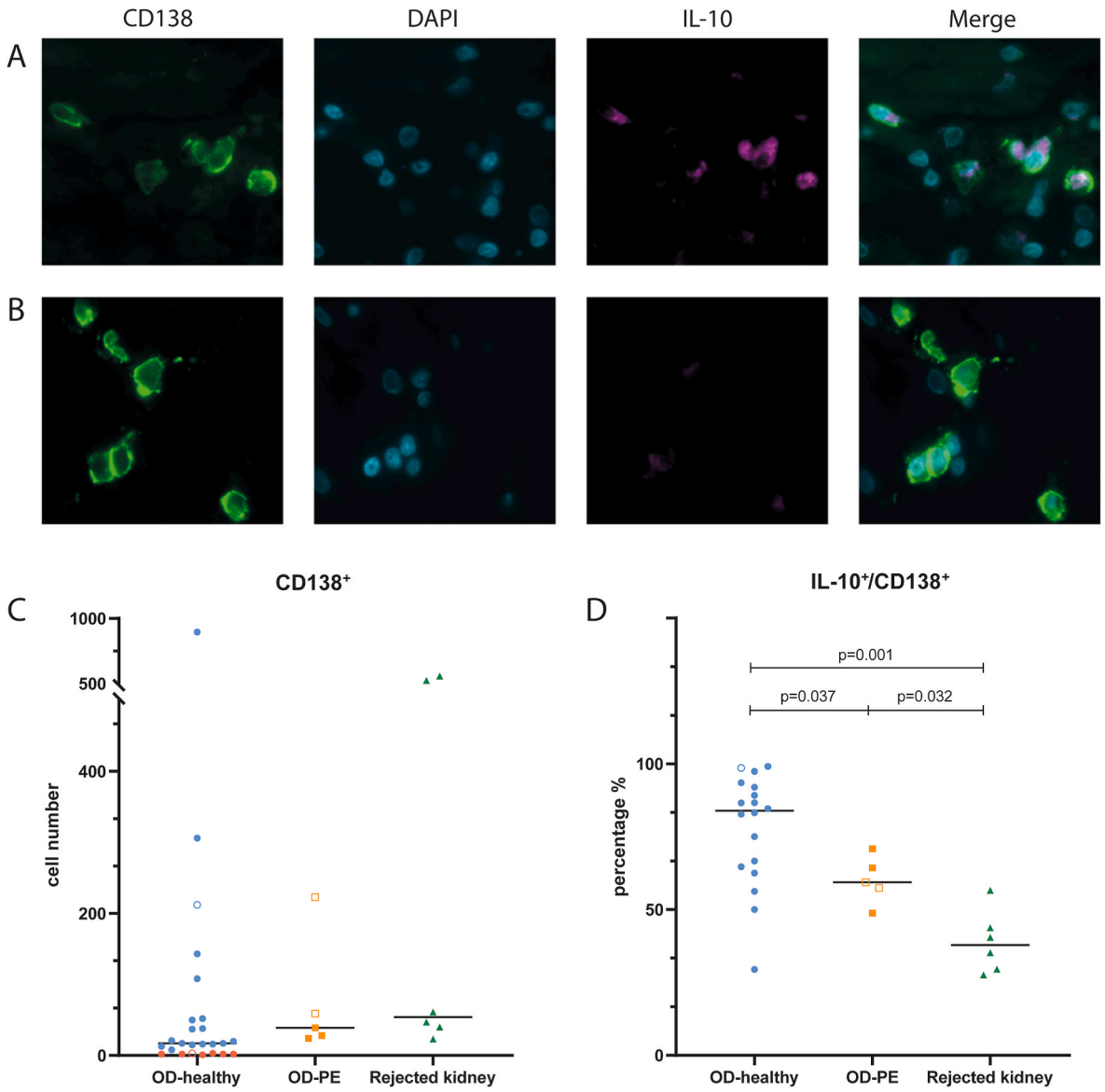


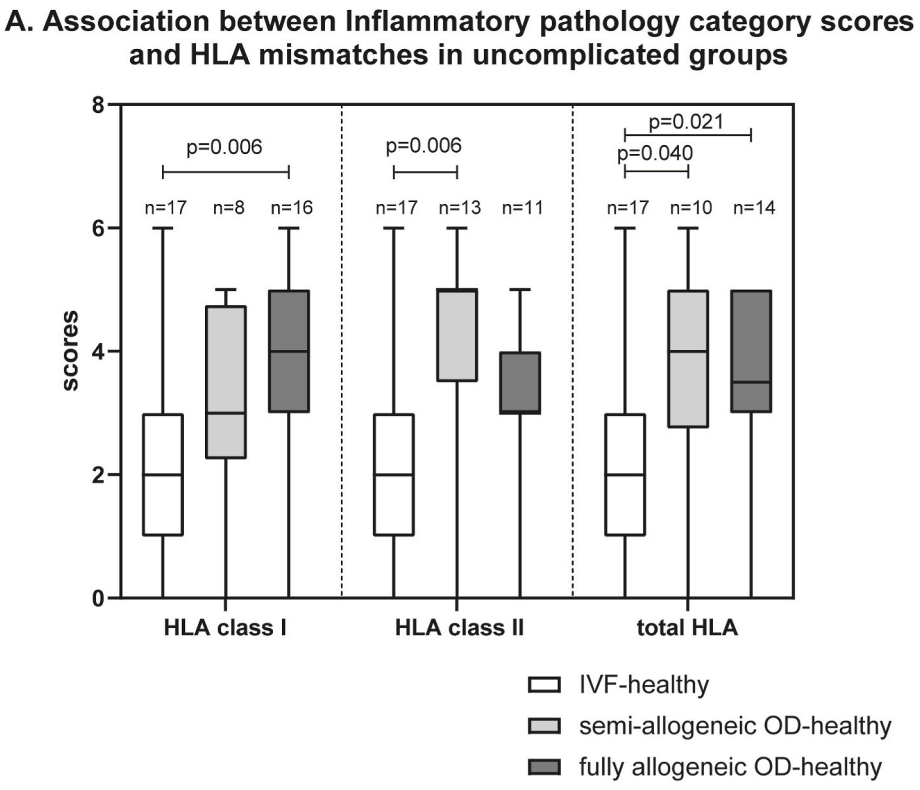
Fig. 4. A, B: Representative images of the expression of IL-10 in plasma cells. CD138+ plasma cells produced IL-10 (A) or did not produce IL-10 (B) in decidua basalis from OD pregnancies. The nucleus is shown in cyan, CD138 is shown in green, IL-10 is shown in magenta. **C:** The cell numbers of $CD138^+$ plasma cells in OD-healthy, OD-PE and rejected kidney transplant groups. Red dots represent samples with $CD138^+$ cell number less than 5. **D:** The proportion of IL-10 producing $CD138^+$ plasma cells among all the $CD138^+$ plasma cells in three groups, after excluding samples with less than 5 $CD138^+$ plasma cells. Mann-Whitney U tests were performed to identify differences between each two groups. P values are shown above. Closed symbols represent singletons, and open symbols represent multiplets.

Fig. 5A), while this difference cannot be found between semi-allogeneic OD-healthy group and IVF-healthy group. Moreover, HLA-total-fully-allogeneic OD-healthy group demonstrated significantly higher inflammatory pathology score than the IVF-healthy group (Fig. 5A). In HLA class II and total HLA, the semi-allogeneic OD healthy group also showed significantly higher scores than the IVF-healthy group. For chronic deciduitis with plasma cells, the HLA-class-I and HLA-total fully allogeneic OD group showed a higher percentage than the IVF-healthy group ($p < 0.05$; Fig. 5B), while only in HLA-class-II, the semi-allogeneic OD healthy group showed a higher percentage than the IVF-healthy group. For the OD-PE group, no significant difference was found for HLA mismatches when compared with the OD-healthy group (data not shown).

4. Discussion

Inflammatory lesions in the placenta are specifically observed in uncomplicated OD pregnancies. By hierarchical clustering analysis we distinguished two patient sample clusters, one characterized by high severity in inflammatory lesions and low frequency in MVM lesions, mainly consisting of samples from the OD-healthy group. This was further confirmed by the category analysis, in which OD-healthy group showed a significantly higher inflammatory pathology score than the other groups. The fully allogeneic OD-healthy pregnancies, especially HLA-class-I fully allogeneic OD-healthy pregnancies, which are related to a high extent of fetal-maternal HLA mismatches, had significantly higher inflammatory pathology scores and higher proportion of chronic deciduitis with plasma cells than those in IVF-healthy pregnancies. In addition, plasma cells in chronic deciduitis are more often producing IL-10 in OD-healthy placentas compared with OD-PE placentas.

Previous studies displayed similar results with a higher frequency of chronic placental lesions in OD pregnancies compared to IVF pregnancies, while comparison of pathology lesions frequencies between OD-healthy and OD-PE pregnancies was not specifically described [27,28].



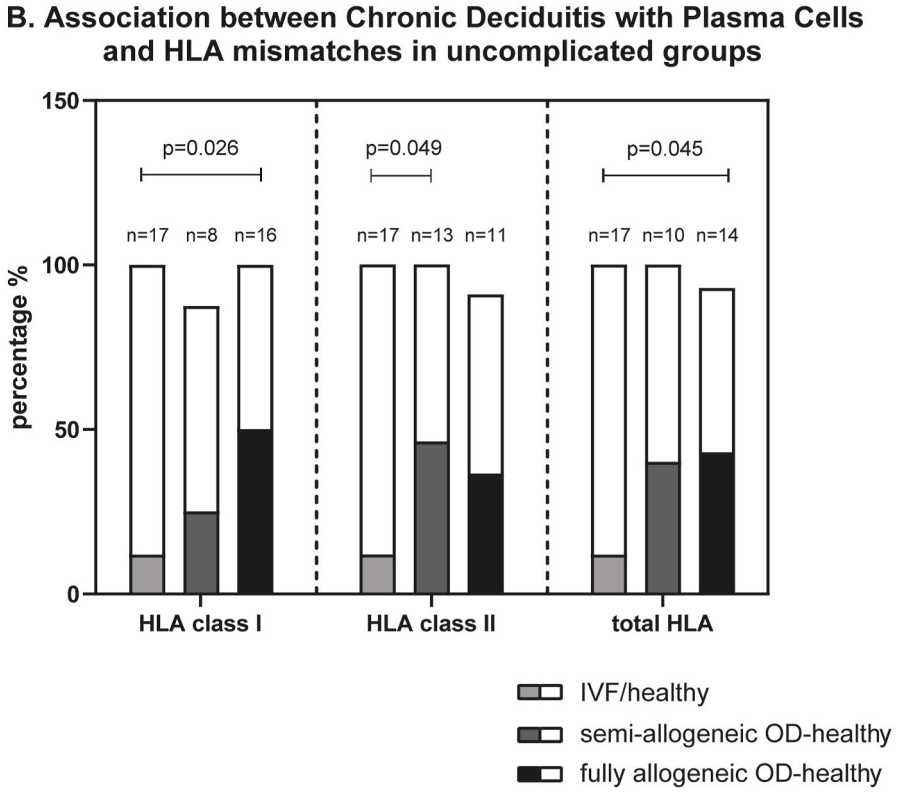


Fig. 5. Association between fetal-maternal HLA mismatches and inflammatory pathology category score/chronic deciduitis with plasma cells in uncomplicated groups. Significant differences are shown with p value. Sample sizes are shown below the p value. **A.** Mann-Whitney U tests were performed to identify differences between groups. The middle horizonal line within the box indicates the median, the ends of the box correspond to the upper and lower quartiles of the data, and the whiskers indicate minimum and maximum values. **B.** Fisher's exact tests were performed to identify lesion proportion differences between groups. On each column, the colored part stands for "Yes", the uncolored part stands for "No".

As OD pregnancies are facing a potentially more extensive fetal-maternal incompatibility than normal pregnancies, a healthy OD pregnancy might require more immune regulation at the fetal-maternal interface. Our study showed that OD-healthy and OD-PE group did not have significant difference in vascular malperfusion pathology lesions, but demonstrated differences in inflammatory lesions, and this difference appeared to be significant after including multiplets to expand the sample size of OD-PE group. As the OD-PE group only had 3 singleton samples, which made this group more susceptible to random variability, we used non-parametric test and included multiplets to correct this bias. Although we are aware that there are more differences between singletons and multiplets, we considered fetuses in multiplets as separate samples in our analysis based on the differences in their gender and/or HLA typing. Lashley et al. showed C4d deposition features between pre-eclamptic OD and autologous pregnancies [29]. Thus, we suggest that the underlying pathophysiology for pre-eclampsia cases in OD pregnancies is not represented by placental malperfusion but rather by a lack of immune regulation.

We have further specified some remarkable individual lesions. Dancey et al. [28] found greater odds ratios of VUE for OD pregnancies compared to IVF pregnancies. We further distinguished villitis on its location. Basal villitis, which is the inflammation involving the villi (fetal portion) that are anchored to the basal plate (maternal portion) [30], was present in a significantly higher proportion in the OD-healthy group than in the NC-PE group. The OD-healthy group also showed a significantly higher proportion of basal villitis than the OD-PE group after including multiplets. This higher presence of basal villitis in OD-healthy samples suggests that this inflammatory change is specifically located and may play a role in fetal-maternal interaction. The positive relationship between basal villitis and chronic deciduitis with plasma cells may suggest an inflammatory infiltration locally.

The exact immune mechanism at the fetal-maternal interface of OD-healthy pregnancies needs to be further defined. Chronic deciduitis with plasma cells, characterized by presence of lymphocytes and plasma cells at the basal plate [20], was more abundant in the OD-healthy group than in the IVF-healthy group. Gundogan et al. [31] demonstrated that $CD4^+$ T helper cells but not cytotoxic $CD8^+$ T cells were increased in OD pregnancies with chronic deciduitis, which suggests regulation rather than attack from maternal immune cells towards fetal cells in OD pregnancies [32]. However, Rudenko et al. [27] found the opposite tendency with respect to decidual T cell differentiation in OD pregnancies with more deciduitis. Meanwhile, both studies showed pronounced CD138 positive signals, a marker for plasma cells, in the decidua basalis of OD pregnancies. Generally, plasma cells are considered to be antibody-secreting cells: in kidney transplant rejection they produce IgG and are related to poor graft survival [33,34]. But certain plasma cell subsets may have immune regulatory functions by producing the cytokine IL-10 [22,35]. Therefore, we performed double-label immunofluorescent staining to reveal the phenotype of plasma cells in samples with chronic deciduitis. As the majority of plasma cells in decidua basalis of OD-healthy samples were producing IL-10, significantly higher than the proportion observed in the OD-PE group, it may be that such feature is related to the healthy outcome of OD pregnancies. In this study we have analyzed rejecting kidney transplant samples and found a low proportion of IL-10 producing plasma cells in the kidney biopsies, confirming that in rejected kidneys the infiltrating plasma cells are less likely to perform immune regulation function. The OD-healthy group showed significantly higher IL-10 producing plasma cells percentage than the rejected kidney group. We speculate that to maintain OD pregnancies healthy, immunosuppressive conditions are required for maternal immune cells when facing fetal cells with a high HLA mismatch load.

Several acute lesions, possibly associated with infection and spontaneous delivery [36], were included in our categories. Microscopic chorioamnionitis, defined as an acute lesion [37], showed significantly higher frequency in OD-healthy- and OD-PE groups compared to the NC-PE group. Histologic acute chorioamnionitis is related to spontaneous labor [38], although in our study no association between these two parameters was found. Since we found no signs of clinical infection in any of the cases, and no association between inflammatory pathology score and delivery methods, the impact of acute lesions to the whole score is minor. Dominated by chronic inflammatory lesions, the inflammatory pathology category in our study tends to be associated with the source of the oocyte and possibly represents the fetal-maternal interaction in the placenta [20].

The observation of a higher frequency of MVM lesions in the NC-PE group compared to the IVF-healthy group fits with the common understanding of pre-eclampsia [39]. No difference in inflammatory lesions was found between these two groups. The OD-PE group demonstrated a lower trend of having MVM lesions than NC-PE group but without statistical significance. It is reported that in preterm birth, singleton pregnancies are characterized by a higher rate of inflammatory and malperfusion lesions in their placentas [40,41]. This might be a part of the reason that the OD-PE group had a significantly lower MVM pathology score than the NC-PE group after including multiplets. But without showing a high frequency in either MVM or inflammatory category, the OD-PE group demonstrated a heterogeneous image in the heatmap, leaving the pathogenesis of pre-eclampsia in OD pregnancies yet unexplained [21,42].

HLA mismatches are potentially associated with a high frequency of inflammatory lesions in the OD-healthy group, as the elevated inflammatory pathology score and chronic deciduitis with plasma cells were found more often in the OD-healthy group than the IVF-healthy group. Especially in HLA class I, only the fully allogeneic but not the semi-allogenic OD healthy group showed significantly higher frequency of inflammatory lesions than the IVF healthy group. HLA-C is the only classical HLA molecule expressed on the extravillous trophoblasts in the fetal-maternal interface [43,44]. Maternal Natural Killer (NK) cells and T cells have the ability to recognize fetal HLA-C allo-antigens, and conversely the HLA-C expression level influences responses by immune cells [44]. Through inhibitory and activating killer cell immunoglobulin-like receptors (KIR) that are present on the NK cell surface, HLA-C molecules adjust decidual NK cells function towards trophoblast invasion and placentation [45]. One study also showed that HLA-C mismatched pregnancies are related to elevated levels of functional $CD4^+CD25^{bright}$ regulatory T cells in the decidual tissue [46]. HLA-DR mismatches have a positive correlation with the number of $CD4^+CD25^{dim}$ cells in maternal peripheral blood of uncomplicated OD pregnancies [47]. Since OD pregnancies with higher HLA mismatches tend to have more inflammatory lesions but still maintain healthy until term, it is possible that these inflammatory lesions are innocuous or even protective. Additional HLA molecules, such as HLA-DP and minor histocompatibility antigens (mHAg), which might be shared with related oocyte donors may also influence immune responses, as was reported for solid organ and hematopoietic cell transplantation [48]. However, since only three samples are confirmed to have related donors in our study, and one of them is found to have high HLA mismatches, we expect this influence to be minor. The exact functional pathway between HLA mismatches and inflammatory lesions needs to be further defined in future studies with a larger cohort.

In conclusion, placental inflammatory lesions are specifically detected in uncomplicated OD pregnancies, and their presence is associated with a higher extent of fetal-maternal HLA mismatching. Immune cells that infiltrate these lesions may play an immunosuppressive role to protect OD pregnancies from complications. Further studies characterizing the underlying pathophysiology of this inflammation will help to understand the reason for adverse pregnancy outcomes and may contribute to pregnancy management.

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Author contributions

XT: data collection, experiment and analysis, draft writing, NNTG: data collection and evaluation, LvdM: data evaluation, JY: experiment and analysis, HK: experiment, EELOL: conceptualization, ME: conceptualization, supervision and manuscript editing, MLPvdH: conceptualization, supervision and manuscript editing.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2023.10.005.

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