

ranging from first to third trimester), six weeks after OD pregnancy ( $n = 16$ , mononuclear cells), and directly after autologous pregnancy with a male fetus ( $n = 15$ , whole blood) were analysed for the presence of male FMc using quantitative polymerase chain reaction (qPCR) for a Y-chromosome-specific sequence (DYS1). In addition, umbilical cord blood samples were collected directly after delivery. Maternal and fetal HLA typing was performed at split level for HLA class I loci A, B, and C and for HLA class II loci DR1 and DQ1 to calculate the number of fetal-maternal HLA mismatches.

**Results:** Male FMc was detected in 14 of 16 (87.5%) women during OD pregnancy, 12 of 16 (75.0%) women at six weeks after OD pregnancy, and 14 of 15 (93.3%) women after autologous pregnancy. The median genome equivalent values for these three groups were 1.368 (0 – 11.947), 1.301 (0 – 9.371), and 4.107 (0 – 26.941), respectively ( $p =$  not significant between groups). When the number of fetal-maternal HLA mismatches of the autologous group and postpartum OD group were taken into account, the amount of FMc was significantly lower after pregnancies with high immunogenetic dissimilarity (6-10 total HLA mismatches) compared to low immunogenetic dissimilarity (0-5 total HLA mismatches;  $p = 0.039$ ). When the number of fetal-maternal HLA mismatches of only the postpartum OD group were taken into account, the amount of FMc was also lowest in high immunogenetic pregnancies (4-6 mismatches for HLA class I, 3-4 mismatches for HLA class II, 6-10 for total HLA mismatches), though without statistical significance.

**Conclusions:** FMc is detectable in maternal blood after OD pregnancy, and a higher degree of fetal-maternal immunogenetic dissimilarity may be related to decreased amounts of FMc. Due to the limited sample size, the study is further expanded to establish a conclusive analysis of the association between the amount of FMc and the number of fetal-maternal HLA mismatching. Nonetheless, this is the first study to investigate this association, which might provide new insights of the maternal immune response and its interaction

<http://dx.doi.org/10.1016/j.jri.2022.103534>

### 16:10-16:20 A combination of immune cell types identified through ensemble machine learning strategy detects altered profile in recurrent pregnancy loss

Marilen Benner<sup>a,2</sup>, Dorien Feytaerts<sup>a,3</sup>,  
Alejandro Lopez-Rincon<sup>b</sup>,  
Olivier WH van der Heijden<sup>c</sup>,  
Marie-Louise van der Hoorn<sup>d</sup>, Irma Joosten<sup>a</sup>,  
Gerben Ferwerda<sup>a</sup>, Renate G van der Molen<sup>a</sup>

<sup>a</sup> Department of Laboratory Medicine, Laboratory of Medical Immunology, Radboud University Medical Center, Nijmegen, The Netherlands

<sup>b</sup> Department of Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands

<sup>c</sup> Department of Obstetrics and Gynaecology, Radboud University Medical Center, Nijmegen, The Netherlands

<sup>d</sup> Department of Gynaecology and Obstetrics, Leiden University Medical Center, Leiden, The Netherlands

**Problem:** The underlying cause of recurrent pregnancy loss (RPL) remains largely unknown. As successful implantation and placentation depends on a tightly regulated immune response to facilitate adequate interaction with trophoblast cells, dysregulation of immunity might account for idiopathic RPL. So far, most studies focused on peripheral blood immune cells and performed single immunological parameter

analysis which up till now did not allow for clear classification of RPL versus healthy pregnancies. Therefore, we here defined immune profiles in menstrual blood (MB) derived endometrial cells as well as peripheral blood (PB) of RPL patients and compared them to those of women who had healthy pregnancies.

**Method of Study:** PB and MB was obtained from women who suffered from at least three consecutive unexplained RPL ( $n=18$ ) and aged-matched women without known disorders of reproduction and analyzed using flowcytometry. The proportions of 63 immune cell types defined by deep immune phenotyping were included in the analysis, next to age and CMV status.

**Results:** By harnessing the combined value of 8 machine learning classifiers in an ensemble strategy and recursive feature selection, we were able to determine a combination of immune parameters that separated RPL from controls. In peripheral blood, the combination of four cell types (non-switched memory B cells, CD8 + CD4-T cells, CD56bright CD16- Natural Killer (NK) cells, CD4 + effector T cells) classified samples correctly to their respective cohort. The identified cell types differed from the results observed in MB, where a combination of 6 cell types (Ki67 + CD8 + T cells, (HLA-DR +) regulatory T cells, CD27 + B cells, NK cells, CD24HiCD38Hi B cells) plus age. Based on the combination of these features, the average area under the curve of a receiver operating characteristics curve and the associated accuracy was  $>0.8$  for both sample sources.

**Conclusion:** A combination of immune subsets for cohort classification allows for robust identification of immune parameters with possible diagnostic value and deserves further large-scale validation. In addition, the non-invasive source of menstrual blood holds many opportunities to assess and monitor reproductive health and further study the pathological mechanism of RPL.

<http://dx.doi.org/10.1016/j.jri.2022.103535>

### 16:20-16:40 HLA sensitization and fetal losses

A. Benachi, Julien Zuber

Hopital antoine Beclère, Clamart, France

The historical conceptualization of the fetus as a semi-allogenic allograft has been vigorously challenged over the past decades. Critics argued that this paradigm overlooked the unique specificities of the maternal-fetal interface, namely the placenta.

However, recent reports have demonstrated that a breakdown in maternal-fetal tolerance could entail severe obstetrical complications, including intrauterine growth restriction (IUGR) and fetus demises. More specifically, immunological mechanisms that fully recapitulate those of allograft rejection have been involved in the pathogenesis of chronic histiocytic intervillitis.

According to the Banff classification of allograft pathology, chronic histiocytic intervillitis meets all the criteria for the diagnosis of antibody-mediated rejection based on the following evidence: 1- histologic evidence of acute tissue injury; 2- evidence of current or recent antibody interactions with targeted tissues; 3- serologic evidence of a fetus-specific antibody targeting the paternal human leukocyte antigen (HLA) at the villous surface.

This finding uncovers that an overt and potent adaptive alloimmune response can develop in pregnancy and can eventually lead to fetus rejection.

We aim to review our current understanding of how the alloimmune response is differently shaped and controlled in pregnancy and transplantation. A better grasp of the complex synergistic pathways involved in maternal-fetal tolerance across HLA barrier, as well as the understanding of the mechanisms underpinning its

<sup>2</sup> Authors contributed equally.

<sup>3</sup> Present address: Stanford University School of Medicine, Department of Anesthesiology, Perioperative and Pain Medicine, Stanford, CA, USA.