

RESEARCH

Open Access



Sex-specific phenotypical, functional and metabolic profiles of human term placenta macrophages

Daniel E. Paparini^{1*}, Esteban Grasso¹, Franco Aguilera^{1,2}, M. Agustina Arslanian³, Victoria Lella³, Brenda Lara¹, Ana Schafir¹, Soledad Gori¹, Fátima Merech¹, Vanesa Hauk¹, Claudio Schuster², Marcelo Martí², Cesar Meller³, Rosanna Ramhorst¹, Daiana Vota¹ and Claudia Pérez Leirós^{1*}

Abstract

Background Placental macrophages, Hofbauer cells (HBC) are the only fetal immune cell population within the stroma of healthy placenta along pregnancy. They are central players in maintaining immune tolerance during pregnancy. Immunometabolism emerged a few years ago as a new field that integrates cellular metabolism with immune responses, however, the immunometabolism of HBC has not been explored yet. Here we studied the sex-specific differences in the phenotypic, functional and immunometabolic profile of HBC.

Methods HBC were isolated from human term placentas ($N=31$, 16 from male and 15 female neonates). Ex vivo assays were carried out to assess active metabolic and endoplasmic reticulum stress pathways by flow cytometry, confocal microscopy, gene expression and in silico approaches.

Results HBC from female placentas displayed a stronger M2 phenotype accompanied by high rates of efferocytosis majorly sustained on lipid metabolism. On the other hand, male HBC expressed a weaker M2 phenotype with higher glycolytic metabolism. LPS stimulation reinforced the glycolytic metabolism in male but not in female HBC. Physiological endoplasmic reticulum stress activates IRE-1 differently, since its pharmacological inhibition increased lipid mobilization, accumulation and efferocytosis only in female HBC. Moreover, differential sex-associated pathways accompanying the phenotypic and functional profiles of HBC appeared related to the placental villi environment.

Conclusions These results support sex-associated effects on the immunometabolism of the HBC and adds another layer of complexity to the intricate maternal-fetal immune interaction.

*Correspondence:

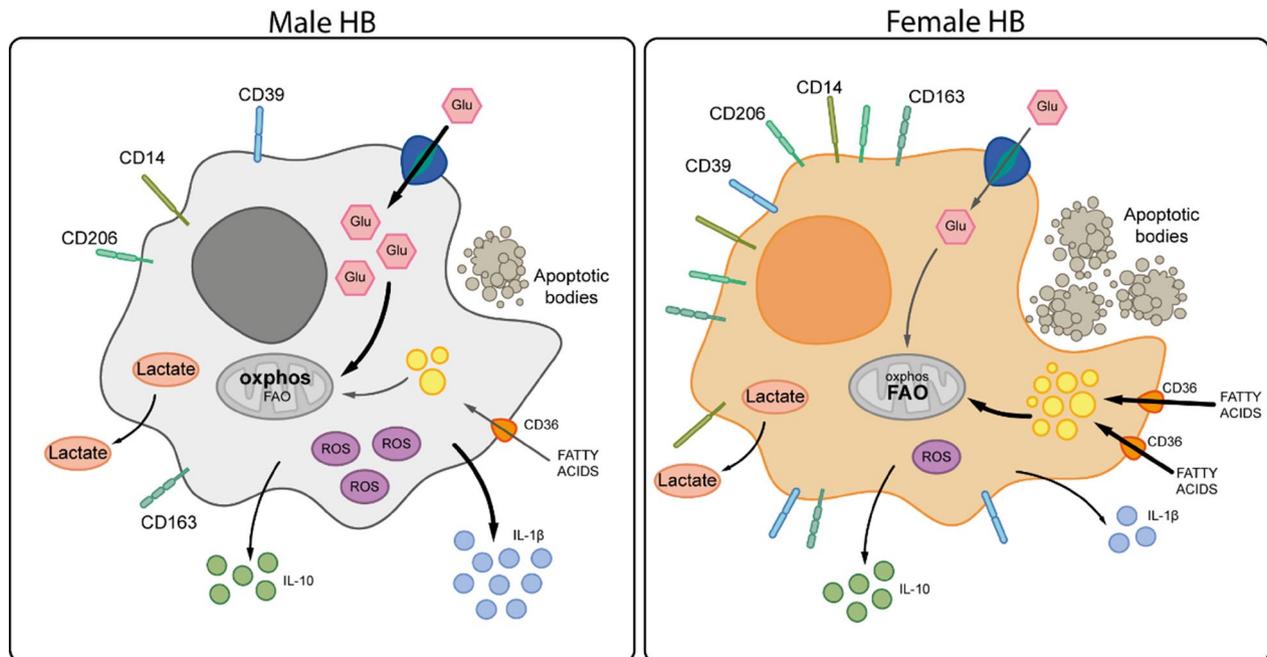
Daniel E. Paparini
daniel.paparini@gmail.com
Claudia Pérez Leirós
cpleiros@qb.fcen.uba.ar

Full list of author information is available at the end of the article



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Graphical abstract



Plain English Summary

Placental macrophages are the only fetal immune cell population within the stroma of healthy placenta along pregnancy and play a central role in contributing to the correct functioning of the placenta for the development of the fetus. Alterations in their metabolism lead to failures in their functions which are associated to pregnancy complications. Although, sex-specific differences were found in placental adaptation to pregnancy complications and outcomes, but the metabolism associated to their functions of placenta macrophages and whether they are associated to the sex of the placenta have not been explored so far. Here we studied human term placenta macrophages with special focus on their metabolism associated with their functions. We found out that macrophages from female placenta got energy from fatty acids whereas male macrophages used glucose. Furthermore, when female macrophages were exposed to a bacterial component, their metabolism or cellular function did not change towards one associated with a classic profile, but male macrophages did. These results might contribute to gain more insight into the immune-placental interactions at term human pregnancy and provide new clues to begin personalizing the pregnancy according to the sex of the fetus in physiological or pregnancy complications of inflammatory nature.

Highlights

- Sex-associated diversity in placental macrophages (HBC) immunometabolism may contribute to the understanding of differential susceptibility to infections and pregnancy complications.
- Under physiological stress, IRE-1 α bias acts differently in male and female HBC in the endoplasmic reticulum and underlies a possible mechanism of HBC immunometabolic profiling.

Keywords Placental-macrophages, Metabolism, Sex-associated differences

Background

Differences in pregnancy development and outcomes associated to the fetal sex have been reported for many years [1–5]. Fetal growth is partly determined by fetal sex: at birth, male neonates tend to be larger and heavier than females regardless of placental weight [4, 6–8]. Interestingly, during gestation, male fetuses grow faster than female fetuses, what makes them more susceptible to

maternal nutritional insults [3, 9, 10], exposure to chemical [11–13] and infectious agents [10, 14, 15]. Moreover, sex-specific differences were found in placental adaptation to pregnancy complications and outcomes [2, 16–18].

Hofbauer cells (HBC) are the only fetal immune cell population within the stroma of healthy placenta [19–21]. These placental cells are transcriptional and

chromosomally like yolk sac macrophages [21–23]. They reside in the placenta from 18 days post-conception until term and are central players in maintaining immune tolerance during pregnancy [19, 20, 24, 25]. HBC are poorly described due to their heterogeneity. Physiologically, HBC are activated in an alternative or M2 profile that sustain tissue repair, remodeling, apoptotic cell removal (efferocytosis), angiogenesis and immunological tolerance with massive antiinflammatory mediators' secretion [21, 25, 26]. In pregnancy complications like preeclampsia or fetal growth restriction, they switch to a predominantly classical or M1 activation and contribute to homeostasis loss at the maternal-fetal unit [24, 27], but in vitro they had showed a resistance to inflammatory cues [28]. Regarding sex-differences in the phenotypical profile of HBC in normal pregnancy, female HBC mounted a stronger and wider response against viral and bacterial ligands than male HBC [28, 29].

Immunometabolism emerged in the last decade as a novel approach that integrates cellular metabolism with immune responses [29, 30]. Macrophages activated in an M1 phenotype derive energy preferentially from glycolysis and pentose phosphate pathway (PPP) whereas proinflammatory stimuli like lipopolysaccharide (LPS), can further increase the glycolytic metabolism. On the other hand, macrophages on the alternative or M2 phenotype obtain energy majorly from oxidative phosphorylation, fatty acid oxidation and amino acid metabolism [29–32]. However, the association between macrophage phenotypic profile and their immunometabolic reprogramming can vary depending on the tissue context and the signals received from the environment. In pregnancy, recent evidence points to the role of immunometabolic reprogramming in maternal monocytes and decidual macrophages [33,33–36]. So far, the immunometabolic profiles of placenta macrophages and whether they are associated to the sex of the placenta have not been explored. Taking into account that in normal pregnancy female HBC are more likely alternative activated macrophages, we hypothesized that this phenotype was associated to a differential immunometabolic profile. Here we studied human term placenta HBC with special focus on metabolic, phenotypic and functional profiles. The potential role of endoplasmic reticulum (ER) stress signals was also analyzed.

Materials and methods

Placenta samples and Hofbauer cell isolation

Term placenta tissue ($N=31$, 16 from male and 15 female placentas regarding to the sex of the singleton born) with gestational age of 39.1 ± 0.6 was collected from women undergoing programmed caesarean birth. Women participating in the study were 26 to 41 (34.5 ± 0.7) years old and all of them had normal BMI (23.6 ± 0.6). They

had negative serology for HIV, HBCV, Chagas disease and syphilis and no clinical records of gestational diseases or more than one pregnancy loss. Samples from women with serious complications during pregnancy such as infections that compromised pregnancy or fetal death were excluded. Neonates with known organ malformations or congenital disorders were also excluded. Approval was obtained from Hospital Italiano of Buenos Aires Research Ethics Committee (HIBA 1681). Written informed consent was obtained from all women undergoing elective caesarean birth. Placental villi immediately beneath *decidua basalis* was dissected as described previously [37, 38] and processed for cell and tissue assays. Briefly, small decidual or villi explants of $5\times 5\times 1$ mm were excised from term placenta's cotyledons, washed with cold PBS and then cultured in 24-well polystyrene plates in 500 μ l DMEM: F12 complete, with 10% FCS and 100 μ g/ml penicillin/ 100 U/ml streptomycin (Life Technologies, Buenos Aires, Argentina) for 20 h. The explants were collected for RNA assays in TRizol and conserved at -80 °C until use. For Hofbauer cell isolation, 20–40 g of villi were washed thoroughly with PBS to minimize the presence of maternal cells. It was digested with collagenase II (1 mg/ml), DNase I (0.1 mg/ml) and Trypsin-EDTA (0.25%, Gibco) for 45 min and enzymatic digestion was finished by 10% FCS and followed by Ficoll-Hypaque centrifugation, as previously reported [39, 40]. Next, to enrich the sample in placental macrophages, an extra adherent step or positive selection CD14 cell isolation kit with magnetic beads coupled antibodies (Miltenyi) was performed with a 95% of purity (90–99%). A potential activation of HBC during purification by CD14 positive selection seems negligible, since cells were responsive to different stimuli with the mild variability expected within each sex.

Blood samples

Blood samples were processed from pregnant women ($N=10$, 4 male and 6 female) at the moment of caesarean birth. Neither donor was under pharmacological treatment for at least 10 days before the day of sampling. Blood was obtained by puncture of the forearm vein, and it was drawn directly into heparin containing sterile plastic tubes. Studies were approved by the Hospital Italiano de Buenos Aires Research Ethics Committee and Research Committee (HIBA 1681). Samples were collected after corresponding written informed consent was signed by the participants.

Flow cytometry and ELISA

4×10^5 HBC were cultured without or with 100 nM lipopolysaccharide (LPS) or 10 μ g/ml STF-083010 (STF), inhibits IRE-1 α endonuclease and mRNA splicing activity in response to endoplasmic reticulum stress but has

no effect on its kinase activity, in RPMI-160 medium (RPMI) 2% FCS and after 20 h cells were recovered by Trypsin-EDTA (0.25%, Gibco) and stained for:

- *Cytokines*: with Fluorescein Isothiocyanate (FITC-), Phycoerythrin (PE-), Phycoerythrin-Cyanine7 (PECy7-) or Allophycocyanin (APC-) conjugated mAbs directed to CD14 (BioLegend Cat. No. 325617, RRID: AB_830690), CD36/FAT (fatty acid translocase. BD Biosciences Cat. No. 555454, RRID: AB_2291112), CD163/SCAR11 (scavenger receptor cysteine-rich SRCR. BioLegend. Cat. No.333605, RRID: AB_1134005), CD39/ENTPD1 (ectonucleoside triphosphate diphosphohydrolase 1. Biolegend Cat. No. 328205, RRID: AB_940423), CD206/MRC1 (mannose receptor C-type 1) and CD209/DC-SIGN (C-type lectin) (BD Pharmingen, San Diego, CA, USA. Cat. No. 17-2099-42, RRID: AB_11039758).
- *Metabolism probes*: 2-NBDG (2-Deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose)-FITC for glucose uptake, DCFH-DA (2',7'-Dichlorodihydrofluorescein diacetate)-FITC for total reactive oxygen species (ROS), BODIPY (4,4-difluoro-3a,4a-diaza-s-indacene)-FL C12-FITC for long chain fatty acid uptake, BODIPY 493/ 503 for lipid droplets accumulation. All probes were obtained from Thermo Fisher Scientific.

Fifty thousand events were acquired in a FACS Aria II cytometer® (Becton Dickinson)

and results were analyzed using FlowJo software (<http://www.flowjo.com/>).

Results were expressed as the percentage of the respective population and the quadrant was set using irrelevant isotype specific Ab and were expressed as mean fluorescence intensity (M.F.I.) or double positive cell frequencies.

Enzyme-Linked ImmunoSorbent Assay (ELISA) was performed to determine IL-1 β and IL-10 levels in HBC supernatant in absence or presence of 100 nM LPS using a commercial kit (BD Biosciences), according to the manufacturer's recommendations and as previously described [41].

Glucose uptake

HBC supernatant was removed after 20 h in RPMI 2% FCS with or without treatments. Cells were incubated with 100 μ M 2-NBDG, a fluorescent glucose analogue, and CD14-PECy7 at 37 °C, 5% CO₂ in glucose-free RPMI for 10 min. Cells were washed with cold PBS and resuspended in 2% FCS PBS (FACS solution) for flow cytometry. Results were expressed as the 2-NBDG M.F.I. in CD14 positive cells.

Lactate secretion

Lactate concentration in cell supernatant was measured 20 h after treatments with L-Lactate kit (Wiener Lab) according to the manufacturer's instructions.

Long chain fatty acids uptake

After treatments, cells were stained with the specific fluorescence probe BODIPY-FL C12 as in [34]. This probe is a 12-carbon chain length saturated fatty acid linked to the fluorophore BODIPY (4,4-difluoro-3a,4a-diaza-s-indacene), resembling an 18-carbon fatty acid [42]. The probe was preincubated with 0.1% fatty acid free bovine serum albumin (FAF-BSA, Sigma) for 30 min at 37 °C. Cells were washed twice with PBS and incubated with 5 μ M BODIPY-FL C12 solution in serum-free RPMI for 5 min at 37 °C, 5% CO₂. Cells were washed with 0.2% BSA, resuspended in FACS solution and data was acquired as for glucose uptake assay. Results were expressed as the BodiPY FL C12 M.F.I. in CD14 positive cells.

Lipid droplets accumulation

Cells were treated as for glucose and LCFAs uptake, then were washed twice with PBS and incubated with a 2 μ M BODIPY 493/503 fluorescent probe in PBS for 15 min at 37 °C, 5% CO₂. Cells were washed with cold PBS, harvested with Trypsin-EDTA (0.25%) and resuspended in FACS solution and flow cytometry was performed as described above. Results were expressed as the BodiPY 493/503 M.F.I. in CD14 positive cells.

Efferocytosis assays

Phagocytosis of autologous apoptotic neutrophils (efferocytosis) in 3×10^5 HBC CD14 positive cells cultured without or with 10 μ M STF-083010 (STF) was carried out as previously reported [43, 44]. Neutrophils were obtained after the Ficoll-Hypaque gradient and subsequent Dextran purification [44, 45]. Apoptotic neutrophils were obtained after 20 h incubation in RPMI (spontaneous apoptosis) and stained with 3 μ M/10⁶ cells of CFSE (Life Technologies, Buenos Aires) for 10 min in RPMI without FCS. Excess of CFSE was eliminated by serial washes with RPMI 10% FCS. The percentage of neutrophil apoptosis was higher than 50% as determined by annexin-propidium iodide staining and flow cytometry [43, 44].

For metabolic pathways inhibition, 10 mM 2-deoxy-d-glucose (2-DG), 100 nM rotenone (ROT) or 10 μ M Etomoxir were added to HBC cultures 90 min before efferocytosis assay to inhibit glucose utilization, mitochondrial electron transport chain or, or fatty acid oxidation. HBC were incubated with apoptotic neutrophils in a 1:5 ratio and after 50 min of efferocytosis cells were collected, immunostained for CD14 and the percentage of CD14/CFSE double-positive cells was analyzed by flow cytometry as described above.

Confocal microscopy

HBC were labeled with the lipid intercalating dye PKH26 (Sigma-Aldrich) according to the manufacturer's instructions. Male or female macrophages were incubated with their autologous apoptotic neutrophils stained with CFSE and performed efferocytosis assay, as previously described. Then, cells were washed, fixed with paraformaldehyde 4%, and stained with DAPI. Cells were visualized and microphotographed with a confocal fluorescence Zeiss-LSM980 microscopy. Photographs were analyzed with Fiji software.

Negative controls were set with each fluorescent dye separately and images displayed were at 60x.

RT-qPCR

Villi gene expression of cytokines [Interleukin (*IL*) -1 β , -6, -10; Indoleamine 2,3-dioxygenase (*IDO*), Transforming growth factor (*TGF*-) β 1, tumor necrosis factor (*TNF*) - α], toll-like 4 receptor (*TLR4*), prostaglandin-endoperoxide synthase 2 (*PTGS2*) and unfolded protein response (UPR) genes (*IRE-1 α* , *PERK*, *ATF6 α* and *ATF4*) was determined by RT-qPCR as previously described [37]. Briefly, total RNA was isolated following manufacturer recommendations with Trizol reagent (Life Technologies, Grand Island, NY, USA), cDNAs generated from 1 μ g of RNA using a MMLV reverse transcriptase, RNAsin RNase inhibitor and oligodT kit (Promega Corporation, Madison, WI, USA). Samples were stored at -20 °C for batch analysis.

RT-qPCR master mix (Biodynamics, Buenos Aires, Argentina) was used according to the manufacturer's recommendation. Real-time PCR was performed on a Bio-Rad iQ5 Real-time PCR system. The relative gene expression levels were determined using the threshold cycle (CT) method (2^{-dCT} method) with reference to two housekeeping genes that did not show sex-influence, Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)

and beta-2 microglobulin (*B2-M*). The primer sequences are described in Table 1.

Bioinformatic studies

Samples classification

Expression matrix was downloaded from NCBI GEO (accession GSE30595 [46]) using the GenePattern platform [47]. We selected samples which underwent canonical differentiation towards M1 macrophages (GM-CSF/LPS/IFN). Consequently, nine samples were selected for subsequent enrichment analysis, differential expression, and gene contrasts.

To determine the sex of each selected sample, the expression of genes associated with the Y chromosome (*UTY*, *TMSB4Y*, *EIF1AY*, *USP9Y*, *RBM1A1*) were detected by the microarray and clustering by PCA on R [48].

Differential gene expression and enrichment analysis

We studied differentially expressed genes (DEG) and contrasted between female vs. male samples in each activation profile using the limma package in R [49] ($\text{Log}_2\text{FC} > 0.58$, adjusted P -value < 0.05). Subsequently, Gene set enrichment analysis (GSEA) dot plots was obtained with gseapy package [50] in Python.

Statistical analysis

The significance of the results was analyzed by Wilcoxon matched-pairs or Mann-Whitney U-test for two non-parametric samples. When multiple comparisons were necessary, One- or two-way ANOVA test followed by Holm-Sidak or Tukey's multiple comparison tests were used. Differences between groups were considered significant at $P < 0.05$ using the GraphPad Prism9 software (GraphPad, San Diego, CA, USA).

Table 1 Primer sequences with their melting temperature

Gene	Primer Forward	Primer reverse	Tm (°C)
ATF4	5'GTCAGTCCCTCCAACAACAGC 3'	5'AGAGATCACAAAGTGTCATCCAACG 3'	60
ATF6 α	5'GCTGCCCTCTCAGAAAACGA 3'	5'CCTGTTCCAACATGCTCATAGG 3'	60
B2-M	5'AAGCAGCATCATGGAGGTTTG 3'	5'GAGCTACCTGTGGAGCAACC 3'	58
GAPDH	5'ATCACCATCTTCCAGGAGCGA 3'	5'GGTTCACACCCATGACGAAC 3'	60
IDO-1	5'AGGCCCTGATTATGAGAACAT 3'	5'TTTCCAACAGCGCCTTTAGC 3'	60
IL-1 β	5'GGATATGGAGCAACAAGTGGTGT 3'	5'CACGACAGGACAGGTACAGATT 3'	60
IL-6	5'TTCGGTACATCCTCGAGGC 3'	5'TCACCCAGGCAAGTCTCCTA 3'	60
IL-10	5'CATCAAGGCGCATGTGAACT 3'	5'CACAGGGAAGAAATCGATGACAG 3'	60
IRE-1 α	5'GGGTCTGAGGAAGGTGATGC 3'	5'AACATACAGAGTGGGCGTCAG 3'	60
PERK	5'AGACGATGAGACAGAGTTGCG 3'	5'TTGCTAAGGCTGGATGACACC 3'	58
PTGS2	5'TGGATGCTTCGTTAATTTGTTT 3'	5'ACCCACAGTGCTTGACAC 3'	60
TGF- β 1	5'GGACACCAACTATTGCTTCAG 3'	5'CCAGGCTCCAAATGTAGGG 3'	56
TNF- α	5'GCCTCTTCTCTTCTGATCG 3'	5'CAGCTTGAGGGTTTGCTACA 3'	60
TRL4	5'ATCCCTGAGGCATTAGGC 3'	5'TTGCTGGATTTACACCTGG 3'	58

Results

Female Hofbauer cells are more prone to an alternative-like profile

HBC cells from female term placentas (FEMALE) showed increased co-expression of CD14 and CD163/SCAR11 (scavenger receptor cysteine-rich SRCR) compared to male HBC cells (MALE) (Fig. 1A-B). Additionally, Female HBC exhibited higher expression of antiinflammatory surface markers such as CD39/ENTPD1 (ectonucleoside triphosphate diphosphohydrolase 1) and CD206/MRC1 (mannose receptor C-type 1) ($P < 0.05$), but not CD209/DC-SIGN (C-type lectin) (Fig. 1C). This profile was accompanied by a decrease in IL-1 β secretion without changing IL-10 secretion and a higher IL-10/IL-1 β ratio in female compared to male HBC (Fig. 1D) ($P < 0.05$). Interestingly, these sex-specific differences in the phenotypical profile of HBC have a correspondence in monocyte-derived M1 macrophages from male volunteers compared to female, as acquired using public data (GSE30595) displayed in Supplementary Fig. 1.

Male HBC display a preferential glycolytic metabolism whereas female HBC rely on fatty acid uptake and accumulation of lipid droplets

On the basis that the immunometabolic reprogramming of macrophages underlies the phenotypic profile acquired [32, 34, 51, 52], we next explored whether the

sex-associated phenotype of HBC was related to a differential metabolic profile. Male HBC showed higher uptake of the glucose analogue, 2-NBDG (Fig. 2B), higher production of total reactive oxygen species (ROS) ($P < 0.01$) and similar levels of lactate secretion compared to female HBC (Fig. 2C-D). On the other hand, Female HBC displayed higher long chain fatty acids (LCFA) incorporation (BodiPY FLC₁₂) (Fig. 2G) accompanied by higher production of CD36 and lipid droplets accumulation (BodiPY 493/503) than male HBC (Fig. 2F, H) ($P < 0.05$). These results are in line with sex-associated metabolic pathways accompanying phenotypic differential profiles.

Higher efferocytic capacity and differential efferocytosis fueling in female HBC

First, we assessed the efferocytic capacity of HBC as the ability to remove maternal apoptotic neutrophils. As shown in Fig. 3, female HBC have almost three times higher efferocytosis than male HBC as assessed by flow cytometry (Fig. 3A) ($P < 0.01$) and visualized by confocal microscopy (Fig. 3B). These results were accompanied by an increase in the expression of the efferocytosis receptors CD36, CD163 and CD206 (Fig. 3C) ($P < 0.05$), congruently with their alternative profile (Fig. 1). To explore further the metabolic preferences of male and female HBC, we studied their efferocytic capacity in the presence of different metabolic inhibitors. To assess glucose

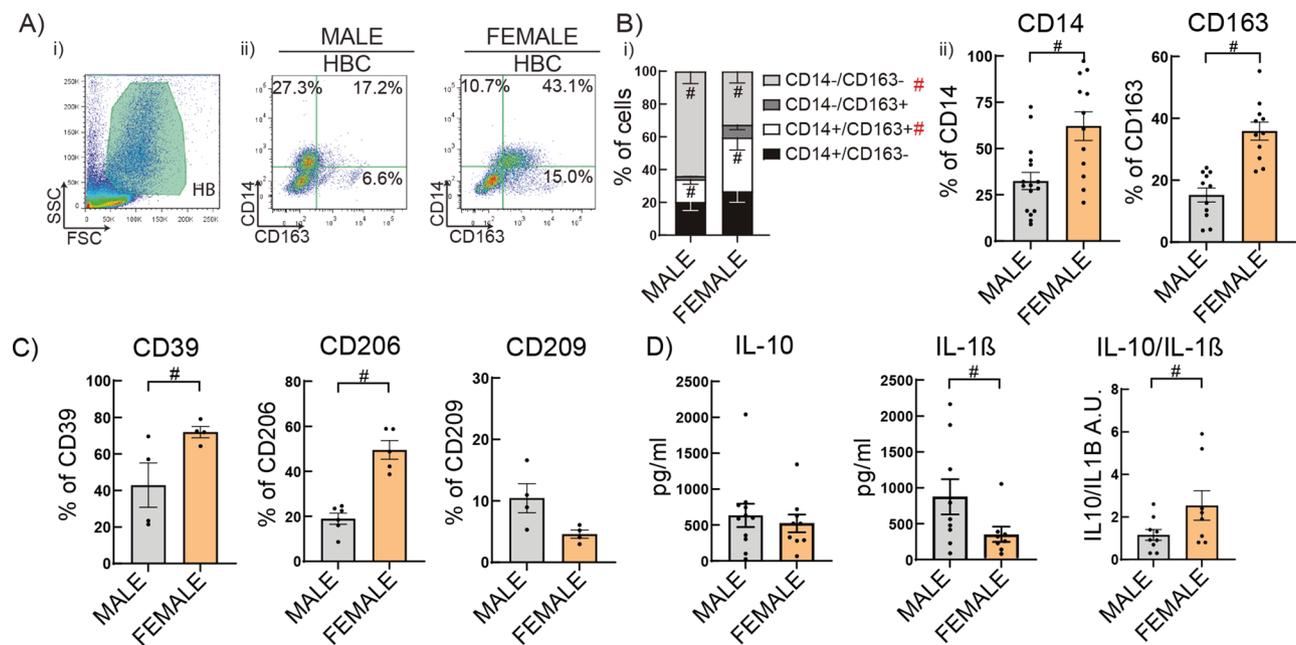


Fig. 1 Female Hofbauer cells are more prone to an alternative-like profile. HBC were isolated from 31 placentas (16 male and 15 female newborn) by enzymatic digestion and cultured with RPMI 2% FCS ON. Cells were collected and analyzed by flow cytometry. **A**) Representative dot plots of (i) HBC gate and (ii) CD14-CD163% of positive cells. **B**) CD14 and CD163 proportion and **C**) CD39, CD206, CD209% of positive cells were shown. **D**) IL-10 and IL-1 β secretion (pg/ml) were measured by ELISA in HBC supernatants. Results are expressed as Mean \pm SEM. Statistically significant differences by placental sex are represented as in B) # (red) $P < 0.05$. Two-way ANOVA, Tukey's multiple comparisons test [Interaction: $F(3) = 8.628$, P -value < 0.0001 ; Sex: $F(1) = 5.99e-008$, P -value = 0.998; Cell population: $F(3) = 20.64$, $P < 0.0001$]. B-D) # $P < 0.05$ (Mann-Whitney test) when only 2 conditions were analyzed. Each dot represents an independent experiment

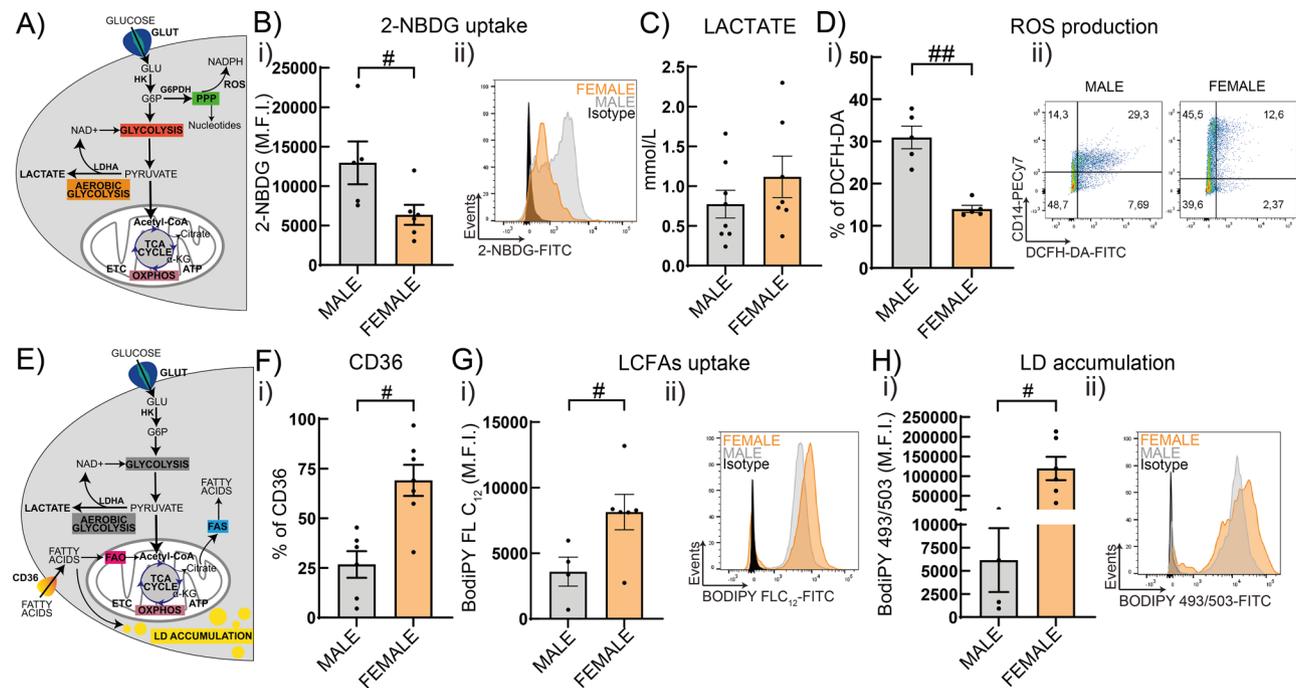


Fig. 2 Differential metabolic preferences in male and female HBC. **A)** and **E)** Simplified schematic overview of glucose and lipid metabolism, respectively. HBC cells were cultured with RPMI 2% FCS ON and collected to perform metabolic assays to study **B)** the glucose analogue 2-NBDG uptake, **D)** ROS production, **F)** CD36 expression, **G)** LCFAs uptake and **H)** Lipid Droplets (LD) accumulation by flow cytometry. Supernatants were used to study **C)** Lactate secretion with a colorimetric assay. Results are expressed as Mean \pm SEM and in ii) representative histogram or dot plots are shown. Statistically significant differences between sex are represented as # $P < 0.05$ (Mann-Whitney test). Each dot represents an independent HBC sample. Abbreviations: Glucose (GLU), hexokinase (HK), glucose-6-phosphate (G6P), G6P dehydrogenase (G6PDH), pentose phosphate pathway (PPP), reactive oxygen species (ROS), Lactate dehydrogenase A (LDHA), Tri-carboxylic acid (TCA), electron transport chain (ETC), oxidative phosphorylation (OXPHOS), fatty acid oxidation (FAO), fatty acid synthesis (FAS)

or mitochondrial dependent efferocytosis, the competitive inhibitor of glycolysis 2-deoxy-d-glucose (2-DG) or Rotenone (ROT) were added to HBC cultures before the efferocytosis assays, as depicted in Fig. 3D). The inhibitor of glucose utilization 2-DG reduced efferocytosis up to 35% only in male HBC ($P < 0.05$), with no changes in female HBC (Fig. 3E). In contrast, the implication of mitochondrial energy for this function was more evident in female HBC, since the electron transport chain inhibitor ROT reduced up to 50% the efferocytosis in female ($P < 0.01$) but not in male HBC (Fig. 3E). Finally, female HBC were cultured with Etomoxir, a CPT1-specific inhibitor to prevent the transport of fatty acids into the mitochondrial matrix for further metabolism. As shown in Fig. 3E, female HBC obtain energy for efferocytosis, at least partially, through fatty acid oxidation (Fig. 3E) ($P < 0.05$).

Differential activation of HBC metabolism by LPS

Next, we studied the metabolic rewiring of male or female HBC when challenged ex vivo with a classical proinflammatory stimulus as lipopolysaccharide (LPS). An enhanced glycolytic metabolism with higher glucose analogue uptake and lactate secretion was observed in male HBC (Fig. 4A) ($P < 0.05$). LPS did not affect free

fatty acid uptake, lipid droplet accumulation and anti-inflammatory surface marker expression (Fig. 4B, C). On the other hand, female HBC cultured with LPS showed lower secretion of lactate ($P < 0.05$) without changes in glucose uptake or ROS production (Fig. 4A). Nevertheless, an increment in lipid droplets (LD) accumulation (Fig. 4B) ($P < 0.05$) and a trend to increase in CD163 was observed (Fig. 4C). Both male and female HBC secreted more IL-1 β and IL-10 upon LPS challenge (Fig. 4D) ($P < 0.05$). These results indicate that male HBC's metabolism challenged by LPS responded similarly to myeloid macrophage, increasing glycolytic metabolism and secretion of proinflammatory mediators, as a classic activation profile. In contrast, female HBC challenged by LPS did not rewire their metabolism to glycolysis, but rather maintained that of an alternative activation profile.

IRE-1 regulates differently the immunometabolic profile of HBC

Components of the unfolded protein response (UPR) regulate various processes, from lipid and cholesterol metabolism and energy homeostasis to inflammation and cell differentiation [53, 54]. One of the sensors of the UPR, the inositol-requiring protein 1 (IRE-1), is widely expressed by macrophages and activated by endoplasmic

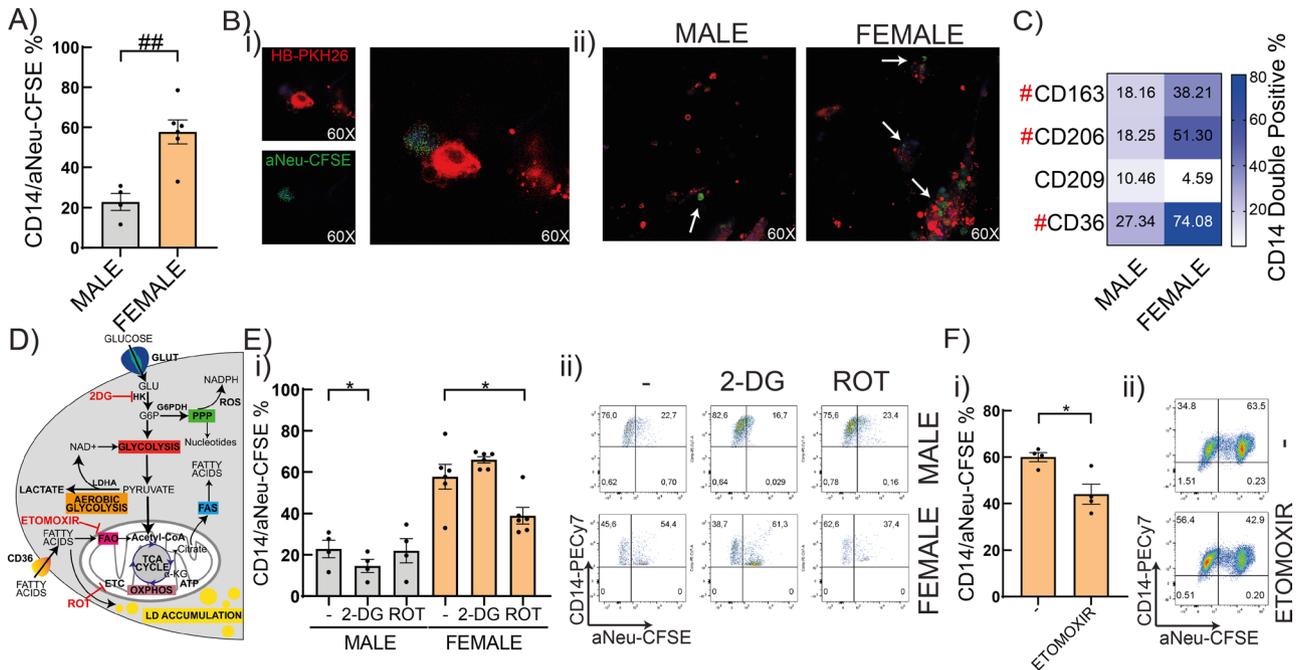


Fig. 3 Sex-associated metabolic pathways in efferocytosis of HBC. **A)** Male or female HBC were challenged with maternal apoptotic neutrophils (aNeu), stained with CFSE, in a 1:5 ratio during 1 h. Then, they were washed and incubated with CD14 antibody. Double positive cells were analyzed by flow cytometry. **B)** Representative microphotographs of efferocytosis process (i) and MALE and FEMALE efferocytosis (ii) were taken by Zeiss LSM980 confocal microscope of 3 independent experiment each. White arrows show apoptotic bodies (green) in HBC-PKH26 (red). **C)** Phagocytic receptors expression (CD163, CD206, CD209 and CD36) were analyzed by flow cytometry. **D)** Simplified schematic overview of metabolic pathways and pharmacological inhibitors. **E), F)** HBC cells were cultured with metabolic inhibitors 2-DG (10 mM), ROT (100 nM) or ETOMOXIR (10 μ M), stimulated with apoptotic neutrophils as in (A) and analyzed by flow cytometry. Results are expressed as Mean \pm SEM. Statistically significant differences: A, C) between HBC sex are shown as # $P < 0.05$, ## $P < 0.01$ (Mann-Whitney test). E) * $P < 0.05$, ** $P < 0.01$. Two-way ANOVA, Holm-Sidak's multiple comparisons test. [Interaction: $F(2) = 11.62$, P -value = 0.0006; Sex: $F(1) = 70.85$, P -value < 0.0001; Treatment: $F(1) = 6.157$, $P = 0.0095$]. F) * $P < 0.05$ (basal vs. ETOMOXIR, Wilcoxon matched-pairs signed rank test). Each dot represents an independent HBC sample

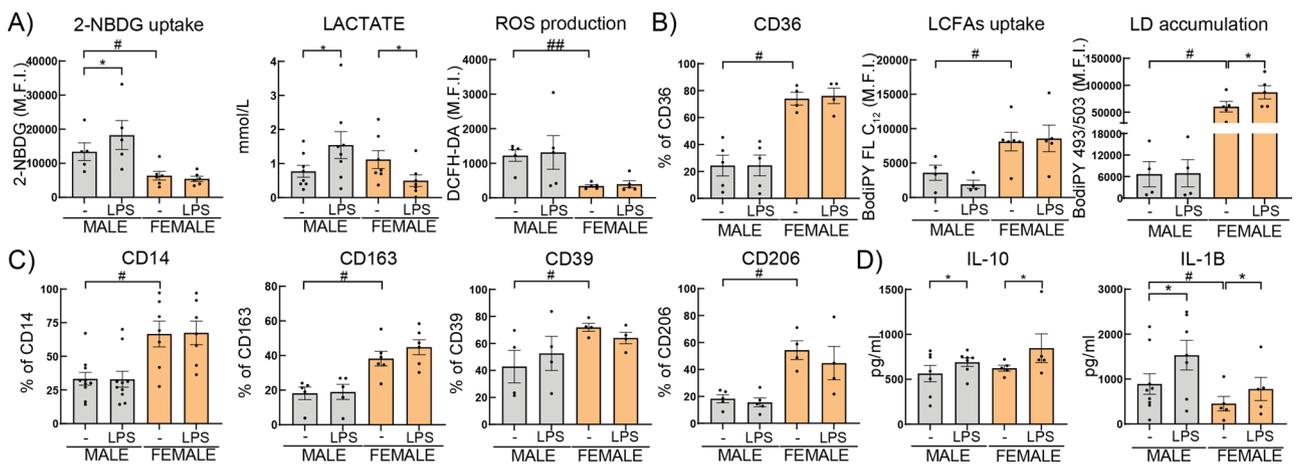


Fig. 4 HBC immunometabolic profile modulated by LPS. Male or female HBC were cultured in RPMI 2% FCS ON. **A)** glucose uptake and ROS production, **B)** CD36, LCFAs uptake, lipid droplets (LD) accumulation and **C)** CD14, CD163, CD206 and CD39, were measured by flow cytometry. In the supernatants **A)** Lactate or **D)** IL-10 and IL-1B secretion were quantified by enzymatic assay or ELISA, respectively. Results are expressed as Mean \pm SEM. Statistically significant differences by the stimuli within each sex are represented as * $P < 0.05$ (Wilcoxon matched-pairs signed rank test) and those parameters that were detected as significantly different between basal male and female HBC in Figs. 1 and 2, are here marked with #. Each dot represents an independent HBC sample

reticulum stress [54–56]. As a potential cellular mechanism for the sex-associated differential immunometabolic profiles, we studied the involvement of IRE-1 in HBC. Since IRE-1 associates with metabolic pathways as glucose fluctuation, lipid and/or cholesterol biosynthesis and regulates cytokines associated to secretion in macrophages [54, 56, 57], we performed experiments with its pharmacological inhibitor STF-083010 (STF). Male HBC treated with STF increased lactate secretion and decreased ROS production ($P < 0.05$) without changing lipid metabolism, efferocytosis or scavenger receptor expression (Fig. 5A–D). In contrast, female HBC cultured with STF decreased lactate secretion ($P < 0.01$) without changing ROS production, but it increased LCFA uptake (Fig. 5A–D). Surprisingly, STF enhanced efferocytosis, CD206 and a slight CD209 increase only in female HBC (Fig. 5A–D) ($P < 0.05$). Altogether, these results suggest that in a physiological stress response, IRE-1 α is regulating the predominant M2 phenotype of female but not of male HBC.

Contribution of the placental microenvironment to sex-associated differences of HBC

To understand if the evidence of sex-specific diversity in the HBC is intrinsic to the cell or it also extends to the surrounding environment of the placental villi, we evaluated the expression of certain selected genes contributing

to pro-, antiinflammatory responses or endoplasmic reticulum stress in placental explants. Villi gene expression was normalized against two housekeeping genes that did not show sex-influence, Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and beta-2 microglobulin (B2-M). In female villi, as expected according to isolated cells data, there was lower expression of proinflammatory genes such as IL-1 β and TNF- α , but higher expression of antiinflammatory genes, IL-10 and TGF- β 1 compared to male villi ($P < 0.05$). Notably, female villi expressed higher expression of TLR4 and ATF4 a UPR gene (Fig. 6A) ($P < 0.05$). Interestingly, IRE-1 α is the only UPR sensor expressed in all samples, whereas PERK and ATF6 α were expressed in a few samples independently of the placenta sex (Supplementary Fig. 2). Moreover, when plotting the expression of IRE-1 α vs. different genes in male and female villi, an inverse correlation was observed for TLR4 and IDO-1 whereas differential steepness was obtained for TNF- α . Taken together, these results support the proposal that villi inherent factors associated to the inflammatory and reticulum stress responses have a role in the sex-specific features described in female and male HBC, with female villi genes contributing mostly to antiinflammatory profiles than male villi.

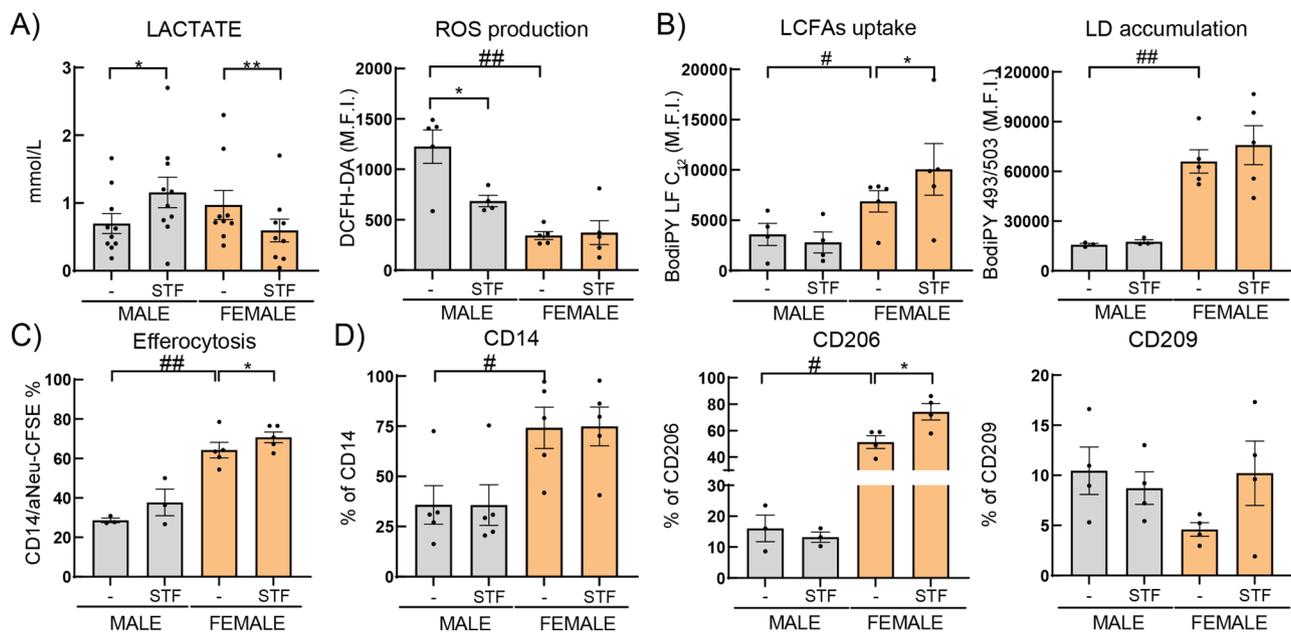


Fig. 5 Pharmacological inhibition of IRE-1 and its association to HBC metabolic profile. HBC were cultured without or with 10 μ g/ml IRE1 α inhibitor, STF-083010 (STF) in RPMI 2% FCS ON. **A**) ROS production, **B**) LCFA uptake and LD accumulation, **C**) Efferocytosis and **D**) CD14, CD206 and CD209 positive cells were evaluated by flow cytometry. In the supernatant **A**) Lactate was quantified by enzymatic assay. Results are expressed as Mean \pm SEM. Statistically significant differences by the stimuli within each sex are represented as * $P < 0.05$ (Wilcoxon matched-pairs signed rank test) and those parameters that were detected as significantly different between basal male and female HBC in Figs. 1, 2 and 3, are here marked with #. Each dot represents an independent HBC sample

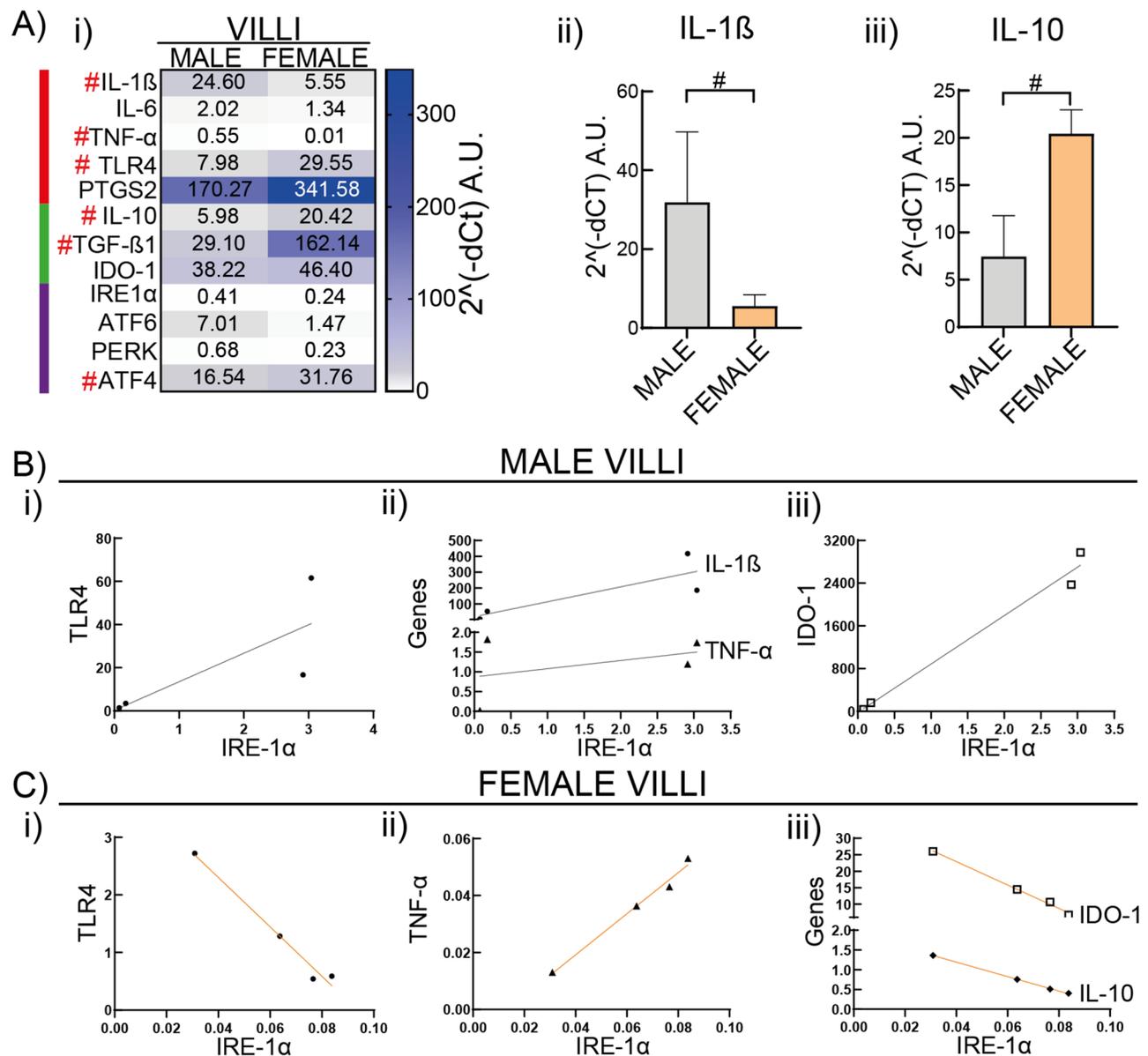


Fig. 6 Sex associated transcriptionally differences in the placental microenvironment. Placentas (from 4 male and 4 female births) were processed to study gene expression in villi explants by RT-qPCR. **A)** Male or female gene expression is shown as their Mean $2^{\Delta(-dCt)}$. Statistically significant differences between sex are shown by # $P < 0.05$ (Mann-Whitney test). Red, green and purple lines mark pro, antiinflammatory and UPR genes, respectively. **B)** Male or **C)** Female gene correlations were evaluated by Pearson's r . The r value was positive (blue) or negative (red) when the gene correlation was positive or negative, respectively. All correlations that are shown have P -values < 0.05

Discussion

Here, we present evidence of sex-specific differential immunometabolic, functional and phenotypical profiles of HBC in human term placentas. Results also point to the preferential metabolic pathways involved in the efferocytic function of HBC. We propose that placental macrophages' function depends on the preferentially selected metabolic pathways, independently of cellular access to nutrients. Our results support that female HBC have a prominent M2 phenotype associated to lipid metabolism; instead, male HBC present a weaker M2 phenotype

associated to glycolytic pathways. These conclusions are based on the following observations. First, a higher expression of CD14 and CD163 was confirmed in female HBC than male HBC [4, 5], along with greater expression of CD39, CD206 as well as lower secretion of IL-1β. Second, male HBC displayed higher glucose analogue uptake and ROS production than female HBC, whereas female HBC presented higher fatty acid uptake and accumulation of lipid droplets, accompanied by higher production of the scavenger receptor CD36. Third, female HBC displayed almost three times more efferocytosis than male

HBC, as well as enhanced expression of CD36, CD163 and CD206. Noteworthy, energy for efferocytosis was obtained, at least partially, from fatty acid oxidation in female but from glycolysis in male HBC, as corroborated with pharmacologic inhibitors. Fourth, the predominant metabolic pathways in male HBC were reinforced upon LPS challenge, since increased glucose uptake and lactate secretion without changing lipid mobilization and accumulation was observed; in contrast, female HBC presented lower secretion of lactate and higher lipid droplet accumulation without changing glucose or fatty acid uptake. Fifth, IRE-1 α inhibitor favored lactate secretion but decreased ROS production without changing efferocytosis or the expression of receptors associated to this process in male HBC. In female HBC, IRE-1 α inhibition reduced lactate secretion, increased fatty acid uptake and strengthened even more the efferocytic capacity in female HBC as well as increased CD206 expression. Sixth, sex-differences studied in isolated HBC were observed in term placenta villi as well: female villi contributed less to a proinflammatory profile while IRE-1 α expression correlated differently with pro- and antiinflammatory genes.

Our data on HBC immunometabolic profiles according to the sex of the placenta fits in the epidemiologically registered sex-associated differences of maternal and fetal outcomes related to the function of the fetoplacental unit. HBC are originated in the placenta and share genetic information with the fetus. Differences among these cells are not only genetical [5, 22, 58] but also environmental [12, 13] and depending on gonadal hormones [1, 12, 59, 60]. Sexual hormones are known to modulate macrophage classical innate function in adult males and females [61–63]. However, hormonal independence in the sex-specific metabolic preferences of macrophages where ovaries-derived hormones appeared unlikely regulators of sex dimorphism [64]. In this work, we opted to focus on factors inherent to the female or male villi, which include the hormonal environment, rather than on the hormones alone. Our results indicate that female villi contributed to antiinflammatory profiles and strongly support a role for inflammatory and endoplasmic reticulum stress mediators in the effect. This was not as evident in the males pointing out that HBC immunometabolic differential profiles rely on a more complex regulatory network that includes inflammatory, UPR and ER stress signals. Moreover, these differential sex-associated pathways of HBC accompanying their phenotypic and functional profiles seem to occur in the villi environment before cell isolation, since the *ex vivo* assays were carried out in the same metabolic experimental settings.

Efferocytosis, macropinocytosis and phagocytosis together with diverse scavenger receptors (including CD36, CD163, CD206) are involved in lipid uptake by

macrophages [65]. The fact that female HBC had more efferocytosis accompanied by a stronger surface expression of CD36, CD163 and CD206 [28, 66] and that rotenone and etomoxir decreased efferocytosis strongly support the preferential use of fatty acid oxidation to get energy for this process. In contrast, male HBC, under the same nutrient supply, preferred the faster glycolytic metabolism to perform functions as efferocytosis *in vitro* (Figs. 2 and 3). This result adds new metabolic evidence to explain the observed higher susceptibility of male fetuses to various insults that alter cellular metabolism [3].

Interestingly, female placental macrophages displayed a more prominent M2 phenotype than male macrophages whereas they better controlled the inflammatory response to LPS. It is conceivable that these mechanisms might underlie the impact of their response against environmental and infectious insults as shown in placental [12] and immune tissues [62]. These results are in line with Pantazi et al. [28] who reported a stronger response of female term HBC to LPS than male HBC. Regarding the differences seen in TLR4 and the response to LPS it is worthy to note that TLR4 is expressed by HBC from early stages of pregnancy until term [25] but its role in the immunometabolism of HBC regarding sex has not been assessed. Remarkably, gene expression relationships in villi indicate that TLR4 is highly expressed and correlates negatively with TNF- α but positively with IDO-1 and IL-10 in female placentas (Fig. Suppl 2). On the other hand, male HBC cultured with LPS showed a reinforced glycolytic metabolism favoring the aerobic glycolysis instead of the oxidative pentose phosphate pathway; and it was accompanied by IL-1 β and IL-10 secretion (Fig. 4). Similarly, TLR4 correlates positively with IL-6 and TGF- β 1 on male villi (Supplementary Fig. 3). This preferential glycolytic metabolic pathway associated to the higher number of proteins related to cholesterol (steroids) and alcohol biosynthesis [28], it may be a link to explain why HBCs are activated in a weaker M2 phenotype than females HBC.

We found a strong association of the IRE-1 pathway to the phenotypic, functional and metabolic profile of isolated HBC and villus tissue as well (Figs. 5 and 6), being more significant in female samples. This may suggest that, given the demanding functions that placental macrophages need to fulfill [21, 67], depending on their metabolism they react differently to the physiological stress. This differential bias in how IRE-1 α acts in male and female HBC should be further explored to understand how it is involved in the immunometabolism of HBC and whether it affects pregnancy outcome.

Immunometabolism and pregnancy is a novel area of research with only a few papers about immunometabolic profiles of decidual macrophages [33, 35, 67] and a

recent report from our group studying maternal monocytes' immunometabolism at 16–20 weeks of pregnancy [34]. To our knowledge, this is the first report on HBC immunometabolism and, especially, the first taking the particular approach of sex-associated differences. Sex-associated diversity in Hofbauer cell immunometabolism may contribute to the understanding of sex-specific susceptibility to infections during pregnancy and other pregnancy complications. Investigating these differences may offer insights into the pathogenesis of conditions such as preeclampsia and intrauterine growth restriction, opening avenues for targeted interventions.

Abbreviations

2-DG	2-deoxy-d-glucose
2-NBDG	2-Deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose
aNEU	Apoptotic neutrophils
ATF	Activating transcription factor
APC	Allophycocyanin
B2M	Beta-2 microglobulin
BMI	Body Mass Index
BODIPY	4,4-difluoro-3a,4a-diaza-s-indacene
CFSE	Carboxyfluorescein succinimidyl ester
DCFH-DA	2',7'-Dichlorodihydrofluoresceindiacetate
DEG	differentially expressed genes
DMEM	Dulbecco's Modified Eagle Medium
EDTA	Ethylenediamine tetraacetic acid
EIF1AY	Eukaryotic Translation Initiation Factor 1 A Y-Linked
ELISA	Enzyme-Linked ImmunoSorbent Assay
ER	Endoplasmic reticulum
ETC	electron transport chain
FCS	Fetal calf serum
FITC	Fluorescein Isothiocyanate
FAF-BSA	fatty acid free bovine serum albumin
FAO	fatty acid oxidation
FAS	fatty acid synthesis
G6P	glucose-6-phosphate
G6PDH	G6P dehydrogenase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GM-CSF	Granulocyte macrophage colony-stimulating factor
GSEA	Gene set enrichment analysis
GLU	Glucose
HBC	Hofbauer cells
HK	hexokinase
IDO	Indoleamine 2,3-dioxygenase
IFN	Interferons
IRE-1	Inositol-requiring enzyme-1
LCFA	Long chain fatty acid
LD	Lipid droplets
LDHA	Lactate dehydrogenase A
LPS	Lipopolysaccharide
MFI	Mean fluorescence intensity
OXPPOS	oxidative phosphorylation
PE	Phycocyanin
PECy7	Phycocyanin-Cyanine7
PERK	Protein Kinase R-like ER Kinase
PPP	Pentose phosphate pathway
RBMY1A1	RNA binding motif protein Y-linked family 1 member A1
ROS	Reactive oxygen species
ROT	Rotenone
RPMI	Roswell Park Memorial Institute 1640
STF	STF-083010
TCA	Tri-carboxylic acid cycle
TGF	Transforming growth factor
TLR	Toll like receptor
TMSB4Y	Thymosin Beta 4 Y-Linked
TNF	Tumor necrosis factor
UPR	Unfolded protein response

USP9Y Ubiquitin Specific Peptidase 9 Y-Linked
 UTY Ubiquitously transcribed tetratricopeptide repeat containing, Y-linked

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13293-024-00652-w>.

Supplementary Material 1

Acknowledgements

Not applicable.

Author contributions

D.E.P. performed all the ex vivo immunometabolic experiments. D.E.P., E.G. F.A. and A.S. carried out the Hofbauer isolation from term placenta. B.L. and V.H. obtained PBMCs from pregnant blood samples and carried out neutrophil isolation and performed the ELISA measurements. D.E.P. and S.G. carried out IRE-1 inhibition experiments. F.A., B.L. and A.S. performed RT-qPCR assays and lactate measurements. F.M. carried out efferocytosis experiments and performed the statistical tests. E.G., F.A., C.S. and M.M. carried out and analyzed bioinformatic assays. M.A.A., V.L. and C.M. recruited and followed up clinically all the pregnant women. D.E.P., R. R., D. V. and C. P. L. discussed the results and reviewed the manuscript. C.P.L. and D.E.P. designed the whole research, analyzed raw data, discussed the results and wrote the manuscript.

Funding

This work was supported by grants from the Agencia Nacional de Promoción Científica y Tecnológica (PICT 2021-I-A-00318 and PICT 2019–1554 to CPL; PICT 2018–2584 to DV; PICT 2020–2192 to DEP), Universidad de Buenos Aires (UBACyT 20020170100317BA), and Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET) (PIBAA287202110101109CO to DV), Buenos Aires, Argentina.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The use of human term placenta with the peripheral blood samples at the moment of cesarean birth were approved and conducted according to Hospital Italiano of Buenos Aires Research Ethics Committee (Ethics Acceptance Number: HIBA 1681). Written informed consent was obtained from all women undergoing elective caesarean birth.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹Immunopharmacology Laboratory, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Universidad de Buenos Aires-Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

²Bioinformatic Laboratory, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Universidad de Buenos Aires-Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

³Obstetric Service, Hospital Italiano, Buenos Aires, Argentina

Received: 30 April 2024 / Accepted: 23 September 2024

Published online: 17 October 2024

References

- Mitchell AM, Palettas M, Christian LM. Fetal sex is associated with maternal stimulated cytokine production, but not serum cytokine levels, in human pregnancy. *Brain Behav Immun*. 2017;60:32–7.
- O'Callaghan JL, Clifton VL, Prentis P, Ewing A, Saif Z, Pelzer ES. Sex-dependent differential transcript expression in the placenta of growth restricted infants. *Placenta*. 2022;128:1–8.
- Eriksson JG, Kajantie E, Osmond C, Thornburg K, Barker DJP. Boys live dangerously in the womb. *Am J Hum Biol*. 2010;22:330–5.
- Sun T, Gonzalez TL, Deng N, DiPentino R, Clark EL, Lee B et al. Sexually Dimorphic Crosstalk at the Maternal-Fetal Interface. *J Clin Endocrinol Metab* [Internet]. 2020;105:e4831–47. <http://www.ncbi.nlm.nih.gov/pubmed/32772088>
- Braun AE, Mitchel OR, Gonzalez TL, Sun T, Flowers AE, Pisarska MD, et al. Sex at the interface: the origin and impact of sex differences in the developing human placenta. *Biol Sex Differ*. BioMed Central Ltd; 2022.
- Melamed N, Yogev Y, Glezerman M. Fetal gender and pregnancy outcome. *J Matern Fetal Neonatal Med*. 2010;23:338–44.
- Verburg PE, Tucker G, Scheil W, Erwich JJHM, Dekker GA, Roberts CT. Sexual dimorphism in adverse pregnancy outcomes - A retrospective Australian population study 1981–2011. *PLoS ONE*. 2016;11.
- Gonzalez TL, Sun T, Koepfel AF, Lee B, Wang ET, Farber CR et al. Sex differences in the late first trimester human placenta transcriptome. *Biol Sex Differ*. 2018;9.
- Elsmén E, Källén K, Maršál K, Hellström-Westas L. Fetal gender and gestational-age-related incidence of pre-eclampsia. *Acta Obstet Gynecol Scand*. 2006;85:1285–91.
- Zazara DE, Arck PC. Developmental origin and sex-specific risk for infections and immune diseases later in life. *Semin Immunopathol*. Springer Verlag; 2019. pp. 137–51.
- Beilby KH, Kneebone E, Roseboom TJ, van Marrewijk IM, Thompson JG, Norman RJ, et al. Offspring physiology following the use of IVF, IVF and ICSI: a systematic review and meta-analysis of animal studies. *Hum Reprod Update*. 2023;29:272–90.
- Gabory A, Roseboom TJ, Moore T, Moore LG, Junien C. Placental contribution to the origins of sexual dimorphism in health and diseases: sex chromosomes and epigenetics. *Biol Sex Differ*. 2013.
- Maxwell A, Adzibololu N, Hu A, You Y, Stemmer PM, Ruden DM et al. Intrinsic sexual dimorphism in the placenta determines the differential response to benzene exposure. *iScience*. 2023;26.
- Falahi S, Abdoli A, Kenarkoobi A. Maternal COVID-19 infection and the fetus: immunological and neurological perspectives. *New Microbes New Infect*. 2023;53:101135.
- Olin A, Henckel E, Chen Y, Lakshminathan T, Pou C, Mikes J, et al. Stereotypic Immune System Development in Newborn Children. *Cell*. 2018;174:1277–e129214.
- Forsen T, Eriksson JG, Tuomilehto J, Osmond C, Barker DJP. Growth in utero and during childhood among women who develop coronary heart disease: longitudinal study. *BMJ*. 1999;319:1403–7.
- Taylor BD, Ness RB, Klebanoff MA, Tang G, Roberts JM, Hougaard DM, et al. The impact of female fetal sex on preeclampsia and the maternal immune milieu. *Pregnancy Hypertens*. 2018;12:53–7.
- Mirembert H, Ganer Herman H, Bustan M, Weiner E, Schreiber L, Bar J, et al. Placental vascular lesions differ between male and female fetuses in early-onset preeclampsia. *Arch Gynecol Obstet*. 2022;306:717–22.
- Reyes L, Golos TG. Hofbauer cells: their role in healthy and complicated pregnancy. *Front Immunol*. Frontiers Media S.A.; 2018.
- Seval Y, Korgun ET, Demir R. Hofbauer Cells in early human placenta: possible implications in Vasculogenesis and angiogenesis. *Placenta*. 2007;28:841–5.
- Ingman K, Cookson VJKW, Jones CJP, Aplin JD. Characterisation of Hofbauer Cells in First and Second Trimester Placenta: incidence, phenotype, survival in vitro and motility. *Placenta*. 2010;31:535–44.
- Thomas JR, Naidu P, Appios A, McGovern N. The Ontogeny and function of placental macrophages. *Front Immunol*. Frontiers Media S.A.; 2021.
- Zulu MZ, Martinez FO, Gordon S, Gray CM. The elusive role of placental macrophages: the Hofbauer cell. *J Innate Immun*. S. Karger AG; 2019.
- Thomas JR, Appios A, Zhao X, Dutkiewicz R, Donde M, Lee CYC et al. Phenotypic and functional characterization of first-trimester human placental macrophages, Hofbauer cells. *J Exp Med*. 2020;218.
- Megli CJ, Coyne CB. Infections at the maternal–fetal interface: an overview of pathogenesis and defence. *Nat Rev Microbiol Nat Res*; 2022. pp. 67–82.
- Tauber Z, Chroma K, Baranova R, Cizkova K. The expression patterns of IL-1 β and IL-10 and their relation to CYP epoxygenases in normal human placenta. *Annals Anat*. 2021;236.
- Schlieffsteiner C, Ibesich S, Wadsack C. Placental hofbauer cell polarization resists inflammatory cues in vitro. *Int J Mol Sci*. 2020;21.
- Pantazi P, Kaforou M, Tang Z, Abrahams VM, McArdle A, Guller S et al. Placental macrophage responses to viral and bacterial ligands and the influence of fetal sex. *iScience*. 2022;25.
- O'Neill LAJ, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nat Rev Immunol Nat Publishing Group*; 2016. pp. 553–65.
- Tabas I, Bornfeldt KE. Intracellular and intercellular aspects of macrophage immunometabolism in atherosclerosis. *Circ Res*. 2020;126:1209–27.
- Wculek SK, Heras-Murillo I, Mastrangelo A, Mañanes D, Galán M, Miguel V, et al. Oxidative phosphorylation selectively orchestrates tissue macrophage homeostasis. *Immunity*. 2023;56:516–e5309.
- Wculek SK, Dunphy G, Heras-Murillo I, Mastrangelo A, Sancho D. Metabolism of tissue macrophages in homeostasis and pathology. *Cell Mol Immunol*. Springer Nature; 2022. pp. 384–408.
- Sheng YR, Hu WT, Shen HH, Wei CY, Liu YK, Ma XQ et al. An imbalance of the IL-33/ST2-AXL-efferocytosis axis induces pregnancy loss through metabolic reprogramming of decidual macrophages. *Cell Mol Life Sci*. 2022;79.
- Merech F, Gori S, Calo G, Hauk V, Paparini D, Rios D, et al. Monocyte immunometabolic reprogramming in human pregnancy: contribution of trophoblast cells. *Am J Physiol Endocrinol Metab*. 2024;326:E215–25.
- Gao L, Xu QH, Ma LN, Luo J, Muyayalo KP, Wang LL, et al. Trophoblast-derived lactic acid orchestrates decidual macrophage differentiation via SRC/LDHA Signaling in early pregnancy. *Int J Biol Sci*. 2022;18:599–616.
- Paparini DE, Choudhury RH, Vota DM, Karolczak-Bayatti M, Finn-Sell S, Grasso EN et al. Vasoactive intestinal peptide shapes first-trimester placenta trophoblast, vascular, and immune cell cooperation. *Br J Pharmacol* [Internet]. 2019;176:964–80. <http://www.ncbi.nlm.nih.gov/pubmed/30726565>
- Paparini DE, Grasso E, Fernandez LDC, Merech F, Weingrill-Barbano R, Correa-Silva S, et al. Decidual factors and vasoactive intestinal peptide guide monocytes to higher migration, efferocytosis and wound healing in term human pregnancy. *Acta Physiol (Oxf)*. 2021;232:e13579.
- Wetzka B, Clark DE, Charnock-Jones DS, Zahradnik HP, Smith SK, Kaufmann. Isolation of macrophages (Hofbauer cells) from human term placenta and their prostaglandin E 2 and thromboxane production (MHC) class I and II antigens (Castellucci. *Human Reproduction vol*. 1997.
- Appios A, Thomas JR, McGovern N. Isolation of first-trimester and full-term human placental Hofbauer cells. *Bio Protoc*. 2021;11.
- Paparini D, Gori S, Grasso E, Scordo W, Calo G, Pérez Leirós C et al. Acetylcholine contributes to control the physiological inflammatory response during the peri-implantation period. *Acta Physiol*. 2015;214.
- Kolahi K, Louey S, Varlamov O, Thornburg K. Real-time tracking of BODIPY-C12 long-chain fatty acid in Human Term Placenta Reveals Unique Lipid Dynamics in Cytotrophoblast Cells. *PLoS ONE*. 2016;11:e0153522.
- Paparini D, Grasso E, Calo G, Vota D, Hauk V, Ramhorst R et al. Trophoblast cells primed with vasoactive intestinal peptide enhance monocyte migration and apoptotic cell clearance through $\alpha\text{v}\beta\text{3}$ integrin portal formation in a model of maternal–placental interaction. *Mol Hum Reprod* [Internet]. 2015;21:930–41. <http://www.molehr.oxfordjournals.org/lookup/doi/https://doi.org/10.1093/molehr/gav059>
- Calo G, Sabbione F, Vota D, Paparini D, Ramhorst R, Trevani A et al. Trophoblast cells inhibit neutrophil extracellular trap formation and enhance apoptosis through vasoactive intestinal peptide-mediated pathways. *Hum Reprod*. 2017;32.
- Calo G, Sabbione F, Pascuali N, Keitelman I, Vota D, Paparini D, et al. Interplay between neutrophils and trophoblast cells conditions trophoblast function and triggers vascular transformation signals. *J Cell Physiol*. 2020;235:592–603.
- Svensson J, Jenmalm MC, Matussek A, Geffers R, Berg G, Ernerudh J. Macrophages at the fetal-maternal interface express markers of alternative activation and are induced by M-CSF and IL-10. *J Immunol* [Internet]. 2011;187:3671–82. <http://www.jimmunol.org/cgi/doi/https://doi.org/10.4049/jimmunol.1100130>
- Reich M, Liefeld T, Gould J, Lerner J, Tamayo P, Mesirov JP. GenePattern 2.0. *Nat Genet*. 2006;38:500–1.
- R Core Team. R Core Team. (2022) R: A language and environment for statistical computing. Vienna; 2022.
- Smyth G, [cre. aut]. llimma. Linear Models for Microarray Data. 2017.

49. Fang Z, Liu X, Peltz G. GSEAPy: a comprehensive package for performing gene set enrichment analysis in Python. *Bioinformatics*. 2023;39.
50. Freerman AJ, Johnson AR, Sacks GN, Milner JJ, Kirk EL, Troester MA, et al. Metabolic reprogramming of macrophages: glucose transporter 1 (GLUT1)-mediated glucose metabolism drives a proinflammatory phenotype. *J Biol Chem*. 2014;289:7884–96.
51. Van den Bossche J, O'Neill LA, Menon D. Macrophage immunometabolism: where are we (going)? *Trends Immunol*. Elsevier Ltd; 2017. pp. 395–406.
52. Verberk SGS, de Goede KE, Gorki FS, van Dierendonck XAMH, Argüello RJ, Van Den Bossche J. An integrated toolbox to profile macrophage immunometabolism. *Cell Rep Methods*. 2022;2.
53. Hetz C. The unfolded protein response: Controlling cell fate decisions under ER stress and beyond. *Nat Rev Mol Cell Biol*. 2012. pp. 89–102.
54. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science*. 2011;334:1081–6.
55. Ribeiro CMP, Lubamba BA. Role of IRE1 α /XBP-1 in cystic fibrosis airway inflammation. *Int J Mol Sci*. MDPI AG; 2017.
56. Huang S, Xing Y, Liu Y. Emerging roles for the ER stress sensor IRE1 in metabolic regulation and disease. *J Biol Chem*. 2019;294:18726–41.
57. Schurz H, Salie M, Tromp G, Hoal EG, Kinnear CJ, Möller M. The X chromosome and sex-specific effects in infectious disease susceptibility. *Hum Genomics*. 2019;13:2.
58. Capone I, Marchetti P, Ascierio PA, Malorni W, Gabriele L. Sexual dimorphism of Immune responses: a new perspective in cancer immunotherapy. *Front Immunol*. 2018;9.
59. Dearden L, Bouret SG, Ozanne SE. Sex and gender differences in developmental programming of metabolism. *Mol Metab*. Elsevier GmbH; 2018. pp. 8–19.
60. Bouman A, Heineman MJ, Faas MM. Sex hormones and the immune response in humans. *Hum Reprod Update*. 2005;11:411–23.
61. Markle JG, Fish EN. SexX matters in immunity. *Trends Immunol*. 2014;35:97–104.
62. Trigunaite A, Dimo J, Jørgensen TN. Suppressive effects of androgens on the immune system. *Cell Immunol*. 2015;294:87–94.
63. Dolfi B, Gallerand A, Firulyova MM, Xu Y, Merlin J, Dumont A, et al. Unravelling the sex-specific diversity and functions of adrenal gland macrophages. *Cell Rep*. 2022;39:110949.
64. Doran AC, Yurdagul A, Tabas I. Efferocytosis in health and disease. *Nat Rev Immunol Nat Res*; 2020. pp. 254–67.
65. Feng J, Han J, Pearce SF, Silverstein RL, Gotto AM, Hajjar DP, et al. Induction of CD36 expression by oxidized LDL and IL-4 by a common signaling pathway dependent on protein kinase C and PPAR-gamma. *J Lipid Res*. 2000;41:688–96.
66. Silverstein RL, Febbraio M. CD36, a scavenger receptor involved in immunity, metabolism, angiogenesis, and behavior. *Sci Signal*. 2009;2:re3.
67. Wei Y, Ding J, Li J, Cai S, Liu S, Hong L, et al. Metabolic reprogramming of Immune cells at the maternal-fetal interface and the development of techniques for Immunometabolism. *Front Immunol*. Frontiers Media S.A.; 2021.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.