

Supplement

Decidua

	Gestational age	Sex	Birth weight	Maternal age	Birthmode
Spearman r	-0.452	0.109	-0.381	0.095	-0.218
p value	0.268	0.886	0.360	0.840	0.686

Supplementary Table S1: Spearman correlation of the percentage of CD68⁺ cells of all isolated decidual cells and different patient characteristics; * significant $p < 0.05$.

Hofbauer

	Gestational age	Sex	Birth weight	Maternal age	Birthmode
Spearman r	0.310	0.327	0.167	0.167	0.109
p value	0.462	0.486	0.703	0.703	0.886

Supplementary Table S2: Spearman correlation of the percentage of CD68⁺ cells of all isolated villous cells and different patient characteristics; * significant $p < 0.05$.

VT

Digestion steps	1	2	3
Time	15min	20min	1h
Temperature	37°C	37°C	37°C
Trypsin concentration	1.25mg/ml	0.72mg/ml	-
Collagenase A concentration	-	-	1mg/ml
DNase I concentration	0.1mg/ml	0.1mg/ml	0.1mg/ml
Dilution medium	HBSS-HEPES	HBSS-HEPES	DMEM

Supplementary Table S3: Digestion steps for villous tissue.

Decidua

Digestion steps	1	2
Time	20min	1h
Temperature	37°C	37°C
Trypsin concentration	0.72mg/ml	-
Collagenase A concentration	-	1mg/ml
DNase I concentration	0.02mg/ml	0.02mg/ml
Dilution medium	HBSS-HEPES	DMEM

Supplementary Table S4: Digestion steps for Decidua.

	FACS (n=8)	IF/IHC (n=9)
Gestational age (weeks+days)	39+3 ± 1+0 (38+0 – 40+6)	37+5 ± 1+5 (35+1 – 40+0)
Maternal Age (years)	35 ± 4 (28 - 40)	32 ± 5 (22-39)
Fetal sex	♂4 ♀4	♂1 ♀8

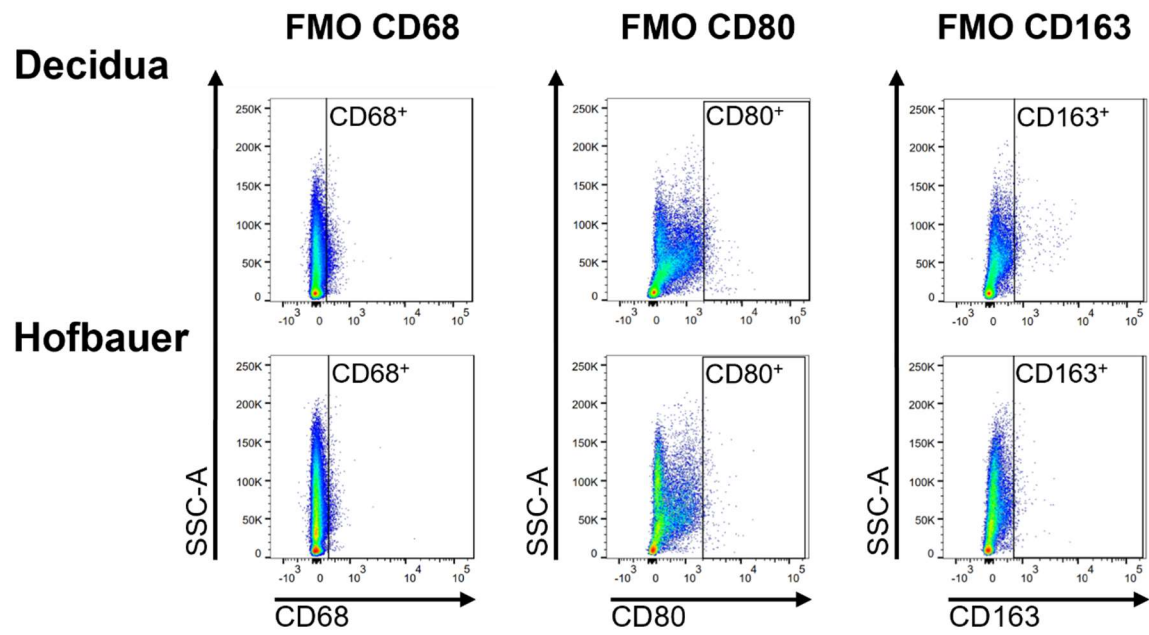
Supplementary Table S5: Patient characteristics of FACS and IF results. All data are shown as mean ± SD (range).

Antibody /Dye	Color	Species isotype	Manufacturer	Clone	Used Concentration
CD45	FITC	Mouse IgG1, κ	BioLegend (San Diego, USA)	HI30	2.0µg/ml
CD163	APC	Mouse IgG1, κ	BioLegend	GHI/61	2.0µg/ml
CD80	BV421	Mouse IgG1, κ	BioLegend	2D10	1.6µg/ml
CD68	APC-Cy7	Mouse IgG2b, κ	BioLegend	Y1/82A	1.6µg/ml
CK7	PE	Rabbit IgG	Abcam (Cambridge, UK)	EPR1619Y	4.0µg/ml
7AAD			BD Biosciences (Franklin Lakes, USA)		
Fixable viability dye eFluor 780			Thermofisher		

Supplementary Table S6: Antibodies and dyes used for the FACS-staining.

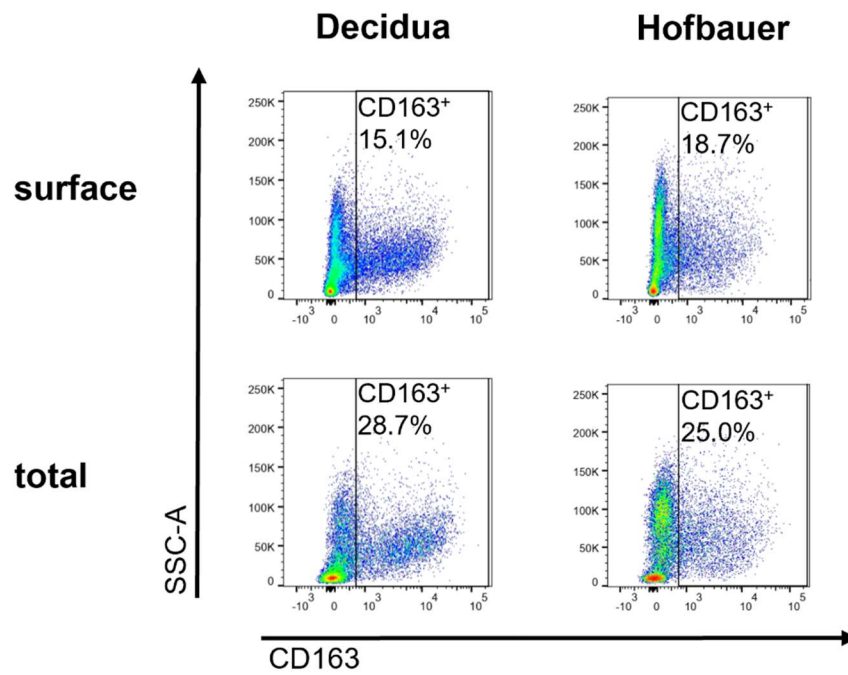
Problem	Possible reason	Solution
Too less isolated macrophages	Tissue is too sticky	Wash with enough HBSS-HEPES between the digestion steps.
	Too less Percoll-layers	Use 4 tube with Percoll layer for 150gr. VT-tissue and 2 tubes for whole (ca. 35gr.) Decidua.
No layer after Percoll centrifugation	Wrong centrifuge setting	Check the centrifuge settings again, you have to use 2100g at RT without brake.
	Pancoll (1.077 gr./ml) was used instead of Percoll (1.130 gr./ml)	Use Percoll or different centrifuge settings; With the same concentrations and settings like for Percoll, all the cells sink to the bottom of the tube with Pancoll.
Trophoblast contamination	Too less Percoll-layers	Use 4 tubes with Percoll layer for 150gr. VT-tissue and 2 tubes for whole (ca. 35gr.) Decidua.
	The red and yellowish top layer from the Percoll tube were not removed	Remove the top red and yellowish layer with a different plastic Pasteur pipette before taking out the macrophages layer.
Too much red blood cell contamination	Erythrocyte layer was also aspirated in the pipette	With the help of the plastic Pasteur pipette, the transparent layer is slowly and carefully aspirated. Too fast aspiration leads to the swirling of the erythrocytes. At the end, a thin transparent macrophages layer must be left.
	Still too much blood in the tissue after washing	Scratch the tissue better before washing. Repeat the washing more often with 0.9% NaCl.

Supplementary Table S7: Trouble shooting.

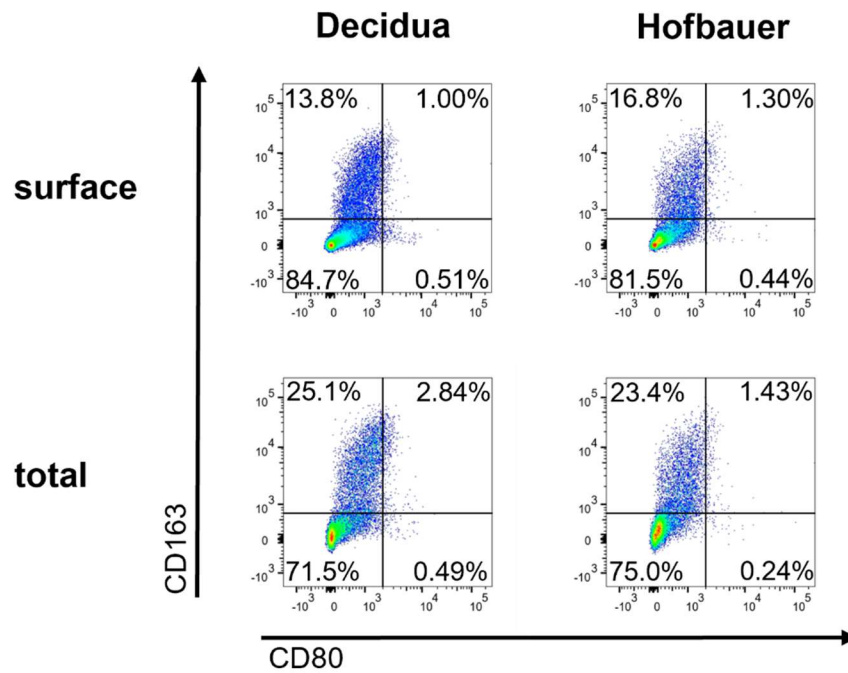


Supplementary Figure S1: FMOs for decidual and Hofbauer macrophages.

A

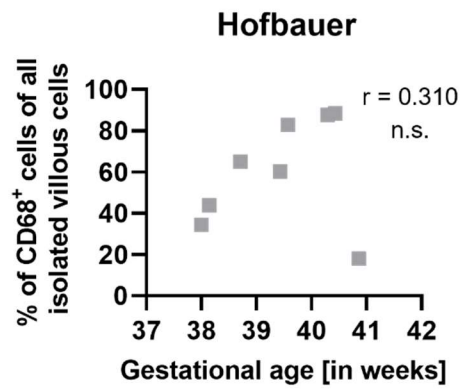
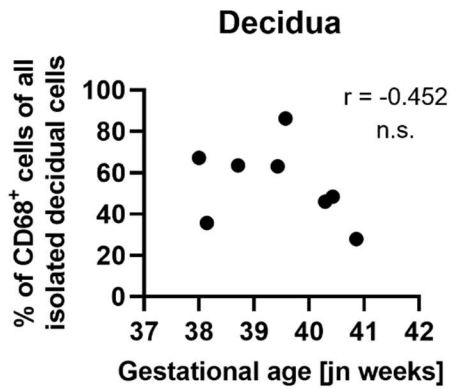


B

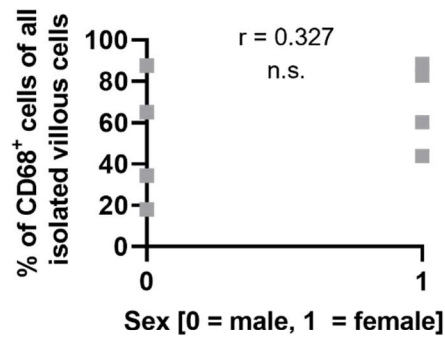
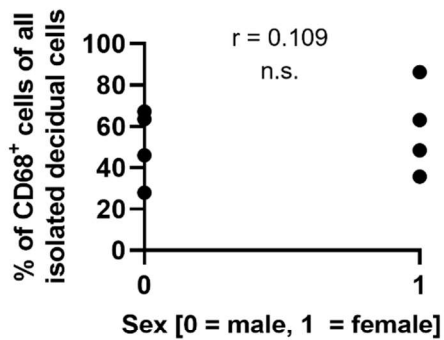


Supplementary Figure S2: Comparison of surface and total staining of CD163: FACS images of (A) CD163⁺ macrophages and (B) CD80⁺CD163⁺ macrophages.

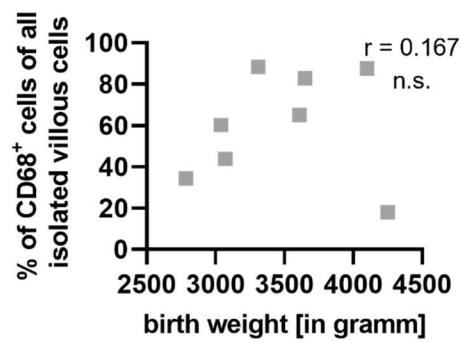
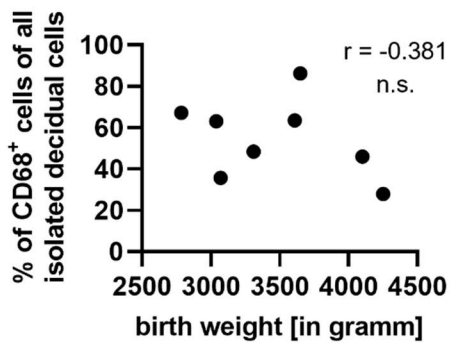
A



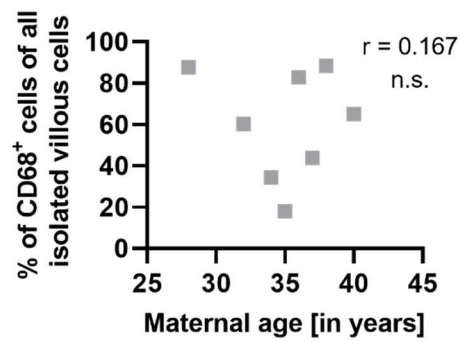
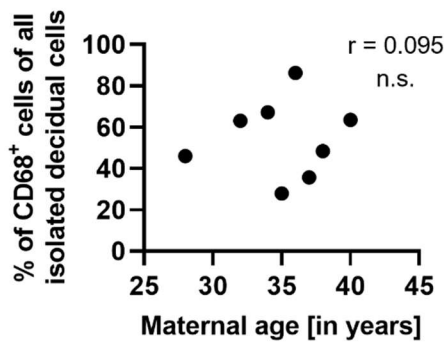
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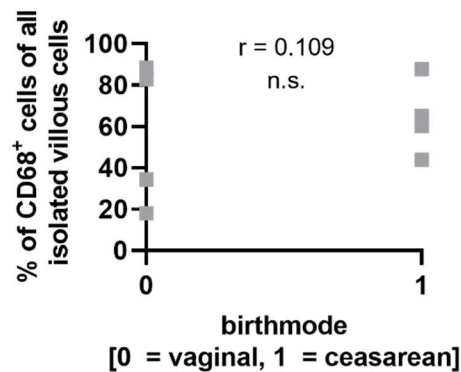
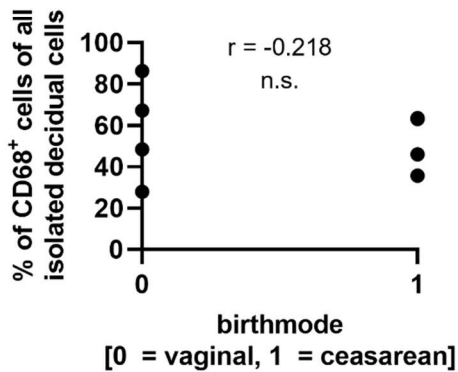
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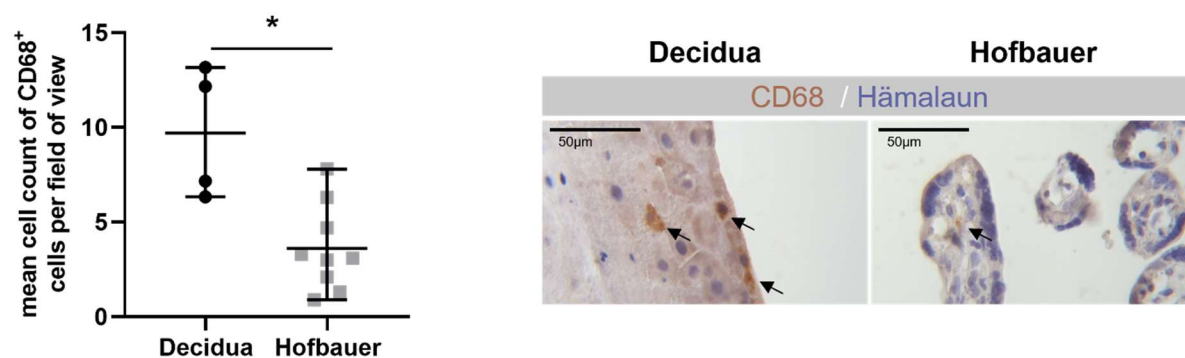
D



E



Supplementary Figure S3: Spearman correlation of the percentage of CD68⁺ of all isolated cells of decidual and villous tissue and (A) the gestational age, (B) fetal sex, (C) birth weight, (D) maternal age and (E) birthmode; * significant $p < 0.05$.



Supplementary Figure S4: Differences in the mean cell count of CD68⁺ cells per field of view in the IHC of CD68; Doplots (as mean \pm range) and representative IHC-images; p-values were calculated with Mann-Whitney-U-Test, * significant $p < 0.05$; $n=9$.