Simmental x Holstein crossbred: comparison of immunological traits with parental breeds during peripartum and early lactation period

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SUPPLEMENTARY FILE

Material and Methods

Cellular markers and flow cytometric gating strategy

Flow cytometric analysis was performed on all animals per time point to evaluate the proportion of innate and adaptive immune cells. Briefly, 50 μL samples of whole blood were incubated for 30 min at 4°C in the dark with conjugated monoclonal antibodies (Supplementary Table S1). Erythrocytes were then lysed with 1 mL of Tris-buffered ammonium chloride solution (0.87% w/v, pH 7.3) for 10 min at room temperature. After a wash with 2 mL of cold phosphate buffered solution (PBS, pH 7.2), the cells were centrifuged at 300g for 5 min, suspended in PBS and collected on a CytoFLEX flow cytometer (Beckman Coulter, USA). Kaluza Analysis Software v 2.1 (Beckman Coulter, USA) was used to analyze flow cytometric data. An electronic gate (FSC *vs* SSC) was created based on morphological features to include total leukocytes population. The proportions of different leukocyte subsets, Granulocytes and Peripheral Blood Mononuclear Cells (PBMC), were assessed based on morphological features (size and granularity) (Figure S1, A). Within granulocytes, eosinophils were easily separated by neutrophils by increased fluorescence in FL1-FITC channel as already described by De Matteis *et al.*, 2016 and Grandoni *et al.*, 2017 (Figure S1, B). Within PBMC, lymphocytes and monocytes were separated based on positivity of CD14 marker, expressed as $CD14^-$ and $CD14^+$ respectively (Figure S1, C) (Panel A, Table S1). The $CD21^+$ B lymphocytes were identified within PBMC as $CD21^+$ and the NK cells as $CD335^+$ (Panel A, Table S1). T lymphocytes subsets were expressed as percentage of $CD3^+$ cells within PBMC, which were positive for each surface marker ($CD4^+$ and $CD8^+$) (Panel B, Table S1).

Legend Figure S1:

Gating strategy for the identification of leukocyte populations by flow cytometric analysis.

(A) Identification of granulocytes and PBMC using a FSC *vs* SSC analysis; (B) Identification of neutrophils and eosinophils selected according to the basal eosinophils autofluorescence in FL-1; (C)

Identification of lymphocytes ($CD14^-$) and monocytes ($CD14^+$), (D) $CD21^+$ B lymphocytes and (E) $CD335^+$ NK cells with Panel A (Table S1); (F) Identification of $CD3^+$ T lymphocytes, (G) $CD4^+$ T helper and $CD8^+$ T cytotoxic with Panel B (Table S1).

Figure S1:

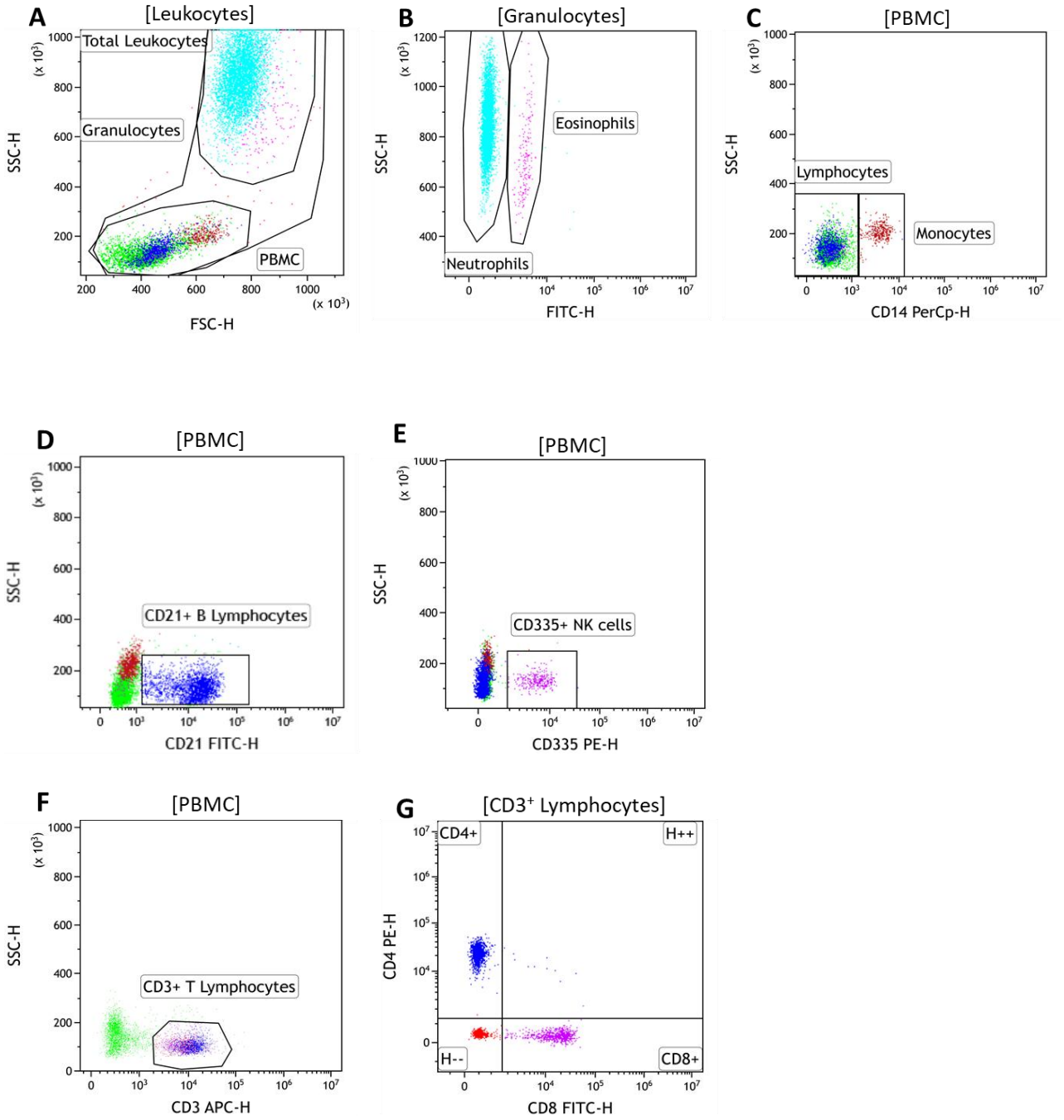

Table legend:

Table S1:

Details of monoclonal antibodies used for flow cytometry assay

Table S2:

Estimated average values of percentage of immunological traits of the three genetic groups at time point

Table S1:

	Specificity	mAb	Isotype	Conjugation	Source
Panel A					
	CD14	TÜK4	IgG2a	Per-CP	ThermoFisher Scientific
	CD21	CC21	IgG1	FITC	Bio-Rad
	CD335	AKS1	IgG1	PE	ThermoFisher Scientific
Panel B					
	CD3	MM1A	IgG1	APC[2]	WSU-MAC[1]
	CD4	CC8	IgG2a	PE	ThermoFisher Scientific
	CD8	CC63	IgG2a	FITC	Bio-Rad

[1] Washington State University Monoclonal Antibody Centre, Pullman, WA-USA
[2] Clone MM1A were available as purified mAb. We used a direct labeling method and the MM1A clone was labelled with Lightning-Link® APC (Allophycocyanin), (Abcam, Cambridge, UK)

Table S2:

Traits	time point[1]	HO		SI		CR	
		mean	**se**	**mean**	**se**	**mean**	**se**
Granulocytes							
	1	38.10	4.86	39.40	4.59	36.60	3.40
	2	47.50	4.45	37.70	5.14	42.20	3.80
	3	55.30	4.44	50.40	4.54	48.60	3.18
	4	46.60	4.14	51.70	4.52	44.90	3.17
	5	54.60[a]	4.14	42.30[b]	4.75	43.20[b]	3.17
	6	46.70	4.14	46.10	4.52	46.60	3.17
Neutrophils							
	1	33.40	4.75	33.70	4.48	32.20	3.33

	2	42.40	4.35	35.20	5.03	38.30	3.72
	3	53.50	4.34	48.40	4.44	47.20	3.11
	4	43.50	4.05	46.10	4.42	42.80	3.10
	5	51.10[a]	4.05	37.90[b]	4.65	39.20[b]	3.10
	6	43.40	4.05	42.40	4.42	42.80	3.10
Eosinophils							
	1	4.60	1.13	5.70	1.07	4.40	0.79
	2	5.20	1.03	2.50	1.19	3.90	0.88
	3	1.80	1.03	2.00	1.05	1.40	0.74
	4	3.10	0.96	2.50	1.10	2.10	0.74
	5	3.40	0.96	4.40	1.10	4.00	0.74
	6	3.30	0.96	3.70	1.05	3.80	0.74
Monocytes							
	1	8.00	1.24	5.40	1.17	6.30	0.87
	2	7.40	1.13	7.00	1.31	6.30	0.97
	3	10.20	1.13	10.20	1.16	8.50	0.81
	4	12.40[a]	1.06	5.90[b]	1.15	7.90[b]	0.81
	5	8.60	1.06	7.50	1.21	7.80	0.81
	6	10.20[a]	1.06	6.80[b]	1.15	6.30[b]	0.81
Lymphocytes							
	1	53.60	4.73	54.90	4.47	56.80	3.31
	2	44.50	4.33	55.30	5.01	51.20	3.70
	3	34.30	4.33	38.90	4.42	42.70	3.10
	4	40.70	4.03	41.80	4.40	46.60	3.09
	5	36.70[b]	4.03	50.30[a]	4.63	48.70[a]	3.09
	6	43.00	4.03	47.00	4.40	46.80	3.09
$CD335^+$							
	1	3.10	0.54	3.00	0.51	2.60	0.38
	2	3.40	0.49	3.20	0.57	3.20	0.42
	3	2.40	0.49	1.90	0.51	1.90	0.35
	4	2.30	0.46	2.70	0.50	2.20	0.35
	5	3.00	0.46	2.00	0.53	2.40	0.35
	6	2.50[ab]	0.46	1.80[b]	0.50	3.10[a]	0.35
$CD21^+$							
	1	19.80[a]	2.01	12.50[b]	1.90	22.60[a]	1.41
	2	19.00[ab]	1.84	15.30[b]	2.13	22.60[a]	1.57
	3	23.00[a]	1.84	14.60[b]	1.88	26.80[a]	1.34
	4	20.60[b]	1.71	13.90[c]	1.87	24.90[a]	1.31
	5	18.30[b]	1.71	13.40[b]	1.97	23.60[a]	1.31
	6	19.20[b]	1.71	14.40[b]	1.87	23.40[a]	1.31
$CD3^+$							
	1	64.80[a]	3.67	63.70[a]	3.46	52.80[b]	2.57
	2	55.00	3.36	45.30	4.29	56.10	3.85
	3	43.90[b]	3.35	56.40[a]	3.42	43.00[b]	2.40
	4	41.60[b]	3.12	57.80[a]	3.41	42.60[b]	2.39
	5	44.40[b]	3.12	59.90[a]	3.44	46.60[b]	2.56
	6	45.00[b]	3.12	58.80[a]	3.41	48.30[b]	2.39
$CD4^+$							
	1	42.20	3.60	44.50	3.80	46.10	2.50
	2	43.70	3.30	41.30	4.20	44.90	3.80
	3	50.10	3.30	50.10	3.40	49.60	2.40

	4	52.00	3.10	49.30	3.40	50.80	2.40
	5	48.50	3.10	52.40	3.40	49.10	2.50
	6	52.80	3.10	52.70	3.40	51.10	2.40
$CD8^+$							
	1	27.20	3.60	32.90	3.40	27.00	2.50
	2	27.50	3.30	29.90	4.20	29.80	3.80
	3	24.30	3.60	28.20	3.40	23.90	2.40
	4	27.20	3.10	26.10	3.40	26.70	2.40
	5	30.80	3.10	26.90	3.40	28.60	2.50
	6	29.20	3.10	31.80	3.40	28.40	2.40
$CD4^+$: $CD8^+$							
	1	1.80	0.38	1.58	0.40	1.87	0.27
	2	1.82	0.35	1.52	0.44	1.86	0.40
	3	2.10	0.37	2.05	0.36	2.34	0.25
	4	2.09	0.32	1.93	0.35	2.33	0.25
	5	1.74	0.32	2.14	0.36	1.93	0.26
	6	2.16	0.32	1.85	0.35	1.99	0.25

[1] time point 1 and 2 correspond at around 30 and 15 days before the expected calving; time point 3 corresponds at calving or at the day after the calving; time point 4, 5 and 6 correspond at 15, 30 and 60 day after calving

[a, b, c] within the same row indicate significant differences between groups ($P < 0.05$)

References

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