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Pre-farrowing gene expression differences in blood of sows with high and low colostrum Brix values

S.A. Collier[1*], A.M. Luttman[1], N.E. Raney[1] and C.W. Ernst[1]

[1]*Michigan State University, East Lansing, USA; Corresponding author: colli838@msu.edu*

Introduction

Colostrum is defined as the first mammary secretions produced after parturition begins, lasting until approximately 24 hours postpartum (Hurley, 2015). During this period, piglets receive essential nutrients and bioactive compounds that support growth, thermoregulation, and gut development (Hurley, 2015; Quesnel *et al*., 2012). Since piglets are born with almost no circulating antibodies, ingestion of colostral immunoglobulins is essential for the development of passive immunity (Rooke and Bland 2002). Immunoglobulin G (IgG) is the primary immunoglobulin in sow colostrum and is positively associated with colostrum Brix values, which are obtained via refractometry and provide an approximation of the percentage of total solids in a liquid (Hasan *et al*., 2016). The ingestion of colostrum with low IgG concentrations may reduce piglet survivability due to impaired passive humoral immunity development (Rooke and Bland, 2002). Therefore, understanding the mechanisms controlling colostral IgG content remains an important objective for reducing piglet mortality. While it is well established that colostrum IgG is entirely derived from maternal circulation during the last 10 days of gestation (Feyera *et al*., 2018), the factors influencing maternal IgG concentrations and their subsequent transfer into colostrum are the focus of few studies (Devillers *et al*., 2004; Feyera *et al*., 2018). Thus, the objective of this study was to use RNA sequencing of pre-farrowing blood samples from primiparous females with high and low Brix values to identify genes and biological pathways potentially associated with serum and colostrum IgG concentrations.

Materials & Methods

The animal housing, care, and protocols used in this study were reviewed and approved by the Michigan State University Institutional Animal Care and Use Committee (PROTO202400145).

Animal housing and sample collection. The study population consisted of 24 Yorkshire primiparous females bred to the PIC800 sire line (PIC, Hendersonville, TN, USA) over two farrowing groups (FG1: n = 14; FG2: n = 10). Housing, diet, and management protocols were identical between groups throughout gestation and were based on farm standard operating procedures. At approximately 110 days of gestation blood samples were collected via jugular venipuncture. Buffy coats were isolated from whole blood samples by erythrocyte lysis using Buffer EL (QIAGEN, Germantown, MD, USA) and subsequently washed with PBS prior to RNA extraction. Serum was isolated by centrifugation, aliquoted, and frozen at -80 °C until analysis. Sows were moved into farrowing crates at approximately 111 days of gestation and once farrowing began, colostrum was collected through manual milking within 0-3 h after of the birth of the first piglet. A 15mL pooled sample was generated for each primiparous female by combining equal volumes of colostrum from the anterior, medial and posterior teats. Brix values of the pooled sample were measured on-farm after collection using a digital Brix refractometer (Atago 3810 PAL-1, Tokyo, Japan). The remaining colostrum was stored at -80 °C.

RNA extraction and RNA sequencing. Total RNA was extracted from buffy coats using the RNeasy mini kit (QIAGEN, Germantown, MD, USA). To compare gene expression between primiparous females with high and low colostrum Brix values, a subset of the three primiparous females with the highest Brix values and three with the lowest were selected from each farrowing group for RNA sequencing (HIGH, n = 6; LOW, n = 6). The extracted RNA was quality checked using RIN scores obtained from the Agilent 4200 TapeStation HS RNA assay (Agilent Technologies, Santa Clara, CA, USA) prior to analysis. Sequencing of the RNA was then performed by the Research Technology Support Facility Genomics Core at Michigan State University using an Element Biosciences AVITI Instrument in a 2x150bp format. Raw reads were quality checked for adaptor content and low base quality using FastQC v0.12.1. Trimming was performed using Trim Galore v0.6.10 with default paired-end settings to remove adaptor content and low-quality bases. Trimmed reads were then aligned to the *Sus scrofa* 11.1 reference genome using HISAT2 v2.2.1 with reverse–forward (RF) strandedness and default settings. Subsequent gene counts were obtained using HTSeq v2.0.7 with the intersection-nonempty mode with features defined as exons and reverse-stranded library settings.

Differential expression and Gene Set Enrichment Analysis. Prior to analysis, data was filtered of undetected and lowly expressed transcripts. Differential expression analysis was performed on R v4.3.2 using the DESeq2 package v1.42.0 with a negative binomial generalized linear model including the fixed effects of Brix group, farrowing group, and order of blood collection. Differentially expressed genes (DEGs) were assessed as HIGH relative to LOW with a threshold of Benjamini-Hochberg False Discovery Rate (FDR) < 0.1 regardless of Log_2fold change (Log_2FC). The g:GOSt function of g:Profiler (https://biit.cs.ut.ee/gprofiler/gost) was used for Gene Set Enrichment Analysis (GSEA) on the DEGs with higher transcript abundance using the Gene Ontology (GO), Kyoto Encyclopaedia of Genes and Genomes (KEGG), and Reactome (REAC) databases for *Sus scrofa* (Pig). The significance threshold was defined as Benjamini-Hochberg FDR < 0.1.

IgG quantification. Serum and colostrum IgG concentrations were quantified using an enzyme-linked immunosorbent assay (ELISA) for swine IgG according to the manufacturer's instructions (Bethyl Laboratories, Montgomery, TX). The serum and colostrum samples were diluted to 1:500,000 and 1:1,000,000, respectively. The subsequent intra-assay CV for serum and colostrum were 3.3% and 3.8%. Differences between HIGH and LOW for Brix values, serum IgG, and colostrum IgG were determined in R v4.3.2 using a two-way ANOVA with the fixed effects of Brix group, farrowing group, and their interaction. The correlation between pre-farrowing serum IgG and colostrum IgG was assessed using Pearson's correlation coefficient.

Results

Brix, serum IgG, and colostrum IgG comparisons. For Brix values, HIGH had a greater mean Brix value than LOW (31.4 ± 0.4 vs. 24.9 ± 0.4; $P < 0.001$) and FG2 had a greater mean Brix value compared to FG1 (29.1 ± 0.4 vs. 27.2 ± 0.4; $P < 0.05$). However, no interaction was detected between Brix group and farrowing group. For serum IgG, there was no interaction or effect of farrowing group, but HIGH tended to have higher serum IgG concentration compared to LOW (7.51 ± 0.97 mg/mL vs. 4.58 ± 0.97 mg/mL; $P = 0.07$). For colostrum IgG, there was a significant interaction between farrowing group and Brix group. For each farrowing group, colostrum IgG concentration was significantly greater in HIGH compared to LOW (FG1: 63.9 ± 8.93 mg/mL vs. 26.2 ± 8.93 mg/mL; $P < 0.001$; FG2: 121.2 ± 8.93 mg/mL vs. 38.7 ± 8.93 mg/mL; $P < 0.05$). For each Brix group, there was a significant difference between HIGH in

FG1 and FG2 (63.9 ± 8.93 mg/mL vs. 121.2 ± 8.93 mg/mL; $P < 0.05$) but not between the LOW groups ($P = 0.36$). Colostrum IgG concentration tended to have a positive correlation with pre-farrowing serum IgG concentration ($P = 0.08$; r = 0.52).

RNA-seq efficiency and differential expression analysis. We obtained an average of 93.2 M raw reads and after trimming the alignment percentage ranged from 85.9% to 90.2%. After differential expression analysis, a total of 33 DEGs were identified when comparing HIGH to LOW, 30 with higher transcript abundance and 3 with lower transcript abundance. A subset of DEGs associated with milk production and the immune system are presented in **Table 1**.

Table 1. DEGs related to milk production and immune system function.

Ensembl ID	Gene ID	Gene Description	Log_2FC[1]	FDR
ENSSSCG00000037579	*PPBP*	pro-platelet basic protein	1.81	0.003
ENSSSCG00000017306	*ITGB3*	integrin subunit beta 3	1.43	0.019
ENSSSCG00000030005	*LGALSL*	galectin like	1.30	0.005
ENSSSCG00000016887	*ITGA2*	integrin subunit alpha 2	1.05	0.006
ENSSSCG00000006288	*SELP*	selectin P	1.01	0.021
ENSSSCG00000038398	*CD226*	CD226 molecule	0.77	0.040

[1] Log_2FC: HIGH relative to LOW

Gene Set Enrichment Analysis. For the 30 DEGs with higher transcript abundance in HIGH relative to LOW, GSEA revealed significant enrichment of 84 GO molecular function terms, 307 GO biological process terms, 68 GO cellular component terms, 17 KEGG pathways, and 15 Reactome pathways. Pathways and GO terms relevant to immune system activation, regulation, and cell migration are highlighted in **Table 2.**

Table 2. Enriched GO, KEGG, and Reactome Pathways relevant to immune system function.

Term Name[1]	Term ID	Enrichment	FDR
GO			
Positive regulation of antigen receptor-mediated signalling pathway	GO:0050857	33.5	0.098
Cell adhesion mediated by integrin	GO:0033627	31.6	0.006
Leukocyte migration	GO:0050900	10.4	0.014
Regulation of immune system process	GO:0002682	3.7	0.045
Positive regulation of immune system process	GO:0002684	3.6	0.091
KEGG			
Platelet activation	KEGG:04611	11.0	0.021
REAC			
Platelet degranulation	REAC:R-SSC-1	19.7	<0.001
Platelet activation, signalling and aggregation	REAC:R-SSC-7	11.7	<0.001

[1] GO: Gene Ontology, KEGG: KEGG metabolic pathways, REAC: Reactome pathways

Discussion

Our classification into HIGH and LOW groups based on Brix values correctly identified primiparous females that had high and low colostrum IgG concentrations, supporting the findings of Hasan *et al*. (2016) that Brix values are influenced by colostrum IgG content. Pre-farrowing serum IgG concentration had a tendency to associate with Brix group and tended to

correlate with colostrum IgG concentration. This finding is different from the results of Devillers *et al*. (2004) who reported no correlation between plasma and colostrum IgG concentration over time in multiparous sows. It should be noted the sample sizes of both studies were limited, with eight multiparous sows in Devillers *et al*. (2004) and twelve primiparous females in our study. Additionally, all pigs in each study were housed on the same farm minimizing environmental differences that could contribute to variation in IgG levels such as herd health status, management strategies, and dietary differences. Our findings suggest that higher availability of IgG within maternal circulation at around 110 days of gestation may have a positive influence on the resulting colostrum IgG concentration. This finding is a promising start toward elucidating the mechanisms that may be driving colostrum IgG content, which could influence piglet passive humoral immunity acquisition and subsequent survivability, as piglet plasma IgG is positively correlated with survival (Rooke and Bland, 2002).

The higher transcript abundance of genes such as *ITGB3, SELP,* and *CD226,* together with enriched GO terms and pathways related to immune system functioning and regulation in HIGH suggest altered transcriptional profiles of immune components that may contribute to the observed differences in colostrum IgG content. Specifically, *CD226* and the platelet associated genes, *ITGB3* and *SELP*, may contribute to immune cell interactions that facilitate conditions that better support increased IgG transfer (Koupenova *et al*., 2018; Liu *et al*., 2025). KEGG and Reactome pathways support this interpretation as highlighted by enriched immune system regulation, trafficking, and activation. Additionally, *LGALSL* which had higher transcript abundance in HIGH and has been associated with higher milk production in dairy cows (Bai *et al*., 2016), may reflect differences that positively contribute to mammary gland functioning.

Conclusions

In summary, pre-farrowing transcriptional differences in blood of primiparous females with high or low Brix values suggest altered immune system mechanisms that may modulate resulting colostrum IgG concentrations.

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