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Circulating biomarkers associated with pelvic organ prolapse risk in late gestation sows

Zoë E. Kiefer,[†] Jamie M. Studer,[†] Amanda L. Chipman,[‡] Malavika K. Adur,[†] Christine Mainquist-Whigham,[∥] Nicholas K. Gabler,[†] Aileen F. Keating,[†] and Jason W. Ross[†,‡,1]

[†]Department of Animal Science, Iowa State University, Ames, IA 50011, USA, [‡]Iowa Pork Industry Center, Ames, IA 50011, USA, [∥]Pillen Family Farms, Columbus, NE 685601, USA

[1]Corresponding author: jwross@iastate.edu

ORCiD numbers: 0000-0002-3064-2384 (Z. E. Kiefer); 0000-0003-1913-3383 (J. M. Studer); 0000-0001-7283-4782 (N. K. Gabler); 0000-0003-4950-2258 (J. W. Ross).

Abstract

Sow mortality, as the result of pelvic organ prolapse (POP), has been increasing in the last decade in the U.S. swine industry. The objective of this study was to identify potential biological markers associated with risk of POP in sows. We hypothesized that sows differing in perineal score (PS) from PS1–PS3 (PS1—a presumed low POP risk; PS2—a presumed moderate POP risk; and PS3—a presumed high POP risk) would differ in circulatory biomarkers of inflammation and hormonal profiles. On gestation week 15, 2,864 individual sows were assigned a PS, and subsequently, 1.0%, 2.7%, and 23.4% of PS1, PS2, or PS3 sows, respectively, experienced POP. During PS assignment at days 107–116 of gestation, blood samples were collected from sows on two farms of similar genetics, feed sources, and health status. Whole blood was subjected to complete blood count (CBC) analysis ($n = 212$) and steroid hormones were measured in serum from a subset ($n = 110$) of animals assigned PS3 parity matched to PS1. Lipopolysaccharide-binding protein (LBP), tumor necrosis factor-alpha (TNF-α), haptoglobin, C-reactive protein (CRP), and creatine kinase (CK) levels were also evaluated. Complete blood count analysis revealed decreased ($P \leq 0.05$) mean platelet volume (3.9%), lymphocytes (6.5%), and monocytes (7.5%) in PS3 compared to PS1 sows. Increased ($P \leq 0.02$) abundance of androstenedione (13.4%), androsterone (18.2%), estrone (24.8%), and 17β-estradiol (26.2%) was observed in PS3 compared to PS1 sows. Additionally, a 25.8% increase ($P = 0.04$) in LBP in PS3 compared to PS1 sows was observed. Many dynamic physiological changes occur in sows during late gestation as they approach farrowing. The data presented herein demonstrate that distinct differences in concentrations of circulating biomarkers exist between late gestation sows at high or low risk for POP and may serve as a useful tool for understanding the etiology of POP and evaluation of mitigation strategies.

Keywords: biomarkers, pelvic organ prolapse, sow, swine

Introduction

Sow survivability and longevity are reduced as the result of pelvic organ prolapse (POP), for which incidence has been consistently increasing in the U.S. swine industry over the past decade (Supakorn et al., 2014). Our group previously conducted a large, U.S. industry-wide survey, collecting data from over 100 farms with varying mortality rates demonstrating that 21% of all sow mortality is due to POP (Ross, 2019). The phenotype of POP is characterized by

Abbreviations

APP	acute phase protein
CBC	complete blood count
CK	creatine kinase
CRP	C-reactive protein
E2	17β-estradiol
ELISA	enzyme-linked immunosorbent assay
IAV	influenza A virus
LBP	lipopolysaccharide-binding protein
LPS	lipopolysaccharide
MHC	major histocompatibility complex
MHP	Mycoplasma hyopneumoniae
MPV	mean platelet volume
P4	progesterone
PED	porcine epidemic disease
POP	pelvic organ prolapse
PRRS	porcine reproductive and respiratory syndrome
PS	perineal score
TLR	toll-like receptor
TNF-α	tumor necrosis factor-alpha

one or more pelvic organs (uterus, rectum, and/or vagina) shifting from their normal location within the pelvic area and descending from the body cavity (Jelovsek et al., 2007). Affected sows typically experience POP around the time of parturition, which almost invariably results in the euthanasia of sows and often in the loss of offspring, furthering the economic loss and animal welfare concerns of this biological phenomenon. The cause of POP in swine is not well-understood, creating a significant barrier in the development of strategies to mitigate its occurrence.

As parturition looms, the perineal area of a sow during late gestation can phenotypically appear red and swollen in comparison to other stages of gestation. It has been shown that sows with greater relative POP risk have an abnormal amount of swelling, indicating a potential inflammatory response (Kiefer et al., 2021). Characterization of a late gestation phenotype and the associated edema in the perineal area has resulted in the development of a scoring system, which effectively distinguishes sows with low and high risk for POP (Kiefer et al., 2021).

With respect to immune modulating factors, the vaginal environment is neither constant nor well defined in most species (Farage et al., 2010) and the immune cell repertoire in the lower genital tract differs between species and during different phases of the reproductive cycle (Ravel et al., 2011; Chu et al., 2017). This may be partly influenced by commensal bacteria colonization of the lower genital tract that can affect immune responses (Mirmonsef et al., 2011). The epithelial cells of the female tract express receptors for factors involved in immune responses, such as toll-like receptors (TLR) and major histocompatibility complex (MHC) molecules, which help identify, process, and initiate cellular and humoral immune responses (Mirmonsef et al., 2011). These epithelial cells can, upon activation, produce a variety of cytokines and chemokines that aid in recruiting and activating cells of the immune system in the female reproductive tract (Franklin and Kutteh, 1999).

The production of steroid hormones is necessary for endocrine regulation of metabolism, inflammation, immune function, and reproductive function in livestock species. Steroid hormones can be synthesized in the adrenal cortex, gonads (testes and ovaries), brain, placenta, and adipose tissues (Falkenstein et al., 2000). Given the steroid hormone production capacity from these tissues in pregnant sows, it is

also possible that steroid hormones may alter inflammation and immune system function. As an example, T-helper lymphocytes, which function to produce cytokines during an inflammatory response (Bouman et al., 2005), can potentially be reduced via testosterone induced apoptosis (Mcmurray et al., 2001). It has also been suggested that estrogen, and possibly progesterone (P4), decreases monocytes (Ben-Hur et al., 1995). However, these hormones have also been observed to induce monocyte release from the bone marrow (Bain and England, 1975). How inflammation or the immune system is associated with POP remains unexplored.

The objectives of this study were to evaluate differences in inflammatory markers and steroid hormone concentrations in sows with high and low POP risk. To accomplish this, we tested the hypothesis that sows with high risk for POP during late gestation would have increased serum biomarkers associated with inflammation and/or immune modulation, in addition to alterations in steroid hormone profiles compared to low-risk sows.

Materials and Methods

Animals
All experiments involving animals were approved by the Iowa State University Institutional Animal Care and Use Committee. All animals were individually housed in commercial sow farms located throughout the Midwest United States.

Perineal scoring system
Sows were of commercial genetics and from two different farms approximately one mile from each other, populated from the same gilt multiplier, fed identically formulated diets from the same feed mill, and housed and managed similarly. Additionally, farms had the same health status of porcine reproductive and respiratory syndrome (PRRS) naïve, *Mycoplasma hyopneumoniae* (MHP) stable, porcine epidemic disease (PED) naïve, and influenza A virus (IAV) stable. Utilizing an established perineal scoring (PS) system, 2,864 pregnant sows (gestation days 107–116), from two different farms, were categorized during late gestation into three PS categories of presumed low to high POP risk (Kiefer et al., 2021). To minimize variation, all animals were scored as PS1 (low risk), PS2, or PS3 (high risk), by the same two individuals, while the sow was laying down. It is important to note that a PS3 sow is considered abnormal for any stage of gestation. Following scoring, sows were monitored for subsequent POP incidence.

Sample collection
On a subset of sows, at the time of PS assignment, biological samples were collected from sows classified as PS3 (n = 127) along with a parity-matched PS1 sow (n = 103). Blood samples were collected by jugular venipuncture into multiple vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ). Blood (3 mL) collected into EDTA tubes was used for complete blood count (CBC) analysis. Blood samples (10 mL) were also collected and allowed to coagulate at room temperature before separation of serum by centrifugation at 20 °C for 15 min at 2,000 × g followed by aspiration and storage at –80 °C until further analysis.

Complete blood count analysis
Whole blood collected into EDTA tubes was used for CBC analysis. Samples were submitted from PS1 (n = 90) and PS3 (n = 122) animals to the Iowa State University Clinical Pathology laboratory (Ames, IA) for CBC with automated differential analysis.

Steroid hormone analysis

Serum samples from PS3 scored sows (n = 60) and parity-matched PS1 scored sows (n = 50) were quantified using a AbsoluteIDQ®Stero17 Kit from Biocrates (Innsbruck, Austria) from a 17-panel quantification of endogenous steroids (Koal et al., 2012). This panel included deoxycorticosterone, deoxycortisol, hydroxyprogesterone, aldosterone, androstenedione, androsterone, corticosterone, cortisol, cortisone, estrone, 17β-estradiol (E2), P4, and testosterone. Sample preparation procedure was based on solid phase extraction technique in a 96-well plate format, which is necessary for cleaning and concentrating of the target steroid hormones. The HPLC–MS/MS analysis was conducted in multiple reaction monitoring mode using a Waters Xevo TQ-S-micro (Waters, Vienna, Austria). For quantification of all 17 steroid analytes, 7-point external calibration curves and 13 stable isotope-labeled internal standards were used as previously described (Koal et al., 2012).

Analysis of markers of inflammation

Serum from PS1 (n = 50) and PS3 (n = 60) sows was used to quantify cytokine and acute phase protein (APP) markers associated with inflammation via ELISA. Lipopolysaccharide-binding protein (LBP) ELISA (Cat. # HK503, HycultBiotech, Plymouth Meeting, PA) was used to measure LBP. The detection range for the assay was 1.6 to 100 ng/mL and the inter-assay CV was 12.21%. Porcine tumor necrosis factor-alpha (TNF-α) quantikine ELISA (Cat. # PTA00, R&D Systems, Minneapolis, MN) was used to measure TNF-α. The detection range for this TNF-α assay was 23.4 to 1,500 pg/mL and the inter-assay CV was 11.02%. Porcine haptoglobin ELISA (Cat. # 41-HAPPO-E01, Alpco Ltd., Salem, NH) was used to measure haptoglobin. The detection range for this assay was 25 to 400 ng/mL and the inter-assay CV was 16.4%. Porcine C-reactive protein (CRP) ELISA (Cat. # 41-CRPPO-E01, Alpco Ltd., Salem, NH) was used to measure CRP. The detection range for this assay was 6.25 to 200 ng/mL and the inter-assay CV was 6.18%. All ELISA assays were conducted in accordance with the manufacturer's protocols. Serum aliquots from a subset of sows, based on serum availability, for those scoring PS1 (n = 47) and PS3 (n = 55) were also submitted to the Iowa State University Clinical Pathology laboratory (Ames, IA) for quantification of creatine kinase (CK) using a dry chemistry method via a VITROS 5000.

Statistical analysis

For CBC, steroid hormones analysis, and markers of inflammation, main effects of PS, Farm, and the interaction between PS and Farm were evaluated in SAS 9.4 (Cary, NC) utilizing the PROC MIXED procedure. Furthermore, parity was used as a covariate and gestation day at the time of sampling as a random effect. Additionally, the effect of POP outcome was evaluated for all parameters using only PS3 animals for analysis. For this analysis, parity and farm were used as covariates and gestation day as a random effect. Data are presented as least squares means and considered significant if $P \leq 0.05$ and a tendency for biological meaning if P is between 0.05 and 0.10. Comparisons between individual effects are presented as differences of least squares means.

Results

Differences in perineal score are associated with differing risks of POP

Of the 2,864 sows scored during late gestation, 1,570, 1,166, and 128 were assigned PS1, PS2, and PS3, respectively. A difference ($P < 0.01$) in POP rate was observed between PS1, PS2, and PS3, with 1.0% of PS1, 2.7% of PS2, and 23.4% of PS3 scored sows, subsequently experiencing POP (Figure 1). Distribution of PS1, PS2, and PS3 at Farm A was 55.5%, 41.3%, and 3.2% of the population, respectively. Similarly, distribution of PS at Farm B was 54.1%, 40.1%, and 5.7% of sows scored as PS1, PS2, and PS3, respectively. Additionally, Farm A had a 1.6% POP rate while Farm B had a 3.7% POP rate during the duration of this study. The current study was performed over the course of 7 consecutive weeks, during winter/spring of 2019, with each farm being scored on subsequent days but the same day of each week. For all the sows used in the study, average parity was 2.2 ± 0.04 and average gestation day was 112.3 ± 0.03 at the time of PS assignment. When considering all animals assigned a PS (n = 2,864), sow parity differed ($P < 0.01$) between PS1 and PS3 scored sows, with an average parity of 1.9 and 3.3 ± 0.18, respectively.

Complete blood count parameters differ in sows with high and low POP risks

During CBC analysis, a total of 16 blood parameters were evaluated and reported in Table 1. Farm A had increased ($P \leq 0.03$) mean corpuscular volume by 1.6%, when compared with Farm B. Farm B had an insubstantial increase (0.7%) in mean corpuscular hemoglobin concentration and a 18.4% increase in eosinophils ($P \leq 0.02$) compared to Farm A. There was an effect of PS on mean platelet volume (MPV), lymphocytes, and monocytes ($P \leq 0.05$) with an increase of 3.9%, 6.5%, and 7.5%, respectively, in PS1 compared to PS3 sows. When evaluating CBC levels in sows assigned PS3 that subsequently did or did not experience POP, only a difference ($P = 0.02$) in MPV was observed with a 6.1% increase in sows that experienced POP compared to those that did not (Table 4).

Steroid hormones in serum differ between sows with high and low POP risks

Serum steroid hormone concentrations are reported in Table 2. Etiocholanolone and dihydrotestosterone were undetectable in all samples and dehydroepiandrosterone-sulfate was detected in only three sows from Farm B, and therefore, they were not analyzed. Of
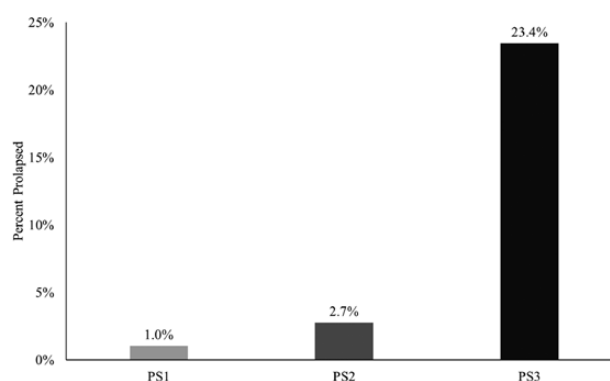
Figure 1. Perineal score (PS) at gestation week fifteen is an indicator of pelvic organ prolapse (POP) risk. Perineal scoring was conducted on sows (n = 2864) during late gestation (days 107–116). Sows assigned a PS1 (n = 1570), PS2 (n = 1166), and PS3 (n = 128) were considered low, medium, and high risks, respectively, for POP. There was a difference in POP rates among PS1, PS2, and PS3 sows, with 23.4% of PS3 sows experiencing POP while 1.0% and 2.7% of PS1 and PS2 sows, respectively, experienced POP ($P < 0.01$). These data demonstrate a method to distinguish differential risk of POP for late gestation sows.

Table 1. Complete blood count analysis differences between late gestation sows with differing risks for pelvic organ prolapse (POP)[1]

Parameter, units	Farm A		Farm B		SEM	P-value		
	PS1	PS3	PS1	PS3		Farm	PS	Farm*PS
White blood cells, $10^3/\mu L$	13.22	12.51	13.23	12.99	0.4	0.50	0.16	0.48
Red blood cells, $10^6/\mu L$	5.20	5.12	5.24	5.17	0.09	0.61	0.27	0.93
Hemoglobin, gm/dL	10.8	10.7	10.8	10.7	0.1	0.89	0.45	0.85
Hematocrit, %	32.6	32.4	32.6	32.2	0.5	0.72	0.44	0.77
Mean corpuscular volume, fl	63.2	63.9	62.5	62.6	0.5	0.03	0.40	0.46
Mean corpuscular hemoglobin, pg	20.9	21.1	20.8	20.8	0.2	0.27	0.40	0.67
Mean corpuscular hemoglobin concentration, gm/dL	33.0	33.0	33.2	33.3	0.1	0.02	0.87	0.55
Red cell distribution width, %	16.4	16.1	16.5	16.7	0.2	0.11	0.84	0.12
Platelets, $10^3/\mu L$	182	187	178	218	28	0.58	0.33	0.44
Mean platelet volume, fl	10.3	10.1	10.5	9.8	0.2	0.72	0.03	0.24
Neutrophils, $10^3/\mu L$	6.50	6.37	6.08	6.24	0.33	0.38	0.96	0.60
Lymphocytes, $10^3/\mu L$	5.13	4.88	5.55	5.12	0.21	0.10	0.05	0.60
Monocytes, $10^3/\mu L$	0.52	0.46	0.51	0.50	0.02	0.42	0.04	0.14
Eosinophils, $10^3/\mu L$	0.93	0.69	0.96	1.00	0.07	0.01	0.11	0.01
Basophils, $10^3/\mu L$	0.05	0.04	0.04	0.04	< 0.01	0.36	0.24	0.08
Absolute large unstained cells, $10^3/\mu L$	0.09	0.08	0.08	0.09	0.01	0.98	0.78	0.51

[1]Sows were assigned a perineal score (PS) based on their relative risk of experiencing POP. Sows assigned PS1 were considered low risk, whereas sows assigned PS3 were considered high risk for POP.

Table 2. Differences in circulating steroid hormones between sows differing in risk for pelvic organ prolapse (POP)[1]

Steroid Hormone, nM	Farm A		Farm B		SEM	P-value		
	PS1	PS3	PS1	PS3		Farm	PS	Farm*PS
Deoxycorticosterone	0.23	0.26	0.29	0.34	0.04	0.06	0.18	0.80
Deoxycortisol	0.41	0.48	0.51	0.63	0.13	0.23	0.33	0.82
Hydroxyprogesterone	0.113	0.128	0.122	0.109	0.012	0.60	0.90	0.15
Aldosterone	0.23	0.34	0.42	0.47	0.09	0.02	0.21	0.63
Androstenedione	0.105	0.134	0.105	0.109	0.009	0.10	0.02	0.07
Androsterone	0.060	0.074	0.056	0.068	0.006	0.40	0.02	0.95
Corticosterone	0.61	0.53	0.72	0.75	0.12	0.09	0.74	0.52
Cortisol	35	34	40	40	5	0.18	0.91	0.96
Cortisone	10.7	12.1	11.7	11.3	0.8	0.89	0.37	0.15
Estrone	8.4	12.4	8.6	10.2	0.8	0.15	< 0.01	0.09
β-Estradiol	0.67	0.97	0.61	0.76	0.06	0.02	< 0.01	0.17
Progesterone	26	23	24	24	1	0.61	0.11	0.45
Testosterone	0.040	0.055	0.050	0.053	0.006	0.48	0.11	0.23

[1]Sows were assigned a perineal score (PS) based on their relative risk of experiencing POP. Sows assigned PS1 were considered low risk, whereas sows assigned PS3 were considered high risk for POP.

the remaining 14 hormones, Farm A had 16.4% greater (*P* = 0.02) levels of E2 in serum and Farm B had a 36.2% increase (*P* = 0.02) in serum levels of aldosterone (Table 2). There was a tendency (*P* ≤ 0.09) for Farm B to have 22.1% and 22.3% higher serum levels of deoxycorticosterone and corticosterone, respectively. Serum androstenedione, androsterone, estrone, and E2 levels were 13.4%, 18.2%, 24.8%, and 26.2% increased (*P* ≤ 0.02) in PS3 compared to PS1 sows, respectively. No significant farm by PS interaction was detected for any of the hormones evaluated (Table 2). Additionally, no differences (*P* ≥ 0.15) in any steroid hormones that were evaluated were detected between PS3 sows that subsequently did or did not experience POP (Table 4).

Acute phase protein and cytokine profiles in sows identified as high risk for POP

Circulating concentrations of LBP were 25.8% higher (*P* = 0.04) in PS3 compared to PS1 sows (Table 3). When comparing PS3 to PS1 sows, no difference in TNF-α concentration (56.5 pg/mL ± 7.1)

was observed between PS, farm, or an interaction between PS and farm (*P* ≥ 0.60; Table 3). Serum CRP concentrations (31.4 μg/mL ± 5.4) were not different among PS, farm, or an interaction between PS and farm (*P* ≥ 0.20; Table 3). Serum haptoglobin concentrations (928.3 ± 138.4) were also not different among PS, farm, or an interaction between PS and farm (*P* ≥ 0.28). Farm A had 55.9% greater (*P* = 0.01) serum CK concentrations compared to Farm B; however, there was no observable difference (*P* = 0.31) between PS or an interaction of PS and farm on CK concentration (Table 3). Additionally, no differences (*P* ≥ 0.22) were observed between PS3 sows that subsequently did or did not experience POP in any serum biomarkers evaluated (Table 4).

Discussion

Although the biological explanation for POP remains unknown, it is critical that mitigation strategies be developed which are

Table 3. Serum biomarker analysis between late gestation sows differing in risk for pelvic organ prolapse[1]

Parameter, units	Farm A		Farm B			P-value		
	PS1	PS3	PS1	PS3	SEM	Farm	PS	Farm*PS
Lipopolysaccharide binding protein, ng/mL	6.089	8.050	6.038	8.294	1.224	0.93	0.04	0.89
Tumor Necrosis Factor alpha, pg/mL	58.0	61.1	62.7	74.6	24.5	0.60	0.64	0.78
C-reactive protein, µg/mL	29.56	32.48	23.84	31.88	5.06	0.48	0.20	0.55
Haptoglobin, µg/mL	847.9	888.7	786.5	791.9	139.1	0.28	0.51	0.54
Creatine Kinase, pg/nm	1,582	1,006	563	580	326	0.01	0.31	0.28

[1]Sows were assigned a perineal score (PS) based on their presumed risk of experiencing pelvic organ prolapse (POP). Sows assigned PS1 were considered low risk, whereas sows assigned PS3 were considered high risk for POP.

Table 4. Circulatory marker analysis between PS3 sows that subsequently did or did not experience POP[1]

	POP outcome			
	No	Yes	SEM	P-value
CBC, units				
White blood cells, 10^3/µL	12.84	12.51	0.43	0.50
Red blood cells, 10^6/µL	5.11	5.13	0.09	0.90
Hemoglobin, gm/dL	10.7	10.7	0.2	0.78
Hematocrit, %	32.2	32.2	0.5	0.93
Mean corpuscular volume, fl	63.3	63.0	0.5	0.68
Mean corpuscular hemoglobin, pg	21.0	20.9	0.2	0.96
Mean corpuscular hemoglobin concentration, gm/dL	33.1	33.2	0.1	0.43
Red cell distribution width, %	16.5	16.4	0.2	0.75
Platelets, 10^3/µL	196	230	38	0.43
Mean platelet volume, fl	9.8	10.4	0.3	0.02
Neutrophils, 10^3/µL	6.48	6.33	0.77	0.71
Lymphocytes, 10^3/µL	5.04	4.87	0.25	0.56
Monocytes, 10^3/µL	0.48	0.47	0.02	0.70
Eosinophils, 10^3/µL	0.92	0.82	0.08	0.24
Basophils, 10^3/µL	0.04	0.04	< 0.01	0.82
Absolute large unstained cells, 10^3/µL	0.08	0.08	0.01	0.79
Steroid hormone, nM				
Deoxycorticosterone	0.32	0.32	0.04	0.93
Deoxycortisol	0.52	0.66	0.10	0.27
Hydroxyprogesterone	0.116	0.110	0.008	0.64
Aldosterone	0.43	0.46	0.08	0.67
Androstenedione	0.118	0.112	0.007	0.56
Androsterone	0.069	0.070	0.006	0.89
Corticosterone	0.59	0.78	0.11	0.15
Cortisol	35	41	4	0.32
Cortisone	11.2	11.8	0.8	0.44
Estrone	11.1	10.5	0.7	0.58
β-Estradiol	0.84	0.81	0.05	0.64
Progesterone	24	24	1	0.57
Testosterone	0.056	0.050	0.005	0.46
Biomarker, units				
Lipopolysaccharide binding protein, ng/mL	8.048	8.279	1.075	0.88
Tumor Necrosis Factor alpha, pg/mL	56.8	87.9	27.3	0.22
C-reactive protein, µg/mL	32.97	30.06	4.5	0.64
Haptoglobin, µg/mL	846.2	854.5	114.1	0.96
Creatine Kinase, pg/nm	756	655	202	0.73

[1]Sows that were assigned PS3 (high risk) that subsequently did (yes) or did not (no) experience POP.

predicated on understanding the biological causes contributing to POP since the incidence of POP accounts for approximately 21% of sow mortality. Sows during late gestation were selected based upon their putative risk for POP using an established scoring strategy (Kiefer et al., 2021) and evaluated for blood markers commonly associated with inflammation and immune system function.

An important component for this study was that the PS system employed was effective in assessing relative risk of POP, as sows scored PS3 had a markedly higher prolapse rate

compared to those assigned PS1, recapitulating our previous findings (Kiefer et al., 2021). The observation that sows with an elevated risk of POP also experience decreased circulating lymphocytes and monocytes, albeit not a marked shift, could be explained by the reallocation of immune cells to target tissues, which has been observed previously (Kratofil et al., 2017). Monocyte recruitment plays a critical role in the host's defense by promoting inflammation or facilitating tissue repair (Kratofil et al., 2017). An additional observation from the CBC analysis was that MPV was decreased in PS3 compared to PS1. Previous studies have associated changes in MPV with inflammatory diseases, such as inflammatory bowel disease, in humans and swine (Järemo and Sandberg-Gertzen, 1996; Kapsoritakis et al., 2001). In humans, a negative correlation between MPV and Crohn's disease is also reported (Shah et al., 1989). The current observations in lymphocyte abundance and MPV alterations between sows with differing POP risk have also been reported in other diseases, along with increases in cytokine abundance (Kapsoritakis et al., 2001; D'Ambrosio et al., 2002). The drastic difference in phenotype along with observable differences in immune cell populations between high and low risk sows led us to investigate other factors associated with the inflammatory response in PS3 sows.

After penetration of protective barriers, bacterial endotoxin can lead to immune activation (Mueller et al., 2005), and phagocytes, such as monocytes, have a role in the pathophysiology of inflammation and associated cytokine production (Heine et al., 1999). The observation of high-risk sows having elevated LBP may help explain, at least in part, the significant swelling and presumed local inflammation observed in PS3 sows. When tissues are injured by bacteria, trauma, toxins, heat, or various other reasons, cells release chemicals that cause swelling as part of the inflammatory response (Mirmonsef et al., 2011). Tumor necrosis factor-alpha is an inflammatory mediator rapidly secreted in response to LPS (Takashiba et al., 1999) and increases during the initial phases of an inflammatory response (Petersen et al., 2004). Given that the timing of sample collection in this study was presumably past initial exposure to potential elicitors of inflammation, this could explain why no difference in TNF-α between sows with differing POP risk was detected. These results support that a bacterial infection may be associated with the inflammatory response observed in PS3 sows.

Creatine kinase is an enzyme found in skeletal muscle and other tissues and is a biomarker found in blood following muscle and tissue damage or muscle usage, such as exercise (Brancaccio et al., 2007; Brancaccio et al., 2010). Serum CK in this study was evaluated with respect to POP risk to determine if CK was a potential marker of the presumed tissue and muscle damage experienced by sows at high risk of POP. Interestingly, CK was not different between sows at low and high risk for POP, but was significantly different between farms. Furthermore, and contrary to our expectations, the farm with the greater level of POP, during the project period and historically, had lower serum CK on average. The observation that CK was different between farms is puzzling given the similarity of the genetics of the sows, facilities, and nutrition. Further investigation would be required to better understand if the differences observed between farms have biological meaning and application.

Haptoglobin is also a modulator of the inflammatory response due to LPS exposure and regulates monocyte activation (Arredouani et al., 2005). Samples were collected at a single time point, and it is difficult to pinpoint the onset of an inflammatory reaction, since the temporal pattern of the immune response cannot be evaluated. Similarly, CRP is a rapid

reaction APP and serum levels have previously been observed to increase in response to inflammation (Gabay and Kushner, 1999; Petersen et al., 2004). The acute-phase response to inflammation can include fever and hormonal changes (Petersen et al., 2004).

Specifically, the production of pro-inflammatory cytokines that LBP binds to can stimulate the production of APP and activate the hypothalamic–pituitary–adrenal (HPA) axis (Steel and Whitehead, 1994; Ulevitch and Tobias, 1995; Nordgreen et al., 2018) and steroid hormone levels have been observed to be altered in response to inflammation (García-Gómez et al., 2013). In sows assigned PS3, the phenotypic observations are consistent with presumed local inflammation; however, it is not known if the alteration in steroid hormones is biologically related to PS. An increase in both LBP and steroid hormones was observed in the PS3 sows compared to PS1. These data support that there is a potential connection between the increased LPB levels in PS3 sows, increased circulating hormones, and the inflammatory response observed in PS3 sows.

Immune responses, as well as changes in the microbiome, have been associated with fluctuations in steroid hormones (García-Gómez et al., 2013). Interestingly, distinct differences in the vaginal microbiome are present in sows at high risk for POP (Kiefer et al., 2021) and increased steroid hormones in PS3 sows were observed in this study. The communication between sex steroids and the host microbiome has the potential to determine the outcome of an infection (Hughes and Sperandio, 2008) and P4, E2, and testosterone can alter the immune response against bacterial infection (Vegeto et al., 1999; García-Gómez et al., 2013). In general, estrogens upregulate proinflammatory cytokines while testosterone has been observed to decrease this response (Ahmed et al., 1985), although the mechanism through which this is accomplished is not established. Another potential beneficial role of E2 is that it has been demonstrated to protect immune cells against apoptosis during in vitro culture, specifically TNF-α induced apoptosis (Sorachi et al., 1993; Vegeto et al., 1999). Modulation of the immune system by steroid hormones may control pro- and anti-inflammatory cytokine expression and regulate the activity of immune cells, specifically lymphocytes (García-Gómez et al., 2013), which were increased in high risk for POP sows. In addition, E2 promotes a pro-inflammatory response and is increased in humans with endometriosis, a disease characterized by excessive inflammation and increased CYP19A1 activity (García-Gómez et al., 2019) and E2 was increased in PS3 compared to PS1 sows. The production of androsterone, estrone, and E2 is all catalyzed by CYP19A1, and were all increased in PS3 compared to PS1 sows, which could indicate that CYP19A1 protein level or function could be associated with POP risk. E2 levels have been observed to increase in humans who have experienced POP (Bai et al., 2002). Relaxin is another hormone of interest in relationship to POP because of its role during parturition (Anderson et al., 1982). Unfortunately, due to the timing of POP in sows, some sows never achieve farrowing making relaxin difficult to evaluate in the current study design.

Another area of investigation involves how the vaginal microbiota may influence POP in sows. Differences in the vaginal microflora relate to alterations in steroid hormones and/or immune system modulation through interactions with cells mediating immunological responses. This remains an important area for discovery as differences in vaginal microbiota have been discovered in sows with variable risk for POP (Kiefer et al., 2021). This is of significance, as one function of the unique mucosal immune system within the female reproductive tract is to identify, and subsequently tolerate or eliminate pathological

microbes (Franklin and Kutteh, 1999). Furthermore, fluctuations of immune responses and inflammation in the reproductive tract can occur through differing mechanisms and are regulated by the sex steroid hormones E2 and P4 (Vegeto et al., 1999; Rakasz and Lynch, 2002; García-Gómez et al., 2013; García-Gómez et al., 2019). In addition to immune cell migration to the reproductive tract, there is a transudation of immunoglobulins from the blood through the extracellular matrix (García-Gómez et al., 2019). Further investigation would be needed to evaluate these potential connections to POP in sows.

Conclusion

This study further validated a phenotypic PS system to effectively categorize sows by putative risk for POP. Additionally, serum biomarkers associated with inflammation and/or immune modulation, in addition to differences in steroid hormone profiles, exist between sows with different relative risks of POP. Collectively, discovery of these differences between sows with different risk levels for POP during late gestation is a critical step in developing the biological understanding of POP in the U.S. sow herd. Furthermore, the measures in this study may prove to be useful indicators as POP mitigation strategies are developed and evaluated.

Conflict of interest statement

Any opinion, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the funding agency. No conflicts of interest, financial, or otherwise are declared by the authors.

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