

# Serum concentrations of immunoglobulins and cortisol around parturition in clinically healthy sows and sows with postpartum dysgalactia syndrome (PDS)

Ewelina Czyżewska-Dors<sup>1</sup>, Karol Wierzchosławski<sup>2</sup>, Małgorzata Pomorska-Mól<sup>3</sup>✉

<sup>1</sup>Department of Swine Diseases, National Veterinary Research Institute, 24-100 Puławy, Poland

<sup>2</sup>Agrobiovet, 62-200 Gniezno, Poland

<sup>3</sup>Department of Preclinical Sciences and Infectious Diseases, Faculty of Veterinary Medicine and Animal Science, University of Life Sciences, 60-637 Poznań, Poland  
mpomorska@up.poznan.pl

Received: March 4, 2022

Accepted: June 20, 2022

## Abstract

**Introduction:** This study aimed to determine the profile of immunoglobulins and cortisol concentrations in serum around the periparturient period in sows suffering from postpartum dysgalactia syndrome (PDS) and in healthy sows. **Material and Methods:** A total of 45 sows with lactation impairment (Group PDS) and 58 clinically healthy sows with a physiological peripartum period (Group H) were subjected to a serological test (ELISA) for measurement of serum immunoglobulins (IgG, IgM, and IgA) and cortisol concentration. **Results:** The serum contents of IgG, IgM and IgA had highly similar profiles in PDS-affected sows and healthy ones. A significantly higher concentration of IgG at 28 and 14 days ante partum compared to days 3 and 7 post partum was only observed in Group H. The mean cortisol content remained at a highly similar level throughout the entire experiment in both groups. **Conclusion:** The results of the study indicate that lactation impairment such as PDS did not influence the immunoglobulin or cortisol concentration in sow serum.

**Keywords:** sows, postpartum dysgalactia syndrome, immunoglobulins, cortisol.

## Introduction

Postpartum dysgalactia syndrome (PDS) is a frequently observed health disorder of sows in modern pig farming (13, 18). The occurrence of PDS in sows can cause large financial losses and affect sow welfare (21). Although PDS directly affects the sows, it has a substantial influence on neonates' vitality and health, which is linked with milk production disturbances. The aetiology of the syndrome is complex and not fully elucidated. The literature identified various risk factors for PDS occurrence, including bacteria of the genera *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella*, belonging to the class of coliforms component and lipopolysaccharides (LPS) endotoxins as a main cause of the syndrome (13). In addition, activation of stress response systems, oxidative stress, metabolic disturbances connected to low energy intake, and inflammation have been suggested to be involved in PDS pathogenesis (9, 18). The primary clinical sign of PDS is lactation impairment with or without evident mastitis during the

first days after farrowing (12, 13). In neonate piglets, insufficient colostrum and milk intake lead to growth retardation, decreased performance and high mortality before weaning (3). Besides colostrum's nutritional and energy providing function, it is the only source of immunoglobulins (Ig) and other factors (lymphocytes, cytokines, and various growth factors) which modulate the development of the postnatal immune system. This is because piglets are born agammaglobulinaemic and immunologically undeveloped because of the epitheliochorial nature of the swine placenta (17, 24, 26). Therefore newborn piglets' survival and health are strictly dependent on the consumption of colostrum and milk. Passive transfer of maternal immunity to newborn piglets delivers protection against infectious agents before the maturation of their own immunity (17, 23). It was previously reported that at least 200 g of colostrum per piglet during the first 24 h of life could significantly decrease the rate of death before weaning (5). Furthermore, porcine milk boosted piglet performance and visceral organ and skeletal muscle protein synthesis in newborns (2).

The sow's serum is a source of the Ig present in the colostrum (24, 29) and colostrigenesis in the mammary glands begins before parturition at about 90 days of gestation; intending to find inferences from this, the study sought to determine the pattern of variation of serum Ig concentrations around the periparturient period in clinically healthy sows and sows suffering from PDS.

Parturition is linked to an inflammatory response as a normal physiological occurrence in sows and other females of domestic species such as mares and cows, and also in human females (6, 20, 22, 25, 28). However, the literature shows that PDS is associated with significant inflammatory overload compared to healthy sows (11, 22, 25, 28). The second objective of our study was to identify whether serum cortisol levels reflected the degree of stress and inflammation as well as sows' welfare and whether differences between healthy and PDS-affected females occurred.

## Material and Methods

**Animals.** This study was carried out in two Polish commercial herds: X and Y, made up of 990 sows and 1,800 sows, respectively. The criteria necessary to qualify the herd for the study included: a full production cycle, proper documentation of production, professional veterinary supervision, and evident lactation problems (together with the clinical manifestation of PDS).

The clinical condition of the sows during the whole peripartum period was monitored by professional veterinary staff. An examination card was created for each sow for recording numerous clinical and production parameters as given in Table 1.

Based on the clinical course of the peripartum period, the sows from both herds were divided into two groups: H ( $n = 58$ ), a group of clinically healthy sows with a physiological peripartum period and PDS ( $n = 45$ ) a group of sows with milk production disorders (hypogalactia) and mastitis. Both groups H and PDS comprised only multiparous sows (from second to sixth parity). For group H and group PDS, gestation length ranged from 112 to 119 days and from 113 to 120 days, respectively. In healthy sows, farrowing lasted from 2 to 5.5 h, whereas in PDS-affected sows, farrowing lasted from 2.9 to 10.5 h. The number of piglets born per litter of sows from group H and group PDS was between 9 and 24 piglets and 9 and 25 piglets, respectively. Sows with complicated parturitions requiring farrowing assistance, inflammations not connected with mammary glands (abscesses or hoof infections), or lameness were excluded from the study.

**Herd X:** The basic herd consisted of 990 Danish sows with DanAvl genetics. The F1 gilts (Landrace × Large White) were all purchased from a nucleus herd in Denmark. Groups of 45 sows were formed weekly. Transfer of the sows to the farrowing facilities occurred 8–10 days before the expected parturition. Piglets were weaned at 26 days of life. The health status of the herd was porcine respiratory and reproductive syndrome virus (PRRSV)-positive and stable; the herd was also positive for *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae* serotype 2, and dermonecrotic *Pasteurella multocida*. The animals were vaccinated against PRRS, porcine parvoviral infection, erysipelas, atrophic rhinitis and colibacillosis of newborn piglets.

**Table 1.** The clinical examination card of experimental sows

Sow no....	Genetics.....		Parity.....		Date of inclusion in examination.....					Treatment:
	Date	Date	Date	Date	Date	Date	Date	Date	Date	
Rectal temperature (°C)	-14	-7	-3	-1	0	+1	+3	+7	+14	
Duration of pregnancy	Duration of parturition									
Number of piglets in a litter	Number of live piglets/litter									
Number of stillbirths/litter	Number of mummified piglets/litter					Number of weaned piglets				
Appetite *										
Preserved	0									
Decreased	1									
(I, II, III)										
Milk production	PARTURITION					Date	Date	Date	Date	Date
Normal						N				
Hypogalactia						H				
Agalactia	A					+1	+3	+5	+7	+14
PDS**						Date	Date	Date	Date	Date
Yes	- 1					PDS severity***				
No	- 0					(I, II, III)				
						+1	+3	+5	+7	+14
Other disorders										
(date, type)										

\* Appetite scores were =0 – preserved (eats more than 80% of daily portion); I – decreased: (eats 50–80% of daily portion); II – decreased (eats less than 50% of daily portion); III – lack of appetite (does not eat)

\*\* If at least 1 teat was inflamed, PDS was defined as 1, and cases with no lesions were defined as 0

\*\*\* PDS severity: I – mild (1–2 teats); II – moderate (3–6 teats); III – severe ( $\geq 7$  teats)

**Herd Y:** The basic herd consisted of 1,800 sows in a confined cycle with Dutch Hypor genetics. The farm had its own nucleus herd of 150 Large White sows; the rest of the reproductive animals consisted of F1 line animals (Large White x Landrace). Groups of 85 sows were formed weekly. The sows were moved to the farrowing pen 8–10 days before the expected parturition. The average lactation period was 27 days (21–30 days). The health status of the herd was PRRSV-positive and stable, the herd was also positive for *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae* serotypes 2 and 9 and dermonecrotic *Pasteurella multocida*. The animals were vaccinated against PRRS (on day 60 of pregnancy), porcine parvoviral infection, erysipelas (2 weeks after parturition), atrophic rhinitis and neonatal colibacillosis (1 month before parturition).

**Sampling.** Blood samples were collected *via vena cava jugularis* venepuncture to serum separator test tubes (Medlab Products, Raszyn, Poland). In the laboratory, serum was obtained by centrifugation ( $3,000 \times g$  for 15 min at  $4^{\circ}\text{C}$ ) and stored at  $-70^{\circ}\text{C}$  until further analysis. Because a month before parturition determination of the exact date of delivery was impossible, blood for laboratory tests was collected from each sow 12 to 16 times, and that practice determination possible of the markers tested at fixed intervals from 4 weeks before up to 4 weeks after parturition. Eleven samples from each of 103 females were selected for appropriate analyses on the following days: -28 (-30 to -25), -14 (-16 to -11), -7 (-8 to -6), -5, -3, -1, 0 (day of delivery), +1, +3, +5, +7, +14 and +28 days before or after delivery.

**Immunoglobulin concentration.** Concentrations of immunoglobulin A (IgA), immunoglobulin M (IgM), and immunoglobulin G (IgG) in serum were measured with a commercial ELISA kit (ELISA Quantitation Kit, Bethyl Laboratories Inc., Montgomery, TX, USA) according to the manufacturer's instructions. The absorbance was measured at 450 nm using a microplate

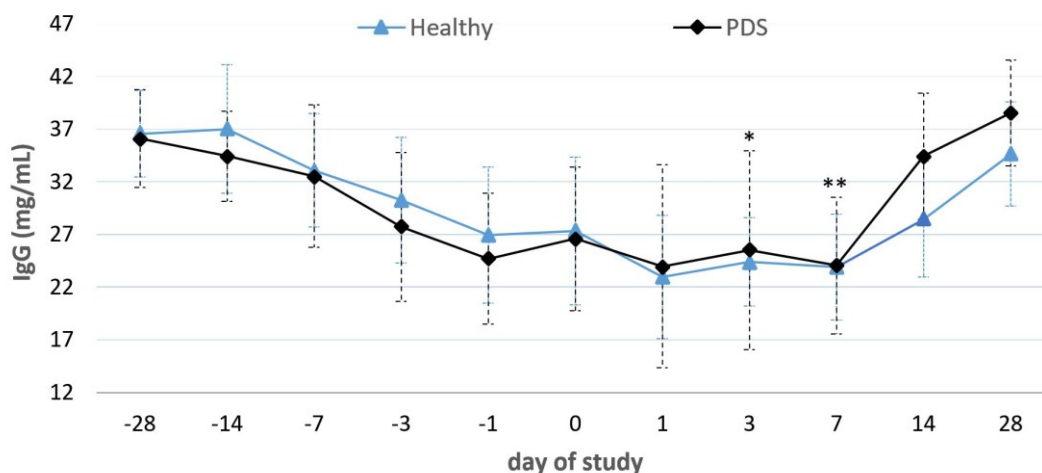
reader (Multiscan RC, Labsystems Diagnostics, Vantaa, Finland). The concentrations of the examined immunoglobulins were recorded on a calibration curve generated using the FindGraph computer programme (Uniphiz Laboratory Software, Tver, Russia).

**Cortisol concentration.** The serum concentration of cortisol was determined by a commercial ELISA kit (Pig Cortisol ELISA Kit, MyBioSource, San Diego, CA, USA) in accordance with the recommendations given by the manufacturer. The absorbance was measured at 450 nm using the same microplate reader as used to evaluate the immunoglobulin concentration. The quantity of studied hormone was calculated based on a standard curve using the FindGraph computer programme once again.

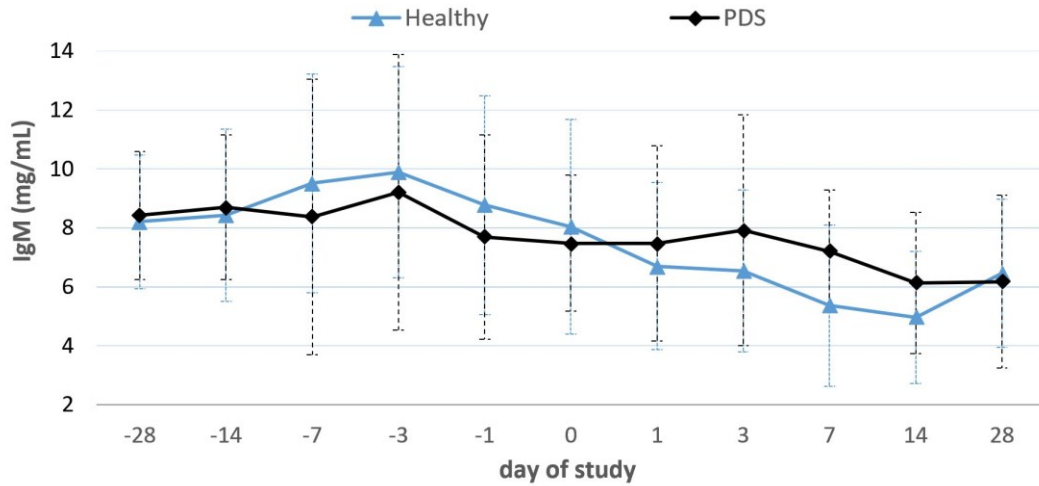
**Statistical analysis.** To evaluate and compare the course of the immunoglobulin and cortisol concentration variability in the serum of the sows from various groups, STATISTICA 13.0 software (StatSoft, Tulsa, OK, USA) was used. The following tests were carried out: the Shapiro–Wilk test for distribution, Levene's test for assessment of homogeneity of variances, the Friedman test for analysis of repeated measures for comparison of immunoglobulins and cortisol concentrations at various time points in the same group of animals, and the Mann-Whitney U test for comparison of the results from both groups.

## Results

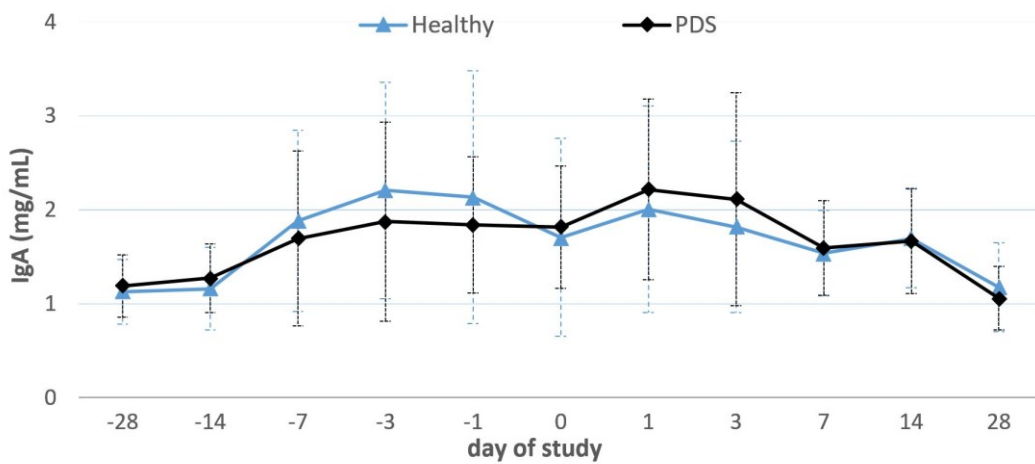
The patterns of variation of the mean concentrations of IgG, IgM and IgA from 28 days before parturition to 28 days postpartum in the healthy and PDS groups are presented in Figs 1–3. In healthy sows, mean serum concentrations of IgG 28 and 14 days before parturition were above 36 mg/mL and then gradually decreased until the first day postpartum (23 mg/mL) and remained relatively stable until 7 days after parturition.



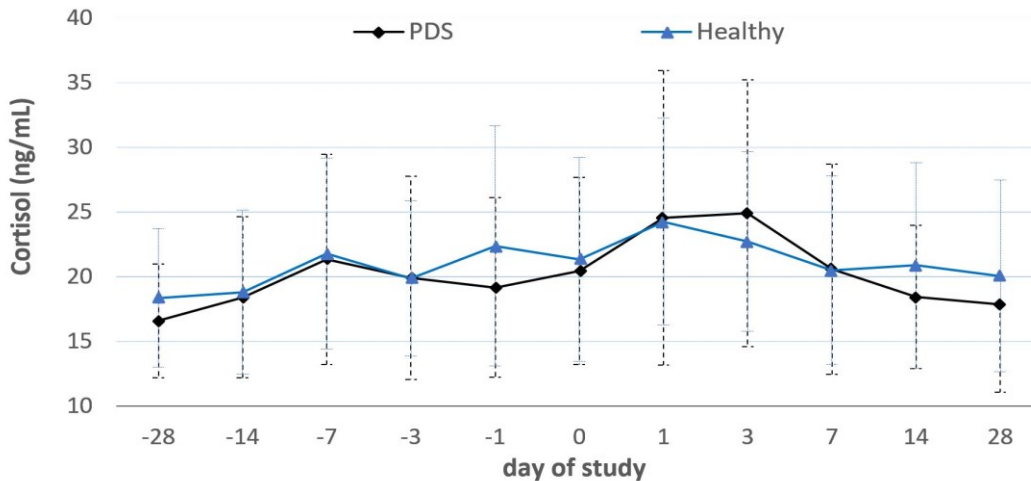
**Fig. 1.** Changes in serum immunoglobulin G (IgG) concentrations (mean  $\pm$  SD) around the peripartum period in sows from individual experimental groups. PDS – postpartum dysgalactia syndrome; \* – statistically significant differences compared to day -28 and day -14 in the healthy group; ( $P < 0.05$ ); \*\* – statistically significant differences compared to day 28 and day 14 in the healthy group; ( $P < 0.05$ )



**Fig. 2.** Changes in serum immunoglobulin M (IgM) concentrations (mean ± SD) around the peripartum period in sows from individual experimental groups. PDS – postpartum dysgalactia syndrome



**Fig. 3.** Changes in serum immunoglobulin A (IgA) concentrations (mean ± SD) around the peripartum period in sows from individual experimental groups. PDS – postpartum dysgalactia syndrome



**Fig. 4.** Changes in serum cortisol concentrations (mean ± SD) around the peripartum in sows from individual experimental groups. PDS – postpartum dysgalactia syndrome

Starting from day 14 of lactation to the end of the study, the content of IgG increased almost 1.5-fold (from 24 mg/mL to 34.6 mg/mL). Moreover, on days 3 and 7 after parturition, the amount of IgG was significantly lower than on days 28 and 14 before

parturition ( $P < 0.05$ ). In the PDS group, the pattern of IgG concentrations was highly similar to those observed in healthy sows. However, no statistically significant difference was observed at any time point ( $P > 0.05$ ).

Regarding IgM, its serum concentrations were relatively stable throughout the entire experiment and did not present any statistically significant variation over time nor between group H and group PDS ( $P > 0.05$ ). Similarly to IgM, the IgA content did not reveal any statistically significant fluctuation at any time point either in analysed groups or between the groups ( $P > 0.05$ ).

Regarding cortisol, its mean content remained at a highly similar level throughout the entire experiment ( $P > 0.05$ ), with a large inter-sow variation in both healthy and PDS-affected sows (Fig. 4). The mean cortisol level ranged between 16.6 ng/mL and 24.9 ng/mL in group PDS and between 18.3 ng/mL to 24.2 ng/mL in group H. Moreover, farrowing itself also did not induce significant changes either in the mean or individual cortisol concentrations.

## Discussion

The present study was undertaken to analyse the pattern of changes in serum IgG, IgM and IgA concentrations in clinically healthy sows and sows with lactation impairment. To the authors' knowledge, this is the first report of parturition-related variations of immunoglobulin in the serum of sows with postpartum dysgalactia syndrome.

Immunoglobulins and other immunomodulatory factors are integral parts of the colostrum and the most important constituents conferring immune competence to newborns (24). It is worth highlighting that colostrum immunoglobulins are non-selectively transported from sow serum into the mammary gland (1). In sows, all colostrum IgG and almost 80% and 40% of colostrum IgM and IgA, respectively, are derived from serum. Therefore, an adequate content of Ig, mostly IgG, in sow serum is of great importance to newborns. Several previous studies identified multiple factors which may affect Ig content in colostrum, *e.g.* parity, season, genotype, and the part of the udder (7, 8, 14). Nonspecific immunostimulators (*e.g.* Isoprinosine) and vaccination administered to pregnant sows at the beginning of colostrogenesis increase the IgG concentration in the colostrum (8, 15). Prenatal maternal stress during late gestation, *per contra*, negatively affects IgG concentrations (27).

In the current study, the IgA and IgM content in the serum showed highly similar profiles in PDS-affected sows and healthy ones. The evaluated groups also presented comparable IgG concentrations. However, in group H, a significantly higher concentration of IgG was observed on 28 and 14 days ante partum than on days 3 and 7 post partum. These observations are partially in line with the results of Devillers *et al.* (4), in which the IgG concentration in the serum of healthy sows gradually decreases around parturition. In addition, Markowska-Daniel *et al.* (17) documented IgG decline over the antepartum period in clinically healthy sows. The decrease in serum IgG concentrations observed in clinically healthy sows and PDS-affected sows around

parturition was connected with its substantial transfer from serum to colostrum. The confirmation of this phenomenon was the detection of very high concentrations of IgG in the colostrum just before parturition (4). The lack of statistically significant differences in IgG content in PDS-affected sows during the follow-up period may result from some more pronounced inter-sow variability observed in this group. It is worth noting that inter-sow variability is one of the most frequently described factors affecting colostrum immunoglobulin concentrations (16, 17).

Cortisol has been the object of previous studies concerning its utility as a marker of various stress conditions and inflammation, also those related to PDS (10). In swine, cortisol is acknowledged as the primary glucocorticoid hormone; its increase is related to hypothalamic–pituitary–adrenal axis activation by stress stimuli such as inflammatory processes (19). In the present study, serum cortisol concentration showed similar kinetics in sows from both analysed groups during the entire follow-up period. In contrast, a previous study on sows (clinically healthy and PDS-affected) revealed significantly higher cortisol concentration in the serum and saliva of PDS-affected sows than in healthy ones (10). The discrepancies in these findings may result from the different methods used in the previous study to evaluate cortisol concentration in the serum. Moreover, the follow-up period and time point of each sampling in the present study were not fully consistent with those of the experimental design of Kaiser *et al.* (10), which may also have contributed to the different outcomes. However, as shown by Devillers *et al.* (4), the cortisol content in the serum of clinically healthy pregnant sows increased slowly from 120 h to 2 h antepartum and robust thereafter, reaching peak values during parturition. Even though in the current study the cortisol amount was measured at 28, 14, 7, 3 and 1 days antepartum, parturition onset (day 0) and then 3, 7, 14 and 28 days postpartum, we were not able to detect a significant peak close to parturition. It bears mentioning that there was a significant individual variation in cortisol content between sows at each time point of sampling, even within the same study group. This finding indicated the existence of individual factors which may affect the cortisol level and may consequently reducing cortisol's utility as a good marker of stress and welfare in sows.

In summary, the present results demonstrated that lactation impairment in PDS did not influence immunoglobulin or cortisol concentrations in sow serum. Future studies are desirable to evaluate immunoglobulin content in colostrum and serum of piglets born to sows suffering from PDS.

**Conflict of Interest Statement:** The authors declare that there is no conflict of interests regarding the publication of this article.

**Financial Disclosure Statement:** This work was supported by the National Science Centre (DEC-

2020/37/B/NZ/00021) and by the Polish Ministry of Science and Higher Education's "Regional Initiative Excellence" Programme 2019–2022, Project No. 005/RID/2018/19.

**Animal Rights Statement:** Animal use and handling protocols were approved by the II Local Ethical Commission for Animal Experiments of the University of Life Sciences in Lublin.

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