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Influence of porcine parity on colostrum cytokine levels and their passive transfer to piglets

A influência da paridade suína na diferença dos níveis de citocinas no colostro e na transferência passiva para leitões

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Highlights

Colostrum and plasma cytokine concentrations are higher in sows.
Concentration of cytokines in colostrum influences sows' offspring.
Piglets nursed by sows have high cytokine levels.
Sow parity can affect colostrum quality.

Abstract

The limited ability of newborn piglets to produce cytokines may influence lymphocyte development and response to antigen exposure. As a result, colostrum intake is crucial because it contains nutrients that contribute to immune system development in piglets. Our goal was to investigate the effect of sow parity on the transfer of maternal cytokines to nursing piglets. Sixty piglets from nine sows were divided into six groups: piglets from gilts or sows kept with their dams and allowed to suckle normally; piglets from gilts or sows having their dams exchanged and then allowed to suckle normally; piglets from gilts or sows isolated from their dams and bottle-fed a commercial milk replacer formula for pigs. All piglets remained in the diet groups for 24 hours after birth. Concentrations of cytokines in colostrum and serum of gilt/sows and serum of piglets were then evaluated. The 13 evaluated cytokines had higher concentrations in colostrum and serum of sows than in gilts. Concentrations of GM-CSF, IFNγ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-18, and TNFα were higher in piglets suckling sows. Piglets that received commercial formula showed higher concentrations of the cytokines IL1-RA and IL-8 than piglets fed colostrum. This outcome can influence piglets' development into adulthood. In short, our findings demonstrated that maternal parity influenced colostrum cytokine composition and its maternal transfer patterns.

Key words: Gilts. Serum. Neonatal immunity. Piglets. Passive immunity.

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Resumo

A capacidade limitada dos leitões recém-nascidos de produzir citocinas pode influenciar o desenvolvimento de linfócitos e a resposta à exposição ao antígeno. Portanto, a ingestão de colostro é importante porque contém nutrientes, que contribuem para o desenvolvimento do sistema imunológico do leitão. O objetivo do estudo foi investigar o efeito da paridade da porca na transferência de citocina materna para leitões lactentes. Sessenta leitões de nove porcas foram divididos em seis grupos: leitões de marrãs/porcas mantidas com suas próprias mães e amamentadas normalmente; leitões de marrãs/porcas que foram trocados de mães e amamentados normalmente; leitões de marrãs/porcas que foram isolados das mães e alimentados com mamadeira com substituto do leite para suínos. Os leitões permaneceram nos grupos por 24 horas após o nascimento. Foram avaliadas as concentrações de citocinas no colostro e plasma das marrãs/porcas e no plasma dos leitões. O colostro e o plasma das porcas apresentaram maiores concentrações das 13 citocinas analisadas do que as marrãs. No mesmo sentido, as concentrações de GM-CSF, IFNγ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-18 e TNFα foram significantemente maiores nos leitões que mamaram o colostro de porcas. Os leitões que receberam fórmula comercial apresentaram, em especial, concentrações das citocinas IL1-RA e IL-8 superiores aos leitões amamentados com colostro. Isso pode influenciar o desenvolvimento até a fase adulta. Portanto, nossos dados demonstraram que a paridade materna influenciou a composição das citocinas do colostro, bem como as características das citocinas na transferência materna.

Palavras-chave: Marrãs. Soro. Imunidade neonatal. Leitões. Imunidade passiva.

Introduction

Newborn piglets are agammaglobulinemic and have immature adaptive immunity (Bandrick et al., 2014). They also lack cell-mediated immunity and effector or memory T lymphocytes at birth; however, they gradually develop a fully functioning immune system by the age of 14 days (Bandrick et al., 2014; Hlavova et al., 2014). Due to the epitheliochorial placenta of sows, maternally derived antibodies and biological components play a crucial role in the survival of their offspring. A newborn piglet will be highly susceptible to disease without passively transferred immunity through colostrum (Bandrick et al., 2014; Le Dividich et al., 2005).

Porcine colostrum contains some biological components essential for stimulating the development of early immune functions in piglets (Donovan et al., 1994). Some of these components are often influenced by dam parity orders (Forner et al., 2021). There is evidence that some cytokines play a role in modulating the development of the newborn immune system. However, the precise nature of these proteins is still debatable. Several authors have investigated the humoral and cellular composition of porcine colostrum, but for a better understanding of its immunology, the expression of cytokines must also be examined (Forner et al., 2021; Nguyen et al., 2007). In this context, cytokines signal intercellular communication and share several functions. As they are present in colostrum, they may affect an array of processes involved in regulating inflammatory responses and the function of the immune system of

piglets, including the regulation of intestinal maturation (Nguyen et al., 2007; Wagstrom et al., 2000).

Considering that maternal porcine colostrum is the gold standard for feeding newborn piglets, the effects of sow parity on colostrum composition are still poorly understood. The goals of this study were to compare the cytokine composition of colostrum and the similarity of the sows, and to investigate the effect of colostrum from gilts, sows, and milk replacers on perinatal characteristics in piglets.

Material and Methods

Animals

This study was performed in compliance with the Animal Use Ethics Committee of Embrapa Swine and Poultry (protocol number 001/2016). The study involved nine crossbred female (Large White × Landrace) dams, which were individually housed on a slatted floor during gestation. Seven days before the expected farrowing, the dams were transferred to the farrowing room. Throughout the study, the dams had ad libitum access to water and were fed twice a day with a traditional gestation diet (3,315 kcal ME/kg, 15.7% crude protein, and 0.9% digestible lysine), totaling 2.4 kg/d. The animals were fed the gestation diet until the second day of lactation.

A total of 35 healthy candidate sows (20 sows and 15 gilts) with similar expected delivery dates were selected. Parturition was induced on day 114 of gestation (after 1st insemination) by intramuscular administration of 10 mg of prostaglandin F2 alpha. Candidate sows and gilts were excluded if the difference in delivery time onset was more than 1 hour. The study included five pregnant multiparous adult sows with synchronized parturition (parity = 4) and four primiparous gilts. All dams were examined to rule out the possibility of puerperal disorders, specifically mastitis-metritis-agalactia syndrome. Some piglets remained with their dams, while others were relocated, resulting in the formation of six groups:

GG: piglets born to a gilt were suckled by a gilt (gilt colostrum, n = 20);

GS: piglets born to a gilt were suckled by a sow (sow colostrum, n = 20);

GMR: piglets from gilt were kept isolated in temperature-controlled containers and bottle-fed a commercial milk replacer formula for pigs (Vetmilk S, Agrifirm-Brasil) every 1 hour during the first 24 hours of life (n = 15).

SS: piglets born to a sow were suckled by a sow (sow colostrum, n = 20);

SG: piglets born to a sow were suckled by a gilt (gilt colostrum, n = 20);

SMR: piglets from the sow were kept isolated in the same condition as GMR (n = 16).

Cross-fostering with colostrum was essential for our research purposes and required only during the first 24 hours of life. The birth weight category was used to cross-foster piglets (10-12 piglets), with all piglets in each litter falling within the same birth weight category of 1.0 to 1.5 kg. After the first round of cross-fostering, during which colostrum and blood samples were collected from both piglets and gilts/sows, this part of the experiment was considered complete, and

the piglets were returned to their respective mothers.

Blood and colostrum samples were collected from the dams on the first day after giving birth, while piglet serum samples were collected on the day after their birth. Colostrum was manually collected immediately after the birth of the first piglet, before suckling, from all functional teats located in the anterior, middle, and posterior parts of the udder. The first few drops of colostrum, approximately 5 mL, were discarded, and 10 mL were collected. Teats were scrubbed with iodine alcohol to minimize contamination (Maciag et al., 2022a).

After collection, colostrum and blood samples were centrifuged for 20 minutes at 1300 x g and room temperature, and the upper-fat layer was discarded. Once treated, serum and colostrum samples were stored at -80°C for further analysis.

Quantification of cytokines in colostrum and serum samples

The levels of various cytokines including Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), Interferon-Gamma (IFNγ), IL-1α, IL-1β, IL-1RA, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, and Tumor Necrosis Factor-α (TNFα) were quantified using the MAGPIX® analyzer (Luminex Corp, Austin, TX, USA). Acquisition and data analysis were performed using the xPONENT version 4.2 (Luminex Corp) and MILLIPLEX® Analyst Version 5.1 (Merck Millipore), and cytokine concentrations were expressed in pg/mL.

Statistical analysis

Group comparisons were performed using the Kruskal-Wallis test, and statistical analyses were conducted using the NPAR1WAY procedure of the Statistical Analysis System [SAS] (2012). The data are expressed as mean ± standard error, and statistical significance was declared at $p \leq 0.05$.

Results and Discussion

Differences in both humoral and cellular immune function were observed in the colostrum of sows and gilts (Forner et al., 2021). Specifically, sow colostrum was found to be positively correlated with higher concentrations of immunoglobulins and lymphocyte cells. While the information on cytokines in swine is still limited, the capacity of farm animals to produce cytokines is believed to play a significant role in determining their overall health and well-being (Groot et al., 2005). The cytokine profile of an animal may also impact its susceptibility to infectious diseases and the effectiveness of vaccine strategies (Carr et al., 2021).

The current study found that sow's colostrum and serum contained significantly higher levels of GM-CSF, IFNγ, IL-1α, IL-1RA, IL-2, IL-4, IL-6, IL-10, IL-12, IL-18, and TNFα compared to gilts (Table 1). Colostrum samples contain high concentrations of various cells capable of producing cytokines, which explains the detection of those cytokines in the samples. In humans, colostrum cells also produce cytokines in the infant's gut (Carr et al., 2021).

Table 1
Levels of plasma and colostrum cytokines (ng/mL) in gilts and sow groups

Cytokine	Plasma		Pr>χ^2	Colostrum		Pr>χ^2
	Gilt	Sow		Gilt	Sow	
GM-CSF	0.090±0.024	2.783±1.882	<.0001	0.005±0.004	0.167±0.064	0.0044
IFN-y	3.400±0.735	244.73±54.1	<.0001	0.472±0.244	53.29±15.1	<.0001
IL-1α	0.161±0.047	0.746±0.104	<.0001	0.039±0.010	0.551±0.130	<.0001
IL1β	0.145±0.056	2.126±0.322	<.0001	0.053±0.017	1.309±0.407	<.0001
IL-1RA	1.088±0.230	7.396±1.158	<.0001	0.574±0.149	4.564±1.297	<.0001
IL-2	0.775±0.188	11.60±2.09	<.0001	0.477±0.144	7.017±4.207	<.0001
IL-4	0.983±0.360	147.72±19.5	<.0001	4.043±1.820	85.25±18.1	<.0001
IL-6	0.286±0.120	4.061±0.753	<.0001	0.094±0.040	7.066±3.975	<.0001
IL-8	0.140±0.029	0.397±0.085	0.0080	8.696±1.886	24.70±5.94	0.0407
IL-10	1.259±0.307	36.69±4.34	<.0001	0.492±0.164	18.09±4.20	<.0001
IL-12	0.767±0.154	3.525±0.418	<.0001	0.303±0.077	2.581±0.706	<.0001
IL-18	1.807±0.368	41.08±6.10	<.0001	0.641±0.255	21.09±5.88	<.0001
TNF-α	0.150±0.034	3.906±1.146	<.0001	0.065±0.018	0.639±0.211	.0001

The analysis was performed with the Kruskal-Wallis tests. Data are shown as Mean ± SEM.

For neonatal piglets, colostrum serves as the primary source of cytokines and chemokines. The concentrations of GM-CSF, IFNγ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-18, and TNFα were the highest in piglets suckling sows (SS and GS), followed by piglets suckling gilts (GG and SG), and the lowest in piglet groups fed milk replacer (Table 2). There was a similar developmental pattern observed for all Th1, Th2, and Th17 cytokine correlates. However, the groups of suckling piglets fed with milk replacer (GMR and SMR) showed stronger production of IL-1RA and IL-8 compared to piglets nursed by gilts or sows (SS, SG, GG, and GS), although the difference was not statistically significant.

In this study, we observed that cytokine concentrations in piglets' serum were detectable 24 h after colostrum feeding and similar to those found in sow and gilt colostrum. These findings suggest that the serum cytokines in neonatal piglets originate from colostrum. As piglets are more susceptible to infection than adult swine due to their immature immune system (Maciag et al., 2022b), the transfer of colostral cytokines to neonates may enhance their immune responses against infections (Nguyen et al., 2007). The transfer of smaller immunomodulatory molecules, such as cytokines, from colostrum occurs before gut closure and enhances general systemic immune responses. This situation could explain the overall increased occurrence of post-weaning diseases in piglets from the first parity (Miller et al., 2013).

Table 2
Levels of plasma cytokines (ng/mL) in piglet groups. Data are shown as Mean ± SEM

Cytokine	Group						
	GG	GS	GMR	SS	SG	SMR	Pr>χ^2
GM-CSF	0.056±0.008^{b}	0.8146±0.166^{a}	0.012±0.006^{b}	0.918±0.345^{a}	0.058±0.011^{b}	0.013±0.010^{b}	0.0004
IFN-y	43.24±8.50^{b}	218.04±56.2^{a}	6.104±1.033^{c}	301.20±30.2^{a}	40.01±8.4^{bc}	11.06±5.79^{bc}	0.0002
IL-1α	0.431±0.058^{b}	1.030±0.0910^{a}	0.020±0.004^{c}	1.310±0.096^{a}	0.590±0.112^{b}	0.030±0.006^{c}	0.0003
IL1β	0.184±0.322^{b}	1.241±0.474^{a}	0.010±0.002^{b}	2.297±0.567^{a}	0.496±0.645^{b}	0.060±0.154^{b}	0.0004
IL-1RA	21.88±5.91	9.69±3.89	34.96±22.7	4.891±0.986	16.99±8.74	30.78±26.9	0.3699
IL-2	1.882±0.656^{b}	4.543±0.789^{a}	0.105±0.034^{c}	7.026±1.701^{a}	1.166±0.447^{b}	0.064±0.102^{c}	0.0002
IL-4	50.14±11.9^{b}	139.39±22.4^{a}	0.086±0.048^{c}	186.60±23.8^{a}	41.92±16.5^{b}	0.134±0.032^{c}	0.0002
IL-6	0.454±0.166^{b}	3.252±0.119^{a}	0.042±0.027^{b}	4.211±1.108^{a}	0.696±0.312^{b}	0.017±0.012^{b}	0.0013
IL-8	0.261±0.050^{c}	0.516±0.089^{abc}	1.006±0.115^{a}	0.642±0.057^{ab}	0.288±0.050^{bc}	0.870±0.155^{abc}	0.0403
IL-10	8,294±3.542^{c}	23.07±6.11^{b}	0.240±0.088^{d}	50.16±1.09^{a}	18.47±2.96^{c}	0.214±0.222^{d}	0.0002
IL-12	1.029±0.410^{b}	2.874±0.063^{a}	0.340±0.036^{b}	4.018±0.712^{a}	1.332±0.484^{b}	0.370±0.068^{b}	0.0014
IL-18	8.120±3.574^{bc}	28.98±2.00^{a}	0.428±0.323^{cd}	38.71±9.25^{a}	10.158±4.550^{b}	0.522±0.336^{d}	0.0003
TNF-α	0.332±0.050^{b}	5.480±0.983^{a}	0.380±0.071^{b}	7.044±1.220^{a}	0.430±0.091^{b}	0.330±0.096^{b}	0.0004

The analysis was performed with the Kruskal-Wallis tests. a, b, c, d Different superscript letters indicate the significant statistical difference between ($p \leq 0.05$).

It has been suggested that management of cross-fostering procedures may have a negative effect on piglets whose siblings are fed milk replacer, as evidenced by higher concentrations of IL-1RA. The mean concentrations of IL-1RA were found to be 30-34 times higher than those of IL-1β. The balance between IL-1 and IL-1RA is thought to play an important role in the normal physiology of various organs and tissues (Arend, 2002). First infant feeding has a significant impact on a newborn's development, which can influence their development into adulthood. That is because excessive concentrations of these cytokines in colostrum could lead to adverse side effects in the neonate. Based on the results obtained and what has been demonstrated in the literature, IL-1RA (Wu et al., 2019) and IL-8 (Sharabiani et al., 2011) are linked to bone fragility and obesity, respectively. Therefore, there is a possibility that piglets fed commercial formula could develop some degree of bone fragility and increase their fat mass.

In contrast to the findings of Prendergast et al. (2012), we found that the levels of T helper 1 (TH1) cytokines, such as interferon-γ and IL-12, were identical to those of TH17 cytokines (IL-6). This difference may be attributed to the type of placenta since alloantigens in maternal cells can cross the placenta in humans, but the fetus is protected from antigenic stimuli by the diffuse epitheliochorial function of the pig's placenta. It should be noted that further investigation is required to confirm these findings. A previous study reported that the production of neonatal swine cytokines is significantly influenced by litter, age, and gender effects, with concentrations increasing as the animals age (Groot et al., 2005).

Our study found that the cytokine profile induced during the fetal phase did not interfere with the profile observed in the first 24 hours of piglets' lives. We discovered that colostrum, rather than milk replacer, and porcine parity were both important factors in determining the cytokine profiles of siblings. However, the mechanisms underlying the distinct functional responses of fetal cytokines remain unclear and require further investigation.

Conclusion

Our study examined a profile of 13 cytokines in porcine colostrum and investigated whether their transfer was primarily influenced by pregnancy or lactation. In contrast to previous studies, our findings suggest that both gestating and lactating sow parity affect cytokine concentrations in colostrum and their transfer to suckling piglets.

Interestingly, our study provides new data indicating that cytokine concentrations in piglets are primarily influenced by colostrum intake parity rather than gestating sow parity. The concentration of the main cytokines was found to be higher in piglet groups that suckled sow colostrum than in their siblings from other groups. Furthermore, to our knowledge, there have been no reports of maternal cytokines persisting in piglets (Nguyen et al., 2007).

Further research studies are needed to gain a comprehensive understanding of the function of immunomodulatory molecules present in porcine colostrum in terms of their impact on both intestinal and systemic immune responses, as well as their effect on infection among pre-weaned piglets.

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Declaration of Conflict of Interest

The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

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