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IMMUNE STATUS OF PIGLETS DURING THE FIRST WEEK OF LIFE: CURRENT KNOWLEDGE, SIGNIFICANCE AND ASSESSMENT – A REVIEW*

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Abstract

The immune system of neonate piglets differs from adult pigs in structure and competence. Although piglets are born immunocompetent, they are genuinely immunologically defenseless. To survive in the environment, piglets need passive protection provided by sow's colostrum and milk when constantly exposed to numerous pathogens. Early assessment of piglets' immune status may enable rapid intervention in case of detection of any deficiencies or disorders. Moreover, awareness of the piglets' immunocompetence and the level of maternally-derived antibodies (MDA) may allow the creation of a proper vaccine schedule. Hence, extending knowledge of prenatal ontogeny of the porcine immune system, the immune status of neonate piglets' and the immunological components of porcine colostrum is crucial. Since animal welfare has become a more critical element of animal production, new, non-invasive sampling methodologies are highly desirable for the evaluation of piglets' immune status.

Key words: neonate, piglets, immune status

The piglet's immune system begins to develop in the early stages of prenatal ontogeny, during the embryonic period of embryogenesis (Šinkora and Butler, 2009). However, neonate piglets' immune systems differ from those of adult pigs in both morphology and function. For example, the mesenteric lymph nodes and Peyer's patches are smaller in piglets and have fewer Ig-secreting cells than do those of adult pigs (Levast et al., 2014). Moreover, the jejunal lymph nodes of newborn piglets consist of a chain of single lymph nodes parallel to the small intestine on both sides of the mesentery; in contrast, in two-week-old piglets the jejunal lymph nodes merge into an almost confluent band of lymphatic tissue (Schnapper et al., 2003). The cellular components of the piglet's immune system are not fully operational by the end of gestation (Stepanova et al., 2007; Pomorska-Mól and Markowska-Daniel, 2011). They have limited external antigen stimulation during the fetal period, which results in a minimal number of peripheral double-positive αβ T cells (effector or memory T cells). Moreover, the lymphocyte B population consists of immature cells (Šinkora and Butler, 2009). In addition, an epitheliochorial structure of the porcine placenta effectively precludes the transfer of immunoglobulins or immune cells (Rooke and Bland, 2002). Colostrum and milk thus constitute the only sources of maternal immunity (Rooke and Bland, 2002). It has been confirmed that fetuses can produce their own antibodies against antigens in the final third of gestation (Martinez-Boixaderas et al., 2022); however, their ability to respond to antigen stimuli is feeble (Martínez-Boixaderas et al., 2022). Therefore, although piglets are born immunocompetent, they are immunologically vulnerable and rely on passive immunity early in life (Pomorska-Mól and Markowska-Daniel, 2009). With the beginning of extrauterine life, piglets are exposed to various environmental antigens, unlike during prenatal life. The development of primary immunity is too slow to protect piglets from pathogens, and piglets' adaptive immunity is primitive compared to adults' (Pomorska-Mól and Markowska-Daniel, 2010 a). The acquisition of appropriate amounts of high-quality colostrum is thus significant. Colostrum, defined as the first secretion of a mammary gland, usually released within 24 hours after farrowing, provides newborn piglets with energy and passive immunity (Inoue and Tsukahara, 2021). Its early ingestion by newborn piglets is crucial for their further healthy development and growth (Inoue and Tsukahara, 2021). The immune components of the colostrum include highly concentrated immunoglobulins and many other elements, such as cytokines, cellular components (e.g., lymphocytes, and phagocytes), and various growth factors (Inoue and Tsukahara, 2021). Piglets' immune

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systems are of great interest to researchers, and gaining knowledge of this subject would allow for improvements in the immune status of piglets, as well their future healthy growth and performance; this all brings measurable effects to the pig production sector (Šinkora et al., 1998 a; Pomorska-Mól and Markowska-Daniel, 2010 b). In addition, a piglet's immunological status shortly after birth is an important question for effective vaccination management. Knowledge of the immune status of piglets may also enable rapid intervention when a deficiency or disorder is detected. The ideal situation is when all litters, as well as all piglets in each litter within a herd, have a similar immune status. Such similarities are desirable both in immunocompetence as well as in the level of colostrum (maternal) antibodies against pathogens that are present in the herd.

In this paper, we review the early prenatal ontogeny of the swine immune system, the immunological components of porcine colostrum, and piglets' immunological status during the first week of life.

Prenatal ontogeny of swine immune system

Morphogenesis, hematopoiesis, and lymphopoiesis

Porcine gestation lasts about 114–116 days (Belt et al., 1971). By the end of porcine organogenesis, on day 35 of gestation, the lymphatic system has already been physiologically formed; nevertheless, when the bone marrow starts its hematopoietic activity, lymphoid components and lymphocytes are rare in the various organs (Šinkora and Butler, 2009). Due to the expansion of peripheral lymphatic organs between days 60 and 90 of gestation, the lymph nodes, including mesenteric lymph nodes, are negligible up to day 70 of gestation. Porcine lymphopoiesis is known as lymphoid hematopoiesis, as the B cells develop in the primary hematopoietic organs and the pro-T cell progenitors that settle the thymus are derived from the same hematopoietic centers; the lymphopoiesis of lymphocytes B and T is thus closely associated (Šinkora and Butler, 2009). The yolk sac is the first lymphopoietic organ. Hematopoiesis can be detected here as soon as the day 16 of gestation (Trebichavský et al., 1996; Šinkora et al., 2002). Nevertheless, its contribution to overall lymphocyte production is rather small, since the yolk sac involutes early, around day 24 or 27 of gestation. Subsequently, first the liver, and later (around day 45 of gestation) the bone marrow, are responsible for lymphopoietic activity and for the development of the preimmune B cell receptor repertoire (Šinkora and Butler, 2009).

Development of individual adaptive immunity components

Šinkora et al. (1998 a) investigated the surface phenotype of fetal lymphoid cells in the thymus, cord blood, spleen and mesenteric lymph nodes at different stages of gestation. This study indicates that the second trimester of gestation is the period when the major subpopulations of lymphocytes begin to appear (Table 1). The first leucocytes with CD45 phenotype were observed in their research on day 35 of gestation in the fetal spleen and cord blood, and at day 40 of gestation in the mesenterium. However, there was an absence of cells with typical lymphocyte markers ($CD3^+$). Among the lymphocyte population, B lymphocytes appeared at the earliest in the periphery. Their presence was detected at day 40 of gestation and they remained the predominant lymphocyte population in lymph nodes, spleen, and blood up to day 55 of gestation. The authors considered that these findings, in combination with those of the study of Cukrowska et al. (1996), indicate that $sIgM^+$ B cells may be one of the first functional lymphocyte subpopulations in porcine fetuses (Šinkora et al., 1998 a). Cukrowska et al. (1996) observed the presence of immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) in fetus plasma and noted the ability of surface immunoglobulin M^+ ($sIgM^+$) $CD5^-$ B cells isolated from the fetal liver to secrete IgM. Every fetal $sIgM^+$ B lymphocyte was similar to an adult $sIgM^+$ B cell on account of CD45RC and SLA-DR positivity. Furthermore, over 90% of $sIgM^+$ cells were $CD2^+$, and this marker was expressed as the B cells developing before sIgM, which is normally considered a B-cell precursor (Šinkora et al., 1998 a). The development of thymic tissues was observed as early as the day 21 of porcine gestation (Šinkora and Butler, 2009). The thymus is a specialized lymphatic organ in which T lymphocytes mature. Around day 40 of gestation, the first T cells can be detected in this organ (Šinkora and Butler, 2009). Subsequently, T cells have been observed on day 45 of gestation in the spleen and umbilical blood and, on day 50 in the mesenterium. In all cases, their appearance was delayed relative to that of the first $sIgM^+$ lymphocytes (Šinkora et al., 1998 a). Among the T lymphocytes, γδ T cells appeared first, developing primarily in the thymus; five days later they were also detectable in the periphery. The occurrence of γδ T cells remains relatively constant up to day 90 of gestation; however, around farrowing, a sharp growth of γδ T cells in the blood and spleen was noted (Šinkora et al., 1998 a). After that time, the blood and spleen selectively accumulated a great percentage of $CD2^+CD8^-$ γδ T cells and $CD2^-CD8^-$ γδ T cells, respectively (Šinkora et al., 1998 a). The count of T-cell receptor $\alpha\beta^+$ ($TCR\alpha\beta^+$) lymphocytes began to increase around day 55 of gestation, and soon after they outnumbered the T cells subset in the thymus and periphery (Šinkora et al., 1998 a). At about day 90 of gestation, the concentration of αβ T cells plateaued (Šinkora et al., 1998 a). A growth in the number of B cells was also observed; hence $sIgM^+$ and TCRαβ T lymphocytes are prominent cells in the fetal periphery at this time (Šinkora et al., 1998 a). During late gestation, the relative proportion of αβ T cells and B cells varies between individual organs. However, they are similar to those observed in neonate piglets (Šinkora et al., 1998 a). $CD4^+CD8^-$ αβ T helper cells were the first prominent subset of αβ T lymphocytes. Moreover, they also made up the greatest αβ T cell subpopulation

within gestation (Šinkora et al., 1998 a). $CD4^{-}CD8^{hi}\alpha\beta$ cytotoxic T cells were absent up to day 55 of gestation; subsequently, their number increased until birth (Šinkora et al., 1998 a). In the study of Cukrowska et al. (1996), serum immunoglobulins of all isotypes were detected in fetuses at day 44 of gestation. The predominant immunoglobulin was IgM, and the overall immunoglobulin concentration was increased during ontogeny, independently of external stimuli. Moreover, antibody activity against autoantigens, phylogenetically conserved proteins, haptens, and bacterial components was found in the sera of fetuses at the end of embryonic life. Preimmune immunoglobulins can display a large spectrum of physiological functions, and many remain unknown. With other natural factors, they constitute the first line of defense during gestation and the early extrauterine stage of life (Cukrowska et al., 1996).

Development of individual innate immunity components

Data on the development and maturation of porcine innate immunity components is limited. Along with the beginning of hematopoietic activity of the primary lymphoid organs, cellular elements of the innate immunological system such as morphonuclear leukocytes, macrophages, and dendritic cells begin to appear (Table 1) (Šinkora and Butler, 2009). For example, macrophages were detected as soon as day 25 of gestation (Trebichavský et al., 1996). Cells with the natural killer (NK) phenotype also appeared early in the ontogeny, and $CD3\varepsilon\ CD8^{lo}\ CD2^{+}$ lymphocytes were observed in the umbilical blood and spleen around day 45 of gestation (Šinkora et al., 1998 a). Their abundance varies from 1% to 10%, with an upward tendency over time (Šinkora and Butler, 2009). Subsequently, NK cells were also detected in mesenteric lymph nodes (MLN) at about day 50 of gestation. However, their concentration was low (Šinkora et al., 1998 a). Later in the ontogeny, at approximately day 70 of gestation, the NK cell concentrations stabilize and then remain relatively constant until birth (Šinkora and Butler, 2009). Previous studies have shown that fetal NK cells lack the ability to kill before birth, and killing was delayed in germ-free piglets. This may suggest that colonization with microflora may be crucial to the complete functional development of these cells (Šinkora and Butler, 2009). Interferon-α secreting cells (IFN-α SC) were found in the fetal liver at a very early stage of fetal development, at day 26 of gestation, when differentiated lymphocytes were still not detectable (Splichal et al., 1994). After that, they were also detected in other tissues such as cord blood, spleen, and bone marrow. The authors suggested that IFN-α SC may represent an early antiviral defense mechanism (Splichal et al., 1994). An *in vitro* study performed on pig fetus macrophages and lymphocytes showed high cytoplasmic expression of tumor necrosis factor (TNF-α) when stimulated with bacterial mitogens (Trebichavský et al., 1995). The main producers of TNF-α at a later stage of development were macrophages. According to those authors, their findings may suggest that lymphocytes and macrophages play a pivotal role in the induction of inflammatory responses in fetuses (Trebichavský et al., 1995). Elevated interferon γ (IFN-γ) and TNF-α mRNA levels were observed in the tissues from porcine fetuses infected with porcine reproductive and respiratory syndrome virus (PRRSV), and corresponded to elevated cytokine proteins in serum (Rowland, 2010).

Table 1. The first appearance of the particular immune components in porcine fetuses during prenatal ontogeny

Immune component	First appearance (day of gestation)	Location	References
Macrophages	25	No data	Trebichavský et al., 1996
CD45+leukocytes	35	Spleen, cord blood	Šinkora et al., 1998 a
	40	Mesenterium	Šinkora et al., 1998 a
sIgM+ cells	30	Liver	Šinkora et Butler, 2009
	40	Periphery	Šinkora et al., 1998 a
γδ T cells	40	Thymus	Šinkora and Butler, 2009
	45	Liver, cord blood, spleen	Šinkora and Butler, 2009
	60	Bone marrow	Šinkora and Butler, 2009
αβ T cells	55	Thymus	Šinkora nad Butler, 2009
	58	Liver, cord blood, spleen	Šinkora and Butler, 2009
	60	Mesenteric lymph nodes, bone marrow	Šinkora and Butler, 2009
NK cells	45	Spleen, umbilical cord	Šinkora et al., 1998 a
	~50	Mesenteric lymph nodes	Šinkora et al., 1998 a
Immunoglobulins	44	Blood	Cukrowska et al., 1996
Interferon α secreting cells	26	Liver	Splichal et al., 1994

Table 2. Characteristic of different classes immunoglobulins in sow's colostrum

Features	IgG	IgA	IgM	References
Origin	Almost 100% derives from sow's serum	60% produced locally in the mammary gland 40% derive from sow's serum	85% derives from sow's serum 15% produced locally in the mammary gland	Maciag et al., 2022; Pomorska-Mól and Markowska-Daniel, 2009
Predominant immunoglobulin	Colostrum	Milk	–	Klobasa et al., 1987; Markowska-Daniel et al., 2010; Markowska-Daniel and Pomorska-Mól, 2010
Influence of sow's parity on colostral concentration	Concentration significantly higher in multiparous sows than in gilts	No significant impact on the colostral concentration Decreased concentration in the lacteal secretions of primiparous sows compared to multiparous sows	No significant effect on the colostral concentration	Klobasa et al., 1986; Cabrera et al., 2012; Forner et al., 2021; Maciag et al., 2022
Highest concentration in sow's colostrum	At farrowing	At farrowing	At farrowing	Klobasa et al., 1987; Markowska-Daniel et al., 2010
The concentration of immunoglobulins in particular teats	Higher amounts in anterior teats	Higher amounts in anterior teats	No data	Ogawa et al., (2014 a)

Table 3. Changes in the concentration of immunoglobulins in the sow colostrum (mg/ml) within time according to different studies

Immunoglobulins concentration	Markowska-Daniel et al., 2010		Klobasa et al., 1987	Bland et al., 1999		
At farrowing	IgG	98.17	IgG	95.6	IgG	58.0
	IgA	23.20	IgA	21.2		
	IgM	9.07	IgM	9.1		
24 hours post parturition	IgG	19.74	IgG	14.2	IgG	8.7
	IgA	9.17	IgA	6.3		
	IgM	4.42	IgM	2.7		

The significance of colostrum

In Suidae species, as mentioned previously, the epitheliochorial nature of the placenta prevents the sows' immunoglobulins from being transferred. Colostrum is thus the sole source of passive immunity for piglets. Besides immunoglobulins, colostrum contains lymphocytes, cytokines, nucleotides, and essential growth factors that stimulate the postnatal development of the immune system and the visceral organs, as well as protein synthesis in skeletal muscles (Burrin et al., 1992).

Prior to the maturation of the piglets' own immunity, the maternally derived antibodies (MDA) confer protection against any infectious agents that the sows had been naturally infected with or vaccinated against (Klobasa et al., 1981; Inoue and Tsukahara, 2021). For example, a previous study indicated that maternally derived rotavirus-specific IgG significantly alleviates the clinical symptoms following experimental rotaviral infection of neonatal piglets (Ward et al., 1996). Another study conducted under field conditions documented that antibodies passively acquired by neonates through the colostrum and milk of sows vaccinated against the *Clostridium perfringens* toxoid substantially reduced piglet losses caused by *C. perfringens* type C (Springer and Selbitz, 1999).

Immunoglobulins

The most important element of the sows' colostrum from an immunological point of view are immunoglobulins. These constitute the most abundant component of whey protein (approximately 80%) (Inoue and Tsukahara, 2021). Because piglets are born with almost no serum antibodies, MDAs play a crucial role in protecting them against infectious agents before they develop their own adaptive immunity (Markowska-Daniel et al., 2010; Forner et al., 2021). However, MDAs can only protect piglets from infections caused by pathogens that the sow's immune system has faced.

To provide a sufficient level of passive immunity and to significantly reduce the risk of early death, the piglet needs to ingest a minimum of 200 mg of colostrum within the first 24 hours after birth; consumption of 250 mg per piglet should provide good future health (Quesnel et al., 2012). Antibodies can pass to the colostrum from sow serum or can be produced directly in the mammary

gland (Table 2) (Pomorska-Mól and Markowska-Daniel, 2009). Almost 100% of colostral IgG derives from sow's serum; meanwhile, more than 50% of IgA is produced locally in the mammary gland (Maciag et al., 2022). A substantial majority (around 85%) of colostral IgM derives from sow's serum (Pomorska-Mól and Markowska-Daniel, 2009). The predominant colostral immunoglobulin is IgG (Klobasa et al., 1987; Markowska-Daniel et al., 2010; Markowska-Daniel and Pomorska-Mól, 2010). This is followed by IgA (the predominant immunoglobulin of milk) and IgM (Klobasa et al., 1987; Markowska-Daniel et al., 2010; Markowska-Daniel and Pomorska-Mól, 2010). Immunoglobulin concentration in colostrum is not constant, declining during the first 24 hours after farrowing; however, the decrease in the level of IgG is more drastic than in IgA or IgM (Table 3) (Klobasa et al., 1987; Markowska-Daniel et al., 2010). For example, in the research of Markowska-Daniel et al. (2010), the highest level of immunoglobulin was noted in the first colostrum, which is acquired at farrowing. The mean initial concentration was 118.5 mg/ml, 23.8 mg/ml, and 12.1 mg/ml for IgG, IgA, and IgM, respectively. After 24 hours, the concentration of IgA and IgM had reduced to 4.59 mg/ml and 4.29 mg/ml, respectively, and the level of IgG decreased to 34% of its initial level (Markowska-Daniel et al., 2010). This is partially consistent with the findings of Klobasa et al. (1987), where the mean IgG concentration was also highest at farrowing, at 95.6 mg/ml; IgA was at 21.2 mg/ml and IgM was at 9.1 mg/ml. These were subsequently reduced to 14.2 mg/ml, .3 mg/ml, and 2.7 mg/ml, respectively, 24 hours after birth (Klobasa et al., 1987). Bland et al. (1999) assessed the mean IgG concentration at farrowing as 58.0 mg/ml; 24 hours later, the level had decreased to 8.7 mg/ml (Bland et al., 1999). These studies also indicated that the concentration of colostral immunoglobulins varies between sows. Kielland et al. (2015) noted that the concentration of colostral IgG varies not only between particular sows, but also between herds (Kielland et al., 2015). Several studies have indicated that the concentration of IgG in the colostrum was significantly higher in multiparous sows than in gilts (Cabrera et al., 2012; Forner et al., 2021; Maciag et al., 2022). However, there was no such relationship for IgA content (Forner et al., 2021). This is consistent with Maciag et al. (2022), where the parity order did not significantly affect the colostral concentration of IgA and IgM (Maciag et al., 2022). On the contrary, Klobasa et al. (1986) showed that the levels of IgA in the lacteal secretions of primiparous sows were lower than in multiparous sows (Klobasa et al., 1986). The level of immunoglobulins in colostrum can also vary between individual teats (Table 2). According to Ogawa et al. (2014 a), a higher amount of colostrum was obtained from anterior teats. Furthermore, a positive correlation between colostrum secretion volume (CSV) and IgG was observed from 6 hours after farrowing and from 12 hours after farrowing between CSV and both IgG and IgA. This may suggest that the anterior teats contain greater amounts of IgA and IgG than the posterior teats (Ogawa et al., 2014 a). Other factors that can affect the final concentration of IgG in colostrum are the mother's genotype, age, and vaccination, as well as endocrine status, feeding, and herd management (Kielland et al., 2015; Maciag et al., 2022). The immunoglobulins ingested by piglets are taken up by nonspecific pinocytosis into the enterocytes (Rooke and Bland, 2002; Kielland et al., 2015). An important role in the absorption of immunoglobulin from the guts is played by trypsin inhibitor, which inhibits the enzymatic activity of trypsin and prevents denaturation of the immunoglobulins; however, its level in colostrum decreases within time (Jensen and Pedersen, 1979). The concentration of IgG in the piglet's serum depends on several factors, such as the amount of available and ingested colostrum, IgG absorption in the piglet's intestine, and gut closure, which occurs 24 to 36 hours after birth (Pomorska-Mól and Markowska-Daniel, 2009; Kielland et al., 2015). The study of Kielland et al. (2015) indicates a strong association between the concentration of colostrum IgG and piglet serum IgG (Kielland et al., 2015). Interestingly, this is not consistent with some other studies, where a lack of correlation is demonstrated between levels of colostral Ig and piglet's serum Ig (Markowska-Daniel et al., 2010). The concentration of piglet IgG decreased with each piglet born. However, this was not significantly associated with litter size, but was more dependent on the time of birth relative to farrowing onset (Kielland et al., 2015). Litter size affected colostrum intake but not the concentration of piglet IgG. This may be due to the flattening of piglets' plasma IgG concentration above a certain level of colostrum IgG (Kielland et al., 2015). Bland et al. (1999) have shown that, despite differences in colostrum intake (and hence IgG) between piglets, the total concentration of IgG in the piglet's plasma did not vary between litters. This may indicate that piglets can regulate the quantity of plasma IgG independently of the quantity of IgG obtained (Bland et al., 1999). In Kielland et al. (2015), a significant association was observed between body mass index (BMI) and piglets' IgG level on day 1. The authors further concluded that piglets with BMIs of 17 kg/m^2 or lower may not be strong enough to obtain sufficient amounts of IgG. (Kielland et al., 2015). Bandrick et al. (2014) demonstrated that levels of IgG and IgA in the serum of piglets that ingested colostrum mimicked the distribution of these immunoglobulins in the sow's colostrum. Furthermore, IgG and IgA were absent from piglet serum before colostrum intake. This may indicate nonselective absorption of colostral immunoglobulins (Bandrick et al., 2014). A positive correlation between IgG levels in the serum of piglets at 7 days and 56 days of age may point to a positive relationship between initial antibody concentration in the piglet's serum and the development of their active immunity (Markowska-Daniel et al., 2010). According to Rooke et al. (2003), naturally suckling piglets start to produce IgG at 7 days of age. Its quantity was positively correlated with the amount of absorbed colostral

IgG (Rooke et al., 2003). Curtis and Bourne (1973) indicate that active IgM production by piglets emerges from 10–12 days of age. Porter and Hill (1970) reported that IgM in suckling piglets begins to increase from 7 days of age. Active synthesis of IgA by suckling piglets up to 12 days of age does not contribute significantly to serum IgA concentrations (Curtis and Bourne, 1973). This suggests that piglets begin to effectively produce their own IgA at a later age, but to date, there is no conclusive data on this issue. Other investigation, with piglets weaned at different times (7, 14, 21 or 28 days) showed that endogenous colostrum/milk factors may be critical to promote IgA synthesis (Levast et al., 2010). Ultra-early weaned piglets (at 7 and 14 days) showed lower serum IgA concentrations at 21 days proving a deficiency in the IgA gut immune response development.

Cytokines

The presence has been noted in porcine colostrum of granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon γ (IFN-γ), interleukin 1 α (IL-1α), interleukin 1 receptor antagonist (IL-1RA), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 10 (IL-10), interleukin 12 (IL-12), interleukin 18 (IL-18), tumor necrosis factor-alpha (TNF-α), and transforming growth factor β (TGF-β) (Table 4) (Nguyen et al., 2007; Ogawa et al., 2014 b; Maciag et al., 2022). Maternal cytokines contained in the colostrum may play an instructive role in the maturation of the neonatal immune system (Nguyen et al., 2007). It is assumed that colostrum is the only source of various cytokines for newborn piglets (Nguyen et al., 2007). The study of Nguyen et al. (2007) indicated that the moderate correlation between sow blood and colostrum for IL-4, IL-6, IL-10, IL-12, and IFN-γ indicates that these cytokines pass to the mammary gland from the sow's bloodstream. Given the lack of such a correlation between TNF-α and TGF-β1 (the concentration of both in the colostrum being higher than in the sow's blood), they must be produced directly in the mammary gland (Nguyen et al., 2007). These cytokines were only present in the blood of piglets that ingested colostrum (Nguyen et al., 2007). The presence of IL-6, TNF-α, INF-γ, IL-4, and IL-10 was not detected in the group of age-matched colostrum-deprived gnotobiotic piglets. The authors thus assumed that these cytokines do not pass through the placenta. However, IL-12 and TGF-β1 were detected in the plasma of colostrum-deprived gnotobiotic piglets. This may indicate that piglets can produce the cytokines by themselves. In the mentioned study, the authors did not detect TNF-α in the plasma of piglets in either group. The lack of this cytokine in the serum of sows and piglets is probably due to some control mechanism that prevents prolonged inflammatory responses, which could further damage tissue (Nguyen et al., 2007). However, data concerning this cytokine is inconsistent: its presence in piglet plasma was observed in Maciag et al. (2022). Nguyen et al. (2007) found the highest concentration of the cytokines in the secretion of the mammary gland on the first and second days after parturition; this correlated with the time of their peak concentrations in the piglet's serum. The predominant cytokine in the sow's colostrum was IL-4, followed by TGF-β1. Other cytokines occurred at lower levels, and the least concentrated were IL-12, IL-10, and TNF-α (Nguyen et al., 2007). One *in vitro* study evaluated the impact of various concentrations of TGF-β1 and IL-4 on porcine neonatal B cell responses (Nguyen et al., 2007). High TGF-β1 levels caused suppressed immunoglobulin-secreting cell responses to LPS and rotavirus, and low TGF-β1 levels led to isotype switching to IgA antibodies. IL-4 provoked inverse dose-dependent isotype switching to IgA. Moreover, it also improved IgM-secreting cell responses to LPS and rotavirus. Furthermore, an elevated concentration of Th2 cytokines or TGF-β in newborn piglets may be crucial for their acquisition of normal commensal microflora in the intestine, given the reduction of immune and inflammatory response in the gut (Nguyen et al., 2007). Maciag et al. (2022) found that concentrations of GM-CSF, IFN-γ, IL-1α, IL-1RA, IL-2, IL-4, IL-6, IL-10, IL-12, IL-18, and TNF-α were significantly higher in serum and colostrum of multiparous sows than in gilts (Maciag et al., 2022). Moreover, these above cytokines occurred at higher levels in the serum of piglets that received colostrum from multiparous sows than in the serum of piglets fed with gilt's colostrum. The lowest concentration of these cytokines was observed in the piglets fed with milk replacer. A greater tendency to innate inflammatory and anti-inflammatory responses, and a greater propensity to specific Th1 and Th2 responses, during the perinatal period was significantly correlated with piglets that suckled sow colostrum (Maciag et al., 2022). Ogawa et al. (2014 b) confirmed the presence of IL-18 in the colostrum, but not in the sow's milk. This cytokine is believed to regulate the immune system of newborn piglets (Ogawa et al., 2014 b). Elahi et al. (2017), using a pertussis model showed that immunization of pregnant sows with heat-inactivated bacteria may lead to the induction of various cytokines, such as TNF-α, IFN-γ, IL-6, IL-8, and IL-12/IL-23p40. Moreover, these cytokines were detectable next to pertussis-specific antibodies, not only in vaccinated sow serum and colostrum, but also in their progeny's serum and bronchoalveolar lavage fluid. Interestingly, active vaccination of newborn piglets with heat-inactivated bacteria led to high levels of IgG and IgA specifically, but no cytokines. Even though the concentration of antibodies in vaccinated piglets and of passively obtained antibodies were similar, the authors did not observe any protection against *Bordetella pertussis* infection in the vaccinated individuals. Elahi et al. (2017) hence conclude that the presence of passively transferred cytokines or antibodies affects newborn piglets' ability to secrete cytokines, and that this may suggest that the vaccinating the sow can affect the newborn's cytokine milieu and impact immune cell differentiation (Elahi et al., 2017).

Table 4. Characteristic of cytokines present in sow's colostrum and piglets' serum

Features	Cytokines	References
Presence in sow's colostrum	GM-CSF, IFN-γ, IL-1α, IL-1RA, IL-2, IL-4, IL-6, IL-10, IL-12, IL-18, TNF-α, TGF-β	Nguyen et al., 2007; Ogawa et al., 2014 b; Maciag et al., 2022
Originating from sow's serum	IL-4, IL-6, IL-10, IL-12, IFN-γ	Nguyen et al., 2007
Production directly in the mammary gland	TNF-α, TGF-β1	Nguyen et al., 2007
Predominant cytokine of sow's colostrum	IL-4	Nguyen et al., 2007
Least concentrated in sow's colostrum	IL-12, IL-10, TNF-α	Nguyen et al., 2007
Concentration higher in multiparous sow than gilts	GM-CSF, IFN-γ, IL-1α, IL-1RA, IL-2, IL-4, IL-6, IL-10, IL-12, IL-18, TNF-α	Maciag et al., 2022
Likely production by neonate piglets	IL-12, TGF-β1	Nguyen et al., 2007

Cellular components

Porcine colostrum, apart from multiple proteins, also contains several types of cells (Wagstrom et al., 2000). It has been estimated that piglets ingest some 500–700 million colostral cells daily (Nguyen et al., 2007), including epithelial cells, lymphocytes (T and B cells), and phagocytes (neutrophils and macrophages) (Wagstrom et al., 2000). In contrast to many mammal species, epithelial cells constitute a great fraction of sow mammary gland secretions, representing approximately 20%–40% of all the colostral cells (Le Jan, 1996). Epithelial cells in the colostrum are small in size and have little or no IgA (Le Jan, 1996). They furthermore exhibit low expression levels of secretory components (Le Jan, 1996). Small epithelial cells can be propagated *in vitro* for at least three passages. When cultured in the presence of the serum of lactating sows, these cells differentiate and begin to produce α-lactoglobulin (Le Jan, 1996). Depending on their differentiation level, they display the ability to express major histocompatibility complex II (MHC II antigen) (Wagstrom et al., 2000). Moreover, it is considered that porcine colostral epithelial cells can produce cytokines and can function as antigen-presenting cells (Wagastrom et al., 2000). Macrophages constitute about 7%–11% of cells (Maciag et al., 2022). 10%–25% of all porcine colostrum cells are lymphocytes, of which 70%–80% are T cells (Hlavova et al., 2014). According to Hlavova et al., (2014) the predominant cell types in colostrum are $CD8^+$ single-positive T cells (53.6%), followed by $CD4^+CD8^+$ double-positive T cells (21.1%), $CD2^+CD8^+$ γδ T cells (15.0%), and NK cells (13.5%). The $CD4^+$ single-positive T cells (4.4%) and other γδ T cell subpopulations were less common. The proportion of individual lymphocytes varies between colostrum and sow peripheral blood: the proportion of cytotoxic and double-positive T cells was significantly higher in colostrum than in peripheral blood (Hlavova et al., 2014). On the contrary, the proportion of helper T cells was higher in peripheral blood (Hlavova et al., 2014). The greatest number of T cells are found in the colostrum obtained around parturition, with their number significantly decreasing after the first eight hours of lactation. Afterwards, the number of T cells remains constant (Hlavova et al., 2014). Forner et al. (2021) assessed the differences in the distribution of colostrum's humoral and cellular composition over 40 gilts and 40 sows (parity orders 3–4). Their results show that parity does not influence the total count of macrophages, granulocytes, or T and B cells. Nevertheless, multiparous sow colostrum contained significantly larger T lymphocyte subsets than gilts (i.e., central and effector memory $CD4^+$ T cells or central memory $CD8^+$ T cells). The authors concluded that parity order may influence the cell population and piglet immune adaptive response, which induces neutralizing antibodies and cellular immune responses (Forner et al., 2021). Williams (1993) demonstrated that the lymphocytes contained in colostrum could cross the intestinal wall and migrate via the piglet's bloodstream to various organs (including the liver, lungs, lymph nodes, and spleen). Moreover, Nechvatalova et al. (2011) demonstrated that these cells possess functional abilities (they can become activated) and display an antigen-specific activity in organs (Nechvatalova et al., 2011). Goubier et al. (2009) detected antigen-specific lymphocytes that were able to produce IFNγ and TNFα after *in vitro* stimulation with circoviral antigens vaccinated against PCV2 sow colostrum (Goubier et al., 2009). Bandrick et al. (2008) demonstrated that lymphocytes obtained by piglets from vaccinated sow colostrum can proliferate and participate in functional response to *Mycoplasma hyopneumoniae* (Bandrick et al., 2008). Hlavova et al. (2014) assessed the activation status of T and NK cells in colostrum using the expression of CCR7, CD11b, CD25, CD45RA, and MHC class II receptors. They managed to observe the expression of markers consistent with an effector memory phenotype on T cells; this might suggest that these were antigen-experienced cells. Based on the phenotype of colostrum-derived T lymphocytes and NK cells, those authors concluded that these components may play a role in mucosal immunity, and potentially in the transfer of passive immunity (Hlavova et al., 2014). A study by Bandrick et al. (2014) demonstrated that colostral lymphocytes are selectively transferred into the suckling bloodstream. These can then influence neonatal piglets' innate and adaptive immune responses (Bandrick et al., 2014). B lymphocytes constitute about 20% of all lymphocytes in mammary secretions (Pomorska-Mól et al., 2010), and

the concentration of SWC7+ CD5+ cells turned out to be significantly higher in multiparous sow colostrum than in gilts (Forner et al., 2021). Maciag et al. (2022) indicated that levels of activated B and T cells were higher in piglets fed multiparous sow colostrum. Lymphocytes can cross the intestinal wall of newborn piglets only when they are viable and have derived from the dam of a particular piglet (Bandrick et al., 2011). This is not the case with immunoglobulins, which can be absorbed across the intestinal mucosa of neonatal piglets, regardless of the maternal source or donor species (Bandrick et al., 2011). As a result, fostered piglets that suckled colostrum from another sow than their natural dam will have deficiencies in maternal cell-mediated immunity (CMI) (Bandrick et al., 2011). Bandrick et al. (2011) had demonstrated that transfer of *Mycoplasma hyopneumoniae*-specific CMI to piglets occurs only when piglets were maintained with their biological dams for at least twelve hours after parturition (Bandrick et al., 2011). To conclude, maternal antigen-specific leukocytes, delivered to piglets via colostrum, may constitute an extra line of active defense against infection for neonatal piglets (Goubier et al., 2009).

Immunity of neonatal piglet

Mucosal immunity

After birth, neonatal piglets are exposed to plenty of various antigens. Most of them enter a host via mucosal membranes (Solano-Aguilar et al., 2001). Thus, these structures are equipped with specialised protective elements called mucosa-associated lymphoid tissue (MALT), distinct from remaining lymphatic tissue (Solano-Aguilar et al., 2001). Based on its distribution in the organism, several types of MALT were distinguished, i.e. gut-associated lymphoid tissue (GALT), nasopharynx-associated lymphoid tissue (NALT) or bronchus-associated lymphoid tissue (BALT) (Mazzoni et al., 2011). Due to the risk related to numerous potential pathogens or food-borne antigens and the presence of commensal microbiota, GALT is well developed and consists of the following structures: the Peyer's patches, mesenteric lymph nodes and intraepithelial lymphocytes and lamina propria lymphocytes (Solano-Aguilar et al., 2001; Mazzoni et al., 2011). GALT of neonatal piglets is a relatively developed structure with pre-existing Peyer's patches (Levast et al., 2014). However, shortly after birth, the piglet's gut is characterized by a small number of lymphocytes T and antigen-presenting cells (Levast et al., 2014). Given exposure to numerous external antigens and commensal microorganisms, GALT enlarges in size and lymphocyte subset content and reaches maturity in conventionally-raised piglets within two months (Levast et al., 2014). At that time, the GALT of conventionally-raised piglets can respond to intestinal antigens with good T and B lymphocyte activation and IgA production (Levast et al., 2014). However, mesenteric lymph nodes and Peyer's patches are still smaller and have fewer Ig-secreting cells than adult ones (Levast et al., 2014). Solano-Aguilar et al. (2001) have described changes in lymphocyte subsets in mucosal tissues with increasing age. According to this study, the GALT of piglets varies from the GALT of adult pigs in the presence or count of the respective lymphocyte subsets. The authors also pointed out that the majority of the phenotypes characterized by increasing trends during the pre-weaning time were located on lymphocytes isolated from mesenteric lymph nodes and ileal Peyer's patches, which can further imply that these lymphocytes are key populations in the early postnatal period during the development of lymphoid cells (Solano-Agilar et al., 2001). Rothkötter et al. (1991) studied lamina propria (LP) of normal and germ-free piglets in early postnatal period. According to this study, the number of LP lymphocytes has increased two-fold between the first and 29th day after farrowing. Moreover, the authors observed that the distribution of lymphocyte subsets exhibited an unusual pattern. Approximately 80% of T cells of piglets aged 1 to 5 days belonged to $CD2^{+}CD4^{-}CD8^{-}$ subset. However, around 12 days post-parturition, this subset started to disappear. Interestingly, 49 days old germ-free piglets displayed similar T cell subset patterns as above mentioned conventionally raised piglets between 1 and 5 days. Ig-positive cells were observed later than T cells. On the first-day post-parturition, a minimal number of IgM^{+} was noted. Forty days later, the number of IgA^{+} was higher than IgM^{+}. The authors conclude that the results obtained in the germ-free piglets may prove that the major changes in the LP are caused by the appearance of microbial antigens in the intestine (Rothkötter et al., 1991). Schnapper et al. (2003) have observed rapid changes occurring in piglet's lymphoid tissue – the weight of Peyer's patches, tonsil of the soft palate, lymph nodes (cranial mesenteric lymph center and bronchial lymph center), spleen and thymus have grown faster than the body weight of piglets and had been significantly enlarged during the first two weeks of life (Schnapper et al., 2003). The mammalian intestine is colonized with normal gut flora during the first few days of life (Butler et al., 2000). This component is believed to have an important role in providing health to its host, by among others inhibiting intestine colonization with pathogenic bacteria (Butler et al., 2000). Colostrum and milk constitute one of the sources that deliver gut microbes to newborn piglets (Mardiaga et al., 2018). Maradiaga et al. (2018) investigated gastrointestinal microflora and mucosal immune gene expression in newborn piglets that were reared in a cross-fostering model. Results of the above study show that although cross-fostering does not affect bacterial communities present in the neonate's intestine, the mRNA expression of TLR and inflammatory cytokines change within the location in the gastrointestinal tract. The authors suggested that the results presented in their study may indicate the influence of colostrum and maternal microbial communities on microflora development as well as mucosal immune gene expression in newborn piglets (Maradiaga et al., 2018). The secondary function of the mucosal system is to discriminate be-

tween pathogen-associated and non-pathogenic antigens. This phenomenon is called mucosal tolerance (Bailey et al., 2005). According to Bailey et al. (2005), data concerning response to intestine microflora are still inconsistent. One of the mechanisms involved in maintaining tolerance may be anergy or deletion of specific T-cells clones. It is grounded on the recognition of antigen on wrong presenting cells by antigen-naive T cells. It is worth emphasizing here the role of dendritic cells, which are responsible for the presentation of antigens to naive T cells. These cells exhibit the ability to switch naive T cells to active response or tolerance. Thus, the interaction between these two types of cells is believed to determine the reaction. However, there are reports showing that the outcome of antigen recognition is determined by antigen-non-specific signals arrived directly at mucosal regulatory T cells (Bailey et al., 2005). As mentioned earlier, the mucosal system of neonates is immature after birth in comparison to adult ones. This agent may be responsible for the lack of active immune responses as well as the development of tolerance (Bailey et al., 2005).

Innate immunity

The components of porcine innate immunity are similar to those in many other mammals (Šinkora and Butler, 2009). Innate immunity is based on two main mechanisms: activation of cellular components, such as macrophages, neutrophils, NK cells, or dendritic cells; and the release of various extracellular mediators – such as cytokines, chemokines, and complement or antimicrobial peptides (Šinkora and Butler, 2009). The total number of leukocytes increases during the first week of neonate life (Talker et al., 2013). Pomorska-Mól et al. (2011) indicate that, on the first day of a piglet's life, the number of granulocytes is similar to the number of lymphocytes (Pomorska-Mól and Markowska-Daniel, 2011). However, the absolute granulocyte size and lymphocyte number predominance decreases during the next three weeks. In addition, a positive correlation between the mean number and mean percentage of granulocytes and the piglet's age was observed (Pomorska-Mól and Markowska-Daniel, 2011). They also showed that piglet granulocytes have decreased phagocytosis and weaker adhesive molecule expression than adults (Pomorska-Mól and Markowska-Daniel, 2010 a). The median value of NK-cell numbers in piglets' blood is lowest at birth and increases from there (Talker et al., 2013). Talker et al. (2013) indicate that the presence of perforin was noted in all NK cells as early as the day of birth. This finding may imply that piglets' NK cells exhibit immediate cytotoxic activity (Talker et al., 2013). Nevertheless, it contrasts with previous research, where NK cells were isolated from pigs for up to two weeks after birth, and showed weak cytolytic activity against K562 target cells (Yang and Schultz, 1986). Moreover, Talker et al. (2013) observed that pigs possess $CD3^{-}CD8\alpha^{+}NKp46^{-}$ cells with entirely functional characteristics of NK cells; this is interesting because NKp46 was believed to be a species-spanning marker for NK cells. However, no evident changes in phenotype or quantity caused by environmental changes or piglet ageing were observed for NK cells (Talker et al., 2013). Llamas Moya et al. (2007) investigated age-related changes in proinflammatory cytokines, acute phase proteins (APP), and cortisol concentrations during the first week of a piglet's life (Llamas Moya et al., 2007). According to this study, the concentration of TNF-α and haptoglobin (Hp) increased with age, while the serum amyloid A (SAA) level decreased. The lowest level of plasma TNF-α was observed on the first day after birth. On day 5, the concentration of TNF-α achieved its peak, and remained elevated on day 7. Similarly, the level of Hp was lowest on day 1, and after that increased with age. On the other hand, the concentration of plasma SAA was elevated on days 1, 3, and 5, but had significantly reduced by day 7. There were no age-dependent changes in the plasma concentration of IL-1β or C-reactive protein (CRP). Interestingly, husbandry practices such as ear notching, teeth clipping, and tail docking did not affect other measured parameters, other than Hp. The authors thus suggested that such management practices did not result in systemic inflammation in the early postnatal life of piglets (Llamas Moya et al., 2007). Similarly, Martin et al. (2005) found that the Hp concentration in neonatal piglets' plasma was also low on day 1 after birth and subsequently increased (Martin et al., 2005). Moreover, an age-dependent increase in the level of plasma major acute-phase protein (Pig-MAP) from birth up to 4 days of age was observed. After that, the concentration of Pig-MAP remained relatively high. The authors suggested that the rapid increase in the levels of proteins that were low at birth suggests some variety of acute-phase response, and that this response may be an evolutionary adaptation of the piglets to managing during the first critical period of extrauterine life (Martin et al., 2005).

Adaptive immunity

The development of the cellular components of the pig immune system is not fully complete by the end of gestation (Stepanova et al., 2007; Pomorska-Mól and Markowska-Daniel, 2011). The T cells, B cells, and peripheral blood mononuclear cells (PMBC) of neonatal piglets have a less developed ability to respond to mitogens and a lower number of antigen-presenting cells (Maciag et al., 2022). The total number of lymphocytes increases during the first week of a piglet's life (Talker et al., 2013). Some studies have indicated that $CD4^{+}$ lymphocytes form the predominant subpopulation of T cells in neonate piglets at farrowing, while $CD8^{+}$ lymphocytes are less common (Stepanova et al., 2007). On the other hand, in Pomorska-Mól and Markowska-Daniel (2011), the number of $CD8^{+}$ cells was greater than the number of $CD4^{+}$ cells. The ratio of $CD4^{+}$ to $CD8^{+}$ cells decreased with age as the $CD8^{+}$ increased, which was accompanied by a proportional decrease in $CD4^{+}$ lymphocyte numbers (Stepanova et al., 2007; Pomorska-Mól and Markowska-Daniel, 2011). Double-positive $CD4^{+}CD8^{+}$ are less fre-

quent in neonate piglets than in adults, and their population increases with age, probably as a result of the antigen-dependent maturation of naive $CD4^+$ T helper lymphocytes into antigen-specific memory T helper lymphocytes (Saalmüller et al., 2002; Stepanova et al., 2007). However, there is some variance, depending on the levels of double-positive $CD4^+CD8^+$ lymphocytes in piglets. According to Stepanova et al. (2007), these cells were rare during the first month of piglets' life, at 0.5%, while Pomorska-Mól and Markowska-Daniel (2011) indicated that the proportion of this subset on the first day of piglets' life was 6.63%. This discrepancy might result from the animals' conditions (experimental conditions versus commercial breeding farm) (Stepanova et al., 2007; Pomorska-Mól and Markowska-Daniel, 2011). Stepanova et al. (2007) have noted changes in the subpopulations of T lymphocytes in the peripheral blood and secondary lymphoid tissue during the first month of the piglet's life. A significant age-dependent increase in the total concentration of γδ T cells was noted in the blood and spleen, but not in the lymph nodes. However, there was significant growth in the percentage of the γδ TCR+CD8+ subpopulation (Stepanova et al., 2007). Strong growth in the total number of γδ T cells since birth was also confirmed in Talker et al. (2013), who observed significant phenotypic changes only within the $CD2^+$ subset. Nevertheless, the increase in overall amounts was proportional to the increase of the CD2-T-cell subset (Talker et al., 2013). Due to the involvement of γδ lymphocytes in innate immune efficiency, they are also considered to constitute part of the innate immunity (Šinkora and Butler, 2009). The pool of B lymphocytes in neonatal piglets is immature (Šinkora and Butler, 2009). The more significant percentage of peripheral $sIgM^+$ B cells consists of $sIgM^+CD2^+$ B cells, and $sIgM^+CD2^-$ are relatively rare (Šinkora et al., 1998 b). This is interesting, as porcine B cells were considered not to express CD2 (Šinkora et al., 1998 a). According to Šinkora et al. (1998 b), after the piglet's gut has been colonized with a complex intestinal microflora, this proportion changes, and the number of $CD2^-$ B cells increases with age, reaching 15%–35% of $sIgM^+$ peripheral blood lymphocytes in week-old piglets. They suggested that the expression of CD2 may be related to the functional status of porcine B cells, but can then be lost as a consequence of the maturation process (Šinkora et al., 1998 b). Thus, the microflora-dependent $sIgM^+CD2^–$ lymphocytes in pigs can be activated/memory B cells (Šinkora et al., 1998 b). Antigen stimulation due to colonization of the intestinal duct, as well as contact with the extrauterine environment, results in the activation of T and B cells (Pomorska-Mól and Markowska-Daniel, 2010 a). Šinkora and Butler (2009) indicate that, in germ-free piglets, the development of the immune system is slower than in an age-matched control group, and these piglets were also unable to perform a humoral response (Šinkora and Butler, 2009). The maturation of the elements of adaptive immunity thus arises from several factors, such as whether maternal immunoglobulins are obtained with the colostrum and whether there is any interaction with environmental microorganisms. These are essential for the appearance of primed T and B cells that subsequently develop into their effector and memory progeny (Šinkora and Butler, 2009). Primed T lymphocytes with elevated expression levels of CD25 meanwhile become effector/memory cells with elevated expression of MHC II and display a $CD4^+CD8\alpha^+$ and $CD2^+CD8\alpha^+$ γδT phenotype (Šinkora and Butler, 2009).

Assessment of piglets' immune status: current possibilities and perspectives

Although piglets are born immunocompetent – i.e. able to respond – they are at the same time immunologically defenseless. To survive in an environment where they are exposed to pathogens, they need their mother's protection, which is provided as passive immunity that comes with the colostrum and milk, in the form of antibodies and other regulatory elements involved in the immune response. Early assessment of a piglet's immune status may enable rapid intervention in case of deficiencies or disorders. In addition, knowledge of a piglet's immunocompetence and MDA levels is crucial to designing a proper vaccination schedule (Martínez-Boixaderas et al., 2022). Piglet protection can be achieved either passively, through the transfer of maternally derived immunity, or actively through vaccination. However, vaccinating piglets in the presence of remaining MDA might interfere with vaccine efficacy. Most works in the literature have dealt with the acquisition of passive humoral immunity by newborns, although the transmission of specific cellular immunity has also been reported (Le Jan, 1996; Nguyen et al., 2007; Pravieux et al., 2007). For example, it has been shown that colostrum-derived T cells could cross the intestinal barrier and enter the systemic circulation and lymphoid organs (Tuboly et al., 1995). These lymphocytes are also a potential source of cytokines and chemokines that may exert a regulatory effect on antigen-presenting cells and specific antigen responses. Mammary gland secretions thus also have immunoregulatory properties. Besides, colostrum contains numerous other components involved in the systemic immune processes, such as cytokines, interferons, lysozyme, lactoferrin, peroxidase, complement components, hormones, and other compounds involved in the mechanisms of innate immunity (Salmon, 1999; Schultz, 2006). These substances participate in the maturation of both local and systemic defense processes, as well as in the induction and orientation of the piglets' active response to the antigen (Salmon, 1999; Schultz, 2006). After being absorbed in the first 24 to 36 hours of life, antibodies and other colostrum components are transferred into the blood, where they provide systemic resistance to infectious agents in piglets (Inoue and Tsukahara, 2021). It is known that Ig plasma concentrations in piglets shortly after birth positively correlate with their chance of surviving the critical perinatal period (Devillers et al., 2011). Cytokines passed to piglets by their mother with

colostrum can play the role of "teacher" in the maturation of suckling piglets' immune system (Nguyen et al., 2007). However, cytokine transmission with colostrum or milk is not well documented.

Studies on the immunological status of piglets are presently performed using blood samples. Blood collection from individual animals has been the method most commonly used in veterinary practice to obtain samples for monitoring and surveying herd health status, including for pigs. However, the technique is time-consuming, laborious, and stressful for both animals and collecting staff. In addition, sampling piglet blood is relatively difficult and carries a high mortality risk. For this reason, farmers and veterinarians often refrain from carrying out such tests, or postpone monitoring until later in production. This can significantly delay the detection of important health problems in the herd, including the identification of lactation problems in sows, irregularities in the vaccination of dams, or transmission of maternal immunity to piglets. A specimen that could be collected noninvasively and without inducing stress in the animal, while still offering accurate diagnostic information, would greatly benefit herd health status and disease monitoring. In the EU, noninvasive sampling methodologies for monitoring the health status of farm animals must comply with the EU's general objectives on food safety policy (Regulation 652/2014), in which animal welfare and wellbeing are major issues. In the assessment of the immunological status of piglets, only one component of the immune response is usually considered – namely, humoral immunity, including its level and duration. It seems that a more detailed analysis of immunological parameters in this period (taking into account specific antibodies, and also the types of particular groups of immunoglobulins, cytokines, APP, and so on) would shed more light on the significance of maternal immunity and its level in piglets in the first days of life for their further development. The duration of maternal MDA refers to the age of the piglet at which their MDA levels fall below the limit of detection of the test used for evaluation (Opriessnig et al., 2004), or the rate of decay ("half-life") of MDA (the time required for a 50% decrease in MDA levels) (Fort et al., 2009). The amount of antibodies and other immune factors transferred from sows to offspring is determined by the sow's immunity level near parturition, the timing of colostrum intake, and the volume of colostrum ingested (Klobasa et al., 1981). Strengthening sow herd immunity through vaccination is an important management tool for preventing clinical signs in piglets and for delaying infection until the piglet immune system is fully developed (Poonsuk and Zimmerman, 2018). Noninvasive sample collection methods are thus desirable and of great research interest. The usefulness of oral fluid (OF) specimens for detecting antibody and pathogen genetic material in pig population is already accepted and commercially used (Prickett et al., 2008; Prickett and Zimmerman, 2010; Ramirez et al., 2012; Kittawornrat et al., 2014; Sattler et al., 2015). Several advantages of the use of such noninvasive sampling have been reported: it allows efficient and low-cost collection of large numbers of diagnostic samples, and it permits repeated sampling without the risk of discomfort or stress (Turlewicz-Podbielska et al., 2020). Recently, processing fluid (PF) has become more widely used in diagnostic practice (Trevisan et al., 2019; López et al., 2022). PF consists of blood and tissue fluids obtained during castration and tail-docking, which are usually performed during the first week of a piglet's life (3–5 days) (Turlewicz-Podbielska et al., 2020). As castration is still performed on many farms, gaining PF does not require additional procedures or animal restraint, and thus does not generate additional stressful situations. PF is considered a promising, practical, and inexpensive specimen that may improve the monitoring of some porcine diseases, such as PRRS (Turlewicz-Podbielska et al., 2020). The idea of using this type of specimen to monitor other swine diseases, as well as immune parameters such as cytokines, immunoglobulins, and APP shows some promise. An additional advantage of PF is that the specimen comes from piglets less than one week old. Studies indicate that collecting PF from such young piglets is very difficult, if not impossible (Jabłoński et al., 2011). Replacing traditional samples, such as blood or serum, with PF has numerous benefits, saving time, work, and costs while minimizing the stress associated with sampling, which positively impacts animal welfare and immunity. However, there is a lack of experimental data on its use for assessing the immunological status of piglets, including the correlation between the concentration of various immunological parameters in PF and the corresponding sera, which until now have been the material used in this type of determination (the gold standard).

Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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