Protective effect of the maternally derived porcine circovirus type 2 (PCV2)-specific cellular immune response in piglets by dam vaccination against PCV2 challenge

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The objective of the present study was to evaluate (i) the passive transfer of maternally derived functional porcine circovirus type 2 (PCV2)-specific lymphocytes of seronegative sows immunized with the PCV2 vaccine to newborn piglets and (ii) the functional role of the maternally derived PCV2-specific cellular immune response in protecting newborn piglets from challenge with PCV2. After ingesting colostrums, piglets from vaccinated sows (PT01 and PT02) have significantly higher numbers of PCV2-specific gamma interferon-secreting cells, an increased PCV2-specific delayed type hypersensitivity response, and a stronger proliferative response of peripheral blood mononuclear cells compared with piglets from non-vaccinated seronegative sows (PT03 and PT04). In the PCV2 challenge study, the number of serum genomic PCV2 copies was significantly less in piglets from vaccinated sows (PT02) compared with piglets from non-vaccinated sows (PT04) at 7–28 days post-inoculation (P<0.05 and P<0.001). The histopathological lesions and immunohistochemical scores were significantly lower in piglets of vaccinated sows compared with those of non-vaccinated sows. To our knowledge, this is the first report of transferring a maternally derived PCV2-specific cellular immune response from vaccinated dams to their offspring.

Maternally derived adaptive cellular immune responses play a critical role in protecting newborn piglets challenged with PCV2 at 3 weeks of age.

INTRODUCTION

Porcine circovirus type 2 (PCV2) is associated with a number of diseases and syndromes collectively known as porcine circovirus-associated disease (PCVAD), including post-weaning multisystemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome (PDNS), reproductive failure, porcine respiratory disease complex and exudative epidermitis (Chae, 2004, 2005). PMWS, the most common and the most severe form of PCVAD, is clinically characterized by wasting, progressive weight loss, enlarged lymph nodes and dyspnoea (Chae, 2004, 2005). Since PCV2 vaccines were introduced onto the world market in 2006, vaccination has become an important tool for controlling PMWS.

Several commercial PCV2 vaccines are available in the global market, including an inactivated PCV2 vaccine that has been administered to sows (Pejsak et al., 2010). Vaccination of sows provides their offspring with maternal antibody protection against PMWS and subclinical infection of PCV2 and may control PCV2 infections (Kurmann et al., 2011; Pejsak et al., 2010). However, the efficacy of the piglet vaccine which is different from sow vaccine used in this study is unrelated to the serum antibody response. Instead, it is due to the cellular immune response induced by the PCV2 vaccine (Fort et al., 2009b; Kim et al., 2011; Opriessnig et al., 2009). These results suggest that the passive transfer of the maternally derived cellular, rather than the humoral, immune response may play a critical role in controlling PCV2 infection in newborn piglets later. The passive transfer of the maternally derived PCV2-specific cellular immune response to piglets has not been studied. Hence, the objective of the present study was to evaluate the (i) passive transfer of maternally derived functional PCV2-specific lymphocytes to newborn piglets from sows immunized with the PCV2 vaccine and (ii) the functional role of the maternally derived PCV2-specific cellular immune response in protecting newborn piglets from challenge with PCV2.

RESULTS

Body weight

Significant differences in mean body weight were not detected among the four groups until 14 days post-inoculation (p.i.).

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Body weight in challenged piglets from non-vaccinated sows (PT04) was significantly lower at 21 (8.4 ± 4.6 kg, P < 0.05) and 28 (10.1 ± 4.3 kg, P < 0.001) days p.i. compared with piglets in groups PT01 (12.9 ± 2.1 kg at 21 days p.i. and 18.3 ± 1.2 kg at 28 days p.i.), PT02 (12.1 ± 1.2 kg at 21 days p.i. and 15.8 ± 1.4 kg at 28 days p.i.) and PT03 (12.5 ± 0.8 kg at 21 days p.i. and 15.5 ± 1.3 kg at 28 days p.i.).

**Anti-PCV2 ELISA and serum virus neutralization titre**

Anti-PCV2 titres were determined by ELISA and serum neutralization (SVN) titre in vaccinated sows between 14 and 0 days antepartum, with a mean titre of 6.5 ± 0.4 log₂ at 0 days antepartum in vaccinated sows. No serum antibody titres (mean ELISA EU titre: 5389 ± 602 for PT01 and 5427 ± 309 for PT02 and mean SVN titre: 6.50 ± 1.80 log₂ for PT01 and 6.40 ± 0.42 log₂ for PT02). From −21 to 28 days p.i., the anti-PCV2 titres in the non-challenged piglets (PT01) decreased gradually from 5389 ± 402 EU to 2532 ± 115 EU and from 6.50 ± 1.80 log₂ to 4.56 ± 1.70 log₂, in ELISA and SVN, respectively. After challenge, seroconversion of SVN titre started at 0–7 days p.i. in the challenged piglets from vaccinated (PT02) and from non-vaccinated (PT04) sows. No SVN titres were detected in serum from non-challenged piglets (PT03) (Fig. 1).

**Quantification of PCV2 DNA**

No genomic copies of PCV2 were detected in any piglets' serum samples at −21, −20 or 0 days p.i. No genomic copies of PCV2 were detected in any of the serum samples from non-challenged piglets (PT01 and PT03). Among challenged pigs from vaccinated sows (PT02), ten were viraemic at 7 days p.i., nine were viraemic at 14 days p.i. and eight were viraemic at 28 days p.i. All challenged pigs from non-vaccinated sows (PT04) were viraemic throughout the experiment. The number of genomic copies of PCV2 in serum was significantly lower in challenged piglets from vaccinated sows (PT02) than in challenged piglets from non-vaccinated sows (PT04), from 7 to 28 days p.i. (P < 0.05 or P < 0.001) (Fig. 2).

**Identifying lymphocyte subsets**

In the flow-cytometry analyses, most changes in the relative proportions of lymphocyte subsets could be attributed to the treatments received. At 1 day post-partum, vaccinated sows showed an increase in the relative proportions of CD4⁺CD8⁺ cells (59.19 ± 3.75 % vs 38.36 ± 7.59 %, P < 0.0001), CD3⁺CD4⁺ cells (44.95 ± 0.86 % vs 41.53 ± 6.32 %, P = 0.044), CD3⁺CD8⁺ cells (73.23 ± 4.00 % vs 60.33 ± 11.68 %, P = 0.029) and CD25⁺ cells (13.79 ± 1.46 % vs 10.18 ± 1.76 %, P = 0.017) compared with non-vaccinated sows. Colostrums of vaccinated sows increased proportions of CD4⁺CD8⁺ cells (30.33 ± 2.52 % vs 25.74 ± 1.48 %, P = 0.003), CD3⁺CD4⁺ cells (19.60 ± 0.86 % vs 17.15 ± 1.52 %, P = 0.040), CD3⁺CD8⁺ cells (49.58 ± 0.73 % vs 40.68 ± 1.78 %, P < 0.0001) and CD25⁺ cells (8.83 ± 1.58 % vs 6.16 ± 0.88 %, P = 0.049) compared with those from non-vaccinated sows.

![Fig. 1. Mean values of the serum neutralization antibody titre in the different groups. Non-challenged piglets (PT01; ▲) and challenged piglets (PT02; ■) from vaccinated sows, and non-challenged piglets (PT03; △) and challenged piglets (PT04; □) from non-vaccinated sows. Variation is expressed as SD. Significant difference is indicated at P-value < 0.05* or < 0.001†.](http://vir.sgmjournals.org)

![Fig. 2. Mean values of the genomic copy number of PCV2 DNA in blood samples from challenged piglets (PT02; ■) from vaccinated sows and challenged piglets (PT04; □) from non-vaccinated sows. Variation is expressed as SD. Significant difference is indicated at P-value < 0.05* or < 0.001†.](http://vir.sgmjournals.org)
After ingesting colostrums, the relative proportions of CD3⁺CD4⁺ cells were significantly different at day −20 ($P<0.001$) and day 0 ($P<0.05$), and the proportions of CD25 cells were significantly different at day 0 ($P<0.001$) in the peripheral blood mononuclear cells (PBMCs) from piglets vaccinated sows (PT01 and PT02) and in those from piglets from non-vaccinated sows (PT04). After challenge with PCV2, piglets from vaccinated sows (PT02) had significantly different relative proportions of all tested lymphocyte subsets except CD4⁺CD8⁺ and CD25 cells at 14 and 21 days p.i. in the PBMCs compared with challenged piglets from non-vaccinated sows (PT04) (Fig. 3).

After ingesting colostrums, the relative proportions of CD3⁺CD4⁺ cells were significantly different at day −20 (28.42 ± 5.50 % for PT01 vs 19.08 ± 1.44 % for PT03, $P<0.001$) and day 0 (20.44 ± 5.18 % for PT01 vs 14.44 ± 4.1 % for PT03, $P<0.05$), and the proportions of CD25 cells were significantly different at day 0 (5.01 ± 1.21 % for PT01 vs 1.21 ± 0.3 % for PT03, $P<0.05$) in the PBMCs from piglets from vaccinated sows (PT01) and those from piglets from non-vaccinated sows (PT03).

**PCV2-specific lymphocyte stimulation assay**

The stimulation index (SI) value for colostrums from vaccinated sows was significantly greater compared with that of non-vaccinated sows. There was no difference in the proliferation response of PBMCs stimulated by phytohaemagglutinin (PHA) in piglets from vaccinated and non-vaccinated sows. However, stimulation with PCV2 antigen resulted in a higher proliferative response of PBMCs in piglets from vaccinated sows (PT01 and PT02) compared with piglets from non-vaccinated sows (PT03 and PT04). The SI value for PT01 and PT02 piglets was significantly higher at −20 days p.i. (1 day of age), than for PT03 and PT04 piglets ($P<0.001$) (Fig. 4).

### PCV2-specific gamma interferon (IFN-γ)-secreting cells

No PCV2-specific IFN-γ-secreting cells (IFN-γ-SCs) were detected in the PBMCs from piglets of either vaccinated or non-vaccinated sows before colostrums ingestion (at −21 days p.i.). After colostrums ingestion, PCV2-specific IFN-γ-SCs were detected in the PBMCs of piglets from vaccinated sows (PT01 and PT02). PCV2-specific IFN-γ-SCs gradually decreased in challenged piglets (PT02) until 0 day p.i. and gradually increased after PCV2 challenge. PCV2-specific IFN-γ-SCs developed in challenged piglets from non-vaccinated sows (PT04) between 14 and 21 days p.i. No PCV2-specific IFN-γ-SCs were detected in the PBMCs from non-challenged piglets (PT03) (Fig. 5).

### Delayed type hypersensitivity (DTH)

At 36 h after intradermal injection of PCV2 antigen, piglets from vaccinated sows (PT01 and PT02) showed skin reactions characterized by circumscribed and often erythematous nodules. The nodules regressed slowly after 48 h and left no scar tissue. No erythematous nodules were observed in piglets from non-vaccinated sows (PT03 and PT04). Piglets from vaccinated and non-vaccinated sows showed DTH responses to the non-specific mitogen PHA. No difference in the magnitude of PHA-induced DTH response size was observed between piglets from the vaccinated and non-vaccinated sows (10.82 ± 0.78 mm vs 10.99 ± 0.41 mm, $P=0.892$). Piglets from vaccinated sows had significantly greater PCV2-specific DTH responses (14.07 ± 2.73 mm) than piglets from non-vaccinated sows (0.80 ± 0.30 mm, $P<0.0001$). No erythematous nodules were observed at saline injection sites for any piglets.
Lymph node lesions

Mild to moderate lymphoid depletion and histiocytic replacement of lymphoid follicles associated with PCV2 infection was observed in the lymph nodes from challenged piglets from non-vaccinated sows (PT04). No histopathological lymph node lesions were observed in piglets from any other group. The morphometric analysis of histopathological changes in the lymph nodes showed significantly reduced scores in challenged piglets from vaccinated sows (PT02) (lesion score 1.45±0.69) compared with piglets from non-vaccinated sows (PT04) (lesion score 3.17±0.75, P=0.001).

The mean number of PCV2-positive cells per unit area of the lymph node in challenged piglets from non-vaccinated sows (PT04) (immunohistochemical score 88.12±29.15) was significantly greater than that of challenged piglets from vaccinated sows (PT02) (immunohistochemical score 29.94±10.69, P<0.001).

Statistical analysis

In challenged piglets from vaccinated (PT02) and non-vaccinated sows (PT04), the number of genomic copies of PCV2 in the blood was correlated with SVN titre (PT02: r²=0.480, P=0.032 and PT04: r²=0.545, P=0.013), PCV2-specific IFN-γ-SCs (PT02: r²=0.532, P=0.007 and PT04: r²=0.799, P=0.001), histopathological lesion score (PT02: r²=0.826, P=0.001 and PT04: r²=0.469, P=0.048) and immunohistochemical score (PT02: r²=0.789, P=0.001 and PT04: r²=0.597, P=0.003).

DISCUSSION

The present study has demonstrated that maternally derived PCV2-specific cellular immunity can be passively transferred to newborn piglets by dam vaccination against PCV2. Antigen-specific DTH responses and lymphocyte proliferation indicate an anti-PCV2-specific adaptive cellular response. Furthermore, piglets that received maternal PCV2-specific memory T lymphocytes mount DTH reactions in response to intradermal injection of PCV2 antigen. The DTH response was PCV2-specific because skin lesions characteristic of the DTH response were only detected upon intradermal injection with PCV2 antigens and not with mock preparations. The PCV2-specific proliferation was also detected by stimulating the PBMCs with PCV2 antigen, whereas colostral lymphocytes from non-vaccinated sows did not proliferate following stimulation. Lymphocytes isolated from piglets before suckling did not respond to PCV2 stimulation (data not shown), which indicates that newborn piglets are naïve to PCV2 at birth. Therefore, the DTH and lymphocyte proliferation responses observed in newborn piglets from vaccinated sows were due to the action of PCV2 antigen-specific maternal colostral lymphocytes.

Serum PCV2 contributes to the development of PCVAD, such as PMWS. Viraemia contributes to viral distribution throughout the lymphoid tissues (Kim et al., 2003). High PCV2 viraemia has been associated with the development of PCVAD (Larochelle et al., 2003; Meerts et al., 2006). Hence, reduction of viraemia may protect piglets from PCV2 infection. Reducing the PCV2 load in the blood coincided with the appearance of both serum virus neutralizing antibodies and PCV2-specific IFN-γ-SCs, as reported previously (Fort et al., 2009a). Moreover, a good correlation was observed between serum neutralizing antibodies and decreased PCV2 replication with a lack of clinical PCVAD under experimental (Meerts et al., 2005, 2006) and field (Fort et al., 2007) conditions when PCVAD pigs were compared with pigs subclinically infected with PCV2. The development of IFN-γ-SCs is another key component of the developing anti-PCV2 adaptive cellular response. In the present study, passively transferred PCV2-specific IFN-γ-SCs were observed in newborn piglets from vaccinated, but not non-vaccinated, sows. The level of immune protection against PCV2 infection depends on the development of mechanisms that reduce PCV2 spread and develop memory T lymphocytes. We confirmed that maternally derived PCV2-specific T lymphocytes mount an immune response, which highlights the clinical importance of protective T lymphocyte-mediated cellular responses to PCV2.

In the present study, the numbers of CD4⁺CD8⁺ and CD3⁺CD4⁺ lymphocytes were decreased, while the number of CD3⁺CD8⁺ was unchanged in the challenged piglets from non-vaccinated sows (PT04) compared with challenged piglets from vaccinated sows (PT02). This observation indicated that CD3⁺ and CD4⁺ lymphocytes were reduced by PCV2 infection. These results agree with previous findings of reduced CD3⁺ and CD4⁺ cells in pigs with PMWS, while absolute and relative CD8⁺ cell numbers are similar in both normal and diseased pigs.
(Segalés et al., 2001). A selective loss of CD3+ and CD4+ cells during PCV2 infection may impair the immune system in pigs and result in co-infections with other viral and bacterial pathogens (Kim et al., 2002; Pallarès et al., 2002).

In conclusion, our results suggest that maternally derived colostral lymphocytes from sows immunized with PCV2 vaccine transferred into the neonates’ circulation and participated in the cellular immune response in a PCV2 antigen-specific manner. Our findings are supported by in vivo DTH responses and in vitro lymphocyte proliferation and the presence of IFN-γ-SCs. To our knowledge, this is the first confirmation of the transfer of a maternally derived PCV2-specific cellular immune response from vaccinated dams to their offspring. These maternally derived adaptive cellular immune responses play a critical role in protecting newborn piglets from PCV2 infection even if PCV2 infection should occur as late as 8–14 weeks of age. Further studies are needed to confirm the duration of maternally derived adaptive cellular immunity to protect newborn piglets against PCV2 infection.

**METHODS**

Sow experiment design. Twenty conventional crossbred pregnant sows with a second parity were obtained from a porcine reproductive and respiratory syndrome virus (PRRSV)-free herd. All sows were serologically negative for PCV2, porcine parvovirus, PRRSV and swine influenza virus. Sows were moved to a research facility, housed individually in separate rooms and randomly allocated into one of two groups. Group 1 (ST01, n=10) sows were vaccinated intramuscularly twice at 5 and 2 weeks antepartum with a 2 ml dose of a commercial inactivated PCV2 vaccine (Circovac) according to the manufacturer’s recommendation. Group 2 (ST02, n=10) sows served as non-vaccinated controls.

At parturition, sows farrowed naturally and no cross-fostering was applied to ensure maternal lymphocyte transfer into blood circulation of piglets. The ten vaccinated sows delivered 105 live and three stillborn piglets. The ten non-vaccinated sows delivered 107 live and two stillborn piglets. A total of 80 newborn piglets (four piglets per sow) were randomly selected on the day of birth and notched in their ear with a unique identification number. After selection, selected piglets stayed with their sows until weaning at 3 weeks of age in a separate room within the facility and unselected piglets were removed from the sow.

Piglet experimental design. At weaning, 40 piglets randomly selected once from the litters of each of the vaccinated sows (ST01) were allocated into group 1 (PT01, n=20) or 2 (PT02, n=20). Another 40 piglets (four piglets per sow) randomly selected from the litters of non-vaccinated sows (ST02) were allocated into group 3 (PT03, n=20) or 4 (PT04, n=20). Piglets in groups 2 (PT02) and 4 (PT04) were intranasally administered 2 ml PCV2b (strain SNUVR000463; 5th passage) pool containing 1.0×10⁷ TCID₅₀ ml⁻¹ (Kim et al., 2003). The same amount of sterile PBS was intranasally administered in non-challenged pigs in groups 1 (PT01) and 3 (PT03). The live weight of each pig was measured at −21, −14, −7, 0, 7, 14, 21 and 28 days p.i.

All piglets were tranquillized by an intravenous injection of azaperon (Stresnil; Janssen Pharmaceuticals) and euthanized by electrocution at 28 days p.i. Tissue samples were collected at necropsy and processed for histopathological examination and immunohistochemistry as described previously (Kim et al., 2011). All methods were approved by the Seoul National University Institutional Animal Care and Use Committee.

Sample collection. Blood samples from each sow were collected by jugular venipuncture at 35, 14 and 0 days antepartum. Ten millilitres of colostrum was manually collected from three udders in 50 ml conical tubes from all sows after disinfection with alcohol. Eighty newborn piglets (four piglets per sow) were randomly selected on the day of birth and subjected to blood collection at −21 (0 h before colostrums ingestion), −20 (24 h after colostrums ingestion; 1 day old), 0 (21 days old), 7 (28 days old), 14 (35 days old), 21 (42 days old) and 28 (49 days old) days p.i.

Serology. Serum samples were stored at −20 °C and tested using a commercially available ELISA (SeraLisa PCV2 Ab Mono Blocking ELISA; Symbiotics), SVN tests were performed as described previously (Allan et al., 1994; Pognachnyy et al., 2000). The air-dried cells were stained for PCV2 antigens using an immunoperoxidase assay (Rodríguez-Arrioja et al., 2000).

Quantification of PCV2 DNA. DNA extraction from collected serum samples was performed using the QIAamp DNA mini kit (Qiagen). DNA extracts were used to quantify PCV2 genomic DNA copy numbers by real-time PCR as described previously (Gagnon et al., 2008).

Flow cytometry. PBMCs were prepared as described previously (Williams, 1993) and were incubated with R-PE- or FITC-conjugated mouse mAbs [anti-swine CD3 (R-PE), CD4a (R-PE and FITC) and CD8a (FITC); SouthernBiotech] or non-conjugated anti-swine CD25 (VMRD) for 30 min at 4 °C in the dark and washed twice. For CD3, CD4a and CD8a staining, cells stained with conjugated antibodies were resuspended immediately in supplemented RPMI 1640 medium. For CD25 staining, cells stained with primary non-conjugated antibodies were incubated with FITC-conjugated goat anti-mouse IgG (Santa Cruz Biotechnology) for 30 min at 4 °C in the dark, washed twice and resuspended in medium before evaluation. Cells were analysed using a FACScalibur flow cytometer (Becton Dickinson) as described previously (Sosa et al., 2009).

PCV2-specific lymphocyte stimulation assay. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay for PCV2-specific lymphocyte stimulation was performed as described previously (Lee & Ge, 2010; Wang et al., 2011) with slight modifications. PBMCs were stimulated with PCV2 antigen. Cells were adjusted to a density of 2×10⁶ cells per well in 180 μl, placed in 96-well tissue culture plates (Corning Laboratories Science Products; Corning) and incubated with 20 μl PCV2 antigen, PHA (Roche Diagnostics GmbH) as a positive control, or PBS as a negative control for 60 h at 37 °C in a 5 % humidified CO₂ atmosphere. Following this incubation, 100 μl MTT (0.5 mg ml⁻¹; aMReSCo) was added to each well and the cells were incubated for another 4 h. The formazan was dissolved with 100 μl DMSO and the plates were shaken lightly for 30 min. The reduction of MTT was quantified by absorbance at a wavelength of 550 nm using a microplate reader (model-550; Bio-Rad). The results were expressed as the mean differential SI (SI=optical density with PCV2 antigen/optical density without the antigen) of each sample.

Enzyme-linked immunospot assay (ELISPOT). The numbers of PCV2-specific IFN-γ-SCs in PBMCs were determined by a commercial linked ELISPOT assay kit (MABTECH) using a commercial mAb (swine IFN-γ Cytosets kits; Biosource) following a previously described protocol (Diaz & Mateu, 2005).

Preparation of PCV2 antigen. PCV2 antigen was prepared as described previously (Bautista & Molitor, 1997). The same PCV2
strain, as for challenge of the pigs, was propagated in PCV-free PK15 cells to a titre of $10^8$ TCID$_{50}$ ml$^{-1}$ and treated with two freeze–thaw cycles. Inactivation was confirmed by the absence of virus antigen in PK15 cells as determined by immunoperoxidase assay (Rodriguez-Arrioja et al., 2000).

**DTH.** DTH test was performed on 80 piglets (four piglets per sow) at 4 days of age. Piglets were injected intradermally on the left inguinal area with 250 μg partially purified PCV2 antigen from infected PK15 cells. PHA (20 μg ml$^{-1}$ in 0.1 ml) and saline (0.1 ml) were used as positive and negative controls, respectively. Induration was measured with a micrometer after 36 h. Orthogonal diameters of the induration were recorded.

**Histopathology.** For the morphometric analysis of histopathological changes in lymph nodes, three superficial inguinal lymph node sections were examined blindly. The scores ranged from 0 (normal, i.e. no lymphoid depletion or granulomatous replacement) to 5 (severe lymphoid depletion and granulomatous replacement) as described previously (Kim et al., 2011).

**Immunohistochemistry.** Immunohistochemistry was performed using a polyclonal anti-PCV2 antibody as previously described (Kim et al., 2011). Single sections (10 randomly selected fields) from each formalin-fixed lymph node sample were used for the virus-infected morphometric analysis, as described previously (Kim et al., 2003).

**Statistical analysis.** Summary statistics were calculated for all groups to assess the overall quality of the data, including normality. For single comparisons, ANOVA with a post-hoc Tukey’s test was used to compare the primary variables (sow and colostrum lymphocyte subsets, DTH response, PCV2-specific lymphocyte stimulation assay, histopathological lesion score and immunohistochemical score) among groups. The continuous data for PCV2 serology, PCV2 DNA quantification, PCV2-specific IFN-γ-SCs and lymphocyte subsets of the piglets were analysed using an ANOVA for each time point. When a one-way ANOVA test had a significance of $P<0.05$, Tukey’s Honestly Significant Difference test was used to determine the significance of individuals between group differences. The Pearson’s correlation coefficient was used to assess the relationship among viraemia, serum virus neutralization titre, PCV2-specific IFN-γ-SCs and the Spearman’s rank correlation coefficient was used to assess histopathological lesion score and immunohistochemical score. A value of $P<0.05$ was considered significant.

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