The effect of porcine circovirus type 2 (PCV2) vaccination of male piglets on sperm quality at the age of puberty

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Abstract

The objective of the study was to investigate the effect of porcine circovirus type 2 (PCV2) vaccination in piglets on sperm quality of young boars. A total of 136 sows were divided in four groups of 34 animals each (17 vaccinated with Circovac®, Merial and 17 unvaccinated in each group). A total of 1 200 piglets were selected, half of which were vaccinated against PCV2 on the 21st day (Porcilis® PCV, MSD) and the other half was left unvaccinated. Four groups of 300 pigs each were formed as follows: PS group (vaccinated sows + piglets), S group (vaccinated sows + unvaccinated piglets), P group (unvaccinated sows + vaccinated piglets), C group (unvaccinated sows + piglets). Furthermore, 80 boars (20 piglets per group) were selected and slaughtered at the age of 5.5 months and weight of 95 ± 5.5 kg and their epididymal sperm was collected and evaluated for motility and kinetics, concentration and morphology. Additionally, 10 pigs from each group were used for blood sampling and serological testing for PCV2 IgM and IgG antibodies at the age of 21, 70, 110 and 150 days. The IgG and IgM patterns suggested that the piglets were coming into contact with PCV2 early in life. The S group demonstrated significantly lower curvilinear velocity (VCL, μm/s), amplitude of lateral head displacement (ALH, μm) and significantly higher head abnormalities (%) compared to other groups ($P < 0.05$). In conclusion, vaccinated young boars showed some improved epididymal sperm kinetic indices and head morphology.

Boar, epididymal sperm, sperm kinetics, head morphology

Porcine circovirus type 2 (PCV2) was first described in 1997 as the causative agent of the post weaning multisystemic wasting syndrome (Clark 1997). The first reports for late-term abortions, birth of stillborn and mummified piglets due to PCV2 infection go back as far as 1999 (West et al. 1999). The main route of transmission of PCV2 in swine populations is faecal-oral (Segales et al. 2005). Detection of PCV2 DNA has been reported in secretions and excretions including faeces, urine, saliva, ocular fluid, nasal secretions, colostrum, and semen of infected pigs (Shibata et al. 2003). The virus infects the embryo, while the sow can be infected either horizontally or by infected boar semen.

Mature boars infected with PCV2 (naturally or experimentally) are often free of clinical signs but they can shed the virus in semen for a long period (Gerber et al. 2010). The PCV2 viral antigen has been detected in the reproductive organs (testes) and tissues (germinal epithelial cells, epididymis) of naturally and experimentally infected boars. When semen samples of naturally infected boars, between 33.9–149.3 weeks of age were tested, it was more likely semen that had been collected from younger boars to be positive to PCV2 DNA. However, PCV2 DNA in semen does not appear to have detrimental effects on sperm morphology (McIntosh et al. 2006).

Several commercial PCV2 vaccines are currently available for use in piglets or adult animals, considered to be able to reduce the clinical signs of a PCV2 infection at a farm level. It has also been reported that vaccination against PCV2 in experimentally (Seo et al.

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2011) or naturally (Alberti et al. 2011) infected mature boars can decrease the duration of viral shedding in semen. Since a very high percentage of boars become naturally infected by PCV2 later in life, it is of importance to assess whether the vaccination early in the boar's life with a commercial PCV2 vaccine still has a beneficial effect. Therefore, the objective of the present study was to determine the effect of vaccination against PCV2 early in the boar's life with or without the vaccination of their mothers on epididymal semen quality.

Materials and Methods

The study was approved by the Ethics Committee on Animal Use (approval number: 6365) of the School of Veterinary Medicine, Aristotle University of Thessaloniki, Greece, and all operations were carried out according to the university's guidelines for animal research.

For the needs of the study, the vaccine Porcilis PCV MSD-Intervet (Madison NY, USA) was used which induces immunity against PCV2. It contains PCV2 ORF2 subunit antigen (≥ 4.5 $\log_2$ ELISA units/2 ml) expressed on baculovirus. It was administered intramuscularly twice at a dose of 2.0 ml. The onset of immunity occurs in 2 weeks, with a duration of 22 weeks (Reg.No.G.3936/Act 36/1947).

A Greek pig farm of 670 sows was selected for the trial. The herd was naturally infected with PCV2 according to routine serological testing performed prior to the beginning of our experiment. A total of 136 sows were divided into four groups of 34 animals each (17 vaccinated and 17 unvaccinated in each group) with an interval of one week between the groups according to the production system of the farm; resulting in 68 vaccinated and 68 unvaccinated sows. The vaccinated sows received double vaccination at 60 and 30 days before farrowing with a commercial vaccine (Circovac®, Merial, Lyon, France) while the unvaccinated sows received placebo (Normal Saline) on the same days.

A total of 1 200 born piglets were selected (600 piglets from vaccinated and 600 piglets from unvaccinated sows) to assess the efficacy of different vaccination schedules against PCV2 on the piglets' health and performance (under publication). Vaccination and placebo (Diluvac Forte®, Diluent, MSD-Intervet, Madison NY, USA) were administered at 21 days of the piglet's life (according to the manufacturer's instructions) with a commercial PCV2 vaccine (Porcilis® PCV, MSD-Intervet, Madison NY, USA) and in accordance with the welfare rules.

This resulted in 4 groups of piglets divided as follows: a) 300 pigs: vaccinated sows - vaccinated piglets (SP group), b) 300 pigs: vaccinated sows - unvaccinated piglets (S group), c) 300 pigs: unvaccinated sows - vaccinated piglets (P group), d) 300 pigs: unvaccinated sows - unvaccinated piglets (C, control group).

Piglets of each group were marked with an ear tag in the left ear in colour according to the experimental group to which it belonged. At the age of 21 days, 20 healthy male piglets from each group (in total 80) were selected for serological and semen tests. All selected piglets were healthy at the beginning of the experimental period (no diarrhoea, coughing, or morphological abnormalities), their body weight was over 5 kg and they came from sows of 4–6 parities. Additionally, ten pigs from each group were selected and marked with red ear tag to be used for blood sampling on days 21, 70, 110 and 150 of life to detect the presence of IgM and IgG against PCV2.

Epididymal sperm sample collection

All male piglets from each group at the age of 5.5 months and weight of 95 ± 5.5 kg were slaughtered and both testes and epididymides contained in scrotal sacs were immediately removed, placed in bags into an isothermal box (4–5 °C) and transferred to the laboratory in less than 2 h. Each epididymal cauda was carefully separated from the testis using a scalpel blade and was placed in a Petri dish containing 5 ml of pre-warmed phosphate buffered saline (PBS) buffer at 37 °C. After that, many sections by the blade were performed in each separated epididymal cauda. Then they were washed by a standard volume of 5 ml of PBS to collect epididymal spermatozoa into the Petri dish. The collected epididymal sperm was washed in PBS by centrifugation (400 × *g*, 10 min), the supernatant was removed, and 1 ml of PBS was added to resuspend the sperm sample for the following analysis process.

Epididymal sperm quality evaluation

Sperm kinetics were evaluated using a computer-assisted semen analysis (CASA-Sperm Class Analyser®, Microptic S.L., Automatic Diagnostic Systems, software version SCA® v.5.2.0.0, Spain) and a microscope (× 100; AXIO Scope A1, Zeiss, Germany) accomplished with a heating stage. Ten µl of each semen sample were placed on the preheated Makler chamber (Makler® counting chamber, 10 µm deep, Sefi Medical Instruments, Israel) at 37 °C, and triplicates of the assessment were performed. The following CASA motility indicators and kinetics were estimated: total motility (%), progressive motility (%), rapid, slow and immotile spermatozoa (%), VCL-curvilinear velocity (µm/s), VSL-straight line velocity (µm/s), VAP-average path velocity (µm/s), ALH-amplitude of lateral head displacement (µm), BCF-beat/cross-frequency (Hz), LIN-linearity (VSL/VCL × 100), STR-straightness (VSL/VAP × 100), and WOB-wobble (VAP/VCL × 100).

The CASA system software was configured as follows: eight fields and > 500 spermatozoa, 25 frames/s, region of particle control 10–18 µm, progressive movement of > 45% of indicator STR, circumferential movement <

50% of indicator LIN, field depth of 10 μm. The objects incorrectly identified as spermatozoa were manually removed from each picture.

Assessment of sperm morphology

Sperm morphology was evaluated by the SpermBlue staining method (SpermBlue® 08029, Microptic S.L., Spain) according to the manufacturer's instructions. Spermatozoa were assessed microscopically (× 400) and classified as normal or with morphological abnormalities (head, neck, tail). Totally, 200 spermatozoa were scored and the % ratio per sample was calculated.

Determination of sperm concentration

The concentration of each epididymal sperm sample was determined according to Pruneda et al. (2005) with some modifications. Briefly, after the transfer of the epididymites in the lab and before any other process, a small piece of 30 mg of tissue was gently cut from each epididymis region to be used for the determination of sperm concentration. Those tissues were bisected further into a Petri dish with PBS. Then a standard volume of 2 ml of PBS was added and the solution was collected and centrifuged at 400 × g for 10 min. After centrifugation, the supernatant was discarded, the sperm pellet was resuspended in 2 ml PBS, and the sperm concentration was determined by an improved Neubauer haemocytometer (Neubauer Improved Counting Chamber, Paul Marienfeld Gmbh & Co. KG, Germany).

Serology

The presence of IgM and IgG against PCV2 was detected at Dierengezondeheidszorg Vlaanderen vzw, Torhout, Belgium using a commercially available ELISA kit (Ingezim Circovirus IgG/IgM ELISA, Ingenasa, Madrid, Spain) based on the use of 3 monoclonal antibodies (MAb) specific for porcine circovirus, porcine IgM specific mAb, porcine IgG specific mAb and a recombinant antigen. With this assay it is possible to differentially detect IgM and IgG antibodies specific to circovirus type 2 in porcine serum samples. The ELISA tests were performed according to the manufacturer's instructions.

The statistical analysis performed using the Statistical Analysis Systems version 9.3 (SAS Institute Inc., Cary, N.C., USA). Normality of the data was tested using Shapiro-Wilk test (PROC UNIVARIATE). Indicators that did not follow normal distribution were normalized by square root transformation. For reasons of clarity, the means and SEM of the data were presented. Statistical analysis was conducted with type III Anova Model of General Linear Models. Pairwise comparisons were performed with Tukey's Studentized Range (HSD) test. Significant difference was defined as $P < 0.05$.

Results

The number of samples positive for IgG antibodies was high in groups P and SP four weeks after vaccination until the start of the finishing period, whereas the samples positive for IgM antibodies were high between 7 and 10 weeks of age. The number of samples positive for IgG antibodies was high in groups S and C during the finishing period, whereas the samples positive for IgM antibodies were high at 7 weeks of age and at the beginning of the finishing period. The relative results are summarized in Figs 1, 2.

The results of epididymal sperm motility are presented in Table 1. Even though the values found in vaccinated groups of piglets were higher, no significant differences were observed between the 4 treatment groups. The results for epididymal sperm kinetics are presented in Table 2. Significantly lower values of ALH were noticed in group S compared to groups

Table 1. Assessment of sperm motility by the computer assisted semen analysis system (CASA) (mean ± SEM).

Indicator	Groups				*P*-value
	C n = 19	P n = 20	SP n = 20	S n = 19	
Progressive motility (%)	25.09 ± 5.16	36.49 ± 5.69	32.97 ± 5.75	23.30 ± 5.74	0.294
Total motility (%)	43.41 ± 7.29	62.05 ± 6.87	55.77 ± 7.06	39.23 ± 7.33	0.093

SP = vaccinated sows - vaccinated piglets group; S = vaccinated sows - unvaccinated piglets group; P = unvaccinated sows - vaccinated piglets group; C = unvaccinated sows - unvaccinated piglets group; SEM - standard error of the mean

P and SP ($P = 0.002$). Similarly, VCL values were lower for group S compared to groups P and SP ($P = 0.045$). No significant differences were found for the remaining indicators between groups. The results for epididymal sperm concentration and morphology are presented in Table 3. Head abnormalities (%) were significantly higher in group S compared to group P ($P = 0.033$). No significant differences were noticed for the remaining tested indicators between groups.

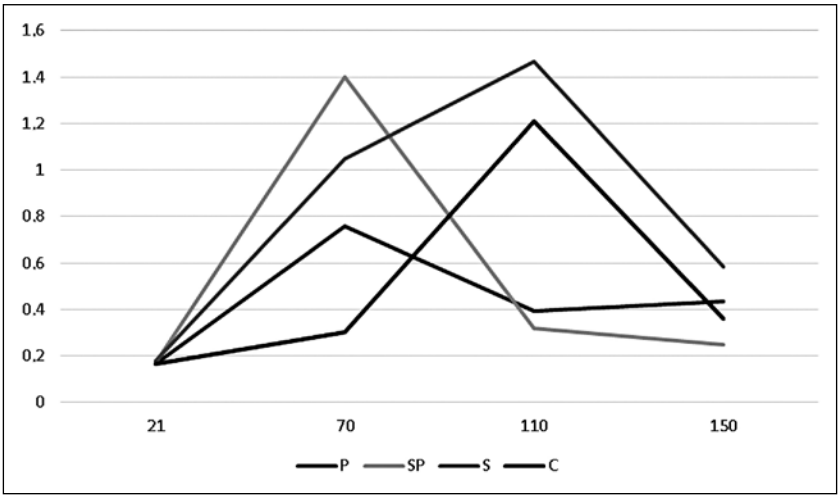

Fig. 1. Mean optical density (OD) values for IgM antibodies by vaccination group and sampling day.
SP = vaccinated sows - vaccinated piglets group; S = vaccinated sows - unvaccinated piglets group; P = unvaccinated sows - vaccinated piglets group; C = unvaccinated sows - unvaccinated piglets group

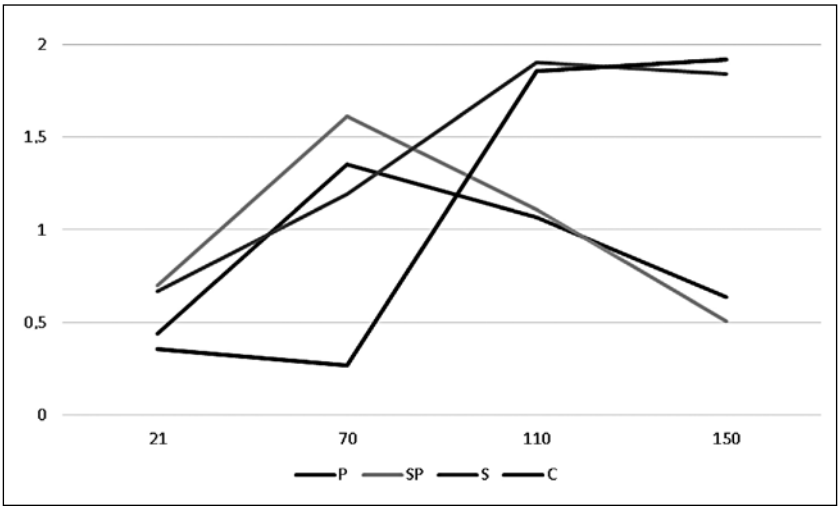

Fig. 2. Mean optical density (OD) values for IgG antibodies by vaccination group and sampling day.
SP = vaccinated sows - vaccinated piglets group; S = vaccinated sows - unvaccinated piglets group; P = unvaccinated sows - vaccinated piglets group; C = unvaccinated sows - unvaccinated piglets group

Table 2. Assessment of sperm kinetics by computer assisted semen analysis system (CASA) (mean ± SEM).

Indicator	Groups				*P*-value
	C n = 19	P n = 20	SP n = 20	S n = 19	
Immotile spermatozoa (%)	56.59 ± 7.29	37.95 ± 6.87	44.24 ± 7.06	60.77 ± 7.33	0.093
Spermatozoa with rapid movement (%)	23.88 ± 5.35	39.34 ± 7.22	35.92 ± 6.51	21.90 ± 6.27	0.149
Spermatozoa with medium movement (%)	9.50 ± 1.53	10.79 ± 1.01	9.17 ± 1.10	8.08 ± 1.42	0.512
Spermatozoa with slow movement (%)	10.02 ± 1.36	11.91 ± 1.23	10.70 ± 1.04	9.25 ± 1.35	0.487
VCL	47.58 ± 3.51 [ab]	56.30 ± 4.82 [a]	58.01 ± 4.81 [a]	41.94 ± 4.59 [b]	0.045
VSL	16.64 ± 1.87	22.35 ± 2.54	20.21 ± 2.70	17.43 ± 2.67	0.345
VAP	25.52 ± 2.48	34.12 ± 3.77	31.53 ± 3.65	24.95 ± 3.68	0.172
LIN	35.10 ± 2.80	38.23 ± 2.22	32.79 ± 2.44	38.33 ± 3.12	0.384
STR	64.33 ± 2.18	64.77 ± 1.61	61.89 ± 1.68	66.43 ± 1.71	0.359
WOB	53.33 ± 2.69	58.34 ± 2.49	51.94 ± 2.60	55.15 ± 3.06	0.371
ALH	2.57 ± 0.14 [ab]	2.82 ± 0.15 [a]	3.00 ± 0.18 [a]	2.11 ± 0.19 [b]	0.002
BCF	7.30 ± 0.20	7.38 ± 0.18	7.23 ± 0.35	6.60 ± 0.40	0.238

Different superscripts (a, b) denote significant differences between groups ($P \leq 0.05$)
SP = vaccinated sows - vaccinated piglets group; S = vaccinated sows - unvaccinated piglets group; P = unvaccinated sows - vaccinated piglets group; C = unvaccinated sows - unvaccinated piglets group; SEM - Standard Error of the Mean; VCL - curvilinear velocity (μm/s); VSL-straight line velocity (μm/s); VAP - average path velocity (μm/s); LIN - linearity (VSL/VCL×100); STR - straightness (VSL/VAP×100); WOB - wobble (VAP/VCL×100); ALH - amplitude of lateral head displacement (μm); BCF - beat/cross-frequency (Hz).

Table 3. Assessment of sperm concentration and morphology (mean ± SEM).

Indicator	Groups				*P*-value
	C n = 19	P n = 20	SP n = 20	S n = 19	
Head abnormalities (%)	13.36 ± 7.72 [ab]	8.00 ± 1.43 [a]	12.29 ± 2.22 [ab]	16.76 ± 2.84 [b]	0.033
Neck abnormalities (%)	10.40 ± 2.69	10.40 ± 3.64	9.11 ± 2.97	8.37 ± 1.93	0.948
Tail abnormalities (%)	16.74 ± 2.14	28.63 ± 5.77	23.74 ± 4.15	18.68 ± 3.72	0.750
Total abnormalities (%)	41.55 ± 3.31	47.23 ± 5.27	44.66 ± 3.84	43.82 ± 4.57	0.829
Concentration (millions/ml)	44.92 ± 7.79	72.51 ± 8.95	59.35 ± 8.73	47.06 ± 7.53	0.122

Different superscripts (a, b) denote significant differences between groups ($P \leq 0.05$)
SP = vaccinated sows - vaccinated piglets group; S = vaccinated sows - unvaccinated piglets group; P = unvaccinated sows - vaccinated piglets group; C = unvaccinated sows - unvaccinated piglets group; SEM - Standard Error of the Mean

Discussion

Previous studies reported that vaccination of mature boars can reduce the virus load and duration of shedding in semen without any major impact on the sperm quality or quantity (Caspari et al. 2011). The importance of the presence of PCV2 in semen of premature boars in relation with its quality after puberty is currently unknown and the purpose of this

study was to expand the knowledge on this topic. Our study suggested that an improvement of some semen characteristics occurs in the sperm of vaccinated piglets, indicating that early vaccination against PCV2 could alter the sperm quality. Groups P and PS tended to differ non-significantly ($P = 0.093$) suggesting that vaccination against PCV2 early in life could have a beneficial effect on the sperm quality of young boars. Other authors did not find a positive relation between boar vaccination against PCV2 and sperm quality (Gava et al. 2008; Madson et al. 2009).

This difference in young compared to mature boars is probably due to the time of vaccination and the age of the boars. Vaccination early in life protects effectively against the systemic disease caused by PCV2 (SD-PCV2) (Fraile et al. 2012; Martelli et al. 2013) and decreases the virus load in the testes and sexual glands (Seo et al. 2013). Frequently in the field, piglets are exposed to PCV2 after the 42nd day of life (Larochelle et al. 2003). This means that when vaccination is administered on the 21st day of life, there is enough time for the vaccine to induce a strong immune response till the time of exposure. PCV2 vaccines provoke a strong humoral and cellular immune reaction. Neutralizing and total antibodies reach maximum titres approximately 14 to 21 days post vaccination. Neutralizing antibodies are responsible for the protection against SD-PCV2 and the subclinical form and reduction of the virus load to target organs and tissues.

Especially in epididymis, PCV2 is identified at a higher frequency than in other accessory glands of naturally infected boars (Ciacci-Zanella et al. 2008). Epididymis dysfunction seems to lead to a high incidence of sperm with single bent tails and low motility (Kunavongkrit et al. 1988).

Serology indicates an early infection of piglets with PCV2 though an early vaccination increases the IgG level. The IgG level rose in the vaccinated group indicating that the increase was due to the vaccine and not due to the infection with the PCV2 field virus. Vaccinated piglets are protected against the clinical manifestation of the SD-PCV2 and the load of PCV2 –DNA in organs and tissues including the testes, epididymis and semen is lower than in the unvaccinated groups (Seo et al. 2011).

It is important to note that good sperm kinetics positively affect fertility. Kinetic indicators are major predictors of the litter size; VCL has a major effect on the farrowing rate and ALH on the litter size (Juonala et al. 1998; Broekhuijse et al. 2012). Some researchers showed that PCV2 infections of sows lead to a smaller litter size (O'Connor et al. 2001; Ladekjaer-Mikkelsen et al. 2001). We probably also need to consider the sperm quality of boars that were infected with PCV2 some time in their lives, as an indicator that could lead to a smaller litter size on farms with a history of PCV2 infection.

Sperm head abnormalities are classified as important morphological defects which affect fertility (Tsakmakidis et al. 2010), sperm motility, and litter size (Gil et al. 2009; McPherson et al. 2014). The lower the percentage of this indicator the better the sperm quality. In our study, a significant difference was found between the groups, and group P revealed a significantly lower percentage compared to group S. This difference could be due to higher levels of IgG antibodies in group P compared to other groups and thus better protection against SD-PCV2. Another probable reason for the difference in the quality of semen between vaccinated and unvaccinated animals is that some of the unvaccinated piglets suffered from a subclinical form of the disease (Kumman et al. 2011; Oliver-Ferrando et al. 2016) which increases susceptibility to other infections (Opriessnig and Halbur 2012; Park et al. 2014). Potential infection of the unvaccinated groups from another pathogen [e.g. porcine reproductive and respiratory syndrome virus (PRRSv), porcine parvovirus (PPV), *Chlamydia* sp., *Brucella suis*, leptospires] during experimentation resulted in alterations in the quality

of semen (Teankum et al. 2006; Althouse et al. 2007). Especially PRRSv which appears more often than any other virus in coinfections with PCV2 (Pallarés et al. 2002) does not cause specific lesions in testes and epididymides but alters the sperm quality causing sperm abnormalities, reduced motility, increased ALH and reduced linearity (Sur et al.1997; Schulze et al. 2013).

During experimentation, all the male piglets were kept under the same environmental (ventilation and temperature) and nutritional conditions (no feed restriction, no protein restriction, same feed ratio and nutritional value) that could influence the boar semen quality (Wettemann et al. 1976). The only difference between the groups was vaccination against PCV2. We can conclude that the PCV2 vaccination can have a positive effect on boar semen quality.

In our observations, alterations in epididymal sperm kinetic properties and morphology after vaccination against PCV2 in young boars are noticeable, an improvement occurs which is, however, non-specific in order to consider the PCV2 vaccination as a tool to improve the sperm quality in mature boars at a field level. In conclusion, vaccination against PCV2 at 21 days of life positively affects the young boar's epididymal sperm quality. Further research is needed to confirm and support the results of the present study on a field fertility level.

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