

# Pathogenesis of type 1 (European genotype) porcine reproductive and respiratory syndrome virus in male gonads of infected boar

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**Abstract** The objective of this study was to determine the pathogenesis of experimental infection with a type 1 porcine reproductive and respiratory syndrome virus (PRRSV) by defining the sites of viral replication and apoptosis in male gonads from infected boars for a period of 21 days after intranasal inoculation. Microscopically, hypospermatogenesis and abundant germ cell depletion and death were observed in the testes. Such germ cell death occurs by apoptosis, as determined by a characteristic histological patterns and evidence of massive DNA fragment detected in situ terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) reaction. PRRSV was detected in the testicular tissue of infected boars only. Viral nucleic acid was localized in spermatogonia, spermatocytes and spermatids but not in the vesicular and bulbourethral gland. In serial sections, PRRSV-positive cells did not co-localized with apoptotic cells. TUNEL-positive apoptotic cells were more numerous than PRRSV-positive cells in testicular sections. The present study demonstrated that type 1 PRRSV infects the spermatogonia and their progeny, and induces apoptosis in these germ cells.

**Keywords** Apoptosis · Boar · Porcine reproductive and respiratory syndrome virus · Reproductive system

Kiwon Han and Hwi Won Seo made an equal contribution to this work, they are co-first authors.

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## Introduction

Porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) is a member of the *Arterivirus* genus (family *Arteriviridae*, order *Nidovirales*) (Cavanagh 1997), and genetic analysis has established two PRRSV genotypes, type 1 (European genotype) and type 2 (North American genotype), which share approximately 60 % nucleotide identity for entire genome (Allende et al. 1999; Nelsen et al. 1999). Recently, type 1 PRRSV has emerged in Asian countries (Amonsin et al. 2009; Chen et al. 2011; Lee et al. 2010).

PRRS, caused by the PRRSV, occurs in 2 major clinical forms: reproductive failure in breeding animals and respiratory disease in nursery and grow-finishing pigs. In the sow, reproductive failure characterized by abortion and delivery of stillborn near-term fetuses or premature and weak piglets reviewed by Zimmerman et al. 2012. In the boar, clinical manifestations include anorexia, lethargy, and loss of libido (Feitsma et al. 1992; Hopper et al. 1992).

The pathogenesis of reproductive disease caused by type 2 PRRSV in boars has been studied: type 2 PRRSV is able to replicate and induce apoptosis in the epithelium of the seminiferous tubules and produce alterations in the reproductive tract (Sur et al. 1997). The pathogenesis of reproductive disease caused by type 1 PRRSV in boars indicates that type 1 PRRSV is shed in the semen of infected boars, and viral replication has been demonstrated within different reproductive tissues (most consistently in the epididymis) by virus isolation and reverse transcription-polymerase chain reaction (RT-PCR) (Prieto et al. 2003). However, the data on the viral distribution and apoptosis caused by type 1 PRRSV are limited; therefore, it is difficult to elucidate the pathogenesis of type 1 PRRSV in boars. Hence, the objective of this study was to determine the pathogenesis of experimental infection with a type 1 PRRSV by defining the sites of viral replication and apoptosis in testes from infected boars.

## Materials and methods

### PRRSV inoculum

Type 1 PRRSV (SNUVR100748) was used as inoculums. The type 1 PRRSV strain was isolated in lung samples from an aborted fetus in 2010 in Kyounggi Province. SNUVR100748 was identified as type 1 PRRSV on the basis of the nucleotide sequences of the open reading frame (ORF) 5 (GenBank accession number JX393303) and ORF7 (GenBank accession number JX393304). Type 1 PRRSV (passage 6) was propagated in MARC-145 cells to a titer of  $1 \times 10^6$  50 % tissue culture infective doses (TCID<sub>50</sub>)/ml.

### Experimental design

At 8 months of age, 10 purebred male Duroc pigs were purchased from a PRRSV-free commercial farm. All boars were negative for porcine circovirus type 2 (PCV2) and PRRSV according to routine serological testing prior to delivery and on arrival. All boars were individually housed throughout the experiment in an environmentally controlled building with pens over completely slatted floors.

The boars were randomly divided into two groups. The boars in group 1 ( $n=8$ ) were inoculated with type 1 PRRSV intranasally (2 mL) with an infectious titer of  $10^6$  TCID<sub>50</sub> per mL. The boars in group 2 ( $n=2$ ) served as negative controls. Following PRRSV inoculation, the physical condition of the boar was monitored daily and their rectal temperatures were daily taken from 0 to 14 dpi.

### Tissue sample collections

Each of four infected and one negative control boars were tranquilized by an intravenous injection of azaperon (Stresnil, Janssen Pharmaceutica, Beerse, Belgium) and then euthanized by electrocution for necropsy at 10 and 21 days post-inoculation (dpi). Reproductive tissues (testicle, epididymis, ductus deferens, prostate gland, bulbourethral gland, and vesicular gland) were collected from each pig at necropsy. These reproductive tissues were used for virus isolation and in situ hybridization (ISH). All of the methods were previously approved by the Seoul National University Institutional Animal Care and Use Committee.

### Serology

Blood samples from boars were collected at 0, 4, 7, 10, 14, 18, and 21 dpi. The serum samples were tested using the commercially available PRRSV enzyme-linked immunosorbent assay (ELISA; HerdCheck PRRS 2XR™, IDEXX Laboratories Inc., Westbrook, Maine, USA). Serum samples were considered positive for PRRSV antibody if the S/P

ratio was greater than 0.4 according to the manufacturer's instructions.

### Virus isolation

Reproductive tissue samples collected from necropsy were used to isolate PRRSV as described by Cheon and Chae (2000). Briefly, MARC-145 cell lines were used to isolate PRRSV from tissue suspensions. A 20–30 % tissue suspension was prepared in cell culture medium (minimal essential medium plus 8 % fetal bovine serum) and then centrifuged ( $1,800 \times g$ ) for 20 min. Five hundred  $\mu$ l of supernatant was filtered (0.45  $\mu$ m) and delivered into individual wells of plates containing a confluent monolayer of MARC-145 cells through a syringe filter (0.45  $\mu$ m). After incubation for 2 h at 37 °C, the supernatant was discarded, fresh maintenance medium (minimal essential medium plus 4 % fetal bovine serum) was added to each well, and the plates were incubated for 9 days with observation for cytopathic effects (CPE). Cultures showing CPE were fixed in 80 % acetone and tested for PRRSV antigen by indirect immunofluorescent antibody test. Wells without CPE after 9 days of incubation were frozen and thawed, and 1 ml of medium and cells were transferred to a new well, this time containing fresh maintenance medium. Second passage cultures were incubated for 9 days and observed as before. After acetone fixation, wells were stained with an anti-PRRSV monoclonal antibody SDOW-17 and fluorescein isothiocyanate (FITC)-conjugated anti-mouse immunoglobulin G, and then viewed with a fluorescence microscope for evidence of specific viral antigen.

### Sequence analysis

PRRSV isolated from testicle and epididymis was further analyzed for the ORF5 sequence. RNA was extracted from PRRSV-infected MARC-145 cell lines (Cheon and Chae 2000) and amplified from the ORF5 region by reverse-transcriptase PCR (RT-PCR) (Oleksiewicz et al. 1998). Sequencing was performed on the purified RT-PCR products of amplified ORF5.

### Quantification of PRRSV RNA in semen and serum samples

RNA extractions from the semen (raw) and serum samples were collected 0, 4, 7, 10, 14, 18, and 21 dpi from boars used in this study. Semen and serum samples were treated with commercial TRIzol reagent (Life Technologies, Carlsbad, CA, USA). Briefly, 500  $\mu$ l of diluted (1:20 in PBS, 0.01 M, pH7.4 for semen and 1:2 dilution in PBS, 0.01 M, pH7.4 for serum) seminal (or serum) and were vortexed with an equal volume of TRIzol (Life Technologies). After the addition of

chloroform (300  $\mu$ l), the RNA in the aqueous phase was precipitated with 500  $\mu$ l of isopropanol for 15 min. The final ethanol-washed RNA pellet was air dried and then dissolved in 30  $\mu$ l of diethyl-pyrocabonate-treated water.

Real-time PCR for the type 1 and type 2 PRRSV was used to quantify PRRSV genomic cDNA copy numbers using RNA extraction from semen and serum samples which were performed as previously described (Wasilk et al. 2004). The real-time PCR was considered to be positive if the cycle threshold level was obtained at  $\leq 45$  cycles as previously described (Wasilk et al. 2004).

#### Preparation of labeled probe

For type 1 PRRSV, a 354-base-pair cDNA fragment representing the region of ORF6 and ORF7 was used as a probe. The forward and reverse primers were 5'-CGCTG TGACAAAGCCCGGAC-3' (nucleotides 14,482 to 14,501) and 5'-TCGATTGCAAGCAGAGGGAG-3' (nucleotides 14,814 to 14,835), respectively. PCR was performed as previous described (Kono et al. 1996). The PCR products were purified with Wizard PCR Preps (Promega Biotech, Madison, Wisconsin, USA). The purified PCR product was labeled by random priming with digoxigenin-dUTP using a commercial kit (Boehringer Mannheim, Indianapolis, USA). A probe for type 2 PRRSV was used as the negative probe (Cheon et al. 1997).

#### In situ hybridization

After fixation, the tissues from each pig were dehydrated through a graded series of alcohol solutions and a xylene step and embedded in paraffin wax. Five serial sections (4  $\mu$ m) were mounted on positively charged slide (Superfrost/Plus slides; Erie Scientific Company, Portsmouth, NH, USA) and then prepared from each tissue, two being further processed for ISH using a type 1 and 2 PRRSV probe without RNase A treatment, two for ISH using a type 1 and 2 PRRSV probe with RNase A treatment and one for haematoxylin and eosin (HE) staining. ISH was performed as previous described (Cheon et al. 1997). The lung tissues from three Korean type 2 PRRSV-inoculated pigs euthanized at 7 dpi (Cheon et al. 1997) were used as negative controls for type 1-PRRSV ISH.

#### In situ TUNEL reaction

Paraffin sections were placed on Superfrost/plus slides (Fisher Scientific, Pittsburgh, PA, USA), deparaffinized, and rehydrated. Sections and slides were first treated with 20  $\mu$ g/ml of proteinase K in phosphate buffered saline (PBS, 0.1 M, pH 7.4) for 20 min at 37 °C. After washing in PBS, sections and slides were covered with 50  $\mu$ l of the TUNEL reaction mixture (Boehringer Mannheim, Indianapolis, IN,

USA) and incubate under a coverslip in a humidified chamber for 1 h at 37 °C. The reaction was stopped by washing slides in PBS for 15 min at room temperature. The sections were then incubated with the anti-fluorescein-alkaline phosphatase conjugate (Boehringer Mannheim) diluted 1/3 in 100 mM Tris-HCl, 150 mM NaCl (pH 7.5), and 1 % blocking agent for 1 h at room temperature. After three washes in PBS, substrate consisting of nitroblue tetrazolium (NBT) and 5-bromocresyl-3-indolylphosphate (BCIP) was layered over the sections. Color was allowed to develop for 5–8 h in the dark, and the reaction was stopped by dipping slides briefly in Tri-ethylenediaminetetraacetic acid buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). Sections were counterstained with 0.5 % methyl green, and the slides were then washed with distilled water for 1 min, and then allowed to dry completely.

#### Morphometric analysis

Five sections of formalin-fixed testes were taken from each virus-infected boar for morphometric analysis. In each testicular slide, 10 fields were randomly selected, and the number of positive cells by ISH and in situ TUNEL per unit area (0.25 mm<sup>2</sup>) was counted.

#### Statistical analysis

Continuous data for the differences of ISH score, in situ TUNEL scores in type 1 PRRSV-infected boars between 10 and 21 dpi, and PRRSV RNA quantification over time between the groups were analyzed at each time point using paired *T* test. The Pearson's correlation coefficient was used to assess the relationship between viremia and shedding in semen. *P* < 0.05 indicated statistical significance.

## Results

#### Serology of PRRSV

Anti-PRRSV IgG antibodies were detected in infected boars as early as 7 dpi only and all infected boars were found seropositive by 10 dpi. Thereafter, all of the infected boars remained seropositive against PRRSV. As expected, no anti-PRRSV IgG antibodies were detected in the serum from the negative control boars throughout the experiment.

#### Virus isolation and sequence analysis

Attempts were made to isolate and identify type 1 PRRSV from the reproductive tissues of infected and negative control boars. Type 1 PRRSV was consistently isolated from testicle (8 boars) and epididymis (7 boars) in infected boars

(Table 1). All isolated PRRSV from 8 infected boars was confirmed by sequence analysis to be the same propagating virus as the challenge stock. No PRRSV was isolated from the reproductive tissues of the negative control boars.

#### Quantification of PRRSV RNA in semen and serum samples

Genomic copies of the type 1 PRRSV were detected in the serum and semen samples from infected boars (Table 2). Genomic copies of the type 1 PRRSV in serum was increased from 0 to 7 dpi ( $P<0.05$ ), but had decreased thereafter. Genomic copies of the type 1 PRRSV in semen was increased from 0 to 10 dpi ( $P<0.05$ ) but had decreased thereafter (Fig. 1). No genomic copies of the type 1 PRRSV were found in the serum or semen samples from negative control boars throughout the course of the experiment.

#### Microscopic lesions

Microscopic lesions were observed in type 1 PRRSV-infected boars but not in negative control boars. A prominent morphological feature in the testes was the presence of apoptotic cells. Apoptotic cells were characterized by basophilic apoptotic bodies and condensed in the nucleus periphery. These cells were usually separated from neighboring cells. Apoptosis was prominent in infected boars at 10 dpi. Another consistently observed lesion was hypospermatogenesis, which was characterized by a complete lack of mature spermatids and a nearly complete absence of germ cells, except for a small number of spermatogonia. The germ cells showed disarray in seminiferous tubules. Hypospermatogenesis was prominent in infected boars at 21 dpi.

#### In situ hybridization

Type 1 PRRSV-infected positive cells were found in the testes of all of the infected boars examined. No hybridization signal

was observed in tissue sections pretreated with RNase A. The distribution of positive cells was focal, found in single or small clusters of germ cells with the virus localized to the spermatogonia, spermatocytes and spermatids (Fig. 2). The number of type 1 PRRSV-infected positive cells was significantly higher in spermatocytes ( $P=0.026$ ) and spermatids ( $P=0.024$ ) from infected boars at 10 dpi than those from infected boars at 21 dpi. At 10 dpi, the number of type 1 PRRSV-infected positive cells was significantly higher in spermatocytes ( $P<0.05$ ) than spermatogonia, spermatids and non-sperm cells in infected boar (Table 3). The virus was not detected in Sertoli cells, Leydig cells, or endothelial cells. Infected cells were rarely noted among the stromal cells and appeared cytologically to be macrophages with large oval nuclei and abundant cytoplasm.

Hybridization signals were also detected in epididymal tissues from infected boars. Most of the infected cells were in the lumen of the efferent ducts. The non-sperm cells contained viral nucleic acid. Occasionally, positive cells were also observed in the stromal connective tissue in epididymis, ductus deferens and prostate gland. The spermatozoa were consistently negative for ISH of type 1 PRRSV in the lumen of ductus deferens. No hybridization signals were detected in the bulbourethral glands or penis of infected boars.

Positive hybridization signals were not detected in sections from negative control boars. Positive hybridization signals were not detected in the testes and epididymis from boars infected experimentally with type 1 PRRSV using the type 2-based PRRSV probe. Positive hybridization signals were not detected in the lungs from pigs infected experimentally with Korean type 2 PRRSV using the type 1-based PRRSV probe.

#### In situ TUNEL staining

Apoptosis induced by type 1 PRRSV was examined in the testes from type 1 PRRSV-infected and negative control

**Table 1** Isolation of type 1 porcine reproductive and respiratory syndrome virus (PRRSV) from reproductive tissues in infected boars at 10 and 21 days post-inoculation (dpi)

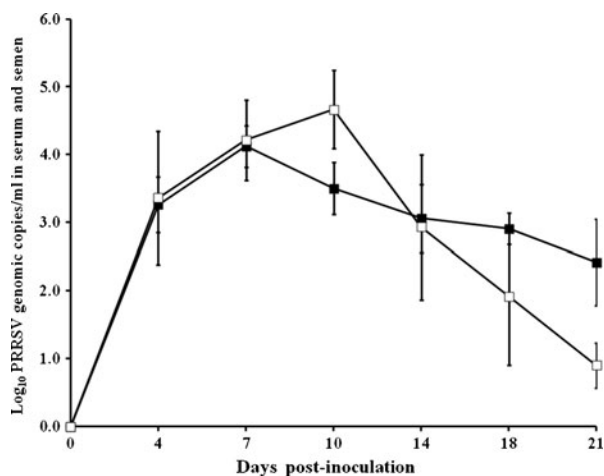
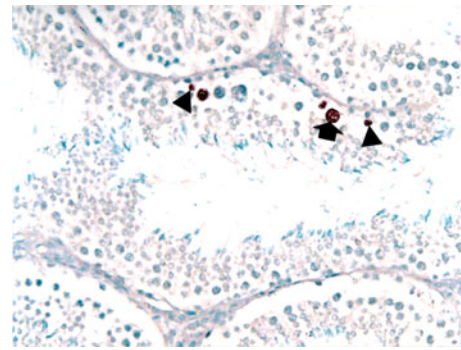
Boar	dpi	Reproductive tissues					
		Testicle	Epididymis	Ductus deferans	Prostate gland	Bulbourethral gland	Vesicular gland
1	10	+	+	–	–	–	–
2	10	+	+	–	–	–	–
3	10	+	+	–	–	–	–
4	10	+	+	–	–	–	–
5	21	+	+	–	–	–	–
6	21	+	–	–	–	–	–
7	21	+	+	–	–	–	–
8	21	+	+	–	–	–	–

**Table 2** Detection of type 1 porcine reproductive and respiratory syndrome virus (PRRSV) by real-time PCR in semen and serum samples from infected boars

Boar	Various days post-inoculation (semen/serum)					
	0	4	7	14	18	21
1	-/-	-/+	+/+	NA <sup>a</sup>	NA	NA
2	-/-	-/+	+/+	NA	NA	NA
3	-/-	+/+	+/+	NA	NA	NA
4	-/-	-/+	-/+	NA	NA	NA
5	-/-	+/+	+/+	+/+	-/+	+/+
6	-/-	-/+	-/+	+/+	+/+	-/+
7	-/-	-/+	+/+	-/+	+/+	+/+
8	-/-	-/+	+/+	+/+	+/+	-/-

<sup>a</sup> NA not applied

boars. TUNEL-positive cells had red-staining nuclei. Apoptotic bodies of various sizes exhibited distinct staining, and the cytoplasm of the apoptotic cells was also often stained, indicating leakage of DNA fragments out of the nucleus. In serial sections, the PRRSV-positive cells did not co-localize with apoptotic cells (Fig. 3a and b). TUNEL-positive apoptotic cells were more numerous than PRRSV-positive cells in testicular sections. The numbers of TUNEL-positive cells were significantly higher in testicular tissues from infected boars at 10 dpi than those from infected boars at 21 dpi ( $P=0.035$ ). Minimal numbers of apoptotic cells were observed in testicular tissues from negative control boars ( $4.3\pm 2.3$  cells/ $0.25$  mm<sup>2</sup> for boar at 10 dpi and  $3.7\pm 1.9$  cells/ $0.25$  mm<sup>2</sup> for boar at 21 dpi).

**Fig. 1** Mean values of the genomic copy number of type 1 porcine reproductive and respiratory syndrome virus (PRRSV) in serum (black square) and semen (white square) samples from boars experimentally infected with type 1 PRRSV**Fig. 2** In situ hybridization of testicular tissues from boars experimentally infected with type 1 porcine reproductive and respiratory syndrome virus (PRRSV) at 10 dpi. Type 1 PRRSV nucleic acids were detected in spermatocytes (arrow) and spermatogonia (arrowheads)

## Discussion

This study demonstrates that type 1 PRRSV replicates in the reproductive organs of infected boars. Our results are in agreement with previous studies that showed that type 2 PRRSV induces microscopic lesions in the reproductive tract, including hypospermatogenesis, desquamation of the seminiferous tubules, formation of abnormal germinal cells, and the death of germinal cells (Sur et al. 1997). Seminal quality was not examined in the present study. The consequences of hypospermatogenesis and death of germinal cells might alter seminal quality in infected boars as previous study (Prieto et al. 1996). Comparative analysis indicates that type 2 PRRSV induces a more severe respiratory disease in growing pigs than does type 1 PRRSV (Martínez-Lobo et al. 2011; Halbur et al. 1995). Although differences in virulence between the two genotypes of PRRSV have not been definitely proven experimentally, further study is needed to determine the differences in virulence between the two genotypes of PRRSV when targeting the male reproductive system.

PRRSV demonstrated an apparent tropism for macrophage lineages in the respiratory and lymphoid systems of the pigs in previous studies (Halbur et al. 1995; Sur et al. 1998; Cheon et al. 1997), but PRRSV infected target cells other than macrophages in the male reproductive system in the present study. Spermatogonia infection most likely occurs secondary to hematogenous spread of the virus. Although the virus is present in infected macrophages have not been determined in the present study, viremia contributes to viral distribution throughout the reproductive tissues (Christopher-Hennings et al. 1998). Monocytes can be infected under some circumstances (Christopher-Hennings et al. 1998; Voicu et al. 1994) and they could be the source of virus for different tissues when they migrate to these tissues to become macrophages (Christopher-Hennings et al. 1998; Prieto and Castro 2005). Direct infection from

**Table 3** Morphometric analysis of testicular tissue from boars experimentally infected with type 1 porcine reproductive and respiratory syndrome virus (PRRSV) at 10 and 21 days post-inoculation (dpi)

Boar	dpi	Number of positive germ cells for in situ hybridization and apoptosis (cells/0.25 mm <sup>2</sup> )				
		Spermatogonia	Spermatocyte	Spermatid	Non-sperm cell	Apoptosis
1	10	1.2±0.5	3.4±0.8 <sup>ab</sup>	2.2±0.8 <sup>b</sup>	2.2±0.5	31.2±5.4 <sup>b</sup>
2	10	1.2±0.3	4.2±0.9 <sup>ab</sup>	3.2±0.7 <sup>b</sup>	2.4±0.7	25.4±4.8 <sup>b</sup>
3	10	1.6±0.4	2.6±0.6 <sup>ab</sup>	2.4±0.5 <sup>b</sup>	1.6±0.4	33.2±5.5 <sup>b</sup>
4	10	1.4±0.7	3.6±0.4 <sup>ab</sup>	2.8±0.3 <sup>b</sup>	1.4±0.6	36.4±6.2 <sup>b</sup>
5	21	0	1.2±0.8	0	1.2±0.3	17.6±6.9
6	21	0	1.4±0.7	1.4±0.6	1.6±0.7	23.2±7.1
7	21	1.2±0.3	1.2±0.6	1.6±0.7	0	21.4±6.5
8	21	1.6±0.2	1.6±0.3	1.2±0.4	1.4±0.5	20.8±8.3

<sup>a</sup> $P < 0.05$  vs. spermatogonia, spermatid, non-sperm cell in each boar at 10 dpi ( $P < 0.05$ )

<sup>b</sup> $P < 0.05$  vs. in boar at 21 dpi ( $P < 0.05$ )

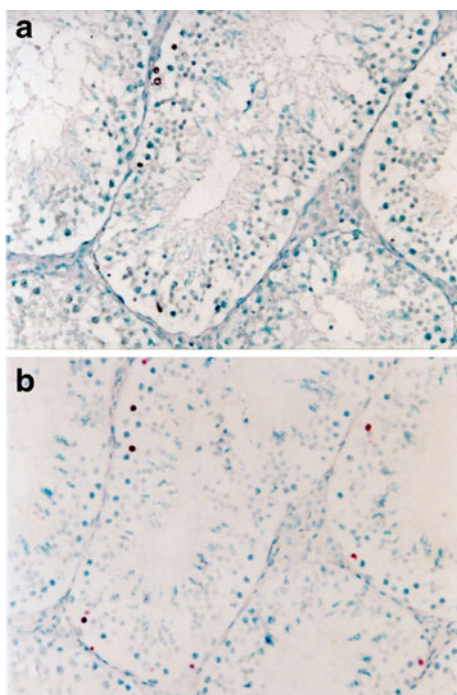
infectious virions in the peripheral blood is also a distinct possibility given the highly vascular nature of the testes.

The observation that the spermatogonia and their progeny are infected by type 1 PRRSV also explains a mechanism whereby the virus is transmitted by artificial insemination (AI). Although viral replication in these cell types could facilitate shedding in the ejaculates, the present study have not been determined the presence of this immature and/or degenerate cells in the ejaculates of the infected boars. However, the transmission of type 1 PRRSV via semen to offspring by AI has also been reported (Guerin and Pozzi

2005; Robertson 1992). Detection of type 1 PRRSV in male gonads has important implications for disease control strategies because the type 2 PRRSV-based modified live vaccine (Ingelvac PRRS MLV, Boehringer Ingelheim Animal Health, St. Joseph, MO, USA) was unable to decrease shedding of type 1 PRRSV in semen after a challenge (Han et al. 2011). These results are clinically meaningful because the type 2 PRRSV-based vaccine could not protect boars from type 1 PRRSV. Furthermore, the commercial type 1 PRRSV-based vaccine is not available yet in most Asian countries, including Korea.

The main characteristic of PRRSV infection in the boar is the intermittent shedding in semen (Christopher-Hennings et al. 1995a, 2001). However, type 1 PRRSV is constantly detectable throughout 21 dpi in semen as did type 2 PRRSV in previous study (Christopher-Hennings et al. 1995b). These results suggested that different patterns of shedding (constant vs. intermittent) may exist due to the strain variation and dose of virus at exposure during the early phase of infection. Additionally, it could be due to difference in detection method (real-time PCR vs. conventional PCR) and genetic susceptibility of boars. Real-time PCR was more sensitive than conventional PCR for the detection of PRRSV in serum and semen samples (Egli et al. 2001; Wasilk et al. 2004). It has been also reported that Landrace boars may be more sensitive than Yorkshire to PRRSV shedding in semen (Christopher-Hennings et al. 2001).

The lack of a close cell-to-cell correlation between PRRSV infection and apoptosis in serial sections from testicular tissues indicated that PRRSV can induce apoptosis via indirect mechanisms. PRRSV appears to kill infected cells by cell lysis in vitro and induces apoptosis in uninfected bystander cells in vivo (Sirinarumit et al. 1998; Sur et al. 1998). In the respiratory disease, tumor necrosis factor (TNF)- $\alpha$  released from PRRSV-infected macrophages may induce apoptosis in uninfected bystander cells. Alternatively, because germ cells in testicular tissues are not able to produce the apoptogenic factors such as



**Fig. 3** Consecutive serial sections of testicular tissues from boars experimentally infected with type 1 porcine reproductive and respiratory syndrome virus (PRRSV) at 21 dpi. **a.** Positive hybridization signals for type 1 PRRSV nucleic acids were detected in spermatocytes (In situ hybridization). **b.** apoptotic signals were detected in germ cells (TUNEL reaction)

TNF- $\alpha$ , it has been shown that the expression of viral glycoprotein 5 (GP5), which is a product of PRRSV open reading frame 5, triggers the apoptotic process in infected cells (Suárez et al. 1996). Further study is needed to elucidate the mechanisms by which PRRSV infection induces apoptosis of germ cells in PRRSV-infected boars.

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