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Vertical transmission of porcine circovirus 2b (PCV2b) to mouse fetuses in maternal uterus by artificial insemination with semen from PCV2b-infected male mouse

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Abstract: In order to investigate whether porcine circovirus 2b (PCV2b) can be replicated in the spermatids of mice and whether semen from PCV2b-infected male mice can induce reproductive failure in female mice, 10 male Kunming mice were intraperitoneally inoculated with PCV2b, and 10 female Kunming mice were artificial inseminated with semen from the PCV2b-inoculated male mice. Semen from inoculated male mice and newborn mice or dead fetuses from inseminated female mice were tested for PCV2b by polymerase chain reaction, transmission electron microscopy, and immunofluorescent histochemistry. The results showed that PCV2b was capable of being replicated in spermatids in Kunming mice and could be transmitted from semen to fetuses by artificial insemination, causing reproductive failure in pregnant Kunming mice and PCV2b vertical infection to the newborn mice.

Key words: PCV2b DNA-positive semen, artificial insemination, PCV2b transmission to fetuses, reproductive failure, Kunming mice

Porcine circovirus (PCV) is a nonenveloped, circular single-stranded DNA virus belonging to the Circoviridae virus family. Two members, the nonpathogenic PCV1 and pathogenic PCV2, have been identified so far. PCV2 has been implicated and associated with many diseases in growing and mature pigs. PCV2-associated reproductive failure is a broad term in swine and manifests as early embryonic death, abortion, and reduced litter size, which are commonly observed on PCV2-contaminated swine farms (1–4). To study this disease, a good animal model is required. A successful model of PCV2 infection in pigs was established by Madson et al. (5), which indicated that PCV2 DNA was detected in semen from experimentally infected boars and PCV2 DNA-positive semen could induce reproductive failure in pregnant sows. According to the aid of a signature motif, which was a short amino acid motif encoded within the capsid protein, PCV2 was separated into at least 2 major groups, PCV2a and PCV2b. This classification received more attention, as it seemed to define PCV2b to be more virulent (6). However, little is known about the role of PCV2b in semen from PCV2b-infected male mice and the transmission of PCV2b from semen to fetuses via artificial insemination. In this study, an artificial insemination technique was used instead of natural mating for avoiding PCV2b infection via contact in Kunming mice, and the results showed that PCV2b was capable of being replicated in spermatids in Kunming mice and transmitted from semen to fetuses by artificial insemination, causing reproductive failure in pregnant Kunming mice and PCV2b vertical infection to newborn mice.

The homogeneous, infectious PCV2b (GenBank accession number EU095020), which was isolated from the lymph nodes and lungs of 11-week-old pigs in a field case of postweaning multisystemic wasting syndrome (PMWS), was generated and cultured with the methods described in a previous study (7). The inoculum was tested for PCV2b by polymerase chain reaction (PCR) and the virus titers were determined by indirect immunofluorescence assay.

Fourteen male and female 8-week-old Kunming mice were purchased from the Experimental Animal Center, School of Medicine, Central South University, Changsha, Hunan, China. Their specific-pathogen-free status was verified by the supplier.

Ten male mice were each intraperitoneally inoculated with a PCV2b inoculum containing 10,000 $TCID_{50}$ in 0.1 mL of cell culture supernatant and kept in 2 cages (5 mice per cage), while another 4 male mice were mock-infected with a cell culture supernatant and kept in 1 cage. At 21 days after infection, both PCV2b-inoculated and mock-infected mice were euthanatized, and semen was collected from the caudae epididymides within 5 min after euthanasia, then diluted with 1 mL of 9% milk for artificial insemination and PCR. Samples of the testis were fixed

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in 10% neutral buffered formalin for PCV2b detection by immunofluorescent histochemical assay.

According to the methods described in the previous study (8), 10 female mice were each inseminated with semen from the inoculated male mice in 0.05 mL of milk suspension as a test group (TG). In the meantime, 4 female mice were inseminated with semen from the mock-infected mice as a control group (CG). All semen for artificial insemination was used within 1 h after collection. When the female mice became pregnant, each of them would be kept in an individual cage. After delivery, 3 newborn mice from each litter were randomly selected immediately after birth and euthanatized. Samples of the lungs, hearts, and spleens were collected for PCR.

PCV2b DNA in the tissues and semen was extracted using a viral DNA extract kit (Nanjing KeyGen Biotech Co. Ltd., Nanjing, Jiangsu, China) following the manufacturer's protocol and recommendations. Using the PCV2b-specific primers (5'-GGAGCTTCCAATCTCCC-3', 5'-TAGGAGCTCCACACTCC-3'), the PCV2b DNA in mice was detected by PCR following the methods described in a previous study (9).

For immunofluorescent histochemical test, antigens in the paraffin tissues were retrieved in 1.0% EDTA at 95 °C for 15 min and cooled to room temperature; endogenous peroxidase activity was quenched with 3.0% H_2O_2 in methanol (v/v) at room temperature for 10 min. The primary mouse anti-PCV2b sera preparation was as described in our previous study (9), and then sections were incubated with a 1:400 dilution of primary mouse anti-PCV2b sera at 37 °C for 2 h. Following this, a 1:50 dilution of secondary anti-mouse IgG antibody with fluorescein (Zhongshan Golden Bridge Co. Ltd., Beijing, China) was added at 37 °C for 1 h; finally, it was photographed under a fluorescence microscope (Olympus, Tokyo, Japan).

In preparation of transmission electron microscopy (TEM) sections, samples of the testis from PCV2b-infected male mice were fixed in 2.5% aldehyde for 24 h, then in osmium tetroxide for 2 h. Tissues from the testis were embedded in Epon 812, and ultrathin sections were cut with a LKB III ultramicrotome (LKB, Bromma, Sweden) and stained with lead citrate and uranyl acetate. Sections were examined with a transmission electron microscope H7500 (Hitachi, Tokyo, Japan).

The results showed that PCV2b DNA was detected by PCR in semen from all virus-inoculated mice, and PCV2b antigen fluorescent signals (Figure 1a) and PCV2b inclusion bodies (Figure 2) could be observed in the spermatids by the immunofluorescent histochemical and transmission electron microscopic assays. No PCV2b DNA, virus antigen signals, or PCV2b inclusion bodies were detected in the controls.

For the inseminated female mice, abortion was not found in either the TG or CG during pregnancy. Seven of the 10 mice in the TG became pregnant after artificial insemination, 2 of which had the embryos' development interrupted within 11 to 15 days post coitus such that 5 of them became pregnant and gave birth at the expected time. Their newborn mice were less active than age-matched litters from the CG. PCV2b DNA was detected in the lungs, hearts, and spleens from all PCV2b-infected litters and dead fetuses by PCR (Figure 3). In the CG, only 1 mouse had failed artificial insemination, while 3 of the 4 mice became pregnant with normal births. No PCV2b DNA was detected in the organs from the control litters.

PCV2 transmission through the placenta in sows was reported in a previous study (10); the results revealed that PCV2-inoculated sows showed abortion and that virus-inoculated sows had dead and/or stillborn piglets, and PCV2 antigen and DNA were detected in the dead fetuses and/or live-born piglets by PCR and/or immunohistochemistry. In a study by Madson et al. (5), results showed that PCV2 DNA was detected in semen from experimentally infected boars, and PCV2 DNA-

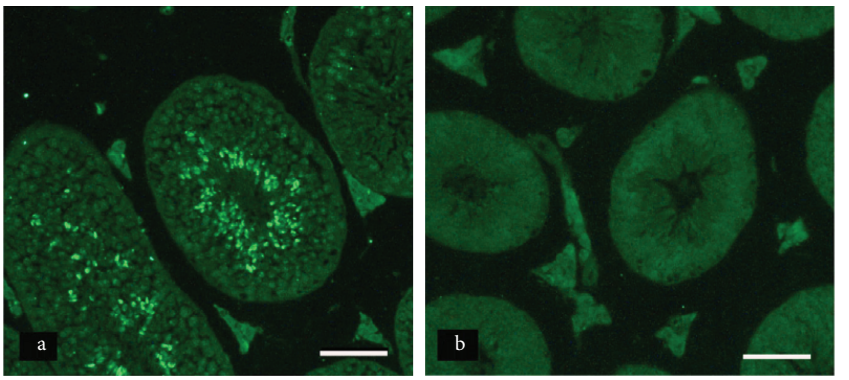

Figure 1. Immunofluorescent histochemical staining of sections for testis. PCV2b antigen fluorescent signals were observed in spermatids by immunofluorescent microscope (a). The control was negative for immunofluorescence (b). Bar = 100 μm.

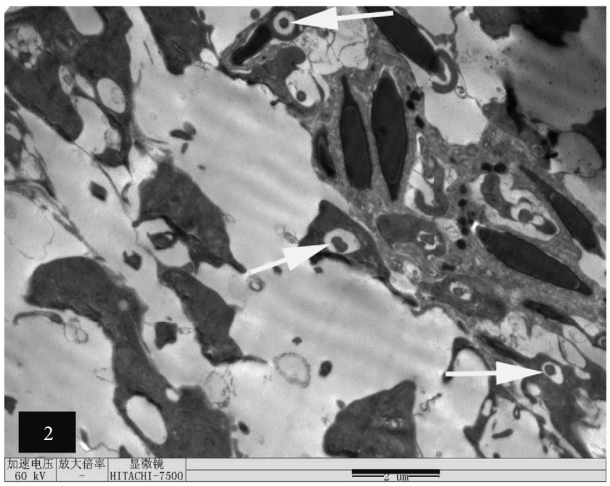

Figure 2. TEM section of the testis from PCV2b-infected male mouse. Viral inclusion bodies in spermatids are shown by arrows. Bar = 2 μm.

positive semen could induce reproductive failure in pregnant sows. In our study, although abortion was not observed in PCV2b-inoculated pregnant mice, 2 mice had embryo development interrupted and PCV2b DNA was detected in the newborn mice of PCV2b-infected litters. Our results were in accordance with those described by Yoon et al. (10) and Madson et al. (5).

Efforts to detect PCV2 transmission in pregnant mice through the placenta were reported by Lőrincz et al. (11); the results showed that PCV2 can be present in house mice on PCV2-contaminated farms, but no fetuses from the pregnant mice were PCV-positive. In our study, semen from PCV2b-inoculated male mice could induce reproductive failure in pregnant female mice and PCV2b could be transmitted to fetuses. These results showed some discrepancy from those described by Lőrincz et al. (11). Different forms of PCV infection in pregnant mice may account for the discrepancy. In our study, the female mice were artificially inseminated with PCV2b DNA-positive semen under experimental conditions. In the study conducted by Lőrincz et al. (11), the pregnant mice were infected by PCV2 via natural infection under field conditions.

Artificial insemination techniques are commonly used in pig species for dam mating to study certain vital virus infections, such as porcine respiratory and reproductive syndrome virus, porcine parvovirus, or PCV infection. In a certain set of circumstances, semen may be contaminated by the above-mentioned viruses or other pathogenic microorganisms and can be transported far distances, resulting in viral and/or bacterial infections on a large scale on pig farms. This has occurred in many cases of viral infection and caused great economic losses in pig breeding practices (12,13). PCV2 potential transmission from semen to fetuses via artificial insemination was evaluated in this study.

In conclusion, PCV2b was capable of being replicated in spermatids in Kunming mice following intraperitoneal inoculation with PCV2b and transmitted from semen to fetuses by artificial insemination, causing reproductive failure in the pregnant Kunming mice and PCV2b vertical infection to newborn mice.

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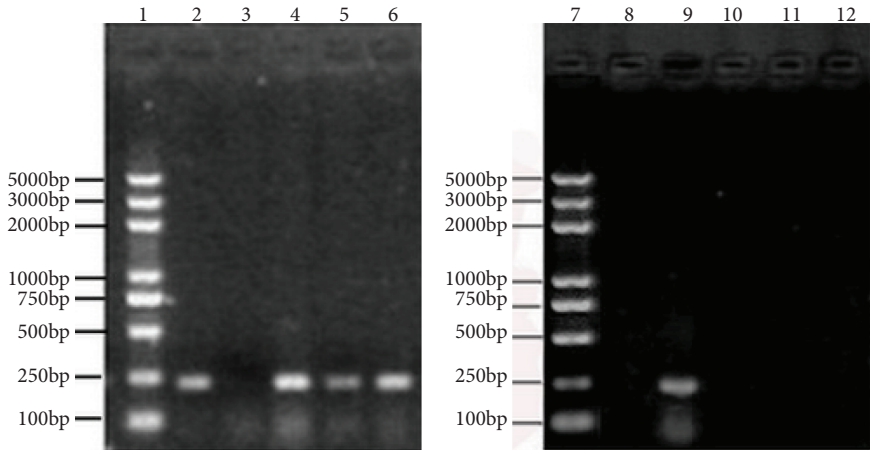

Figure 3. PCR results. Lanes 1 and 7, DNA markers; lanes 2 and 9, positive controls; lanes 3 and 8, negative controls; lanes 4 to 6, PCR products (positive) from lung, heart, and spleen of the PCV2b-infected litter; lanes 10 to 12, PCR products (negative) from lung, heart, and spleen of the control litter. The PCV2b DNA target fragment was 234 bp.

References

1. Hansen, M.S., Hjulsager, C.K., Bille-Hansen, V., Haugegaard, S., Dupont, K., Høgedal, P., Kunstmann, L., Larsen, L.E.: Selection of method is crucial for the diagnosis of porcine circovirus type 2 associated reproductive failures. Vet. Microbiol., 2010; 144: 203–209.
2. Maldonado, J., Segalés, J., Martínez-Puig, D., Calsamiglia, M., Riera, P., Domingo, M., Artigas, C.: Identification of viral pathogens in aborted fetuses and stillborn piglets from cases of swine reproductive failure in Spain. Vet. J., 2005; 169: 454–456.
3. Nathues, H., Tegeler, R., Grummer, B., grosse Beilage, E.: [Infectious agent detection in reproductive disorders in swine herds. Retrospective evaluation of diagnostic laboratory examinations]. Tierarztl Prax. Ausg. G Grosstiere Nutztiere, 2011; 39: 155–161 (article in German).
4. Sharma, R., Saikumar, G.: Porcine parvovirus- and porcine circovirus 2-associated reproductive failure and neonatal mortality in crossbred Indian pigs. Trop. Anim. Health Prod., 2010; 42: 515–522.
5. Madson, D.M., Patterson, A.R., Ramamoorthy, S., Pal, N., Meng, X.J., Opriessnig, T.: Reproductive failure experimentally induced in sows via artificial insemination with semen spiked with porcine circovirus type 2. Vet. Pathol., 2009; 46: 707–716.
6. Wiederkehr, D.D., Sydler, T., Buergi, E., Haessig, M., Zimmermann, D., Pospischil, A., Brugnera, E., Sidler, X.: A new emerging genotype subgroup within PCV-2b dominates the PMWS epizooty in Switzerland. Vet. Microbiol., 2009; 136: 27–35.
7. Fenaux, M., Halbur, P.G., Haqshenas, G., Royer, R., Thomas, P., Nawagitgul, P., Gill, M., Toth, T.E., Meng, X.J.: Cloned genomic DNA of type 2 porcine circovirus is infectious when injected directly into the liver and lymph nodes of pigs: characterization of clinical disease, virus distribution, and pathologic lesions. J. Virol., 2002; 76: 541–551.
8. Duselis, A.R., Vrana, P.B.: Harvesting sperm and artificial insemination of mice. J. Vis. Exp., 2007; (3): 184.
9. Deng, Z.B., Wang, N.D., Xu, D.J., Yuan, A.W., Ge, M., Luo, W., Xue, L.Q., Yu, X.L.: Viral distribution and lesions in Kunming mice experimentally infected with porcine circovirus type 2b. Vet. Res. Commun., 2011; 35: 181–192.
10. Yoon, K.J., Jepsen, R.J., Pogranichniy, R.M., Sorden, S., Stammer, R., Evans, L.E.: A novel approach to intrauterine viral inoculation of swine using PCV type 2 as a model. Theriogenology, 2004; 61: 1025–1037.
11. Lőrincz, M., Cságola, A., Biksi, I., Szeredi, L., Dán, A., Tuboly, T.: Detection of porcine circovirus in rodents: short communication. Acta Vet. Hung., 2010; 58: 265–268.
12. Guerin, B., Pozzi, N.: Viruses in boar semen: detection and clinical as well as epidemiological consequences regarding disease transmission by artificial insemination. Theriogenology, 2005; 63: 556–572.
13. Madson, D.M., Opriessnig, T.: Effect of porcine circovirus type 2 (PCV2) infection on reproduction: disease, vertical transmission, diagnostics and vaccination. Anim. Health Res. Rev., 2011; 12: 47–65.