การตรวจหาดีเอ็นเอของเชื้อเซอร์โคไวรัสชนิดที่ 2 ในรังไข่และมดลูกจากสุกรสาวที่ถูกคัดทิ้ง
เนื่องจากปัญหาทางระบบสืบพันธุ์

นายพชระ เพียรอดวงษ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาวิทยาการสืบพันธุ์สัตว์ ภาควิชาสูติศาสตร์-เธนุเวชวิทยาและวิทยาการสืบพันธุ์
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2556

DETECTION OF PORCINE CIRCOVIRUS TYPE 2 DNA IN OVARY AND UTERUS FROM GILTS CULLED DUE TO REPRODUCTIVE DISTURBANCE

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Theriogenology
Department of Obstetrics Gynaecology and Reproduction
Faculty of Veterinary Science
Chulalongkorn University
Academic Year 2013

Thesis Title	DETECTION OF PORCINE CIRCOVIRUS TYPE 2 DNA IN OVARY AND UTERUS FROM GILTS CULLED DUE TO REPRODUCTIVE DISTURBANCE
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Field of Study	Theriogenology
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พชระ เพียรอดวงษ์ : การตรวจหาดีเอ็นเอของเชื้อเซอร์โคไวรัสชนิดที่ 2 ในรังไข่และมดลูกจากสุกรสาวที่ถูกคัดทิ้งเนื่องจากปัญหาทางระบบสืบพันธุ์. (DETECTION OF PORCINE CIRCOVIRUS TYPE 2 DNA IN OVARY AND UTERUS FROM GILTS CULLED DUE TO REPRODUCTIVE DISTURBANCE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. น.สพ. ดร. เผด็จ ธรรมรักษ์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. น.สพ. ดร. คมกฤช เทียนคำ, 45 หน้า.

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อหาความสัมพันธ์ระหว่างการตรวจพบดีเอ็นเอของเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกร ในเนื้อเยื่อรังไข่และมดลูก กับสมรรถภาพทางการสืบพันธุ์ สาเหตุการคัดทิ้ง พยาธิสภาพ และระดับแอนติบอดีต่อเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกร และเพื่อหาการปรากฏของแคพซิดโปรตีนของเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกร ในเนื้อเยื่อรังไข่และมดลูกของสุกรสาวที่ถูกคัดทิ้งเนื่องจากปัญหาทางระบบสืบพันธุ์ โดยใช้ตัวอย่าง รังไข่ 70 ตัวอย่าง มดลูก 102 ตัวอย่าง ที่ถูกฝังในพาราฟินและซีรั่ม 102 ตัวอย่าง โดยเก็บข้อมูลประวัติของสุกรสาวซึ่งประกอบด้วย น้ำหนักตัว อายุที่เข้าทดแทน อายุที่ถูกคัดทิ้ง น้ำหนักที่เพิ่มขึ้นเฉลี่ยต่อวัน และข้อมูลสมรรถภาพทางการสืบพันธุ์ ซึ่งประกอบด้วย อายุที่เป็นสัดครั้งแรก อายุที่ได้รับการผสมพันธุ์ครั้งแรก และจำนวนวันสูญเสีย สาเหตุการคัดทิ้งสุกรสาวประกอบไปด้วยอาการ แท้ง หนองไหล ไม่เป็นสัด ผสมซ้ำ และปัญหาที่ไม่เกี่ยวกับระบบสืบพันธุ์ พยาธิสภาพของรังไข่ถูกแบ่งเป็นกลุ่มต่างๆดังนี้ ปกติ พบซิสต์ 1 ซิสต์ พบซิสต์ ≥2 ซิสต์ และอื่นๆ ส่วนพยาธิสภาพของมดลูกถูกแบ่งเป็นกลุ่มต่างๆดังนี้ ปกติ มดลูกอักเสบ และอื่นๆ ดีเอ็นเอถูกสกัดจากเนื้อเยื่อโดยใช้ชุดสกัดสำเร็จรูป ดีเอ็นเอของเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกรถูกตรวจโดยวิธีพีซีอาร์ซึ่งใช้ไพร์เมอร์ที่จำเพาะต่อ ORF1 ของของเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกร แคพซิดโปรตีนของเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกร ในเนื้อเยื่อนั้นจะถูกตรวจหาโดยใช้วิธีการอิมมูโนฮิสโตเคมีซึ่งใช้แอนติบอดีทีจำเพาะต่อแคพซิดโปรตีนของเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกร ตัวอย่างซีรั่มจะถูกตรวจหาระดับแอนติบอดีต่อเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกรโดยใช้ชุดตรวจอิไลซ่าสำเร็จรูป ผลการศึกษาพบว่า ดีเอ็นเอของเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกร สามารถตรวจพบได้ใน 21/70 (30.0%) ของรังไข่ และ 46/102 (45.1%) ของมดลูก สมรรถภาพทางการสืบพันธุ์ของสุกรสาวที่ตรวจพบและไม่พบเอ็นเอของเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกรนั้นไม่แตกต่างกันทางสถิติ ร้อยละของการตรวจพบเอ็นเอของเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกร ในมดลูกของสุกรสาวที่ถูกคัดทิ้งจากปัญหาที่ไม่เกี่ยวกับระบบสืบพันธุ์ 4/20 (20.0%) มีค่าต่ำกว่าสุกรสาวที่ถูกคัดทิ้งจากอาการ แท้ง 6/7 (85.0%) หนองไหล 19/40 (47.5%) และไม่เป็นสัด 15/28 (53.5%) ($p<0.05$) ร้อยละของการตรวจพบดีเอ็นเอของเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกร ในรังไข่หรือมดลูกนั้นไม่สัมพันธ์กับพยาธิสภาพของทั้งรังไข่และมดลูก ระดับแอนติบอดีต่อเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกรเฉลี่ยในซีรั่มมีค่าเท่ากับ 1,271±867 มีค่าตั้งแต่ 150 ถึง >2,484 ร้อยละของการตรวจพบดีเอ็นเอของเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกรที่มีระดับแอนติบอดีต่อเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกรสูง (23.0%) มีค่าน้อยกว่าสุกรสาวที่มีแอนติบอดีต่อเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกรต่ำ (57.6%) และมีระดับแอนติบอดีต่อเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกรเป็นลบ (64.5%) ($p<0.05$) แคพซิดโปรตีนของเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกรถูกตรวจพบในเนื้อเยื่อมดลูกโดยวิธีการอิมมูโนฮิสโตเคมี โดยปรากฏอยู่ทั้งในไซโทพลาซึมและนิวเคลียสของเซลล์เยื่อบุมดลูก ลิมโฟไซต์และมาโครฟาจในชั้นเนื้อเยื่อเกี่ยวพันใต้เยื่อบุมดลูก การศึกษานี้สรุปได้ว่าดีเอ็นเอของเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกร สามารถตรวจพบได้ใน 21/70 (30.0%) ของรังไข่ และ 46/102 (45.1%) ของมดลูกของสุกรสาวที่ถูกคัดทิ้งจากปัญหาทางระบบสืบพันธุ์ โดยสาเหตุการคัดทิ้งมีความสัมพันธ์กับการตรวจพบดีเอ็นเอของเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกร ไม่พบความสัมพันธ์ระหว่างสมรรถภาพทางการสืบพันธุ์ พยาธิสภาพและการตรวจพบดีเอ็นเอของเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกร แคพซิดโปรตีนของเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกรถูกตรวจพบได้ในเซลล์เยื่อบุมดลูก ลิมโฟไซต์และมาโครฟาจในชั้นเนื้อเยื่อเกี่ยวพันใต้เยื่อบุมดลูก สุกรสาวที่มีระดับแอนติบอดีต่อเชื้อเซอร์โคไวรัส ชนิดที่ 2 สูงนั้นมีการตรวจพบดีเอ็นเอของเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกรน้อยกว่าสุกรที่มีระดับแอนติบอดีต่ำกว่าและมีระดับแอนติบอดีเป็นลบ

ภาควิชา	สูติศาสตร์-เธนุเวชวิทยาและวิทยาการสืบพันธุ์	ลายมือชื่อนิสิต	.....
สาขาวิชา	วิทยาการสืบพันธุ์สัตว์	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก	.....
ปีการศึกษา	2556	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม	.....

5575311431 : MAJOR THERIOGENOLOGY
KEYWORDS: GILT / OVARY / PCV2 / REPRODUCTIVE DISTURBANCE / UTERUS

PACHARA PEARODWONG: DETECTION OF PORCINE CIRCOVIRUS TYPE 2 DNA IN OVARY AND UTERUS FROM GILTS CULLED DUE TO REPRODUCTIVE DISTURBANCE. ADVISOR: ASSOC. PROF. PADET TUMMARUK, Ph.D., CO-ADVISOR: ASST. PROF. KOMKRICH TEANKUM, Dr.Med.Vet., 45 pp.

The objectives of present study were 1) to determine the association between porcine circovirus type2 (PCV2) DNA detection in ovary and uterine tissues and reproductive performance, reason for culling, gross morphology, and PCV2 ELISA antibody titer and 2) to locate the PCV2 capsid protein in the ovary and uterus of gilts culled due to reproductive disturbance. In total, formalin-fixed paraffin-embedded ovaries (n=70), uteri (n=102), and serum samples (n=102) were included. Historical data (i.e., body weight, age at enter into the herd, age at culling, average daily gain (ADG)) and reproductive performances (i.e., age at first observed estrus, age at first mating, and non-productive days (NPD)) were collected. Reasons for culling (abortion, abonormal vaginal discharge, anestrus, repeat service, and non-reproductive reason), gross morphology of the ovary (normal, single or multiple cyst, miscellaneous) and the uterus (normal, endometritis, and miscellaneous) were classified. DNA were extracted by using a commercial extraction kit. The polymerase chain reaction was performed by using ORF1 of PCV2 specific primers. The PCV2 capsid protein localization was identified by using polyclonal anti-PCV2 primary-antibody. Serums were test for PCV2 antibody by using the commercial ELISA. It was found that PCV2 DNA were detected in 21/70 (30.0%) of the ovary and in 46/102 (45.1%) of the uterus. Reproductive performances of gilts that had PCV2 DNA in their reproductive organs and those without the PCV2 DNA were not significantly difference. The percentage of PCV2 detection in the uterus in gilts culled due to non-reproductive problem 4/20 (20.0%) was lower than that in gilts culled due to abortion 6/7 (85.0%), abnormal vaginal discharge 19/40 (47.5%), and anestrus 15/28 (53.5%) ($p<0.05$). The percentage of PCV2 DNA detection in either ovarian or uterine tissues were not related with gross morphologies of both organs. The average PCV2 antibody titer of the gilts were 1,271±867 (range 150 to >2,484). The percentage of PCV2 DNA detection in the gilts with a high antibody titer (23.0%) was lower than that in the gilts with low antibody titer (57.6%) and seronegative gilts (64.5%) ($p<0.05$). PCV2 capsid proteins were detected in uterine tissues by immunohistochemistry. The PCV2 were appeared both in the cytoplasm and the nucleus of the cells. In addition, the PCV2 antigens intranuclear staining were found in the endometrial cells, while intracytoplasmic and the intranuclear staining were found in the lymphocytes and macrophages. In conclusion, PCV2 was detected by 30.0% of the ovary and 45.1% of the uterus of gilts culled due to reproductive disturbances. The reasons for culling were associated with PCV2 DNA detection. No association between reproductive performance, and gross morphology and PCV2 DNA detection were observed. The PCV2 capsid protein were found in the endometrial cells, subepithelial lymphocytes and macrophages. The PCV2 DNA detection in the ovary of gilts with high positive PCV2 antibody titer were lower than the gilts with lower PCV2 antibody titer.

Department:	Obstetrics Gynaecology and Reproduction	Student's Signature .....
Field of Study:	Theriogenology	Advisor's Signature .....
Academic Year:	2013	Co-Advisor's Signature .....

ACKNOWLEDGEMENTS

These studies were carried out at the Department of Obstetrics, Gynecology and Reproduction, in co-operation with Department of Pathology, and Veterinary Diagnostic Laboratory, Faculty of Veterinary Science, Chulalongkorn University.

There are many persons who dedicated their time supporting my studies.

I would like to thank these following people:

Assoc. Prof. Dr. Padet Tummaruk, Head of Department of Obstetrics, Gynecology and Reproduction, my thesis advisor who gave me the opportunity to be his student and encouraged me to be an active researcher. Not only the knowledge and skills that he gave me, but the vision of a researcher that I absorbed from him was also one of the most important thing that I have earned.

Asst. Prof. Dr. Komkrich Teankum, Head of Pathology Department of Pathology, one of my thesis co-advisors. I would like to thank him for providing me a chance to do the immunohistochemistry at the Pathology Department and also for interesting comments on my immunohistochemistry results. Lastly, I would like to thank him for helping me on selecting the PCV2 antibody.

Dr. Rachod Tantilertcharoen, Head of Vetrinary Diagnostic Laboratory, another thesis co-advisor of mine. I will not forget the warm welcome which he provided to me when using the his laboratory. He instructed me to many laboratory techniques especially the excellent deparafinization technique, conventional DNA extraction and PCR technique, immunohistochemistry, and histopathological examination.

Asst. Prof. Dr. Sayamon Srisuwatanasagul, she is my excellent invited immunohistochemistry co-advisor. I would like to thank for her suggestions in adjusting the immunohistochemistry condition.

Mr. Sitthichok Lacharoje, for the professional cutting of 4 um thick slide.

Dr. Narong Tiptanavattana, for his suggestion in adjusting the PCR condition to make the more clearer PCR band.

Finally, I would like to thank for the helpful suggestion and encouragement from my mother.

Without their guidance and help, this dissertion would not have been possible.

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ABBREVIATIONS

ADG	average daily gain
ADV	Aujezsky's disease virus
AI	artificial insemination
AMs	alveolar macrophages
Ab1	antiPCV2 antibody
BFDV	beak and feather disease virus
bp	base pair
NPD	non productive day
ºC	degree celsius
CAV	chicken anemia virus
CL	corpora lutea
cm	centimeter
CoCV	columbid circovirus
conj-HRP	anti-PCV2/peroxidase conjugate
DCs	dendritic cells
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assays
GCV	goose circovirus
g	gram
h	hour
H_2O_2	hydrogen peroxide
IFATs	indirect fluorescent antibody test
IFN- α	interferon alpha
IgG	immunoglobulin G
IHC	immunohistochemistry

IL10	interleukin 10
IPMA	immunoperoxidase monolayer assay
ISH	in situ hybridization
kg	kilogram
M	Molarity
min	minute
ml	milliliter
m^2	square metres
MCP-1	monocyte chemoattractant protein-1
mg/10 µl	milligram per ten microliter
mm	millimeter
mmol/l	millimole per litre
nm	nanometer
OD	optical density
ORFs	open reading frames
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PCV	porcine circovirus
PCV1	porcine circovirus type1
PCV2	porcine circovirus type2
PCV2a	porcine circovirus type 2a
PCV2b	porcine circovirus type 2b
PCV2c	porcine circovirus type 2c
PCV2d	porcine circovirus type 2d
PCV2e	porcine circovirus type 2e
PCVAD	porcine circovirus associated disease
pDCs	plasmacytoid dendritic cells
PDNS	porcine dermatitis and nephropathy syndrome

pH	power of hydrogen ion
PK-15	porcine kidney cell line 15
PMWS	post weaning mutisystemic wasting syndrome
PPV	porcine parvovirus
PRRSV	porcine reproductive and respiratory syndrome virus
SD	standard deviation
sec	second
TNF-α	tumor necrosis factor alpha
µl	microliter
USA	United States of America
UV	ultravioletCHAPTER

CHAPTER I
INTRODUCTION

Important and rationale

Porcine circovirus associated disease (PCVAD) is referred as clinical symptoms involving PCV2 infection in pigs including postweaning multisystemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome (PDNS), enteric diseases, respiratory diseases, and reproductive disorders (Segales, 2012). The clinical symptoms of PCVAD are varied depending on many factors, e.g., age, breed, immune status, and concurrent infections (Krakowka et al., 2001; Opriessnig et al., 2006; Opriessnig and Halbur, 2012; Shen et al., 2012). Madson et al. (2009) found that artificial insemination (AI) with semen spiked with PCV2 in naïve sows causes reproductive failures, i.e., mummified fetuses and stillborn piglets. Interestingly, the virus can also be detected in up to 88% of the live-born piglets in sows experimentally challenged with PCV2 during AI (Madson et al., 2009). In addition, reproductive failure associated with PCV2 can also be manifested as irregular return to estrus, abortion, and low litter size at birth (Madson and Opriessnig, 2011). The PCV2 antigen is commonly detected in the myocardium of the dead fetuses by immunohistochemistry (IHC) (West et al., 1999). In Thailand, a study on PCV2 detection in PWMS pigs indicate that PCV2 DNA could be detected from formalin-fixed paraffin-embedded tissues of the pigs (Kiatipattanasakul-Banlunara et al., 2002) Tummaruk et al. (2009a) found that PCV2 antibody was detected in 10% of the PCV2 non-vaccinated gilts culled due to reproductive disturbance (e.g., anestrus and abnormal vaginal discharge). Furthermore, a co-infection between PCV2 and other viruses (e.g., porcine parvovirus (PPV), porcine reproductive and respiratory syndrome virus (PRRSV), and Aujezsky's disease virus (ADV) has also been reported (Kim et al., 2004).

In general, 40-60% of sows in swine breeding herds are replaced by gilts annually (Tummaruk et al., 2010). Therefore, the health of the replacement gilts is important for both the reproductive performance and the herd health status. Under

field condition, a number of gilts are culled before they can successfully produce their first litter. The common culling reasons include anestrus, abnormal vaginal discharge, repeat service, and not being pregnant (Tummaruk et al., 2009b). Our previous study has demonstrated that PCV2 infection was found in the gilts culled due to anestrus and abnormal vaginal discharge (Tummaruk et al., 2009a). To our knowledge, no comprehensive study on the association between the reproductive failure in replacement gilts and the PCV2 DNA detection in their ovary and uterus has been done.

CHAPTER II
LITERATURE REVIEW

History

Porcine circovirus (PCV) has been identified for the first time in the contaminated porcine kidney cell line (PK 15) (Tischer et al., 1982). In 1980s, PCV was recognized as a non-pathogenic virus. It has been demonstrated that experimentally infected pig developed antibodies against PCV and the vial shedding can be identified in nasal swab and fecal samples (Tischer et al., 1986). Nevertheless, none of the animal shows signs of illness or pathological changes after PCV infection (Tischer et al., 1986). Furthermore, PCV antibodies can be detected in wild boar in the forest and in 77-95% of the slaughtered pigs (Tischer et al., 1986), indicating that PCV is a common virus in swine populations.

In 1990s, PMWS was recognized as a new disease in swine herds in western Canada (Ellis et al., 1998). The disease is usually found in nursery pigs and the clinical signs of the disease include progressive weight loss (wasting), tachypnea, dyspnea, and jaundice. The gross and histopathological lesions of the infected pigs include lymphadenopathy, interstitial pneumonia, hepatitis, enteritis, and nephritis (Ellis et al., 1998; Harding et al., 1998). Using electron microscopic examination and in situ hybridization (ISH) in lung, liver, pancreas, spleen, and lymph node of PMWS pigs, PCV-like nucleic acid is identified (Allan et al., 1998). After that, PCV is classified into two types consist of PCV type 1 (PCV1, non-pathogenic PCV) and PCV type 2 (PCV2, pathogenic PCV) (Meehan et al., 1998).

Circovirus

In general, the Circoviridae family consists of chicken anemia virus (CAV), beak and feather disease virus (BFDV), columbid circovirus (CoCV), goose circovirus (GCV), and porcine circovirus (PCV). The CAV is classified in the genus Gyrovirus, while PCV, BFDV, CoCV, and GCV are classified in the genus Circovirus (Pringle, 1999; Mankertz et al., 2000; Todd et al., 2001). The size of PCV is 17 nm diameter (Tischer et al., 1982), and contain a covalently closed circular single stranded DNA with 1,759 neucleotides (Meehan et al., 1997). The nucleotide sequence of PCV2 is 68% homology with that PCV1 (Hamel et al., 1998). Up to date, the PCV2 is divided into five subtypes, i.e., PCV2a, PCV2b, PCV2c, PCV2d, and PCV2e (Jantafong et al., 2011; Trible and Rowland,

2012; Buapaichit et al., 2013). PCV2 genome contains 3 open reading frames (ORFs), i.e., ORF1, ORF2, and ORF3 (Olvera et al., 2007; Faurez et al., 2009). The ORF1 encodes proteins that is important for viral replication, ORF2 encodes the antigenic capsid protein (Olvera et al., 2007), and ORF3 encodes protein that can induce apoptosis (Liu et al., 2005).

Pathogenesis of PCV2

PCV2 exists in pig population for over 10 years since late 1990s. In general, PCV2 is able to transmit for a long time by many routes and it is spread within the swine population both horizontal and vertical transmission (Rose et al., 2012). The most effective infectious route of PCV2 is direct contact. The virus can be infected to susceptible pigs via respiratory, digestive and urinary secretions (Rose et al., 2012). PCV2 can infect into endothelial cells, epithelial cells, macrophages, dendritic cells (DCs), and lymphocytes (Darwich et al., 2004; Perez-Martin et al., 2007; Lin et al., 2008). PCV2 can be detected in the oral fluid, nasal secretion, tonsil, blood, and urine until 125 days post infection (Bolin et al., 2001; Prickett et al., 2011). It has been demonstrated that PCV2 reduce proliferative activity of lymph node and lead to cell depletion in PMWS pigs (Mandrioli et al., 2004). In addition, PCV2 infection causes lymphopenia and neutrophilia in gnotobiotic pigs (Gauger et al., 2011). After intranasal inoculation of sucking piglets, PCV2 can induce apoptosis of hepatocytes. This suggest that PCV2-induced apoptosis is important in the pathogenesis of PCV2 associated disease (Sinha et al., 2012). Gilpin et al. (2003) demonstrated that PCV2 is detected in the cytoplasm of the monocyte and pulmonary macrophage. However, they suggest that these monocytic cells may not represent the primary target for PCV2 replication. Histologically, a hallmark lesion of PCV2 infection is granulomatous inflammation in many organs including lymph node, liver, spleen, tonsil, thymus, and Peyer's patches (Chae, 2004). However, to our knowledge, no histopathological lesion of PCV2 infection in the reproductive organs has been done. Park et al. (2009) found a simultaneous detection of the PCV2 antigen and DNA in the mammary gland from experimentally infected sows. However, the clinical implication of the PCV2 detection in the mammary gland of sows has not been comprehensively investigated. In nursery pigs with a clinical symptoms of PMWS, the detection of monocyte chemoattractant protein-1(MCP-1) in the granulomatous lesion of the lymph node suggest that MCP-1 plays a role in the pathogenesis of the PCV2 infection (Kim and Chae, 2003). Chang et al. (2006) found that the presence of PCV2 antigen in alveolar macrophage (AMs) reduced the phagocytosis capability of the

cells. PCV2 can also showed the immune evasion and the escaping of DCs degradation pathway by persists in DCs in the absence of virus replication or degradation (Vincent et al., 2003). The infected DCs provide a potent vehicle for transport of the virus throughout the host (Vincent et al., 2003). The PCV2 infection of plasmacytoid dendritic cells (pDCs) impairs the induction of interferon (IFN)-α and tumor necrosis factor (TNF)-α (Vincent et al., 2007). In the experimentally inoculated pigs, levels of interleukin 10 (IL10) is elevated at 10^{th} day post infection in the PMWS piglets higher than that in the subclinically infected piglets (Stevenson et al., 2006). The level of IL10 induced by PCV2 infection disappeared at 10 weeks after infection (Darwich et al., 2008). In general, a single infection of PCV2 rarely induces clinical symptoms in pigs, however the virus is usually act as a primary causative agent of PCVAD (Opriessnig and Halbur, 2012; Meng, 2013). PCV2 infection can also cause subclinical disease in the swine herd (Segales, 2012). The clinical symptoms of PCVAD are varied depending on many factors, e.g., age, breed, immune systems, and concurrent infections (Krakowka et al., 2001; Opriessnig et al., 2006; Opriessnig and Halbur, 2012; Shen et al., 2012). For instance in PDNS cases, an abnormality of cytokine production is found (Sipos et al., 2005). However, in PMWS pigs, it is hypothesized that long term activation of immune system may reduce the nutrient absorbtion via neuro-immunoendocrine network (Suradhat, 2006).

PCV2 detection in Thailand

In 1999, PCV has been detected for the first time in three pigs in Nakhon Pathom province with dyspnea, diarrhea, and cachexia (Tantilertcharoen et al., 1999). The necropsy results showed 3-4 times generalized lymph node enlargement, enterocolitis, and cranioventral pneumonia (Tantilertcharoen et al., 1999). Lymphoid depletion and intracytoplasmic inclusion bodies has also been found in macrophage (Tantilertcharoen et al., 1999). PCV2 can also be detected in the formalin-fixed paraffin-embedded tonsil, spleen, and lymph node tissues of 6 to 14 weeks old PMWS cases by nested PCR (Kiatipattanasakul-Banlunara et al., 2002). In the lymphoid tissues, the granulomatous inflammation, homogeneous, amphophilic intracytoplasmic inclusion bodies in macrophage are also detected (Kiatipattanasakul-Banlunara et al., 2002). PCV2 associated reproductive failure in Thailand has not been comprehensively investigated, but the sero-prevalence of PCV2 in replacement gilts at average age of 300 days that were culled due to reproductive failure has been reported (Tummaruk et al., 2009a). Tummaruk et al. (2009a) found that PCV2 antibody was detected in 10% of the PCV2 non-vaccinated gilts culled due to

reproductive failure. Furthermore, the PCV2 co-infection with PPV, PRRSV, and ADV has also been reported (Kim et al., 2004). Under field conditions, AI is commonly practiced in swine commercial herds in Thailand. The PCV2 detection in the boar semen has been reported in 92 non-vaccinated boars from 3 provinces in Thailand since 2008 (Nuntaprasert et al., 2008). An earlier study found that maternally derived PCV2 passive immunity remain in a high level until 4 weeks of age then decline between 5 and 7 weeks of age (Paphavasit et al., 2009). Under field conditions, a seroconversion of PCV2 is commonly found between 9 and 12 weeks of age due to natural infection (Paphavasit et al., 2009). Up to date, four subtypes of PCV2 have been found in Thailand, i.e., PCV2a, PCV2b, PCV2d, and PCV2e (Jantafong et al., 2011; Buapaichit et al., 2013).

Culling of gilt due to reproductive disturbance

In general, 40-60% of sows in swine breeding herds are replaced by gilts annually (Tummaruk et al., 2010). After selection, the replacement gilts are commonly culled due to many problems (Tummaruk et al., 2009b). Tummaruk et al. (2006) found that 47% of the replacement gilts in swine commercial herds in Thailand are culled due to reproductive disturbance. The common reasons for culling associated with reproductive disturbance include anestrus (44.0%), repeat service (15.5%), and abnormal vaginal discharge (20.5%) (Tummaruk et al., 2009b) In practice, various factors influencing reproductive performances of the gilt include climate or season, nutrition, breed, diseases, and management (Tummaruk et al., 2007; Olanratmanee et al., 2011; Iida and Koketsu, 2013). Infectious diseases are one among of an important reasons causing the removal of gilts and sows from swine commercial herds in USA (Lucia et al., 2000). The percentage of the culling due to infectious diseases varies from 12.9% to 22.5% among parities of sows (Lucia et al., 2000). To our knowledge, these figures have never been investigated in the Thai swine herds.

Effect of PCV2 infection on embryo and fetal mortality

PCV2 can infect and replicate in hatched blastocysts leading to embryonic death (Mateusen et al., 2007). Madson et al. (2009) found that AI with semen spiked with PCV2 in naïve sows causes viremia within 7 days in sows and subsequently result in mummified fetuses, stillborn piglets, and PCV2 viremia live-born piglets. Furthermore, it is found that the crown-rump length of the mummified fetuses varies

between 7 and 27 cm (Madson et al., 2009). Interestingly, the virus can also be detected up to 88% of the live-born piglets and in 100% of the dead fetuses in the sows experimentally challenged with PCV2 (Madson et al., 2009). Heart, liver, lung, kidney, and lymphoid organs are the major target organs of PCV2 replication (West et al., 1999; Saha et al., 2010). The target cells of PCV2 replication change from cardiomyocytes, hepatocytes, and macrophages during fetal life to be only macrophages in live-born piglets (Sanchez et al., 2003). PCV2 can induce gross pathological lesions in the fetuses, including distended abdomen, oedema, haemorrhage, and congestion of internal organs (Saha et al., 2010). The clinical manifestation of the fetal death (i.e., mummification, abortion, and stillborn piglets) depends on the timing of PCV2 infection (O'Connor et al., 2001; Johnson et al., 2002; Kim et al., 2004; Madson and Opriessnig, 2011). In pregnant sow, the in utero infection can be divided into PCV2-associated reproductive failure and subclinical PCV2 in utero infection. In the subclinical PCV2, PCV2 antigen or antibody can be detected in the serum of pre-suckling piglets without histopathological lesion (Madson and Opriessnig, 2011). The diagnostic criteria for PCV2-associated reproductive failure have not been established (Segales et al., 2005). Up to date, the diagnostic criteria for reproductive failure associated with PCV2 infection is generally relied on clinical manifestation and pathological evidences (Madson and Opriessnig, 2011).

Effect of PCV2 infection in gilt and sow

The clinical symptoms of PCVAD are varied depending on many factors, e.g., age, breed, immune systems, and concurrent infections (Krakowka et al., 2001; Opriessnig et al., 2006; Opriessnig and Halbur, 2012; Shen et al., 2012). In general, the clinical symptoms of PCV2 infection in sows are absent, but anorexia is occasionally observed in aborted sows (Park et al., 2005). Pittman (2008) found that PCV2-associated enteritis, pneumonia, and systemic infection were also observed in gilts during the time of reproductive failure. The PCV2 DNA has been detected in the tissues of the PCV2 seropositive pigs (i.e., 84% in serum, 22% in follicular fluid, 56% in oviductal cells, 36% in uterine wash and 11% in oocytes) (Bielanski et al., 2004). Microscopic lesions of the ovarian tissues after experimental inoculation of PCV2 included arteritis in the tubaric branches of the ovarian artery, thickened by spindle cells and acute hemorrhage (Langohr et al., 2010).

PCV2 detection

The methods for detection of PCV2 in tissue sample are based on the viral genome. Polymerase chain reaction (PCR) is the standard PCV2 DNA detection method that has been used to detect the PCV2 DNA in fresh samples (Paphavasit et al., 2009). Furthermore, formalin fixed paraffin embedded tissue has also been used in retrospective study about PCV2 infection using PCR assay (Morandi et al., 2012). In general, the sensitivity of PCR assay is higher than Immunohistochemistry (IHC) assay (Kim and Chae, 2004). The PCR method can be used to detect PCV2 infection with a relatively high sensitivity for a long time span. Additionally, it has a potential to find the PCV2 DNA in the tissue without any lesion (Kiatipattanasakul-Banlunara et al., 2002; Hansen et al., 2010). Serological diagnosis is the method commonly used for the herd monitoring purpose to determine the time of PCV2 infection (Opriessnig et al., 2007). Up to date, the serological diagnosic assay that most reliable are immunoperoxidase monolayer assay (IPMA) and indirect fluorescent antibody test (IFATs). Nevertheless, these methods are expensive and highly time consuming (Jittimanee et al., 2012). Recent study indicated that the enzyme-linked immunosorbent assays (ELISA) based on viral protein are more convenient to be used for PCV2 diagnosis (Kurmann et al., 2011; Jittimanee et al., 2012).

IHC is a common diagnostic tool to investigate the location of PCV2 antigen in the tissue section. Either monoclonal or polyclonal antibodies can be used to detect the specific antigen in the tissue sections (McNeilly et al., 1999; Sorden et al., 1999). The antigen-antibody bindings are expressed by the color of immunohistochemical reaction and visible under light microscope (Ramos-Vara, 2005). Up to date, IHC is still considered as a gold standard for PCVAD diagnosis (Ghebremariam and Gruys, 2005). In general, the diagnosis for PCVAD consists of three main criteria: first, the presenting of clinical sign, second, the presenting of lesion and third, the presenting of PCV2 DNA or antigen (Opriessnig et al., 2007).

Objectives

1. To determine the prevalence of PCV2 DNA positive uterine and ovarian tissues in gilts culled due to reproductive disturbance in relation to age at culling, reasons for culling, and gross morphology of the reproductive organs
2. To determine the association between the frequency of PCV2 DNA detection in the uterine and ovarian tissues in gilts and PCV2 ELISA antibody titer
3. To determine the localization of PCV2 antigen in the uterine and ovarian tissues of gilts

Expected output

1. The percentage of PCV2 DNA positive ovarian and uterine tissues is associated with reproductive performances, reason for culling, gross morphology of the reproductive organs
2. The percentage of PCV2 DNA positive ovarian and uterine tissues in gilts with a high PCV2 antibody titer will be lower than those with a low antibody titer
3. PCV2 antigen will be found in the ovarian and uterine tissues of gilts.

CHAPTER III
MATERIALS AND METHODS

Animals and tissues samples

The present study was carried out in ovarian (n=70) and uterine (n=102) tissues samples from gilts culled due to reproductive disturbance from four swine herds (A, B, C and D) in Thailand. All of the samples were obtained from our previous study (Tummaruk et al., 2009b). Briefly, historical data and blood samples were collected from the gilts before culling. The collected historical data include the breed, gilt identity, date of birth, date of entry into the herd, date of first observed estrus, mating date, date at culling, reason for culling, birth weight, and culling weight. The historical data were calculated for age at first observed estrus, age at entry, age at first mating, and age at culling. The ovary and uterus were collected, place on ice and sent to the laboratory within 24 h after the gilts were slaughtered. The ovary and uterus had been fixed in 10% neutral buffer formalin for 48 h before paraffin embedment. The paraffin embedding were cut by sterile blade and submitted for DNA extraction. The average daily gain (ADG) from birth to culling was calculated by using the following formula: ADG (g/day) = (weight at culling-1.5/age at culling) x 1,000. Non-productive days (NPD) was defined as the interval from date at entry into the herd to date at culling.

General management

Herds in the present study are breeding herds located in the northeastern (A), middle (B) and eastern (C, D) parts of Thailand. Herds A produce replacement gilts and use their own grandparent stock, while herd B, C and D brought replacement from other breeders. The number of sow-on-production ranged between 900 and 3,500 sows. The sows are kept in a conventional open-housing system. The heat stress is reduced by using a water sprinklers and fans. The health of the animals is monitored by veterinarians. The gilts were vaccinated against foot-and-mouth disease

virus, classical swine fever virus, Aujeszky's disease, and porcine parvovirus between 154 and 210 days of age. No PCV2 vaccination has been done in these herds. Gilts were kept in each pen with a group size of 10-15 gilts per pen with a density of 1.5-2.0 m^2 per gilt. The herds were recommended to breed the replacement gilts at 224 days of age onwards with a body weight of at least 130 kg at the second or later observed estrus. The mating technique for all herds was performed by artificial insemination.

Definitions

The examination of ovary and uterus were performed grossly. The structures of ovary, corpora lutea (CL) and follicles were examined to define the estrus cycle and gross morphology. The CL was defined as structures on the ovary of pink or tan or yellow color, with a diameter of 7-12 mm. The follicles were defined as transparent, fluid-containing structure in ovary. The ovary was classified into cyclic and non-cyclic ovary. The non-cyclic ovary was defined if the ovary has only follicles (<7mm) with no evidence of CL. The cyclic ovary was defined if the ovary has CL or preovulatory follicle 7-12 mm diameter. Follicular phase was defined by the appearance of follicle (7-12 mm). Luteal phase was defined by the appearance of CL. Cystic ovary was defined as transparent wall with diameter larger than 1.5 cm. The cystic ovary was characterized by presence of single cyst or multiple cyst (≥2 cyst/gilt). The others pathological changes such as hemorrhage in the follicle, atresia of the ovary were classified as miscellaneous group. Subsequently, the uterus was dissected longitudinally from uterine horn to uterine body before the gross morphological examination. Endometritis was diagnosed if signs of inflammation are visible, i.e. severe edema and congestion, dark, red color, and purulent exudate in lumen. Edema of endometrium was defined as normal. The pathology, i.e. segmental aplasia, double uterine horn was classified as miscellaneous group. Others gross appearances was defined as normal. Abortion was defined as gilts that showed expulsion of dead fetus between 35-112 days after insemination. Anestrus was defined as gilts that were culled because the sign of standing estrus could not be observed. Abnormal vaginal discharge was defined as gilt that showed the clinical

sign of purulent vaginal discharge. Repeat service was defined as the gilts that showed repeat return to estrus after insemination for ≥ 2 estrus cycles. Non reproductive reason was defined as culling of gilts form non reproductive problems including inverted nipples, legs problems, and poor conformation.

Classification of gilts

The culled gilts were classified according to age at enter into the herd, at first observed estrus, at first mating, at culling, body weight at culling, ADG, and NPD. Also, reason for culling (i.e., abortion, abnormal vaginal discharge, anestrus, repeat service, and non-reproductive reason) and gross morphology findings of the ovary (normal, single or multiple cyst, miscellaneous) and uterus (i.e., normal, endometritis, and miscellaneous) were classified. The age at enter into the herd (day) were categorized into four groups: <200, 200-220, 221-240, >240. The age at first estrus (day) were categorized into three groups: <210, 210-225, >225. The age at first mating (day) were categorized into two groups: ≤250, >250. The age at culling (day) were categorized into three groups: <250, 250-280, >280. The body weight at culling (kg) were categorized into three groups: <140, 140-160, >160. For ADG (g/day), the gilts were divided into four groups: <500, 500-540, 541-590, >590. The NPD (day), were divided into four groups: <35, 35-50, 51-100, >100. The classification criteria were based on both frequency distribution of the parameters and the number of observations in each group. Each class of the parameters represented different characters of the gilts (e.g., from low to high) and included at least 6 gilts per group.

PCV2 DNA detection by PCR

The paraffin-embedded tissues were deparaffinized by incubating in xylene for 10 min 3 times at 37 ºC then incubated in 50% xylene and ethanol solution for 10 min at 60 ºC then incubated in ethanol for 2 times, 50% ethanol and distill water for 10 min at room temperature. The deparaffinized tissues (0.01 g.) were incubated in 990 µl of lysis buffer (100 mmol/l NaCl, 10 mmol/l Tris HCl, 25 mmol/l EDTA and 0.5% sodium dodecyl sulfate) and 10 µl of 0.4 mg/10 µl of proteinase k (Proteinase k, USbiological, USA) for 24-48 h. at 56 ºC then the DNA in the suspension were

extracted by using a commercial extraction kit (NucleoSpin® RNA virus, MACHEREY-NAGEL, Germany).

The forward primer sequence was ATG CCC AGC AAG AAG AAT GGA AGA AG and reverse primer sequence was AGG TCA CTC CGT TGT TGT CCT TGA GAT C (Paphavasit et al., 2009). These primers are specific to ORF1 conserved region. The PCR was performed in 20 µl mixture of ready-to-use buffer solution (Go taq® Green Master Mix, Promega®, USA), 0.5 µl of each primers, 3 µl of DNA template and distilled water. The PCR condition consisted of initial denaturing at 95 ºC for 4 min. for 1 cycle, followed by 35 cycles of denaturation at 94 ºC for 2o sec. annealing at 56 ºC for 20 sec. and extension at 72 ºC for 20 sec. followed by final extension for 2 min. to amplify 350 bp product. After expanding process, the products were run on the 2% agarose gel in tris-borate EDTA buffer, stained with nucleic staining solution (RedSafeTM iNtRON, Biotechnology, Switzerland) and visualized under a UV trans-illuminator.

Serological test

In total of 102 serum samples, individual serum was test for PCV2 antibody following the manufacturer's instructions of commercial competitive enzyme-linked immunosorbent assay test kit (SERELISA® PCV2 Ab Mono Blocking, Synbiotics, France). Briefly, the controls and samples were placed in wells sensitized with anti-PCV2 antibodies (Ab1) bound specifically to purified PCV2 antigen. After a wash step to eliminate the non-associated fractions, an anti-PCV2/peroxidase conjugate (conj-HRP) is added. If there was no specific anti-PCV2 antibody in the sample, the anti-PCV2/peroxidase conjugate is free to attach forming the following complex: (Ab1)-(Ag-PCV2)-(conj-HRP). After a second washing step, the coupled enzyme conjugate was revealed by the addition of a substrate, which transforms it into a colored product. The optical density (OD) was measured for calculate the quantity of serum titer. The PCV2 titer below 550 indicates seronegative. The antibody titer that higher than 2,484 was categorized in the high positive group.

Immunohistochemistry

This immunohistochemistry was modified from previous study (Paphavasit et al., 2009). The PCV2 DNA positive uterine samples (n=2) were selected to find the PCV2 localization. The 4-µm-thick sections of paraffin-embedded tissue were placed on 3-aminopropyl-triethoxysilane-coated slides. The sections were deparaffinized by xylene and rehydrated by graded ethanol. The antigen retrieval was carried out by heating 3 times in the microwave oven at 250 watts for 5 min in the 0.01 M citrate buffer, pH 6.0. Blocking of peroxidase reaction by using fresh-prepared 5 ml of 30% H_2O_2 in 45 ml of methanol at room temperature for 20 min. Nonspecific antigens were blocked by 1% of normal goat serum (Vector Laboratories, CA, USA) for 30 min at room temperature. The sections were incubated with rabbit polyclonal anti-PCV2 primary-antibody GTX128120 (GeneTex, Inc., USA) at dilution of 1:200 and incubated at 4°C for 24 h. After that, sections were washed with PBS 3 times and incubated with the biotinylated goat anti-rabbit secondary antibody (Vector Laboratories, CA, USA) at dilution of 1:200 for 30 min. The avidin-biotin-peroxidase complex method was used to evaluated the presentation of PCV2 in the tissue samples. The solution of diaminobenzidine (ImmPACT™ DAB, Vector Laboratories, Inc., Burlingame, CA, USA) was used to develop the visualization of PCV2. After this, sections were counterstained with hematoxylin for 30 sec and washed in running water for 5 min and mounted for investigation under light microscope. The presentations of PCV2 were demonstrated by presenting of brown intranuclear staining on the section under light microscope. The intestine of PMWS pigs was served as positive control. For the negative control the primary antibody was omitted.

Statistical analysis

The statistical analyses was carried out by using SAS (SAS Inst. Inc., Cary, USA). Categorical data was expressed as percentage and was compared by using Chi-square test. Continuous data was presented as mean±SD. Multiple analysis of variance was conducted to analyze continuous data by using general linear model procedure of SAS (PROC GLM). Least squares means was calculated and was compared by using Tukey-Kramer test. $p<0.05$ was considered statistically significant.

CHAPTER IV
RESULTS

Reproductive data

Table 1 displayed the reproductive data of the slaughtered gilts. On average, the gilts entered the gilt pools at 219 ± 49 days of age. The gilts showed first estrus at 225 ± 24 day of age. The 38 of 102 gilts (37.2%) have been mated and the age at first mating was 260 ± 21 days. The gilts were culled at 280 ± 37 day of age and a body weight of 144.6 ± 18 kg. ADG from birth to culling was 511 ± 76 g/day and NPD (i.e., the interval from entering the herd to culling) was 61 ± 44 day.

Table 1 Descriptive statistics on reproductive data of the slaughtered gilts

Parameters	Number of gilts	Mean ± SD	Range
Age at entry (day)	95	219.8 ± 49.3	94.0 – 292.0
Age at first estrus (day)	83	225.5 ± 24.9	156.0 – 321.0
Age at first mating (day)	38	260.8 ± 21.2	230.0 – 323.0
Age at culling (day)	101	280.4 ± 37.8	209.0 – 406.0
Bodyweight at culling (kg)	102	144.6 ± 18.6	92.0 – 203.0
ADG (g/day)	101	511.2 ± 76.2	150.0 – 665.0
NPD (day)	95	61.5 ± 44.5	4.0 – 178.0
Ovarian weight (g)	102	6.5 ± 3.0	1.8 - 17.7
Uterine weight (g)	101	599.6 ± 285.8	56.0 - 1,523.0
Number of ovulation	70	15.2 ± 4.1	1.0-27.0

PCV2 DNA detection

The ORF1 region of PCV2 was amplified and the PCR products were shown in Figure 1. The PCV2 DNA were detected in 21 of 70 (30%) of the ovarian tissues and in 46 of 102 (45.1%) of the uterine tissues. Of the 70 gilts that both uterus and ovary were determined, PCV2 DNA was detected in 38 gilts (54.3%). Of these 70 gilts, 15 gilts (21.4%) had PCV2 DNA in both the uterus and the ovary. Seventeen gilts (24.3%) had PCV2 DNA only in the uterus and 6 gilts (8.6%) had PCV2 DNA only in the ovary.

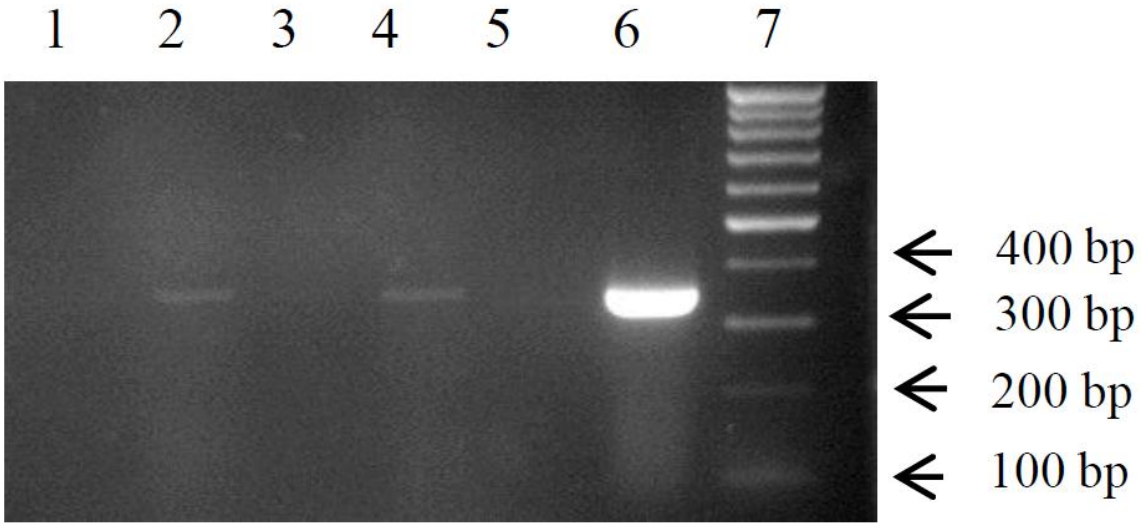

Figure 1 Polymerase chain reaction of ORF1 PCV2, lane 1, negative and lane 6, positive control, lane 2 and 4, PCV2 positive samples, and lane 7, 100 bp DNA ladder.

PCV2 DNA detection in relation with historical data and reproductive performances

Historical data and reproductive performances of gilts that had PCV2 DNA in their reproductive organs compared to those without the PCV2 DNA were displayed in Table 2.

Table 2 Historical data and reproductive performances of gilts (n=70) by PCV2 DNA detection

Parameter	Number of gilt	PCV2 DNA detection	
		Negative	Positive
Historical data			
Age at entering the herd (day)	69	201.7 ± 53.9 [a]	220.8 ± 46.3 [a]
Age at culling (day)	69	270.2 ± 36.7 [a]	271.5 ± 28.7 [a]
Bodyweight at culling (kg)	70	146.5 ± 18.4 [a]	147.2 ± 15.7 [a]
ADG (g/day)	69	540.5 ± 50.3 [a]	519.1 ± 94.6 [a]
Reproductive performances			
Age at first estrus (day)	60	217.1 ± 18.2 [a]	219.4 ± 17.3 [a]
Age at first mating (day)	20	250.5 ± 13.4 [a]	250 ± 9.6 [a]
Number of ovulation	50	12.6 ± 3.6 [a]	14.2 ± 3.3 [a]
NPD (day)	69	68.5 ± 49.3 [a]	52.1 ± 36.9 [a]

[a] Different superscript within row differ significantly ($p<0.05$)

The percentage of PCV2 DNA detection in different group of gilts classified by their historical data and reproductive performances were displayed in Table 3 and Table 4. The percentage of PCV2 DNA detection was not significantly different in any of the reproductive classification groups.

Table 3 Percentage of PCV2 DNA detection in different groups of gilts classified by their historical data

Parameter	Number of gilt	PCV2 DNA detection (%)
Age at entering (day)		
<200	13	8 (61.5%) [a]
200-220	17	8 (47.0%) [a]
221-240	20	9 (45.0%) [a]
>240	19	13 (68.4%) [a]
Age at culling (day)		
<250	21	9 (42.8%) [a]
250-280	28	18 (64.2%) [a]
>280	20	11 (55.0%) [a]
Bodyweight at culling (kg)		
<140	24	13 (54.1%) [a]
140-160	28	14 (50.0%) [a]
>160	18	11 (61.1%) [a]
ADG (g/day)		
<500	16	8 (50.0%) [a]
500-540	20	12 (60.0%) [a]
541-590	22	12 (54.5%) [a]
>590	11	6 (54.5%) [a]

[a] Different superscript within column differ significantly ($p<0.05$)

Table 4 Percentage of PCV2 DNA detection in different group of gilts classified by their reproductive performances

Parameter	Number of gilt	PCV2 DNA detection (%)
Age at first estrus (day)		
<210	18	11 (61.1%) [a]
210-225	19	9 (47.3%) [a]
>225	23	12 (52.1%) [a]
Mating history		
Mated	20	12 (60.0%) [a]
Not mated	50	26 (52.0%) [a]
Age at first mating (day)		
≤250	10	7 (70.0%) [a]
>250	10	5 (50.0%) [a]
NPD (day)		
<35	19	9 (47.3%) [a]
35-50	22	14 (63.6%) [a]
51-100	15	8 (53.3%) [a]
>100	13	7 (53.8%) [a]

[a] Different superscript within column differ significantly ($p<0.05$)

PCV2 DNA detection in relation with reason for culling

In this study, gilts were culled from abortion (n=7), anestrus (n=28), repeat service (n=7), abnormal vaginal discharge (n=40), and non-reproductive problem (n=20). The percentage of PCV2 detection in the uterus and ovary by different culling reason were displayed in Table 5. The percentage of PCV2 detection in gilt culled due to clinical sign of reproductive disturbances 42/82 (51.2%) was higher than gilt culled due to non-reproductive disturbances 4/20 (20%) ($p=0.01$). The percentage of PCV2 detection in the uterus of gilts that culled due to non-reproductive problem 4/20 (20%) was lower than gilts that culled due to abortion 6/7 (85%), abnormal vaginal discharge 19/40 (47.5%), and anestrus 15/28 (53.5%) ($p<0.05$). The PCV2 could also detected in the ovary but the detection were not significantly different between groups. The percentage of PCV2 seropositive gilts were not significantly different between reason for culling.

Table 5 Percentage of PCV2 DNA detection in the ovarian and uterine tissues and PCV2 seropositive gilt by different reason for culling

Reason for culling	PCV2 DNA positive ovary	PCV2 DNA positive uterus	PCV2 seropositive gilt
Abortion	4/7 (57.1%) [a]	6/7 (85.7%) [a]	7/7 (100.0%) [a]
Abnormal vaginal discharge	2/8 (25.0%) [a]	19/40 (47.5%) [ab]	29/40 (72.5%) [a]
Anestrus	9/28 (32.1%) [a]	15/28 (53.5%) [ab]	15/28 (53.5%) [a]
Repeat service	1/7 (14.2%) [a]	2/7 (28.5%) [bc]	5/7 (71.4%) [a]
Non-reproductive causes	5/20 (25.0%) [a]	4/20 (20.0%) [c]	14/20 (70.0%) [a]
total	21/70 (30.0%)	46/102 (45.1%)	70/102 (68.6%)

[a,b,c] Different superscript within column differ significantly ($p<0.05$)

PCV2 DNA detection in relation with gross morphologies

The percentage of PCV2 DNA detection in ovary and uterus in different gross morphologies were displayed in Table 6 and Table 7. For the ovary, percentage of PCV2 detection was 20.0% (1/5) of single cyst, 44.4% (4/9) of multiple cyst, and 26.4% (14/53) of normal ovary. For the uterus, the percentage of PCV2 detection was 40.0% (6/15) of endometritis uterus, and 50.0% (37/74) of normal uterus.

Table 6 Percentage of PCV2 detection in the ovary in different gross morphology

Morphology	Number	PCV2 DNA positive ovary
Single cyst	5	1 (20.0%) [a]
Multiple cyst	9	4 (44.4%) [a]
Miscellaneous	3	2 (66.6%) [a]
Normal	53	14 (26.4%) [a]

[a] Different superscript within column differ significantly ($p<0.05$)

Table 7 Percentage of PCV2 detection in the uterus in different gross morphology

Morphology	Number	PCV2 DNA positive uterus
Endometritis	15	6 (40.0%) [a]
Miscellaneous	13	3 (23.0%) [a]
Normal	74	37 (50.0%) [a]

[a] Different superscript within column differ significantly ($p<0.05$)

PCV2 DNA detection in relation with PCV2 antibodies titer

In this study, average antibody titer of culled gilts were 1,271±867 and ranged from 150 to more than 2,484. In these 102 gilts, 32 gilts were seronegative and 70 gilts were seropositive. In the 70 seropositive gilts, 25 gilts were high seropositive and 45 gilts were seropositive. In Table 8, the averaged antibody titer of the PCV2 DNA negative gilts was 1,279±942 while the average antibody titer of PCV2 DNA positive gilts were 1,006±709 ($p>0.05$).

Table 8 PCV2 antibody titer of PCV2 DNA negative and positive gilts

PCV2 DNA detection	Number of gilt	Seropositive gilt (%)	PCV2 antibody titer
Negative	32	21 (65.6%) [a]	1,279±942 [a]
Positive	38	23 (60.5%) [a]	1,006±709 [a]

[a] Different superscript within column differ significantly ($p<0.05$)

In the different levels of antibody titer, the percentage of PCV2 DNA detection in uterus or ovary were displayed in Table 8. The percentage of PCV2 DNA detection in the gilts with a high antibody titer (23.0%) was lower than that in the gilts with low antibody titer (57.6%) and in those with a seronegative PCV2 (64.5%) ($p<0.05$).

Table 9 The percentage of PCV2 detection in different groups of antibody titer

Antibody titer	PCV2 DNA positive (%)		
	Ovary	Uterus	Gilt
High positive (>2484)	0/13(0.0%) [a]	9/25(36.0%) [a]	3/13 (23.0%) [a]
Low positive (550-2484)	13/31(41.9%) [b]	23/45(51.1%) [a]	20/31(64.5%) [b]
Negative (<550)	8/26(30.7%) [b]	14/31(43.7%) [a]	15/26(57.6%) [b]
Total	21/70	46/102	38/70

[a] Different superscript within column differ significantly ($p<0.05$)

Localization of PCV2 antigens in the uterine tissues of gilt

PCV2 capsid proteins were detected in PCV2 DNA positive uterine tissues by IHC (Figure 2). The PCV2 positive cells were appeared as a dark brown staining both in the cytoplasm and in the nucleus of the cells (Figure 2A, C, D). In addition, the intranuclear staining of PCV2 antigens were found in the endometrial cells and epithelial cell of the uterine gland. In the subepithelial connective tissue layer of the endometrium, intranuclear staining was also detected in the lymphocytes and macrophages.

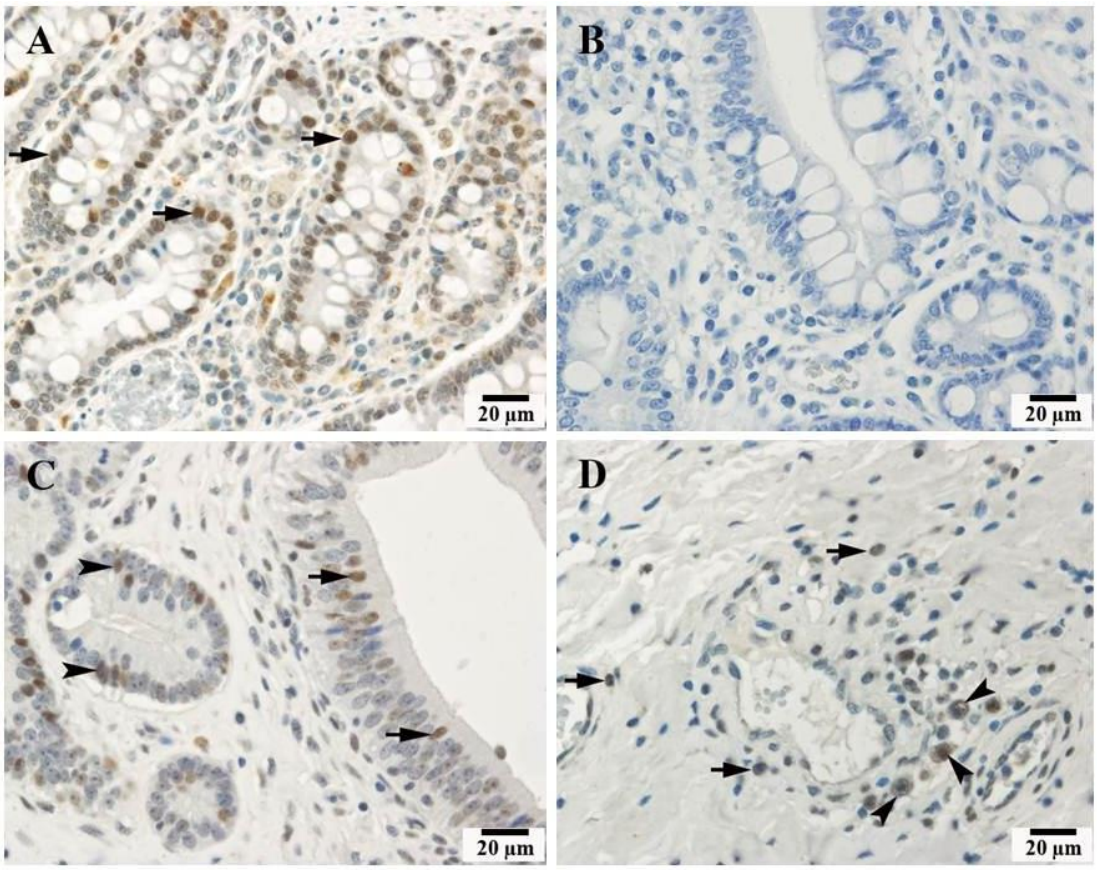

Figure 2 Immunohistochemistry localization of PCV2 antigens in the uterine tissue of the PCV2 DNA positive gilts: A) positive control (intestine) *Black arrows* indicated PCV2 positive epithelial cells; B) negative control (intestine); C) uterine tissue of PCV2 DNA positive gilt *Black arrows* indicated intranuclear staining of PCV2 positive endometrial cells *Black arrow head* indicated intranuclear staining of PCV2 positive epithelial cell of uterine gland; D) Subepithelial layer of uterine tissue of PCV2 DNA positive gilt, *Black arrows* indicated intranuclear staining of PCV2 positive lymphocytes, *Black arrow head* indicate intranuclear staining of PCV2 positive macrophages.

CHAPTER V
DISCUSSION

The detection of PCV2 in the paraffin-embedded tissues by using PCR technique is in agreement with a previous study on the PCV2 detection in the fresh ovarian and uterine tissues in gilts (Bielanski et al., 2004). This result indicated that the paraffin-embedded uterine and ovarian tissues can be practically used as materials for PCV2 detection. In the present study, a relatively high proportion of PCV2 DNA remains in both the ovarian and uterine tissues of the culled gilts. Moreover, these reproductive tissues have been embedded in the paraffin section for over five years (Tummaruk et al., 2009). These findings implied that additional retrospective studies on the PCV2 DNA in the reproductive organs can be performed. Under field conditions, the samples collected from the reproductive failure cases is time consuming and a rather limited number of cases was followed by the veterinarians. This leads to a limitation of the laboratory diagnosis to achieve final diagnosis for many reproductive failure cases observed under field conditions.

Interestingly, our study revealed that the PCV2 antigen appear in many cell types of the porcine endometrium. In general, the PCV2 can infect to many cell types including fetal cardiomyocytes, hepatocytes, macrophages, lymphocytes, endothelial cells, and epithelial cells (Sanchez et al., 2003; Perez-Martin et al., 2007; Yu et al., 2007). Macrophage and lymphocytes were found in both uterus and ovary in all stages of oestrus cycle (Standaert et al., 1991; Teamsuwan et al., 2010). Thus, the detection of PCV2 in both the uterus and ovary is not surprised. Nevertheless, the detection of PCV2 in many cells types of the porcine reproductive organs highlights the importance of this virus on the reproductive functions in gilts. Moreover, in the present study, the percentage of PCV2 DNA detection in the uterus tend to be higher than in the ovary. This possibly due to the fact that the uterine tissues contains numerous immune cells infiltration achieved from blood vessel within the sow's endometrium (Teamsuwan et al., 2010).

To our knowledge, the association between PCV2 DNA detection in reproductive organ and the gilt's reproductive performances has not been investigated before. In the sow, a previous study found that long term PCV2 vaccination could improve the reproductive performances, e.g., number of piglets born alive per litter, piglet's birth weight, and number of piglets weaned per litter (Pejsak et al., 2012). This suggested that the PCV2 infections might be involved in the

reproductive performances of sows, e.g., affecting the fetal mortality (Madson et al., 2009). In the present study, the reproductive performances of both PCV2 DNA negative and positive culled gilts are within a normal range and are in accordance with previous studies in Thailand (Tummaruk et al., 2009b; Tummaruk, 2012b). Nevertheless, the timing of PCV2 infection in the replacement gilts was not known in the present study. The infection may occur after the gilts enter the herd or during acclimatization. On average, the gilts enter the herd at 220 days of age and were culled at 280 days of age. Therefore, the infection and/or re-infection maybe occur between 220 and 280 days of age. Bolin et al. (2001) found that PCV2 DNA can be detected in the secretion of gilts up to 125 days after infection. These findings indicated that management of replacement gilts should be modified to minimize the PCV2 infection and reduce the remaining of the virus in the reproductive organs before insemination. For instance, early acclimatization and extend cool down period (i.e., leave the gilts alone without acclimatization), enhance immunity and/or vaccination are all recommended. However, the present study could not find any association between PCV2 DNA detection in reproductive organs and reproductive performances of replacement gilts. This implies that PCV2 infection may not cause dramatically changes on the reproductive function of the gilts. Therefore, reproductive functions of the ovary and uterus after the PCV2 infection should be further investigated.

The efficacy of the viral transmission depends on many factors including the susceptibility of the pig, the virulent or stability of the virus, the type of exposure, and the sufficient infectious dose and contact time (Rose et al., 2012). PCV2 are stable in the environment, under high temperature (85°C) and acid condition (pH3) (Allan et al., 1994; O'Dea et al., 2008). The large amount of PCV2 could be shed through feces, urine, oral, nasal, and tonsillar swab. In the present study, PCV2 can be detected in the replacement gilts population. In the previous study, the prevalence of PCV2 detection between 2006 to 2010 varied between 34% and 65% (Pearodwong et al., 2013). High prevalence of PCV2 in all range of age and weight indicating the importance of horizontal transmission and disease control strategy. To reduce the risk of PCV2 horizontal transmission, disinfectants (i.e., quaternary ammonium compound, oxidizing agent containing potassium peroxomonosulfate, glutaraldehyde and quaternary ammonium compound, and sodium hypochlorite compound) are suitable for PCV2 reduction in the environment (Patterson et al., 2011).

The investigation of PCVAD should be based on the presenting of clinical sign, histopathological lesion, and presenting of PCV2 in that lesion (Sorden, 2000). The clinical signs including abortion, anestrus, and abnormal vaginal discharge associated with uterine PCV2 detection in present study. Therefore, the histopathological lesion of the uterus should be further investigated for PCVAD. Twenty eight of 102 gilts were culled due to anestrus at 264 ± 20 day of age. These gilts were not delay puberty by showed the first estrus sign at 211 ± 23 day of age (Roongsitthichai et al., 2013). That information suggested that PCV2 DNA detection in the ovary and uterus were not associate with first estrus showing. In the other hands, the PCV2 DNA detection in the uterus may associate with second and third estrus sign.

In general, stress from acclimatization, overcrowding, hot and humid climate could induce the increasing of plasma cortisol (Roongsitthichai et al., 2009). The immunosuppression may be occurred and leading to be infected by PCV2. In general, PCV2 could also induce apoptosis of lymphocytes (Gauger et al., 2011) and cause acute and chronic vasculitis of ovarian arteries (Langohr et al., 2010). The abnormal vaginal discharge may be inflammation of urinary tract, vaginitis, cervicitis, metritis, and endometritis (Dee, 1992). Ascending bacterial infection was the common cause of the disease. The housing system, environment, restricted drinking water, lack of exercise, overcrowding, and stage of estrus cycle could be the pre-disposing factors of this symptom. In the 40 gilts that culled due to abnormal vaginal discharge, the gross morphology of the endometritis and normal uterus were 12/40 (30%) and 26/40 (65%), respectively. In abnormal vaginal discharge gilts, PCV2 DNA were detected in 16/26 (61.5%) of normal uterus while PCV2 DNA were detected only in 3/12 (25%) of endometritis uterus. In addition, the PCV2 DNA detection in the uterus associated with abnormal vaginal discharge but did not associate with endometritis. That result suggested that most of the gilts showed clinical sign of abnormal vaginal discharge were not likely to cause by endometritis. The pathogenesis pathway should be carried out. Recent study found that PCV2 DNA were detected by 6.0% of the uterus, in which the most common gross pathology was endometritis (Ritterbusch et al., 2012). Sarli et al. (2012) also found the uterine mucosal congestion and edema in 1/6 gilts that were inseminated with PCV2 spiked semen.

For the uterine gross morphology, PCV2 DNA could also be detected in the normal and endometritis uterus without significantly different. The PCV2 DNA could also be found in the ovary and uterus of gilts that culled due to non-reproductive cause suggesting there were 20-25% of subclinical cases of PCV2 infected pig in the herd.

For the ovarian gross morphology, PCV2 DNA could also be detected in cystic ovary and normal ovary without significantly different. The result suggested that PCV2 infection were not associate with cyst formation in the ovary. For the cystic ovary in pig, the mechanism has not completely understood. There were many factors could influenced on cystic formation, e.g., insufficient of gonadotropin-releasing hormone or luteinizing hormone producing, development of follicular stimulating hormone receptor, stress from hot and raining climate (Liptrap and McNally, 1977; Castagna et al., 2004; Tummaruk, 2012a). That result suggested that PCV2 infection in the ovary may not directly associate with those factors. In addition, there was no association between number of ovulation and PCV2 DNA detection in ovary and uterus, indicating that presenting of PCV2 DNA in the reproductive organs may not interfere the ovulation mechanism. In contrast, current study showed that the average number of CL of PCV2 experimental infected gilts (CL=17.2) was significant lower than naïve gilts (CL=30.2) (Bielanski et al., 2013).

The severity of lesion in the organ not depends on only PCV2 infection, but infectious and non-infectious cofactor may be the importance role of PCVAD (Silva et al., 2011). The lymphocytic-histiocytic and, granulomatous inflammation were the key of PCV2 effected lesion (Chae, 2004). For the immunohistochemistry of the uterus and ovary, PCV2 antigens were detected in the endometrial cells, lymphocytes and macrophages in subepithelial connective tissue layer without any lesion. That finding suggested that the reproductive organs could be the accumulation site for PCV2.

In present study, the level of antibody titer of culled gilts were similar to the unvaccinated dam from previous report (Kurmann et al., 2011). In general, PCV2 antibody could be detected within 7 days post-infection and specific antibody rose 2-3 week thereafter and still detected until more than 98 days after infection (Steiner et al., 2009; Prickett et al., 2011). In PMWS affected farm, viral load in the serum can be detected in serum of pigs at 6 week of age and the viral concentration are highest at 10 week of age then gradually decrease until 25 weeks of age and remain stable in the sow (Carasova et al., 2007). That implied the persistent infection of PCV2. This study, there were 70 of 102 (68.6%) seropositive gilts. Interestingly, there were 21 gilts were seropositive without PCV2 DNA detection in the reproductive organs. The previous study showed that in the total of 55 PCV2 seropositive gilts, there were only 46 gilts were PCV2 DNA positive to PCV2 DNA (Bielanski et al., 2004). Among the tissues of gilts that were inoculated intranasally with PCV2, the percentage of PCV2

DNA detection in bronchial lymph node, liver, lung, and spleen were 95.2-100% while the percentage of PCV2 DNA detection in the ovarian CL and uterine endometrium were 79% and 93%, respectively. That results suggested that the reproductive organs may not the target organs of viral replication. The seropositive gilts without PCV2 DNA detection in reproductive organs may keep the PCV2 in others lymphoid organs. In the present study, antibody titer of gilts that PCV2 DNA negative in the ovary was tend to higher than PCV2 DNA positive gilts. Meerts et al. (2005) found that low neutralizing antibody titer was associated with increasing of PCV2 replication. These indicated that gilts with high PCV2 antibody titer might have more capability to reduce the viremia of PCV2.

Previous study had demonstrated that the PCV2 antibody (IgG) of the dam were detected at 112 days after intrauterine insemination of PCV2 spiked semen (Madson et al., 2009). This study, the gilts were inseminated approximately 60 days before culling. Although this study didn't have the result of PCV2 detection in the semen. From the antibody titer result, the artificial insemination may not be the route of infection. The presenting of PCV2 DNA in the lymphocytes or macrophages in the uterus may indicate the spreading of the PCV2 by viremia stage or using these cells as a vehicle.

In conclusion, PCV2 was detected by 30.0% of the ovary and 45.1% of the uterus of gilts culled due to reproductive disturbances. The reason for culling including abortion, abnormal vaginal discharge, and anestrus in gilt were associated with PCV2 DNA detection in the uterus. No significant associations between reproductive performances, and gross morphology and PCV2 DNA detection in the ovary and uterus were found. The PCV2 antigens were detected in the endometrial cells, uterine gland, subepithelial lymphocytes and macrophages. The PCV2 DNA detection in the ovary of gilts with a high PCV2 antibody titer were lower than that in the gilts with a low PCV2 antibody titer.

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APPENDIX

APPENDIX A

Table 10 Individual reproductive data of 102 slaughtered gilts

N	Culling reason	Weight	Age at				ADG	NPD	Cycle	CL	AI
			culling	Enter	1st estrus	mate					
1	abortion	168	266	221	190	230	626	45	L	24	1
2	abortion	166	316	242	216	256	521	74	L	16	1
3	anestrus	113	234	98	170	.	476	136	P	.	0
4	anestrus	123	270	99	178	.	450	171	P	.	0
5	anestrus	148	277	99	.	.	529	178	P	.	0
6	anestrus	142	267	96	.	.	526	171	P	.	0
7	anestrus	105	209	94	.	.	495	115	L	15	0
8	vaginal	136	264	95	210	236	509	169	L	13	1
9	repeat	168	309	242	243	257	539	67	L	18	1
10	anestrus	150	235	98	.	.	632	137	P	.	0
11	anestrus	152	246	95	.	.	612	151	P	.	0
12	anestrus	137	245	94	.	.	553	151	P	.	0
13	vaginal	182	358	242	209	252	504	116	L	17	1
14	repeat	159	312	245	239	262	505	67	L	21	1
15	abortion	169	332	237	211	247	505	95	L	18	1
16	abortion	166	295	228	201	241	558	67	L	23	1
17	vaginal	136	290	250	218	258	464	40	L	14	1
18	repeat	203	406	267	216	279	496	139	L	14	1
19	repeat	185	329	239	228	249	558	90	F	.	1
20	vaginal	124	272	232	204	243	450	40	L	17	1
21	anestrus	175	304	250	221	.	571	54	L	1	0
22	anestrus	152	290	236	207	.	519	54	L	20	0
23	anestrus	165	287	247	239	.	570	40	F	.	0
24	anestrus	157	279	233	224	.	557	46	L	14	0
25	anestrus	150	264	233	203	.	563	31	F	.	0
26	anestrus	166	262	99	.	.	628	163	P	.	0
27	repeat	164	312	231	205	245	521	81	F	.	1
28	abortion	151	287	249	217	258	521	38	L	21	1
29	vaginal	132	253	204	.	.	516	49	F	.	0
30	vaginal	156	279	226	197	236	554	53	F	.	1
31	abortion	164	315	249	221	260	516	66	L	18	1
32	anestrus	164	292	253	245	.	557	39	L	12	0
33	anestrus	145	295	256	229	.	486	39	L	17	0
34	anestrus	150	272	244	.	.	150	58	L	13	0

35	repeat	164	312	239	231	252	521	73	L	15	1
36	anestrus	150	252	94	156	.	589	158	P	.	0
37	anestrus	146	262	230	204	.	552	32	L	17	0
38	anestrus	146	264	232	221	.	547	32	F	.	0
39	anestrus	161	253	235	207	.	630	18	L	14	0
40	abortion	148	296	292	203	246	495	4	L	18	1
41	anestrus	140	269	230	225	.	515	39	L	13	0
42	repeat	162	336	249	243	267	478	87	L	17	1
43	vaginal	128	274	242	212	.	462	32	P	.	0
44	anestrus	131	274	242	215	.	473	32	L	14	0
45	anestrus	129	258	233	222	.	494	25	L	14	0
46	anestrus	158	259	241	231	.	604	18	L	9	0
47	anestrus	132	240	222	196	.	544	18	L	14	0
48	anestrus	128	262	237	210	.	483	25	L	10	0
49	anestrus	147	274	242	235	.	531	32	L	23	0
50	vaginal	148	256	224	215	234	572	32	L	17	1
51	nonrep	124	251	201	231	.	488	50	F	.	0
52	nonrep	129	215	193	202	.	593	22	L	16	0
53	nonrep	140	248	198	229	.	558	50	L	10	0
54	nonrep	139	253	231	228	.	543	22	L	14	0
55	nonrep	147	240	211	210	.	606	29	L	17	0
56	nonrep	161	240	203	206	.	665	37	L	17	0
57	nonrep	138	234	203	226	.	583	31	L	17	0
58	nonrep	132	.	.	.	.	.	.	L	18	0
59	nonrep	141	235	204	229	.	594	31	L	13	0
60	nonrep	142	235	204	225	.	598	31	L	12	0
61	nonrep	127	262	211	230	.	479	51	F	.	0
62	nonrep	142	246	209	235	.	571	37	P	.	0
63	nonrep	129	245	208	237	.	520	37	L	11	0
64	nonrep	141	245	201	241	.	569	44	L	14	0
65	nonrep	144	253	216	240	.	563	37	L	13	0
66	nonrep	129	248	209	221	.	514	39	L	12	0
67	nonrep	127	242	203	235	.	519	39	L	5	0
68	nonrep	132	241	203	235	.	541	38	L	15	0
69	nonrep	140	239	202	229	.	579	37	L	13	0
70	nonrep	130	238	202	213	.	540	36	L	27	0
71	vaginal	140	405	245	321	323	342	160	F	.	1
72	vaginal	149	371	214	260	260	398	157	P	.	1
73	vaginal	146	266	260	.	.	543	6	F	.	0
74	vaginal	146	302	261	256	274	478	41	L	11	1
75	vaginal	111.5	283	.	.	.	389	.	L	15	0
76	vaginal	111.5	254	234	.	235	433	20	F	.	1

77	vaginal	138	285	251	241	261	479	34	L	12	1
78	vaginal	135	320	244	204	247	417	76	L	17	1
79	vaginal	145	273	.	.	.	526	.	L	17	0
80	vaginal	92	240	.	.	.	377	.	P	.	0
81	vaginal	121.5	318	247	226	.	377	71	F	.	0
82	vaginal	164	260	.	241	.	625	.	F	.	0
83	vaginal	171	318	270	239	281	533	48	L	13	1
84	vaginal	157	279	259	251	.	557	20	L	11	0
85	vaginal	166	353	277	268	313	466	76	L	18	1
86	vaginal	159	309	247	237	259	510	62	L	17	1
87	vaginal	145	316	261	253	273	454	55	L	18	1
88	vaginal	122.5	268	243	250	250	451	25	F	.	1
89	vaginal	151	308	253	243	263	485	55	L	12	1
90	vaginal	162.5	366	254	301	301	440	112	L	12	1
91	vaginal	155	303	262	236	276	507	41	L	16	1
92	vaginal	173	292	272	265	.	587	20	L	8	0
93	vaginal	156	266	246	241	.	581	20	L	20	0
94	vaginal	110	291	241	266	266	373	50	F	.	1
95	vaginal	135	283	.	.	.	472	.	L	19	0
96	vaginal	143	309	289	242	.	458	20	L	18	0
97	vaginal	138	282	.	.	.	484	.	L	15	0
98	vaginal	104.5	275	241	.	.	375	34	L	14	0
99	vaginal	144	294	251	.	.	485	43	P	.	0
100	vaginal	133	285	249	184	264	461	36	F	.	1
101	vaginal	130.5	356	252	233	299	362	104	L	17	1
102	vaginal	119.5	323	245	197	258	365	78	F	.	1

vaginal : vaginal discharge; repeat : repeat service; nonrep : non-reproductive
L : luteal phase; F : follicular phase; P : prepuberty

APPENDIX B

Table 11 Individual gross morphology of gilt's ovary and uterus

N	Gross morphology		N	Gross morphology	
	Ovary	Uterus		Ovary	Uterus
1	M_Cyst	Normal	53	Normal	Normal
2	M_Cyst	Normal	54	Normal	Normal
3	Normal	Normal	55	Normal	Normal
4	Normal	Normal	56	Normal	Normal
5	Normal	Normal	57	Normal	Normal
6	Normal	Normal	58	Normal	Normal
7	miss	Normal	59	Normal	Normal
8	Normal	Normal	60	Normal	Normal
9	Normal	Normal	61	S_Cyst	Normal
10	Normal	Normal	62	Normal	Normal
11	miss	miss	63	M_Cyst	Normal
12	Normal	Normal	64	Normal	Normal
13	S_Cyst	miss	65	Normal	Normal
14	Normal	Normal	66	Normal	Normal
15	Normal	inflame	67	Normal	miss
16	M_Cyst	miss	68	Normal	Normal
17	S_Cyst	Normal	69	Normal	Normal
18	Normal	Normal	70	M_Cyst	Normal
19	Normal	miss	71	M_Cyst	inflame
20	Normal	Normal	72	Normal	inflame
21	M_Cyst	Normal	73	Normal	inflame
22	Normal	miss	74	Normal	Normal
23	Normal	miss	75	Normal	Normal
24	Normal	Normal	76	M_Cyst	inflame
25	M_Cyst	Normal	77	Normal	Normal
26	Normal	Normal	78	Normal	Normal
27	Normal	Normal	79	Normal	inflame
28	Normal	Normal	80	Normal	Normal
29	Normal	Normal	81	Normal	inflame
30	Normal	Normal	82	Normal	inflame

31	Normal	Normal	83	Normal	inflame
32	M_Cyst	Normal	84	S_Cyst	inflame
33	Normal	miss	85	miss	miss
34	M_Cyst	Normal	86	S_Cyst	Normal
35	Normal	miss	87	miss	Normal
36	Normal	inflame	88	Normal	Normal
37	Normal	Normal	89	Normal	Normal
38	Normal	miss	90	M_Cyst	Normal
39	Normal	Normal	91	Normal	Normal
40	Normal	Normal	92	M_Cyst	inflame
41	Normal	miss	93	Normal	Normal
42	S_Cyst	Normal	94	Normal	Normal
43	Normal	Normal	95	Normal	Normal
44	Normal	Normal	96	Normal	Normal
45	Normal	Normal	97	Normal	Normal
46	Normal	Normal	98	Normal	inflame
47	Normal	inflame	99	Normal	Normal
48	S_Cyst	Normal	100	Normal	Normal
49	Normal	miss	101	Normal	Normal
50	miss	Normal	102	Normal	inflame
51	Normal	Normal			
52	Normal	Normal			
M_Cyst : multiple cyst; S_Cyst : single cyst, inflame : endometritis Miss : miscellaneous					

APPENDIX C

Table 12 Individual result of PCV2 DNA detection in ovary and uterus, and PCV2 antibody titer

N	OPCV2	UPCV2	PCV2 titer	N	OPCV2	UPCV2	PCV2 titer
1	0	1	1688	53	0	1	436
2	1	1	1132	54	1	0	675
3	1	1	1417	55	0	0	1407
4	0	0	>2484	56	0	1	>2484
5	0	0	>2484	57	0	0	>2484
6	0	1	>2484	58	0	0	1220
7	0	0	>2484	59	0	0	1846
8	0	1	2223	60	0	0	1492
9	1	1	1625	61	1	0	680
10	0	0	>2484	62	0	0	>2484
11	1	1	777	63	0	0	601
12	0	1	>2484	64	0	0	955
13	0	0	>2484	65	0	1	464
14	0	0	636	66	1	0	347
15	1	1	1021	67	0	0	>2484
16	0	0	>2484	68	1	0	242
17	0	1	356	69	0	0	508
18	0	0	259	70	1	0	519
19	0	0	1592	71	.	0	505
20	0	0	>2484	72	.	0	>2484
21	0	0	348	73	.	1	>2484
22	0	0	354	74	.	1	>2484
23	1	0	1510	75	.	0	>2484
24	0	0	1592	76	.	0	541
25	1	1	150	77	.	0	1333
26	0	1	309	78	.	1	>2484
27	0	1	1933	79	.	0	>2484
28	0	1	836	80	.	0	249
29	1	1	343	81	.	0	2274
30	0	1	150	82	.	0	1239

31	1	1	1718	83	.	0	1736
32	0	1	214	84	.	1	1536
33	1	1	1497	85	.	0	1909
34	1	1	1281	86	.	0	2372
35	0	0	301	87	.	1	>2484
36	0	1	493	88	.	1	1185
37	0	0	237	89	.	1	764
38	0	0	150	90	.	0	237
39	1	1	320	91	.	1	372
40	1	1	1391	92	.	1	150
41	0	0	284	93	.	0	>2484
42	0	0	993	94	.	1	>2484
43	0	0	260	95	.	1	900
44	0	1	1021	96	.	1	>2484
45	0	0	150	97	.	1	1097
46	1	1	1550	98	.	0	657
47	0	1	631	99	.	0	>2484
48	0	0	288	100	.	1	1071
49	1	1	417	101	.	0	576
50	1	1	424	102	.	0	>2484
51	0	1	998				
52	0	0	624				

OPCV2 : Ovarian PCV2 DNA detection

UPCV2 : Uterine PCV2 DNA detection

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Publication

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