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**Conclusions**: This is the first case of PDD associated with ABV infection in Korea. To prevent spread of ABV and outbreak of PDD in Korea, epidemiological survey was warranted.

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**P-118**

## Development of Direct PCR Preventing DNA Carryover Contamination for the Detection of Porcine Circovirus

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**Introduction**: Porcinecircovirus disease (PCVD) caused by PCV2 is the most common disease incommercial pork production worldwide.Laboratorydiagnosis of PCV2 is carried out on tissues of infected animals usinghistopathology associated with the detection of PCV2 DNA by in situ hybridizationor viral antigens by immunohistochemistry or indirect immunofluorescence. Although these techniques have a good sensitivity and specificity, they must beperformed on post-mortem specimens and can be time-consuming. Alternatively, polymerasechain reaction (PCR) can be used to detect PCV2 DNA in tissue samples and alsoin a broad range of body fluids such as blood, nasal and semen. PCR assay hasbeen now widely used as a specific and sensitive diagnostic method for thedetection of PCV2 in field samples AlthoughPCR provided a valuable approach for detection of pathogens, the high level ofsensitivity of these assays also makes them prone to false positive result. Especially,contamination by amplified DNA products and primers from previous PCRs isserious. Therefore, the main objective of this study was to develop a directPCR (dPCR) with UDG for rapid detection of PCV2 and prevention of DNAcontamination in entire PCR process.

**Materials and Methods**: Primersets that could detect the PCV2 were designed. Nucleotide sequence data forPCV2 strains from GenBank were aligned by using Clone Manager 6 to identifyregions that equal between the genotype. The direct PCR was used as a DNA templatethat lysates directly extracted from field sample (tissue, blood) without any DNApurification. The conventional PCR was performed with DNA template as genomicviral DNA. Genomic viral DNA from field sample was extracted and purified byusing Inclone™ RNA/DNA mini Extraction kit (Inclone biotech, Korea) as describedin the manufacturer's manual. PCR reactions were optimized based on primer concentrationselection criteria. The amplified PCR products were analyzed by 1.5% agarosegel electrophoresis and examined under ultraviolet light. The sensitivity ofthe dPCR was examined in comparison with conventional PCR. The DQ629135(PCV2-b) strains were serially diluted in phosphate-buffered saline from $10^0$to $10^{-7}$. DNA was extracted from the diluted virus solution andapplied for the assay. Inorder to confirm the effectof UDG on the carryover contamination, dPCR with/without the UDG systemwere performed on samples containing serially diluted pre- amplified DNA ofPCV2 ORF2 gene.

**Results**: Inthis study, we developed a dPCR method without DNA extraction process for thedetection of PCV2 DNA by applying uracil DNA glycosylase (UDG) system. Thesensitivity of the dPCR was confirmed that the same level or higher compared tothe conventional method with DNA extraction process. The dPCR applied with/withoutthe UDG system were performed on samples containing serially diluted pre-amplified DNA of PCV2 ORF2 gene. The results of dPCR with the UDG system was proven tounaffected by pre-amplified DNA contamination, but the results of dPCR withoutthe UDG system was not.

**Conclusions**: The developed dPCR with UDG system for thedetection of PCV2 was simple and rapid compared to the conventional PCR, andwas proven to prevent DNA carryover contamination that can occur in the PCRprocess. It is expected that the use of this dPCR method will be very useful andcost-saving for diagnosis of PCV2 in pig disease diagnostic laboratories.

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**P-119**

## Generation of Diploid Cloned Embryos from Porcine-Induced Pluripotent Stem Cells Synchronized to Metaphase

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**Introduction**: Pigs provide outstanding models of human genetic diseases due to the striking similarities to human anatomy, physiology and genetics. Pig induced pluripotent stem cells (piPSCs) have been generated for several years but the cloning efficiency using unsynchronized piPSCs was extremely low. Here, we reported a method to produce diploid cloned embryos from piPSCs which were synchronized to metaphase.

**Materials and Methods**: The piPSCs cell line was established using a drug-inducible system and exhibit similar morphology to mouse embryonic stem cells with normal karyotype. The piPSCs were first synchronized by a 2-step block method with aphidicolin and nocodazole to mitotic metaphase. Then the

cells were used as donor cells for nuclear transfer. 6DMAP was added just after activation or pseudo-second polar body (p2PB) extrusion to examine its effect on piPSCs nuclear transfer (piPSNT) efficiency. Effect of immediately activation (IA) and delayed activation (DA) methods were also compared.

**Results**: After synchronization, 77.6 % piPSCs were arrested at G-2/M phase. Round cells ranged from 17-19 μm were used as donor cells. After activation, 81.3 % of reconstructed embryos extruded one p2PB. The immune-fluorescent results confirmed that half chromatids were extruded with the p2PB. However, 2mM 6DMAP treatment post activation blocked the p2PB extrusion. Moreover, IA method yielded significantly more blastocysts than DA (31.3% vs. 16.0%, based on fused embryos). 6DMAP treatment post p2PB extrusion also didn't improve the blastocyst formation rate. Karyotyping of the blastocysts indicated that 59.7 % blastocysts were diploid.

**Conclusions**: This study demonstrated a new efficient way to produce cloned embryos from piPSCs which synchronized to mitotic metaphase. It will help to study the gene expression profile of piPSNT embryos and the feasibility of using these embryos to produce cloned or transgenic pigs.

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## P-120

## Effect of Ganglioside GT1b Treatment during Porcine *In Vitro* Maturation

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**Introduction**: Ganglioside is acidic glycosphingolipid it has sialic acids residue. b-series ganglioside GT1b was reported that suppressing damage of mtDNA by reactive oxygen species (ROS) in mouse brain. Recently, ganglioside discovered not only central nervous system but also mouse embryos. The purpose of this study is to investigate the effect of exogenous addition of ganglioside GT1b on *in vitro* maturation (IVM) of porcine oocytes and to confirm the related bradykinin 2 receptors (B2R).

**Materials and Methods**: Ganglioside GT1b were treated on IVM that concentration was 0 (control), 5, 10 and 20nM. Data were analyzed by ANOVA followed by Duncan using SPSS (Statistical Package for Social Science) mean ± SEM. After IVM, we evaluated intracellular glutathione (GSH) levels and ROS levels in matured oocytes. And, we examined developmental capability (parthenogenesis, *in vitro* fertilization) using matured oocytes with Gt1b in IVM stage. To analyze the mechanism of GT1b effect on IVM, we examined the expression of apoptosis-associated genes (Bax, BCl2, Caspase-3), B2R and CaMK2G gene in matured cumulus cells. B2R known to increase calcium concentration stimulated by GT1b using.

**Results**: Intracellular GSH levels in oocytes matured with 5nM and 20nM GT1b decreased significantly ($P<0.05$) compared with those in the other groups. The 10nM and 20nM groups showed a significant ($P<0.05$) decrease in intracellular ROS levels compared with control group. However, developmental capability were no significant ($P<0.05$) developmental difference in the two experiments. The treatment of 20nM GT1b significantly ($P<0.05$) decreased the expression of PCNA and CaMK2G, and BCl2, an anti-apoptotic gene, was increased significantly ($P<0.06$). GT1b significantly ($P<0.05$) decreased expression of B2R.

**Conclusions**: In conclusion, these results indicated that ganglioside GT1b play an important role in decreasing the intracellular ROS levels during IVM. But it did not effect on development capability.Further studies are needed to show the mRNA expression of apoptosis-associated genes and CaMK2G in matured oocytes.

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## P-121

## Synergistic Antibacterial and Antiquorum Sensing Activity of *Nymphaea Tetragona* (Water lily) Extract in vitro

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**Introduction:** *Salmonella* species are the leading cause of bacterial gastroenteritis in humans and animals all over the world. Food animals and water are the most important reservoirs of the bacteria. *Salmonella* typhimurium infect a wide range of animal hosts which usually cause a self-limited gastroenteritis. The use of antibiotics is a major strategy and is commonly used to treat *S.* typhimurium infections. However, increased antimicrobial resistance is exacerbating impact on public health worldwide. Development of alternative antibacterial therapies is necessary to overcome this outbreak. The possible therapeutic use of *Nymphaeaceae* may be a good alternative of traditional antibacterial. The purpose of this study was to investigate the antimicrobial activity of *Nymphaea tetragona* alone and