PCV2 detection in in vitro-produced blastocysts after virus sperm exposure

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Introduction
Porcine circovirus type 2 (PCV2) is a small, non-enveloped, circular, single stranded DNA virus. By phylogenetic analysis PCV2 can be further divided into two main genotypes, PCV2a and PCV2b, both distributed worldwide. Despite the fact that PCV2 is now being regarded as an important swine pathogen, information on its interaction with oocytes and embryos should be further investigated. This research has been designed to study the capability of PCV2-added semen to produce infected blastocysts from IVM (in vitro maturation) - IVF (in vitro fertilization) oocytes.

Materials and Methods
Exposition of boar spermatozoa to PCV2b. PCV2 Real-Time PCR negative sperm rich fraction of ejaculates were collected from two mature boars and extended in equal volume of Androhep (Minitüb, Tiefanbach, Germany). Sperm suspension containing 3x10^9 spermatozoa/100 ml of Androhep, was incubated for 2h at 16°C with 10 ml of a PCV2b viral suspension containing 10^3.9TCID50/ml.

IVM and IVF of cumulus-oocyte-complexes (COCs). Ovaries were collected from prepubertal gilts at a local slaughterhouse. COCs were aspirated from 4 to 6 mm antral follicles, selected and matured as previously described (3). For IVF, sperm suspensions were washed twice in IVF medium (Bracket&Olifant medium). Matured oocytes were fertilized with infected (infected group; I) and non-infected (control group; C) sperm (1x10^6 sperm/ml) in IVF medium. After 90 min of co-culture, oocytes were transferred to fresh IVF medium and grown for an additional 18 h period. After IVF, oocytes were washed twice in NCSU-23 and cultured in the same medium as previously described (3). At 168 h post-fertilization, embryos were examined at stereomicroscope and blastocysts were treated with pronase, in order to remove zona pellucida, rinsed twice in nuclease free water, individually frozen in 5 µl water and stored at -80°C until PCR analysis.

Virus detection. Blastocysts were lysed as previously described (4). The detection of PCV2a or PCV2b was conducted by nested PCR. In the first round, the outer primers used were amplifying a 627 bp product (forward: 5’-CAGGATTTGTAGTCCTGTT-3’; reverse: 5’-CCGCACCTTGCAGTTATCTGTC-3’). DNA amplification parameters consisted of 35 cycles at 95°C for 1 min, 55°C for 1 min and 72°C for 1 min. In the second PCR run, the forward primer, (5’-CACGGATTTGTAGTCCTGTT-3’) was designed to amplify both PCV2a and PCV2b, while the reverse either PCV2a (5’-GGGGGACCAAAACATCTC-3’) or PCV2b (5’-GGGGGCTCAAACCCCCGCTC-3’). Amplification of DNA was achieved by 35 cycles of 95°C for 1 min, 62°C for 1 min and 72°C for 1 min. The expected PCR product was 374 bp. The amplicons were analyzed on 2% agarose gel, stained with ethidium bromide and visualized under UV light. The specificity of the primers was demonstrated amplifying PCV2a and PCV2b control viruses.

Results
Exposition of spermatozoa to PCV2b did not exert any effect on the percentage of embryos that developed to blastocyst stage (C group: 19.7%; I group: 21.3%). In C group, 4 out of 10 blastocysts tested were PCV2a positive. In I group, 1 out of 15 blastocysts screened were PCV2b positive and 2 PCV2a positive.

Discussion
This preliminary study suggests as follows:
1. Oocytes, collected from slaughterhouse gilts, can be PCV2 contaminated cells as previously reported (5); in our experiment only PCV2b was added to PCV2 free spermatozoa, thus the presence of PCV2a in the blastocysts can be only explained by COCs natural infection.
2. PCV2 positive oocytes or spermatozoa can produce infected blastocysts by-passing the zona pellucida protection that other authors (1) proved to be an obstacle to the virus entrance.
3. In our experimental conditions the virus seems not to easily infect sperm cells and therefore to be brought into the oocyte at fertilization. Further research will be focused to set up sperm integration of the virus.

References