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*Short communication*

# Obtaining healthy offspring from PRRSV-positive pig breeding herds

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## Abstract

In this study, we propose a possible way of obtaining reproductive and respiratory syndrome virus (PRRSV) free offspring from genetically valuable lines of Přeštice black-pied boars coming from PRRSV-positive pig breeding herds with the use of artificial insemination (AI). The ejaculates were collected from 4 different lines of boars. Samples of fresh semen were not detected with the virus and 12 sows were inseminated. Blood samples of sows and their offspring were repeatedly tested for the virus but the results were negative. We managed in this way to maintain the endangered population of this breed and obtain PRRSV-free offspring.

**Key words:** boar semen, PRRS, insemination

## Introduction

Přeštice black-pied pig is an original national breed and has been included in the program of preservation of Animal Genetic Resources in the Czech Republic since 1992. In the Czech Republic, after area testing, the occurrence of the porcine reproductive and respiratory syndrome virus (PRRSV) was recorded in some of the important breeding. The statute of PRRSV-free breeding was recorded in only 4 of the total number of 20-25 Přeštice black-pied pigs. The occurrence of PRRSV has not been observed for long time and the findings were rather immunological in nature than clinical manifestations. Out of 10 genealogical living lines, there were 4 lines at the time of the experiment which were represented by only one breeding boar from PRRSV-positive breeding. The virus strain and its virulence play a role, but animal-individual factors also

have an influence on the presence and quantity of virus in the semen. Shedding of the virus can be continuous or intermittent (Nathues 2014) and it is possible to obtain a negative virus sample of semen (Prieto et al. 2005). This possibility was confirmed in our preliminary study (Lustyková et al. 2015). Therefore, the aim of this study was to obtain offspring without PRRSV from boars of positive breeding using artificial insemination to maintain not only the endangered line of boars, but also the population of this breed.

## Materials and Methods

The study did not require the approval of the Ethical Commission on Animal Use because samplings and handling of animals were within normal breeding and veterinary activities.

Table 1. Boar semen quality parameters.

Item	Line 1	Line 2	Line 3	Line 4
Semen volume (ml)	200	100	280	100
Sperm motility (%)	75	75	75	80
Sperm concentration ( $\times 10^3/\text{mm}^3$ )	436	282	556	369
MAS – total (%)	54.5	77	11.5	64
PPD	4	37	1.1	12
DPD	42	25	8.1	49
Folded tail	5.5	7	2.1	0.5
Coiled tail	0	8	0.1	1
Other abnormalities	3	0	0.1	1.5
Sperm motility 1+1 (%) after dilution	65	70	70	70
after 48h storage time	50	45	60	45
after 72h storage time	50	40	60	45
Dilution rate – insemination dose	1+3	1+3	1+2	1+4

MAS – Morphologically abnormal spermatozoa

PPD – Proximal protoplasmic droplet

DPD – Distal protoplasmic droplet

Table 2. Reproductive performance of sows (mean  $\pm$  SD).

Item	Line 1	Line 2	Line 3	Line 4
Sows inseminated (n)	4	1	4	3
Conception rate (%)	50	100	75	33.33
Farrowing rate (%)	50	100	75	33.33
Total born/litter size (n)	8.50 $\pm$ 0.50	10.00 $\pm$ 0.00	10.33 $\pm$ 0.47	6.00 $\pm$ 0.00
Live born/litter size (n)	7.00 $\pm$ 0.00	10.00 $\pm$ 0.00	8.00 $\pm$ 0.82	4.00 $\pm$ 0.00
Litter size at weaning (n)	7.00 $\pm$ 0.00	10.00 $\pm$ 0.00	8.00 $\pm$ 0.82	4.00 $\pm$ 0.00

Four different genealogical lines of the Preštice black-pied boars bred on two farms in the Czech Republic where boars were used regularly in natural breeding. Line 1 – Sokolík (age 3.5 years), Line 2 – Vis-kont (age 8 years), Line 3 – Pirát (age 2.25 years) were housed on the first farm and Line 4 – Sudet (age 1.5 years) from the second farm. Sperm rich fractions were collected using the gloved-hand technique from boars on the farms. The basic semen parameters were evaluated. The sperm motility was assessed using phase contrast microscopy at 200 $\times$  magnification. Sperm concentration was measured using a Chroma Colorimeter 254 (Sherwood Scientific Ltd., Cambridge, England). Morphologically abnormal spermatozoa (MAS) were assessed according to the staining method of Čeřovský (1976). The categories of abnormalities were proximal protoplasmic droplet (PPD), distal protoplasmic droplet (DPD), tail defects and other abnormalities. Semen was pre-diluted (1+1) with

Androhep extender (Minitübe, Germany) and stored at 17°C up to 72 h. Sperm motility was evaluated at 48 h and 72 h.

Twelve selected and synchronized virus-free sows were inseminated with virus-free insemination doses (ID) on the 3rd to the 4th day after collection. The dilution rate for the ID is shown in Table 1. Samples of fresh semen were tested for the detection of PRRSV using SOP VIR.16 RT-PCR method in accredited testing laboratory No. 1129 of the Jihlava State Veterinary Institute in the Czech Republic. Blood samples from the sows and their offspring were tested by commercial ELISA Ab test in the 4th month after farrowing before inclusion of the offspring into breeding and then once a year. Basic statistical characteristics of the results were calculated using the QC Expert program (TriloByte Statistical Software, s. r. o., Pardubice, Czech Republic).

## Results and Discussion

The initial semen quality parameters of the four different genealogical lines of Preštice black-pied pig are presented in Table 1. Sperm motility  $\geq 70\%$ , sperm concentration  $\geq 150 \times 10^3/\text{mm}^3$ , semen volume  $\geq 100$  ml and MAS  $\leq 25\%$  are the recommended requirements for boar semen quality parameters for AI in the Czech Republic. The initial semen quality parameters were in accordance with these requirements; only the MAS limit was exceeded and there was a high incidence of PPD and DPD. The occurrence of MAS was tolerated because the boars were commonly used in natural breeding. Schulze et al. (2013) found that infection with PRRSV in boars induced a significant decrease in intact acrosomes and membranes in spermatozoa samples. All tested samples of boar semen for the detection of PRRSV were negative; synchronized sows were therefore inseminated using prepared ID. Results for the reproductive performance of the sows are shown in Table 2. No complications were detected in the sows during pregnancy and post-partum in this study. Conception rate and farrowing rate were 58.33%. Václavková et al. (2012) noted similar results in reproductive performance in this breed. Blood samples from sows and their offspring for detection of virus were always negative after the 4th month of farrowing following the selection of animals for breeding, and then once a year. In conclusion, this study demonstrated a method for obtaining PRRSV-free offspring that could not be obtained by other standard breeding procedures. We managed in this way to maintain the

endangered population of this breed and obtain healthy PRRSV-free offspring.

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