

Detecting PRRSV in boar semen

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Transmission of PRRSV

Since the initial outbreaks of porcine reproductive and respiratory syndrome (PRRS) in 1987 and subsequent identification of the causative virus in 1991,^{1,2} the primary concern of the swine industry has been the routes of PRRS virus (PRRSV) transmission. Initial epidemiological studies, particularly in Europe, indicated that airborne transmission, even over significant distances, was important.^{3,4} However, recent experimental studies at Iowa State University found that aerosol transmission of PRRSV was difficult to achieve.⁵ They found that contact between pigs—possibly through such secretions as urine, feces, or nasal droplets—may be more important in the spread of PRRSV.⁵

Transmission of PRRSV in boar semen

Since 1993, epidemiologic investigations have implicated boar semen as a potential mode of PRRSV transmission.^{6,7} In a large farrow-to-finish operation in South Dakota, semen was implicated as a source of PRRSV transmission.⁶ A second case implicated semen transmission of PRRSV from several boar stud operations to recipient herds in the United Kingdom.⁷ Since then, experimental studies have shown direct transmission of PRRSV to sows or gilts through raw or extended semen. Yaeger, et al.,⁶ showed seroconversion of two gilts artificially inseminated with PRRSV-contaminated raw semen. In 1995, Swenson, et al.,⁸ isolated PRRSV from the reproductive tract of gilts infected with extended PRRSV-contaminated semen. These epidemiological and experimental studies indicate that PRRSV is now one of at least 14 viruses (e.g., adenovirus, African swine fever, pseudorabies virus (PRV), cytomegalovirus, enterovirus, foot and mouth disease, hog cholera, Japanese encephalitis, porcine parvovirus, reovirus, swine influenza, swine vesicular disease, and transmissible genital papilloma virus) that have been identified in boar semen. Three of these (e.g., African swine fever, PRV, and porcine parvovirus), including PRRSV, are also known to be transmitted through boar semen.⁹

Clinical signs and semen quality in PRRSV-infected boars

Clinical signs in boars infected with PRRSV are usually mild, transient, and sometimes non-existent.^{6,10,11} Depression and anorexia for 3 days post challenge can occur,¹² as well as a fever during the first 2 weeks after inoculation.^{6,11,13} Mild sneezing and coughing have also been re-

ported for 1 day's duration.¹³ Semen volume may be decreased after PRRSV inoculation,^{6,14} while motility, morphology, concentration, and color may remain within normal limits.^{6,12} However, other studies have shown decreased sperm motility in PRRSV-infected boars, along with an increased number of sperm with distal cytoplasmic droplets and abnormal acrosome structure.¹⁵ In one study of five PRRSV-infected AI centers, which housed approximately 230 boars each, only 25% of boars showed clinical signs.¹⁰ Boars without clinical symptoms showed no decrease in sperm motility while PRRSV-affected boars showed variable decreases of sperm motility. Morphological abnormalities, particularly damaged acrosomes and abnormal headshapes, were only noted in ejaculates from those boars with decreased sperm motility.¹⁰

Diagnostic techniques to identify PRRSV in boar semen

Identifying PRRSV-infected semen may be difficult because clinical signs and decreased semen quality can be mild, variable, or non-existent in PRRSV-infected boars. With the increasing use of artificial insemination (AI) in the United States, diagnostic techniques are becoming more important for detecting PRRSV in boar semen. Some studies have used a "swine bioassay" to identify PRRSV in semen.^{8,12,13,19} In the "swine bioassay," 13–15 mL of whole semen is injected intraperitoneally into 4- to 8-week-old PRRSV-seronegative piglets. Piglets are then monitored by weekly blood sampling for a total of 5 weeks to detect seroconversion to PRRSV. If the piglets seroconvert, this indicates the presence of PRRSV in the sample of boar semen tested. While this bioassay is an effective technique for detecting PRRSV in boar semen, it is quite laborious, requiring many piglets and at least 5 weeks of serologic monitoring.

Virus isolation has also been used to identify PRRSV in boar semen. However, as for other viruses in boar semen, this technique is not very sensitive because toxic factors in boar semen can destroy the cell monolayer the virus needs for replication.¹⁶

A third method for detecting PRRSV in semen is the polymerase chain reaction (PCR).¹⁷ The PCR procedure is much more sensitive than virus isolation, detecting as few as 10 virions per mL in boar semen.¹⁷ Other advantages are that it is a very specific test for PRRSV RNA. It can identify all field and experimental PRRSV isolates tested to date, and it is less expensive than the "swine bioassay," and it takes approximately 2 days to run (per eight semen samples). Even though the PCR test specifically identifies PRRSV RNA and not necessarily replicating virus as detected by the "swine bioassay," there appears to be good correlation between these two tests.¹⁷ The disadvantages of the PCR procedure is that due to its extreme sensitivity, several quality control mea-

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tures are required to prevent false-positive reactions and only a few diagnostic laboratories are currently performing this assay. If semen samples are being sent to the laboratory for PCR analysis, the sample should consist of 10 mL of whole, non-extended semen from each boar. The semen should be shipped cold on wet ice or ice packs.

The relationships of duration of PRRSV shedding in semen with indirect-fluorescent antibody (IFA) titers and viremia

Once the “swine bioassay” and PCR procedure were developed, further studies were performed correlating the duration of PRRSV shedding in boar semen with IFA titers and viremia.^{11,13} These studies indicated that all experimentally infected boars shed PRRSV in semen after intranasal inoculation of PRRSV and that the duration of shedding by individual boars was variable and in some cases intermittent.¹¹ In our study of four boars, each boar shed PRRSV in semen for 25 (two boars), 56 (one boar), or 92 (one boar) days after experimental intranasal inoculation of the PRRSV.¹¹ In an ISU study of five additional boars, PRRSV was shed through 8, 13, 25, 27, or 43 days post-intranasal inoculation of PRRSV,^{12,13} while a University of Minnesota study detected two boars that shed PRRSV through 50 or 57 days post inoculation.¹⁸ All 11 boars in these three studies started shedding PRRSV in semen between 2–7 days after inoculation and the average duration of PRRSV shedding in semen was 39 days post-inoculation.

Prior to the release of a commercial enzyme-linked immunosorbant assay (ELISA), the IFA test was the most commonly used test for identifying PRRSV antibodies in exposed pigs. In the South Dakota State University (SDSU)¹¹ and ISU studies,¹³ there was a small “window” of time (approximately 1 week immediately after inoculation) when boars were shedding the PRRSV in semen and were IFA negative. Since IFA titers were positive for the remainder of both studies, there was also a significant amount of time when IFA titers were positive and boars were not shedding PRRSV in semen (several months). Viremia was detected from 1–14 days after inoculation,^{11,13} while boars shed PRRSV in semen for an average of 39 days post-inoculation. This would indicate that neither viremia nor IFA titers adequately indicate when boars might be shedding PRRSV in semen. As a practical method of preventing boars that are shedding PRRSV in semen from entering a boar stud or seronegative herd, some producers are placing seropositive boars in quarantine for 45–60 days and then testing a semen sample for PRRSV by PCR. If the boar remains seronegative during this time, PCR testing may not be necessary. However, if some boars in the quarantine group are still seropositive, then testing of semen by PCR would be advisable. This quarantine method reduces the risk of introducing boars which are shedding PRRSV and other disease agents into a herd. An alternative protocol to quarantine is to require PCR testing of 2–3 weekly semen samples from a single boar.

Vaccination of boars with a modified-live (MLV) PRRSV vaccine

With the licensing of the MLV RespPRRSTM vaccine for use in 3- to 18-

week-old pigs, many producers are using the vaccine for extralabel use in adult boars. This has raised some questions as to whether vaccination protects boars from shedding the virus in semen after PRRSV challenge. To date, three groups of researchers have shown that the MLV vaccine can be shed in boar semen after a 2-mL intramuscular dose in adult boars. Researchers at the University of Minnesota demonstrated that PRRS vaccine virus was present in semen from three vaccinated boars at 6–15, 9–12, and 15–21 days post vaccination using PCR.¹⁸ Another group in Denmark also demonstrated PRRSV vaccine shedding in boar semen using the “swine bioassay” detection method.¹⁹ Four of five vaccinated boars in that study shed PRRSV in semen 141 days after vaccination.¹⁹ At SDSU, four of five boars shed the PRRSV within the first 14 days after vaccination while the fifth boar shed viral RNA intermittently through 39 days postvaccination.²⁰ When vaccinated boars were then challenged intranasally with PRRSV at 28 or 35 days postvaccination, seminal PRRSV shedding was not detected.^{14,19} However, when boars were challenged at 50 days postvaccination, PRRSV could be detected in the semen.²¹ This was probably challenge virus that was being shed, since vaccine virus had not been detected in semen for an average of 4 weeks prior to challenge. Therefore, the MLV vaccine is shed in boar semen and subsequent challenge may cause PRRSV shedding in semen. More research is necessary to determine the most effective method to vaccinate boars to reduce the risks of PRRSV being shed in semen.

Significance of PRRSV in boar semen

It is now well established that PRRSV can be shed and transmitted through boar semen. Transmission, however, as determined by virus isolation and/or seroconversion, may not always occur after insemination with PRRSV-contaminated semen.¹² This may reflect a requirement for a higher exposure dose by this route, as opposed to intranasal or intramuscular routes. Further investigation is needed to determine whether transmission is dependent on the immune status of the dam and whether protection from PRRSV-contaminated semen could be conferred to the sow or gilt via vaccination.

Preliminary studies on the effect of insemination with PRRSV-contaminated semen and subsequent fertility have been performed.^{6,8,22} In these three experimental studies, decreased pregnancy rates in gilts exposed to PRRSV-contaminated semen were reported. However, these results were not statistically significant since too few animals were included in these experiments. Cumulatively, however, they suggest that intrauterine exposure to PRRSV at the time of breeding reduces pregnancy rates. The mechanism by which PRRSV reduces fertility is not known. Swenson, et al.,⁸ artificially inseminated five gilts with extended semen from a boar shedding PRRSV. At the time of euthanasia on day 5 after breeding, all five gilts were virus-isolation positive and four of five gilts were pregnant. These results indicated that conception can occur in the presence of PRRSV. Further studies to determine how PRRSV affects subsequent events in pregnancy are needed.

Implications

- Boars that are intranasally inoculated with PRRSV can shed virus in semen for variable periods of time.
- Neither viremia nor the serologic status of the boar are good indicators of when PRRSV is being shed in semen.
- The PCR technique is a sensitive and reliable method to identify PRRSV RNA in boar semen.
- Intermittent shedding of PRRSV can occur in boar semen. Testing a single semen sample, in which PRRSV is not detected, does not indicate that previous or subsequent samples will also be free of virus.
- At least 2–3 semen samples from a single boar should be tested approximately 1 week apart. Boars should be quarantined for 1–2 months. A semen sample should then be tested to identify boars that are shedding PRRSV in semen.
- MLV PRRSV vaccine can be detected in boar semen.
- Further studies are needed to determine the significance and control of PRRSV shedding in boar semen.

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