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Artificial Insemination and Its Role in Transmission of Swine Viruses

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1. Introduction

Artificial insemination (AI) in swine is not a new technique and reports as early as the 1930s (Lush, 1925) describe collecting semen for AI. However, because of farm structure changes, increasing farm sizes and separation of production stages, interest in intensive pig production is growing and AI has become a critical component in modern pig production. In 2001, nearly 60% of North American swine herds utilized AI (Singleton, 2001), a drastic increase from the estimated 5% in the 1990's (Flowers & Esbenshade, 1993). This is still relatively low compared to the 90% or greater use of AI in Western Europe (Madsen, 2005; Maes et al., 2008). The extensive use of AI in pig reproduction in the last decade has facilitated the exchange of desirable genetic characteristics at an international level, allowing producers to make greater use of superior genetics at a lower cost than some natural-service systems (Gerrits et al., 2005). However, the growth in use of AI has increased the risk of quick and widespread transmission of venereally transmissible pathogens (Thacker et al., 1984). It has been reported that the porcine male reproductive tract is highly susceptible to viral infections (Phillips et al., 1972; Spradbrow, 1968). This, coupled with the ability of boars to produce tens to thousands of insemination doses per week and the widespread distribution of the processed semen (both nationally and internationally), further increases the risk of wide transmission of viral pathogens by semen.

Many viruses have been reported to be present in boar semen (Yaeger et al., 1993; Lucas at al., 1974, Madson et al., 2008) and have the potential of being transmitted to susceptible breeding animals during AI, particularly when the boar is viremic or clinically sick. Viral shedding may also continue long after clinical signs have abated. In addition, infertility or reduced reproductive performance have been reported in boars with detectable virus in their semen (Guerin & Pozzi, 2005; Larsen et al., 1980). The potential economic impact and liability associated with transmission of diseases through semen has created great interest and investment in testing boars for viral diseases prior to entry and while at AI centers.

In many countries, foot-and-mouth disease virus (FMDV), porcine reproductive and respiratory syndrome virus (PRRSV), Japanese B encephalitis virus (JBEV), pseudorabies virus (PRV), classical swine fever virus (CSFV) and African swine fever virus (ASFV) are of particular importance. Accurate monitoring of boars in AI stations is essential to reduce the
risk of virus transmission. Monitoring for the presence of other ubiquitous viruses that can be found in semen, such as porcine circovirus type 2 (PCV2), torque teno sus virus (TTSuV), porcine parvovirus (PPV) and porcine adenovirus (PAV) is commonly not a high priority in most countries despite the fact that some of these viruses have been associated with sporadic reproductive failure in breeding animals.

Due to differences in pathogenicity, economic consequences, geographic localization, and epidemiological parameters, different viruses are commonly assigned different levels of importance. The aims of this review are to summarize the information on swine viruses that can be transmitted via AI, their shedding characteristics in boars, effects of these viruses on naïve breeding animals, testing that is currently done to reduce or prevent the risk of transmission, and current best practices being used to reduce or eliminate virus shedding in semen.

2. Impact of viral contamination of semen

Microbial contamination of boar semen not only reduces semen quality but can also reduce conception rate resulting in considerable economic loss. It is not uncommon that a single boar stud serves multiple breeding herds with an average of 200 to 10,000 sows on each site. The risk for breeding herds becoming infected via AI increases with the number of pathogens present in the semen, the dose of the pathogen(s) in the semen, the number of sows inseminated with the contaminated semen, and the level of protective immunity in the breeding herd. From a practical point of view, most economically important viral diseases are associated with clinical signs, so semen collection from sick boars will not always take place, and consequently the risk of pathogen transmission into susceptible breeding herds by this route may be lower than expected. However, when clinical signs are mild or absent the risk of viral transmission via contaminated semen is a concern.

Artificial insemination is now widely recognized as a route for spread of swine diseases (Thacker et al., 1984) and continues to be a major concern to pig breeders and regulatory authorities in countries where AI is practiced. Many viruses known to contaminate semen have been shown to be highly infectious via the uterine route, and wide dissemination of porcine pathogens by semen could occur when semen is shipped globally. Transmission of viruses via the semen route to breeding females has been experimentally proven for CSFV (de Smit et al., 1999), PRRSV (Yaeger et al., 1993), and PPV (Lucas et al., 1974). It is of importance to note that, although virus-contaminated semen indeed constitutes a serious risk for transmission, it does not guarantee that transmission to the sow or gilt by AI will consistently occur (Swenson et al., 1994b; Yaeger et al., 1993). The conditions required for establishment of infection in the breeding female are complex, and lack of transmission might be explained by sow immunity or failure to reach the minimum infectious dose. In this regard, much research has been conducted concerning the risk of transmission of PRRSV by semen, and the minimum dose necessary to establish infection in the sow (Benfield et al., 2000; Prieto et al., 1996b).

Contamination of semen with virus can occur through three possible routes: 1) fecal contamination of semen during collection, 2) systemic viral infection, or 3) local viral infection (testes, accessory gland, etc.). Once virus-contaminated semen is utilized to inseminate a breeding female, the outcome can vary depending on the time of fetal
infection. Early embryonic death may result from direct invasion of the embryo by the pathogen or by uterine epithelial alterations in response to the pathogen (Wrathall & Mengeling, 1979). Until six to seven days after conception, an embryo is surrounded and protected by an impervious barrier called the zona pellucida, which helps the embryo avoid pathogen invasion (Mateusen et al., 2004). However, after entering the blastocyst stage, embryos may become susceptible to viral infections. Fetuses that are infected prior to 70 days of gestation usually die (mummies) and contribute to smaller litter sizes or early termination of pregnancy. Fetuses that are infected around or after 70 days of gestation are able to mount an active immune response against the pathogen and survive intrauterine infection.

As the risk of rapid virus spread via semen is well known, sanitation protocols in AI centers are constantly being reviewed to assure customers that current best practices are in place. A new categorization of viruses has been proposed to further address different risk levels (Guerin & Pozzi, 2005): Category 1 includes viruses for which there is scientific proof of transmission by semen, but without any risks for AI due to official national eradication programs such as FMDV, PRV, CSFV, and ASFV. Category 2 includes viruses for which there is scientific proof of transmission by semen and which can be commonly encountered such as PRRSV. Category 3 includes viruses for which additional scientific proof is needed to better assess the risk of transmission by semen (PCV2, porcine rubulavirus). In addition, some viruses have not been shown to be present in semen and thus are considered non-hazardous to AI.

3. Swine viruses that can be present in boar semen

3.1 Porcine Reproductive and Respiratory Syndrome Virus

Porcine reproductive and respiratory syndrome virus (PRRSV) is a single-stranded, positive-sense, enveloped RNA virus that belongs to the family Arteriviridae, genus Arterivirus (Cavanagh, 1997). PRRSV was first recognized in the late 1980’s (Cavanagh, 1997; Meulenberg et al., 1993) and today is found globally in swine producing countries. As the name of the virus implies, PRRSV infection is associated with reproductive failure in pregnant sows and respiratory disease in pigs of all ages. It is less commonly associated with neonatal diarrhea (Albina et al., 1994; Bierk et al., 2001; Neumann et al., 2005; Rossow, 1998; Wensvoort et al., 1991). Although clinical disease associated with PRRSV in growing pigs can be quite severe, no or mild symptoms are typically seen in boars (Wensvoort et al., 1991). One of the main characteristics of PRRSV is its high transmissibility, making it difficult to maintain pig populations free of PRRSV (Prieto & Castro, 2005). The 50% tissue culture infective dose (TCID\textsubscript{50}) for exposure via oral, intranasal and AI routes was determined to be $10^{5.3}$, $10^{4.0}$ and $10^{4.5}$, respectively (Benfield et al., 2000). Experimental infection in boars has demonstrated seminal shedding of PRRSV (Prieto et al., 1996b; Swenson et al., 1994a), and epidemiological evidence confirms that transmission of PRRSV from fresh semen of acutely infected boars into breeding herds is possible (Robertson, 1992; Yaeger et al., 1993). However, successful transmission is not always achieved (Swenson et al., 1994b; Yaeger et al., 1993) and appears to depend largely on the amount of PRRSV present in the semen (Benfield et al., 2000). In semen of adult boars, PRRSV can persist for variable periods (Christopher-Hennings et al., 1995a; Swenson et al., 1994) suggesting that the virus continues to replicate in the one or more tissues of the reproductive tract. Several
studies have since been carried out to determine the temporal localization of the virus in different organs and tissues (Prieto et al., 2003; Sur et al., 1997) and vascular dissemination and replication of PRRSV in tissues of the reproductive tract was confirmed (Sur et al., 1997). In addition, migration of infected monocytes and macrophages directly from blood and lymph of the reproductive tract into the semen has also been suggested as a mechanism for PRRSV contamination of semen (Christopher-Hennings et al., 1998; Prieto et al., 2003). PRRSV has been detected in semen samples of experimentally infected boars ranging from 4 to 92 days post infection (Christopher-Hennings et al., 1995a, 1998). This marked variability may be due to individual host factors (Christopher-Hennings et al., 1995a, 2001; Swenson et al., 1994a), the breed of the boars (Christopher-Hennings et al., 2001), and the strain of virus used in the experimental inoculation. European and North American PRRSV strains differ genetically and antigenically (Bautista et al., 1993; Meng et al., 1995). A bioassay has been used to determine the presence of infectious PRRSV in semen samples for a period of time lasting from 4 to 42 days post infection (Swenson et al., 1994a). In semen, PRRSV is sometimes associated with drastic changes in quality (reduced motility, abnormal acrosomes, morphological alterations, etc.) and volume of the semen (Teuffert et al., 1998). Conversely, some researchers have found that the quality of semen remains within normal limits after infection with PRRSV (Swenson et al., 1994a; Yaeger et al., 1993). The etiological role of PRRSV in reproductive failure of swine is firmly established (Christianson et al., 1993; Lager & Mengeling, 1995; Mengeling et al., 1994; Prieto et al., 1996a; Terpstra et al., 1991). The effect of PRRSV on reproductive parameters was found to be highly related to strain pathogenicity (Prieto et al., 1997). Sows and gilts bred by infected boars (Gradil et al., 1996; Yaeger et al., 1993) or with experimentally contaminated semen (Prieto et al., 1997; Swenson et al., 1994b) typically show seroconversion to PRRSV, even in the absence of detectable viremia (Christopher-Hennings et al., 1995a, 2001). It has been reported that insemination of seronegative or pre-immunized gilts with boar semen containing PRRSV often has little to no effect on conception rates, but may result in early embryonic infection and death (Prieto et al., 1997). In addition, PRRSV can be a major cause of prenatal death in commercial swine herds and often preweaning mortality is also increased.

3.2 Porcine Parvovirus

Porcine parvovirus (PPV) is a small, single-stranded, non-enveloped DNA virus (Molitor et al., 1984) that belongs to the family Parvoviridae, genus Parvovirus. PPV is considered ubiquitous in the pig population. The main routes of transmission of PPV are oronasal and transplacental. In general, infection of growing pigs and mature boars with PPV alone is not associated with clinical disease (Allan et al., 2000; Brown, Jr. et al., 1980; Kennedy et al., 2000; Krakowka et al., 2000). There is evidence that PPV-isolates vary in pathogenicity (Mengeling & Cutlip, 1976; Oraveerakul et al., 1993). PPV-isolates have been classified as non-pathogenic (Mengeling & Cutlip, 1976; Paul et al., 1979), pathogenic to non-immunocompetent fetuses leading to death (Mengeling, 1979), pathogenic to immunocompetent fetuses and inducing dermatitis (Choi et al., 1987; Kresse et al., 1985; Lager et al., 1992; Lager & Mengeling, 1994), and enteric PPV-strains (Dea et al., 1985; Duhamel et al., 1991). PPV has previously been isolated from semen of naturally infected boars (Cartwright & Huck, 1967). Boars can shed the virus in semen during the acute phase of infection (Gradil et al., 1990); shedding beyond this phase has not been demonstrated, but the possibility of immunotolerant carriers of PPV as a result of early in utero infection has
been suggested (Cartwright et al., 1971). Semen may also become contaminated with PPV from feces or from the male reproductive organs (Biront & Bonte, 1983; Lucas et al., 1974). Changes in sperm output, ejaculate volume, motility, or morphologic defects have not been observed when the semen quality was evaluated after experimental inoculation of boars with PPV (Thacker et al., 1987a). Acute infection of breeding females with PPV is usually subclinical (Johnson et al., 1976). During initial infection, PPV replicates extensively and can be found in many tissues including lymphoid tissues (Cutlip & Mengeling, 1975a). The major and often only clinical response to PPV infection is reproductive failure. Return to estrus, fewer pigs per litter, and increased numbers of mummified fetuses are often observed (Cartwright & Huck, 1967; Joo et al., 1976). The consequence of PPV infection depends on timing of infection of the fetus: Death and reabsorption are usually observed in 10-30 gestation day embryos, death and mummification are seen when 30 to 70 day gestation fetuses are infected, and an active immune response and survival in utero is seen in fetuses when infected after 70 days of gestation (Bachmann et al., 1975; Cutlip & Mengeling, 1975b). The role of semen contamination with PPV leading to clinical reproductive problems has not been clearly established (Lucas et al., 1974; Mengeling & Paul, 1986; Thacker et al., 1987b; Wrathall & Mengeling, 1979).

3.3 Pseudorabies Virus

Pseudorabies virus (PRV) is a double-stranded DNA virus that belongs to the subfamily Alphaherpesvirinae of the family Herpesvirus (Mettenleiter, 2000). PRV is distributed worldwide; however, in recent years eradication efforts have been successful to eliminate PRV from the domestic pig population in parts of Europe, Canada, New Zealand and the United States. PRV replication typically occurs in the nasal and pharyngeal mucosa and primary transmission occurs via the nasal route. Viral replication has also been reported to take place in the genital tract and transmission by copulation is therefore possible (Hall, Jr. et al., 1984a; Vannier & Gueguen, 1979). Differences in pathogenicity and duration of virus shedding exist among PRV strains (Maes et al., 1983). Clinically affected boars are often unable to mount a dummy (Guerin & Pozzi, 2005) and can additionally develop respiratory disease (Hall, Jr. et al., 1984b). PRV can be isolated infrequently from urine, preputial membranes, and semen after either natural (Medveczky & Szabo, 1981) or experimental infection (Vannier & Gueguen, 1979). Very high viral concentrations have been reported in semen ranging from $10^8$ to $10^9$ TCID$_{50}$ per ml (Medveczky & Szabo, 1981) to $10^{3.2}$ to $10^{4.9}$ TCID$_{50}$ per ml (Vannier & Gueguen, 1979). Transient viral excretion for up to 12 days usually occurs during the acute phase of the disease (Ressang, 1973) but viral excretion has been observed for long periods of time after natural infection (Wittmann, 1985). After experimental PRV infection by the intratesticular route, testicular degeneration and transient elevation in sperm abnormalities have been reported (Hall, Jr. et al., 1984a; Larsen et al., 1980). Sows inseminated with contaminated semen may develop vaginitis or endometritis resulting in embryonic death (Maes et al., 2008). Sows infected in the first trimester of pregnancy often reabsorb their fetuses and return to estrus. If sows get infected in the later stages of pregnancy they may abort or have larger numbers of stillborn and weakborn piglets. If susceptible females are infected with PRV close to parturition, there are increased numbers of weakborn pigs with signs of nervous system disease.
3.4 Porcine Rubulavirus

Porcine rubulavirus (PoRV), also known as La-Piedad-Michoacan paramyxovirus (LPMV), is associated with blue eye disease in pigs. This virus was first isolated in Mexico in the early 1980’s. PoRV belongs to the genus Rubulavirus and family Paramyxoviridae. Only one serotype has been recognized to date. Subclinically infected pigs are the primary source for PoRV transmission which occurs primarily via oronasal route. Boars, like other adult animals infected with PoRV, generally do not show clinical signs except for epididymitis and orchitis and in some cases loss of libido (Maes et al., 2008). Transmission of the virus through semen has not been proven experimentally; however, PoRV has been recovered from semen, testes, and other tissues of the reproductive tract for up to 49 days after inoculation (Solis et al., 2007). Based on semen evaluation in herds naturally infected with PoRV, approximately 30% of boars develop temporary or permanent infertility. Semen abnormalities include a decrease in concentration, increased morphologically abnormal sperm, decreased sperm motility and viability, and azoospermia (Maes et al., 2008). Most sows are clinically normal with a few animals developing corneal opacity. Infected breeding herds may experience increased returns to estrus and a reduction in farrowing rates.

3.5 Porcine Enteric Picornaviruses

Porcine enterovirus (PEV) and porcine teschovirus (PTV) are single-stranded positive-sense RNA viruses in the family Picornaviridae. PEV and PTV infections are commonly transmitted between pigs by oral exposure to contaminated feces; however, contamination of semen via aerosol during semen collection cannot be excluded (Guerin & Pozzi, 2005). PTV has been isolated from the male genital tract (Phillips et al., 1972); however, insemination of gilts with PTV contaminated semen had no effect on their fertility (De Meurichy & Pensaert, 1977). PEV can cause seminal vesiculitis, sperm abnormalities, and decrease libido (Phillips et al., 1972). Usually, there are no clinical signs in the sows. Although evidence is limited, there are reports that suggest that semen contaminated with PEV and PTV could cause embryonic and neonatal death (Dunne et al., 1969).

3.6 African Swine Fever Virus

African swine fever virus (ASFV) is a double-stranded, linear DNA virus in the genus Asfivirus of the family Asfarviridae (Tabares et al., 1980). ASFV replicates in monocytes and macrophages of the lymph nodes near the site of viral entry and subsequently spreads through the blood system, the lymphatic system, or both (Maes et al., 2008). ASFV transmission is generally vector-borne via tick bites (Parker et al., 1969); however, infections can also occur through direct interaction of sick and naïve animals (Plowright et al., 1970). Clinical signs range from subclinical to severe acute systemic disease that can resemble other hemorrhagic diseases in pigs. In naïve pigs, acute disease is characterized by high fever, loss of appetite, and hemorrhages in the skin (Moulton & Coggins, 1968). ASFV has been experimentally isolated from semen (Schlafer & Mebus, 1987; Thacker et al., 1984). Effect on semen quality and volume has to the authors knowledge not been reported. Under experimental conditions, ASFV has been shown to induce abortion when sows were infected between 38 and 92 days of gestation (Schlafer & Mebus, 1987). It was determined that abortion resulted from the effect of the virus on the dam (clinical illness) rather than direct effect on the fetuses (Schlafer & Mebus, 1987).
3.7 Classical Swine Fever Virus

Classical swine fever virus (CSFV) is a small, enveloped, positive-sense, single-stranded RNA virus in the genus *Pestivirus* of the family *Flaviviridae* (Becher et al., 1999). CSFV has been eradicated from many countries including Australia, New Zealand, North America, and Western Europe; however, it has been periodically reintroduced into domestic pigs that are in contact with wild boars. During the CSFV epizootic of 1997-1998 in the Netherlands (de Smit et al., 1999), two AI centers became infected and more than 100,000 sows from approximately 1,700 farms were affected (Hennecken et al., 2000) highlighting the economic importance of disease spread though AI centers. The main transmission route of CSFV is oronasally with primary virus replication in tonsils. Data from natural infection (Hennecken et al., 2000) and experimental inoculations (Choi & Chae, 2003; Floegel et al., 2000) have demonstrated that CSFV can be excreted in semen from infected boars. Boars experimentally infected with CSFV shed the virus in semen for up to 53 days post-infection (Choi & Chae, 2003). The virus does not affect the semen quality and motility and concentration are within normal range. Sows that were inseminated with contaminated semen seroconverted; however, the virus was shown to cross the placental barrier and infected the fetuses causing embryonic mortality (de Smit et al., 1999). CSFV continues to be important, especially in areas where CSFV is endemic, because the virus is highly contagious and infection of pregnant sows, in contrast to acute infections in piglets, may not be apparent (Hare et al., 1985; Ressang, 1973). If clinically normal CSFV immunotolerant piglets are born, they can spread virus for months without showing signs of disease or developing an antibody response (de Smit et al., 1999).

3.8 Foot and Mouth Disease Virus

Foot and mouth disease virus (FMDV) is a small single-stranded positive-sense RNA virus which belongs to the genus *Apthovirus* in the family *Picornaviridae* (Carrillo et al., 1990). FMDV is endemic in Asia, some areas in South America and in Africa. FMDV can be isolated from all excretions and secretions including urine, feces, milk and semen (Lubroth & Brown, 1995). Infected pigs develop fever, lethargy and lameness which is associated with vesicular lesions in the hoof area (Mebus, 1978). Infection with FMDV leads to viremia, with subsequent systemic dissemination of the virus, including the genital tract and the skin around the preputial orifice (Alexandersen et al., 2001). FMDV has been detected in boar semen before and during manifestation of clinical signs of the disease. The viral concentration in semen has been found to be low (Guerin & Pozzi, 2005). After natural infection, FMDV has been isolated from infected boar semen for up to 9 days, but AI with contaminated semen failed to transmit the disease to sows (McVicar et al., 1977). To the authors’ knowledge, an effect on semen quality has not been described. Abortion storms can be observed and are mainly due to the high fever and clinical illness of the dams.

3.9 Japanese B Encephalitis Virus

Japanese B encephalitis virus (JBEV) is a member of the *Flavivirus* genus of the family *Flaviviridae* (Solomon et al., 2003). JBEV is a mosquito-borne pathogen affecting humans and animals. This virus represents an economically important reproductive pathogen of breeding pigs, especially in Asia and Northern Australia, and is a common cause of
infertility in Japanese pigs (Habu et al., 1977). Infection of susceptible boars resulted in edematous, congested testes and semen with numerous abnormal spermatozoa and significantly decreased total and motile sperm counts (Habu et al., 1977). These changes are usually temporary and most boars recover completely. JBEV has been isolated from the testicles of boars with orchitis and also can be shed in the semen for 5 weeks (Habu et al., 1977). JBEV infection can easily be transmitted if gilts are inseminated with infected semen (Guerin & Pozzi, 2005; Habu et al., 1977).

3.10 Porcine Circovirus type 2

Porcine circovirus type 2 (PCV2) is a small, single-stranded, ambi-sense DNA virus that belongs to the family Circoviridae, genus Circovirus (Tischer et al., 1982). PCV2 is a ubiquitous virus and most herds worldwide are seropositive for PCV2 (Dulac & Afshar, 1989; Edwards & Sands, 1994; Segalés et al., 2008; Tischer et al., 1986). When first described in 1998, PCV2 was linked to disease mainly characterized by wasting and generalized lymphadenopathy in growing pigs (Allan et al., 1998; Morozov et al., 1998). Since that time, PCV2 has been associated with several disease manifestations in pigs commonly referred to as PCV-associated disease (PCVAD) which includes systemic disease, respiratory disease, enteric disease, porcine dermatitis and nephropathy syndrome (PDNS) and reproductive failure in dams (late term abortions and stillbirths) (Choi & Chae, 2001; Harms et al., 2002; Kim et al., 2004a; Kim et al., 2004b). PCV2-associated reproductive disease under field conditions is rare. The main route of transmission has been postulated to be the fecal-oral route (Segalés et al., 2005); however, due to the rapid spread and the extensive use of AI, semen transmission has been suggested as a potentially significant route of dissemination of PCV2 (Horlen et al., 2007; Lawton et al., 2004; Rose et al., 2003; Sibila et al., 2004; West et al., 1999). Mature boars infected with PCV2 generally lack clinical signs and lesions (Larochelle et al., 2000; Madson et al., 2008). The virus has been detected in semen of naturally and experimentally infected boars, even after the appearance of antibodies in the serum (Larochelle et al., 2000). In the acute phase of infection, PCV2 DNA was detected in serum and semen at two and five days post inoculation, respectively (Larochelle et al., 2000; Madson et al., 2008). Detection of PCV2 viremia commonly precedes the detection of semen-associated virus (Madson et al., 2008), but semen shedding has been reported in the absence of viremia (Larochelle et al., 2000). Anti-PCV2 antibodies typically develop by two weeks post inoculation (Larochelle et al., 2000; Madson et al., 2008). Following intranasal inoculation, intermittent semen shedding of PCV2 DNA was observed during a 47 day observation period (Larochelle et al., 2000). Intermittent semen shedding was confirmed by a different study (Grasland et al., 2008) after testing semen samples for 56 days in four inoculated boars. In contrast, continuous semen shedding was observed following intranasal and intramuscular inoculation following a 90 day observational period (Madson et al., 2008). In addition, naturally infected boars were found to sporadically shed PCV2 DNA in semen for up to 27.3 weeks in a PCV2 positive boar stud (McIntosh et al., 2006). Peak PCV2 shedding in semen occurs between nine and 20 days post inoculation (Grasland et al., 2008; Madson et al., 2008). Changes in morphology, motility, or quantity are not commonly associated with natural or experimental PCV2 infection (Madson et al., 2008; McIntosh et al., 2006). Clinical signs of PCV2 infection in the dam are typically absent or unapparent; however, a low percentage of females may abort due to systemic illness.
Pyrexia and anorexia are frequently observed in aborting dams (Park et al., 2005). The virus is also able to replicate in the zona pellucida-free embryos, leading to embryonic death (Mateusen et al., 2004, 2007). Delayed farrowing (>118 days of gestation) (Ladekjær-Mikkelsen et al., 2001) or pseudopregnancy (Josephson & Charbonneau, 2001) is less frequently observed with PCV2-associated reproductive failure. PCV2 affected litters are commonly composed of increased numbers of non-viable fetuses (mummified and stillborn) at parturition (Madson & Opriessnig, 2011).

3.11 Other viruses that are of minor importance

3.11.1 Swine Vesicular Disease Virus

Swine vesicular disease virus (SVDV) is a small, non-enveloped single-stranded positive-sense RNA virus, in the family Picornaviridae, genus Enterovirus (Nardelli et al., 1968). Documented outbreaks of this disease have been limited to selected countries in Asia, Europe and Central America. Clinical signs include appearance of vesicles around the coronary bands, snout, tongue and lip (Kanno et al., 1995) making this disease an important differential for FMDV. After natural infection, SVDV has been isolated from infected boar semen for up to four days, but AI with contaminated semen failed to transmit the disease to sows (McVicar et al., 1977).

3.11.2 Non-Classical Swine Fever Virus pestiviruses

Pigs are also susceptible to non-CSFV pestiviruses, including bovine viral diarrhea virus (BVDV) and border disease virus (BDV), which are associated with disease in cattle and sheep, respectively. Pigs congenitally infected with these viruses may shed large amounts of virus. Previously, BVDV has been isolated from oropharyngeal fluid, urine and semen of a congenitally infected, infertile boar (Terpstra & Wensvoort, 1997).

3.11.3 Porcine retroviruses

Retroviruses are RNA viruses that exist in two main groups: endogenous or exogenous retroviruses. Endogenous retroviruses are thought to be present in all vertebrates accounting for approximately 8% of their genomes (Tucker et al., 2006). All pigs carry PERV in their genome (Wilson, 2008) and three porcine endogenous retrovirus (PERV) subtypes have been identified based on their envelope sequence and tropism in cell culture (Le et al., 1997; Wilson et al., 2000): PERV-A, PERV-B and PERV-C (Martin et al., 2000a, 2000b; Patience et al., 1997; Specke et al., 2001; Takeuchi et al., 1998). More recently, the possible existence of exogenous porcine retrovirus in pigs was proposed (Scobie et al., 2004; Wood et al., 2004). It was demonstrated that a human-tropic recombination between PERV-A and PERV-C (designated as PERV-A/C) was not a product of in vitro recombination; instead PERV-A/C appeared to exist in vivo as an exogenous virus (Wood et al., 2004). Furthermore, a PERV-A/C recombinant was isolated from porcine peripheral blood mononuclear cells and was not present in a proviral form in the miniature swine genome (Scobie et al., 2004a; Wood et al., 2004). The PERV A/C virus is a recombination within the env region and is thought to arise from exogenous recombination of mRNA (Martin et al., 2006). Shedding via the semen route occurs, since PERVs are embedded in the genome.
3.11.4 Swine Influenza Virus and transmissible gastroenteritis virus

Swine influenza virus (SIV), a negative-sense, single-stranded RNA virus in the genus *influenzavirus A* of the family *Orthomyxoviridae* (Lamb & Krug, 2001; Wright & Webster, 2001), and transmissible gastroenteritis virus (TGEV), a positive-sense, single-stranded RNA virus in the *Coronaviridae* family (Lai & Cavanagh, 1997) have not been isolated from semen. Contamination of semen and transmission of SIV and TGEV have not been demonstrated to date; however, these viruses are highly contagious and could be easily transmitted via aerosol at the time of collection and preparation of semen. Boars in the acute stages of SIV or TGEV infection are often clinically ill on examination (Guerin & Pozzi, 2005).

3.11.5 Porcine Cytomegalovirus

Porcine cytomegalovirus (PCVM), a double-stranded DNA virus, is classified in the genus *Cytomegalovirus* in the subfamily *Betaherpesvirinae* of the family *Herpesviridae* (Roizman, 1982). Infection with PCMV is usually subclinical in adults including boars. Following infection in boars, the virus was detected in the testis and the epididymis (Shirai et al., 1985); however, semen shedding of the virus has not been determined.

3.11.6 Porcine Adenovirus

Porcine adenovirus (PAV) is a double-stranded, linear DNA virus which belongs to the family *Adenoviridae*. PAV is distributed worldwide and associated with gastroenteric disease. As such, its importance in AI is due to the potential of fecal contamination of semen.

4. Diagnostic approaches to prevent virus transmission by the semen route

The concern of spreading diseases on a global scale via semen has placed restrictions on international movement of semen. From the early 1990s onwards, major improvements in detection methods particularly in terms of sensitivity have better enabled diagnostic laboratories to detect viruses in boar semen. Today, various methods are available for demonstration of viruses in boars and include monitoring for presence of clinical signs, demonstration of virus (virus isolation, bioassay), nucleic acids, and demonstration of antibodies against the virus. However, it needs to be considered that the results of any of these methods may vary substantially, depending on individual sample type and the sensitivity and specificity of a chosen diagnostic method. Accurate diagnosis of semen associated viruses (correctly identified positive or negative status of any given sample) is extremely important as false positive as well as false negative test results can cause substantial economic damage. For example, with PRRSV, any false positive sample out of a boar stud has the following consequences: (1) Immediate hold on all semen samples from the stud to breeding herds resulting in missed cycles and associated production losses in hundreds to thousands of sows supplied by the boar stud. (2) Retesting of boars. (3) Culling of suspect positive boars.

4.1 Importance of sample type

Samples that are commonly collected in boar studs for routine surveillance include semen (raw or extended), blood swabs, serum and more recently oral fluids. Blood swabs have
become a popular sample type in recent years. For blood swab collection, the ear is pricked with a needle during semen collection and a cotton swab is used to catch the resulting blood drop (Reicks et al., 2006). Pathogens that cause systemic disease are typically identified in serum or blood first before they enter the semen and this should be taken into consideration when collecting appropriate samples. A negative result on semen only means that the tested sample does not contain virus, and that the particular semen sample is likely to be virus-free. It does not provide certainty that there will be no risk of contamination in the future. On the other hand, a negative result on serum does not guarantee that the semen is free of the virus as the viremic stage may have been very short.

4.2 Pooling of samples

Economic aspects are important to consider. For interpretation of diagnostic results at the boar stud level, in addition to the detection limit of the diagnostic method used, it is extremely important to test a representative number of semen samples, and to include evaluation of more than one parameter, such as presence of antibodies, viremia, or clinical symptoms. Because of the potential high cost of this approach, producers commonly rely on testing pooled samples. This strategy allows testing a larger number of animals while running the same number of tests, thus decreasing the cost per boar stud. Pooling can be successfully applied if the assay utilized has a high analytical sensitivity; however, because of a dilution effect, the sensitivity of the test when run on pooled samples is lower than its sensitivity when run on individual samples (Munoz-Zanzi et al., 2006).

4.3 Detection methods

4.3.1 Clinical evaluation of the boar

As a very basic and broad approach, in order to avoid spread of disease via contaminated semen, the health status of the animals should be checked daily. In case of any abnormality or clinical disease signs, semen collection should be halted until the animal has recovered. However, as indicated before, clinical examination alone is insufficient for most viral infections, since clinically normal boars can shed pathogens in their semen for extended periods of time.

4.3.2 Detection of viable virus

For demonstration of viable virus in semen two options exist including virus isolation and conducting bioassays. Virus isolation is often difficult as bacterial contamination of semen can be substantial. Virus isolation can be further complicated by the existence of cytotoxic factors in semen that destroy cell culture systems, and antiviral factors that nonspecifically neutralize virus (Christopher-Hennings et al., 1995b, 1997; Prieto et al., 1996b, 2003). In many cases swine bioassays have been found to be more sensitive than cell culture systems for demonstrating viable viruses in semen. However, animal inoculations cannot be justified to be used routinely for large numbers of samples and turnaround time for test results is poor.

4.3.3 Direct detection of RNA/DNA

Much progress has been made in recent years to improve the quality of molecular diagnostic assays used on semen. Numerous PCR techniques are now available for detecting
pathogens in boar semen. Many of these are highly sensitive, specific and rapid (Christopher-Hennings et al., 1995b). In the case of PRRSV, reverse transcriptase-PCR is today considered to be the most sensitive diagnostic technique. It allows for the detection of as little as $10^0 \text{TCID}_{50}$ per seminal dose (Christopher-Hennings et al., 1995b; Gradil et al., 1996), 20 times less virus than what has been shown experimentally to result in transmission of PRRSV in gilts (Shin et al., 1997).

4.3.4 Detection of antibodies

Serology is a simple and relatively inexpensive way to survey for pathogens that should not be present in the boar stud. The main disadvantage of serology is the time period between pathogen exposure and detectable levels of antibodies in a boar which may range from a few days to weeks during which time the boar is not identified as being positive and may shed the virus via semen.

5. Prevention of introduction of viruses into boar studs

The best way to prevent disease transmission via semen is to assure pathogens are not introduced to the boar studs by maintaining very strict biosecurity measures (Madec et al., 1999) and routine surveillance. Due to the high risk of dissemination of disease via AI, the most important goal is to provide pathogen-free semen, which is feasible with adequate control measures.

5.1 Biosecurity

Strict biosecurity measures should be maintained, involving all of the following: (1) **Location of the boar stud:** To avoid introduction of pathogens via aerosol routes, boar studs are often located in remote areas away from pig dense populations. (2) **Protection from aerosol contamination:** High traffic areas and location next to heavily travelled roads have shown to be a risk factor for exposure to airborne viruses (Otake et al., 2002). Boar stud owners often attempt to protect the boars by implementing filter systems for incoming air in AI centers to safeguard boars against the entry of airborne pathogens (Dee et al., 2005, 2006). (3) **Incoming replacement boars:** Replacement boars are typically isolated for 30 to 60 days and housed in a separate off-site facility. During the isolation time, the health status of the boars is evaluated. Animals that test positive for any disease of concern are typically not allowed to enter. During the isolation time, incoming boars are often vaccinated against diseases present in the resident boars. The annual boar replacement rate in AI centers is typically 60% (Singleton, 2001). (4) **Employees and visitors:** Strict regulations are typically in place for people entering AI centers. Often, there is 24 to 72 hour down-time required during which time people are not allowed to have contact with other live pigs. In addition, there is often a shower in/shower out requirement for all visitors and employees. (5) **Deliveries:** Any deliveries (clothes, mail, supplies, feed, tools, and equipment) are brought to a special entrance with no direct contact with the boar stud and are fumigated prior to entry into the boar studs. (6) **Rodent and insect control:** Rodents and insects have been shown to be capable of carrying several pig viruses (Lorincz et al., 2010; Otake et al., 2003a, 2003b; Plowright et al., 1970) and appropriate prevention measures need to be in place. (7) **Implementation of a regular and appropriate cleaning and disinfectant procedure.**
5.2 Routine surveillance testing

Commercial AI centers need be regularly checked for conformity with specific criteria, assuring that their products are free of certain pathogens and contain a minimal or acceptable number of microorganisms (Guerin & Pozzi, 2005; Prieto et al., 2004). Many veterinary diagnostic laboratories took this need into account and started to offer daily service for boar studs.

5.3 General semen handling and storage

To reduce the unavoidable presence of bacteria in the ejaculate and to prolong in vitro longevity of sperm, use of antimicrobials in semen is a common part of most semen extenders. Apart from a possible dilution effect of pathogens, semen processing and addition of antimicrobials does not eliminate viruses. The use of effective antiviral agents to render semen virus-free has so far not been adopted in the swine AI industry. However, several best practices for handling, storage conditions, and use of fresh semen have been described to reduce the potential for transmission of viral pathogens (Guerin & Pozzi, 2005). For boars, the immediate use of fresh semen increases the risk of pathogen transmission (Prieto et al., 2004).

5.4 Vaccination

The use of modified-live and inactivated-virus vaccines in boars can be highly effective in eliminating or decreasing shedding of viruses and with that decreasing the risk of virus transmission by AI. For example, vaccination against PPV may help to reduce shedding of the virus following infection. This has also been shown for PCV2 under experimental conditions (Opriessnig et al., 2011). In many countries, vaccination is done as part of a PRV eradication program (Siegel & Weigel, 1999). In the case of PRRSV, the use of a modified live virus vaccine shortened or eliminated virus shedding in boars challenged with wild-type virus for 50 days after vaccination (Christopher-Hennings et al., 1997; Nielsen et al., 1997). However, based on other studies it appears that PRRSV vaccination provides only partial protection (Nielsen et al., 1997). Moreover, semen shedding has been demonstrated after vaccination with a modified live vaccine virus (Christopher-Hennings et al., 1997). In addition, the modified live PRRSV vaccine virus has been shown to be shed in the semen in low levels (Christopher-Hennings et al., 1995b). In contrast, an inactivated vaccine, did not clearly reduce subsequent shedding of wild-type virus in semen (Christopher-Hennings et al., 1997; Stevenson et al., 1994). The presence of PRRSV in semen was demonstrated by PCR in most of the vaccinated boars during an interval of 7–21 days post-vaccination, although some boars sporadically shed the virus for longer periods of time (Christopher-Hennings et al., 1997). Similarly, when a swine bioassay was used, the presence of infectious virus in semen samples of vaccinated boars was confirmed 14 days post-vaccination (Nielsen et al., 1997).

6. Summary

Virus contamination of boar semen poses a great risk for breeding herds worldwide due the possibility of fast introduction of viruses into large naïve and susceptible populations.
Producers in some countries have led the way in establishing and maintaining specific pathogen-free AI centers in which incoming and resident boars are systematically screened for specific viruses to prevent introduction of viruses and if viruses are introduced to prevent spread of the virus within the boar studs and to breeding herds that receive semen from the studs. Serious attention is being given to appropriate location of boar studs and major investments are being made in air filtration equipment and building design and strict adherence is given to biosecurity protocols to further decrease the risks of introduction of diseases, particularly PRRSV, to boar studs in North America.

7. References


Artificial Insemination and Its Role in Transmission of Swine Viruses


Veterinary medicine is advancing at a very rapid pace, particularly given the breadth of the discipline. This book examines new developments covering a wide range of issues from health and welfare in livestock, pets, and wild animals to public health supervision and biomedical research. As well as containing reviews offering fresh insight into specific issues, this book includes a selection of scientific articles which help to chart the advance of this science. The book is divided into several sections. The opening chapters cover the veterinary profession and veterinary science in general, while later chapters look at specific aspects of applied veterinary medicine in pets and in livestock. Finally, research papers are grouped by specialisms with a view to exploring progress in areas such as organ transplantation, therapeutic use of natural substances, and the use of new diagnostic techniques for disease control. This book was produced during World Veterinary Year 2011, which marked the 250th anniversary of the veterinary profession. It provides a fittingly concise and enjoyable overview of the whole science of veterinary medicine.

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