Proceeding of the Biennial Conference of the Association for Applied Animal Andrology

July 28-29, 2012 - Vancouver, Canada

9th Association for Applied Animal Andrology Biennial Conference will be held in 2014. Visit www.animalandrology.org for more information and updates

Reprinted in the IVIS website with the permission of the Association for Applied Animal Andrology - AAAA
Biosecurity and quality assurance in equine semen production centers

Paul R. Loomis*
Select Breeders Services, MD, USA

Abstract

In the United States there are very few regulations concerning the collection, evaluation, processing, distribution, and insemination of stallion semen. Over the last two decades there has been a significant increase in the use of transported cooled and frozen semen as advances in semen preservation techniques and mare management have allowed for improved pregnancy rates. Transported cooled and frozen semen can be an effective means for the spread of equine diseases if the causative agents can be found in semen. Lack of donor stallion testing and poor hygiene at collection facilities leads to the potential for the spread of venereally transmitted diseases. International trade of semen to most countries requires testing of donor stallions for a variety of equine diseases and for some countries inspection and approval of semen collection facilities. Within the member states of the European Union, semen processing and AI stations must be inspected and licensed by governmental agencies in order to ship semen. In the United States, a voluntary program of independent auditing of health testing, production center management and semen quality monitoring in the cattle has allowed the industry to develop without direct government regulatory involvement. Currently there are no universally accepted standards for semen quality within the equine industry and this has led to a great deal of variation in the quality of semen on the commercial market. A breeder that purchases frozen semen or contracts to receive cooled transported semen should be provided with assurance that the semen will contain sufficient sperm of sufficient quality to provide for a reasonable expectation of fertility. There should also be an expectation that use of the semen does not pose a health risk to mares from exposure to mare pathogens and significant environmental contaminants that may be present in the semen. Breeders and professionals concerned with the responsible development of the equine AI industry should consider implementing a program of oversight to help minimize the spread of disease and provide some level of quality control for stallion semen production and distribution in the U.S.

Keywords: Equine semen production; Biosecurity; Equine AI industry; Quality assurance

1. Introduction

Over the last 25 years transported semen has fundamentally changed the way horses are bred in North America. In the US, cooled and frozen semen is routinely shipped around the country to inseminate mares from all but one of the major horse breeds. There are no regulations,
certifications or licenses required to collect, process, and distribute semen within the US or Canada and as such the potential for the spread of disease exists. Recent occurrences of both equine viral arteritis (EVA) and contagious equine metritis (CEM) in the US have illustrated the potential for the spread of such diseases in the equine breeding population. In addition to concerns for disease transmission, no standards for semen processing or quality control exist, which has led to wide variation in the quality of semen received and expectation of success following insemination.

In the last two decades, there has been a marked increase in the use of frozen semen. One of the main reasons is for international trade. Many breed associations are actively promoting growth of the breed through expansion into other countries. For example, since the early 1990’s frozen semen from Standardbred race horses has been exported to Europe, Australia, and New Zealand while semen from European Warmblood sport horses has been imported and used by breeders in the North America since the mid 1980’s. More recently, the American Quarter Horse and American Paint Horse Associations have permitted the registration of foals conceived by frozen semen which has led to an active and growing market for the export of semen from the US to numerous countries around the world. Government veterinary agencies from most countries have established specific requirements for the importation of semen. These range from minimal health testing of donor stallions to extensive health testing and quarantine of donor stallions, detailed requirements governing the collection and processing of semen, and biannual inspection and certification of the collection centers by the USDA or similar government authority.

This paper will provide a brief general overview of diseases potentially transmitted in semen and propose standards and guidelines for the production of quality semen by equine semen collection centers.

2. Biosecurity for semen production facilities

Direct transmission of infectious disease between horses may occur via respiratory (primarily nose-to-nose contact) or venereal (natural mating or AI) routes. Indirect transmission can occur through respiratory exposure by inhalation of aerosolized infective agents, ingestion of feed or water contaminated with infective agents, contact with contaminated feces or other bodily fluids and exposure via insect or rodent vectors. Another mode of disease transmission important in semen collection facilities is through exposure to contaminated fomites such as semen collection equipment, phantom, halters and stud shanks, water buckets, vehicles, wash buckets, hoses, clothing, and hands of collection center personnel. The 2008/2009 occurrence of CEM in the US in which 23 stallions were found to be contaminated with \textit{Taylorella equigenitalis} was likely the result of indirect transmission from infected to non-infected stallions via exposure to contaminated collection equipment [1].

As with any equine operation, biosecurity measures to prevent the spread of all contagious diseases should be followed for semen production facilities. Veterinary examinations of new animals entering the facility and isolation from other animals in the center are general precautions that should be implemented in order to safeguard the resident equine population. However, in many cases stallions and mares may be short-term borders or trailer-in, trailer-out non-residents and therefore no isolation period is possible before they are exposed to the collection or insemination facility. In this case, pre-arrival health testing is critical to minimize
risk of contaminating facilities. This discussion will focus primarily on diseases potentially transmitted in semen.

3. Health testing of donor stallions

Equine Viral Arteritis (EVA) is an acute, contagious disease of horses caused by the equine arteritis virus (EAV). The virus is spread primarily via the respiratory route and is rarely fatal to adult horses, but in some cases may cause abortion in pregnant mares or be fatal to very young foals. Many horses are asymptomatic following primary exposure to EAV whereas others may exhibit one or several of the following symptoms: fever, depression, anorexia, dependent edema (particularly the scrotum, prepuce and lower hind legs of stallions), conjunctivitis, supra-or periorbital edema, nasal discharge, and urticarial type skin reaction. Outbreaks have been reported in North America and Europe and may occur in all breeds but seem to be most prevalent in Standardbred and Warmblood breeds.

Of particular concern to semen production centers is that stallions infected with the EAV are often asymptomatic after primary exposure and become carriers of the virus in their reproductive tracts. Stallions may harbor and continuously shed virus in their semen. The virus survives quite well in cooled or frozen semen and can therefore infect mares that are inseminated with contaminated semen. Most mares exposed this way will seroconvert and may shed the virus in nasal discharges after insemination. This is generally not a significant problem for the mare and presence of the virus does not appear to have a negative effect on fertility or cause endometritis [2]. However, there is a significant risk of abortion to pregnant mares or health concerns with young foals exposed to the virus shed by the inseminated mare.

Prior to arrival at the collection facility, all stallions should be serologically screened for the presence of EAV antibodies. We require this negative EVA to be obtained within 30 days of arrival. Stallions that are seropositive must have the absence of virus in the semen confirmed by attempted virus isolation on the semen by a USDA approved laboratory. Stallions may be seropositive from prior exposure or they may have been vaccinated for EVA. In North America, a highly attenuated modified live vaccine is approved for use in stallions and non-pregnant mares. Vaccination of stallions is recommended as a means to control the disease as the asymptomatic carrier stallion is thought to be the natural reservoir for the virus. In order to avoid potential problems with exportation to some countries, it is critical that prior to initial vaccination stallions are confirmed seronegative for EAV antibodies, preferably from a blood sample taken immediately prior to vaccination. A veterinary certificate attesting to this should be obtained and maintained with the stallion’s records as proof that the stallion was negative prior to vaccination. Annual boosters of this vaccine are recommended.

For semen that is being collected for export, EVA testing is mandatory and may be required after the stallion has entered and been resident in the collection center for 14 days. Depending on the importing country and the testing protocol chosen by the collection facility, serological testing within a specific period of time after the last collection of semen for export may also be required before semen can be released from quarantine. As is always the case, specific health testing requirements for the importing country must be adhered to strictly and current regulations obtained from that country prior to the start of a frozen semen exporting program. The American Association of Equine Practitioners (AAEP) has published “Biosecurity Guidelines for Control of Venereally Transmitted Diseases” [3], which provides a more thorough set of recommendations for control of EVA and other potential venereal diseases.
Interestingly, the United States does not have a negative EVA requirement for stallions or frozen semen entering the country and there have been several documented cases of EVA-positive frozen semen being imported over the last 25 years. In 2004 there was a severe outbreak of EVA in a relatively naïve Quarter horse population in the western US. An asymptomatic carrier stallion was being collected and his cooled semen shipped to farms in several states for AI of unvaccinated mares. The outbreak caused clinical disease and abortions at several locations and had a significant financial impact. This outbreak could have been avoided had there been a requirement for EVA testing and/or vaccination for domestic distribution of cooled or frozen semen. As a result, a few of those western states have instituted requirements for EVA testing of stallions whose semen will be brought into the state. With the apparent increase in the reported occurrences of EVA worldwide perhaps more states should institute a program of EVA vaccination and testing.

Contagious equine metritis is a true venereal disease of horses caused by the bacterium *Taylorella equigenitalis*. The bacterium causes endometritis in mares, is not systemic or fatal and can usually be treated successfully with intra-uterine antibiotic therapy. The endometritis and discharge that follows is similar in clinical appearance to endometritis in mares caused by more common reproductive pathogens such as *klebsiella pneumonia*, *pseudomonas aeruginosa*, and *streptococcus zooepidemicus*. The stallion does not usually develop any symptoms after exposure but may become a carrier of the bacteria on the external genitalia. Outbreaks of CEM were first reported in Thoroughbreds in the United Kingdom and Ireland in 1977 and in the United States in 1978. The disease is considered foreign in North America and positive cases must be reported to the USDA or CFIA.

Despite strict quarantine and testing of breeding aged mares and stallions entering the country, many carrier stallions and mares have been identified in quarantine and also in the general population following routine screening prior to collection of semen for export. In the 2008/2009 breakout in the US, 23 carrier stallions and five infected mares were identified during an intense trace back study that identified a single imported stallion from Europe in 2004 as the source of contamination. Although most effectively spread in natural mating populations, almost all of the stallions in this outbreak were not bred naturally and were infected via lateral transmission from stallion to stallion by exposure to contaminated fomites at semen collection facilities. It was also concluded that some of the positive mares became infected following artificial insemination with transported cooled semen from a carrier stallion.

Since the US is considered CEM-free and the consequences resulting from detection of a positive animal are extreme, we require that all stallions entering our collection facility have been tested negative from a set of swabs taken from the external genitalia and pre-ejaculatory fluid within 30 days of arrival. Nearly all countries, including the US, have strict CEM testing requirements for stallions whose semen is being imported. Since CEM is a reportable disease in the US and is believed to be fairly well controlled if not eliminated in the general population and because it is very difficult to culture, we do not specifically culture each batch of frozen semen post-thaw for the presence of the bacterium. Stallions used in artificial insemination programs that are only collected at our facility must be tested at the start of each breeding season. If the stallion is collected at another facility or breeds any mares by natural service, then we require that the cultures are repeated before the stallion can re-enter the collection facility.

As mentioned earlier, *klebsiella pneumonia*, *pseudomonas aeruginosa* and *streptococcus zooepidemicus* are much more common bacterial pathogens that cause endometritis in mares and can be found on external genitalia and in the semen of stallions. Stallions are not screened for the
The presence of these bacteria prior to entering the facility; however, each batch of frozen semen produced in our laboratories is cultured post-thaw to confirm the absence of bacterial pathogens or significant environmental contaminants.

Equine coital exanthema is a highly contagious venereal disease caused by equine herpesvirus 3 that causes ulcerative lesions on the penis of stallions and perineum or vulva of mares. The virus can be easily transmitted by natural mating, through AI with contaminated semen or indirectly via contaminated fomites. Diagnosis is primarily by clinical detection of the lesions and infected stallions or mares should not be bred until all lesions have healed.

While not generally considered to be spread venereally, a number of other diseases are of concern and should be considered. Equine infectious anemia (EIA) and piroplasmosis are two systemic diseases of horses that theoretically could be spread in semen of carrier stallions that hemorrhage during ejaculation and experience some degree of hemospermia. Equine encephalomyelitis, West Nile encephalitis, vesicular stomatitis, and African horse sickness could theoretically be transmitted in semen of infected stallions the same way and as a result some government agencies have required testing of stallions whose semen will be imported for a number of these diseases as well.

4. Sanitary procedures to minimize sample contamination

Stallions producing semen for transported cooled or fresh semen AI programs should be cultured prior to the start of the season and periodically during the season to confirm that they are not harboring and shedding potential pathogens. In an AI program, bacteria cultured from semen can usually be successfully treated with appropriate antibiotics in the semen extender. The incidence of bacterial contaminants in commercial cooled semen has been reported in the literature and reveals a high percentage of contaminated samples [4]. Even though commercial cooled semen extenders contain prophylactic antibiotics, they do not typically control the proliferation of bacteria in a contaminated sample present in extended semen [5]. Minimizing the bacterial load in the sample through careful collection techniques and sanitary handling of semen in the laboratory will decrease the chance of inseminating semen containing pathogens or significant contaminants. The presence of significant amounts of contaminating bacteria illustrates a lack of hygiene in many commercial semen processing laboratories. Frozen semen permits post-processing, pre-insemination testing to confirm the absence of potential mare pathogens or significant environmental contaminants prior to use, whereas transported cooled semen is typically inseminated before any culture results of extended, cooled samples could be obtained. If post-thaw culture of test straws from every ejaculate of frozen semen is adopted as standard practice then the incidence of mare pathogens or contaminating bacteria present in frozen semen should be greatly reduced and help minimize spread of disease in the breeding industry.

There have been few reports on the bacterial load in commercial equine frozen semen. A retrospective study published in 2009 [6] on semen frozen in several laboratories in two European countries between 1990 and 2004 reported contamination prevalence rates of 72 to 100% from 55 banked semen samples of 11 stallions. Quality control screenings of samples collected and processed in the North American Select Breeders Service Affiliate Network during the period from 2008 to 2011 revealed a contamination prevalence rate of 7.7% for 3,886 samples submitted (unpublished data). The difference in bacterial load reported in these two populations may be due to the degree of hygienic quality control practiced by the different laboratories.
laboratories, the type of antibiotics used in semen extenders, and variations in the techniques used by different microbiology laboratories.

Adhering to strict hygiene protocols in the collection facility is critical to prevent the potential spread of disease between animals at the center and to ensure that processed semen samples are not contaminated with environmental bacteria. There are many potential sources of bacterial contamination of extended, cooled or frozen semen samples in the collection center. Heavy loads of commensal bacteria on the stallion’s external genitalia can contaminate semen collected by artificial vagina and proliferate in extended semen samples. Stallions should be washed thoroughly prior to collection with warm water to reduce the potential bacterial load and associated dirt and debris commonly found on the penis, in the glans fossa, urethral diverticulum, and prepuce. Water is a common source of contaminating bacteria and should be monitored frequently. Hoses attached to faucets used to dispense water for washing stallions and cleaning collection equipment have often been identified as reservoirs for bacteria and provide a very efficient method of spreading bacteria from stallion to stallion and contaminating collected samples.

The breeding phantom or “dummy mount” should be wrapped with disposable plastic on the back end of the mount and discarded and replaced between collections. The remainder of the mount should also be disinfected between collections. Stallion shanks, halters, and other animal handling equipment should be unique for each horse and, if not, should be properly disinfected between horses. Collection equipment must be new and disposable or thoroughly cleaned, rinsed of any soap residues, and properly disinfected between uses. All personnel involved in the collection process and especially technicians that will then be involved in the further handling of the semen should wear gloves and protective clothing and employ frequent hand washing with bactericidal soap and/or alcohol based hand sanitizers.

Whenever possible single use disposable products such as collection bags or bottles, specimen cups, pipets, centrifuge tubes, tubing for straw filling, should be employed when handling semen. Water baths used in the laboratory are very common sources of contamination. The warm water provides a perfect environment for proliferation of many bacteria and should be cleaned and disinfected frequently. Every time a tube, specimen cup or extender bottle is placed in a water bath, the bacteria on the hands of the technician are transferred to the water and will proliferate in the bath. Prior to thawing semen in a water bath, it should be cleaned and disinfected and fresh clean laboratory grade deionized water should be used. Periodic swabbing of equipment and laboratory surfaces should be performed to ensure that cleaning protocols are sufficient. The effectiveness of hygiene protocols can be monitored by frequent screening for the presence of contaminating bacteria in extended or frozen thawed semen samples.

Currently there are no universally accepted standards for the production of quality semen by AI centers in North America. The European Union has established regulations concerning the production of semen to be used between member states; however, these regulations do not address the issues of semen quality other than from the perspective of preventing disease transmission. There are no regulations concerning the quantity (number of sperm) or quality (post-thaw motility or other assay of sperm function) of sperm provided in a dose of semen. A European organization called the World Breeding Federation for Sport Horses published recommended guidelines for semen production and quality standards for its member breeding stations. The details of these guidelines can be found at: http://www.wbfsf.org/files/Semen%20standards.pdf. Member laboratories of the Select Breeders Affiliate Network have also agreed to
follow a set of guidelines for quality standards for frozen semen. Those guidelines are presented below.

5. Recommended quality standards for equine frozen semen

Stallion health testing:
I. Equine arteritis virus
   a. SBS currently requires negative EVA (serology) within 30 days prior to arrival at collection facility.
   b. Seropositive stallions must provide negative virus isolation test results on semen within 30 days prior to arrival or proof of vaccination with annual booster.
   c. Stallions that are shedding EAV in semen are not admitted into SBS collection facility.
   d. Semen from EAV shedding stallions can be frozen for domestic distribution but only at client location and using separate equipment and storage tanks. Any frozen semen from shedding stallions is only distributed along with paperwork that thoroughly describes the recommended procedures for testing, vaccination and insemination of mares with EVA positive semen.

II. Taylorella spp.
   a. SBS currently requires that all stallions entering collection facility be cultured negative for the causative agent of CEM (*T. equigenitalis* and *asigenitalis*) within 30 days of arrival into collection facility.

III. Equine infectious anemia virus
   a. SBS currently requires that all stallions be tested negative for EIA within 6 months prior to entering collection facility.

IV. Other diseases of concern: there are other diseases that may be of concern to animal health officials of countries importing semen from the US. Often the importation requirements for frozen semen will require that stallions be tested for other diseases. If a stallion is being presented to an SBS facility for export freezing then all of those health testing requirements for the importing country must also be met. These may include negative tests for vesicular stomatitis, dourine, glanders and statements of vaccination for equine encephalomyelitis and rhinopneumonitis.

Post-thaw semen quality:
I. SBS’s current recommendation for commercial distribution of frozen semen are as follows:
   a. Each dose of semen should contain a minimum of 200 million progressively motile sperm (after thawing).
   b. The post-thaw progressive motility of thawed semen should be ≥ 30%. This is measured using an objective computer assisted motility analyzer (CASA). Progressive motility is defined as those sperm moving with an average path velocity (VAP) > 50 microns/second and a straightness value (STR) > 75%. In our system, two straws (0.5 mL) are thawed for each frozen ejaculate, diluted in a standard milk-sugar type extender, and incubated for 30 minutes at 37°C. The post-thaw motility is then assessed following the thermal stress test of incubation.
c. Thawed semen should be free of potential pathogens and significant environmental contaminants. Semen from the test straws for every ejaculate frozen is cultured.

II. These are SBS recommended minimum requirements for commercial distribution of frozen semen. Since we do not own the semen these are recommendations rather than absolute requirements; however, if a client (stallion or semen owner) asks SBS to distribute semen that does not meet these requirements we will do so but only if accompanied by a transaction report that details the results of the post-thaw analysis. The paperwork accompanying the shipment also contains a notice which indicates that a post-thaw analysis of the semen from this ejaculate was performed and the results indicate that the semen quality does not meet SBS recommended standards for commercial distribution. The goal here is to ensure that all parties involved are aware of the semen quality. We recognize that semen from some stallions/ejaculates may meet these requirements but result in poor fertility while some that do not meet these requirements may, if managed properly, result in acceptable pregnancy rates. Post-thaw sperm motility is not always well correlated with fertility. Our goal is to try and eliminate semen from commercial circulation that has a high likelihood of resulting in poor fertility.

Over 30 years ago the bovine AI industry made a decision to self regulate the production and distribution of frozen bull semen. The National Association of Animal Breeders formed a subsidiary organization in 1976 with the goal of providing oversight and quality assurance for bull studs. Certified Semen Services (CSS) has been successful in implementing this voluntary program throughout the industry. The success of the CSS program has enabled the national animal breeding industry to essentially regulate itself without direct government involvement. Whether a voluntary oversight program such as that provided by CSS in the cattle industry or a mandatory program instituted by a governmental regulatory body similar to the EU licensing program or nothing at all would benefit the horse breeding industry is a topic of controversy among breeders and veterinarians. However, the increased popularity of transported cooled and frozen semen and the increased risk of disease transmission associated with international movement of equine semen warrants serious discussion among breeders and professionals concerned with the responsible development of the equine AI industry.

References
