

BIOSECURITY AND FROZEN SEMEN

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Introduction

Concern about the biosecurity of frozen semen dates back to 1947. Two articles described the transmission of *Brucella abortus* and *Trichomonas fetus* by artificial insemination (AI) with semen collected from infected bulls (1). Efforts to control the potential for seminal transmission of these diseases were probably considered as preventive medicine rather than biosecurity. Regardless, this early research illustrated that semen used for AI had to be considered as a point source for transmission of disease. Numerous studies regarding the potential for other diseases to be transmitted by AI have been conducted on many species since 1947 (2,3,4) along with extensive research to evaluate, enhance, and refine diagnostic methods for each disease considered.

Biosecurity and risk analysis

There are probably numerous definitions of biosecurity, but at its basic level relative to animal husbandry it implies protection from disease and protection from recognized hazards that may cause disease in a herd of animals. Relative to the animal genetics and artificial insemination industries, biosecurity has two primary points of concern: the herds of animals from which the genetic products are produced and the end-user of the genetic product.

When a dairy producer, for example, inseminates a cow with semen, biosecurity is not his foremost consideration. The genetics provided by the donor of the semen is his primary concern. However, he has introduced a biological entity from a remote source into the herd. Accordingly, the semen used for AI would be one of a myriad of components a dairy producer should consider when implementing a biosecurity program. The reason that most dairy producers and ranchers do not consider semen a biosecurity hazard is due to the integrity established by the organized bovine AI industry to monitor and control disease in herds of donor bulls. Members of the National Association of Animal Breeders (NAAB) and its subsidiary Certified Semen Services (CSS) comply with industry regulations to detect and control specific diseases of cattle considered to be of primary concern for transmission by AI (5). The development of a thorough biosecurity program for cryopreserved semen is a multi-faceted topic.

One approach to establish a biosecurity program is with risk analysis. The three primary components of risk analysis are risk assessment, risk management, and risk communication (6). The first step is *risk assessment*.

When a genetics company is developing its business plan it should determine the potential problems, or hazards, that may be encountered when procuring its animals and producing its products. This includes an evaluation of the geographic location of the farms from which the bulls originate, the herd health history of those farms, the timely cooperation of the farm managers to get animals evaluated and tested, and how animal

transport will be conducted on a regular basis. It also includes an evaluation of personnel traffic, vendor access and traffic on the AI Center (such as feed deliveries, fuel deliveries, waste and refuse removal), and visitor policies. Consideration for the manner by which emergencies of animal health, employee injuries, and premises problems will be handled is also important. These are achieved by studying the literature, talking to colleagues in the industry, and discussing potential scenarios with appropriate persons. It should also be recognized that business problems and their management become more numerous and complex when an international component is included in the company's marketing structure.

The next component of risk analysis is *risk management*. It is the most significant component relative to the efforts required and expenses incurred. Risk management includes the pragmatic methods employed to detect and control those diseases and related hazards that were identified as critical to business success in the risk assessment. It consists of the strategic decisions by which the numerous risks determined in the assessment phase will be approached and managed. Risk management considers the methods necessary to prevent incursion of disease into the herd, to control transmission of disease within the established herd of genetic donor animals, and to prevent contamination of the genetic product to be offered for sale to the public.

Risk management recognizes that diseases do not all have the same *risk* of transmission. The relative risk for horizontal transmission and the modes of transmission are variable among diseases of concern for any species of livestock. Preventive medicine programs recognize such differences; appropriate control programs are established to achieve the needed level of biosecurity for each disease. Similarly, the potential for seminal transmission is variable among the diseases of concern for any species from probable, to sporadic, to highly unlikely. Accordingly, the relative risks for semen to become contaminated with specific microbial agents must be identified and appropriate control measures instituted. Persons responsible for the animal health and biosecurity of a genetics business should understand the typical epidemiological patterns for each disease of concern and the pathogenesis of those diseases in relation to male genital tract physiology. Even though control of a specific disease may be important from a typical herd health perspective, the potential for seminal contamination and the likelihood for transmission by AI may or may not be meaningful.

Risk communication represents the manner by which the risk management program is described to others so that it is correctly implemented and recognized by persons outside of the risk management team. For example, the farm managers and animal husbandry personnel must understand the basic features of the risk management program so its numerous components are appropriately implemented and practiced. The risk management program is also communicated when international health documents are prepared that confirm compliance with specific protocol stipulated by the importing regulatory agency or business entity.

Risk management relative to biosecurity of frozen semen

Within any species of domestic livestock, numerous diseases will be recognized as potential hazards to the continued health and well being of the resident herd of genetic-donor animals and as potential agents that may contaminate semen. Diseases have been categorized by their potential to contaminate semen (3,7) and that is important relative to risk assessment, but that does not necessarily provide a guide for risk management or for developing a biosecurity program. In 1976, Dr. Bartlett provided a framework by which diseases could be categorized based upon current epidemiological control measures and available diagnostic capabilities (2). This concept will be adopted in this article to illustrate one manner by which biosecurity of cryopreserved semen is achieved. It must be recognized that the categorization of diseases in this article is not necessarily definitive and adjustments in categorization are possible depending on the business needs of the AI Center and the attending regulatory environment of the countries to which semen may be exported. The following discussion will be restricted to disease control in cattle.

Freedom from disease by territorial status, or regionalization

The primary bovine diseases to be considered in this category relative to the United States include **contagious bovine pleuropneumonia** (caused by *Mycoplasma mycoides*), **foot and mouth disease**, **rinderpest**, and **lumpy skin disease**. Additional microbial agents recognized as “foreign animal diseases” could be included.

Bovine spongiform encephalopathy (BSE) could also be included in this category because the disease has not been detected in the United States. However, it is preferable that BSE *not* be considered in a discussion about biosecurity of cryopreserved semen because there has been no evidence that abnormal prions are in semen (7). Furthermore, the International Animal Health Code of the Office of International Epizootics (OIE) states that “regardless of the BSE status of the exporting country, Veterinary Administrations should authorize without restriction the import or transit through their territory of the following commodities: 2) semen and embryos” (8).

Freedom from disease by herd negative status

The primary bovine diseases to be included in this category are **tuberculosis** (*Mycobacterium bovis*), **brucellosis** (*Brucella abortus*), **bovine genital trichomoniasis** (*Trichomonas fetus*), and **bovine genital campylobacteriosis** (*Campylobacter fetus* subsp. *venerealis*). Each of these diseases has been found to be potentially transmitted by semen used for AI. Most established AI Center herds are maintained free of tuberculosis and brucellosis by compliance with the Uniform Methods and Rules of the United States Department of Agriculture. New bulls to the AI Center are tested according to required protocol; bulls in resident herds are tested at six-month intervals for tuberculosis (intradermal caudal fold test using bovine PPD tuberculin) and brucellosis (complement fixation and a buffered antigen test). For bovine genital trichomoniasis and campylobacteriosis, all bulls entering the AI Center should be repeatedly tested by

culture of preputial material according to the protocol stated in the CSS Minimum Requirements for Disease Control of Semen used for AI, and then retested at six-month intervals as long as they remain in a semen production herd (5). In this manner, entire herds of bulls are maintained free of genital trichomoniasis and campylobacteriosis.

Bulls resident in an AI Center herd may also be recognized free of persistent infection (PI) with **bovine viral diarrhea virus** (BVDV), based on repeated testing for PI at the time of entry to the AI Center and testing of semen for virus when first collected (1,5). According to CSS protocol, bulls are evaluated for BVDV-PI prior to entry to the admittance isolation facility of the AI Center and again during the isolation interval (5). In this manner, bulls that are PI for BVDV will be precluded from entering the admittance facility; the subsequent test conducted during the isolation interval by the AI Center veterinarian confirms that all bulls entering the AI Center are free of BVDV-PI. To confirm that bulls do not have a persistent testicular infection with BVDV, the semen of all bulls is tested for evidence for BVDV, or, the semen of those bulls found to be seropositive for BVDV is tested (5). A test of semen from only BVDV-seropositive bulls is appropriate because the persistent testicular infection is considered to occur near the age of puberty (1). Regular retests for BVDV at annual or semiannual intervals are not considered necessary.

Resident herds of bulls may also be maintained free of bluetongue virus (BTV), bovine leukosis virus (BLV), and infectious bovine rhinotracheitis virus (IBRV) based on repeated serological testing and strict animal entry policies. In the United States, herds of dairy bulls seronegative for these diseases have been developed and are maintained to comply with specific international regulatory protocol. However, extenuating circumstances exist for each of these diseases such that herd negative status, and even donor animal seronegative status, is *not required* to assure the end user that semen used for AI will not transmit disease to cows inseminated. This will be discussed in greater detail in the following section.

Control of disease by surveillance of the semen donor animal

Specific diseases may be adequately controlled by repeated diagnostic surveillance of the semen donor animal, or even of individual ejaculates. This level of biosecurity may be acceptable when a disease is considered ubiquitous in a country or vaccination is widely practiced within the national herd. In other circumstances, the available diagnostic technology lends itself better to control programs on an individual animal basis. Consistent with this approach, the level of biosecurity achieved is dependent on the sensitivity and specificity of the diagnostic techniques employed. For some of the diseases to be mentioned in this category, the potential for seminal transmission is highly unlikely.

Because vaccination for **IBRV** is widely practiced in the United States, it is not critical that a semen donor bull be IBRV-seronegative when its semen is used domestically. This is particularly applicable to the beef segment of the industry wherein most bulls are vaccinated as calves or when they enter a performance evaluation program. Evaluation

of tens of thousands of ejaculates collected from both IBRV-seropositive and IBRV-seronegative donor bulls by *in vitro* and *in vivo* virus detection techniques, with no evidence of virus, indicates that semen can be safely collected from bulls regardless of their IBRV-serologic status (9). To provide assurance for semen marketed internationally, importing countries (that have a control program in their AI Centers or national herd for IBRV) can require the donor bull be tested seronegative within six months prior to collection or at least 21 days after collection, or, have the semen collection being exported tested for the virus.

Bluetongue virus is a disease that lends itself toward regionalization programs. Bluetongue virus is enzootic in the southern and western regions of the United States, but the northeastern and Great Lakes region of the United States has a low prevalence for BTV because of the absence of competent arthropod vectors. Furthermore, BTV does not cause latent infection in exposed cattle; the viremic interval is typically 60 days or less. Seminal contamination in bulls can occur, but only sporadically and only during the interval of viremia. Accordingly, bulls resident in the low prevalence region of the United States will not be exposed to BTV and therefore do not present a risk for seminal transmission. Bulls previously exposed to BTV will typically remain seropositive for life, but because the viremic interval is short-lived, they do not pose a risk for seminally transmitting BTV after the viremic interval if they are no longer subject to viral exposure (10). Pertinent control programs include residence of bulls in the low prevalence region of the United States; this may or may not include serologic monitoring to assure continued negative status of seronegative donor bulls. It may also include testing whole blood of a previously exposed and seropositive semen donor bull to confirm a BTV-aviremic status. For bulls resident in other regions of the US, serologic monitoring to assure continued seronegative status of BTV-seronegative donor bulls is appropriate, or testing whole blood of a BTV-seropositive semen donor bull when it is collected during the vector season.

It is widely recognized that **bovine leukosis virus** is unlikely to be transmitted by AI (1,2,3,4,7). Accordingly, donor bull and herd health programs for BLV are not included in the CSS Requirements. Despite the scientific evidence that BLV is not seminally transmitted, many international regulations stipulate continued diagnostic surveillance for BLV and require freedom on either a donor animal or herd basis. There has recently been recognition of a PCR-based test of semen, which is available to evaluate semen collected from BLV-seropositive bulls and confirm its negative virus status for export.

Leptospirosis has been a component of the CSS Requirements for many years (5); it has also been categorized as a disease able to be controlled on a herd basis (2). To qualify for several international markets, AI Centers maintain herds of bulls seronegative for the five primary serotypes of leptospirosis present in the U.S.: *L. canicola*, *L. grippotyphosa*, *L. hardjo*, *L. icterohemorrhagiae*, and *L. pomona*. However, there are problems with controlling leptospirosis on a herd basis by diagnostic serology alone. The primary problem is that a small percentage of cattle previously infected with the host adapted-serovar, *L. hardjo*, may not demonstrate a serologic response at the 1:100 titer dilution. Accordingly, several commercial AI Centers have adopted a program wherein *all*

incoming animals are treated with an antibiotic that eliminates the renal carrier state and the shedding of leptospires in urine; such antibiotics are tilmicosin, oxytetracycline, and ceftiofur sodium. In this manner, the serologic response for leptospirosis is not important relative to the potential for seminal transmission. Furthermore, recent research has determined that the combination of antibiotics added to the semen per the CSS protocol, glycerolization, and freezing of semen eliminate the infectivity of any leptospires that may have contaminated semen (11).

Diagnostic tests for *Mycobacterium avium* subsp. *paratuberculosis* (*Map*), or Johnes Disease, are included in most AI Center health programs in the US. Bulls are tested so that diagnostically responsive animals may be segregated and prevented from entering the semen production herds of the AI Center. Despite current evidence that seminal transmission of *Map* is unlikely (12), several international regulations require the AI Center herd be negative to one of several diagnostic tests. Research continues to search for a diagnostic test or diagnostic program for young animals that is predictive for probable infection at a later age. But because this diagnostic program remains elusive, a preventive program that precludes the entry of all *Map*-infected bulls does not yet exist. Because the potential for seminal transmission is unlikely, diagnostic testing of the donor bull should be sufficient for international compliance. Because the potential for transmission of *Map* among post-pubertal animals in an AI Center environment is unlikely, international regulations stipulating freedom of disease in the herd for extended time intervals of six months to five years provide no additional security to that provided by tests of the individual donor bull.

Many AI Centers vaccinate bull calves several times during the admittance isolation interval for the virus causing fibropapillomas. The intent of such a vaccination program is to reduce the prevalence of penile fibropapillomas, or warts. Relative to concerns about seminal transmission of fibropapillomas virus, bulls with penile fibropapillomas should not be collected until the wart has been surgically excised and evidence of regrowth is absent (2).

Control of seminal transmission by addition of antibiotics to semen

About 50 years ago, antibiotics were added to semen to control the seminal transmission of *Campylobacter fetus* subsp. *venerealis*. Because of improved diagnostic capabilities, bulls and entire herds are now maintained free of this disease. Accordingly, antibiotics in semen provide a secondary, but important, control of *Campylobacter fetus* subsp. *venerealis* (2).

Extensive research in the mid-1980s evaluated the efficacy of numerous antibiotics for their control of several potential pathogens and also their toxicity to bovine spermatozoa. From this research it was confirmed that primary control of *Mycoplasma spp* could be achieved with the addition of lincomycin and spectinomycin to semen. Control of *Ureaplasma spp* was achieved with tylosin. These antibiotics, along with gentomycin sulfate for control of *Campylobacter fetus* subsp. *venerealis*, also provided control of *Hemophilus somnus*. The current antibiotic regimen for bovine semen includes 50

mcg/ml tylosin, 250 mcg/ml gentamycin sulfate, 150 mcg/ml lincomycin, and 300 mcg/ml spectinomycin (13). Recent research has determined that this antibiotic combination, along with glycerolization and the freezing of semen, afforded control of leptopirosis in semen (11).

Concluding considerations

The biosecurity programs employed at AI Centers include testing of all bulls prior to entry to an admittance isolation facility, repeated testing and clinical surveillance of bulls during an isolation interval that may last for 30 to 60 days, and regular retesting of all resident bulls at six month intervals thereafter. Control of disease is achieved by diagnostic surveillance for most diseases rather than vaccination programs. Movement of bulls into the resident herd and within the AI Center herds is closely controlled and monitored; stall location histories for each bull are maintained on computer database. Perimeter fencing and restricted access points permit additional control of animal and people contacts. Extensive diagnostic test histories on each bull are also maintained by a computer database for ready access and relational assessments. Control of pests, such as rodents and birds, is also conducted. Accordingly, semen collected for domestic and international use is from bulls that have controlled animal contacts and reside in an environment with a comprehensive, ongoing biosecurity program. The potential for seminal transmission is controlled by programs specifically developed for each disease of concern.

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