

CRYOGENIC PRESERVATION OF BULL SEMEN – SANITARY, VETERINARY AND LEGAL ASPECTS

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Abstract

In the presented study, the authors present the applicable legal and sanitary aspects in relation to cryogenic protection of semen of bulls in its collection centers. The current legislation regulates the production, treatment and preservation of biological material, as well as provides guidelines on the health status of sperm donors. The use of cryopreserved semen for insemination, collected from healthy donors while maintaining sanitary rigour, ensures safe breeding and poses no risk to inseminated females.

Keywords: storage centers, bull semen, health status

Introduction

The scope and importance of artificial insemination in cattle is enormous, and a factor that favours the use of this method is the possibility of effective cryogenic preservation of biological material in the form of bull semen. The use of low temperatures in reproductive biotechnology allows cryopreservation and long-term storage of biological material from farm animals in the form of semen, embryos and oocytes. Collecting frozen biological material from male and female donors allows it to be used both during life and after the death of the animal. In the case of males, especially bulls, the development of an effective cryopreservation method has allowed them to better exploit their reproductive potential and influenced the practical use of frozen sperm in artificial insemination worldwide. The ability to transport frozen semen over long distances has facilitated the use of prominent individuals from home and abroad for mating.

The beginnings of insemination in Poland are associated with the activities of the Polish Society of Animal Husbandry, which in 1946, on the initiative of the Ministry of Agriculture and Agrarian Reforms, organized the first cattle insemination course in Pawłowice near Leszno. Intensive development of cattle insemination took place in the early sixties of the twentieth century with the establishment in 1956 of the State Insemination Plants, where cryopreservation and collection of semen from selected bulls were implemented on a large scale. Insemination with frozen semen of such sires improved the musculature of cattle, increased cow productivity and milk quality, and also facilitated the fight against diseases such as brucellosis, tuberculosis or other diseases spread by natural mating (Kondracki et al., 2013).

Biological risks associated with artificial insemination in cattle

Biological material, especially semen, can pose a high risk of spreading animal diseases because it is obtained from a limited number of donors and is used to inseminate a large animal population (Rola i Polak, 2007). For this reason, bulls from which semen is collected and cryopreserved must be under constant veterinary care and their health status must comply with current legislation.

Research conducted by Wierzbowski (1981) showed that the most numerous group of potentially pathogenic microorganisms isolated from the semen of bulls are *P. aeruginosa*, *Streptococcus* spp., *Staphylococcus* spp., *Proteus* spp. and *Bacillus* spp. In special conditions, e.g. weakening of the body's immunity, these microorganisms can pose a threat to the health and even life of the animal (Althouse, 2008; Andrabi, 2007). In the case of other opportunistic microorganisms present in the bull's semen, i.e. *Ureaplasma diversum*, *Histophilus somni*, *Chlamydophila abortus*, *Escherichia coli* and *Staphylococcus* sp. their transfer to the reproductive system of females during artificial insemination causes a decrease in female fertility (Kilburn et al., 2013). Heifers inseminated with semen containing *Mycoplasma bovis* develop inflammation of the uterus and fallopian tubes (Nielsen et al., 1987). In contrast, Saed and Al-Aubaidi (1983) found papular vulvovaginitis in females inseminated with semen showing the presence of *Mycoplasma bovinegenitalium*. Transmission of *infectious bovine rhinotracheitis/infectious pustular vulvovaginitis* IBR/IPV during insemination of cows and heifers causes vesicular vulvovaginitis, repeated oestrus and may also induce abortions. The transmission of bovine viral diarrhoea (mucosal disease BVD-MD) during insemination with semen of an infected bull induces embryo death and repeated estrus in cows (Virakul et al., 1988). The effectiveness of transmission of BVD-MD virus infection by insemination with such semen can be up to 100% (Rola i Polak, 2007).

In bulls infected with brucellosis, inflammation of the testicles and epididymis occurs, and the vascularization of females with the semen of a bull whose genitals are covered by infection causes catarrhal inflammation of the vagina and uterus (Żmudziński, 1996). Infection with *Campylobacter fetus* ssp. *venerealis* in bulls is asymptomatic, but performing insemination with infected semen causes inflammatory changes in the uterus and abortions in females. In turn, the main sources of BLV (Bovine leukemia virus) infection belonging to the retrovirus family are blood, milk, colostrum, but also saliva, urine, faeces, amniotic waters and semen (Gillet et al., 2007).

Due to the possibility of transmission of various diseases via the semen, it is important to strictly comply with the current regulations governing the health status of donors of biological products and to eliminate from reproduction animals that are a potential source of infection.

Legal regulations regarding the sperm collection center

In accordance with current regulations, the production, processing and conservation of biological matter takes place in acquisition centers. Commission Regulation (EU) 2020/686 of 17 December 2019 supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as regards the approval of establishments handling biological material and traceability and animal health requirements for the movement within the European Union of biological material of certain farmed terrestrial animals details the veterinary requirements applicable to bovine semen and specifies, that a semen collection centre is an establishment,

situated in the territory of one of the Member States of the European Union or a third country, where semen for artificial insemination is collected and treated, approved and supervised by an official veterinarian.

Regulation (EU) 2016/429 aims to create in a single piece of legislation a regulatory framework that includes animal health requirements, disease management, prevention and eradication of animal diseases.

Currently, according to the data of the Chief Veterinary Inspectorate (<https://www.wetgiw.gov.pl/handel-eksport-import/listy-centrow-i-zespolow->), there are 9 approved cattle semen collection centres in Poland, where the selection of sires takes place, and the collected and packaged biological material is used for artificial insemination. The semen collection centre must be under the constant supervision of a veterinarian and should consist of separate lockable rooms for the permanent presence of animals, the quarantine of animals, the isolation of sick animals, the collection, treatment, maintenance and storage of semen, and the cleaning, disinfection or sterilisation of instruments and equipment.

Health requirements for donors of germinal products

Detailed guidelines on animal health requirements for bovine animals from which germinal products are collected and requirements for quarantine and laboratory testing or other testing of these animals are regulated by Commission Regulation (EU) 2020/686 of 17 December 2019.

Requirements for testing animals before they are admitted to the centre

According to the Regulation, only animals that have not been used for natural mating during the period of at least 30 days prior to the date of the first collection of semen and during the period of semen collection may be present in the semen collection centre. Animals shall, within 30 days prior to entry into the premises in which they are quarantined at the semen collection centre, be tested for infection with:

- *Mycobacterium tuberculosis* complex (*M. bovis*, *M. caprae* and *M. tuberculosis*) by intradermal tuberculinisation test,
- *Brucella abortus*, *Brucella melitensis* and *Brucella suis* by serological test,
- enzootic bovine leucosis (EBL) by serological test,
- infectious bovine rhinotracheitis/infectious bovine infectious IBR/IPV by serological test,
- BVD-MD bovine viral diarrhoea by a test for the presence of the virus genome or antigen.

The supervising veterinarian may admit to the centre a sperm donor from an establishment which was not free of enzootic bovine leucosis, only on condition that the animal is less than 2 years old and has been born by a mother who has undergone a serological test for EBL after taking the animal from its mother and has obtained a negative result. Another derogation for the admission of an animal from an establishment that was not free of enzootic bovine leukaemia to the centre is if the animal is negative in a serological test for the disease when it has reached the age of two years.

Obtaining negative results for the above-mentioned infections by donors allows them to be admitted to a sperm collection center and introduced into quarantine rooms.

Requirements for animals in quarantine

Only other cloven-hoofed animals of at least the same health status are kept in the quarantine room located in the bull semen collection centre. No sooner than 21 days after the animals are admitted to quarantine, bulls shall be tested for infectious diseases for:

- *Brucella abortus*, *Brucella melitensis* and *Brucella suis* by serological test,
- bovine rhinotracheitis/infectious IBR/IPV by serological test,
- BVD-MD bovine viral diarrhoea by a virus isolation test or a virus antigen test and a serological test to determine the presence or absence of antibodies,
- bovine turbidity disease (*Campylobacter fetus ssp. venerealis*),
- bovine trichomonas (*Trichomonas foetus*).

Testing for bovine cloudy disease (*Campylobacter fetus ssp. venerealis*) and trichomonas plague (*Trichomonas foetus*) may be carried out on a sample of artificial vaginal or foreskin washings taken once or three times at intervals of at least 7 days.

If any animal is positive for *Brucella abortus*, *Brucella melitensis*, *Brucella suis* or IBR/IPV, it is immediately removed from the quarantine facility. The remaining animals in the same group shall remain in the quarantine accommodation and shall be retested for the abovementioned infections, but not earlier than 21 days after the animals have been removed with positive results.

Any seronegative or seropositive animal after testing for bovine viral diarrhoea may be admitted to a semen collection centre only if there is no seroconversion in those animals which have tested positive before introduction into the quarantine accommodation. If seroconversion occurs, all animals which are still seronegative shall be kept in the quarantine facility for an extended period until a case of seroconversion appears in the group of animals for a period of 3 weeks. Seropositive animals may be admitted to a semen collection centre. In addition, before the first shipment of semen, a bull that has tested positive for BVD is subjected to a virus isolation test or an enzyme-linked immunosorbent assay-ELISA for virus antigen. If a positive result is obtained, the bull is removed from the centre and the semen obtained from it destroyed.

If the result of any of the tests carried out during the quarantine of animals is positive, the animal in question shall be immediately removed from the quarantine site. If the entire group of animals is tested positive for mandatory diseases during quarantine, the district veterinarian determines how to handle the animals and the procedure for reintroducing them into the semen collection centre.

Requirements for animals kept at a semen collection centre

At the sourcing centre, all bulls are subjected at least once a year to mandatory tests for:

- Mycobacterium tuberculosis complex infection (*M. bovis*, *M. caprae* and *M. tuberculosis*), infections of *Brucella abortus*, *Brucella melitensis* and *Brucella suis*,
- enzootic bovine leucosis,
- infectious bovine rhinotracheitis/infectious bovine vulvovaginitis,
- bovine viral diarrhoea, a serological test for the detection of antibodies which is used only for seronegative animals. If an animal is seropositive, all semen of that animal collected from the last negative test shall either be rejected or tested negative for virus or virus genome,
- bovine turbidiosis,
- trichomonas blight of cattle.

In addition, in the case of bulls from which semen has been re-collected after a break of more than 6 months, tests for trichomonas contagion and bovine turbulense disease shall be carried out in the 30 days prior to the resumption of semen production. If the result of any of the tests is positive, the animal is isolated from other animals and semen collected from that animal since the last test in which a negative result was obtained may not be the subject of trade.

Where the result of a test for bovine viral diarrhoea and mucosal disease is positive, semen collected from that animal since the last negative test shall be tested to exclude the presence of the virus.

Sanitary protection of the premises of the centre and semen production

In addition to verifying the health status of donors of biological material, it is important to provide sanitary protection of the rooms in which semen is collected, processed (thinning, cryopreservation), as well as appropriate protection of equipment and personnel in contact with it. The collection, processing and storage of semen should take place in separate rooms designated for this purpose, with strict hygiene. In addition, rooms for the processing, handling and cryopreservation of biological material may not have a direct connection to the permanent animal accommodation. All instruments in contact with the donor during collection and processing of semen should be cleaned and disinfected or sterilised before use, except for new disposable instruments. All biological products of animal origin used in the processing of semen, including thinners, additives or thinners, should come from sources that do not present a risk or have been subjected prior to use to a treatment that excludes such a risk. Cryogenic agents used during freezing must be of appropriate quality and used once for the treatment of biological material. Staff employed at the semen collection centre must receive appropriate training in disinfection techniques and hygiene in preventing the spread of animal diseases.

Semen collection and processing are not sterile procedures, so high sanitary and hygienic standards must be maintained during semen production to minimise the risk of contamination. In addition, the processing of semen involves the use of diluents, which must obligatorily contain bactericidal and/or bacteriostatic compounds in their composition (Goularte et al., 2020). In accordance with current sanitary and veterinary standards, additives used in diluents must not pose a threat to animal health. Such a risk seems real, as additives can be a source of pathogens and are also an excellent medium for pathogens. This is important in a situation where most commercially available diluents are produced on the basis of animal substitutes, mainly milk and egg yolk. Commercial diluents used in insemination practice in the European Union must have an effective antibacterial effect. Commission Delegated Regulation (EU) 2020/686 maintains the provisions on the use of antibiotics laid down in Directives 88/407/EEC, 90/429/EEC and 92/65/EEC, as well as those recommended by the OIE. In accordance with Directive 88/407/EEC the EEC is obliged to add antibiotics to bovine semen that act against campylobacter, leptospirae and mycoplasmas. Antibiotics reduce the possibility of transmission of infection via semen only in the case of bacterial infections, while in the case of viral infections, the addition of antibiotics does not provide any protection (Wierzbowski, 1990).

Summary

Continuous monitoring of the health of sperm donors at the semen collection centres and the performance of cyclic tests in accordance with the legislation allows for obtaining high quality biological material free of epidemiological risks. The existing health standards for animals received and located at the semen collection centre make it possible to avoid economic losses and maintain high efficiency in cattle breeding.

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SUMMARY

In the article, the authors present the applicable legal and sanitary aspects related to the cryogenic protection of semen in collection centres. The current regulations contain information about the production, treatment and preservation of biological material as well as provide guidance on the health status of donors. The use of semen collected from healthy donors and cryopreserved in accordance with sanitary requirements ensures the safety of breeding and it does not pose a threat to inseminated females.

Keywords: storage centers, bull semen, health status