Opinion

Bovine herpes virus-1: comparison of methods for removal of a commercially important pathogen from cattle sperm, oocytes and pre-implantation embryos

Abstract

Bovine herpesvirus 1 (BoHV-1) is a pathogen of major veterinary importance, causing principally reproductive failure, genital and respiratory disease in cattle. Since embryo transfer is a rapidly growing commercial venture, BoHV-1 has a significant negative impact on cattle breeding by both natural and artificial service, and thereby on the global livestock industry. Clinical infection of the reproductive tract causes infertility, early embryonic death and abortion. BoHV-1 may infect an embryo by either of two means. The first is through entry of contaminated sperm into the oocyte at the point of fertilization, while the second is via contact with either contaminated follicular fluid, oviductal or uterine tissues. In addition, the virus may infect the recipient cow if an infected embryo is transferred by assisted reproduction technology. This article briefly examines the two principal methods that are routinely available to eliminate BoHV-1, performed in order to prevent infection of bovine embryos. Although each offers considerable benefits, it is also imperfect. Even after multiple trypsin washes BoHV-1 can adhere to the zona pellucida of oocytes and pre-implantation stage embryos; likewise, cryopreservation fails to eliminate all infectious virus particles. A more experimental technique, sperm processing, shows considerable promise but requires further validation as an effective way to remove BoHV-1 from bull semen before it can be recommended for industry-wide use.

Keywords: bovine herpesvirus 1, cattle, fertility, assisted reproduction, oocyte, semen, embryo, trypsin, swim-up, cryopreservation, sperm processing

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Introduction

Bovine herpesvirus 1 (BoHV-1) is a member of the genus *Varicellovirus* in the subfamily Alphaherpesvirinae.[1] Infection of cattle with this double-stranded DNA virus imposes trading restrictions and produces worldwide economic losses to the livestock industry.[2] BoHV-1 causes predominantly respiratory and genital disease in cattle, although it is also associated with meningoencephalitis.[3] Infection has been causally linked to reproductive failure, through such clinical manifestations as infectious pustular vulvovaginitis, endometritis, salpingitis, shortened oestrous cycles, early embryonic death and abortions, and temporary infertility in susceptible cows.[2–4] Cattle of either gender may also carry the virus with no clinical signs and thus provide an unrecognized reservoir of infection within a herd.[4] Primary infection with BoHV-1, which is triggered by viral replication in nasal, vaginal or preputial mucosa, may not be cleared completely and result in latent infection of neuronal ganglia, like the other Alphaherpesvirinae viruses, notably the varicella-zoster virus that causes chickenpox and its recurrence as shingles in humans. Latent BoHV-1 infection causes an animal to become a life-long carrier and potential disseminator of the virus.[4] This mechanism plays a pivotal role in maintaining the BoHV-1 epidemiological cycle. Any factor that triggers host immunosuppression may result in reactivation of BoHV-1 followed by episodes of virus re-excretion.[4–6]

Transmission and epidemiology of infection

There are two major ways by which BoHV-1 may infect a cattle embryo. The first route is via entry of virus-contaminated spermatozoa into the oocyte during fertilization, which depends mostly on the virus strain and the concentration of virus particles in the insemination dose. The second route is through contact with virus-contaminated follicular fluid, oviductal and/or uterine tissues.[2,7] There are a considerable information regarding the effect of BoHV-1 on breeding by both artificial insemination and natural service.[2,5,7,8] BoHV-1 infection can increase the frequency of sperm abnormalities such as sperm head decondensation. The pioneering work of Bielanski and Dubuc demonstrated that oocytes recovered from BoHV-1-infected cows could mature and be fertilized *in vitro*, thus yielding transferable embryos.[9] However, the proportion of morphologically normal transferable blastocysts was reduced. Subsequent studies have reported that BoHV-1 exerts negative effects on the *in vitro* embryo production system.[5,7,8] Different strains of BoHV-1, including a live attenuated vaccine, can remain in a latent state in clinically healthy bulls, only to be reactivated and excreted in their semen, thereby promoting transmission of infection to seronegative cattle. This requires restrictions on the use of semen from potentially infected bulls to produce embryos.[2] Oliveira et al. showed that both fresh and frozen semen samples can contain infectious virus.[3] The fact that cattle, sheep and goat embryos can all be successfully frozen or vitrified and stored for long periods has enabled the exchange of genetic material between and across countries and continents.[10] As embryo transfer has become an expanding business enterprise in recent years, questions have arisen concerning the risk of virus transmission through embryos and the epidemiological complications of this assisted reproduction technique.[11]

Virus entry into oocytes and embryos

Mammalian oocytes and pre-implantation stage embryos are

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surrounded by an outer layer of granulosa cells, the cumulus oophorus, and an inner zona pellucida (ZP).[12] This glycoprotein extracellular matrix plays an important role in many physiological functions, including fertilization, blocking polyspermy, facilitating the passage of an embryo through the oviduct, containment of blastomeres and protecting the embryo during early stages of development.[11,12] The ZP is an effective barrier against penetration of most potential pathogens into the ovum during fertilization.[13] However, the risk remains that some viruses and bacteria can bind strongly to the outer layers of the ZP.[11,13] Queiroz-Castro et al. showed that an intact ZP was not able to prevent the entry of BoHV-1 into the bovine oocyte.[4] It is worth emphasizing that oocytes containing BoHV-1 adhered to the ZP also allow for possible infection of the oviduct epithelial cells by the virus, due to its epitheliotropic nature, leading to cell lysis and thus poor embryo development.[5] The observed adherence of BoHV-1 to the ZP after the maturation period may enable its penetration into the oocyte at the moment of fertilization.[5] In addition, the virus may infect the recipient cow if a contaminated embryo is transferred.

Methods for virus removal from embryos

The ability of BoHV-1 to adhere to the ZP has been reported, even after taking the precautionary measure of trypsin washes that is recommended by the International Embryo Technology Society.[14,15] At least under experimental conditions, this procedure provides a means to remove BoHV-1 from the surface of embryos. However, the effectiveness of trypsin washing for decontamination of *in vitro*-produced bovine embryos is controversial.[16] In support of this contention, while several studies demonstrated that trypsin treatment is very effective for the removal or inactivation of BoHV-1,[7,10,17] other investigators found that the inclusion of trypsin solution in the washing procedure may be sufficient to strip BoHV-1 from the surface of the embryo but has no such effect on virus particles once inside.[14,18,19] Bielanski and Lalonde demonstrated that embryo cryopreservation may be regarded as a safety measure to decrease the potential risk or even mitigate disease transmission by embryo transfer.[20] Nevertheless, it is considered highly unlikely that either slow freezing or vitrification as a stand-alone precaution could eliminate all infectious BoHV-1 particles attached to the ZP of an embryo.[20]

Methods for virus removal from semen

Concurrently reported studies at the start of this century concluded that centrifugation on a density gradient followed by a 'swim-up' procedure removes pathogens such as hepatitis C and human immunodeficiency type 1 viruses from human semen and recovers a highly functional motile sperm population for *in vitro* fertilization.[21–23] Similar effects were observed using this double processing method with porcine circovirus type 2 from the Swedish Yorkshire pig breed and with equine arteritis virus from stallion semen.[12,24] However, at present it is not known whether the technique would be suitable for all viruses or for only those of a certain size/density. Furthermore, the use of density gradient centrifugation is limited to small volumes of semen or for preparing sperm for the application of assisted reproduction techniques. It is therefore impractical for processing, for instance, the large volume of boar ejaculate for artificial insemination.[12] A focus of future investigation should be the use of a sperm processing technique for the elimination of infectious BoHV-1 in bovine semen that do not show clinical signs of viral infection and, therefore, are asymptomatic for BoHV-1 infection. The double processing method, consisting of single layer centrifugation followed by a swim-up, is a priority for further study.

Conclusion

BoHV-1 infection of cattle has a significant impact on livestock production. Hence, there is a pressing need to restrict to a minimum virus contamination of sperm and oocytes for fertilization by natural and artificial means. The collective evidence indicates the incomplete effectiveness of either trypsin washing or cryopreservation to eliminate BoHV-1 and hence alternative methods need to be considered. It is recommended that the use of a sperm processing technique, such as the double step method of single layer centrifugation followed by a swim-up, be investigated for the removal of infectious BoHV-1 in bovine semen.

Authors' contributions

FS and AWTR jointly conceived the concept of the paper and researched available literature. FS wrote the first draft. AWTR provided supervision and critically revised various versions. Both authors contributed to preparation of the final version and agreed to its submission.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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