Infectious Bovine Rhinotracheitis (IBR) Virus in Bovine Semen: Current Concepts

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The realization that infectious bovine rhinotracheitis (IBR) (15,20,28,30) or other viruses (3) can occasionally be found in bovine semen has raised questions about possible effects on breeding programs (11), about the feasibility of testing semen for viruses, about recommending IBR vaccines for bulls and about IBR control procedures for bull studs (23). Because uncertainty prevails, confusing and conflicting recommendations have appeared. Some purchasers of purebred bulls and semen have imposed unrealistic requirements thereby excluding valuable genetic material from breeding programs. This paper presents current thoughts derived from several years experience investigating IBR virus in semen.

Contamination of bovine semen with IBR virus is most likely when bulls have clinical IBR infection of the preputial mucosa (2). (Infectious bovine balanoposthitis [IPB]) (29). It may also result from reactivation of latent infections which persist for long periods (27) in clinically normal bulls (2,6,24,25). If contaminated semen is used in artificial insemination a number of possibilities exist, the recipient cow may not be become infected (23), or if infection occurs, the outcome can vary from inapparent infection to clinical signs of infectious pustular vulvovaginitis (IPV) (12,15), endometritis (12,13,15), salpingitis, infertility (15,17,30), and shortened estrous cycles (12,15). The outcome of exposure is probably determined multifactorially by the biotype of the viral contaminant, the dosage of infective virus present in the semen and by the immunologic status of the inseminated female (23). Each of these determinants deserves discussion.

The agents causing IBR, IPV and IBP are usually regarded as IBR virus. They are indistinguishable by most virologic and serologic tests. However, all IBR strains are not identical in biologic characteristics (8,9,14). Vaccine strains are manipulated to alter their virulence and field strains probably vary in their capacity for infecting and causing disease in various tissues. Some strains replicate most efficiently in reproductive mucosa (1) while others have predilection for respiratory, ocular or nervous (7) tissues. Strains with variant biologic properties are usually referred to as *biotypes*. Most are immunologically similar but subtle differences between biotypes can sometimes be demonstrated by tedious techniques involving neutralization kinetics (16).

Like most infections, the outcome of intrauterine or intravaginal exposure to IBR is also affected by the *dose of free infective virus* present in the inoculum. Most descriptions of serious clinical disease following insemination with IBR contaminated semen have been associated with high viral dosage (usually greater than 10,000 tissue culture infective doses [$TCID_{50}$]) (12,15). Conversely, studies involving low dosages (around 100 $TCID_{50}$) have resulted in no infection or mild inapparent infection manifested only by seroconversion (23).

When exposure occurs, the biotype and dosage of IBR virus in semen determine the severity of the challenge and immunologic factors largely determine the resistance of the inseminated cow.

Both humoral and cell mediated immune systems respond to IBR infection (5,17,18,19). Indications are that previously exposed or vaccinated cattle with appropriate immunity can resist interuterine exposure. However, serum antibody alone may not confer protection (23) particularly if the exposure constitutes high doses of virulent biotypes possessing strong affinity for reproductive mucosa.

In addition to reproductive disorders, there is concern that insemination with IBR contaminated semen could introduce IBR virus into hitherto uninfected herds, establishing latent infections which upon later reactivation, could spread to susceptible cattle causing clinical disease or abortion (11,23,26).

The overall impact of these possible consequences is unknown. Very few problems have been conclusively associated with IBR virus in semen. Nevertheless, the artificial insemination industry is understandably concerned and seeks guidance from veterinarians who are expected to be up-to-date on the matter.

Efforts to detect IBR virus in semen have been most successful in situations where very large quantities of virus are present. These situations include experimental reactivation of latent infection with massive

steroid treatment (24,25) and some naturally occurring cases (15,27). Isolation is based on inoculation of suspect semen into cell cultures with subsequent daily examination for cytopathic effects (CPE) characteristic of IBR (26). This procedure, sometimes known as the "Cornell Semen Test" (23,26), is complicated by the fact that when undiluted semen is placed in cell cultures, a cytotoxicity occurs (11) which makes the test unreadable. This toxicity can be eliminated by dilution (as occurs in extending semen) or by addition of 5 to 10 parts of a trypsin inhibiting substance to one part of raw semen (11,26). Both procedures dilute any virus present, reducing the viral concentration and sometimes lowering the dose below the detectable threshold so false negative test results may be obtained. Some workers suggest tedious repeated passage may be needed (21,27). In addition, some semen specimens may contain anti-IBR substances (2,4) which make virus identification difficult. It must be appreciated that any factors reducing the chance of detecting the virus in cell cultures probably also reduce the likelihood that the semen would successfully infect cattle.

To date, in examining semen for export and in routine surveillance programs, thousands of semen specimens (11,23) from normal healthy bulls (many of which were IBR seropositive) have been examined in the USA without detection of IBR virus.* As a check on the correlation between the results of the "Cornell Semen Test" and the ability of semen to infect cattle, semen that was negative to this test has been inoculated intranasally and intravenously into susceptible (seronegative) heifers without producing disease or seroconversion (23). In addition, specimens containing quantities of virus adequate to be detected by the "Cornell Semen Test" have produced seroconversion and sometimes IPV, endometritis and altered estrus periods (12,15,23). The tentative conclusion of these studies is that IBR virus in concentrations adequate to cause clinical manifestations or interfere with fertility rarely appears in semen from normal healthy bulls and commercial semen from studs with a sound herd-health program represents a minimal hazard of transmitting of IBR. It is not known how frequently undetectable amounts of IBR virus are present in semen.

The question of whether to vaccinate bulls or male calves reared for breeding purposes is a difficult one. The three forms of IBR vaccine available appear to have different effects on possible IBR contamination of semen. The *inactivated products* may limit the severity of clinical disease but they do not seem to prevent vaccinated bulls from becoming latent carriers of field strains if exposed after vaccination (23,25,26). The *modified live virus* (MLV) vaccines that are prepared for intramuscular inoculation can produce latent vaccine virus infections which can subsequently be reactivated with shedding of the abortifacient vaccine strain. The intranasally administered vaccines are apparently prepared from less virulent seed stocks and preputial shedding of vaccine virus is highly unlikely in the period immediately following vaccination or as a result of later reactivation of latent infections (23). Intranasally vaccinated bulls appear sufficiently protected to resist preputial infection after virulent challenge 4-6 months after vaccination (23).

Many purchasers of purebred bulls are semen discriminate against IBR seropositive bulls so we recommend not vaccinating purebred bulls on the farm. On a population basis, the impact of leaving a few bulls unvaccinated is probably minimal.

The vaccination decision for artificial insemination units is another issue. Some bull studs endeavor to keep the population free of IBR by serologic testing and quarantine measures (20). These units usually will not purchase seropositive bulls because of the potential of latent infection. Because IBR virus is virtually ubiquitious, maintenance of seronegative studs is nearly impossible. Furthermore, some seronegative bulls can shed virus (6,10,22,23). Exclusion of bulls from studs because of IBR serotiters constitutes a serious loss of valuable genetic material that cannot be justified by the minimal hazard associated with the transmission of the virus through semen. Some insemination units conduct periodic intranasal vaccination of all bulls in an effort to maintain high titers as protection against introduction of field strains of virus and development of latently infected carriers of virulent virus (23). Thus, in bull studs, the vaccination decision must be based upon the serologic status of the population, the requirement of foreign importers of semen and the disease control philosophy of the management and the veterinary consultants.

CONCLUSION

Current knowledge indicates there is little likelihood of IBR virus in concentration adequate to cause clinical manifestations or interfere with fertility being present in semen unless the semen is collected from bulls with clinical IBR or IBP infection or the bull has recently received prolonged corticosteroid therapy. Semen collected from such bulls should not be used. Commercially processed semen from a stud with a rigorous veterinary-supervised herd health program has very low probability of being the source of IBR infections.

In those instances where massive IBR contamination of semen has occurred, the effects on fertility were readily evident. Because of the discrimination against seropositive bulls, it is recommended that male cattle not be vaccinated on farms. This decision enables purchasers to make the vaccination decision for themselves.

Seronegativity *per se* is not an adequate indicator of freedom from IBR virus in semen. Currently available methods for detecting IBR virus in

*During discussion of a paper on this topic at the 1976 Meeting of the American Association of Veterinary Laboratory Diagnosticians in Miami, FL, (11) workers from several laboratories reported never finding IBR in a commercially processed extended semen examined for export purposes.

raw or extended semen are imperfect but are adequate for detecting high concentrations of IBR virus in semen.

The method of IBR control in bull studs is dependent upon the serologic status of the stud and the disease control philosophy of its managers. Efforts to keep the stud free of IBR virus or frequent repeated intranasal vaccination of all bulls are the alternatives.

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Practice Methods

Surgical Treatment of Necrotic Laryngitis (Calf Diptheria)

Following limited success in treating necrotic laryngitis with broad spectrum antibiotics (Blood & Henderson, 1974), I have found that by doing a tracheotomy using a 12-14 mm plastic disposable tracheotomy tube, a far better and more rapid recovery can be expected. The procedure is carried out with the calf in dorsal recumbency and using local anaesthesia. It appears that complete removal of a segment of a cartilage ring does not subsequently affect the animal. After placement of the tube, the skin is closed (No. 3 Vetafil) either side of the tube. The tube is left in place for 3-4 days, but is removed daily for cleaning. The animal is treated with Trimethoprim IM at the rate of 1 ml/15 kg for four days. After removal of the tube, the wound is allowed to close and heal by itself. Skin sutures are removed 7-10 days later. Some superficial infection of the wound occurs but does not appear to be any real problem. I have treated 12 animals using this technique and in only one case where the condition had pre-existed for one month and had been treated medically with no response was the response not rapid and entirely satisfactory.

Reference: Blood & Henderson (1974) - 4th Ed. Bailliere Tindall - Page 433.

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