HYGIENIC PRODUCTION OF BOVINE SEMEN

Rajendra Singh and RS Chauhan
Centre for Animal Disease Research and Diagnosis
Indian Veterinary Research Institute, Izatnagar–243 122, Bareilly (UP)

Introduction

The germplasm including semen, embryo and/or ova is an essential commodity for continuance of species. The mammoth bovine population (272.5 million cattle and buffaloes; either pure, indigenous/graded or crosses) in India is the result of natural selection and/or breeding policies implemented by the Veterinary Authorities. Further with the introduction of Frozen Semen for artificial insemination in 1975, after replacing the liquid semen, the country has achieved greater success in animal production at the same time preserving desirable traits in animals; with the result today the country ranks first in milk production in the world. Today all states in the country are flooded with Deep Frozen Semen Stations maintaining exotic, crossbred and indigenous breeding bulls for collection of semen.

After proper processing, the semen is stored and distributed through vast network of Frozen Semen Banks and supply of liquid nitrogen and frozen semen straws to the Artificial Insemination Centers. Many private firms and NGOs are also actively involved in such activities. With such an intricate network of germplasm collection and related activities in the country, the dangers of infectious agents being transmitted between animals via the venereal route or by the use of infected semen in artificial insemination are likely to occur if hygienic production of semen is not adhered as per the laid down norms (OIE, 2004). The infectious diseases, which are known to be transmitted through bovine semen by AI, have been classified as category 1, with moderate to high risk of transmission (FMD, IBR, BVD/MD, Campylobacteriosis, Trichomonas, Mycoplasmosis and E. coli, Pseudomonas spp.), category 2 with low risk of transmission (Bluetongue, Enzootic bovine leucosis, bovine ephemeral fever, leptospirosis) and category 3, with little or no information regarding the risk of transmission (bovine immunodeficiency virus, bovine paratuberculosis, contagious bovine pleuropneumonia). The risk of BSE and scrapie transmission by semen has been found negligible if procedures for collection, processing and storage semen are followed strictly.

Semen and its contamination

The bovine semen consists of 10% v/v spermatozoa and the seminal plasma. The spermatozoa right from their site of production in testicular compartments to the site of final destination in oviduct undergo tortuous journey and meet with different kinds of secretions including tubular and accessory sex glands secretions that favour their survival within and outside the genitalia. Unfortunately during this long journey, the semen gets infected with various kinds of pathogens, which are present in different locations in the genitalia of breeding bulls/females or gets contaminated from external sources, if not hygienically collected, processed, stored and distributed. With the expanding global trade, the possibility of various potential pathogens being transmitted between animals via the venereal route or by the use of semen cannot be ruled out in various farm animals, particularly in cattle. The sexual transmission of viruses, bacteria, mycoplasma, ureaplasma and other potential
pathogens may at times cause epidemics, that may be spread further worldwide by export of the gametes and embryos of these animals. Further, the diseases in which clinical signs are rarely evident such as IBR/IPV, BVD/MD and BT, but the detection of the same in semen is of great importance. Under most circumstances, the semen is free of bacterial pathogens. However, bacterial contamination with *E. coli, Pseudomonas* can occur prior to semen deposition into the cervix and uterus as several steps are involved in collection, processing and storage of the semen. These pathogens and their endotoxins can affect the quality and quantity of the viable sperms. The antibiotics used in semen extenders though check the growth of the pathogens but get ineffective in long usage if not checked periodically for their minimum inhibitory concentration for determination of antibacterial sensitivity. Thus infected semen not only reduces the overall success of AI but also transmits the disease in the population and affects the trade.

**Effects of semen contamination**

The presence of various potential pathogens in bovine reproductive tract and semen not only affect the semen quality and spread of the disease, but also have various other functional consequences within infected organs leading to reproductive disorders like infertility/sterility. These disorders may occur due to changes in testicular compartments (Germ cells, Sertoli cells, Leydig cells), orchitis, azoospermia and testicular carcinoma, sperm abnormalities, infiltration of leukocytes into the reproductive tract or semen, cachexia by drop in testosterone production, incorporation of the viral genome into germ cell genome, infection of ova and embryo, miscarriage and embryonic and foetal abnormalities. Because of the effective blood-testis barrier, the testis acts as a reservoir of viruses protected against antiviral antibodies and, therefore, plays an important role in the context of viral infection and STD’s. A number of agents are detected in semen associated with cells (spermatozoa, macrophages/mononuclear cells) or in seminal plasma. Semen contamination with pathogens, particularly viruses, is very common in bulls and since frozen semen is widely distributed. It is essential to follow the existing guidelines for establishing disease free bull studs producing semen free from potential pathogens.

**Hygienic semen production**

The hygienic production of semen is only possible if the procedures at all levels are strictly followed with no sloppy short cuts (OIE, 2004). Hygienic semen from bulls is derived when these are in perfect health and properly cared with negative results of STDs testing and fulfill minimum health requirements; and are well separated in properly maintained semen collection facilities by committed personals. The artificial insemination centers including bull accommodation, semen lab, storage area, administration officials along with distantly located quarantine area, approved by Veterinary Authorities, should be under supervision of veterinary officer. The facilities should be cleaned and disinfected properly everyday. The semen laboratory with separate area for AI, cleaning and preparation of artificial vagina, semen evaluation and processing, semen pre-storage and storage should be looked and handled by technically competent personals who himself care for personal hygiene and follow good laboratory practices. The artificial vagina preparation, and other equipments should be properly cleaned and sterilized. Particular attention should be focused on proper cleaning of water baths as these provide good source for bacterial contamination. Each day working area should be effectively cleaned and disinfected. Prior to making semen straws, the qualities and
quantities of semen are evaluated for sperm motility, sperm count, normal and abnormal percentage and bacterial counts. The following organisms can be used as marker for contaminant: *Actinomyces pyogenes bovis*, *Staphylococcus aureus*, *Streptococci* (Lancefield groups A and D), *Escherichia coli* and *Pseudomonas aeruginosa*. Bacterial counts (CFU/ml) carried out on the processed semen give a useful indication of the hygienic standard of the semen laboratory. The count should not exceed $5 \times 10^3$ per ml. The antibiotics used in the semen extenders should be regularly looked for minimum inhibition concentration (MIC) for determination of antibacterial sensitivity.

So, overall success of the AI programme rests on the hygienic production of the semen. The purpose of hygiene is to maintain health of animals on AI Centers at the level, which permits the international distribution of semen having negligible risk of infecting other animals and humans with specific pathogenic organisms that can be transmitted by semen (OIE, 2004). Keeping the whole scenario in mind, recently a brainstorming session was organized by the CADRAD at IVRI and the status of sexually transmissible diseases was discussed. On this occasion a Handbook of TB, JD and STDs in bovines was released for the users. This Centre as Central Disease Diagnostic Laboratory with its 5 wings located in five regional laboratories (RDDLs) is doing regular screening of sexually transmitted diseases in cattle and buffaloes. The screening of blood/ serum, preputial washings and tuberculin testing is being carried out by OIE recommended tests, which are performed according to the specifications in the Terrestrial Manual in order to avoid any differences between the exporting and importing countries or within the country in the interpretation of the results. In the context of our country, only diseases including TB, JD and STDs out of 14 have been included for regular monitoring in the bovine population. The Center has all infrastructure facilities and technical know-how to test the bulls, teaser animals and females during pre-quarantine, quarantine and in other facilities. The animals found positive for TB, JD and STDs are segregated as per the recommendations emerged out in brain storming session.

**Table 1. Prescribed diagnostic tests of TB, JD and STDs in animals**

<table>
<thead>
<tr>
<th><strong>Terrestrial Code chapter No.</strong></th>
<th><strong>Terrestrial Manual chapter No.</strong></th>
<th>Disease Name</th>
<th>Prescribed tests</th>
<th>Alternative tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3.1.</td>
<td>2.3.1.</td>
<td>Bovine brucellosis</td>
<td>BBAT, CF, ELISA, FPA</td>
<td>–</td>
</tr>
<tr>
<td>2.3.2.</td>
<td>2.3.2.</td>
<td>Bovine genital campylobacteriosis</td>
<td>Agent id.</td>
<td>–</td>
</tr>
<tr>
<td>2.3.3.</td>
<td>2.3.3.</td>
<td>Bovine tuberculosis</td>
<td>Tuberculin test</td>
<td>–</td>
</tr>
<tr>
<td>2.3.5.</td>
<td>2.3.5.</td>
<td>Infectious bovine rhinotracheitis/ infectious pustular vullovaginitis</td>
<td>VN, ELISA, Agent id. (Semen only)</td>
<td>–</td>
</tr>
<tr>
<td>2.3.6.</td>
<td>2.3.6.</td>
<td>Trichomonosis</td>
<td>Agent id.</td>
<td>Mucus agg.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JD</td>
<td>Johnin test</td>
<td>–</td>
</tr>
</tbody>
</table>

Precautions

- Use strict hygienic measures in all stages of semen collection, processing and insemination. Never become careless with hygiene.
- Get the bulls/dams tested for STDs before introducing to AI Stations.
- Avoid bacterial contamination.
- Evaluate the efficacy of antibiotics used in the semen extenders.
- The animals tested positive for TB, JD and STDs should be excluded from the semen collection centers.
- Entry should be restricted in semen collection Centers.

Suggested Reading