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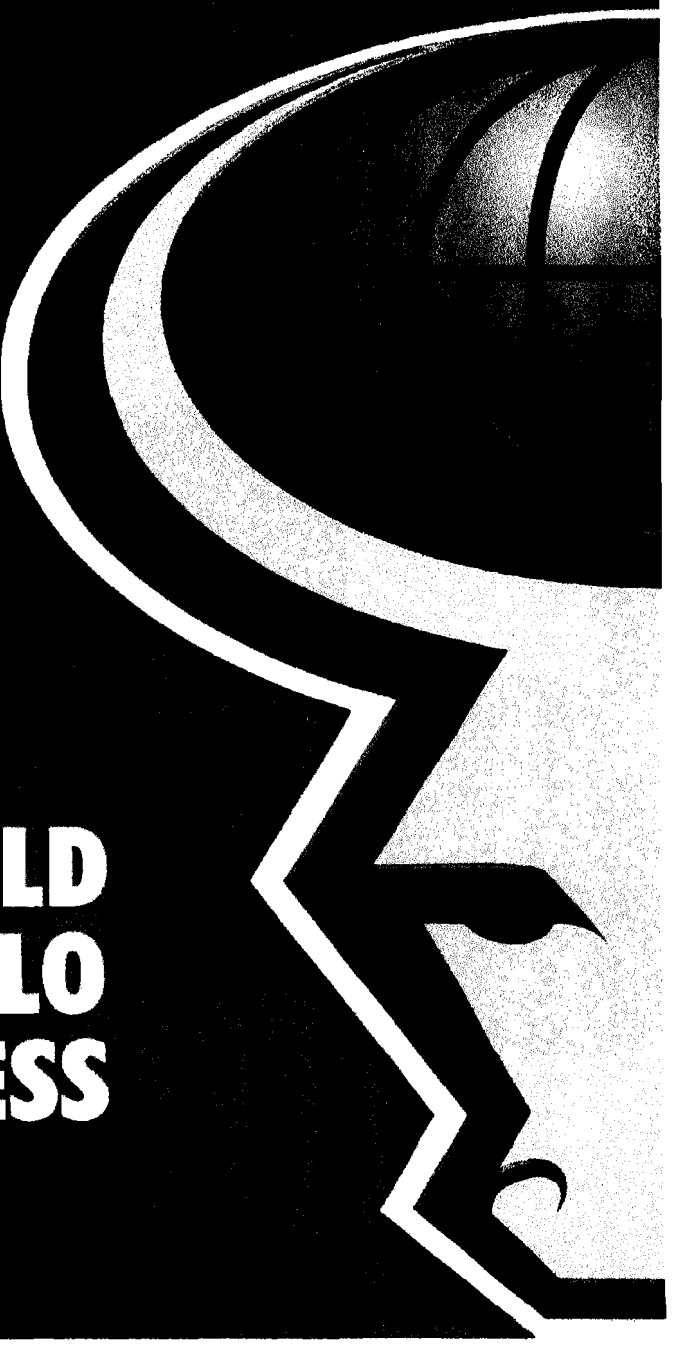

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SANITARY RISK OF ARTIFICIAL INSEMINATION AND EMBRYO TRANSFER IN BUFFALOES

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ABSTRACT
Commonly by "bio-technologies" we mean: "programmed used of biological systems and processes to improve production". In the last years, thanks to the advantages they bring to the production development, biotechnologies have been applied in the buffalo sector too. In fact, artificial insemination, embryo transfer, superovulation, in vitro fertilization, cloning, transgenesis, have became words and techniques commonly used in the buffalo sector.
The advantages that may be achieved from the application of new animal breeding technologies are: a)genetic improvement; b) sanitary control; c) reproductive control. Among the few disadvantages caused by these techniques, connected with the first remarkable economical investments and to their successive management, the risk of transmitting specific infective pathologies, has to be included. The adoption of inappropriate manual procedures may transform these techniques from pathology controllers into dangerous disease spreaders.
In this brief review the main infective diseases due to the use of artificial insemination and embryo transfer will be listed and commented in order to provide a useful tool to all the technicians that have to be informed about the possible dangers, analyse the risks and single out the weak points to control and prevent the transmission of pathogenic agents.

ARTIFICIAL INSEMINATION

Potential risks

The organisms present in the semen may be viruses, bacteria, and fungi and generally they are classified as pathogenic, potentially pathogenic, and not pathogenic. Their presence in the semen may be due to do nor bull's systemic or local specific infections; it may also come from the normal prepuce flora or from the contamination that follows the semen collection because of inappropriate manual procedures or contaminated equipment. Many of these microorganisms may survive during the storing carried out at low temperatures and they may represent a real danger for the spread of diseases if we consider that the semen is inserted directly into the uterus without undergoing the bactericidal action of the vaginal and cervical secretions produced during oestrus.

Bubaline Herpes Virus 1 (BuHV-1) and Bovine Herpes Virus 1 (BoHV-1)

Even if the pathogenic role of the BuHV-1 and BoHV-1 have not been clarified yet, the isolations and the serologic results lead us to believe that this infections are particularly spread among buffaloes. In all probability, the viruses are eliminated through the bulls semen both during the acute phase of the disease and during latent infections in clinically normal animals. The excretion may occur even in lack of antibody answer, therefore only seronegative and viruses-free bulls have to be used for artificial insemination. The diagnosis is carried out through Elisa, serum neutralization test, viral isolation or direct demonstration of the viral DNA through PCR (Polymerase Chain Reaction).

Bovine Viral Diarrhoea Virus (BVDV)

Although cattle are the primary hosts, the BVDV can infect most even-toed ungulates. Interspecies spread of this virus has been demonstrated but is epidemiological significance is uncertain. There are too few data to clarify the pathogenic role of BVDV in buffalo. Further research could shed light on the role of this virus and make it possible to determine pathogenesis, clinical characteristics and lesions.

Brucella

Buffaloes are susceptible to infection with *Brucella abortus* and *Brucella melitensis*. *B. melitensis* biovar 3 and B. *abortus* biovars 1 and 6 predominates in Italian buffalo herds. *Brucella* infection in bulls may affect the testicles, the epididymis, the seminal vesicle, and the ampulla. The infected males eliminate the *Brucella* spp. with the semen, so they play an active role in sthe spread of the disease. On this regard, the artificial insemination represents a good tool to control this pathology on condition that the donors are carefully monitored. Since the infected bulls may result serologically negative bacteriological tests are necessary. They have to be applied to the

semen and carried out in successive stages. The seminal plasma has to undergo the serum-agglutination test. National eradication schemes are based on the detection and slaughter of infected buffaloes. Two vaccines have been important in the control of brucellosis: attenuated vaccine Buck 19 strain and RB51 vaccine a rifampinresistant mutant of *B. abortus* which induces good protection and does not result in serological responses detectable in tests used in brucellosis surveillance programmes. Preliminary experiments suggest that RB51 can be effective for the profilaxis of *B. abortus* infection in buffalo. In contrast this vaccine seems does not confer protection against B. *melitensis*.

Arcanobacterium

Arcanobacterium pyogenes is commonly present on the nasopharyngeal mucosa of buffalo, in the bulls the usual habitat is the preputial mucosa. *Arcanobacterium pyogenes* is a common cause of suppurative lesions in buffalo. This bacterium has been associated with mastitis, metritis, pyometra and abortion in buffalo cows. In bulls *Arcanobacterium pyogenes* is an important cause of orchitis, epididymitis or seminal vesiculitis, so the organism can be eliminated with the semen. Specimens suitable for diagnostic laboratory procedures include exudates, aspirates, tissue samples and semen. *Arcabacterium pyogenes* is a Grampositive pleomorphic rod; produces a characteristic haemolytic pin-point colonies 48 hours of incubation.

Campylobacter

The infection of buffaloes with *Campylobacter foetus* may be widespread. The disease causes abortion. The use of communal bulls and the use of males that have not tested for *Campylobacter foetus* at artificial insemination centres are important factors in spreading infection. The disease has been recorded in India, Malaysia and former U.S.S.R The animals used for the artificial insemination have to undergo a quarantine, and result negative to three consecutive cultural tests carried out on preputial scraping. Afterwards their sanitary conditions have to be checked every six months.

Leptospira

Many serologic researches demonstrate that the buffalo population has antibodies against several *Leptospira* spp.. Since these micro-organisms cause hypo fertility and abortion and survive in the frozen semen, particular attention has to be paid to the bulls involved in the artificial insemination. The seminal vesicles of the bull are considered to be a major site for the localization of *Leptospira interrogans* serovar *hardjo*.The isolation of *Leptospira spp.* from the semen is not easy; therefore the microagglutination test has to be used even if it does not allow to distinguish the vaccinated animals from the infected ones. Because of these problems bulls should not be vaccinated.

Chlamydophila

The *Chlamydophila abortus* infection may cause abortion and hypo-fertility. The micro-organism is eliminated through the semen of sick bulls that appear clinically normal, even if, sometimes, their semen has a large number of leukocites and a low concentration of sperm with poor motility and high percentage of sperm cell abnormalities. To isolate or demonstrate *Chlamydophila abortus* from the semen, preputial or urethral swabs, Elisa, PCR, embryonated eggs or culture tissue are the techniques generally used.

Mycobacterium

Mycobacterium bovis can be responsible of orchitis in buffalo male. Therefore the buffalo donors' semen has to be tested before using it and then once a year. The tests applied are the single intradermal test and, if necessary, the comparative intradermal test. Blood based assays which have been developed for use in conjunction with the tuberculin test include Gamma interferon test, Elisa and PCR

Trichomonas

Trichomoniasis is caused by *Trichomonas foetus*, piriform protozoan, that causes premature abortions, pyometra and infertility.

This flagellate is transmitted to the female buffalo during her mate with an infected bull and vice versa. *Trichomonas foetus* infection of the genital tract of buffaloes was recorded only in India and Egypt. This suggest that the buffalo is an unusual host for this parasite and is not generally susceptible to infection. The infected animals may be carriers for their whole life. The parasite resists in the diluited semen and to freezing; therefore the donors have to be tested frequently either through a microscopic examination or through a cultural test in order to excluded any infection.

Other preventive measures

The adoption of appropriate preventive measures does not concern only the control of the above illustrated bacteria, but it regards also the right semen collecting technique to reduce the contaminating flora represented by *Listeria monocytogenes, Pseudomonas aeruginosa, Bacillus licheniformis, Streptococcus* spp., *Staphilococcus* spp., *E. coli, Proteus* spp., *Aeromonas* spp., yeast and moulds. Since the highest amount of the bacteria present in the semen comes from the prepuce, from the skin and from the equipment used, the semen collecting procedures have to be carried out using sterilised tools and materials, and cleaning constantly the bulls preputial cavities. In spite of these preventive measures, sometimes the ejaculate presents a not specific microbial flora = 5 x IO6 CFU/ml. These concentrations may be reduced administrating antibiotics, diluting the semen, that lowers the number of viruses and bacteria of about 100 times, and freezing until the following count is obtained: 8 x 102 CFU/ml (considered normal).

EMBRYO TRANSFER

Potential risks

The transfer or embryo transplantation consists in the removal of an embryo in the beginning stadium of its development and in its introduction in the uterus of a receiver where the foetal development and later the delivery will take place. Embryo-transfer is a biotechnology that has met a great success in the bovine sector and even if there are still some problems that have to be resolved it seems to have a great potential in the buffalo sector too.

The application of this technology may cause the transmission of pathogen agents. This happens when the micro-organism is in the embryo or on its surface during the transplantation or in the fluids transferred with the embryo.

Guidelines for the safe international movement of livestock embryos are given in the *International Animal Health Code of the Office International des Epizooties.*

The transmission of the pathogens to the embryo may occur in three different ways:

1) Through infected gametes

In this case the microorganism has to be in the ovum or in the spermatozoon or adhere to its surface and be able to replicate in the embryo, in the foetus or in the animal after birth;

2) Uterine contamination

Infective agents on the uterus, due to oviduct or uterus infections, caused by seminal fluids may adhere or get over the glycoproteic membrane (zona pellucida) that surrounds the ovum and the embryo in the be ginning phases of its development, or infect the embryo cells directly when the embryo has already lost its zona pellucida.

3) By contamination of the external environment

The pathogen agents may come in contact with the ovum or the embryo during the manual procedures to which they are subjected out of the maternal environment.

Bubaline Herpes Virus 1 (BuHV-1) and Bovine Herpes Virus 1 (BoHV-1)

It has been demonstrated that embryos without zona pellucida resulted infected and die after in vitro BoHV-1 exposure. This viruses does not kill the embryo if the zona pellucida is intact. Nevertheless BoHV-1 is able to adhere to the zona pellucida even if it is not able to go through it and to reproduce itself in the embryo cells. The washings to which the embryo undergoes do not remove the viral particles that adhere to the zona pellucida; therefore the embryos have to be treated with trypsin or with solutions containing specific antibodies.

Bovine Viral Diarrhoea Virus (BVDV)

The bovine viral diarrhoea virus is not able to penetrate or to adhere the zona pellucida. A potential danger may derive from the use of contaminated sera used to prepare embryo flushing and washing fluids.

Foot and Mouth Disease Viruses (FMDVs)

The risk is mainly connected to the importation of embryos from countries where the FMD is endemic. The current information demonstrates that the FMD viruses does not penetrate nor attach to the zona pellucida and they are not isolable from the embryos of infected donors. FMD viruses may be isolated again from 33% of the bovine embryos without zona pellucida and experimentally exposed to the infection. Therefore the zona pellucida has to result intact to offer the necessary guarantees and exclude the risk of transmission.

Brucella

Experimental studies have clarified that seropositive bovine donors do not transmit the disease through embryo-transfer neither to the receiver nor to their offspring. Moreover the freezing procedures for the preservation of the

embryo cause a 64% decrease of the *Brucella abortus* vitality. The administration of the appropriate antibiotics causes a 99% inactivation of the micro-organism.

Prophylaxis

In order to avoid the transmission of infective diseases through embryo-transfer, the sanitary conditions of the donator and of the embryo have to be checked. In the first case, the preventive strategy considers the fact that if the animals are not infected the embryos wont either, on the condition that the collecting phase is carried out respecting asepsis.

The prophylaxis based on the sanitary state of the embryo implies preventive diagnostic controls and the utilisation of sterilised tools necessary to avoid any contamination. The drawn embryos have to be watched through a microscope in order to be sure that the zona pellucida is intact and does not present adhered material. Then the embryos have to undergo serial washings and trypsin solution treatment. The flushing fluids, the washing fluids and the embryos not fit for transplantation should always be controlled to check it there are any pathogen agents. Another thing that has to be considered is the receiver's sanitary conditions. The receiver has to be healthy and therefore not affected with the above mentioned diseases that may compromise the embryo and the foetus.

CONCLUSIONS

From the data above illustrated it appears that diseases may be transmitted more easily through natural or instrumental insemination than through embryo-transfer. Since the embryo is drawn during the first days of its development, the possibilities to be infected within the mother's uterus are quite scanty. When the embryo is transferred into the receiver it is still enclosed in the zona pellucida that surrounds and protects it. Only some viruses, particularly the BoHV-1, are able to adhere and multiply themselves on the glycoprotein membrane of bovine embryo. As already said, the washings and the successive trypsin treatment have resulted efficacious in destroying the viral particles and in reducing the sanitary risks.

The development of the bio-technologies in the buffalo sector has to keep up with the progressive improvement of the semen and embryo genetic and sanitary quality. The sanitary guarantees imposed by national and international rules that regulate the biologic material market, will allow to use the new technologies even to carry out an efficacious preventive program against

several patho logies that otherwise, with the current natural reproduction systems, will be hard to control.

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