Infectious Causes of Infertility in Buffalo Bull (*Bubalus bubalis*)

P. Perumal[1*], J. K. Chamuah[1], N. Srivastava[2] , Kezhavituo Vupru[1] and S. K. Srivastava[2]

[1]National Research Center on Mithun, Jharnapani, Medziphema, Nagaland (797 106), India
[2]Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh (243 122), India

Article History

Manuscript No. 314
Received in 23rd May, 2012
Received in revised form 3rd August, 2012
Accepted in final form 4th March, 2013

Correspondence to

E-mail: perumalponraj@gmail.com

Keywords

Buffalo bull, infectious causes of infertility, artificial insemination

Abstract

The artificial insemination (AI) technique plays important roles in improvement of conception rate, prevention of sexually transmitted diseases and carryout of genetic material to next generation. The breeding soundness evaluation (BSE) plays an important step in selection of buffalo bull for the said above purpose. This evaluation is an effective, inexpensive and easy method for selection of breeding buffalo bull. In buffalo breeding management programme, the seasonal variation, nutrition, congenital defect, hormonal changes and hereditary plays a critical role in determining the reproductive efficiency in buffalo bull. The information regarding to the reproductive health of buffalo bull is meager for breeding soundness evaluation. The venereally transmitted infections cause early embryonic death and infertility or sterility in both male and female buffaloes. To avoid these infective conditions, proper and careful investigation of reproductive tract, evaluation of semen and treatment are essential.

1. Introduction

The buffaloes are in the order of *Artiodactyla*, the cloven-hooved mammals, genus *Bubalus* and species *bubalis*. Two main species of buffalo are found in the world: the Asiatic (water) buffalo (*Bubalus bubalis*) and the African buffalo (*Syncerus caffer*). The two buffalo types are have different habitat and chromosome numbers. There are about 170 million buffaloes in the world (Perera et al., 2005). Out of this, 97% of them are water buffaloes, mainly found in the Asian Region. Riverine buffaloes are characterized by black colour and have long curled horns (e.g. Murrah breed) and swamp buffaloes are dark grey, but may also be black, black and white, or even all white, have long, gently curved horns. Riverine buffaloes (70% of the total world population) are reared in high numbers in South Asia, especially in India and Pakistan. Swamp buffaloes are found in South-east Asia and Southern China, mainly Thailand, Philippines, Indonesia, Vietnam, Burma (Myanmar), Laos, Sri Lanka, Cambodia and Malaysia (Chantalakhana and Falvey, 1999). Riverine buffaloes are predominantly used for milk production and also used for meat, fuel, fertilizer production as well as for draught power, where as swamp buffaloes are traditionally kept as draught animals and are also (to a lesser recognition) used for meat production. Improvement of buffalo reproduction helps the production to enhance significantly the economy and living standards of many rural communities throughout the world. The purpose of this paper is to describe the different type of infectious disturbance affecting the reproductive performance of buffalo bull.

2. General View of Infectious Diseases in Buffalo Bulls

The infectious microorganisms affecting the fertility in two ways, such as direct effect on the reproductive system and the quality of semen and in another way it affects the other systems to prevent the semen production and the libido, mating ability and prolonged reaction time. Lagerlof (1934: cited in Rao, 1997) established the relationship between semen charactertics and testicular pathology. The condition of testis is clearly reflected in the type of semen produced. Semen charactertics and fertility have a close relationship. Bacterial contaminants of the semen lead to the production of number macrophages and polymorphonuclear granulocytes as the first line of defense against the bacteria in the semen and this in turn leads to the generation of reactive oxygen species (ROS) (Ochsendorf, 1998) and also production of more number of dead sperm will enhance ROS generation in semen (Aitken, 1995). Increased ROS generation impairs sperm functions and fertilizing capacity (Aitken, 1995; Griveau et al., 1995). The common disease conditions such

as balanitis, posthitis, seminal vesiculits, prostatitis, urethral inflammation, testicular degeneration, orchitis, epididymitis and ampulitis have resulted male infertility in buffaloes. Under practical conditions, it is not possible to produce semen free from microorganisms as contamination with few non- pathogenic organisms is unavoidable (Binda et al., 1994). The commonsaul micro-organisms infect the reproductive tract and affect the sperm motility and viability of sperm are *E. coli* (Schirren and Zander, 1966; Huwe et al., 1998), *Streptococcus faecalis* (Bisson and Czyglick, 1974) and *Streptococcus faecalis* (Makler et al., 1981; Huwe et al., 1998).

The common micro-organisms present in the semen are bacteria, viruses, protozoa, chlamydia, rickettsia and fungi and are generally classified as pathogenic, potentially pathogenic and not pathogenic (Gangadhar et al., 1986). Their presence in the semen is due to systemic or local specific infections of bulls and it may also come from the normal prepuce flora or from the contamination that follows the semen collection procedure 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 the semen is inserted directly into the uterus without undergoing the bactericidal action of the vaginal and cervical secretions produced during estrus. Mean microbiological load was increased by four fold in second ejaculate (Gangadhar et al., 1986), hence use same artificial vagina for two successive ejaculates is not expected under clean semen production. The bacterial contamination of semen is major concern for semen production laboratories as the pathogens adversely affect the semen quality (Diemer et al., 1996). Bacteria are contaminating approximately 50% of frozen semen doses (Wierzbowski et al., 1984). Moreover, the higher microbial load in the frozen semen of the buffalo bull (Shukla, 2005) ($4.860 \pm 0.73 \times 10^2$ ml^{-1}); Rathnamma et al. (1997) ($5.05\text{-}171.4 \times 10^3$ ml^{-1}); Jaisal et al. (2000) ($1.0\text{-}50.0 \times 10^2$ ml^{-1}) has a highly significant negative ($p<0.01$) correlation of standard plate count with progressive sperm motility (Shukla, 2005), live sperm (Shukla, 2005; Ahmed and Mohan, 2001) and HOS% (Shukla, 2005) both in neat as well as cryopreserved semen, whereas a positive correlation of sperm abnormalities with standard plate count was also recorded. The standard plate count (SPC) and type of micro organism vary among the different bulls (Shukla, 2005). The common microorganisms are isolated from buffalo bull semen are *Pseudomonas* sp., *Streptococcus* sp., *Staphylococcus* sp., *E. coli*, *Bacillu* sp., *Aeromonas* sp., and *Yeast* (Gangadhar et al., 1986; Ramaswamy et al., 2002) and sensitivity [chloramphenicol (100%), ciprofloxacin (100%) gentamicin (100%), neomycin (100%), streptomycin (86.62%), tobramycin (80%), co-trimoxazole (73.33%), erythromycin (53.33%), polymycin-B (13.33%), cephalexin (6.67%), nitrofurantoin (6.67%), tetracycline (6.67%)] and resistant [penicillin-G (100%), Oxytetracycline (100%), carbenicillin (100%), amoxicillin (100%), ampicillin (100%)] to microorganism have been reported for buffalo semen in fresh and frozen thawed semen (Ramaswamy et al., 2002; Singh et al., 1992). Balakrishnan et al. (2006) had isolated microorganism from frozen thawed buffalo semen were *Staphylococcus* sp. (56%), *Bacillus* sp. (45%), *Micrococcus* sp. (5%), *E. coli* (5%), *Klebsiella* sp. (3%), *Proteus* sp. (3%) and *Pseudomonas* sp. (10%). Balakrishnan et al. (2006) had reported that the sensitivity test for buffalo semen were perfloxacin (95.2%), ciprofloxacin (94.4%), gentamicin (93.60%), enrofloxacin (92.8%), penicillin (31.2%), streptomycin (16.8%), oxytetracycline (40.8%), ampicillin (34,8), amoxicillin (38.4%), chloramphenicol (36.8%) and triple sulpha (27.2%). Sharma et al. (1994) reported that addition of gentamicin sulphate (500 μg ml^{-1}), Chloramphenicol sodium succinate (500 μg ml^{-1}) or ampicillin sodium (500 μg ml^{-1}) in Tris egg yolk glycerol proved to be more beneficial rate than combination of streptomycin sulphate (500 μg ml^{-1}) and penicillin-G sodium (500 IU ml^{-1}) at every stage of cryopreservation.

3. Infectious Bovine Rhinotracheitis (IBRT)

The disease is caused by Bubaline Herpes Virus 1 (BuHV-1) and Bovine Herpes Virus 1 (BoHV-1) is member of the Alphaherpesvirinae. BHV-1 (Gibbs and Rweyemamu, 1977) infects the respiratory and genital tracts of cattle and buffaloes causing infectious bovine rhinotracheitis, infectious pustular vulvovaginitis and infectious pustular balanoposthitis, abortion, mastitis, infertility, tracheitis, conjunctivitis-keratoconjunctivitis, encephalitis and fatal disease in newborn calves (Gibbs and Rweyemamu, 1977) and is one pathogen responsible for causing significant losses in the livestock industry, through the failure of reproduction and increased mortality in cattle (Cortez et al., 2001; De Carlos et al., 2004). Phylogenically, this virus is closest to Bovine Herpes Virus-5 (BoHV-5), which causes encephalitis in cattle. On the basis of current knowledge, it cannot be claimed that BuHV-1, is responsible for abortion in buffalo cows. By contrast, BuHV-1 is suspected of being responsible for cross reactions with the IBR virus, when Bovine Herpes Virus-1 (BoHV-1) or its parts (glycoproteins) are used as an antigen in serological tests (Galiero, 2007). But the pathogenic role of the BuHV-1 and BoHV-1 has not been clarified yet and the isolations and the serologic tests results lead us to believe that this infection is particularly spread among buffaloes (De Carlos et al., 2004). This disease is transmitted through respiratory contact, ocular and reproductive secretions, with the latter route seemed to be the most important entry into herds (Afshar and Eaglesome, 1990). In all probability, the viruses are eliminated through the semen of bulls both during the acute

phase of the disease and during latent infections in clinically normal animals and bulls may shed virus in semen during both clinical and subclinical infections (Van Oirschot et al., 1993). Viral reactivation from the latent state is generally thought to be stress-induced but can also be induced by the injection of corticosteroids (Pastoret et al., 1982). The excretion may occur even in lack of antibody answer, therefore only sero-negative and viruses-free bulls have to be used for artificial insemination. There is breed differences for susceptibility of the disease has been reported that the Murrah breed (85.8%) is more susceptible (p=0.0004) than Mediterranean type (67.7%). Moreover, the age is also a major factor for susceptibility of this disease and progressively increasing rates of infection with advancing age (Ferreira et al., 2010). The diagnosis is carried out through ELISA, fluorescent antibody tests (FAT) of tissues, serum neutralization test, viral isolation or direct demonstration of the viral DNA through PCR (Polymerase Chain Reaction).

4. Foot and Mouth Disease (FMD)

FMD virus could be transmitted in bull semen before the infected bull shows any signs of disease. The virus carrier stage has been shown to persist in some cattle for many months following recovery from the disease or following prophylactic immunization. The disease has been shown to be transmissible when susceptible females were inseminated with semen from virus shedding bulls. It is found that FMD virus can survive in semen and freezing procedure in liquid nitrogen. Foot and mouth disease causes severe degeneration of germinal epithelium of testes of bulls. The semen picture was severely adversely affected. The motility was poor and the total sperm abnormalities ranging from 34-62%. Significant FMD virus type specific antibody titers (IgG1, IgG2 and IgA) were detected in milk and serum of female buffaloes and serum of male buffalo. FMD virus type specific IgG1 was found to be the predominant subclass as compared to IgG2 and IgA both in milk and serum of vaccinated buffaloes. Milk and serum IgG1, IgG2 and IgA antibody titres were positively correlated with values of regression coefficient (R) as 0.506, 0.434 and 0.396, respectively (Yadav et al., 2007).

5. Bovine Viral Diarrhea (BVD)

This disease is caused by bovine viral diarrhoea virus (BVDV) and the virus belongs to genous Pestivirus and family Togaviridae (Andrews et al., 1978). Although cattle are the primary hosts, the BVDV can infect most even-toed ungulates. The main mode of transmission is through the oral route, but also transmitted by inhalation, artificial insemination using semen of persistently infected bulls. Interspecies spread of this virus has been demonstrated but is epidemiological significance is uncertain. Seroepidemiological studies seem to indicate that these viruses circulate to some extent within the buffalo population in Italy (Galiero, 2007). Till date, however, there is no conclusive scientific proof that the same viruses isolated in cattle are not also present in the buffalo, nor that they act through the complex pathogenic mechanisms that have long been known to operate in cattle (Galiero, 2007). Analysis of the molecular characteristics of the two strains enabled to classify the isolates as BVDV-1, sub-genotype 1b. Evidence suggests that this virus is present within the buffalo population and is associated with abortion, brings to light a previously unknown health problem for the buffalo (Galiero, 2007).

6. Brucellosis

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 the spread of the disease (Eaglesome and Garcia, 1992). With regard to the brucellosis, it is a disease infectiocontagiosa that concerns to bovine and other domestic traditional and not traditional species as the buffalo, which is characterized by the production of abortions in the last third of gestation, retention of placenta, metritis, unfertility, birth of natimortos, mastitis, minor production and quality of milk and in male, arthritis, orchitis and epididymitis (Radostis et al., 2003). Moreover, the freezing procedures for the preservation of the embryo cause a 64% decrease of the *Brucella abortus* vitality (Martinez et al., 2007). The administration of the appropriate antibiotics causes a 99% inactivation of the micro-organism and the national eradication schemes are more beneficial to eradicate this organism based on the detection and slaughter of infected buffaloes.

7. Arcanobacteriosis

Arcanobacterium pyogenes is commonly present on the nasopharyngeal mucosa of buffalo and in the bulls the usual habitat is the preputial mucosa (Radostis et al., 2003). *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 *A*. *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. *A*. *pyogenes* is a gram positive pleomorphic rod, produces a characteristic hemolytic pin-point colony in 48 h of incubation. The diagnosis is based on bacteriological examination of the

organs. *A. pyogenes* develops in 24-48 h, forming hemolytic colonies on agar supplemented with 5% sheep erythrocytes. Biochemical tests yield definitive microbial identification (Galiero, 2007).

8. Campylobacteriosis

The infection of buffaloes with C*ampylobacter fetus* is widespread. *C. fetus* subsp. *venerealis* causes a type of venereal diseases in cow that transmitted through natural service or artificial insemination. The disease is characterized by infertility, prolonged estrous, premature embryo death and in some instance, untimely abortion with placental retention (Das and Paranjape, 1987). The use of communal bulls and the use of males that have not tested for *C. fetus* at artificial insemination centers are important factors in spreading infection. It has been reported that there are seven strains of *C. sputorum* sub-sp. *Bubulus* (Modulo et al., 1997) and *C. fetus*, *C. fetus* sub-sp. *venerealis* and *C. fetus* sub-sp. *fetus* (Joshi et al., 2006) have isolated from prepuce of buffalo bulls. The disease has been recorded in India, Malaysia and former USSR (Eaglesome and Garcia, 1992). The animals used for the artificial insemination have to undergo 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.

9. Leptospirosis

The micro-organisms belonging to this genus are mobile, helical bacteria, the terminal part of the bacterial body being hook-shaped. Although cytochemically gram-negative, they do not stain well with the conventional bacterial stains and are normally observed under the dark-field microscope. In the past, leptospires were sub-divided on the basis of serological reactions into two species: *L. interrogans* and *L. biflexa*. In nature, leptospires survive in ponds, puddles and wet earth. They can be hosted by animals and humans, causing diseases of the urinary and genital apparatus or serious systemic diseases. In the animal reservoir, the micro-organism is hosted in the renal tubules or genital tracts. The pathogenic role of *L. hardjo* has long been known in cattle, in which it causes abortion, stillbirth and agalactia. Serological studies and the sporadic isolations described in the literature seem to suggest that various serotypes of *Leptospira* spp. are present in many buffalo herds. Many serologic researches demonstrate that the buffalo population has antibodies against several *Leptospira* spp. (Eaglesome and Garcia, 1992). 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 *L. Pomona*, *L. canicola* and *L. hardjo* serotypes are also found in several foetal buffalo kidneys (Galiero, 2007). The isolation of *Leptospira spp.* from the semen is not easy; therefore the micro-agglutination test has to be used even if it does not allow distinguishing the vaccinated animals from the infected ones. Because of these problems, bulls should not be vaccinated.

10. Chlamydophilosis

Chlamydiae are members of the family *Chlamydiaceae*, a group of obligate intracellular bacteria. Their developmental cycle comprises two forms: infecting elementary bodies and non-infecting reticular bodies. The former are small and metabolically inert and penetrate the host cell by means of endocytosis. The reticular bodies are metabolically active and replicate by means of binary fission inside an endosome. Two genera are recognized on the basis of ribosomal RNA analysis: *Chlamydophila* spp. and *Chlamydia* spp. Some of the species that cause chlamydiosis are zoonotic: *Chlamydophila abortus*, *Chlamydophila psittaci*, *Chlamydophila felis* and *Chlamydophila pneumoniae*, while others are not: *Chlamydophila caviae*, *Chlamydophila pecorum*, *Chlamydia suis*, *Chlamydia muridarum* and *Chlamydia trachomatis*. Ruminants can be infected by two species: *Chlamydophila abortus* and *Chlamydophila pecorum*. *Chlamydophila abortus* causes abortion in small ruminants. This pathogen is also deemed to be responsible for abortion in buffaloes. Abortion, which may even become epidemic, occurs in the second half of pregnancy. The *Chlamydophila abortus* infection may cause abortion and hypo-fertility. *Chlamydophila pecorum* has long been recognized as the etiological agent of encephalomyelitis in buffalo calves. Recent studies conducted by means of molecular biology techniques on positive foetal tissues from archives have enabled the species involved to be typed as *pecorum*. It can therefore now be claimed that *Chlamydophila pecorum* is the main agent responsible for abortion in buffalo cows, as well as for encephalomyelitis (Galiero, 2007). 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 leukocytes 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.

11. Mycobacteriosis

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 intra-dermal test and, if necessary, the comparative intra-dermal test. Blood based assays which have been developed

for use in conjunction with the tuberculin test include Gamma interferon test, ELISA and PCR.

12. Trichomoniasis

Trichomoniasis is caused by *Trichomonas foetus*, pyriform 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 (Eaglesome and Garcia, 1992). This suggests 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 diluted 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 exclude any infection.

13. Mycoplasmosis

Various workers have been reported the recovery of mycoplasma from bovine semen. It is established fact that mycoplasma is common in cattle and buffalo bull semen and survives during semen processing, freezing and storage. The survivals of mycoplasma in frozen semen at -196°C for 18 months have been reported. Mycoplasma cause granular vulvo-vaginitis and damage the inner lining of the oviduct in females, while impaired infertility in males. The role mycoplasma species especially, *bovigenitalicum* and *agalactiae* and the frequency with which they are associated with seminal vesiculitis in male and metritis and salpingitis in female.

14. Fungi (Moulds and Yeasts) Infections

Several genera of fungi have been cultured from raw and extended semen and preputial washings. These fungi may contribute to reproductive failure under certain condition. Their source may be semen or anatomical loci within the male or female reproductive tract or contaminated semen collection equipments. *Candida trapicalis*, *Candida stellatoidea*, C. *albicans*, *Torulopsis femata* and *Aspergillus fumigatus* have been isolated prominently by culture isolation and *Aspergillus* sp., *Fuzerium* sp., *Penicillum* sp., *Mucor* sp., with were isolated by staining examination from buffalo semen and found to be associated with reduced fertility (Kodagali, 1979). Management practices leading to lowered resistance, feed fortified with antibiotics and nutritional disorders are supposed to make liable for mycotic infection.

15. *Escherichia coli* Infections

E. coli is a gram-negative, non-endospore producing and facultative aerobic bacillus. Its antigenic structure comprises the lipopolysaccharides of the cell wall (O antigen), the polysaccharides of the capsule (K antigen) and the flagellar and fibrillar proteins (H and F antigens, respectively). Although about 50,000 serotypes have been identified, only a limited number of strains are able to cause disease. The pathogenic action is linked to the ability of the clone to produce so-called virulence factors, which may be either structural (flagellae, capsule, lipopolysaccharides, adhesins or secreted (cytotoxic and cytotonic toxins, haemolysins). A wide variety of different serotypes of *E. coli* can be found in buffalo herds. Many of these are pathogenic in newborns, such as enterotoxaemic and enterohaemorrhagic *E. coli*, which produce heat-stable toxins, verocytotoxins and necrotising cytotoxic factor. The buffalo is an important reservoir of verocytotoxic *E. coli* serotypes, especially O157. *E. coli* may also cause abortion, albeit sporadically. Till date, it is not certain whether abortion is caused by the bacterium and its structural antigens or by the cytolytic action of its toxins. The diagnosis is based on the serological examination of the foetal organs. *E. coli* develops in MacConkey agar medium, fermenting lactose to produce reddish-pink colonies. Any haemolytic activity can be evaluated by means of blood agar. PCR is a useful tool in detecting, from isolated strains, the gene sequences responsible for coding virulence factors or toxins (Galiero, 2007).

16. Miscellaneous Micro-organisms

The other micro-organisms that involved in infectious infertility in buffaloes are *Usteria monocytogenes*, *Pseudomonas aeruginosa*, *Baci1lus licheniformis*, *Streptococcus* spp., *Staphilococcus* spp., *Proteus* spp., *Aeromonas* spp., yeast and moulds. Fungal infections may develop during processing of semen for preservation. Fungal infections of prostate and other accessory sex glands may result in presence of fungal infections in semen, which reduces quality of semen. The principal part of contamination of semen consists of saprophytic or opportunistic organisms from prepuce and upper parts of genital organs. 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 sterilized tools and materials, and cleaning constantly the bull's preputial cavities. In spite of these preventive measures, sometimes the ejaculate presents a not specific microbial flora (5×10^6 CFU ml^{-1}). These concentrations may be reduced by 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: 8×10^2 CFU ml^{-1} (considered normal) (Visintin et al., 1997). A permissible count of 500 bacteria per dose of semen straw is the international standard. Accordingly, it is recommended that preputial washings (maximum 50,000 count) and neat semen (maximum 1,000-5,000 count) should carry lowest

minimum bacterial load with no count for artificial insemination (AI) equipments and dilutors as they are to be used only after strict sterilization (Visintin et al., 1997). Liquid nitrogen used for storage of frozen semen doses may act as vehicle for contaminant pathogens with variety of organisms and at various degrees. The same thus serves as source of infection to cows and buffalos during AI. Even fresh liquid nitrogen carries *Staphylococcus aureus*. Certain bacterial contaminants acquire a level of resistance to antibiotics and they are able to survive at -190^{o}C in liquid nitrogen (Ronald and Prabhakar, 2001).

17. Conclusion

The breeding soundness evaluation (BSE) plays an important step in selection of male buffalo for the purpose of artificial insemination. In buffalo breeding management program, the infectious agents play a critical role in determining the reproductive efficiency in buffalo bull. The infectious agents have spread to cows and calves during artificial insemination will cause congenital defect, malformation of the fetus and death of the offspring. To avoid the infectious diseases in buffalo bulls, careful screening of the reproductive as well as other system of the bulls before breeding program. The breeding soundness evaluator should take care of infectious and disease condition of buffalo bulls, used for breeding program.

18. References

Afshar, A., Eaglesome, M.D., 1990. Viruses associated with bovine semen. Veterinary Bulletin 60, 93-99.

Ahmed, K., Mohan, G., 2001. Effect of antibiotics on the bacterial load and quality of semen of Murrah buffalo bulls during preservation. Indian Journal of Animal Science 22, 79-80.

Aitken, R.J., 1995. Free radicals, lipid peroxidation and sperm function. Journal of Reproduction and Fertility 7, 659-668.

Andrews, A., Pereira, H.G., Wildy, P., 1978. Virus of Vertebrates (4th Edn.). Baillere Tindall, London, 171.

Balakrishnan, G., Saravanabava, K., Dorirajan, N., 2006. Microbial quality and antibiotic sensitivity pattern of buffalo semen. Indian Veterinary Journal 83, 1225-1226.

Binda, D.S., Pangaonkar, G.R., Matharoo, J.S., 1994. Effect of preputial washing on semen quality of buffalo bulls. Indian Journal of Animal Reproduction 15, 75-76.

Bisson, J.P., Czyglick, F., 1974. Retentissement de I" infection genitor-urinaire sur les spermazoides. Journal of Urology and Nephrology 8, 631-635.

Chantalakhana, C., Falvey, L., 1999. Small holder dairying in the tropics. International Livestock Research Institute, Nairobi, Kenya, 462.

Cortez, A., Heinemann, M.B., Alfieri, A.A., Medici, K.C., Alfieri, A.F., Oliveira, D.B., Meyer, A.D., Soares, R.M., Sakamoto, S.M., Amaral, R., Baruselli, P.S., Fujji, T., Richtzenhain, L.J., 2001. Comparação das tecnicas de ELISA indireto de soroneutralizão da detecção de anticorpos contra o BHV-1 em amostras de soro bubalino (*Bubalus bubalis*). Brazilian Journal of Veterinary Research and Animal Science 38, 146-148.

Das, A.M., Paranjape, V.L., 1987. Association of *Vibryo* like organism in abortion in buffaloes. Indian Journal of Animal Science 57, 408-410.

De Carlos, E., Re, G.N., Letteriello, R., Del Vecchio, V., Giordanelli, M.P., Magnino, S.,Fabbi, M., Bazzocchi, C., Bandi, C., Galiero, G., 2004. Molecular characterization of a field strain of bubaline herpevirus isolated from buffaloes (*Bubalus bubalis*) after pharmacological reactivation. Veterinary Record 154, 171-174.

Diemer, T., Weidner, W., Michelmann, H.W., Sciefer, H.G., Rovan, E., Mayer, F., 1996. Influence of *E.coli* on mortality parameters of human spermatozoa *in vitro*. International Journal of Andrology 19, 271- 277.

Eaglesome, M., Garcia, M., 1992. Microbial agents associated with bovine genital tract infections and semen, Part I: *Brucella abortus*, Leptospira, *Campylobacter fetus* and *Tritrichomona foetus*. Veterinary Bulletin 62, 743-775.

Ferreira, M.S., Fernandes, L.C., Pfrimer, I.A.F., Pichiteli, C.R., Tambourgi, D.V., de Souza Lino (Jr), R., Carvalhaes, M.S., 2010. *Lagochilascaris minor*: susceptibility and resistance to experimental infection in mice is independent of H-2^{a} Haplotype and correlates with the immune response in immunized animals. Journal of Parasitology Research 2010, 8. doi:10.1155/2010/610457.

Galiero, G., 2007. Causes of infectious abortion in the Mediterranean buffalo. Italian Journal of Animal Science 6, 194-199.

Gangadhar, K.S., Ramamohana Rao, A., Krishnaswami, S., Umamaheswara Rao, S., 1986. Bacterial and fungal types and their load in the frozen semen of buffalo bulls. Indian Veterinary Journal 63, 48-53.

Gibbs, E.P.J., Rweyemamu, M.M., 1977. Bovine herpes viruses, Part I: Bovine herpes virus 1. Veterinary Bulletin 47, 317-343.

Griveau, J.F., Domount, E., Renard, P., Challegani, J.P., Lelannou, D., 1995. Reactive oxygen species lipid peroxidation and enzymatic defense system in human spermatozoa. Journal of Reproduction and Fertility 103, 17-26.

Huwe, P., Diemer, T., Ludwig, M., Liu, J., Schiefer, H.G., Weidner, W., 1998. Influence of different uropathogenic microorganisms on human sperm motility parameters in an in vitro experiment. Andrologia 30, 55-59.

Jaisal, S., Katoch, R.C., Chachara, D., Mahajan, A., 2000. Evaluation of bacterial load in fresh ejaculates of bovine and buffalo bull semen in Himachal Pradesh. Indian Journal of Animal Science 70, 465-467.

Joshi, K., Sharma, N.S., Jand, S.K., Oberoi, M.S., 2006. Prevalence of *Campylobacter fetus* in cattle and buffalo breeding bulls in Northern India. Indian Journal of Animal Science 76, 609-611.

Kodagali, S.B., 1979. Prevalence of fungi in buffalo semen. Indian Veterinary Journal 56, 807-809.

Makler, A., Urbach, Y., Lefler, D.S., Merch, D., 1981. Factors effecting sperm motility VI: sperm viability under the influence of bacterial growth in human ejaculates. Fertility and Sterility 35, 666-670.

Martinez, J.L., Baquero, F., Andersson, D.I., 2007. Predicting antibiotic resistance. Natural Review Microbiology 5, 958-965.

Modulo, J.R., Bisping, W., Lopes, C.A.M., Gottschalk, A.F., Fava, C.D., 1997. Characterization of *Campylobacter* in genitals of buffalo bulls. Indian Journal Animal Science 67, 682-683.

Ochsendrof, F.R., 1998. Infection and reactive oxygen species. Andrologia 30, 81-86.

Pastoret, P.P., Thiry, E., Brochier, B., Derboven, G., 1982. Bovid herpes virus 1 infection of cattle: pathogenesis, latency, consequences of latency. Annales de Recherches Veterinaires 13, 221- 235.

Perera, B.M.A.O., Abeygunawardena, H., Vale, W.G., Chantalakhana, C., 2005. Livestock and wealth creation-improving the husbandry of animals kept by poor people in developing countries. In: Buffalo Livestock Production Program. Natural Resources International Ltd., United Kingdom, 601.

Radostis, O.M., Gay, C.C., Blood, D.C., Hinchcliff, K.W., 2003. Veterinary Medicine (9th Edn.). WB Saunders Elsevier Science Ltd., 867-891.

Ramaswamy, V., Latha, N., Gnanasubramanian, T., Manickam, R., 2002. Aerobic bacteria in buffalo semen and their metabolism. Indian Journal of Animal Reproduction 23, 117-119.

Ranold, B.S.M., Prabhakar, T.G., 2001. Bacterial analysis of semen and their antibiogram. Indian Journal of Animal Science 71, 829-831.

Rao, A.R., 1997. Reproductive Disorders in Indian Livestock (1st Edn.). Indian Council of Agricultural Research, New Delhi, India, 128.

Rathnamma, D., Rao, M.S., Ramanatha, K.R., Raghavan, R., 1997. Assessment of bacterial load in semen of Holstein Friesian bulls. Current Research 26, 205-207.

Schirren, C., Zander, H.A., 1966. Genitalinfectionen des Mannes und ihre Auswirkungen auf die spermatozoenmotilitat. Medizinische Welt 45, 45-47.

Sharma, R.K., Pangawkar, G.R., Matharoo, J.S., 1994. Efficacy of certain antibiotics on the bacterial load of buffalo semen during cryopreservation. Indian Journal of Animal Science 64, 468-470.

Shukla, M.K., 2005. Correlation of microbial load of cryopreserved semen with quality of neat and cryopreserved Murrah buffalo bull semen. Buffalo Bulletin 24, 84-87.

Singh, R.S., Tomar, N.S., Sharma, K.C., Sharma, K.B., 1992. Studies on acrosomal abnormalities of cattle and buffalo spermatozoa in relation to other semen characteristics and fertility. Indian Veterinary Journal 69, 267-268.

Van Oirschot, J.T., Straver, P.J., Van Lieshout, J.A.H., Quak, J., Westenbrink, F., Van Exsel, A.C.A., 1993. A subclinical infection of bulls with bovine herpes virus type 1 at an artificial insemination center. Veterinary Record 132, 32-35.

Visintin, J.A., Assumpcao, M.E.O.A., Baruselli, P.S., Mello, M.R.B., Tavares, L.M.T., 1997. Use of biotechnologies in buffalo bred in Brazil. In: Proceedings of 3rd Course on Biotechnologies of Reproduction in Buffaloes, Caserta, Italy, October 6-10, 217-235.

Wierzbowski, S., Nowakowski, W., Wayda, E., Kuzniak, S., 1984. Antibiotic level and bacterial contamination of frozen bull's semen. Medyeyay-Westerynaryjna 40, 284-287.

Yadav, V., Sharma, A., Sharma, A., 2007. Detection of FMD virus type specific IgG1, IgG2 and IgA antibodies in milk and serum of buffaloes vaccinated with oil adjuvant polyvalent FMD vaccine. Italian Journal of Animal Science 6, 869-871.